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# Temperature-controlled ionic liquid dispersive liquid–liquid microextraction combined with fluorescence detection of ultra-trace Hg<sup>2+</sup> in water†

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Significant efforts have been devoted to developing trace-level quantification of Hg<sup>2+</sup>. In this work, a novel fluorescence detection (FD) approach combined with preconcentration of Hg<sup>2+</sup> in ionic liquid (IL), *N*-octylpyridinium tetrafluoroborate ([OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>−</sup>), was developed for the determination of trace Hg<sup>2+</sup> in water. The temperature-controlled ionic liquid dispersive liquid–liquid microextraction (TC-IL-DLLME) technique was used to improve the enrichment factor. After extraction and centrifugal separation, precipitated IL was diluted with water and acetonitrile containing the fluorescent probe. Under optimum conditions, the photoluminescence intensity of the fluorescence emission peak was found to be linear with the concentration of Hg<sup>2+</sup> in the range from 0.1 to 0.5 μg L<sup>−1</sup>. The limit of detection for the TC-IL-DLLME-FD method was calculated to be 0.0342 μg L<sup>−1</sup> (S/N = 3). Further, the selectivity of the sensor was measured by evaluating its response to other heavy-metal ions and was shown to be excellent. Recoveries were assessed by spiking water samples with different Hg<sup>2+</sup> standard stock solutions, giving satisfactory recoveries from 80 to 110%.

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## 1. Introduction

The mercury ion (Hg<sup>2+</sup>), once ingested by organisms or human beings, is highly hazardous, leading to organ damage, nervous system disorders, cognitive deterioration, and even death.<sup>1,2</sup> There is strong evidence that Hg<sup>2+</sup>, even at trace levels in soil and water, is potentially risky due to its extremely high bioaccumulation factor (up to 10<sup>6</sup>) in the food chain.<sup>3</sup> According to the United States Environmental Protection Agency (US-EPA) regulation, the Hg<sup>2+</sup> ion in potable water should be under 2 μg L<sup>−1</sup>.<sup>4</sup> As a result, accurate and highly sensitive sensors of trace Hg<sup>2+</sup> are in high demand. Various methods have been reported for the detection of Hg<sup>2+</sup> in the ng L<sup>−1</sup> range, among which atomic absorption spectrometry (AAS),<sup>5,6</sup> atomic fluorescence spectrometry (AFS),<sup>7–9</sup> and inductively coupled plasma

mass spectrometry (ICP-MS)<sup>10,11</sup> are popular methods. However, these methods require very expensive, complex and bulky instrumentation. Therefore, novel strategies that provide low-cost, simple and selective detection of trace-level Hg<sup>2+</sup> have been an important research focus in recent years.

Many strategies were developed for mercury analysis, such as electrochemical sensing,<sup>12</sup> capillary electrophoresis<sup>13,14</sup> and optical sensing.<sup>15,16</sup> For optical sensing, fluorescence-based detection with Hg<sup>2+</sup>-responsive probes represents a promising approach for simple, low-cost, rapid, and selective monitoring of the mercury ion in water samples.<sup>17</sup> It has been extensively applied for the determination of heavy metal ions, such as copper, lead, cadmium and mercury.<sup>18,19</sup> The designed fluorescent probes are mainly based on rhodamine,<sup>20</sup> boron-dipyrromethene,<sup>21</sup> thymine,<sup>22</sup> noble metal nanoparticles,<sup>23</sup> and quantum dots.<sup>24</sup> Chen *et al.* proposed novel Hg<sup>2+</sup> sensors using tetraphenylethene derivatives as donors and a rhodamine core as the acceptor with high energy transfer efficiency.<sup>25</sup> However, the detection limit of 1 μg L<sup>−1</sup> with the *para*-position-tetraphenylethene–rhodamine derivative (PTR) probe was insufficient to meet the requirement for environmental monitoring.

A practical path to achieve better sensitivity and selectivity for the determination of ultra-trace Hg<sup>2+</sup> is to introduce sample extraction prior to fluorescence detection. Jin *et al.* succeeded in fluorescence detection of Hg<sup>2+</sup> by designing a bifunctional fluoroionophore–ionic liquid hybrid with a synergistic extraction

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effect.<sup>26</sup> The achievable detection limit was 160 ng L<sup>-1</sup> by liquid–liquid extraction with the aqueous-to-ionic liquid phase volume ratio of 10 : 1. In comparison, dispersive liquid–liquid microextraction (DLLME), as a sample-preparation technique, exhibits simpler operation, provides higher enrichment (three orders of magnitude) and requires smaller amounts of solvents, and lower cost.<sup>27,28</sup> At the same time, it is facile to combine DLLME with many detection techniques, such as capillary electrophoresis,<sup>13,14</sup> electrochemical methods,<sup>29</sup> fluorescence analysis<sup>30</sup> and gas chromatographic-mass spectrometry.<sup>31</sup> Therefore, it has been widely used as an attractive choice for preconcentrating very low-concentration metal ions and metalloids.<sup>32–34</sup> It is important to select an appropriate extraction solvent in order to achieve efficient extraction. However, the extraction solvents used in DLLME are often toxic, flammable and environmentally damaging.

Ionic liquids (ILs) are nonvolatile, nonflammable, thermally stable, and environmentally friendly solvents, which are applied in both solid-phase microextraction and DLLME.<sup>35,36</sup> They are promising replacements for traditional organic solvents,<sup>37</sup> especially for the extraction of metal ions.<sup>38–40</sup> In the past few years, the use of ILs as extraction solvents for the preconcentration of metals in DLLME with a high enrichment factor has attracted considerable attention.<sup>41,42</sup> Our group first studied dispersive liquid–liquid microextraction of Hg<sup>2+</sup> with four kinds of ILs with *N*-octylpyridinium cations ([OPy]<sup>+</sup>).<sup>29</sup> Temperature-controlled dispersive liquid–liquid microextraction (TC-DLLME) is based on the solubility change of ILs with temperature, as first reported by Majid<sup>43</sup> and Qingxiang.<sup>44</sup> And TC-IL-DLLME was employed to trap Hg<sup>2+</sup> with a high enrichment factor (344) and high selectivity against Zn<sup>2+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup>. However, as for electrochemical stripping voltammetry determination of Hg<sup>2+</sup>, ILs were diluted with organic media 20 times in order to liberate mercury ions, which greatly reduced the whole enrichment factor (17.2).

Based on that work, this paper presents a hyphenation technique by combining a high preconcentration technique with a sensitive and selective detection technique for ultra-trace Hg<sup>2+</sup> determination in water. To our knowledge, extracting ultra-trace mercury with ILs followed by fluorescence detection (FD) in the organic and aqueous phase has not been reported in the literature. Liquid *N*-octylpyridinium tetrafluoroborate ([OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup>) at room temperature was found to be an efficient and selective extractant for the mercury ion. PTR<sup>25</sup> with excellent selectivity for Hg<sup>2+</sup> was chosen as a model fluorescent probe. In this TC-IL-DLLME-FD protocol, Hg<sup>2+</sup> in water sample was first trapped in [OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> by TC-IL-DLLME. After separation, Hg<sup>2+</sup> within the [OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> was then mixed with the PTR probe solution. Finally, the fluorescence response of the PTR probe upon addition of various trace concentrations of Hg<sup>2+</sup> was found to be in good linearity.

## 2. Experimental

### 2.1 Chemicals and instruments

[OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> was purchased from Shanghai Chengjie Chemical Co., Ltd. Mercury standard stock solution (100 mg L<sup>-1</sup> Hg<sup>2+</sup> in

3% nitric acid) was purchased from the China National Research Centre for Certified Reference Material. Wahaha (Hangzhou, China) deionized water was used throughout this work. Acetonitrile was of HPLC grade (Fisher Scientific, Hampton, USA). Other reagents including HgCl<sub>2</sub> and absolute ethanol were of analytical grade and used as received. The ultraviolet-visible (UV-vis) absorption and photoluminescence (PL) spectra were recorded on a UV SP-752 spectrophotometer (Shanghai, China) and Hitachi F-4600 FL Spectrophotometer (Tokyo, Japan), respectively. The Zonkia HC-3018 high speed centrifuge (Hefei, China) was used for separating the IL phase and water phase. The trace-level mercury in the water phase after centrifugation was measured by atomic fluorescence spectrometry (AFS). The mercury concentrations of prepared mercury solutions were examined with ICP-MS or AFS in the Pony Testing International Group. All the experiments were operated at room temperature without specific instructions.

### 2.2 Procedures of TC-DLLME and separation

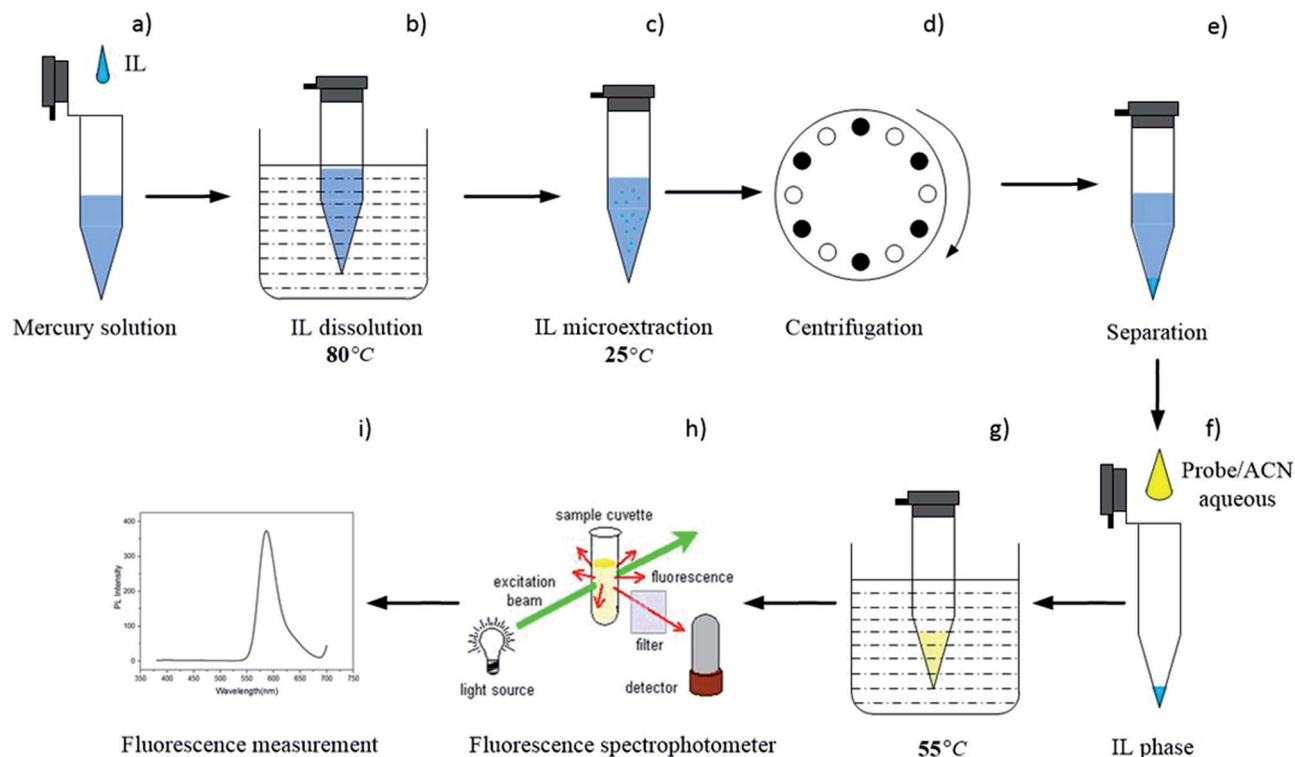
Initially, 1 mL [OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> (2.4% mass ratio) was added into 45 mL water to reach saturation, during which the 50 mL centrifuge tubes were water bathed at 80 °C and continuously shaken until complete dissolution. Then the tubes were cooled naturally to room temperature along with the rapid formation of cloudy solutions. A large quantity of fine [OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup> droplets with enriched Hg<sup>2+</sup> formed and dispersed in water. After centrifugation at 3000 rpm for 30 minutes, the water/IL micro emulsions were separated into the aqueous and IL phase.

### 2.3 Fluorescence detection

For the determination of Hg<sup>2+</sup>, the separated IL phase with enriched Hg<sup>2+</sup> was dissolved in acetonitrile (ACN) solution containing 10 μM PTR. The conditions were optimized, including the composition of the test solution and the reaction time. The mixture was water bathed at 55 °C for a controlled amount of time and then quickly transferred to a 1 cm quartz cuvette. The wavelength scan speed was set at 2400 nm min<sup>-1</sup>, so it only took 3.75 seconds from 530 nm to 680 nm for one fluorescence measurement. The emission spectra were recorded at the excitation wavelength of 355 nm. Only 200 μL was required to do each fluorescence measurement. After each measurement, the quartz cuvette was cleaned with absolute ethanol and deionized water and dried with nitrogen gas. Each measurement was repeated at least three times.

### 2.4 Mercury solution test procedures

For the preconcentration and fluorescence tests of mercury, initial mercury stock solutions were prepared in deionized water at different concentrations and qualified by ICP-MS or AFS. The entire procedure is presented in Fig. 1. First, 1 mL of IL was added into 45 mL of mercury solution and saturated IL solution developed at 80 °C. As the solution cooled down to room temperature, Hg<sup>2+</sup> was extracted in IL microdroplets. The emulsion was then centrifuged at 3000 rpm for 30 minutes with IL as the residue at the bottom of the centrifuge tube. The supernatant was pipetted off carefully, leaving 125 μL of IL



**Fig. 1** Graphical illustration of the TC-IL-DLLME-FD method. The mercury solution (a) dissolved IL achieving saturated IL aqueous solution at 80 °C (b); then temperature decreased to 25 °C (c) with IL droplets suspending in the emulsion. After (d) centrifugation and (e) separation, (f) the IL phase was mixed with ACN and aqueous solution containing the PTR probe. (g) After adequate reaction at 55 °C, (h) mercury ions were quantified through a fluorescence spectrophotometer, generating (i) the fluorescence spectrum.

residue. Then, 375  $\mu\text{L}$  of ACN and aqueous solution containing 10  $\mu\text{M}$  PTR was injected into the dilute IL phase at a dilution ratio of 3. After adequate reaction between PTR and  $\text{Hg}^{2+}$ , fluorescence measurement was performed and recorded.

### 3. Results and discussion

#### 3.1 Preconcentration

For the TC-IL-DLLME method, IL acted as an extractant in a very small volume. When the solution cooled down with rapid dispersion of the immiscible IL in water, fine droplets formed. Not only was the contact area between the IL and mercury extremely large, but also the microextraction process was very fast. The enrichment factor (EF), which is defined as the ratio between the final concentration of analytes in the extractant phase and the initial concentration of analytes in the aqueous sample, is calculated as:

$$\text{EF} = \frac{C_{\text{in}} - C_{\text{fin}}}{C_{\text{in}}} \times \frac{V_{\text{aq}}}{V_{\text{IL}}}$$

where  $C_{\text{in}}$  is the concentration of mercury in the initial solution,  $C_{\text{fin}}$  is the concentration of mercury in the final aqueous phase,  $V_{\text{aq}}$  is the volume of the aqueous phase, and  $V_{\text{IL}}$  is the volume of the settled IL phase.  $C_{\text{in}}$  and  $C_{\text{fin}}$  are measured by ICP-MS and AFS. The maximum enrichment factor (EF<sub>max</sub>, theoretically) of the TC-IL-DLLME method without dilution was about 360 due to the large phase ratio of the sample volume and IL volume (45

$\text{mL} : 125 \mu\text{L}$ ). But not the total mercury in the sample can be extracted into the IL phase. The microextraction took several minutes and the average EF was calculated to be 252.5 and the extraction efficiency was 70.14%.

#### 3.2 Microextraction mechanism

In contrast to standard DLLME, no dispersive solvents (*e.g.*, acetone<sup>7</sup> or ethanol<sup>45</sup>) and complexing agents (*e.g.*, ammonium pyrrolidinedithiocarbamate (APDC)<sup>46</sup> or 2-mercaptoethanol (2-ME)<sup>9</sup>) were used in this method. Since  $\text{Hg}^{2+}$  could not be back-extracted from  $[\text{OPy}]^+[\text{BF}_4]^-$  by strong acids, even in the presence of 1 M hydrochloric, sulfuric, nitric and perchloric acid, the extraction for  $\text{Hg}^{2+}$  would not be affected.

In order to investigate the interaction between  $[\text{OPy}]^+[\text{BF}_4]^-$  and  $\text{Hg}^{2+}$ , the UV-vis spectral characteristics of 0.1% IL and 0.1% IL and 0.1%  $\text{Hg}(\text{NO}_3)_2$  were monitored and compared from 200 to 300 nm. As shown in Fig. 2, there are two characteristic absorption bands, one at 210 nm and the other at 259 nm, reflecting the presence of the pyridinium ring.<sup>47</sup> The pyridine ring in the cation is the only site that may coordinate with metal ions. However, no difference in UV-vis absorption spectra at the 259 nm peak was observed between these two solutions. In addition, the difference value of these two curves from 200 nm to 240 nm was in accordance with the UV absorption spectrum of the nitrate ion.<sup>48</sup> So  $\text{Hg}^{2+}$  and the

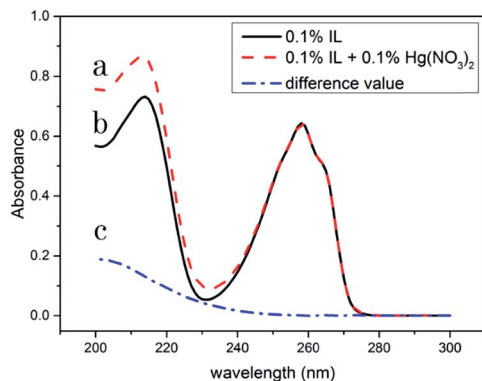


Fig. 2 UV-vis absorption spectra of (a) 0.1% IL and (b) 0.1% IL and 0.1%  $\text{Hg}(\text{NO}_3)_2$ . (c) Difference value.

pyridine ring existed independently in the homogeneous solution without interaction.

As IL was oversaturated in the mercury solution, the mercury concentration in the aqueous phase decreased, which means  $\text{Hg}^{2+}$  was extracted by microdroplets of pure IL. It is assumed that pure IL in the form of neutral molecule clusters can extract mercury efficiently. Therefore, the binding force with  $\text{Hg}^{2+}$  only lies within the cluster structure of IL.

### 3.3 Optimization of the fluorescence detection

In fluorescence detection, IL with enriched  $\text{Hg}^{2+}$  was diluted to liberate  $\text{Hg}^{2+}$ . Parameters that would affect the reaction of  $\text{Hg}^{2+}$  and the PTR probe were further studied to improve the DLLME-FD method. For optimal experiments, solid  $\text{HgCl}_2$  was directly dissolved in IL as a replacement for separated IL with enriched  $\text{Hg}^{2+}$ . For fluorescence tests of mercury ions, initial mercury solutions with different concentrations were prepared in  $[\text{OPy}]^+[\text{BF}_4]^-$  by stepwise careful dilution.

**3.3.1 Effect of detection solution system.** As for the detection system, IL with  $10 \mu\text{M}$   $\text{Hg}^{2+}$  was first dissolved into ACN with  $10 \mu\text{M}$  PTR probe as the detection solution. A series of solutions with different volume ratios of IL from 1% to 50% were studied. Also, the same test solutions without  $\text{Hg}^{2+}$  were prepared as the blank solutions. The volume ratio of IL (IL%) means the volume percentage of IL in the test solution. Each test solution was transferred into a quartz cuvette for fluorescence detection. However, there was almost no difference in photoluminescence (PL) spectra between mercury solutions and blank solutions at the same IL%. All the test solutions were stored in airtight and moisture-free centrifuge tubes away from light. However, 16 days later, the PL intensity of the mercury solution with 1% IL increased dramatically (Fig. S1<sup>†</sup>), while PL spectra of all the other solutions were almost unchanged. It was evident that the PL intensity could be enhanced by lowering the volume ratio of IL and extending the reaction time.

Thus,  $10 \mu\text{M}$  mercury solutions with lower IL% (less than 5%) were prepared and mixed with ACN solution containing  $10 \mu\text{M}$  PTR probes. Fig. 3 depicts the PL intensity of mercury solutions at the emission wavelength of 586 nm within 60 hours. As the experiment continued, the PL intensity of all

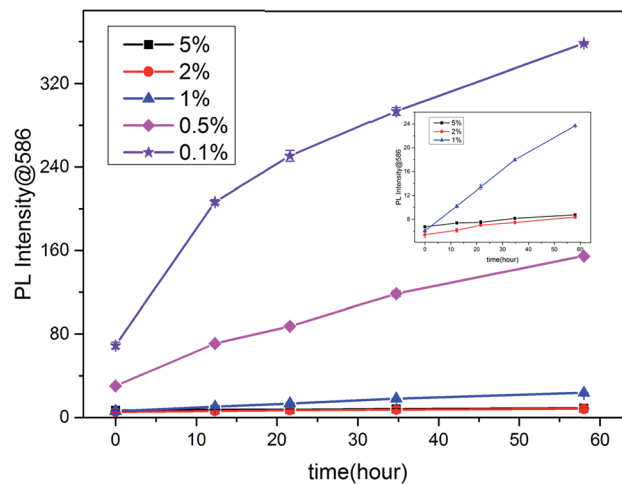


Fig. 3 PL intensity of  $10 \mu\text{M}$  PTR and  $10 \mu\text{M}$  mercury solutions with different IL% and ACN.

mercury solutions increased progressively, particularly for 0.1% IL. There was no obvious increase for 2% IL and 5% IL after 36 hours. The PL intensity gradually rose as the volume ratio of IL decreased down to 0.1% IL. It appeared that IL clusters partly existed in ACN, which prevented mercury ions from taking part in the fluorescence reaction. It was demonstrated that the reaction between PTR and  $\text{Hg}^{2+}$  was slow and related to the composition of solvents, which was consistent with the result of the electrochemical method.<sup>29</sup> However, IL% should be as large as possible, taking the dilution ratio in fluorescence detection into consideration. In order to accelerate the reaction and increase IL%, two strategies were adopted: one was to raise the temperature of the reaction solution and the other was to introduce water into the solvents to promote the liberation of  $\text{Hg}^{2+}$  from the IL.

**3.3.2 Effect of volume ratio of IL and water fraction.** The water fraction means the volume percentage of water in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  mixtures. First,  $10 \mu\text{M}$  mercury solutions with 15% IL and 30% water fraction were prepared. As displayed in Fig. S2,<sup>†</sup> PL intensity continuously rose for the initial 7 hours. Also, the repeatability was very poor among those tests, which could be attributed to the different initial values upon mixing. However, after 10 hours, the PL intensity fluctuated in an acceptable range. As shown in Fig. S3,<sup>†</sup> the probe displayed very weak fluorescence at 589.2 nm in blank solutions ( $0 \mu\text{M}$   $\text{Hg}^{2+}$ ). Upon binding  $10 \mu\text{M}$   $\text{Hg}^{2+}$ , remarkable fluorescence enhancements at 589.2 nm (40-fold) occurred. The steady state was reached after 10 hours, even with different IL% (Fig. S4<sup>†</sup>), which was especially advantageous for multiple fluorescence measurements with good repeatability. Thus, the detection solution was kept in a water bath at  $55^\circ\text{C}$  for 10 hours when optimizing IL% and the water fraction.

In order to achieve a high whole enrichment factor ( $E_F$ ) and high PL intensity, IL% from 5% to 50% was studied.  $E_F$  means the final signal enhancement factor of the TC-IL-DLLME-FD protocol. So  $E_F$  was equal to the product of EF in the DLLME process and IL% in the FD process. The PL intensity difference



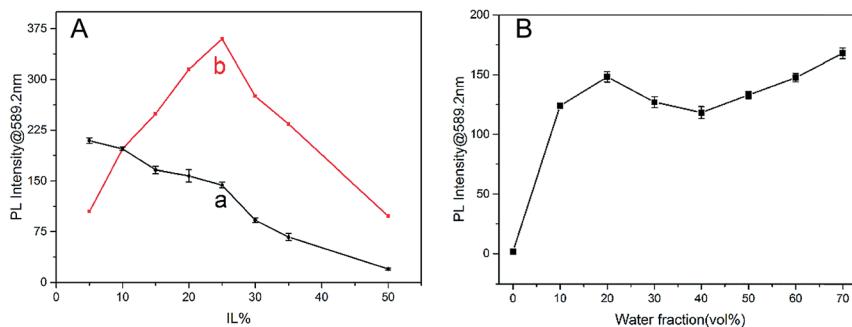


Fig. 4 Effects of (A) IL% and (B) water fraction on fluorescence detection.

between 10  $\mu\text{M}$   $\text{Hg}^{2+}$  and blank solutions with 30% water fraction is shown in Fig. 4A(a). IL showed some inhibition in the fluorescence reaction of mercury and the probe. The low IL% signified high PL intensity but low  $E_F$ . As for the final PL intensity, both should be taken into consideration. In order to display the two curves clearly in a graph, we assumed that the EF was 10 in the DLLME process, so the final PL intensity was the product of PL intensity difference and  $E_F$ . As shown in Fig. 4A(b), it was found that the fluorescence intensity rose as the IL% was increased to 25%, and then declined. The protocol exhibited the best signal enhancement at a dilution ratio of 4. Therefore, 25% IL was chosen for the detection solution. Thus, the  $E_F$  value was calculated to be 63.13.

Water fractions from 0 to 99% were also studied. As the water fraction increased, the dissolution of IL became more difficult. When the water fraction exceeded 70%, the experiment could not proceed in a controllable manner. The PL intensity difference between 10  $\mu\text{M}$   $\text{Hg}^{2+}$  and blank solutions with 25% IL is shown in Fig. 4B. When water was introduced (less than 20% water fraction) into the detection solution, it was observed that the PL intensity dramatically increased. Both 20% and 70% displayed higher PL intensity, so they were chosen for further optimization of reaction time.

**3.3.3 Effect of reaction time.** The reaction time between the PTR probe and  $\text{Hg}^{2+}$  was defined as the time interval between

mixing IL with the PTR probe solution and the fluorescence measurement. Fig. 5 depicts the influence of reaction time on the PL intensity. It was found that it is long to reach the steady state, which may be attributed to the moderate release of mercury from IL in the ACN/ $\text{H}_2\text{O}$  solution. The reaction equilibrium was reached after mercury was completely released from the cluster structures of IL. The PL intensity was nearly independent of reaction time after 5 hours. The optimum reaction time was then determined to be 5 hours. In addition, the error bars represented the standard deviation of three measurements. Clearly, 20% water fraction displayed small error bars and a stable fluorescence response compared with 70% water fraction. Thereby 20% water fraction was adopted for the following mercury detection.

### 3.4 Analytical performance

Based on the experimental conditions determined above, the PL spectrum of PTR was collected in 25% IL and 20% water fraction after a 5 hour water bath at 55  $^\circ\text{C}$ . First, the  $\text{Hg}^{2+}$  standard solutions were prepared in  $[\text{OPy}]^+[\text{BF}_4]^-$  with addition of the probe into ACN and water for fluorescence detection. As shown in Fig. S5,<sup>†</sup> a gradual increase in the PL intensity of the PTR probe was observed as the concentration of  $\text{Hg}^{2+}$  ion was

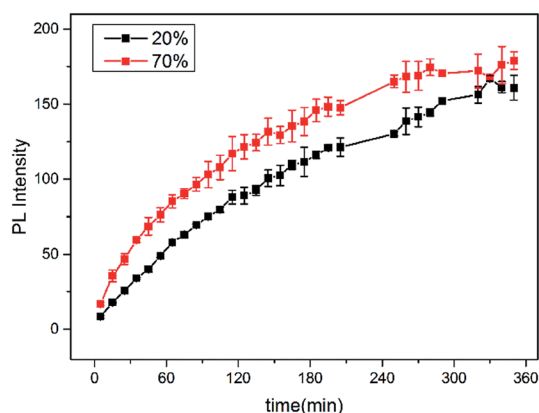


Fig. 5 PL intensity of 10  $\mu\text{M}$  PTR and 10  $\mu\text{M}$  mercury solutions with 25% IL, and water fractions of 20% and 70%, in a water bath (55  $^\circ\text{C}$ ).

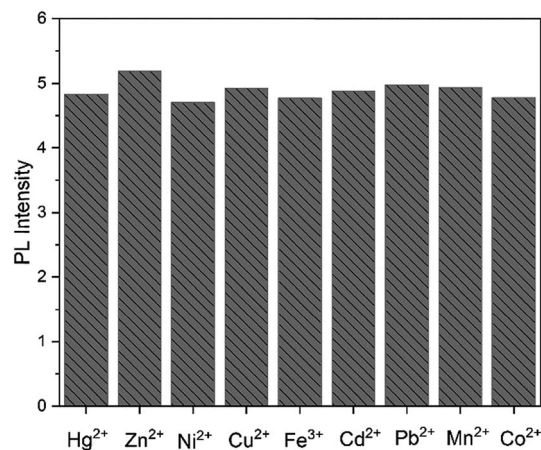


Fig. 6 PL intensity in the presence of 50  $\mu\text{g L}^{-1}$  marked metal ions followed by addition of 5  $\mu\text{g L}^{-1}$   $\text{Hg}^{2+}$  with the blank solution subtracted.

**Table 1** Analytical performances for the determination of Hg<sup>2+</sup> by the TC-IL-DLLME-FD method

Analytical performances	TC-IL-DLLME-FD method <sup>a</sup>
Linear equation	$y = 2.8213x + 10.266$
Slope	$2.8213 \pm 0.0610$
Intercept	$10.266 \pm 0.0113$
Linear range	0–0.5 $\mu\text{g L}^{-1}$
Regression correlation coefficient ( $R^2$ )	0.9981
Limit of detection ( $S/N = 3$ )	$0.0342 \mu\text{g L}^{-1}$
Limit of quantification ( $S/N = 10$ )	$0.114 \mu\text{g L}^{-1}$

<sup>a</sup> Calibration levels ( $n = 5$ ) and mean  $\pm$  standard deviation ( $n = 3$ ).

**Table 2** Analytical results of Hg<sup>2+</sup> by the TC-IL-DLLME-FD method in water samples<sup>a</sup>

Samples	Added ( $\mu\text{g L}^{-1}$ )	Certified ( $\mu\text{g L}^{-1}$ )	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)
Tap water	0	nd	$0 \pm 0.01$	—
	0.2	$0.22 \pm 0.01$	$0.24 \pm 0.05$	110%
	0.45	$0.46 \pm 0.01$	$0.48 \pm 0.06$	104.4%
Pond water	0	nd	$0 \pm 0.01$	—
	0.2	$0.23 \pm 0.01$	$0.19 \pm 0.1$	80%
	0.45	$0.49 \pm 0.01$	$0.44 \pm 0.08$	88.9%

<sup>a</sup> nd: not detectable ( $<0.01 \mu\text{g L}^{-1}$ ).

increased. The linear equations are given in Fig. S6,<sup>†</sup> in the range of 0–0.125  $\mu\text{M}$  and 0.125–1.25  $\mu\text{M}$ , respectively. Based on this finding, selectivity and analytical figures of merit of the TC-IL-DLLME-FD method were evaluated in aqueous solutions. Desirable mercury solutions for this work were progressively diluted from the standard stock solution with deionized water.

**3.4.1 Selectivity studies.** The effect of coexisting metal ions regularly found in water samples was studied together with mercury detection. In these experiments, solutions of 5  $\mu\text{g L}^{-1}$  Hg<sup>2+</sup> spiked with 50  $\mu\text{g L}^{-1}$  various metal ions were investigated to demonstrate the selectivity of the proposed TC-IL-DLLME-FD

procedure. Fig. 6 shows the fluorescence responses of 10  $\mu\text{M}$  PTR to the presence of mercury and coexisting metal ions, with PL intensity of the blank solution subtracted. The fluorescence intensity almost remained unchanged with the addition of 10-fold concentration of other competitive metal ions, including Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Mn<sup>2+</sup> and Co<sup>2+</sup>. Since Zn<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup> and Pb<sup>2+</sup> were rarely extracted into the IL phase,<sup>29</sup> and the rhodamine moiety of the PTR probe had good anti-interference against Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Pb<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup> and so on,<sup>25</sup> the TC-IL-DLLME-FD method based on the PTR probe displayed excellent selectivity for mercury detection.

**3.4.2 Analytical figures of merit.** Under optimum conditions, the proposed method was calibrated with 0–0.5  $\mu\text{g L}^{-1}$  Hg<sup>2+</sup> solutions in deionized water. Good linearity was achieved within the test range (linear regression coefficient of  $R^2 = 0.9981$ ). Error bars were ascribed to variations in manually operating microliter IL in the TC-DLLME process. This may be improved by further automation with microfluidic devices. Table 1 summarizes the analytical performance obtained by the TC-IL-DLLME-FD procedure. According to the IUPAC recommendation,<sup>49</sup> the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated as the concentration yielding a peak area equal to the blank signal plus 3- and 10-times the blank standard deviation, respectively. The LOD of Hg<sup>2+</sup> was estimated to be 34.2 ng L<sup>-1</sup>, which was low enough to satisfy the threshold limit established by the WHO and EPA. The LOD was 30 times better than that of fluorescence detection using the PTR probe under its optimum conditions.<sup>25</sup>

**3.4.3 Application to real water samples.** In order to investigate the application of the new approach for preconcentrating and determining ultra-trace Hg<sup>2+</sup> in real water samples, tap water collected in our lab and pond water collected from a nearby pond were analyzed under the optimal conditions. They were firstly spiked with 0.2  $\mu\text{g L}^{-1}$  and 0.45  $\mu\text{g L}^{-1}$  Hg<sup>2+</sup> from standard stock solutions. Then the concentration of Hg<sup>2+</sup> was certified using ICP-MS and tested with our method. The results are shown in Table 2. The recoveries of Hg<sup>2+</sup> ranged from 80% to 110%, which was satisfactory for the TC-IL-DLLME-FD method in real water samples with complex matrices.

**Table 3** Comparison of analytical performances with other IL-DLLME methods for the determination of trace mercury in aqueous samples

IL-DLLME	Extractant	Sample volume/mL	IL volume/ $\mu\text{L}$	Detection instruments	Diluent	Whole enrichment factor	Analysis time	LOD ( $\mu\text{g L}^{-1}$ )	References
TC-IL-DLLME	[OPy] <sup>+</sup> [BF <sub>4</sub> ] <sup>-</sup>	45	125	FD (PTR probe)	Acetonitrile & water	63.13	5 hours	0.034	This work
TC-DLLME	[OPy][BF <sub>4</sub> ]	45	125	Stripping voltammetry	Acetonitrile	17	6 min	0.050	29
Vortex-assisted DLLME	[Hmim][NTf <sub>2</sub> ]	10	43.8	Stripping voltammetry	HCl	20–31	4 min	0.500	46
DLLME	[HMIM][PF <sub>6</sub> ]	5	70	HPLC-DAD	Methanol	21.4	—	0.320	50
TSIL-USA-DLLME	TOMAS	10	30	CV-AAS	Ethanol	37.2	—	0.030	5

TC-DLLME: temperature controlled dispersive liquid–liquid microextraction; [Hmim][NTf<sub>2</sub>]: 1-hexyl-3-methylimidazolium bis[(trifluoromethyl) sulfonyl]imide; [HMIM][PF<sub>6</sub>]: 1-hexyl-3-methylimidazolium hexafluorophosphate; HPLC-DAD: high performance liquid chromatography with diode array detection; TSIL-USA-DLLME: task specific ionic liquid-based ultrasound-assisted dispersive liquid–liquid microextraction. TOMAS: methyltrioctylammonium thiosalicylate; CV-AAS: cold vapor-atomic absorption spectrometry.

**3.4.4 Comparison with other methods.** In comparison to other IL-DLLME methods for the determination of trace mercury in aqueous samples, the proposed method requires lower volumes of reagents and provides a higher whole enrichment factor (Table 3). In addition, the LOD in our work is better than those of most methods and comparable to that of TSIL-USA-DLLME-CV-AAS. Our work focused on exploring a higher whole enrichment factor, since it directly represented the enriched concentration before coupling with other instrumentation. Although much lower detection limits were achieved by the TSIL-USA-DLLME-CV-AAS method, it is much more expensive and complex than the proposed approach in this work.

## 4. Conclusion

In summary, a novel method based on TC-IL-DLLME-FD was proposed and experimentally demonstrated for the detection of mercury at ultra-trace levels in water. Ionic liquid, [OPy]<sup>+</sup>[BF<sub>4</sub>]<sup>-</sup>, was used for the preconcentration of ultra-trace mercury with a high enrichment factor. Effects of the volume ratio of IL, water fraction and reaction time were optimized. Due to the high enrichment capability of TC-IL-DLLME and the selective response to Hg<sup>2+</sup> by using the PTR probe, the developed method in this work provided a high whole enrichment factor, excellent selectivity and very low limit of detection. It holds great potential for the determination of ultra-trace mercury in real-world environmental samples with complex matrices.

## Conflicts of interest

There are no conflicts to declare.

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