DEVELOPMENT OF ENVIRONMENTALLY FRIENDLY
DISPERSE LIQUID-LIQUID MICROEXTRACTION TECHNIQUES

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NATIONAL UNIVERSITY OF SINGAPORE

2013
DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety.

I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Zhang Yufeng

29 January 2013
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SUMMARY

Sample preparation is a key procedure in modern chemical analysis, particularly in dealing with complex sample matrices; this procedure concentrates the target analytes to adequate levels for measurement and removes contaminants to yield clean, informative chromatograms. In recent years, the trend has been toward the development of microscale sample preparation procedures. Liquid-phase microextraction (LPME) is a sample preparation technique which is based on the use of a small amount of extraction solvent to extract analytes from minimal amounts of sample matrices.

This thesis focuses on one of the major challenges associated with sample preparation, developing miniaturized and environmentally friendly LPME methodologies. The work described involves the development of different novel modes of dispersive liquid-liquid microextraction (DLLME) techniques for some important analytes of environmental concern. To avoid the use of large amount of toxic dispersive solvent (up to hundred microliters) which is often applied in traditional DLLME, and ensure sufficient dispersion of extraction solvent to the aqueous sample and high extraction efficiency, a simple solvent microextraction method termed vortex-assisted dispersive liquid-liquid microextraction (VADLLME) is studied. This is described in Chapter 2. In order to avoid the use of relatively high toxic and high density chlorinated solvent in traditional DLLME and our previous work on VADLLME, the application of relatively low toxic ILs and lighter-than-water solvent as the extraction solvents in DLLME have been explored in Chapters 3
and 4, respectively. To further improve the dispersion of low-density organic solvent to the aqueous sample in an even faster and more efficient way, in Chapter 5, low-density solvent-based vortex-assisted surfactant-enhanced dispersive liquid-liquid microextraction (LDS-VSDLLME) has been investigated.

In Chapter 2, a simple and environmentally friendly microextraction method termed VADLLME coupled with gas chromatography-mass spectrometry (GC-MS) is reported and used for the analysis of six benzophenone ultraviolet (UV) filters in water samples. In this method, no dispersive solvent was used; with the aid of vortex agitation, good extraction solvent dispersion and high extraction efficiency were achieved. Moreover, no centrifugation was required in this microextraction procedure.

In Chapter 3, a rapid, highly efficient and environmentally friendly sample preparation method named ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME), followed by high performance liquid chromatography (HPLC) is described for the extraction and preconcentration of four benzophenone-type UV filters from three different water matrices. In Chapter 4, the application of low toxic, low-density organic solvents in DLLME is reported. In this study, a low-density organic solvent-based ultrasound-assisted dispersive liquid-liquid microextraction (LDS-USA-DLLME) was successfully developed for the extraction of trace level of organochlorine pesticides (OCPs) in water samples and followed by GC-MS analysis. To achieve easy collection of the final low-
density organic extract, a cheap, flexible and disposable polyethylene Pasteur pipette has been used as a convenient extraction device. No dispersive solvent was required in this procedure; ultrasound radiation was applied to accelerate the dispersion of low-density organic solvent in aqueous solutions to enhance the microextraction efficiency of OCPs in water samples. This method provided the combined advantages of the polyethylene Pasteur pipette, low-density organic solvents and ultrasound-assisted emulsification microextraction (USAEME). Significantly, fast analysis and high extraction efficiency were achieved.

In Chapter 5, LDS-VSDLLME combined with GC-MS has been established for the determination of six phthalate esters (PEs) in water samples. This method combined the advantage of surfactant and vortex agitation to make a full dispersion of the extraction solvent, thus fast and high efficient extraction was achieved. The use of the surfactant in the VSDLLME method could enhance the dispersion of extraction solvent into aqueous sample and also favorable for the mass-transfer of the analytes from aqueous sample to the extraction solvent. Moreover, using a relatively less toxic surfactant as the emulsifier agent overcame the disadvantages of traditional organic dispersive solvents that are usually more toxic.

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<th>Full Name</th>
<th>Description</th>
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<tr>
<td>BBP</td>
<td>Butyl benzyl phthalate</td>
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<tr>
<td>BEHP</td>
<td>bis-2-Ethyl hexyl phthalate</td>
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<tr>
<td>BH</td>
<td>Benzhydrol</td>
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<tr>
<td>[BMPL][FAP]</td>
<td>1-Butyl-1-methylpyrrolidinium</td>
<td>tris(pentafluoroethyl)trifluorophosphate</td>
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<tr>
<td>BP</td>
<td>Benzophenone</td>
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<td>BP-1</td>
<td>2,4-Dihydroxybenzophenone</td>
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<td>BSTFA</td>
<td>N,O-bis-(trimethylsilyl)trifluoroacetamide</td>
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<td>[BTMA][NTf₂]</td>
<td>Butyltrimethylammonium</td>
<td>bis(trifluoromethanesulfonyl)imide</td>
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<td>CE</td>
<td>Capillary electrophoresis</td>
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<td>CFME</td>
<td>Continuous-flow microextraction</td>
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<tr>
<td>CTAB</td>
<td>Cetyltrimethyl ammonium bromide</td>
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<td>DnBP</td>
<td>Di-n-butyl phthalate</td>
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<td>o,p'-DDD</td>
<td>1,1-Dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane</td>
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<td>p,p'-DDE</td>
<td>1,1’-(2,2-dichloroethylidene)bis(4-chlorobenzene)</td>
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<tr>
<td>DEP</td>
<td>Diethyl phthalate</td>
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<td>DLLME</td>
<td>Dispersive liquid-liquid microextraction</td>
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<tr>
<td>D-LPME</td>
<td>Dynamic liquid-phase microextraction</td>
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<td>DMP</td>
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<td>DnOP</td>
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<td>EF</td>
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<td>Ethylhexyl salicylate</td>
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<td>[HMIM][FAP]</td>
<td>1-Hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate</td>
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<td>[HMIM][PF$_6$]</td>
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<td>Limit of quantification</td>
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<td>MS</td>
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<td>NaCl</td>
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<tr>
<td>OCPs</td>
<td>Organochlorine pesticides</td>
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<tr>
<td>PAH</td>
<td>Polycyclic aromatic hydrocarbon</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyl</td>
</tr>
<tr>
<td>PEs</td>
<td>Phthalate esters</td>
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<tr>
<td>RR</td>
<td>Relative recovery</td>
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<td>RSD</td>
<td>Relative standard deviation</td>
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<td>SBME</td>
<td>Solvent-bar microextraction</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td>SIM</td>
<td>Selective ion monitoring</td>
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<td>Supported liquid membrane</td>
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Chapter 1 Introduction

One main and important objective of analytical chemistry is to provide methods for determining the presence of elements and chemicals to understand nature. Currently, with the increasing concern of environmental pollution by chemicals, the analysis of chemical compounds in environmental water, pharmaceutical, biological, food and agrochemical fields plays an important role in the development of analytical science.

In chemical analysis, analytical methods involve various processes such as sampling, sample preparation, separation, detection and data analysis. In order to obtain accurate results, each step of the analysis processes is crucial. In an attempt to improve the separation and quantification efficiency, great improvements have been made in the measurement techniques such as gas and liquid chromatography, spectroscopy and sensor over the last few decades. However, most instruments cannot handle samples directly due to the complexity of the sample matrices. As a result, an appropriate sample preparation step is critical to clean up, isolate and concentrate the analytes of interest to render them in a form that is compatible with the analytical instruments.

To achieve the aim of sample preparation, two classical sample preparation methods liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are popular choices. However, both of these two techniques are time-consuming, tedious and labor intensive. The disadvantages of these conventional
extraction techniques have led to the development of miniaturized sample preparation methods, which use small volumes of organic solvent. Therefore, many efforts in the past decades have been devoted to the adoption of existing methods and the development of new techniques which are environmentally friendly, economical, accurate and with high extraction efficiency. As alternatives to LLE and SPE, environmentally friendly and cost-effective miniaturized sample preparation methodologies such as liquid-phase microextraction (LPME) [1] and solid-phase microextraction (SPME) [2] have been developed. LPME was first introduced in middle-to-late 1990s [3-5] and it is a big breakthrough in the development of sample preparation methods. A latter development of LPME was based on a droplet of organic solvent hanging at the end of a microsyringe needle (single drop microextraction, SDME), followed subsequently by the advent of hollow fiber LPME (HF-LPME) [6], dynamic LPME (D-LPME) [7], continuous-flow microextraction (CFME) [8] and solvent bar microextraction (SBME) [9]. Due to their low consumption of organic solvents, simplicity in experimental setup and high extraction efficiency, these techniques became widely applied in the past few years [10,11].

Dispersive liquid-liquid microextraction (DLLME) was introduced by Rezaee et al. in 2006 [12]. Due to its important advantages such as speed, cost-effective and ease of operation, this technique has been widely used by many researchers in recent years. Subsequently, different modes of DLLME (i.e. temperature-controlled ILDLLME, ultrasound-assisted DLLME, vortex-assisted DLLME and surfactant-assisted DLLME) have been successfully
developed to enhance the extraction efficiency, simplify the operation procedure, minimize the impact on the environment and reduce the operation cost. So it is worthwhile to continue to develop different kinds of miniaturized environmentally friendly DLLME sample preparation methods and widen their applications for the analysis of environmental pollutants.

In the following section, traditional sample preparation techniques and modern miniaturized sample preparation methods are briefly reviewed.

1.1 Sample preparation techniques

To achieve the aim of sample preparation, two classical sample preparation methods LLE and SPE are popular choices. LLE is a traditional technique for extracting organic compounds from aqueous samples. The extraction principle is based on the partition of the dissolved target analytes between the organic solvent and the aqueous sample solution according to their partition coefficients. The selectivity of LLE can be easily adjusted by changing the polarity of extraction organic solvent, the pH of the aqueous sample or the salts content depending on the natural properties of the analytes. Although LLE has been widely used, it has some disadvantages, such as time-consuming, tedious, and utilizes large amounts of high purity organic solvents, which are potentially toxic and expensive. In addition to these, the formation of emulsions in LLE procedure leads to the difficult separation of the organic phases and the aqueous phases. Due to all these drawbacks, it is being replaced by other methods.
SPE is a more modern extraction technique and based on the sorption of analytes on the sorbent. In this procedure, organic compounds are initially trapped on the sorbent (cartridges, precolumns, and disks) while the aqueous sample passed through the cartridge or disk. Then the target analytes are eluted with a suitable solvent. Therefore, separation and enrichment can be achieved. Compared to LLE, SPE consumes much smaller amounts of organic solvent. However, SPE requires column conditioning which is tedious and is relatively expensive. The disadvantages of these conventional extraction techniques have led to the development of miniaturized sample preparation methods, which use small volumes of organic solvent. And recent research has been oriented towards the development of efficient, economical, and environmentally friendly sample preparation methods.

As a result, many efforts in the past decades have been devoted to the adoption of existing miniaturized sample preparation methods and the development of new techniques in this field. As alternatives to LLE and SPE, environmentally friendly and cost-effective miniaturized sample preparation methodologies such as liquid-phase microextraction (LPME) [1] and solid-phase microextraction (SPME) [2] have been developed in the needs of times.

SPME was introduced as a solvent-free sample extraction technique by Arthur and Pawliszyn [2] in 1990. It has been more and more widely used in sample preparation, especially since the first fiber was commercialized in 1993. The basic SPME format is a polymeric stationary phase coated onto a
stainless steel or fused silica fiber. The extraction is based on the establishment of equilibrium between the target analytes and the coating. The analytes are then desorbed from the fiber into a suitable separation and detection system, usually a gas chromatography. Currently, there are three modes of SPME operation: direct immersion, headspace and the less commonly-used membrane-protected SPME. Till now, SPME has been extensively applied for the analysis of organic compounds in pharmaceutical, food and environmental samples. The main advantage of this technique is the ease of operation, which incorporates sampling, extraction, concentration and sample introduction into a single step. Additionally, SPME completely eliminates the usage of organic solvent, thus it can provide good quantitative results over wide range concentrations of analytes and is sensitive for low concentration analytes. However, there are still some limitations for this technique. Firstly, it suffers from carry-over problems, which may be difficult to eliminate in some cases, even though fibers are normally reconditioned at high temperature. Secondly, the SPME fibers are very fragile, which leads to a short lifetime for some applications. In addition, SPME fibers are expensive, which increases the sample preparation cost. Furthermore, it lacks selectivity when extracting analytes in complex matrices and the reproducibility is relatively poor.

To overcome these shortcomings, another novel microextraction method termed LPME as an alternative miniaturized sample preparation approach was developed. In the following section, LPME is fully introduced, including the
development of LPME, especially its different operational modes, such as SDME, CFME, HF-LPME and DLLME.

1.2 Liquid-phase microextraction (LPME) techniques

In order to reduce the consumption of organic solvents, much work has been devoted to the development and application of miniaturized or microscale LLE during the last 20 years. LPME, as an alternative miniaturized sample preparation approach emerged in the mid-to-late 1990s [3, 5], has gained widespread acceptance and witnessed incessant growth in the range of applications of sample preparation for trace organic and inorganic analysis from different sample matrices since its introduction. A latter development of LPME was based on a droplet of organic solvent hanging at the end of a microsyringe needle (SDME) [5], followed subsequently by the advent of HF-LPME [6, 13-19], D-LPME [7, 20-23], CFME [8, 24-27], SBME [9, 28-32] and DLLME [10, 33]. As its name indicates, LPME uses only a small amount of solvent for concentrating analytes from sample matrix. It overcomes many disadvantages of LLE, SPE as well as SPME, and shows many merits such as ease of operation, low organic solvent consumption and high extraction efficiency. Moreover, it is characterized by its affordability, and reliance on widely available apparatus.

In the following parts, the development of LPME is described in detail, based on its different modes, with focus on the development of DLLME. This thesis focuses on the development of different kinds of DLLME methods, i.e.
vortex-assisted dispersive liquid-liquid microextraction (VADLLME), ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME), low-density solvent-based ultrasound-assisted dispersive liquid-liquid microextraction (LDS-USA-DLLME), and low-density solvent-based vortex-assisted surfactant-enhanced dispersive liquid-liquid microextraction (LDS-VSDLLME). These four kinds of DLLME methods and the analytical results will be discussed in detail in Chapters 2, 3, 4 and 5, respectively.

1.2.1 Single drop microextraction (SDME)

SDME, characterized by its simplicity of operation and high extraction efficiency, has attracted considerable attention over the last 15 years. Since the introduction of SDME in 1996 [1, 3], different modes of SDME have been developed, catering to various analytical applications, such as direct immersion-SDME and headspace-SDME (HS-SDME). Based on these various implementations, various approaches have been taken by researchers to improve selectivity, stability of the microdrop, expand the application range of the procedure, introduce a degree of automation, and make it more compatible with more analytical techniques.

In 1996, Liu and Dasgupta [1] reported a novel drop-in-drop system to extract sodium dodecyl sulfate. In this report, a water-immiscible organic microdrop (1.3 μL) was suspended inside a flowing aqueous drop from which the analytes were extracted. At almost the same time, Jeannot and Cantwell [3]
introduced another type of solvent microextraction. In this study, a small drop (8 μL) of water-immiscible organic solvent 1-octanol containing an internal standard was located at the end of a Teflon rod and suspended in a stirred aqueous sample solution. After extraction for a prescribed time, the Teflon rod was withdrawn from the aqueous solution; the organic solvent was then sampled with a microsyringe and injected into GC system for analysis. In this work, the equilibrium and kinetic theories were also discussed in detail. Although acceptable analysis results were obtained, limitations such as tedious microextraction procedures and special care of operation were still existed.

In 1997, Jeannot and Cantwell [5] introduced another novel microextraction technique, which used a microsyringe as the organic solvent holder instead of a Teflon rod, thus realizing the combination of extraction and injection in a single device. It is a milestone of the LPME development history and greatly improved the previous LPME techniques for only a microsyringe needle is employed for sampling, extraction and injection. For this work, as shown in Figure 1-1, a microliter of organic solvent was first withdrawn into a microsyringe, and then the needle of the microsyringe was passed through the sample vial septum and immersed in the liquid sample. At last, a droplet of organic solvent was suspended at the tip of the syringe needle in a stirred aqueous sample by pushing the plunger of the microsyringe. It represents a desirable convenience of the microextraction operation. In 2001, Jeannot and co-workers [34] developed HS-SDME to analyze volatile organic compounds in aqueous matrix. Figure 1-2 shows the basic setup of HS-SDME, the extraction organic solvent was suspended in the headspace of the sample vial
which just above the aqueous sample, model compounds evaporated to the headspace of the bottle and were conveniently and rapidly preconcentrated in the microdrop.

Figure 1-1 Single drop microextraction (SDME)

Figure 1-2 Headspace single drop microextraction (HS-SDME)

In order to enhance the stability of the drop, efforts were devoted to modify the needle tip of the microsyringe [35] and investigate the use of novel solvent
ILs as the extraction solvent [36]. The modification of the needle tip enlarged its cross-sectional area, resulting in stronger adhesion between the needle tip and the organic drop. With this modification, the organic drop was able to withstand a higher stirring speed, up to 1700 rpm, and enhanced EFs ranging from 540 to 830-fold for organophosphorus pesticides (OPPs) achieved. ILs have recently been investigated as SDME extraction solvents [37-41], and these are generally considered due to their environmentally friendly behaviors and unique characteristics (e.g. no effective vapor pressure, adjustable viscosity and immiscibility in water and some other organic solvents). In 2003, Liu and Jiang [36] introduced the first application of IL as an extraction solvent in SDME. In this paper, an IL 1-octyl-3-methylimidazolium hexafluorophosphate was adopted for the analysis of polycyclic aromatic hydrocarbons (PAHs). The tunable physical properties of IL enable the use of IL in separation science. Compared with organic solvent, IL provided higher enrichment factor because of its non-volatility and adequate viscosity which made longer extraction time possible. The interaction between IL and target compounds, enhance the extraction and make IL a favorable choice in developing new extraction techniques. Using ILs as extraction solvent, chlorobenzene [39], UV filters [42], aromatic amines [43], sulfonamides [44] and phenols [45] have been determined. ILs were demonstrated to be compatible with many detection techniques, apart from HPLC, such as cold-vapor atomic fluorescence spectrometry and AAS.

In order to further improve the extraction efficiency and achieve fast analysis, dynamic SDME and dynamic HS-SDME were developed
successively. In 1997, He and Lee [46] compared the extraction efficiency based on EF, and reproducibility between the two modes. In both modes, chlorobenzenes were used as model analytes and extracted by toluene. In dynamic SDME, the microsyringe was used as a separatory device, which involved the repeated movement of the syringe plunger. It was indicated that for this dynamic mode extraction primarily occurred in the thin organic film formed on the wall of the microsyringe barrel and needle. As a result, faster analysis and higher EFs were achieved for the increased surface area between sample solution and extraction organic solution. In 2003, Hou and Lee [47] extended this dynamic mode to dynamic HS-SDME for the analysis of 5 chlorobenzenes in soil. In this microextraction, when the syringe plunger was pushed and pulled, the organic solvent film was formed in the microsyringe barrel and served as the extraction interface. This method was shown to be a fast and simple extraction method for volatile compounds.

SDME has emerged as a viable sample preparation method with which one could obtain generally acceptable analytical results. It has been shown to be routinely applicable to real world samples. SDME is accessible to almost all laboratories due to its ease of operation, simplicity and insignificant startup cost. However, some limitations are still existed, firstly, in its most basic (direct immersion) mode, it requires careful and elaborate manual operation because of the problem of drop dislodgment and instability; secondly, an extra filtration step of the sample solution is usually necessary since more complex matrixes will compromise the stability of the extraction organic solvent drop, this problem can be alleviated by carrying out HS-SDME; thirdly, not
withstanding the acceptable analytical performance mentioned above, the
sensitivity and precision of SDME methods need further improvement. The
main issue lies with the adverse consequences of prolonged extraction time
and fast stirring rate, since they may result in drop dissolution and/or
dislodgement; finally, relatively long extraction time is still a problem. To
address these problems, another novel LPME approach, termed CFME was
developed.

1.2.2 Continuous-flow microextraction (CFME)

CFME evolved from conventional SDME, and was first reported by Liu and
Lee in 2000 [48]. This approach appeared to be an effective combination of
Lin and Dasgupa’s [49] and Jeannot and Cantwell’s [3] earlier works. As
shown in Figure 1-3, briefly, an aqueous sample (typically of total volume 3
mL or less) was pumped continuously at a constant flow rate (0.05 mL/min, or
above) into a 0.5-mL glass extraction chamber via connecting PEEK tubing.
After the chamber had been filled with sample solution, the required volume
(1-5 μL) of water-immiscible extraction solvent was introduced into the
system through the injector. The drop then traveled to the outlet of the PEEK
tubing when it remained attached. The sample solution was continuously
pumped “around” the drop, allowing the target analytes to be extracted
efficiently. At the end of the extraction, a microsyringe was introduced into
the chamber to collect an appropriate amount of the extraction solvent for
analysis. As a result, high EFs ranging from 260- to 1600-fold were achieved
within 10 min of extraction of trace nitroaromatic compounds and
chlorobenzene in environmental samples. In the combination with GC-electron capture detection (ECD), the sample preparation method allowed analytes to be detected at fg/mL levels. An alternative way is to use a microsyringe and the drop, being formed at the end of the needle, placed just above the PTFE tubing outlet in the extraction chamber [27]. This extraction setup avoided the use of solvent injector and two separate microsyringes. Another modification (termed, cycle-flow microextraction) was to return the effluents of extraction chamber back to the aqueous sample reservoir and use it repeatedly for extraction [50]. The re-circulation of sample solution permitted analysis on further reduced sample volume (1-2 mL), thus avoided running the sample reservoir dry accidentally.

**Figure 1-3** Continuous flow microextraction (CFME). Modified from ref [46].

CFME differs from other extraction methods and affords some advantages as follows. For CFME, the extraction solvent drop fully and continuously makes contact with a fresh and flowing sample solution, thus both diffusion
and molecular momentum resulting from mechanical forces contribute to its effectiveness. Another advantage is that since high preconcentration can be achieved, smaller volumes of aqueous samples were needed for extraction. Finally, a direct comparison of CFME and static direct immersion SDME has proved the latter to yield superior detection limits and precision. However, most procedures making use of CFME are limited to extraction of nonpolar or slightly polar semivolatile compounds, such as PAHs [51] and pesticides [8], owing to the fact that only nonpolar extracting solvents are stable in the flowing system and the extent of their dissolution in the flowing aqueous sample is small. Another shortcoming of this mode is the need for additional equipment, such as a microinfusion pump.

1.2.3 Hollow fiber - protected liquid-phase microextraction (HF-LPME)

In order to improve the stability and reliability of SDME, Pedersen-Bjergaard and Rasmussen introduced HF-LPME in 1999 [52]. In this concept the extraction solvent was placed inside the lumen of a porous polypropylene hollow fiber. A supported liquid membrane was formed by dipping the hollow fiber into the organic solvent. The solvent penetrated the pores of the hollow fiber and was bound by capillary forces to the polypropylene network comprising the fiber wall. The high porosity enabled immobilization of a certain volume of solvent as thin film. The extraction solvent which was placed in the lumen of the fiber was mechanically protected inside the hollow fiber and it was separated from the sample by the supported liquid membrane (SLM). This prevented dissolution of the extraction solvent phase into sample.
In HF-LPME, analytes are extracted from an aqueous sample, into the organic solvent immobilized as a supported liquid membrane, and into the acceptor solution placed inside the lumen of the hollow fiber. Subsequently, the acceptor solution is removed by a micro-syringe and transferred to final instrument for analysis. With the protection of hollow fiber, acceptor phase is not in direct contact with the sample solution, which can avoid large molecule in sample matrices from entering to the acceptor phase, thus high sample clean-up performance could be achieved. The basic set-up for HF-LPME is illustrated in Figure 1-4. HF-LPME can be performed either in the 2- or 3-phase mode. If the acceptor solution is an organic solvent (the same as used for the SLM), resulting in a 2-phase extraction system, if the acceptor solution is an acidic or alkaline aqueous solution, it is a 3-phase extraction system. A short piece of a porous hollow fiber is used for HF-LPME, and this may either be a rod configuration with a closed bottom [53] or a u-configuration [52] where both ends of the hollow fiber is connected to guiding tubes.

Figure 1-4 Hollow fiber liquid phase microextraction (HF-LPME). Modified from ref [52, 54].
Later, in order to increase the extraction efficiency and reduce the extraction time, dynamic 2-phase and 3-phase HF-LPME were introduced by Zhao et al. [55] and Hou et al. [56], respectively. Since the enhanced contact surface areas between sample solution and organic solvent, higher enrichment factor can be achieved. Subsequently, SBME as an improved mode of HF-LPME was developed by Jiang et al. [9]. In this method, the organic extraction solvent was confined within a short length of a hollow fiber (heat-sealed at both ends) and then was placed in a stirred aqueous sample solution. Tumbling of the extraction device within the sample solution upon stirring facilitated extraction. After extraction, the solvent bar was taken out, and one end of it was trimmed off. A 1 μL of analyte enriched extract was subsequently retrieved and injected into the GC system for analysis. It was a simple and sensitive method for sample preparation.

In addition to high analyte enrichment and excellent sample clean-up, a major advantage of HF-LPME is that the sample can be stirred or vibrated vigorously without any loss of the extracting liquid, as it is mechanically protected, thus low consumption of organic solvent can achieved. Typically, the volume of organic solvent immobilized in the pores of a hollow fiber segment range from 5 to 30 μL [54]. Further more, LPME enables a high degree of flexibility. With the same extraction device, either 2- or 3- phase extractions can be performed, providing compatibility with GC, HPLC, and capillary electrophoresis (CE). However, a relatively long extraction time is the main problem.
1.2.4 Dispersive liquid-liquid microextraction (DLLME)

Recently, a novel and popular LPME method named DLLME was developed by Rezaee et al. [12], which opened a new horizon on fast sample analysis and greatly reduced sample preparation time and cost. It was another milestone in the developmental history of LPME.

Generally, DLLME is based on a ternary component solvent system resembling homogeneous liquid-liquid extraction (HLLE) and cloud point extraction (CPE). It is a simple and fast microextraction technique based on the use of an appropriate amount of high-density extraction solvent such as chlorobenzene, chloroform or carbon disulfide and a dispersive solvent, i.e., methanol, acetonitrile or acetone with high miscibility in both extraction organic solvent and aqueous phase. The extraction, as shown in Figure 1-5, including the injection of an appropriate mixture of an extraction solvent and a dispersive solvent rapidly into the sample solution, after which the extraction solvent is fully dispersed into the aqueous sample as very fine droplets by gently shaking and a cloudy solution is formed, into which the analytes are enriched. Owing to the considerably large surface area between the extraction solvent and the aqueous sample, the equilibrium is achieved quickly and the extraction is independent of time, which is the principal advantage of DLLME. After centrifugation of the cloudy solution, the extractant organic phase enriched with analytes settles at the bottom of the vial and can be collected for instrumental analysis. DLLME is a modified solvent extraction method and its acceptor-to-donor phase ratio is greatly reduced compared with other
extraction methods. It possesses some other advantages, such as ease of operation, rapidity, low sample volume, low cost and high EF. Since its introduction, DLLME has been widely used by many researchers for the determination of many kinds of organic, inorganic and organicmetallic species such as PAHs [57], chlorobenzenes [58], PEs [59], chlorophenols [60], triazine herbicides [61], phenols [62], cholesterol [63], trihalomethanes [64], aromatic amines [65], OPPs [66], polybrominated diphenyl ethers (PBDEs) [67], chlorophenoxyacetic acids [68], carbendazim and thiabendazole [69], clenbuterol (CB) [70], OCPs [71], selenium [72, 73], copper [74] and lead [75], cadmium [76] and organotin [77] in liquid samples.

![Diagram of DLLME](image)

**Figure 1-5** Dispersive liquid-liquid microextraction (DLLME)

However, relatively toxic halogenated organic solvents are applied for these works, which may cause health problems for workers and bad for the environment. To address this problem, many efforts have been contributed to introduce less toxic low-density organic solvents [78-82] to DLLME. For the application of low-density organic solvents, some researchers [78, 82, 83]
were focused on the development of solidification of the extraction solvent drop by ice bath to get a solid drop, which was easily withdrawn after extraction, at the same time some other researchers [79, 80, 84] contributed to design new extraction devices to benefit the collection of low-density organic solvent. In 2008, Leong and Huang [83] introduced DLLME based on solidification of floating organic droplet (SFO-DLLME) and successfully applied it to the determination of halogenated organic compounds in aqueous samples.

Some other efforts have been spent in introducing ILs [85-91] to DLLME, which is another approach to avoid the use of high toxic organic solvent. Room temperature ILs are an interesting alternative to organic solvents because of their unique physicochemical properties, which depend on the nature and size of their cationic and anionic constituents. The main advantages of ILs include good thermal stability, negligible vapor pressure, tunable viscosity and miscibility with water and organic solvents and thus an environmentally friendly extraction phase; therefore, they are useful as extraction solvents for DLLME technique. In 2008, for the first time, Zhu and co-workers [91] developed IL-DLLME combined with HPLC for the extraction of 2-methylaniline, 4-chloroaniline, 1-naphthylamine and 4-aminobiphenyl from water samples. This method combines the merits of both DLLME and ILs, providing good analytical results and environmentally friendly behavior.
Although fast and simple analysis can be achieved by the aforementioned DLLME, moreover, with the introduction of low-density solvent and ILs, better environmentally friendly behaviors can be realized. However, relatively large volume (several hundred microliters) of dispersive solvent is required, which not only add the organic solvent consumption, but also decrease the partition coefficient of analytes into the extraction solvent, thus reducing the extraction efficiency to some extent. More recently, to address this problem, temperature-controlled IL-DLLME, USA-DLLME, vortex-assisted dispersive liquid-liquid microextraction (VADLLME) and surfactant-assisted dispersive liquid-liquid microextraction (SADLLME) were developed sequentially, instead of dispersive solvent as applied in DLLME, the dispersion of the extractant phase to the aqueous solution was achieved by using temperature, ultrasound, vortex and surfactant, respectively. In the following part, the development of these four kinds of DLLME will be described in detail.

1.2.4.1 Temperature-controlled ionic liquid dispersive liquid-phase microextraction (Temperature-controlled ILDLLME)

Temperature-controlled ILDLLME evolved from IL-DLLME, and was first described by Zhou et al. [92] in 2008. Generally, Temperature-controlled ILDLLME is based on temperature change that enables ILs to completely disperse into the aqueous phase and increase the mass transfer of the analytes into the IL phase. Phase separation is achieved upon cooling and centrifugation. In this method, 45 μL 1-hexyl-3-methylimidazolium hexafluorophosphpate [HMIM][PF₆] was completely dissolved in 10 mL aqueous sample by heating the sample in a water bath with the temperature
controlled at 70 °C, after which, the analytes would migrate into the IL phase adequately. Then the solution was centrifuged to achieve phase separation after it was cooled with ice water for a fixed time. Finally the extract was injected to HPLC system for analysis. Satisfactory LODs ranging from 0.28 to 0.6 μg/L and linearity in the range of 1.5-100 μg/L with correlation coefficients ranging between 0.9725 and 0.9931 for all the analytes were achieved for the extraction of pyrethroid pesticides.

Since its introduction, Temperature-controlled ILDLLME has been successfully examined for the determination of OPPs [93], vanadium species [94], dichlorodiphenyltrichloroethane and its metabolites [95], dicofol and DDT [96], triclosan and triclocarban [97], chlorobenzenes [98], pyrethroid insecticides [99], phenols [100] and PEs [101] in liquid samples and satisfactory analysis results were achieved. However, ILs are incompatible with GC, when applied to HPLC, further dilution with mobile phase is necessary for their relatively high viscosities.

1.2.4.2 Ultrasound-assisted dispersive liquid-liquid microextraction (USA-DLLME)

It is well-known that ultrasonic radiation is a powerful aid in the acceleration of various steps, such as emulsion forming, homogenizing and mass transferring between immiscible phases, in the processes of separation and extraction. The application of ultrasonic radiation in LLE methods (USALLE) was first reported by de Castro and Priego-Capote [102]. A latter development (USA-DLLME), which applied a miniaturized approach to
USALLE by using a micro volume of organic phase to provide the advantage of both DLLME and USALLE, was introduced by Regueiro et al. [103] in 2008. In general, USA-DLLME is based on the dispersion of a microvolume of water-immiscible extraction solvent in the sample aqueous solution by ultrasound radiation which accelerates the mass-transfer process of the analytes between the two immiscible phases. The two phases can then be readily separated by centrifugation. In their work, 100 μL chloroform was first introduced into 10 mL water sample, placed in a 15 mL conical-bottom glass centrifuge tubes. The tube was then immersed into an ultrasonic water bath, after which, extraction was performed at 40 kHz of ultrasound frequency and 100 W of power for 10 min at room temperature. As a result, chloroform was fully dispersed to the aqueous sample with the aid of ultrasound radiation. Phase separation was then achieved by centrifugation and the sedimented phase was retrieved for further analysis. Due to the full dispersion of the organic solvent into water sample with the help of ultrasound radiation, mass transfer between the two phases was accelerated, thus resulting in high extraction efficiency. As a result, low LODs to pg/mL level were achieved for the determination of some emergent contaminants and pesticides in environmental waters. Since its introduction, Fontana et al. applied this method for the determination of polybrominated flame retardants [104] in water samples by GC-MS. Under optimum conditions, EFs higher than 319 were achieved. They demonstrated that USA-DLLME is an efficient, simple, and rapid as well cheap extraction technique prior to the GC analysis. Subsequently, Zhou et al. [105] dispersed an IL [HMIM][PF$_6$] by ultrasonic radiation to determine some aromatic amines in real water samples. This
developed method provides many merits such as excellent EFs, simplicity, easy of operation, low cost and consumption of organic solvents.

Till now, USA-DLLME has been successfully applied to the determination of PAH [104, 105], chlorinated phenoxyacetic acids [108], trichloroanisole [109], phenolic preservatives [110], polychlorinated biphenyls (PCBs), triclosan[111], diethofencarb and pyrimethanil fungicides [112], nitroaromatic explosives [113], geosmin and 2-methylisoborneol [114], antidepressant drugs [115], pyrethroids [116], OPPs [117], PEs [118], copper [119] and gold [120] in different sample matrices.

**1.2.4.3 Vortex-assisted dispersive liquid-liquid microextraction (VADLLME)**

VADLLME has been introduced by Yiantzi et al. [121] in 2010 whereby dispersing the extraction solvent into water by using vortex mixing, which is a mild emulsification procedure compared with USA-DLLME. In this method, after the extraction solvent was introduced into the aqueous sample, which was placed in a round-bottom glass vial, the mixture was then vigorously shaken using a vortex agitator for a fixed time. As a result fine droplets were formed facilitating mass transfer of the target analytes into the organic acceptor phase. After centrifugation the two phases separated, the floating organic solvent phase was then retrieved and used for HPLC analysis. This method had been successfully applied to the determination of octylphenol, nonylphenol and bisphenol-A at trace levels in water samples and afforded advantages such as fast, ease of operation and economical. Since its introduction, it has been successfully applied in the determination of pesticides
in tap and snow water [122], perfluorooctane sulfonate in tap, well and river water [123], PCBs in wastewater [124] and OPPs in wine and honey samples [125] due to its simplicity and high efficiency in the extraction process.

1.2.4.4 Surfactant-assisted dispersive liquid-liquid microextraction (SADLLME)

It is well known that surfactants are organic compounds that are amphiphilic. They contain both hydrophilic heads and hydrophobic tails. The tail is generally a hydrocarbon chain with a different number of carbon atoms, which maybe linear or branched, and may also contain aromatic rings [126]. Therefore, they can soluble in both organic solvent and water. Surfactant reduces the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface [127]. So, surfactant could serve as an emulsifier instead of relative large amount of toxic dispersive solvent as applied in DLLME to enhance the dispersion of the water-immiscible phase into the aqueous phase.

The first application of a surfactant as an emulsifier in LPME was developed by Wu et al. [126] in 2010 and proved to be efficient, simple, rapid and inexpensive. In this method, appropriate amount of extraction solvent and surfactant (Tween 20) were first added to water sample, which placed in a screw cap glass tube. The resulting mixture was then immersed into an ultrasonic bath to sonicate for a fixed time. Subsequently, phase separation was achieved by centrifugation and the sedimented organic phase was
retrieved. After reconstituted with methanol, the extract was injected into HPLC system for analysis. Under the optimum conditions, high EFs in the range of 170 and 246 were achieved. With the use of surfactant instead of dispersive solvent, high extraction efficiency, fast analysis and environmentally friendly behavior were achieved. Lately, this method has been successfully applied to the determination of carbamate pesticides [126, 128], OPPs [125, 129] and estrogens [130] in different environmental matrices. Subsequently, SADLLME without any other external forces [127] or with the help of vortex [125] were developed. All these techniques are approved to be fast, simple and efficient.

1.3 Principle of DLLME

Generally, different modes of DLLME consist of two basic steps: (1) Injection of an appropriate of extraction solvent or an appropriate mixture of extraction and dispersive solvents into aqueous sample, containing the analytes. In this step, the extraction solvent is dispersed into the aqueous sample as very fine droplets by different approaches, and the analytes are enriched into it. Owing to the large surface area between the extraction solvent and the aqueous sample, equilibrium state is achieved quickly. This is the most important advantage of these methods. (2) Phase separation of the two immiscible phases by centrifugation or gravity.

In DLLME, the main factors that affect extraction efficiency are as follows: suitable extraction and dispersive solvents, appropriate volume of extraction
and dispersive solvents. Selection of an appropriate extraction solvent is the major parameter for DLLME process. Halogenated hydrocarbon such as chlorobenzene, chloroform and tetrachloroethylene are usually selected as extraction solvents because of their high density and good extraction capability of the target compounds. In order to avoid the use of high toxic halogenated solvents, less toxic low-density organic solvent and ILs also was introduced to DLLME.

Miscibility of dispersive solvent in both extraction solvent and aqueous phase is essential in selection of it. Acetone, methanol and acetonitrile are usually selected as dispersive solvents. The extraction solvent volume has important effect on the EFs. By increasing of the extraction solvent volume, the volume of sedimented phase obtained by centrifugation increases, resulting in a decrease on EF. Therefore, the optimal extraction solvent volume should ensure both high EFs and enough volume of the sedimented phase for the subsequent analysis after centrifugation. The dispersive solvent volume directly affects the formation of cloudy solution, the dispersion degree of the extraction solvent in aqueous phase and, subsequently, the extraction efficiency. Variation of dispersive solvent volume affects the volume of sedimented phase. The suitable volume of dispersive solvent for well cloudy solution depends on the volume of both aqueous phase and extraction solvent. In DLLME, the important factors affecting the volume of sedimented phase are: (1) solubility of extracting solvent in water, (2) aqueous sample volume, (3) dispersive solvent volume and (4) extraction solvent volume.
In DLLME, extraction time is defined as an interval between the injection of mixture of an extraction solvent and dispersive solvent and centrifugation. The surface area between extraction solvent and aqueous phase is infinitely large. Thereby, transfer of analytes from aqueous phase to organic solvent is fast.

In DLLME, EF is defined as the ratio of the analyte concentration in the sedimented phase \( C_{sed} \) and the initial concentration of analyte \( C_{aq} \) in the aqueous sample:

\[
EF = \frac{C_{sed}}{C_{aq}}
\]

\( C_{sed} \) is obtained from a suitable calibration graph. The extraction recovery (ER) is defined as the percentage of total analyte amount \( n_0 \), extracted to the sedimented phase \( n_{sed} \):

\[
ER = \frac{n_{sed}}{n_0} \times 100 = C_{sed} \times V_{sed} / C_{aq} \times V_{aq} \times 100
\]

\[
ER = (V_{sed} / V_{aq}) \ EF \times 100
\]

Where \( V_{sed} \) and \( V_{aq} \) are the volumes of sedimented phase and sample solution, respectively.

1.4 This work: Objective and organization

In view of the above review, it is worthwhile to point out that all the LPME methods apply microscale organic solvents which greatly reduce the organic solvents consumption compared with traditional sample preparation methods. Moreover, they offer advantages such as high extraction efficiency and simplicity. However, for SDME, HF-LPME and CFME, the main
disadvantage is the long extraction time. In addition, the operation procedures are complex and some steps are hard to control. While for DLLME, although it is fast, the addition of large amount of organic dispersive solvent reduces the extraction efficiency and increases the sample preparation cost. Furthermore, the use of toxic high-density chlorinated organic solvents in DLLME is bad for both the operators and the environment. So it is of great importance to continue to develop different kinds of miniaturized environmentally friendly DLLME sample preparation methods to overcome the above-mentioned disadvantages, and widen their applications for the analysis of environmental pollutants.

To overcome the shortcomings of the previous LPME methods, the main objective of this thesis was to develop different kinds of DLLME sample preparation methods (including VADLLME (Chapter 2), IL-USA-DLLME (Chapter 3), LDS-USA-DLLME (Chapter 4) and LDS-VSDLLME (Chapter 5)) that reduce the use of dispersive solvent or apply relatively low toxic low-density organic solvents and ILs to determine and monitor organic hazardous pollutants (e.g. UV filters, OCPs and PEs) in environmental water samples. In Chapter 2, VADLLME is described which avoided the use of relatively large amount (several hundred microliters) of dispersive solvents, and high extraction efficiency was achieved. However, relatively high toxic and high density chlorinated solvent was applied. To overcome the shortcoming of VADLLME, IL-USA-DLLME and LDS-USADLLME were explored with the use of relatively low toxic ILs and lighter-than-water solvent as the extraction solvents. For IL-USA-DLLME, due to the relatively high viscosity of IL, a
small amount of organic solvent would be added to aqueous sample and combined with ultrasound radiation to achieve fast and fully extraction solvent dispersion. For LDS-USADLLME, polyethylene Pasture pipette would be introduced as a convenient device to achieve easily collection of the low-density extraction solvent. To further increase the extraction efficiency and decrease the extraction time of VADLLME, IL-USA-DLLME and LDS-USA-DLLME, LDS-VSDLLME was developed. LDS-VSDLLME applied a little amount of surfactant instead of toxic and expensive organic solvent as the dispersive solvent, which was more environmentally. Moreover, vortex agitation was introduced to further aid the dispersion of extraction solvent to aqueous sample in a short time. As a result, with the combination of surfactant and vortex agitation, higher extraction efficiency and shorter extraction time would be achieved. The specific objectives of this thesis are as follows:

- Develop a novel vortex-assisted dispersive liquid-liquid microextraction (VADLLME) followed by GC-MS method for the determination of benzophenone UV filters in water samples.

- Introduce ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME) followed by high-performance liquid chromatography for determination of UV filters in environmental water samples.

- Apply low-density organic solvent in ultrasound-assisted dispersive liquid-liquid microextraction (LDS-USA-DLLME) to determine OCPs in water samples.
Develop low-density solvent-based vortex-assisted surfactant-enhanced dispersive liquid-liquid microextraction (LDS-VSDLLME) combined with GC-MS for the fast determination of PEs in water samples.

As a result, the analytical thesis may have significant influence on the development of different kinds of DLLME. All these proposed microextraction methods should be useful for the determination of the target analytes at trace levels in environmental water samples, and may afford many advantages such as being fast, simple, robust, accurate, economical, highly efficient and environmentally friendly.

The sample preparation methods presented here are limited to the development of DLLME, although some different kinds of LPME methods as background knowledge were introduced in the previous section. Since DLLME shows numerous prominent merits over other LPME methods, it is worthwhile to further investigate and expand its application to the detection of different kinds of hazardous organic compounds.
Chapter 2 Vortex-assisted dispersive liquid-liquid microextraction of ultraviolet filters from water samples

2.1 Introduction

UV filters are widely applied to sunscreen, cosmetics and other pharmaceuticals and personal care products in order to protect the skin from solar radiation. However, excessive use of UV filters would lead to environmental pollution as well as accumulative negative effect on human beings [131, 132]. It is difficult to determine this kind of organic pollutants in real samples due to their low concentration and the co-existing interference. As a result, an appropriate sample pretreatment and enrichment procedure is necessary before analysis. Recent techniques include SPE [133], SPME [134], pressurized liquid extraction [135], CPE [136], SBSE [137], membrane-assisted liquid-liquid extraction [138], pressurized membrane-assisted liquid extraction [139], SDME [140, 141], HF-LPME [142], DLLME [80, 143-145] and IL-USAEME [146] have been reported to be successfully applied to extract UV filters from aqueous samples.

Since DLLME was introduced by Rezaee et al. [12] in 2006, many applications based on DLLME have been reported to deal with different kinds of samples such as biological, environmental samples, and foodstuff due to its faster operation, solvent economy, easy of operation and high EFs. DLLME is based on a ternary component solvent system resembling homogeneous LLE combined with CPE. A mixture of extractant and organic dispersive solvent is
injected rapidly into an aqueous sample by syringe, and then a cloudy solution is formed, which markedly increases the contact surface between phases and thus reduces the extraction times with enhanced extraction efficiency. However, the drawback of the inconvenience in separating the extract after extraction and the necessity of using a third component (dispersive solvent) that usually decreases the partition coefficient of the analytes into the extractant solvent limit its application to some extent.

Recently, vortex mixing as an efficient emulsification procedure was introduced in DLLME by Yiantizi [121], which was termed VALLME. In VALLME, no dispersive solvent was used, the extraction solvent is dispersed into water by vortex agitation, and thus high extraction efficiency can be achieved. Since its introduction, it has been successfully applied to the determination of OCPs [147], OPPs [125], PCBEs [124] and perfluorooctane sulfonate [123] in different samples. However, the centrifugation step is still necessary and this step cannot handle large volume samples since current conical centrifuge tubes are of limited capacities.

Herein, in this chapter, for the first time, a fast, simple, cost-effective and environmentally friendly VADLLME method has been developed for the extraction of UV filters in water samples. This method is a great improvement in DLLME and VALLME, and combined their advantages together. No dispersive solvent and centrifugation step were necessary. To facilitate the mass transfer of the analytes from the aqueous samples to the extractant, vortex mixing was used during extraction, after which, the extractant was
easily separated from the water sample by leaving the extraction system to stabilize for a very short time (~1 min). Several factors influencing the extraction efficiency of the VADLLME including extraction solvent, volumes of the extraction solvent, vortex time, salt addition and pH were investigated.

2.2 Experimental

2.2.1 Reagents and materials

Tetrachloroethene (purity > 99%) and chlorobenzene (purity > 99.9%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The six UV filters benzhydrol (BH) (purity 99%), 2,4-dihydroxybenzophenone (BP-1) (purity 99%), benzophenone (BP) (purity 99%), 2-hydroxy-4-methoxybenzophenone (BP-3) (purity 98+%), ethylhexyl salicylate (EHS) (purity 99%) and homosalate (HMS) (purity 99%) were purchased from Alfa Aesar (Karlsruhe, Germany). Their structures and relevant physico-chemical properties are given in Table 2-1. Concentrated hydrochloric acid, bought from Merck was used to adjust the pH of the water sample and was diluted to 0.1 M before use. Sodium dodecyl sulfate (SDS) (purity 99%) was purchased from BDH Laboratory Supplies (Poole, England). HPLC–grade methanol, acetonitrile, acetone, ethanol and chloroform were purchased from Tedia Company (Fairfield, OH, USA). Sodium chloride (NaCl) was from Goodrich Chemical Enterprise (Singapore). Ultrapure water, used in all experiments, was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water purification system.
BSTFA containing 1% trimethylchlorosilane and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) from Sigma-Aldrich were evaluated as derivatization reagents for GC analysis.

A 10-µL microsyringe used for GC-MS injection and a 100-µL HPLC microsyringe used for the addition of extraction solvent were purchased from SGE (Sydney, Australia).

A stock solution containing six UV filters (BH, BP, EHS, HMS, BP-3 and BP-1) at 10 mg/L concentration was prepared in methanol. Water samples were prepared by spiking ultrapure water with analytes at known concentrations (10 µg/L of each analyte) daily to study the VADLLME performance under different conditions. Local river (Singapore river, pH 8) and reservoir (Pandan reservoir, pH 8.2) water samples were used for evaluation of the application of this method to real world samples. All these samples were stored in dark at 4 °C before use, and then processed and analyzed without filtration.
Table 2-1 Structures and some physico-chemical properties of the target compounds

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Chemical structure</th>
<th>Chemical structure</th>
<th>CAS number</th>
<th>Log $K_{ow}$</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH</td>
<td><img src="image1" alt="BH structure" /></td>
<td>C_{13}H_{12}O</td>
<td>91-01-0</td>
<td>2.71</td>
<td>13.5</td>
</tr>
<tr>
<td>BP</td>
<td><img src="image2" alt="BP structure" /></td>
<td>C_{13}H_{10}O</td>
<td>119-61-9</td>
<td>3.38</td>
<td>-</td>
</tr>
<tr>
<td>EHS</td>
<td><img src="image3" alt="EHS structure" /></td>
<td>C_{15}H_{22}O_3</td>
<td>118-60-5</td>
<td>5.97</td>
<td>8.13</td>
</tr>
<tr>
<td>HMS</td>
<td><img src="image4" alt="HMS structure" /></td>
<td>C_{16}H_{22}O_3</td>
<td>118-56-9</td>
<td>6.16</td>
<td>8.09</td>
</tr>
<tr>
<td>BP-3</td>
<td><img src="image5" alt="BP-3 structure" /></td>
<td>C_{14}H_{12}O_3</td>
<td>131-57-7</td>
<td>3.79</td>
<td>7.56</td>
</tr>
<tr>
<td>BP-1</td>
<td><img src="image6" alt="BP-1 structure" /></td>
<td>C_{13}H_{10}O_3</td>
<td>131-56-6</td>
<td>2.96</td>
<td>7.53</td>
</tr>
</tbody>
</table>

$^a$ $K_{ow}$, octanol-water partition coefficient

2.2.2 Instrumentation

Analysis was carried out on a Shimadzu QP2010 (Kyoto, Japan) GC-MS system with a DB-5MS fused silica capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm) (J&W Scientific, Folsom, CA, USA). Helium was employed as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set at 280 °C. The GC oven temperature was initially held at 110 °C for 2 min and then programmed to 170 °C at 20 °C/min. Then the temperature was programmed to 210 °C at 6 °C/min. Finally it was
programmed to 290 °C at 20 °C/min and held for 4 min. The GC-MS interface was maintained at 280 °C. The solvent cut time was 8 min (to bypass the solvent peak). All injections were in splitless mode. Selective ion monitoring (SIM) mode was adopted for quantitative determination of the analytes. The masses monitored by the detector were set as follows: 8-9.19 min, m/z 256, 179, 167, 166 (BH); 9.19-10 min, m/z 182, 181, 105, 77 (BP); 12.5-13.5 min, m/z 196, 195, 151, 120 (EHS); 13.6-14 min, m/z 210, 196, 195, 193 (HMS); 14.1-14.7 min, m/z 286, 285, 242, 135 (BP-3); 14.8-15.0 min, m/z 345, 344, 343, 164, 105 (BP-1).

A vortex agitator (Scientific Industries, Bohemia, NY, USA) was used during the extraction process.

2.2.3 VADLLME procedure

An aliquot of 10 mL water sample (previously adjusted to pH 4) was placed in a 15 mL screw cap polyethylene centrifuge tube with conical bottom and 40 µL tetrachloroethene (as extraction solvent) were rapidly injected into it. The mixture was then vigorously shaken on the vortex agitator at 3200 rpm for 3 min. Fine droplets were formed during the vortex agitation process which facilitated mass transfer of the analytes from water sample to the organic solvent. Separation of the two phases automatically occurred after a short time (~1min) and the extraction solvent was left at the bottom of the aqueous solution in the form of microdrop. Twenty microliters of the microdrop were collected and then transferred to a 200-µL glass insert.
2.2.4 Derivatization step

The extract from the VADLLME procedure was dried under a gentle nitrogen gas stream, and then reconstituted in 30 µL of BSTFA. Then the glass insert with extract and BSTFA was placed into a 2.0 mL GC injection vial. The vial was sealed and shaken vigorously by vortex and then left to react for 30 min at 75 °C in an oven. After that, it was left aside for 5 min to reach room temperature before GC-MS analysis.

2.3 Results and discussion

2.3.1 Derivatization

For GC analysis, it was necessary to derivatize the low volatility UV filters to convert them into more volatile derivatives. Among the different derivatization methods (e.g., silylation, alkylation, esterification, acylation, etc.), silylation is by far the most widely used method for compounds containing labile hydrogens which exhibit tailing and low sensitivity in GC analysis, since the derivatization can be easily performed and there are a large number of silylation reagents available. Amongst various silylation reagents for the derivatization of the hydroxyl group, such as nitrogen-containing silyl ethers, trimethylsilyl ether, bis(trimethylsilyl)trifluoroacetamide, bis(trimethylsilyl)acetamide, and pentafluorophenylsilyl ether, BSTFA has been widely used because of its fast and quantitative reaction under moderate conditions [148].
A previous report [144] showed that for the derivatization of UV filters, BSTFA gave better derivatization results compared with MSTFA. Therefore, in this study, BSTFA and MSTFA were evaluated under the same derivatization conditions in order to confirm and corroborate the results of the previous work. Fifty microliters of a standard solution (10 mg/L of each analyte in methanol) were first introduced to a 200-µL glass insert. After evaporation to dryness under a gentle nitrogen gas steam, the residue was derivatized as described above, except that 50 µL derivatization agent was used to ensure full derivatization. The mixture was retrieved from the oven and then put aside for 5 min to equilibrate to room temperature. Finally, the extract (1 µL) was injected into the GC-MS system for analysis. The peak areas (data not shown) obtained for the six analytes were slightly higher with BSTFA than with MSTFA, in line with the previous report [142], BSTFA thus was selected as silylation reagent for further experiments. Fig. 2-1 shows a comparison of chromatograms of BP standard solutions (10 mg/L) without and after derivatization by BSTFA. Without derivatization (Fig. 1 (a)), BP, EHS, HMS and BP-3 could be detected, but BH and BP-1 were not detected conceivably due to their higher polarity which was not amenable to GC-MS analysis. After derivatization (Fig. 1 (b)), peaks of the modified BH and BP-1 (BH’ and BP-1’, respectively) could be detected. Moreover, the peaks of other silylated BPs were sharp and their peak intensities greatly increased, due to the further derivatization of the phenolic hydroxyl groups.
Figure 2-1 Comparison of chromatograms of UV filters obtained (a) without and (b) after derivatization at a concentration of 10 mg/L for each analyte. (BH': silyl derivative of BH; BP: non-derivatized; EHS': silyl derivative of EHS; HMS': silyl derivative of HMS; BP-3': silyl derivative of BP-3; BP-1': silyl derivative of BP-1).
2.3.2 Optimization of extraction performance

2.3.2.1 Extraction solvent

The selection of an appropriate extraction solvent is of great importance in VADLLME in order to emphasize high extraction efficiency. In this method, a suitable solvent has to meet the following requirements: (1) having a good solubility for the target analytes to ensure high enrichment; (2) immiscibility with water; (3) having a higher density than water; (4) having a relatively low boiling point to ensure fast evaporation. To select the most suitable solvent, three organic solvents, including chloroform (density 1.483 g/mL), chlorobenzene (density 1.11 g/mL) and tetrachloroethene (density 1.622 g/mL), which met the above criteria, were investigated in preliminary experiments. This was carried out by using 50 µL of each solvent in a 10 mL water sample containing 10 µg/L of each target analyte for VADLLME. However, only a very limited volume of chloroform could be retrieved after extraction. This may be due to that chloroform has a much higher solubility in water (~8.0 g/L) than tetrachloroethene (~0.15 g/L) and chlorobenzene (~0.47 g/L), even though it is relatively water immiscible. For chlorobenzene and tetrachloroethene, after extraction, an aliquot of a fixed volume (15 µL) of the analytes-enriched extract instead of the entire amount was easily retrieved while avoiding the collection of some of the aqueous phase. The extract was then reconstituted with 20 µL BSTFA and derivatized as above. Under these extraction conditions, chlorobenzene and tetrachloroethene gave comparable results (data not shown). However, chlorobenzene (~5 min) could not be separated from the aqueous sample as quickly as tetrachloroethene (~1 min), and this is most probably due to its relative lighter density. Taking all these
factors into account, tetrachloroethene was deemed to be the most suitable solvent in regard of its extraction performance and handling characteristics.

2.3.2.2 Effect of extraction solvent volume

In order to study the effect of the volume of the extraction solvent on extraction, the volume of tetrachloroethene was varied over the range from 40 to 70 µL. However, it was observed that with volumes lower than 40 µL, insufficient extract settled at the bottom of the extraction vial. Fig. 2-2 shows that extraction efficiency continuously decreased for all the analytes when higher volumes were used probably due to the dilution effect. Therefore, 40 µL tetrachloroethene was selected as the most suitable volume for subsequent experiments.

![Figure 2-2: Effect of organic solvent volume on extraction (extraction conditions: sample volume, 10 mL; vortex time, 3 min; extraction solvent, tetrachloroethene).](image_url)
2.3.2.3 Vortex time

The role of vortexing was to disperse the organic solvent into the aqueous phase which was dependent on both the rotational speed and vortex time. To achieve the best dispersion of the extraction solvent, the maximum speed setting of the vortex agitator (3200 rpm) was applied in all experiments. Thus, the effect of different vortex durations within a range of 0.5-5 min on the extraction efficiency was studied at a rotational speed at 3200 rpm. The results (Fig. 2-3) show that by increasing the vortex time, the extraction efficiency increased, reaching the maximum value at 3 min, and remaining constant after that. This indicated that the transfer of the analytes from the sample solution to the organic solvent was fast because of the fine dispersal drops of extractant created by vortex agitation. In this way, equilibrium was achieved within a few minutes. A vortex time of 3 min was thus most suitable. A comparison of the time to equilibrium of the proposed method against some other reported microextraction methods for the extraction of UV filters from water samples [78, 134] showed that the present method was very fast (within 3 min).

![Figure 2-3](image.png)

**Figure 2-3** Effect of vortex time on the preconcentration of UV filters (extraction conditions: sample volume, 10 mL; extraction solvent, 40 µL tetrachloroethene).
2.3.2.4 Effect of the salt

The influence of the ionic strength of the water sample on the performance of VADLLME was investigated by adding different amounts of NaCl (0-200 g/L) into water samples while the other conditions were kept constant during extraction. The salt effect on LPME and SPME has been widely reported. Salt addition to water sample may have several different effects on extraction (salting-out, salting-in or no effect). Usually, depending on the solubility of the target analytes, adding salt to water sample enhances extraction of the relatively more polar analytes. In our study, a slight increase in extraction efficiency was observed for BH and BP-1 when the NaCl concentration was increased from 0 g/L to 50 g/L (as shown in Fig. 2-4) which might be due to their relative higher polarity. For the other analytes no change was observed, indicating salt addition had little influence on the extraction performance. Thus, as a compromise and for operational convenience, no salt was added in subsequent experiments.

![Figure 2-4](image)

**Figure 2-4** Effect of salt addition on extraction (extraction conditions: sample volume, 10 mL; extraction solvent, 40 µL tetrachloroethene; vortex time, 3 min).
2.3.2.5 Effect of the pH

Since the target compounds are weak acids with low ionization constants (BP-1 (pKₐ = 7.53), BP-3 (pKₐ = 7.56), HMS (pKₐ =8.09) , EHS (pKₐ =8.13), BH (pKₐ =13.5)) [138, 139], the pH of the aqueous sample is an important parameter in extraction because it determines the existing state of the analytes. The analytes can be better extracted by organic solvent when they are in their molecular forms. Therefore, the influence of the pH on the extraction efficiency was investigated in the range of 2.0 to 6.0 by adding hydrochloric acid to the water sample. Fig. 2-5 depicts the influence of the pH of the sample solution on the extraction efficiency. The extraction efficiency was enhanced when the pH increased from 2.0 to 4.0, and then decreased slightly when the pH was further increased to 6.0. This could be explained that a lower pH, the analytes probably existed in their neutral forms, which was beneficial for their distribution into the organic phase. At higher pH values, the analytes underwent ionization and/or hydrolysis, resulting in decreased extraction. Since these analytes are weak acids, they undergo ionization in water. Under acidic conditions, the ionization is suppressed, leading to better extraction. At higher pH, the analytes are ionized (loss of their protons), leading to lower extraction. From the Fig. 2-5 we can see that, the extraction efficiency for BP-3, EHS and HMS was significantly increased with the pH increased from 3 to 4. This can be explained by their lower pKₐ values; they were probably existed in their neutral form when pH was 4. For BH and BP no much change was observed, not surprisingly since they are relatively neutral and hydrophobic. For BP-1, its pKₐ is comparable with BP-3, EHS and HMS, but only slight extraction efficiency increase was achieved when the pH increased from 3 to 4.
This may be due to its less hydrophobic properties. Therefore, pH 4 was considered most favorable for extraction.

**Figure 2-5** Effect of sample pH on extraction (extraction conditions: sample volume, 10 mL; extraction solvent 40 µL tetrachloroethene; vortex time, 3 min; salt concentration, 0 g/L).

2.3.3 Final considerations

The obtained volume of the extractant after extraction was ca. 20 µL, and this entire amount was retrieved for the following derivatization step in the following experiments to ensure good sensitivity. The most suitable amount of BSTFA for the derivatization in the range of 20-50 µL on VADLLME was studied. The results (data not shown) indicated that the derivatization efficiencies were better when 30 µL of BSTFA was used. Excessive amount (>30 µL) of BSTFA caused poor GC resolution of the analytes and low precision of the analysis. Fig. 2-6 shows a chromatogram of spiked ultrapure water sample (10 µg/L of each analyte) after extraction with derivatization by the developed method under the optimum conditions.
2.3.4 Method validation

A series of experiments with regard to the linearity, repeatability, LODs, limits of quantification (LOQs) and EFs were performed to validate the proposed method at the developed working conditions. The results are listed in Table 2-2. Depending on the compounds, calibration curves gave satisfactory linearity in the range of 0.1-10 µg/L and 0.05-10 µg/L with correlation coefficients ranging between 0.9983 and 0.9999 for all the analytes, indicating the method could be used for the determination of UV filters at trace level concentration. The LODs for the analytes, calculated at a signal-to-noise (S/N) ratio of 3, ranged from 8.0 to 45.0 ng/L. The LOQs, calculated at S/N=10, were from 20.0 to 100.0 ng/L. The results are comparable with those from a previous report, where DLLME was used for analysis of the UV filters [144]. Compared with DLLME, VADLLME affords advantages such as the avoidance of the need of a dispersive solvent and centrifugation, which greatly simplifies the operation of procedure.

Figure 2-6 Chromatogram of spiked ultrapure water sample extract under the most favorable extraction conditions.
The repeatability of the method, expressed as RSD, was studied for five replicate experiments by spiking ultrapure water with UV filters at low concentrations. The RSDs for the UV filters were below 12.9%, illustrating satisfactory repeatability was achieved by the proposed method.

The EFs for all the analytes, which were obtained by comparing the calibration graphs before and after the extraction process, were in the range from 310 (for BP) and 51 (for BH). These values highlight the good extraction performance of the new technique.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (µg/L)</th>
<th>r²</th>
<th>RSDa (%) (n=5)</th>
<th>LOD (ng/L)</th>
<th>LOQ (ng/L)</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH</td>
<td>0.1-10</td>
<td>0.9996</td>
<td>12.9</td>
<td>45</td>
<td>100</td>
<td>51</td>
</tr>
<tr>
<td>BP</td>
<td>0.05-10</td>
<td>0.9987</td>
<td>12.1</td>
<td>8</td>
<td>20</td>
<td>310</td>
</tr>
<tr>
<td>EHS</td>
<td>0.1-10</td>
<td>0.9983</td>
<td>11.4</td>
<td>22</td>
<td>75</td>
<td>171</td>
</tr>
<tr>
<td>HMS</td>
<td>0.1-10</td>
<td>0.9984</td>
<td>11.6</td>
<td>30</td>
<td>75</td>
<td>133</td>
</tr>
<tr>
<td>BP-3</td>
<td>0.05-10</td>
<td>0.9999</td>
<td>6.1</td>
<td>17</td>
<td>50</td>
<td>152</td>
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<tr>
<td>BP-1</td>
<td>0.05-10</td>
<td>0.9992</td>
<td>10.1</td>
<td>20</td>
<td>50</td>
<td>53</td>
</tr>
</tbody>
</table>

a RSD: five replicate analysis of a standard solution containing 0.5 µg/L of each analyte

2.3.5 Analysis of real samples

The proposed method was applied to analyze two environmental water samples collected from a local river and reservoir under the most favorable extraction conditions. The results showed that the concentration of the target analytes was found to be below the LOD of the proposed method. Therefore, all the water samples were fortified with the target analytes to study matrix effects on the extraction efficiency. The performance of VADLLME in spiked river and reservoir waters is shown in Table 2-3. As can be seen, for the two
matrices, the relative recoveries of the UV filters from river and reservoir water ranged from 76.5% to 120.0%. These results showed that the matrix types had a slight effect on the extraction, but the overall analysis was not significantly affected.

### Table 2-3 Analytical results and recoveries obtained from analysis of spiked genuine water samples by the proposed method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>River water Added (µg/L)</th>
<th>RR&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>RSD (%) (n=3)</th>
<th>Reservoir water Added (µg/L)</th>
<th>RR (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH</td>
<td>0.5</td>
<td>111.2</td>
<td>10.9</td>
<td>0.5</td>
<td>112.4</td>
<td>13.8</td>
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<td></td>
<td>0.25</td>
<td>82.5</td>
<td>6.3</td>
<td>0.25</td>
<td>79.0</td>
<td>14.8</td>
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<tr>
<td>BP</td>
<td>0.5</td>
<td>120.0</td>
<td>9.1</td>
<td>0.5</td>
<td>100.5</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>118.5</td>
<td>9.1</td>
<td>0.25</td>
<td>71.0</td>
<td>13.0</td>
</tr>
<tr>
<td>EHS</td>
<td>0.5</td>
<td>93.8</td>
<td>13.3</td>
<td>0.5</td>
<td>83.7</td>
<td>12.1</td>
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<tr>
<td></td>
<td>0.25</td>
<td>97.9</td>
<td>14.4</td>
<td>0.25</td>
<td>90.7</td>
<td>12.9</td>
</tr>
<tr>
<td>HMS</td>
<td>0.5</td>
<td>78.1</td>
<td>11.2</td>
<td>0.5</td>
<td>78.1</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>104.7</td>
<td>12.7</td>
<td>0.25</td>
<td>76.5</td>
<td>4.1</td>
</tr>
<tr>
<td>BP-3</td>
<td>0.5</td>
<td>86.1</td>
<td>13.3</td>
<td>0.5</td>
<td>77.9</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>110.4</td>
<td>5.1</td>
<td>0.25</td>
<td>76.1</td>
<td>15.8</td>
</tr>
<tr>
<td>BP-1</td>
<td>0.5</td>
<td>108.1</td>
<td>3.9</td>
<td>0.5</td>
<td>87.7</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>104.0</td>
<td>5.8</td>
<td>0.25</td>
<td>88.5</td>
<td>11.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Defined as the ratio of the GC peak areas of spiked real water extracts and the peak areas of spiked ultrapure water extracts at the same concentration.

2.3.6 Comparison of VADLLME with other sample preparation techniques

Comparisons between the present VADLLME method and other sample preparation techniques such as DLLME [144], IL-DLLME [145], sorptive extraction followed by solvent desorption (SE-SD) [141], dispersive solid-phase extraction with magnetic nanoparticles (dSPE-MNPs) [149] and magnetic stirring-assisted dispersive liquid-liquid microextraction (MSA-DLLME) [80] from the viewpoint of linearity, LOQs, RSDs, EFs and extraction time are shown in Table 2-4. It can be observed that the analytical performance (linearity, LOQs, and RSDs) of the present VADLLME-GC-MS
is comparable with other reported microextraction methods coupled with GC-MS for the determination of the UV filters and much better than that coupled with high performance liquid chromatography and UV detection (HPLC-UV). In comparison with IL-DLLME, SE-SD, dSPE-MNPs and MSA-DLLME, the extraction time for VADLLME is greatly shortened since the extraction can reach equilibrium very rapidly due to the large surface area between the organic droplets and the aqueous sample solution. Compared with DLLME, no centrifugation and dispersive solvent were required in VADLLME, which was simple, economical and environmentally friendly. Moreover, high extraction efficiency could be achieved. Furthermore, the present technique provided higher EF in comparison with most of the other methods except dSPE-MNPs. Although dSPE-MNPs gave much higher EF [149], a much larger aqueous sample volume of 75 mL was used, while for the present method, only 10 mL was needed. All these results indicate that VADLLME is a fast, environmentally friendly, highly efficient, reproducible and simple technique for the enrichment and determination of UV filters in water samples.
Table 2-4 Comparison of the proposed VADLLME-GC-MS method with other methods for the determination of UV filters

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample volume (mL)</th>
<th>Linearity (µg/L)</th>
<th>LOQ (ng/L)</th>
<th>Extraction time (min)</th>
<th>RSD (%)</th>
<th>RR (%)</th>
<th>EF</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLLME-GC-MS</td>
<td>5</td>
<td>0.2-1</td>
<td>108-168</td>
<td>-</td>
<td>5.6-6.2</td>
<td>65-222</td>
<td>58-65</td>
<td>[144]</td>
</tr>
<tr>
<td>IL-DLLME-HPLC-UV</td>
<td>10</td>
<td>10-1000</td>
<td>-</td>
<td>14</td>
<td>4.1-8.0</td>
<td>-</td>
<td>18.9-26.8</td>
<td>[145]</td>
</tr>
<tr>
<td>SE-SD-LVI&lt;sup&gt;a&lt;/sup&gt;-GC-MS</td>
<td>100</td>
<td>0.01-10</td>
<td>3-40</td>
<td>840</td>
<td>1.0-9.9</td>
<td>49-108</td>
<td>19-102</td>
<td>[143]</td>
</tr>
<tr>
<td>dSPE-MNPs&lt;sup&gt;b&lt;/sup&gt;-GC-MS</td>
<td>75</td>
<td>0.02-1</td>
<td>0.5-20</td>
<td>13</td>
<td>5.6-16</td>
<td>63-125</td>
<td>453-748</td>
<td>[149]</td>
</tr>
<tr>
<td>MSA-DLLME&lt;sup&gt;c&lt;/sup&gt;-HPLC-UV</td>
<td>-</td>
<td>5-20000</td>
<td>-</td>
<td>25</td>
<td>1.4-4.8</td>
<td>91.3-97.1</td>
<td>59-107</td>
<td>[80]</td>
</tr>
<tr>
<td>VADLLME-GC-MS</td>
<td>10</td>
<td>0.05-10</td>
<td>20-100</td>
<td>~4</td>
<td>6.1-12.9</td>
<td>76.5-120</td>
<td>51-310</td>
<td>This method</td>
</tr>
</tbody>
</table>

<sup>a</sup> SE-SD-LVI = sorptive extraction followed by solvent desorption with large volume injection  
<sup>b</sup> dSPE-MNPs = dispersive solid-phase extraction with magnetic nanoparticles  
<sup>c</sup> MSA-DLLME = magnetic stirring-assisted dispersive liquid-liquid microextraction
2.4 Conclusion

In the present study, a fast and simple method termed as vortex-assisted dispersive liquid-liquid microextraction (VADLLME) has been developed for the extraction of benzophenone-type UV filters from water samples. The extraction solvent could be rapidly retrieved in the centrifuge tube after letting it stand for a short time, without need of centrifugation. The proposed microextraction method has advantages such as economical, speed, low LOQs, good repeatability and simplicity in experimental set-up. The important benefit of this approach is the avoidance of the centrifugation step. In addition, no dispersive solvent is applied; thus high extraction efficiency is achieved. Furthermore, this method opens up a potentially new horizon for on-site DLLME. If portable agitator is available or high agitation can be achieved manually, on-site DLLME is easily accessible. Combined with portable GC-MS, on-site sample analysis is also achievable. The performance of the proposed method in the extraction of UV filters from spiked genuine water samples was acceptable, which also demonstrates the feasibility of the proposed method for environmental aqueous sample analysis.
Chapter 3 Ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction of ultraviolet filters from environmental water samples

3.1 Introduction

Recently, to avoid the use of the large amount of dispersive solvent (up to several hundred microliters) that is often applied in DLLME, USAEME [150] was developed. USAEME is based on the emulsification of a microvolume of water-immiscible extraction solvent in the sample aqueous solution by ultrasound radiation which accelerates the mass-transfer process of the analytes between the two immiscible phases. Ultrasound radiation is a powerful aid in the acceleration of emulsion forming and mass transferring between immiscible phases, thus high extraction efficiency and extraction equilibrium in a very short time could be obtained. Up to now, USAEME have been successfully applied to the extraction and preconcentration of PAH [107], triclosan, triclocarban [110], pyrethroid pesticides [116], OPPs [129] and OCPs [151] in aqueous samples.

ILs, which are composed of organic cations and organic or inorganic anions, are liquids near room temperature (or by convention below 100 °C). ILs provide desirable characteristics for the different combination of a great variety of cations and anions of different sizes. They are relatively thermally-stable and provide no detectable vapor pressure, thus avoiding environmental and safety problems due to volatilization. Therefore, ILs are regarded as “green solvents”. ILs are now widely used as replacement solvents in sample
preparation, due to their unique physical and chemical properties such as non-flammability, negligible vapour pressure, good extractability for a wide spectrum of inorganic, organic and organometallic compounds, as well as tunable viscosity and miscibility with water and organic solvents. For example, ILs have been investigated as extraction solvents for SDME [152], HF-LPME [153] and DLLME [154]. They could replace the commonly used highly toxic chlorinated extraction solvents, with direct extract injection into HPLC systems for analysis.

In order to avoid the use of highly toxic chlorinated extraction solvents in not only traditional DLLME but also our previous work (VADLLME), the aim of the present study reported in this chapter was to develop a new method named IL-USA-DLLME to combine the merits of ILs, DLLME and USAEME for the extraction and determination of four UV filters in environmental water samples. A simple, environmentally friendly and rapid IL-USA-DLLME procedure using [HMIM][FAP] as the IL extraction solvent and only a small amount of methanol as dispersive solvent was first conducted. Then an ultrasound-assisted process was applied to accelerate the extraction and reduce equilibrium time. Various factors affecting extraction efficiency (such as type and volume of extraction and dispersive solvent, ionic strength, pH and extraction time) were evaluated and optimized.

3.2 Experimental

3.2.1 Reagents and materials
1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF$_6$]) and butyltrimethylammonium bis(trifluoromethanesulfonyl)imide ([BTMA][NTf$_2$]) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 1-butyl-1-methylpyrrolidinium tris(pentafluoroethyl)trifluorophosphate ([BMPL][FAP]) and 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ([HMIM][FAP]) were purchased from Merck (Darmstadt, Germany). The four UV filters BP (purity 99%), BP-3 (purity 98+%), EHS (purity 99%) and HMS (purity 99%) were purchased from Alfa Aesar (Karlsruhe, Germany). Their structures and relevant physico-chemical properties are given in Table 2-1.

Concentrated hydrochloric acid, bought from Merck (Darmstadt, Germany) was used to adjust the pH of the water sample and was diluted to 0.1 M before use. HPLC–grade methanol, acetonitrile, acetone and ethanol were purchased from Tedia Company (Fairfield, OH, USA). Sodium chloride (NaCl) was from Goodrich Chemical Enterprise (Singapore). Ultrapure water, used in all experiments, was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water purification system.

Another stock solution containing four UV filters (BP, BP-3, EHS and HMS) (50 mg/L each) was prepared in acetonitrile. The four UV filter water samples were prepared by spiking ultrapure water with analytes at known concentration (50 µg/L) daily to study the IL-USA-DLLME performance under different conditions. Tap, river and swimming pool water samples were used for evaluation of the application of this method to real world samples. Tap water samples were freshly collected from our laboratory. River and
swimming pool water were collected in amber glass containers and stored in the dark at 4 °C until use. All these samples were analyzed unfiltered.

3.2.2 Instrumentation

Separation of UV filters was performed on a Waters (Milford, MA, USA) HPLC system which consisted of a Rheodyne (Cotati, CA, USA) 7725i injector equipped with a 20- µL sample loop, a Waters 1525 µ binary pump, and a Waters 2487 Dual λ Absorbance Detector. Data was collected and processed by Empower version 5.0 (Waters) data analysis software.

A Phenomenex (Torrance, CA, USA) BDS RP-C<sub>18</sub> column (250 mm × 4.6 mm, particle size 5 µm) was used for separations. The HPLC conditions for the separation of the studied UV filters were optimized. The mobile phase consisted of acetonitrile and water, and was applied in gradient mode. The separation gradient was started with 70% acetonitrile and held for 9 min, followed by a linear increase to 100% in 6 min at a flow rate of 1.0 mL/min. After 5 min at 100%, the gradient was reversed to the initial condition in 5 min and equilibrated for an additional 5 min before the next sample was injected. Injection volume was 10 µL for every analysis. BP-3, EHS and HMS were detected at 305 nm and BP at 254 nm.

A 35 kHz and 0.32 kW ultrasonic water bath (Ultrasonic LC 30, Germany) was used in this work.
3.2.3 Ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME) procedure

Fig. 3-1 shows the IL-USA-DLLME procedure. Ten milliliters of water sample (pre-adjusted to pH 4) were placed in a 15 mL screw cap polyethylene centrifuge tube and then a mixture of 20 µL of extraction solvent ([HMIM][FAP]) and 0.1 mL of dispersive solvent (methanol) was injected rapidly into the sample. The mixture was gently shaken for several seconds and then immersed into an ultrasonic water bath for extraction. The extractions were performed at 35 kHz of ultrasound frequency and 320 W for 3 min at room temperature to form the cloudy state, which contained fine droplets of extraction solvent dispersing entirely in the aqueous phase. Phase separation was performed by a centrifugation immediately at 4000 rpm for 5 min. The sedimented phase (~17 µL) was retrieved and 10 µL was injected into the HPLC system.

![IL-USA-DLLME procedure](image)

**Figure 3-1** The IL-USA-DLLME procedure.
3.3 Results and discussion

3.3.1 Optimization of IL-USA-DLLME

3.3.1.1 Effect of type and volume of the extraction solvents

Preliminary experiments were conducted with the aim of selecting the best extraction solvent for the preconcentration of the analytes. The extraction solvent in USA-DLLME has to meet several requirements: low volatility, low water solubility, good chromatographic behavior, higher density than water and high extraction capability of organic compounds. Most of the halogenated solvents applied in DLLME possess all of above properties, but these solvents are highly toxic and their use is not desirable. ILs represent superior and efficient alternatives for conventional organic halogenated solvents. In this study, taking into account all these properties, four hydrophobic ILs, including [HMIM][PF$_6$], [BTMA][NTf$_2$], [BMPL][FAP] and [HMIM][FAP] were examined. The densities of the selected ILs are 1.29 g/mL, 1.41 g/mL, 1.60 g/mL and 1.56 g/mL at room temperature. A series of sample solutions using 0.5 mL of acetone as the dispersive solvent, and with different volumes of extraction solvent to obtain 35 µL of the sedimented phase were studied; accordingly, 100, 108, 41 and 40 µL of [HMIM][PF$_6$], [BTMA][NTf$_2$], [BMPL][FAP] and [HMIM][FAP], respectively, were selected. The extraction efficiencies for different ILs are shown in Fig. 3-2. The results revealed that there were no significant difference among the extractions obtained by different ILs and all the ILs showed good enrichment for all the analytes. This may be due to their good solubilities and extraction capabilities towards the target analytes. The main interaction takes place through π-π interaction
between the aromatic part of the analytes and the imidazolium ring, as well as hydrophobic interaction between the aromatic part of the analytes and the long hydrocarbon chain form the ILs. However, compared with [HMIM][PF₆] and [BTMA][BF₄], the water solubilities of [BMPL][FAP] and [HMIM][FAP] are much lower such that much less of these solvents were consumed to result in the same volume (35 µL) of extract. Considering the lower viscosity of [HMIM][FAP] compared with [BMPL][FAP] [155], and for more convenient handling (a high viscosity solvent is difficult to retrieve by a microsyringe), [HMIM][FAP] was selected as extraction solvent in subsequent experiments.

The volume of extraction solvent was another crucial parameter that could affect the extraction efficiency. To examine the effect of the extraction solvent volume, solutions containing different volumes of IL were subjected to the same USA-DLLME procedure. Thus, the effect of IL volume on the extraction efficiency was studied by using 0.5 mL of acetone containing different volumes (20, 30, 40, 50 and 60 µL) of [HMIM][FAP]. The volume of [HMIM][FAP] determined the extent of the cloudy state of the sample solution, and thus the efficiency of extraction. When the volume of the extraction solvent was less than 20 µL, the amount of the sedimented phase was too small to be removed by a microsyringe and was insufficient for the HPLC analysis. Fig. 3-3 depicts the extraction efficiency versus volume of extraction solvent for the target analytes. As can be seen, the peak areas decreased with the increasing volume of extraction solvent from 20 µL to 60 µL. Based on LLE principle, the extraction efficiency is directly related to the interfacial area between the sample solution and the extraction solvent and
inversely related to the organic phase volume. Therefore, by increasing the drop volume, the effect of the interfacial area predominates first and then solvent volume, and the analytical signals will increase to a point and decreases thereafter. Twenty microliter might give the highest extraction efficiency and dilution effect occurred when the [HMIM][FAP] increased to 60 µL. Consequently, 20 µL was used as the optimum quantity for the extraction in further experiments since the highest extraction efficiency was obtained at this condition, and the reproducibility was also acceptable.

Figure 3-2 Effect of extraction solvent on extraction.
3.3.1.2 Effect of type and volume of the dispersive solvent

The extraction solvent will disperse as very fine droplets when rapidly injected with a dispersive solvent into the aqueous sample. The addition of dispersive solvent decreases the interfacial tension between the two phases and accelerates the formation of droplets in water samples, correspondingly increasing the surface area for the extraction of the target analytes [156]. To form a fine dispersive phase for the extraction, a suitable dispersive solvent is required. Therefore, methanol, ethanol, acetone and acetonitrile as dispersive solvents on the extraction efficiency were investigated by applying 0.5 mL of each solvent and 20 µL of [HMIM][FAP] under the same extraction conditions. All these results were compared with extractions under the same conditions but without the use of dispersive solvent. According to the results in Fig. 3-4, extraction efficiency was significantly enhanced by the use of dispersive solvent, which served to facilitate mass transfer of the analytes to
the organic extraction solvent. The highest extraction efficiency was obtained by using methanol as dispersive solvent.

![Effect of dispersive solvent on extraction](image)

**Figure 3-4** Effect of dispersive solvent on extraction.

In DLLME, the volume of dispersive solvent is another important parameter to be considered. At lower dispersive solvent volume, tiny droplet formation may not be effective thereby lowering the extraction efficiency. However, at higher dispersive solvent volume, the solubility of the analytes increases in the aqueous phase leading to a decrease in extraction efficiency [157]. On the one hand, it is expected that the dispersive solvent volume should be as low as possible to achieve the highest extraction efficiency; on the other hand, it should be sufficient to disperse effectively the extraction solvent to fine droplets satisfactorily. Therefore, different volumes of methanol (0.1, 0.3, 0.5, 0.8 and 1.0 mL) were tested to obtain the best results in terms of extraction efficiency. As can be seen in Fig. 3-5, the results indicated that the extraction efficiency decreased with the increase of methanol volume from 0.1 mL to 1.0
mL for the reason mentioned above. Therefore, 0.1 mL methanol was applied for the further work.

Figure 3-5 Effect of dispersive solvent volume on extraction.

3.3.1.3 Effect of the salt

The salt effect in DLLME has been widely studied and salting-out (increasing the extraction efficiency by the addition of salts), salting-in (decreasing the extraction efficiency), or no effect has all been reported. The influence of the ionic strength on the performance of IL-USA-DLLME was investigated by adding different amounts of NaCl (0 to 200 g/L) into water samples while the other conditions were kept constant. The results are presented in Fig. 3-6. It can be observed that there is no significant peaks increase with the addition of salt. Therefore, salt addition was deemed to be unnecessary in this work.
3.3.1.4 Effect of the pH

Since the target compounds, except for BP, are weak acids with low ionization constants (BP-3 ($pK_a = 7.56$), HMS ($pK_a = 8.09$), EHS ($pK_a = 8.13$)) [138], the pH of the sample solution is of great influence on the extraction because it determines the ionic state of the analytes upon which extraction is dependent. The influence of the pH on the extraction efficiency was investigated in a range of 2.0 to 6.0. The results are displayed in Fig. 3-7. It can be observed that as the pH increased from 2.0 to 4.0, the peak areas for all the analytes increased accordingly. When the pH was further increased to 6.0, the peak areas decreased slightly. This could be explained that at a lower pH, the analytes probably existed in their neutral forms, which was beneficial for their distribution into the organic phase. The highest extraction efficiency was found to be at pH 4. At higher pH values the analytes were ionized; this
reduced their migration into the extraction solvent. Therefore, pH 4 was considered optimal for extraction.

**Figure 3-7** Effect of pH on extraction.

### 3.3.1.5 Effect of ultrasonic time

Compared with commonly used traditional organic solvents, [HMIM][FAP] is relatively viscous, which is problematical for the mass transfer of the analytes through the interfaces. In IL-USA-DLLME, the interface between the sample solution and the extraction solvent was significantly enlarged by the formation of the cloudy emulsion. An ultrasound-assisted process can promote fine droplets of extraction solvent and accelerate the formation of cloudy solution. However, there are disadvantages in prolonging ultrasonication time, such as wear and tear of equipment, loss of extraction solvent and analytes, and the tendency of decline in ultrasonic power [144]. Thus, the addition of dispersive solvent to reduce the ultrasonication time to an optimum value is
desirable. Different ultrasonication times ranging from 0 to 5.0 min were investigated. The results (Fig. 3-8) show that the analyte peak areas increased from 0 to 3.0 min, then only changed slightly when ultrasonication continued until 5 min. This might be due to the maximum amount of fine droplets of the extraction solvent being formed by ultrasonic vibration within the first 3 min, and this did not increase further even at increased ultrasonication time. Therefore, 3.0 min was set as the optimum ultrasonication time.

![Figure 3-8 Effect of ultrasonication time on extraction.](image)

3.3.2 Method validation

A series of experiments with regard to the linearity, precision, accuracy and sensitivity were performed to validate the proposed method at the developed working conditions. The results are listed in Table 3-1. The calibration graph for each analyte was constructed by extracting seven standard solutions in triplicate containing all the analytes at concentrations ranging from 1 µg/L to
500 µg/L. The calibration curve was linear in the range 1-500 µg/L for BP, 5-500 µg/L for BP-3 and HMS, 10-500 µg/L for EHS. The calculated calibration curves gave a high level of linearity, yielding coefficients of estimation of ($r^2$) 0.9996, 0.9996, 0.9946 and 0.9946 for BP, BP-3, EHS and HMS, respectively. The LODs for the analytes, calculated at a S/N ratio of 3, ranged from 0.2 to 5.0 µg/L. The LOQs, calculated at S/N=10, were from 0.5 to 10 µg/L.

The repeatability of the method, expressed as RSD, was evaluated for five replicate experiments with spiked ultrapure water with UV filters at concentrations of 50 µg/L of each UV filter. The RSDs were below 6.3%, illustrating satisfactory repeatability. The EFs for the four UV filters ranged from 354- to 464-fold.

**Table 3-1** Quantitative results of the proposed IL-USA-DLLME method

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (µg/L)</th>
<th>$r^2$</th>
<th>RSD(^a) (%) (n=5)</th>
<th>LOD (µg/L)</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>1-500</td>
<td>0.9996</td>
<td>4.0</td>
<td>0.2</td>
<td>464</td>
</tr>
<tr>
<td>BP-3</td>
<td>5-500</td>
<td>0.9996</td>
<td>5.5</td>
<td>0.5</td>
<td>412</td>
</tr>
<tr>
<td>EHS</td>
<td>10-500</td>
<td>0.9946</td>
<td>6.3</td>
<td>5.0</td>
<td>354</td>
</tr>
<tr>
<td>HMS</td>
<td>5-500</td>
<td>0.9946</td>
<td>5.4</td>
<td>1.0</td>
<td>400</td>
</tr>
</tbody>
</table>

\(^a\) %RSD: five replicate analysis of a standard solution containing 50 µg/L of each analyte

3.3.3 Analysis of real samples

The current method was applied to the determination of the four UV filters in swimming pool, tap and river water under the optimum conditions. However, none of the target analytes was detected in all the three matrices, indicating the analytes were not present or were below the LODs of this
method. Therefore, all the water samples were fortified with the target analytes at two concentration levels (30 µg/L and 50 µg/L) to study matrix effects on the extraction recovery. A typical chromatogram of a spiked river water sample (50 µg/L of each analyte) obtained after IL-USA-DLLME is shown in Fig. 3-9. The performance of IL-USA-DLLME in spiked real water samples is shown in Table 3-2. As can be seen, the RRs for the four UV filters from the three different matrices ranged from 81 to 117% for tap water, 81 to 118% for river water and 71 to 117% for swimming pool water. The results suggest that the matrix has little, if any, significant effects on the proposed method for the various types of samples. The proposed method could be an effective sample preparation method for the determination of UV filters in real water samples.

Figure 3-9 HPLC trace of extract of spiked river water sample (50 µg/L of each analyte) under the most favorable IL-USA-DLLME conditions. (A) Detection wavelength at 254 nm and (B) detection wavelength at 305 nm. Peaks: (1) [HMIM][FAP]; (2) BP; (3) BP-3; (4) EHS; (5) HMS.
Table 3-2 Analytical results and recoveries obtained from analysis of real water samples spiked with the UV filters by the proposed method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Tap water</th>
<th>River water</th>
<th>Swimming pool water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 µg/L spiking level</td>
<td>50 µg/L spiking level</td>
<td>30 µg/L spiking level</td>
</tr>
<tr>
<td>BP</td>
<td>117</td>
<td>91</td>
<td>118</td>
</tr>
<tr>
<td>BP-3</td>
<td>105</td>
<td>82</td>
<td>114</td>
</tr>
<tr>
<td>EHS</td>
<td>93</td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td>HMS</td>
<td>87</td>
<td>81</td>
<td>81</td>
</tr>
</tbody>
</table>

*Defined as the ratio of the peak area of an analyte in real water sample and the peak area of the analyte in ultrapure water sample spiked at the same concentration.*

3.3.4 Comparison of IL-USA-DLLME with other sample preparation techniques

Comparisons between the present IL-USA-DLLME method and other sample preparation techniques such as CPE, SDME, DLLME and MSA-DLLME from the viewpoint of linearity, LOD, RSDs, EFs and extraction time are shown in Table 3-3. It can be observed that the analytical performance of the present IL-USA-DLLME-HPLC method is comparable with the other reported microextraction methods coupled with HPLC for the determination of the UV filters. In comparison with SDME, CPE and MSA-DLLME, the extraction time for IL-USA-DLLME is greatly shortened since the extraction can reach equilibrium extremely quickly due to the large surface area between the organic droplets and the aqueous sample solution. Compared with conventional DLLME, an IL such as [HMIM][FAP] is generally considered to be more environmentally benign than volatile organic solvents. Furthermore, less dispersive solvent was needed in IL-USA-DLLME, thus resulting in higher EFs than those obtained with all the other reported extraction methods.
<table>
<thead>
<tr>
<th>Method</th>
<th>Linearity (µg/L)</th>
<th>LOD (µg/L)</th>
<th>Extraction time (min)</th>
<th>RSD (%)</th>
<th>RR (%)</th>
<th>EF</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPE-HPLC-UV</td>
<td>0.5-30</td>
<td>0.14-1.27</td>
<td>30</td>
<td>3.9-5.2</td>
<td>98.5-102</td>
<td>50-80</td>
<td>[136]</td>
</tr>
<tr>
<td>DLLME-HPLC-UV</td>
<td>10-1000</td>
<td>1.9-6.4</td>
<td>-</td>
<td>4.1-8.0</td>
<td>-</td>
<td>21.7-26.8</td>
<td>[145]</td>
</tr>
<tr>
<td>MSA-DLLME-HPLC-UV</td>
<td>5-20000</td>
<td>0.2-0.8</td>
<td>25</td>
<td>1.4-4.8</td>
<td>91.3-97.1</td>
<td>59-107</td>
<td>[80]</td>
</tr>
<tr>
<td>SDME-HPLC-UV</td>
<td>1-300</td>
<td>0.06-3.0</td>
<td>37</td>
<td>2.8-7.9</td>
<td>92-115</td>
<td>8-98</td>
<td>[141]</td>
</tr>
<tr>
<td>DLLME-GC-MS</td>
<td>0.2-50</td>
<td>0.032-0.05</td>
<td>&gt;30</td>
<td>5.6-6.2</td>
<td>65-222</td>
<td>58-64</td>
<td>[144]</td>
</tr>
<tr>
<td>IL-USA-DLLME-UV</td>
<td>1-500</td>
<td>0.2-5.0</td>
<td>3</td>
<td>4.0-6.3</td>
<td>71-118</td>
<td>354-464</td>
<td>This method</td>
</tr>
</tbody>
</table>

Table 3-3 Comparison of the proposed IL-USA-DLLME method with other methods for determination of UV filers
3.4 Conclusion

A fast, simple and environmentally-friendly sample preparation method termed ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME) has been developed for the extraction of benzophenone-type UV filters from different types of water samples. In this procedure, an ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution using less dispersive solvent compared with conventional DLLME, which significantly increased extraction efficiency and reduced extraction time. Under the optimum conditions, the EFs for the UV filters ranged from 354- to 464- fold. The method represented here has acceptable RRs (71-118%), good repeatability (RSDs 4.0-6.3%) and a wide linear range (1 to 500 µg/L). When compared to other methods, such as CPE-HPLC-UV, SDME-HPLC-UV and DLLME-GC-MS, this new extraction method reduces the exposure to toxic solvents as used in the conventional extraction procedures, and also has much faster extraction time with high extraction efficiency. The performance of the proposed method was satisfactory in the determination of UV filters in spiked water samples from different sources. IL-USA-DLLME-HPLC-UV was demonstrated to be a simple, fast and efficient technique for the enrichment and determination of UV filters in water samples.
Chapter 4 Low-density solvent-based ultrasound-assisted dispersive liquid-liquid microextraction of organochlorine pesticides from water samples

4.1 Introduction

OCPs are some of the most persistent organic pollutants present in the environment and they are hazardous to both human health and the environment. They are believed to be potential carcinogens or mutagens as well as endocrine disruptors that may affect the normal functioning of the endocrine system. Therefore, determination and monitoring of OCPs are of great importance, the development of new and appropriate methods, for the analysis of OCPs in environment samples should continue to be encouraged.

To avoid the application of high toxic high-density organic solvents as the extraction solvent, some efforts have been made to apply low toxic low-density organic solvents [84, 115, 118, 158, 159] other than ILs. Among all these applications, to collect the final low-density extract, additional devices such as home-made glass vial [84], self-made sealing nut and self-scaled capillary tube [158], microtube [159] and additional cooling procedures [115, 118] were necessary which were complex, tedious and might cause potential carryover and cross-contamination.

In this chapter, intended to improve DLLME and our previous work to avoid using dispersive and high toxic solvent, we introduced a cheap, flexible
and disposable polyethylene Pasteur pipette as a new extraction device and proposed a polyethylene Pasteur squeeze-type pipette–based LDS-USA-DLLME for the extraction and preconcentration of OCPs from water samples with GC-MS analysis, providing the combined advantages of the polyethylene Pasteur pipette, low-density organic solvents and USAEME. For this pipette, no modification was needed, and it could be used straight from the box. This study aims to present a new extraction alternative that provides a simple and fast way to collect less toxic low-density organic extraction solvent from an extraction unit which avoids inconvenient and cumbersome procedures as mentioned above, and reported in previous studies. The present approach made use of a disposable flexible polyethylene Pasteur pipette as an extraction unit, and employed ultrasound radiation to disperse effectively the extraction solvent into the aqueous sample without using any disperser solvent. Furthermore, ultrasound radiation also facilitated the mass transfer between the aqueous and organic solvent phases, thus increasing extraction efficiency. The effects of various experimental parameters, such as the type of extraction solvent and extraction solvent volume, sonication and centrifugation time, temperature and ionic strength were investigated. Genuine water samples, including river and tap water, were analyzed to demonstrate the applicability of the proposed method.

4.2 Experimental

4.2.1 Reagents and materials
Nonane, 1-octanol and the OCP standards (hexachlorobenzene (HCB), heptachlor, p,p’-DDE, dieldrin, endrin and o,p’-DDD) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isooctane and cyclohexane were purchased from Merck (Darmstadt, Germany). HPLC-grade ethanol and methanol were obtained from Fisher (Loughborough, UK). Ultrapure water used in all experiments was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water purification system.

The soft polyethylene Pasteur pipette (ca. 8-mL capacity) is manufactured by Continental Lab Products (San Diego, CA, USA) and was purchased from Practical Mediscience Pte., Ltd. (Singapore). A 100-µL syringe used for injection of extraction solvent and a 10-µL microsyringe used for GC-MS injection were purchased from SGE (Sydney, Australia). The 10-mL plastic syringe was bought from HSW (Tuttlingen, Germany).

Stock solutions containing all analytes (at 10 mg/L of each) were prepared in methanol and stored at 4 °C before use. Water samples were prepared by spiking ultrapure water with analytes at known concentrations (25 µg/L of each) daily to study extraction performance under different conditions. River and tap water samples were used for evaluation of the method as applied to real world samples. River water sample was collected from a local river. Tap water samples were collected from our laboratory, after allowing the water to flow for about 3-4 min. Both types of water samples were stored in the dark at 4 °C before use, and then analyzed unfiltered.
4.2.2 Instrument conditions

GC-MS analysis was carried out on the Shimadzu QP2010 system. Helium was employed as the carrier gas at a flow rate of 1.8 mL/min. The injector port temperature was set at 250 °C. The GC oven temperature was initially held at 50 °C for 2 minutes and then increased to 158 °C at a rate of 36 °C/min. Finally, the temperature was increased to 293 °C at a rate of 9 °C/min. The GC-MS interface temperature was maintained at 300 °C. SIM mode was adopted for quantitative determination of the analytes. The masses monitored by the detector were: 9.0-9.7 minutes, m/z 288, 286, 284, 282, 251, 249, 247 and 216 (hexachlorobenzene); 11.4-11.7 minutes, m/z 372, 337,301, 274, 272, 270, 237 and 235 (heptachlor); 14.1-14.5 minutes, m/z 320, 318, 316, 281, 248, 246, 210 and 176 (p,p'-DDE and dieldrin); 14.6-14.9 minutes, m/z 368, 345, 319, 317, 281, 250, 209 and 196 (endrin); 15-15.2 minutes, m/z 320, 283, 237, 235, 212, 199, 165 and 151 (o,p'-DDD).

A 35 kHz and 0.32 kW ultrasonic water bath was used in this work.

4.2.3 Polyethylene Pasteur pipette–based LDS-USA-DLLME procedure [160, 161]

The schematic of the polyethylene Pasteur pipette–based LDS-USA-DLLME procedure is shown in Fig. 4-1. Briefly, an aliquot of 6 mL sample solution was placed in an 8-mL Pasteur pipette using a 10-mL plastic syringe. Thirty microliters of the extraction solvent (isooctane) were injected into the sample solution. After the injection, the pipette was placed in an ultrasonic water bath. An emulsion formed during the ultrasonication. After a 30 s
sonication at 35kHz of ultrasound frequency and 0.32 kW of power at 25 ± 2°C, the emulsion was separated into two phases by centrifugation at 4000 rpm for 5 min. The pipette bulb was then squeezed slightly, and the upper layer comprising the organic extract moved into the narrower stem of the pipette. This permitted the convenient retrieval of the extract using a 10-µL GC microsyringe. One microliter of the extract was injected into the GC-MS system for analysis. The used pipette was discarded to avoid carryover problems.

![Figure 4-1 Schematic of polyethylene Pasteur pipette-based LDS-USA-DLLME. (a). Introduction of aqueous sample and extraction solvent; (b). Ultrasonication for 30 s; (c). Phase separation after centrifugation; (d). Squeezing of the pipette bulb; (e). Collection of the extract.](image)

### 4.3 Results and discussion

#### 4.3.1 Optimization of microextraction performance

**4.3.1.1 Selection of organic solvent**

To achieve satisfactory USA-DLLME, some factors should be considered on selecting the appropriate extraction solvent. Firstly, the tested analytes
should have good solubility in it to achieve high extraction efficiency. Secondly, the solvent must have low water solubility. Additionally, it should have a low vapor pressure to prevent loss during extraction and to be compatible with GC-MS. Most reports on USA-DLLME have indicated the use of toxic denser-than-water chlorinated solvents such as carbon tetrachloride, chlorobenzene and tetrachloroethene. In this study, solvents less dense than water were tested due to their environmental friendliness and relatively less hazardous properties compared to chlorinated solvents. They included nonane, 1-octanol, isoctane, cyclohexane (their properties are listed in Table 4-1). Fig. 4-2 shows the extraction efficiency exhibited by each solvent. Fifty microliters of each solvent were used for the extraction of 25 µg/L of each OCP in 6 mL water sample under USA-DLLME with 3 min ultrasound sonication and 5 min centrifugation. Cyclohexane and isoctane gave better analytical signals. However, the former could not form a clear interface with the water sample after centrifugation, making it more difficult to retrieve. Additionally, it was observed that the cyclohexane evaporated more quickly due to its relatively higher vapor pressure. Isooctane did not have these problems and was therefore preferred for this work.

Table 4-1 Properties of extraction solvents evaluated.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Density, 20 °C (g/mL)a</th>
<th>Water solubility, 20 °C (g/L)b</th>
<th>Boiling point (°C)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonane</td>
<td>0.72</td>
<td>0.07</td>
<td>151</td>
</tr>
<tr>
<td>Octanol</td>
<td>0.82</td>
<td>0.0003</td>
<td>195</td>
</tr>
<tr>
<td>Isooctane</td>
<td>0.69</td>
<td>0.0014</td>
<td>99.3</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0.78</td>
<td>0.055</td>
<td>80.7</td>
</tr>
</tbody>
</table>

a From reference [84]
b From reference [158]
4.3.1.2 Effect of extraction solvent volume

The extraction solvent volume was also investigated for the optimization of this method. The results of the extraction of 6 mL of aqueous solution containing 25 µg/L of each OCP with 20-60 µL of isoctane under ultrasonic-assisted emulsification for 3 min and centrifugation for 5 min are shown in Fig. 4-3. The analytical signals first increased when the extraction solvent volume was increased from 20 to 30 µL, and then decreased when the volume was further increased to 60 µL; the highest extraction efficiency was obtained with 30 µL of extraction solvent. The reason for the observation may lie in the fact that a dilution effect is applicable here, that thus affected the enrichment factor, which is related to the final concentration in the extraction phase [162]. Although slight peak area increases were observed for hexachlorobenzene, heptachlor and p,p’-DDE when the solvent volume increased from 40 to 50 µL, they were within the error range. Similar observations have been reported previously [160, 163]. On the other hand, when the initial volume of
extraction solvent was less than 30 µL, it was difficult to collect the extract after extraction. Therefore, 30 µL of isoctane was considered most suitable as the extraction solvent volume.

![Figure 4-3](image_url)  

**Figure 4-3** Effect of extraction solvent volume on the preconcentration of OCPs.

4.3.1.3 Effect of extraction time

Ultrasonication affects both the emulsification and mass transfer processes, and thus influences the extraction efficiency. The extraction time was defined as the elapsed time from the beginning of the emulsification and the end of the sonication. For the present study, the extraction time was varied within the range of 30 s to 10 min. The results are shown in Fig. 4-4. From the figure we can see that there was no significant change in signal intensities in the first 3 min, but extraction efficiency decreased when the extraction time was further increased to 10 min. This indicated that the mass transfer (of the analytes from the sample solution to the organic solvent) was fast because of a cloud of fine drops of extractant created by ultrasonic radiation, which increased
significantly the contact surface between the two phases. Therefore, equilibrium was achieved in a few seconds. However, the emulsion was unstable and it would delaminate (divide into layers) in the course of prolonged extraction time, which could disturb the equilibrium and lead to lower extraction efficiency [164]. Moreover, potential analyte losses might occur over a relatively longer extraction time. To ensure complete extraction and prevent analyte loss, an extraction time of 30 s was selected. A comparison of the equilibrium time of the proposed method and some other reported microextraction methods for the extraction of OCPs from water samples [151] indicated that the present method was very fast.

4.3.1.4 Effect of salt addition

The salting out effect has been widely used in LPME and SPME. As is well known, the addition of salt to an aqueous sample can potentially increase analyte extraction. On the other hand, as the ionic strength of the medium...
increases, the viscosity and density of the aqueous solution are also enhanced, leading to a reduction of the efficiency of the mass transfer process and thus the extraction efficiency of the procedure [150]. The effect of the addition of NaCl for the salting-out effect was examined in this study by varying its amount (in the range of 0 and 200 g/L) in the sample. The results (data not shown) showed that there was no significant influence on the extraction of the OCPs.

4.3.1.5 Effect of extraction temperature

Temperature can have an effect on the distribution coefficient and the mass transfer of the analytes, and also on the emulsification process, thus influencing the extraction efficiency. The effect of temperature (from 10 to 45 °C) was investigated. Fig. 4-5 depicts the influence of the temperature of the sample solution on the extraction. At relatively low temperature (< 25 °C) low extraction efficiency was observed. This may be because at temperatures < 25 °C it was difficult to obtain a homogeneous emulsion in a very short time (30 s), resulting in an almost instantaneous phase separation. The viscosity of isooctane increased at lower temperatures affecting the emulsification negatively. This means that there was minimal dispersion of the extractant solvent in the water sample, and the mass transfer process was limited to a very short duration, leading to poor extraction. In the 25-45 °C range, emulsification was easily achieved and remained invariant during the extraction process; the highest extraction efficiency was achieved at 25 °C. At temperatures between 25-45 °C, the extraction efficiency was observed to decrease. This might be due to the enhanced solubility of OCPs in the water.
At a temperature higher than 45 °C (e.g. 55 °C) isoctane was completely dissolved in the aqueous sample. It was not possible to achieve a homogeneous emulsion at this temperature and phase separation was only achieved by firstly cooling down the tube, followed by centrifugation. The most favorable extraction temperature was therefore at 25 +/- 2 °C, the ambient temperature.

![Figure 4-5 Extraction temperature profile.](image)

**4.3.1.6 Effect of centrifugation time**

Centrifugation was required to break down the emulsion and accelerate phase separation. Centrifugation times at 4000 rpm were examined in the range of 1 to 15 min. The results showed that extraction efficiency increased when the centrifugation time was increased from 1 to 5 min. No obvious changes were observed when the time was further increased to 15 min (as shown in Fig. 4-6). This indicated that 5 min of centrifugation was adequate to break down the emulsion, and effect phase separation.
4.3.2 Method validation

To evaluate the proposed polyethylene Pasteur pipette–based LDS-USA-DLLME method, some parameters such as linearity, reproducibility, and EF were determined under the described extraction conditions. The results are listed in Table 4-2. The linearity of the method was studied with a series of analyte solutions of different concentrations. Depending on the compounds, calibration curves gave satisfactory linearity in the range of 0.01 to 50 µg/L for hexachlorobenzene, dieldrin, endrin and o,p'-DDD, 0.05 to 25 µg/L for heptachlor and 0.005 to 50 µg/L for p,p'-DDE with correlation coefficients > 0.9935. The LODs for all compounds, calculated at a S/N ratio of 3, ranged from 0.8 to 10 ng/L. The intraday and interday (3 consecutive days) reproducibility of the method was evaluated, by extracting and analyzing spiked water samples at a concentration of each OCP at 5 µg/L. The EFs for all the analytes, which were obtained by comparing the calibration graphs before and after the extraction process, were in the range of 128 (for hexachlorobenzene) and 328 (for heptachlor). The RSD values varied from
2.7% to 12.4% and 2.7% to 13.6%, respectively, illustrating the satisfactory reproducibility achieved by the procedure.

The Pasteur pipettes, as mentioned previously, are made of low-density polyethylene. It cannot be discounted that they may contain plasticizers (phthalates) which might affect the analysis. However, in our experiments, there were no observable traces of phthalates that might have originated from the pipettes.

**Table 4-2** Quantitative results of polyethylene Pasteur pipette-based LDS-USA-DLLME.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (µg/L)</th>
<th>r²</th>
<th>RSDa (%) (n=4)</th>
<th>RSDb (%) (n=4)</th>
<th>LOD (ng/L)</th>
<th>EFc</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexachlorobenzene</td>
<td>0.01-50</td>
<td>0.9997</td>
<td>7.4</td>
<td>6.3</td>
<td>1.5</td>
<td>128</td>
</tr>
<tr>
<td>heptachlor</td>
<td>0.05-25</td>
<td>0.9995</td>
<td>4.1</td>
<td>13.5</td>
<td>10</td>
<td>328</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>0.005-50</td>
<td>0.9986</td>
<td>4.7</td>
<td>13.6</td>
<td>0.8</td>
<td>179</td>
</tr>
<tr>
<td>dieldrin</td>
<td>0.01-50</td>
<td>0.9980</td>
<td>12.4</td>
<td>2.7</td>
<td>0.8</td>
<td>200</td>
</tr>
<tr>
<td>endrin</td>
<td>0.01-50</td>
<td>0.9998</td>
<td>4.4</td>
<td>7.2</td>
<td>1.5</td>
<td>268</td>
</tr>
<tr>
<td>o,p'-DDD</td>
<td>0.01-50</td>
<td>0.9935</td>
<td>2.7</td>
<td>5.1</td>
<td>0.8</td>
<td>176</td>
</tr>
</tbody>
</table>

a RSDs and b RSDs are intraday and interday reproducibility calculated separately from sample spiked at 5 µg/L of each OCP.

C Calculated from sample spiked at a level of 50 µg/L of each OCP.

4.3.3 Analysis of OCPs in real water samples

To eliminate possible matrix effects, the standard addition method was adopted for the quantitative determination of the OCPs in different water samples. Three aliquots of each of the genuine samples were analyzed in parallel, with the results presented in Table 4-3. The RSDs were generally satisfactory (<11.9%). It can be observed that 0.21 µg/L p,p'-DDE, 0.08 µg/L dieldrin and 0.11 µg/L o,p'-DDD were detected in river water. No analyte was found in the tap water sample. As several OCPs were detected in some
genuine samples, the procedure developed was suitable for genuine environmental applications.

Recovery was studied by considering the spiked water samples at two different concentration levels (0.1 µg/L and 1 µg/L). Satisfactory recoveries in the range of 77.7-120.3% were obtained, with RSDs ranging from 2.2 to 15.2% (as shown in Table 3). These results demonstrated that the proposed method was reliable for the analysis of the OCPs in environmental water samples.
Table 4-3 Analytical results and recoveries obtained from analysis of real water samples by the proposed method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Detected&lt;sup&gt;a&lt;/sup&gt; (µg/L)</th>
<th>RSD (%) (n=3)</th>
<th>Added (µg/L)</th>
<th>RR&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>RSD (%) (n=3)</th>
<th>Added (µg/L)</th>
<th>RR (%)</th>
<th>RSD (%) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>hexachlorobenzene</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1</td>
<td>80.3</td>
<td>2.2</td>
<td>1.0</td>
<td>79.1</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heptachlor</td>
<td>ND</td>
<td>0.1</td>
<td>95.8</td>
<td>8.6</td>
<td>1.0</td>
<td>92.3</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p,p'-DDE</td>
<td>0.21</td>
<td>11.9</td>
<td>0.1</td>
<td>108.7</td>
<td>15.2</td>
<td>1.0</td>
<td>113.8</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>dieldrin</td>
<td>0.08</td>
<td>9.8</td>
<td>0.1</td>
<td>108.6</td>
<td>11.7</td>
<td>1.0</td>
<td>120.3</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>endrin</td>
<td>ND</td>
<td>0.1</td>
<td>90.8</td>
<td>14.2</td>
<td>1.0</td>
<td>81.5</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o,p'-DDD</td>
<td>0.11</td>
<td>10.7</td>
<td>0.1</td>
<td>88.7</td>
<td>5.8</td>
<td>1.0</td>
<td>90.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Tap water</td>
<td>hexachlorobenzene</td>
<td>ND</td>
<td>0.1</td>
<td>108.1</td>
<td>7.7</td>
<td>1.0</td>
<td>118.1</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heptachlor</td>
<td>ND</td>
<td>0.1</td>
<td>90.8</td>
<td>3.6</td>
<td>1.0</td>
<td>96.5</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p,p'-DDE</td>
<td>ND</td>
<td>0.1</td>
<td>99.3</td>
<td>6.8</td>
<td>1.0</td>
<td>106.6</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dieldrin</td>
<td>ND</td>
<td>0.1</td>
<td>82.1</td>
<td>7.5</td>
<td>1.0</td>
<td>77.7</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>endrin</td>
<td>ND</td>
<td>0.1</td>
<td>98.9</td>
<td>10.2</td>
<td>1.0</td>
<td>92.3</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o,p'-DDD</td>
<td>ND</td>
<td>0.1</td>
<td>106.6</td>
<td>9.6</td>
<td>1.0</td>
<td>107.2</td>
<td>3.8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The samples were analyzed directly; <sup>b</sup> ND-not detected.
4.3.4 Comparison of polyethylene Pasteur pipette-based LDS-USA-DLLME-GC-MS with other analytical methodologies

A comparison between the proposed LDS-USA-DLLME technique and different published techniques for the extraction of OCPs using DLLME [165], static LPME [166], SDME [167] conventional USA-DLLME [151] and SFD-LPME [168] methods are presented in Table 4-4. The LODs, EFs and precision determined by the present method are comparable with the other microextraction methods. The extraction time for this method was only a few seconds, which was much shorter than the sampling methods listed in Table 4-4, except for DLLME. The EF of this proposed LDS-USA-DLLME method was comparable with DLLME which uses relatively larger volumes of sample, and also dispersive solvent. Moreover, the isoctane, used as extraction solvent in the proposed method, is much less toxic in comparison with the chlorinated solvents widely used as extraction solvents in conventional DLLME.
### Table 4-4 Comparison of the proposed LDS-USA-DLLME method with other methods of extraction in the determination of OCPs

<table>
<thead>
<tr>
<th>Method</th>
<th>Extraction solvent</th>
<th>Sample (mL)</th>
<th>LOD (ng/L)</th>
<th>Extraction time (min)</th>
<th>RSD (%)</th>
<th>RR (%)</th>
<th>EF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLLME GC-MS</td>
<td>tetrachloroethylene/acetone</td>
<td>10</td>
<td>1-25</td>
<td>0.5</td>
<td>5-15</td>
<td>70-120</td>
<td>46-316</td>
<td>[165]</td>
</tr>
<tr>
<td>Static-LPME GC-ECD</td>
<td>n-hexane</td>
<td>3</td>
<td>20-200</td>
<td>20</td>
<td>3.2-10.7</td>
<td>83.3-98.3</td>
<td>&gt;20</td>
<td>[166]</td>
</tr>
<tr>
<td>SDME GC-MS</td>
<td>toluene</td>
<td>10</td>
<td>22-101</td>
<td>37</td>
<td>5.9-9.9</td>
<td>34-118</td>
<td>-</td>
<td>[167]</td>
</tr>
<tr>
<td>Conventional USA-DLLME GC-ECD</td>
<td>chloroform</td>
<td>10</td>
<td>2-16</td>
<td>15</td>
<td>&lt; 9</td>
<td>75-103</td>
<td>-</td>
<td>[151]</td>
</tr>
<tr>
<td>SFD-LPME GC-ECD</td>
<td>1-dodecanol</td>
<td>20</td>
<td>7-19</td>
<td>30</td>
<td>&lt; 7.2</td>
<td>-</td>
<td>708-1337</td>
<td>[168]</td>
</tr>
<tr>
<td>LDS-USA-DLLME GC-MS</td>
<td>isoocctane</td>
<td>6</td>
<td>0.8-10</td>
<td>0.5</td>
<td>2.7-12.4</td>
<td>77.7-120.3</td>
<td>128-328</td>
<td>This method</td>
</tr>
</tbody>
</table>
4.4 Conclusion

In the present study, a polyethylene Pasteur squeeze-type pipette was used as an extraction device in which LDS-USA-DLLME was conducted with a low-density solvent, for the extraction and determination of OCPs in genuine water samples. The use of the widely available pipettes meant that emulsification, centrifugation and the organic solvent collection procedures were conducted very conveniently. The results of optimization showed that the ionic strength, extraction time and the emulsification temperature had no significant effects on the extraction. The independence of extraction efficiency of these parameters afforded a more precise and robust method that was suitable for the analysis of the OCPs in complex matrices. Under the optimized working conditions, EFs of up to 328 were obtained and the LODs for all the analytes were of the order of ng/L with acceptable precision. The proposed method was an efficient and rapid extraction method for OCPs that is an alternative to normal DLLME and USA-DLLME methods that employ potentially toxic high-density organic solvents as extractants. However, the performance of the proposed method is possibly limited by the volume of the aqueous sample since the current Pasteur pipettes used are of limited (8 mL) capacities. Better performance could conceivably be achieved, if larger size pipettes were available that would allow the extraction of bigger volumes of aqueous samples.
Chapter 5 Low-density solvent-based vortex-assisted surfactant-enhanced emulsification liquid-liquid microextraction of phthalate esters from water samples

5.1 Introduction

PEs are used primarily as plasticizers in polymeric materials to increase their workability and flexibility. Since they are physically bound to the polymer chains, they can be released easily from plastic products and migrate into the water or food that comes into direct contact with them [169, 170]. Certain PEs, as well as their degradation products and metabolites, can cause adverse effects on human health, especially on the kidney, liver and testicles [171]. Recently, the potential endocrine disrupting properties of PEs and food products contaminated with PEs were also reported [172] due to the use of plastics as food containers and packaging. Therefore, the development of sensitive and reliable analytical methods to evaluate and monitor trace amounts of PEs in different water samples are desirable for human health protection and environmental control.

Surfactants are amphiphilic organic compounds which contain both hydrophilic heads and hydrophobic tails. A surfactant can reduce both the surface tension of water by adsorbing at the liquid-gas interface, and the interfacial tension between oil and water by adsorbing at the liquid-liquid interface, thus serving as an emulsifier to enhance the dispersion of the water-immiscible phase into the aqueous phase. The application of a surfactant as an
emulsifier in LPME was developed by Wu et al. [126] and proved to be efficient, simple, rapid and cost-effective. In 2011, Yang et al. [125] applied a surfactant Triton X-114 as an emulsifier in VALLME and developed VSLLME to combine the advantages of surfactant, VALLME and DLLME. In this work, the addition of surfactant Triton X-114 as emulsifier greatly enhanced extraction efficiency and reduced extraction time. VALLME is usually carried out for 2 min [121, 123, 147], while for this work only 30 s was enough for the extraction. After extraction, the two phases could be separated by centrifugation and the sediment phase could be easily collected for further analysis. However, high-density solvent chlorobenzene was used, which is undesirable since it is potentially toxic. In addition, the use of a high-density solvent limits the wider applicability of the method due to a more limited choice of solvents.

In this work, LDS-VSDLLME combined with GC-MS was for the first time applied to the fast determination of six PEs in bottled water samples. In the LDS-VSDLLME procedure, the addition of surfactant cetyltrimethyl ammonium bromide (CTAB) enhanced the dispersion of low-density extraction solvent (toluene) in aqueous sample and was also favorable for the mass transfer of the PEs from the aqueous sample to the toluene. Moreover, using a relatively less toxic surfactant CTAB as the emulsifier agent overcame the disadvantages of traditional organic dispersive solvents that are usually more toxic and might conceivably decrease extraction efficiency to some extent since they are not as effective as surfactants themselves in generating an emulsion. With the aid of surfactant and vortex agitation to achieve good
organic extraction solvent dispersion, extraction equilibrium was achieved within 30 s, indicating it was a fast sample preparation technique. After extraction, the supernatant (extraction solvent) was collected at the conical bottom of the tube after removing the aqueous sample by a syringe. This method avoids the necessity of a special homemade device for the collection of low-density organic solvents [84], which is tedious and troublesome to fabricate. In order to evaluate the proposed method, conventional DLLME, LDS-DLLME and USAEME were carried out for comparison with the performance of LDS-VSDLLME. Under the optimized microextraction conditions, the developed method was applied to analyze bottled water samples.

5.2 Experimental

5.2.1 Structures of analytes

The PE standards (dimethyl phthalate (DMP), diethyl phthalate (DEP), (di-n-butyl phthalate (DnBP), benzyl butyl phthalate (BzBP), di-2-ethyl hexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP)) were bought from Supelco (Bellefonte, PA, USA) in the form of a methanolic stock solution containing 2000 mg/L of each compound. Their structures are shown in Table 5-1. HPLC-grade methanol (purity 99.9%), acetone (purity 99.9%) and toluene (purity 99.9%) were purchased from Tedia Company (Fairfield, OH, USA). 1-Octanol (purity >99%), toluene (purity 99.9%), CTAB (purity >99%), polyoxyethylene octyl phenyl ether, Triton X-100 (C_{14}H_{22}O(C_{2}H_{4}O)_{n}) (n=9~10) (purity >99%) and polyethylene glycol tert-
octylphenyl ether, Triton X-114 \((C_{14}H_{22}O(C_2H_4O)_n)\) \((n=7-8)\) (purity >99%) were bought from Sigma-Aldrich (St. Louis, MO, USA), while chlorobenzene (purity 99.9%), cyclohexane (purity 99.9%) and isoctane (purity 99.9%) were from Fisher (Loughborough, UK). Sodium chloride (NaCl) was obtained from Goodrich Chemical Enterprise (Singapore). Sodium dodecyl sulfate (SDS) (purity 99%) was purchased from BDH Laboratory Supplies (Poole, England). Ultrapure water was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water purification system.

Both of the 100-µL HPLC microsyringe used for the addition of extraction solvent and surfactant, and the 10-µL microsyringe used for GC-MS injection were purchased from SGE (Sydney, Australia). The 5-mL plastic syringe was bought from HSW (Tuttlingen, Germany).

A stock solution containing all analytes (at 10 mg/L of each) was prepared in methanol and stored at 4 °C. Water samples were prepared by spiking ultrapure water with analytes at known concentration (5 μg/L) daily to study extraction performance under different conditions. Bottled water samples were bought from a local market and were stored in dark at 4 °C and then analyzed without filtration.
### Table 5-1 Chemical structures of PEs considered in this work

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CAS number</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>DEP</td>
<td>84-66-2</td>
<td><img src="image" alt="DEP Structure" /></td>
</tr>
<tr>
<td>DnBP</td>
<td>84-74-2</td>
<td><img src="image" alt="DnBP Structure" /></td>
</tr>
<tr>
<td>BzBP</td>
<td>85-68-7</td>
<td><img src="image" alt="BzBP Structure" /></td>
</tr>
<tr>
<td>DEHP</td>
<td>117-81-7</td>
<td><img src="image" alt="DEHP Structure" /></td>
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<tr>
<td>DnOP</td>
<td>117-84-0</td>
<td><img src="image" alt="DnOP Structure" /></td>
</tr>
</tbody>
</table>

5.2.2 Instrument and conditions

Analysis was carried out on the Shimadzu QP2010 GC-MS system. Helium was employed as carrier gas at a flow rate of 1.65 mL/min. The injector temperature was set at 250 °C. The GC oven temperature was initially set at 100 °C and then programmed to 280 °C at 10 °C/min and then held for 4 min.
The GC-MS interface was maintained at 270 °C. All injections were in splitless mode. SIM mode was adopted for quantitative determination of the analytes. The masses monitored by the detector were set as follows: 6-7 min, m/z 135, 163, 164, 194 (DMP); 8-8.5 min, m/z 149, 150, 176, 177 (DEP); 12-12.5 min, m/z 149, 150, 205, 223 (DnBP); 15.5-16 min, m/z 91, 149, 150, 206 (BBP); 17-17.5 min, m/z 149, 150, 167, 279 (BEHP); 18.5-20 min, m/z 149, 150, 167, 279 (DnOP).

5.2.3 LDS-VSDLLME procedure

Fig. 5-1 shows the LDS-VSDLLME procedure. Briefly, 30 µL toluene and 50 µL 2.0 × 10⁻² mol/L aqueous solution of detergent CTAB were first injected into a 10 mL glass tube with a conical bottom, using a 100-µL HPLC microsyringe. A 5 mL water sample was added in the tube subsequently. The resulting mixture was then vigorously shaken on a vortex agitator at 3200 rpm for 1 min. As a result, an emulsion containing fine droplets was formed facilitating mass transfer of the target analytes into the extraction solvent. The emulsion was disrupted by centrifugation at 4000 rpm for 5 min, the organic phase formed a layer at the top of the aqueous sample. The aqueous phase was then completely removed using a 5.0 mL syringe first, leaving behind the organic solvent (~ 15 µL) at the bottom of the conical tube. One microliter of the extract could be easily withdrawn using a 10-µL GC syringe and injected into the GC-MS system for analysis.
5.2.4 Comparative studies

The performance of LDS-VSDLLME was compared with conventional DLLME, LDS-DLLME and USAEME. Spiked ultrapure water samples (3 μg/L of each PE) were used for the comparative extractions.

5.2.4.1 Conventional DLLME

For DLLME, a 5 mL water sample was placed in a 10 mL glass tube with a conical bottom. A mixture of 500 μL acetone (dispersive solvent) and 30 μL chlorobenzene (extraction solvent) was rapidly injected into the aqueous solution. Immediately, an emulsion was formed. After centrifugation at 4000 rpm for 5 min, the organic extract (~ 11 μL) settled at the bottom of the centrifuge tube. One microliter of extract was injected into the GC-MS system for analysis. The conditions used here were most favorable for extraction.
5.2.4.2 LDS-DLLME

For LDS-DLLME, the procedure was similar to that for DLLME as described above, except that the extractant solvent was toluene (30 µL). The extract was left at the top of the water sample, which was removed as described above (Section 5.2.3), leaving behind ca. 7 µL of the extract. One microliter of the extract was injected into the GC-MS system for analysis.

5.2.4.3 USAEME

Thirty microliters of toluene were rapidly injected into a 5 mL water sample in a 10 mL glass tube with a conical bottom. After injection, the tube was immersed in an ultrasonic water bath. Upon application of 35 kHz of ultrasound frequency, an emulsion formed in the tube. After 1 min of ultrasonication/extraction, the emulsion was separated into two phases by centrifugation at 4000 rpm for 5 min. The upper layer (organic extract, ~23 µL) was collected as described above (Section 5.2.3) and 1 µL of the extract was analyzed by GC-MS.

5.3 Results and discussion

It is well known that the most important problem concerning PE analysis is the risk of contamination, resulting in over-estimated concentrations. The sources of contamination can be present in any step of the analytical procedure. In this work, special care was taken to avoid the contact of reagents and solutions with plastic materials. Laboratory glassware was washed prior to use
with ultrapure water, acetone and methanol, and then dried at 100 °C overnight. They were stored in heat-treated aluminum foil to minimize exposure. Despite these precautions, a problem with PE contamination was encountered. In a blank run (LDS-VSLLME of ultrapure water), DEP, DnBP and DEHP were detected. In isolating the problem, it was discovered that the toluene was the source of the contamination. Direct injection of the toluene used indicated that it was contaminated with DEP, DnBP and DEHP. The concentrations of these PEs were similar to those detected after the blank analysis (data not shown). This result indicated that the extraction procedure apparently did not introduce extraneous PEs, and all the contaminants were from the toluene. To address the problem, toluene from another supplier was evaluated. However, the contamination problem was even worse. As a result, we continued to use the first brand of toluene, but taking into account the PE contamination in the solvent in the subsequent optimization and validation experiments, and genuine sample analysis.

5.3.1 Comparison of LDS-VSdllme with conventional DLLME, LDS-DLLME and USAEME

It can be clearly seen from Fig. 5-2 for the 6 representative PEs that LDS-VSLLME gave the best extraction results (the total peak area is defined as the product of GC peak area of 1 µL extract multiplied by the volume of final extract for a particular extraction method), followed by DLLME, USAEME and LDS-DLLME. Moreover, the toluene, which is used as extraction solvent in the proposed method, LDS-VSLLME, is much less toxic in comparison with the chlorinated solvents widely used in conventional DLLME. The
simple low-density solvent collection approach expands the applicability of DLLME. Compared with USAEME, much higher extraction efficiency was obtained; this may be due to that the combination of surfactant and vortex agitation was highly efficient for the dispersion of the organic extraction solvent, and thus extraction equilibrium could be achieved in a short time. Most importantly, the proposed technique employed a surfactant as a substitute for the large amount of dispersive solvent (up to several hundred microliters) that is often applied in DLLME. This also addresses the issue of a reduced partition coefficient when a large volume of dispersive solvent is used in the latter method which inhibits the mass transfer of analytes to the extraction solvent. Moreover, a surfactant is relatively less toxic compared to an organic dispersive solvent, especially one that is as volatile as acetone.

![Figure 5-2 Comparisons of DLLME, LDS-DLLME, USAEME, and LDS-VSDLLME](image)

**Figure 5-2** Comparisons of DLLME, LDS-DLLME, USAEME, and LDS-VSDLLME
5.3.2 Determination of the most favorable extraction conditions

To achieve the best LDS-VSDLLME conditions, the effect of different extraction parameters including the type and volume of extraction solvent, the type and concentration of the surfactant, salt addition, and vortex time were studied in terms of the peak areas of analytes. All experiments were performed at least in triplicate. To achieve the best dispersion of the extraction solvent, the maximum speed setting of the vortex agitator (3200 rpm) was applied during all the experiments.

5.3.2.1 Effect of extraction solvent

The selection of an appropriate extraction solvent is of great importance in LDS-VSDLLME. The organic extraction solvent determines the partition coefficient between the extraction phase and donor phase. In this method, a suitable solvent has to meet the following requirements: (1) having a good extraction affinity for the target analytes to ensure high enrichment; (2) being immiscible with water; (3) having a lighter density than water; (4) having good chromatographic behavior. To select the most suitable one, four common low-density organic solvents were evaluated as extraction solvent including cyclohexane (density 0.78 g/mL), 1-octanol (density 0.82 g/mL), isoctane (density 0.69 g/mL) and toluene (density 0.87 g/mL). Peak areas were compared and the results for all the PE analyses are shown in Fig. 5-3. The figure shows that toluene and 1-octanol have comparable extraction efficiencies which were higher than those obtained with the other solvents; this may be accounted for by their better solvation capabilities towards the
target analytes. However, considering the better GC-MS peak shapes (not shown) achieved when using toluene as solvent, it was preferred.

![Figure 5.3](image_url)  
**Figure 5.3** Effect of extraction solvent on extraction. Extraction conditions: sample volume, 5.0 mL; extraction solvent volume, 40 µL; Triton X-100 concentration, 0.2 mmol/L; vortex time, 1 min.

### 5.3.2.2 Effect of extraction solvent volume

In LDS-VSDLLME, the volume of extraction solvent is also an important parameter, as it impacts on the EF. To study the effect of extraction solvent volume on extraction, the volume of toluene was varied over the range of 20 and 60 µL, and the results are shown in Fig. 5-4. According to Fig. 5-4, the extraction efficiencies decreased when the volume of toluene used was increased from 20 to 60 µL. This can be explained by the dilution effect. At smaller volumes of the extraction solvent, higher extraction efficiency was obtained as expected. However, when the volume of toluene was 20 µL, it was relatively difficult to retrieve it reproducibly after extraction. Thus, in consideration of the extraction efficiency, volume and reproducibility, 30 µL
of toluene were selected as the most suitable volume for subsequent experiments.

**Figure 5-4** Effect of the solvent volume on extraction. Extraction conditions: extraction solvent, toluene; sample volume, 5.0 mL; Triton X-100 concentration, 0.2 mmol/L; vortex time, 1 min.

5.3.2.3 **Effect of the type and concentration of surfactant**

The surfactant, which serves as an emulsifier, accelerates the emulsification of the water-immiscible organic extraction solvent in the aqueous solution under vortex mixing. Three different types of surfactants (anionic (SDS), cationic (CTAB) and non-ionic (Triton X-100 and Triton X-114)) were investigated. Their critical micellar concentrations (CMCs) are 7, 0.91, 0.24 and 0.21 mmol/L, and hydrophile-lipophile balance (HLB) values are 40, 15.8, 13.4 and 12.4, respectively. Fig. 5-4 shows the variation of the extraction efficiency with, and without the surfactants under consideration. According to Fig. 5-5, Triton X-100 and CTAB gave comparably good extraction efficiency for all the analytes. The effect of different surfactants on the extraction
efficiency could be related to the hydrophobicity and polarity of the analytes and the HLB value of the surfactants. When the HLB value of a surfactant is between 8 and 18, it can be used as an emulsifier [126]. This suggests that SDS is not suitable for use as an emulsifier since its HLB value is much higher than 18. Triton X-100 and CTAB might have a suitable hydrophobicity for the PEs, thus resulting in better and comparative extraction efficiency. Based on the experimental results, selection of either Triton X-100 or CTAB as the surfactant was reasonable. However, after extraction, for some reason that we have yet to determine, the extract volume when CTAB was used (~15 µL) was relatively larger than that (~4 µL) when Triton X-100 was used. Thus, for this reason, and for more convenient handling (the preparation of a CTAB solution is much easier due to its solid state compared with Triton X-100 which is a high viscosity liquid), CTAB was favored as the optimal surfactant (see also the following paragraph).

Surfactant concentration also plays an important role in the emulsification and mass transfer process, which affect the extraction efficiency. In order to study the influence of the concentration of CTAB, different concentrations ranging from 0 to 0.5 mmol/L were investigated. As shown in Fig. 5-6, the extraction efficiency increased when surfactant concentration was increased from 0 to 0.2 mmol/L. After that, the extraction efficiency began to decrease. A possible explanation is that when the surfactant concentration was increased from 0 to 0.2 mmol/L, the free surfactant monomer increased, resulting in an improved dispersion process. Eventually, however, aggregation of pre-micelles occurred as the level of surfactant reached the CMC, which caused a
decrease in extraction efficiency, possibly as a result of stronger interaction between the analytes and the pre-micelles (i.e. there was competition between the pre-micelles and the extraction solvent for the analytes) [127]. In addition, there was formation of foam observed when the concentration of CTAB was further increased to 0.5 mmol/L. Concomitant with the increase in the amount of surfactant, the volume of the organic solvent phase decreased, making the retrieval of the extract problematical. In view of this, a concentration of 0.2 mmol/L of CTAB was found to be suitable.

Figure 5-5 Effect of different surfactants on extraction. Extraction conditions: sample volume, 5.0 mL; extraction solvent, 30 μL toluene; surfactant concentration, 0.2 mmol/L; vortex time, 1 min.
5.3.2.4 Effect of the salt

Salt addition to the water sample may have several different effects on extraction (salting-out, salting-in or no effect). Usually, depending on the solubility of the target analytes, adding salt to water sample normally enhances extraction of the relatively more polar analytes. For investigating the influence of the salt addition on the performance of LDS-VSDLLME, different amounts of NaCl (in the range of 0 to 200 mg/mL) were added into water samples while the other conditions were kept constant. The results are shown in Fig. 5-7. The observation indicated that with the increase of the salt content (from 0 to 200 g/L), the extraction efficiency decreased for most of the target analytes except in the case of the more polar DMP (whose extraction generally remained constant). An explanation for this is that with the increase of the salt content, the viscosity and density of the solution increased, leading to an inhibition of the mass transfer process, thus overcoming the salting-out effect.
Thus as a compromise and for operational convenience, no salt was added in subsequent experiments.

Figure 5-7 Effect of salt addition on extraction. Extraction conditions: sample volume, 5.0 mL; extraction solvent, 30 µL toluene; concentration of CTAB: 0.2 mmol/L; vortex time, 1 min.

5.3.2.5 Effect of vortex time

Vortex time (duration of the vortexing) is one of the main factors in LDS-VSDLLME. It affects both the emulsification and mass transfer processes, and thus influences the extraction efficiency of the method. For the present study, the effect of the vortex time was studied over the time range of 30 s to 5 min. Fig. 5-8 shows the extraction efficiency for PEs versus vortex time. It can be observed that the extraction efficiencies increased with the increase of vortex time from 30 s to 1 min; beyond 1 min, there was either a flattening out of the profile, or a slight decrease, depending on the analytes. This is due to the fact that the contact surface between extraction solvent and aqueous sample was greatly enhanced by the addition of surfactant and the vortex agitation, thus
greatly increasing the mass transfer. As a result, the equilibrium state could be achieved within 1 min.

Figure 5-8. Effect of vortex time. Extraction conditions: sample volume, 5.0 mL; extraction solvent, 30 µL toluene; concentration of CTAB: 0.2 mmol/L.

On the basis of the above discussion, the most suitable extraction conditions for LDS-VSDLLME were as follows: 30 µL toluene as extraction solvent, 0.2 mmol/L of CTAB selected as the surfactant, vortex time of 1 min; and without salt addition. All the following experiments were carried out under these conditions. Fig 5-9 shows a chromatogram of a spiked water sample (25 µg/L of each analyte) after extraction by the developed method under the described conditions.
Figure 5.3.3 Method validation

A series of experiments with regard to the linearity, repeatability, LODs and EFs were performed to validate the proposed method at the developed working conditions. The results are listed in Table 5-2. The linearity of the method was explored at PE concentrations from 0.05 or 0.1, to 25 μg/L with good squared regression coefficients ($r^2$) of between 0.9823 and 0.9992. The LODs ranged between 8 and 25 ng/L. The results were comparable with those reported in previous microextraction studies, where SPME [173] or HF-LPME [174] was used for the extraction of PEs.

The repeatability of the method, expressed as RSD, was studied for five replicate experiments with spiked ultrapure water with PEs at concentrations of 5 μg/L. The RSDs for the PEs were below 11.9%, illustrating satisfactory repeatability was achieved by the proposed method. The EFs for the six PEs ranged from 200 (for DnBP) to 290 (for BEHP). These values highlight the good extraction performance of the new technique.
Table 5-2  Quantitative results of the proposed LDS-VSDLLME-GC-MS method

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity (μg/L)</th>
<th>r²</th>
<th>RSD (%) (n=5)</th>
<th>LOD (ng/L)</th>
<th>EF</th>
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<tbody>
<tr>
<td>DMP</td>
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<tr>
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</table>

5.3.4 Analysis of genuine samples

The proposed method was applied for extraction of PEs from a 5 mL bottled water sample. To eliminate matrix effects, the standard addition method was adopted. The concentrations of PEs in the genuine samples were calculated using standard addition calibration after subtraction of the blank values. Three aliquots of the water sample were analyzed in parallel, with the results presented in Table 3. As expected, since PEs are ubiquitous in plastic packages, the water samples showed individual concentrations ranging from undetected to 0.4 μg/L. RRs, which indicate the effect of the sample matrix on extraction, were determined. Relative recoveries are defined as the ratios of analyte peak areas of spiked bottle water sample extracts and those of spiked ultrapure water extracts, with both types of samples spiked at the same concentrations of analytes (in this case, 0.1 μg/L and 1 μg/L). The RRs varied between 73.5% and 106.6%, and RSDs (n=3) were below 11.7%. The results indicated that the present method was suitable for the determination of PEs in environmental water samples, although matrix effects could arise when dealing with more complex samples.
Table 5-3 PEs in unspiked and spiked bottled water samples determined by LDS-VSDLLME and GC-MS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration of PEs in bottle water (μg/L)</th>
<th>Spiked bottle water (1 μg/L of each analyte)</th>
<th>Spiked bottle water (0.1 μg/L of each analyte)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RR (%)</td>
<td>RSD (%) (n=3)</td>
<td>RR (%)</td>
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<tr>
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<td>BnOP</td>
<td>0.12</td>
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</table>

5.4 Conclusion

In the present study, low-density solvent-based vortex-assisted surfactant-enhanced-emulsification liquid-liquid microextraction (LDS-VSDLLME) was developed and for the first time applied for determining of PEs in bottled water samples. A low-density solvent, toluene, which is less toxic than chlorinated solvents widely used in conventional DLLME, was successfully used in conjunction with a simple low-density solvent collection procedure. The use of low-density solvents expands the applicability of DLLME. The important benefit of this approach was the elimination of a relatively large amount (several hundred microliters) of organic dispersive solvent. With the aid of surfactant and vortex agitation, the organic extraction solvent was better dispersed and mass transfer was increased, resulting in extraction equilibrium being achieved in only 1 min, and high extraction efficiency. Overall, LDS-VSDLLME was shown to be a fast, efficient, simple and cost-effective method for the determination of PEs in environmental water samples.
Contamination of the toluene used in the procedure was addressed by using the standard addition method with background subtraction for quantitative measurements.
Chapter 6 Conclusions and Outlook

Four different kinds of miniaturized environmentally friendly DLLME sample preparation methods, including vortex-assisted dispersive liquid-liquid microextraction (VADLLME), ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction (IL-USA-DLLME), low-density solvent-based ultrasound-assisted dispersive liquid-liquid microextraction (LDS-USA-DLLME) and low-density solvent-based vortex-assisted surfactant-enhanced dispersive liquid-liquid microextraction (LDS-VSDLLME) have been developed in this thesis. VADLLME was studied in which no organic dispersive solvents were used (Chapter 2). Low toxicity and environmentally friendly solvents, such as ILs and low-density solvents were introduced for IL-USA-DLLME and LDS-USA-DLLME, respectively (Chapter 3 and 4). In Chapter 5, a study was reported in which surfactant was applied to enhance the dispersion of organic solvent into aqueous sample for LDS-VSDLLME. It was found that all these novel microextraction methods afforded excellent analytical performances and environmentally friendly behaviors for the extraction of several classes of water contaminants and pollutants (including UV filters, OCPs and PEs).

In Chapter 2 a novel technique named VADLLME was developed, for which the dispersion of organic solvent into aqueous sample is achieved by vortex agitation. No dispersive solvent and centrifugation were required in this microextraction procedure. As a result, high extraction efficiencies were achieved in a short analysis time.
To avoid the application of high-density chlorinated organic solvent in our previous work, IL-USA-DLLME and LDS-USA-DLLME were investigated. In Chapter 3, IL-USA-DLLME followed by HPLC-UV detection has been developed for the determination of four benzophenone UV filters. In this procedure, an ultrasound-assisted process was applied to accelerate the formation of the fine cloudy solution using less dispersive solvent compared with conventional DLLME, which significantly increased extraction efficiency and reduced extraction time. To solve the problem of collecting low-density organic extraction solvents, we introduced and reported in Chapter 4, a novel collection procedure by employing a flexible polyethylene Pasteur pipette as a convenient extraction device. As a result, all the analytical results are comparable with previous reports [12, 71, 165, 175] that used high-density chlorinated organic solvents and larger volume of sample solution.

To further improve the dispersion of the extraction solvent to aqueous sample in a fast and high efficient way, in Chapter 5, LDS-VSDLLME has been coupled with GC-MS for the determination of six PEs in bottle water samples. With the aid of surfactant and vortex agitation to achieve good organic extraction solvent dispersion, extraction equilibrium was achieved within 1 min. Another prominent feature of the method was the simple procedure to collect a less dense than water solvent by a microsyringe.

The significance of the development of different novel modes of DLLME methods in this study have been successfully applied to the determination of different kinds of environmental pollutants. They extend previous work on
DLLME and expand their applications for the analysis of environmental pollutants. Especially, the introduction of the new extraction device for the collection of low-density solvent widens the applicability of DLLME to a wider range of solvents. For IL-USA-DLLME, LDS-USA-DLLME and LDS-VSDLLME, low toxic environmentally friendly ILs and low-density organic solvents were applied, which provided good alternatives to traditional DLLME. Furthermore, a key advantage of all these techniques lies in the application of disposable polyethylene Pasteur pipettes or centrifuge tubes, which avoids the carry-over problems. For VADLLME, the important benefit, which is the avoidance of the centrifugation step, opens up a potentially new horizon for on-site dispersive liquid microextraction. Last but not the least, all those superior features prove that the developed microextraction methods are promising techniques in the sample preparation fields.

The limitation of the sample preparation methods developed in this work is the difficulty of automation, because centrifugation is usually necessary to achieve phase separation after extraction for most modes of DLLME. Future work should be devoted to the implementation of partial or full automation of some of these microextraction processes, which can further reduce labor and analysis time. One possible approach is to disperse organic solvent by vortex agitation or other external power rather than adding organic dispersive solvent, and then phase separation can be achieved by gravity. This may add cost and complexity, but in order to attract widespread commercial and industrial utilizations of these techniques, the trend towards automation is inevitable. Overall, all the developed microextraction methods in this thesis have been
demonstrated to be economical, simple, efficient and environmentally friendly, as well as accessible to even those with only basic facilities in their laboratories.
References


List of Publications


Conference presentations

1. “6th Mathematical and Physical Science Graduate Congress”, University of Malaya, Malaysia, 2010
   Poster presentation “Liquid phase microextraction - Using knitting wool as the extractant phase holder to determine UV filters in swimming pool water”

   Poster presentation “Liquid phase microextraction - Using knitting wool as the extractant phase holder to determine UV filters in swimming pool water”

   Oral Presentation “Ionic liquid-based ultrasound-assisted dispersive liquid-liquid microextraction followed high-performance liquid chromatography for the determination of UV filters in environmental water samples”