Recent Development and Application of Dispersive Liquid-Liquid Microextraction

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Abstract: Dispersive liquid-liquid microextraction (DLLME) has become a very popular environmentally benign sample-preparation technique, due to its simplicity, rapidity of operation and low consumption of solvent and reagent. It has attracted much interest from scientist working in separation science and much improvement has been made since its introduction in 2006. It has been combined with different extraction techniques such as floating organic drop, solid-phase extraction, and supercritical fluid extraction. The present review has focused on the recent development of DLLME and its application in different samples, such as water, soil, food and biological material with different analytical techniques. An outlook on the future of the technique is given also.

Keywords: dispersive liquid-liquid microextraction; ionic liquid; extraction; separation; determination; food; water; biological material.

1. Introduction

In spite of substantial technological advances in analytical field, most instruments cannot directly handle complex sample matrices yet. So, a sample-preparation step is commonly involved before instrumental analysis and is possibly the most important step in analysis. The main aim of sample preparation is to clean up and concentrate the analytes of interest, while rendering them in a form that is compatible with the analytical system (Zgola-Grzeskowiak and Grzeskowiak, 2011).
Classical sample pre-treatment techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are widely employed for sample preparation, even today. However, they have plenty of drawbacks such as expensive, time-consuming and labor intensive. Often extensive amounts of hazardous organic solvents are used and large amounts of pollutants are generated in LLE, which made it environmentally unfriendly. Although the solvents used in SPE are much less than LLE, the usage can still be considered significant, and normally an extra step of concentrating the extract down to a small volume is needed. Moreover, SPE cartridge is used once only in ultra-trace analysis. It is not only expensive but also generates a great deal of waste (Rezaee et al., 2010a). Nowadays, minimizing sample and reagent consumption and speeding up the sample-treatment process is currently considered the bottleneck of analysis. Substantial efforts have been made in the past time to improve the existing sample-preparation methods and develop new approaches to save time, labor and materials (Herrera-Herrera et al., 2010; Rezaee et al., 2010a).

One of the sample-preparation techniques attracting special attention is dispersive liquid-liquid microextraction (DLLME), which was introduced by Rezaee and co-workers in 2006 (Rezaee et al., 2006). It is a simple and fast microextraction technique, generally based on a ternary component solvent system, in which extraction and disperser solvents are rapidly introduced into the aqueous sample to form a cloudy solution. Then extraction equilibrium is quickly achieved, due to the extensive surface contact between the droplets of the extraction solvent and the aqueous sample. Therefore, the extraction time is very short. This is also the principal advantage of DLLME (Rezaee et al., 2010a). After centrifugation of the cloudy solution, extraction solvent is normally sedimentated at the bottom of the tube (if the density is above that of water) and taken with a micro syringe for its later analysis with appropriate analytical technique. DLLME has potential advantages which include the simultaneous extraction and preconcentration of target analyte, cost effectiveness, high enrichment factor and environmental benignity when compared to other sample preparation methods reported earlier (Mudiam et al., 2012). In recent years, DLLME has been applied to determine some substances in water, soil, food and biological samples, such as polycyclic aromatic hydrocarbons, phthalate esters, rhamnolipids, polychlorinated biphenyls, organochlorine pesticides, triazine herbicides, metal ions, and phenols. The present review focuses on the updated development and application of DLLME during the last several years. In addition, some limitations and an outlook on further developments of DLLME are discussed also.

2. Principles of DLLME

In DLLME, extraction of analytes takes place in dispersion of the extracting solvent made in water and a second solvent (the dispersing solvent) is used to achieve the dispersion. The extraction
process consists of two steps, briefly described as follows. Firstly, the mixture of extracting and dispersing solvents is rapidly injected to a water sample. In this step, the extracting solvent is dispersed into the aqueous sample as very fine droplets and the analytes are enriched into it. Then the dispersion is removed by centrifugation or other separation method and the extracting solvent containing analytes is taken for analysis with a micro syringe (Zgola-Grzeskowiak and Grzeskowiak, 2011). In general, the extraction efficiency of DLLME is influenced by several factors such as types and volumes of extraction and disperser solvents, sample amount, pH, and salt addition. The conditions of extraction should be optimized before the main experiment is conducted.

Selection of an appropriate extracting solvent is the major parameter for DLLME process. The extracting solvent has to fulfill several requirements. Firstly, it has to possess good capability for extracting interested analytes. Also, it has to be soluble in the dispersing solvent while its solubility in water has to be very low. Besides, the density of the extracting solvent has to differ greatly from the density of water to enable phase separation (Rezaee et al., 2010a). Originally, only solvents with higher density than aqueous samples were used in order to ease their collection as they settle below the aqueous phase by centrifuging. Halogenated hydrocarbons such as chloroform, chlorobenzene, tetrachloroethylene and carbon tetrachloride, which have higher density than water, are usually selected as extracting solvents. However, use of these solvents limits the complete removal of the upper phase for using the sedimented phase for gas chromatography (GC) analysis. To overcome this, a new method was adopted by a group of researchers in which organic solvents with densities less than 1 g/mL were used. Low density solvents were commonly employed for extraction of analytes from aqueous solution as they settle at upper phase to aqueous phase (Mudiam et al., 2012). The common used low-density extraction solvents are n-hexane and n-hexadecane (Moreno-Gonzalez et al., 2012). The dispersing solvent has to be fully soluble with the water phase and soluble in the extraction solvent, thus enabling the formation of fine droplets of the extraction solvent in the aqueous phase. Usually acetone, acetonitrile and methanol are used for this purpose. Selection of both extracting and dispersing solvents is important to obtain a high enrichment factor.

Moreover, the volumes of these solvents have to be optimized, and that is usually performed by a central composite design (Zgola-Grzeskowiak and Grzeskowiak, 2011). The disperser solvent volume directly affects the formation of cloudy solution, the dispersion degree of the extracting solvent in aqueous phase and the extraction efficiency subsequently. For the variation of disperser solvent volume would change the volume of sedimentated phase, it is necessary to change the volumes of disperser solvent and extracting solvent simultaneously to achieve a constant volume of sedimentated phase. The suitable volume of disperser solvent for well cloudy solution depends on the volume of both aqueous phase and extracting solvent (Rezaee et al., 2010a).
Other parameters affecting DLLME also have to be optimized. Two most frequently optimized parameters are the pH of the sample and the amount of a salt added to the sample (salting-out effect). The former is particularly important in extraction of polar analytes. The salt is chosen so as to make the analyte less soluble in the water phase and subsequently improve the extraction efficiency. On the other hand, the salting-out would increase the volume of extraction phase, which result in a decrease of enrichment factor. Therefore, the amount of a salt added to the sample is determined according to specific conditions (Zgola-Grzeskowiak and Grzeskowiak, 2011).

3. Development of DLLME

The first DLLME procedure proposed by Assadi and co-workers required use of 1 mL of acetone as the dispersing solvent and 8 µL of tetrachloroethylene as the extracting solvent for isolation of polycyclic aromatic hydrocarbons (PAHs) from 5 mL of water sample (Zgola-Grzeskowiak and Grzeskowiak, 2011). This technology immediately attracted plenty of attention of related researchers in the world since it was published and then many papers on applications of DLLME to pre-concentration of organophosphorus pesticides, phthalate esters and chlorophenols and so on were published in next years (Rezaee et al., 2010a). However, a few flaws of this approach appeared in the further applications in analyses of some compounds in different matrix. In order to promote the widespread applications of DLLME, substantial efforts have been made to improve this procedure.

3.1. Ionic Liquids Based on DLLME

The traditional solvents used in DLLME cannot be considered as environmentally friendly, and the danger connected with toxic properties of chlorinated solvents led to the introduction of ionic liquids as the extracting solvents. Ionic liquids (IL), which consist of various organic cations and anions, are a new type of organic salt that have melting points less than or equal to 100 °C and have favorable solvating properties for a range of polar and non-polar compounds. ILs are less hazardous than conventional organic solvents and are generally considered as green solvents because of their unique properties of high viscosity, negligible vapor pressure, excellent thermal stability, adjustable miscibility, and polarity. Ionic liquids can be injected directly to high performance liquid chromatography (HPLC), although the high viscosity of compounds used led to the idea of diluting them before analysis (Zgola-Grzeskowiak and Grzeskowiak, 2011). Moreover, ionic liquids used in DLLME are heavier than water and are deposited on the bottom of the centrifuge tube. This makes it easier to handle them than long-chained alcohols, hydrocarbons and other solvents less dense than water (Rezaee et al., 2010a). In recent years ionic liquids dispersive liquid-liquid microextraction (IL-DLLME) is more and more popular used due to its advantages described above.
An environmentally friendly IL-DLLME method coupled with HPLC for the determination of antihypertensive drugs irbesartan and valsartan in human urine samples was developed by Li et al. (2013a). Six ionic liquids were tested for the first time as solvents in the extraction and preconcentration of deoxyribonucleic acid (DNA) using an in situ DLLME approach. The highest extraction efficiencies of DNA were obtained using 1-(1, 2-dihydroxypropyl)-3-hexadecylimidazolium bromide and N, N-didecyl-N-methyl-D-glucaminium bromide respectively. Extraction efficiencies higher than 97% were obtained using small amounts of ionic liquids (0.50 mg) for each extraction (Li et al., 2013b). In another study, a novel automatic on-line sequential injection dispersive liquid-liquid microextraction method, based on 1-hexyl-3-methylimidazolium hexafluorophosphate ionic liquid as an extractant solvent was developed and demonstrated for trace thallium determination by flame atomic absorption spectrometry. The ionic liquid was on-line fully dispersed into the aqueous solution in a continuous flow format. No specific conditions like low temperature are required for extractant isolation. The results showed that for 15 mL of sample solution, an enhancement factor of 290, a detection limit of 0.86 µg/L and a precision (RSD) of 2.7% at 20.0 µg/L Tl(I) concentration level, were obtained. The developed method was evaluated by analyzing certified reference materials while good recoveries from environmental and biological samples proved that present method was competitive in practical applications (Anthemidis and Ioannou, 2012).

It is noteworthy that ionic liquids used in DLLME must be insoluble in water. This greatly reduces the list of extracting solvents. Typically, ionic liquids containing hexafluorophosphate ion are used, such as 1-butyl-3-methylimidazolium hexafluorophosphate, 1-hexyl-3-methylimidazolium hexafluorophosphate, 1-octyl-3-methylimidazolium hexafluorophosphate, 1-decyl-3-methylimidazolium hexafluorophosphate, 1,3-dibutylimidazolium hexafluorophosphate and 1-octyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (Zgola-Grzeskowiak and Grzeskowiak, 2011). With the developments of IL-DLLME, more and more ionic liquids are attempted for being used as extracting solvents. Recently, 1,3-dipentylimidazolium hexafluorophosphate [PPIm][PF6] was used as an alternative extractant in a study, where an approach was developed for the determination of a group of pesticides and metabolites (2-aminobenzimidazole, carbendazim/benomyl, thiabendazole, fuberidazole, carbaryl, 1-naphthol, and triazophos) from soils. A comparison of the performance of [PPIm][PF6] versus that of the so-common 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIm][PF6]) was accomplished. Results indicate a comparable extraction efficiency with both ILs, being slightly higher with [HMIm][PF6] for the metabolite 2-aminobenzimidazole, and slightly higher with [PPIm][PF6] for triazophos. In all cases, the LODs were in the low ng/g range (0.021 ng/g for [HMIm][PF6] and 0.026 ng/g for [PPIm][PF6]). As a result, this study constitutes a starting point for the use of the IL [PPIm][PF6] for further analytical approaches (Asensio-Ramos et al., 2012).
The final precaution is that, as an alternative, sample heating was used occasionally in IL-DLLME. Sometimes, the sample with added ionic liquid is heated until homogenous liquid is obtained. The ionic liquid droplets containing analytes are centrifuged after cooling down. This procedure is called temperature-controlled ionic liquid dispersive liquid-phase microextraction (TC-IL-DLLME) (Zgola-Grzeskowiak and Grzeskowiak, 2011). In a study, TC-IL-DLLME was introduced to analyze malachite green (MG) and crystal violet (CV) in environmental water by coupling with HPLC. In this method, 1-octyl-3-methylimidazolium hexafluorophosphate ([C8MIM][PF6]) and methanol were selected as appropriate extraction and dispersive solvents, respectively. Target compounds were extracted into the IL phase (dispersed completely in the aqueous phase) at a proper temperature. Under the optimum conditions, the established method offered good linear range (0.25-20 µg/L), low detection limits (MG, 0.086 µg/L; CV, 0.030 µg/L), good reproducibility (relative standard deviation, MG, 9.4%; CV, 7.6%; n = 5), good recoveries (91.7% for MG and 97.2% for CV, respectively; n = 5) and high enrichment factor (254 for MG, 276 for CV), which makes the method suitable to monitor low concentrations of MG and CV in aqueous systems (Zhang et al., 2012a).

3.2. DLLME Coupled with Auxiliary Instruments

Though DLLME is a relatively rapid procedure compared with traditional extraction methods, efforts have been given to make it coupled with some auxiliary instruments for improving its extraction efficient. Microwave and ultrasound are often used with DLLME. Besides, some other auxiliary instruments are attempted to couple with DLLME in recent several years.

3.2.1. Microwave and ultrasound assisted DLLME

Nowadays, microwave and ultrasound are main assisted methods used in DLLME. The high polarity of ILs results in them easily absorbing microwave energy, which can decrease the extraction time compared with conventional extraction methods. In a recent study, a simultaneous ionic liquid-based microwave-assisted dispersive liquid-liquid microextraction (IL-based MADLLME) method was developed for the determination of plasticizers in water using HPLC with ultraviolet detection. The parameters affecting the extraction efficiency, such as type and volume of ionic liquid and disperser solvent, microwave time and temperature were optimized. The optimal values were determined to be an extraction volume of 110 µL, a dispersive solvent volume of 0.26 mL, and a microwave irradiation temperature and time of 60 °C and 2 min, respectively. Under the optimum conditions, the limits of detection of these plasticizers were in the range of 0.71-1.94 µg/L. Then validation of the methodology was carried out by the method of standard addition at two concentration levels for three water samples and the recoveries of the analytes were in the range of 85.2-103.3%.
with the relative standard deviations (RSDs, n = 6) ≤ 5.9%. The results showed that IL-based MADLLME is a suitable method for the determination of these five plasticizers in water. An in-situ IL-based MADLLME followed by HPLC was developed for determination of the triazine herbicides ametryne, prometryne, terbutylazine and terbutryn in water samples. The type and volume of IL, the type and volume of disperser, microwave irradiation temperature, extraction time and salt concentration were optimized. The results showed that the recoveries were 88.4-114 %. The relative standard deviations were in range of 1.6-6.2 %, and limits of detection were between 0.52 and 1.3 µg/L (Zhong et al., 2012).

It is well-known that ultrasound is a powerful aid in the acceleration of various steps in the processes of separation and extraction, such as emulsion forming, homogenizing and mass transferring between immiscible phases (Zhang and Lee, 2012a). In recent years, ultrasound-assisted IL-based DLLME was developed and widely applied to analytical chemistry, which could obtain high extraction efficiency and extraction equilibrium in a very short time.

A sample preparation method, namely ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME), followed by HPLC has been developed for the extraction and preconcentration of four benzophenone-type ultraviolet filters from three different water matrices. The ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution, which markedly increased the extraction efficiency and reduced the equilibrium time. The parameters that affected the extraction efficiency, such as type and volume of extraction and dispersive solvents, ionic strength, pH and extraction time, were evaluated. Under the optimal conditions, the proposed method provided good enrichment factors in the range of 354-464, and good repeatability (RSDs below 6.3%, n = 5). Furthermore, the limits of detection were in the range of 0.2-5.0 ng/mL and relative recoveries were in the range of 71.0-118.0%. The results showed that the proposed method was successful for the determination of ultraviolet filters in river, swimming pool and tap water samples (Zhang and Lee, 2012a). In another study, IL-USA-DLLME procedure was developed for the extraction of eight fluoroquinolones including marbofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, oxolinic acid and nalidixic acid in groundwater, using high performance liquid chromatography with fluorescence detection (HPLC-FLD). The ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution using a small volume of disperser solvent (0.4 mL of methanol). For the DLLME procedure, the IL 1-octyl-3-methylimidazolium hexafluorophosphate and methanol were used as extraction and disperser solvent, respectively. Under the optimum conditions, linearity of the method was observed over the range 10-300 ng/L with correlation coefficient > 0.9981. The proposed method has been found to have excellent sensitivity with limits of detection between 0.8 and 13 ng/L and precision with relative standard
deviation values between 4.8 and 9.4% (n=5). The enrichment factors and recoveries were 122-205 and 85-107%, respectively. This simple and economic method has been successfully applied to analyse real groundwater samples with satisfactory results (Vazquez et al., 2012). What’s more, IL-USA-DLLME coupled with spectrophotometry detection was developed for determination of linear alkylbenzene sulfonates (LASs) in environmental water samples. Different operational conditions affecting extraction efficiency of LASs including pH, volume of extraction solvent, methylene blue concentration, salt concentration and sonication time were studied via central composite design optimization method. Under the optimal conditions, a calibration curve was obtained with linear dynamic range between 0.8 and 100 ng/mL and detection limit of 0.37 ng/mL. The results showed that the proposed method was feasible for determination concentrations of LASs in different non-spiked aqueous samples and satisfactory results were obtained (Arvand et al., 2012a).

Moreover, IL-USA-DLLME combined with in-situ metathesis reaction was developed for the determination of five phenylurea pesticides (i.e., diuron, diflubenzuron, teflubenzuron, flufenoxuron, and chlorfluazuron) in environmental water samples. In the developed method, 360 µL LiNTf2 aqueous solution (0.162 g/mL) was added to the sample solution containing a small amount of [C6MIM]Cl (0.034 g) to form a water-immiscible ionic liquid, [C6MIM]NTf2, as extraction solution. The mixed solutions were placed in an ultrasonic water bath at 150 W for 4 min and centrifuged at 3500 rpm for 10 min to achieve phase separation. The quantity of [C6MIM]Cl, the molar ratio of [C6MIM]Cl and LiNTf2, ionic strength, ultrasound time, and centrifugation time, were optimized. The optimized technique provides good repeatability (RSDs 2.4 to 3.5%), great enrichment factor (244 to 268), linearity (0.5 to 500 µg/L), and low limits of detection (0.06 to 0.08 µg/L). The developed method can be applied in routine analysis for the determination of phenylurea pesticides in environmental samples (Zhang et al., 2012b).

It should be pointed out that microwave and ultrasound not only are used in IL-based DLLME but also in routine DLLME. Microwave-assisted extraction (MAE) coupled with DLLME followed by semi-automated in-syringe back-extraction technique was used for extraction of five chlorophenols from soil and marine sediment samples (Naeeni et al. 2012). Microwave-assisted extraction was performed by using 2.0 mL of alkaline water at pH 10.0. After extraction, the pH of extraction solution was adjusted at 6.0 and DLLME procedure was done using 1.0 mL of acetone as a disperser solvent and 37.0 µL of chlorobenzene as extraction solvent. The obtained recovery and preconcentration factors for the analytes were in the range of 68.0%-82.0% and 25-30, respectively, with relative standard deviations ≤ 7.6%. The limits of the detection were found in the range of 0.0005-0.002 mg/kg. The method provides a simple and fast procedure for the extraction and determination of chlorophenols in soil and marine sediment samples (Naeeni et al., 2012). A simple and efficient
method was developed using MAE-DLLME coupled with GC-MS for the extraction and quantification of 16 polycyclic aromatic hydrocarbons (PAHs) in smoked fish. Several parameters, including the types and volume of hydrolysis, extracting and disperser solvents, microwave time and pH, were optimized. Under the optimum condition, the MAE-DLLME method coupled with GC-MS provided excellent enrichment factors (in the range of 244-373 for 16 PAHs) and good repeatability (with a relative standard deviation between 2.8 and 9%) for spiked smoked fish. The calibration graphs were linear in the range of 1-200 ng/g, with the square of the correlation coefficient ($R^2$) > 0.981 and detection limits between 0.11 and 0.43 ng/g. The recoveries of those compounds in smoked fish were from 82.1% to 105.5%. A comparison of this method with previous methods demonstrated that the proposed method is an accurate, rapid and reliable sample-pretreatment method that gives very good enrichment factors and detection limits for extracting and determining PAHs from smoked fish (Ghasemzadeh-Mohammadi et al., 2012). Moreover, a method based on MAE in combination with DLLME followed by gas chromatography-electron capture detection (GC-ECD) has been proposed as a new approach for the sensitive determination of cork taint responsible compounds in cork stoppers and oak barrel sawdust. Under the optimal condition, the proposed method showed satisfactory linearity (correlation coefficients over 0.991), good repeatability (below 10.4%) and inter-day precision (below 11.2%). Detection limits obtained were similar or even lower than previously reported. The results obtained proved the suitability of MAE-DLLME as a sensitive sample preparation method for the analysis of haloanisoles and halophenols in solid enological matrices (Pizarro et al., 2012).

Nowadays, an increasing number of papers concerned about the application of USA-DLLME in analytical chemistry have been published. In a recent study, USA-DLLME coupled with gas chromatography-flame ionization detector was developed for the determination of benzene, toluene and xylenes isomers (BTX) in water samples. The effects of different experimental parameters in the extraction step including type and volume of extraction and dispersive solvents, extraction time and sample volume were studied using two techniques, namely one-variable-at-a-time and response surface method. The optimal conditions were determined to be a volume of extraction solvent (chloroform) of 51 µL, volume of dispersive solvent (methanol) of 514 µL and volume of sample of 12 mL. Under the optimum conditions, the enrichment factors of 241.2-305.1, the limits of detection of 205-382 ng/L were obtained for the BTX. In addition, the relative standard deviations for 50 µg/L of the BTX in the water samples were found to be in the range of 1.9 %-5.7 % ($n = 5$). The developed procedure was then applied for the extraction and determination of BTX in the water samples (Khajeh et al., 2012). In another study, USA-DLLME method combined with GC-MS has been developed for the simultaneous determination of flavoring compounds, including safrole, coumarin, 6-methylcoumarin, 7-
metheoxycoumarin, estragole, methyleugenol, pulegone and thujone, in tobacco additives. In this method, the targets were extracted from tobacco additive sample using ethanol by UAE. Then, 0.5 mL of the extract was used for the DLLME. The parameters affecting the DLLME, including type and volume of extraction and disperser solvents, salt effect and extraction time, were investigated and optimized. Under the optimum conditions, the enrichment factors ranged from 140 to 208. The limits of detection were between 0.04 and 0.24 ng/mL and the average recoveries were between 89.9 and 99.7% with relative standard deviations ranging from 2.5 to 6.7%. The linear relationship was obtained in the range of 0.4-928 ng/mL, which showed satisfactory linearity with correlation coefficients over 0.9989 for all the analytes. The results showed that this method was feasible for the preconcentration and determination of these target compounds in tobacco additives (Li et al., 2012a). In addition, USA-DLLME method combined with GC-MS has been used for the determination of six commonly found synthetic polycyclic musks in aqueous samples. In this study, the limits of quantitation were less than 0.6 ng/L and the relative standard deviations were less than 11% for both intra-and inter-day analyses. Accuracy, expressed as the mean extraction recovery, was between 71 and 104% (Yang and Ding, 2012).

3.2.2. Other instruments assisted DLLME

Lastly, magnetic stirring-assisted and ortex-assisted DLLME were reported. A method, namely magnetic stirring-assisted DLLME followed by HPLC with ultraviolet detection, was developed for the determination of phthalate esters (PEs) in water samples. This novel microextraction method is based on the fast injection of extracting solvent into the aqueous solution, which is being stirred by a magnetic stirrer, to form a cloudy binary component solvent system. The extraction parameters such as type and volume of extracting solvent, pH of sample, salt addition, extraction time and stirring rate were optimized. The result showed that the optimal stirring rate is 1000 rpm. Under the optimal condition, the limits of detection and quantification were ranged from 0.13 to 0.38 µg/mL and 0.43 to 1.27 µg/mL, respectively. The ranges of intra-day and inter-day precisions (n=5) at 100 µg/mL of PEs were 1.50-2.65% and 2.31-3.35%, respectively. The results indicated that MSA-DLLME method was successful for preconcentration of PEs in drinking and environmental water samples (Ranjbari et al., 2012a).

Moreover, vortex-assisted DLLME coupled with GC-MS has been developed and used for the analysis of six benzophenone ultraviolet filters in water samples. The obvious advantage of this new method is that no centrifugation and disperser solvent were required in this microextraction procedure. Meanwhile, short extraction time and high extraction efficiency were achieved. This method opens up a potentially new horizon for on-site dispersive liquid-liquid microextraction. Under the optimum
conditions, the proposed method provided good enrichment factors up to 310, with relative standard deviations ranging from 6.1 to 12.9%. The limits of quantification were in the range of 20-100 ng/L. The results showed that the proposed method was feasible for the determination of UV filters from spiked genuine water samples and acceptable recoveries over the range of 71.0-120.0% were obtained (Zhang and Lee, 2012b).

3.3. Combination of DLLME with Other Extraction Techniques

3.3.1 DLLME combined with SFO

As shown above, the concerns connected with toxicity of chlorinated solvent led to use of less toxic solvents such as long-chained alcohols or hydrocarbons. These solvents are lighter than water and must be collected from the surface of the water sample, so different methods of collecting the extracting solvent had to be invented (Rezaee et al., 2010a). In 2007, a new mode of liquid-phase microextraction based on solidification of floating organic droplet (LPME-SFO) was developed (Rezaee and Mashayekhi, 2012). In this method, no specific holders such as the needle tip of microsyringe, hollow fiber or polychloroprene rubber tube is required for supporting the organic microdroplet due to the using organic solvents with low density and proper melting point and the extractant droplet can be collected easily by solidifying it at low temperature. However, the extraction time was somewhat long, thus it cannot satisfy the demand of fast analysis. Therefore, a new method based on DLLME and LPME-SFO was proposed, which overcomes the problems mentioned above. The large contact surface between the sample and the droplets of the extractant speeds up mass transfer, as fast as DLLME, and shorter extraction time than that of LPME-SFO. In DLLME-SFO, lower toxicity extracting solvents can be used and the floated extractant is solidified and easily collected from the top of the solution for analysis. As a result, with these merits, more and more papers on the applications of DLLME-SFO are published during past several years (Zgola-Grzeskowiak and Grzeskowiak, 2011).

Recently, a method based on DLLME-SFO combined with GC-MS method was developed for the analysis of antidepressant drugs in water samples. In the method, the disperser solvent (0.5 mL acetonitrile) containing 30 µL of n-hexadecane was rapidly injected using a syringe into 5.0 mL of water sample in a glass tube. After centrifugation, the mixture was cooled in ice bath for 5 min. The solidified n-hexadecane was transferred into a conical vial, where it melted rapidly at room temperature. Under optimized conditions, good linearity in the range of 0.04 - 0.12 µg/mL for amitriptyline and chlorpromazine with correlation of determination in the range of 0.992 - 0.995 was achieved. The limits of detection were in the range 0.0085 - 0.0285 µg/mL and the extraction recoveries of amitriptyline and chlorpromazine from water samples at spiking level of 0.08 µg/mL
were 71.34 - 73.52% and 73.83 - 91.09%, respectively, with relative standard deviations in the range of 4.97 - 6.85% for amitriptyline and 4.84 - 7.49% for chlorpromazine. The results showed that this method was feasible for the determination of the analytes in drinking water, lake water and tap water samples (Sanagi et al., 2013). In another study, DLLME-SFO was used for simultaneous extraction of trace amounts of nickel, cobalt and copper followed by their determination with electrothermal atomic absorption spectrometry. The 300 µL of acetone and 1-undecanol was injected into an aqueous sample containing diethyldithiocarbamate complexes of metal ions. For a sample volume of 10 mL, enrichment factors of 277, 270 and 300 and detection limits of 1.2, 1.1 and 1 ng/L for nickel, cobalt and copper were obtained, respectively. Furthermore, this method has been applied to the extraction and determination of these metals in different water samples (Amirkavei et al., 2013).

Moreover, DLLME-SFO was applied to the simultaneous separation and preconcentration of iron in water samples (Moghadam et al., 2011). For determining chlorpyrifos in environmental water samples, DLLME-SFO followed by GC with flame photometry detection has been adopted and improved (Xiong et al., 2012). A method for the analysis of 14 anilines in water samples by DLLME-SFO prior to GC-MS was developed and optimized (Diao and Wei, 2012). Furthermore, a novel surfactant-assisted dispersive liquid-liquid microextraction (SADLLME) based on SFO combined with HPLC-UV has been proposed for extraction and determination of amphetamine and methamphetamine in urine samples. The application feasibility of SFO-SADLLME-HPLC-UV in real sample was investigated by analyzing different real samples and satisfactory results were obtained (Tehrani et al., 2012).

3.3.2. DLLME combined with SPE

SPE is a widely used sample preparation technique for the isolation of selected analytes, usually from a gas, fluid or liquid phases. The principal goals of SPE are trace enrichment (concentration), matrix simplification and medium exchange. However, DLLME is not suitable for complex matrices such as highly saline solution. So, a combination of SPE and DLLME was introduced by Rezaee and co-workers (Rezaee et al., 2006). SPE-DLLME is an efficient hyphenated technique that offers the advantages of both methods such as simplicity, low solvent usage and exposure, low disposal cost and extraction time, with high recovery and enrichment factor, and it can be also used in complex matrices.

In a study, SPE-DLLME is established to determine carbamazepine in biological fluids and water samples. After concentration and purification of the sample using SPE C-18 sorbent, 1.5 mL of acetonitrile containing 60.0 µL of chloroform was injected into 5.0 mL pure water. After extraction and centrifuging, the sedimentated phase was evaporated and the residue was dissolved in 30 µL
methanol and injected into the HPLC system. This new method provides detection limits of 0.8 µg/L and 1.7 µg/L in urine and plasma samples, respectively. The calibration graphs are linear in the range of 2.5-500 µg/L and 5.0-500 µg/L in urine and plasma, respectively. The results show that SPE-DLLME is a suitable method for the determination of carbamazepine in biological and water samples (Rezaee and Mashayekhi, 2012). For the detection of cypermethrin and permethrin in river water, SPE-DLLME coupled with gas chromatography-electron capture detection (GC-ECD) was developed. The samples were firstly extracted using a large-volume SPE procedure and the eluents of SPE were further purified and enriched by the following DLLME. Good linearity and high enrichment factors were obtained under optimized conditions. The proposed method was successfully used to detect the two pyrethroid residues in river water samples (Yan et al., 2012). In another study, SPE-DLLME was applied to the preconcentration and analysis of short-chained dodecyl alcohol ethoxylates and dodecyl alcohol. The results showed that the analytes were preconcentrated 700 times with the use of small sample volume. The developed method was used for the analysis of short-chained dodecyl alcohol ethoxylates and dodecyl alcohol in both sewage effluent from sewage treatment plants and river water samples (Zgola-Grzeskowiak and Grzeskowiak, 2012).

3.3.3. DLLME combined with SDME

The single-drop microextraction (SDME) is performed by suspending a microliter drop of water-immiscible solvent in the stirred aqueous solution or placing the drop in the headspace of sample bottle. Its extreme simplicity motivates many successful applications. However, this technique suffers much from the instability of the suspending drop and approaching high signal intensity of analyte usually takes quite some time for extraction. The integration of these two techniques, i.e. DLLME and SDME is presented as a new extraction method for environmental analysis. This proposed process allows the individual shortcomings of each technology to be overcome and leads to an optimum process configuration (Zgola-Grzeskowiak and Grzeskowiak, 2012). Furthermore, DLLME is used mainly for the pre-concentration of the analytes into an extraction organic solvent which afterwards using GC as detection system and SDME is used for the back extraction of the analytes into the water samples which can provide situation for using HPLC method. Therefore, in a combination of these two methods, HPLC can be used as detection system. The combination of DLLME and SDME as a new pre-concentration technique is developed for the separation and determination of acidic non-steroidal anti-inflammatory pharmaceutical compounds, namely, naproxen, diclofenac, and ibuprofen, in water samples using HPLC-UV. Good linearity range of 0.11000 µg/L, acceptable reproducibilities (RSDs, 4.5-8.8%), low limits of detection (0.03-0.2 µg/L), and satisfactory relative recoveries were obtained.
The results showed that developed method was acceptable for the determination of anti-inflammatory drugs in river and waste water samples (Sarafraz-yazdi et al., 2012).

Moreover, the low-density solvent-based DLLME combined with SDME was developed in a new format of fast three-phase microextraction for the first time. In a study, a volume of low-density solvent (toluene) was used as organic phase and injected into the aqueous sample (donor phase) with disperser (methanol). The analytes were pre-extracted into the organic sample within 2 min. Afterwards the layer of the organic phase was formed on the top of the aqueous phase by a 2 min centrifugation. Then a drop of acceptor solution was introduced into the upper layer of toluene and the SDME was carried out for back extraction. After extraction, the acceptor drop was withdrawn and directly injected into a HPLC-UV for analysis. In this procedure, the high speed and efficiency of DLLME make the typical stirring step in SDME unnecessary and the total extraction time noticeably short (Li et al., 2013c).

3.3.4. DLLME combined with SFE

Supercritical fluid extraction (SFE) has been adopted to extract different substances from solid matrices since three decades ago. As is well known, in spite of substantial advantages of DLLME, it is not suitable for extraction of compounds from solid samples and sometimes extra steps such as drying and filtering processes in sample preparation before DLLME are time-consuming. Also, sometimes it is impossible to do DLLME for the extraction of analytes from complex matrices (Zgola-Grzeskowiak and Grzeskowiak, 2012). For the first time, a combination of SFE and DLLME, as a sample-preparation method was developed for determination of ten PAHs in marine sediment samples. In SFE-DLLME, the collecting solvents such as methanol and acetonitrile in SFE can be used as disperser solvent in DLLME. After performing SFE and collecting the extracted analytes in the disperser solvent, a suitable volume of the extracting solvent was added into the collecting solvent. Finally, the mixture was injected to the aqueous sample. The other steps were similar to DLLME method. SFE-DLLME leads to high preconcentration factor for determining organic compounds in solid samples, easy use of DLLME in solid samples and can eliminate the need to evaporate the collecting solvent at the end of SFE. The performance of SFE-DLLME in the extraction of polycyclic aromatic hydrocarbons (PAHs) from different marine sediment samples with various matrices was excellent. PAHs were employed as model compounds to assess the extraction procedure and were determined by gas chromatography-flame ionization detection (GC-FID). SFE of PAHs was performed at 313 K and 253.2 bar, at static and dynamic time 10 and 30 min, respectively. The extracted PAHs were collected in 1 mL of acetonitrile. Subsequently, 16 µL of chlorobenzene (as extraction solvent) was added to collecting solvent (1.0 mL of acetonitrile). Then, the mixture was injected rapidly into
5.0 mL of aqueous solution. After centrifugation, the PAHs in the sedimentated phase were analyzed by GC-FID. Under the optimum conditions, the calibration plots were linear in the range of 0.4-41.6 mg/kg and the limits of detection were 0.2 mg/kg for all of the analytes. Analysis of PAHs in different solid samples showed that the improved technique has great potential for PAHs analysis in marine sediments (Rezaee et al., 2010b).

This method possesses a great potential in the analysis of trace organic compounds in real solid samples (Rezaee et al., 2010a). For the first time, in order to extract and determine ultra-trace amounts of seven organophosphorus pesticides (o,o,o-triethyl phosphorothioate, thionazin, sulfotepp, disulfoton, methyl parathion, parathion, and famphur) in soil and marine sediment samples, SFE-DLLME followed by GC-FID was adopted. In this procedure, supercritical CO$_2$ at 150 bar, 60 °C, 10 min static and 30 min dynamic extraction time were used to extract the pesticides. The extraction recoveries for the target analytes were in the range of 44.4% and 95.4% and relative standard deviations for four-replicate measurements were below 7.5%. The detection limits of the method for the determination of the pesticides were in the range of 0.001-0.009 mg/kg (Naeeni et al., 2011).

4. Applications

4.1. Applications of DLLME for Environmental Water Samples

DLLME has been successfully applied to extraction and concentration of a wide variety of organic compounds and metal ions, mainly from water of different types, such as tap, river, well and lake waters. Among them, pesticide analysis is probably the field in which DLLME has found its major applications. Substantial papers were devoted to development of DLLME for this group of analytes. Such a great number of papers concerning one group of analytes can be attributed to high interest in this field connected with food and environmental pollution caused by these compounds. Different methods based on DLLME were developed for the determination of different kinds of pesticide in water samples.

Recently, some new methods based on DLLME were developed for the determination of different kinds of pesticide in water samples. DLLME based on SFO multi-residue method for the simultaneous determination of polychlorinated biphenyls, organochlorine, and pyrethroid pesticides in tap water, lake water, and industrial waste water were established by Zuo et al. (2012). Meanwhile, determination of chlorpyrifos in environmental water samples by DLLME-SFO followed by GC with flame photometry detection was developed (Xiong et al., 2012). A new DLLME method in a narrow-bore tube for preconcentration of triazole pesticides from aqueous samples was developed (Farajzadeh et al., 2012). DLLME using a low density extraction solvent for the determination of 17 N-
methylcarbamates by micellar electrokinetic chromatography-electrospray-mass spectrometry employing a volatile surfactant was studied (Moreno-Gonzalez et al., 2012). For the determination of phenylurea pesticides in water samples, in-situ metathesis reaction combined with USA-IL-DLLME followed by HPLC was developed (Zhang et al., 2012b). As regards with pyrethroid pesticides in environmental samples, IL-DLLME combined with HPLC-UV was established (Wu et al., 2012). What’s more, a novel mixed ionic liquids DLLME method was developed for rapid enrichment and determination of environmental pollutants in water samples (Wang et al., 2012). SPE-DLLME coupled with gas chromatography-electron capture detection (GC-ECD) was established for the detection of cypermethrin and permethrin in river water (Yan et al., 2012). Soisungnoen et al. has devoted to determine organophosphorus pesticides using DLLME combined with reversed electrode polarity stacking mode-micellar electrokinetic chromatography (Soisungnoen et al., 2012). DLLME using nonhalogenated solvents combined with GC-MS was applied to the determination of five organophosphorous pesticides including prophos, diazinon, chlorpyrifos methyl, fenchlorphos, and chlorpyrifos in water samples (Alves et al., 2012). Moreover, for ultra-preconcentration and determination of thirteen organophosphorus pesticides in water samples, a method using SPE-DLLME and GC with flame photometric detection was developed (Samadi et al., 2012). In another study, an alcoholic-assisted DLLME for extraction of pentachlorophenol in water was studied (Hadjmohammadi et al., 2012). Besides, a novel DLLME method followed by HPLC analysis, termed sequential DLLME, was developed for the preconcentration and determination of aryloxyphenoxy-propionate herbicides (i.e. haloxyfop-R-methyl, cyhalofop-butyl, fenoxaprop-P-ethyl, and fluazifop-P-butyl) in aqueous samples (Li et al., 2012b).

Except for pesticides analysis, DLLME has been used for analysis of some other compounds such as pharmaceuticals and antibiotics in environmental water samples. DLLME combined with ultra-high performance liquid chromatography (UHPLC) for the simultaneous determination of 25 sulfonamide and quinolone antibiotics in water samples was developed by Herrera-Herrera et al. (2012). And a simple, rapid, and sensitive method for determination of β-blockers in environmental water using DLLME followed by liquid chromatography with fluorescence detection was developed (Vazquez et al., 2012a). For determination of eight fluoroquinolones in groundwater samples, a method with USA-DLLME prior to HPLC-FLD was established (Vazquez et al., 2012b). Moreover, IL-DLLME was successfully applied to the determination of formaldehyde in wastewaters and detergents, and IL-USA-DLLME followed HPLC was adopted for the determination of ultraviolet filters in environmental water samples (Arvand et al., 2012b; Zhang and Lee, 2012a). In another study, DLLME combined with micro-volume spectrophotometry for determining phenols in water and wastewater was developed (Lavilla, 2012). A procedure based on SPE-DLLME followed HPLC with
tandem mass spectrometry analysis was established for trace determination of short-chained dodecyl alcohol ethoxylates and dodecyl alcohol in environmental water samples (Zgola-Grzeskowiak and Grzeskowiak, 2012). For the determination of orthochlorophenol in environmental water samples, DLLME coupled with GC was developed by Yang and Ding (2012). Extraction optimization of polycyclic aromatic hydrocarbons by alcoholic-assisted DLLME was studied (Fatemi et al., 2012). As regards with the determination of parabens, an approach based on in-situ derivatization and DLLME combined with GC was developed (Prichodko et al., 2012). Besides, for the determination of trace level estrogens in water sample, SPE-DLLME and pre-column derivatization were used (Li et al., 2012c).

Last but not least, we have to mention the applications of DLLME on the preconcentration of heavy metals, especially at trace levels. DLLME for the simultaneous separation of trace amounts of zinc and cadmium ions in water samples was carried out by Mohammadi et al. (2012). Spectrophotometric determination of mercury in water samples was conducted after preconcentration using DLLME (Lemos et al., 2012a). In another study, DLLME for preconcentration and determination of nickel in water was developed (Lemos et al., 2012b). Moreover, a method for the determination of cadmium at ultratrace levels in environmental water samples by means of total reflection X-ray spectrometry after DLLME was developed (Margui et al., 2013).

4.2. Applications of DLLME for Soil and Sediment Samples

Traditionally, DLLME is not suitable for soil samples. With the developments of DLLME combined with other extraction techniques, more and more papers have been published concerning about the applications of DLLME for soil and sediment samples. In the application to solid matrices, DLLME has acted as a cleaning and/or concentration step in the sample pre-treatment procedure. In some cases, the solvents used to extract the analytes from the solid matrix were utilized directly as the disperser in the DLLME process with good recoveries and clean sediment phases (Campone et al., 2012). Microwave-assisted extraction coupled with DLLME followed by semi-automated in-syringe back-extraction technique was used for extraction of five chlorophenols from soil and marine sediment samples (Naeeni et al., 2012a). A simple and miniaturized pretreatment procedure combining matrix solid-phase dispersion (MSPD) with USA-DLLME technique was proposed for simultaneous determination of three pyrethroids (fenpropatrin, cyhalothrin and fenvalerate) in soils. The solid samples were directly extracted using MSPD procedure, and the eluent of MSPD was used as the dispersive solvent of the followed DLLME procedure for further purification and enrichment of the analytes before GC-ECD analysis (Wang et al., 2012b). Moreover, in another work, the use of 1,3-dipentylimidazolium hexafluorophosphate ([PPIm][PF6]) as an alternative extractant for IL-DLLME
of a group of pesticides and metabolites from soils is described. After performing an initial USA, the IL-DLLME procedure was applied for the extraction of these organic analytes from soil extracts (Asensio-Ramos et al., 2012).

Furthermore, a method based on the combination of SFE with DLLME for extracting organophosphorus pesticides from soil and marine sediment samples was developed. In this work, supercritical CO$_2$ at 150 bar, 60 °C, 10 min static and 30 min dynamic extraction time were used to extract the pesticides. The method was successful for analysis of organophosphorus pesticides in real soil and marine sediment samples and satisfactory results were obtained (Naeeni et al., 2012). Besides, the SFE followed by the DLLME coupled with gas chromatography-flame ionization detection (GC-FID) has been developed for extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in marine sediments. In this work, SFE of PAHs was performed at 313 K and 253.2 bar, at static and dynamic time 10 and 30 min, respectively (Rezaee, Mohammad). In addition, for the extraction of PAHs in sediment samples, a simple method based on vortex-assisted extraction followed by DLLME has been developed prior to analysis by HPLC-FLD. Acetonitrile was used as collecting solvent for the extraction of PAHs from sediment by vortex-assisted extraction. In DLLME, PAHs were rapidly transferred from acetonitrile to dichloromethane (Leng et al., 2012).

4.3. Applications of DLLME for Food Samples

Relatively few applications have been devoted to the analysis of organic compounds in highly complex matrices, such as food and biological samples, due to the interference of matrix components in these kinds of samples. Usually, the extracts of complex matrices may not be compatible with the DLLME process due to the interaction of matrix components with the extractant. An appropriate extractant should possess a higher extraction capacity for the analytes than the interferences. This higher extraction capacity is possible only if the analyte has a chemical behavior very different from the interferences. If raw extracts contain lipophilic matrix components, the hydrophobic nature of conventional DLLME extraction solvents is unable to discriminate among analytes and interferences, compromising the efficiency and selectivity of the process. Two kinds of procedures were used for this purpose. In the first, the sample was homogenized and then centrifuged, or filtered juice was taken for DLLME. In the second group of procedures, samples were pre-extracted from the food matrix and the extract was used for DLLME. The QuEChERS technique (Quick, Easy, Cheap, Effective, Rugged, and Safe) was used several times in this field (Zgola-Grzeskowiak and Grzeskowiak, 2011). About food samples, it can be divided into two categories: fluid food and solid matrix food. Applications of DLLME on fluid food such as wine, juice, and milk are more than solid matrix food.

A highly efficient separation and pre-concentration method for arsenic species determination in
mono-varietal wines, based on IL-DLLME implemented in a flow analysis system is proposed by Escudero et al. (2013). Under optimal conditions, As(III) extraction efficiency was 100% and an enhancement factor of 46 was obtained with only 4.0 mL of sample. Determination of ochratoxin A in wines using DLLME combined with capillary liquid chromatography with laser induced fluorescence detection was conducted (Arroyo-Manzanares et al., 2012). For the determination of hydroxylated stilbenes in wine, a method based on DLLME followed by GC-MS was developed. In this procedure, DLLME was preceded by an acetylation step and satisfying results were achieved (Rodriguez-Cabo et al., 2012).

For the quantification of β-carotene, retinol, retinyl acetate and retinyl palmitate in enriched fruit juices, DLLME coupled to liquid chromatography with fluorescence detection and atmospheric pressure chemical ionization-mass spectrometry was used (Vinas et al., 2013a). An evaluation of cis- and trans-retinol contents in juices using DLLME coupled to liquid chromatography with fluorimetric detection was carried out by Vinas et al. (2013b). Moreover, DLLME using extraction solvents lighter than water combined with high performance liquid chromatography for determination of synthetic antioxidants in fruit juice samples was proposed. The designed method was successfully applied for the preconcentration and determination of the studied synthetic antioxidants in different fruit juice samples, and satisfactory results were obtained (Biparva et al., 2012).

Magnetic stirring-assisted DLLME followed by HPLC for determination of phthalate esters in drinking samples was proposed (Ranjbari and Hadjmohammadi, 2012). The determination of the dye rhodamine B in water samples and soft drinks was conducted by a new approach for the integration of various analytical steps inside a syringe (Maya et al., 2012). For the determination of macrocyclic lactones in milk, DLLME followed by liquid chromatography with diode array detection and atmospheric pressure chemical ionization ion-trap tandem mass spectrometry was developed (Campillo et al., 2013). What’s more, a three phase DLLME technique for the extraction of antibiotics in milk was proposed (Adlnasab et al., 2012). USA-DLLME coupled with GC was developed for determination of volatile components of green, black, oolong and white tea (Sereshti et al., 2013). DLLME for the determination of organochlorine pesticides residues in honey by GC-electron capture and ion trap mass spectrometric detection was established (Zacharis, Constantinos K.). Besides, application of DLLME for the determination of selected organochlorine pesticides in honey by GC-MS was attempted (Kujawski et al., 2012). Furthermore, a novel method for high preconcentration of ultra trace amounts of B-1, B-2, G-1 and G-2 aflatoxins in edible oils by DLLME followed HPLC coupled with fluorescent detector was developed. In this study, a new approach which uses immunoaffinity column clean-up combined with DLLME is proposed for the preconcentration of ultra trace amounts of aflatoxins. Samples are separated by immunoaffinity column, and their eluents are used as
dispersants of the subsequent DLLME, for further enrichment of aflatoxins. The results show that dispersive liquid-liquid microextraction combined with HPLC is a selective, simple, sensitive, and effective analytical method for the preconcentration and determination of ultra trace amounts of aflatoxins in edible oils (Afzali et al., 2013).

As regards with applications of DLLME on solid matrix food, recently, there has been a tendency toward rising amounts of published papers. A simple, inexpensive, and reliable analytical method was developed to determine the benzimidazole residues in tomato using HPLC. In this study, sample pretreatment using SPE to remove the matrix and DLLME to enrich the analytes were performed (Han et al., 2013). A multiclass and multi-residue method was optimized and validated for analysis of 19 pesticides of 16 chemical classes in greenhouse cucumber and tomato followed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). In this study, DLLME technique was applied for extraction and pre-concentration of pesticide residues from QuEChERS (quick, easy, cheap, effective, rugged and safe) extracts (Dashtbozorgi et al., 2013). Moreover, monitoring pesticide residues in greenhouse tomato by combining acetonitrile-based extraction with DLLME followed by GC-MS was conducted (Melo et al., 2012). For determination of deoxynivalenol in wheat flour, DLLME-HPLC-UV was proposed (Karami-Osboo et al., 2013). IL-based vortex-assisted DLLME of organophosphorus pesticides in apple and pear (Zhang et al., 2012c) and DLLME combined with HPLC of some carbamate pesticides in watermelon and tomato samples were developed (Liu et al., 2012). In addition, DLLME followed by sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples was proposed (Zhang et al., 2012d).

A new simple and reliable method combining an acetonitrile partitioning extractive procedure followed by dispersive solid-phase cleanup with DLLME and further GC-MS analysis was developed for the simultaneous determination of bisphenol A and bisphenol B in canned seafood samples. The final DLLME extractive step was designed in order to allow the simultaneous acetylation of the compounds required for their GC analysis (Cunha et al., 2012). In another study, pH-controlled DLLME for the analysis of ochratoxin A in cereals was developed. This DLLME mode is based on two successive DLLMEs conducted at opposite pH values. The hydrophobic matrix interferences in the raw methanol extract were removed by a first DLLME performed at pH 8 to reduce the solubility of ochratoxin A in the extractant (CCl₄). The pH of the aqueous phase was then adjusted to 2, and the analyte was extracted and concentrated by a second DLLME (extractant, C₂H₄Br₂). The results showed that the proposed method is feasible for the analysis of ochratoxin A in cereals (Campone et al., 2012). In another study, USA-IL/IL-DLLME followed by HPLC was developed and applied to the extraction, separation and determination of sulfonamides in infant formula milk powder samples. The
hydrophobic IL and hydrophilic IL were used as extraction solvent and dispersion solvent, respectively. The purification of sample and concentration of target analytes were performed simultaneously and the introduction of ion-pairing agent (NH4PF6) was beneficial to the improvement of recoveries for IL phase and analytes (Gao et al., 2012).

4.4. Applications of DLLME for Biological Samples

A few papers have reported the application of DLLME on determination of drug mainly in urine. For the first time, DLLME-SFO was combined with field-amplified sample injection in capillary electrophoresis (CE) to determine four 2-agonists including cimbuterol, clenbuterol, mabuterol, and mapenterol in bovine urine. The applicability of the proposed method was successfully confirmed by determination of the four 2-agonists in spiked bovine urine and accuracy higher than 96.0% was obtained (Us et al., 2013). Moreover, DLLME prior to field-amplified sample injection method has been developed and validated for the sensitive analysis of 3,4-methylenedioxymethamphetamine, phencyclidine and lysergic acid diethylamide by capillary electrophoresis in human urine (Airado-Rodriguez et al., 2012). A novel method for the determination of sertraline using DLLME coupled with CE was developed. Acetone and dichloromethane were used as the disperser solvent and extraction solvent, respectively. This method was successfully applied to the determination of sertraline in human urine (Huang et al., 2012). Besides, the combination DLLME with CE and a time-of-flight mass spectrometer (TOF-MS) was evaluated for the toxicological compounds (i.e., amphetamines and their derivatives, opiates, cocaine and its metabolites and pharmaceuticals) screening in urine samples. The combination of DLLME and CE was particularly attractive because of the small amount of organic solvents required (Kohler et al., 2013).

An environmentally friendly IL-DLLME method coupled with HPLC for the determination of antihypertensive drugs irbesartan and valsartan in human urine was developed (Li et al., 2013a). In another study, temperature controlled IL-DLLME combined with HPLC has been developed for the simultaneous determination of di(2-ethylhexyl)phthalate (DEHP) and its metabolite mono(2-ethylhexyl) phthalate (MEHP) in human urine. Under the optimized extraction conditions, the method showed 115- and 164-fold enrichment factors for MEHP and DEHP in urine, respectively. This method can be expected to monitor the human exposure to the plasticizer agent by determining its metabolites in human urine (Sun et al., 2013). Furthermore, a multi-residue pretreatment technique, termed temperature-assisted IL-DLLME, and its application to simultaneous extraction of polychlorinated biphenyls and polybrominated diphenyl ethers in urine was reported. In this method, an ionic liquid was used as the extraction solvent and dispersed into the liquid sample with the help of methanol and at elevated temperature (Zhao et al., 2012).
For the simultaneous determination of quinocetone and three of its synthesized desoxy metabolites in swine urine via HPLC, a method based on USA-DLLME was developed. The results showed that USA-DLLME has a potential application in the pharmacokinetic and residue studies of quinoxaline-N-dioxides derivatives in biological fluid samples (Zhang et al., 2012e). For preconcentration and determination of ecstasy compounds such as 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethylamphetamine, 3,4-methylenedioxyethylamphetamine and 3,4-methylene-dioxypropylamphetamine in urine, a new method based on DLLME and GC-FID was developed. The method was based on the formation of tiny droplets of an organic extractant in prepared sample solution using water, immiscible organic solvent (CS₂) dissolved in water-miscible organic disperser solvent (acetone). The results showed that method was successfully for the determination of analytes in urine (Mashayekhi and Rezaee, 2012). In another study, DLLME followed by HPLC with diode array detection has been developed as sample preparation method for simultaneous determination of seven organic UV filters in urine. The proposed method was successfully applied to different volunteer urine samples and it was shown that the extraction efficiency was not affected by the type of urine samples (Vosough et al., 2012). Moreover, DLLME followed by HPLC was used for the extraction and determination of oxazepam, diazepam, and alprazolam in human urine (Vardini et al., 2012).

Furthermore, a novel surfactant-assisted dispersive liquid-liquid microextraction (SADLLME) based on SFO combined with HPLC-UV has been proposed for extraction and determination of amphetamine and methamphetamine in urine (Tehrani et al., 2012). In another study, a novel method for the determination of macrolide antibiotics in human urine using DLLME coupled to surface-assisted laser desorption/ionization mass spectrometric detection was developed (Chen et al., 2012). Moreover, a developed technique was reported for extraction and pre-concentration of methamphetamine and 3,4-methylenedioxymethamphetamine from urine samples using molecularly imprinted-solid phase extraction (MISPE) along with simultaneous derivatization and DLLME (Djozan et al., 2012). In another study, the preparation and evaluation of a molecularly imprinted polymer as SPE sorbent and simultaneous ethyl chloroformate derivatization and pre-concentration by DLLME for the analysis of t,t-muconic acid in urine using GC-MS was described (Mudiam et al., 2013).

An analytical method, namely SPE-DLLME, is established to determine carbamazepine in urine and plasma. In this work, after concentration and purification of the samples using SPE C-18 sorbent, 1.5 mL of acetonitrile containing 60.0 µL of chloroform was injected into 5.0 mL pure water. After extraction and centrifuging, the sedimentated phase was evaporated and the residue was dissolved in 30 µL methanol and injected into the HPLC system. The new method provides detection limits of 0.8 µg/L and 1.7 µg/L in urine and plasma, respectively. The results show that SPE-DLLME
is a suitable method for the determination of carbamazepine in biological samples (Rezaee and Mashayekhi, 2012). In another study, a method for the extraction of quercetin as well as its determination in urine and plasma samples using inverted dispersive liquid-liquid microextraction (IDLLME) and HPLC-UV was developed. The extraction method is based on the application of an extracting solvent lighter than water in the ternary component solvent (aqueous solution: extracting solvent: disperser solvent) system (Ranjbari et al., 2012a). Analysis of losartan and carvedilol in urine and plasma samples using a DLLME coupled with HPLC-UV was conducted (Soltani et al., 2012).

For the first time, combination of electromembrane extraction (EME) and DLLME followed by GC-FID was developed for determination of tricyclic antidepressants in untreated human plasma and urine. The results showed that EME-DLLME-GC/FID is a promising combination for analysis of tricyclic antidepressants present at low concentrations in biological matrices (Seidi et al., 2013). Surfactant-based USA-DLLME and derivatization were applied for the pretreatment of three corticosteroids prior to determination by high-performance liquid chromatography-diode array detector (HPLC-DAD). An appropriate mixture of Triton X-100 (disperser solution), CCl₄ (carbon tetrachloride; extraction solvent), and salicylaldehyde (derivatization reagent) was added to human urine and plasma samples. The results showed that the method is applicable to the determination of trace corticosteroids in human urine and plasma (Qin et al., 2013). A method based on ultrasound-enhanced surfactant-assisted dispersive liquid-liquid microextraction (UESA-DLLME) followed by HPLC has been developed for extraction and determination of ketoconazole and econazole nitrate in human blood. In this method, a common cationic surfactant, cetyltrimethylammonium bromide, was used as dispersant. Chloroform (40 µL) as extraction solvent was added rapidly to 5 mL blood containing 0.068 mg/mL cetyltrimethylammonium bromide (Xia et al., 2012).

A method to determine the cypermethrin (CYP) insecticide in rat tissues (kidney, liver and brain) and blood has been developed for the first time using low density solvent DLLME followed by gas chromatography-electron capture detector (GC-ECD) analysis. Firstly, tissue samples containing CYP were homogenized in acetone. Subsequently, homogenate was mixed with n-hexane (extraction solvent) and the mixture was rapidly injected into water. Afterward, the upper n-hexane layer was collected in a separate microtube and then injected into GC-ECD for analysis. Blood samples were diluted with ultrapure water and subjected to DLLME through similar procedure. The results showed that this method is a simple, rapid and efficient technique for extraction and determination of CYP in rat tissues and blood (Mudiam et al., 2012). In another study, DLLME-SFO technique was developed for the determination of duloxetine in human plasma by HPLC-FLD. During the extraction procedure, plasma protein was precipitated by using a mixture of zinc sulfate solution and acetonitrile (Suh et al., 2012). Moreover, extraction and determination of warfarin, a widely used anticoagulant drug, in
human plasma were performed using a new generation of DLLME and HPLC (Ghambari and Hadjmohammadi, 2012). Besides, for the detection of trace amounts of methadone in human urine, plasma, saliva and sweat samples, DLLME followed by HPLC was used (Ranjbari et al., 2012b).

5. Conclusions and Prospects

DLLME has emerged as a viable sample-preparation approach with the advantages of simplicity of operation, rapidity, low cost, high recovery, and high preconcentration factor. Since it was invented in 2006, many improvements have been introduced such as conjunction with different extraction technique. Meanwhile, the application of DLLME has been expanded to some complex matrices samples such as environmental, food and biological samples, which not be confined to water samples. In the future, connection of DLLME to other extraction techniques is still the trend as they allow obtaining both better selectivity and lower limits of detection. Moreover, although some progress has been made to automate DLLME, further research is still needed.

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