



Effective indirect enrichment and determination of nitrite ion in water and biological samples using ionic liquid-dispersive liquid–liquid microextraction combined with high-performance liquid chromatography

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ABSTRACT

An ionic liquid dispersive liquid–liquid microextraction high-performance liquid chromatography (IL-DLLME-HPLC) method for effective enrichment and determination of nitrite ion in water and biological samples was developed. The method was based on the reaction of nitrite ion with p-nitroaniline in the presence of diphenylamine in acid media and IL-DLLME of azo product. The optimization of reaction and extraction conditions, such as kind and concentration of acid, reaction time, volume of reaction solvent, temperature, kind of extraction and dispersive solvent, volume of extraction and dispersive solvent, addition of salt, extraction and centrifugal time were studied. Under the optimal conditions, 1-octyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide IL-DLLME procedure provided high enrichment factor of 430 and good extraction recovery of 91.7% for nitrite ion. The linearity was observed in the range of 0.4–500.0 $\mu\text{g L}^{-1}$ with good correlation coefficient ($r^2 = 0.9996$). The relative standard deviations (RSDs) for five replicate measurements varied between 1.5% and 4.8%. The limit of detection of the method ($S/N = 3$) was 0.05 $\mu\text{g L}^{-1}$. The interference effect of some anions and cations was also tested. The developed method allowed achieving an excellent enrichment factor, yielding a lower LOD in comparison with other methods. Moreover, the proposed method was able to analyze nitrite ion in water and biological samples with satisfactory recovery ranged from 96.5% to 107.3%.

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1. Introduction

Nitrite ion is an important intermediate in the biological nitrogen cycle and is present in soils and surface waters due to biological denitrification, fertilizers degradation, acid water and industrial waste. However, high levels of nitrite ion can lead to a wide range of detrimental health effects [1] and ecosystem degradation [2]. On the other hand, dietary nitrite may be an essential component of host defense against many common pathogens. Nitrite ion in saliva is regarded as an important antimicrobial substance, especially for oral cavity and gastrointestinal tract [3]. It is very important to determine the content level of nitrite ion for the environment and the public health. Several analytical techniques have been proposed for the determination of nitrite ion, such as spectrophotometry [4], flow injection analysis (FIA) [5], high performance liquid chromatography (HPLC) [6], ion chromatography [7] and so on. Sample preparation plays an important role in the nitrite ion analysis, especially when isolating and preconcentrating trace object from

complex matrices. Solid phase extraction (SPE) [4], cloud point extraction (CPE) [8] and headspace liquid phase microextraction (HS-LPME) [9] were reported for isolating and enrichment of nitrite ion from some water, fruit and biological samples.

Dispersive liquid–liquid microextraction (DLLME), introduced by Assadi and co-workers in 2006, has become a powerful alternative sample preparation technique for extraction and pre-concentration of various analytes due to the advantages of short extraction time, high enrichment, ease of operation and small amounts of solvents [10]. The selection of an extraction solvent with appropriate water immiscibility and higher density than water is essential for the establishment of a DLLME procedure. Based on these criteria, CCl_4 , C_2Cl_4 , $\text{C}_6\text{H}_5\text{Cl}$, CS_2 , $\text{C}_2\text{H}_3\text{Cl}_3$, CHCl_3 and $\text{C}_2\text{H}_2\text{Cl}_4$ were used as extraction solvent to effectively extract organic pollutant including polycyclic aromatic hydrocarbons, pesticides and phenols [11–13], as well as heavy metal ions such as cadmium and lead [14,15] in water and biological samples.

Ionic liquids (ILs) are a class of ionic compounds with low melting point. In comparison with traditional organic solvents, ILs have a variety of unique physicochemical properties including negligible vapor pressures, good thermal stabilities, non-flammability and good extractabilities for organic compounds, allowing many

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of them to be sustainable green solvents in the sample preparation. Besides these, “tunable structure” is another most remarkable feature of ILs. ILs have been broadly used as extraction solvent in extraction separation techniques and reviewed as substitutes for conventional organic solvents [16,17]. The usually employed ILs in DLLME were 1-alkyl-3-methylimidazolium hexafluorophosphate [RMIM][PF₆] (R = Butyl, Hexyl, Octyl) [18–20]. Compared with ILs incorporating imidazolium cation and PF₆⁻, ILs with bis[(trifluoromethyl)sulfonyl]imide anion (Tf₂N⁻) would be more propitious to be used as extraction solvent in DLLME because of their higher hydrophobicity and lower viscosity [21,22]. Up to date, all of the IL-DLLME procedures described have been focused on the extraction and enrichment of organic compounds and inorganic cations [20,23,24], but the preconcentration of inorganic anion by ILs-DLLME has received limited application.

In this work, 1-octyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([OMIM][Tf₂N]) is synthesized and is for the first time employed as extraction solvent in DLLME combined with HPLC for determination of nitrite ion in water and biological samples. The method is based on the color reaction of nitrite with p-nitroaniline in the presence of diphenylamine in acid media and DLLME of an azo product. Various factors that influence extraction performance including reaction and extraction conditions were investigated. The comparison study between the [OMIM][Tf₂N]-DLLME-HPLC and other methods for determination of nitrite ion was performed.

2. Experimental

2.1. Reagents and standards

p-Nitroaniline and diphenylamine were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). 1-Methylimidazole was obtained from Kaile Chemical Engineering Factory (Linhai, China). 1-Octylbromide was bought from Shanghai Jingchun Reagent Co., Ltd. (Shanghai, China). Lithium bis(trifluoromethane sulfonyl)imide (LiTf₂N) was provided by Wanxiang Technology & Trade Co., Ltd. (Zhengzhou, China). Sodium nitrite was acquired from Zhengzhou First Chemical Reagent Factory (Zhengzhou, China). 1-Octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF₆]) was obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China).

Other chemical reagents including methanol, ethanol, acetonitrile and acetone were of HPLC grade. Doubly distilled water was used to prepare the mobile phase and doubly quartz sub-boiling water was used to obtain stock and working solution. Stock nitrite solution (100 mg L⁻¹) was prepared by dissolving an appropriate amount of its sodium salt in water, into which a drop of chloroform was added for stabilization of nitrite solution. The working solutions were prepared from the stock solution by suitably diluting with doubly quartz sub-boiling water. p-Nitroaniline (0.5%, m/v) and diphenylamine solution (0.4%, m/v) were prepared in ethanol.

2.2. Instrumentation

A LC-VP liquid chromatography (Shimadzu, Kyoto, Japan) with a Shimadzu LC-10AT pump, a Shimadzu SPD-10A UV-vis detector (operated at 533 nm) and a 7725i six-port injection valve with 20 μL sample loop (Rheodyne, Rohnert Park, CA, USA) was employed. A Shimadzu VP-ODS column (150 mm × 4.6 mm i.d., 5 μm particle size) and methanol/water (90:10, v/v) at a flow rate of 1.0 mL min⁻¹ were used for separation. Data acquisition and processing were accomplished with Shimadzu LC solution software. ILs were weighed with a Shimadzu AUJ220 electronic balance. A 80-1

centrifuge was bought from Huafeng Instrument Co., Ltd. (Jintan, China). The ¹H NMR spectra of [OMIM][Tf₂N] were measured using DPX-400 (Bruker, Optics Inc., Ettlingen, Germany).

2.3. Preparation of [OMIM][Tf₂N]

1-Octyl-3-methylimidazolium Bromide ([OMIM]Br) was prepared by the reaction of 0.5 mol 1-methylimidazole and 0.5 mol 1-octylbromide in 50 mL of acetonitrile in a nitrogen atmosphere at 90 °C for 48 h with stirring and refluxing. The obtained viscous [OMIM]Br was cooled and then washed with 200 mL of ethyl acetate for five times, followed by drying at 80 °C under vacuum for 24 h.

0.4 mol of [OMIM]Br was mixed with equimolar LiTf₂N in H₂O (200 mL). After decanting the upper solution, the resulting viscous liquid was washed with H₂O until no bromide ion was detected by the silver (I) nitrate titration test. The obtained [OMIM][Tf₂N] was dried at 80 °C under vacuum for 24 h. The ¹H NMR data of the product was as follows: δ, 0.86 (t, 3H), 1.25 (s, 10H), 1.77 (t, 2H), 3.84 (s, 3H), 4.15 (t, 2H), 7.77 (d, 2H), 9.10 (s, 1H).

2.4. Real samples

The tap, well and Yellow River water were collected from Zhengzhou City of Henan Province (China). Bottled purified water was purchased from the local supermarket. Overnight plain boiled water was from our laboratory. Saliva was collected from a 25-year-old woman and diluted as 1:4 with doubly quartz sub-boiling water. All samples were filtered through a 0.45 μm membrane prior to use for DLLME procedure. For recovery determinations, samples were spiked with nitrite ion at three different concentration levels and analyzed using the proposed method.

2.5. DLLME procedure

The DLLME procedure was carried out according to our previous literatures [20,25]. A 5.0 mL of water sample solution containing 20 μg L⁻¹ nitrite ion was spiked with sulfuric acid (2 mol L⁻¹, 0.3 mL), p-nitroaniline (0.5%, 50 μL) and diphenylamine (0.4%, 0.11 mL), and then the mixture solution were placed in a 10 mL glass centrifugal tube with conical bottom for 10 min. Methanol (1.0 mL) as disperser solvent, containing 22.0 μL [OMIM][Tf₂N] (0.0315 g) as extraction solvent was injected rapidly into the sample solution, and a cloudy solution consisting of water, methanol and [OMIM][Tf₂N] was quickly formed and the insoluble diazo-coupling reaction product in water phase was extracted into the dispersed fine droplets of [OMIM][Tf₂N]. After the extraction, the cloudy solution was further separated by centrifuging at 4000 rpm for 5 min, and the [OMIM][Tf₂N] phase was settled at the bottom of centrifugal tube, then removed by microsyringe and directly analyzed by HPLC.

3. Results and discussion

The proposed method for detection of nitrite ion is based on the diazotization of p-nitroaniline by nitrite in acid media, with the subsequent coupling reaction providing a colored azo product (Fig. 1), which was enriched into the extraction phase in the IL-DLLME procedure. The concentration of nitrite ion was indirectly determined from azo product. Thus, both reaction and extraction conditions would have influences on extraction efficiency. To obtain optimum reaction and extraction parameters, enrichment factor (EF) and extraction recovery (ER) were used to evaluate the extraction performance. EF was expressed as the ratio of the analyte concentration in the extraction phase (*c*_{sed}) to that in the sample phase (*c*₀). ER is defined as the percentage of total analyte amount

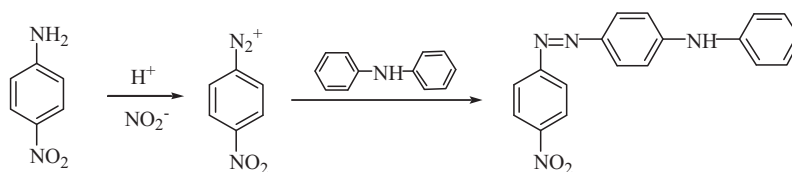


Fig. 1. Scheme demonstrating the composite diazotization coupling reaction of p-nitroaniline and diphenylamine in acid media.

extracted to the extraction phase and can be calculated according to the following equation.

$$ER = \frac{n_{\text{sed}}}{n_0} \times 100\% = \frac{c_{\text{sed}}V_{\text{sed}}}{c_0V_0} \times 100\% = EF \times \frac{V_{\text{sed}}}{V_0} \times 100\%$$

where n_0 is the initial amount of analyte in the sample, n_{sed} is the amount of analyte in the extraction phase, V_0 and V_{sed} are the volume of the sample and the sediment phase, respectively.

Triplicate extractions were performed for all experiments and the average of the results was used in plotting of curves or in tables.

3.1. Optimization of reaction conditions

The diazotization reaction of p-nitroaniline and nitrite requires a strong acidic condition, however, the coupling reaction of diphenylamine and diazonium salt is usually carried out under the weak acidic condition. Sulfuric acid, hydrochloric acid, phosphoric acid and acetic acid were tested and the best result was obtained with sulfuric acid. The effect of the concentration of sulfuric acid was also studied. Both EF and ER increased dramatically by increasing sulfuric acid up to 1.0 mol L^{-1} and remained nearly constant at higher concentration, and then decreased with concentration of sulfuric acid greater than 4 mol L^{-1} . It can be reason that the diazotization reaction was inhibited at lower acid concentration, resulting in the decrease of the extraction performance. While at higher acid concentration, the coupling reaction was influenced, inducing a decrease in extraction efficiency. To acquire high extraction efficiency and stable results, 2 mol L^{-1} of sulfuric acid was used in the subsequent experiment.

Reaction time plays an important role in diazo-coupling reaction. The effect of reaction time in the range from 2 to 14 min was investigated. A distinct increase in EF and ER was observed up to a reaction time of 10 min and a slight increase occurred with reaction time above this value. Based on the obtained results, a reaction time of 10 min was used for further experiment.

The amount of p-nitroaniline has impact on the formation of azo product, subsequently influencing the extraction efficiency for nitrite ion. Both EF and ER increased rapidly with increase of p-nitroaniline volume from 10 to $50 \mu\text{L}$, while, changing the volume of p-nitroaniline in the range of $50\text{--}90 \mu\text{L}$ had no effect on the EF and ER. Therefore, $50 \mu\text{L}$ p-nitroaniline was used for further work. The effect of the volume of diphenylamine on the EF and ER was also investigated. The results showed that the EF and ER increased dramatically by increasing the diphenylamine volume up to 0.11 mL and then it increased slightly at bigger volume. Therefore, 0.11 mL of diphenylamine was applied in the proposed method.

In general, the mass transfer of anyte from sample phase to extraction phase is enhanced by increasing the temperature. But the diazotization reaction often occurs under low temperature condition. The effect of temperature on the proposed procedure was studied in the range of $0\text{--}40^\circ\text{C}$. Both EF and ER reached their highest value at 25°C , which was close to environmental temperature. Therefore, the following experiment was carried out at environmental temperature.

3.2. Optimization of IL-DLLME

3.2.1. Comparison of [OMIM][Tf₂N] with conventional organic solvents and [OMIM][PF₆]

The widely applied extraction solvents in DLLME technique are some conventional organic solvents such as CCl_4 , $\text{C}_2\text{H}_4\text{Cl}_2$ and $\text{C}_6\text{H}_5\text{Cl}$. Ionic liquids were also reported as extraction solvent such as [RMIM][PF₆] (R = hexyl, octyl). In order to evaluate the advantage of [OMIM][Tf₂N]-DLLME of nitrite ion, it is very necessary to provide the comparison between [OMIM][Tf₂N] and traditional organic solvents as well as other ionic liquids. For this purpose, [OMIM][PF₆] and three organic solvents including CCl_4 , $\text{C}_2\text{H}_4\text{Cl}_2$ and $\text{C}_6\text{H}_5\text{Cl}$ were also investigated as extraction solvent under the same experimental conditions. The extractions were conducted with 1 mL methanol containing different types and volumes of extraction solvent.

Table 1 shows some results obtained with the five extraction solvents. The required volume of [OMIM][Tf₂N] was the least among all above-mentioned solvents when the obtained amount of sediment phase was similar (about $11.5 \mu\text{L}$). Moreover, [OMIM][Tf₂N] could provide highest EF and ER for nitrite ion. To further evaluate the predominance of [OMIM][Tf₂N] as extraction solvent, five solvents with the same volume ($22.0 \mu\text{L}$) were investigated. The settled phase was not obtained using [OMIM][PF₆] as extraction solvent due to its relative high miscibility with water. The obtained volume of settled phase using three organic solvents as extraction phase was less than that using [OMIM][Tf₂N]. Although [OMIM][Tf₂N] had lower EF than CCl_4 and $\text{C}_6\text{H}_5\text{Cl}$, it provided much higher ER. Besides, when the volume of sediment phase was low, withdrawing sediment phase was difficult and the precision would be poor. The better extraction efficiency of [OMIM][Tf₂N] was clear compared with $\text{C}_2\text{H}_4\text{Cl}_2$, in terms of higher EF and ER. On the basis of the above results, it was evident that [OMIM][Tf₂N] was superior to [OMIM][PF₆] and conventional organic solvents for extraction and enrichment of nitrite ion in DLLME procedure.

3.2.2. Selection of disperser solvent

The type of disperser solvent used in DLLME is an essential consideration for successful extraction. The disperser solvents should possess appropriate miscibility with both extraction phase and sample phase to achieve a high EF and ER. On the basis of these considerations, methanol, ethanol, acetonitrile and acetone were tested. A series of sample solutions were studied using 1.0 mL of each solvent containing $22.0 \mu\text{L}$ [OMIM][Tf₂N]. The results indicated that the extraction efficiency were different for respective solvent. Methanol provided the highest EF and ER for nitrite ion among all the candidate solvents. Thus, methanol was chosen as the disperser solvent in this work.

3.2.3. Effect of the volume of [OMIM][Tf₂N]

To test the effect of the volume of extraction solvent, a constant volume (1.0 mL) methanol containing different volumes of [OMIM][Tf₂N] (14.0 , 18.0 , 22.0 , 26.0 and $30.0 \mu\text{L}$) was investigated in the same DLLME procedure. As seen from Fig. 2, a gradual decrease in EF was observed with an increase of [OMIM][Tf₂N] volume, while the ER increased constantly with increasing [OMIM][Tf₂N] from 14.0 to $22.0 \mu\text{L}$ and increased only

Table 1
Comparison of [OMIM][Tf₂N] with [OMIM][PF₆] and traditional organic solvents.

Extraction solvent	Volume of extraction solvent (μL)	Volume of settled phase (μL)	EF	ER%
[OMIM][Tf ₂ N]	22.0	11.5	413	94.2
[OMIM][PF ₆]	43.0	11.3	378	87.0
CCl ₄	37.0	11.8	330	77.9
C ₆ H ₅ Cl	30.0	12.0	326	78.1
CH ₂ Cl ₄	24.0	12.0	353	84.8
[OMIM][PF ₆]	22.0	-	-	-
CCl ₄	22.0	5.5	536	58.9
C ₆ H ₅ Cl	22.0	6.0	437	52.4
CH ₂ Cl ₄	22.0	10.5	374	78.5

Reaction conditions: nitrite ion, $20 \mu\text{g L}^{-1}$; the concentration of sulfuric acid, 2 mol L^{-1} ; p-nitroaniline, $50 \mu\text{L}$; diphenylamine, 0.11 mL ; reaction time, 10 min . Extraction conditions: water sample volume, 5.0 mL ; disperser solvent (methanol), 1.0 mL ; centrifugal time, 5 min .

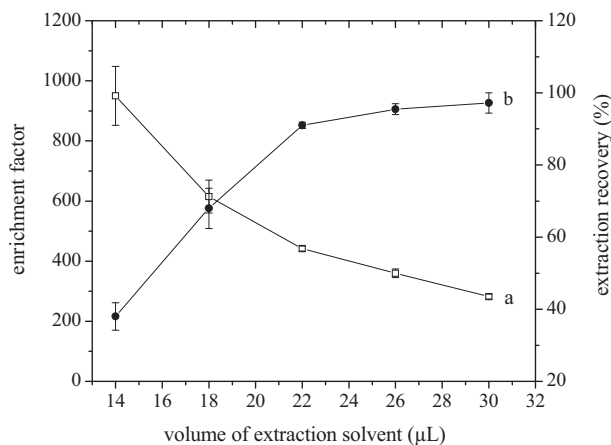


Fig. 2. Effect of extraction solvent volume on EF (a) and ER (b). Reaction conditions: nitrite ion, $20 \mu\text{g L}^{-1}$; sulfuric acid concentration, 2 mol L^{-1} ; p-nitroaniline, $50 \mu\text{L}$; diphenylamine, 0.11 mL ; reaction time: 10 min . Extraction conditions: water sample volume, 5.0 mL ; extraction solvent, [OMIM][Tf₂N]; disperser solvent (methanol), 1.0 mL ; centrifugal time, 5 min .

slightly after $22.0 \mu\text{L}$. The results could be explained by the change of sediment phase volume. With the enlargement of [OMIM][Tf₂N] volume, the volume of sediment phase increased, resulting in a decreased EF and an increased ER. To obtain high EF and satisfactory ER, the volume of [OMIM][Tf₂N] chosen for further experiment was $22.0 \mu\text{L}$.

3.2.4. Effect of the volume of methanol

The effect of the volume of disperser solvent was investigated by changing its volume between 0.6 , 0.8 , 1.0 , 1.2 and 1.4 mL containing $22.0 \mu\text{L}$ [OMIM][Tf₂N], respectively. The results in Fig. 3 show that larger volumes of disperser solvent give higher EF and lower ER. At lower volumes of the disperser solvent, tiny droplet extraction solvent may not be effectively formed, resulting in a reduced EF. Then with increasing the volumes of disperser solvent, the solubility of the extraction solvent in aqueous phase increased and the volume of settled phase decreased, leading to a decrease in ER. In order to ensure high EF and good ER, 1.0 mL methanol was chosen in this study.

3.2.5. Effect of extraction and centrifugal time

Extraction time is one of the most important factors in DLLME as in other extraction procedures. In DLLME, extraction time is defined as the time interval between injection the mixture of disperser solvent and extraction solvent, and the time starting to centrifuge. The effect of extraction time was studied in the range 0 – 20 min . No obvious change for the extraction efficiency was observed with the increase of extraction time, indicating that the extraction equilibrium was attained in a very short time. This could be attributed

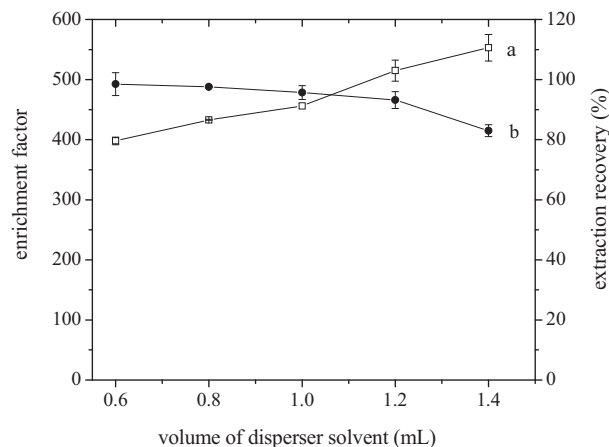


Fig. 3. Effect of disperser solvent volume on EF (a) and ER (b). Reaction conditions: nitrite ion, $20 \mu\text{g L}^{-1}$; sulfuric acid concentration, 2 mol L^{-1} ; p-nitroaniline, $50 \mu\text{L}$; diphenylamine, 0.11 mL ; reaction time: 10 min . Extraction conditions: water sample volume, 5.0 mL ; extraction solvent ([OMIM][Tf₂N]), $22.0 \mu\text{L}$; disperser solvent, methanol; centrifugal time, 5 min .

to the large contact surface area between extraction solvent and aqueous phase with aid of disperser solvent. Therefore, the transfer process of analyte from aqueous phase to extraction solvent was very fast. The short extraction time is one of the remarkable advantages of the DLLME compared with other extraction techniques.

Centrifugation plays an important role in the phase separation procedure. The ionic liquid phase was settled at the bottom of tested tube during this process. Centrifugal time was studied in the range 2 – 14 min at the interval 3 min at a rate of 4000 rpm . Both EF and ER increased from 2 to 5 min while a slight decrease was observed after 5 min . Therefore, 5 min was selected as centrifugal time.

3.2.6. Salt addition

The addition of salt had a complex influence on the extraction efficiency for analyte in IL-DLLME procedure. The extraction efficiency could be increased due to the increase of ionic strength of aqueous solution, but decreased because of the electrostatic interaction and possible ion exchange process. In addition, the generating azo product could be influenced by the addition of salt, reducing the partition of analyte into the extraction phase. To investigate the effect of salt addition in this study, a series of experiments were performed by adding various concentration of NaCl from 0 to 36% (w/v). However, the sediment phase was wrapped by NaCl and not withdrawn as the concentration of NaCl was over 25% . Thus, the range of 0 – 25% was actually examined.

Both EF and ER of nitrite ion increased slightly from 0 to 1% while a continuous decrease was observed afterward. The reasons for this phenomenon may be explicated as follows. At a low concentration of NaCl, the increase of ionic strength could play an

Table 2
Tolerance ratio of various ions to the determination of 20 $\mu\text{g L}^{-1}$ nitrite ion.

Ion	Tolerance ratio (ion/nitrite ion)
NH_4^+ , Cr^{3+} , CH_3COO^- , Al^{3+} , Ni^{2+} , Co^{2+} , Na^+ , K^+ , CO_3^{2-} , Ca^{2+} , Mg^{2+} , Cd^{2+} , Zn^{2+} , NO_3^- , F^- , Cl^- , PO_4^{3-}	10,000
Ba^{2+} , Cu^{2+} , Br^- , Ag^+ , tartrate	5000
I^- , Fe^{3+} ^a	1000

^a In the presence of 1 g L^{-1} tartrate.

important role in extraction process, enhancing the extraction efficiency of nitrite ion. At a higher concentration of NaCl, the decrease in extraction performance could be ascribed to the negative effect of salt addition on the diazo-coupling reaction. It should be noted that the dependence of NaCl amount on the volume of sediment phase using [OMIM][Tf₂N] as extraction solvent was different from the case of [OMIM][PF₆] and [BBIM][PF₆] as described in our previous literatures [20,25]. For [OMIM][Tf₂N], the volume of sediment phase increased constantly, while for [BBIM][PF₆], the volume initially decreased then increased in the investigated range of 0–3% and 3–36%(w/v), respectively, and for [OMIM][PF₆], the volume decreased in the investigated range (0–2% NaCl, w/v).

After a comprehensive consideration of the results, salt addition was not used in the subsequent experiments.

3.3. Interference effect of foreign ions

In order to evaluate the selectivity of the proposed IL-DLLME-HPLC method, the effect of various cations and anions on the determination of nitrite ion was investigated by adding known quantities of each ion to a solution containing 20 $\mu\text{g L}^{-1}$ of nitrite ion. Each substance was considered not to interfere, if it caused a variation less than $\pm 5\%$ in the analytical signal. The results obtained were given in Table 2. The results indicated that the investigated ions except Fe^{3+} were tolerated in rather large amounts and did not interfere in the determination of nitrite ion by the proposed method. Fe^{3+} could be masked by adding 1 g L^{-1} tartrate, which had no effect on the determination from Table 2.

3.4. Analytical features of the proposed method

Under the optimized experimental conditions, some parameters (i.e., linear range, repeatability and limit of detection) were investigated. The linearity was observed in the range of 0.4–500.0 $\mu\text{g L}^{-1}$ with the correlation coefficient (r^2) of 0.9996. The repeatability study was performed by five replicative extractions of water samples spiked at three different concentration levels of 1, 10 and 20 $\mu\text{g L}^{-1}$. The relative standard deviations (RSDs) were calculated to be from 1.5% to 4.8%. The limit of detection of the proposed method, defined as the ratio of signal to noise at 3 ($S/N=3$), was 0.05 $\mu\text{g L}^{-1}$, and the method quantity limit ($S/N=10$) was 0.4 $\mu\text{g L}^{-1}$.

Table 3
Comparison of the proposed method with other methods for the determination of nitrite.

Enrichment technique	Analytical method	Sample volume (mL)	LOD ($\mu\text{g L}^{-1}$)	EF	Precision (RSD%)	Linear range ($\mu\text{g L}^{-1}$)	Reference
Column preconcentration	Spectrophotometry	10	<1.4	–	<0.48	7–131	[26]
SPE	UV-Vis	100	5	>140	<1	0–140	[4]
SPE	FIA-UV-Vis	2.5	0.46	93	3.8	1–100	[27]
Micro-phase sorbent extraction	Spectrophotometry	10	–	–	–	1.5–30	[28]
CPE	UV-Vis	10	0.87	20	2.57	2.0–40.0	[8]
SPE	SIA-UV-Vis	150	0.05	–	1.44	0.71–42.9 (nmol L^{-1})	[29]
HS-LPME	UV-Vis micro-spectrophotometry	10	1.5	193	3.5	10–100	[9]
DLLME	HPLC-UV-Vis	5.0	0.05	430	<4.1	0.5–500	This work

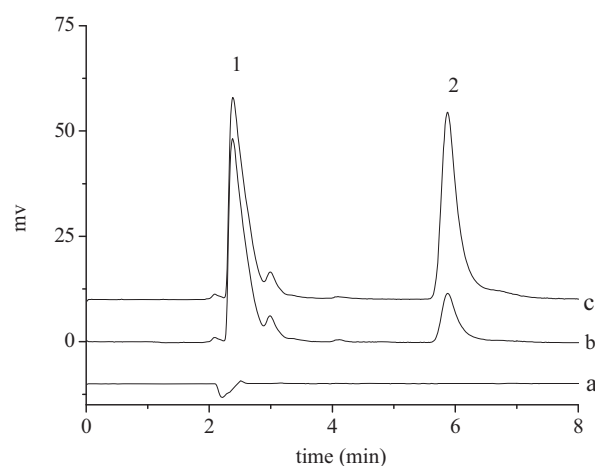
**Fig. 4.** Chromatogram for direct injection of tap water (a), and extraction of blank (b) and spiked at 20 $\mu\text{g L}^{-1}$ nitrite ion (c) by the proposed IL-DLLME procedure. Chromatographic condition was described as Section 2.2, peak: 1. [OMIM][Tf₂N]; 2. azo product.

Table 3 presented a comparison of some analytical parameters obtained for the proposed method with other methods including SPE-UV-Vis, SPE-FIA, CPE-UV-Vis, SPE-SIA and HS-LPME-UV-vis. As can be seen, the proposed method provided highest EF among the above-mentioned methods, and lower or equivalent LOD compared with other methods. The high sensitivity could be ascribed to the two factors. One was the high extraction ability of [OMIM][Tf₂N] for analyte, another was the high enrichment capability of DLLME procedure, in which the extraction efficiency of object could be enhanced owing to the large contact surface area between extraction solvent and sample phase. In addition, wide linear range, acceptable repeatability and low sample volume were achieved with the developed method.

3.5. Determination of nitrite ion in real samples

The proposed IL-DLLME-HPLC method was applied to determine nitrite ion in some water and biological samples. Five water samples including tap water, overnight plain boiled water, bottled purified water, Yellow river water and well water, and one biological sample, namely, saliva were all spiked at three different concentration levels of 0, 20 and 100 $\mu\text{g L}^{-1}$, respectively. For each sample, the extraction was repeated for three times. The corresponding analytical results were listed in Table 4. As can be seen, all real samples presented detectable nitrite ion content. The nitrite ion concentration was found to be close to 100 $\mu\text{g L}^{-1}$ in Yellow river water and be below 6.0 $\mu\text{g L}^{-1}$ in other water samples. The obtained recoveries and RSDs were in the range of 96.5–107.3% and 1.3–5.8%, respectively, which indicated that the complex matrices of real samples had little effect on the enrichment and determination of nitrite ion using the developed method. Fig. 4 shows the

Table 4
Determination of nitrite ion in the real samples by the proposed method.

Sample	Nitrite ion added ($\mu\text{g L}^{-1}$)	Nitrite ion found ^a ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)
Tap water	0.0	0.2 ± 0.01	–	4.6
	20.0	20.6 ± 0.2	101.7	1.5
	100.0	98.5 ± 2.2	98.3	2.0
Overnight plain boiled water	0.0	4.7 ± 0.2	–	4.6
	20.0	24.1 ± 0.7	96.8	3.5
	100.0	104.5 ± 5.8	99.8	5.4
Bottled purified water	0.0	2.0 ± 0.04	–	2.1
	20.0	22.6 ± 0.2	103.1	1.3
	100.0	103.1 ± 5.4	101.1	5.0
Yellow river water	0.0	99.4 ± 1.5	–	1.5
	20.0	119.4 ± 1.2	100.0	5.6
	100.0	201.2 ± 2.7	101.8	2.4
Well water	0.0	5.7 ± 0.3	–	5.8
	20.0	27.2 ± 0.4	107.3	1.8
	100.0	104.9 ± 2.9	99.2	2.7
Saliva ^b	0.0	43.5 ± 1.4	–	3.5
	20.0	65.0 ± 0.3	107.1	1.5
	100.0	140.0 ± 1.9	96.5	1.8

^a Average of three determinations ± standard deviation.

^b Diluted as 1:4 before analysis.

chromatogram for tap water obtained by direct injection, and by extraction of blank and solution spiked with 20 $\mu\text{g L}^{-1}$ nitrite ion with the proposed IL-DLLME procedure.

4. Conclusions

In this study, an ionic liquid ([OMIM][Tf₂N]) was synthesized and was for the first time employed as extraction solvent in DLLME for indirect extraction and enrichment of nitrite ion in water and biological samples. The determination has been carried out by the combination of DLLME with HPLC on the basis of the diazo-coupling reaction. The color azo product generated in situ was extracted and enriched into [OMIM][Tf₂N] phase. Results indicated that the proposed [OMIM][Tf₂N]-DLLME-HPLC method provided high EF, low LOD, wide linear range, acceptable reproducibility and low sample volume. This developed method was employed to determine nitrite ion in five real water samples and one biological sample with satisfactory recovery and repeatability.

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