Chemistry
Communication
and Chemical
Engineering

ce.ccerci.ac.ir
Research Articles

Corresponding Author Emails:
n.shokoufi@ccerci.ac.ir
mirzaei@ccerci.ac.ir

Orcid.org/0000-0000-0000-000

Keywords:
ionic liquid, tuning of ILs, rotative liquid-liquid microextraction, polycyclic aromatic hydrocarbons

Ionic liquid based rotative liquid liquid microextraction combined with high performance liquid chromatography for determination of trace levels of polycyclic aromatic hydrocarbon

Mojtaba Mirzaei[a]* , Naeimeh Hadadi Heris[a], Koroush Tabar Heidar[a], Nader Shokoufi[a]*

[a] *Chemistry & Chemical Engineering Research Center of Iran, Tehran, Iran*

Abstract: A fast, simple and eco-friendly microextraction method termed ionic liquid-rotative liquid-liquid microextraction (IL-RLLME), coupled with high performance liquid chromatography (HPLC) was developed for preconcentration and determination of polycyclic aromatic hydrocarbons (PAHs) in environmental waters and food samples. The combination of ionic liquid-rotative liquid-liquid microextraction method with HPLC-DAD makes it possible to use of the less volume of the extraction phase and also simultaneous preconcentration and determination of several analytes. Different ionic liquids (ILs) were evaluated for preconcentration procedure and 1-hexyl-3-methylimidazolium bis (trifluoromethylsolfonyl) imid [C_6mim][NTf_2] was chosen as most suitable extraction solvent. In addition, various parameters affecting the extraction efficiency such as, volume of extraction solvent, extraction time, and speed were optimized. Under the optimized conditions, the calibration curves gave high levels of linearity having correlation coefficients (R) greater than 0.99 and the limits of detection (LODs), ranging from 0.005-0.013 µg L^{-1}. The relative standard deviations (RSDs) were in the range of 1.36-2.40 % (n = 8) for 100 mg L^{-1} mixed solution of PAHs. The proposed method provided high enrichment factors in the range of 590–1431 for PAHs.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed by a group of chemical compounds known carcinogenic. These compounds that contain two or more fused aromatic rings are from incomplete burning or pyrolysis of organic materials during various industrial processes.[1, 2] Due to the toxic nature, preconcentration and determination of PAHs in food products are important analytical problems. The most used techniques for separation and quantification of PAHs in different samples are chromatographic techniques. However, direct determination of PAHs in various samples needs to sample preparation techniques in order to achieve the required sensitivity and selectivity.[3-6]

Liu and Dasgupta[7] as well as Jeannot and Cantwell[8] presented the first papers on solvent microextraction into a drop in 1996. Later, Liquid-phase microextraction (LPME) as a miniaturized sample preparation approach was introduced by He and Lee.[9] As its name suggests, in LPME, only a micro volume of the extraction solvent is consumed to extract analysts from the aqueous samples. Several methodologies that are different in extraction performance have evolved including single drop microextraction (SDME)[8], hollow fiber liquid-phase microextraction (HF-LPME)[10], and dispersive liquid-phase microextraction (DLPME)[11]. To improve the extraction efficiency in LLME, solidification of floating organic drop microextraction (SFODME) [12], vortex-assisted liquid phase microextraction (VALLME)[13,14] , ultrasound-assisted emulsification microextraction (USAEME)[15-17], air–assisted liquid–liquid microextraction (AALLME)[18-21], and other methods have been developed. Recently, rotative liquid-liquid microextraction (RLLME) was introduced as a new LPME method by our group.[22] In the last years, microextraction techniques were employed increasingly for preconcentration and determination of PAHs[23], however in these methods, organic solvents had been used as extraction solvent particularly in DLLME. Organic solvents have toxic nature and are environmentally unfriendly. Moreover, these unstable solvents have high vapor pressure and very low viscosity. In order to overcome these drawbacks, the replacement of harmful organic solvents by ILs in different extraction processes was proposed. The peculiar characteristics of ILs such as high thermal stability, negligible vapor pressure, tunable viscosity and miscibility with water and organic solvents, make them attractive alternatives to environmentally unfriendly solvents.[24-26] Hence in 2003, Liu et al. reported the first study on the use of ILs such as 1-octyl-3-methylimidazolium hexafluorophosphate ([C_8mim][PF_6]) as an extraction solvent in liquid-phase microextraction (LPME).[27] Subsequently, Zhou and Ye introduced IL-enhanced SDME for determination of aromatic amines by designing a small ball mouth device to increase the contact area between the needle tip and suspended drop.[28] Furthermore, Yao and Anderson proposed an in situ metathesis reaction as an innovation in IL-DLLME, in which 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]) as a water-miscible IL through an ion exchange reaction with $LiNTf_2$ was converted to 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_4mim][NTf_2]) as a water-immiscible IL which used for the extraction of 13 of PAHs.[29] Pena developed IL-DLLME coupled by HPLC for determination of PAHs using 1-octyl-3-methylimidazolium hexafluorophosphate ([C_8mim][PF_6]) as an extraction solvent. In this method, dispersion was achieved via cooling step to form cloudy solution before centrifugation.[30] Moreover, De Boeck et al. have investigated the applicability of IL-DLLME coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the analysis of benzodiazepines in postmortem blood.[31] Avan and Hayati used the IL-DLLME for the spectrophotometric detection of bilirubin where bilirubin was analyzed through reacting with periodate in the presence of hydrochloric acid.[32] Given the importance of vitamin E in public health, determining its level in human serum is essential for routine clinical work, in this regard, Yin et al. used ionic liquid-based liquid-liquid microextraction combined with HPLC for simultaneous determination of vitamin E isomers in human serum.[33]

Herein, we introduced a novel and simple microextraction method named rotative liquid-liquid microextraction (RLLME) to preconcentration and determination of cobalt in pharmaceutical samples.[22] RLLME overcomes the main drawbacks of other microextraction methods like needing to the dispersive solvent and centrifugation step. The aim of this study is preconcentration and determination of the PAHs using IL based-rotative liquid-liquid microextraction (IL–based RLLME) method coupled with HPLC as a green microextraction technique.

Result and Discussion

The properties of ILs such as nonvolatility, appropriate viscosity, and thermal stability, make them attractive alternative for organic solvents. Nowadays, ILs are increasingly used in sample-preparation techniques. We proposed rotative mechanism for liquid phase microextraction by IL as a simple, effective and safe method.

Optimization of IL-RLLME-HPLC

The extraction efficiency of IL-RLLME-HPLC depends on some important experimental parameters which should be investigated in detail. The effect of type and volume of IL, extraction speed and time along with the salt addition were studied.

Selection of extraction solvent

An appropriate extraction solvent, in this case IL, is a crucial factor in extraction performance of the target analytes. The cation containing the alkyl part and anion of the IL has significant influence on its physical and chemical properties such as viscosity, solubility and density that might affect the extraction efficiency of target analytes. In IL-RLLME, the extraction solvent should have special characteristics including low viscosity, low solubility in water, higher density than water, extraction capability of interested compounds. Our surveys for selection of suitable cation and anion were done according to the physical parameters of different type of ILs. The viscosity change with anion change in [C6mim][X] was seen in Table 1 and Fig. 1A and the dependence of viscosity on side chain length change in [$C_n$mim][NTf_2] was shown in Table 2 and Fig. 1B. In Table 3 and Fig. 1C, the water solubility of [C_6mim][X] was drawn.

Table. 1. Dependence of viscosity on the type of anion in [C_6mim][X] ILs at 298.15 K

X	Viscosity (cP)	Refs
$B(CN)_4$	49.58	(Neves et al., 2013)[34]
$N(CN)_2$	49.99	(Neves et al., 2013)[34]
NTf_2	70.10	(Harris & Kanakubo, 2021)[35]
BF_4	205.90	(Harris & Kanakubo, 2021)[35]
PF_6	496.00	(Harris & Kanakubo, 2007)[36]
NO_3	454.00	(Khedri et al., 2019)[37]
CH_3CO_2	1270.00	(Fillion et al., 2016)[38]

Table. 2. Dependence of viscosity on the alkyl chain length in [$C_n$mim][NTf_2] ILs at 298.15 K

C_n	Viscosity (cP)	Refs
C_2	32.83	(Harris & Kanakubo, 2021)[35]
C_4	51.00	(Harris & Kanakubo, 2007)[36]
C_6	70.10	(Harris & Kanakubo, 2021)[35]
C_8	88.70	(Aljasmi et al., 2022)[39]
C_{10}	108.20	(Hosseini & Scurto, 2008)[40]

Table. 3. Water solubility of [C_6mim][X] ILs at 293.15 K

X	$10^6 x_{IL}$
PF_6	386.04×10^{-6}
NTf_2	66.06×10^{-6}
$C(CF_3SO_3)_3$	9.12×10^{-6}
$(C_2F_5)_3PF_3$	1.17×10^{-6}

(Ranke et al., 2007)[41]

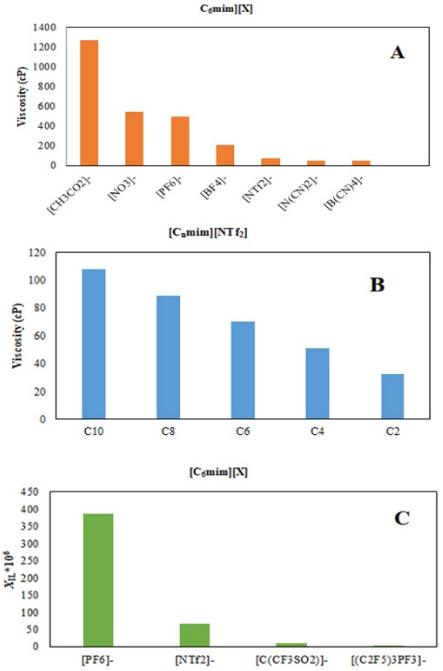


Fig. 1. Physical parameters of different ionic liquids; A) viscosity change with anion change in [C_6mim][X] ILs; B) viscosity change with alkyl length change in [$C_n$mim][NTf_2] ILs; C) water solubility change with anion change in [C_6mim][X] ILs

In this study, the four [C$_6$mim][PF$_6$], [C$_6$mim][NTf$_2$], [C$_4$mim][NTf$_2$] and [C$_6$Py][NTf$_2$] ILs were selected and compared as extraction solvents. From an analytical chemistry point of view, a larger peak area is desirable to get a lower detection limit. Thus, as seen in Fig. 2, [C$_6$mim][NTf$_2$] was selected as an effective solvent in the following investigation.

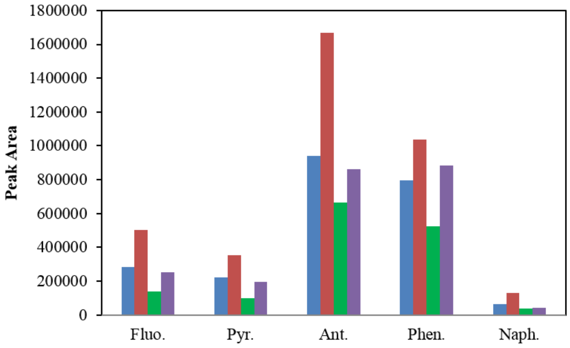


Fig. 2. Effect of IL's type on the peak area of PAHs. 100 µg L^{-1} PAHs extracted in 1800 rpm and 90 s using 40 µL solvent, [C$_4$mim][NTf$_2$] (■), [C$_6$mim][NTf$_2$] (■), [C$_6$Py][NTf$_2$] (■), [C$_6$mim][PF$_6$] (■)

Affecting factors in extraction efficiency

Volume of the extraction solvent

Another important factor in extraction efficiency is the volume of extraction solvent. As shown in Fig. 3, different volumes of the extraction solvent [C$_6$mim][NTf$_2$] (30, 40, and 50 µL) were investigated. The results showed that increasing the volume of the extraction solvent [C$_6$mim][NTf$_2$] increases the process efficiency. However, by the increase of the extraction solvent from 40 µL to 50 µL, the volume of the sedimented phase increases and consequently concentration of the analytes in the collected solvent decreases and leads to a decrease in the efficiency of the extraction process. Therefore, 40 µL was selected as the suitable extraction solvent volume.

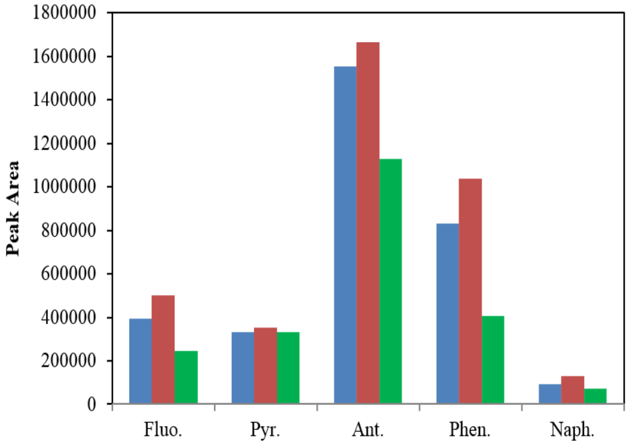


Fig. 3 Effect of the extraction solvent volume on the RLLME of PAHs. RLLME conditions: extraction solvent, [C$_6$mim][NTf$_2$]; rotative rate, 1800 rpm; extraction time, 90s; 30 µL (■), 40 µL (■), 50 µL (■)

Extraction time and Rotation speed

The test tube and its contents were rotated around a fixed axis at difference speed from 1000-2000 rpm. The maximum peak area was obtained in 1800 rpm (Fig. 4).

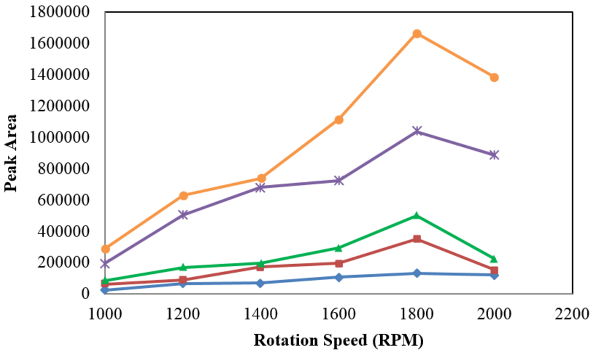


Fig. 4 Effect of the rotation speed on the peak area of PAHs. Sample: 5 mL of 1µg/L spiked PAHs in aqueous solution. RLLME conditions: extraction solvent, [C$_6$mim][NTf$_2$]; extraction solvent volume, 40 µL; extraction time, 90s, Ant (●), Phen (ж), Fluo. (▲), Pyr (■), Naph (♦)

Furthermore, the extraction time of RLLME is an important factor. In this experiment, extraction time means the time from the moment that the extraction solvent contact with aqueous samples and start to rotation. Thus, in order to evaluate the optimum extraction time, different rotation times (30, 40, 70, 90, 100, 120 s) were investigated at 1800 rpm rotational speed (Fig. 5). For extraction times longer than 90 s, the peak area was decreased and the reproducibility was lost significantly, therefore, 90 s was selected as extraction times.

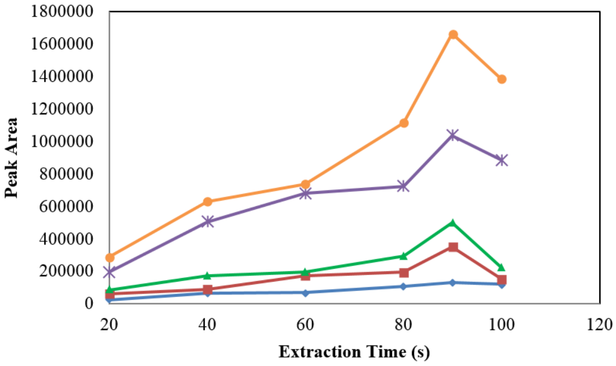


Fig. 5 Effect of extraction time on the peak area of PAHs. Sample: 5 mL of 1µg/L spiked PAHs in aqueous solution. RLLME conditions: extraction solvent, [C$_6$mim][NTf$_2$]; extraction solvent volume, 40 µL; rotative rate, 1800 rpm, Ant (●), Phen (ж), Fluo. (▲), Pyr (■), Naph (♦)

Effect of ionic strength

In general, addition of salt to water samples increases the ionic strength of the solutions where the salting effect improves the extraction efficiency by decreasing the solubility of analytes in the aqueous sample and enhances their partitioning into the acceptor phase for liquid-phase microextraction. Thus, in this study, the effect of salt addition on LPME of PAHs by IL was investigated by adding different amounts of NaCl to the sample. However, it may have negative effect when ILs are used as the extraction solvent and the experimental results showed that with increasing NaCl concentration from 0 to 3%, the efficiencies of IL-RLLME for PAHs extraction and peak area of analyte noticeably decreased. The reduction of the peak area could be related to the addition of salt, which increases the solubility of IL in water and reduces the diffusion rate of PAH molecules from the bulk sample solution to the IL phase.

Table. 4. Analytical performance of the IL-RLLME method for extraction of 5PAHs

Method	Extraction solvent	LOD (µg L^{-1})	R.S.D (%)	EFs	Time (min)
IL-SDME/HPLC-PDA[42]	[C_6mim][PF_6]	1.2-3	6		4
IL-HS-SDME/GC-MS[43]	[C_8mim][NTf_2]	0.005-0.400	6.1-11.8	80-110	-
IL-DLLME/HPLC-UV[29]	[C_4mim][Cl]	0.02-0.30	3.7-6.9	184-935	5
DLLME-SFO/HPLC-VWD[44]	1-Dodecanol	0.045-1.100	2.3-5.4	88-118	2
MAE-DLLME/GC-MS[45]	Tetrachloroethylene	0.11-0.43		244-373	10
SFE-DLLME/GC-FID[46]	Chlorobenzene	0.2		88-268	40
IL-DLLME /HPLC-FLU[30]	[C_8mim][PF_6]	0.0001-0.007	1.2-5.7	301-346	10
HF-LPME/HPLC[29]	[C_4mim][Cl]	0.25		90.9-109	15
IL-RLLME/HPLC-PDA	[C_6mim][NTf_2]	0.00075-0.013	1.37-2.4	590-1431	15

Analytical characteristics

In the optimum extraction condition, using 40 µL of [C_6mim][NTf_2] for 5 mL of the sample containing PAHs (100 µg L^{-1}) and extraction time 90 s at 1800 rpm, the performance of the proposed method was validated by a series of experiments with regard to the linearity range (LR), square of correlation coefficients (R^2), limit of detection (LOD), limit of quantification (LOQ), extraction recovery (ER), enrichment factor (EF), and repeatability (Table 4). The LOQ and LOD were calculated experimentally, being the minimum detectable amounts of analytes which showed a signal-to-noise ratio of 10 (LOQ) and signal-to-noise ratio of 3 (LOD), on analyzing the extract of the spiked blank matrix. EF is defined as the ratio of the concentration of analytes in the extraction phase (C_{sed}) to that in the sample phase (C_0). ER is defined as the ratio of analytes amount extracted to the [C_6mim][Tf_2N] phase (n_{sed}) to total analyte amount (n_0) and is a function of EF and the phase volume ratio (V_{sed}/V_0, where Vsed and V_0 are the volumes of the extraction phase and the sample phase, respectively) as described by the Eq. (1). Because of the high volume ratio between the aqueous sample and the extraction solvent, high enrichment factors can be using the following equation.

$$ER = \frac{n_{sed}}{n_0} = \frac{C_{sed}}{C_0} \times \frac{V_{sed}}{V_0} = EF \times \frac{V_{sed}}{V_0} \quad (1)$$

Analyte	Linear range (µgL^{-1})	Linear equation Y=	LOD (µgL^{-1})	LOQ (µgL^{-1})	EFs	RSD (% n=10)
Naph.	0.20-300.00	1647x +477.7	0.0130	0.0420	750.35	1.74
Pyr.	0.10-275.00	1907x+2980	0.0120	0.0396	590.00	1.37
Phen.	0.15-180.00	12923 + 1220	0.0028	0.0068	1431.23	1.70
Fluo.	0.05-275.00	2848x +7081	0.0020	0.0070	1773.57	2.30
Ant.	0.10-120.00	63348x + 11411	0.00075	0.0024	1934.13	2.40

Table. 5. Comparison of the developed IL-RLLME method with other procedures described in literature for the determination of PAHs

Comparison of IL-RLLME with other methods

The extraction efficiencies of PAHs by IL-RLLME/HPLC-PDA detection were compared with other reported methods based on the parameters such as extraction solvent, LOD, RSD, EF and extraction time. As listed in Table 5, the comparison of the results shows that the extraction time for this work is lower than that other methods. Moreover, in comparison with other methods it has higher enrichment factor. In addition to the advantages of DLLME, it does not require dispersion and centrifuge for collection. Therefore, proposed method is very simple, sensitive, rapid, reproducible, and easy to use and automation.

Real sample analysis

The optimized method was applied to the determination of 5 PAHs in seawaters, sunflower oil and olive oil samples. The results of the analysis are summarized in Tables 6 and 7. The analytical determinations were carried out in triplicate for each sample. The obtained results showed that the water samples were free of naphthalene and pyrene contaminations. To ensure the absence of matrix effects, samples were spiked with standard solutions of the analytes, and were extracted under the optimized conditions. These results demonstrated that the different matrices of seawater used in this experiment had a negligible effect on the result.

Table 6 Results for determination of PAHs in non-spiked and spiked water samples from Persian Gulf and Amirabad port.

	Analyte	Non spiked (µg L^{-1})	Spiked (µg L^{-1})	Founded (µg L^{-1})	Recovery (%)
Persian Gulf	Naph.	-	1.5	1.13	-
	Phen.	0.89	0.5	1.45	112
	Ant.	0.22	0.5	0.79	114
	Fluo.	14.40	1.5	14.59	12
	Pyr.	-	0.5	0.54	-
Amirabad port	Naph.	-	1.5	1.20	-
	Phen.	0.81	0.5	1.29	96
	Ant.	0.35	0.5	0.86	102
	Fluo.	18.20	1.5	18.28	7
	Pyr.	-	0.5	0.62	-

Table 7 Results for determination of PAHs in non-spiked and spiked food oil samples:

Sunflower Oil

Analyte	**No Spiked ($\mu g\ L^{-1}$)**	**Spiked ($\mu g\ L^{-1}$)**	**Founded ($\mu g\ L^{-1}$)**	**Recovery (%)**
Naph.	3.55	1.5	5.12	104
Phen.	2.73	0.5	3.26	106
Ant.	0.33	0.5	0.81	96
Fluo.	0.77	1.5	1.25	32
Pyr.	-	0.5	-	-

Olive Oil

Analyte	**No Spiked ($\mu g\ L^{-1}$)**	**Spiked ($\mu g\ L^{-1}$)**	**Founded ($\mu g\ L^{-1}$)**	**Recovery (%)**
Naph.	**1.50**	**1.5**	**2.8**	**86.6**
Phen.	**0.128**	**0.5**	**0.63**	**100.4**
Ant.	**1.52**	**0.5**	**1.94**	**84.0**
Fluo.	**2.01**	**1.5**	**2.72**	**47.3**
Pyr.	**-**	**0.5**	**0.28**	**56.4**

Experimental

The Chemicals and reagents

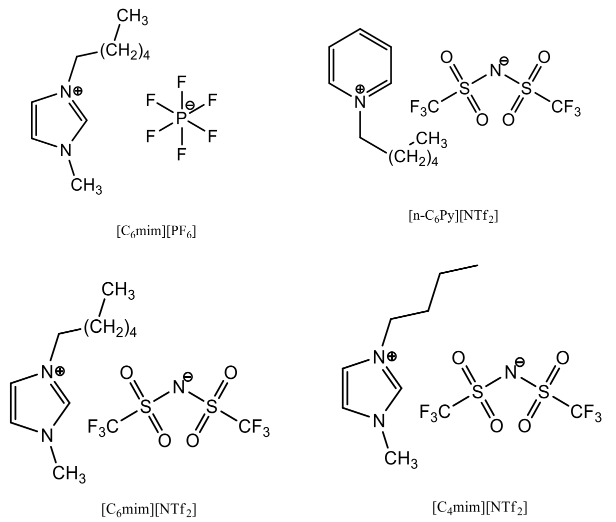
Analytical grade reagents were utilized during the experiments. naphthalene (Naph.), phenantherene (Phen.), anthracene (Ant.), fluoranthene (Fluo.), and pyrene (Pyr.) were purchased from Fluka (Buchs, Swiezerland). Stock solutions (100 mg/L) of each analyte were prepared in acetonitrile. The working standards were prepared by the subsequent dilution of stock solutions. HPLC grade solvents acetonitrile and sodium chloride (NaCl) were supplied by Merck (Germany). N-Methylimidazole (98%), pyridine (C5H5N, 98%), sodium nitrate ($NaNO_3$, 98%), silver nitrate ($AgNO_3$, 99.8%), phosphorus pentoxide (P_2O_5, 98%), 1-chlorobutane (C_4H_9Cl, 98%), 1-chlorohexane ($C_6H_{13}Cl$, 98%), and organic solvents (acetonitrile, ethyl acetate, ether, dichloromethane, acetone, anhydrous ethanol and chloroform) were all obtained from Merck Company. potassium hexafluorophosphate (KPF6, 99%) and lithium bis (trifluoromethylsulfonyl) imide ($LiNTf_2$, 99%) were purchased from Sigma-Aldrich Company. 1-hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide $[C_6mim][NTf_2]$, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide $[C_4mim][NTf_2]$, N-hexylpyridinium bis(trifluoromethylsulfonyl)imide $[C_6Py][NTf_2]$, and 1-hexyl-3-methylimidazolium hexafluorophosphate $[C_6mim][PF_6]$ were synthesized in our Lab, according to known procedure. The water used was deionized by a Nano pure water purification system Barnsted (Miliford, MA, USA). Water samples were collected from Persian Gulf and Amirabad port in glass bottles and stored in the dark at -4 °C before analysis and analyzed without any prior treatment or filtration to avoid loss of PAHs.

Instruments and chromatographic conditions

The ILs were characterized as $CDCl_3$ solutions by 1H NMR spectroscopy (Bruker Advance spectrometer, 500 MHz). Density and viscosity of the ILs were measured with densitometer model DEM-5000-Anton paar (± 0.01 Kg/m3) and viscometer model Anton paar SVM 3000) ± 0.0001 Pa.S(. The water content of the ILs was measured by Karl-Fischer (KF-Coulometer 831). The total chloride content of the ILs was determined potentiometrically using a chloride ion selective electrode (ISE) connected to a pH meter (Philips PW 9420). Chromatographic analysis was carried out by a Knauer/Smartline HPLC with Chromgate software version 3.1.7, equipped with Smartline 1000-1 and Smartline 1000-2 binary pumps, Jetstream Oven (Column thermostat), diode array detector (DAD) DAD-UV detector, a A1366 six-port two channel sample injection valve and a 20-μL loop (Knauer, Germany). A 25-μL HPLC and a 50-μL syringe were purchased from Hamilton (Switzerland). Vortex agitator from Reax Control was used Heidolph (Germany). Two adjustable samplers, (10–100 μL) and (100–1000 μL), were prepared from Eppendorf (Hamburg, Germany). The chromatographic separations were performed at 25°C by injecting 20 μL on an analytical Vydac 20ITD54C18 column (30 cm×5 mm, with 5 mμ particle size). A mixture of water–acetonitrile (30:70 v/v) with a flow rate of 1 ml/min was used as a mobile phase in isocratic elution.

Synthesis of ILs

We used four synthesized ionic liquid in our investigation (Fig. 6). The ILs were synthesized in two-steps. In the first step, the quaternized 1-Butyl-3-methylimidazolium chloride $[C_4mim][Cl]$, 1-Hexyl-3-methylimidazolium chloride $[C_6mim][Cl]$, and N-hexylpyridinium chloride $[C_6Py][Cl]$ were produced through a common method by interaction of 1-methylimidazole with a little excess of the corresponding 1-chloroalkane.[47]


$[C_6mim][PF_6]$

$[n\text{-}C_6Py][NTf_2]$

$[C_6mim][NTf_2]$

$[C_4mim][NTf_2]$

Fig. 6. The Ionic liquids used in this study

For example, to synthesis $[C_4mim][NTf_2]$, A mixture of 1-methylimidazole (5 mL, 63 mmol) and 1-chlorobutane (8 mL, 76 mmol) was dissolved in dry acetonitrile (30 ml) and refluxed for 48 h under inert atmosphere. The volatile materials were removed under deep vacuum and the remaining yellow oil was dissolved in acetonitrile and was added dropwise to a flask containing dry ethyl acetate to remove unreacted materials and obtaining $[C_4mim][Cl]$ as a white crystal (m.p: 62 °C) with approximately 85% yield. In the second step, the pure $[C_4mim][Cl]$ (8 g, 46 mmol) was undergone an ion exchange with lithium bis(trifluoromethansulfonyl)imide $LiNTf_2$ (14 g, 50 mmol) through 5 h in water (30 mL) at room temperature. Then, in order to remove unreacted substances, the water phase was decanted and the IL phase was washed several times by deionized water until no precipitation of AgCl observed by addition of $AgNO_3$ solution (0.01M) to filtrate. To absorb the remaining water, sodium sulfate was added to the IL solution in dichloromethane. After filtration, the dichloromethane was recycled by rotary evaporator and the residual was dried under vacuum for 6 h to give 15.20 g of $[C_4mim][NTf_2]$ as a light white liquid with a purity of 99% (Fig. 7). The structure of $[C_4mim][NTf_2]$ was confirmed by 1H Nuclear Magnetic Resonance (1H NMR) spectrum, recorded by a Bruker Advance spectrometer, 500 MHz in CD_3Cl at 25°C. 1H NMR ($CDCl_3$, δ ppm) 8.73 (s, 1 H), 7.30 (s, 1H), 7.29 (s, 1H), 4.16 (2H, J= 7.5 Hz, 2H), 3.93(s, 3H), 1.84 (m, 2H), 1.36 (m, 2H), 0.95 (3H, J= 7.4 Hz).

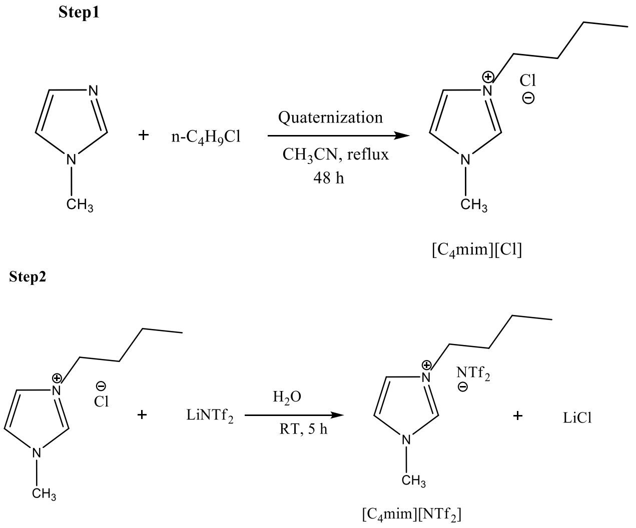


Fig. 7. Synthesis of $[C_4mim][NTf_2]$

The physical properties of the synthesized ILs are shown in Table 8.

Table. 8. Density, viscosity along with chloride ion and water contents of synthesized ILs

Ionic Liquid	Molecular Weight(Mw)	Density (kgm^{-3}) (25°C)	Viscosity (Pa.S) (25°C)	[Cl^-] ppm	H_2O ppm
$[C_4mim][NTf_2]$	419.40	1432.22	0.0522	20	50
$[C_6mim][NTf_2]$	447.42	1371.03	0.0708	25	40
$[C_6Py][NTf_2]$	444.41	1384.41	0.0962	20	46
$[C_6mim][PF_6]$	312.24	1294.11	0.4880	28	52

IL-RLLME Procedure

The IL-RLLME system contains two liquid phases: the aqueous sample containing the target analytes and the IL as the solvent extraction. 5.0 mL of the sample solution with all target analytes were placed in a 15 mL conical test tube (diameter: 2 cm), then 40 µL of the suitable IL $[C_6mim][NTf_2]$ was introduced as the extraction solvent. Afterwards, the test tube contents were rotated around a fixed z axis for 90 s at 1800 rpm by vortex agitator. The principles of RLLME were given in previous work.[22] Due to different velocities of the organic phase and the sample solution, the aqueous phase was rotated with higher velocity than the organic solvent. Because of different viscosity and density of the IL and sample solution, the aqueous phase was rotated with high velocity over the IL. Therefore, the microextraction was performed by increasing the number of collisions per unit time between two phases. Separation of the two phases automatically occurred on the conical test tube immediately after several seconds and the IL was settled to the conical bottom of the glass test tube (Fig. 8). In order to reduce the viscosity and easy transfer, the settled phase at the bottom of the conical test tube was redissolved in 5 µL acetonitrile. Thus, 30µL of IL could be collected with a micro syringe and injected directly into the HPLC for analysis. As shown in Fig. 9, the proposed PAHs passed through the column after 20 minutes and a chromatogram with clean separation was readily achieved.

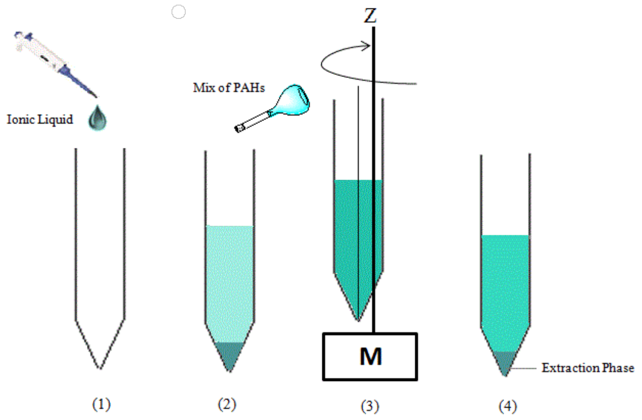


Fig. 8. Schematic of rotative liquid-liquid microextraction

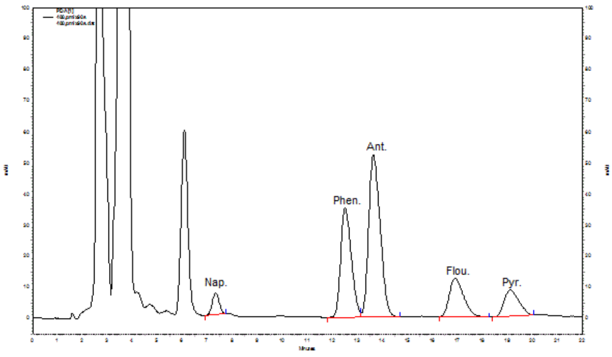


Fig. 9. Chromatogram related to the microextraction of the target analytes by IL-RLLME – HPLC at the concentration level of Ant.: 20 µg L^{-1}; Phen.: 30 µg L^{-1} ; Naph., Fluo. and Pyr.: 50 µg L^{-1}

Conclusions

Ionic liquid-rotative liquid liquid microextraction (IL-RLLME) in combinations with HPLC-DAD, have been developed successfully for the determination of trace amounts of PAHs. Polycyclic aromatic hydrocarbons (PAHs) are a class of potentially mutagenic substances which was fond in food oils and environmentally waters with different origins. The optimized liquid phase microextraction method using ionic liquid was shown good precision in the developed application. These methods are environmentally friendly due to the no consumption of toxic organic solvent. The IL-RLLME method has shown to be much more efficient than SDME and DLLME to carry out and presenting better time, low detection limits, and extraction recoveries. In addition, the IL-RLLME method is very rapid, around 90s for the overall process when compared to the necessity of 10 min in DLLME and 60 min in SDME. The proposed method provided high enrichment factors in the range of 590–1431 for different compounds of PAH. Thus, we propose this method as a fast, simple, safe, effective, and selective for analysis of PAHs. Moreover, it is possible to tune different ILs to be used in IL-RLLME/ HPLC-DAD. On the other hand, it will have more potential for on-line preconcentration and automation for sample treatment processes.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Research Council of Chemistry & Chemical Engineering Research Center of Iran. We many thanks Dr. Behnaz Abasgolinejad Asbagi for the kind cooperation.

References

1. IARC Monograph. Evaluation of carcinogenic risk of polynuclear aromatic compounds. *IARC*, **1983**, *5*, 169–195.
2. Lee, M. Analytical chemistry of polycyclic aromatic compounds. *Elsevier*, **2012**.
3. Srogi, K. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environmental Chemistry Letters*, **2007**, *5*, 169–195.
4. Pandey, S.K., Kim, K.H., Brown, R.J.C. A review of techniques for determination of polycyclic aromatic hydrocarbons in air. *Trends in Analytical Chemistry*, **2011**, *30*, 1716–1739.
5. Lawrence, J.F., Weber, D.F. Determination of polycyclic aromatic hydrocarbons in Canadian commercial fish, shellfish, and meat products. *Journal of Agricultural and Food Chemistry*, **1984**, *32*, 789–794.
6. Shariati-Feizabadi, S., Yamini, Y., Bahramifar, N. Headspace solvent microextraction and GC determination of PAHs in water samples. *Analytica Chimica Acta*, **2003**, *489*, 21–31.
7. Liu, H., Dasgupta, P.K. Analytical chemistry in a drop: solvent extraction in a microdrop. *Analytical Chemistry*, **1996**, *68*, 1817–1821.
8. Jeannot, M.A., Cantwell, F.F. Solvent microextraction into a single drop. *Analytical Chemistry*, **1996**, *68*, 2236–2240.
9. He, Y., Lee, H.K. Liquid-phase microextraction using a microsyringe. *Analytical Chemistry*, **1997**, *69*, 4634–4640.
10. Pedersen-Bjergaard, S., Rasmussen, K.E. Liquid–liquid–liquid microextraction prior to capillary electrophoresis. *Analytical Chemistry*, **1999**, *71*, 2650–2656.
11. Rezaee, M., et al. Dispersive liquid–liquid microextraction for determination of organics in water. *Journal of Chromatography A*, **2006**, *1116*, 1–9.
12. Hu, L., et al. In situ metathesis reaction with liquid-phase microextraction. *Talanta*, **2015**, *144*, 98–104.
13. Zhang, Y., Lee, H.K. UV filters in water by dispersive LLE and GC-MS. *Journal of Chromatography A*, **2012**, *1249*, 25–31.
14. Xue, J., et al. Fungicide residues in chrysanthemum by DLLE. *Journal of Chromatography A*, **2015**, *975*, 9–17.
15. Tuzen, M., Pekiner, O.Z. Selenium speciation via IL-DLLE. *Food Chemistry*, **2015**, *188*, 619–624.
16. Ezoddin, M., et al. Supramolecular IL-DLLE for palladium. *Journal of Molecular Liquids*, **2015**, *209*, 515–519.
17. Gong, A., Zhu, X. DLLE-HPLC for ulipristal acetate. *Talanta*, **2015**, *131*, 603–608.
18. Farajzadeh, M.A., Afsharmogaddam, M.R. Air-assisted DLLE of phthalates. *Analytica Chimica Acta*, **2012**, *728*, 31–38.
19. Barfi, B., et al. Solvent-free air-assisted DLLE of dyes. *Journal of Chromatography B*, **2015**, *998*, 15–25.
20. You, X., et al. Air-assisted DLLE of fungicides in juice. *Analytica Chimica Acta*, **2015**, *875*, 54–60.
21. Farajzadeh, M.A., et al. DLLE of parabens in personal care products. *Journal of Separation Science*, **2013**, *36*, 3571–3578.
22. Shokoufi, N., Asbaghi, B.A.N. Rotative LLE for cobalt determination. *Separation Science and Technology*, **2015**, *50*, 2327–2334.
23. Guo, L., Lee, H.K. Demulsification DLLE for PAHs. *Journal of Chromatography A*, **2011**, *1218*, 5040–5046.
24. Zhou, Q.X., et al. Ionic liquid SDME of phenols. *Chinese Chemical Letters*, **2006**, *17*, 1073–1076.
25. Ye, C.L., et al. IL-HS-LPME for phenols. *Journal of Separation Science*, **2007**, *30*, 42–47.
26. Liu, R., et al. Ionic liquids in sample preparation. *Analytical and Bioanalytical Chemistry*, **2009**, *393*, 871–883.
27. Liu, J.F., et al. IL-LPME of PAHs. *Analytical Chemistry*, **2003**, *75*, 5870–5876.
28. Zhou, Q.X., Ye, C.L. IL-SDME for aromatic amines. *Microchimica Acta*, **2008**, *162*, 153–159.
29. Yao, C., Anderson, J.L. In situ metathesis IL for preconcentration. *Analytical and Bioanalytical Chemistry*, **2009**, *395*, 1491–1502.

30. Pena, M.T., et al. IL-DLLE for PAHs in water. *Journal of Chromatography A*, **2009**, *1216*, 6356–6364.
31. De Boeck, M., et al. IL-LLE for benzodiazepines in postmortem blood. *Journal of Forensic Sciences*, **2018**, *63*, 1875–1879.
32. Avan, A.A., Hayati, F. IL-DLLE and spectrophotometry for bilirubin. *Current Analytical Chemistry*, **2020**, *16*, 652–659.
33. Yin, S., et al. IL-LLE-HPLC for vitamin E isomers. *Journal of AOAC International*, **2020**, *103*, 989–996.
34. Neves, C.M.S.S., et al. Thermophysical properties of ILs. *Journal of Physical Chemistry B*, **2013**, *117*, 10271–10283.
35. Harris, K.R., Kanakubo, M. Viscosity of imidazolium ILs. *Journal of Chemical Engineering Data*, **2021**, *66*, 4618–4628.
36. Harris, K.R., et al. Viscosity of ILs at elevated pressure. *Journal of Chemical Engineering Data*, **2007**, *52*, 1080–1085.
37. Khedri, Z., et al. Thermodynamic properties of IL-alkanol mixtures. *Journal of Chemical Engineering Data*, **2019**, *64*, 4465–4473.
38. Fillion, J., et al. Phase transitions and properties of ILs. *Journal of Chemical Engineering Data*, **2016**, *61*, 2897–2914.
39. Aljasmi, A., et al. Physicochemical properties of ILs. *Journal of Chemical Engineering Data*, **2022**, *64*, 858–868.
40. Hosseini, A., Scurto, A.M. Viscosity of imidazolium ILs. *International Journal of Thermophysics*, **2008**, *29*, 1222–1243.
41. Ranke, J., et al. Lipophilicity of IL cations and cytotoxicity. *Ecotoxicology and Environmental Safety*, **2007**, *67*, 430–438.
42. Vidal, L., et al. IL-SDME for benzophenone-3 in urine. *Journal of Chromatography A*, **2007**, *1174*, 95–103.
43. Hsieh, Y.N., et al. Nafion-supported IL-SPME for PAHs. *Analytica Chimica Acta*, **2006**, *557*, 321–328.
44. Xu, H., et al. IL-DLLE for PAHs in aqueous samples. *Analytica Chimica Acta*, **2009**, *636*, 28–33.
45. Ghasemzadeh-Mohammadi, V., et al. MAE and IL-DLLE for PAHs in smoked fish. *Journal of Chromatography A*, **2012**, *1237*, 30–36.
46. Rezaee, M., et al. SFE with IL-DLLE for organics in solids. Journal of Supercritical Fluids
47. Mirzaei, M., Mokhtarani, B., Badiei, A., & Sharifi, A. Solubility of carbon dioxide and methane in 1-hexyl-3-methylimidazolium nitrate ionic liquid, experimental and thermodynamic modeling. *The Journal of Chemical Thermodynamics*, **2018**, *122*, 31–37