DEVELOPMENTS IN SORPTION-BASED MICROEXTRACTION OF ORGANIC CONTAMINANTS IN WATER

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NATIONAL UNIVERSITY OF SINGAPORE

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Thesis declaration

I hereby declare that the work in this thesis is my original work and it has been written by me in its entirety under the supervision of Professor Lee Hian Kee, (in the laboratory of Microscale Analytical Chemistry), Department of Chemistry, National University of Singapore, between Jan 2011 and Jan 2015. I have duly acknowledged all the sources of information which have been used in the thesis. This thesis has not been submitted for any degree in any university previously.

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Summary

Among the recently developed sample preparation methods, solvent-minimized and environmentally friendly microextraction approaches have attracted the greatest attention. Porous membrane-assisted micro-solid phase extraction (μ-SPE) is a recently introduced sample preparation method that integrates microextraction configurations and membrane microfiltration with solid-phase extraction. It involves a two-step process and enabled simultaneous analyte extraction, sample clean-up and enrichment. μ-SPE of targeted analytes was examined to gain awareness into sorbent designs and selection conditions that could enhance vital parameters of μ-SPE namely, enrichment factor (EF) and relative recovery (R).

Recently, electro membrane extraction (EME) was proposed as a new concept for analytical sample preparation. In EME, charged analytes are extracted from an aqueous sample through an organic solvent (known as supported liquid membrane, SLM) immobilized in the pores of a thin polymeric membrane, and into a microlitre volume of an organic extractant phase. The driving force for the extraction is a direct current electrical potential sustained over the SLM. The method can be used for complex sample matrices, since membrane containing the extraction solvent can act as a filter to prevent the co-extraction of matrix interferences. In this work, EME involving a porous membrane filled with electroconductive sorbent and SLM is presented and fully discussed.

This thesis begins with a review of technical literature featuring the development and recent applications of microextraction techniques. Then, firstly, μ-SPE device using as a stirrer bar is reported and discussed. A novel sorbent, magnetic chitosan

functionalized graphene oxide was synthesized and characterized for use in μ-SPE. The π-π interaction between sorbent and analyte containing benzenoid ring facilitates adsorption of analytes onto the sorbent. Additional adsorption was due to the coarse surface and non-polarity of the cross-linked chitosan. During extraction, the μ-SPE device filled with sorbent was agitated in the sample solution serving as a stirrer bar by itself. Ultrasonication assisted desorption using organic solvent was then carried out to retrieve analytes. The extract was injected into a gas chromatography-mass spectrometric (GC−MS) system for analysis. This method, magnetic μ-SPE -GC-MS provided limits of detection (LODs) as low as 0.2 ng L^{-1} and EFs up to 302 for the determination of five polycyclic aromatic hydrocarbons in water.

Subsequently, a procedure based on μ-SPE followed by the more sensitive thermal extraction coupled with GC-mass selective detector is reported and discussed. This double-extraction method was validated for the determination of five polybrominated diphenyl ethers (PBDEs) in environmental water. Chitosan-graphene oxide (CS-GO) composite was prepared by CS and GO in aqueous solution using ultrasonicaton. The CS in the composite was then cross-linked with glutaraldehyde. The membrane protected sorbent CS-GO composite was used for the μ-SPE of PBDEs. The latter were extracted thermally in a thermal desorption unit tube combined with a cooled injection system for an analysis. The analytes possessing relatively lower molecular masses exhibited the higher extraction efficiencies. This method achieved LODs in the range 0.007 and 0.016 μg L^{-1} and EFs of up to 188. The selectivity of the sorbent is conceivably the reason for the enhanced performance of sorption-based microextractions including μ-SPE. Finally, in this thesis, EME-solid-liquid-phase microextraction (SLPME) to determine phenolic contaminants in water is reported. The extraction system consisted of a solid/liquid interface made up of three-phase

microextraction: an aqueous (donor phase), organic solvent/sorbent (membrane), and organic solvent (extractant phase), operated in a direct immersion sampling system. The sorbent, reduced graphene oxide/polyvinyl alcohol (r-GO/PVA), was first synthesized: by dispersing GO in PVA, which was then chemically reduced in situ using aqueous solution. The prepared sorbent dispersed in 1-octanol was immobilized in the pores of the porous polypropylene membrane by the aid of sonication. The membrane filled with organic solvent/sorbent was in contact with the aqueous donor and organic extractant solutions (1-octanol was used as SLM and extractant solution). The analytes were transported by application of an electrical potential difference of 100V over a SLM/sorbent into the organic extractant phase. After extraction, the analytes in the extract was derivatized prior to analysis by GC−MS. The LODs were in the range of between 0.003 and 0.053 μg L^{-1} with EFs up to 193. EME-SLPME was an effective technique to reduce extraction time (only 5 min). The shorter extraction time could reduce the potential co-extraction of other undesirable compounds, resulting in enhanced EF and R. The results indicated that this approach was suitable for the determination of polar contaminants in water. These sorbents are therefore favourable platforms for synthesizing sorbents for sorption–based microextractions of organic contaminants in water. Chapter 5 summarizes the results of this work, and a future perspective is presented for further work in this area.

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List of Abbreviations

Ace	Acenaphthylene
AF-SPME	Agarose film-solid phase microextraction
Ant	Anthracene
BDE-47	2,2´,4,4´-tetrabromodiphenyl ether
BDE-49	2,2´,4,5´-tetrabrodiphenyl ether
BDE-99	2,2´,4,4´,5-pentabromodiphenyl ether
BDE-153	2,2´,4,4´,5, 5´-hexabromodiphenyl ether
BDE-154	2,2´,4,4´,5, 6´-hexabromodiphenyl ether
BPA	Bisphenol A
BSTFA	*N,O*-bis-trimethylsilyltrifluoroacetamide
CAR	Carboxen
CIS	Cooled injection system
CP	Chlorophenol
CPE	Cloud-point extraction
CS	Chitosan
CW	Carbowax
DAD	Diode array detector
DI-SDME	Direct immersion-single drop microextraction
DLLME	Dispersive liquid-liquid microextraction
DVB	Divinylbenzene
ECD	Electron-capture detection
ED	Endocrine disruptor
EF	Enrichment factor (*E*)

EG	Ethylene glycol
EME	Electro membrane extraction
FESEM	Field emission scanning electron microscopy
FID	Flame ionization detector
FLD	Fluorescence detection
Flt	Fluoranthene
Flu	Fluorene
FTIR	Fourier transform infrared
GC−MS	Gas chromatography-mass spectrometry
GO	Graphene oxide
Hep P	*para*-n heptyl phenol
Hex P	*para*-n hexyl phenol
HF-LPME	Hollow fibre-liquid-phase microextraction
HPLC	High-performance liquid chromatography
HRMS	High resolution magnetic sector
HS-SDME	Headspace-single drop microextraction
HSSE	Headspace sorptive extraction
HS-SPME	Headspace-solid phase microextraction
IL	Ionic liquid
ILD	Ionic liquid dispersion
INCAT	Inside needle capillary adsorption trap
LC	Liquid chromatography
LDH	Layered double hydroxide
LLE	Liquid-liquid extraction
LLLME	Liquid-liquid-liquid microextraction

LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
MCFG	Magnetic chitosan functionalized graphene oxide
MeOH	Methyl alcohol
MEPS	Microextraction by a packed sorbent
MISPE	Molecularly imprinted solid-phase extraction
MOF	Metal-organic framework
MPS	Multipurpose sampler
μ-SPE	micro-solid-phase extraction
MSD	Mass selective detector
MWCNT	Multi-walled carbon nanotube
m/z	Mass-to-charge ratio
Nap	Naphthalene
NP	Nanoparticle
OP	*para*-n octyl phenol
PA	Polyacrylate
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
PDMS	Polydimethylsiloxane
Phe	Phenanthrene
POP	Persistent organic pollutant
PP	Polypropylene
PTV	Programmable temperature vapourizer
PVA	Polyvinyl alcohol

Pyr	Pyrene
r^2	Coefficient of determination
R	Relative recovery (*R*)
rpm	Revolution per minute
RSD	Relative standard deviation
r-GO	Reduced graphene oxide
SBME	Sorption-based microextraction
SBSE	Stir bar sorptive extraction
SDBS	Sodium dodecyl benzene sulfonate
SFO	Solidification of floating organic drop
SIM	Selective ion monitoring
SLM	Supported liquid membrane
SLPME	Solid-liquid phase microextraction
S/N	Signal to noise ratio
SPDE	Solid-phase dynamic extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SWCNT	Single-walled carbon nanotube
TA	Temperature-assisted
TE	Thermal extraction
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TMBP	*para*-(1,1,3,3-tetramethylbutyl)-phenol
TMCS	Trimethylchlorosilane
USA	Ultrasonication assisted

UV-Vis	Ultraviolet-Visible
VWD	Variable wavelength detector
XRD	X-ray diffraction
ZIF	Zeolite imidazole framework

Chapter 1 Introduction

1.1 Sample preparation

1.1.1 Preamble

Environmental contamination or pollution is widespread since it originates from both natural and anthropogenic sources. Natural emissions including volcano eruptions and forest fires can release pollutants into the environment. However, worldwide man-made pollutants from combustion, construction, mining, agriculture and warfare are increasingly substantial. These ubiquitous contaminants are found in all environmental compartments i.e. air, water and soil.

Water pollution, by the release of wastewater from commercial and industrial waste (deliberately or through spills) into surface waters; discharges of untreated domestic sewage, and chemical contaminants, such as chlorine, from treated sewage; release of waste and contaminants into surface runoff flowing to surface waters (including urban runoff and agricultural runoff, which may contain chemical fertilizers and pesticides); waste disposal and leaching into groundwater; leads to eutrophication.

Perhaps, the most important objective of analytical chemistry is to provide a means of determining the presence of elements and chemicals in the environment. Since environmental samples comprise a wide variety of complex matrices, it shows low concentrations of analytes and presence of several interferences. Therefore, sampling and sample preparation are of crucial importance in an analytical procedure, which basically consists of separation, quantitation, data collection and evaluation. In this list of significant steps, each has its own vital role for the achievement of a successful analysis. It is worth describing the difference between sampling, which consists of the

selection of the most homogeneous sample to be taken from the raw matrix, so to be appropriately representative of it; and sample preparation, which prepares the collected sample into a laboratory-suitable extract, ready to be analysed. In fact, it has been reported that around 80% of the analysis time is spent in sampling and sample preparation [1, 2].

The objectives of sample preparation are: isolation of target analytes, pre-concentration or sample enrichment so as to improve limits of detection (LODs) and limits of quantification (LOQs), and elimination of interfering substances from "dirty" samples (sample clean-up) [1]. For analytical approaches, development and validation include optimization of some critical analytical parameters such as accuracy, sensitivity, reproducibility, simplicity, cost effectiveness, flexibility and speed [3-5]. Moreover, recently introduced sample preparation techniques are challenged to meet the new criteria: miniaturization, higher sensitivity and selectivity, and automation. Currently, automation represents a desirable trend of handling all the various sample preparation steps involved: extraction, preconcentration, derivatization, and injection of target analytes [1, 6], including the integration with the analytical operation itself.

1.1.2 Sample preparation techniques

Conventional sample preparation techniques such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE), are major sample preparation techniques. However, these techniques are often laborious, and dependent on large volumes of samples. In addition, they are not environmentally friendly in that they require large and therefore wasteful amount of organic solvents. In recent years, the focus on development of green analytical procedures has prompted introduction of rapid and non-polluting techniques

of sample preparation [7]. In fact, sample preparation is considered the most polluting phase of analysis, with its reliance, conventionally, on organic solvents, which are harmful to both humans and the environment. In this respect, at least three options are available to the analyst; solventless or virtually solventless extraction, use of less harmful solvents, and use of extraction aids. The reduction or complete removal of solvents is a characteristic feature of the so-called miniaturized techniques, which basically use microextraction devices with small extracting surfaces. In solvent-free techniques, a small amount of sorbent material is directly exposed to the sample matrix, either a liquid or headspace. In virtually solventless techniques, drops of solvents are used as extraction phases. However, recently introduced miniaturized sample preparation techniques can be grouped in two main categories; extraction based on the use of sorbents and those based on the use of solvents [1, 8]. Sorbent-based or, perhaps more correctly sorption-based miceoextraction (SBME) including various modes of fibre-based solid-phase microextraction (SPME), microextraction by packed sorbent (MEPS), stir-bar sorptive extraction (SBSE), and micro-solid phase extraction (μ-SPE) have shown growing applicability [9-15]. Conversely, of the recently developed methods for liquid-phase extractions that require minimal solvent amounts, liquid-phase microextraction (LPME) is attractive due to the ease of the method and low requirement of the extracting organic solvent [16-20]. These two techniques, SBME and LPME, are the most suitable, reliable and useful methods as far as miniaturized extraction techniques are concerned in the contemporary laboratory. In this preliminary part of the thesis, advances in extraction designs and novel sorbent materials for SBME are given an overview. Furthermore, the improvement of LPME, especially its different operational modes, is also highlighted.

1.2 Sorption-based microextraction techniques

1.2.1 Solid-phase microextraction (SPME)

This miniaturized technique was introduced in the 1990s by Pawliszyn's group [21]. SPME is a solventless technique operated based on the adsorption/desorption of analytes onto a sorbent-coated fibre, which can be immersed either in the headspace or in the liquid matrix. The fibre is a fused silica or stainless steel rod covered with a thin film (7-100 μm) of stationary phase. The extraction is based on the creation of equilibrium between the target analytes and the coating. After the extraction is considered complete, the fibre is withdrawn and injected into the gas chromatography (GC) or liquid chromatography (LC) injector for analyte desorption and the chromatographic run [1]. The commonly used commercially available sorbents are: polydimethylsiloxane (PDMS), carboxen (CAR)-PDMS, divinylbenzene (DVB)-CAR-PDMS, polyacrylate (PA), PDMS-DVB, carbowax (CW)-DVB, and CW-templated resin. Many applications of SPME have been reported in the pharmaceutical, food, cosmetic, forensic, medical, environmental etc., fields.

SPME is a simple and sensitive solvent-free sample preparation technique. It integrates sampling, extraction and preconcentration in one step. However, while the application of SPME has covered many fields, there are also some limitations. The carryover effect is the main problem in SPME, which need to be eliminated [22]. Furthermore, the limitations on commercially available fibre coatings, extraction capacity, and lifetime of fibres, their brittleness (especially after multiple use) and their relatively high cost are considered as weaknesses of SPME.

For in-tube SPME, unlike fibre-based SPME, a capillary is internally coated with a polymeric film. The early work on this was reported by Eisert et al in 1997 [23]. This

method is based on the distribution of analytes between the sample solution and the stationary phase. This extraction device is particularly suitable for the enrichment of volatile compounds, since a continuous flow of the gaseous sample can be supplied through the extraction needle. Nevertheless, they can also be employed for water samples [24]. After extraction, the analytes can be desorbed by a flow of an appropriate mobile phase. In-tube SPME is fast and inexpensive, and it can overcome the drawbacks of fibres used in SPME. Moreover, this method is suitable for convenient automation which provides fast analysis and better precision and accuracy compared to manually operated techniques [25].

1.2.2 Microextraction by packed sorbent (MEPS)

Solid-phase dynamic extraction (SPDE), analogous to SPME, uses a syringe with a modified hollow needle whose inner wall is coated with a sorbent material. It is also known as the inside needle capillary adsorption trap (INCAT) (Fig 1-1). As reported initially, the extraction device is based on either a short fragment of capillary column or a layer of carbon being placed inside the hollow needle [26]. Sampling occurs by pushing the syringe plunger back and forth making the extraction process active, faster, and more sensitive. Thermal desorption takes place in MEPS is considered to be within the realm of SPDE technology, in which a gas-tight syringe is used with a micro-SPE device that is placed between the barrel and the needle (Fig 1-2). The available sorbents for SPE cartridges include derivatized silica or molecularly imprinted polymers. The sample preparation technique was first reported by Abdel-Rehim [27, 28]. MEPS consists of four different steps: sampling by pushing the syringe plunger back and forth; successive washing of cartridge with water for the elimination of interferences; withdrawal of the selected solvent, which dissolves target analytes; and finally, the

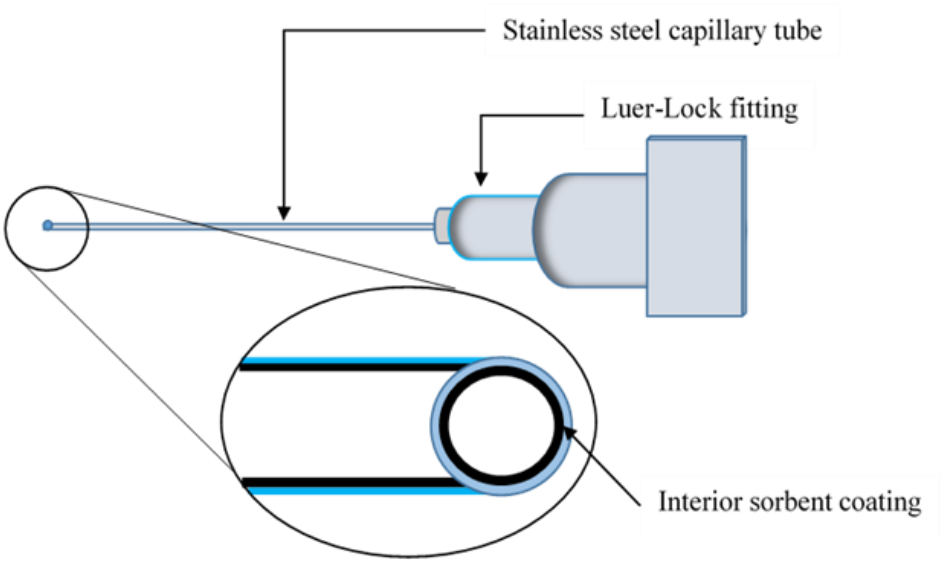

Fig 1-1 Schematic of INCAT device

injection of the extract into a GC or LC system. The miniaturization of SPE in the MEPS technique makes it able to handle sample volumes as small as 10 µL in a sorbent bed incorporated in a micro volume syringe (100-250 µL)

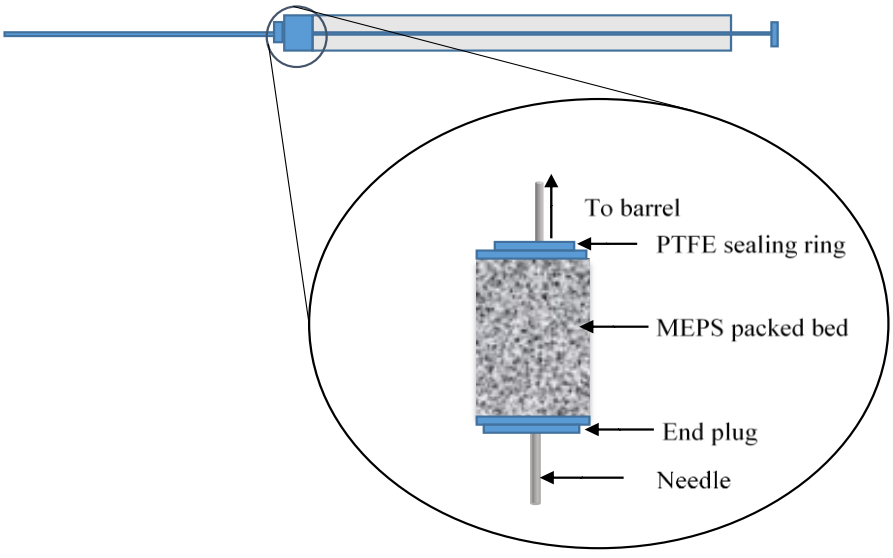

Fig 1-2 Schematic of MEPS device

[29, 30]. Furthermore, MEPS is fully automated and reduces sample preparation time and organic solvent consumption compared to SPE or LLE. This technique has been

used in environmental analysis of various groups of compounds, such as pharmaceuticals and personal care products, endocrine disruptors (EDs), aromatic amines, pesticides and polycyclic aromatic hydrocarbons (PAHs) [30].

1.2.3 Stir bar sorptive extraction (SBSE)

SBSE was originally reported by Baltussen et al in 1999 [31] based on the use of magnetic stirrer encapsulated in glass on which a sorbent material was coated. Operationally, the stir bar is submerged in the liquid sample matrix.

The technique can also be employed for headspace analysis: the stirrer bar is suspended in the sample headspace by means of stainless steel wire in a vial screw cap. In both, in-sample and headspace analysis, at the end of the extraction the stirrer bar is removed from the sample vial and transferred into the thermal desorber, which is located in the GC injector port.

The magnetic stir bar is usually 10-40 mm long, with the amount of PDMS (one of only three types of sorbents available commercially) varying between 55 and 220 mL. The fundamental theory of SBSE is the same as for headspace (HS)-SPME [32]. It operates based on sorptive extraction. The analytes migrate into the sorbent phase, and therefore, the total amount of extraction phase is important. However, compared to SPME, SBSE possesses higher concentration capability since the amount of PDMS is 50-250 times larger in SBSE. This feature allows the pre-concentration efficiency to be improved compared to SPME, which is the main advantage [33]. Alternatively, with its wide variety of fibre coatings at different degrees of polarity, SPME is considerably more selective than SBSE. Nevertheless, in recent years, developments have been made in this area in SBSE technology, including one approach called “dual-phase Twister” [34].

In fact, accompanied by the PDMS coating, a mixture of PDMS/EG (ethylene glycol) is accessible, coated onto an inert metal grid for mechanical stabilization. Hitherto, the commercially available coatings for stir bars include PDMS, EG-silicone and polyacrylate (PA), still a limited range [35]. The most popular and successful applications are the analysis of emerging environmental contaminants such as persistent organic pollutant (POPs), alkyl phenols, sunscreen agents, EDs, and some pharmaceuticals [7].

1.2.4 Micro-solid phase extraction (µ-SPE)

The first µ-SPE was reported in 2006 by Basheer et al. [36] in which multi-walled carbon nanotubes (MWCNTs) sorbent enclosed in a porous polypropylene (PP) membrane envelope was used to extract organophosporous pesticides from a sewage sludge sample.

During extraction, µ-SPE device tumbles freely in the sample solution stirred by a magnetic stirrer, facilitating extraction. The porous membrane performs as a filter to prevent the extraction of interferences in sample matrix and protect the sorbent as well. Therefore, further cleanup of the extract was not necessary. The consumption of organic solvent was much less compared to conventional SPE. Furthermore, µ-SPE has also been demonstrated with advantages to overcome some drawbacks associated with SPME. Since the µ-SPE device consists of the sorbent enclosed in a porous polypropylene (PP) membrane envelop, its significant benefit is that a wider range of different sorbent materials can be used for the extraction of different analytes. The choice of a suitable sorbent is crucial to determine the selectivity of the extraction.

In subsequent studies, different materials have been employed as sorbent by Basheer et al. for the µ-SPE of a variety of compounds in different samples, such as C_{18} sorbent to extract acidic drugs from water and carbamate pesticides in soil samples [37, 38], while HayeSep A and C_{18} sorbents were used to extract POPs in tissue samples [39]. In other studies, authors have also used a varieties of sorbents, depending on targets and samples [40-47] for µ-SPE.

1.2.4.1 Selection of novel materials applicable to µ-SPE

The main objectives of exploring for novel sorbents are: higher selectivity and specificity for unambiguous analytes, better sorptive and adsorptive capacity to obtain better sensitivities, along with enhanced thermal, chemical or mechanical stability. As mentioned above, the use of commercially available sorbents with different polarities, has been reported by several research groups for their ability to extract target analytes from samples [36, 47].

Silica-based sorbents such as C_2, C_8, C_{18} and HayeSep A and B were commonly involved in these studies. The non-polar characteristics (hydrophobicity) of these alkyl-functionalized silica sorbents were helpful when extracting non-polar analytes from aqueous matrices. If a less retentive phase (such as C_2 and C_8) is used, the analytes will still be sufficiently retained, but can be eluted more easily in minimal elution volumes. Bulky, very non-polar analytes, although well retained on C_{18} sorbents, can be difficult to elute as the non-polar interactions between analyte and sorbent are very strong. On the other hand, HayeSep B is highly polar, with HayeSep A being of the intermediate polarity.

Metal-organic framework (MOF) materials have drawn special attention in analytical applications in recent years. They are featured by higher surface areas, nanoscale porosity, tenable pore sizes, in-pore functions, and out-surface modification [48, 49]. Ge and Lee used zeolite imidazole framework 8 and 4 (ZIF-8 and ZIF-4) as the sorbent for μ-SPE of PAHs and antidepressant drugs from environmental water samples [50, 51]. Polychlorinated biphenyls in water sample were extracted using hollow fibre-protected MOF materials, in which (MIL-101) was used as μ-SPE adsorbents by Zang et al [49].

Carbonaceous materials have been extensively employed as sorbents since they have high surface area with a constant pore structure, leading to high adsorption capacity. Moreover, they are geometrically stable and inert in common organic solvents and at extreme pH conditions. Nano-structured materials such as nanoparticles, nanowires and nanotubes have become the focus of attention since their discovery in 1991 [52]. CNTs, especially MWCNTs, have been reported to be a powerful sorbent due to their excellent mechanical and electronic properties, and large surface area. These properties allow them to have effective sorption properties for the extraction of various organic compounds particularly with the benzenoid rings [53-56]. For example, Guo and Lee [57] reported the use of MWCNTs as μ-SPE sorbent to extract PAHs in environmental water.

Graphene, an allotrope of carbon, consists of sp^2 hybridized carbon atoms packed into a honeycomb crystal structure with extremely large surface area, and has high electron and thermal conductivity, which may make it attractive as sorbent. The improvement of numerous simple and easy methodologies to synthesize graphene and chemical-modified graphene in recent years, has promoted studies of their promising applications in various fields. Subsequently, significant developments in graphene chemistry have

also attracted the attention of researchers in analytical chemistry [9]. The functionalization of graphene has been considered to be important for improving their solubility and stability to avoid agglomeration in aqueous solution. Zhang and Lee had reported [58] in 2012 that sulfonated graphene sheets could be used as sorbent for the μ-SPE of PAHs in water. This water-soluble graphene sheets which possess the extensive π-electron system can interact strongly with benzene rings [59].

A report by Liu et al. [60] represented the first utilization of graphene powder as SPE sorbent. In this work, the cartridges packed with graphene powder were used to extract eight chlorophenols (CPs) from water which were determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The method revealed favourable results with high sensitivity and good reproducibility. The graphene in the packed cartridge stayed intact upon cleaning with water and various organic solvents, demonstrating its robustness. Comparative studies confirmed that graphene was superior to other sorbents including C_{18}, silica, graphitic carbon, single walled (SW) CNTs and MWCNTs along with sorption capacity, capability of elution and recovery for the extraction of the analytes considered. The advantages are attributed to not only the large surface area of the graphene but also the highly delocalized π-conjugate electrons on its surface, which offer strong π-π interaction between the target molecules and the sorbent material. Furthermore, the remaining hydrophilic groups and the easily accessible planar surface could increase retention and elution of polar molecules. Their work revealed the significant extractive capability of graphene towards aromatic compounds and reliability for reuse of the material [9, 60].

1.3 Solvent-based microextraction

1.3.1 Liquid-phase microextraction (LPME)

Loosely, LPME is a miniaturized form of LLE. Jeannot and Cantwell first introduced LPME technique, termed as solvent microextraction, in 1996 [61]. A microdrop (microliter of volume) suspended at the tip of a GC syringe needle is used to extract analytes from the sample solution. The water-immiscible drop is submerged in an aqueous solution. Once the extraction is completed, the microdrop is withdrawn into the needle and injected directly into the separation system such as GC or LC. There are several different operational modes in LPME: direct immersion-single-drop microextraction (DI-SDME), HS-SDME, hollow fibre (HF)-LPME, continuous flow-microextraction and liquid-liquid-liquid microextraction (LLLME) [62- 64]. More recently, the approach on dispersive liquid-liquid microextraction (DLLME) was introduced in 2006 by Rezaee et al [65]. In DLLME, the droplet acting as the extractant is mixed with an extra water-soluble solvent, defined as a "disperser", with both rapidly injected together into a water sample where droplets of the extractant are formed and dispersed. The extraction performance is facilitated by this increased contact surface area between droplets and the sample. Typical extractants are dichloromethane, chlorobenzene, and tetrachloroethane whereas acetone, acetonitrile and methanol (MeOH) are most often employed as dispersers [66]. Another form of LPME is HF-LPME which features a porous wall-hollow fibre, whose wall pores and channels are filled with the extracting solvent. Typical application of HF-LPME include the analysis of drugs in human urine [67-68].

In general, the advantages of LPME can be defined by simplicity, sensitivity, low cost, automation, and its ready-to-inject mode. Alternatively, the undesired phenomenon of

"drop-off" can occur due to the partial miscibility of the extractant with the aqueous sample solution. Therefore, a recent report by Zhang and Lee [69] presented a new approach for trace analysis using knitting wool as solvent holder. In their work, UV filters were used as the target analytes and good LOQs and linearity were obtained.

1.3.2 Electro membrane extraction (EME)

EME can be considered as an evolution of the HF-LPME technique. It is a relatively recent sample preparation technique, proposed by Pedersen-Bjergaard and Rasmussen in 2006 [70], focused on the extraction of electrically charged analytes. EME extractions are electrically driven. For alkaline analytes, an anode is placed in the sample aqueous solution and a cathode in the acceptor phase. The chemical environment is pH controlled due to the presence of ions. Therefore, the direction of the electrokinetic migration is governed by the pH gradient. The application of an electric potential enhances HF-LPME performance in terms of velocity of analyte mass transfer, thus the analysis time in EME is much reduced. When the EME is completed, the liquid acceptor phase is well-suited with analytical techniques such as LC or capillary electrophoresis (CE).

The advantages of EME thus includes: shorter analysis time, improved extraction capability, low consumption of solvents, selectivity (only charged species are extracted) and sensitivity. Apart from practical applications involving real samples, the kinetics of migration and development of membrane technology have also been the subject of investigation in EME. The technique fits well with the analysis of biological fluids, such as urine, plasma, breast milk, saliva, amniotic liquid and post mortem blood for forensic science [71-76].

1.4 Thesis purpose and scope

In the past few years, research in the analytical sample preparation field has been with respect to the discovery and the application of novel materials as sorbent for analyte extraction. Rapid, more selective extraction and high enrichment of analyte have been the desired goals that can be achieved with the use of appropriate sorbents. These newly developed sorbents help to address some of the limitations of the commercial ones. Therefore, the main objective of this work was to synthesize and characterize potential sorbents affording high selectivity and efficiency.

Although research on graphene dates back to about half-a-century ago, the new class of allotropic carbon nanomaterial began to initiate great interest in 2004, when an easy method to provide high quality graphene was invented [77]. The graphene nanosheets which possess planar geometry are in close interaction with the surrounding environment which is important in sorptive processes. The crumpled graphene surface can thus connect well with adsorbed targets. Moreover, functionalized graphene maintains the high surface area of the original material. Thus, the new sorbent is considered to possess the twin features of high surface area for sorption, and the existence of extended π-electrons with enhanced analyte π-π interaction capabilities. For these reasons, graphene-based sorbents in SPE and SPME have attracted great attention from analytical chemists in recent years [78, 79].

The first part of thesis reports the synthesis and characterization of a novel adsorbent, magnetic chitosan functionalized graphene oxide (MCFG). The application of this material was demonstrated by μ-SPE of PAHs in water followed by separation and detection using GC−MS. In a manner similar to that of SBSE, this μ-SPE device acts as the coated magnetic stir bar. Except that, normal stirring allows it to tumble freely in the sample solution. A single-step extraction (clean-up and enriching) procedure

involving ultrasonication assisted (USA)-µ-SPE was developed. This novel method demonstrates its applicability to real environmental water matrices.

Additionally, cross-linked chitosan-graphene oxide (CS-GO) was prepared and characterized and used as sorbent in µ-SPE to determine polybrominated diphenyl ethers (PBDEs) in water (reported in Chapter 3). In this work, a highly efficient two-step extraction method; USA-µ-SPE followed by thermal extraction (TE) was developed. The analytes in the extract (obtained by sonication of µ-SPE using organic solvent) were thermally extracted in thermal diffusion unit (TDU) tube at ~300ºC, which was combined with the cooled injection system (CIS) and GC injector port of an autosampler system. Finally, EME-solid-liquid phase microextraction (SLPME) was developed and reported in Chapter 4. Three-phase EME was developed based on a sorbent filled membrane bag. The highly electro-conductive sorbent, reduced GO/ polyvinyl alcohol (r-GO/PVA) composite, was prepared and characterized, which was then used to fill the pores of the membrane. Electrically charged moieties of alkyl phenols were extracted from water to an extractant organic solution, within a short duration. The procedure was evaluated using alkyl phenols as model analytes.

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Chapter 2 Magnetic Micro-Solid-Phase Extraction of Polycyclic Aromatic Hydrocarbons in Water

2.1 Introduction

Among conventional extraction techniques, SPE has several advantages such as relatively lower cost, reduced consumption of organic solvents as described in Chapter 1. However, the procedure consists of several steps, which can be time consuming and suffers from possible loss of analytes during the extraction process. Since the selection of the sorbent plays an important role for sorbent based analytical techniques, exploring new sorbents for enrichment is a new direction in environmental analytical chemistry and pollutant monitoring [1, 2].

In recent years, nano-magnetic materials have attracted much interest due to their unique size and physical properties. Because of the superparamagnetic property, the nanoparticles can be attracted by a magnet but do not retain magnetism after the field is removed. The magnetic nanoparticles on which organic contaminants are adsorbed can therefore be separated from the matrix by applying a magnetic field, and simply decanting the solution. Hence, the nanoparticles can be reused or recycled [3, 4].

SBSE has been widely applied to environmental monitoring [5] (see Chapter 1). However, the procedure has some major drawbacks: the commonly used sorptive stir bar frequently suffers from the loss of coating by friction, and as described in previous chapter, the commercially available SBSE has only limited types of sorbents. In order to overcome these difficulties with magnetic separation and commercial SBSE, a μ-SPE alternative to SBSE was developed in this work. As indicated in Chapter 1, the μ-SPE device consists of a porous PP membrane envelope in which a few milligrams of sorbent were enclosed. This device acted as a stir bar, rotating in the sample solution during extraction by application of magnetic stirring, facilitating mass transfer during

extraction of analytes from the aqueous sample solution. It is a relatively simple and fast extraction technique that requires minimal amount of solvent. The membrane of the device minimizes potential interferences in the sample and hence the extraction can be accomplished without additional sample clean-up. The major advantage of μ-SPE is that the extraction and enrichment during the extraction process take place in single step and without any wear-and-tear effect on the sorbent itself since it is protected by the membrane [6].

CS, a natural biopolymer obtained by hydrolysis of the aminoacetyl groups of chitin, is affordable and due to the presence of a large number of amino and hydroxy groups, which allows a wide range of chemical modifications to be made. As described in Chapter 1, graphene is a single layer sp^2 bonded carbon materials with honeycomb and two dimensional lattices. This greatly attributed to its excellent physicochemical properties including a large surface area, high dispersibility and hydrophilicity [7, 8]. GO is an oxidized form of graphene consisting of various functional groups such as hydroxyl, carboxyl, and epoxy groups [9, 10] (see Chapter 1). One of the major advantages with GO is that it is hydrophilic with very high negative charge density arising from the oxygen containing functional groups. In solution phase, GO exists as single layer and can act as weak acid cation exchange resin because of the ionisable carboxyl groups, which allow ion exchange with positively charged organic molecules. Despite the steric hindrance and diffusion restrictions, directly coupling the functional groups on polymer chains with hydroxyls, epoxides, or carboxylic acids on GO sheets still remains a facile and promising way to functionalize graphene [11, 12]. Moreover, a feasible and effective means of improving the dispersion of graphene mainly depends on the chemical groups of graphene oxide. Based on favourable adsorption properties of CS and the inherent properties of GO, possibilities were explored to apply the CS-

GO composite as an adsorbent. In this work, MCFG was prepared, in which the carboxyl group of GO was chemically reacted with the amine group of magnetic CS with the consequent formation of a covalent bond between the GO and the biopolymer [8]. However, since CS is soluble in acidic solution, it is necessary to crosslink the biopolymer in order to make it more stable at low pH. The cross-linking CS between functional groups of CS and different kinds of cross-linking agents conceivably converts CS into a hydrophobic polymer. Most of the CS-based sorbents are submicron to micron-sized [13-15]. The combination of magnetic CS with GO conceivably possessed the higher extraction capacity due to the hydrophobicity and the large delocalized π-electron system which can facilitate the π-π stacking interactions with aromatic compounds such as PAHs [16]. PAHs are ubiquitous pollutants in the environment including water. Thus they were chosen for a proof of concept application of MCFG. This represented the first time MCFG was being used as μ-SPE sorbent for contaminants in environmental water. The aim of this work was to design a sorbent impregnated μ-SPE device capable of agitating by itself the sample solution, and facilitating extraction of analytes, without any additional stir device. The applicability of the approach was evaluated by considering PAHs, with analysis by GC−MS. Real water samples collected from Singapore River were analysed.

2.2 Experimental

2.2.1 Chemicals, materials and instrumentation

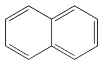
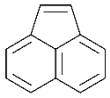
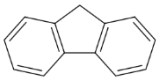
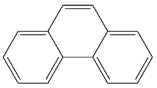
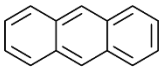
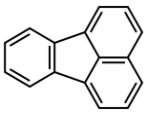
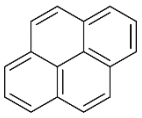
Pure PAHs standard; naphthalene (Nap), acenaphthylene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), and pyrene (Pyr), were purchased from Sigma Aldrich (Milwaukee, WI, USA). Some physicochemical properties of these PAHs were shown in Table 2-1. These analytes were dissolved in

MeOH to prepare 1mg/mL stock solutions. Working methanolic standard mixtures were prepared by dilution with water from the stock solution at 20 µg/mL. Solutions were kept at 4º C in the refrigerator. Q3/2 Accurel 2E HF (R/P) PP sheets (157 µm thickness, 0.2 µm pore size) were purchased from Membrana (Wuppertal, Germany). The CS powder (made from crab shell) and the cross linking agent, glutaraldehyde, were obtained from Sigma-Aldrich (St. Louis, MO, USA). The desorption solvent, n-hexane was supplied from Tedia (Fairfield, OH, USA). The graphite powder and hydrochloric acid (HCl 37%) were purchased from Merck (Darmstadt, Germany). Sodium nitrate ($NaNO_3$), hydrogen peroxide (H_2O_2 30%) and sulfuric acid (H_2SO_4 98%) was obtained from Sigma Aldrich (Milwaukee, WI, USA). Potassium permanganate ($KMnO_4$) was bought from Comak Chemicals Ltd. (Hertfordshire, UK). Ultrapure water was obtained from an ELGA Purelab Option-Q (High Wycombe, UK) water purification system. A freeze dryer (Martin Christ, Osterode am Harz, Germany) was used for drying prepared sorbent sample. Fourier transform infrared (FTIR) spectra were obtained by using an ALPHA FT-IR Spectrometer, Bruker (Berlin, Germany). Field emission scanning electron microscope (FESEM) was performed by JEOL JSM-6701F (JEOL, Tokyo, Japan). Real water samples from Singapore River were collected in glass bottles pre-cleaned with acetone. The bottles were filled without leaving a headspace and wrapped with aluminium foil, before transportation to the laboratory. The sample bottles were kept in the refrigerator at 4 ºC until use. The river water sample was extracted with proposed method without further filtration and prior treatment.

2.2.2 Preparation of GO

Graphite powder was used to prepare GO as reported in literature [17]. Briefly, 1g of graphite and 1g of $NaNO_3$ were placed in a flask. Forty eight millilitres of H_2SO_4 98 % was added to the mixture while stirring in an ice-bath. Six grams of $KMnO_4$ was slowly added followed by vigorous stirring for 1.5 h. Then, the temperature was raised to 35ºC and stirring was continued for 2 h. Forty millilitres of water was poured dropwise to the solution within the time interval of 30 min. The temperature rose to 90º C during the addition of water. The addition of 100 mL water was subsequently carried out, and H_2O_2 30% (10 mL) was finally added to the solution. Centrifugation and repeated rinsing with HCl 5% was carried out thoroughly. Washing with water was performed for final cleaning of the precipitate. Freeze drying the graphite oxide residue was carried out at -78º C for 48 h. Exfoliated GO was prepared by sonicating the graphite oxide in water for 1 hr. Repeated washing and centrifugation were carried out with 2M HCl and water until pH~7 was reached. The GO was freeze dried at -78ºC for 24 h.

Table 2-1 Some physicochemical properties of PAHs considered in this work.

Name	Abbreviation	Empirical formula	Chemical structure	Log K_{ow}
Naphthalene	Nap	$C_{10}H_8$		3.34
Acenaphthylene	Ace	$C_{12}H_8$		4.07
Fluorene	Flu	$C_{13}H_{10}$		4.18
Phenanthrene	Phe	$C_{14}H_{10}$		4.34
Anthracene	Ant	$C_{14}H_{10}$		4.56
Fluoranthene	Flt	$C_{16}H_{10}$		4.50
Pyrene	Pyr	$C_{16}H_{10}$		4.88

2.2.3 Preparation of MCFG

CS powder (0.4 g) was dissolved in 20 mL of 2% (V/V) acetic acid solution under ultrasonic stirring for 2 h. The colloidal solution of CS was added with different amount of magnetic nanoparticles (Fe_3O_4) (0.05 g, 0.07 g and 0.1g respectively). Each mixture was stirred for 1.5 h. Hexadecane (paraffin oil) was slowly dispersed in the mixture solution under stirring for 30 min. Then, the CS in the mixture was crosslinked with 3 mL of glutaraldehyde. Accurately weighed GO (0.3 g) was added to the above mixture and stirred continuously for 1.5 h in a water bath at 50ºC. The solution was adjusted to pH 9 using 3M sodium hydroxide (NaOH). The mixture was kept in the water bath

which temperature was set to 80ºC for 1 h. The MCFG was precipitated as black particles and washed with ethanol and water until the pH of the precipitate was at ~7. The MCFG obtained was dried in an oven at 60ºC for 24h.

2.2.4 µ-SPE

The µ-SPE device was prepared as reported by Basheer et al [6]. Briefly, sorbent (100 mg) was enclosed inside the porous PP membrane envelope (made up of 2 overlapped pieces of sheet membrane) whose final dimension was 1.5 cm x 0.8 cm. The edges of the device were heat-sealed to secure the enclosed sorbent. The device was conditioned by ultrasonication in water and then n-hexane for 5 min each and kept in the latter solvent until use.

2.2.5 Extraction and desorption

After drying in air for 5 min, the magnetic µ-SPE device was placed in a 10 mL pure water sample solution spiked at 50 μgL^{-1} of each PAH for extraction. The magnetic stirrer was set at 1250 revolution per minute (rpm) for 30 min with the stirring provided by the µ-SPE device itself (Fig 2-1).

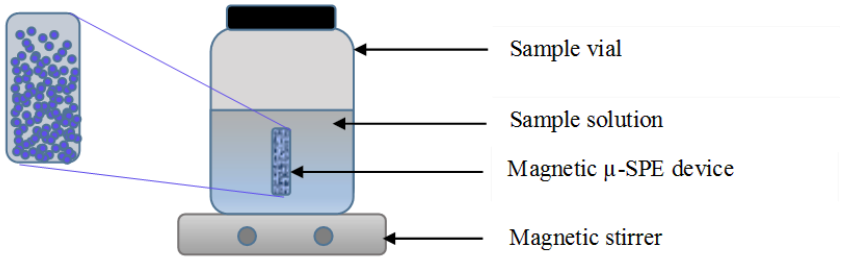


Fig 2-1. Schematic of magnetic µ-SPE.

After extraction, the device was removed from the sample solution and dried thoroughly with lint free tissue. It was immediately placed in a 1.5-mL centrifuge tube and eluted

with 150 µL of n-hexane under ultrasonication for 10 min. The extract was transferred to a GC−MS autosampler vial. One microliter of the extract was injected into the GC−MS system using the autosampler. The used µ-SPE device was then ultrasonicated for 5 min with 2 mL n-hexane to eliminate preferential carryover by re-extracting the µ-SPE device. The carryover effect was randomly tested using 150 µL n-hexane followed by GC−MS. No analyte peaks were detected. This study clearly indicated that the device was reusable. A series of tests proved that the device was reusable up to 15 times without impacting its extraction efficiency negatively.

2.2.6 GC−MS

A Shimadzu (Kyoto, Japan) QP2010 GC−MS system equipped with a Shimadzu AOC-20i auto sampler and a DB-5 MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m x 0.025 mm internal diameter (i.d), 0.025 µm film thickness) were used for analysis. Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 2 mL/min. The injector temperature was set at 280º C and the GC−MS interface temperature at 150º C. The GC oven was initially set at 80º C for 4 min. The temperature was increased to 260º C at 10º C/min. The final temperature was held for 3 min. Injector was in splitless mode. The standard PAH solution and the real water extracts were analysed in selective ion monitoring (SIM) mode. The masses monitored by the detector were set as: m/z 128,129 for Nap, m/z 153,152 for Ace, m/z 166, 167 for Flu, m/z 188, 179 for Phe, m/z 178,179 for Ant, m/z 202, 203 Flt and m/z 202, 203 Pyr (m/z: mass-to-charge ratio). The fragments of the ions monitored in SIM mode were selected based on good selectivity and high sensitivity.

2.3 Results and discussion

2.3.1 Characterization of MCFG

The FT-IR spectrum of pristine GO showed the frequency peaks: ~1097 cm^{-1} (C−O stretching), ~1384 cm^{-1} (C−H stretching), ~1630 and ~1726 cm^{-1} (C=O stretching) and a broad band around 3438 cm^{-1} (the H-bond associated with−OH). The cross-linked CS possesses the bands: −N−H stretching vibration (~ 3423 cm^{-1}),−N−H stretching of –NH_2 and –NH_2 bending vibration (~1570 cm^{-1}) (also known as −NH_2 scissoring). These bands in the cross-linked chitosan spectrum were related to the vibration from the primary amine –NH_2 of the free amino groups (some portion of the amine was left un-reacted). The spectrum of the MCFG provided combined spectral characteristics similar to that of the cross-linked CS and GO. The broad band appearing around 3400 cm^{-1} refers to the mixture of the N−H stretching in amine groups of CS and to the O−H stretching of −OH groups in GO. It can be seen that there are increased intensities at around 1630 cm^{-1} and decreased intensities at around 1570 cm^{-1}. These bands are attributed to the C=O stretching in the amide and N−H bending of the free –NH_2. These prove the formation of –NHCO−groups which appeared after the reaction of some –NH_2 groups on chitosan chains with –COOH groups on the surface of the GO sheet. The carboxyl groups of GO react the amine groups of chitosan forming a covalent bond. Additionally, the frequency band around 580 cm^{-1} corresponds to the existence of Fe−O stretching in Fe_3O_4. The FTIR results thus demonstrated that the GO was successfully functionalized with magnetic CS (Fig 2-2).

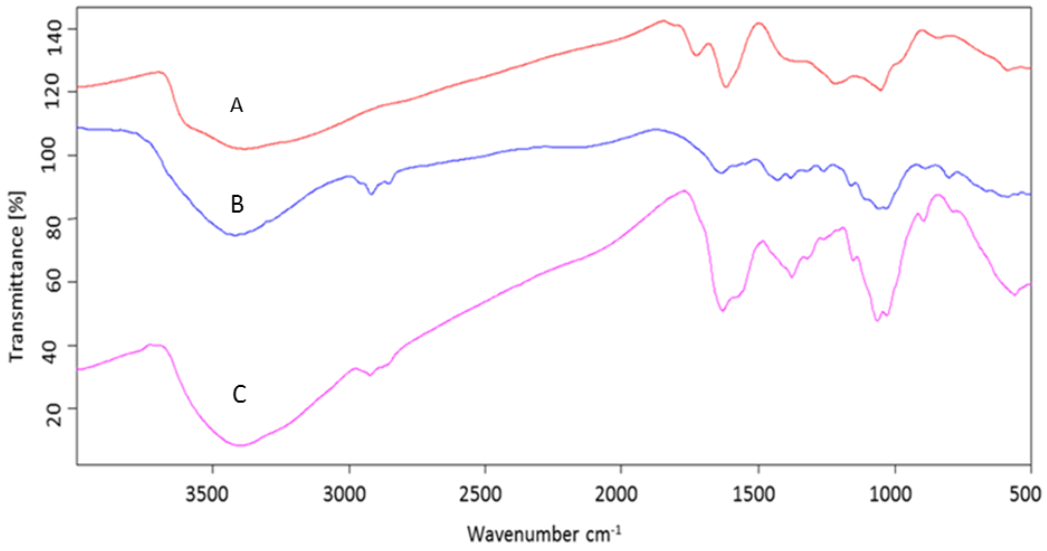


Fig 2-2. FTIR spectra of (A) GO (B) cross-linked CS and (C) MCFG.

The FESEM images of GO showed the sheet-like structure with smooth surfaces and crumpled edges. However, cross-linked CS revealed the rough wrinkled surface. The MCFG composite with a much coarser surface indicated that magnetic chitosan had been densely distributed on the surface of GO layers (Fig 2-3).

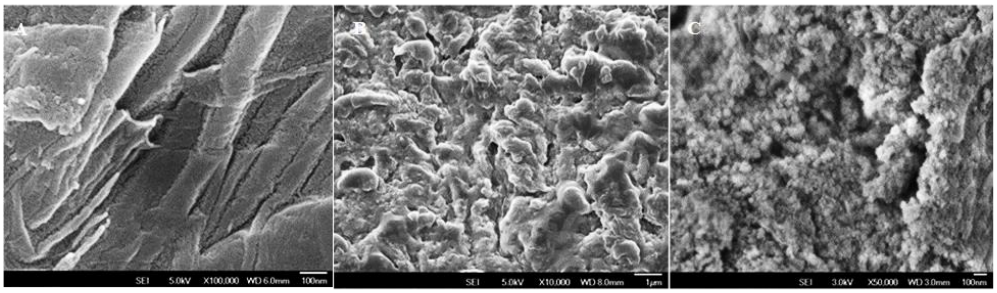


Fig 2-3. FESEM images of (A) GO sheet (B) cross-linked CS and (C) MCFG

2.3.2 Optimization of magnetic µ-SPE

Since the adsorption of analytes on the sorbent is a reversible phenomenon, adsorption and desorption, they occur simultaneously and until an equilibrium is established. The

movement of µ-SPE device under magnetic force in the sample solution was conceivably the most important factor in facilitating mass transfer. In order to achieve the highest analyte enrichment, extraction factors such as type of sorbent, volume of solution, extraction time, desorption time and desorption solvent were evaluated.

2.3.2.1 Selection of sorbent

In order to examine the most promising another new µ-SPE abbreviation and acronym, the three types of sorbents prepared with different amounts of Fe_3O_4 (0.05g, 0.07g and 0.1g) were subjected to the extraction procedure. Fig 2-4 clearly demonstrates the highest chromatographic response was for the sorbent prepared with 0.1g of Fe_3O_4. It appears that because of the magnetic nanoparticles constituted in the sorbent, the optimum stirring was associated with the sorbent prepared with a higher ratio of these nanoparticles. The equilibrium mass transfer during extraction is mainly based on the optimum movement of this µ-SPE device, leading to the most favourable enrichment. Lower chromatographic responses were recorded for those sorbents with lower amount of magnetic nanoparticles. The evaluation of analyte enrichment was also carried out by varying the amounts of MCFG (10, 50, 80 and 100 mg) in the respective µ-SPE devices. The results (data not given) indicated that 100 mg of MCFG gave the highest response peak for most of the PAHs, which is unsurprisingly given the greater capacity for analyte extraction provided by the biggest amount of the sorbent. In order to minimize the amount of sorbent used, in terms of environmental friendliness, no attempt to load >100 mg of the material was considered.

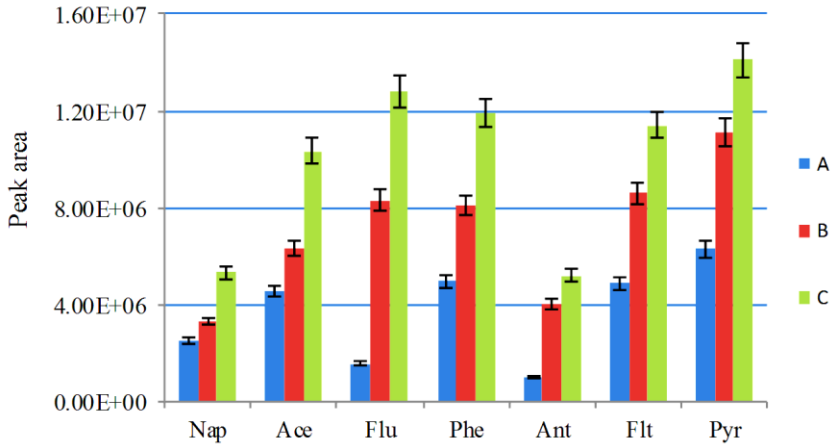


Fig 2-4. The comparison of extraction efficiencies of sorbent composites containing (A) 0.05 g (B) 0.07 g and (C) 0.1 g of Fe_3O_4.

2.3.2.2 Volume of sample solution

The effect of sample volume on µ-SPE efficiency was investigated by considering 5, 7, 10, 15 and 25 mL of water. Higher analyte enrichments were observed with increasing sample volumes. However, as expected, the enrichment was limited by the adsorption sites of the sorbent becoming fully saturated with the analytes [18]. As shown in Fig 2-5, the maximum chromatographic signal was reached at 10 mL of sample solution with optimized stirring. Therefore, 10 mL of the sample solution was deemed to be the most favourable sample solution for the proposed method.

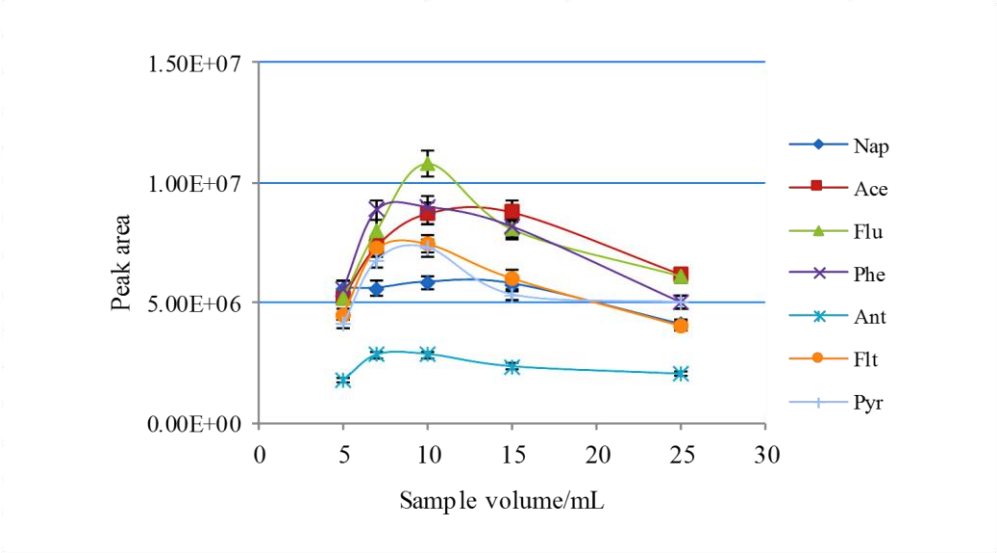


Fig 2-5. Effect of sample volume at 50 µg L^{-1}. Desorption solvent, 150 µL n-hexane; extraction time, 30 min; desorption time, 10 min.

2.3.2.3 Extraction time profile

The effect of extraction time on PAH extraction was evaluated by monitoring the chromatographic peak area response over 5-40 min. Since extraction is a time-dependant mass transfer process, continuous stirring at 2500 rpm at room temperature was carried out to minimize equilibrium time. The partition coefficient of the analyte between the aqueous sample and the sorbent plays an important role in relation to the amounts of analytes that are extracted from the sample solution. Rapid partitioning between these two phases was observed in the initial extraction profile, followed by a slower one. As shown in Fig 2-6, the peak areas steadily increase with sampling time in the range of 5-30 min. The decrease in peak area after 30 min is believed due to desorption of analytes from the sorbent over time. Thus, back-diffusion has been frequently observed in many microextraction procedures [19, 20]. Hence, the optimized extraction time was selected as 30 min since it was deemed to be sufficient for current method.

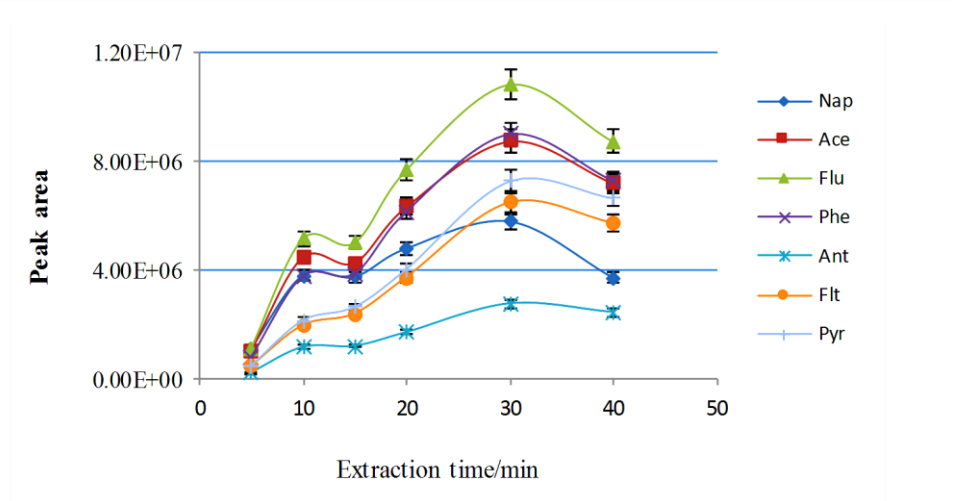


Fig 2-6. Effect of extraction time at 50 µg L^{-1}. Desorption solvent, 150 µL n-hexane; desorption time, 10 min.

2.3.2.4 Desorption time profile

After extraction, the analytes were desorbed from the µ-SPE device with a suitable organic solvent with the aid of ultrasonication. Desorption time was investigated the range of 5 and 30 min. The peak areas of analytes desorbed were not significantly increased after 10 min of ultrasonication (Fig 2-7). An optimized desorption time of 10 min appeared to be a reasonable compromise for subsequent experiments. However, there were slight increases in peak areas of some PAHs after 10 min but not to the extent reached at 10 min. After the first desorption, the device was further desorbed to determine the carryover effects, if any. No analyte carryover was observed, indicating that analytes were completely desorbed from the sorbent after one iteration [21].

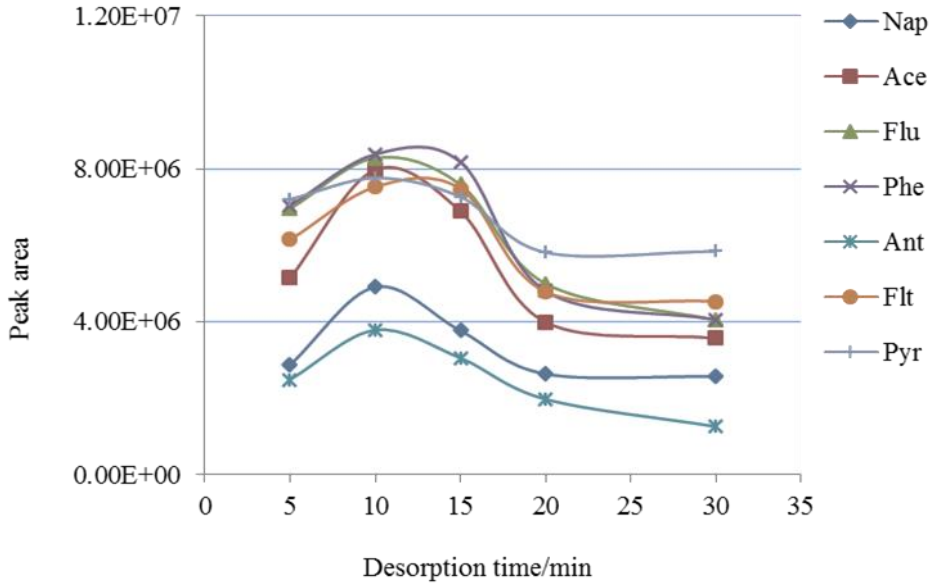


Fig 2-7. Effect of desorption time at 50 µg L^{-1}. Desorption solvent, 150 µL n-hexane; extraction time, 30 min.

2.3.2.5 Selection of desorption solvent

Desorption capabilities of solvents were evaluated by considering three solvents including n-hexane, toluene and o-xylene. The results of the comparative studies are shown in Fig 2-8.

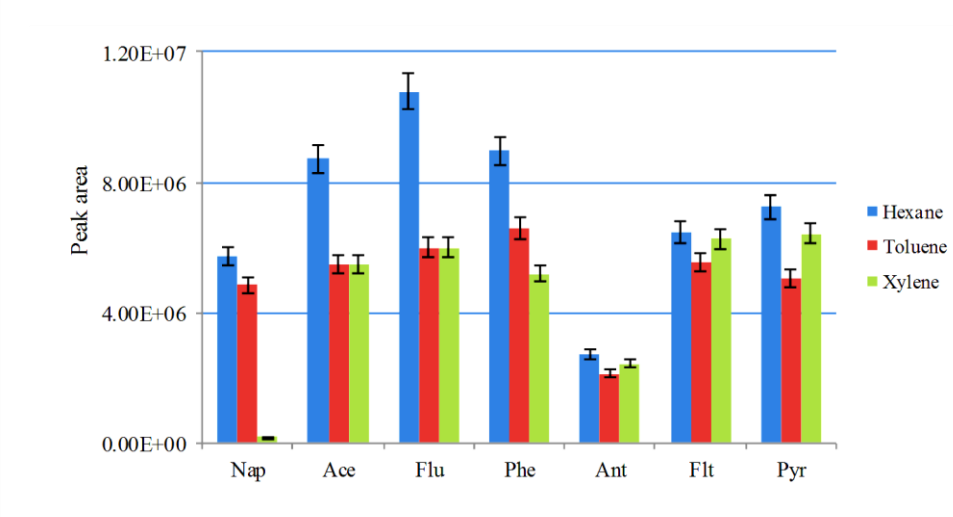


Fig 2-8. Effect of desorption solvent at 50 µg L^{-1}. Sample volume, 10 mL; desorption solvent volume, 150 µL; extraction time, 30 min; desorption time, 10 min.

It is observed that n-hexane provided the highest chromatographic response, followed by toluene and o-xylene. The strong hydrophobic interaction between the analytes and n-hexane may thus be responsible for this observation. The hydrophobicity of PAH generally increases with increasing molecular weight [22]. The octanol-water partition coefficient (log P_{ow}) is the most useful factor to explain the solubility of analyte in desorption solvent especially for those with higher hydrophobicities. Since all seven PAHs possess relatively higher hydrophobic character with log P_{ow} >1.5, their solubilities in n-hexane (log P_{ow}=3.8) are higher than in toluene and o-xylene (P_{ow} = 2.69, 3.0) [23, 24]. Therefore, the selection of n-hexane to be desorption solvent for PAHs analysis in this current method is borne out by the experimental observations.

2.4 Quantitative results

In order to assess the applicability of the proposed method, linearity, repeatability, LODs and LOQs were evaluated using the most favourable extraction conditions. The analytical data obtained are tabulated in Table 2.2.

Table 2-2. Linear ranges, coefficients of determination, LODs, LOQs, precision and enrichment factors.

Analyte	Linear range (μg L^{-1})	Coefficient of determination (r^2)	LOD (ng L^{-1})	LOQ (ng L^{-1})	RSD (%) [a]	EF [b]
Nap	0.01-100	0.997	0.71	2.33	2.9	67
Ace	0.05-100	0.990	0.52	1.82	3.7	281
Flu	0.01-50	0.987	1.80	5.90	4.0	302
Phe	0.05-100	0.996	1.52	5.03	3.4	194
Ant	0.01-50	0.999	1.12	3.61	3.2	91
Flt	0.01-100	0.989	0.23	0.83	2.3	138
Pyr	0.01-100	0.990	0.33	0.84	2.1	69

[a, b] Calculated from each sample spiked at a concentration level of 5 μg L^{-1}

The linearity was studied with series of concentrations by spiking ultrapure water samples. The calibration plots were linear in the range 0.01 and 100 µg L^{-1} for Nap, Flt and Pyr while for Ace and Phe, the linear range was in the range of 0.05 and 100 µg L^{-1}. For Flu and Ant, the range of between 0.01 and 50 µg L^{-1} was relevant. The coefficients of determination (r^2) based on the above linearity ranges for the seven PAHs were between 0.987 and 0.999. The LODs for all target analytes were determined by injecting successively decreasing concentrations of analytes until the signals were detected at a signal-to-noise ratio (S/N) of 3. The LODs obtained were in the range 0.23 and 1.80 ng L^{-1} whereas the LOQs were in the range of between 0.83-5.90 ng L^{-1} at S/N=10.

In comparison with the LODs of established methods reported in the literature for PAH determination, the proposed method is one of the most sensitive (Table 2.3). Previously reported methods include SPME-LC [25], MWCNT-HS-SPME-GC-FID [26], PDMS-HSSE-SBSE-HPLC-FLD [27], SPME-GCMS (orthogonal array) [28], MEPS-GCMS [29], AF-LPME-GCMS [30], Zn/Al-SDBS-LDH-SPE-GCMS [31] and NPs-SPE-UV-vis [32] (Table 2-3 for explanations of the abbreviations). The method using PDMS-HSSE-SBSE-HPLC-FLD utilized a commercial extraction device and provided relatively lower LODs. However, the current method is independent of such commercial devices. EFs are defined as the ratios of the final analyte concentrations in the acceptor phase and the initial concentrations of analytes in the sample. The analyte EFs were calculated to be in the range of between 67 and 302. These results indicated that the MCFG enabled highly hydrophobic and π-π intractions with PAHs with the highly active surface area, as has been reported previously [33].

2.5 Real water sample analysis

The performance of the developed method was tested by the determination of the considered PAHs in a river water sample. As expected, the sample was found to contain two PAHs at trace levels since PAHs are ubiquitous in the environment. The concentrations of Nap and Phe were found to be 0.15 and 0.17 μg L^{-1} respectively. The other PAHs were non-detectable or their concentrations were below the LOQs. Extraction recoveries and reproducibility of the method were also determined by triplicate analysis of samples spiked at concentration levels 1 and 5 μg L^{-1}. The results obtained are listed in Table 2.4. The R (%) in relation to river water sample were calculated to be between 67.5 and 106.9% with RSDs below 15%. These results clearly demonstrate that real water sample matrices had little effect on the performance of the developed method indicating that it is applicable to the analysis of trace levels of PAHs in real world environmental water samples.

Table 2-3. Comparison of the LODs of magnetic-µ-SPE-GC-MS with other previously reported methods for the extraction and determination of PAHs from water samples.

Analysis method	Analyte [a]	LOD (ng L^{-1})	Reference
SPME-LC	Flu, Phe, Ant, Flt, Pyr	14-80	[25]
MWCNT-HS-SPME-GC-FID	Nap, Ace, Flu, Phe, Flt	30-70	[26]
PDMS-HSSE-SBSE-HPLC-FLD	Nap, Ace, Phe, Ant, Flt, Pyr	0.03-2.23	[27]
PDMS-SPME-GC-MS (orthogonal array)	Nap, Ace, Flu, Phe, Ant, Flt, Pyr	3.10-18.02	[28]
Silica-C_8-MEPS-GC-MS	Nap, Ace, Flu, Phe, Ant, Flt, Pyr	0.5-1.6	[29]
AF-LPME-GC-MS	Flu, Phe, Flt, Pyr	10-40	[30]
Zn/Al-SDBS-LDH-SPE-GC-MS	Ace, Flu, Flt, Pyr	1.2-3.2	[31]
NPs-SPE-UV-Vis	Flu, Phe, Ant, Pyr	0.21-1.66 (ng mL^{-1})	[32]
Magnetic -µ-SPE-GC-MS	Nap, Ace, Flu, Phe, Ant, Flt, Pyr	0.2-1.8	present work

Method abbreviations:

SPME, solid-phase microextraction; LC, liquid chromatography; MWCNT, multiwalled carbon nanotube; HS, headspace; GC, gas chromatography; FID, flame ionization detector; PDMS, polydimethylsiloxane; HSSE, headspace sorptive extraction; SBSE, stir bar sorptive extraction; HPLC, high-performance liquid chromatography; FLD, fluorescence detection; MS, mass spectrometry; MEPS, microextraction by packed sorbent; AF-LPME, agarose flim liquid phase microextraction; Zn/Al-SDBS-LDH, Zn/Al layered double hydroxide intercalated sodium dodecyl benzene sulfonate; SPE, solid-phase extraction; NPs, nanoparticles; UV-Vis, Ultraviolet-visible; µ-SPE, micro-solid phase extraction.

[a] Only analytes also considered in the present work are listed.

Table 2.4. Analytical results of PAHs in river water sample by magnetic µ-SPE-GC-MS

Analyte	Concentration in river water (µg L^{-1})	R (%)	
		spiked at 1µgL^{-1}	spiked at 5µgL^{-1}
Nap	0.15	99.8 (13.4)[b]	106.9 (9.7)[c]
Ace	<LOQ[a]	86.5 (2.9)	106.7 (7.1)
Flu	<LOQ	67.5 (7.2)	75.3 (12.4)
Phe	0.17	86.2 (11.1)	101.3 (14.3)
Ant	<LOQ	99.0 (15.0)	92.7 (9.3)
Flt	<LOQ	96.1 (5.0)	102.9 (11.5)
Pyr	<LOQ	96.6 (11.8)	74.2 (8.4)

[a] Below LOQ

[b,c] RSD (%)

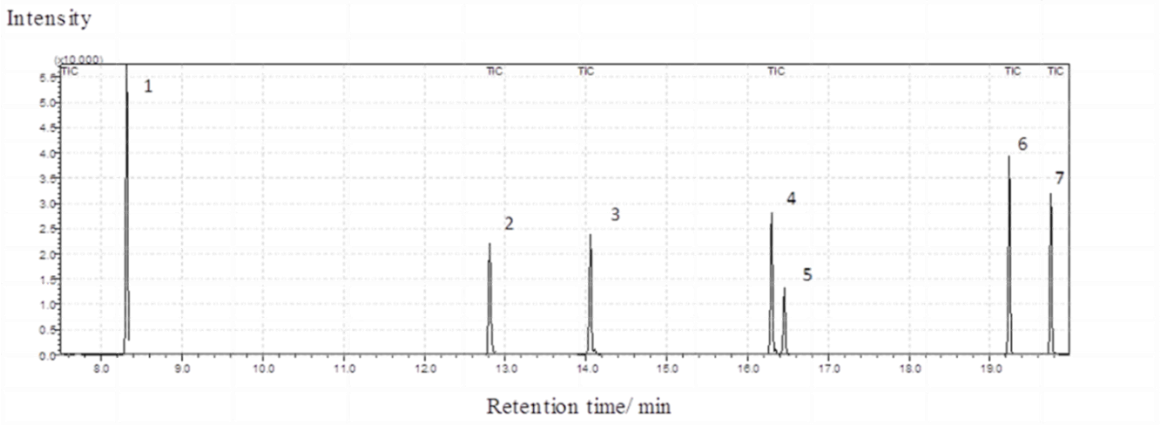


Fig 2-9. GC−MS-SIM trace of PAHs in river water sample spiked with standards at 1µg L^{-1} after µ-SPE: Sample volume: 10 mL: extraction time, 30 min: desorption time, 10 min: desorption solvent 150 µL n-hexane: Peak identities: (1) Nap (m/z 128), (2) Ace (m/z 153), (3) Flu (m/z 166), (4) Phe (m/z 188), (5) Ant (m/z 178), (6) Flt (m/z 202), and (7) Pyr (m/z 202).

2.6 Conclusion

In this research, a magnetic sorbent was designed and prepared, and used in a µ-SPE device for the extraction of PAHs in environmental water. This proposed procedure was

simple, easy to operate and user friendly. The analytical performance of the μ-SPE in combination with GC−MS analysis was evaluated. The important features of the proposed extraction method are that the MCFG sorbent was compatible with water giving its phenyl moiety and adsorption sites provide good compatibility with the PAHs due to hydrophobic and π-π interactions. The superparamagnetic (Fe_3O_4) CS doped on the surface of crumpled GO provided the μ-SPE device with an independent means of stirring the solution. The proposed method was applied to the analysis of seven PAHs in river water and demonstrated its potential as an alternative to conventional SBSE. Conceivably, the procedure may also be applied to other non-polar water contaminants.

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Chapter 3 Micro-Solid-Phase Extraction Followed by Thermal Extraction for Environmental Contaminants in Water

3.1 Introduction

In recent years, the monitoring of trace pollutants in the environmental water is required to estimate and manage the risks associated with the presence of these compounds in the environment. There are two types of sources of contaminants and pollutants: sewage treatment plants and wastewater treatment plants; others include urban storm water, agricultural runoff, and wet and dry deposition from the atmosphere. The determination of the hydrophobic organic compounds in the aquatic environment is challenging due to their trace concentrations in complex sample matrices [1, 2].

PBDEs, the anthropogenic chemicals widely used as flame retardants, are used in polymers for textiles, plastics, paints and electronic components [3, 4]. They can be discharged from these manufactured products to environmental matrices, leading to their accumulation, while resisting degradation, for several years [5]. Due to their highly hydrophobic character (high K_{ow}), PBDEs can be accumulated in fats and proteins, posing health risks such as endocrine disruption, reproductive toxicity, neurobehavioral effects and probable carcinogenesis [6-8]. The current pre-concentration techniques of PBDEs from environmental water consist of SBSE [9, 10], SPME [11, 12] and DLLME [13]. However, these techniques have obvious advantages over classical extraction methods, they do have some drawbacks (see Chapter 1). µ-SPE, a relatively simple, fast sampling technique that requires minimal amount of solvents with good clean-up properties, was developed to overcome most of these problems. In the present work, extraction and detection carried out along with µ-SPE,

TE and GC-mass selective detector (MSD). TE was carried out for liquid samples using TDU coupled with GC−MSD system. Viscous or matrix-containing liquid samples are automatically injected to µ-vials in TDU, subsequent TE was carried out at higher temperature in 20-mL standard headspace vials (in TDU liner). Then, Analytes are trapped on adsorbent in the CIS liner and subsequent desorption takes place at elevating temperature in the CIS-4 connected to TDU (Fig 3-1). The dirty µ-vials are discarded and replaced to make GC−MSD system clean.

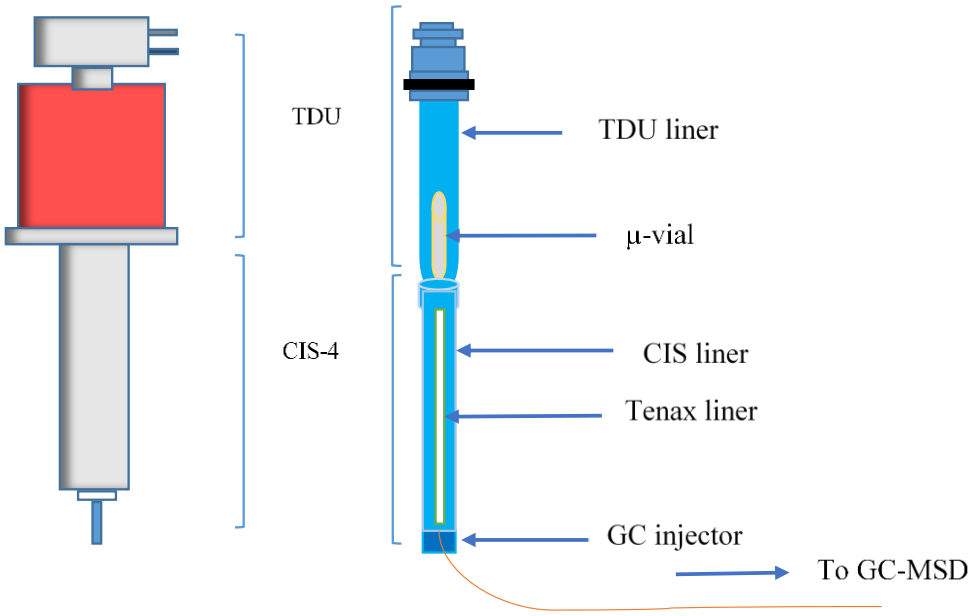


Fig 3-1 Schematic of TDU, CIS-4 and GC injector.

In the present work, the organic extract obtained by µ-SPE was introduced into the µ-vial placed in TDU tube at high temperature. Analytes were transported and trapped in Tenax adsorbent which were placed in CIS liner connected to TDU. Thereafter, the analytes were conveyed into GC−MSD injector port for separation and detection. This step could make further enrichment of analytes by removing matrix residue.

CS-GO composite were prepared as sorbent. The advantages of using this composite are mentioned in Chapter 2. Since GO can be dispersed in water with polymer matrix, the epoxy group in GO react favourably with primary amine group of polymer. This modification process of GO is commonly used to form a new mixture of CS-GO composite [14-16].

The purpose of this research work was to develop a more sensitive novel method based on a µ-SPE technique using CS-GO composite as sorbent. After µ-SPE, the extract was subjected to thermal extraction. This step involved additional extract clean-up before GC-MSD. This procedure was successfully applied to the determination of trace PBDEs in water.

3.2 Experimental

3.2.1 Chemicals and materials

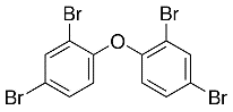
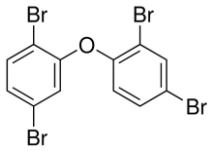
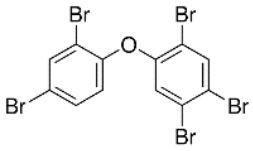
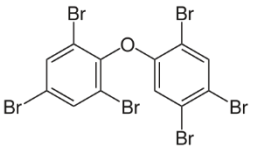
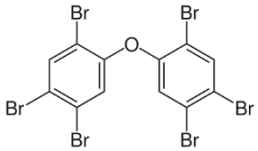
AccuStandard (New Haven, CT, USA) supplied the five PBDE standards (50 mg L^{-1} in isooctane for each): 2,2´,4,4´-tetrabromodiphenyl ether (BDE-47), 2,2´,4,5´-tetrabromodiphenyl ether (BDE-49), 2,2´,4,4´,5-pentabromodiphenyl ether (BDE-99), 2,2´,4,4´,5, 5´-hexabromodiphenyl ether (BDE-153) and 2,2´,4,4´,5, 6´-hexabromodiphenyl ether (BDE-154). Some physicochemical properties of these PBDEs were shown in Table 3.1 [17, 18]. Common laboratory reagents and DI water were obtained as mentioned in Chapter 2. Stock solution containing mixed standard solution (10 mg L^{-1} of each analyte) was prepared by combining five PBDE standards (each at 50 mg L^{-1} in isooctane). The subsequent standards were prepared from standard mixture using n-hexane as solvent and a stock solution of 0.1 mg L^{-1} was prepared using MeOH respectively. They were kept at 4˚C in refrigerator. Genuine water samples were

collected from Kallang and Jurong rivers, Singapore. The collection and transportation processes of these samples were the same as reported in a previous chapter.

3.2.2 Apparatus and instrumentation

An Agilent 7890A GC system coupled with inert MSD 5975C (Wilmington, DE, USA) was used for separation and detection of analytes. The GC−MSD system was equipped with Gerstel MultiPurpose Sampler 2 (MPS-2 XT with Dual Head) having a TDU and CIS-4 (Mülheim an der Ruhr, Germany). The capillary column and carrier gas helium used were the same as mentioned in a previous chapter. Helium gas at a flow rate of 1.1 mL/min was used and purified air for functioning TDU. GC was performed under programmable temperature vaporizer (PTV) - solvent vent mode with 10.5 min sampling time. The CIS-4 in which a liner packed with Tenax TA sorbent was kept at 10 ^{o}C for 0.01 min and heated up to 320 ^{o}C at a rate of 12 ^{o}C/s, with the final temperature held for 5 min. GC was set a splitless time of 2 min, and vent flow was set at 50 ml/min. GC oven was initially held at 60˚C for 2 min, then increased to 220˚C at a rate of 40˚C/min and further increased to 300˚C at a rate of 10˚C, with the final temperature held for 7 min. Fig 3-1 shows the schematic setup of TDU, CIS-4 and GC injector. Bruker-AXS (Siemens) D5005 Diffractometer (Karlsruhe, Germany) was used to characterize the prepared CS-GO composite by X-ray diffraction analysis. Other common instruments are the same as reported in a previous chapter.

Table 3.1 Some physicochemical properties of PBDEs considered in this work

Name	Abbreviation	Empirical formula	Structure	Log K_{ow} at 25˚C [a]
2,2´,4,4´-Tetrabromodiphenyl ether	BDE-47	$C_{12}H_6Br_4O$		7.3
2,2´,4,5´-Tetrabromodiphenyl ether	BDE-49	$C_{12}H_6Br_4O$		7.3
2,2´,4,4´,5-Pentabromodiphenyl ether	BDE-99	$C_{12}H_5Br_5O$		7.6
2,2´,4,4´,5, 6´-Pexabromodiphenyl ether	BDE-154	$C_{12}H_4Br_6O$		7.8
2,2´,4,4´,5, 5´-Hexabromodiphenyl ether	BDE-153	$C_{12}H_4Br_6O$		7.9

[a][17,18]

3.2.3 Preparation of CS-GO composite

Graphite powder was used to prepare GO as reported in the literature. The preparation of GO has already been reported in Chapter 2. The CS-GO composite was prepared as reported by Liu et al [19]. The prepared GO was dispersed in 20 mL 1% (v/v) acetic acid solution followed by ultrasonication for 30 min at room temperature forming suspension. The CS powder (0.5 g) was added while stirring and ultrasonicated again for 1 h to thoroughly dissolve the powder and mixed it with GO. The mixture was kept at room temperature for 12 h. The well-distributed suspension (20 mL) was dropped into 3.0% (w/v) aqueous NaOH solution using a 0.05-mm i.d disposable syringe with an injection speed 60 drops per min. The beads submerged in the solution were solidified by leaving them for 24 h. Repeated washing with ultrapure water was carried

out until the pH of the water was neutral. Thirty millilitres of MeOH and 1.5 mL of 50% aqueous glutaraldeyde were added to the beaker in which the filtered beads were placed. They were stirred gently at room temperature for 5 h. Thereafter, the beads were filtered and washed several times with ethanol and ultrapure water and dried in air at room temperature for 24 h.

3.2.4 µ-SPE followed by TE

As described in previous chapter, a µ-SPE device was prepared as reported by Basheer et al [20]. The µ-SPE device containing sorbent was dried in air for 15 minutes. Then, it was introduced into a 5-mL sample solution and allowed to tumble freely with stirring at 1000 rpm for 15 min. After that, the device was taken out from the sample solution and dried thoroughly with lint free tissue. Immediately after, the device was placed in a 1-mL centrifuge tube. Seventy micro litres of o-xylene was charged into the tube and ultrasonicated for 15 min for desorption of the analytes. Then, 1µL of the extract was injected into the µ-vial placed in TDU tube for thermal extraction at ~ 300ºC via Gerstel MPS2 autosampler. Thereafter, thermally extracted analytes were transported to the CIS-4 (fitted with Tenax liner) connected to the TDU. Subsequently, the analytes trapped in the Tenax adsorbent were desorbed by elevating the temperature and transported to the GC injector. Finally, GC–MSD analysis was carried out. As indicated in a previous chapter, further desorption of µ-SPE with o-xylene was carried out randomly to confirm that there were no contamination and carry-over effects suggesting that the µ-SPE device could be reused. In this work, each device could be used for up to 15 times. All experiments were performed in triplicate.

3.3 Results and discussion

3.3.1 Characterization of prepared sorbent

GO, CS and CS-GO composite were characterized by FT-IR and results are shown in Fig. 3-2. GO showed peaks at ~1700 cm^{-1} (C=O str.), ~1620 cm^{-1} (C=C str.), and ~1060 cm^{-1} (C−O str.). For CS, the peaks at ~3400 cm^{-1} correspond to the vibration of N−H whereas the peaks at ~1010 and ~1160 cm^{-1} refer to the primary and secondary alcoholic groups (C−OH str.). The peak at ~1640 cm^{-1} is due to the C=O str. The peaks related to CS-CO, similar to the peaks appearing for CS and CO, are located at ~3400 cm^{-1} (combination of vibrations of N-H groups of CS and –OH groups of GO). The peak at ~1680 cm^{-1} is slightly down shifted due to the formation of hydrogen bonding between –COOH from GO and hexatomic ring of CS, and ~1600 cm^{-1} (C=C groups from GO). Thus, the FT-IR results reveal the presence of the interaction between CS and GO.

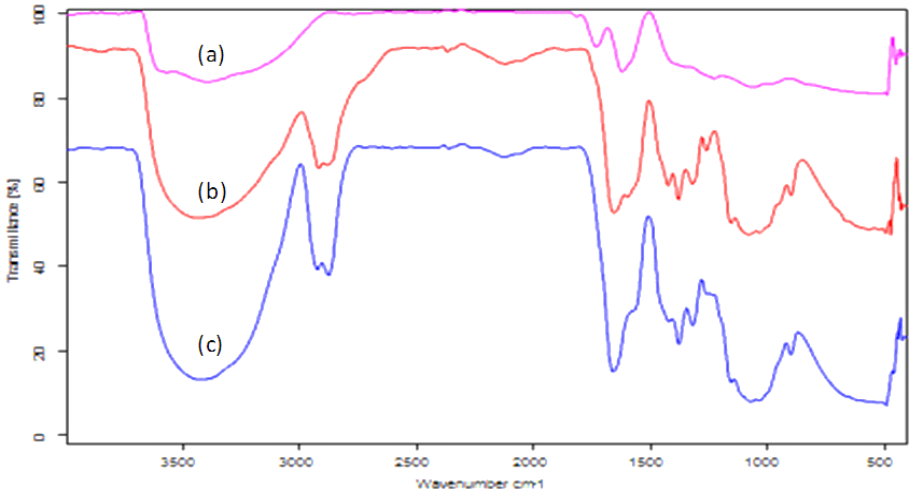


Fig 3-2. FT-IR spectra of (a) GO (b) CS-GO and (c) CS

The XRD patterns of GO and CS-GO composite are shown in Fig 3-3. The XRD peaks appearing at ~ 2θ=11.4˚ and ~20.3˚refer to the GO and CS-GO. The diffraction peak of

GO is not observed in the CS-GO composite. This imply that, the degree of crystallinity of GO decreased after mixing with CS. Thus, there is presumably hydrogen bonding between CS and GO rather than bond formation from a chemical reaction [13, 19].

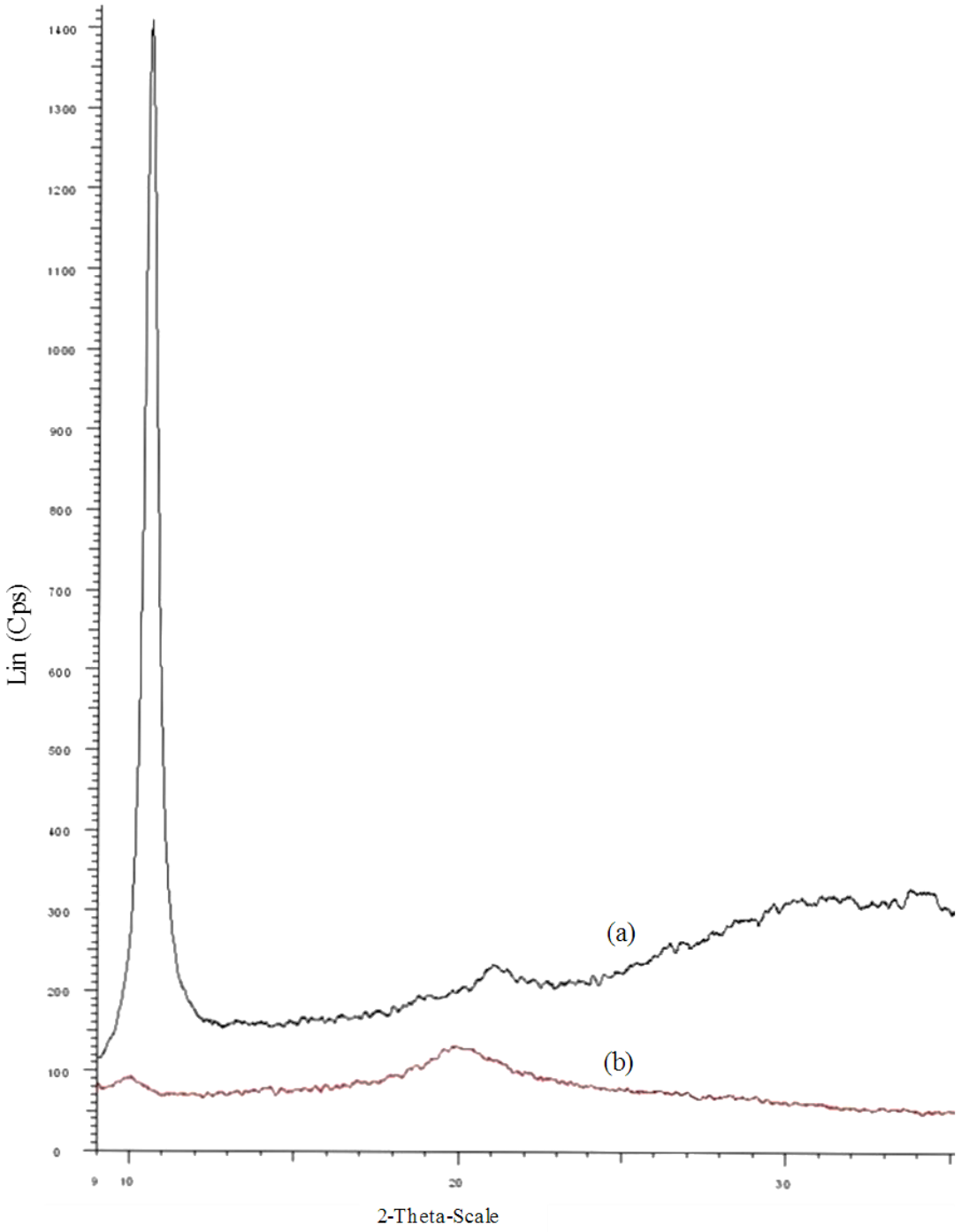


Fig. 3-3. XRD pattern of (a) crystal form of GO and (b) amorphous GO in CS-GO.

3.3.2 Optimization of µ-SPE followed by TE

3.3.2.1 Selection of desorption solvent

Toluene, o-xylene, MeOH and 1-octanol were studied for their desorption efficiencies for this work. Fig 3-4 shows the highest peak areas for all analytes were obtained using o-xylene as solvent followed by toluene which gives slightly lower peak area. Since the analytes possess high partition coefficients [17, 18] (Table 3.1.), they desorbed better in non-polar solvents such as o-xylene and toluene. Additionally, as o-xylene possesses relatively lower vapour pressure, analytes dissolved in it showed higher peak areas compared with those in which analytes were dissolved in toluene.

Therefore, o-xylene was chosen as the desorption solvent for subsequent experiments.

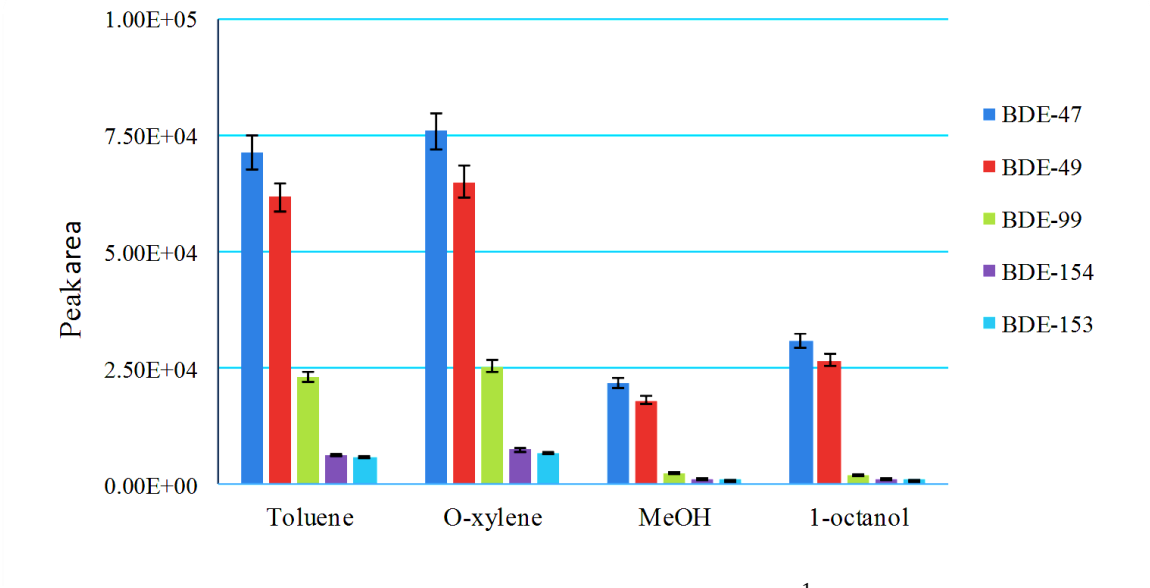


Fig 3-4. Selection of desorption solvent for 5 µg L^{-1} PBDEs in water sample. Conditions: sorbent weight, 1 mg; extraction time, 15 min; desorption time, 15 min.

3.3.2.2 Extraction time

Experiments were run for between 5 and 30 min to evaluate the effect of time on extraction efficiency. The results are shown in Fig 3-5 which depicts that the peak areas

of all analytes increased from 5 to 15 min. Subsequently, for most analytes the peak area reached a plateau, and then decreased slightly. Since µ-SPE is an equilibrium-based extraction process, the extraction efficiency depends on analytes transferring from sample solution to sorbent which is time-dependent. Thus, in this case, equilibrium was achieved at 15 min, selected as the extraction time. After 15 min, due to back-diffusion, peak areas registered a decrease for the next 10 min before slightly increasing again, but not to the levels reached earlier. Nevertheless, by this time (30 min) the extraction would have been too long to be practical.

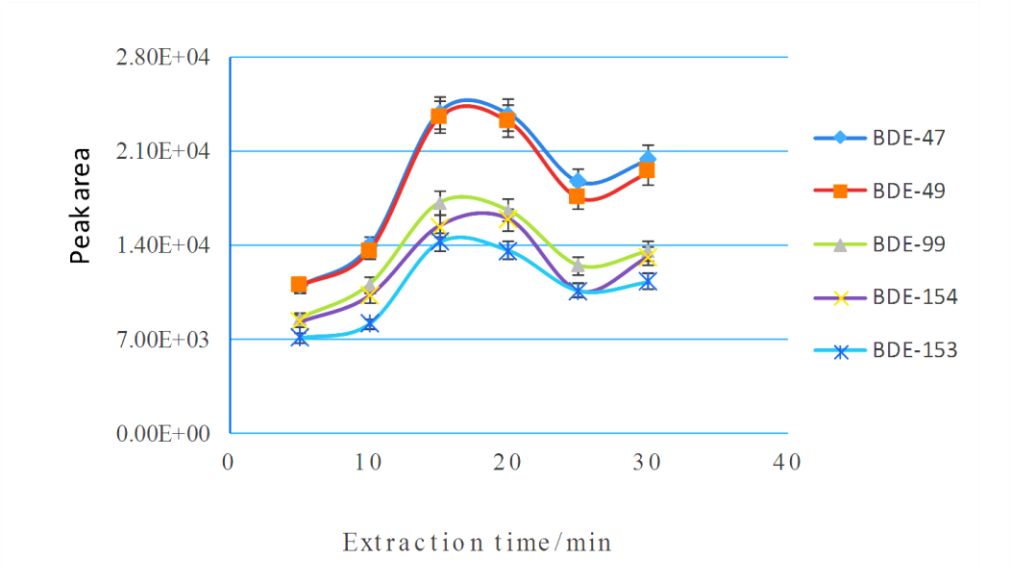


Fig 3-5. Extraction time for 5 µ g L^{-1} PBDEs in a water sample. Conditions: sorbent weight, 1 mg; desorption time, 20 min; desorption solvent, o-xylene.

3.3.2.3 Desorption time

O-xylene was used as the solvent to study the effect of desorption time of analytes with assistance of ultrasonication from 5 to 30 min. As shown in Fig 3-6, the peak area profiles related to the chromatographic response demonstrate a slope after 20 min of sonication time. This slight decrease in desorption profile can conceivably be explained

as analytes were re-adsorbed by the sorbent material when desorption time was increased [21, 22]. The optimum time for desorption was thus chosen as 20 min.

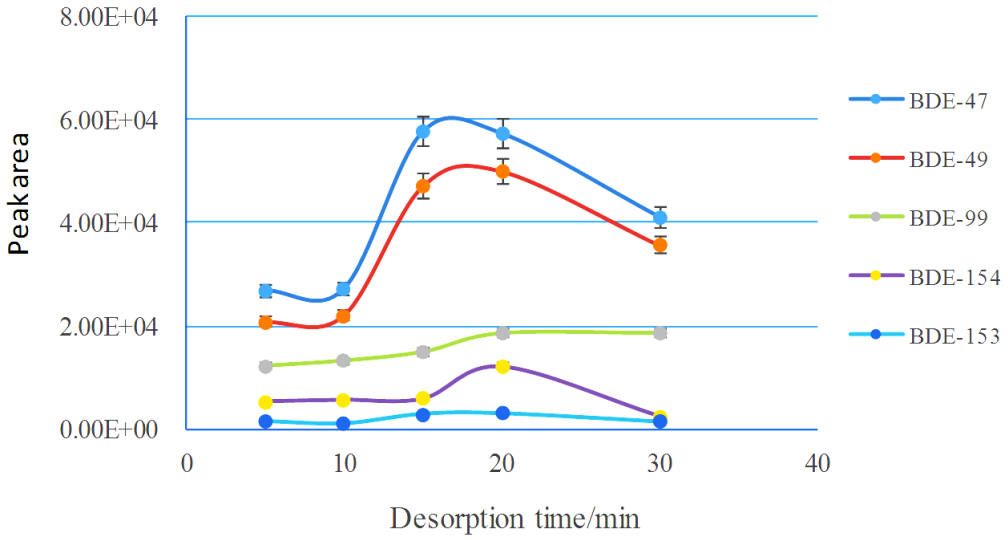


Fig. 3-6. µ-SPE desorption time for 5 µ g L^{-1} PBDEs in a water sample. Conditions: sorbent weight, 1mg; extraction time, 15 min: desorption solvent, o-xylene.

3.3.2.4 Comparative study with other sorbents

Commercial sorbents, C_{18}, HayeSep A, HayeSep B, Porapak were in parallel with CS-GO for their extraction performances using 5µg L^{-1} of PBDE standard solutions. One milligram of each sorbent was used. Extraction was carried out using the optimized parameters. As shown in Figure. 3-7, CS-GO was found to be the most effective sorbent for extracting PBDEs in water as it showed the highest chromatographic response, followed by C_{18} sorbent. The sorbent $C_{18,}$ the most hydrophobic among the sorbents, and possessing a silica surface covered with linear octylsilyl chains, similar to the bristles of a brush, thereby providing a relatively larger surface for adsorption of target analytes, gave slightly lower peak areas. HayeSep-A possessing intermediate polarity and HayeSep-B of the highest polarity amongst the group possess compact structures and bulky aromatic rings, whereas Porapak R which is of intermediate polarity consists

of cross-linked polystyrene [20, 22]. Based on peak area analysis, relatively poor extraction efficiencies were observed for HayeSep-A, HayeSep-B and Porapak R. Among the sorbents, the superior performance of CS-GO for all PBDE analytes could be due to the π-π interaction between the GO moiety of the composite, and the target analytes. Moreover, the cross-linked CS part of the composite might possess higher surface area for more efficient adsorption of the analytes. The same parameters were used for other sorbents.

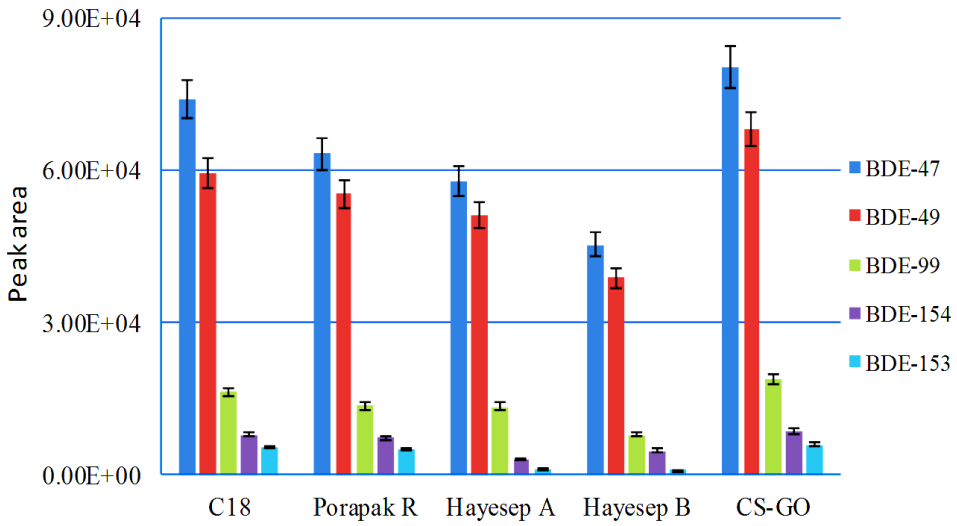


Fig 3-7. Comparative data on extraction efficiency of CS-CO with different commercial sorbents using 5 µg L^{-1} standard PBDEs in a water sample. Conditions: sorbent weight, 1 mg; extraction time, 15 min; desorption time, 15 min; desorption solvent, o-xylene.

3.3.2.5 Comparison with other methods

In order to compare the performance of the proposed procedure with those of other methods capable of being operated by the Gerstel MPS-2 system such as liquid injection GC−MSD, standard sample solution (for GC−MSD) or liquid extract (for µ-SPE-GC-MSD) were directly injected into the GC-MSD systems. The CIS fitted with hollow

baffled liner was used for GC−MSD and µ-SPE-GC-MSD without TDU while TDU equipped with the CIS fitted with Tenax liner was used for TE-GC-MSD and µ-SPE-TE-GC-MSD. In TE-GC-MSD method, the sample solution was first injected into micro-vial placed in TDU tube. As the temperature of the TDU was set at ~300ºC, the analytes diffused into the liner in the CIS where they were trapped in Tenax sorbent. The cold-trapped analytes in the Tenax sorbent were again thermally desorbed at elevated temperature, and the analytes then diffused into the GC−MSD for separation and detection. A similar protocol was carried out for the method developed in this work that included the µ-SPE step. One µL of the extract (from the µ-SPE step) was injected into the TDU.

Among the methods, the developed procedure was capable of extracting and enriching PBDEs. Interestingly, its extraction was drastically improved for those PBDEs with relatively lower molecular mass with lower boiling points. The finding shows that the developed method may be more attractive for the selective isolation of lower molecular mass. It is obvious that the procedure including TE give relatively higher chromatographic response (Fig 3-8). This is considered to be the effect of further cleaning step which may remove the matrix effects and residues.

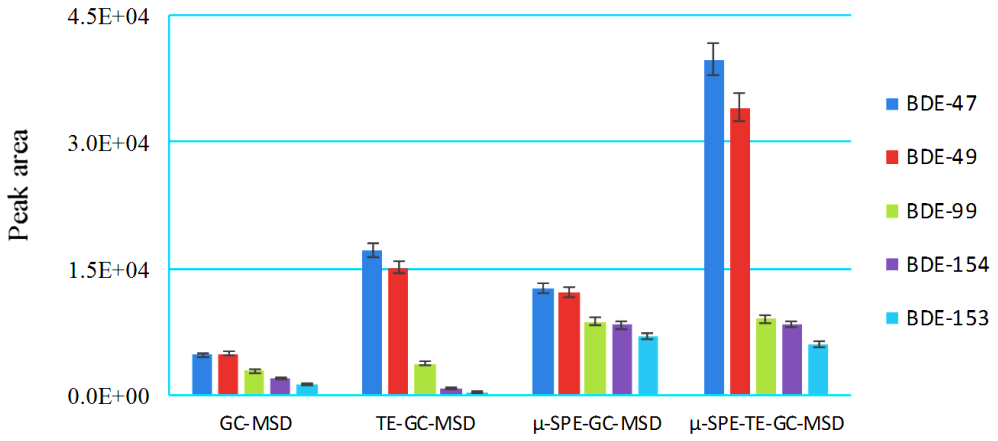


Fig 3-8. Comparison of the extraction efficiency of μ-SPE-TE-GC-MSD with other extraction methods using 5 μg L^{-1} PBDEs in water

3.4 Method validation

By using the optimized parameters, quantitative analysis was performed to assess the applicabilityof the proposed method. The performance characteristics such as reproducibility, linearity, LODs and LOQs were obtained. As indicated in Chapter 2, EFs are defined as the ratios of the final analyte concentrations in the acceptor phase and the initial concentrations of analytes within the sample, were also measured. They were in the range of between 121 and 188. As Table 3-2 summarizes, linear ranges of 0.1-20 μg L^{-1} for BDE-47 and BDE-49 and 0.5-20 μg L^{-1} for the other three analytes, are associated with r^2 of up to 0.9995, which is satisfactory. The precision of the method (%RSD) determined by performing three consecutive extractions from an aqueous solution, was between 3.54 and 11.36% indicating the good repeatability of the method. LOD and LOQ, calculated [20] based on S/N ratios 3 and 10, were in the ranges between 0.007 and 0.016 μg L^{-1}, and between 0.068 and 0.163 μg L^{-1} respectively.

Table 3-2. Linear ranges, coefficients of determination, LODs, LOQs, precision and enrichment factors.

Analyte	Linear range (µg L^{-1})	Coefficient of determination (r^2)	LOD (µg L^{-1}) (S/N=3)	LOQ (µg L^{-1}) (S/N=10)	RSD (%) (n=3)	EF
BDE-47	0.1-20	0.9988	0.007	0.071	4.63	188
BDE-49	0.1-20	0.9986	0.007	0.068	7.09	183
BDE-99	0.5-20	0.9995	0.016	0.162	3.54	142
BDE-154	0.5-20	0.9982	0.016	0.163	11.36	121
BDE-153	0.5-20	0.9992	0.014	0.137	7.85	123

3.5 Real water analysis

Analytical data obtained from real water analysis with the developed method are tabulated in Table 3-3. Experiments were performed on waters collected from the Kallang and Jurong rivers of Singapore. Traces of some PBDEs in unspiked water samples in both Kallang and Jurong rivers were detected and the concentrations of other congeners were non-detected or below the LODs of the method. Fig 3-9 shows the chromatograms of water and spiked water samples of Jurong River which were extracted and analysed using the present method under the most favourable conditions. These genuine samples were spiked to a level of 5 µg L^{-1} of each compound and processed to access matrix effects. R for water samples were determined to be in the range of between 71.52 and 96.15%. The calculation of R was performed based on the following equation:

$$(\%)\ R = C_{sw} - C_w / C_s \times 100$$

where C_{sw} is the concentration of the analytes in the spiked water sample, and C_w and C_s refer to the measured analyte concentrations in the water sample and spiked concentrations in water.

Table 3-4 illustrates the comparative data reported using different methods regarding the determination of PBDEs in water. SFOME-HPLC-VWD method was developed by

Liu et al [2] to determine PBDE congeners in water whereas Zhao et al. implemented TA-ILD-LLME-HPLC-VWD method [23]. LLE-GC-MS was proposed by Xiang and co-workers to detect PBDEs in water [24] and Barco-Bonilla et al [25] validated a method based on SPE-GC-HRMS. Only the last-named method exhibited lower LODs than the present procedure; it used commercial Extra Bond C_{18} cartridges with a large amount of sample solution (500 mL) needed. These comparative data demonstrate that the developed methodology provides sufficient and acceptable sensitivity with a small amount of sample (5 mL).

Table 3-3. Concentrations of analytes and relative recoveries of water samples from different sites

Analyte	Unspiked real sample concentration (μgL^{-1})		R (%) (real sample spiked at 5 $\mu g\ L^{-1}$)	
	Kallang river	Jurong river	Kallang river	Jurong river
BDE-47	0.008	N.D	88.78	75.81
BDE-49	N.D	0.013	82.54	74.93
BDE-99	N.D	0.015	80.40	75.08
BDE-154	N.D	N.D	71.52	88.34
BDE-153	0.015	N.D	96.15	91.60

N.D: not detected or below the limits of detection

Table 3.4. Comparison of LODs obtained by different methods

Method	Sample volume (mL)	Analyte *	LODs (ng L^{-1})	Reference
SFOME-HPLC-VWD	40	BDE-47, BDE-99, BDE-154	10-40	Ref [2]
TA-ILD-LLME-HPLC-VWD	5	BDE-47, BDE-99, BDE-154	0.1-0.4 (ng mL^{-1})	Ref [23]
LLE-GC-MS	1L	BDE-47, BDE-99, BDE-153	0.2 (µg L^{-1}) (for each BDE)	Ref [24]
SPE-GC-HRMS	500	BDE-47, BDE-99, BDE-154, BDE-153	0.02-0.05	Ref [25]
µ-SPE-TS-GCMS	5	BDE-47, BDE-49, BDE-99, BDE-154, BDE-153	7-16	present work

Method abbreviations:

SFOME-HPLC-VWD: Solidification of floating organic drop microextraction-high performance liquid chromatography-variable wavelength detector. TA-ILD-LLME-HPLC-VWD: temperature-assisted ionic liquid dispersive-liquid-liquid microextraction-high performance liquid chromatography-variable wavelength detector. LLE-GC-MS: Liquid-liquid extraction-gas chromatography-mass spectrometry. SPE-GC-HRMS: solid-phase extraction- gas chromatography-high resolution magnetic sector mass spectrometer.

*Only analytes related to the present work are mentioned

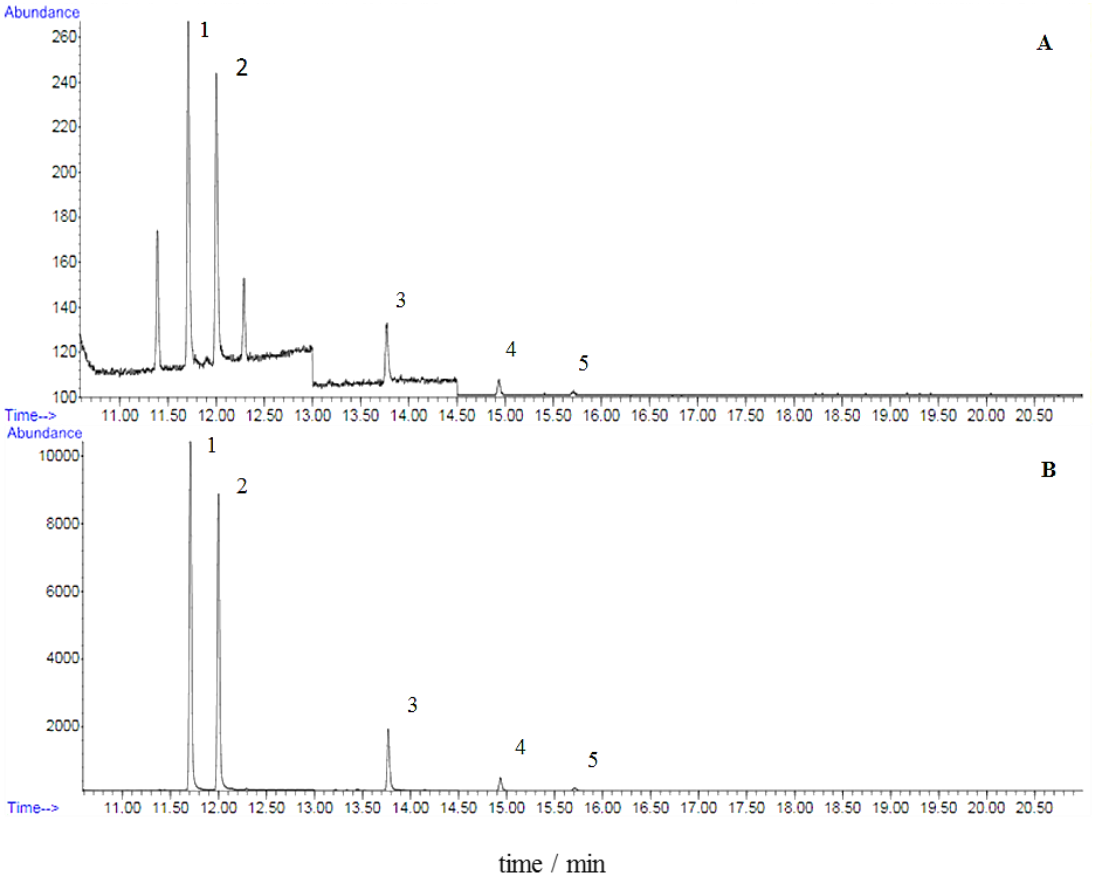


Fig. 3-9. GC−MSD traces of extracts: (A) unspiked water sample collected from Jurong river (B) water sample spiked with 5 µg L^{-1} PBDE standards (extracted via µ-SPE-TE-GC-MSD under optimized conditions). Peak identification: (1) BDE-47 (2) BDE-49 (3) BDE-99 (4) BDE-154 (5) BDE-153.

3.6 Conclusion

The proposed two-step extraction method revealed that the comparatively lower molecular mass compounds were more amenable to be isolated and detected. Although more research is required for better understanding of sorbent based µ-SPE in conjunction with TE, the developed technique has definitely presented several advantages such as high sensitivity, simplicity, short analysis time, ease of operation and low consumption of sample and solvent. Finally, the feasibility of the method to extract PBDEs selectively was demonstrated successfully for genuine water samples.

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Chapter 4 Electro Membrane Extraction Using Sorbent Filled Porous Membrane Bag

4.1 Introduction

As indicated in Chapter 1, SPME and LPME are two miniaturized extraction techniques that have emerged in the past 15-20 years [1, 2]. SPME overcomes the difficulties of conventional extraction methods by eliminating the use of organic solvents and allowing sample extraction and preconcentration to be performed in a single step. LPME is based on the use of very low volumes (microlitre range) of solvent and had its origin in the use of a drop of extraction solvent. In spite of all the advantages of this design, single-drop LPME (SDME) is not a very robust system due to the problems related to the instability of the microdrop held in the sample solution or in its headspace. To address this issue, HF-LPME was introduced by Rasmussen and Pederson-Bjergaard [3] in the form of two- and three-phase microextraction. This technique especially overcomes several drawbacks of SDME in complex matrices like biological and environmental solutions. Pedersen-Bjergaard et al [4] had also proposed electrokinetic HF-LPME (see Chapter 1). This electrokinetic migration technique known as EME was established to effectively increase extraction speed [5-8].

The simultaneous extraction of acidic and basic drugs using EME was carried out by Basheer et al [9]. In their work, the extraction time was determined to be only 10 min. Lee et al [10] had also achieved the same extraction time for determination of CPs in water using EME. Like HF-LPME, in EME, the extractant organic phase is protected by the porous membrane when dealing with complex sample matrices [11-13]. EME analysis has been successfully carried out on a wide range of biological fluids, such as drugs from untreated human plasma and whole blood [14, 15].

As described in previous Chapters, graphene, monolayer aromatic sheets composed of

sp^2 carbon atoms with a two- dimensional honeycomb lattice, possess considerably high electrical and thermal conductive and mechanical properties which has attracted much attentions. Owing to its hydrophilicity and ease of formation of stable colloidal suspensions, graphene can be utilized as an effective conductive nanofiller in graphene-based polymer composites [16]. Moreover, in merging the graphitic nanoflakes into elastomeric polymer cast, high performance composites with improved mechanical and functional properties can be generated [17-19].

Graphene has been used as the sorbent for SPME, µ-SPE and other sample preparation procedures [20, 21] for benzenoid organic analytes such as PAHs, PBDEs and phenols in environmental samples. The idea to use graphene materials like r-GO in EME was considered in this work. The r-GO/PVA, a biocompatible polymer, was prepared through direct chemical reduction of the GO/PVA film [22]. PVA hydrogels swell upon contact with water which is an important property for its applications in electrolytes. Based on this swelling behavior, the solution containing reducing agent (e.g. sodium dithionite, $Na_2S_2O_4$) can disperse into the GO/PVA composites without destroying the structure. The GO/PVA was then chemically reduced. An arranged nanostructure of r-GO could be formed in PVA, without any surface changes of r-GO [22, 23]. EME-SLPME was developed in this work. r-GO/PVA was introduced into the pores of a polypropylene membrane supported by organic solvent, EME-SLPME may be effected. This procedure was used to extract phenols from water and its performance was evaluated in the present study.

4.2 Experimental

4.2.1 Chemicals, materials and instrumentation

All phenol analytes: p-n hexyl phenol (Hex P), p-n heptyl phenol (Hep P), p-n octyl

phenol (OP), p-(1,1,3,3-tetramethylbutyl)-phenol (TMBP) and bisphenol A (BPA) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The derivatization agent *N,O*-bis-trimethylsilyltrifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). PVA and $Na_2S_2O_4$ were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Other common materials and chemicals were purchased as mentioned in previous chapters. The stock standard solutions of each analyte were prepared separately in methanol at 1000 mg L^{-1} and stored at 4ºC. Genuine water samples were collected as reported in previous chapters. The d.c power supply was generated by a multichannel electrophoresis system MCE-PS468 from CE Resources with software driven applied voltage in the range 0-5 kV. A transmission electron microscope (TEM) JEOL JEM 2010-F & JEOL JEM 3010-F (JEOL, Tokyo, Japan) was used to examine the nanostructure of the composites. The thermal properties of the composites were studied by thermogravimetric analysis (TGA) (Universal V3.9A, TA Instruments, New Castle, DE, USA). Other instruments used in this work were the same as reported in Chapter 1 & 2.

4.2.2 GC−MS

A Shimadzu QP2010 GC−MS system which has been mentioned in Chapter 1 was used in this work. Helium at a flow rate of 1.7 mL/min was used. The injector and interface temperatures were set at 280ºC and 300ºC respectively. The GC oven was initially held at a temperature of 90ºC for 5 min. The temperature was then increased to 120ºC at a rate of 20ºC/min, followed by another increase to 150ºC at 5ºC/min. A final increase to 300ºC at 20ºC/min was then enabled; this temperature was held for 5 min. The solvent cut time was set at 9.0 min. Extracts derivatized with BSTFA + 1% TMCS were injected under splitless mode. The derivatized analytes were analyzed quantitatively under

selective ion monitoring (SIM) mode. The ions of the derivatives were selected as: Hex P, m/z 179, 250; TMBP, m/z 207, 263; Hep P, m/z 179, 182; O P, m/z 179, 182; and BPA, m/z 213, 225. Triplicate experiments were performed.

4.2.3 Preparation of r-GO/PVA

Graphite oxide was first prepared from graphite powder by means of the protocol as described elsewhere. An account regarding the protocol for the preparation of graphite oxide was given in Chapter 2.

The sorbent r-GO/PVA was prepared using submicron GO and PVA as described by Yang et al [22]. In order to prepare submicron GO, GO (3% by weight in water) was sonicated by alternately taking 5 min intervals for every 10 min sonication. This process was repeated for 2 h. The prepared sub-micron GO suspensions were directly added to a PVA solution.

PVA (8% by weight in water) was heated at 80ºC for 2 h and continuously ultrasonicated for 30 min at 70ºC. Then, the prepared sub-micron GO (predetermined 14% by weight in PVA) was added as explained above. The mixture was stirred at 600 rpm for 6 h at room temperature. The viscous solution of GO/PVA was dried at the 60ºC for 24 h. Then, the GO/PVA film obtained was submerged in the aqueous solution of the reducing agent (15 mg mL^{-1} $Na_2S_2O_4$ and 50 mg mL^{-1} NaOH) at 60ºC for 2 h. After reduction, the yellow colour of the solution turned black. The r-GO/PVA was obtained after washing the residue with water and dried at 60ºC for 6 h.

4.2.3.1 Filling of porous membrane with r-GO/PVA.

The polypropylene membrane bag was made by cutting the sheet into two rectangular

pieces with dimension 2.5 x 0.8 cm. These two pieces were superimposed on each other. By using a heat sealer, two long and one short edges were thermally sealed leaving one open side to form a membrane envelope. The bag was then cleaned with dichloromethane for 5 min.

Two miligrams of r-GO/PVA was ultrasonicated for 1 h in 1 mL of 1-octanol to produce a dispersed sorbent suspension. One hundred microlitres of the dispersed solution was placed into the membrane bag. Then, the bag was ultrasonicated for 10 min so as to retain the sorbent/solvent in the pores of porous membrane. After sonication, the excess solvent in the bag was cautiously removed using a microsyringe. The bag was dabbed with tint-free tissue and was then ready for EME-SLPME.

4.2.4 EME-SLPME

Three millilitres of the aqueous sample solution was used for EME; its pH was adjusted to 11 using 0.1M NaOH solution. The membrane bag whose walls were impregnated with 1-octanol (which also acted as the supported liquid membrane, SLM) and r-GO/PVA sorbent, was filled with 50 µL of 1-octanol (extractant phase) and suspended in the sample solution secured by a plastic hook. One-mm diameter platinum wires were used as electrodes. The positive electrode was placed in the 1-octanol in the bag, while the negative electrode was inserted in the sample solution (Fig 4-1). A potential difference of 100V was applied for 5 min for extraction with the sample solution agitated with a magnetic stirrer bar at 800 rpm. Thereafter, the membrane bag was taken out from sample solution with tweezers and the extract containing the analytes was retrieved using a microsyringe. Ten microlitres of extract was mixed with the same volume of derivatization agent BSTFA+1%TMCS. The mixture was then heated at 70ºC in a waterbath for 30 min. One microlitre of the derivatized extract was injected

into the GC−MS system. The membrane bag was rinsed with 1-octanol after each run. Random analyses of the 1-octanol washings revealed no carryover effects. Our experiments indicated that each membrane bag could be reused for five times with no variation in the analytical results.

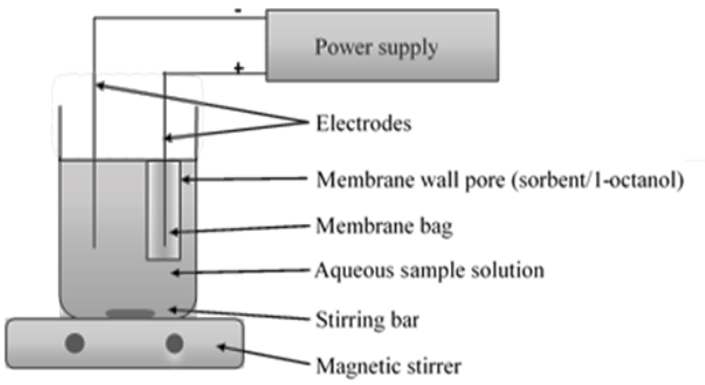

Fig 4-1. Schematic of EME-SLPME setup

4.3 Results and discussion

4.3.1 Sorbent filled membrane

The blending process using graphene or r-GO is the most common method to improve the conductivity of the polymer [24, 25]. Thus, the GO/PVA film was chemically reduced to form the r-GO/PVA. The network nanostructure of r-GO was observed due to the fine dispersivity of r-GO in the PVA matrix [22]. The images regarding the sub-micron r-GO and the dispersed nano network structure of r-GO in PVA films were observed through TEM (Fig 4-2). FESEM images showing the membrane wall before and after filling with r-GO/PVA in 1-octanol are shown in Fig 4-3 (a) and (b). Because of the thermal conductivity of fine r-GO in the r-GO/PVA composite, it revealed an apparently lower temperature TGA curve than that of GO/PVA (Fig 4-4).

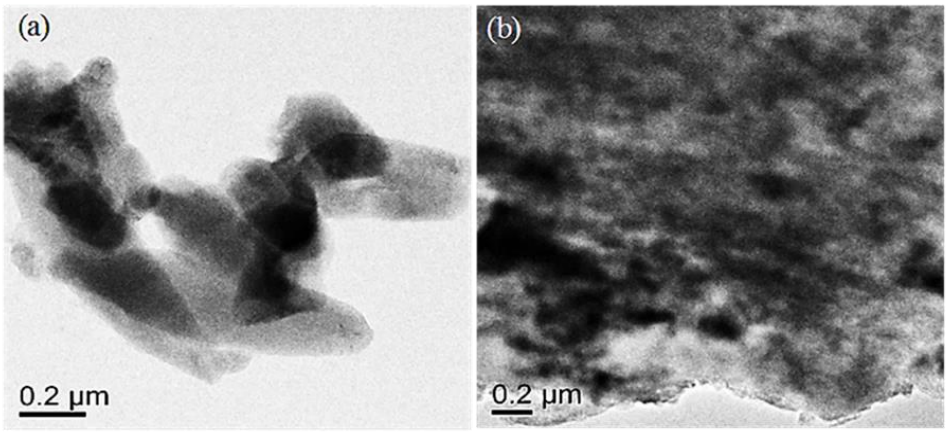

Fig 4-2. TEM images for (a) submicron r-GO and (b) r-GO dispersed in PVA.

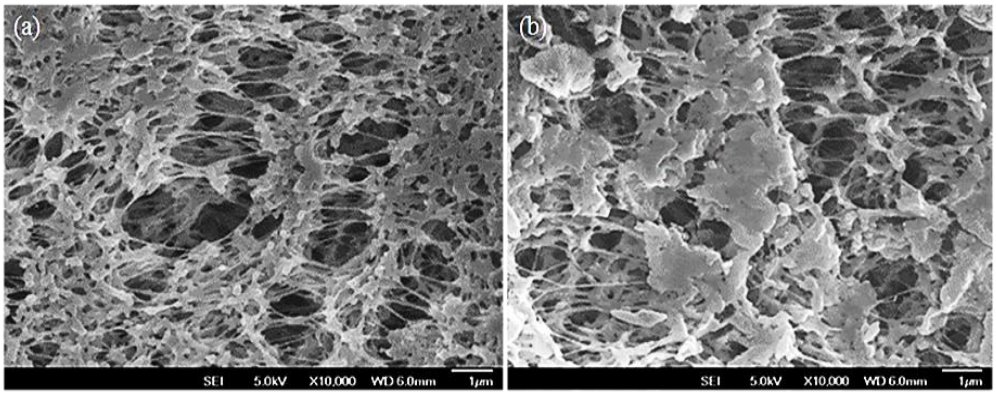

Fig. 4-3. FESEM images for polypropylene membrane (a) before and (b) after filling with dispersed r-GO/PVA, with 10 min ultrasonication in 1-octanol.

4.3.2 Method optimization

4.3.2.1 Derivatization conditions

It is necessary to derivatize phenols for GC−MS analysis [26, 27]. The time required for derivatization of phenols had previously been optimized by Basheer et al [9] and Guo and Lee [11]. In their work, derivatization time was reduced by heating at 70ºC in

a water bath. This temperature was applied for 30 min in the present work. In addition, the influence of different volumes of derivatization agent added was also investigated. Best results were attained with a 1:1 ratio of extract: derivatization agent. If a higher ratio of derivatization agent was employed, column bleeding took place as a result of the derivatization of the siloxane groups on the GC column [9, 11]. Based on our own experiences and literature reports, the optimized conditions for the proposed method were chosen at stated above.

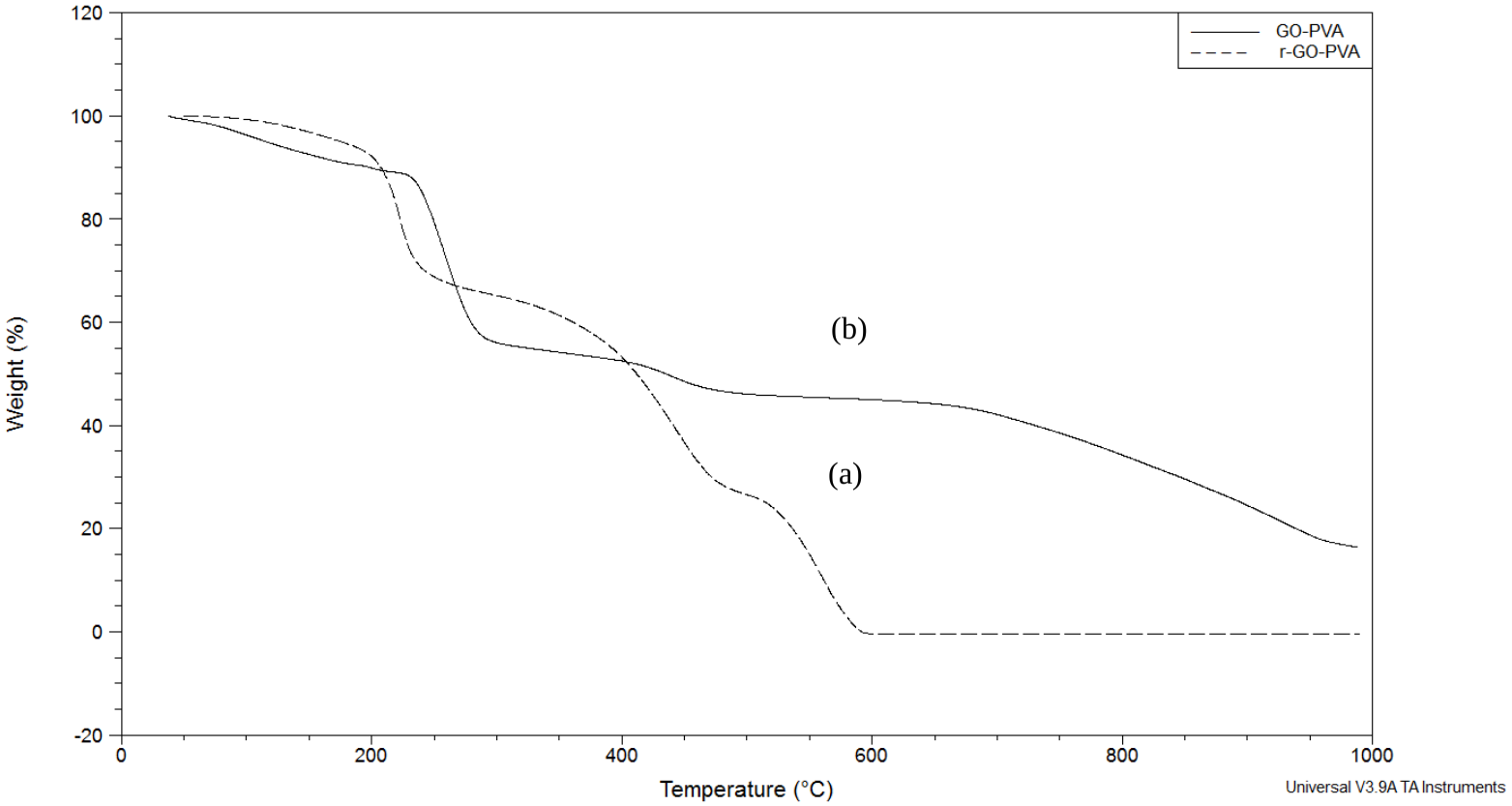

Fig. 4-4. TGA curves for (a) r-GO/PVA and (b) GO/PVA at heating rate 10ºC under nitrogen gas.

4.3.2.2 Type of organic solvent for SLM/Sorbent

During the EME procedure, the SLM served as an intermediary phase to support the transfer of analytes from the sample solution to the extractant phase [9, 10]. A number of factors on deciding on a suitable organic solvent to use as SLM includes: (1) the solvent should have adequate electrical conductivity to allow an electrical field to be created between the donor and acceptor solutions; (2) the chemical properties of the organic solvent should be appropriate for the analytes, facilitating their electrokinetic migration and transfer across the membrane; (3) the solvent should be well-accommodated within the pores of membrane so as to be well saturated within the membrane; (4) and the solvent should be immiscible in water to avoid analyte transfer back to the sample solution.

Moreover, a solvent with a lower boiling point is also undesirable; otherwise, solvent loss will possibly take place owing to Joule heating produced under the electrical potential [28]. The octanol-water partitioning coefficient, pK_{ow}, would also have to be considered as it plays an important role in the selection of the SLM and acceptor solutions. Since the pK_{ow} values of the para-alkylated phenols are estimated to be 4.1-5.5 and that of BPA to be 3.2 [29, 30], all these analytes are therefore considered to be hydrophobic compounds [31].

The extraction of these analytes with organic solvent is expected to be more favourable. In view of the factors mentioned above, some common organic solvents were examined for SLM. Amongst these solvents, 1-octanol demonstrated the highest efficiency for all analytes. Other solvents with relatively lower boiling points and densities showed lower chromatographic signals using EME [9]. Hence, 1-octanol was selected as the SLM for subsequent experiments.

The sorbent enforced porous membrane was prepared by filling of dispersed r-

GO/PVA in 1-octanol into the pores. Considering the adsorption on the r-GO, aromatic molecules were able to interact by π-π stacking with the graphene surface of fine r-GO [32]. This allowed efficient preconcentration of phenols from water.

4.3.2.3 Extraction time

To examine the electrokinetic migration of the phenols over time, different extraction times set in the range of between 3 and 20 min in optimizing experiments were investigated. EME is a non-exhaustive, time-dependant extraction procedure in which the equilibrium is established relatively rapidly due to the electrokinetic mass migration of analytes. The EME time was thus expected to be shorter than conventional LPME procedures [33]. As shown in Fig 4-5, the amount of extracted BPA increased from 3 to 5 min but a significant decline in the signal intensity was observed after 5 min. An analogous trend was observed for Hex P and TMBP in which these analytes revealed a reduced intensity after 5 min. This observation was most likely owing to the back-extraction of analytes into the organic SLM, possibly stimulated by the decrease in pH of the acceptor solution after 5 min extraction time. The optimized extraction time was, therefore, set as 5 min.

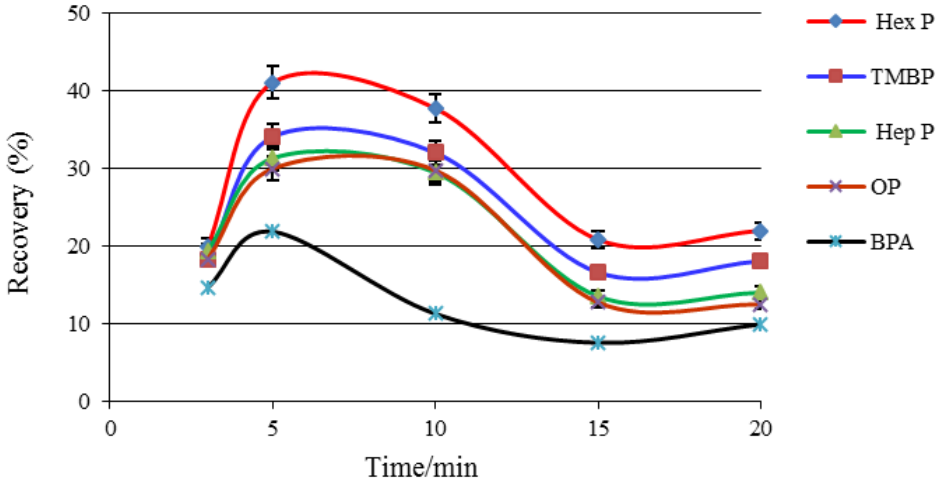

Fig 4-5. Extraction time profile of phenols. Extraction voltage, 50 V; SLM, 1octanol; extractant, 1-octanol; analytes at 100 µg L^{-1}

4.3.2.4 Electrokinetic potential

During EME, as mentioned previously, the passage of analytes from the donor solution migrating across the SLM into the acceptor solution is significantly enhanced by electrical potential. The effect of electrical potential on the extraction was studied by a series of experiments performed with the voltage in the range of between 0 and 500V. The effect of potential difference on analyte migration is demonstrated in Fig 4-6. The signal intensity increased as the voltage was changed from 0 to 100 V. Kjelsen et al [34] had depicted the fluctuation of analytes driven by electrical potential across the SLM. In accordance with the modified Nernst–Planck equation, the flux of the analytes over the membrane was considered to be enhanced with the increased electrical potential applied [35]. However, the decrease in signal intensity of analytes with a further increase of voltage was observed in a series of experiments. This could be explained in terms of pH changes in the donor and acceptor solution due to the increased applied voltage. The electrolysis of water at the positive electrode increased at higher voltages, leading to a rise of hydronium ion content; thus, a decrease in the pH of the

acceptor solution was expected. This conceivably facilitated back distribution of analytes from the acceptor solution to the SLM, reversing the extraction process [36]. Moreover, the creation of bubbles at the electrodes might also occur during extraction process. This might further reduce the extraction efficiency [9, 37]. Employing the relatively higher volumes of the donor and the extractant phase in this proposed method could moderately counteract these negative influences on extraction under a higher voltage [12].

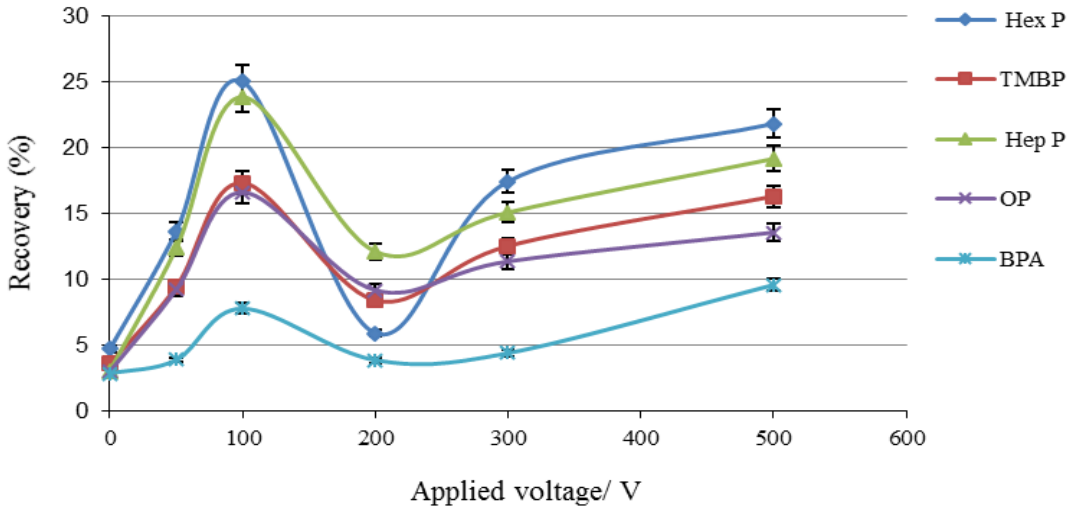

Fig 4-6. Effect of applied voltage. Extraction time, 5 min; SLM, 1octanol; extractant, 1-octanol; analytes at 100 µg L^{-1}

4.3.2.5 pH of sample solution

Analytes should be in their ionized forms in the sample solution during the EME process so as to allow their electrokinetic migration through SLM. To achieve this, pH control was necessary. The pH of the sample solution was studied in the range of from pH 8 to 12. This pH range was set on the basis of the pK_a values of all analytes in an effort to ensure they were in their ionized forms. The pK_a values of p-alkyl substituted phenols are estimated to be between 9.9 and10.9 whereas that of BPA is 9.7 [29, 30]. The chromatographic response for all analytes increased as the pH of the donor solution

was increased from 8 to 11 (Fig 4-7). This can be explained by the complete ionization of analytes in a higher pH solution, which is favorable for the migration of analytes under electrical potential. At pH>11, the ionic strength of the solution might be affected greatly by back-extraction induced by the adjustment the pH of the solution, thus hindering the migration of the analytes [28]. The adjustment of higher pH of the extractant solution, leading to an excess amount of hydroxyl ions, might presumably affect the further derivatization of the extract using BSTFA, before injection into the GC−MS system.

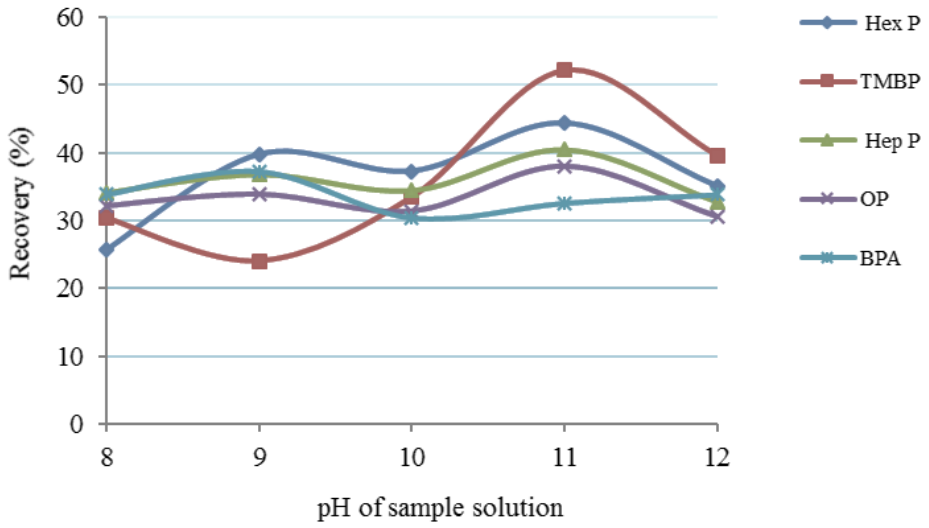

Fig 4-7. Effect of pH of sample solution. Voltage, 100V; extraction time, 5 min; SLM, 1-octanol; extractant, 1-octanol; analyte at 100 µgL^{-1}.

4.3.2.6 Sonication time

The sonication time with solvent/sorbent in the porous membrane was another parameter to be evaluated for EME-SLPME. A uniform distribution of the solvent/sorbent in the pores of membrane was necessary. To prepare the sorbent impregnated membrane, 100 µL sorbent dispersed in 1-octanol was charged into the membrane bag manually followed by ultrasonication until the mixture was well

dispersed and distributed within the membrane pores. As illustrated in Fig 4-8, no significant increase in signal intensity was observed after 10 min sonication time. Therefore, the favoured sonication time was selected as 10 min.

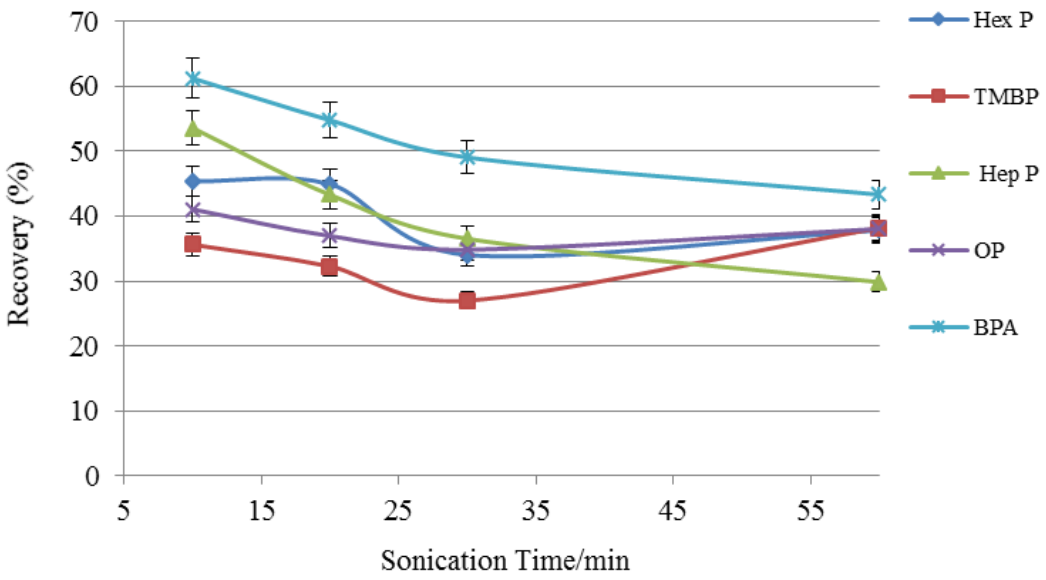

Fig 4-8. Effect of sonication time of sorbent mixture held in internal surface of porous membrane bag. Voltage, 100 V; extraction time, 5 min; extractant, 1-octanol; pH, 11; analytes at 100 µg L^{-1}.

4.3.2.7 Comparative study with other sorbents

Graphene related sorbents such as GO/PVA, r-GO, GO and amine functionalized reduced graphene oxide (r-GO-NH_2) were used for comparative study of the efficiency of the proposed method. As shown in Fig 4-9, the proposed method with sorbent r-GO/PVA showed the highest signal response. In addition to the π-π stacking force of analyte onto the phenyl moiety of graphene in sorbent, the network dispersion of fine r-GO in r-GO/PVA composite film with high conductivity presumably facilitated the speedy electrokinetic migration of analyte ions into the extractant phase (Fig 4-9).

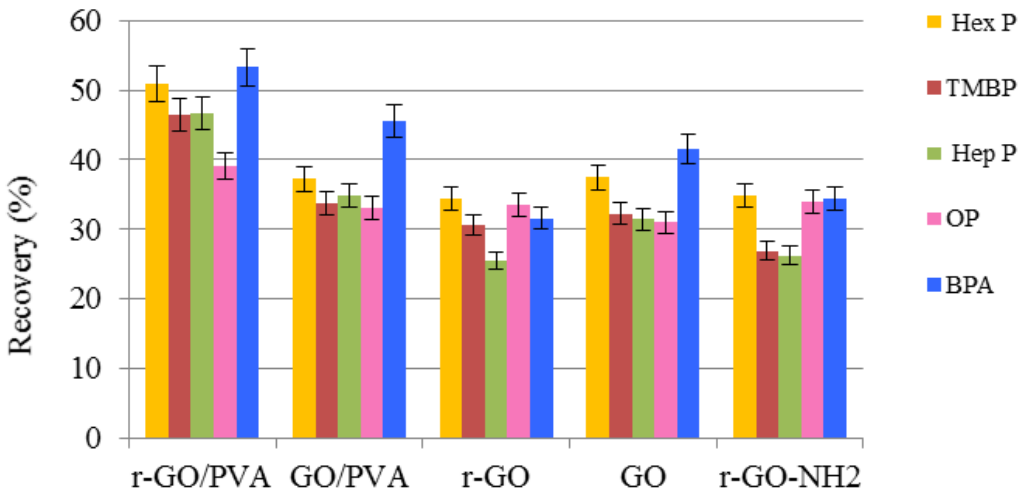

Fig 4-9. Comparison of recoveries with different sorbents. Voltage, 100 V; extraction time, 5 min; extractant, 1-octanol; pH, 11; analytes at 100 µg L^{-1}.

4.3.2.8 Comparative study with other methods

A comparative study amongst LPME, EME, and EME-SLPME methods was carried out. In order to produce comparable data, two-phase LPME extraction using the following: aqueous solution (donor phase), 1-octanol (SLM), 1-octanol solution (extractant phase) was carried out with the same apparatus and extraction conditions, exclusive of the use of electrodes and power supply. The sample solution was also treated in different way: in EME, the alkaline sample solution was prepared to make sure that ionization of the acidic analytes could occur easily; this was essential to support electrokinetic migration in the system. For LPME, the sample solution was prepared to maintain the neutrality of the analytes and promote their distribution into the organic SLM by stirring the solution. The organic solvent, the extractant phase, with a volume of 50 µL was placed in the membrane bag as in EME. The bag was then submerged in 1-octanol for a few seconds to fill the pores of the membrane wall to create the SLM. It was then dabbed gently with tint-free tissue paper to remove excess 1-octanol. The bag was then submerged in the sample solution. During extraction, the

sample solution was agitated at 800 rpm with a magnetic stirrer. After 5 min, the extractant phase was collected in a 100-µL micro-vial and derivatized with BSTFA+1%TCMS. One microlitre of this solution was injected into the GC−MS system for analysis. Although the experiments were similar in terms of the apparatus required, the extraction principle is different. In LPME, mass transfer is based on passive diffusion (although aided by stirring), instead of electrokinetic migration as in EME [28]. The results in Fig. 4-10 revealed that the developed EME-SLPME procedure was capable of extracting and enriching the analytes due to the π-π stacking interactions of aromatic benzenoid ring with graphene in sorbent and the relatively high electroconductive property of r-GO/PVA polymer. This result confirms that electroconductive sorbent consisting of a conjugated π system is more efficient in driving large charged hydrophobic compounds across an SLM/sorbent interface.

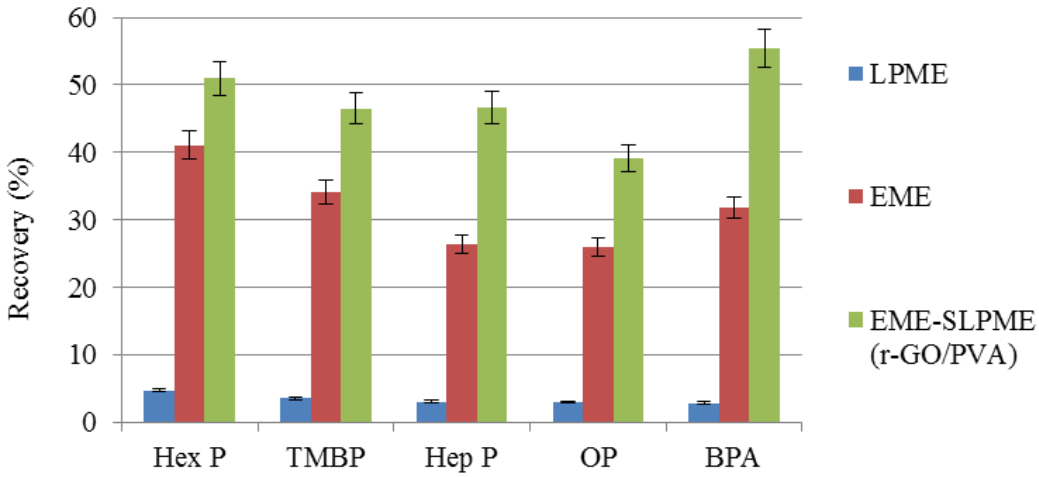

Fig 4-10. Comparison of recoveries of EME-SLPME with different methods. voltage, 100V; extraction time, 5 min; acceptor, 1-octanol; sorbent content, 2 mg mL^{-1} in 1-octanol; analytes at 100 µg L^{-1}

4.4 Method validation

The extraction efficiency and enrichment were examined using a range of parameters influential to the extraction such as SLM selection, pH of sample solution, applied voltage and extraction time which were optimized through a univariate approach. Enrichment (*E*) and percent recovery (*R*) were calculated by the following equations for each analyte:

$E=C_f/C_i$

$R= (V_a/V_s)\ (C_f/C_i)$ x 100%

where C_f and C_i refer to the final concentration of analyte in the acceptor phase and the initial analyte concentration within the sample, and V_a and V_s are the volume of the extractant phase, and the volume of the sample, respectively.

Using the optimized parameters as stated above, quantitative analysis for EME-SLPME was assessed with spiked deionized water samples. As summarized in Table 4-1, linear ranges for analytes were of from 0.05 to 50 μgL^{-1} for Hex P, from 0.05 to 100 μgL^{-1} for TMBP and O P, and from 0.1 to 100 μgL^{-1} for Hep P, and from 0.5 to 100 μgL^{-1} for BPA. All these linear ranges were associated with r^2 (coefficient of determination) values in the range of between 0.987 and 0.996, and with precision (RSDs) in the range of between 6.8 and 15.6%. LODs and LOQs were calculated [38] to be in the ranges of between 0.003 and 0.053 μgL^{-1} (LODs) and between 0.009 and 0.178 μgL^{-1} (LOQs) respectively. *E* values were calculated to be up to 193.

Table 4-1. Coefficient of determination, linear range, LOD, LOQ, precision and enrichment of EME-SLPME.

Analyte	Coefficient of determination (r^2)	Linear range (μg L^{-1})	LOD (μg L^{-1}) (S/N=3)	LOQ (μg L^{-1}) (S/N=10)	%RSD	*E*
p-n Hex P	0.992	0.05-50	0.003	0.009	15.6	148
TMBP	0.990	0.05-100	0.015	0.051	8.5	157
p-n Hep P	0.991	0.1-100	0.007	0.024	6.8	125
p-n-OP	0.987	0.05-100	0.008	0.026	10.0	152
BPA	0.996	0.5-100	0.053	0.178	12.1	193

For calculations of spiked water samples, the following equations were used:

$E = (C_f - C_{f,f}) / Ci$

$R = (V_a/V_s)(C_f - C_{f,f})/C_i \times 100\%$

where C_f is the final analyte concentration in the acceptor solvent, $C_{f,f}$ is the analyte concentration found from unspiked real sample and C_i is the initial analyte concentration within the sample solution, and V_a and V_s are the volumes of extractant phase and sample solutions.

In order to assess the applicability of the current method for phenol extraction, experiments were conducted on water samples collected from the Kallang and Jurong rivers in Singapore. The matrix effect was found to be relatively higher in the water of latter river since some of the analytes in this water showed relatively lower relative recovery values.

The water sample was first extracted using the developed method without prior filtration. Most of the phenols in water samples were not detected or below the LOQs. Only BPA and TMBP were found in these samples at concentration of 0.02 and 0.01μg L^{-1}. The *E* and *R* were calculated [9] with real water samples spiked with standard analytes at a concentration level of 1 μgL^{-1}. The results are shown in Table 4-2.

Table 4-2. Percent recoveries of unspiked and spiked real samples collected from different sites.

Analyte	Concentration in water (µg L^{-1})		Spiked real sample at 1 µg L^{-1}	
	Kallang river	Jurong river	Kallang river	Jurong river
			R (%)	*R* (%)
Hex P	N.D[a]	N.D	99.60	75.88
TMBP	N.D	0.01	96.26	41.37
Hep P	N.D	N.D	46.63	45.17
OP	N.D	N.D	56.39	41.65
BPA	0.02	N.D	60.58	35.34

[a] N.D: Not detected

The analysis of CPs and alkyl phenols had previously been carried out by Kojima et al [29] using SPE-GC-MS, in which the LODs for p-alkyl phenols were found to be from 0.00045 to 0.0021 µg L^{-1}. These relatively lower LODs than those achieved in the present work, were obtained using commercial Oasis MAX SPE cartridges based on the relatively larger sample volumes (100 mL). The LODs obtained for the extraction of analytes by the present method were comparable to or better than MISPE-HPLC-DAD method [39] for both surface and ground waters, i.e, 0.04 and 0.03 µg L^{-1}. In a SPME-HPLC method [40], the LOD for BPA was determined to be 1.1 µg L^{-1}. Jiang et al [41] calculated the LOD for BPA using IL-DLLME-HPLC method to be 0.58 µg L^{-1}. In another study, the LOD for BPA using DLLME-SFO-HPLC was determined to be 1.02 µg L^{-1} [42] (Table 4-3). These studies did not consider the other phenols.

Table 4-3. Comparison of LODs for EME-SLPME with those of different methods

Method	Extraction time (min)	Extraction solvent	Analyte*	LOD (μg L^{-1})	References
SPE-GC-MS	-	-	Hex P, Hep P OP	2.1, 0.45, 2.3 (ng L^{-1})	[29]
MISPE-HPLC-DAD	-	-	BPA	0.04 (surface) 0.03 (ground)	[39]
SPME-HPLC	20.00	-	BPA	1.10	[40]
IL-DLLME-HPLC	3.00	$[C_8MIM][PF_6]$	BPA	0.58	[41]
DLLME-SFO-HPLC	1.00	1-dodecanol	BPA	1.02	[42]
EME-SLPME	5.00	rGO-PVA/1-octanol	Hex P, Hep P TMBP, OP, BPA	0.003-0.053	this work

Method abbreviations:

SPE-GC-MS: solid-phase-extraction-gas chromatography-mass spectrometry. MISPE-HPLC-DAD: molecularly imprinted solid phase extraction-high performance liquid chromatography-diode array detector. SPME-HPLC: solid-phase microextraction-high-performance liquid chromatography. IL-DLLME-HPLC: ionic liquid-dispersive liquid-liquid microextraction-high-performance liquid chromatography. DLLME-SFO-HPLC: dispersive liquid-liquid microextraction-solidification of floating organic drop-high-performance liquid chromatograph

* Only analytes related to the present work are mentioned.

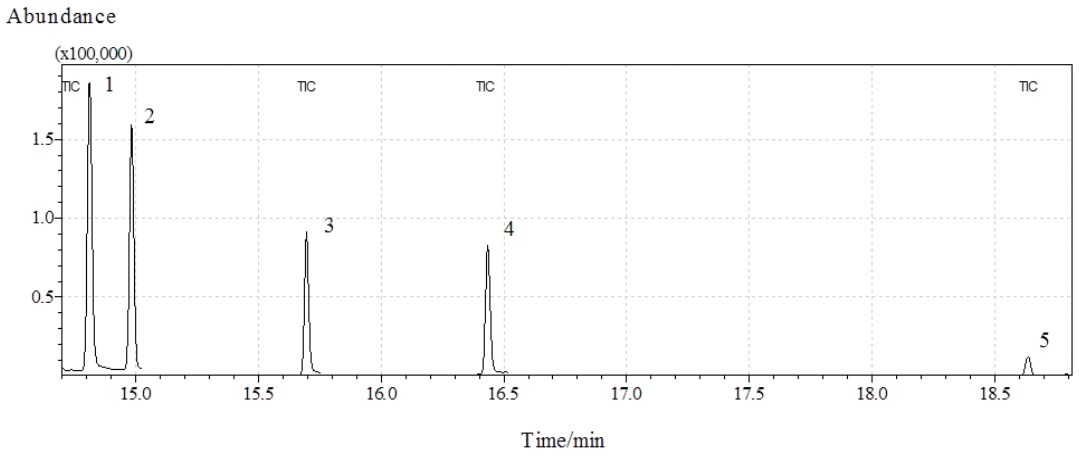

Fig 4-11. Gas chromatogram of real water sample spiked with analytes at 1 μgL^{-1} after EME-SLPME. Peak identities: (1) Hex P, m/z 179, 250 (2) TMBP, m/z 207, 263(3) Hep P, m/z 179,182 (4) OP, m/z 179,182 (5) BPA, m/z 213, 225.

4.5. Conclusion

The results from this preliminary study with EME-SLPME-GC-MS demonstrated that r-GO/PVA composite could be used advantageously as a novel sorbent for the extraction of phenols from water. Due to the highly electro conductive effect and π-π staging interaction of this prepared sorbent, the present method exhibited relatively higher extraction ability in relation to these ionizable benzenoid compounds. When evaluated against other microextraction methods, the proposed technique exhibited comparable selectivity towards p-alkyl substituted phenols and BPA. Good precision, reproducibility, and linear range responses over a wide range of concentrations were obtained with this method. Although more research is required for better understanding of the electrokinetic extraction of hydrophobic analytes across membrane filled with SLM/sorbent, the procedure has advantages such as simplicity, short time, cost effectiveness, ease of operation and low consumption of solvent.

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Chapter 5 Concluding remarks and future work

The objectives of the work described in this thesis include fast, more selective extraction and high enrichment of analytes that can be accomplished with the use of appropriate sorbents. The properties and applications of some graphene based sorbents have been described in this work. Graphene has been reported to be superior to other sorbents including silica, graphitic carbon and carbon nanotubes for the extraction of the analytes [1, 2]. Thus, in this thesis, the performance of graphene based sorbents in the microextraction of aromatic analytes was investigated and discussed. First, μ-SPE device impregnated with MCFG, acting as a self-contained stir bar, was used for extracting PAHs. Extraction took place as the μ-SPE device rotated in the sample solution assisted by magnetic power. This stir bar sorption extraction-like method exhibited low LODs and high Rs.

In another project, a two-step extraction method, μ-SPE followed by TE was investigated using CS-GO composite as sorbent for PBDEs. This method gave low LODs with high Rs especially for analytes with low molecular mass. Corroborating the results of previous studies, the membrane bag sewed as a protective sheath for the sorbent, shielding it from interferences present in the sample matrix. This allowed even complex matrices to be processed directly. The functional groups inhibited aggregation of GO in solution. This led to the high surface area associated with these materials, made accessible for sorptive-based extraction of the analytes from solution.

Since co-extraction of undesirable substances from the sample matrix is a common problem in any procedure, the development of sorption-based microextraction of a highly selective nature is therefore necessary. The longer the procedure of sample

preparation, the lower the sensitivity recorded. Moreover, an increase in possible analytes loss takes place with a large number of sample preparation steps.

An efficient method, EME-SLPME-GC-MS was therefore developed to minimize such problems. In this method, analytes were extracted from the sample solution into the extractant phase under electrical potential. The highly electroconductive sorbent (r-GO/PVA) was filled in the pores of the membrane together with the SLM. This three-phase microextraction approach consisting of aqueous donor phase, membrane (filled with sorbent and SLM), and organic solvent extractant phase, was successfully employed. Relatively higher extraction efficiency with lower LODs for five alkyl phenols were identified. In principle, EME-SLPME is an integration of two different miniaturized techniques. It was demonstrated to be a relatively faster (~5 min), precise and convenient pretreatment procedure for environmental water samples.

The procedures developed in this work are currently lacking in full automation. The potential reasons for automation generally arise from the requirement for analytical precision, increase of sample throughput, and reduced consumption of materials and energy, and reduction or elimination of manual labour. The potential of total on-site analysis, to obviate the pitfalls of preservation, storage and sample transportation [3, 4] is also an anticipated benefit. Future work should focus on this feasibility.

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