DEVELOPMENT AND APPLICATION OF MINIATURIZED SAMPLE PREPARATION PROCEDURES FOR ENVIRONMENTAL WATER CONTAMINANTS

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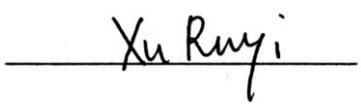
DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety, under supervision of Professor Lee Hian Kee, (in the Microextraction, Separation Science and Enviroanalytics laboratory), Chemistry Department, National University of Singapore, between 08/2009 and 07/2013.

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



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SUMMARY

This thesis focuses on the development and application of microextraction methods for rapid determination of organic contaminants from environmental water samples.

Despite all of the merits of solid-phase microextraction (SPME) and liquid-phase microextraction (LPME), drawbacks including time consuming, tedious, labor intensive and requirement of large amount of organic solvent still exist. One major problem is the relatively long extraction time. Sometimes the extraction process may take 30 min or even several hours. The extraction speed depends mainly on passive diffusion, and it requires a certain time to reach the distribution equilibrium between the donor phase and the acceptor phase. In 2006, a novel microextraction technique termed dispersive liquid-liquid microextraction (DLLME) was developed. It was based on the use of a small amount of high density organic solvent and a dispersive solvent which was miscible to both the organic solvent and the aqueous solution. Extraction of target analytes is considered as independent of time since the surface area between the donor phase and the acceptor phase is considerably large. Another promising approach is electromembrane microextraction (EME). In EME, the mass transfer of charged analytes could be accelerated under an external electrical field, greatly reducing the extraction time.

Therefore, the development and modification of DLLME and EME is the main target of this thesis.

For modification of DLLME, a method called low-density solvent based solvent demulsification-dispersive liquid-liquid microextraction (LDS-SD-DLLME) was developed in Chapter 2 to extract polycyclic aromatic hydrocarbons (PAHs). Non-chlorinated solvent was used since it is environmentally friendlier. The entire extraction took place in 2 min in a disposable syringe. It was easy to collect the organic extract since it would float on the top of the solution. Therefore, no centrifugation was needed in this procedure. In another work described in Chapter 3, a microextraction procedure termed low-density solvent based ultrasound-assisted surfactant-enhanced dispersive liquid-liquid microextraction (LDS-UASEME) was developed to extract organochlorine pesticides (OCPs). This method combines the merits of the application of low-density solvent, ultrasonic radiation and surfactant. A soft flexible polypropylene Pasteur pipette was employed to retrieve low-density solvent after extraction. Unlike customized extraction devices, Pasteur pipettes are commercially available and were disposed of after each extraction to avoid cross contamination. The whole emulsification process took place in only 2 min. By using gas chromatography-mass spectrometry (GC-MS), good limits of detection (LODs)

(down to 0.06 μg/L) were achieved.

Another modification of DLLME termed sonication-assisted emulsification microextraction coupled with vortex-assisted micro-solid-phase extraction (SAEME-VA-μ-SPE) was explored in Chapter 4. This technique was based on the application of a μ-SPE device to retrieve organic solvent. In this approach, the target analytes were first extracted to 1-octanol under ultrasonic water bath, and then further extracted into a μ-SPE device. This method provided another solution for the retrieval of low-density organic extract. Additionally, the porous membrane of the μ-SPE device served as a filter between the sorbent and other interferences in the donor solution. Therefore, it could be adopted in samples with complex matrices.

EME has been proven to be an effective and efficient method to extract charged compounds from aqueous solution. Normally, EME is considered to be more compatible with high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). However, for some compounds, better LODs could be achieved using gas chromatography-mass spectrometry (GC-MS). Therefore, exploration on the connection of EME and GC-MS could be worthwhile. Additionally, development of electro-enhanced technique could reduce extraction time, overcoming disadvantages of some

existed techniques (e.g. SPME). One method termed electro-enhanced solid-phase microextraction (EE-SPME) was described in Chapter 5. In this method, some tricyclic antidepressants (TCAs) were extracted to a commercially available SPME fiber with the application of an external electrical potential. The needle sleeve of the SPME holder served as the anode and one platinum wire served as the cathode. After a certain time, the SPME fiber was transferred to the injection port of GC-MS and the target analytes were thermally desorbed for analysis. Some parameters influencing the extraction efficiencies were investigated. The LODs of TCAs ranged between 0.079 and 0.296 μg/L. Last but not least, a novel approach termed electromembrane microextraction coupled with vortex-assisted micro-liquid-liquid extraction (EME-VA-μ-LLE) was reported in Chapter 6. Four non-steroidal anti-inflammatory drugs (NSAIDs) were used as models. In the first step, NSAIDs were extracted from the donor solution to the acceptor phase (located in a porous polypropylene membrane envelope) and then further extracted to a small volume of organic solvent. The extraction time required in EME process was 10 min. And due to fast mass transfer between the acceptor phase of EME and the final organic solvent, the second step took only 1 min. Then the organic extract was collected and injected into GC-MS system with 1 μL of derivatization reagent N-methyl-N-*tert*-butyldimethylsilyltrifluoroacetamide (MTBSTFA). The

LODs obtained ranged between 0.012 and 0.037 μg/mL.

To summarize, DLLME and electro-enhanced techniques were explored and developed in this thesis. They were favored due to their short extraction time according to their extraction principles. The modification of DLLME was mainly on the utility of low-density solvents, facilitation of emulsion and convenience of retrieval of organic extract. The modification of electro-enhanced techniques was mainly on its connection with another technique to overcome some drawbacks. The results presented in this thesis showed that these proposed methods could serve as alternative approaches to conventional sample preparation techniques for the fast determination of organic contaminants from environmental water samples.

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LIST OF ABBREVIATIONS

Ace	Acenaphthene
ACN	Acetonitrile
Ant	Anthracene
BSTFA	Bis-(trimethylsily) trifluoroacetamide
CAR/PDMS	Carboxen/Polydimethylsiloxane
CE	Capillary electrophoresis
CMC	Critical micelle concentration
2-CP	2-Chlorophenol
4-CP	4-Chlorophenol
CTAB	Cetyltrimethyl ammonium bromide
DAD	Diode array detection
2, 4-DCP	2, 4-Dichlorophenol
pp'-DDE	p, p'-Dichlorodiphenldichloroethylene
DEHP	di-(2-Ethylhexyl) phosphate
DLLME	Dispersive liquid-liquid microextraction
DLLME-SFO	Dispersive liquid-liquid microextraction based on solidification of a floating organic drop
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane
EA	Ethyl acetate
EE	Electro extraction

EE-SPME	Electro-enhanced solid-phase microextraction
EF	Enrichment factor
EME	Electromembrane microextraction
EME-VA-μ-LLE	Electromembrane microextraction coupled with vortex-assisted micro-liquid-liquid extraction
EMI	Electromembrane isolation
ER	Extraction recovery
ET-AAS	Electrothermal atomic absorption spectrometry
EU	European Union
Flt	Fluoranthene
Flu	Fluorene
GC-ECD	Gas chromatography coupled with electron capture detection
GC-MS	Gas chromatograph-mass spectrometry
HCH	Hexachlorocyclohexane
HPLC	High performance liquid chromatography
HPLC-FLD	High performance liquid chromatography coupled with fluorescence detector
HF-LPME	Hollow fiber-protected liquid-phase microextraction
HLB	Hydrophilic-lipophilic balance

ICP-MS	Inductively coupled plasma-mass spectrometry
IPNB	1-Isopropyl-4-nitrobenzene
ITIES	Interface between two immiscible electrolyte solution
LDS-SD-DLLME	Low-density solvent based solvent demulsification dispersive liquid-liquid microextraction
LDS-UASEME	Low-density solvent based ultrasound-assisted surfactant enhance emulsification microextraction
LDS-USAEME	Low-density solvent based ultrasound-assisted emulsification microextraction
LLE	Liquid-liquid extraction
LLLME	Liquid-liquid-liquid microextraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
MIP	Molecularly imprinted polymer
MOF	Metal-organic framework
MSTFA	N-methyl-N-(trimethylsilyl)-trifluoroacetamide
MTBSTFA	N-(*tert*-butyldimethylsilyl)-N-methyl-trifluoroacetamide
MWCNTs	Multiwalled carbon nanotubes

NaCl	Sodium chloride
NaOH	Sodium hydroxide
Nap	Naphthalene
3-NP	3-Nitrophenol
NPOE	2-Nitrophenyl octyl ether
NSAID	Non-steroidal anti-inflammatory drug
OCP	Organochlorine pesticide
OPP	Organophosphorus pesticide
PA	Polyacrylate
PAH	Polycyclic aromatic hydrocarbon
PDMS	Polydimethysiloxane
PDMS/DVB	Polydimethylsiloxane/Divinylbenzene
Phe	Phenanthrene
POP	Persistent organic pollutant
PP	Polypropylene
Pry	Pyrene
RSD	Relative standard deviation
SAEME-VA-μ-SPE	Sonication-assisted emulsification microextraction combined with vortex-assisted micro-solid-phase extraction
SBME	Solvent bar microextraction

SBSE	Stir bar sorptive extraction
SDME	Single-drop microextraction
SDS	Sodium dodecyl sulfate
SFOD	Solidification of floating organic drop
SIM	Selective ion monitoring
SLM	Supported liquid membrane
S/N	Signal-to-noise
μ-SPE	Micro-solid-phase extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SD-DLLME	Solvent demulsification dispersive liquid-liquid microextraction
ST-DLLME	Solvent terminated dispersive liquid-liquid microextraction
TCA	Tricyclic antidepressant
2, 4, 6-TCP	2, 4, 6-Trichlorophenol
UASEME	Ultrasound-assisted surfactant-enhanced emulsification microextraction
UASEME-SFO	Ultrasound-assisted surfactant-enhanced emulsification microextraction with solidification of floating organic solvent

USAEME	Ultrasound-assisted emulsification microextraction
US EPA	United States Environmental Protection Agency
UV	Ultraviolet detection
ZIF-8	Zeolite imidazolate framework 8

Chapter 1 Introduction

1.1 Sample preparation

An analytical method typically consists of various processes including sampling, sample preparation, isolation of target compounds, detection and data analysis. Among them, sample preparation is possibly the most time-consuming process. It is usually the primary source of errors and discrepancies between different laboratories as well [1]. Therefore, it is commonly considered as the bottleneck in an analytical process.

Environmental analysis has gained increasing attention due to potential risk concerns about chemical contaminants to human health as well as the wildlife. Generally, the concentration of target analytes in environmental matrix can be very low, down to parts per million (ppm), parts per billion (ppb), or even in some cases parts per trillion (ppt). Therefore, it becomes a challenging task to sensitize analytical techniques. Another problem is that environmental samples usually cannot be directly handled by detection instruments. Based on these characteristics of environmental analysis, sample preparation is crucial to the whole analytical procedure. The main purpose of sample preparation is to clean up, isolate and preconcentrate target compounds in a medium that is compatible with the detection instruments. Despite many efforts devoted to

improve instrumental techniques, the standard sample pretreatment procedures remained unchanged until past 20 or so years. Until recently, liquid-liquid extraction (LLE) was still the most commonly used procedure. It should be noted that large amount of organic solvent is required in LLE and the subsequent evaporation of the extract down to a small volume also entails additional effort. Therefore, LLE is regarded as expensive, tedious and environmentally unfriendly.

Solid-phase extraction (SPE) became commercially available in the mid 1970s as an alternative approach. Even though it does not require as much solvent as LLE, drawbacks such as tedious column conditioning and relatively high cost still exist.

To overcome these disadvantages, a lot of effort has been devoted to develop simple, fast and miniaturized sample pretreatment methods over the past two decades. Among them, liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) have received considerable attention, due to their low consumption of organic solvent, simplicity, short extraction time and high enrichment capabilities. They have been successfully adopted to extract different classes of compounds from various kinds of environmental samples.

SPME is almost a solvent-free process. It is reliable, simple and easy to automate. However, one major disadvantage is its relatively long extraction time. Other problems including fragility of SPME fiber, short life span and analyte carry over issues also limits its application. LPME was introduced in 1990s, and regarded as a big breakthrough in the development of sample preparation techniques. It can be classified as different modes including single-drop microextraction (SDME), dynamic LPME, solvent-bar microextraction (SBME), and dispersive liquid-liquid microextraction (DLLME). Among them, DLLME, which was developed by Rezaee et al. in 2006 [2], has been successfully adopted to extract many contaminations such as PAH [3], organophosphorus pesticides (OPPs) [4], chlorophenols [5], etc. Due to the considerably large surface area between extraction solvent and the aqueous sample, the equilibrium state can be achieved very rapidly and the extraction time can be greatly shorten (less than 5 min in most cases). However, the solvents involved in this process must have a density higher than water, so that they can be easily collected after centrifugation. Normally these kinds of solvent are chlorinated ones, which are not beneficial for the health of the operator as well as the environment. Due to these disadvantages, a lot of effort has been devoted to the modification of conventional DLLME.

The disadvantage of a long extraction time also led to the development of

EME. It is a promising microextraction approach, greatly reducing extraction time owing to the acceleration of mass transfer under external electrical potential. Extraction parameters including voltage applied, the component of SLM, as well as the ionization degree of target analytes are very important to EME development. Analytes normally are extracted from the donor phase through the SLM to the acceptor phase. Since the compounds should be in their ionized forms both in the donor and acceptor phase, this approach is more compatible with detection instruments such as HPLC or CE. However, some compounds may show better LODs when detected by GC-MS (with or without derivatization). Therefore, the connection of EME with other instruments like GC-MS could be worthwhile.

In the following section, the development of SPME and LPME are briefly reviewed. Different modes of LPME are explained in detail, especially the development of DLLME and EME,

1.2 Solid-phase microextraction

SPE is a well-established sorbent based extraction method, in which target compounds can be retained in a sorbent and separated from other compounds. The sorbent should have strong affinity towards target compounds relating to

their physical or chemical interaction. Therefore, the choice of an appropriate sorbent is crucial to the whole process. It has long been used to isolate target analytes from a variety of matrices, including water samples, food, soil, bio-fluid, animal tissue, etc.

Figure 1-1 shows the basic procedure of SPE. In SPE, some solvent is first introduced to condition the cartridge, wetting the surface and washing through the column. The sample is then loaded onto the cartridge; target analytes interact with the sorbent in the cartridge and are retained while other impurities and solvent itself pass through the cartridge. Later, the cartridge is washed by a buffer solution to further remove impurities. Finally, the analytes are eluted using an appropriate solvent.

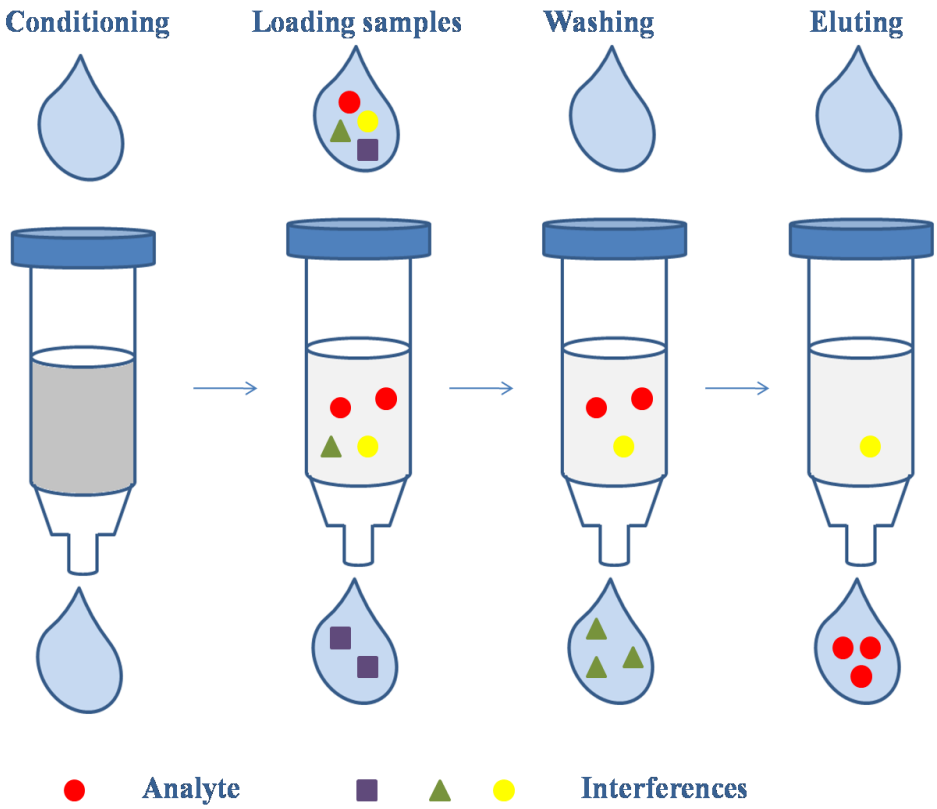


Figure 1-1 Basic process of SPE

Compared to LLE, SPE uses less organic solvent and could avoid some problems such as emulsification. However, the volume of solvent required is still significant and the extraction time is relatively long. A miniaturized sample pretreatment method termed SPME was then developed as an alternative. SPME was first introduced by Pawliszyn's group [6] and quickly commercialized in the early 1990s. It is almost a solvent-free technique, incorporating sampling, extraction and sample introduction in one step. It overcomes many drawbacks of conventional SPE and has lots of advantages including portability, simplicity to operate, fast and ease of automation. SPME process is based on the establishment of distribution equilibrium of target

analytes between the sample and an SPME fiber, which consists of a stationary phase commonly coated onto a fused silica fiber or a stainless steel. After extraction, the analytes can be desorbed from the fiber thermally, which is compatible with GC-MS. Alternatively, the analytes could also be solvent-desorbed, making the procedure accessible to HPLC or CE. SPME can be classified into several types, including on-fiber SPME, in-tube SPME, SBSE, microextraction in a packed syringe and micro-solid-phase extraction (μ-SPE). Among them, on-fiber SPME is the most popular one. There are several kinds of commercially available SPME fibers. SPME fibers produced by Supelco have coatings such as polydimethysiloxane (PDMS) for volatiles, polyacrylate (PA) for polar semi-volatiles, Carbowax for alcohols and polar compounds, Carboxen/polydimethylsiloxane (CAR/PDMS) for gases, polydimethylsiloxane/divinylbenzene (PDMS/DVB) for amines, and divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) for flavor compounds, etc. SPME has gained a huge amount of attention, with thousands of articles related to SPME being published in the last two decades. It has been successfully applied to extract persistent organic pollutants (POPs) from different sources including environmental samples [7-10], food [11], biological fluids [12-14], tissue samples [15, 16], etc. It is highly effective, reliable, simple to operate, and can be easily automated when coupled online with detection instruments, especially GC.

However, SPME still has some limitations. First of all, the commercially available SPME fibers are not suitable to extract every class of compounds. The fiber is relatively fragile and could easily be broken. They cannot be used under extreme conditions like high temperature or extreme pH. The swelling of coatings in organic solvent is another problem. The concentration of non-polar organic solvent must be controlled at a very low level to ensure the reproducibility and reliability. Secondly, the quality of fibers differs from batch to batch, greatly influencing its reproducibility. Conditioning according to the manufacturer is needed in the first time of usage or when it has been put aside for a period of time. Even use with care, the life span of the fiber is still limited. After a certain time, bleeding of the coating may occur and affects the extraction performance. Another problem is analyte carry-over, which is commonly observed in SPME. Normally, the fiber will be reconditioned at high temperature or dissolved in solvent between extractions to reduce its influence, but this might also affect the life span of the fibers.

These problems mentioned above lead to poor reproducibility and narrow linearity in SPME. Thus, a lot of effort has been devoted to improve SPME by either modifying the fiber coating or developing novel modes of SPME.

Several innovative modes of SPME have been developed, including SBSE

[17], microextraction in a packed syringe (MEPS) [18], μ-SPE [19], polymer-coated hollow fiber membrane microextraction, etc. The processes are similar, the major differences being how the stationary phase or sorbent is coated and the nature of the coating material. Among all the new materials, molecularly imprinted polymer (MIP) [20] and nanomaterials like carbon nanotubes or metal-organic framework (MOF) [21] have gained considerable interest.

Aside from the coating materials, one promising SPME mode termed μ-SPE was first introduced in our laboratory [22]. Basically, a sealed polypropylene (PP) membrane envelope containing small amount of sorbent (6 mg of multiwalled carbon nanotubes (MWCNTs)) was used to extract OPPs. The porous PP membrane aids to protect the sorbent, acting as a filter to exclude extraction of extraneous compounds from sewage sludge samples. The extraction performance of μ-SPE was compared against other miniaturized methods like hollow fiber protected solid-phase microextraction and headspace SPME. This method was demonstrated to be fast, accurate and cost-effective. According to the authors, the device could be used up to 30 times without obvious carryover problems, thus overcoming some disadvantages of on fiber SPME. To date, this method has been successfully applied to extract estrogens, berberine, PAH, acid drugs, sulfonamides and

other compounds from different matrices [23-27]. For example, Wang and coworkers had combined μ-SPE with microwave-assisted extraction by using activated carbon not only as the sorbent in μ-SPE, but also as the microwave absorption medium. It was successfully adopted to extract OPPs in vegetables and fruits [28].

1.3 Liquid-phase microextraction

A complementary miniaturized sample pretreatment technique, LPME, was developed in the 1990s. In LPME, extraction is based on the distribution equilibrium between a small amount of water-immiscible extraction solvent and aqueous sample. Normally, the volume of the extraction solvent is in microliter range. Therefore, the enrichment factors could be relatively high due to the high ratio of sample volume to acceptor phase volume. LPME can be classified mainly into SDME, hollow fiber protected liquid-phase microextraction (HF-LPME), liquid-liquid-liquid microextraction (LLLME), DLLME and a special mode called EME. EME works like three-phase LPME with the driving force from electrical field. In the following section, the development of various types of LPME is discussed.

1.3.1 Single drop microextraction

SDME is one of the earliest modes of LPME. The extraction is performed using a single droplet of extraction solvent suspending in or above the aqueous donor solution. The volume needed (typically 0.5-8 μL) is very small, therefore greatly increasing enrichment factors. The mass transfer of target analytes from the donor solution to the microdrop of solvent is based on passive diffusion. After extraction, the droplet is retracted back into the syringe and injected directly into the analytical instruments such as GC-MS.

SDME could be carried out using headspace mode (HS mode) or direct immersion mode (DI mode). As the name suggests, HS mode is performed by suspending the extraction solvent droplet in the headspace of the sample solution. This mode is suitable to extract volatile or semi-volatile compounds, since the target analytes can be easily vaporized and exist in the headspace. On the other hand, DI mode is performed by suspending the extraction solvent directly into the aqueous solution. It is suitable for compounds with medium polarity. DI mode can also be divided into two types: one is the conventional way with solvent immersion into aqueous solution; the other is using an aqueous acceptor phase immersed in the organic phase floating on top of an aqueous donor solution [29]. For example, in Zhu et al.'s work, aromatic amines was first extracted to 150 μL of ethyl acetate from 2 mL of water

sample and then further extracted into 2 μL of acidic aqueous solution within a Telfon ring. The enrichment factors obtained ranged between 218 and 378, with LODs ranging from 0.85 to 1.80 μg/L.

SDME was first introduced by Jeannot and Cantwell in 1996 [30]. Later in 1997 He and Lee suggested that SDME using conventional microsyringe with an angled-cut needle tip could increase drop stability. The improvement could be explained by the increase of cross sectional area and consequent increase of adhesion force between the needle tip and the droplet [31]. Other modes of SDME includes dynamic SDME, static SDME and ionic liquid based SDME which were reported by Zhang, He and Lee [32, 33]. This extraction technique is considered to be simple, cost-effective and environmentally friendly (due to the low consumption of organic solvent). It has been widely applied to extract POPs, coupled with GC, LC, CE as well as inductively coupled plasma-mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ET-AAS) [34, 35]. However, despite all the advantages mentioned above, its application could be limited by some reasons: (i) the instability of the microdrop, (ii) drop dissolution or dislodgement due to long extraction time or fast stirrer speed, (iii) low sensitivity or reproducibility, etc.

1.3.2 Hollow fiber protected LPME

Low cost, porous hollow fiber (typically made of PP) was introduced to enhance the stability of organic solvent in SDME, and a new method termed HF-LPME was developed. As can be seen from the Figure 1-2 [36], it can be classified into two modes: two-phase HF-LPME or three-phase HF-LPME.

In two-phase HF-LPME, the organic solvent used to fill the pores of hollow fiber (HF) is the same as the extraction solvent in the lumen. In three-phase HF-LPME, the target analytes are first extracted to the thin film of organic solvent immobilized in the pores and then further extracted into the aqueous solution in the lumen of the HF. Therefore, this mode is more commonly combined with HPLE or CE analysis.

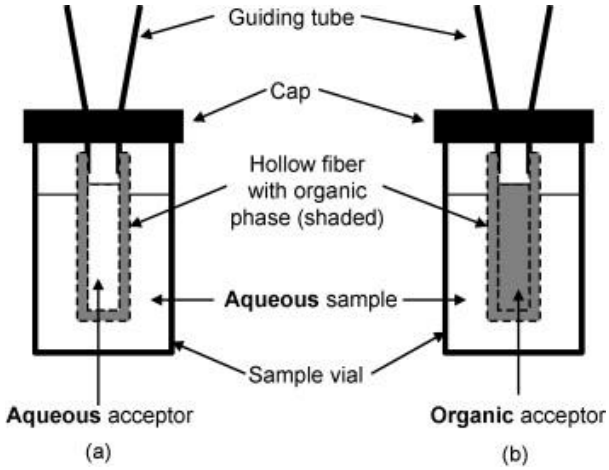


Figure 1-2 Schematic of HF-LPME [36].

HF-LPME could be performed in either static or dynamic mode. In static

mode, HF containing the acceptor phase is immersed into the donor solution using a syringe, while in the dynamic mode, HF is attached to a syringe connected to a programmable pump [37, 38]. Zhang and Lee used a programmable syringe pump to move the acceptor phase participating in the extraction so as to facilitate the extraction process. Extract together with 1 μL of derivatization reagent were injected directly into a GC-MS system for analysis, providing satisfactory LODs (down to 0.01 μg/L). Comparison between dynamic LPME and static LPME indicated that dynamic LPME provided much higher enrichment factors.

In HF-LPME, the HF served as a filter separating the acceptor phase from the donor solution, resulting in possible application on analysis of complex sample matrices. Another advantage of this method is that good extraction stability and reproducibility were obtained against high agitation speed or solvent loss since the acceptor phase is protected by HF. However, HF-LPME still has some drawbacks including long extraction time (typically 20 to 60 min), difficulty in automation and extra work related to the preparation of the HF. More recently, studies have demonstrated its practicality for preconcentration of compounds of divergent polarity and extraction times could be shorten with the application of a potential difference between the donor and acceptor phase [39, 40]. This method is referred as EME and is

discussed in detail in Chapter 1.3.4.

1.3.3 Dispersive liquid-liquid microextraction

DLLME was introduced by Assadi and co-workers in 2006 [2] and regarded as a milestone in the development of miniaturized sample preparation techniques. It quickly attracted a huge amount of attention and has become a well-used procedure. To date, it has been adopted to extract PAHs [41, 42], phenols [43-46], pesticides [47-49], inorganic elements [50-53], pharmaceutical compounds [54-57], emerging pollutants [58, 59] and other POPs in different matrices. DLLME involves several steps: (i) rapid injection of the mixture of organic solvent and disperser into the sample solution, (ii) formation of a cloudy solution containing fine droplets of solvent, (iii) subsequent centrifugation, breaking down the emulsion and enhancing phase separation, and (iv) collection of sedimented organic solvent. Figure 1-3 depicts the basic procedure of DLLME [60].

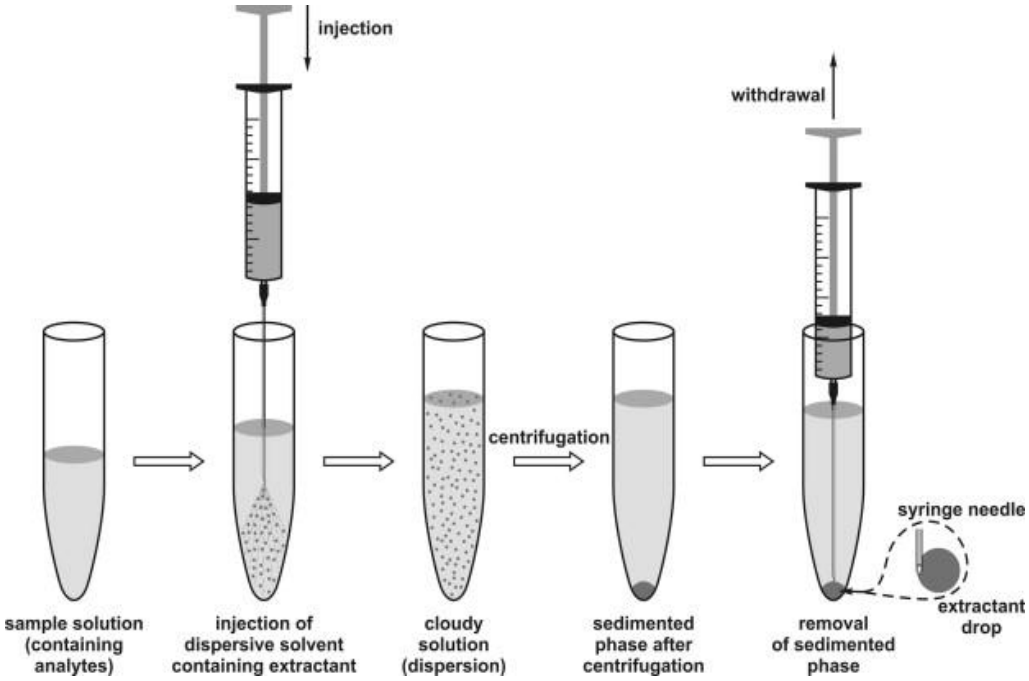


Figure 1-3 Dispersive liquid-liquid microextraction procedure [60].

The major advantage of DLLME is short extraction time due to a very large surface area between the fine droplets of the solvent and the sample solution. The equilibrium state is achieved quickly and the extraction is considered independent of time. DLLME is simple, easy to operate, and highly efficient. The volume of organic solvent required is very low; therefore the enrichment factors obtained are usually high.

In DLLME, an appropriate dispersive solvent is required to help the formation of fine extractant droplets and their dispersion into the aqueous solution. The disperser should be miscible with both the extraction solvent and the donor solution. However, on the other hand, the use of a third component (the disperser) could decrease the partition coefficient of analyte into the extraction

solvent [61] and reduce extraction efficiency. Another limitation of DLLME is that the phase separation is achieved by centrifugation. Therefore, the organic solvents employed should have densities higher than water to facilitate extract collection. Thus, majority of extraction solvents used in DLLME are limited to these halogenated hydrocarbons, (e.g. chlorobenzene, carbon tetrachloride, tetrachloroethylene, etc.) which are not desirable due to health and environmental concerns.

To overcome these abovementioned drawbacks, several promising modifications are developed. They can be classified into three approaches and reviewed in the following sections: (i) extraction using low-density solvent and development of appropriate extractant retrieval method such as modification of device or application of dispersive μ-SPE, (ii) modification of phase separation method such as SD-DLLME and DLLME-SFO, and (iii) facilitation of emulsion, such as with aid of ultrasound, vortex and surfactant.

1.3.3.1 Approaches involving retrieval of low-density solvent

One modification of DLLME involves the use of low-density solvents which is environmentally friendlier than their chlorinated counterparts and the development of its retrieval using customized extraction devices. In Farajzadeh et al. ’s work [62], a mixture of cyclohexane (extraction solvent)

and acetone (disperser) was used. As shown in Figure 1-4 A, after centrifugation, 0.4 μL of the cyclohexane was collected on the upper layer of the extraction vessel and injected directly into GC system. Hashemi et al. introduced a device containing a home-made narrow-necked glass tube into a centrifuge tube [63] (as shown in Figure 1-4 B). The glass tube was about 10 cm in length, 12 and 4 mm in body and neck diameter. After extraction and centrifugation, the extract *n*-hexanol containing target analytes glycyrrhizic acid would float to the narrow neck by carefully adding an appropriate amount of water. Then the extract was withdraw and injected into HPLC system. The principle of the third device developed by Saleh et al. [64] is similar to those discussed earlier. In their work, ultrasonic radiation was employed instead of a disperser to accelerate emulsion formation. After centrifugation, the solvent (toluene) transferred to the capillary tube attached to the top of the vial and was collected with addition of water into the vial through another tube. It was applied to analyze PAHs using GC-FID, with enrichment factors (EFs) reaching 2714 fold. Later in 2011, Zhang [65] et al. used a modified round-bottomed flask (shown in Figure 1-4 D) to retrieve 1-octanol after extraction. In their work, after injection of the organic solvent, the mixture was agitated by a magnetic stirrer for 20 min. 1-Octanol floated above the aqueous solution within 5 min once the stirrer stopped. By tilting the flask and adding some water into the device through the top-port, the liquid level

elevated. At the same time, the extract solvent (1-octanol) would accumulate in the narrow branch tip of the flask, and could be easily collected. This method was successfully used to extract UV filters from environmental samples.

The retrieval of low-density solvents in these approaches was based on accumulation of extraction solvent after phase separation in a narrow-diameter part of the device, and they were published almost at the same time. The differences amongst these approaches are very small, only in the size and form of the narrow upper part of the devices. The home-made devices facilitate the use of low-density organic solvents for DLLME, but increase operational complexity due to the special design and manufacture of the devices.

More recently, a flexible and disposable polyethylene pipette was introduced by Guo and Lee [66, 67] as an extraction device. In their work, the collection of organic solvent could be easily achieved by squeezing the bulb of the upside down pipette after centrifugation. The organic solvent floating on the upper layer of the solution was pushed to the narrow stem of the pipette with an appropriate pressure to the body of the device. It could be employed in other DLLME based techniques including solvent demulsification dispersive liquid-liquid microextraction (SD-DLLME) [66], ultrasound-assisted

emulsification microextraction (USAEME) [67] and ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) [68]. The polyethylene pipette is commercially available and cost-effective, serving as a convenient extraction device when using less toxic, low-density solvents.

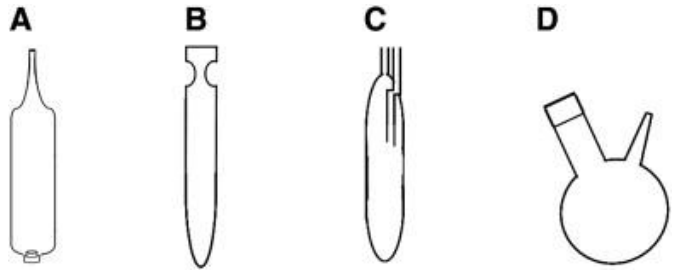


Figure 1-4 Special customized extraction devices for LDS-DLLME. [69]

Another approach for easy retrieval of low-density solvent is μ-SPE or dispersive μ-SPE. In Shi et al.'s work [70], magnetic nanopaticles were served as dispersive μ-SPE sorbent to extract PAHs enriched 1-octanol. After extraction, a magnet was used to isolate the magnetic nanopaticles, and the sample solution was simply discarded. Then 100 μL of acetonitrile was introduced to desorb 1-octanol as well as PAHs under sonication. The supernatant was collected using a pipette with the magnet placed again next to the vial to immobilize the nanoparticles. In Ge et al.'s work [23, 24], a μ-SPE device was prepared by introducing small amount of sorbent (zeolite imidazolate framework 8 (ZIF 8)) into a heat-sealed polypropylene membrane envelope and used to retrieve organic extract. After extraction, the device was

simply removed from the aqueous solution. Analyte desorption was conducted under sonication using a small volume of organic solvent. This method has been successfully applied to extract acidic drugs and PAHs.

1.3.3.2 Solvent demulsification DLLME

In 2010, Chen et al. described a new method termed low-density solvent-based solvent terminated dispersive liquid-liquid microextraction (ST-DLLME) for the determination of carbamate pesticides in water samples [71]. In their work, the emulsion formed by adding mixture of organic solvent and a disperser, and was broken down by adding a second portion of disperser as the demulsifier. The extraction solvent applied was toluene (with density lower than water). Toluene floated on the top and was retrieved easily after the emulsion cleared to give two phases. Later in the same year, Zacharis and co-workers introduced a very similar procedure called SD-DLLME [72], the only difference being that the extraction vial (a 10 mL volumetric flask) was agitated with a magnetic stirrer instead of using a dispersive solvent. Later, a flexible and disposable polyethylene Pasteur pipette was used as the extraction device in SD-DLLME by Guo and Lee [73].

The main advantage of SD-DLLME is that the second portion of disperser

which works as the de-emulsifier facilitating phase separation after extraction. Thus centrifugation is no longer a necessary process. This makes the entire analysis more convenient and faster. There is no requirement for any special home-made extraction devices. However, an obvious disadvantage has also been reported: the use of relatively large amount of disperser could cause a partial dissolution of the target analyte in the aqueous solution, leading to a relatively low recovery and reproducibility.

1.3.3.3 DLLME based on solidification of a floating organic drop

A novel liquid-phase microextraction method based on the solidification of a floating organic drop was first introduced by Zanjani et al. [74]. Eight microliters of 1-undecanol was injected into the sample solution. After a certain extraction time, the sample vial was cooled under ice bath. 1-Undecanol solidified in 5 min and was collected conveniently. By using special extraction solvents like 1-dodecanol, 1-undecanol, etc., which can be easily solidified in an ice bath, the extract can be simply collected without centrifugation. In the method proposed by Zanjani, a magnetic stirrer was used to facilitate extraction. Subsequently, a method combining the benefits of DLLME and utility of easily-solidified floating organic solvent was developed [75, 76]. In this method, a disperser was used to replace the magnetic stirring,

and this novel technique was termed as dispersive liquid-liquid microextraction based on solidification of a floating organic drop (DLLME-SFO). It combines merits of DLLME and SFO techniques. However, a major disadvantage which is the limitation of solvent choice still exists. Solvents in DLLME-SFO need fulfill some requirements: (i) good extraction capability towards the target analytes; (ii) low volatility to avoid loss of solvent during extraction; (iii) an appropriate melting point near room temperature; (iv) good chromatography performance and (v) compatibility with detection systems. 2-Dodecanol, 1-dodecanol, hexadecane, 1-undecanol are commonly used solvents.

DLLME-SFO could be performed with ultrasonic radiation [77, 78] or surfactant [79] to facilitate emulsion formation. Recently, Jia et al. reported a novel method combing in situ benzoylation and DLLME-SFO for the determination of biogenic amines [80]. In Kamarei et al.'s work, a ternary mixture consisting of a disperser, an extraction solvent, and a derivatization reagent was used for the simultaneous derivatization and extraction of aliphatic amines [81]. Coupled with various instruments including HPLC, GC, CE, and electrothermal atomic absorption spectrometry (EAAS), DLLME-SFO based methods have been successfully applied to extract PAHs, OCPs, phenols, hormones, OPPs, polychlorinated biphenyls (PCBs) and

inorganic compounds [41, 77, 82-86].

1.3.3.4 Improving emulsion formation without a disperser

As mentioned before, the use of a third component (the disperser) could decrease the partition coefficient of analytes into the extraction solvent [61]. To overcome this disadvantage, much attention has been paid to perform DLLME without a disperser. A novel microextraction technique, USAEME, was introduced by Garcia-Jares and co-workers [87]. In USAEME, ultrasonic waves were alternatively applied to facilitate a water-immiscible extraction solvent disperse into the aqueous sample. It enhanced the mass transfer between the two immiscible phases, accelerating emulsion formation without a disperser. This method was successfully applied to detect emerging compounds including synthetic musk fragrances, phthalate esters and lindane in water samples. LODs down to pg/mL level were obtained for most of the compounds. The procedure was demonstrated to be an efficient, simple and cost-effective approach. Subsequently, it was used to extract polybrominated diphenyl ethers (PBDE)s [88], PAHs [64], phenols [89], metals [90], pesticides [91] and other contaminants [64, 88-93].

Normally, the extraction time of USAEME is around 10 min at 25°C [88] and

5 min at 35 °C [94] according to early reports. The time needed is relatively short compared to some SPME and LPME processes. But it was found that the extraction time could be further shortened with the addition of an emulsifier. A surfactant was added in USAEME by Wu et al., and a new microextraction technique called UASEME was developed [4]. Surfactants, or surface-active agents, are usually amphiphilic organic compounds. They contain both hydrophobic tails and hydrophilic heads [95]. Therefore, they can enhance the dispersion of the water-immiscible extraction solvent into the aqueous sample, accelerating the formation of cloudy turbulence, resulting in a shorter extraction time. In Wu et al.'s work [95], 30 μL of Tween 20 at a concentration of 6.0×10^{-5} mol/L together with extraction solvent (150 μL of chloroform/chlorobenzene mixture ($CHCl_3$:C_6H_5Cl, 1:1, v/v)) was rapidly injected into a conical-bottomed glass tube. The extraction was completed within 3 min under sonication. After centrifugation, the sedimented phase was collected and injected into HPLC system. This method was demonstrated to be simple, efficient and robust to extract carbamates from water samples. Good repeatability, high enrichment factors and good recoveries were obtained. Later, it was adopted to extract OPPs by the same group [96]. Different types of surfactants were tested including sodium dodecyl sulfate (SDS), Tween 20, Triton X-100, Triton X-114, and their performance were compared with extraction without a surfactant. Among the surfactants investigated, Triton

X-100 gave the highest extraction recoveries. The authors explained that the effect of different surfactants on the extraction efficiency could be related to the hydrophilic-lipophilic balance (HLB) value of the surfactants and the hydrophobicity and polarity of the analytes. The surfactant serving as an emulsifier should has a HLB value between 8 and 18. The extraction efficiencies using SDS was even lower than those without a surfactant. This might due to an inappropriate HLB value (higher than 18). Triton X-100 might have a suitable hydrophobicity for most of the target OPPs, and selected as the final surfactant.

Figure 1-5 shows a schematic of UASEME using a lower-density solvent, introduced by Cheng and coworkers [97]. In their work, 20 μL of cyclohexane together with 10 μL of Tween 20 (0.5 g/L) was injected into the solution. The mixture was immersed into an ultrasonic bath for 1 min and the test tube was sealed using a rubber plug. The whole device was then placed upside down and centrifuged for 3 min. The organic solvent accumulated at the conical bottom, was collected and injected into the HPLC system. This was the first report using low-density solvent in UASEME to extract PAHs from water samples. Good LODs (down to 0.6 ng/L) were obtained. The procedure was also used to extract phthalate and estrogens, but the solvent used were conventional halogenated solvents [98, 99]. Then, very recently, a new method

was developed combining with solidification of floating organic drop (SFOD). Ultrasound-assisted surfactant-enhanced emulsification microextraction with solidification of floating organic solvent (UASEME-SFO) was applied to determine strobilurin fungicides in fruit juice samples.

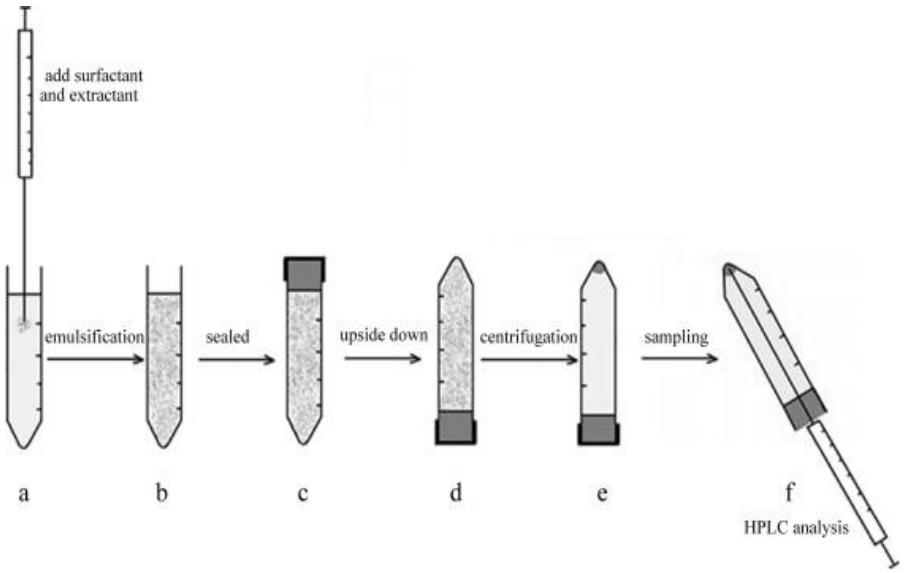


Figure 1-5 Schematic of UASEME [97].

1.3.4 EME

Mass transfer in LLE is basically dependent on the distribution coefficients of the target analytes between the donor phase and the acceptor phase. The migration through liquid-liquid interface is mainly controlled by passive diffusion. Therefore, the extraction time is relatively long, typically in the range of 30 to 60 min. Due to the requirement of short extraction time and high throughput of sample analysis in some field, some analytical chemists started to investigate electro-enhanced extraction system and it was found that

mass transfer of charged compounds could be accelerated with driving force from an electrical field.

The early attempt to involve external electrical field was carried out by Tjaden et al. [100-102] and the method was termed liquid-liquid electro extraction (EE). The target analytes were extracted from the organic media (ethyl acetate) into an aqueous media (aqueous solution adjusted to pH 5 using acetic acid-methanol) and analyzed by CE. An electrical field of 15 KV was required and some other disadvantages limited its applicability. For example, the analytes need to be prepared in an organic media; therefore an extra sample pretreatment step such as LLE is required. The small liquid-liquid interface, (8×10^{-5} cm^2 in the CE capillary) also limits the analyte fluxes. Later, in 2005, Arrigan et al. introduced another LLE approach involving electrical potential, where the target analytes were extracted from aqueous solution to an organic-gel phase (pseudo-liquid). It works similarly to a earlier reported system used for electrochemistry as the so called “interface between two immiscible electrolyte solution” (ITIES) and was termed ITIES extraction [103]. The extraction was performed by applying electrical potential over the phase boundary and the analytes were analyzed electrochemically by plotting the ion-transfer current in hydrodynamic voltammograms. The potential required is typically from -1 V to 1 V, much lower than those used in EE. This

difference is according to the difference of the conductivity of acceptor phase: the organic acceptor phase in EE serving as the donor solution was almost non-conductive while the organic gel served as the acceptor phase in the ITIES system could provide very high electrical conductance. The application of ITIES on the determination of several charged compounds included drugs and additives have been reported [104-106].

Later, a concept termed electro membrane isolation (EMI) or EME based on mass migration through a SLM under an electrical field was introduced by Pedersen-Bjergaard and Rasmussen in 2006 [107]. This system was quite similar to a three phase HF-LPME, except that the external electrical potential was applied between the donor phase in one side of the SLM and the acceptor phase in the other side. In their work, a polypropylene porous hollow fiber was used for immobilization of the artificial membrane. Basic drugs (pethdine, nortriptyline, methadone, and haloperidol) were extracted under a 300 V direct current (d.c.). To ensure complete ionization of the target analytes, the donor and acceptor phases were both acidified with 10mM hydrochloric acid (HCl). 2-Nitrophenyl octyl ether (NPOE) immobilizing in the pores of the hollow fiber and served as a stable SLM. Seventy to seventy nine percent of target analytes were extracted within 5 min. It was then tested on water samples, human plasma and human urine. Good recoveries were obtained. Therefore,

EME was demonstrated to be a very powerful alternative for the isolation, preconcentration, and clean-up of drugs from complex biological samples.

Later, this system was applied to extract some more polar basic drugs [108]. It was found that more polar basic compounds (with log P below 1.7) could not penetrate the interface between the sample and NPOE based SLM. However, di-(2-ethylhexyl) phosphate (DEHP) could work as an ion-pair reagent and facilitate the mass transfer of the ion-paired substance (with cationic analyte) across a thin SLM containing a mixture of NPOE and DEHP (75:25, w/w). It was also found that this NPOE/DEHP membrane was not suitable for other non-polar substance, suggesting the selectivity of this system could be easily controlled by selecting an appropriate SLM. Moreover, EME was also applied to extract acidic drugs [109]. In this work, the system differed slightly from the abovementioned ones since the negative electrode was placed in the donor phase while the positive one in the acceptor phase. NPOE was indicated to be ineffective and long-chain alcohols with strong proton acceptor properties including 1-hexanol, 1-heptanol, 1-octanol, 1-nonano, and 1-decanol were tested as membrane liquids. Good extraction efficiency was reported when 1-heptanol was selected. The equilibrium of extraction was achieved in 5 min under 50 V d.c. with recoveries ranging from 8 to 100%, indicating great potential of EME for rapid extraction of acidic drugs. More recently, EME was

extended to extract various compounds including biological anions [110], chlorophenols [111, 112], nerve agent degradation products [113], and peptides [114, 115].

In the first few works conducted by Pedersen-Bjergaard and Rasmussen, the extraction was carried out under 300 V [107, 116]. Then more recently, other works related to EME have been published using lower voltages such as 50 V [117] or even 10 V [118]. In one article, the EME was demonstrated to be highly effective to extract basic drugs from human plasma, urine and breast milk using a common 9 V battery [119]. The SLM in this report was immobilized with 1-isopropyl-4-nitrobenzene (IPNB), with which the drugs could more easily penetrate the sample/SLM interface. Low voltage is favored due to safety concerns, portability, and especially suitable to those compounds prone to electrolysis under high electrical potential.

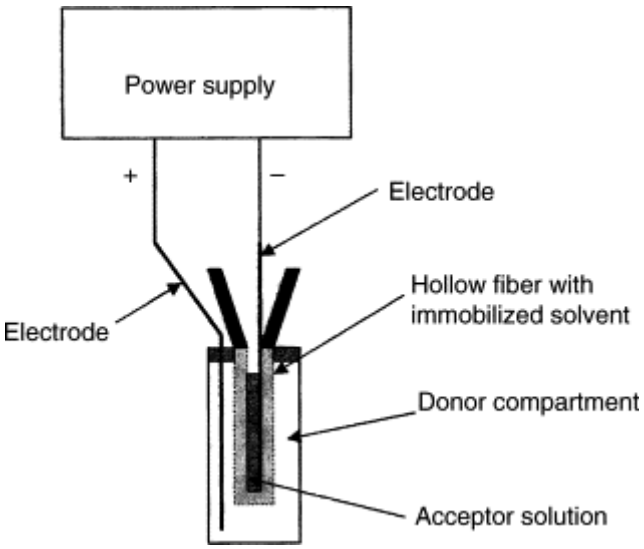


Figure 1-6 Schematic of EME [97].

1.4 Aims of this work

Microextraction technique represents a significant trend of sample preparation and it is favored by many researchers due to its simplicity, low consumption of organic solvent and minor labor-intensity. Enrichment factors are usually high due to the high ratio of sample volume to extraction solvent volume. However, some disadvantages still exist and improvements are urgently needed. For example, long extraction time is the major disadvantage for SDME, SPME and HF-LPME. Limitation of solvent choice in SFO based techniques also imposes restrictions on their application. While for DLLME, extraction solvent should have a density higher than water to facilitate extract retrieval. These halogenated hydrocarbons are usually not favored due to their potential risks on human health and the environment. In addition, centrifugation is

required, also limiting its application. Therefore, further development and investigation of miniaturized sample preparation techniques is of great importance and worthwhile.

To overcome the abovementioned shortcomings, the main objective of this thesis was to develop different modes of DLLME (LDS-SD-DLLME in Chapter 2, LDS-UASEME in Chapter 3 and SAEME-VA-μ-SPE in Chapter 4) and electro-enhanced techniques (EE-SPME in Chapter 5 and EME-VA-μ-LLE in Chapter 6). DLLME and electro-enhanced techniques are favored due to their excellent performance to reducing extraction time. These methods were used to monitor different kinds of POPs (e.g. PAHs, OCPs, phenols, TCAs, and NSAIDs) from environmental samples.

In Chapter 2, LDS-SD-DLLME was developed and demonstrated to be fast, efficient, simple to operate and robust. Environmentally friendlier low-density solvent was used. Additionally, phase separation was conducted by addition of a second portion of organic solvent. Therefore, centrifugation is no longer necessary, broadening its application (such as in on-site monitoring). However, a relatively large amount of solvent was used as emulsifier and demusifier, lowering the participation coefficients of analytes into the extraction solvent. This may also negatively influence the recovery and reproducibility. Therefore,

LDS-UASEME was investigated in Chapter 3, in which surfactant and ultrasonic waves were used to facilitate emulsion formation instead of a disperser. This method combines the merits of low-density solvent, ultrasonic radiation and surfactant. In addition, a polyethylene Pasture pipette was adopted as the extraction device, allowing a convenient retrieval of extract after extraction. An alternative way (μ-SPE) to retrieve low-density solvent was used in USAEME and SAEME-VA-μ-SPE was developed to extract substituted phenols (in Chapter 4). Mass transfer of analyte-enriched 1-octanol to μ-SPE sorbent was facilitated by vortex and then solvent desorption was conducted under sonication. There is no requirement of centrifugation compared with LDS-UASEME. In the second part of this thesis, two electro-enhanced microextraction methods were explored. EE-SPME (described in Chapter 5) overcomes a main disadvantage of SPME (viz. long extraction time) while remaining other advantages such as solventless, high sensitivity, and ease to automation. Lastly, EME-VA-μ-LLE was developed to extract NSAIDs from environmental water samples. EME and μ-LLE was connected to not only further concentrate analytes but also increase its sensitivity by detection using GC-MS after derivatization by MTBSTFA. The specific objectives of this thesis are as follows:

- Develop LDS-SD-DLLME followed by GC-MS for the determination of PAHs from environmental water samples

- Explore LDS-UASEME followed by GC-MS for the determination of OCPs from environmental water samples
- Apply SAEME-VA-μ-SPE followed by HPLC-UV for the determination of substituted phenols in environmental aqueous samples
- Introduce a novel EE-SPME followed by GC-MS for the fast determination of TCAs in environmental water samples
- Develop a two-step EME-VA-μ-LLE for the determination of NSAIDs from water samples

To Summarize, several different methods were developed for the fast determination of POPs in environmental water samples. The method studied in this thesis on the various modifications of DLLME and electro-enhance techniques. They are effective alternatives for the analysis of target analytes at trace levels from environmental water samples and offer several advantages such as extraction speed, simplicity, cost-effect, and environmental friendliness. Therefore, it is worthwhile to further investigate DLLME modification and electro-enhanced techniques and expend its application on fast determination of different kinds of POPs in environmental analysis.

Chapter 2 Low-density solvent based solvent demulsification-dispersive liquid-liquid microextraction

2.1 Introduction

PAHs have long been recognized as carcinogenic environmental contaminants. They could accelerate tumor activity or cause endocrine disruption [120, 121]. PAHs can be detected in many types of environmental matrices since they are discharged by many processes including fossil fuel combustion, oil spills, industrial processes and also through natural means [122]. Therefore, the EU (European Union) and the United States Environmental Protection Agency (US EPA) listed them as priority pollutants. However, due to their low concentration and complexity of environmental sample matrices, preconcentration and sample cleanup is crucial to improve the accuracy and sensitivity of any method used to analyze PAHs [123, 124].

Several sample preparation methods have been applied to extract PAHs, including LLE, SPE, SPME, and a few modes of LPME. Among them, DLLME has rapidly attracted attention since its introduction. In 2010, Zacharis and co-workers found out that the disperser can serve as a de-emulsifier and introduced a method called SD-DLLME. The second portion of disperser behaving as the de-emulsifier could promote physical

phase separation; thus centrifugation is no longer a necessary process, making the analysis easier and quicker [72].

In this work, we conducted SD-DLLME followed by GC-MS to extract PAHs using a disposable polyethylene 10 mL syringe instead of a volumetric flask. One main feature of the present procedure is that the syringe is used as the extraction device itself with no other vessel required. Disposable syringes are commercially available and were disposed after each extraction to avoid cross contamination. It should be noted that since the whole extraction process was performed in a single syringe. Therefore, when appropriately connecting it to a commercial autosampler system such as the CTC Analytics Combi PAL or Gerstel Multi-Purpose Sampler systems, the whole extraction could be achieved by just pressing one button, providing a promising solution for the automation of DLLME.

2.2 Experiment

2.2.1 Reagents and materials

PAHs (naphthalene (Nap), phenanthrene (Phe), anthracene (Ant), acenaphthene (Ace), pyrene (Pry), fluoranthene (Flt) and fluorene (Flu)) were purchased from Supelco (Bellefonte, PA, USA). The structures of these PAHs

were shown in Figure 2-1. High-performance liquid chromatography (HPLC)-grade acetone, methanol, acetonitrile, and *n*-hexane were purchased from Tedia Company (Fairfield, OH, USA). 1-Octanol was bought from Merk (Darmstadt, Germany), toluene and cyclohexane were purchased from Fisher (Loughborough, UK), and *o*-xylene was bought from Sigma-Aldrich (St. Louis, MO, US). Ultrapure water used in the experiment was obtained from a Nanopure water purification system (Barnstead, Dubuque, IA, USA).

The 10 mL disposable syringe used in this work is manufactured by Norm-Ject (Tuttlingen, Germany). A 10 μL microsyringe (purchased from SGE Company (Sydney, Australia)) was used for retrieval and injection of the organic extract.

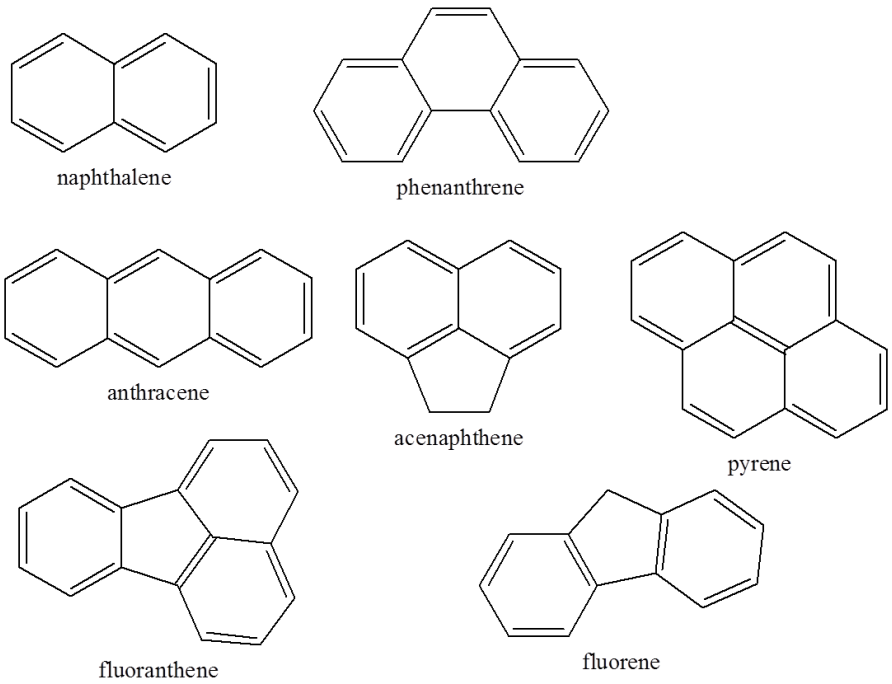


Figure 2-1 Chemical structures of PAHs considered in this study.

2.2.2 GC-MS analysis

GC-MS analysis was carried out on a Shimadzu (Kyoto, Japan) QP2010 system, equipped with a DB-5MS (J&W Scientific, Folsom, CA, USA) silica capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 μm). Helium (purity 99.9999%) was employed as carrier gas at a flow rate of 1.7 mL/min. The injection port temperature was set to 280 °C. The GC oven temperature was first set to 70 °C, held for 2 minutes, then increased to 190 °C at the rate of 15 °C/min, held for 1 minute, then programmed to 285 °C at 5 °C/min and held for another 5 minutes, and then programmed to 300 °C at 20 °C/min and held for 4 minutes. The interface temperature was maintained at 300 °C and the solvent cut time was 6 minute. One microliter of the sample was injected in splitless mode and selective ion monitoring (SIM) mode was adopted for quantitative determination of the compounds. The target ions selected were as follows: Nap, *m/z* 128; Phe, *m/z* 178; Ant, *m/z* 178; Ace, *m/z* 152; Pry, *m/z* 202; Flt, *m/z* 202; Flu, *m/z* 166.

2.2.3 Sample preparation

A stock solution containing all 7 PAHs (10 μg/mL) was prepared in methanol and stored in the refrigerator at 4 °C. The water samples were prepared daily by spiking ultrapure water with analytes at 25 μg/L. River water was collected

from the Singapore River, and rain water was collected at the Kent Ridge Campus, National University of Singapore. Tap water was collected in our laboratory. All these real environmental water samples were collected using pre-cleaned glass bottles, and stored in the refrigerator at 4 °C in dark without any pretreatment or filtration until analysis.

2.2.4 SD-DLLME protocol

As illustrated in Figure 2-2, in SD-DLLME, 10 mL of an aqueous solution containing all the 7 analytes was withdraw into a 10 mL disposable syringe. One milliliter of acetonitrile (as disperser) containing 40 μL cyclohexane (as extraction solvent) was then rapidly withdrawn into the disposable syringe. The mixture was then gently shaken and a cloudy solution was formed. After 2 minutes, a second portion of 1000 μL acetonitrile (as de-emulsifier) was withdrawn into the solution to promote phase separation. The cloudy solution separated into two phases quickly and the organic solvent floated on the top of the solution. By gently pushing the push rod, the organic solvent level elevated. Then the organic solvent was easily collected using a microsyringe. One microliter of the extract was then injected into the GC-MS system.

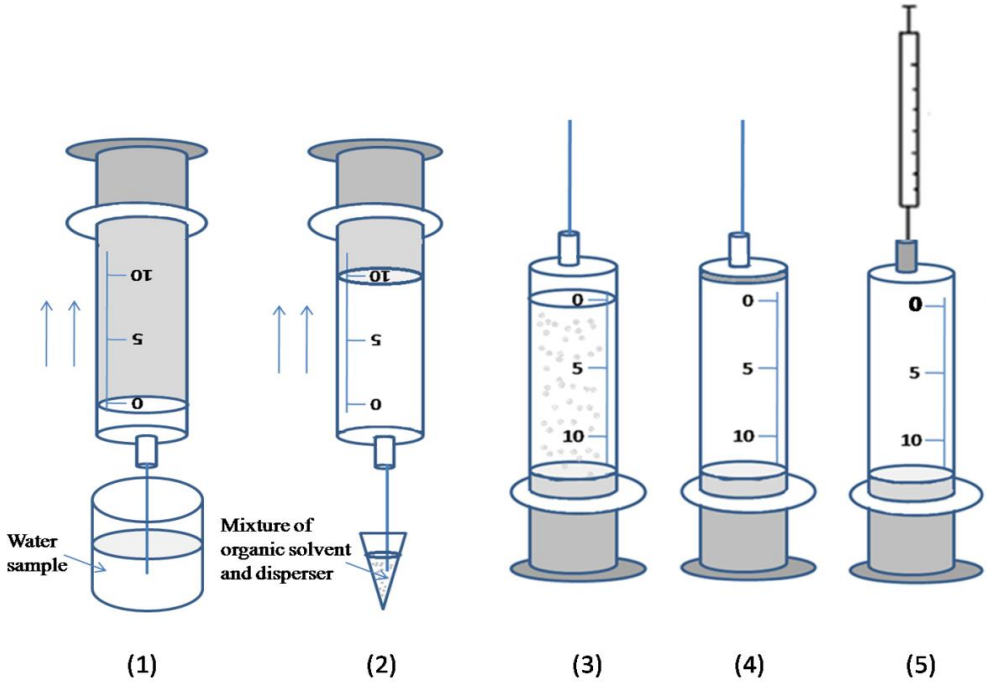


Figure 2-2 Schematic of SD-DLLME: (1) sample loading; (2) injection of mixture of disperser and organic solvent; (3) formation of cloudy solution; (4) injection of demulsifier, leading to phase separation; (5) collection of organic solvent.

2.3 Results and discussion

The extraction efficiency of SD-DLLME can be affected by various parameters such as type and volume of the extraction solvent, type and the volume of the disperser and de-emulsifier and the extraction time. We optimized the extraction conditions by investigating the extraction recovery (ER, %) under different extraction conditions using the "one-parameter-at-a-time" approach. All experiments were performed in triplicate. The extraction recovery (ER) is defined as the percentage of total analyte amount (n_0), extracted to the upper layer (n_{up}):

$$ER = n_{up}/n_0 \times 100 = \frac{C_{up} \times V_{up}}{C_0 \times V_{aq}} \times 100$$

C_{up}, C_0, V_{up}, and V_{aq} are the concentration of analytes in the upper layer, the spiked concentration of analytes in the aqueous solution, the volume of the upper layer (organic extract), and the volume of the aqueous sample, respectively.

2.3.1 Extraction condition optimization

2.3.1.1 Type of the extraction solvent

The choice of extraction solvent is of great importance in determining the overall efficiency of DLLME. The extraction solvent should have the following characteristics: (i) low solubility in water, (ii) high extraction efficiency, (iii) good GC performance, and (iv) low density (lower than the density of water) due to the requirement of the retrieval of the extract after the extraction. Therefore, the following solvents, toluene (density, *d*=0.865 g/mL), *o*-xylene (*d*=0.88 g/mL), *n*-hexane (*d*=0.659 g/mL), cyclohexane (*d*=0.779 g/mL), *iso*-octane (*d*=0.692 g/mL) were investigated. The extraction efficiencies of the above mentioned solvents were studied using standard aqueous solutions spiked with 25 μg/L of each PAH. Fifty microliters of each organic solvent together with 1000 μL acetonitrile (ACN) were withdrawn into the syringe. The solution was manually shaken gently for 2 min, and then

another 1000 μL acetonitrile was injected to break down the emulsion. Then the upper layer (organic extract) was collected and injected into the GC system. As can be seen from Figure 2-3, *n*-hexane gave highest extraction recoveries for most of the 7 PAHs. The high extraction efficiency of *n*-haxane is probably due to its low polarity. Based on this observation, *n*-hexane was chosen as the most suitable extraction solvent for the subsequent experiments.

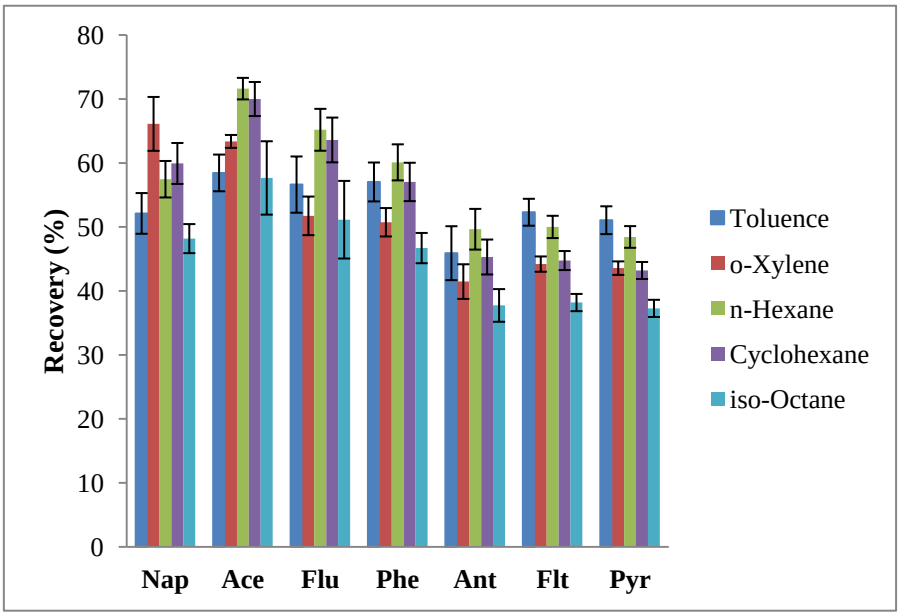


Figure 2-3 Effect of extraction solvent

2.3.1.2 Volume of the extraction solvent

Another important parameter is the volume of the extraction solvent because it influences the EFs. Extraction recoveries were studied as a function of different volume of *n*-hexane (ranged from 40 to 70 μL), and the results are shown in Figure 2-4. It can be seen that, for most of the 7 PAHs, the recoveries

increased with the increase of extraction solvent volumn within the range of 40-50 μL. The recoveries of the analytes remianed unchanged from 50 to 60 μL, and then dropped from 60 to 70 μL, which is consistent with observations reported in previous studies [71, 125]. Too little organic solvent (40 μL) might be problematical for the retrieval of organic solvent after extraction, while too much organic solvent would lead to dilution (reduction in EFs), and might compromise the sensitivity of the method. Based on these considerations, 50 μL was selected as the optimal volume of the extraction solvent.

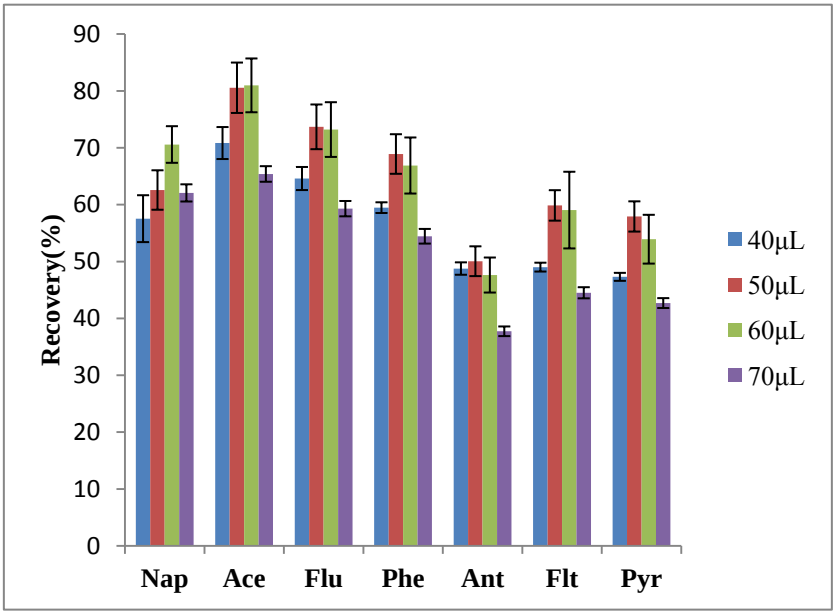


Figure 2-4 Effect of the extraction solvent volume

2.3.1.3 Type of the disperser and de-emulsifier

The emulsification and de-emulsification processes are important during SD-DLLME. Thus, the choice of the disperser and de-emulsifer which

influences the rate of extraction and the separation of the two phases after extraction, should be carefully considered. The miscibility with extraction solvent and the sample solution is the main consideration when choosing a suitable disperser and de-mulsifier. Therefore, in our work, three commonly used solvents were tested, namely acetone, acetonitrile, methanol. Each solvent was divided into two portions serving as both the disperser and de-emulsifier for the sake of simplicity. One milliliter of each disperser with 50 μL *n*-hexane were used for the extraction and a second portion of 1 mL was used to faciliate phase separation.

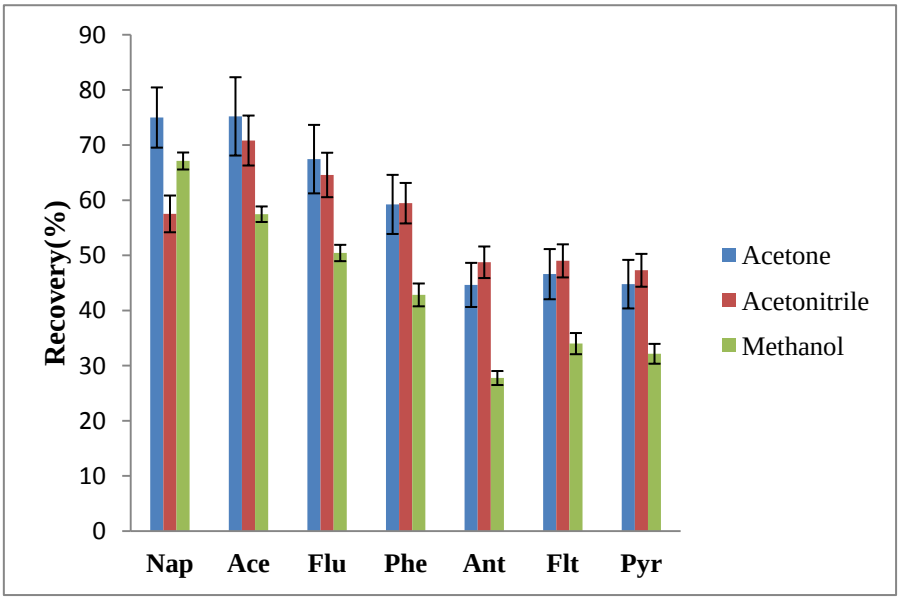


Figure 2-5 Effect of the disperser and de-emulsifier

From Figure 2-5, we can see that acetone and acetonitrile gave similar recoveries, followed by methanol. Then results indicated that acetone and acetonitrile were both suitable to assist the formation of cloudy solution as

well as phase separation, probably due to their low surface tension and high surface activity. Considering acetone's lower price, it was selected as the disperser and demulsifier.

2.3.1.4 Volume of the dispersive solvent and demulsifer

Furthermore, the effect of the volume of the disperser and demulsifer was examined by adding acetone at different volumes (500+500 µL, 750+750 µL, 1000+1000 µL, 1250+1250 µL). The first portion of disperser was mixed with 50 µL *n*-hexane and withdrawn into the syringe. Then after gently shaking for 2 min, the second portion of the acetone was introduced to demulsify the cloudy solution. The results revealed that higher extraction efficencies were obtained by adding 1000 µL+1000 µL acetone.

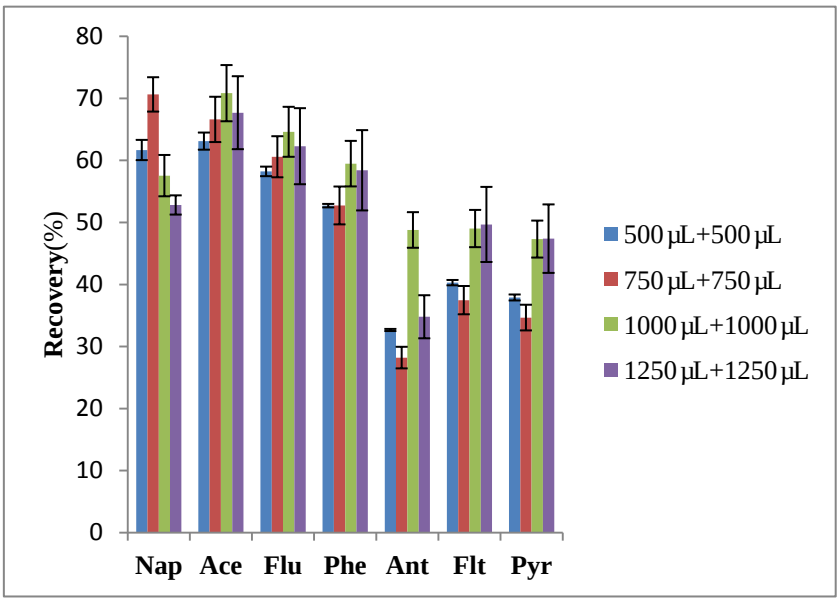


Figure 2-6 Effect of the disperser and emulsifier solvent volume

2.3.1.5 Extraction time

In SD-DLLME, the extraction time is defined as the time between the injection of the mixture of extraction solvent and disperser, and the addition of the second portion of solvent (the demulsifier solvent) [71]. Extraction times were evaluated based on 1-10 min durations.

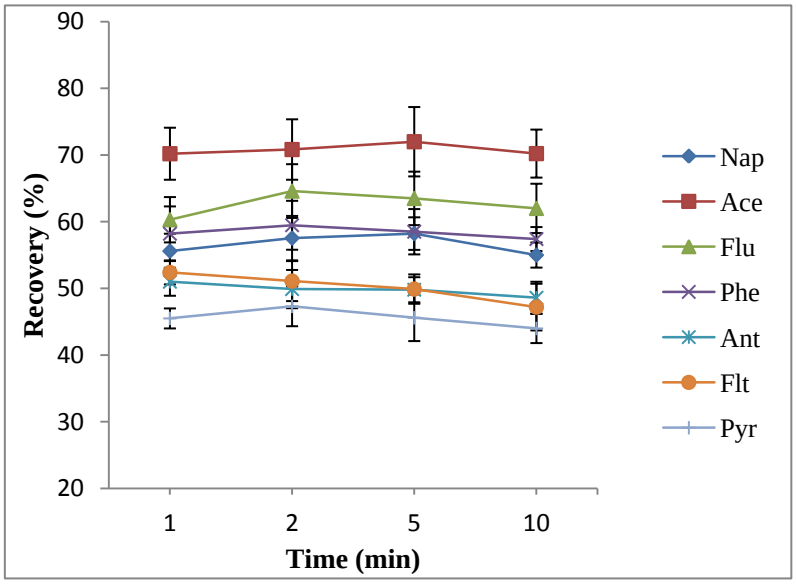


Figure 2-7 Effect of the extraction time

As can been seen from Figure 2-7, the extraction time has no significant influence on the recoveries. It was expected since one major advantage of DLLME based techinique is the short extraction time. Extraction solvent was in the form of fine droplets and the contact area between aqueous solution and the extraction solvent droplets was extremely large, greatly enhacing analytes migration to the extraction solvent. The extraction was considered to be

independent of time. The results showed that one minute appeared to be enough for the extraction of the PAHs. However, to ensure complete extraction, 2 min was selected as the most suitable extraction time.

Overall, the optimized SD-DLLME conditions were as follows: 50 μL of *n*-hexane as extraction solvent together with 1000 μL of acetone into 10 mL of aqueous solution, extraction for 2 min, a second portion of 1000 μL acetone as de-emulsifier solvent was introduced for phase separation.

2.3.2 Method validation

The applicability of the proposed method was evaluated under the most favourable extraction conditions. Validation parameters including the linear range, LODs, LOQs, relative recoveries and relative standard deviation (RSD) were calculated. The results are summarized in Table 2-1. The linearity was studied by plotting the mean peak area against sample concentration, and satisfactory linearity was obtained in the range of 0.1-100 μg/L for Nap, 0.05-50 μg/L for Ace, Flu, Phe, Flt and Pyr, 0.1-50 μg/L for Ant, with coefficients of determination ranging from 0.988 to 0.999.

The LODs calculated at a signal-to-noise ratio (S/N) of 3, ranged from 0.003 to 0.035 μg/L. The LOQs calculated at a S/N of 10, ranged between 0.01 and

0.1 μg/L. The reproducibility was examined in terms of intraday and interday precision, by studying water samples spiked with 5 μg/L of each analyte on the same day or on three consecutive days. As can been seen from Table 2-1, interday or intraday RSDs varied from 4.6% to 11.2% and 4.13% to 10.76% respectively.

Table 2-1 Linear range, LODs, LOQs and precision of the proposed method

Analyte	Linear range (μg/L)	Correlation coefficient (r^2)	LOD (μg/L)	LOQ (μg/L)	RSD[a] (%,n=5)	RSD[b] (%,n=5)
Nap	0.1-100	0.989	0.035	0.10	7.77	7.28
Ace	0.05-50	0.997	0.025	0.08	6.80	9.44
Flu	0.05-50	0.992	0.015	0.08	11.20	8.20
Phe	0.05-50	0.994	0.003	0.01	9.54	9.05
Ant	0.1-50	0.988	0.018	0.06	8.97	10.76
Flt	0.05-50	0.997	0.008	0.03	4.60	6.60
Pyr	0.05-50	0.999	0.008	0.04	5.52	4.13

RSD [a] s and RSD [b] s are intraday and interday reproducibility calculated from the sample spiked at 5 μg/L individually.

Table 2-2 shows a comparison between the proposed method and different published methods for the extraction of PAHs using DLLME, DLLME-SFO, HP-LPME, SBSE, and UASEME. As can be seen from the table, this proposed method showed comparable LODs with extraction using SBSE [126] and DLLME [127], and better than those using DLLME-SFO [128] and HF-LPME [129]. Compared with SBSE and HF-LPME, the extraction time needed in the proposed method was greatly reduced. Additionally, compared with conventional DLLME, the solvent used in this method was fast and efficient, and robust. Low-density solvent, *n*-hexane was employed, which was environmentally friendlier than halogenated solvents. Furthermore, no centrifugation or ultrasonic radiation was needed.

Table 2-2 Comparison of the proposed method with other methods for the determination of PAHs

Methods	Linear range (μg/L)	EF	LOD (μg/L)	RSD (%)	Ref.
DLLME-SFO-HPLC-UV	5.0-50	88-118	0.045-1.1	1.3-4.4	[128]
DLLME-HPLC-UV	0.01-100	296-462	0.001-0.01	1.0-11.5	[127]
HF-LPME-GC-MS	10-2000	-	0.01-0.95	3.4-14.9	[129]
SBSE-GC-MS	0.1-50	-	0.002-0.01	2.0-26	[126]
UASEME-HPLC-FLD	0.01-10	90-247	0.6-62.5	1.8-10.8	[97]
SD-DLLME-GC-MS	0.1-50	120-197	0.003-0.035	4.1-10.7	-

2.3.3 Analysis of real water samples

The applicability of the proposed method for the determination of PAHs in environmental samples was evaluated by analyzing different types of samples including tap water, reservior water and river water. Three aliquots of each sample were analysed in parallel and then the original samples were spiked with 5 μg/L of each PAH, extracted and analyzed again. The results are listed in Table 2-3.

As can be seen from the data, small amount of Nap, Ace and Phe were detected in the river samples, while Nap and Ace were detected in reservoir water samples. In tap water, the only detected analyte was Nap. The relative recoveries were satisfactory, ranging from 75.7 to 117.8%, with RSDs ranging from 3.6 to 12.1%. The results indicated that the proposed method is suitable for the analysis of PAHs in genuine water samples.

Table 2-3 Results of the genuine water samples analyzed by the proposed sample

Sample	Analyte	Detected	RSD (%, n=3)	Added (μg/L)	Recovery (%)	RSD (%, n=3)
River water	Nap	0.44	8.2	5	103.6	8.9
	Ace	0.15	9.9	5	77	5.4
	Flu	N.D. [a]		5	88.4	6.5
	Phe	0.09	5.8	5	93.6	11.2
	Ant	N.D.		5	80.4	7.7
	Flt	N.D.		5	82.5	7.9
	Pyr	N.D.		5	76.9	5.8
Tap water	Nap	0.32	9.4	5	114.2	9.7
	Ace	N.D.		5	88.7	9.5
	Flu	N.D.		5	90.5	6.2
	Phe	N.D.		5	95.8	12.1
	Ant	N.D.		5	77.9	3.6
	Flt	N.D.		5	117.8	7.7
	Pyr	N.D.		5	85.7	9.3
Reservoir water	Nap	0.52	9.2	5	112.7	5.6
	Ace	0.12	7.7	5	106.2	4.8
	Flu	N.D.		5	88.4	9.5
	Phe	N.D.		5	75.7	6.7
	Ant	N.D.		5	90.4	10.2
	Flt	N.D.		5	88.6	9.9
	Pyr	N.D.		5	79.9	5.9

[a] Not detected

2.4 Conclusion

In this study, an efficient sample preparation approach termed LDS-SD-DLLME was applied to extract PAHs from genuine water samples. A water miscible organic solvent was used both as disperser and de-emulsifier in this procedure, facilitating both emulsion formation and phase separation. This method makes use of low-density solvent, which is favored over more hazardous chlorinated solvents. The application of a disposal syringe in SD-DLLME makes retrieval of the organic extract simple and convenient, broadening the application of low-density solvent based DLLME. The results demonstrated that this method was efficient and reliable. Good linearity, sensitivity and repeatablity were obtained. The significant benefit of the proposed method is that the extraction can be achieved in a short time, and no special instrument is required such as centrifugator, ultrasonic water bath or vortex, allowing its application in on site monitoring. Moreover, it also opens a new horizen on automation: it may be possible to integrat sample concentration, analytes enrichment and even sample introduction of the extract into GC–MS system into one step and achieved by pressing a single button, with the help of an autosampler system (for example, CTC Analytics Combi PAL or Gerstel Multi Purpose Sampler) to control the syringe plunger movement. One minor disadvantage might be that the relatively large amout of disperser and demulsifier might potentially cause slight dissolution of analytes in the water sample. Overall, the proposed SD-DLLME method was demonstrated to be a useful tool for fast and effective determination of PAHs. It is conceivable that other environmental aqueous contaminants could also be

considered using this procedure.

Chapter 3 Low-density solvent based ultrasound-assisted surfactant-enhanced emulsification microextraction

3.1 Introduction

OCPs are one of the major classes of pesticides widely used since the 19th century. They are commonly used to prevent, repel or mitigate the effects of pests. However, it was later discovered that they could also exhibit harmful effects on both humans and wildlife due to their mutagenic, carcinogenic and endocrine-disrupting properties [130]. Although most of the OCPs have been now banned in many countries, OCPs and their metabolites can still be detected today in different matrices like plants, water [131], milk [132], honey [133], wines and biological fluids [134]. Thus, they are on the priority list of the United States Environmental Protection Agency (US EPA). In the European Union (EU), the maximum admissible concentration of each OCP in environment and drinking water is 0.1 μg/L, and that of the total amount of all compounds is 0.5 μg/L [135].

GC coupled with electron capture detection (GC-ECD) is one of the most widely employed techniques to analyze OCPs, considering ECD's specificity and sensitivity to electronegative chlorine atom [136]. However, this technique has drawbacks including poor discrimination of co-eluted analytes and a narrow dynamic range [137]. An alternative method is GC-MS, which has been successfully applied to separate and determine OCPs from different matrices [138, 139].

Due to the low concentration of OCPs in aqueous environmental samples and

the complexity of the sample matrix, a thorough pre-concentration and extraction method is crucial. As mentioned before, SD-DLLME proposed in last chapter shows advantages including rapidity, simplicity, low cost and high EFs. However, it should be noted that the use of a third component (the disperser) could decrease the partition coefficient of analytes into the extraction solvent [61]. To overcome this disadvantage, disperser was replaced by ultrasonic waves and it was proved beneficial for the formation of emulsion in DLLME. In Garcia-Jares et al.'s work [87], a novel microextraction technique, termed USAEME was used to extract emerging contaminants and pesticides from environmental water samples. Early reports showed the extraction time was around 10 min under 25 °C [88] and 5 min under 35 °C [94] in the USAEME process. The time required was much less compared to SPME or some LPME approaches. However, it was later found surfactant could also affect the formation of emulsion. Very recently, a surfactant was used as emulsifier in USAEME by Wu et al., and a new microextraction technique called UASEME was developed [4]. Surfactants were thought to enhance the dispersion of the water-immiscible extraction solvent into the aqueous sample, accelerating the formation of cloudy turbulence and therefore reducing extraction time. It was then successfully applied to extract compounds like fungicides [140], OPPs [4], carbamate pesticides [95] and PAHs [97].

Additionally, it should be noted that the majority of extraction solvents used in DLLME, USAEME or UASEME are those having densities higher than that of water. Phase separation after extraction is enabled by centrifugation.

However, these kinds of solvents are mostly halogenated hydrocarbons, which are relatively more toxic. More recently, Guo and Lee developed techniques termed as LDS-DLLME [66] and LDS-USAEME [143]. In these studies, a flexible plastic Pasteur pipette was shown to be a simple and convenient device to allow the use and subsequent retrieval of a low-density solvent in DLLME based techniques. These Pasteur pipettes are commercially available, and could be disposed of after each extraction to avoid cross-contamination problems.

Herein, in this chapter, the aim of this study was to develop a new method named LDS-UASEME which combines the merits of application of a non-chlorinated solvent, ultrasonication, surfactant, and polyethylene Pasteur pipette. The effects of various LDS-UASEME parameters, such as type and volume of the extraction solvents, type and concentration of the surfactant, extraction time and salt addition, were investigated and optimized. The proposed method was tested on environmental water samples to evaluate its feasibility.

3.2 Experimental

3.2.1 Reagents and materials

OCPs, hexachlorocyclohexane (HCH), heptachlor, aldrin, heptachlor epoxide, pp'-dichlorodiphenydichloroethylene (pp'-DDE), dieldrin and endosulfan sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Surfactants (Triton X-100, SDS, cetyltrimethyl ammonium bromide (CTAB))

were also purchased from Sigma-Aldrich. Sodium chloride (NaCl) used to adjust ionic strength was purchased from Merck (Darmstadt, Germany). Other chemicals and reagents used were the same as those described in Chapter 2.

3.2.2 Apparatus

A 35 kHz, 0.32 kW ultrasonic water bath (Ultrasonic LC 30, Germany) was employed to help the formation of the emulsion during extraction, and a Model 5810R centrifuge from Eppendrof (Hamburg, Germany) was used to promote phase separation. The polyethylene Pasteur pipette was manufactured by Continental Lab Products (San Diego, CA, USA) and purchased from Practical Mediscience Pte., Ltd (Singapore). A 100 μL syringe was bought from Hamilton Bonaduz AG (Bonaduz, Switzerland). A 10 μL microsyringe used for GC injection was purchased from SGE (Sydney, Australia).

Analysis was carried out on a Shimadzu (Kyoto, Japan) QP2010 GC-MS system equipped with a DB-5 MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm internal diameter (i.d.), 0.25 μm film thickness). Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1.5 mL/min. Samples (1 μL) was injected in splitless mode. The injection temperature was set at 250 °C and the interface temperature was maintained at 300 °C. The GC oven was initially held at a temperature of 70 °C for 2 min and then programmed to 200 °C at 25 °C/min, and further programmed to 280 °C at 8 °C/min; the final temperature was held for 3 min. The interface temperature was maintained at 300 °C and the solvent cut time

was 5 min. OCPs were analyzed in selective ion monitoring (SIM) mode for quantitative determination. The target ions selected in SIM were as follows: HCH, *m/z* 219; heptachlor, *m/z* 272, 100, 337, 274; aldrin, *m/z* 263, 293, 329; heptachlor epoxide, *m/z* 353, 272, 289; p, p'-DDE, *m/z* 248, 246; dieldrin, *m/z* 263, 79; endosulfan sulfate, *m/z* 272, 387, 229.

3.2.3 Sample preparation

A series of standard solutions were prepared by diluting the stock solutions (containing 1000 mg/L of each analyte) with methanol. Water samples were prepared by spiking ultrapure water with the analytes at known concentrations in volumetric flasks. Water samples were prepared daily and all the solutions were kept in the refrigerator at 4 °C. All environmental water samples were collected as described in Chapter 2.

3.2.4 LDS-UASEME

Figure 3-1 shows the LDS-UASEME procedure. Briefly, a 6 mL aliquot of water sample was placed in a soft polyethylene Pasteur pipette. The mixture of extraction solvent and the surfactant was quickly injected into the aqueous sample using the 100 μL syringe. The pipette was sealed with parafilm and immersed in an ultrasonic water bath for a prescribed time. (In preliminary experiments, no leaching of any interfering contaminants from the parafilm was observed.) The extraction solvent was efficiently dispersed into the sample to form a cloudy solution, where the extraction process took place. After extraction, the pipette and its contents were centrifuged at 4000 rpm for

4 min. Since the extraction solvents we selected had densities lower than that of water, they remained as the upper layer and could be easily retrieved by holding it upside down and manually exerting some finger-pressure to the main body of the pipette. One microliter of the extract was retrieved using the 10 μL syringe and then injected into the GC-MS system for analysis.

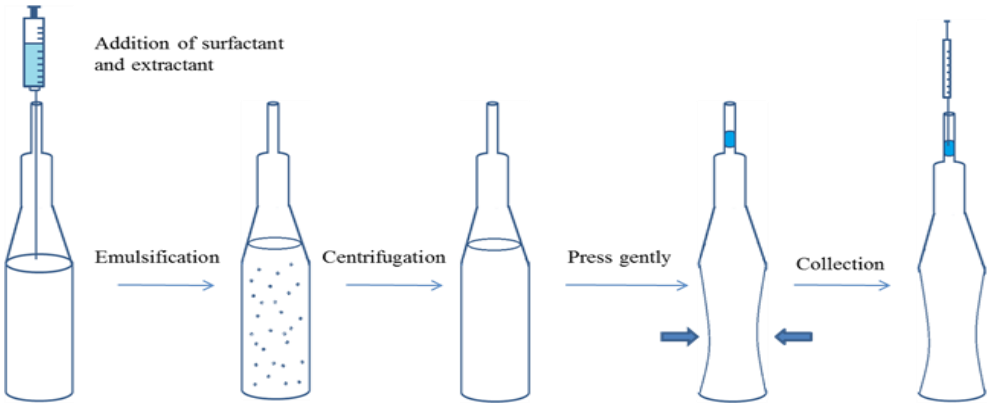


Figure 3-1 Schematic of LDS-UASEME.

3.2.5 LDS-USAEME and LDS-DLLME procedure

The LDS-USAEME procedure was similar to that of LDS-UASEME, the only difference being that no surfactant was added in the extraction process. In LDS-DLLME (classical DLLME but using a low-density solvent), 6 mL of aqueous sample was placed into a soft polyethylene Pasteur pipette. A mixture of acetonitrile (0.5 mL, as the dispersive solvent) and the extraction solvent was rapidly injected into the aqueous solution. After gentle shaking for a certain time, a cloudy solution was formed. After extraction, the pipette and its contents were also centrifuged at 4000 rpm for 4 min. The upper layer was collected as described previously, and 1 μL of the extract was injected into the GC-MS system for analysis.

3.3 Results and discussion

3.3.1 Comparative studies

The performance of LDS-UASEME was compared with that of LDS-USAEME and LDS-DLLME. Spiked water samples (containing 25 µg/L of each OCP) were used to evaluate their performance. Figure 3-2 shows that the extraction efficiencies obtained by LDS-UASEME was higher than that using LDS-USAEME and LDS-DLLME. Compared to LDS-DLLME, no dispersive solvent was needed in LDS-UASEME, meaning less solvent consumption and better phase separation after centrifugation. Compared to LDS-USAEME, the formation of the emulsion in LDS-UASEME was quicker, translating to a shorter overall extraction time. Another advantage is that *o*-xylene employed in this method, is relatively less toxic than chlorinated solvents used in conventional DLLME.

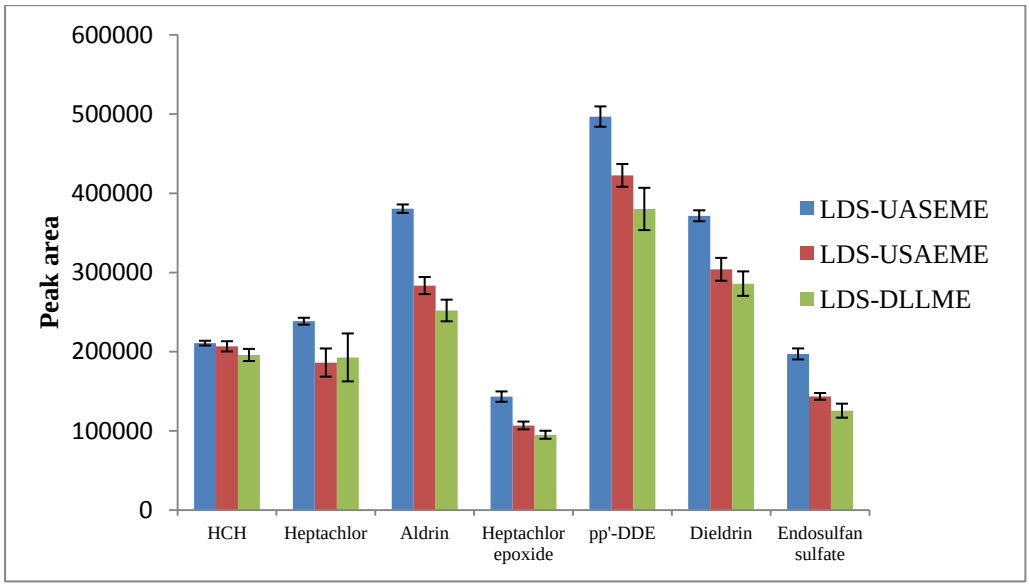


Figure 3-2 Comparison amongst LDS-UASEME, LDS-USAEME and LDS-DLLME.

3.3.2 Optimization

To determine the most suitable conditions of LDS-UASEME, the effect of different extraction parameters including type and volume of extraction solvent, type and concentration of surfactant, extraction time, and ionic strength were studied using spiked aqueous solutions (at 25 μg/L of each analyte). All experiments were performed in triplicate.

3.3.2.1 Selection of extraction solvent

In LDS-UASEME, the extraction solvent should have the following characteristics: (i) low solubility in water; (ii) good extraction capability in relation to target compounds; (iii) good chromatographic behavior; and (iv) lower density than water based on the objective of the present work. Therefore, in this study, 4 non-chlorinated solvents were investigated, i.e. *n*-hexane (density, *d*=0.659 g/mL), toluene (*d*=0.865 g/mL), cyclohexane (*d*=0.779 g/mL) and *o*-xylene (*d*=0.88g/mL). As can be seen in

Figure 3-3, the highest peak areas for most of the analytes were obtained by using *o*-xylene. Comparable extraction efficiency except for HCH and endosulfan sulfate was observed using *n*-hexane. However, it should be noted that compared to *o*-xylene, *n*-hexane has a higher vapor pressure at room temperature and thus evaporated more quickly. Based on these considerations, *o*-xylene was selected as the extraction solvent for the subsequent experiments.

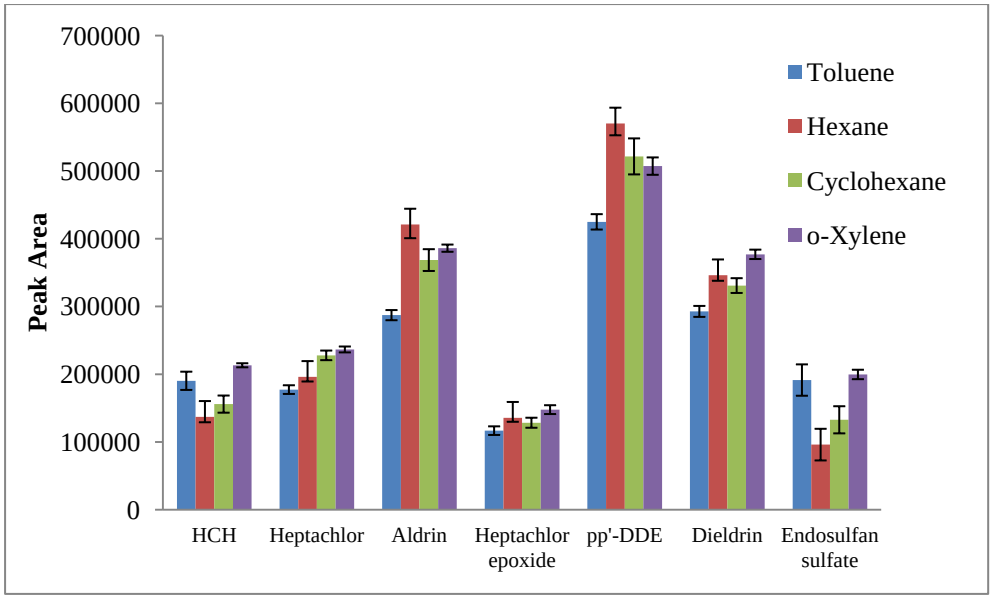


Figure 3-3 Effect of extraction solvent type on the peak areas of OCPs. Extraction conditions: extraction solvent volume, 50 μL; surfactant: Triton-X100 at 2×10^{-4} mol/L; extraction time, 2 min; centrifugation, 4 min at 4000 rpm; salt concentration, 0% (w/v).

3.3.2.2 Effect of extraction solvent volume

The extraction solvent volume could affect both the enrichment factor and recovery. Different volumes of *o*-xylene (ranging between 40 and 80 μL) were evaluated. As can be seen from Figure 3-4, for all analytes, the peak areas dropped gradually with the increase of extraction solvent volumes from 50 to 80 μL, most probably as a result of the dilution effect. However, when increasing *o*-xylene volume from 40 to 50 μL, comparable or even slightly higher peak areas for aldrin, heptachlor and p, p'-DDE were obtained, meaning that higher recoveries for these three OCPs were achieved when 50 μL extraction solvent was used. It could be explained by the greater extraction capability predominating over the dilution effect. Therefore, 50 μL was selected as the final extraction solvent volume.

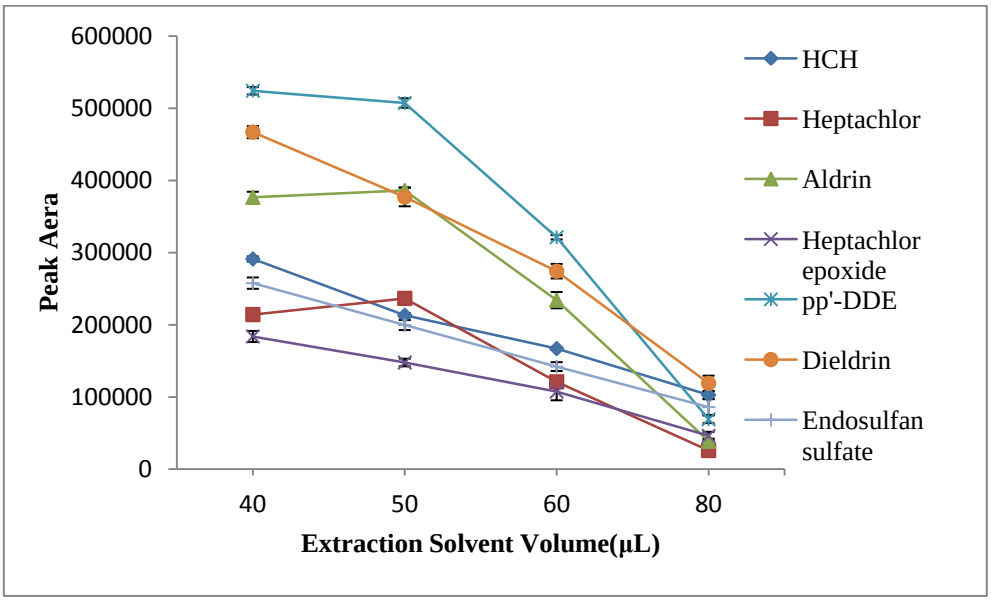


Figure 3-4 Effect of extraction solvent volume on the peak areas of OCPs. Extraction conditions: extraction solvent, *o*-xylene; surfactant, Triton X-100 at 2×10^{-4} mol/L; extraction time, 2 min; centrifugation, 4 min at 4000 rpm; salt concentration, 0% (w/v).

3.3.2.3 Selection of surfactant

The surfactant type is another key parameter for the USAEME process. It serves as the emulsifier, accelerating the emulsification process by facilitating the extraction solvent's dispersion into the aqueous solution. Three different types of surfactants (anionic type, SDS, cationic type, CTAB and non-ionic type, Triton X-100) were investigated and compared with extraction without use of a surfactant. From

Figure 3-5, it can be seen that highest peak areas were obtained using Triton X-100. The peak areas were comparable when using SDS and CTAB, which were higher than those extracted without use of a surfactant. The effect of different surfactants on the extraction efficiency might be related to the hydrophilic-lipophilic balance (HLB) value of the surfactants, the

hydrophobicity and polarity of the analytes. HLB values for SDS, CTAB and Triton X-100 are 40, 15.8 and 13.4 respectively. It has been earlier reported that surfactant with HLB values from 8 to18 appeared to be effective emulsifier. The results indicated that Triton X-100 might have a suitable hydrophobicity for most of the OCPs [87] . Therefore, Triton X-100 was selected as the preferred surfactant.

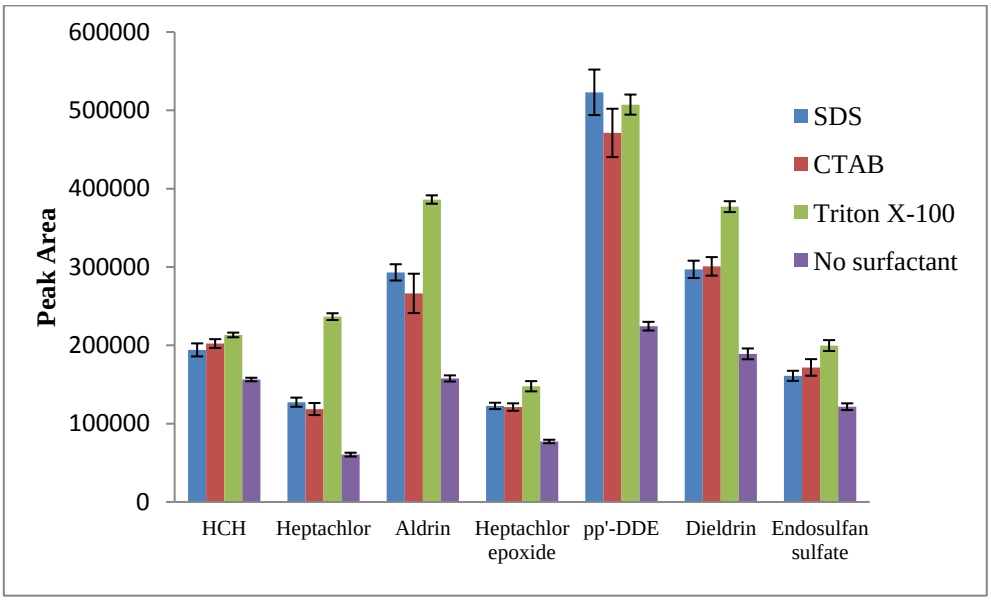


Figure 3-5 Effect of the type of surfactant on the peak areas of OCPs. Extraction conditions: extraction solvent, *o*-xylene; extraction solvent volume, 50 μL; surfactant, Triton X-100 at 2×10^{-4} mol/L; extraction time, 2 min; centrifugation, 4 min at 4000 rpm; salt concentration, 0% (w/v).

3.3.2.4 Effect of surfactant concentration

The concentration of surfactant is also crucial to the emulsification process, and the influence of different Triton X-100 concentrations (0, 0.5, 1.0, 2.0, 3.0×10^{-4} mol/L) was investigated. The results (Figure 3-6) revealed that the peak areas increased when the concentration of Triton X-100 was increased

from 0 to 2.0×10^{-4} mol/L, but decreased when the concentration was further increased to 3.0×10^{-4} mol/L. This observation might be related to the critical micelle concentration (CMC) of the surfactant [4, 5, 95, 140, 144]. CMC is the minimum concentration of the surfactant molecules to aggregate and form micelles. The results indicated that when the concentration of Triton X-100 was higher than its CMC (2.4×10^{-4} mol/L) [4], the extraction efficiency decreased. When the surfactant concentration reached its CMC, a fraction of the analytes might incorporate into the micelles, leading to a decrease of the analyte concentration in the aqueous solution. Therefore, the concentration of Triton X-100 selected was 2.0×10^{-4} mol/L.

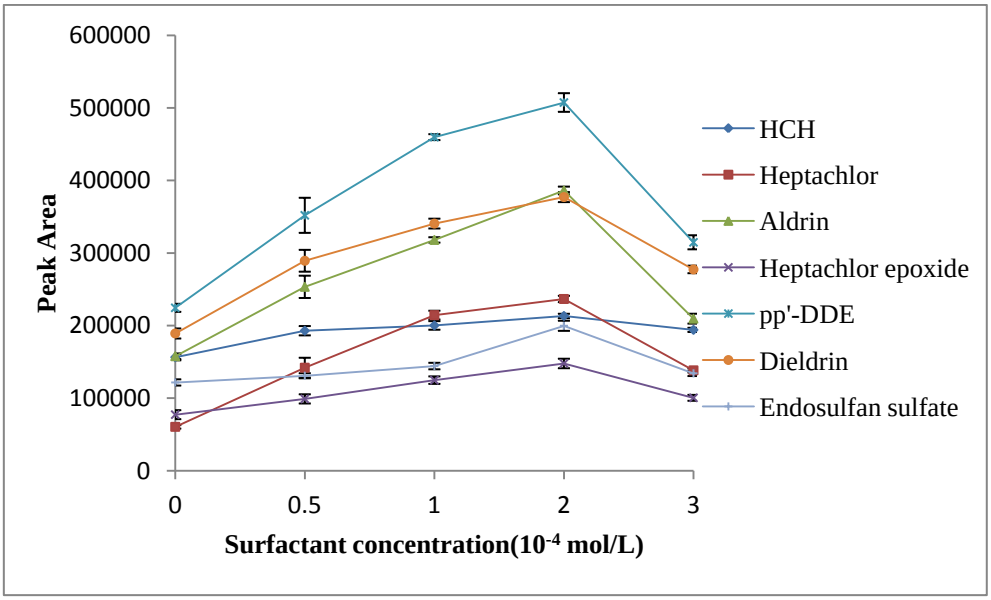


Figure 3-6 Effect of surfactant concentration on the peak areas of OCPs. Extraction conditions: extraction solvent, *o*-xylene; extraction solvent volume, 50 µL; surfactant, Triton X-100 10 µL ; extraction time, 2 min; centrifugation, 4 min at 4000 rpm; salt concentration, 0% (w/v).

3.3.2.5 Salt addition

The influence of salt addition was studied by adding NaCl to the sample to

obtain a final salt concentration from 0 to 18% (w/v). The results demonstrated that the peak areas dropped when the salt concentration increased. It was observed that the volume of the final extract increased, most probably due to a decrease in aqueous solubility of the extraction solvent in the presence of salt [145]. The increase of extract volume therefore caused a dilution effect. The increase of salt concentration could also lead to a higher viscosity of the solution. In this way, the ultrasonic energy can be absorbed by the viscous resistance of the solution and dispersed as calorific energy [146]. This might hold back the dispersion of the extraction solvent into the aqueous solution, and interfere with emulsion formation. Both of these phenomena ultimately lead to lower extraction efficiency. Therefore, based on these considerations and operational convenience, no NaCl was used in further experiments.

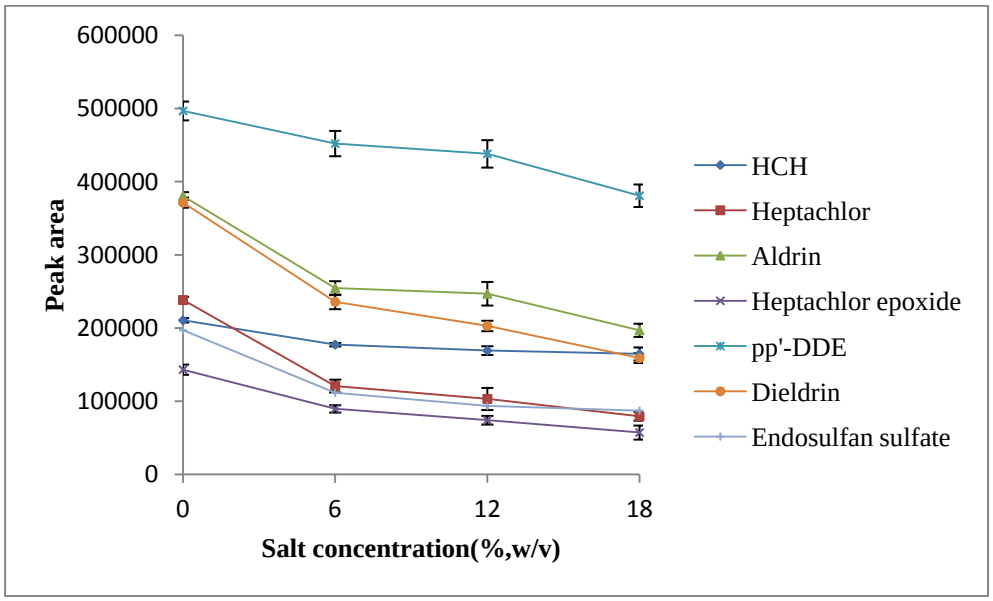


Figure 3-7 Effect of salt concentration on the peak areas of OCPs. Extraction conditions: extraction solvent, *o*-xylene; extraction solvent volume, 50 μL surfactant, Triton X-100 at 2.0×10^{-4} mol/L; extraction time, 2 min; centrifugation, 4 min at 4000 rpm;

3.3.2.6 Extraction time profiles

A series of extraction times (1, 2, 3, and 5 min) was investigated to evaluate their influence on extraction efficiency. In LDS-UASEME, the extraction time was defined as the time interval between the addition of extraction solvent and surfactant to the moment before centrifugation. The results indicated that the peak area increased slightly from 1 to 2 min and no significant improvement thereafter. An explanation is that the contact surface area between the organic extraction solvent and the aqueous solution was greatly enhanced with aid of the surfactant and ultrasonic waves, and achieved its maximum rapidly. Therefore, the mass transfer to organic solvent was greatly increased, subsequently reducing extraction time. Based on this observation, 2 min was selected as the optimal extraction time.

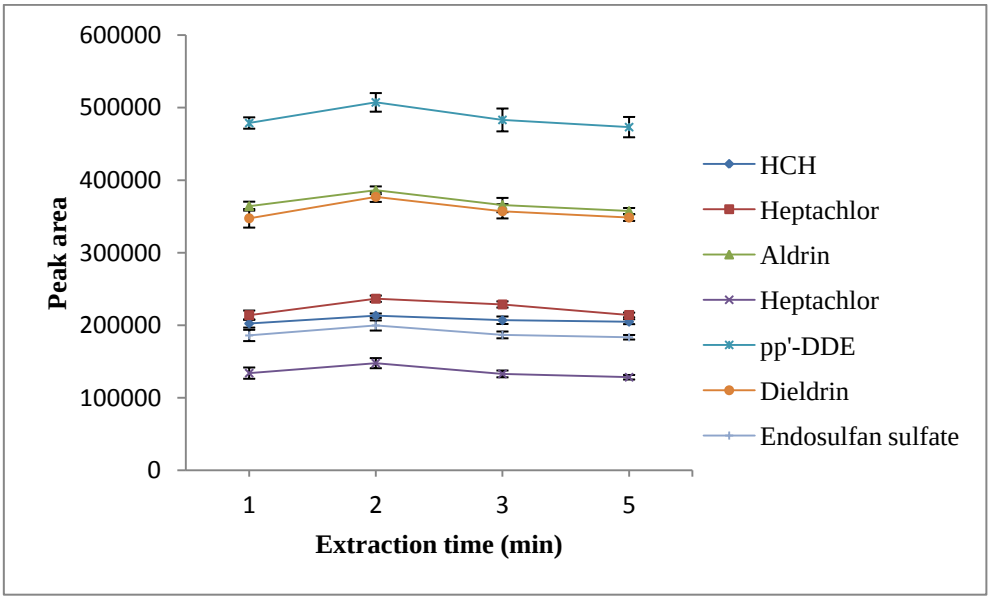


Figure 3-8 Effect of extraction time on the peak areas of OCPs. Extraction conditions: extraction solvent, *o*-xylene; extraction solvent volume, 50 μL; surfactant, Triton X-100 at 2.0×10^{-4} mol/L; centrifugation, 4 min at 4000 rpm; salt concentration, 0% (w/v).

Based on the above discussion, the most favorable LDS-UASEME conditions were as follows: 50 μL *o*-xylene as the extraction solvent, Triton X-100 at 2.0 10^{-4} mol/L as surfactant, 2 min as extraction time without NaCl addition. All the following experiments were performed under the described conditions.

3.3.3 Method validation

The linearity, repeatability, precision, LODs and LOQs were evaluated to assess the performance of the proposed LDS-UASEME. The results are listed in Table 3-1. It can be seen that the current method exhibited good calibration linearity over the concentration range of between 0.1 and 50 μg/L for aldrin, dieldrin, and endosulfan sulfate, between 0.2 and 25 μg/L for HCH, heptachlor epoxide and p, p'-DDE, and between 0.05 and 25 μg/L for heptachlor, with coefficients of determination (r^2) higher than 0.989 for all of the analytes. The relative standard deviations (RSDs) for five replicate experiments (10 μg/L) were found to be lower than 8.8%, indicating good repeatability. The LODs and LOQs, determined at a concentration at which signal-to-noise ratios were 3 and 10, ranged between 0.006 and 0.057 μg/L, and between 0.023 and 0.194 μg/L respectively.

Table 3-1 Regression data, LODs and LOQs of the proposed method

Analyte	Linear range (μg/L)	r^2	LOD (μg/L)	LOQ (μg/L)	RSD [a] (%, n=5）
HCH	0.2-25	0.997	0.045	0.176	8.4
Heptachlor	0.05-25	0.989	0.006	0.023	6.4
Aldrin	0.1-50	0.997	0.023	0.101	3.4
Heptachlor epoxide	0.2-25	0.998	0.057	0.194	8.3
pp'-DDE	0.2-25	0.998	0.041	0.174	8.8
Dieldrin	0.1-50	0.998	0.018	0.079	5.3
Endosulfan sulfate	0.1-50	0.997	0.029	0.088	6.2

[a] Calculated from samples spiked at LOQ levels.

3.3.4 Comparison of LDS-UASEME-GC-MS with other reported techniques.

Some other sample pretreatment methods for determination of OCPs including LPME [147], SDME [148], LPME-SFO [149], DLLME [150], DLLME-SFO [151] and USAEMEF [146] are shown in Table 3-2. From the table, it can be observed that the LODs of the proposed method were comparable with other reported methods. However, compared with static-LPME, SDME, LPME-SFO and USAEME, the extraction time required by the present method was much shorter since the addition of a surfactant accelerated the formation of the emulsion. Compared to DLLME-SFO, the present process was also faster and simpler since no cooling down process was required to solidify the extract. Furthermore, the extraction solvent used in LDS-USAEME was *o*-xylene, which is relatively less toxic compared to chlorinated solvents.

Table 3-2 Comparison of the proposed LDS-UASEME method with other methods for the determination of OCPs.

Method	Extraction solvent	Extraction time	LOD (ng/L)	RSD (%)	Reference
Static LPME GC-ECD	*n*-Hexane	20 min	20-200	3.2-10.7	[147]
SDME GC-MS	Toluene	10 min	22-101	5.9-9.9	[148]
LPME-SFO GC-ECD	1-Dodecanol	30 min	7-19	<7.2	[149]
DLLME GC-MS	Tetrachloroethylene	0.5 min	1-25	5-15	[150]
DLLME-SFO GC-ECD	1-Docanol	2 min	2.8-18.5	2.6-11.8	[151]
USAEME GC-ECD	Chloroform	15 min	2-16	<9	[146]
LDS-UASEME-GCMS	*o*-Xylene	2 min	6-57	<8.8	This work

3.3.5 Genuine water sample analysis

To examine the matrix effects and investigate the applicability of the method to the determination of environmental samples, the developed method was used to detect OCPs in three kinds of genuine water samples: tap water, rain water and river water (Table 3-3). The results showed that 0.18 μg/L heptachlor epoxide and 0.05 μg/L dieldrin were detected in river water, and the concentration of heptachlor epoxide and dieldrin detected in rain water were 0.21 μg/L and 0.05 μg/L respectively. No target analyte was observed in the tap water samples. The relative recoveries, which were defined as the ratios of analyte peak areas of spiked genuine water sample extracts and that of spiked ultrapure water extracts (spiked at 1 μg/L), ranged between 78.8% and 110.1% with RSDs lower than 10.8%. These results indicated that this method was suitable for the determination of OCPs in environmental aqueous samples.

Table 3-3 Result of genuine water samples analyzed by the proposed method

Sample	Analytes	Detected [a] (μg/L)	RSD (%)	Added (μg/L)	Recovery (%)	RSD (%)
Tap water	HCH	N.D.[b]		1.0	96.9	3.3
	Heptachlor	N.D.		1.0	97.2	5.5
	Aldrin	N.D.		1.0	93.9	8.1
	Heptachlor epoxide	N.D.		1.0	100.6	7.3
	p, p'-DDE	N.D.		1.0	110.1	7.6
	Dieldrin	N.D.		1.0	105.7	7.0
	Endosulfan sulfate	N.D.		1.0	108.3	8.9
River water	HCH	N.D.		1.0	81.8	2.3
	Heptachlor	N.D.		1.0	82.7	8.7
	Aldrin	N.D.		1.0	95.1	7.5
	Heptachlor epoxide	0.18	9.8	1.0	79.2	6.8
	p, p'-DDE	N.D.		1.0	86.3	1.9
	Dieldrin	0.05	8.4	1.0	78.8	9.4
	Endosulfan sulfate	N.D.		1.0	85.9	6.0
Rain Water	HCH	N.D.		1.0	84.0	1.7
	Heptachlor	N.D.		1.0	94.9	10.8
	Aldrin	N.D.		1.0	88.3	5.1
	Heptachlor epoxide	0.21	10.2	1.0	87.5	2.5
	p, p'-DDE	N.D.		1.0	93.6	4.9
	Dieldrin	0.05	8.8	1.0	88.1	2.7
	Endosulfan sulfate	N.D.		1.0	106.5	1.9

[a] The samples were analyzed directly.

[b] Not detected

3.4 Conclusion

In this study, a novel LDS-UASEME method was developed, for the preconcentration and determination of OCPs from environmental water samples. The proposed method was simple, accurate, cost effective and time saving. Furthermore, a solvent with density lower than water which is

environmentally friendlier was employed in this process without any home-made device. Instead, a widely available flexible plastic pipette was used. Ultrasonication and a surfactant were used to facilitate emulsion to improve the extraction efficiency. Satisfactory LODs ranging between 0.006 and 0.057 μg/L and good RSD values were achieved. Hence, LDS-UASEME combined with GC-MS, was demonstrated to be a rapid and efficient way to analyze OCPs from environmental water samples.

Chapter 4 Sonication-assisted emulsification microextraction combined with vortex-assisted porous membrane protected micro-solid-phase extraction

4.1 Introduction

Substituted phenols are dangerous pollutants for the environment. They are toxic, persistent and bio-accumulative in animals and vegetables. They are also potential co-carcinogens or promoters [152]. Substituted phenols are found in environmental water mainly due to effluent discharges of paper and pesticide industries. Therefore, it is important to develop simple, effective and robust methods to examine substituted phenols in environmental samples.

The retrieval of organic extract in low-density solvent based UASEME method presented in last chapter was achieved by using a plastic pipette. Convenient collection of low-density extract could also be achieved using other techniques. As mentioned before, Shi and Lee proposed a method using dispersive SPE to retrieve analytes-enriched 1-octanol after DLLME. It is time-saving and highly efficient. However, one major limitation of this method is that a magnetic solid sorbent is required. Another problem is potential interferences from matrix co-extractives, limiting its applicability in samples with complicated matrices.

Micro-SPE device was first introduced in our laboratory by Basheer et al. A μ-SPE device is based on packing of a small amount of sorbent in a sealed porous polypropylene membrane envelope. The membrane serves as a filter,

protecting the sorbent from contaminants in the sample. Micro-SPE could therefore be used in relatively “dirty” matrices. This technique has been demonstrated to be a fast, accurate and effective sample preparation method, successfully applied to extract POPs from water samples, soils, food [25], and biological matrices [27]. However, one disadvantage of this technique is that the extraction time is relatively long (normally more than 30 min).

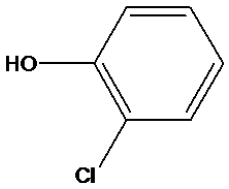
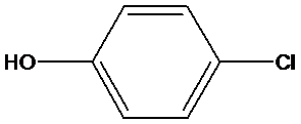
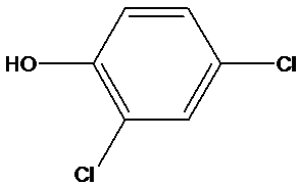
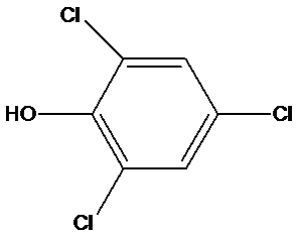
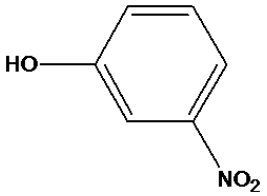
Therefore, in the present work, μ-SPE instead of dispersive SPE was applied to couple with SAEME. This new sample preparation technique termed SAEME-VA-μ-SPE was applied to extract substituted phenols in environmental water samples. The aim of this study is to develop a method combing the merits of SAEME and μ-SPE: (i) μ-SPE device made the retrieval of low-density solvent simple and convenient, (ii) sonication was employed to facilitate emulsion, increasing the contact areas between the extraction solvent and sample solution. Fine droplets of 1-octanol containing substituted phenols could then be rapidly extracted to the μ-SPE device, under vortex agitation. Multiwalled carbon nanotubes (MWCNTs) were selected as sorbent in μ-SPE device due to their extremely large surface area [153]. They were proved to have effective sorption properties with many organic components, especially aromatic hydrocarbons with benzenoid rings [154]. Another merit is possible applicability to samples with complex matrices due to the protection provided by the PP membrane. The proposed method might provide a promising and innovative horizon to solve analytical problems by combining different micro-scale sample preparation methods.

4.2 Experimental

4.2.1 Chemical and reagents

4-Dichlorophenol (2, 4-DCP) were obtained from Sigma–Aldrich (Milwaukee, WI, USA) while 2-chlorophenol (2-CP) and 2, 4, 6-trichlorophenol (2, 4, 6-TCP) were purchased from Fluka (Buchs, Switzerland). Chemical structures and physical properties of target phenolic compounds are listed in Table 4-1 [155]. 1-Octanol and hydrochloric acid were bought from Merck (Darmstadt, Germany). Phosphoric acid was purchased from Carlo Erba (Milan, Italy). Sodium hydroxide (NaOH) was acquired from Chemicon (Temecula, CA, USA). MWCNTs (external diameter, ~20-25 nm; length, ~1-5 μm) were obtained from Strem Chemicals (Newburyport, MA, USA). Other chemicals and reagents were the same as mentioned in Chapter 2 and 3. All standard solutions of the analytes were prepared in methanol at a concentration of 1000 μg/mL and stored in fridge at 4°C. Working solutions were prepared daily by diluting the stock solution with ultrapure water to known concentrations.

Table 4-1 Structures of the substituted phenols.

Analyte	CAS number	Structure	pK_a
2-CP	95-57-8		8.56
4-CP	106-48-9		8.81
2,4-DCP	120-83-2		7.89
2,4,6-TCP	87-86-5		5.99
3-NP	554-84-7		9.3

4.2.2 Apparatus

An Agilent Technologies (Palo Alto, CA, USA) 1200 series HPLC system, including G1311A quaternary pumps, a G1322A degasser, a dynamic mixing chamber and a G1315D diode array detector, was used for separation and determination of the analytes. The separation was performed on a 250 mm × 4.6 mm i.d., 5 μm, Phenomenax Luna C_{18} column (Torrance, CA, USA). The mobile phase consisted of acetonitrile and water. Phosphoric acid was used to

adjust the pH of the mobile phase to pH 3.0. Chromatographic separations were performed using isocratic elution with a flow rate of 0.8 mL/min for each pump while detection was carried out at a wavelength of 220 nm. The chromatographic data were collected and recorded using Agilent ChemStation software.

4.2.3 Extraction Procedures

4.2.3.1 Preparation of μ-SPE device

The μ-SPE device was simply a small amount of sorbent packed in a porous polypropylene (PP) membrane envelope. All sides of the envelope were heat sealed. The content in the device was 3 mg of MWCNTs. The dimension of the envelope was 0.7 cm × 1.0 cm. To prepare the device, a sheet of the PP membrane of about A4 size was first cut off from the sheet membrane. The longer edge of the membrane was folded over to a length about 2 mm greater than 1.0 cm. The fold-over section was then cut off from the mainsheet and further cut into individual rectangular pieces with a width about 4 mm greater than 0.7 cm. The 4 mm of additional width was to provide for allowance for heat sealing of the edge of the two open ends of each piece. One of the open ends was heat sealed and then 3 mg of the MWCNTs was placed through the remaining open end. Finally, this open end was heat-sealed to give the μ-SPE device. All μ-SPE devices prepared were sonicated in acetone for 5 min to remove any possible contamination, and dried completely in air before use. After each extraction, the same cleaning and drying procedure was applied to allow reuse of the devices.

4.2.3.2 Extraction procedure

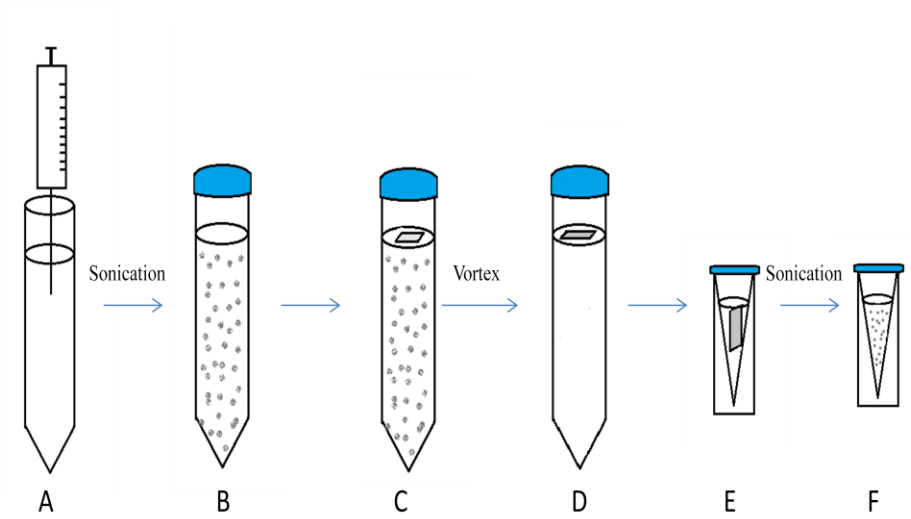


Figure 4-1 Schematic of SAEME-VA-μ-SPE. (A) Introduction of extraction solvent; (B) sonication under a ultrasonic water bath for 2 min; (C) introduction of a μ-SPE device; (D) vortex agitation for 2 min; (E) removal of the μ-SPE device to a 300 μL-glass insert for solvent desorption; (F) sonication for 5 min.

Figure 4-1 shows SAEME-VA-μ-SPE procedure. Briefly, a 10 mL aqueous sample solution containing 250 μg/L of each substituted phenol was added into a conical-bottomed test tube. The solution was then adjusted to pH 2 using 1.0 mol/L HCl followed by rapid injection of 40 μL of 1-octanol using a 50 μL syringe. The resulting solution was sonicated using an ultrasonic water bath for 2 min to give a homogenous cloudy solution. The ultrasonic water bath was operated at 50–60 Hz with maximum output power of 270 W at room temperature. Immediately after sonication, the prepared μ-SPE device was placed in the cloudy solution to extract the 1-octanol through vigorous agitation on a vortex agitator (KylinBell Lab Instruments Co. Ltd., Jiangsu, China) at 4500 rpm for 2 min. After extraction, a clear solution was observed indicating that the immiscible solvent 1-octanol has been removed from the

sample. A pair of tweezers was used to remove the μ-SPE device from the solution and placed it in a 300 μL-glass insert. Fifty microliters of methanol were added to the glass insert to desorb the extracted substituted phenols with the aid of sonication for 5 min. After desorption, the μ-SPE device was removed from the glass insert using the tweezers. The resulting methanol which contained the extracted substituted phenols was directly injected into the HPLC system for analysis.

4.3 Results and Discussion

4.3.1 Optimization

4.3.1.1 Effect of pH of sample solutions

Phenolic compounds are slightly acidic and will ionize in aqueous solution. Therefore it is necessary to adjust the pH of the solution in order to maintain the neutrality of the target analytes. This is so that they will remain in their molecular form and get extracted more readily into the organic extraction solvent. Based on these considerations, the effects of varied pH of the sample solution (2, 4, 6, 8 and 10) were studied. The results are shown in Figure 4-2. As predicted, the peak areas decreased when the pH values increased from 2 to 10. At pH 2, highest peak areas were obtained for all analytes. Thus, the sample solutions were adjusted to pH 2 for subsequent experiments.

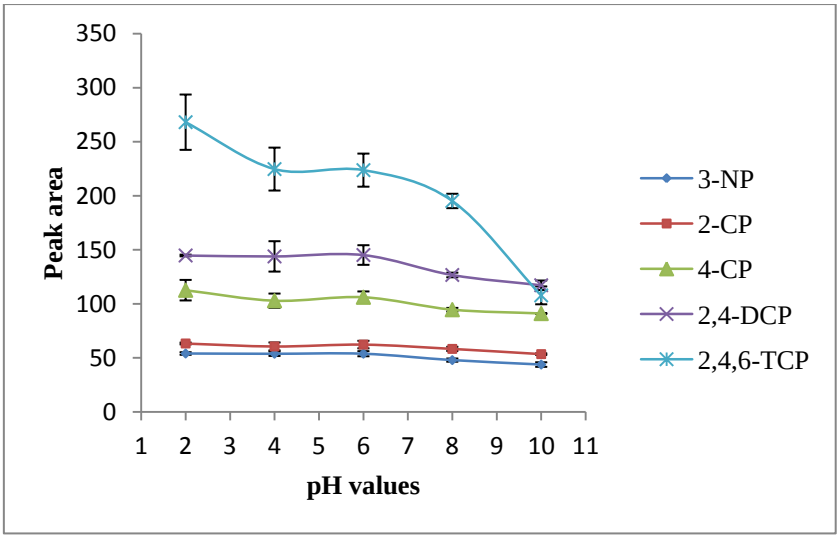


Figure 4-2 Influence of pH of sample on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent, 1-octanol, 40 μL; sonication time, 2 min; extraction time, 2 min; desorption solvent, 50 μL of methanol; desorption time, 5 min.

4.3.1.2 Selection of extraction solvent

Selection of extraction solvent is of great importance for efficient extraction. Five organic solvents, namely, *n*-hexane (density, d = 0.66 g/mL, 20^{o}C), *o*-xylene (d = 0.88 g/mL), toluene (d = 0.87 g/mL), cyclohexane (d = 0.78 g/mL) and 1-octanol (d = 0.82 g/mL) [156], were investigated. Studies were performed by using 40 μL of these solvents. The results (

Figure 4-3) shows that the highest peak areas were obtained by 1-octanol and relatively better HPLC performance (better peak shapes) was observed. Peak areas obtained when toluene and *n*-hexane were used were not depicted since the data seemed not reliable (due to the high RSDs and low peak areas). Hence, 1-octanol was deemed to be most suitable extraction solvent in this work.

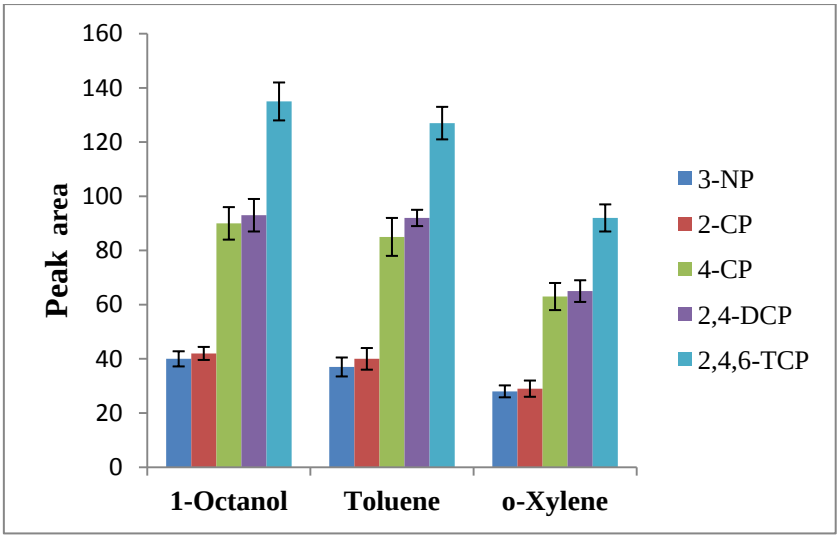


Figure 4-3 Influence of extraction solvent on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent volume, 40 μL; emulsification time, 2 min; extraction time, 2 min; desorption solvent, methanol, 50 μL; desorption time, 5 min.

4.3.1.3 Volume of extraction solvent

The effect of the volume of extraction solvent was examined. As shown in Figure 4-4, the highest peak areas were obtained when the solvent volume was 40 μL. The peak areas decreased when the volumes were greater than 40 μL, probably due to dilution effects predominating over higher extraction capacity. And too little organic solvent would increase difficulty in the retrieval of organic extract after extraction. Therefore, 40 μL of 1-octanol was selected and applied in the subsequent experiments.

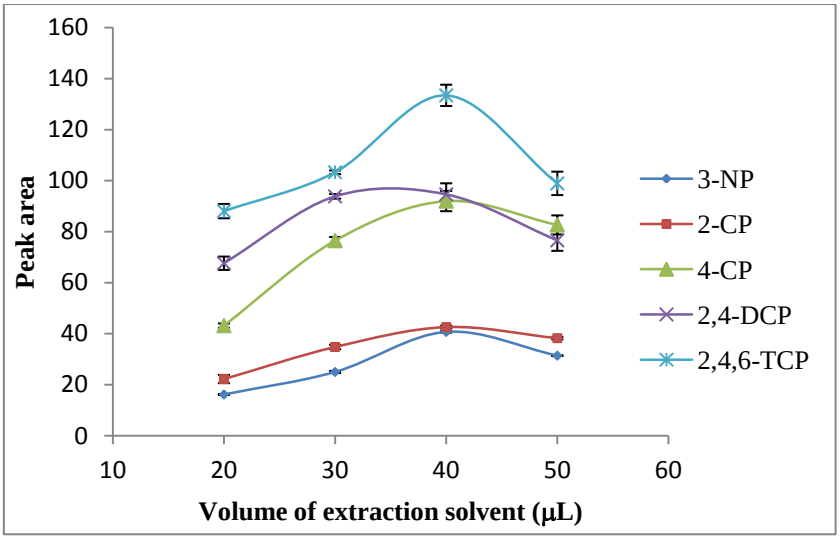


Figure 4-4 Influence of volume of extraction solvent on SAEME-VA-µ-SPE. Extraction conditions: extraction solvent, 1-octanol; sonication time, 2 min; extraction time, 2 min; desorption solvent, methanol, 50 µL; desorption time, 5 min.

4.3.1.4 Selection of desorption solvent and solvent volume

The effect of desorption solvent was examined by considering three possible desorption solvents, methanol, acetone and acetonitrile.

Figure 4-5 shows that the peak areas obtained by acetone and methanol were comparable while acetonitrile gave the lowest peak areas. As methanol gave better chromatographic peak shapes than acetone, it was selected as the desorption solvent. Then the effect of the volume of desorption solvent was examined by evaluating the performance using 40 to 90 µL of methanol. As shown in Figure 4-6, the highest peak areas were obtained when the volume was 50 µL. Too little desorption solvent (40 µL) might not be enough to completely desorb 1-octanol. Too much desorption solvent was also disadvantageous due to the dilution effect which might lead to lower

enrichment factors. Based on these considerations, 50 μL of methanol was selected as the optimal desorption solvent.

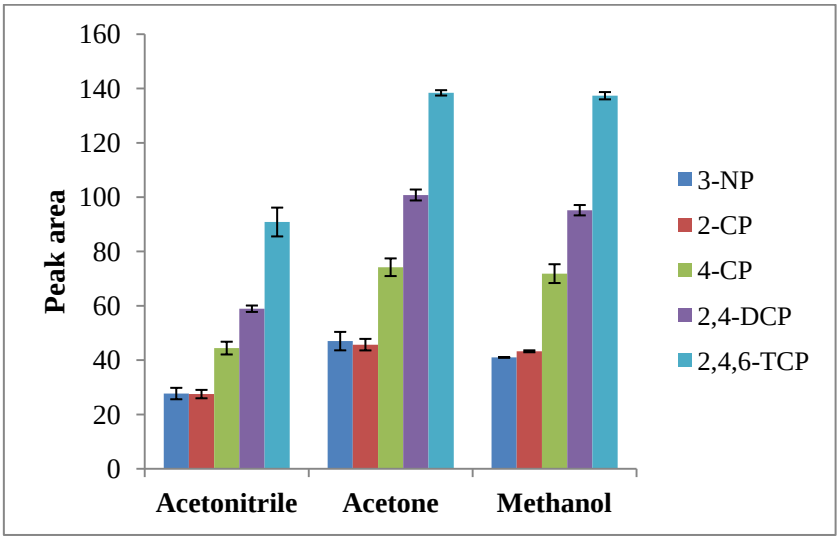


Figure 4-5 Influence of desorption solvent on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent, 1-octanol, 40 μL; sonication time, 2 min; extraction time, 2 min; desorption solvent volume, 50 μL; desorption time, 5 min.

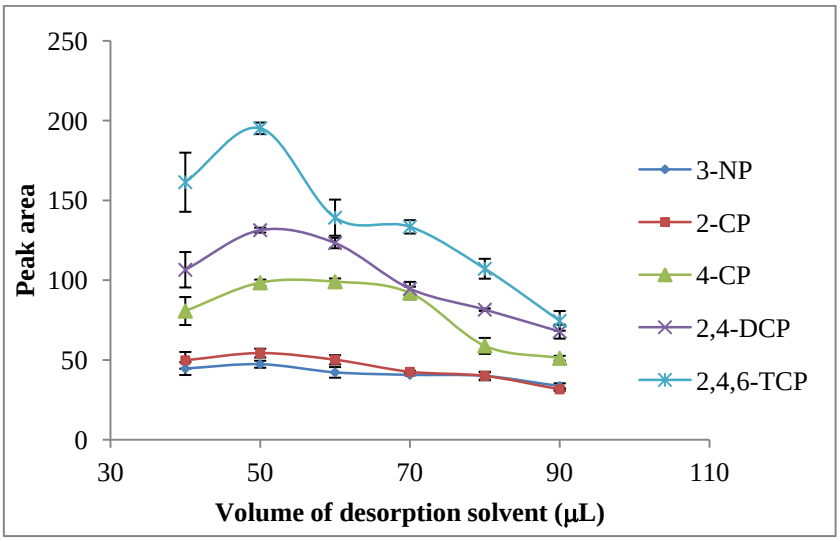


Figure 4-6 Influence of volume of extraction solvent on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent, 1-octanol, 40 μL; sonication time, 2 min; extraction time, 2 min; desorption solvent, methanol; desorption time, 5 min.

4.3.1.5 Extraction time

In the proposed SAEME-VA-μ-SPE, extraction time was defined as the time taken for the adsorption of the low-density organic extraction solvent, 1-octanol by the μ-SPE device. As shown in Figure 4-7, the peak areas increased when the extraction time was increased from 1 to 2 min. When the extraction time was further increased, the peak areas generally remained constant. The results indicated that 2 min was enough for the mass migration to μ-SPE device.

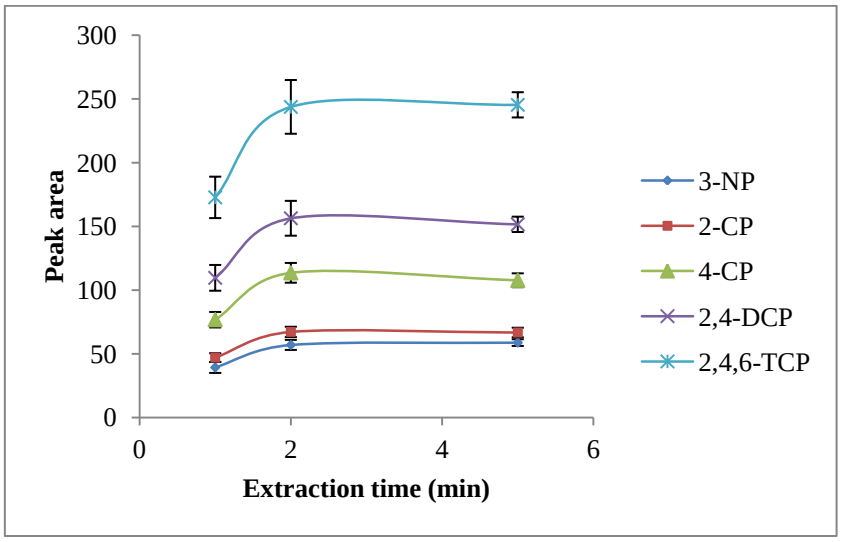


Figure 4-7 Influence of extraction time on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent, 1-octanol, 40 μL; sonication time, 2 min; desorption solvent, methanol, 50 μL; desorption time, 5 min.

4.3.1.6 Desorption time

The effect of desorption time (2–20 min) under sonication was examined. When desorption time increased from 2 to 5 min, the peak areas increased slightly (as seen in Figure 4-8). The peak areas decreased slightly when

desorption time was further increased. The results showed that the proposed method allowed the desorption equilibrium to be reached within a short time.

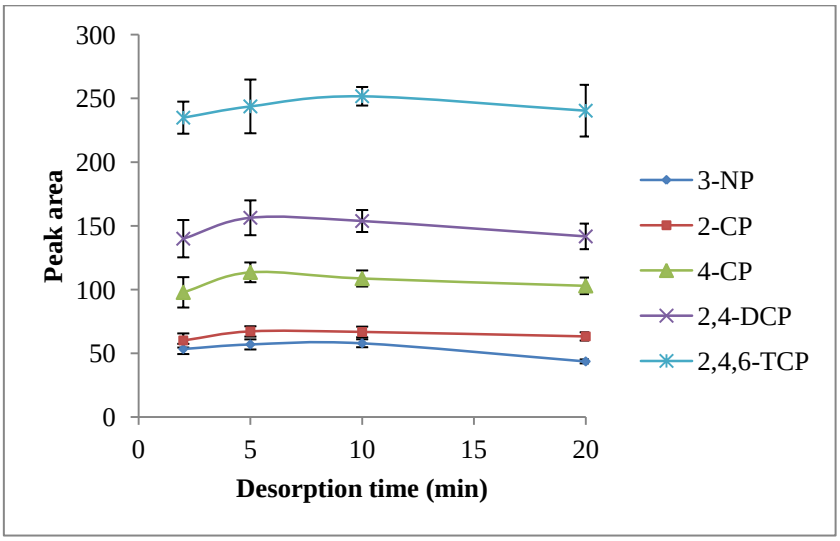


Figure 4-8 Influence of desorption time on SAEME-VA-μ-SPE. Extraction conditions: extraction solvent, 1-octanol, 40 μL; sonication time, 2 min; extraction time, 2 min; desorption solvent, methanol, 50 μL.

Based on the aforementioned discussion, the most favorable extraction conditions for SAEME-VA-μ-SPE were as follows: extraction solvent, 1-octanol, 40 μL, emulsification time, 2 min, extraction time, 2 min, desorption solvent, methanol, 50 μL, desorption time, 5 min. All of the following experiments were carried out under these conditions.

4.3.2 Method validation

To validate the proposed technique, the following validation parameters, including linearity, LODs, LOQs and repeatability were investigated using spiked ultrapure water samples. The results obtained are summarized in Table 4-2.

Table 4-2 Linear range, LODs, LOQs, correlation coefficients, and precision of SAEME-VA-μ-SPE

Analyte	Linear range	Correlation coefficient	LOD (μg/L)	LOQ (μg/L)	RSD (%, n=5)[a]
3-NP	1 - 1000	0.9920	0.171	0.819	7.1
2-CP	0.5 - 1000	0.9931	0.120	0.414	5.7
4-CP	5 - 1000	0.9996	1.200	3.899	10.5
2,4-DCP	1 - 1000	0.9990	0.253	0.775	4.9
2,4,6-TCP	5 - 1000	0.9986	1.084	3.987	9.0

[a] Spiked at 10 μg/L

The linearity of the technique was investigated over the range of 0.5–1000 μg/L for 2-CP, 1–1000 μg/L for 3-NP and 2, 4-DCP, and 5–1000 μg/L for all other analytes. The calibration plots were drawn by plotting the mean peak area against the concentration of the aqueous solution of the substituted phenols. A statistical regression model was applied to the calibration plots obtained and the correlation coefficients (*r*) were between 0.9931 and 0.9996 for all analytes. The relative standard deviations (RSDs) obtained were between 0.5% and 9.7%, illustrating good repeatability of the method.

The LODs of the substituted phenols based on S/N of 3, were in found to be in the range of 0.120 and 1.200 μg/L. The LOQs based on S/N = 10, were determined to be from 0.819 to 3.987 μg/L. As seen from Table 4-3, the LODs obtained by the present proposed method were comparable or even lower than those previously reported with surfactant assisted DLLME-HPLC-UV [156], ionic liquid membrane extraction-HPLC–UV [157], EME-HPLC-UV [112], temperature-controlled ionic liquid DLLME-HPLC–UV [158], SPME-micellar desorption-HPLC–DAD [159] and cloud point extraction-HPLC–UV–EC [160]. From this comparison, it is clear that the proposed technique has satisfactory sensitivity and repeatability for the target

substituted phenols. Compared with SPME [159] and cloud point extraction [160], the proposed method provided lower LODs. Compared with ionic liquid membrane extraction [157] and temperature-controlled ionic liquid DLLME [158], no viscous ionic liquid was required, which might increase operational complexity or have negative influence on HPLC chromatogram. And compared with LLLME [161], (which provides better LODs), the time required for the proposed method is only 2 min, much shorten than it of LLLME (60 min). Furthermore, this method provide an alternative way (μ-SPE) to retrieve solvent with desity lower than water after SAEME, which could broaden the application of LDS-DLLME.

Table 4-3 Comparison of LODs obtained by different methods.

Method	Solvent and volume	LODs (mg/L)	Ref.
Surfactant assisted DLLME-HPLC–UV	35 μL 1-octanol	0.1	[156]
Ionic liquid membrane extraction-HPLC–UV	10 μL NaOH solution	0.5–1.0	[157]
Liquid–liquid–liquid microextraction coupled to ion-pair HPLC–DAD	12.5 μL NaOH solution	0.049–0.081	[161]
SPME-micellar desorption-HPLC–DAD		1.1–5.9	[159]
Cloud point extraction-HPLC–UV–EC	0.5% Triton X114 in sample solution	2–5	[160]
EME-HPLC–UV	100 μL NaOH solution	0.1	[112]
Temperature-controlled ionic liquid DLLME-HPLC–UV	$[C_8MIM][PF_6]$, 50 μL	0.27–0.68	[158]
SAEME-VA-μ-SPE	40 μL 1-octanol	0.171-1.084	this work

4.3.3 Genuine water sample analysis

Sample matrix effects were investigated by analyzing tap water using the proposed technique. There was no target analytes found which can be deduced that the target analytes are either not present in the genuine water samples or they were below the LODs.

Genuine water samples spiked at 50 and 250 μg/L of each analyte were tested. Matrix effects were indicated by the relative recoveries of the substituted phenols, and results are shown in Table 4-4. Relative recovery is defined as the ratio of the peak areas of the analytes in the genuine water sample extracts to peak areas of the analytes in ultrapure water extracts spiked at the same concentrations [162]. The relative recoveries of the five substituted phenols at two different concentrations ranged from 72% to 98%, with RSDs less than 15.7%. From the results obtained, it can be concluded that matrix has negligible effect on this technique. This could be due to the use of the μ-SPE device. The PP membrane provided a barrier between the sample matrix and the MWCNTs in the device by allowing only the 1-octanol with the extracted target analytes to be extracted by the MWCNTs, keeping out the other interferences that might be present in the genuine water samples. Figure 4-9 shows the chromatogram of spiked tap water sample analyzed under the most favorable extraction conditions.

Table 4-4 Summary of results from analysis of chlorophenols in spiked tap water samples by SAEME-VA-μ-SPE

Analyte	Tap water Relative Recovery [a] (%)	RSD (%)	Tap water Relative Recovery [b] (%)	RSD (%)
3-NP	72	8.8	80	6.5
2-CP	78	15.7	84	10.7
4-CP	82	7.6	81	0.6
2, 4-DCP	79	8.3	80	6.1
2, 4, 6-TCP	86	14.2	98	7.0

Relative recovery [a], spiked at 5 μg/L; Relative recovery [b], spiked at 50 μg/L

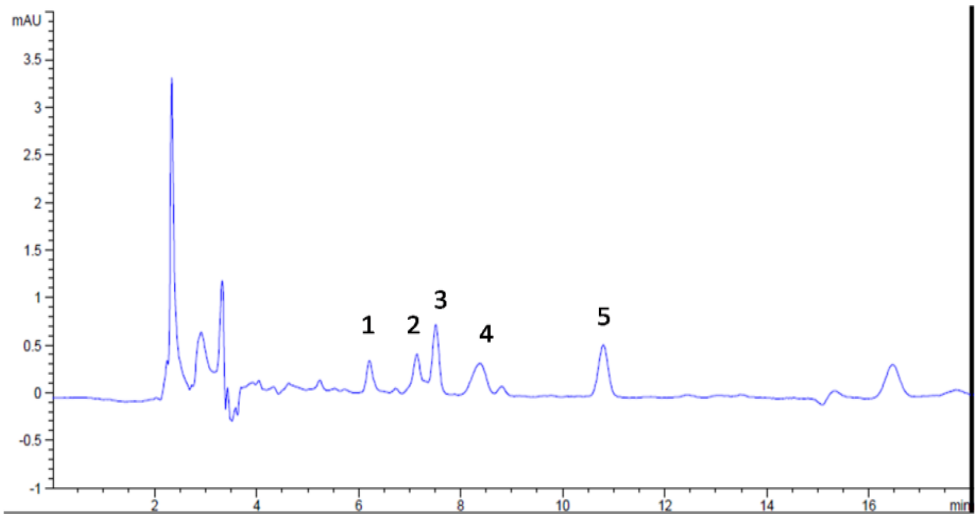


Figure 4-9 Liquid chromatogram of spiked tap water (50 μg/L) sample extracts under the most favorable SAEME-VA-μ-SPE conditions as described in the text. Peak identification: (1) 3-NP, (2) 2-CP, (3) 4-CP, (4) 2, 4-DCP and (5) 2, 4, 6-TCP.

4.4 Conclusion

In the present study, a two-step extraction technique based on a combined approach of USAEME and μ-SPE was proposed. This combined method was applied to the pre-concentration and sample clean-up of substituted phenols in environmental samples. 1-Octanol used as extraction solvent was less toxic and hence environmentally friendlier than the chlorinated solvents

that are commonly used in classical DLLME technique. In addition, the use of ultrasonic radiation facilitated the dispersion of 1-octanol in the aqueous sample solution. Hence there is no need for a disperser solvent. A μ-SPE device was employed successfully to retrieve organic solvent in this study when MWCNTs was heat-sealed in a PP membrane envelope. This study shows that organic solvent with density lower than water can be used in DLLME without additional processing steps (such as centrifugation or cooling process used in DLLME-SFO) or any customized extraction vessel. Compared to dispersive μ-SPE introduced by Shi, the sorbent, MWCNTs were packed in a porous membrane, making the separation of sorbent and donor solution easier and more convenient. Moreover, the membrane served as a filter, allowing extraction from samples with complex matrices. Under the most favorable extraction conditions, the LODs were comparable with other microextraction techniques used for the extraction of substituted phenols. Good linearity and repeatability were obtained. In general, this study showed that SAEME-VA-μ-SPE was a fast, simple and cost effective method for the sample preparation of environmental water samples.

Chapter 5 Electro-enhanced solid-phase microextraction

5.1 Introduction

Tricyclic antidepressants (TCAs) have long been used as reference for treatment of depression and psychiatric disorders like phobias and anxiety [163]. They perform as inhibitors of the reuptake of the neurotransmitter norepinephrine (as in the case of desipramine, nortriptyline and protriptyline secondary amines) or serotonin (as in the case of amitriptyline, imipramine, clomipramine and doxepine tertiary amines) in the central nervous system [12]. These drugs are used extensively especially in developed countries and can enter the aquatic environment mainly through human excretion [164]. Many TCAs cannot be completely removed or degraded during the sewage treatment process and therefore it is important to develop methods to determine their concentrations for the purpose of monitoring their presence in environmental samples [165].

Several chromatographic methods like GC-MS [163, 166-170], HPLC [171-175], HPLC-MS/MS [176-178], CE [165, 179-183], have been developed for the analysis of TCAs in different matrices including serum, whole blood, urine, plasma and waste water [165, 167, 175, 176, 183-188]. Sample pretreatment or preconcentration is a crucial step to obtain good selectivity and sensitivity when determining TCAs in biological fluid or environmental samples.

SPME is almost a solvent free approach, allowing reusing the fibers and

obtaining cleaner extracts. It is comparable with GC-MS, since the compounds extracted to the thin polymeric layer can be thermally desorbed in the injection port. However, normally the whole process of SPME is relatively long, ranged from 30 min to one hour or even several hours, much longer compared to techniques like DLLME [167, 170] or EME [107, 109].

EME is a microextraction technique based on analyte migration from a sample solution to an acceptor solution (extract) with electrical potential as the driving force. The speed of the extraction is dependent on the characteristics of the analytes and the magnitude of the electrical potential. In Davarani's work, it took 20 min to extract imipramine and clomipramine under 200 V [166]. However, 200 V is relatively large and may cause electrical accidents if not handling properly. Recently, some reports indicated that low voltage EME could also give satisfactory results [189-191]. A limitation of EME is that since the acceptor solution is aqueous (because the target analytes should be in their ionized forms in both donor and acceptor phases), only reversed phase HPLC and CE can be used for analysis. It would be advantageous if compounds could be analyzed with GC-MS after EME, and this might expand the applicability of EME. It has been reported that the application of a potential to an SPME fiber could accelerate the migration of some analytes with a charge opposite to that of the fiber, therefore enhancing extraction efficiencies and permitting the use of GC-MS as the determination technique at the same time [192]. So far, there have been a few reports on EE-SPME [192-196].

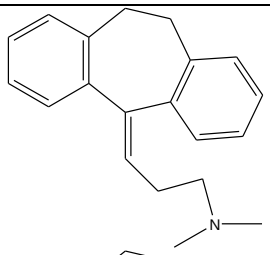
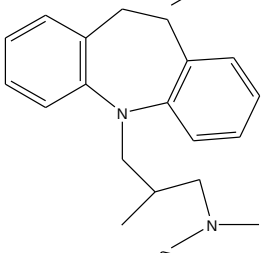
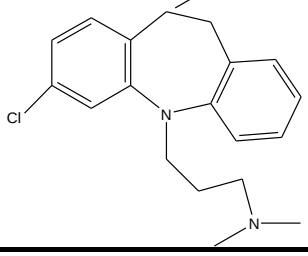
In this study, EE-SPME followed by GC-MS was applied to determine TCAs in environmental water samples. The mass transfer of target analytes from the sample solution to an SPME fiber was accelerated by the electrical field, improving extraction selectivity and efficiency. Extraction conditions such as SPME fiber type, pH value of the sample solution, voltage applied, extraction time, stirring speed, desorption temperature were evaluated. The procedure was then tested on environmental water samples.

5.2 Experimental

5.2.1 Reagents and materials

TCAs, amitriptyline, trimipramine, clomipramine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Their structures and characteristics were listed in Table 5-1. Sodium hydroxide (NaOH) was obtained from Chemicon (Temecula, CA, USA). Other chemicals and reagents used were the same as mentioned in previous chapters.

Table 5-1 Characteristics of TCAs

Analytes	Molar mass	Molecular structure	p*K*a
Amitriptyline	277.41		9.42 ±0.37
Trimipramine	294.43		9.38 ±0.28
Clomipramine	314.30		9.49 ±0.28

5.2.2 Apparatus

The commercial SPME fiber holder and fibers coated with polydimethylsiloxane (PDMS, 100 μm); polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm); Carboxen/polydimethylsiloxane (CAR/PDMS, 85 μm); polyacrylate (PA, 85 μm); and divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50 μm) were bought from Supelco (Bellefonte, PA, USA). Prior to use, the fibers were conditioned in the GC injection port as recommended by the supplier. A voltage adaptor which was used to control the electrical field (from 3 to 15 V) and a voltmeter were bought from the local market. A platinum wire with a diameter of 0.5 mm was used as the positive electrode.

Analysis was performed with a Shimadzu (Kyoto, Japan) QP2010 Ultra

GC-MS system, equipped with a Shimadzu AOC-2000 autosampler and a DB-5 MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1.0 mL/min. The injection port temperature was set as 240°C, and the GC-MS interface temperature was set as 250°C. For chromatography, the GC oven was initially held at a temperature of 80°C for 3 min, programmed to 240°C at 30°C/min, held for 5 min, and further programmed to 280°C at 20°C/min and held at the final temperature for 3 min. Samples were injected in splitless mode and sampling time was set as 3 min. The TCAs were analyzed in selective ion monitoring (SIM) mode. Based on selectivity and sensitivity concerns, the monitored ions were selected as follows: amitriptyline, *m/z*, 58, 202, 193; trimipramine, *m/z*, 58, 193, 249; clomipramine, *m/z*, 58, 85, 269.

5.2.3 Sample preparation

Standard solutions were prepared by diluting stock solutions (containing 1000 mg/L of each analyte) with methanol. Working solutions were prepared by spiking ultrapure water with the analytes at known concentrations in volumetric flasks. All the solutions were kept in the refrigerator at 4°C before use, and working solutions were prepared daily. Tap water, reservoir water samples were collected as described earlier and kept in the dark at 4°C until use.

5.2.4 Electro-enhanced SPME procedure

Figure 5-1 shows the set-up of the EE-SPME procedure. An aliquot of 10 mL aqueous sample solution (pH 4.0) was introduced into a glass vial. A platinum wire was used as the positive electrode and inserted into the sample solution. The immersed stainless needle sleeve of the SPME holder served as the negative electrode. The electrical voltage was applied using the adaptor and the extraction was carried out for 10 min, at a stirring rate of 500 rpm. After extraction, the fiber was retracted into the needle and immediately inserted into the GC injection port for thermal desorption of the analytes at 240 °C for 3 min.

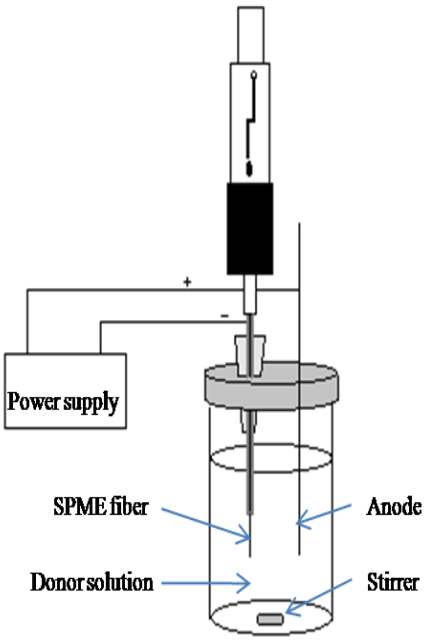


Figure 5-1 Schematic for EE-SPME

5.3 Results and discussion

5.3.1 Optimization

To determine the most suitable conditions of EE-SPME, factors including fiber type, pH value of the sample solution, voltage applied, extraction time, stirrer speed, desorption temperature, desorption time were studied and evaluated. All experiments were performed in triplicate.

5.3.1.1 Selection of SPME fibers

Different types of commercially available SPME (PDMS/DVB, 65 μm; CAR/PDMS, 85 μm; PA, 85 μm; DVB/CAR/PDMS, 50 μm; PDMS, 100 μm) fibers were evaluated. For extraction, 10 mL of the aqueous solution, spiked with 10 μg/mL of each TCA, and adjusted to pH 4.0, was placed in a small glass vial. Different SPME fibers were immersed into the extraction cell and 15 V was applied between the stainless needle sleeve and the platinum wire. The stirring rate was kept at 500 rpm. Stirring could enhance mass transfer during extraction. However, the enhanced mechanical agitation vortex generated in the sample solution if the stirring rate was set too high (>500 rpm), probably influencing the extraction negatively. As illustrated in Figure 5-2, extraction using 100 μm PDMS gave best extraction efficiency, better than those of the fibers with other coatings. This observation is in agreement with those reported in previous direct SPME studies (without applying potentials) [197]. Based on this observation, 100 μm PDMS fiber was selected as the most suitable SPME fiber.

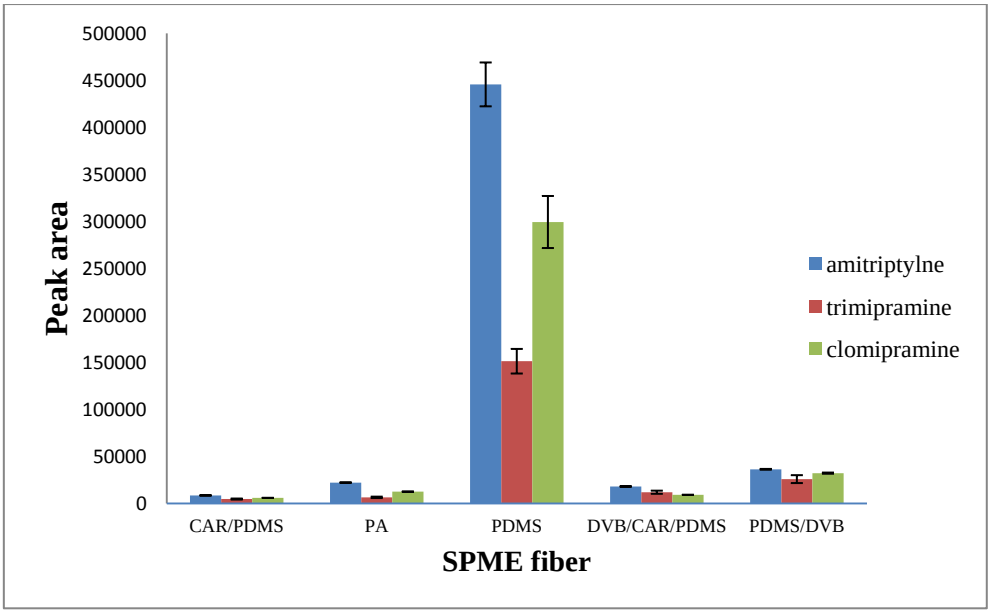


Figure 5-2 Effect of coatings of SPME fiber on extraction of TCAs. Extraction conditions: pH, 4; voltage, 15 V; extraction time, 10 min; agitation speed, 500 rpm; no salt addition; room temperature.

5.3.1.2 Adjustment of sample pH

The pH value of the sample solution is of great importance in the EE-SPME process. It determines the forms of the analytes in the aqueous solution. Figure 5-3 depicts the influence of different pH values (pH 3.0-7.0) of the sample solution on extraction efficiencies. It indicated that pH 4.0 gave best extraction efficiencies. The results could be explained that at lower pH, H^+ predominated in electrostatic migration to the negative electrode, and hydrogen gas bubbles might appear on the surface of the electrode, affecting the extraction efficiencies negatively [192]. On the other hand, at relatively high pH, the extraction decreased because more of the TCAs might be present as the free base (pK_a 9.38-9.49) [198]. On this basis, pH 4.0 was selected as the most favorable pH.

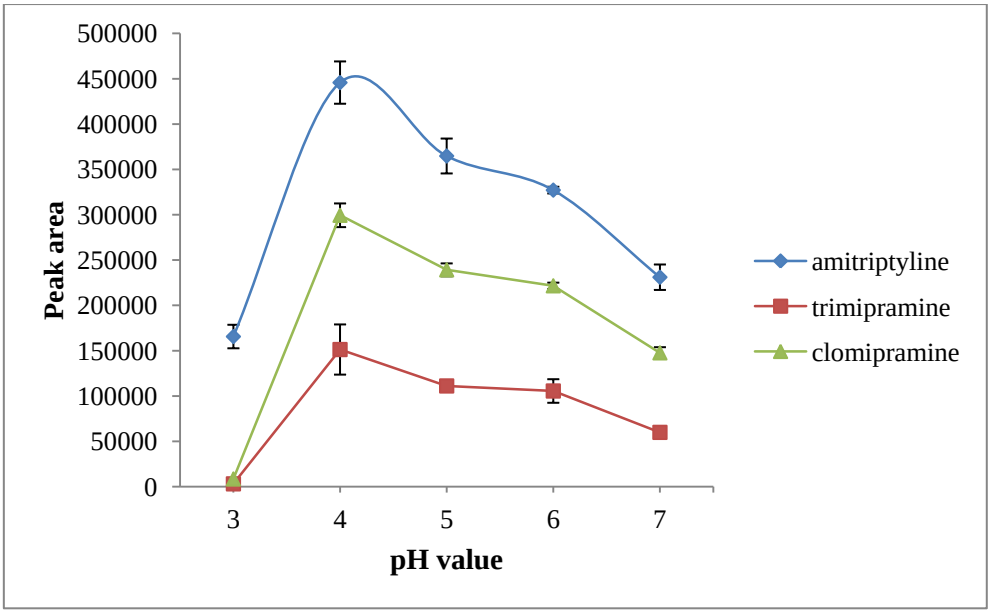


Figure 5-3 Effect of pH value of the donor solution on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; voltage, 15 V; extraction time, 10 min; agitation speed, 500 rpm; no salt addition; room temperature.

5.3.1.3 Extraction voltage and time

As expected, peak areas increased rapidly with the increase of voltage applied (Figure 5-4). In EME, the main driving force of mass transfer is the electrical field. Therefore, higher electrical potential enhances the migration efficiencies of the target analytes, consistent with what the modified Nernst-Planck equation indicates [199].

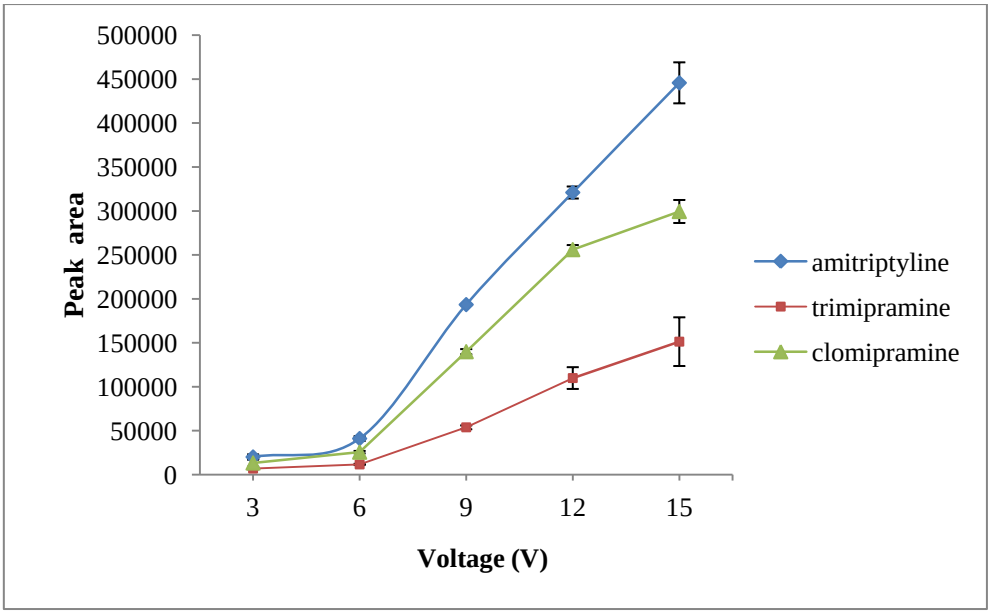


Figure 5-4 Effect of electrical potential applied on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; pH, 4; extraction time, 10 min; agitation speed, 500 rpm; no salt addition; room temperature.

Extraction time is another important factor, and a series of extraction times (5, 10, 15, and 20 min) was investigated. As shown in Figure 5-5, the extraction efficiencies increased from 5 min to 10 min, but no further increase from 10 min to 20 min was observed. The results indicated that the equilibrium could be reached within 10 min. The decrease in extraction efficiencies might be caused by the formation of bubbles at the fiber over longer extraction times. Another possible explanation could be due to the decrease of pH value after a certain time, leading to re-extraction of analytes back to the aqueous solution. Taking these factors into account, 10 min was selected as the most favorable extraction time.

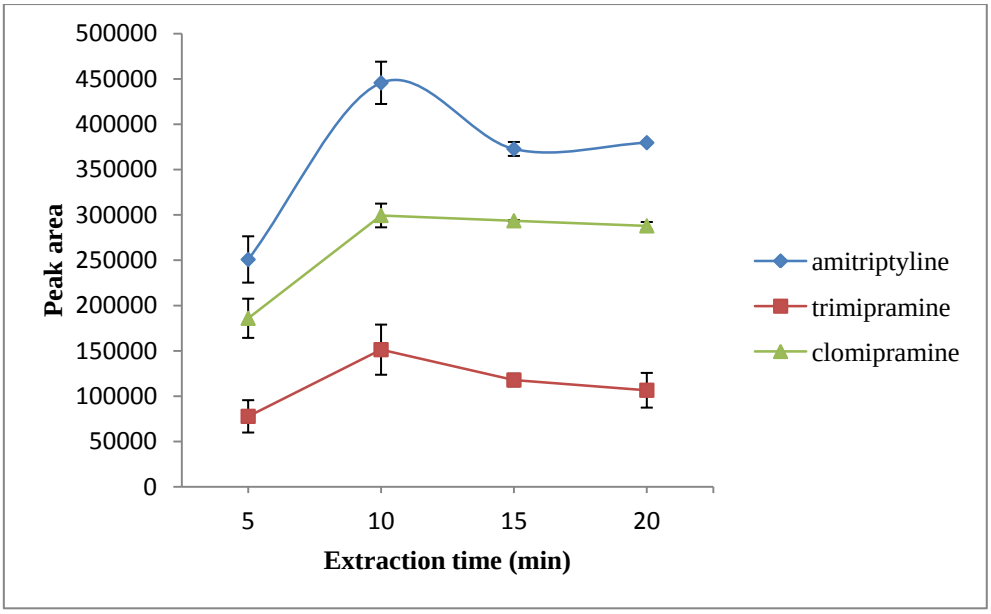


Figure 5-5 Effect of extraction time on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; pH, 4; voltage, 15 V; agitation speed, 500 rpm; no salt addition; room temperature.

5.3.1.4 Agitation speed

The extraction efficiency of EE-SPME depended on the equilibrium reached between compounds in the sample solution and those on the SPME fiber. The stirring rate could influence the mass transfer in this process, therefore affecting the final extraction efficiencies. Different stirring rates (250, 500, 750, and 1000 rpm) were studied, and the results are shown in Figure 5-6. It can be seen that the extraction efficiencies increased with the stirring rate, reached their maximum levels at 500 rpm and decreased thereafter. This observation was in agreement with Djozan‘s work [192]. Stirring reduces the thickness of the boundary layer between the aqueous solution and the surface of the SPME fiber, enhancing mass transfer. However, at higher stirring speed, electrostatic adsorption of the ionized target analytes on the surface of the SPME fiber might be disturbed by the substantial motion of the sample

solution and the vortex generated. Therefore, 500 rpm was adopted for subsequent experiments.

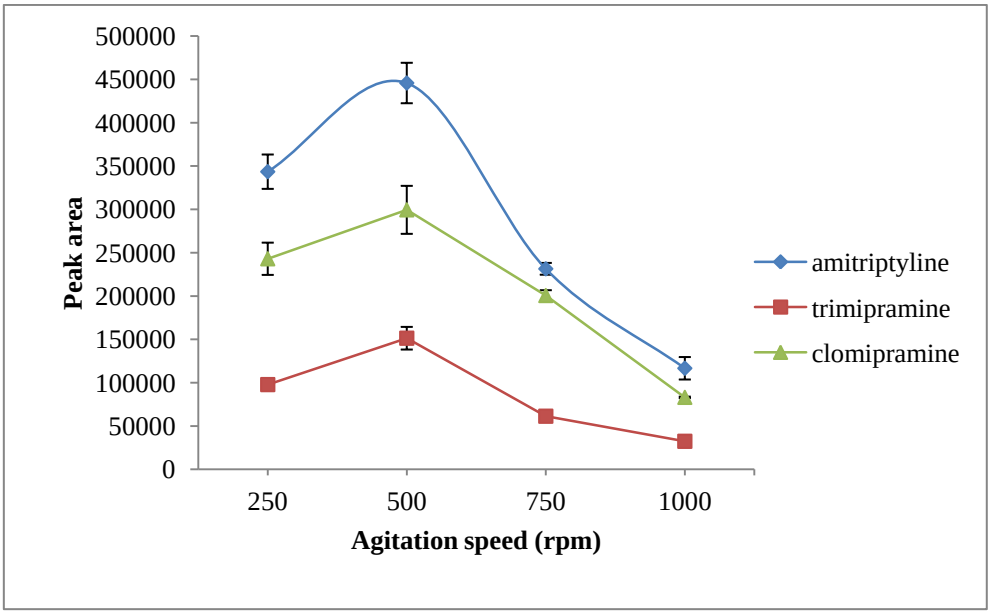


Figure 5-6 Effect of agitation speed on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; pH, 4; voltage, 15 V; extraction time, 10 min; no salt addition; room temperature

5.3.1.5 Desorption temperature and time

The desorption temperature and time may not only influence the sensitivity of the method, but also lead to problems such as sample carry-over if analytes are not desorbed thoroughly during analysis. Desorption time was fixed to 5 min while temperatures between 220 and 280 °C were studied (since 280 °C was the highest temperature recommended by the SPME fiber supplier). The results (Figure 5-7) indicated that desorption at 240 °C gave the best results. Desorption times (1, 3 and 5 min) were studied when desorption temperature was set as 240 °C. As can be seen from Figure 5-8, extraction efficiencies increased from 1 to 3 min, and then remained constant or slightly decreased

from 3 to 5 min. The results indicated that 3 min at 240 ˚C was adequate for complete desorption, and was therefore selected as the optimal desorption conditions.

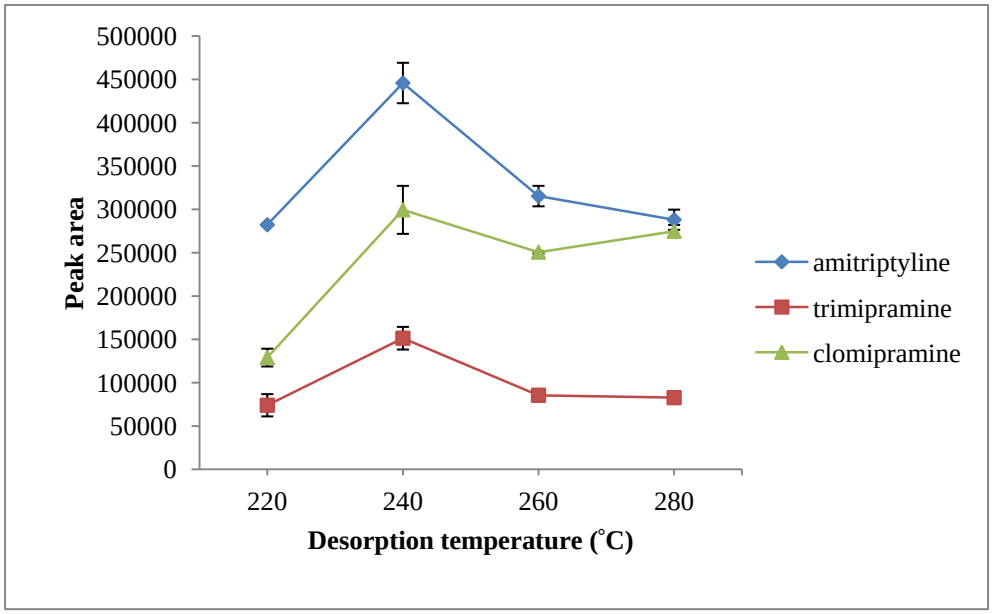


Figure 5-7 Effect of desorption temperature on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; pH, 4; voltage, 15 V; extraction time, 10 min; agitation speed, 500 rpm; no salt addition; extraction temperature, room temperature; desorption time, 3 min.

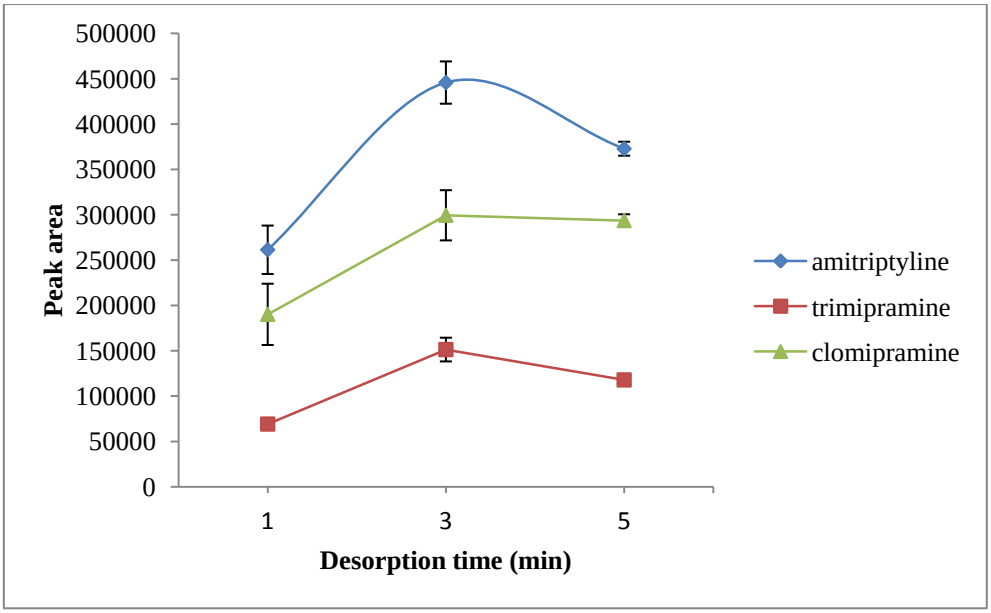


Figure 5-8 Effect of desorption time on extraction efficiencies. Extraction conditions: SPME fiber, PDMS, 100 μm; pH, 4; voltage, 15 V; extraction time, 10 min; agitation speed, 500 rpm; no salt addition; extraction temperature, room temperature; desorption temperature, 240 ˚C.

5.3.1.6 Other factors

Additional factors like salt addition and extraction temperature may also influence EME or SPME based on previous reports [200-203]. It was found in the present work that when NaCl was added to the sample solution, significant bubble formation occurred. This could be related to the migration of Cl^- (as a major competitor), and subsequent formation of chlorine gas bubbles on the electrode surface. Electrical migration of the analytes appeared to be more efficient without salt addition, which was in agreement with EME studies reported by Eskandari et al. [202] and Fotouhi et al. [203]. Therefore, no salt was added in the subsequent experiments. The extraction temperature was studied by preconditioning the extraction vial for 5 min in a water bath at different temperatures. The results showed no obvious increase in peak areas, suggesting that the mass transfer was mainly due to electrokinetic migration instead of passive diffusion which could be affected by the change of temperature.

Based on the above discussion, the most favorable EE-SPME conditions were: PDMS (100 μ m) coated SPME fiber, 10 mL aqueous solution, pH adjusted to 4, 15 V as electrical potential, extraction for 10 min at room temperature, stirring rate of 500 rpm, no salt addition, desorption for 3 min at 240 °C.

5.3.2 Method validation

Linearity, reproducibility, LODs and LOQs were evaluated to assess the

practical applicability of the developed EE-SPME procedure. The repeatability was studied (n=5) with spiked ultrapure water containing 10 μg/L of each analyte. As shown in Table 5-2, relative standard deviations (RSDs) were in the range of between 5.2 and 9.2 %, which was acceptable and comparable with other reported EME or SPME methods. The linearity was investigated in the concentration range of between 0.5 and 500 μg/L for amitriptyline and clomipramine, and between 1 and 500 μg/L for trimipramine, with coefficients of determination (r^2) ranging from 0.993 to 0.999. The LODs and LOQs, determined at a concentration at which signal-to-noise ratios were 3 and 10, ranged from 0.079 to 0.296 μg/L, and from 0.316 to 1.134 μg/L, respectively.

Table 5-2 Quantitative results of EE-SPME.

Analyte	LOD (μg/L)	LOQ (μg/L)	LR (μg/L)	r^2	RSD (%,n=5)
Amitriptyline	0.079	0.316	0.5-500	0.999	5.2
Trimipramine	0.296	1.134	1-500	0.993	8.6
Clomipramine	0.189	0.707	0.5-500	0.997	9.2

A comparison of LOD, linearity and extraction times between the proposed method and other published techniques for extracting TCAs including stirring bar sorptive extraction (SBSE), hollow fiber protected liquid-liquid-liquid microextraction (HF-LLLME), hollow fiber protected liquid-phase microextraction (HF-LPME), DLLME, EME and SPME are presented in Table 5-3. It can be seen that LODs obtained from this work were acceptable, being much lower than those of SBSE, DLLME and SPME, and comparable with those of HF-LLLME and HF-LPME. However, the extraction time required by the present method was only 10 min, much shorter than these other LPME or LLLME methods.

Table 5-3 Comparison between proposed method and other reported methods for determination of TCAs.

Extraction	Detection	LOD (μg/L)	Linearity (μg/L)	Extraction time	Ref.
SBSE	HPLC	40	10-1000	60 min	[17]
HF-LLLME	GC-MS	0.04	0.2-200	40 min	[198]
HF-LPME	HPLC	0.5	5-500	40 min	[171]
DLLME	GC-MS	2.0	2-100	-	[167]
EME	GC-FID	0.8	5-1500	20 min	[204]
SPME	LC	3	5-500	180 min	[205]
EE-SPME	GC-MS	0.08	1-500	10 min	This work

5.3.3 Genuine water sample analysis

To evaluate matrix effects, the proposed method was applied to determine TCAs in tap water and reservoir water under the most favorable extraction conditions. Since no TCA was detected in tap water or reservoir water samples, genuine water samples spiked with two levels of concentration (10 and 1 μg/L) were tested. As shown in Table 5-4, the relative recoveries, defined as the ratios of the analyte peak areas of the spiked genuine water sample and those of the spiked ultrapure water after EE-SPME, ranged from 82.4% to 103.9%, at the 10 μg/L level, and from 93.4% to 113.8 %, at the 1 μg/L level for tap water. The relative recoveries ranged from 85.5% to 97.3%, at the 10 μg/L level, and from 85.7% to 105.3%, at the 1 μg/L level for reservoir water. Figure 5-9 shows a chromatogram of the analytes spiked in tap water at 1 μg/L, which was extracted using the proposed method under the most favorable conditions. Therefore, the developed EE-SPME method was demonstrated as a suitable and robust method for the determination of TCAs from environmental aqueous samples.

Table 5-4 Relative recoveries and precision of EE-SPME from tap water and reservoir water spiked with TCAs at two levels of concentration (1 μg/L and 10 μg/L).

	Analyte	1 μg/L		10 μg/L	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Tap water	Amitriptyline	93.4	2.5	99.3	8.3
	Trimipranmine	113.8	15.5	82.4	11.5
	Clomipramine	107.6	10.6	103.9	1.4
Reservoir water	Amitriptyline	85.7	6.6	96.4	3.5
	Trimipranmine	105.3	11.1	85.5	4.6
	Clomipramine	89	11.2	97.3	5.7

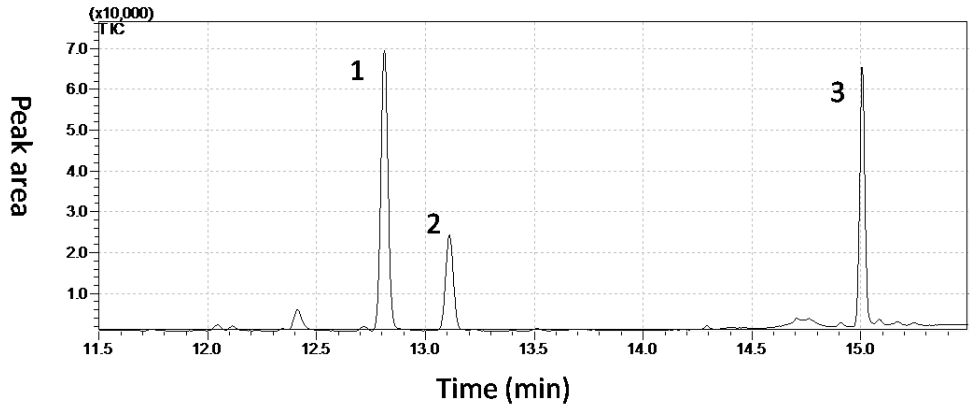


Figure 5-9 Chromatogram of extract of a spiked tap water (10 μg/L) under the most favored extraction conditions. Peaks: (1) amitriptyline (2) trimipramine (3) clomipramine

5.4 Conclusion

The present work illustrated that EE-SPME was a suitable approach for the isolation, preconcentration and clean-up of charged compounds from tap and reservoir water samples. The procedure combined the merits of SPME and electrokinetic migration. Compared with passive diffusion in conventional SPME, the electrical field enhanced extraction efficiencies significantly. Compared with conventional EME, EE-SPME is compatible with GC-MS, which can provide relatively low LODs for some compounds. Moreover, no consideration of supported liquid membrane (SLM) is needed which is necessary in conventional EME, making the whole process simpler, more convenient, and like conventional SPME, solventless. The proposed method showed good LODs, linearity, reproducibility, and could represent a fast and efficient alternative approach for extracting charged compounds without the consumption of organic solvents.

Chapter 6 Electromembrane extraction coupled with vortex-assisted micro-liquid-liquid extraction

6.1 Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of pharmaceutically active compounds which have long been used to treat inflammation and pain associated with various forms of arthritis. They have been widely used in human medicine due to the reason that unlike opioids, they do not produce sedation, respiratory depression or addiction [206]. However, severe nephrosis and gastrointestinal ulcer had been reported as side effects [207, 208], causing increasing concerns about their potential risks to human health and the environment. Most of these drugs can be discharged into the environment through human waste or drug manufacturing process [209, 210]. These compounds or their metabolites have been reported in wastewater, sewage water, surface water or even drinking water [57, 211-217]. Therefore, it is necessary to develop sensitive and efficient methods for the determination of these residues in environmental water samples.

Several analytical techniques including HPLC coupled with ultraviolet detection (UV) [14, 218], diode array detection (DAD) [216, 219], mass MS [213], and CE [220] have been developed to detect NSAIDs in different matrices. However, lower LODs are reported using GC-MS after derivatization of the native compounds to improve their volatility and chromatographic separation [211, 221]. N-(*tert*-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA),

N-methyl-N-(trimethylsilyl) trifluoacetamide (MSTFA) and bis-(trimethylsily) trifluoroacetamide (BSTFA) are the most commonly used derivatization reagents for compounds containing hydroxyl or carboxyl function groups [221-225]. Among them, MTBSTFA is favored since it has been successfully used to perform in situ derivatization [226-229]. In situ derivatization is convenient and time-saving because the entire derivatization process takes place in the GC injection port.

Sample preparation is of great importance in the determination of NSAIDs in environmental samples due to the low concentration of the target compounds and the complexity of the matrices. Several sample pretreatment methods have been used to extract NSAIDs from different matrices including SPME [230], SDME [212], HF-LPME [210, 231], dynamic HF-LPME [232], SBME [228], and LLLME [233]. However, one major disadvantage for most of these approaches is that the time required to extract target analytes is relatively long, usually from 30 minutes to several hours.

Electromembrane extraction (EME) has been demonstrated as an effective and efficient microextraction technique for analytes which could be ionized easily. In the first few studies, extractions were carried out under 300 V [107, 116]. Then more recently, other reports related to EME have been published in which lower voltages such as 50 V [117] or even 10 V were used [118]. According to these reports, mass transfer could be accomplished within 5 to 15 min, much shorter than those using SPME or LPME. Furthermore, the membrane used in EME serves as a filter, protecting the acceptor phase from

samples with complex matrices.

EME is more compatible with HPLC or CE, since the target analytes should be in their ionized forms in both the donor and acceptor phase. Recently, a two-step approach, electromembrane extraction combined with low-density solvent based ultrasound-assisted emulsification microextraction (EME-LDS-USAEME) was developed by Guo and Lee [111]. In their work, the acceptor phase from EME was used as the sample solution in the subsequent LDS-USAEME step and the organic solvent employed in LDS-UASEME could be injected into a GC system directly. However, the sample volume required in this particular study was relatively large (100 mL).

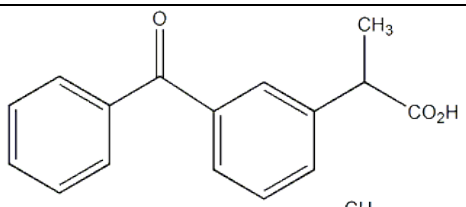
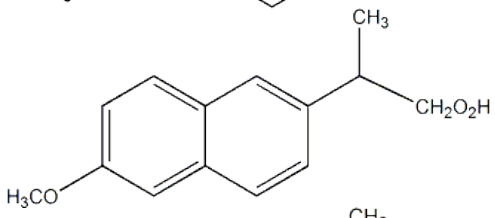
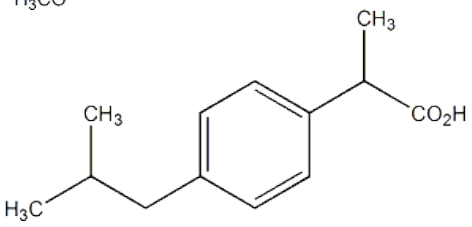
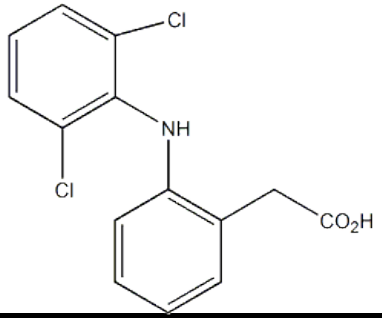
In this work, a highly efficient and rapid two-step approach, EME-VA-μ-LLE coupled with GC-MS after the in situ derivatization, was developed for the determination of NSAIDs in water samples. The VA-μ-LLE worked similarly to a very small-scale LLE or DLLME. The acceptor solution of EME served as the sample solution in the second step, and the analytes were further extracted to the organic solvent, which, together with the derivatization reagent, MTBSTFA, was then injected into GC-MS system. The main parameters affecting extraction performance were evaluated and the optimized method was applied to extract NSAIDs in environmental water samples.

6.2 Experimental

6.2.1 Reagents and materials

Non-steroidal anti-inflammatory drugs, ketoprofen, naproxen, ibuprofen, diclofenac, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Their structures and characteristics were listed in Table 6-1. Organic solvent, ethyl acetate (EA) was HPLC-grade and purchased from Fisher (Loughborough, UK). N-(*tert*-Butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) was brought from Sigma-Aldrich (Buchs, Switzerland). Other chemicals or reagents were the same as those mentioned in previous chapters.

Table 6-1 Characteristics of NSAIDs

Analyte	Molar mass	Molecular structure	p*K*a
Ketoprofen	254.28		4.45
Naproxen	230.26		4.15
Ibuprofen	206.29		4.91
Diclofenac	296.15		4.15

6.2.2 Apparatus

A voltage adaptor and a multimeter were bought from the local market. Two platinum wires were used as electrodes. A 100 μL syringe used for retrieval of the acceptor phase and a 25 μL syringe used for collection of the organic extract were purchased from SGE (Sydney, Australia). Q3/2 Accurel 2E HF (R/P) polypropylene membrane sheets (157 μm thickness, 0.2 μm pore size) used for fabricating envelopes for EME were purchased from Membrana GmbH (Wuppertal, Germany). A vortex agitator was bought from Scientific Industries (Bohemia, NY, USA) and used for VA-μ-LLE.

Analysis was performed using a Shimadzu (Kyoto, Japan) QP2010 Ultra GC-MS system, coupled with a DB5-MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm internal diameter (i.d.), 0.25 μm film thickness). A Shimadzu AOC-2000 autosampler was used to automate in situ derivatization. Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1.0 mL/min. The injection port temperature was set at 240 °C, and the GC-MS interface temperature was set at 300 °C. For chromatography, the GC oven was initially held at a temperature of 80 °C for 2 min, programmed to 300 °C at 20 °C/min, and held at the final temperature for 5 min. The solvent cut time was 8 min. The derivatized NSAIDs were analyzed in selective ion monitoring (SIM) mode. The monitored ions selected based on good selectivity as well as sensitivity were as follows: ibuprofen, *m/z* 161, 263, 319; naproxen, *m/z* 185, 287, 344; ketoprofen, *m/z* 295, 267, 311; diclofenac, *m/z* 214, 352, 409.

6.2.3 Sample preparation

A stock solution was prepared with acetone containing 1000 mg/L of each of the analytes and was stored in the dark at 4°C. Working solutions and environmental water samples were prepared the same as described before. All samples were kept in dark at 4°C until use.

6.2.4 EME-VA-μ-LLE procedure

Figure 6-1 shows the schematic of EME-VA-μ-LLE. In the first step of this method (EME), 5 mL of water sample solution was placed in a glass vial. A polypropylene envelope (1.2 cm length × 1.0 cm width) was prepared by heat-sealing the three edges of a folded membrane sheet. Seventy five microliters of the acceptor solution were introduced into the envelope which was then immersed in 1-octanol for 15 seconds to form the SLM in the membrane wall pores. A platinum wire was placed into the envelope and served as the positive electrode. Another platinum wire, serving as the negative electrode, was placed in the donor solution. A potential was applied between these two electrodes and the voltage was adjusted using the adaptor. The sample solution was agitated using a magnetic stirrer during EME. After extracting for a specified time, 60 μL of the acceptor solution was retrieved using a 100 μL syringe, and placed in a 100 μL glass insert. HCl was added to adjust the pH to neutral. Then, in the second step of extraction, a small amount (ca. 15 μL) of organic solvent was injected into the acceptor solution and the whole vial (with glass insert inside the vial) was vigorously shaken on a vortex agitator for a certain time. After extraction, the organic extract (of density

lower than water) which formed the upper layer was collected using a 25 μL microsyringe. One microliter of the extract combined with 1 μL of the derivatization reagent was then injected into the GC-MS system, for in situ derivatization and analysis.

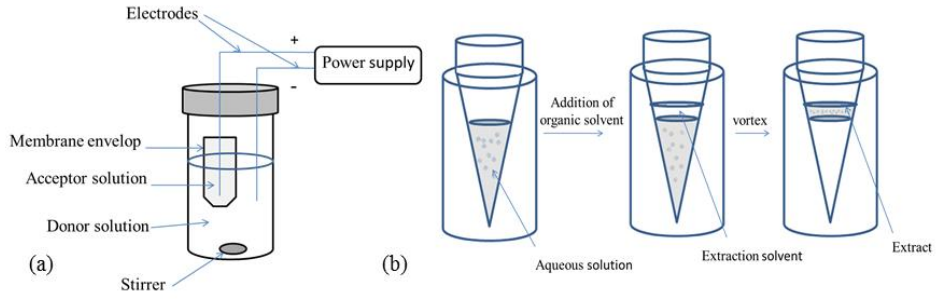


Figure 6-1 Schematic of EME-VA-μ-LLE: (a) EME (b) VA-μ-LLE.

6.3 Results and discussion

6.3.1 Optimization

In EME-VA-μ-LLE, parameters including pH of the donor solution, pH of the acceptor solution, voltage applied, extraction time, stirring rate, extraction solvent, extraction solvent volume, and vortex time were investigated. To determine the most favorable conditions, chromatographic peak areas were used to evaluate the efficiencies and all experiments were performed in triplicate. In all optimization experiments, the concentration of each NSAID was 5 μg/L.

6.3.1.1 Adjustment of pH of the donor and the acceptor solution

The pH of the donor solution is crucial since in EME, the analytes should be in their ionized forms. In order to investigate the influence of pH of the donor solution, extraction efficiencies were studied as a function of pH (pH 7, 9, 10, 11, 12) of the donor solution while keeping the pH of the acceptor phase constant at 12. Results showed that the maximum amount of each NSAID was extracted when the pH of the donor solution was adjusted to 10. This could be explained by the fact that in a solution with a higher pH, the analytes could be ionized completely, which is beneficial for their migration towards the acceptor phase. However, when too much NaOH was added, the ionic strength of the solution was affected, and this impacted the analyte migration process. [234] The pH of the acceptor phase was studied by investigating peak areas of the analytes over various pH values (pH 7, 9, 11, 12, 13) of the acceptor solution while keeping the pH of the donor solution at 10. The results showed that highest extraction efficiencies were achieved by maintaining the pH of the acceptor solution at 12. One possible explanation could be that a higher pH was required to maintain the analytes in their ionized forms, preventing them from being back extracted to the SLM. However, the competitive ions introduced during pH adjustment might also influence the migration of target analytes and decrease the final extraction efficiencies. Therefore, in subsequent experiments, the pH of the donor solution was adjusted to 10 and the pH of the acceptor solution was set at 12.

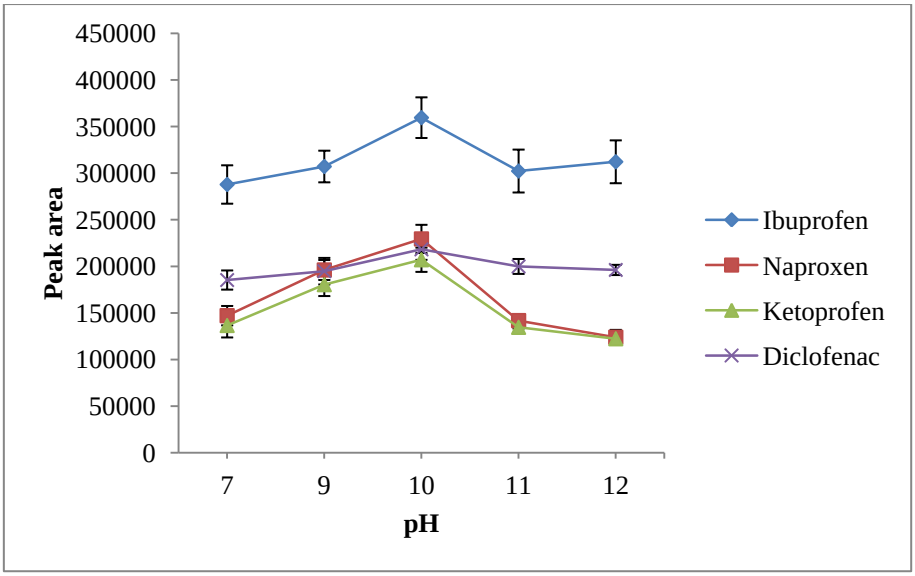


Figure 6-2 Influence of pH of the donor solution. Extraction conditions: pH of the acceptor solution, 12; EME, 9 V for 10 min; stirring rate, 1000 rpm; VA-μ-LLE extraction solvent, EA, 15 μL; vortex time, 1 min;

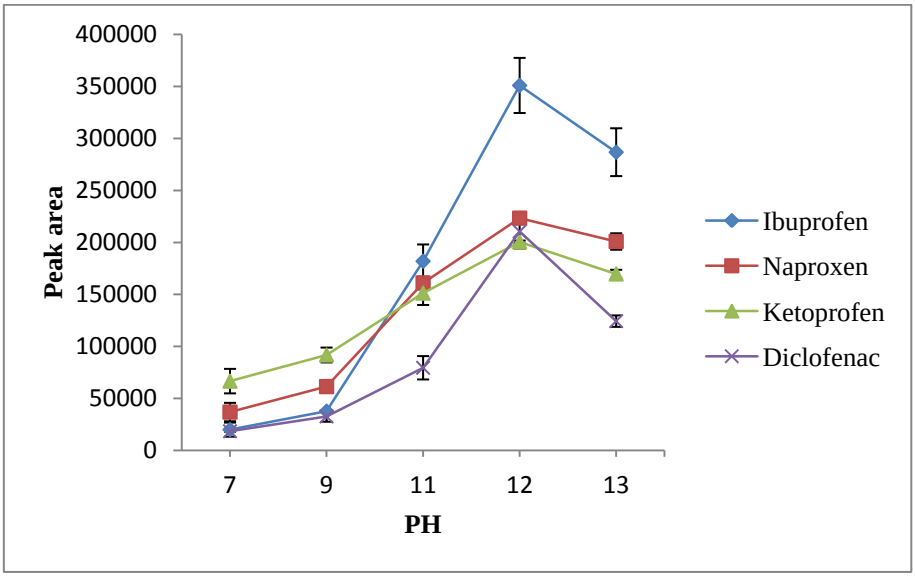


Figure 6-3 Influence of pH of the acceptor solution. Extraction conditions: pH of the donor solution, 10; EME, 9 V for 10 min; stirring rate, 1000 rpm; VA-μ-LLE extraction solvent, EA, 15 μL; vortex time, 1 min;

6.3.1.2 Voltage and time profile of EME

In EME, the electrical potential is the major driving force for migration of

ionized analytes, greatly influencing the extraction efficiencies. In order to find the optimal potential, a series of extractions was performed under varied electrical potentials (3, 6, 9, 12, 18 V). The results indicated that the efficiencies increased when the potential was raised from 3 to 9 V whereas no further increase was observed when the voltage was raised from 9 to 18 V. This phenomenon could be explained by back-extraction of target analytes into the SLM under lower pH, since the pH of the acceptor solution might decrease slightly due to electrolysis [190]. Under a higher voltage, small bubbles were generated at the electrodes and these negatively influenced the migration of the target analytes.

Extraction time is another significant parameter in EME. Extraction efficiencies were studied as the function of extraction time, and the results are summarized in Figure 6-5. The results showed that the extraction efficiencies increased with the increase of EME duration up to 10 min. After 10 min, efficiencies remained almost constant or even decreased. This could be caused by mass transfer resistance and build-up of a boundary layer of ions at the interface on both sides of the SLM at a longer duration [190]. The saturation of analytes in acceptor phase could also lead to back-extraction to the SLM and decrease the final extraction efficiencies [235]. Based on these observations, 9 V was selected as the optimal voltage and 10 min as the optimal extraction time.

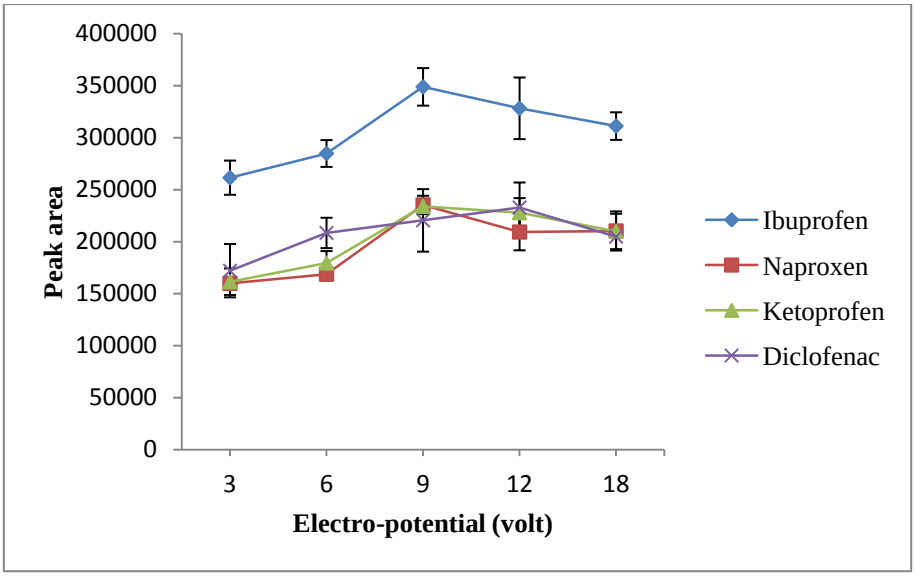


Figure 6-4 Influence of voltage of EME. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME duration, 10 min; stirring rate, 1000 rpm; VA-μ-LLE extraction solvent, 15 μL EA; vortex time, 1 min;

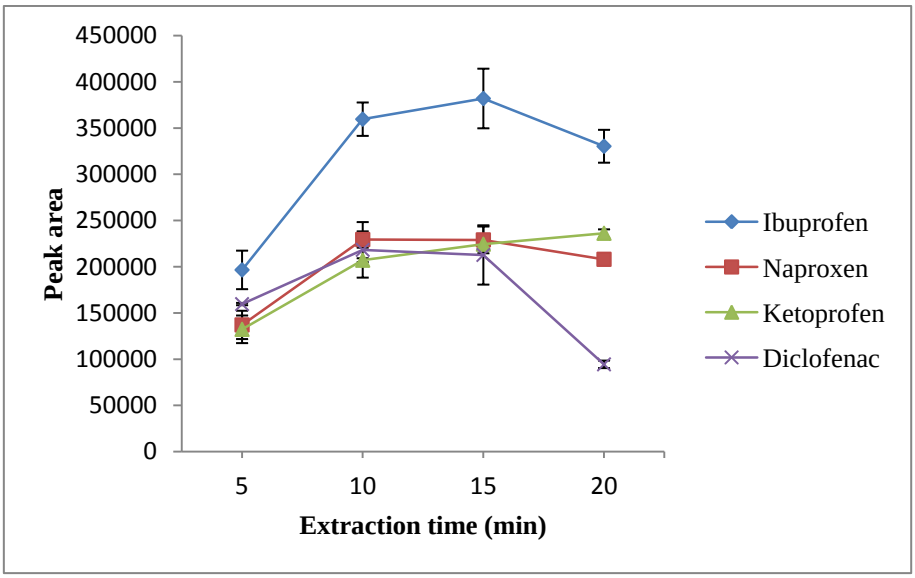


Figure 6-5 Influence of duration of EME. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME voltage, 9 V; stirring rate, 1000 rpm; VA-μ-LLE extraction solvent, 15 μL EA; vortex time, 1 min;

6.3.1.3 Stirring rate

The stirring rate from 0 to 1000 rpm was studied. The results indicated that

stirring was a significant parameter influencing extraction efficiencies despite the fact that extraction could take place in the absence of stirring. The extraction efficiencies increased correspondingly with enhanced stirring speed. Higher stirring rate could promote mass transfer and reduce the thickness of the boundary layer of interface at both sides of SLM at the same time [107]. However, when the stirring rate was set higher than 1000 rpm, some loss of SLM was observed, most probably due to mechanical action, resulting in a decrease of extraction efficiencies and reproducibility. For this reason, 1000 rpm was selected as the optimal stirring rate for the rest of this study.

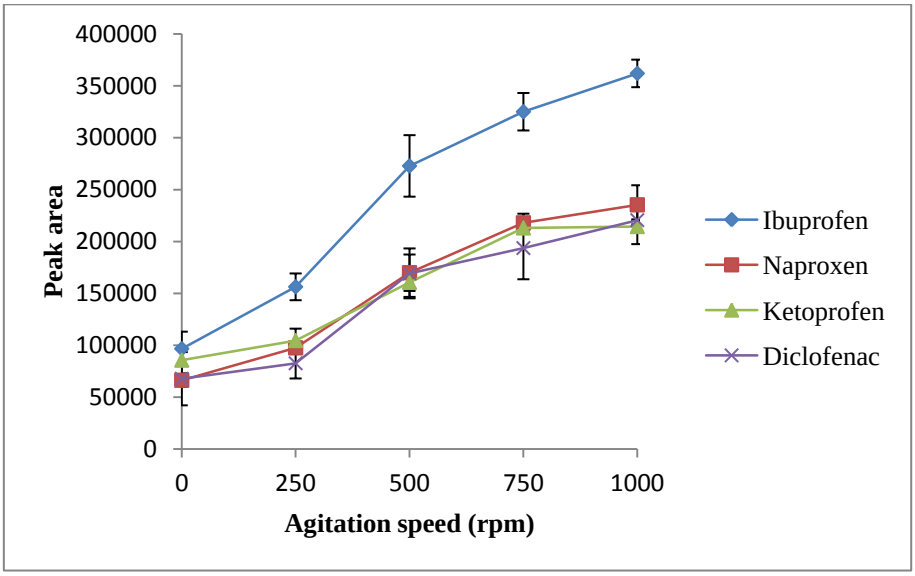


Figure 6-6 Influence of stirring rate. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME voltage, 9 V for 10 min; VA-μ-LLE extraction solvent, 15 μL EA; vortex time, 1 min;

6.3.1.4 Extraction solvent and volume of VA-μ-LLE

The extraction solvent used was selected based on the following requirements: (i) good extraction capability for the target analytes; (ii) low solubility in the aqueous solution; (iii) compatibility with GC-MS system; (iv) non-reactivity

with the derivatization reagent; and (v) no or minimal known toxicity. Accordingly, 5 low-density organic solvents, ethyl acetate (EA), *n*-hexane, cyclohexane, *o*-xylene, and toluene were evaluated. As can be seen in Figure 6-7, EA gave the highest extraction efficiencies for all of the analytes, followed by toluene and *o*-xylene (except when ibuprofen was concerned). Based on this observation, EA was selected as the most favorable extraction solvent for this work.

The influence of the extraction solvent volume during VA-μ-LLE was investigated by using EA at different volumes (15, 20, 25, 30 μL). As expected, the peak areas decreased correspondingly with increasing EA volumes. This could be explained by the dilution of the final extract. Nevertheless, since it was problematic to retrieve sufficient extract when the initial extraction solvent volume was less than 15 μL, this volume of EA (15 μL) was selected as the most favorable for the extraction process.

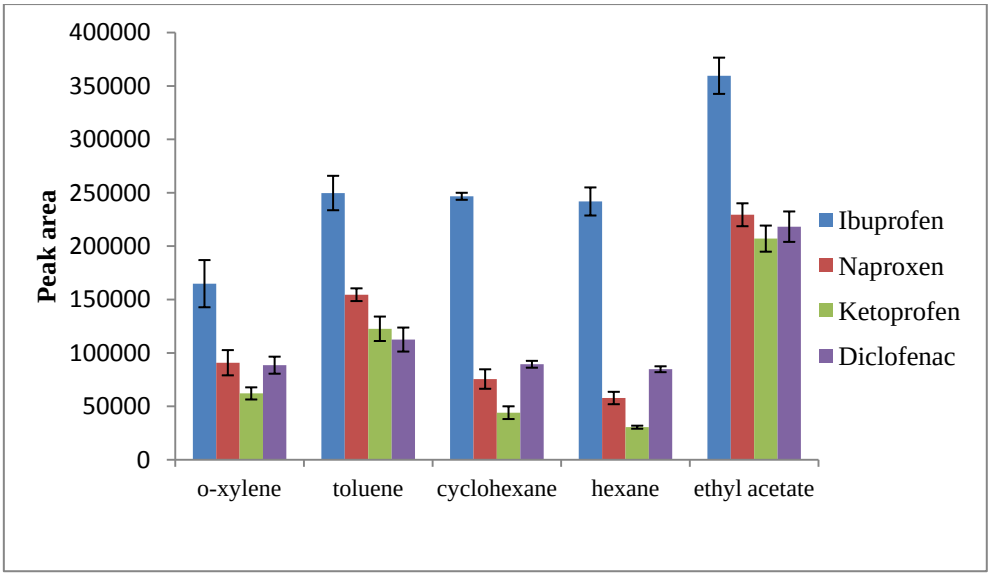


Figure 6-7 Influence of extraction solvent in VA-µ-LLE. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME voltage, 9 V for 10 min; stirring rate, 1000 rpm; VA-µ-LLE extraction solvent volume, 15 µL; vortex time, 1 min;

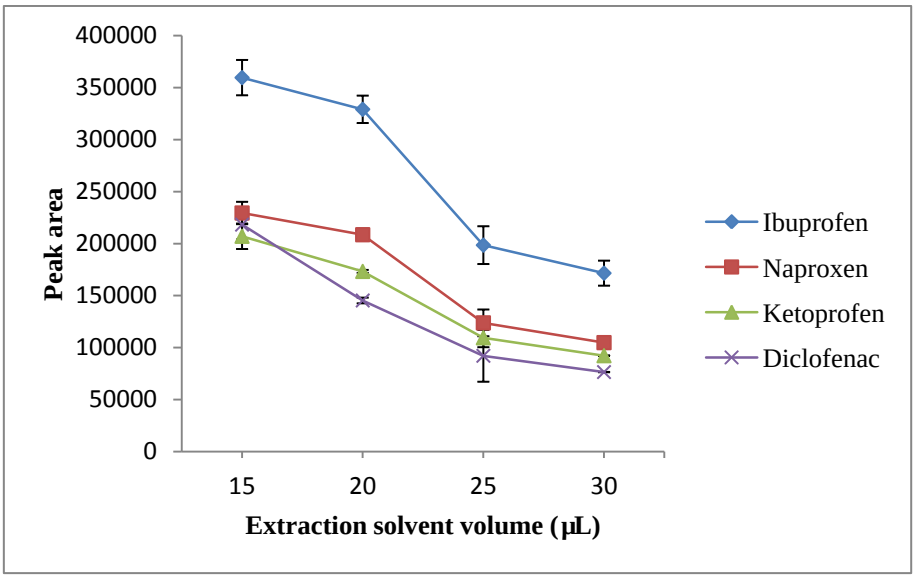


Figure 6-8 Influence of extraction solvent volume in VA-µ-LLE. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME voltage, 9 V for 10 min; stirring rate, 1000 rpm; VA-µ-LLE extraction solvent, EA; vortex time, 1 min;

6.3.1.5 Vortex time

In the second part of the extraction (VA-µ-LLE), the extraction solvent was

injected directly into the acceptor phase obtained from EME. The whole process was performed in a 0.1 mL glass insert within a 1.5 mL vial. During this process, a vortex agitator was used to facilitate mass transfer of the target analytes from the acceptor phase of the first step to an organic extraction solvent. Hence, the vortex time was also a significant parameter. The effect of vortex time (0.5, 1, 3, 5 min) was studied while keeping the agitation fixed at 3200 rpm. The results showed that the extraction efficiencies increased from 0.5 to 1 min, but no further increase was observed from 1 to 5 min. This observation indicated the mass transfer of the analytes from the aqueous solution to the organic solvent was very fast due to the fine dispersal of droplets of extractant created by vortex agitation. Therefore, 1 min was adequate to achieve extraction equilibrium and thus selected as the optimal vortex time.

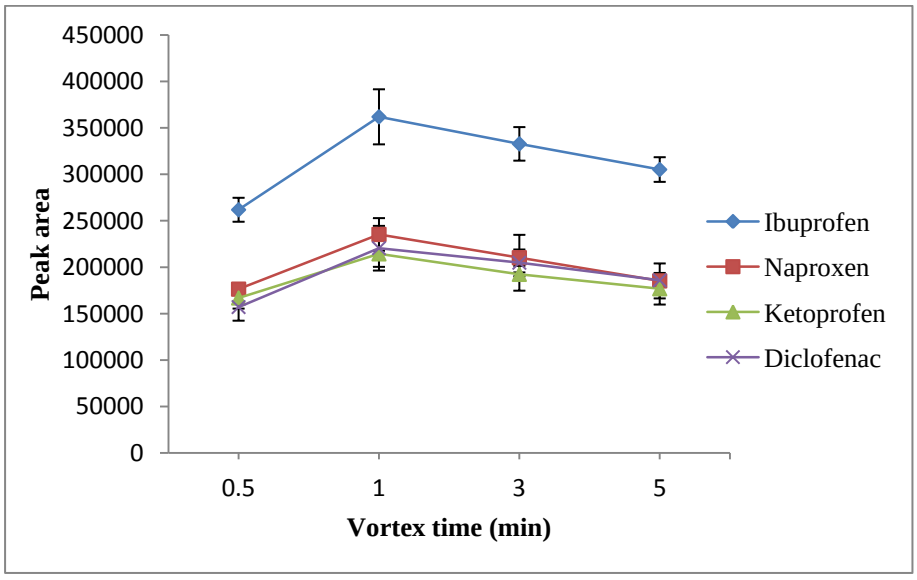


Figure 6-9 Influence of vortex time in VA-μ-LLE. Extraction conditions: pH of the donor solution, 10; pH of the acceptor solution, 12; EME voltage, 9 V for 10 min; stirring rate, 1000 rpm; VA-μ-LLE extraction solvent, 15 μL EA;

Based on the discussion above, the most favorable extraction conditions for EME-VA-μ-LLE were as follows: EME was performed under 9 V for 10 min with pH of the donor solution adjusted to 10 and that of the acceptor solution maintained at 12; 1-octanol was employed as the SLM; stirring rate was set at 1000 rpm; pH of the acceptor phase after EME was adjusted to neutral and 15 μL of EA was used as extraction solvent for VA-μ-LLE for 1 min; finally, 1 μL of the extract combined with 1 μL of derivatization reagent was injected into the GC-MS system.

6.3.2 Method validation

Under the described extraction conditions, the method was validated in terms of the usual parameters including linearity, LODs, LOQs, and repeatability. They were studied by using spiked ultrapure water samples and the results are summarized in Table 6-2. Satisfactory linearity of response was observed over a concentration of 0.145 to 10 μg/L for all of the analytes with coefficients of determination (r^2) ranging from 0.991 to 0.999. When the concentrations of NSAIDs in the test solution were higher than 10 μg/L, 1 μL of MTBSTFA was not adequate to effect complete derivatization. Therefore when dealing samples with analyte concentrations higher than 10 μg/L, dilution would be needed. (Alternatively, a higher volume of MEBSTFA could be used, but derivatization reagents are normally very expensive, thus dilution of samples would be preferred.)

From Table 6-2, it can be seen that the LODs and LOQs, determined at a

concentration at which signal-to-noise ratios were 3 and 10, ranged from 0.012 to 0.037 μg/L, and from 0.031 to 0.146 μg/L respectively. Repeatability was evaluated based on 5 consecutive analyses (concentrations at 5 μg/L of each analyte) at the most favorable operational parameters and good relative standard deviations (RSDs) were obtained (lower than 7.7% for all analytes).

A comparison of LODs, extraction time and sample volume between the proposed method and other published approaches for extracting NSAIDs are summarized in

Table 6-3. It can be seen that the LODs obtained from this method were lower than those using HF-LPME followed by CE with diode array detection [236], hollow fiber protected SPME followed by GC with flame ionization detection [237] and EME followed by HPLC with diode array detection [118], comparable with those achieved in SPME-GC-MS [224], continuous-flow-HF-LPME-HPLC [238], and slightly higher than those using SBME followed by GC-MS [228] and solid-phase extraction followed by ultra-performance LC-tandem mass spectrometry [239]. However, it should be noted that the sample volume required in the proposed method was only 5 mL, much less than those required in SPE or HF-LPME. Furthermore, the extraction time was only 10 min, much shorter than the SPME, LPME or SBME-based methods.

Table 6-2 Validation parameters from spiked ultrapure water samples.

Analyte	LOD (μg/L)	LOQ (μg/L)	LR (μg/L)	r^2	RSD (%,n=5)
Ibuprofen	0.017	0.046	0.046-10	0.999	7.1
Naproxen	0.037	0.146	0.145-10	0.997	5.0
Ketoprofen	0.012	0.031	0.031-10	0.991	7.7
Diclofenac	0.024	0.09	0.09-10	0.999	4.8

Table 6-3 Comparison of LODs obtained from different methods.

Method	Extraction time	Sample volume	LOD (μg/L)	Ref.
HF-LPME-CE-DAD	20 min	50 mL	0.25-0.86	[236]
SPME-GC-MS	40 min	22 mL	0.012-0.04	[240]
HF-SPME-GC-FID	80 min	3 mL	0.03-0.07	[237]
Continuous-flow-HF-LPME-HPLC	45 min	-	0.01-0.05	[238]
SBME-GC-MS	40 min	10 mL	0.006-0.022	[228]
SPE-UPLC-MS/MS	-	100 mL	0.009-0.974	[239]
EME-HPLC-DAD	10 min	10 mL	0.08-3.36	[118]
EME-VA-μ-LLE-GC-MS	10 min	5 mL	0.012-0.037	this work

6.3.3 Genuine water sample analysis

In order to evaluate matrix effect of the proposed method, experiments were carried out to determine NSAIDs in tap water and river water under the optimum conditions. However, there were no target analytes detected in the tap water or river water samples, indicating either these analytes were not present or the concentrations were below LODs of the proposed method. Therefore, all water samples, fortified with target analytes at tow concentration levels (0.1 μg/L and 1 μg/L) were analyzed to study the relative recoveries, which are defined as the ratios of peak areas of the target analytes in genuine water samples to peak areas of the analytes in ultrapure water samples spiked at same concentration after extraction. As can be seen in Table 6-4, the recoveries were in the range between 83.4% and 106.4%, with RSDs lower than 10.9%. Obviously, the matrix has only a minor effect on this method. Hence, the proposed method was demonstrated as a rapid and robust method for the determination of NSAIDs from environmental water samples.

Figure 6-10 shows a chromatogram of spiked water sample extracts under most favorable extraction condition.

Table 6-4 Summary of results from analysis of NSAIDs in spiked genuine water samples.

		0.1 μg/L		1 μg/L	
	Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Tap water	Ibuprofen	99.4	4.9	96.5	4.2
	Naproxen	91	3.5	92.3	10.9
	Ketoprofen	89.5	6.4	88.5	10.5
	Diclofenac	97	3.5	102	2.7
River water	Ibuprofen	98.7	6.2	89	6.3
	Naproxen	92.2	5.8	83.4	3.7
	Ketoprofen	97.2	4.9	89.7	10.2
	Diclofenac	96.5	2.5	106.4	4

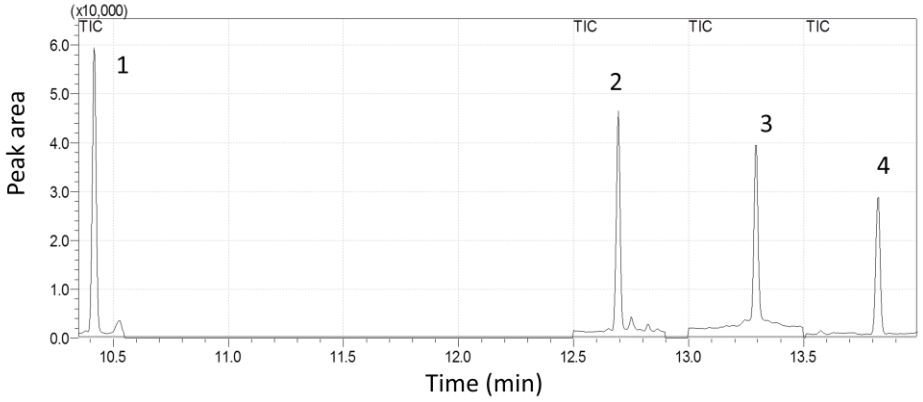


Figure 6-10 Chromatogram of spiked tap water sample (spiked at 1 μg/L) after EME-VA-μ-LLE under the optimal conditions. Compounds: (1) ibuprofen, (2) naproxen, (3) ketoprofen, (4) diclofenac.

6.4 Conclusion

In this study, electromembrane extraction followed by vortex-assisted micro liquid-liquid extraction (EME-VA-μ-LLE) coupled with in situ derivatization followed by GC-MS was developed for the determination of NSAIDs in environmental water samples. The proposed method was simple to operate, accurate and rapid. Furthermore, the membrane used in EME could protect the acceptor solution against potential interference, and was beneficial when dealing with complex sample matrices. The performance of this method was

studied and low LODs (down to 0.012 μg/L) and good repeatability (RSDs lower than 7.7%) were obtained. It was then tested on genuine water samples and the results indicated that the proposed method was an effective and efficient approach for the trace determination of NSAIDs in environmental water samples.

Chapter 7 Conclusions and outlook

Several miniaturization methods for rapid determination of several classes of organic contaminants from environmental water samples were developed in the present dissertation. The novel extraction methods reported in this thesis provide feasible alternative approaches to conventional sample preparation techniques.

The main focus of this thesis is about modification of conventional DLLME and electro-enhanced techniques. They are favored since the extraction time is greatly reduced according to their extraction principles. The modification of DLLME involves the utility of low-density solvent (environmentally friendlier than conventional chlorinated organic solvents), convenient collection of low-density and facilitation of emulsion formation.

In Chapter 2, LDS-SD-DLLME was shown to be fast, simple and convenient to extract PAHs from real water samples. In LDS-SD-DLLME, a second portion of disperser served as the demulsifier, quickly breaking down the emulsion after extraction. No centrifugation is needed. A disposable syringe served as the extraction device, allowing easy collection of the organic extract after extraction. No specific home-made device is required. As a result, high extraction efficiencies were achieved in a very short period of time.

Despite all the merits of SD-DLLME, adding relatively large amount of disperser and demulsifier reduces the participation coefficients of analytes in extraction solvent. Therefore, surfactant and ultrasonic radiation were adopted

to facilitate emulsion instead of a disperser, and a novel method termed LDS-UASEME was investigated in Chapter 3 for extracting OCPs. A disposable pipette was used as the extraction device and low-density extract could be easily retrieved. Combined with GC-MS, satisfactory LODs from 6 to 57 ng/L and good RSDs were achieved. Matrix effects were evaluated and satisfactory relative recoveries were obtained. This work showed that LDS-UASEME combined with GC-MS, could serve as an alternative method for rapid and efficient determination of OCPs in environmental water samples.

Micro-SPE served as an alternative way for convenient retrieval of low-density extract and SAEME-VA-μ-SPE was proposed in Chapter 4. A μ-SPE device was used to collect analyte-enriched 1-octanol (extraction solvent) in UASEME, thus centrifugation is no longer needed. Ultrasonic waves were employed to facilitate emulsion formation, and vortex agitator was employed to accelerate mass transfer of 1-octanol from the cloudy solution to the μ-SPE device. MWCNTs were proved effective to absorb analyte-enriched 1-octanol, probably due to its extremely large surface area. The membrane used to build μ-SPE device protected the sorbent, which is beneficial to samples with complex matrices such as waste water, sea water or even biological samples. The results demonstrate that μ-SPE is a suitable cleanup method, paving the way for application of low-density solvent based DLLME methods.

To accelerate extraction of more polar compounds, EE-SPME and EME-VA-μ-LLE were investigated. In Chapter 5, EE-SPME followed by

GC-MS has been developed for the determination of three TCAs. This method overcomes one major drawback of SPME (viz. long extraction time) while remains its advantages including simplicity, solvent free and high sensitivity. This method opens up a novel practical way for fast determination of easily-charged compounds. Lastly, a two-step approach combining EME and vortex-assisted LLE was developed in Chapter 6. The extraction of target analytes was first accelerated to the acceptor phase within a PP membrane envelope under an electrical field. The membrane served as a barrier against potential interference, allowing its application in samples with complex matrices. Then the analytes were further concentrated using VA-μ-LLE. Vortex was adopted to enhance mass transfer from the aqueous acceptor phase of EME to the extraction solvent. Combined with in situ derivatization, satisfactory LODs could be achieved. Extraction conditions were investigated and the applicability of this method to real aqueous matrix was evaluated. The results indicate that this proposed method is a simple, rapid, effective and robust approach to extract acidic pharmaceutical compounds from environmental water samples.

To summarize, the results of this present study may have significant influence on providing alternative ways for overcoming some limitations of currently reported miniaturized methods. For example, conventional DLLME uses chlorinated extraction solvent, which is environmental unfriendly. While in this thesis, the application of a disposable syringe, a Pasteur pipette or a μ-SPE device allowed the use of low-density extraction solvent. No centrifugation was needed in SD-DLLME or SAEME-VA-μ-SPE, allowing possible

application in on site monitoring and broadening their application on environmental analysis. The proposed method in this thesis illustrates some promising modification of DLLME and electro-enhanced techniques for fast determination of POPs in environmental water samples.

The primary limitation of these approaches is possibly lack of automation. Future work should be devoted to the implementation of partial or full automation of some of these microextraction methods, with commercial autosampler systems such as those from CTC Analytics or Gerstel. Automation might increase expense and complexity in the initial stage, but it would ultimately be more convenient for the operator and increase sample preparation throughput. Moreover, lower RSDs should be achieved. Therefore, in order to encourage and facilitate commercial and industrial use, automation may be an important development.

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LIST OF PUBLICATION

1. R.Y. Xu, H.K. Lee, Application of electro-enhanced solid-phase microextraction combined with gas chromatography-mass spectrometry for the determination of tricyclic antidepressants in environmental water samples, *Journal of Chromatography A*, 1250 (2014) 15-22

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