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Combination of ionic liquid-based dispersive liquid–liquid micro-extraction with stopped-flow spectrofluorometry for the pre-concentration and determination of aluminum in natural waters, fruit juice and food samples

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ABSTRACT

In this research, we combined ionic liquid-based dispersive liquid–liquid micro-extraction (IL-based DLLME) with stopped-flow spectrofluorometry (SFS) to evaluate the concentration of aluminum in different real samples at trace level. 1-Hexylpyridinium hexafluorophosphate [Hpy][PF_6] ionic liquid and 8-hydroxyquinoline (oxine), which forms a highly fluorescent complex with Al^{3+}, were chosen as the extraction solvent and chelating agent, respectively. The hydrophobic Al–oxine complex was extracted into the [Hpy][PF_6] and separated from the aqueous phase. Then, the concentration of the enriched aluminum in the sediment phase was determined by SFS. Some effective parameters that influence the SFS signals and the micro-extraction efficiency, such as the suction and sending time, the concentration of the chelating agent, pH, the amount of the ionic liquid, the type of disperser solvent and diluting agent, ionic strength, extraction time, equilibration temperature and centrifugation time were investigated and optimized. In the optimum experimental conditions, the limit of detection (3 s) and enrichment factor were 0.05 $\mu g\,L^{-1}$ and 100, respectively. The relative standard deviation (RSD) for six replicate determinations of 6 $\mu g\,L^{-1}$ Al was 1.7%. The calibration graph using the pre-concentration system was linear in the range of 0.06–15 $\mu g\,L^{-1}$ with a correlation coefficient of 0.9989. The developed method was validated by the analysis of certified reference materials and applied successfully to the determination of aluminum in several water, fruit juice and food samples.

1. Introduction

It is believed that aluminum is a toxic metal to which humans are frequently exposed. Aluminum may enter the human body by mouth, intravenous infusion and by environment, drinking water, food and pharmacological products. This element was indicated to be involved as a causative factor in several clinical and neuropathological diseases, such as Alzheimer's, Parkinson's, Parkinson–Guam's disease, amyotrophic lateral sclerosis, diabetes and cancer [1]. Aluminum also has an effect on red blood cells, parathyroid glands and chromosomes. The World Health Organization (WHO) has proposed a guideline value of 0.2 $mg\,L^{-1}$ for the maximum permissible concentration of aluminum in drinking water [2]. Therefore, in order to protect human health and the environmental safety, it is essential to establish simple, rapid, sensitive and environment-friendly methods for monitoring of aluminum at trace and sub-trace levels in biological, environmental and food samples.

Spectrofluorometry is a low cost, sensitive, simple, powerful and well-established technique for the determination of Al in complex matrices. The chelating agent 8-hydroxyquinoline (oxine) is one of the most sensitive organic ligands used for the determination of Al^{3+} by fluorimetric detection [3]. It forms a highly fluorescent complex without showing any intrinsic fluorescence itself. Nevertheless, the direct determination of Al at very low concentrations is often difficult because of insufficient sensitivity of this technique and due to the fact that the matrices of real samples are often complex. Therefore, an initial sample pretreatment is often necessary.

Several methods have been developed for separation and pre-concentration of metal ions, such as co-precipitation, ion exchange, liquid–liquid extraction (LLE), solid-phase extraction (SPE) and cloud point extraction (CPE). Among these methods, LLE is the most widely used sample pretreatment technique in routine analysis for trace metal determination due to its simplicity and adaptability. However, some shortcomings like the use of large sample volumes and toxic organic solvents make LLE expensive, time-consuming, laborious and environmentally

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unfriendly. In recent years, efforts have been focused on miniaturizing LLE procedure by reducing the organic solvent, leading to the development of micro-extraction methodologies [4]. Liquid-phase micro-extraction (LPME), including single-drop micro-extraction (SDME) and hollow fiber supported LPME, is based on traditional LLE technique but utilizes only a few μL of organic solvent as the extracting phase [5]. Assadi and co-workers have recently developed a novel modality of LPME called dispersive liquid–liquid micro-extraction (DLLME) [6]. In this method, the appropriate mixture of extraction solvent and dispersive solvent is rapidly injected in an aqueous sample by means of a syringe, and a cloudy solution is formed. The analyte in the sample is extracted into the fine droplets of extraction solvent. After extraction, phase separation is accelerated by centrifugation, and the enriched analyte in the sediment phase is determined by suitable analytical methods. The simplicity of operation, rapidity, low cost, high recovery and high enrichment factor are among the main advantages of DLLME.

Ionic liquids (ILs) are a class of low melting point ionic compounds, which have a variety of properties allowing many of them to be sustainable green solvents in the sample preparation. Besides their low melting points, ILs have many other unique physicochemical properties, such as broad liquid ranges, negligible vapor pressures, good thermal stabilities, non-flammability, and good extractabilities for various organic compounds and metal ions as neutral or charged complexes, as well as tunable viscosity and miscibility with water and organic solvents, which make them very attractive in separation processes [7–9].

Several methods have been developed based on extraction with an ionic liquid for the pre-concentration and determination of trace and sub-trace levels of metal ions, i.e., Hg, Zn, Pb, Cd, Co, Ni, Mn, V, U, etc., after combination with some suitable analytical methods. These include ionic liquid-based liquid–liquid micro-extraction (IL-LLME) [10–16] ionic liquid-based single-drop micro-extraction (IL-SDME) [17–21], hollow fiber-based liquid-phase micro-extraction (HF-LPME) using ionic liquid [22], cold induced aggregation micro-extraction (CIAME) [23,24], in situ solvent formation micro-extraction (ISFME) [25], ionic liquid-based ultrasound-assisted dispersive liquid–liquid micro-extraction (IL-based USA-DLLME) [26], on-line temperature-assisted ionic liquid dispersive liquid-phase micro-extraction (on-line TILDLME) [27] and ionic liquid-based dispersive liquid–liquid micro-extraction (IL-DLLME) [28,29]. To the best of our knowledge, so far no attempt has been made to combine IL-based DLLME with stopped-flow spectrofluorometry (SFS) for the pre-concentration and determination of metal ions. Therefore, in the present work, a new IL-based DLLME–SFS method has been developed for the pre-concentration and determination of Al. In this study, 1-hexylpyridinium hexafluorophosphate [Hpy][PF_6] ionic liquid and oxine, which forms a very stable fluorescent complex with Al^{3+}, were chosen as the extraction solvent and chelating agent, respectively. The factors influencing micro-extraction efficiency and SFS signals were systematically studied. To evaluate the applicability of the proposed method, it was then applied to the analysis of natural waters, fruit juice and food samples.

2. Experimental

2.1. Apparatus and instruments

Fluorescence spectra and intensity measurements were carried out using a FP-6200 spectrofluorometer (JASCO Corporation, Tokyo, Japan) with a wavelength range of 220–730 nm (with 1 nm intervals) for excitation and emission. The instrument equipped with a 150 W xenon lamp, dual monochromaters (silicon photodiode for excitation and photomultiplier for emission), Peltier thermostatted single cell holder (model ETC-272) and peristaltic sipper (model SHP-292) containing a quartz micro-flow cell (16 μL capacity) and supported with PC-based Windows® Spectra Manager™ software for JASCO Corporation version 1.02. The slit widths for both excitation and emission were set at 5 nm and the fluorescence spectra were recorded at a scan rate of 250 nm min^{-1}. Fluorescence intensities were measured at 507 ± 3 nm with excitation at 370 ± 3 nm against a corresponding reagent blank at room temperature.

A centrifuge (Beckman GS-6, USA) was used to accelerate the phase separation process. The pH values were measured with a Metrohm pH-meter (model 827, Switzerland), supplied with a glass-combined electrode. A thermostated water bath (Julabo, GMBH D-77960, Germany) was used for the study of temperature effect in DLLME experiments. An electronic analytical balance (Mettler Toledo, PB303, Switzerland) was used for weighing the solid materials.

2.2. Standard solutions and reagents

All chemicals used were of analytical-reagent grade and all solutions were prepared with high purity deionized water (Ghazi Co., Tabriz, Iran). Stock solutions of aluminum(III) and those used for the interference study (1000 μg mL^{-1}) were prepared by dissolving appropriate amounts of their respective pure nitrate salts (Merck, Darmstadt, Germany) in deionized water. The working standard solutions were prepared by serial dilutions of the stock solution with deionized water immediately prior to analysis.

1-Hexylpyridinium hexafluorophosphate [Hpy][PF_6] (97%) (Acros organics, Belgium) was employed as an extractant solvent without further purification. The chelating agent, 8-hydroxyquinoline (oxine), acetone, tetrahydrofuran (THF), ethanol, acetonitrile, methanol, and all salts were purchased from Merck. Suprapur® HNO_3 (65%), H_2SO_4 (95–98%) and H_2O_2 (30%) were used for sample digestion.

A solution of 2.75×10^{-3} mol L^{-1} oxine was prepared by dissolving appropriate amount of this reagent in 5 mL acetonitrile and diluting to 25 mL with deionized water and was kept in refrigerator (4 °C) for 1 week. [Hpy][PF_6] ionic liquid is solid at room temperature (melting point: 45–48 °C) and requires dissolution with an organic solvent. Therefore, a solution of 0.485 mol L^{-1} [Hpy][PF_6] was obtained by dissolving appropriate amount of this IL in acetonitrile. A stock buffer solution (0.2 mol L^{-1}) was prepared by dissolving appropriate amounts of sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$) (Merck) in deionized water and adjusting to pH 7.0 by adding diluted NaOH solution. A 2 mol L^{-1} $NaNO_3$ solution was used for ionic strength study. Two standard reference materials, SRM 1549 (Non-Fat Milk Powder) and SRM 1643e (Trace Elements in Water) (both from National Institute of Standards and Technology (NIST), Giathersburg, MD, USA) were used for validation of the proposed method. The pipettes and vessels used for the trace analysis were kept in 10% (v/v) nitric acid at least overnight and subsequently washed three times with deionized water.

2.3. Preparation of water samples

Water samples including bottled mineral water and rainwater were chosen for the analysis. The bottled mineral water was purchased from local market and rainwater was collected in PTFE container at Tabriz city, Iran. On arrival to laboratory, both of them were stored in a dark place at 4 °C and aliquots of 25.0 mL from each sample solution were analyzed within 24 h of collection without previous treatment or filtration. In the case of NIST SRM 1643e (Trace Elements in Water), suitable aliquot of this sample was diluted 50-fold and then the concentration of aluminum in 25.0 mL of sample solution was determined by following the procedure described in Section 2.6.

2.4. Preparation of wheat flour and milk samples

An accurately measured amount (50 mg) of wheat flour, powdered milk (Humana) and NIST SRM 1549 (Non-Fat Milk Powder) or 1.0 mL of packed cows' milk was heated on a hot plate at a fairly low temperature in the glass beaker containing mixture of concentrated sulphuric acid (10 mL) and nitric acid (4 mL) to dryness. After that, the sample was cooled down to room temperature and the residue was dissolved in 1.0 mL of HNO_3 of 0.1 mol L^{-1} [30]. After dilution with deionized water, the pH was adjusted to nearly 7 by adding diluted NaOH solution. Then, the solution was transferred into a 50.0 mL volumetric flask and diluted to the mark with deionized water. The concentration of aluminum in 25.0 mL of sample solution was determined by following the procedure described in Section 2.6.

2.5. Preparation of fruit juice samples

Packed fruit juice samples including orange, sour cherry and grape juices were purchased from the local market. For determination of aluminum in each sample, a 1.0 mL sample portion, 3 mL of HNO_3 (65%) and 5 mL of H_2O_2 (30%) was heated on a hot plate at a fairly low temperature in the glass beaker to dryness. After that, the sample was cooled, and 3 mL of H_2O_2 was added and the heating was repeated to obtain about 0.5 mL sample solution. After cooling down the resulting solution to room temperature and dilution with deionized water, pH was adjusted to nearly 7 by adding diluted NaOH solution [31]. Then, the solution was transferred into a 25.0 mL volumetric flask and diluted to the mark with deionized water. Amount of Al in 25.0 mL of sample solution was then determined as described in Section 2.6.

2.6. General micro-extraction and pre-concentration procedure

Aliquots of 25.0 mL sample or standard solution containing Al^{3+} in the range of 0.06–15 μg L^{-1}, 2.3 mL of 0.2 mol L^{-1} phosphate buffer solution ($H_2PO_4^-/HPO_4^{2-}$, pH 7.0) and 0.7 mL of 2.75×10^{-3} mol L^{-1} oxine solution (chelating agent) were placed in a screw-cap conical-bottom polypropylene centrifuge tube. Then, 2 mL of acetonitrile (disperser solvent) containing 0.3 g of $[Hpy][PF_6]$ ionic liquid (extraction solvent) was added. Afterward, the tube was simply shaken obtaining a dispersion of the IL into the aqueous media. After shaking, the resultant solution became immediately turbid at room temperature, extracting the Al–oxine complex into the fine droplets of IL. In order to accelerate phase separation, the cloudy solution was centrifuged at 4000 rpm for 5 min. As a result, the IL-phase settled at the bottom of the centrifuge tube. The upper aqueous phase was manually removed with a syringe centered in the tube without need to cooling in an ice bath. Finally, in order to reduce the viscosity of the IL-phase and facilitate sample handling prior to SFS analysis, the extract in the tube was made up to 300 μL by adding the ethanol. A 100 μL of the resultant solution was introduced into the spectrofluorometer by the peristaltic sipper. A reagent blank was prepared using a similar procedure but without adding aluminum. The optimized conditions are listed in Table 1. In the optimization procedure of impact parameters, the limits marked on the figures were obtained from three experiments that repeated under the same circumstances.

Table 1
Optimization of variables for Al determination.

Instrumental variables	Range studied	Optimum value
Excitation wavelength (nm)	250–400	370
Emission wavelength (nm)	425–700	507
Excitation bandpass (nm)	5–20	5
Emission bandpass (nm)	5–20	5
Temperature (°C)	15–80	Room temperature
Suction time (s)	0.1–1	0.4
Sending time (s)	0.1–2	0.7
Delay time (s)	0.5–4	1
Drain time (s)	5–20	10
Wavelength scan rate (nm min^{-1})	60–1000	250

IL-DLLME variables	Range studied	Optimum value
Working pH	3–12	7
Oxine concentration (mol L^{-1})	0.9–9.0 ($\times 10^{-5}$)	6.4×10^{-5}
Amount of $[Hpy][PF_6]$ (g)	0.09–0.48	0.3
Buffer concentration (mol L^{-1})	0.003–0.03	1.5×10^{-2}
Centrifugation time (min)	1–20	5
Ionic strength (mol L^{-1})	0.0–0.5	<0.3
Equilibration temperature (°C)	4–50	Room temperature
Extraction time (min)	up to 60	<1

2.7. Stopped-flow measurements and fluorescence data processing

The stopped-flow measurements were carried out by means of a peristaltic sipper equipped with a quartz micro-flow cell (3 mm ID × 3 mm length and 16 μL capacity) and controlled by PC-based Windows® Spectra Manager™ software. Inserting the nozzle of sipper into the tube containing the 300 μL diluted IL-phase solution and pressing the nozzle lever activated the peristaltic pump to start suction of 100 μL of the diluted IL-phase solution for 0.4 s. Then, the suctioned sample solution was sent toward the micro-flow cell for 0.7 s. Afterward, the pump was stopped and the delay time (wait time required for the sample to get stabilized in the micro-flow cell and measurable) was automatically started. Finally, after a delay time of 1.0 s, measurements were started and with completion of measurement, the pump was rotated to start draining for 10.0 s.

Each conventional or three-dimensional (3D) spectrum was recorded by the instrument's software and was saved as a TXT format file. The resulting file was then transported into Microsoft Office Excel 2003 or MATLAB (version 7, Mathworks Inc., USA) for preparation of conventional and three-dimensional spectrum, respectively.

3. Results and discussion

In this work, we combined IL-based DLLME method with stopped-flow spectrofluorometry to evaluate the concentration of Al^{3+} in different real samples at trace levels. For this purpose, the effect of several factors influencing the extraction (DLLME) and determination (SFS) conditions were investigated and optimized. All fluorescence spectra or intensity measurements, except study of the effect of temperature on emission spectrum of Al–oxine complex, were carried out by injection of a 100 μL of the diluted IL-phase into the spectrofluorometer by the peristaltic sipper equipped with a micro-cell.

3.1. Study of the spectral characteristics

3.1.1. Three-dimensional (3D) fluorescence spectra of $[Hpy][PF_6]$ ionic liquid

In order to obtain the accurate fluorescence intensity of an analyte, it is necessary to investigate the effect of sample matrix on fluorescence intensity. Therefore, the spectral characteristics of $[Hpy][PF_6]$ ionic liquid as an extraction

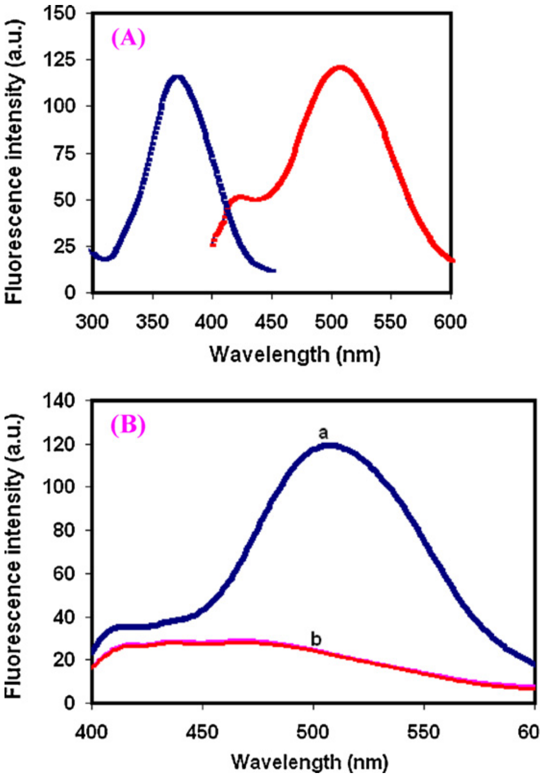

Fig. 1. Spectral characteristics: (A) excitation (left) and emission (right) spectra of Al–oxine complex in [Hpy][PF_6] ionic liquid; (B) emission spectra of Al–oxine complex (a) and blank (b) in [Hpy][PF_6] ionic liquid. Other conditions are as indicated in Table 1.

solvent were studied. For this reason, we employed the three-dimensional excitation–emission matrix (EEM) spectroscopy (3D-spectrofluorometry). The fluorescence EEM spectroscopy involved scanning and recording 31 individual emission spectra (425–700 nm) at sequential 5 nm increments of excitation wavelength between 250 and 400 nm. The bandwidths for both excitation and emission were 5 nm, with emission wavelength increments of 1 nm and an integration time of 0.2 s. These 31 scans were used to generate three-dimensional plots of fluorescence intensity as a function of excitation and emission wavelengths. Based on the obtained spectra, at excitation wavelength between 250 and 400 nm [Hpy][PF_6] IL has no considerable emission between 425 and 750 nm.

3.1.2. *Excitation and emission spectra of Al–oxine complex in [Hpy][PF_6]*

In order to determine the optimum excitation and emission wavelengths (λ_{ex} and λ_{em}), the excitation and emission spectra of Al–oxine complex were recorded after extraction into [Hpy][PF_6] IL and dilution with ethanol as described in Section 2.6. The obtained spectra are given in Fig. 1A. From these spectra, the analytically useful maximum fluorescence intensity of the Al–oxine complex, was measured at $\lambda_{ex} = 370 \pm 3$ nm and $\lambda_{em} = 507 \pm 3$ nm. Comparison of Fig. 1A (right) and three-dimensional fluorescence spectra of [Hpy][PF_6] IL showed that the fluorescence intensity measurement of Al–oxine complex in the presence of [Hpy][PF_6] IL could be carried out without considerable background effect of [Hpy][PF_6] on the fluorescence intensity. On the other hand, as well known, oxine is a fluorescent chelating agent and for the analytical purpose, it is necessary to subtract its emission (or total blank emission) from Al–oxine complex emission at the λ_{em} (507 nm). Therefore, after DLLME procedure, the blank (without Al^{3+}) and Al–oxine complex emission spectra were individually recorded under the same conditions with excitation at λ_{ex} (370 nm). These spectra are shown in Fig. 1B. As can be seen, there is a little background signal from reagent blank that is mainly related to oxine.

3.1.3. *Effect of temperature and time*

The effect of temperature on the fluorescence intensity of Al–oxine complex in IL media was studied. For this purpose, Peltier thermostatted single cell holder containing 1.00 cm quartz cell was employed as temperature controller and 14 individual emission spectra (400–600 nm) were scanned and recorded at sequential 5 °C increments of temperature between 15 and 80 °C with excitation at λ_{ex} (370 nm). These 14 scans were used to generate three-dimensional plots of fluorescence intensity as a function of temperature and emission wavelengths. Based on the obtained results, the fluorescence intensity decreases when the temperature increases from 15 to 80 °C. This can be ascribed to an increase in the kinetic energy of the molecules and hence the probability of their colliding; as a result, radiationless deactivation through the internal conversion prevailed and the fluorescence quantum efficiency decreased. On the other hand, it is well known that temperature has a great effect on complex formation and the decrease in fluorescence intensity of Al–oxine complex at temperatures higher than 70 °C may be attributed to the complex breakdown. Therefore, room temperature was chosen for further experiments.

In order to investigate the stability of the complex, the fluorescence intensity measurement of Al–oxine complex, using $\lambda_{ex} = 370$ nm and $\lambda_{em} = 507$ nm at various times (0–150 min with 300 s interval) was performed. In all of them, the fluorescence intensity of the Al–oxine complex stayed stable and maximum fluorescence intensity was obtained. From this observation, it can be concluded that the Al–oxine complex remained stable for a relatively long time.

3.2. *Study of the IL-based DLLME conditions*

In order to obtain a high extraction efficiency, the effect of different parameters affecting the complex formation and extraction conditions such as the amount of IL, pH, concentration of the chelating agent, extraction time, ionic strength, temperature and centrifugation time were investigated and optimized. One variable at a time optimization was used to obtain the optimum conditions for the IL-based DLLME.

3.2.1. *Selection of disperser solvent and diluting agent*

The most important point for the selection of the disperser solvent in IL-based DLLME procedures is its miscibility with both IL (extraction solvent) and aqueous (sample solution) phases. For this purpose, different solvents such as acetonitrile, acetone, THF, ethanol and methanol were tested. Several sample solutions were studied using 2.0 mL of each disperser solvent, which contains 0.3 g [Hpy][PF_6] IL. The enrichment factor of 90 ± 2 was obtained in the presence of the ethanol. However, in the case of other disperser solvents, the enrichment factor values were between 99 ± 1 and 102 ± 1 and acetonitrile was finally selected as disperser solvent in all of the subsequent experiments. At low volumes of acetonitrile, dispersion was incomplete, while for volumes exceeding 3.0 mL the enrichment factor decreased. Therefore, an optimal volume of 2.0 mL of acetonitrile was chosen to achieve a better and more stable cloudy solution.

Due to its high viscosity, the IL-rich phase had to be conditioned before its introduction into the sipper of spectrofluorometer by diluting agent. The viscosity of the IL-rich phase is drastically decreased using diluting agents. Different solvents such as methanol, ethanol, acetone, THF, and acetonitrile were tried in order to select the one that can dissolve the IL-rich phase completely and gives the best sensitivity. The nature of the organic

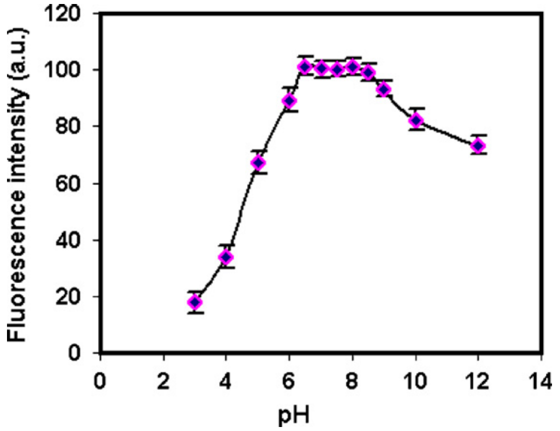

Fig. 2. Effect of pH on the extraction efficiency of aluminum. Utilized conditions: Al^{3+}, 6 μg L^{-1}; oxine, 7.0×10^{-5} mol L^{-1}; [Hpy][PF$_6$], 0.40 g; centrifugation time, 10 min.

solvent had no significant effect on the λ_{em} value, and the strongest fluorescence intensity. So, ethanol was chosen as diluting agent.

3.2.2. Effect of pH

Separation of metal ions by the IL-based micro-extraction methods firstly involved the formation of a complex with sufficient hydrophobicity to be extracted into the small volume of IL-phase. It is well known that the pH of media has a great effect on the existing form of the reagent and plays an important role on metal–chelates formation and subsequent extraction. Therefore, it is necessary to determine the pH of the system that will give the maximum complex formation. In this experiment, the effect of pH upon the extraction of Al^{3+} ions from the solution was studied within the range of 3–12 by adding appropriate volumes of HCl or NaOH solution to the samples. As shown in Fig. 2, the optimum pH value lies in the interval of 6.5–8.5. Therefore, samples and standards were adjusted at pH 7.0 before extraction. The reduced analytical signal at higher pH values could be due to the hydroxide formation of aluminum ions, resulting in decreased concentration of free Al^{3+} ions in sample solution. Thus, in order to maintain a constant working pH that allows complex formation and stability, pH was adjusted at 7.0 by a 1.5×10^{-2} mol L^{-1} $H_2PO_4^-/HPO_4^{2-}$buffer solution in subsequent experiments.

3.2.3. Effect of chelating agent concentration

In this work, oxine was used as chelating agent due to the highly hydrophobic nature of its metal–chelates. The concentration of chelating agent is a critical variable to be optimized. Fig. 3 shows the effect of oxine concentration on the micro-extraction of aluminum ions. The tested concentrations of oxine ranged from 9.0×10^{-6} to 9.0×10^{-5} mol L^{-1}. The extraction efficiency for Al^{3+} ions increased as the concentration of oxine increased from 9.0×10^{-6} to 4.5×10^{-5} mol L^{-1}, and then remained constant up to an oxine concentration of 9.0×10^{-5} mol L^{-1}. Therefore, an oxine concentration of 6.4×10^{-5} mol L^{-1} was chosen to account for other extractable species that potentially interfere with the assaying of Al. The concentrations above this value had no significant effect on the performance of the micro-extraction system.

3.2.4. Effect of IL amount

The type of extraction solvent used in DLLME is essential for obtaining an efficient extraction. This solvent should have a higher density than water, a higher extraction capability of the compounds under study, and a lower solubility in water. The attempt of our research group has currently focused on the application of ILs in DLLME of metal ions [32] and in the present work [HPy][PF$_6$] IL was used as an extraction solvent. The extraction system was carefully studied in order to obtain the lowest IL-phase mass necessary for achieving the highest pre-concentration factor possible. The variation in the analytical signal as a function of the amount of IL, which was added to 30.0 mL sample, was investigated within the range of 0.09–0.48 g. It was found that the extraction efficiency of this pre-concentration procedure was remarkably affected by the IL amount. Fig. 4 highlights that the extraction efficiency rapidly increased with the amount of IL, and then leveled off for an IL amount greater than 0.27 g, using a single step extraction procedure. Therefore, in order to achieve a good pre-concentration factor, a 0.30 g amount of IL was chosen as the optimum value.

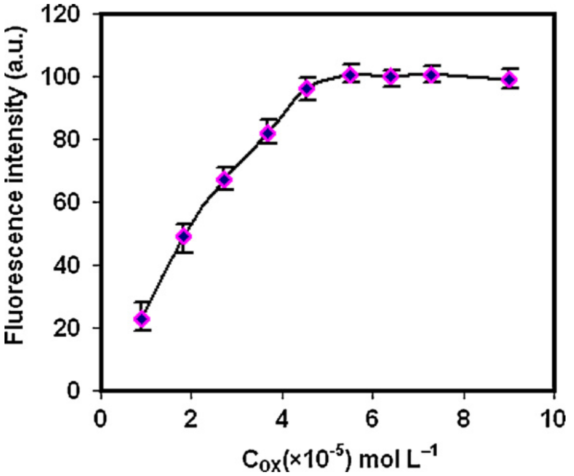

Fig. 3. Effect of oxine concentration on the extraction efficiency of aluminum. Utilized conditions: Al^{3+}, 6 μg L^{-1}; $H_2PO_4^-/HPO_4^{2-}$ buffer, 1.5×10^{-2} mol L^{-1} (pH 7.0); [Hpy][PF$_6$], 0.40 g; centrifugation time, 5 min.

3.2.5. Effect of ionic strength

To investigate the influence of ionic strength on the developed micro-extraction system, various experiments were performed by adding different amounts of $NaNO_3$ (0–0.5 mol L^{-1}) while the rest of the experimental conditions were kept constant. The obtained results showed that the addition of $NaNO_3$ within the interval of 0.0–0.3 mol L^{-1} had no considerable effect on the extraction efficiency. It was also observed that a portion of the IL-phase was dissolved into the aqueous phase when an excessive amount of salt was added to the extraction system. Moreover, at higher $NaNO_3$

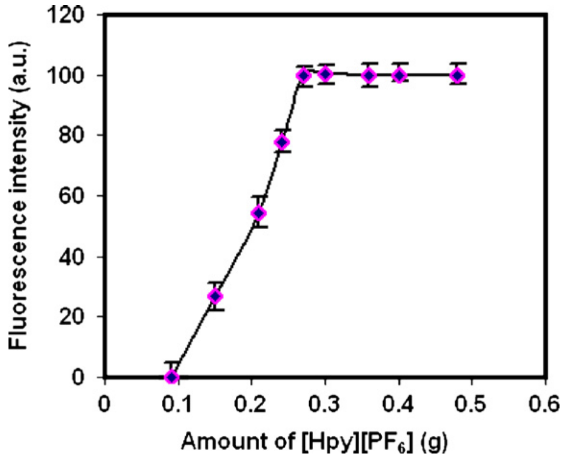

Fig. 4. Effect of amount of [Hpy][PF$_6$] on the extraction efficiency of aluminum. Utilized conditions: Al^{3+}, 6 μg L^{-1}; $H_2PO_4^-/HPO_4^{2-}$ buffer, 1.5×10^{-2} mol L^{-1} (pH 7.0); oxine, 6.4×10^{-5} mol L^{-1}; centrifugation time, 5 min.

Table 2
Tolerance limits of interfering ions in the determination of 6 $\mu g\,L^{-1}$ of Al.

Coexisting ions	Foreign ion to analyte ratio
Na^+, K^+, Cs^+, Ca^{2+}, Sr^{2+}, Sn^{2+}, Cl^-, Br^-, I^-, $SO_4{}^{2-}$, $NO_3{}^-$, $CO_3{}^{2-}$, $PO_4{}^{3-}$	1000:1
Mg^{2+}, Cr^{3+}, Bi^{3+}, Pb^{2+}, Ba^{2+}, Co^{2+}, F^-	500:1
$VO_3{}^-$, Ag^+, Zn^{2+}, Cd^{2+}	200:1
Ni^{2+}, Fe^{3+}, Cu^{2+}	50:1

concentration, the density of aqueous solution probably became higher than that of IL, so the IL-phase did not settle at the bottom of the centrifuge tube. In the tested range, the addition of $NaNO_3$ could not enhance the extraction efficiency. Thus, salt addition was not adopted, as it would significantly affect the formation of the biphasic system.

3.2.6. *Effect of temperature and extraction time*

Optimization of equilibration temperature and extraction time is necessary to achieve complete reaction and easy phase separation and efficient pre-concentration. Moreover, in IL-based DLLME method, the temperature plays an important role in complete solubilization and dispersion of ILs into the aqueous solutions. Therefore, the effect of the equilibration temperature was investigated from 4 to 50 °C before shaking the IL-containing solutions. It was found that the increase of temperature had no significant effect upon the extraction efficiency, and, consequently, room temperature was used in the extraction procedure.

In DLLME methodology, extraction time is defined as the time elapsing from the incorporation of the mixture of extraction and disperser agents to the moment centrifugation began. Keeping the room temperature, the effect of extraction time was examined in the range of 20–3600 s under constant experimental conditions. The obtained results demonstrated that the extraction time had no significant influence on the signal of Al. This would be due to the very large contact surface between the IL droplet and aqueous phase after the formation of the cloudy mixture, which resulted in very rapid processes of complexation and extraction. So, in order to keep analysis time as short as possible, the turbid solution was centrifuged immediately after the preparation at room temperature.

3.2.7. *Effect of centrifugation time*

In DLLME method, the most time-consuming step is the centrifuging of sample solution in the extraction procedure. Centrifugation accelerates the phase separation and the final performance would benefit from a full phase separation. Therefore, the effect of centrifugation time upon extraction efficiency was studied for the range of 1–20 min. The obtained results showed that, over 4 min, extraction efficiency was constant indicating complete transfer of IL-phase to the bottom of centrifuge tube. So, a centrifugation time of 5 min at 4000 rpm was selected for the entire procedure, since complete separation occurred for this time and longer times would not give much larger extraction efficiencies. Consequently, extraction performance without need to heating for solubilization or dispersion of extractant solvent, easy generation of cloudy solution and phase separation without need for cooling before or after centrifugation and short extraction and centrifugation time are the advantages of this IL-based DLLME technique.

3.2.8. *Sample volume and pre-concentration factor*

Sample volume is one of the most important parameters to be studied when real samples are analyzed by a pre-concentration technique, since it conditions the sensitivity enhancement of the method. In order to obtain the best pre-concentration factor, the extraction system was studied to allow the highest volume ratio between sample solution and IL-phase. Thus, the effect of sample volume was examined in a range of 10–45 mL for 6 $\mu g\,L^{-1}$ Al. After addition of an IL constant amount into different volumes of aqueous samples, the extraction procedure was performed for each solution. It was observed that the extraction efficiency of aluminum was quantitative in the range of 10–35 mL, and that, for higher sample volumes, the extraction efficiency decreased. This could be due to the IL-phase partial dissolution in the aqueous phase. On the other hand, when the amounts of IL and sample volumes increased in parallel, the extraction efficiency was constant. Thus, the magnitude of pre-concentration factor is limited by the solubility of $[Hpy][PF_6]$ in aqueous media. It was found that the solubility of $[Hpy][PF_6]$ IL in the proposed extraction system that contain 2 mL acetonitrile, as a disperser solvent, was nearly 6.5 $g\,L^{-1}$. Hence, a 30 mL sample volume was recommended to work with 0.3 g IL. The obtained pre-concentration factor for a sample volume of 30 mL and a final IL-phase volume of 300 μL was 100.

3.2.9. *Study of interferences*

In order to demonstrate the selectivity of the developed micro-extraction system, the effect of coexisting ions in real samples on the recovery of aluminum was also evaluated. The interferences

Table 3
Determination of aluminum in different real samples (results of recoveries of spiked samples and certified reference materials analysis).

Samples	Added Al ($\mu g\,L^{-1}$)	Found Al[a] ($\mu g\,L^{-1}$)	Recovery (%)
Mineral water[b]	–	18.46 ± 2.01	–
	10.0	28.17 ± 1.07	97.1
Rainwater[c]	–	20.44 ± 0.87	–
	10.0	30.08 ± 0.69	96.4
Orange juice[d]	–	276.50 ± 3.43	–
	100.0	369.20 ± 4.40	92.7
Sour cherry juice[d]	–	160.50 ± 2.44	–
	100.0	258.50 ± 3.10	98
Grape juice[e]	–	347.50 ± 2.86	
	100.0	443.80 ± 2.34	96.3
Cow's milk[f]	–	1099.00 ± 7.32	–
	100.0	1200.50 ± 8.11	101.5
Samples	Added Al ($\mu g\,g^{-1}$)	Found Al[a] ($\mu g\,g^{-1}$)	Recovery (%)
Milk powder[g]	–	14.82 ± 0.81	–
	10.0	24.74 ± 1.27	99.2
Wheat flour[h]	–	16.25 ± 0.86	–
	10.0	26.01 ± 0.64	97.6
NIST SRM	Certified values	Assayed values	Recovery (%)
1549	141.8 ± 8.6 ($\mu g\,g^{-1}$)	142.9 ± 6.7 ($\mu g\,g^{-1}$)	100.7
1643e	2[i] ($\mu g\,mL^{-1}$)	2.25 ± 0.30 ($\mu g\,mL^{-1}$)	–

[a] Mean of three experiments ± standard deviation.
[b] From Sahand Suo Co., Tabriz, Iran.
[c] Collected at Tabriz city, Iran (29 June 2009).
[d] From Zarnush Co., Urmia, Iran.
[e] From Aftab Co., Urmia, Iran.
[f] Obtained from the local market (Goldam Co., Azarshahr, Iran).
[g] Obtained from the local pharmacy (Humana milk powder).
[h] From wheat flour of Pirchupan village, Iran.
[i] Information value.

Table 4
Comparison of the proposed method with other pre-concentration methods.

Method	Linear range (μg L^{-1})	LOD (μg L^{-1})	EF[a]	Sample consumption (mL)	RSD (%)	Ref.
CPE–spectrofluorometry	2–200	0.79	10	25	2.74	[33]
CPP–FI-ICP-OES[b]	Up to 200	0.25	200	50	3.1	[34]
LLE–CL[c]	130–670	42.7	85	0.0018	4.5	[35]
On-line SPE–spectrophotometry	4.9–300	4.9	–	–	0.55	[36]
CPE–spectrofluorometry	10–200	0.5	20	10	5.41	[37]
CPE–FIA–Spectrophotometry	5–130	3.02	50	10	0.9	[38]
IL-based DLLME–SFS	0.06–15	0.05	100	30	1.7	This work

[a] Enhancement or enrichment factor.

[b] Cloud point pre-concentration–flow injection inductively coupled plasma optical emission spectrometry.

[c] Liquid–liquid extraction–chemiluminescence.

under study were those related to the pre-concentration step, i.e., cations other than Al^{3+} might react with oxine, and decrease the extraction efficiency, provoking interferences in the fluorescence intensity measurements. In these experiments, different amounts of ions were added to the test solutions containing 6 μg L^{-1} of aluminum and then followed according to general procedure. The tolerance level was defined as the maximum concentration of the foreign ion causing a change in the analytical signal no higher than 5%, when compared with the signal of 6 μg L^{-1} aluminum alone. The results are given in Table 2. It can be seen that commonly encountered concomitant ions such as alkali and alkaline earth elements do not interfere at high concentrations. Whereas, some of the species tried such as Cu^{2+}, Ni^{2+} and Fe^{3+} probably interfered at ratios higher than 50-fold with the determination of Al^{3+}. These interferences were eliminated by using an excess of oxine reagent in sample solutions in order to force the equilibrium towards the formation of Al–oxine complex. As shown later, these results allow the interference-free determination of Al in some natural waters, fruit juice and food samples.

3.2.10. Analytical figures of merit

Under the experimental conditions, a series of experiments were designed for obtaining linear range, precision, detection limit, enrichment factor and other characteristics of the proposed IL-based DLLME method. Extraction efficiency higher than 99.9% was achieved and three replicate extractions were performed for each concentration level. After micro-extraction procedure, the IL-phase was diluted with ethanol and the fluorescence intensity of the Al–oxine complex was measured. In all cases, linear relationships were attained between the fluorescence intensity and the Al concentration in the standard solutions, standard reference materials and real samples. The calibration graph using the pre-concentration system for Al^{3+} was linear between 0.06 and 15 μg L^{-1}, with a correlation coefficient of 0.9989. The regression equation was $I_F = 14.91\,C_{(Al)} + 6.78$, where I_F is the fluorescence intensity and $C_{(Al)}$ is aluminum concentration (μg L^{-1}). The limit of detection (LOD) calculated, as three times the standard deviation of the blank signal, was 0.05 μg L^{-1} for the pre-concentration of 30 mL of sample solution. The relative standard deviation (RSD) resulting from the analysis of 6 replicates of 30 mL solution containing 6 μg L^{-1} Al^{3+} was 1.7%. Regarding the frequency of analysis, although pre-concentration of the analyte for a single sample could take more than 5 min, it was possible to simultaneously treat as many samples as can be placed in the centrifugation equipment. Practically, about 40 analyses could be performed within a 1-h overall time.

3.2.11. Analysis of real samples

To test the reliability of the proposed procedure, the combined IL-based DLLME–SFS method was employed to determine the trace amounts of Al^{3+} in different real samples containing water samples (i.e., bottled mineral water and rainwater), packed fruit juice samples (i.e., orange, sour cherry and grape juices), milk samples (i.e., packed cows' milk and milk powder) and wheat flour sample. In order to verify the accuracy of the established procedure, recovery experiments were also carried out by spiking the samples with different amounts of aluminum before any pretreatment. Table 3 shows the obtained results. As can be seen, recoveries between 92.7% and 101.5% were obtained, which confirm the accuracy of the proposed method. Additionally, the accuracy of the proposed methodology was evaluated by analyzing two standard reference materials, NIST SRM 1643e (Trace Elements in Water), and NIST SRM 1549 (Non-Fat Milk Powder). The certified value of aluminum in SRM 1549 is 141.8 ± 8.6 μg g^{-1} and information value in SRM 1643e is 2 μg mL^{-1}. The corresponding values obtained by using the proposed method were 142.9 ± 6.7 μg g^{-1} and 2.25 ± 0.30 μg mL^{-1} (mean of three determinations ± standard deviation), respectively, which is in good agreement with the certified concentrations. It can be concluded that the proposed method is accurate and free from systematic errors. Statistical analysis of these results using Student's *t*-test showed that there was no significant difference between actual and found concentrations at 95% confidence level.

3.2.12. Comparison of the IL-based DLLME with other methods

We compared in Table 4 the linear range, limit of detection (LOD), enhancement or enrichment factor, relative standard deviation (RSD) and sample volume of the proposed technique with those of other related pre-concentration methods for the extraction and determination of aluminum. Compared to the other reported methods, ionic liquid was used instead of a volatile, possibly toxic organic solvent, as the extraction solvent. The proposed method has relatively low LOD (0.05 g L^{-1}), good enrichment factor (100) and short extraction procedure (less than 10 min) with a sample volume of 30 mL. Simple operation procedure makes the sample preparation very easy and rapid, only a few minutes are needed before instrumental analysis. In addition, owing to high viscosity of ILs, removing bulk aqueous phase is easier and this method is more suitable for extraction of heat-susceptible species in comparison with CPE. In conclusion, IL-based DLLME presents a sensitive, reproducible, simple, low cost and environment-friendly technique that can be used for the pre-concentration of aluminum in routine analytical laboratories.

4. Conclusions

In this study, we proposed the use of stopped-flow spectrofluorometry (SFS) as an alternative determination method after a new ionic liquid-based dispersive liquid–liquid micro-extraction and then this combined method was used for the pre-concentration and determination of trace levels of aluminum in different real samples. Our current hyphenated system using the peristaltic sipper equipped with a micro-cell (16 μL capacity) allows determining the analytes in small volume of the remaining IL-phase. The use of an ionic liquid as the extraction solvent in DLLME reduces the exposure danger to toxic organic solvents used in the conventional extraction procedures, increases the stability of extraction without suspending related to single-drop micro-extraction, enhances the sensitivity because of the larger volume of extraction solvent used and simplifies the extraction procedure. On the other hand [Hpy][PF_6] IL is a good choice for fluorescent measurements because it has very negligible background signal in a wide wavelength range. The results of this work show the possibility of using the oxine–[Hpy][PF_6] IL system for Al pre-concentration, since quantitative extraction and a pre-concentration factor of 100 were achieved. This IL-based DLLME–SFS method was proved to be simple, sensitive, fast and efficient for extraction and is environmentally friendly. The pre-concentration method was successfully applied to monitor low concentrations of aluminum in water, fruit juice and food samples with good accuracy and precision.

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