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Determination of 3-nitroaniline in water samples by directly suspended droplet three-phase liquid-phase microextraction using 18-crown-6 ether and high-performance liquid chromatography

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ABSTRACT

Liquid–liquid–liquid microextraction (LLLME) with directly suspended droplet in high-performance liquid chromatography (HPLC) has been applied as a new, rapid and easy method for the determination of 3-nitroaniline in environmental water samples. The target compound was extracted from the aqueous sample solution (donor phase, pH 13) into an organic phase and then was back-extracted into a directly suspended droplet of an acidic aqueous solution (acceptor phase, pH 0.3). In this method, without using a microsyringe as supporting device, an aqueous large droplet is freely suspended at the top-center position of an immiscible organic solvent, which is laid over the aqueous sample solution while being agitated. Then, the droplet was withdrawn into the microsyringe and directly was injected into the HPLC system with UV detection at 227 nm. Up to 148-fold enrichment of the analyte could be obtained under the optimal conditions [i.e. donor phase: 0.1 M sodium hydroxide solution (4.5 mL); organic phase: *o*-xylene/1-octanol (90:10, v/v; 250 μL); acceptor phase: 0.5 M hydrochloric acid and 500 mM 18-crown-6 ether (6 μL); extraction time: 60 s; back-extraction time: 6 min and stirring rate: 600 rpm]. The limit of detection was 1 μg/L ($n = 7$) and the relative standard deviation (RSD, $n = 5$) was 4.9 at S/N = 3. The calibration graph was linear in the range of 5–1500 μg/L with $r = 0.9983$. All experiments were carried out at room temperature (22 ± 0.5 °C).

1. Introduction

Aromatic amines such as aniline and its derivatives are widely used in industry, production of dyes and antioxidants, cosmetics, pesticides, pharmaceuticals and as the intermediate in many chemical syntheses [1,2]. Nowadays most of these amines are known to be highly mutagenic and carcinogenic and to form adducts with proteins and DNA [3]. Furthermore, it is well known that these compounds are carcinogens and have been implicated in inducing cancer of the bladder [4,5]. Therefore, with the growing use of these compounds in different industries, monitoring of their levels in environmental waters is very important for the protection of health and the environment. These compounds have been included in the US Environmental Protection Agency (EPA) list of priority pollutants [6,7].

These compounds are thermo-labile and polar, and a derivatization step is often required to obtain a good GC performance [2,8,9]. Hence, analytical technique based on reversed-phase high-performance liquid chromatography (RP-HPLC) seems to be a good alternative to GC analysis for the determination of the aromatic amines in environmental analysis [10]. Amines are present in the environment at low level of parts per billion or less. Thus, a preconcentration step is generally required for the determination of their trace levels as the pollutants [10]. Sample preparation is traditionally carried out by liquid–liquid extraction (LLE) or by solid-phase extraction (SPE) techniques [11,12], which need a substantial amount of organic solvents. However, both techniques require the evaporation of the solvent to dryness and the reconstitution of the dry residue in a suitable solvent for HPLC or capillary electrophoresis (CE). These manual procedures are normally tedious and prone to loss of the analytes through evaporation and reconstitution. Therefore, the extraction and clean up of the sample has been performed using a number of different purification techniques such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). The most common sample isolation and preconcentration technique is solid-phase microextraction, which is a solvent free technique [13–15]. But this method has some disadvantages such as, the SPME fibers, which are coated with

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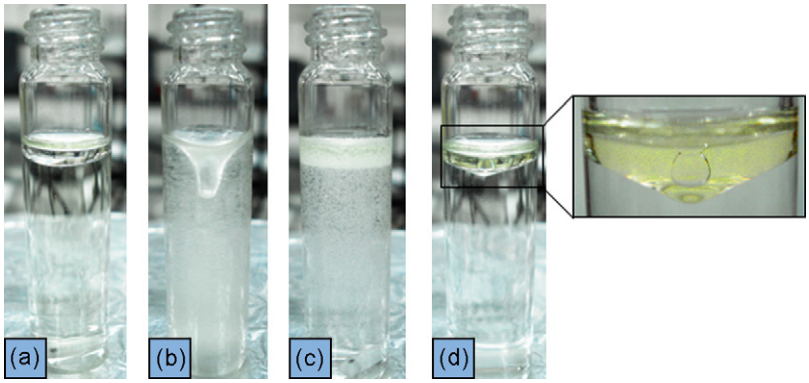

Fig. 1. Illustration of the microextraction apparatus for directly suspended droplet LLLME: (a) addition of the organic solvent to the aqueous sample solution, magnetic stirrer is off; (b) the mixture is being agitated, extraction procedure; (c) separation of the tiny drop of the organic solvent and aqueous sample solution and then addition of the acceptor phase into the organic solvent, magnetic stirrer is off; (d) back-extraction procedure, magnetic stirrer is on.

selective polymer, are fragile and still comparatively expensive. Furthermore, when SPME is coupled to HPLC, a special SPME–HPLC interface device has to be used for solvent desorption to recover all absorbed analytes and to avoid carry-over. Moreover, SPME techniques suffer extensively from the analyte carry-over due to the incomplete analyte desorption [16]. Because of these problems, an alternative miniaturized sample preparation approach, i.e., liquid-phase microextraction (LPME), emerged in the mid-to-late 1990s [17,18]. In LPME, only a small amount of the solvent (microliter) is needed for concentrating of the analytes from the aqueous samples. This method overcomes many of the disadvantages of LLE and SPME, which are mentioned above. In two-phase LPME, extraction takes place between a small amount of a water-immiscible organic solvent and an aqueous phase containing the analytes. However, if the analytes are further back-extracted into a third (aqueous) phase, the procedure is termed three-phase LPME or liquid–liquid–liquid microextraction (LLLME) which is usually used for the organic acids and bases [19–21].

In the present work, we used a new design of liquid–liquid–liquid microextraction method for the preconcentration of the target compound from environmental water samples combined with HPLC. In this method, contrary to the conventional single drop liquid-phase microextraction technique (SD-LPME), an aqueous droplet is directly suspended on the surface of the organic solvent, without using a microsyringe as supporting device. Therefore, a larger droplet with a higher lifetime than conventional one can be used. Because, in this modification, there is a high contact area between the aqueous microdrop and the organic solvent, in comparison with the mode of using the needle tip of the microsyringe. Thus, as the drop surface increases with the increase of the drop volume, this results in a larger enrichment factor [22]. On the other hand, this large and self-stable droplet is freely suspended in the organic solvent and can be rotated around a symmetrical axis during the extraction procedure, which causes an increase in mass transfer process and decrease in equilibrium time. Compared to the most conventional extraction procedures, this extraction technique is very fast, easy and simple.

2. Experimental

2.1. Chemicals and reagents

3-Nitroaniline (3-NA, analytical standard) and 18-crown-6 ether were obtained from Riedel de Haën (Steinheim, Germany). Analytical reagent-grade methanol, benzene, toluene, *o*-xylene, *n*-heptane and hydrochloric acid were purchased from Merck (Darmstadt, Germany). 1-Octanol was purchased from Fluka (Buchs, Switzerland). These compounds were all HPLC grade and were used without further purification. Sodium hydroxide was from Farabi (Tehran, Iran).

Stock solution of analyte (0.1 mg/mL) was prepared in methanol and stored at 4 °C. Standard sample solutions were provided daily at different concentrations by diluting the stock standard solution with distilled water, which was purified on a Milli-Q ultra-pure water-purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

The HPLC system consisted of a Waters 600 E (Millipore, Milford, MA, USA), LC-600 pump, C1 Cheminert injector valve equipped with a 20 μL sample loop (Switzerland), a Waters 486 tuneable UV–Vis detector and a Waters 746 integrator. A C_{18} column (125 mm length, 4.0 mm diameter, and 5 μm particle size) was used for separation. This column was packed in our laboratory with a Knauer packing system including a Knauer pneumatic HPLC pump (Berlin, Germany), using packing material (Eurospher 100, C_{18}). The degassed mobile phase was a mixture of methanol–pure water optimized on (30:70, v/v). The mobile phase flow-rate was 1 mL/min and the UV detection wavelength was set at 227 nm. The column was used at ambient temperature (22 ± 0.5 °C).

2.3. Directly suspended droplet LLLME

The basic experimental apparatus is shown in Fig. 1. Briefly, 4.5 mL of aqueous sample solution (adjusted to pH 13 with NaOH) was placed in a 6 mL sample vial, along with a (7 mm × 3 mm) magnetic stirring bar. The sample vial was placed above the heating-magnetic stirrer (0–1200 rpm) for stirring the extraction mixture. A 25 μL flat-cut HPLC microsyringe (Hamilton, Reno, NV, USA) was used to introduce the acceptor phase and it also served as a sample introduction device into the HPLC. Extraction was performed according to the following procedure. Sample solution was added to the glass vial and the magnetic bar was placed into the vial. Organic solvent (250 μL, *o*-xylene/1-octanol 90:10, v/v) was then added to the sample solution by a 1000 μL syringe (Knauer, Gaithersburg, MD, USA). Then the mixture was agitated for 60 s at 1200 rpm and a cloudy mixture of the sample solution and the tiny drops of the organic solvent was obtained. After then, the mixture was allowed to be quiescent for few seconds to gather the drops of the organic solvent together up to the aqueous sample solution. Afterward, the acceptor phase (6 μL 0.5 M HCl, pH 0.3) was delivered at the top-center position of the immiscible organic solvent. After stirring the mixture in the rate of 600 rpm for 6 min, the microdroplet was withdrawn-back by the HPLC microsyringe and then was injected into the HPLC system with UV detection at 227 nm.

Table 1
Characteristics of organic solvents [25] and enrichment factors (EF).

Solvent	Density (g/cm^3)	Solubility in water (g/L)	Viscosity	EF
1-Octanol	0.824	Insoluble	6.490	52.2
Benzene	0.878	1.87	0.604	85.5
Toluene	0.867	0.50	0.778	69.9
o-Xylene	0.880	0.20	0.812	69.1
n-Heptane	0.683	0.003	0.408	–

3. Results and discussion

3.1. Theory of LLLME

Liquid–liquid–liquid-phase microextraction consists of two processes and three phases: extraction from donor phase (P1) into an organic solvent (P2), and finally back-extraction from the organic phase into an aqueous acceptor phase (P3). In such cases, the pH of the sample is adjusted to make the analytes neutral and thus extractable into the organic solvent. After reaching the equilibrium of phase separation, the analytes that are mostly transferred into the organic phase are back-extracted into the second aqueous phase (acceptor) set to a pH at which, the analytes are charged. This back-extraction step introduces extra selectivity since neutral compounds will preferably stay in the organic phase [10,23]. The theory of the method is well defined by the others [19,24].

3.2. Optimization method

To obtain the optimal extraction conditions for the best efficiency, various parameters like organic solvent, extraction and back-extraction times, different volumes of phases, stirring speed, pH, salting effect and addition of crown ether were tested, which can be discussed as follows:

3.2.1. Organic solvent selection

In LLLME, the type of the organic solvent is an essential factor for achieving the efficient analyte preconcentration. There are several requirements for obtaining the selected organic solvent. The appropriate organic solvents in this work should have lower density than the water to float on the top of the aqueous sample solution. It should be immiscible with water to avoid dissolution in two aqueous phases, because it serves as a barrier between them. The organic solvent should have high viscosity to hold the microdroplet at its top-center position (Fig. 1) without using a microsyringe as supporting device. During this experiment, several organic solvents were tested to investigate their effect on the extraction efficiency.

Five organic solvents such as, 1-octanol, toluene, benzene, *o*-xylene and *n*-heptane have been examined. The characterizations of these solvents are shown in Table 1. Benzene has the highest enrichment factor among the other solvents, but nowadays due to the high toxicity, it is not a preferable solvent for the extractions in laboratories. Toluene and *o*-xylene have the best conditions for the extraction and showed the higher analyte enrichment factors than the others. Among them, *o*-xylene was selected, because it has higher viscosity. Since the microdroplet was not very stable inside it, 1-octanol (with higher viscosity) was used along with *o*-xylene as the extractant. The various percents of 1-octanol in binary mixtures were tested. The best extraction efficiency was obtained when the binary mixture of *o*-xylene/1-octanol (90:10, v/v) was used. Therefore, this mixture was selected as the organic solvent for further studies.

3.2.2. Volume of phases

In the present work, the volume of the acceptor phase was changed while the volume of the donor phase was kept constant at 4.5 mL. In three liquid-phase systems, the enrichment factor can be improved by the increase in the volume ratio of the donor and acceptor phases [18]. However, the volume of the acceptor phase may also be adjusted, related to the analytical instrument which is used. For example, usually in HPLC contrary to GC, injected sample volumes are in the range of 10–25 μL. Therefore, the use of a larger drop results in an increase of the analytical response, but very large drop causes a decrease in the enrichment factor due to the dilution of the analytes in these large droplets. On the other hand, these large droplets are not very stable especially at the high stirring rates. The volumes of the acceptor phase were changed from 3 to 7 μL, and with a 6 μL droplet the best enrichment factor was obtained.

In this work, the volume of the organic phase is too important, due to the special design of the extraction device and must be carefully optimized. The organic solvent keeps the aqueous droplet at the top of its surface. The volume of the organic layer will affect the lifetime of the aqueous droplet and the extraction efficiency. The best volume of the organic solvent was found to be 250 μL. Smaller volumes of the organic solvent tend to cause instability of the aqueous droplet during agitation, and higher volumes (more than 250 μL) cause lower enrichment factors. Consequently, a 250 μL volume of the organic solvent was chosen for the subsequent extractions.

3.2.3. Extraction time

The extraction of 3-nitroaniline from the aqueous sample into the organic phase can be described as a slow equilibrium process. Therefore, the extraction time is expected to be an important factor in the extraction process. According to the theoretical model of the mass transfer for the solvent microextraction [26], increasing of the stirring speed causes an increase in the mass transfer coefficient. In this work, we used a binary mixture of *o*-xylene (very low solubility in water) and 1-octanol (insoluble in water) as extractant, which have lower density than water. Consequently, before addition of the microdroplet, the stirring speed was fixed at 1200 rpm for agitating the donor and organic phase vigorously. Thus, a cloudy mixture of the sample solution and the tiny droplets of the organic solvent was obtained (Fig. 1b) and the intersections between the donor phase and these organic drops were broad and the mass transfer occurred very fast. Our results of the LLLME support this explanation too. The range of extraction times investigated here was between 15 and 90 s. When 1200 rpm of stirring speed was applied, the changes of the HPLC signals became steady after 60 s, and the increase of the signals with elapsed extraction time was very slow. Therefore, higher agitation can increase the extraction efficiency along with a decrease in the extraction time.

3.2.4. Back-extraction time

Maximum efficiency is obtained at the equilibrium, and usually it takes too long. If we use the equilibrium time for the extraction, the droplet will not be stable due to the dissolution, loss or fall. Therefore, the back-extraction from the organic solvent (*o*-xylene/1-octanol 90:10, v/v) into the aqueous microdroplet (6 μL 0.5 M HCl) should not be too long. It took only 6 min for the back-extraction to attain equilibrium. The enrichment factor did not increase significantly after 6 min and after this length of time the droplet was not stable and fell down in the vortex, which is created in the organic solvent while being agitated. Thus, the back-extraction time for further experiments was chosen as 6 min.

3.2.5. The pH of the donor and acceptor phases

In three-phase microextraction process, the pH of the donor phase is adjusted to produce molecular forms of the analytes, and the acceptor phase is adjusted to ionize them. The difference in pH between the donor and acceptor phases can promote the extracted analytes from donor to acceptor phase. Since the 3-nitroaniline is a

Table 2
Effect of pH of donor and acceptor phases on enrichment factors[a].

0.1 M HCl as acceptor phase[b]				0.1 M NaOH as donor phase[c]			
0.001 M NaOH	0.01 M NaOH	0.1 M NaOH	1 M NaOH	0.001 M HCl	0.01 M HCl	0.1 M HCl	0.5 M HCl
18.5	19.7	27.2	27.8	1.2	4.2	27.2	68.5

[a] The concentration of 3-nitroaniline was 1 μg/mL.
[b] The concentration of HCl in the acceptor phase is fixed, the concentration of NaOH in donor phase is varied.
[c] The concentration of NaOH in the donor phase is fixed, the concentration of HCl in acceptor phase is varied.

weak base (pK_a = 2.47) [25,27], protonation is an important reaction. First, the donor phase was investigated by using the various concentrations (0.001–1 M) of NaOH, to adjust the alkaline for deionizing of the 3-nitroaniline. The results, shown in Table 2, indicate that increasing of the NaOH concentrations dose provide some, but not significant enhancement of the extraction efficiency. The difference of enrichment factors between 0.1 and 1 M NaOH was not so significant; therefore, 0.1 M NaOH was used as the donor media.

While the pH of the donor sample solution is not a critical factor, the extraction efficiency is more depended on the pH of the acceptor phase. As shown in Table 2, the HCl concentrations were studied in the range of 0.001–0.5 M. The higher concentration of acid was not used to avoid the possibility of the column bleeding. The results show that the pH of the droplet is a very important factor and has high influence on the EFs. Therefore, 0.5 M HCl was used as the acceptor phase for further analysis. After each injection, rinsing the HPLC injector with pure water should be carried out to prevent possible acid erosion of the stainless steel injector.

3.2.6. Stirring speed

As described before, increasing of the stirring speed caused an increase in the mass transfer and the extraction kinetic. In the present work, the procedure adopts a symmetrical rotated flow field created by a stirring bar, placed at the bottom of the cylindrical sample cell and the single drop is delivered at the top-center position of the organic solvent. Thus, it forms a self-stable single microdroplet system, easy to operate and control. Furthermore, the rotation of the microdroplet around a symmetrical axis may cause an internal recycling and intensify the mass transfer process inside the droplet. Therefore, the stirring speed was also optimized for better extraction, while the back-extraction was performed. The range of stirring speed was 360–720 rpm. Agitation increases the extraction efficiency but in high speed (more than 720 rpm), the droplet is not stable and falls down in the vortex, which is created in the organic solvent by agitation. There are no significant differences in the enrichment factors for speeding rate at 600 and 720 rpm. Consequently, the stirring speed was selected at 600 rpm for further analysis.

3.2.7. Addition of salt to donor phase

Usually, by addition of a salt, the extraction efficiency was enhanced due to salting out effect, whereby water molecules form hydration spheres around the ionic salt molecules. These hydration spheres reduce the amount of water available to dissolve analyte molecules in water [28]; thus, it is expected that the target compounds will drive into the organic solvent. For this purpose, sodium chloride is normally used [29]. In the current work, NaCl was added into the donor sample solution in the range of 2–20%. The results of the NaCl addition showed no significant effect on the extraction efficiencies. This unusual behavior may be due to the form of the extraction procedure. Here, we used organic solvents, which are insoluble in water. The aqueous sample solution was vigorously agitated with the organic solvent through the extraction process. Thus, the mixture became cloudy, because the tiny droplets of the organic solvent were dispersed throughout the aqueous sample solution (Fig. 1b and c). The created intersections between the donor phase and these tiny organic drops were so broad and therefore the mass transfer process occurred very fast without imparting of salting out effect. Based on the above explanation, due to the homogeneous dispersion of the organic solvent droplets through the donor aqueous phase, the salting effect cannot be accounted as a serious parameter in this procedure.

3.2.8. Addition of crown ether to acceptor phase

Protonated aniline can form complex with the crown ether in solutions [30]. In this work, besides protonation, the influence of the analyte complexation on LLLME was investigated by adding 18-crown-6 ether into the microdroplet (aqueous receiving phase) to increase the extraction efficiency. The addition of the crown ether appears to help in facilitating the back-extraction and stabilizing the compounds in the aqueous droplet. Different concentrations (0–600 mM) of 18-crown-6 ether are used in the aqueous acceptor phase (0.5 M HCl) and the results are shown in Fig. 2. These results indicate that no significant effect on extraction was achieved at lower concentrations; but by increasing the concentration of the crown ether, the EF was increased. At 600 mM concentration of the 18-crown-6 ether the droplet was too heavy and not stable; thus, 500 mM 18-crown-6 ether in the acceptor phase was used as optimal concentration.

3.3. Comparison with the conventional SD-LLLME

Directly suspended droplet LLLME (proposed method) and the conventional SD-LLLME were performed for the comparison of their respective efficiencies. In the proposed method, the microdroplet is freely suspended at the top-center position of the organic solvent without using a microsyringe and the larger volume of the aqueous acceptor phase can be used, due to high intersection between them. But, in the conventional one an aqueous microdrop is suspended on the needle tip of a microsyringe immersed in the organic solvent, which is stirred above the aqueous sample solution. In this method, use of a large drop is impracticable, due to its instability and short

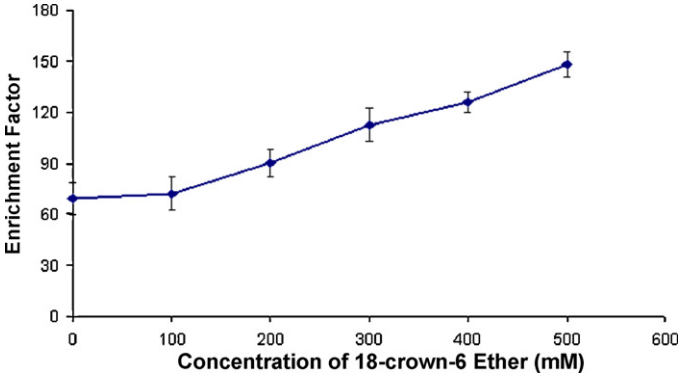

Fig. 2. The effect of the concentration of 18-crown-6 ether in the aqueous receiving phase on the extraction efficiency; P1, 4.5 mL of the pH 13 water sample; P2, 250 μL of *o*-xylene/1-octanol (90:10, v/v); P3, 6 μL of 0.5 M HCl receiving aqueous phase containing 18-crown-6-ether in different concentrations. Time for the extraction is 60 s; for back-extraction is 6 min.

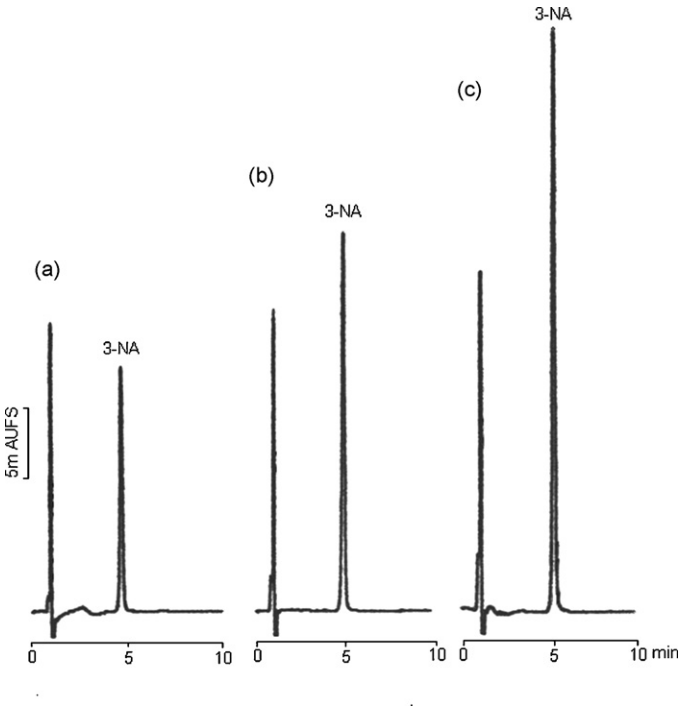

Fig. 3. The chromatograms which were obtained after extraction of 3-nitroaniline: (a) conventional SD-LLLME using a 2 μL droplet as acceptor phase; (b) directly suspended droplet LLLME using a 2 μL droplet as acceptor phase; (c) directly suspended droplet LLLME using a 6 μL droplet as acceptor phase. The experimental conditions were indicated in the text.

lifetime. Therefore, for the comparison of two methods, a smaller volume of microdrop (2 μL) was used. Identical conditions for both procedures were used: donor phase 4.5 mL of 0.1 M NaOH consisting 1 μg/mL of 3-nitroaniline, *o*-xylene/1-octanol (90:10, v/v) as organic solvent, acceptor phase 2 μL of 0.5 M HCl and 500 mM of 18-crown-6 ether solution, stirring rate 600 rpm, extraction time 60 s and back-extraction time 6 min. The results indicate the higher extraction efficiency for our proposed method. The enrichment factors, which are obtained for the extraction of 3-nitroaniline using proposed and conventional method, are 112 and 97, respectively.

Another comparative study was done by using the different volumes of the aqueous acceptor solution in directly suspended droplet LLLME. In this purpose, a larger volume of microdroplet (6 μL) compared to the smaller one (2 μL) as acceptor phase. The results show that using the large droplet leads to the higher extraction efficiency (EF = 149). These comparisons are shown in Fig. 3.

3.4. Quantitative consideration

The evaluation of the practical applicability of the proposed method, repeatability, linearity, limit of detection and limit of quantification under the optimal extraction conditions were investigated by utilizing the standard solutions of 3-nitroaniline in water. The calibration curve for the target compound was obtained by plotting peak area vs. the sample concentrations. The linearity was evaluated within the range of 5–1500 μg/L with correlation coefficient $r = 0.9983$. The limit of detection (LOD) was estimated on a signal-to-noise ratio of 3 (S/N = 3) and was 1 μg/L ($n = 7$). The repeatability of the analytical performance was studied for five replicate experiments with relative standard deviation (RSD, $n = 5$), which was 4.9 and the enrichment factor (EF) was 148.6.

3.5. Real water analysis

Three real environmental water samples including ground, river and tap water spiked with 10 μg/L of 3-nitroaniline were extracted using directly suspended droplet LLLME under the optimal conditions. Tap water was collected from Ferdowsi University of Mashhad (Iran), river water and ground water were collected from Kardeh (a village near Mashhad). The results show that the contents of 3-nitroaniline in the three samples are all under the detection limit. Therefore, separate samples were spiked with 10 μg/L of the target compound. Fig. 4 shows the chromatograms obtained after enriching 4.5 mL of river water without and with spiking 10 μg/L of 3-nitroaniline, using our proposed method. The relative recoveries, which are defined as the ratios of the peak areas of the analyses in the spiked real samples and the peak area of the analyses in pure distilled water sample spiked with the same amount of the analyte, for three samples were, 96% for tap water, 98% for ground water and 101% for river water.

4. Conclusions

The present work describes the possibility of using a new method of LLLME in the extraction of 3-nitroaniline from water samples prior to HPLC by utilizing a simple, rapid and cheap extraction device. In this method, contrary to the ordinary single drop liquid-phase microextraction technique [31], an aqueous large droplet is freely suspended on the surface of the organic solvent [32], without using a microsyringe as supporting device. This large droplet causes an increase in mass transfer process and decrease in equilibrium time. Compared to the most conventional extraction procedures, this extraction technique requires a very little aqueous sample solution and very little expensive and toxic organic extractant. On the other hand, this method is very fast, easy and simple. Using this technique, the analyte can be extracted from real water samples quantitatively. The high enrichment factor and excellent

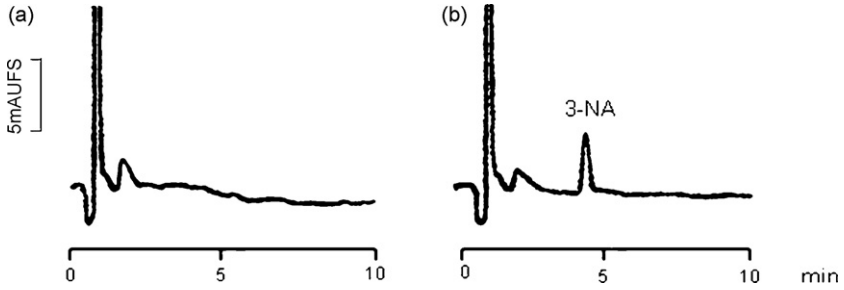

Fig. 4. Chromatograms generated after LLLME of a river water sample: (a) 4.5 mL river water, (b) 4.5 mL river water spiked with 10 μg/L of 3-nitroaniline, the pH of the water sample was adjusted to 13 using 0.1 M NaOH. Organic phase is 250 μL, binary mixture of *o*-xylene/1-octanol (90:10, v/v); acceptor phase is 6 μL, 0.5 M HCl containing 500 mM 18-crown-6 ether. Time for the extraction is 60 s; for back extraction is 6 min.

clean up of the sample are attainable by using 18-crown-6 ether in the aqueous acceptor phase. The good linearity and reasonable relative recovery have been also obtained.

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References

[1] R.J. Lewis, Sax's Dangerous Properties of Industrial Materials, 8th ed., Van Nostrand Reinhold, New York, 1992.
[2] H. Kataoka, J. Chromatogr. A 733 (1996) 19.
[3] W. Kleiböhmer (Ed.), Handbook of Analytical Separation, vol. 3, Environmental Analysis, Elsevier, Amsterdam, 2001.
[4] S. Laha, R.G. Luthy, Environ. Sci. Technol. 24 (1990) 363.
[5] A. Dasgupta, J. Chromatogr. B 716 (1998) 354.
[6] EPA Method 1625, Fed. Reg. US Government Print Office, Washington, DC, September 1994.
[7] EPA Method 8270B, Fed. Reg. US Government Print Office, Washington, DC, September 1994.
[8] R. Haas, T.C. Smidt, K. Steinbach, E. von Low, Fresen. J. Anal. Chem. 359 (1997) 497.
[9] M. Longo, A. Cavallaro, J. Chromatogr. A 753 (1996) 91.
[10] L. Zhao, L. Zhu, H.K. Lee, J. Chromatogr. A 963 (2002) 239.
[11] D. Puig, D. Barcelo, Trends Anal. Chem. 15 (1996) 362.
[12] J. Patsias, E. Papadopoulou-Mourkidou, J. Chromatogr. A 904 (2000) 171.
[13] L. Müller, E. Fattore, E. Benfenati, J. Chromatogr. A 791 (1997) 221.
[14] L.S. DeBriun, P.D. Josephy, J.B. Pawliszyn, Anal. Chem. 70 (1998) 1986.
[15] A. Fromberg, T. Nilsson, B.R. Larsen, L. Montanarella, S. Facchetti, J.Ø. Madsen, J. Chromatogr. A 746 (1996) 71.
[16] R. Doong, S. Chang, Y. Sun, J. Chromatogr. A 879 (2000) 177.
[17] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[18] H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817.
[19] A. Sarafraz-Yazdi, Z. Es'haghi, J. Chromatogr. A 1082 (2005) 136.
[20] L. Xu, C. Basheer, H.K. Lee, J. Chromatogr. A 1216 (2009) 701.
[21] Y. Tao, J.F. Liu, T. Wang, G.B. Jiang, J. Chromatogr. A 1216 (2009) 756.
[22] J. Liu, G. Jiang, Y. Chi, Y. Cai, Q. Zhou, J. Hu, Anal. Chem. 75 (2003) 5870.
[23] L. Hou, H.K. Lee, Anal. Chem. 75 (2003) 2784.
[24] J.Å. Jönsson, L. Mathiasson, J. Sep. Sci. 24 (2001) 495.
[25] R.C. Weast, CRC Handbook of Chemistry and Physics, 59th ed., CRC Press, Boca Raton, FL, 1979.
[26] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 2935.
[27] P.H. Howard, W.M. Meylan, Handbook of Physical Properties of Organic Chemicals, CRC Press/Lewis Publishers, Boca Raton, FL, USA, 1997.
[28] A.A. Boyd-Boland, J.B. Pawliszyn, J. Chromatogr. A 704 (1995) 163.
[29] H.L. Lord, J. Pawliszyn, Anal. Chem. 69 (1997) 3899.
[30] H.J. Buschmann, L. Mutihac, K. Jansen, E. Schollmeyer, An. Quim., Int. Ed. 94 (1998) 211.
[31] L. Xu, C. Basheer, H.K. Lee, J. Chromatogr. A 1152 (2007) 184.
[32] A. Sarafraz-Yazdi, F. Mofazzeli, Z. Es'haghi, Chromatographia 67 (2008) 49.