MICRO-SCALE MEMBRANE EXTRACTION FOR MONITORING ORGANIC POLLUTANTS AT ULTRA-TRACE LEVEL

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เยื่อสกัดจุลภาคเพื่อการตรวจวัดมลพิษอินทรีย์ในระดับปริมาณน้อยมาก

หม่อมหลวงศิริพัสตร์ ไชยันต์

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Hollow-fiber based liquid phase micro-extraction (LPME) is a relatively new extraction technique that combines the miniature of the solid-phase micro-extraction to the principle of liquid-liquid extraction (LLE). Fundamental study comparing the extraction efficiency of LPME and LLE using a simple mathematic model suggested that the analyte partition coefficient controls the extraction in LPME. The data also pointed to other parameters that govern the partition process such as molecular shape, size, and functional groups that control the molecular hydrophobicity. The simple mathematic model is limited to the thermodynamics of ideal partition and not includes kinetics parameters. Acid–base characteristics of the analyte strongly influence the extraction. These characters can be predicted, controlled, and manipulated with good accuracy to maximize the enrichment by simple chemistry concepts. pH has no influence on neutral compounds. Parameters affecting the enrichment were determined to be choices of the extracting solvent, choices of the acceptor solution, extraction time, sample volume, ionic properties of both donor and acceptor solutions, as well as other parameters that can affect the mass transfer rate such as solvent viscosity, temperature, and carrier concentration. The fundamentals were applied to some current pollutants. Three extraction methods were developed for high water soluble pesticides (glyphosate and its metabolite, quaternary nitrogen herbicides) and drinking water disinfection by-products. All methods were tested for their analytical performances and demonstrated excellent capability surpassing many published standard methods. LPME principle employs microliters of extracting solvent to concentrate the analytes without additional solvent evaporation steps. Extractant can be analyzed directly by simple analytical instrument. Three developed LPME methods coupled to analytical procedures for three groups of current organic pollutants surpassed strict validation protocol of trace analysis and deem fit for routine environmental analysis.

Field of Study : Environmental Management   Student's Signature _____

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หม่อมหลวงศิริพัสตร์ ไชยันต์ : เยื่อสกัดจุลภาคเพื่อการตรวจวัดมลพิษอินทรีย์ในระดับปริมาณน้อยมาก. (MICRO-SCALE MEMBRANE EXTRACTION FOR MONITORING ORGANIC POLLUTANTS AT ULTRA-TRACE LEVEL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.ณัฐชนัญ ลีพิพัฒน์ไพบูลย์., 110 หน้า.

เยื่อสกัดจุลภาคเป็นเทคนิคการสกัดใหม่ที่รวมหลักการของการสกัดแบบจุลภาคด้วยวัฏภาคของแข็งและการสกัดด้วยตัวทำละลายเข้าไว้ด้วยกัน งานวิจัยนี้ทำการเปรียบเทียบประสิทธิภาพการสกัดของเยื่อสกัดจุลภาคกับการสกัดด้วยตัวทำละลาย โดยใช้โมเดลทางคณิตศาสตร์พบว่า สัมประสิทธิ์การกระจายของสารในสารละลายสกัดเป็นตัวควบคุมสำคัญ ตัวแปรอื่นที่มีผลต่อการกระจายของสารได้แก่ รูปร่าง ขนาดของโมเลกุล หมู่ฟังก์ชันที่มีผลต่อสมบัติการละลายของสาร โมเดลคณิตศาสตร์นี้จำกัดอยู่เฉพาะระบบที่สมดุลทางเทอร์โมไดนามิกส์เท่านั้นและไม่ครอบคลุมพารามิเตอร์ทางจลนศาสตร์ของระบบ สมบัติความเป็นกรดเบสของสารมีอิทธิพลต่อการสกัดมาก สามารถนำมาใช้ในการทำนาย การควบคุมการสกัดให้มีความแม่นและประสิทธิภาพสูงได้ ค่าความเป็นกรดเบสไม่มีผลต่อการสกัดสารที่เป็นกลาง ตัวแปรที่ควบคุมประสิทธิภาพการเพิ่มความเข้มข้นของการสกัดคือชนิดของสารสกัด สารตัวรับ เวลา ปริมาณตัวอย่าง สมบัติไอออนิก รวมถึงปัจจัยอื่นๆที่ควบคุมการกระจายของสารในระบบด้วย เช่น ความหนืดของตัวทำละลาย อุณหภูมิ และความเข้มข้นของสารตัวพา หลักการพื้นฐานนี้นำไปใช้ในการสกัดมลพิษอินทรีย์ 3 กลุ่มคือไกลโฟเสตและสารเมทาโบไลท์ สารกำจัดวัชพืชประเภท quaternary nitrogen และผลิตภัณฑ์ข้างเคียงจากกระบวนการฆ่าเชื้อในน้ำดื่มทางเคมี พบว่าเยื่อสกัดจุลภาคมีประสิทธิภาพการสกัดสูงกว่าวิธีการสกัดที่ใช้กันอยู่ทั่วไป การปรับภาวะของการสกัดสามารถทำได้เป็นขั้นตอนและเนื่องจากมีการเพิ่มความเข้มข้นของสารสกัด จึงสามารถนำตัวอย่างไปวิเคราะห์ต่อได้โดยเครื่องมือวิเคราะห์ทั่วไป ขีดจำกัดของการวัดต่ำ ผลการประเมินความสามารถของวิธีการสกัดมลพิษอินทรีย์ทั้ง 3 กลุ่ม โดยเยื่อสกัดจุลภาค พบว่าดีกว่าเกณฑ์ที่กำหนดในการวิเคราะห์สารปริมาณน้อยมาก เยื่อสกัดจุลภาคเป็นเทคนิคที่ง่าย มีประสิทธิภาพสูงและเหมาะสำหรับงานวิเคราะห์ด้านสิ่งแวดล้อม

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ABBREVIATIONS

AA	Atomic absorption Aliquat-336*N*-methyl-*N*,*N*-dioctyloctan-*1*-ammonium chloride
AMPA	Aminomethylphosphonic acid
BIAA	Bromoiodoacetic acid
°C	Degree celcius
CQ	Chlormequat
D	Distribution coefficient
DBAA	Dibromoacetic acid
DBPs	Disinfection byproducts
DEHPA	Di(*2*-ethylhexyl)phosphoric acid
DHE	Di-*n*-hexyl ether
DI-SDME	Direct immersion single-drop micro-extraction
DIACID	(*E*)-*2*-iodo-*3*-methylbutenedioic acid
DQ	Diquat
E	(*E*)-*3*-bromo-*3*-iodopropenoic acid
EF	Enrichment factor
ESy	Extraction syringe
GC	Gas chromatography
GC/ECD	Gas chromatography/electro capture detector
HF-LPME	Hollow fiber liquid-phase micro-extraction
HILIC	Hydrophilic. Interaction. LIquid. Chromatography
HPLC	High performance liquid chromatography
HPLC/UV	High performance liquid chromatography/ultraviolet detector
HS-SDME	Head space single-drop micro-extraction
HSE	Hot solvent extraction
IAA	Iodoacetic acid
ICP/MS	Inductively coupled plasma mass spectroscopy
J	Flux
k	Boltzman's constant
K_D	Partition coefficient
K_{oc}	Soil organic carbon-water partitioning coefficient (mg/L)

K_{ow}	Octanol-water partition coefficient
LC/MS	Liquid chromatography/mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
log P	Partition coefficient
LOQ	Limit of quantitation
LPME	Liquid-phase micro-extraction
μ-SLME	Liquid-liquid membrane micro-extraction
m/z	Mass over charge ratio
MAE	Microwave assisted extraction
MASE	Membrane-assisted solvent extraction
MCL	Maximum concentration limit
MMLLE	Microporous-membrane liquid-liquid extraction
MQ	Mepiquat
MS	Mass spectrometry
M_w	Molecular weight (g/mol)
n	Mole
η	Viscosity
OPA	o-Phthaladehyde
P&T	Purge and trap
PAHs	Polynuclear aromatic hydrocarbons
PLE	Pressurized liquid extraction
POPs	Persistent organic pollutants
PQ	Paraquat
R	Recovery (%)
SBSE	Stir bar sorptive extraction
SDME	Single-drop micro-extraction
SFE	Supercritical fluid extraction
SIM	Select ion monitoring
SLM	Supported-liquid membrane
SLME	Supported-liquid membrane extraction
SPE	Solid-phase extraction
SPME	Solid-phase micro-extaction

S_w	Solubility in water (g/L)
T	Absolute temperature (K)
TBAA	Tribromoacetic acid
TCAA	Trichloroacetic acid
THMs	Trihalomethanes
V_a	Acceptor volume
V_d	Donor volume
V_r	Phase ratio
Z	(*Z*)-*3*-bromo-*3*-iodopropenoic acid

CHAPTER I

INTRODUCTION

1.1 Background and Research Rationale

1.1.1 The Process of Environmental Analysis

Environmental analysis is one of the most difficult analyses to carry out. This is because the measurement needs to be performed at extremely low concentration; the pollutants are commonly monitored as groups of compounds of diverse properties; the matrix is complex; and very often, there is little or no information about the samples. Due to these reasons and despite of recent advancements in the analytical technique, it is not possible to directly introduce environmental samples into an instrument. The pollutants must be separated from the samples prior to the analysis, often by some sort of extraction. Figure 1.1 shows overlaid chromatograms of polynuclear aromatic hydrocarbons (PAHs) extracted from a soil sample compared to a chromatogram of standard solution analyzed by HPLC under the same conditions. The chromatogram of the sample shows heavy interferences from the matrix components. These interferences can completely obstruct the detection of the chromatographic signals or partially superimpose with the analyte chromatographic peak causing error in peak integration. Examples of instruments that require ready samples are HPLC, GC, LC/MS, GC/MS, AA, ICP/MS, etc. The sample preparation process helps in removing the interferences from the sample whilst at the same time raising the concentration to a detectable level.

The purpose of an analytical study is to obtain information about an object or a substance. The analytical process can be broken down into steps as illustrated in Figure 1.2. Sample preparation is one of the most important steps for a successful analysis. The *complete analysis* often extends to the sampling where samples are collected in a way that will represent the original object in question. Once collected, the samples are preserved and transported to the laboratory where they are extracted and analyzed.
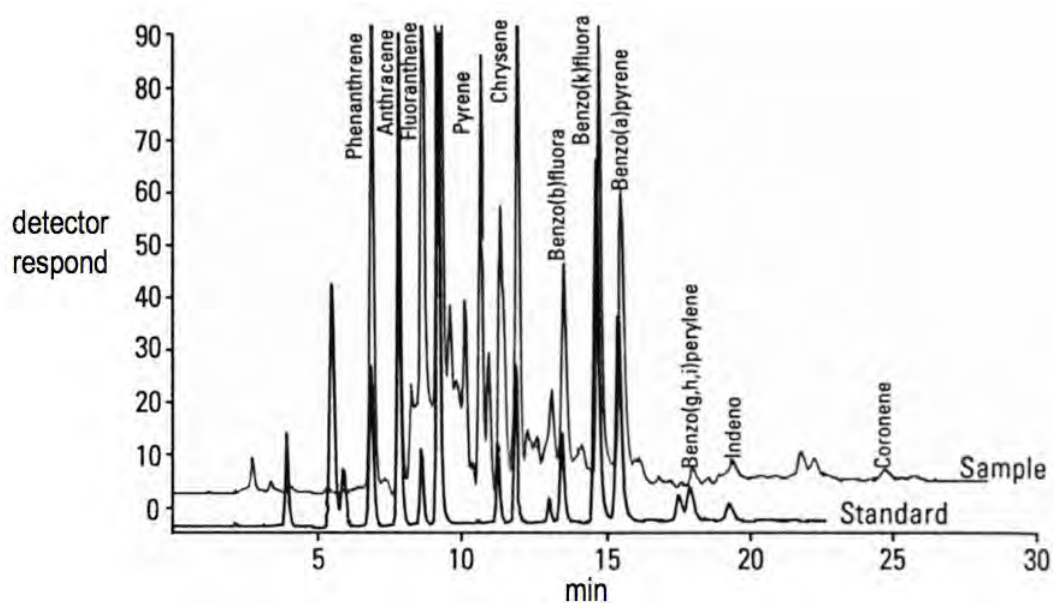
Figure 1.1 Overlaying chromatograms of polynuclear aromatic hydrocarbons extracted from a soil sample compared with standard solution analyzed by HPLC under the same conditions [1]
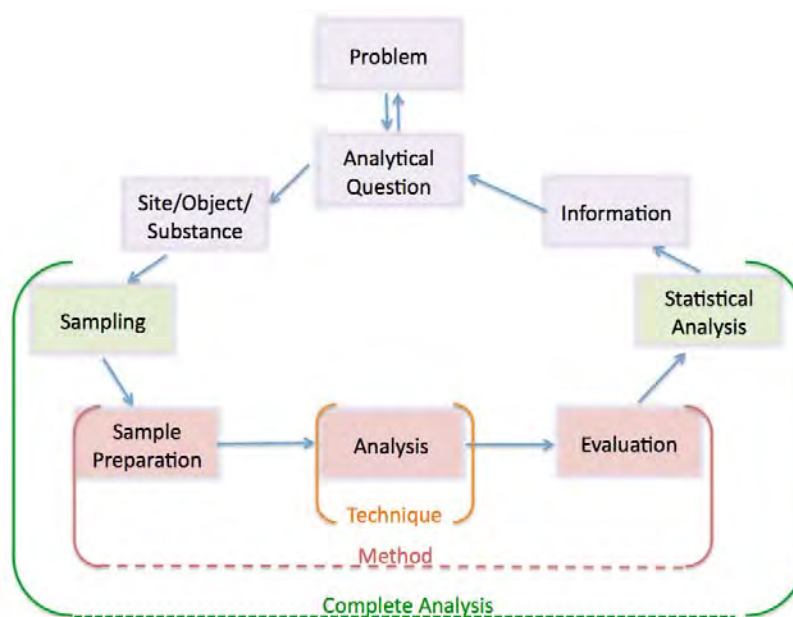
Figure 1.2 Steps of the analytical process

The results are evaluated and a statistical analysis performs on sets of data to project meaningful answers that connect to the original problem [2]. Sample preparation procedures are major source of inaccuracies and time consumption in the total analytical process.

Sample preparation can be achieved by several techniques which share some common goals [3]:

- To remove potential interferences;
- To increase the concentration of the analyte;
- To convert the analyte into a measurable form;
- To provide a reproducible method that is robust and independent of variations;

And recently, there are increasing demands on sample preparation technique to also consider:

- Employ smaller sample size;
- Employ less or no organic solvents;
- Provide greater specificity and selectivity;
- Possibility for automation and increase sample throughput
- Allow on-site analysis
- Time efficiency

Sample preparation must also be tailored to the final analysis, keeping the choice of analytical instrument, degree of accuracy required, and information required (qualitative or quantitative).

1.1.2 New World Pollutants and Their Analysis Problems

This past decade has seen some major successes in terms of global measurement and regulation of persistent, bioaccumulative, and toxic organic pollutants. The challenges have been associated with the releases of undesirable chemicals into the air, water, and soil. Sometimes such releases occur in a context of desirable acts such as disinfection of drinking water with chlorine, which can release potentially harmful byproducts. Hydrophobic persistent organic pollutants are now banned, replaced, or strictly controlled to limit their exposure to the environment.

As analytical instruments evolve, detection limits improve, and the capability to detect new compounds of potential concern emerges. Similar trends were observed in the organic trace analysis of water that is used to mainly focus on persistent organic pollutants (POPs) that can easily bioaccumulate and biomagnify through aquatic trophic chains. Current interests are now centered on the fate and role of polar organic contaminants in the aqueous environment. Many of these compounds are employed as household chemicals such as pharmaceutical drugs, disinfection agents, pesticides, and different personal care products [4]. They are commonly employed in our daily life and are increasingly discharged into the environment. Examples of these pollutants are: brominated flame retardants, endocrine disruptors, halonitromethanes, iodoacid disinfection byproducts, fragrances, perchlorate, pharmaceutical compounds, sunscreens and UV filters, phthalate esters, heavy metals, pesticides and their degradation products, and many more [5-8].

These new pollutants of concern are different to historic persistent organic pollutants in their physicochemical properties. Researchers often detect these emerged pollutants in environmental waters as they are not completely mineralized with the current removal technologies. Therefore, the identification of intermediates and degradation products is as important as the evaluation of their levels and toxicities in treated waters. To achieve these goals, selective and sensitive analytical methods are required.

1.1.3 Extraction Techniques for Environmental Samples

The simple approach of "dilute and shoot" is not usually compatible with environmental analysis. This is due to the fact that the analyte disperses in complex matrices at a very low level. An extraction step is required to isolate and enrich trace level analytes from the sample matrix. The traditional method such as liquid-liquid extraction (LLE) is still one of the most popular procedures in routine sample preparation. LLE is recognized as an attractive method for screening tests of unknown chemicals because of its simplicity, robustness, good efficiency, and only requires minimal training of the operator. LLE procedures are commonly recommended in standard methods. However, this age old technique requires large volumes of high purity solvents, which are hazardous, yields small sample to solvent volume ratios,

and is time consuming. Other disadvantages of LLE are its multistage operation and problems of emulsions formation that counteract its capability [9].

Sample preparation steps contribute largely to analytical method performance. In response to these challenges, new reduced solvent techniques such as solid-phase extraction (SPE) [10], supercritical fluid extraction (SFE) [11], pressurized liquid extraction (PLE) [12], and microwave assisted extraction (MAE) [13] have been developed to address the issues. These new reduced solvent techniques fail to improve the inaccuracy issue, require a large amount of time, and the extracted volume is often much larger than the volumes required for most chromatographic or other analysis methods. Schematic diagrams showing components of these extraction devices are displayed in Figure 1.3.

New movement focused on micro-extraction techniques to address issues of miniaturization, automation, on-site analysis, and time efficiency of the extraction method. The movement was initiated by the invention of solid-phase microextraction (SPME) [14]. Since then the interest has led to many new miniaturized extraction procedures such as stir bar sorptive extraction (SBSE) [15], some miniature liquid extractions such as single-drop micro-extraction (SDME) [16] that can be applied in 2 modes-direct immersion (DI)-SDME, and headspace (HS)-SDME. In DI-SDME mode, the drop of solvent is immersed in sample solution. HS-SDME mode involves placing the drop in headspace of a sample (Figure 1.4c). The analytes are transferred into an organic solvent and after extraction the drop is withdrawn into syringe and then directly injected into GC. The solvent has to be immiscible with the sample matrix (usually water) and not very volatile. SDME uses very small amount of solvent, inexpensive, simple, compatible with the GC-injection system, and has low environmental impact.

The instability of SDME led to the development of liquid-phase micro extraction (LPME) [17] where the extracting solvent is protected in a hollow fiber piece. Analytes are transferred from the donor phase (usually aqueous) to a receptor phase through an   organic phase immobilized in pores of the hollow fiber. In two-phase mode, the solvent in the pores of the fiber is the same as that present in the lumen. In the three-phase mode,

a) Solid-phase extraction (SPE)
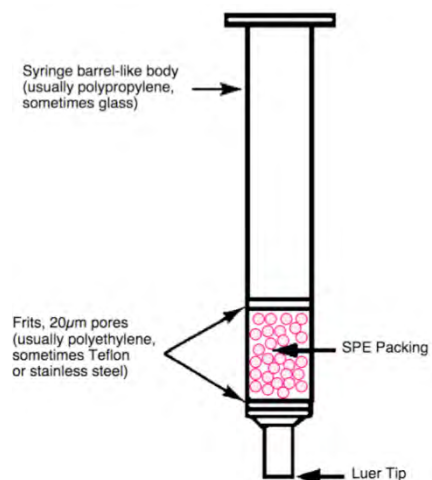
b) Supercritical-fluid extraction (SFE)
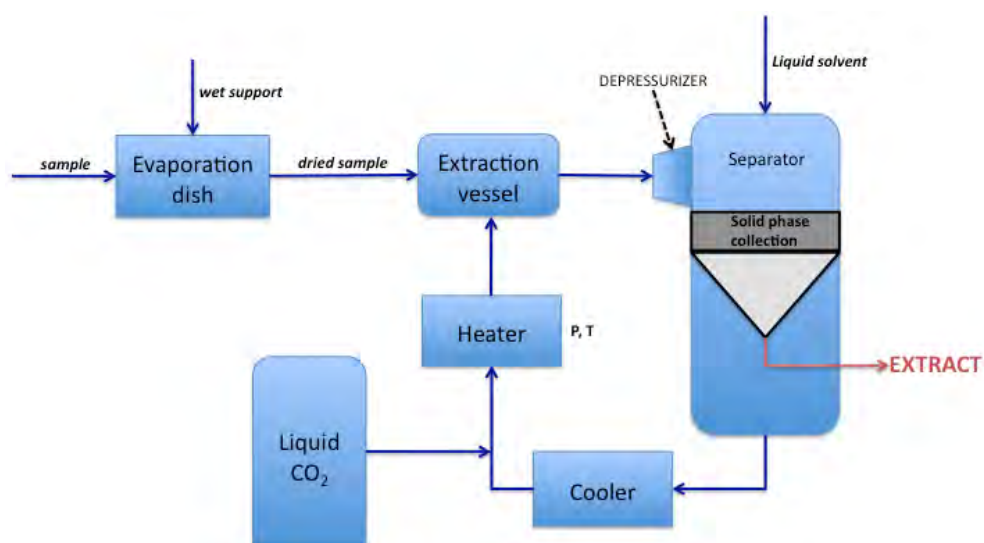
Figure 1.3 Schematic diagrams of reduced solvent extraction techniques: a) solid-phase extraction (SPE); b) supercritical-fluid extraction (SFE); c) presurrized-liquid extraction (PLE); d) microwave-assisted extraction (MAE)

c) Presurrized-liquid extraction (PLE)
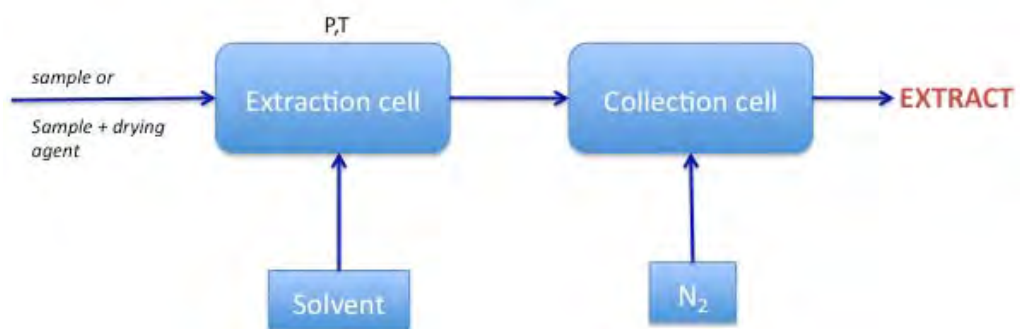
d) Microwave-assisted extraction (MAE)
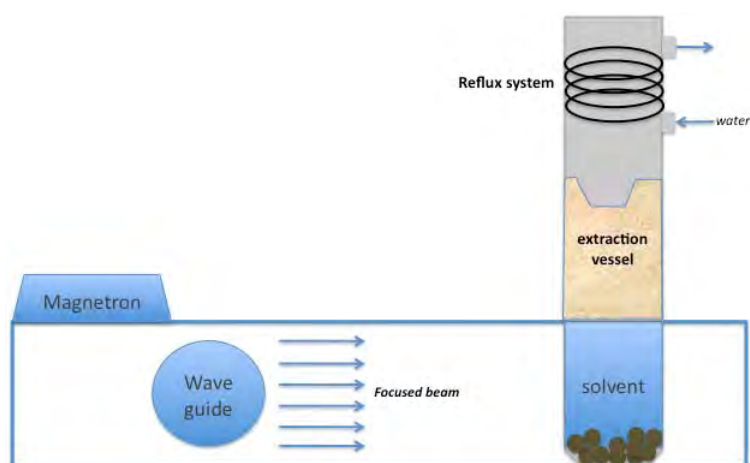
Figure 1.3 (continued)

a) Solid-phase microextraction (SPME)
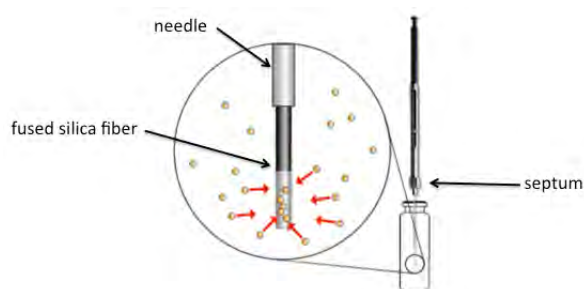
b) Stir bar sorptive extraction (SBSE)
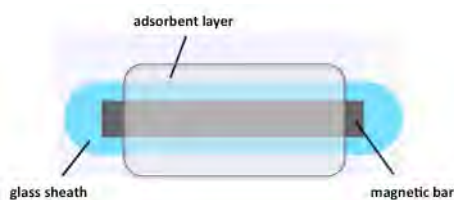
c) Head-space single-drop microextraction (HS-SDME)
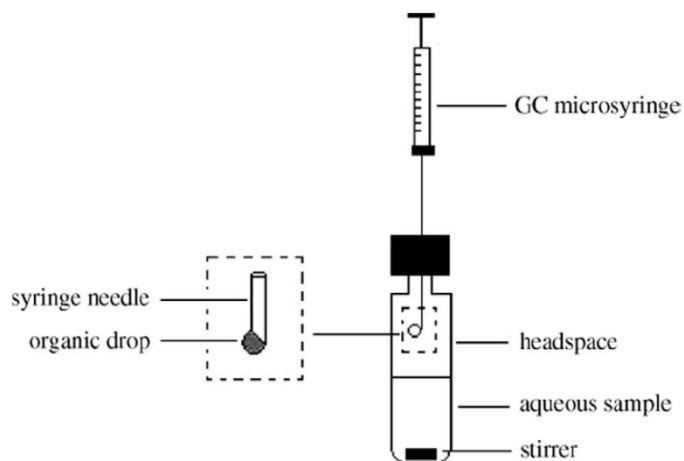
Figure 1.4 Schematic diagrams of microextraction techniques: a) solid-phase microextraction (SPME); b) stir bar sorptive extraction (SBSE); c) single-drop microextraction (SDME); d) Direct immersion single-drop microextraction (DI-SDME); e) Hollow fiber liquid phase extraction (HF-LPME)

d) Direct immersion single-drop microextraction (DI-SDME)

GC microsyringe

syringe needle

organic drop

aqueous sample

stirrer

e) Hollow fiber liquid phase extraction (HF-LPME)

10 µL HPLC Syringe

donor phase

porous hollow fiber

acceptor phase

magnetic stirrer

rod configuration

Needle for injection of acceptor solution

Needle for collection of acceptor solution

Screw top

Vial

Sample solution (donor solution)

Porous hollow fiber (acceptor solution)

u-shape configuration
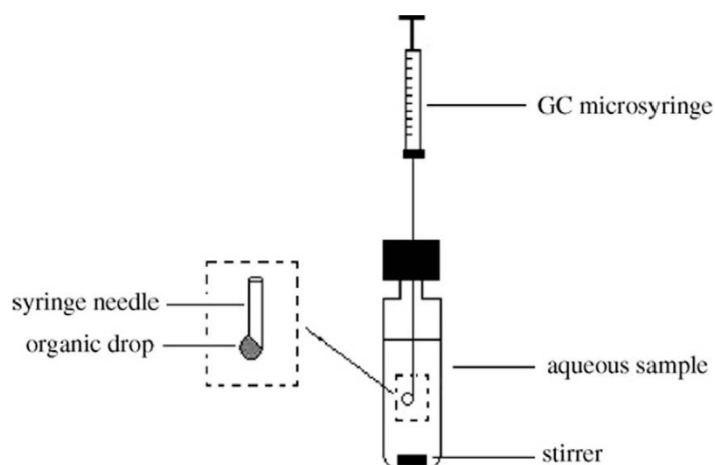
Figure 1.4 (continued)

the solvent placed in the pores of fiber differs from that inside the fiber. The three-phase mode is applied for extracting polar analytes, whereas the two-phase mode is applied for extracting non-polar and semi-polar analytes. The amount of solvent in the fiber is about 10–25 μL. The advantage of LPME over SDME is the presence of the fiber that supports organic solvent, so the process of dissolution or evaporation (in HS-SDME mode) of the solvent is minimized. Other micro-extraction techniques include extracting-syringe technique (ESy) [18], supported-liquid membrane extraction (SLME) and microporous-membrane liquid/liquid extraction (MMLLE) [19], and membrane-assisted solvent extraction (MASE) [20]. These micro-extraction techniques use the principle of liquid-liquid extraction and provide simplicity, speed, and use only microliters of toxic solvents. Their principles of operations are illustrated in Figure 4. Microscale extraction techniques can be divided according to their mode of operation into two general methodologies:

1. Techniques that suspend a discrete drop of immiscible solvent into sample (SDME, HS-SDME, DI-SDME), and
2. Techniques that employ membrane (LPME, HF-LPME, Esy, SLME, MMLLE, MASE).

Microscale extractions are inexpensive and offer considerable freedom in selecting appropriate solvents for the extraction of different analytes. They combine extraction, concentration, and sample introduction in one step. Since very little solvent is used, chemist's exposure to toxic organic solvent is also minimized. In a similar fashion to SPME, micro-extraction technique is non-exhaustive, and only a small fraction of the analytes is extracted/pre-concentrated for the final analysis. However, the suspended drop techniques have some drawbacks:

- Limited drop volume confines the extractable amount of the analyte and directly affect the enrichment,
- The equilibrium time is longer because strenuous agitation is not possible,
- Choice of organic extractant is limited to water immiscible organic solvent with suitable density and viscosity,
- Poor extraction reproducibility and accuracy is often observed due to partial dissolution of the microdrop that is in direct contact with the sample or dislodging of the microdrop during the extraction,

a) Extracting-syringe technique (ESy)
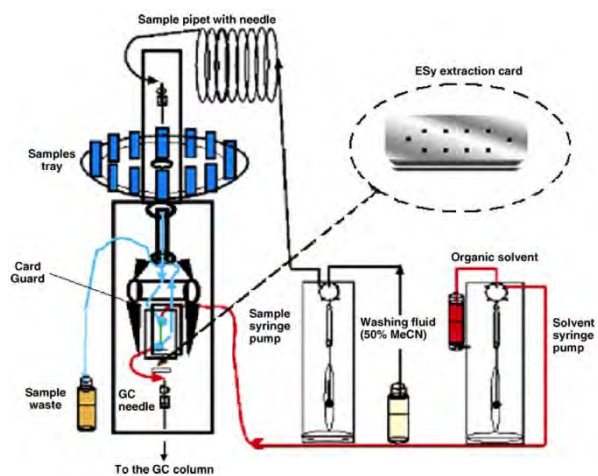
b) Supported-liquid membrane extraction
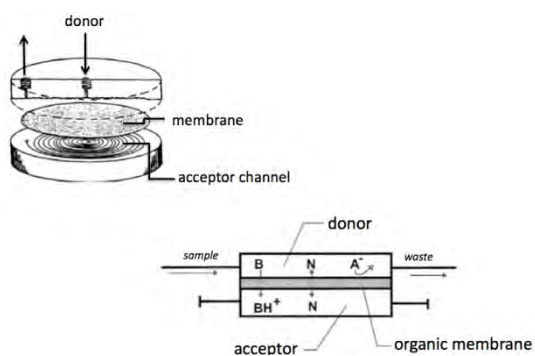
c) Membrane-assisted solvent extraction (MASE)
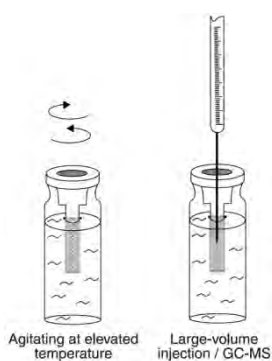
Figure 1.5 Other microscale extraction techniques: a) extracting-syringe technique (ESy); b) supported-liquid membrane extraction; c) membrane-assisted solvent extraction (MASE)

- Incompatible with dirty sample or sample with heavy matrix because suspended particles decrease the drop's stability.

1.1.4 Membrane Based Micro-Extraction

Liquid-phase micro-extraction (LPME) is a new principle in sample preparation that combines LLE with the miniaturized nature of SPME to take advantage of both techniques. The use of a hydrophobic membrane to separate phases and to contain the organic phase extends the capability of the LLE principles [21]. The membrane is impregnated with organic solvent which is then exposed to the sample. The sampling mode can be carried out in two approaches [22]. In an aqueous-organic two phase system, the analytes are extracted from the aqueous sample (donor solution) into the organic solvent (acceptor phase). A two-phase LPME is suitable for the extraction of neutral or non-ionized solutes in aqueous sample due to their preferred affinity with the organic liquid membrane. Because the acceptor phase is an organic solvent, acceptor solution can be injected directly into a GC or a NPLC. Partition coefficient of the analyte between aqueous and organic phases mainly drives the mass transfer of the two-phase system. Two-phase LPME is sometime referred to as *liquid-liquid membrane micro-extraction* (*μ-LLME*). The two-phase system can be slightly modified for the extraction of charged or ionized analytes by replacing an organic acceptor with another aqueous solution. The three-phase LPME consists of two aqueous phases sandwiched a middle organic liquid phase in the membrane pores. In this approach, the pH of the donor phase is adjusted to a value that keeps the solutes in non-ionized forms and is thus able to partition through the organic solvent filled membrane pores. Once the solutes diffuse through the membrane, it will face with an aqueous acceptor solution that is kept at a pH that will convert the solutes into new forms that cannot be extracted back into the membrane. This technique is also known as *supported-liquid membrane micro-extraction* (*μ-SLME*). A three-phase system can be simply tuned and controlled to maximize the extraction efficiency, selectivity, and solute enrichment by changing the membrane liquid and pH of the donor and acceptor solutions. The system operates under the principle of two independent LLE systems and therefore involves two

equilibria. To further augment the selectivity in the three-phase LPME, a carrier molecule with high affinity to target analyte may be added. This is commonly referred to as *facilitated or carrier-mediated transport* and involves reversible complex formation between the carrier and the analyte.

All mentioned micro-scale membrane extraction methodologies proved to be attractive as alternatives to micro-drop techniques because, apart from being simple, inexpensive, fast, and virtually solvent-free, the membranes are disposable and capable of accommodating larger volumes of acceptor solvent phase. This results in an increase in method sensitivity and reproducibility and eliminates carry-over effects. Furthermore, small pores act as a filter that screens larger molecules and interfering matrix components in the donor solution from being extracted. In conclusion, LPME is not only good at enrichment but also provides simultaneous sample cleanup that is beneficial for the analysis of complex matrix such as environmental samples.

1.2 Research Objectives

As already elaborated, a successful environmental monitoring program requires sensitive and selective analytical methods that can provide accurate results. Sample preparation steps contribute largely to the performance of these methods. Recently, alternative sample preparation techniques such as micro-scale membrane extraction attract great attention of the analytical community. Analytical microextraction can be defined as equilibrium non-exhaustive sample preparation using very small volume of extracting phase relatively to the bulk sample. The techniques are friendly to the environment, offer high selectivity and enrichment, and can be further developed and integrated into a portable miniaturized device for environmental monitoring purposes via simple interface with variety of analytical instruments. Therefore, it was the objective of this research to explore the potential of micro-scale membrane extraction techniques in two aspects:

1. Explore the fundamentals of membrane extraction in comparison to a benchmark extraction technique with an aim to identify key extraction parameters so that comprehensive extraction schemes can be developed for simultaneous enrichment of different analytes;

2. Test these comprehensive schemes on real world pollutants such as high water soluble pesticides and water disinfectant byproducts.

1.3 Research Boundary

1. Due to some drawbacks of suspended drop techniques, this research employed micro-scale membrane extraction apparatus constructed with a single strand of a hollow fiber membrane that hold microlitres of organic extractant and is fit for analytical sample preparation purposes.

2. Target pollutants are emerged pollutants that are polar, highly soluble in water, difficult to detect, and often present at low concentration in natural water resources. Their successful analyses require good cleanup (to remove interfering matrix) and preconcentration (to raise method sensitivity).
   a. Group I: high water soluble pesticides such as paraquat, diquat, glyphosate and their other derivatives or metabolites.
   b. Group II: high priority water disinfection byproducts iodoacetic acid that is highly toxic and is a byproduct of a chloramination process.

1.4 Expected Outcomes

1. Fundamental understanding on membrane extraction processes especially on parameters affecting analyte enrichment and selectivity in comparison to benchmark extraction technique.

2. Generalize analyte enrichment schemes that are effective for a range of chemicals of diverse physicochemical characteristics.

3. New applications of micro-scale membrane extraction techniques that are fit for the analysis of emerging pollutants such as high water soluble pesticides and water disinfection byproducts.

4. Promote micro-scale membrane extraction techniques as green alternative extraction technique in the analytical community.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Classification of Extraction Techniques

Extraction techniques can be classified into exhaustive and non-exhaustive methods. The exhaustive method is accomplished by using overwhelming volumes of the extracting phase so that they do not require any calibration and are preferred by practitioners and regulatory agencies. To reduce the amount of solvent, flow-through equilibrium methods can be employed. In a similar way, the flow-through method can be either exhaustive or non-exhaustive as illustrated in Figure 2.1. Non-exhaustive approaches can be designed based on equilibrium, pre-equilibrium, and permeation principles [23]. Among all of these modern extraction techniques, the equilibrium non-exhaustive ones are fundamentally analogue to the equilibrium exhaustive techniques. They use much less solvent and allow for simple extrapolation to determine the total number of analytes. Examples of these techniques are SPME and LPME.

lid-phase e...........,
SFE = supercritical fluid extraction, HSE = hot solvent extraction
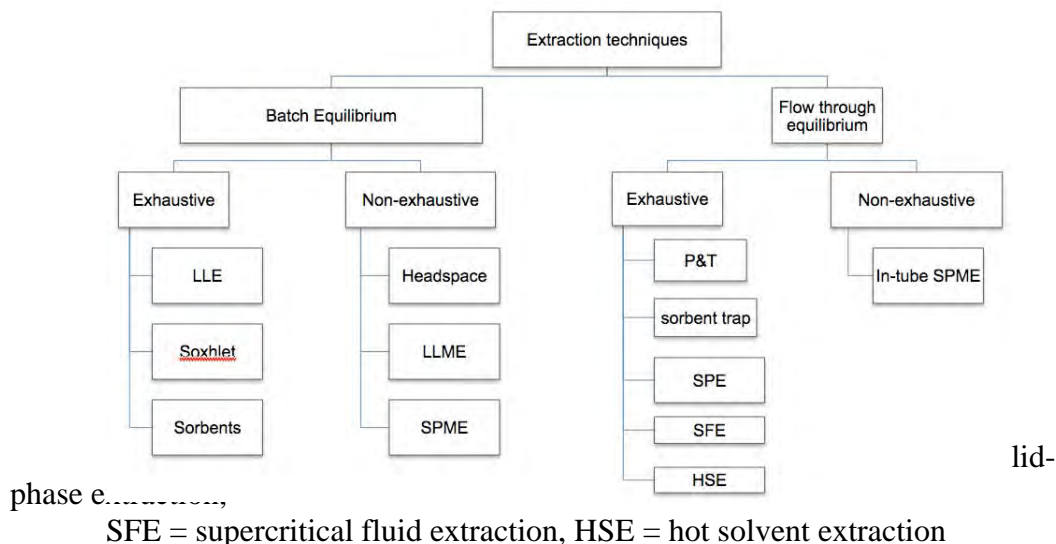
Figure 2.1 General classification of extraction techniques

2.2 BenchmarkExtraction Technique vs. Analytical Microextraction

Solvent extraction or liquid-liquid extraction (LLE) has been the most widely used techniques for sample preparation for qualitative and quantitative analysis. Due to many practical problems associated with the separatory funnel mode of operation, other batch phase separation techniques such as solid-phase extraction (SPE) and solid-phase microextraction (SPME) rapidly replaced routine operation of LLE [24]. Very recently, non-classical modes of LLE that circumvent these functional problems of classical LLE emerged as high potential in sample preparation alternatives [3]. For example, liquid membranetechniques are very promising alternatives to LLE and SPE methods because of the high selectivity and relatively high flux values that can be obtained [25]. Some interest has been directed toward liquid-phase microextraction (LPME) for sample preparation prior to analysis by chromatography and electroporesis techniques[26]. In LPME, the principle of LLEis combined with the miniaturized nature of solid-phase microextraction (SPME) [22]. Several parameters govern the extraction in such a system.

2.2.1 Benchmark Technique -- Liquid-Liquid Extraction

LLE is used for sample cleanup and/or analytepreconcentration. For cleanup purposes, high selectivity of the analyte over the potential interferences is important. To obtain high preconcentration of the anlayte from a large volume of sample into a small volume of extractant, a high distribution ratio is required. Because the extractant is in liquid phase, it is ready for direct measurement with no further transferring steps. LLE is a common method for extracting non-volatile organic compounds from aqueous media or from other liquid matrices. The aqueous sample is usually shaken with a portion of immiscible organic solvent. The movement of a chemical species from one phase to another is driven by a thermodynamic force that could be in a chemical potential (neutral species) or an electrochemical potential (ionic species). The mechanism by which the species move from one phase to another is convectional-diffusive mass transfer. The analytes distribute in both phases until an equilibrium is reached and a constant concentration ratio is obtained as can be stated as:

$$K_D = \frac{[X]_{org}}{[X]_{aq}} \qquad (2.1)$$

where a constant K_D is the partition coefficient, $[X]_{org}$ and $[X]_{aq}$ are the equilibrium concentration of analyte in organic phase and aqueous phase, respectively. The number of moles of the analyte that was extracted is equaled to:

$$n_{org} = \frac{[X]_{org}V_{org}}{[X]_{org}V_{org} + [X]_{aq}V_{aq}} \qquad (2.2)$$

divide equation (2) by $[X]_{org}V_{org}$ gives

$$n_{org} = \frac{1}{1 + \dfrac{[X]_{aq}V_{aq}}{[X]_{org}V_{org}}} = \frac{1}{1 + \dfrac{V_r}{K_D}} \qquad (2.3)$$

where V_r is the phase ratio (V_{aq}/V_{org}) of the system. To obtain large fraction of extracted analyte, V_{org} should be large and select solvent that provides large K_D. Improve extraction efficiency of LLE can be done by successive extraction using small constant volumes of organic solvent [27].

after 1
extraction
$$[X]_{aq}^1 = [X]_{aq}^0 \left[\frac{V_{aq}}{K_D V_{org} + V_{aq}} \right]^1 \qquad (2.4)$$

$$[X]_{aq}^n = [X]_{aq}^0 \left[\frac{V_{aq}}{K_D V_{org} + V_{aq}} \right]^n \qquad (2.5)$$

It is very common to express the extraction efficiency in terms of extraction recovery which expressed the percent mole of analyte extracted by the procedure:

$$\%R = \frac{n_{org}}{n_{aq}} \times 100 = \frac{[X]_{org}V_{org}}{[X]_{aq}V_{aq}} \times 100 \qquad (2.6)$$

LLE employs a full sample volume and therefore offers large linear sample capacity. In a classical approach of LLE (separatory funnel) relatively large volume or organic solvent is used in LLE,

therefore the recovery in LLE is large even for a compound with relatively low K_D.

2.2.2 SolidPhase Micro-Extraction

SPME is anon-exhaustive equilibrium multiphase extraction technique. An extracting phase coated on a piece of fiberis exposed to either gas or liquid samples. If a sampling time is long enough for the analytes to partition into the stationary phase until equilibrium is achieved, the analyte's concentration can be quantified.The extraction system is usually complex and involves several partition processed between phases.The amount of analyte adsorbed by the coating at equilibrium is directly related to the concentration of the analyte in the sample. If the extracting polymer phase is exposed to the headspace of an aqueous sample, the equilibrium between the three phases(aqueous, polymer, headspace) can be described as:

$$K_{pw} = \frac{C_p^{eq}}{C_w^{eq}}, \qquad K_{pg} = \frac{C_p^{eq}}{C_g^{eq}}, \qquad K_{gw} = \frac{C_g^{eq}}{C_w^{eq}} \tag{2.7}$$

K_{pw} is partition coefficient between polymer/aqueous; K_{pg} is partition coefficient between polymer/gas; and K_{gw} is partition coefficient between gas/aqueous. And the system mass conservation can be considered as:

$$C_0 V_w = C_p^{eq} V_p + C_g^{eq} V_g + C_w^{eq} V_w \tag{2.8}$$

whereC_0 is the initial analyte concentration in the sample; V_w, V_p, V_g are volumes of the aqueous phase, polymeric coating, and headspace, respectively; C^{eq} are the equilibrium concentration of the analyte in each phase [14].

If the extraction occurs by adsorption to the coating alone, the mass of the extracted analytecan be derived as:

$$n_p = \frac{K_{pg} K_{gw} V_p C_0 V_w}{K_{pg} K_{gw} V_p + K_{gw} V_g + V_w} \tag{2.9}$$

If the effect of moisture in the system is negligible,

$$n_p = \frac{K_{pw}V_pC_0V_w}{K_{pw}V_p + K_{gw}V_g + V_w}$$

(2.10)

If there is no headspace, then:

$$n_p = \frac{K_{pw}V_pV_wC_0}{K_{pw}V_p + V_w}$$

(2.11)

Normally, the fiber is very small (V_w>>V_p) and has the nature of non-exhaustive extraction.

$$n_p = K_{pw}V_pC_0$$

(2.12)

equation(2.12) states that the adsorbed analytes is directly proportional to the original analyteconcentration in the bulk [28]. Another important aspect that equation (2.12) implies is that the adsorbed analyte is independent to the volume of the sample which is very important for field sampling[29].

2.2.3 Liquid-Phase Microextraction

Liquid-phase microextraction (LPME) shares a common feature of liquid-liquid extraction (LLE) and the miniature of solid-phase microextraction (SPME). It can be used to extract various types of analytes present in liquid samples such as waters, biological fluids, etc. The method is flexible and can be adapted to extract a wide range of analytes from hydrophobic compounds to hydrophilic ones [28]. LPME proves to be very useful for polar analytes which are very difficult to extract by traditional techniques such as LLE and solid phase extraction (SPE) by increasing selectivity.LPME can be operated in a single drop mode (SDME) which has high sample-to-drop volume ratio leading to excellent enrichment of the analyte. Due to the instability of the drop that can be lost during the extraction, the organic solvent can be contained within a piece of hollow fiber and is not in direct contact with the bulk solution [30]. The configuration of LPME can be classified into 2 groups

according to the number of liquid phase presented: 1) two-phase LPME where organic extractant is exposed to an aqueous sample and the analytes partition into the organic phase; and 2) three-phase LPME where an organic extractant is sandwiched between the sample solution and another aqueous acceptor solution. Schematic diagrams of two-phase LPME and three-phase LPME are illustrated in Figure 2.2. The chemical principle of hollow fiber LPME is similar to the principle of supported liquid membrane [31], however, both techniques differ significantly in terms of instrumentation and operation.

2.2.3.1 Two-Phase LPME

In a two-phase LPME, analyte X moves from sample solution (donor) across the liquid membrane into an organic receiving phase (acceptor). This process is driven by partition between the aqueous and the organic phase and at equilibrium, the system is dynamic with solutes distributed across both phases.
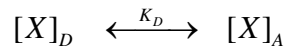
$$[X]_D \quad \xleftarrow{\quad K_D \quad} \quad [X]_A$$

$$K_D = \frac{[X]_A}{[X]_D} = \frac{[X]_{org}}{[X]_D} = K_{D1} \qquad (2.13)$$

K_D is the partition coefficient of analyte X in donor (aqueous) and acceptor (organic) phases. In a similar way to SPME, the extraction is non-exhaustive and once an equilibrium is reached, the extracted amount is constant within the limit of experimental error and remains constant regardless of an extended extraction time. Therefore, the equilibrium condition of SPME can be used to describe the equilibrium condition of LPME such that:

$$n_A = \frac{K_D V_A V_D [X]_i}{K_D V_A + V_D} \qquad (2.14)$$

where n_A is the number of analyte moles in acceptor phase and $[X]_i$ is initial concentration.

a) Two-phase LPME
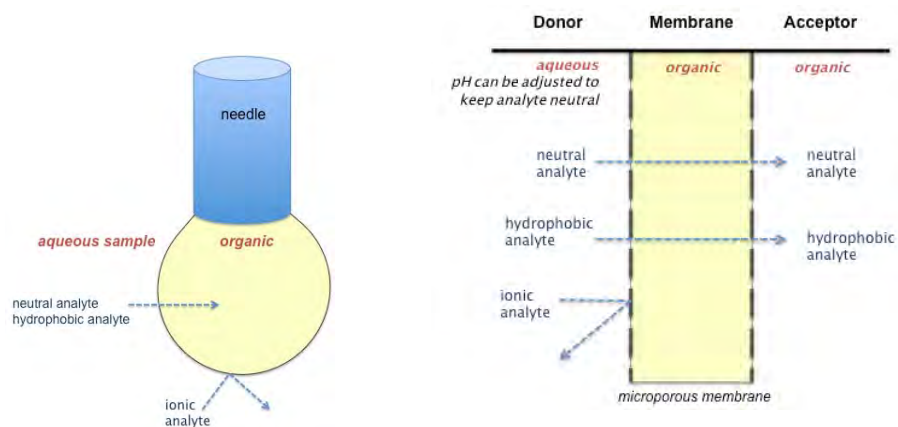
b) Three-phase LPME
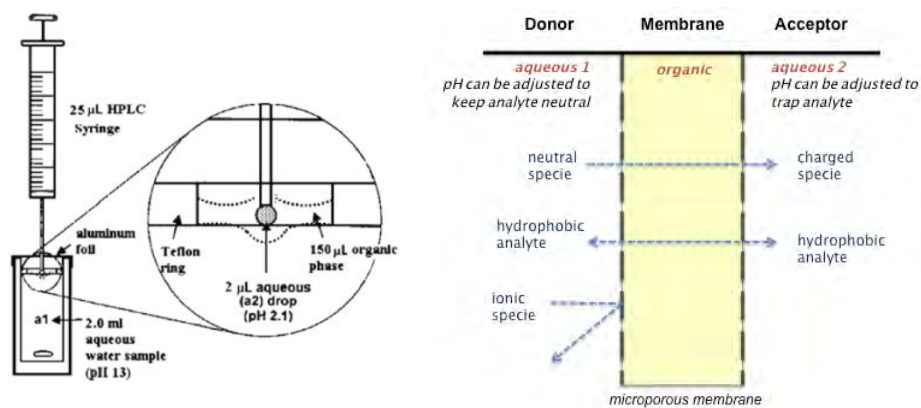
c) Carrier-mediated three-phase LPME
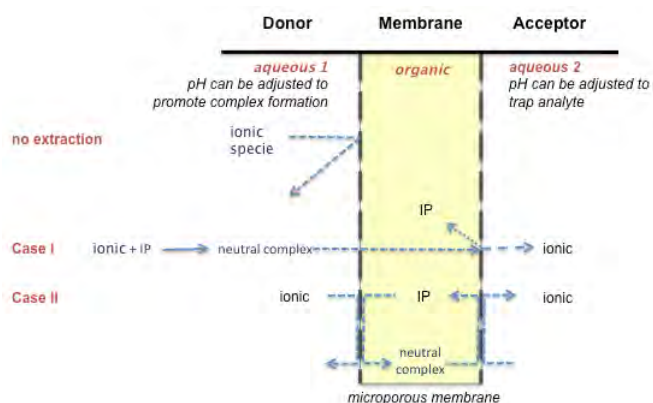
Figure 2.2 Schematic diagram of analyte partition in a) two-phase LPME and b) three-phase LPME in single drop and hollow fiber protected modes c) carrier-mediated transport three-phase HF-LPME

Efficiency of the extraction is commonly expressed as percent recovery (%R).

$$\%R = \frac{n_A}{n_s} \times 100 = \frac{[X]_A V_A}{[X]_s V_s} \times 100 \tag{2.15}$$

$$\%R = \frac{K_D V_s}{K_D V_A + V_s} \times 100 \tag{2.16}$$

Analyte enrichment (*EF*) can be calculated by:

$$EF = \frac{[X]_A}{[X]_s} = \frac{PV_{sd}}{100V_A} \tag{2.17}$$

In two-phase LPME, a fraction of organic solvent remains in the membrane pores leading to reduced *EF* from the calculated value.

2.2.3.2 Three-Phase LPME

Two-phase LPME is very efficient in the extraction of neutral organic analytes in aqueous media. However, the system is not effective for the extraction of polar compounds or ionizable species because they are incompatible with the organic liquid membrane. The solubility of many of these compounds are pH dependent which means that a pH of the aqueous sample can be adjusted to a value that promotes neutral species that can be extracted by organic solvent. The flux of these neutral species can be further forced to move into the aqueous acceptor solution in the lumen that has suitable pH to convert the analytes back to their charged forms that cannot be extracted by the organic liquid membrane and therefore are trapped in the acceptor solution. This process is very selective and the pH can be selected for specific extraction of any analyte. A schematicof a three-phase LPME process can be shown as:
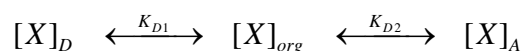
$$[X]_D \quad \xleftrightarrow{K_{D1}} \quad [X]_{org} \quad \xleftrightarrow{K_{D2}} \quad [X]_A$$

where
$$K_{D1} = \frac{[X]_{org}}{[X]_D} \qquad K_{D2} = \frac{[X]_A}{[X]_{org}} \tag{2.18}$$

$$K_D = K_{D1} K_{D2} \tag{2.19}$$

$$n_A = \frac{K_D V_A [X]_i V_D}{K_D V_A + K_{D1} V_{org} + V_D} \tag{2.20}$$

$$\%R = \frac{n_A}{n_i} \times 100 = \frac{[X]_A C_A}{[X]_i C_i} \times 100 \tag{2.21}$$

$$= \frac{K_D V_A}{K_D V_A + K_{D1} V_{org} + V_D} \times 100$$

$$EF = \frac{[X]_A}{[X]_i} = \frac{R V_D}{100 V_A} \tag{2.22}$$

2.2.3.3 Three-Phase LPME--Carrier Mediated Transport

Three-phase LPME exploits the acid-base characteristic of the analytes to achieve simultaneous enrichment and cleanup. In extreme cases such as when the analytes are strong acid, strong base, or carry high charge density; it is not possible to neutralize the compoundcompletely by simply modifying the donor and acceptor solutions. In this situation, poor enrichment was observed [32]. To enhance the transport of polar analyte in the organic liquid membrane, a hydrophobic ion-pair reagent can be added to the donor solution (sample) to form extractable complex with the analyte. At the membrane-acceptor interface, the analyte is released from ion-pair complex and partition into the acceptor solution. This can happen in the present of high concentration of counter-ions in the acceptor solution. Counter-ions can be protons (low pH) or other ions that can form complex with ion-pair reagent. In another configuration of carrier-mediated transport, an ion-pair reagent can be added to the supported liquid membrane where analyte-carrier complex is formed at the donor-membrane interface and the reversible reaction that break this complex occurs at the membrane-acceptor interface. The extraction efficiency of a carrier-mediated transport LPME can be calculated in a similar way to the normal three-phase system using equations (2.21) and (2.22). Schematic diagram of a carrier-mediated transport LPME is illustrated in Figure 2.2c).

2.2.3.4 Extraction Effectiveness in LPME

The effectiveness of an extraction is considered as percent recovery (%R) in exhaustive method such as LLE and SPE where percent recovery is calculated from the mole ratio of the extracted analyte over the total mole in the sample.

$$\%R = \frac{n_{org}}{n_{aq}} \times 100 = \frac{[X]_{org} V_{org}}{[X]_{aq} V_{aq}} \times 100 \qquad (2.23)$$

However, in a non-exhaustive scenario such as SPME and LPME, only a small fraction of the analyte is extracted and a concept of recovery cannot be applied here. Percent recovery can be calculated from the above equation but the value is far from 100% unless K_D is large. The nature of a non-exhaustive system is equilibrium based where the transfer of analyte from a much larger sample volume into a very small volume enriches the extracted concentration. The concentration ratio is more suitable for the evaluation of a non-exhaustive system and is called the enrichment factor (*EF*).

$$EF = \frac{[X]_A}{[X]_i} = \frac{RV_D}{100 V_A} \qquad (2.24)$$

2.3 Application of LPME in Environmental Analysis

LPME has been successfully applied for the analyses of pollutants in soil, food, biological fluids, and water matrices. Both single-drop and hollow fiber protected modes were employed. The analyses were completed on several instruments such as HPLC/UV, CE, and GC with specific detectors or MS. Selected examples of LPME conditions are collected in Table 2.1. LPME procedures were developed for the extraction of nine haloacetic acids in water[37]. Haloacetic acids are polar and have pK_a values below 3.0, the sample pH was adjusted to keep fractional composition of all haloacetic acids close to 1.0 so that they can be extracted by liquid membrane. Limit of detections obtained were comparable or better than the values of the US EPA standard method. Enrichment ranged from 300 to more than 3000 fold with good analytical performance. Three-phase LPME was also described for the analysis of four common phenolic compounds in environmental waters [38].

Table 2.1 Selected examples of microscale membrane extraction of environmental pollutant

Pollutants	Matrix	Organic	V_d	V_a	Detection	LOD (µg/L)	EF	Reference
Carbamates	distilled, tap, and waste	1-octanol	4 mL	2 µL	GC/MS			[33]
Carbaryl	waters					0.2	89	
Chlorpropham						0.6	83	
Methiocarb						0.2	144	
Promecarb						0.2	124	
Propham						0.8	37	
Organochlorines	water and sea water	toluene	4 mL	2 µL	GC/MS			[34]
Aldrin						0.006	not reported	
α-Chlordane						0.002	not reported	
β-Chlordane						0.003	not reported	
Dieldrin						0.001	not reported	
p,p'-DDD						0.001	not reported	
p,p'-DDT						0.001	not reported	
Endosilfan sulfate						0.003	not reported	
α-HCH						0.001	not reported	
β-HCH						0.005	not reported	
γ-HCH						0.003	not reported	
δ-HCH						0.002	not reported	
Heptachlor						0.007	not reported	
Organophosphorous	distilled and river water	toluene	5 mL	3 µL	GC/FID			[35]
Carbofenothio						0.005	not reported	
Chlopyrifos methyl						0.001	not reported	
Dichlorvos						0.032	not reported	
Ethyoprophos						0.004	not reported	
Phenthoate						0.002	not reported	
Methidathion						0.003	not reported	

Table 2.1 (continued)

Pollutants	Matrix	Organic	V_d	V_a	Detection	LOD (μg/L)	EF	Reference
Mevinphoscis						0.004	not reported	
Triazines	DI water	toluene	3 mL	3 μL	GC/MS			[36]
Atrazine						0.014	141	
Desmetryn						0.009	170	
Prometryn						0.007	208	
Propazine						0.010	178	
Secbumeton						0.021	165	
Sebuthylazine						0.010	190	
Simazine						0.063	42	
Simetryn						0.012	179	
Haloacetic acids	DI and tap waters	dihexyl ether	236 mL	30 μL	HPLC/UV			[37]
MCAA						2.69	324	
DCAA						0.25	1413	
MBAA						0.23	366	
BCAA						0.04	1153	
DBAA						0.06	1260	
TCAA						0.05	2411	
BDCAA						0.02	1910	
DBCAA						0.02	1250	
TBAA						0.03	3298	
Dinitrophenols	Environmental waters	undecane	200 mL	500 μL	HPLC/UV			[38]
2,4-dinitrophenol						0.006	not reported	
4,6-*o*-dinitrocresol						0.006	not reported	
4,6-dinitrophenol						0.006	not reported	
2-t-butyl-4,6-dinitropheol						0.006	not reported	

2.4 Analytical Problems Associated with LPME

In LPME, the principle of LLE is combined with the miniaturized nature of solid-phase microextraction (SPME). Several parameters govern the extraction in such a system. Usually, an extraction procedure for analytical purposes is optimized for groups of target analytes which may contain compounds of similar or different physicochemical properties [38]. The optimized extraction procedure is normally selective to some compounds but not to all in the group. Another concern is poor precision which roots from the instability of the liquid membrane or loss of the extracting solvent during the extraction [39].

The data in Table 2.1 showed range of enrichmentsobtained on different pesticides that were extracted under the same condition [33-36]. The data imply that **ONE** single method is unlikely to be sufficiently effective and alternative approach should be considered for the extraction of group of different chemicals regardless of their similarity in chemistry. And very recently, a comprehensive approach LPME was attempted on an extraction of four antibiotics (sulfamethoxazole, ciprofloxacin, trimetroprim, clarithromycin) in reagent water [40].These antibiotics are from different classes with different chemical and physical characteristics. Interestingly, the LPME data indicated that the extraction parameters could be optimized to enrich as high as 2,700 fold of a single compound with acceptable analytical performance. The data confirmed our observation that **ONE** single LPME condition is inadequate for the enrichment of every analytes and that a comprehensive approach would be an attractive way to obtain high extraction efficiency for simultaneous extraction.

2.5 High Water Soluble Pesticides

Pesticide analysis is very difficult because pesticides belong to many different groups of chemical substances of varied physicochemical properties. Most pesticides are volatile and thermally stable, for this reason, their preferred analysis is carried out by a GC with specific detectors or with MS [41]. For difficult pesticides such as those that are thermally unstable or those that are ionic, their analyses required tedious derivatization to convert them into thermally stable compounds that can be analyzed by a GC. The most difficult groups are the high water soluble compounds

because they contain a high charge and cannot be extracted by conventional techniques and required lengthy method of ion-exchange chromatography for their extraction and pre-concentration.Figure 2.3 classified pesticides into groups according to their polarity and volatility.
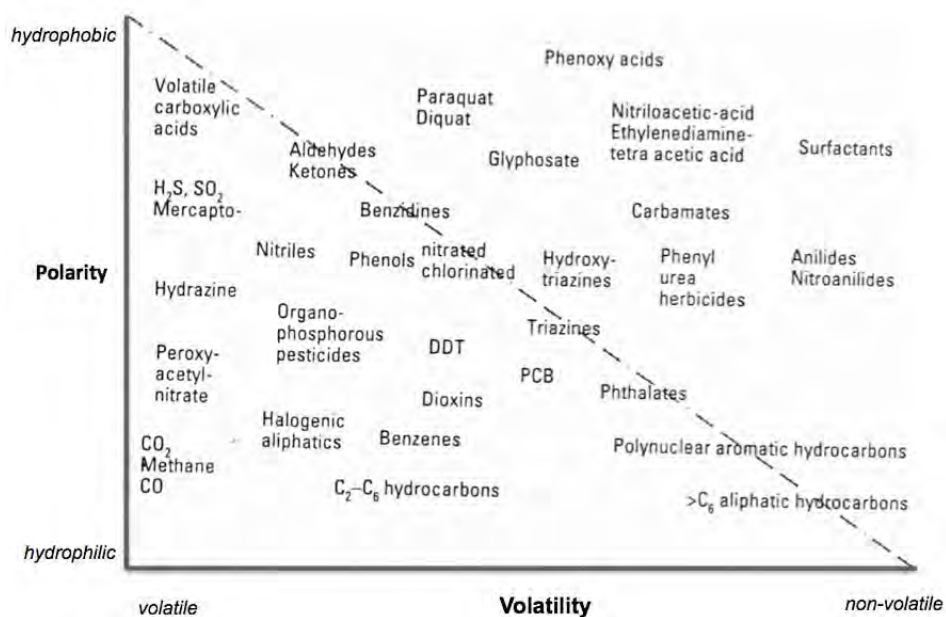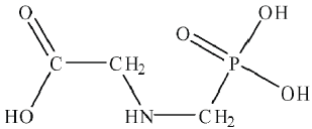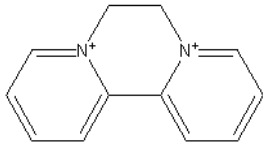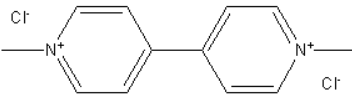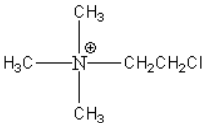
Figure 2.3 Classification of pesticides according to their polarity and volatility

Popular herbicides such as glyphosate, paraquat, and diquat are commonly used in Thailand (Table 2.2). These chemicals are stable, polar, and tend to follow the rainwater on its way to the water table. The analyses are very challenging because they have very high water solubility which make extraction from field samples very difficult; often with low recovery. These herbicides mainly exist in ion forms over a wide range of pH and can be divided into 2 categories: cationic herbicide (paraquat, diquat, chlormequat, and mepiquat), and anionic herbicide (glyphosate).

In recent years, works have been concentrated on how to enhance detection sensitivity and method reproducibility of high polarity herbicides. Pesticides residues exist in the environment at very low concentrations, from part-per-trillion (ng/L) to part-per-million (mg/L), sample preconcentration is very important for their possible detection. Cleanup of sample matrix is often necessary to enhance detection sensitivity.

Table 2.2 Chemical and physical properties of common high water soluble herbicides**.**

Compound	Chemical Structure	M_w	Log P pH 7, 20 °C	K_{oc} (mg/L)	pK_a	S_w 20 °C (g/L)	Aq. Photolysis, pH 7 (day)
Glyphosate		168.07	-3.2	21699	2.34	10.5	69
Paraquat		186.25	-4.50	1 x 10^6	na	620	stable
Diquat		184.24	-4.60	2.2 x 10^6	na	718	7
Chlormequat		122.61	-3.47	168	na	886	stable
Mepiquat		144.21	-3.55	890	na	487	stable

Data obtained from Pesticide Properties Database, IUPAC Agrochemical Information[42]

Traditional method such as liquid-liquid extraction (LLE) is not very suitable for polar organic compounds because LLE employs organic solvents and thus is not very efficient for extracting polar analytes. Solid-phase extraction (SPE) and ion-exchange resin have been regularly employed for the extraction and preconcentration [43].
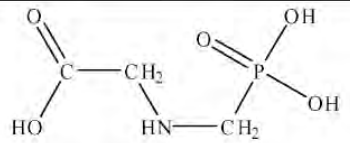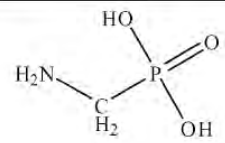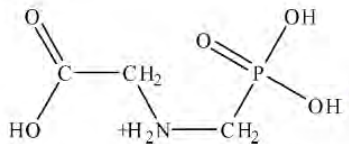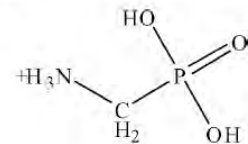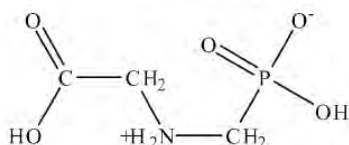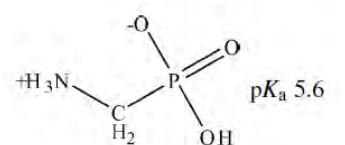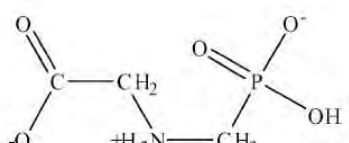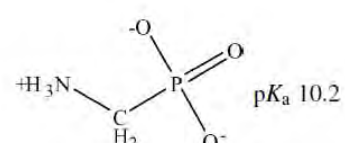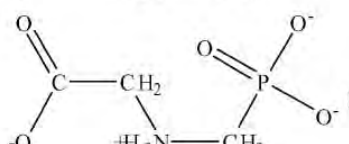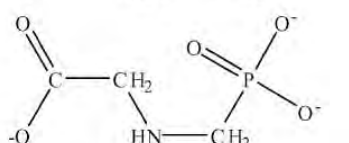
2.5.1 Glyphosate

Glyphosate (N-(phosphonomethyl) glycine) is a nonselectivepostemergence herbicidethat is highly effective against emerged grasses, brush, and broad-leaf weeds. In Thailand, more than twenty-seven thousandtons of glyphosate was imported in 2007 and the annual usagehas been increasing and currently tops the country's pesticideusage list [44].Aminomethylphosphonic acid (AMPA) is a major degradation product of glyphosate. Glyphosate and AMPA have phosphate group and share similarities in chemical structures (Table 2.3). Due to theirhigh solubility in water, leaching and runoffs are detected longafter application at a site far away from the original application. Joint FAO/WHO Meeting on Pesticide Residues (JMPR) concluded that the two compounds have similar toxicologicalprofiles. The maximumresidue level of glyphosate inwater, soil, and food is currently set at0.10µg/L according to Directive 91/414/EEC by the European Union.

2.5.1.1Analytical Methods for Glyphosate

Monitoring of glyphosate and AMPA has been of greatinterest. Many different chromatographic methods have beendeveloped for the analysis of glyphosate and AMPA. Detection of glyphosate and AMPA at trace levels and in a complex matrix is difficult because they are small molecules of highly ionic, with low volatility, and lack of chromophore. All of these factors contribute to the difficulty of their detection. To enhance their detection, either pre- or post-column derivatization is required. The most popular method applies ion-exchange chromatography with post-column derivatization[45-47]. To enhance the limit of detection, the analysis procedure is often included sample preparation and enrichment steps. A popular sample preparation is solidphase extraction (SPE) [46-54]. Enzyme-linked sorbent immunoassay [55] and matrix solid-phase dispersion [56] were also employed, but these techniquesrequire specialized equipments and specific skills.

Table 2.3 Chemical structures, pK$_a$, and ionized species of glyphosate and aminomethylphosphonic acid (AMPA)

	Glyphosate		AMPA	
General structure	(structure)		(structure)	
Undissociated species	H$_4$A	(structure) pK$_a$ 0.8	H$_3$A	(structure) pK$_a$ 0.9
1st dissociated species	H$_3$A$^-$	(structure) pK$_a$ 2.3	H$_2$A$^-$	(structure) pK$_a$ 5.6
2nd dissociated species	H$_2$A^{2-}	(structure) pK$_a$ 6.0	HA^{2-}	(structure) pK$_a$ 10.2
3rd dissociated species	HA^{3-}	(structure) pK$_a$ 11.0	A^{3-}	(structure)
4th dissociated species	A^{4-}	(structure)		

Table 2.4 Comparison of various analytical methods developed for the analysis of glyphosate and AMPA in different matrices

Sample preparation	Enrichment	Matrix	Analysis/detection	LOD (µg/L)	*EF*	Ref.
no	no	water	IEC/PCD-FLD	(G) 6.00	na	45
no	no	water	CE/indirect FLD	(G) 1.301, (A) 1.765	na	60
no	no	juices	LC/coulometric	(G) 100	na	61
no	no	reagent water	CE/laser induced FLD	(G) 32.2, (A) 3.6	na	62
SPE	combined	ground water	IEC/PCD-FLD	(G) 2, (A) 4	na	46
SPE	combined	natural waters	CE/UV-vis	(G) 85, (A) 60	na	48
SPE	combined	water	CE/laser induced FLD	(G) 0.007 reagent water (G) 0.270 river water	na	63
SPE	evaporation	rice, soybean	GC/FPD	(G) 20, (A) 30	na	64
Extraction, SPE	evaporation	Ground water, soil	GC/MS	(G, A) 0.05 water (G, A) 0.003 soil	na	51
Online SPE	combined	waters	PrCD/LC/MS	(G, A) 0.03	na	52
Online SPE	combined	river water	IEC/PCD-FLD	(G) 0.02, (A) 0.1	na	47
Online SPE	combined	waters, soil	PrCD/LC/MS-MS	(G, A) 0.005 water (G, A) 0.005 soil	na	53
Online SPE	combined	waters	PrCD/LC/MS	(G) 0.084	na	54
Imuno assay	no	waters	PrCD/LC/MS	(G) 0.1	na	55
Matrix solid-phase dispersion	evaporation	tomato	PrCD/LC/FLD	(G) 0.05, (A) 0.03	na	56
SLM (flat sheet)	combined	reagent water	CE/UV-vis	(G) 84,500	(G) 15	57
SLM (flat sheet, HFM)	combined	reagent water	PrCD/LC/UV-vis	(G) 500	(G) 8.1, (A) 2.2	58
SLM (flat sheet)	combined	juices	PrCD/LC/PCD-UV-vis	(G) 25	(G, A) 6.41-10.27	59

PrCD = pre-column derivatization, PCD = post-column derivatization

Supported liquid membrane (SLM)was successfully developed for glyphosate and AMPA in water and fruit juices [57-59]. Summary of analytical methods and their performances are compared in Table 2.4.
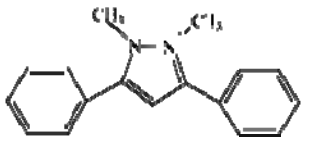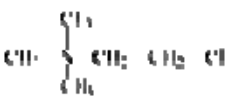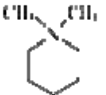
2.5.2 Quaternary Nitrogen Herbicides

Gramoxone (a commercial formulation of paraquat), one of the most dangerous and controversial herbicides in the world, is heavily used in Thailand. Paraquat belongs to a group of quaternary nitrogen herbicides that are divided into two classes. Class one consists of bipyridiliumcompounds which include paraquat (PQ), diquat (DQ), difensoquat (DF), cyperquat, diethamquat, and morphamquat. Class two includes growth inhibitors such as chlormequat (CQ) and mepiquat (MQ). In agricultural areas, these herbicides are sprayed over the fields or applied directly to soil. Because of abusive and uncontrolled usage, they are common pollutants of soil and water resources in Thailand [65,66]. For example, 1.5-18.9 μg/L of PQ was detected in groundwater, and 0.14-87.0 μg/L was detected in surface water around Thailand [67]. Chemical structures and some properties of these chemicals are listed in Tables 2.5. The U.S. Environmental Protection Agency only controls DQ in drinking and ground waters, setting the maximum contaminant level at 20μg/L. The European Union banned PQ and applied a general maximum concentration limit (MCL) at 0.1 μg/L for individual pesticide with a total combined concentration of 0.5 μg/L in drinking water. International maximum concentration limits for common quats in drinking water are summarized in Table 2.5.

2.5.2.1 AnalyticalMethods for Quaternary Nitrogen Herbicides

The analysis of quaternary nitrogen herbicides is difficult due to their high charges and high solubility in water. Techniques such as spectrophotometry[68], electrochemistry [69,70], flow injection analysis [68], capillary electrophoresis [69,70], and ion chromatography [71] have been used for the analysis of these compounds. The most popular method is ion-pair liquid chromatography with UV detection [72,73].

Table 2.5 Chemical structures and general information of quaternary nitrogen herbicides

Chemical structure (ion)	Common name	IUPAC name, (Chemical formula)	Registered trade names, Patent holder	[a]MCLs in drinking water (μg/L)	
	Paraquat (PQ)	1,1'-dimethyl-4,4'-bipyridinium $(C_{12}H_{14}N_2)$	Gramoxome, Zeneca	EU Th US WHO [b]JMPR	0.1* nr nr nr ADI = 4 μg/kg bw/d
	Diquat (DQ)	6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium $(C_{12}H_{12}N_2)$	Reglone, Zeneca	EU Th US WHO [b]JMPR	0.1* nr 20 10 ADI = 2 μg/kg bw/d
	Difenzoquat (FQ)	1,2-dimethyl-3,5-diphenyl-1H-pyrazolium $(C_{17}H_{17}N_2)$	Finaven, Supervene Cyanamid	EU Th US WHO [b]JMPR	0.1* nr nr nr ADI = 0.5 μg/kg bw/d
	Chlormequat (CQ)	2-chloroethyltrimethyl ammonium $(C_5H_{13}ClN)$	Cycocel, BASF and Cyanamid	EU Th US WHO [b]JMPR	0.1* nr nr nr ADI = 40 μg/kg bw/d
	Mepiquat (MQ)	1,1-dimethylpiperidinium $(C_7H_{16}N)$	Pix, BASF	EU Th US WHO [b]JMPR	0.1* nr nr nr ADI = 200 μg/kg bw/d

[a] = maximum concentration limits, [b] = Joint FAO/WHO Meeting on Pesticide Residues

* = EU set MCLs of single pesticide at 0.1 μg/L and total pesticide at 0.5 μg/L in drinking water, nr = not regulat

Recently, dielectrophoretic field-flow fractionation was attempted on PQ [74]; in addition, a fluorescence chemosensor designed to target PQ and DQwas also reported [75]. CQ and MQ cannot be directly analyzed by HPLC-UV because their structures lack a chromophore but successful analyses were reported by Fourier transform Raman spectroscopy [76], ion chromatography [71], and LC/-MS/MS [77]. Hyphenated techniques such as liquid chromatography coupled with mass spectrometry (LC-MS) or with tandem mass spectrometry (LC-MS/MS) were recently developed for quaternary nitrogen herbicides [78-82]. These MS-based techniques provide improved selectivity and sensitivity for detection at trace levels and in complex matrices.

Because the concentration of pesticides in the environment can be extremely low, from parts-per-trillion (ng/L) to parts-per-billion (μg/L), sample preconcentration is required for detection. Cleanup of the sample matrix is often necessary as well. A traditional method such as liquid-liquid extraction (LLE) is not suitable for polar organic compounds such as quats because LLE employs organic solvents and thus is not very efficient for extracting polar analytes. Solid phase extraction (SPE) is the most common sample preparation technique employed. For example, the standard EPA method uses C8 SPE cartridges or discs, followed by ion-pair liquid chromatography with UV detection for the determination of PQ and DQ [73]. Supercritical fluid extraction was also used to extract PQ and DQ from olive oil samples [83]. A supported-liquid membrane (SLM) procedure using a flat-sheet membrane was attempted for the extraction of PQ and DQ in water matrix [84]. The procedure required a pump to circulate a large amount of sample in a lengthy extraction process. This information suggested a possibility of an LPME system for the enrichment of PQ, DQ, MQ, and CQ in water. The detection of PQ and DQ is straightforward. However, CQ and MQ are small molecules with no UV chromophores, detection is only possible with cumbersome derivatization technique.

2.6 Emerging Pollutants

Highly chlorinated compounds such as organochlorine pesticides and other commercial chloroorganic chemicals were major sources of environmental contaminants in the 1960s. Beginning in the 1970s, many organochlorine pesticides

were banned or restricted in North America, western Europe, and Japan. Since then, international actions have developed into global ban of many persistent, bioaccumulatives, and toxic substances. The example is the Stockholm Convention that sets criteria for screening of new persistent organic pollutants (POPs) in terms of persistent, bioaccumulation, potential for long-range transport, and toxicity [88]. However, the vast majority of the approximately 30,000 chemical substances in wide commercial use are not measured in the environmental media and their emission and fate are unknown [89]. Better analytical methods have allowed for the low level detection of emerging contaminants, such as pharmaceuticals, antibiotics, fluorochemical, and other polar organic chemicals that cannot be observed with GC. Other dilemma include unknown metabolites and degradation products of these chemicals that may have a higher toxicity than the parent compounds. These emerging pollutants can exist in any of the environmental matrices, air, soil, and water. Their successful monitoring requires suitable sample preparation and sensitive analytical method. Examples of these emerging chemicals are:

- Perfluorinated surfactants: perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA)
- Parmaceutical products, hormones, endocrine disrupting compounds
- Sunscreens and UV filters
- Drinking water disinfection byproducts
- Polybrominateddiphenyl ethers and new flame retardants
- Benzotriazoles
- Napthenic acids
- Algal toxins
- Perchlorate
- Chiral contaminants
- Pesticide degradation products and new pesticide

2.6.1 Water Disinfection Byproducts

Providing microbially safe drinking water is an important public health issue.Drinking water disinfection has decreased the number of outbreaks of waterborne diseases, such as cholera and typhoid. A production of safe drinking water

combines many purification steps such as oxidation, coagulation, settling, disinfection, and filtration. As an extra measure, a secondary disinfection step is implemented at the end to ensure that the water remains free from microbial contamination during distribution. Chlorine has been the sole chemical disinfectant for over 100 years until an important public health issue was recognized in 1970s when ubiquitous amount of chloroform and trihalomethanes (THMs) were discovered in chlorinated water [90]. The toxicity data also linked chloroform and THMs to cancer in test animals.

Chlorine and other chemical disinfectants are powerful oxidants that are very effective in killing harmful microorganisms. They are used for (a) removing taste and color; (b) oxidizingiron and manganese; (c) improving coagulationand filtration efficiency; (d) preventing algalgrowth in sedimentation basins and filters, and (e)preventing biological regrowth in the water distribution system. These chemical oxidants can also oxidize organic matters,antropogenic contaminants, bromide, and iodide naturally present in the source water into different disinfectant byproducts (DBPs) such as THMs andhaloacetic acids (HAAs) that are the most prevalent classes of DBPS in chlorinated finished water [91]. DBPs can cause unintended health hazards such as cancer, and reproductive and developmental effects in humans, if present at significant concentrations. To minimize consumer exposure to DBPs without risking inadequate disinfection, control limits are normally regulated (Table 2.6).The tightened regulations and increased public health concerns on THMs, forced the water utilities to switch to alternative disinfectants. Water utilities may use ozone, UV light, or chlorine dioxide as primary disinfectants at the treatment plant.

Monochloramine is commonly used as a secondary disinfectant to protect the water as it travels from the treatment plant to consumers. In the chloramination process, anhydrous or aqueous ammonia (NH_3) is added to water before or after the addition of chlorine (HOCl) to produce monochloramine (NH_2Cl).

$$NH_3 + HOCl = NH_2Cl + H_2O$$
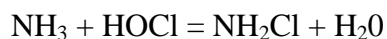
Monochloramine is 200 times less effective than chlorine, but the process does not form THMs. However, in a recent occurrence study in the United States, iodide-containing compounds (Table 2.7) were identified in chloraminated drinking-water extracts using methylation with GC/high resolution-mass spectrometry (MS) [92,93].

Preliminary data suggested that these compounds are formed at high levels together with iodo-THMs in chloramination process.

Table 2.6 Standards or guidelines related to DBPs (mg/L) in various jurisdictions of the world.

Compound	[a]US EPA	[b]WHO	[c]EU	[d]Thailand	Aus-NZ
Regulated DBPs					
Trihalomethanes	0.080		0.1	nr	0.250
Chloroform		0.2			
Bromodichloromethane		0.06			
Chlorodibromomethane		0.1			
Bromoform		0.1			
Haloacetic acids	0.060		nr	nr	
Chloroacetic acid					0.150
Bromoacetic acid					
Dichloroacetic acid		0.05			0.100
Dibromoacetic acid					
Trichloroacetic acid		0.2			0.100
Oxyhalides					nr
Bromate	0.010	0.01	0.01	nr	
Chlorite	1.0	0.7		250	
Others DBPs				nr	nr	nr
Trichloroacetaldehyde		0.01			
Dichloroacetonitrile		0.02			
Dibromoacetonitrile		0.07			
Cyanogen chloride		0.07			
2,4,6-Trichlorophenol		0.2			
Formaldehyde		0.9			
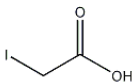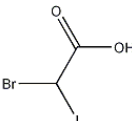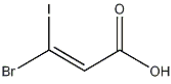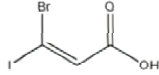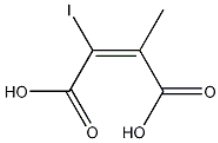
[a]U.S. Environmental Protection Agency, national primary drinking water regulations: stage 2 disinfectants and
disinfection byproducts rule, Fed. Reg 71 (2006): 387-493.
[b]http://www.who.int/water_sanitation_health/dwq/guidelines/en/index.html
[c]Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
[d]ประกาศกระทรวงอุตสาหกรรมฉบับที่ 332 (พ.ศ. 2521) ออกตามความในพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511 เรื่องกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรมน้ำบริโภคตีพิมพ์ในราชกิจจานุเบกษาเล่ม 95 ตอนที่ 68 ลงวันที่ 4 กรกฎาคม 2521

Table 2.7 Chemical structures and physical properties of five iodoacids priority DBPs

Structure	Name	M_W (g/mol)	[a]K_{ow}	[a]pK_a	[a]S_w (mg/L)
	Iodoacetic acid (IAA)	185.95	5.37	2.90	0.52
	Bromoiodoacetic acid (BIAA)	264.84	5.89	1.60	0.076
	(Z)-3-Bromo-3-iodopropenoic acid (Z)	276.86	42.66	3.20	0.023
	(E)-3-Bromo-3-iodopropenoic acid (E)	276.86	42.66	3.20	0.023
	(E)-2-iodo-3-methylbutenedioic acid (DIACID)	256.00	5.50	1.80	0.087

[a]Calculated with ALOGPS version 2.1, Virtual Computational Chemistry Laboratory, Munich, Germany

Because of the similarities in their structures to THMs and haloacetic acids, the researchers are very concerned abour their potential health risks. The toxicity study data confirmed that one of these new iodinated DBPs, iodoacetic acid (IAA), is the most genotoxic to mammalian cells of any known DBPs. This fact raises a concern that IAA is likely to be more hazardous to humans than THMs [94].

These five iodinated-DBPs represent a new class of potent drinking water contaminants. Due to their highly cytotoxic and genotoxic natures, the U.S. EPA categorized them in a high priority group under review. These iodoacidDBPs are generally presented at sub-µg/L or low-to-mid µg/L levels in finished water [94]. Because many have just recently been discovered, standard compounds are not largely available and the analysis required sophisticated and sensitive instruments such as high-resolution GC/MS or derivatization prior to detection [93]. Upon their first discovery, 39 liters of water was passed through an XAD column to concentrate the analytes to detectable levels. Moreover, the concentrations were so low that a highly sensitive instrument such as a GC/high resolution-mass spectrometry was required [95].

HAAsare regulated in drinking water in the United State. The standard method for their analyses is the EPA method 552.3 [96] which employs liquid-liquid extraction of acidified water sample (40 mL) with methyl-*t*-butyl ether followed by derivatization with acidic methanol to methyl esthers that can be analyzed by GC/ECD. The sample preparation steps are lengthy (3 h) and complicated. The analytical procedure by GC requires another 50 minutes. This GC/ECD method was not sensitive enough to detect IAA in finished water and a GC/MS method with negative chemical ionization was used for the analysis [94]. This is due mainly to the low concentration of IAA in finished water (low and sub-ng/L).

A LPME system was successfully developed for the extraction of HAAs in aqueous media, up to 3000-fold of enrichment were obtained [94]. Because the structures of IAA shares some similarity to HAAs, we were interested to see if a similar LPME system can be developed for these iodinated DBPs in water so that a simple and inexpensive analysis method such as a HPLC/UV, can be employed instead of using high-resolution GC/MS. Moreover, the LPME procedure is simple, uses very little amounts of organic solvent, and can be fine-tuned to enrich the analyte hundreds or thousands fold.

CHAPTER III
METHODOLOGY

3.1 Analytical Instrument

    3.1.1 High performance liquid chromatography (HPLC): Agilent model 1100 consisted of an automatic degasser, a binary pump, an autosampler, a column thermostat, a diode-array detector, and a fluorescence detector (Agilent Technologies, Palo Alto, CA, U.S.A.)

    3.1.2 Liquid chromatography/mass spectrometry (LC/MS): Agilent model 1100TM LC system coupled to mass spectrometer model G1946D and equipped with an automatic degasser, an autosampler, a quaternary pump, a column thermostat connected to a mass spectrometry detector with an atmospheric pressure electrospray ionization (AP-ESI) interface (Agilent Technologies, Palo Alto, CA, U.S.A.)

    3.1.3 Gas chromatography/mass spectrometry (GC/MS): Agilent model 6890N GC system coupled to HP 5973 mass spectrometry detector. The GC was equipped with an autosampler and connected to the MSD via an EI interface. (Agilent Technologies, Palo Alto, CA, U.S.A.)

    3.1.4 Post-column derivatization instrument: Model PCX5200 consisted of two post-column pumps, a column thermostat, a heated reactor, and an ambient reactor. (Pickering Laboratory, Mountain View, CA, U.S.A.)

3.2 LPME Device and Extraction Procedure

A LPME device (Figure 3.1) was constructed with a piece of hollow fiber membrane (Accurel PP Q3/2, Membrana, Wuppertal, Germany). The membrane has inner diameter of 600 μm, wall thickness of 200 μm with 75% distribution of 0.2 μm pores. A piece of membrane (total length 54 cm) was cut into a segment of desired length to hold an acceptor solution. The piece was immersed in an extractant of choice overnight. Prior to use, excess solvent was removed from the lumen by blowing air through a few times with a medical syringe. The extraction device was constructed by piercing the septum of a glass vial with two hypodermic needles. The needles inserted through a silicone septum of the screw cap were used to hold the two

ends of the membrane piece during the extraction. The acceptor solution was displaced into the lumen by a microsyringe and the membrane was placed in an extracting flask filled with the donor solution. Stirring was performed with magnetic stirrer for the desired length of time. Replicate extractions were performed simultaneously with IKAMAG magnetic stirrer with multiple reacting vessels (IKA Werke, Staufen, Germany). After which the acceptor solution was transferred into an HPLC micro insert vial and subjected to final analysis. The fiber piece was discarded after each use.

Figure 3.1 LPME device and multi-station magnetic stirrer

3.3 Chemicals

3.3.1 Standard Compounds

- Glyphosate: 99.2%, Riedel-de Haën, Sleeze, Germany
- Aminomethylphosphonic acid: 99.0%, Fluka, Steinheim, Germany
- Paraquat dichloride: 98.5%, DSQ, Augsburg, Germany
- Diquat dibromide hydrate: 99.5%, DSQ, Augsburg, Germany
- Chlormequat chloride: 99.8%, Riedel-de Haën, Seelze, Germany
- Mepiquat chloride: 98.7%, Kanto Chemical Co., Inc., Tokyo, Japan
- Iodoacetic acid: 99.0%, Fluka, Buchs, Switzerland

- Dibromoacetic acid: 97 %, Aldrich, Switzerland

- Trichloroacetic acid: 99.5%, Riedel-de Haën, Sleeze, Germany

- Tribromoacetic acid: 99.0%, Aldrich, Germany

- Dichlobenil: 96.5%, Fluka/Riedel-de Haën, Sleeze, Germany

- EPTC: 95%, Sigma-Aldrich, Buchs, Switzerland

- Molinate: 98.5%, Sigma-Aldrich, Buchs, Switzerland

- Chlorpropham: 99.0%, Sigma-Aldrich, Buchs, Switzerland

- Fenclorim: 98.2%, Sigma-Aldrich, Buchs, Switzerland

- Pirimicarb: 99.1%, Fluka/Riedel-de Haën, Sleeze, Germany

- Benfuresate: 99.0%, Sigma-Aldrich, Buchs, Switzerland

- Metribuzin: 97.5%, Sigma-Aldrich, Buchs, Switzerland

- Simetryn: 99.2%, Sigma-Aldrich, Sleeze, Germany

- Prometryn: 99.4%, Sigma-Aldrich, Sleeze, Germany

- Terbutryn: 99.0%, Sigma-Aldrich, Sleeze, Germany

- Penconazole: 99.5%, Sigma-Aldrich, Buchs, Switzerland

- Benalaxyl: 99.4%, Sigma-Aldrich, Buchs, Switzerland

3.2.2 Other Chemicals, Solvents, and Reagents

All of the chemicals and reagents used were analytical grade. Water was purified with a Milli-Q system (Millipore, Bedford, MA, U.S.A.). HPLC and LC/MS solvent are of ultra-residue analytical grade.

- Di-*n*-hexyl ether: Sigma-Aldrich, Sleeze, Germany

- Di(2-ethylhexyl)phosphoric acid: Fluka, Buchs, Switzerland

- Trioctylphosphine oxide: Fluka Chemika, Germany

- 1-Octanol: Sigma-Aldrich, U.S.A.

- Dodecane: Fluka Chemika, Germany

- Hydrochloric acid: Merck, Darmstadt, Germany

- Disodium hydrogen phosphate: Merck, Darmstadt, Germany

- Ammonium formate: Merck, Darmstadt, Germany

- Formic acid: Fisher Scientific. U.K.

- Potassium dihydrogen phosphate: Merck, Darmstadt, Germany

- Phosphoric acid: Merck, Darmstadt, Germany

- Sodium hydroxide: Carlo Erba, Italy

3.4 Preparation of Standard Solutions

Single stock solutions were prepared at 1000 mg/L or 500 mg/L in methanol for most compounds except for glyphosate, AMPA, paraquat, diquat, mepiquat, and chlormequat were prepared with Milli-Q water. IAA, DBAA, TCAA, and TBAA were prepared in methanol. The stock solutions were kept in a refrigerator at 4 °C. Working standards were freshly prepared daily using the stock solutions.

3.5 Analytical Methods

3.5.1 HPLC and Post-Column Derivatization Method for the Analysis of Glyphosate and AMPA [98]

Analytical column: glyphosate column, K^+ (PN 1954150, Pickering Laboratory)

Mobile phase: A: potassium phosephase buffer, pH 2.0

B: 0.3% wt/v potassium hydroxide in water

Flowrate: 0.4 mL/min

Column temperature: 55 °C

Injection volume: 5 μL

Detector: Fluorescence λ_{ex} 330 nm, λ_{em} 465 nm

Gradient program:

Time (min)	%A	%B
0	100	0
15.0	100	0
15.1-17.0	0	100
17.1-25	100	0

Post-column condition: Reagent 1: oxidizing reagent

Pump 1:   0.3 mL/min

Reactor 1: 36 °C

Reagent 2: OPA reagent

Pump 1:   0.3 mL/min

Reactor 1: ambient

3.5.2 HPLC/UV Method for the Analysis of Iodoacetic Acid

Analytical column: Symmetry Shield RP-C18 column (150 x 2.1
                                   mm, 3.5 μm, Waters)

Mobile phase: A: 20 mM phosphate buffer pH 2.2, 65%
                                   B: methanol, 35% isocratic

Flowrate: 0.4 mL/min

Column temperature: 30 °C

Injection volume: 5 μL

Detector: diode array monitored at 210 nm

3.5.3 LC/MS Method for the Analysis of Quaternary Nitrogen Herbicides

Parameters	Conditions
Analytical column	Atlantis HILIC silica column, 150 x 2.1 mm I.D., 3.0 μm
Mobile phase	A: 10 mM ammonium formate buffer pH 3.0, 60% B: acetonitrile, 40% isocratic
Flowrate	0.35 mL/min
Column temperature	35 °C
Injection volume	1 μL
Detector	Mass Spectrometer, SIM mode
SIM fragment	m/z 114 and 115 for mepiquat m/z 122 and 124 for chlormequat m/z 183 and 184 for diquat m/z 185 and 186 for paraquat
Auxiliary	350 °C

3.5.4 GC/MS Method for the Analysis of Mixed Pesticides

Parameters	Conditions
Analytical column	30 m × 0.25 mm i.d. × 0.25 μm HP-5MS
Oven	Initial 60 °C for 1 min; 15 °C/min to 130 °C, hold 2 min; 15 °C/min to 280 °C hold 5 min.
Injection mode	Split, split ratio 20:1, split flow 20.0 mL/min
Injector	300 °C, solvent delay 9.40 min
Carrier gas	Helium, 1 mL/min
Detector	Mass Spectrometer, SIM mode
SIM fragment	m/z 171 for dichlobenil, t_R 9.703 min
	m/z 128 for EPTC, t_R 9.769 min
	m/z 126 for molinate, t_R 11.782 min
	m/z 127 for chlorpropam, t_R 12.865 min
	m/z 189 for fenclorin, t_R 13.322 min
	m/z 166 for pirimicarb, t_R 14.427 min
	m/z 163 for benfuresate, t_R 14.579 min
	m/z 198 for metribuzin, t_R 14.667 min
	m/z 213 for simetryn, t_R 14.808 min
	m/z 241 for prometryn, t_R 14.894 min
	m/z 226 for terbutryn, t_R 15.084 min
	m/z 248 for penconazole, t_R 15.902 min
	m/z 148 for benalaxyl, t_R 17.573 min
Auxiliary	230 °C

3.6 Study of Parameters Influencing Enrichment of LPME

3.6.1 Verification of the Mathematical Model of K_D

A group of diverse pesticides were selected for this study to mimic real world environmental samples that consist of many components of diverse physicochemical characteristics and make the extraction and the analysis by one method unfeasible. List of pesticides and their characteristics selected for this study are in Table 3.1

Table 3.1 Diverse pesticides and their physicochemical properties

Compound	M_W (g/mol)	pK_a	K_{oc}	S_w (g/L)	log P
Penconazole	284.18	1.51	2205	73	3.72
Simetryn	213.3	4	200	450	2.8
Fenclorim	225.07	4.23	3655	2.4	4.17
Terbutryn	241.36	4.3	2000	22	3.65
Pirimicarb	238.39	4.4	388	3100	1.7
Prometryn	241.36	9.95	400	33	3.34
Metribuzin	214.29	13	38	1165	1.65
Molinate	187.3	*na*	190	110	2.86
Benfuresate	256.3	*na*	214	261	2.41
Dichlobenil	172.01	*na*	237	21.2	2.7
EPTC	189.3	*na*	300	370	3.2
Chlorpropham	213.66	*na*	340	110	3.76
Benalaxyl	325.4	*na*	4998	28.6	3.54

The pesticides were prepared as mixed standard according to the procedure in section 3.4 and subjected to LPME extraction according to procedure in section 3.2.

3.6.2 Correlation Between K_D and K_{ow}

In this study, the pesticide mixture (0.1 ppm) was extracted by two-phase LPME using *n*-octanol as the membrane liquid. The experimental enrichment values were obtained in comparison to calculated value using pesticide's K_{ow}. Parameters influencing the deviation of enrichment from theoretical value were evaluated. The second part of the study compared di-*n*-hexyl ether to *n*-octanol as the membrane liquid. The extractions were repeated 3-5 times.

3.6.3 Influence of Solution pH and Analyte pK_a

Two-phase LPME was first employed to study the influence of donor pH on analyte enrichment. The pesticide mixture (0.1 ppm) was extracted by two-phase LPME using *n*-octanol as the membrane liquid. The donor solution was varied from pH 2-13 and enrichment factor of each pesticide was calculated. In the second part of the study, three-phase LPME was employed to study the influence of the acceptor pH on analyte enrichment. The pesticide mixture (0.1 ppm) was extracted by three-phase LPME using *n*-octanol as the membrane liquid. The donor pH was fixed at the

optimum value determined previously. The extraction was conducted (n=3) over a range of 0.2-14.0 pH units and the enrichment factors were calculated for each pesticide.

3.6.4 Influence of System Volume Ratio

The volume ratio or phase ratio is extremely important for the enrichment of LPME and its influence was carefully studied. Three-phase LPME incorporating carrier molecule was used to extract glyphosate and quaternary nitrogen herbicides. The donor volume was varied while the acceptor volume stayed constant and vice versa for the acceptor volume.

3.6.5 Influence of Equilibrium Time

In order to obtain good reproducibility and to minimize bias of non-exhaustive extraction that is driven by thermodynamics equilibrium but also influence by kinetic factors as well, it is important to determine system equilibrium time. Optimized LPME conditions for glyphosate and quaternary nitrogen herbicides were studied for this purpose. The same condition was repeated at different length of time (n=3-5) and the graph of *EF* vs. extraction time was plotted.

3.6.6 Other Optimization Parameters

3.6.6.1 Carrier Molecule

The optimum concentration of the carrier molecule in the liquid membrane must be determined. Carrier molecule makes selective extraction of target analyte possible. The addition of the carrier often increases the liquid membrane viscosity that affects the mass transfer process. To study the influence of the carrier concentration, an LPME system using DEHPA as the carrier molecule in di-*n*-hexyl ether was employed for the extraction of quaternary nitrogen herbicides. The concentration of the carrier was varied from 30-100%. Because the slow kinetics of the carrier to dissolve into the liquid membrane, immersion time was also studied.

3.7 Application of LPME in Environmental Analysis

3.7.1 High Water Soluble Pesticides

Carrier-mediated three-phase LPME was selected for the extraction of glyphosate and its main metabolite, aminomethlyphosphonic acid (AMPA) in water. Full evaluation of all parameters influencing the enrichment was conducted in this order: membrane liquid, carrier molecule, donor pH, donor volume, acceptor type and concentration, acceptor volume, agitation, and extraction time. The optimum LPME condition was validated and tested on spiked groundwater.

3.7.2 Water Disinfection Byproducts

Carrier-mediated three-phase LPME was selected for the extraction of iodoacetic acid (IAA) and three haloacetic acids: dibromoacetic acid (DBAA), tribromoacetic acid (TBAA), and trichloroacetic acid (TCAA) in water. Full evaluation of all parameters influencing the enrichment was conducted in this order: membrane liquid, carrier molecule, donor pH, donor volume, acceptor type and concentration, acceptor volume, extraction time, and agitation. The optimum LPME condition was validated and tested on real world water samples

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Study of Parameters Influencing Enrichment of LPME

The goal of any extraction process is to extract as much of the analyte as possible. This can be manipulated in many ways such as controlling the chemical conditions of the sample by changing pH, changing extractants, or adding ion-pair reagents, etc. These manipulations mainly influence the speciation (charged/uncharged) of the target analytes. Most of the published works on LPME considered the extraction effectiveness byvalues of obtained enrichment factors (EF_{exp}) aiming at maximizing C_A.

$$EF_{exp} = \frac{C_A}{C_D} \qquad (4.1)$$

whereC_A and C_D are equilibrium concentrations of the analyte in the acceptor and donor solutions, respectively. Enrichment is also influenced by the system volume ratio as stated in equation 2.24.

$$EF = \frac{RV_D}{100V_A} \qquad (2.24)$$

$$R = \frac{K_D V_A}{K_D V_A + V_D} \qquad \text{in two-phase LPME}$$

$$R = \frac{K_D V_A}{K_D V_A + K_{D1} V_{org} + V_D} \qquad \text{in three-phase LPME}$$

where$R$ is the recovery, V_D and V_A are volumes of donor and acceptor, respectively. Equation 2.24 is a simple mathematical expression saying that the enrichment of two-phase LPME is dependent to the system phase ratio and K_D. While in three-phase LPME, phase ratio, K_{D2}, and the volume of an organic layer also rule the enrichment.

*EF*_{*exp*}is usually obtained experimentally via an optimization process, any factor influencing changes in C_A, C_D, K_D, and V_{org}will also influence*EF*_{*exp*}.Fundamental study of these governed constrains would provide understanding on influencing factors of enrichment, recovery, and selectivity of LPME.

4.1.1 The Influence of the Partition Coefficient (K_D)

4.1.1.1 Predicted Enrichment (*EF*_{*cal*})

According to equation 2.14 and 2.20, numbers of extracted solute is a function of K_D in both two-phase and three-phase LPME.

Two-phase LPME
$$n_A = \frac{K_D V_A V_D [X]_i}{K_D V_A + V_D} \qquad (2.14)$$

Three-phase LPME
$$n_A = \frac{K_D V_A [X]_i V_D}{K_D V_A + K_{D1} V_{org} + V_D} \qquad (2.20)$$

Table 4.1 shows calculated values of recovery and enrichment of two-phase LPME at different K_D values in comparison to LLE.The data show excellent recovery in LLE even at very low K_D. This is the nature of an exhaustive technique that consumes the whole sample. However, the data point to low enrichment of the technique that does not improve over the increasing trend of K_D. To the contrary, the non-exhaustive nature of two-phase LPME provides poor recovery if K_D is too low. This is due to the usage of a very small volume of the organic phase. Nevertheless, LPME can provide excellent enrichment even at medium range K_D. However, high recovery can be obtained only with large K_D. In two-phase configuration, some of the analyte may be lost within the membrane. When accounting for this loss, percent recovery is even lower but this does not affect the overall enrichment. Trends of percent recovery and enrichment in two-phase LPME are illustrated in Fig 4.1.

Table 4.1 Predicted values of recovery and enrichment in two-phase LPME in comparisonto two-phase LLE at different partition coefficients

K_D	Two-phase LPME			Two-phase LLE	
	[a]%Recovery_corr	[b]%Recovery	[c]Enrichment	[d]%Recovery	Enrichment
1	0.08	0.22	1.00	66.67	33.33
5	0.40	1.09	4.95	90.91	45.45
10	0.78	2.15	9.79	95.24	47.62
50	3.60	9.91	45.09	99.01	49.50
100	6.56	18.03	82.05	99.50	49.75
200	11.11	30.56	139.03	99.75	49.88
500	19.05	52.38	238.33	99.90	49.95
1000	25.00	68.75	312.81	99.95	49.98
5000	33.33	91.67	417.08	99.99	50.00
10000	34.78	95.65	435.22	100.00	50.00
15000	35.29	97.06	441.62	100.00	50.00

[a]based on equation (2.16), available V_A=20 μL, total V_A=55 μL, V_D=25 mL
[b]based on equation (2.16) total V_A=20 μL, V_D=25 mL
[c]based on equation (2.17), available V_A=20 μL,V_D=25 mL
[d]based on equation (2.6), total V_A= 20 mL, V_D= 20 mL

a)

b)
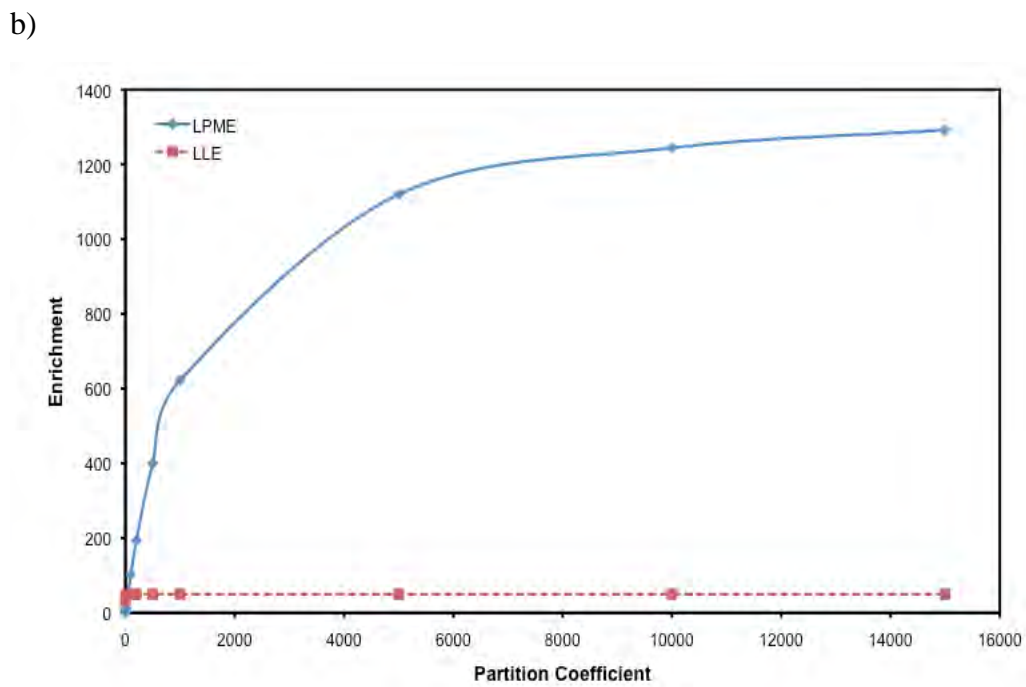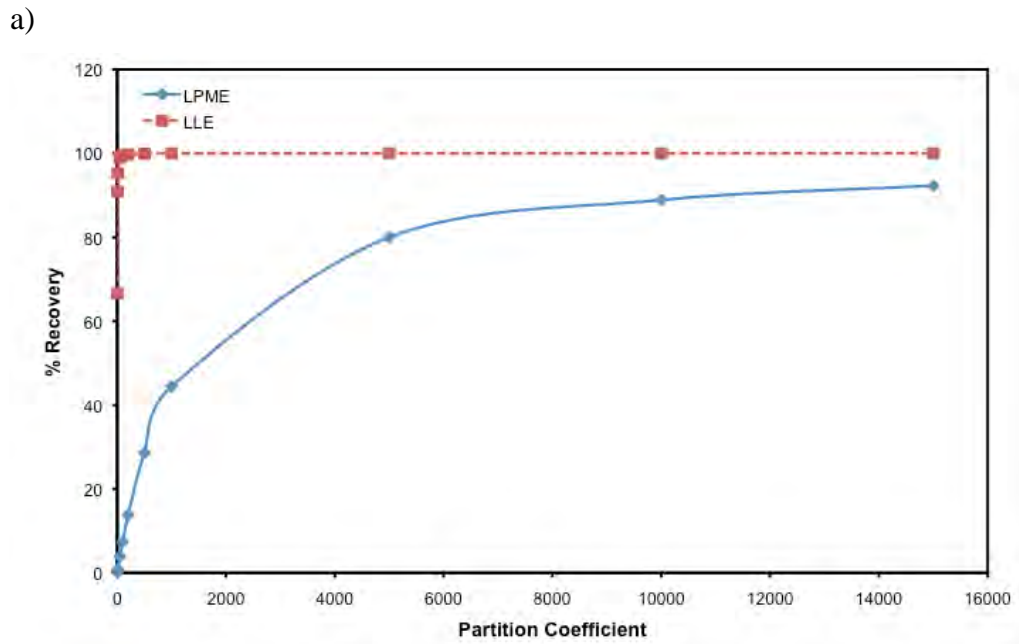
Figure 4.1 Trends of a) percent recovery and b) enrichment in two-phase LPME according to data in Table 4.1

Table 4.2 Predicted values of recovery, total recovery, and enrichment of three-phase LPME (*n*-octanol) in comparison to three-phase LLE at different partition coefficient

K_D	K_{D1}	K_{D2}	Three-phase LPME		Three-phase LLE	
			[a]%Recovery	[b]Enrichment	[a]%Recovery	[b]Enrichment
1	1	1	0.08	1.00	3.23	0.32
5	1	5	0.40	4.97	14.29	1.43
	5	1	0.40	4.95	4.35	0.43
10	1	10	0.79	9.91	25.00	2.50
	5	2	0.79	9.85	8.33	0.83
	10	1	0.78	9.78	4.55	0.45
50	1	50	3.84	48.01	62.50	6.25
	5	10	3.82	47.76	31.25	3.13
	10	5	3.80	47.44	19.23	1.92
	50	1	3.60	45.05	4.72	0.47
100	1	100	7.40	92.47	76.92	7.69
	10	10	7.31	91.41	32.26	3.23
	50	2	6.96	86.96	9.01	0.90
	100	1	6.56	81.97	4.74	0.47
500	1	500	28.54	356.79	94.34	9.43
	10	50	28.29	353.61	70.42	7.04
	50	10	27.21	340.14	33.11	3.31
	100	5	25.97	324.68	19.92	1.99
	500	1	19.05	238.10	4.76	0.48
1000	1	1000	44.41	555.12	97.09	9.71
	10	100	44.10	551.27	82.64	8.26
	100	10	41.24	515.46	33.22	3.32
	500	2	32.00	400.00	9.08	0.91
	1000	1	25.00	312.50	4.76	0.48
10000	1	10000	88.88	1110.94	99.70	9.97
	50	200	88.20	1102.54	90.83	9.08
	100	100	87.53	1094.09	83.26	8.33
	500	20	82.47	1030.93	49.98	5.00
	1000	10	76.92	961.54	33.32	3.33
	10000	1	34.78	434.78	4.76	0.48
15000	1	15000	92.30	1153.72	99.80	9.98
	100	150	91.32	1141.55	88.18	8.82
	500	30	87.59	1094.89	59.98	6.00
	1000	15	83.33	1041.67	42.84	4.28
	10000	1.5	44.44	555.56	6.98	0.70
	15000	1	35.29	441.18	4.76	0.48

[a]based on equation (2.21), V_A=20 μL, V_{org}=35 μL,V_D=25 mL
[b]based on equation (2.22), V_A=20 μL, V_{org}=35 μL,V_D=25 mL
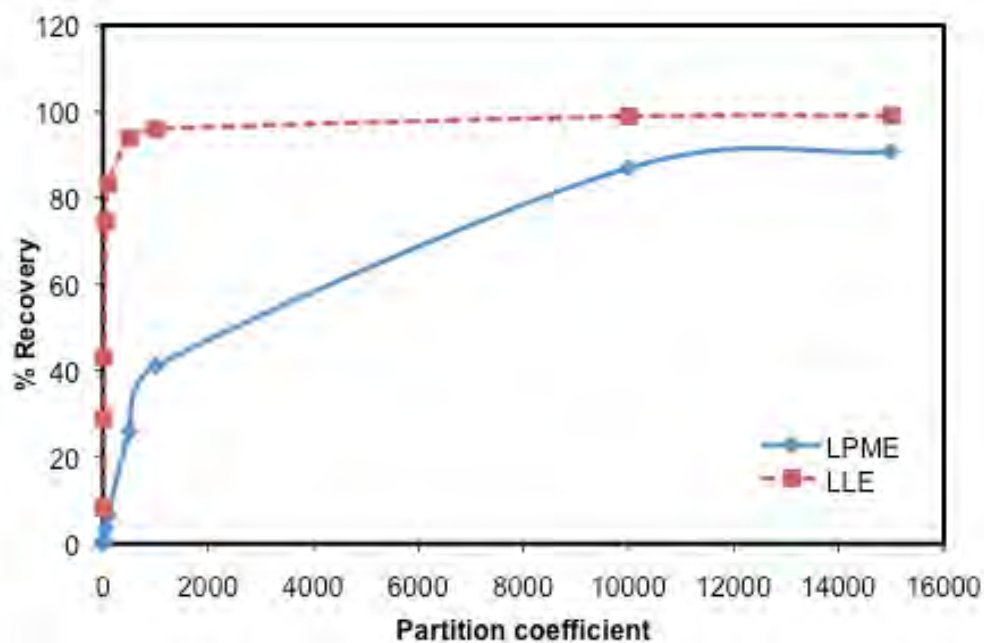
a) $K_{D2} \geq K_{D1}$

b) $K_{D2} < K_{D1}$
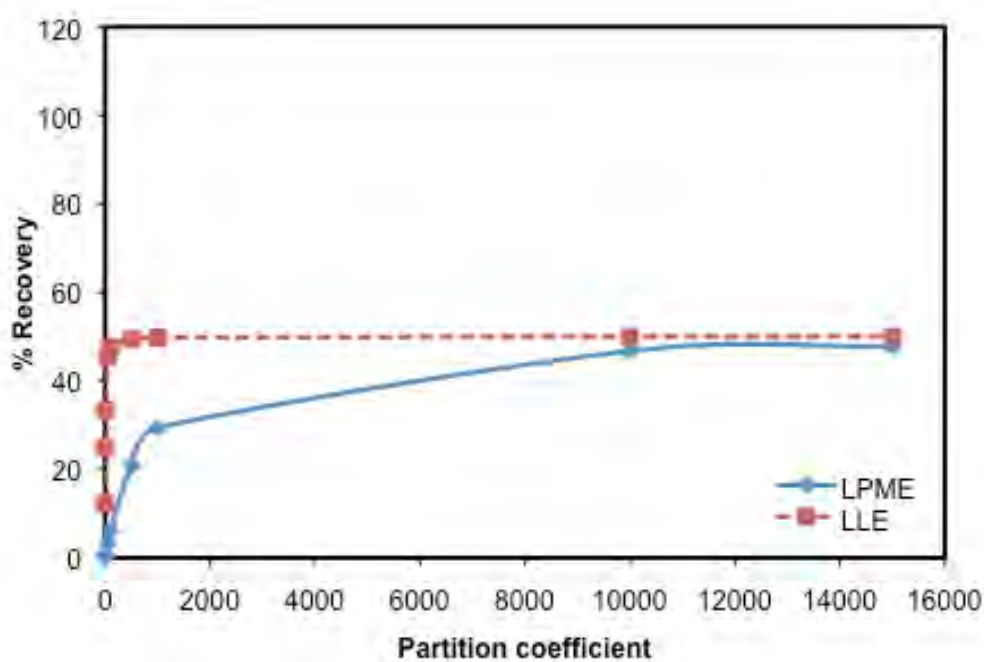
Figure 4.2 Trends of percent recovery and enrichment in three-phase LPME according to data in Table 4.2. a) percent recovery when $K_{D2} \geq K_{D1}$, b) percent recovery when $K_{D2} < K_{D1}$, c) enrichment when $K_{D2} \geq K_{D1}$, d) enrichment when $K_{D2} < K_{D1}$

c)$K_{D2} \geq K_{D1}$
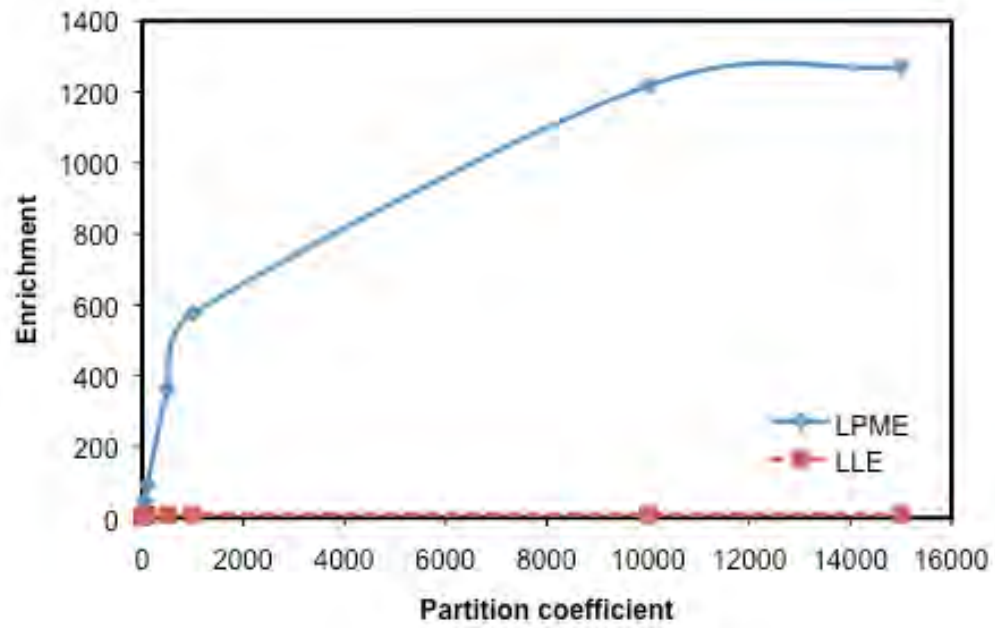
d)$K_{D2} < K_{D1}$
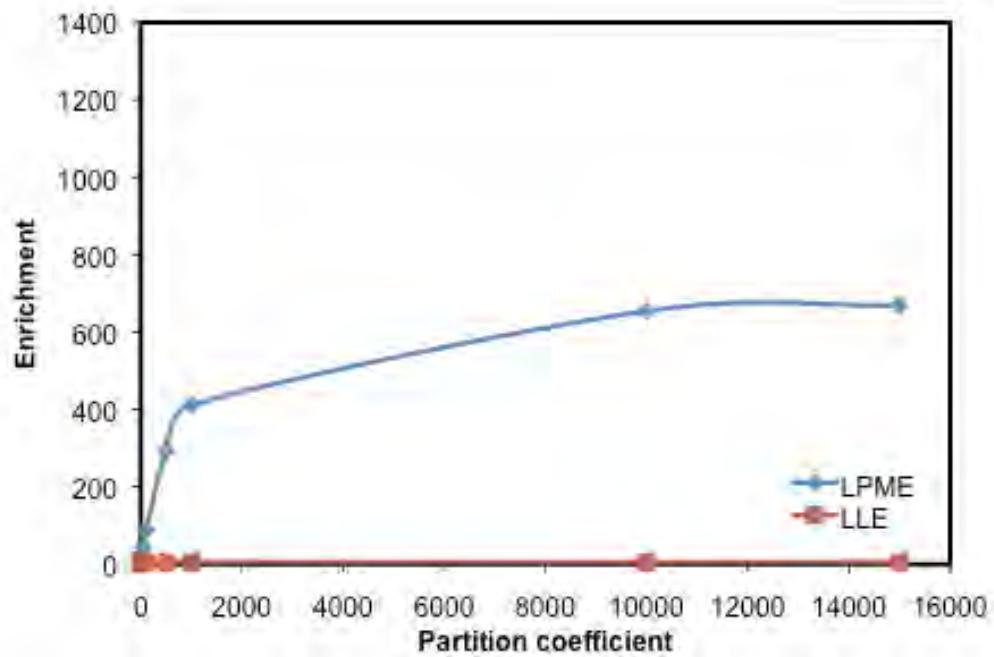
Figure 4.2(continued)

Table 4.2compares predicted recovery and enrichment of three-phase LPME and LLE over a range of K_D. The simulated data suggested that LLE does not enrich and the recovery of three-phase LLE is mainly driven by K_{D2}. As K_{D2} gets larger, percent recovery increases. The data show that K_{D2} drives the enrichment in three-phase LPME. K_{D1} can be low but if K_{D2} is high enough, the trapping of analyte in the acceptor is favored and drives the equilibrium forward resulting in larger enrichment.

In conclusion, the mathematical descriptions of two-phase and three-phase LPME suggest the following:

1. There are correlations between K_D and enrichment and recovery of analyte in LPME. When K_D gets larger, the extraction efficiency improves.

2. K_D governs the enrichment in both two-phase and three-phaseLPME, and therefore choice of membrane liquid should be chosen in a way to provide high partition coefficient of the analytes.

3. Two-phase LPME can provide excellent analyte enrichment and recovery if $K_D$is sufficiently high.

4. K_{D2} drives the recovery in three-phase LPME. If K_{D2} is large enough, it is possible to obtain good recovery in three-phase LPME.

5. It is important to remember that LPME is an equilibrium method ($V_D >> V_A$). Any constrain that violates this nature may deviate the stated correlations.

6. Both two-phase and three-phase LPME can provide powerful enrichment by using much less organic solvent than LLE.

4.1.1.2Correlation Between K_D and K_{ow}

In previous section, it was predicted that $K_D$drives the enrichment in both two- and three-phase LPME. SinceK_D depends on the nature of organic solvent and is not easy to obtain, it was interested to see if the analyte's octanol-water coefficients (K_{ow})could be used to predict the extraction efficiency of LPME. The objectives of the study were 2 fold: 1) to verify the data in section 4.1.1.1 that predicted enrichment with simple mathematical model of K_D, and 2) to investigate the correlation between K_D and $K_{ow}$of some common membrane organic solvents and establish a relationship between analyte partition coefficients in different organic solvents.

Table 4.3 Structures and physicochemical properties of selected pesticides
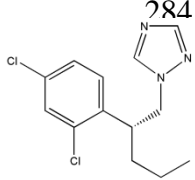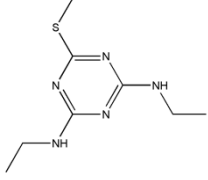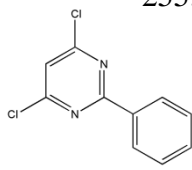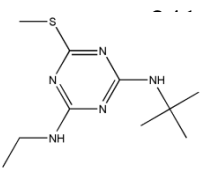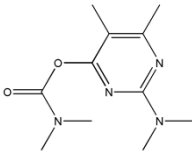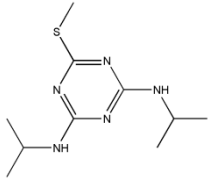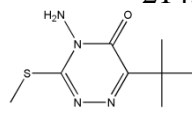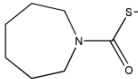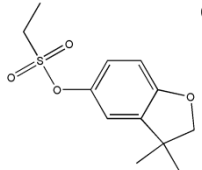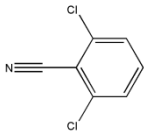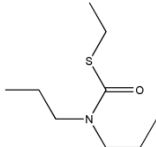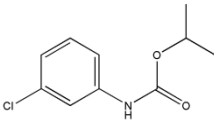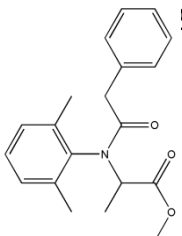
Pesticides	M_w (g/mol)	K_{ow} pH 7, 20 °C	pK_a	S_w (g/L), 20 °C	**Diameter,** *deviation from plane*
Penconazole	284.18	5250	1.51	73	9, *0.577*
Simetryn	3	631	4	450	8, *0.571*
Fenclorim	255.07	14800	4.23	2.4	7, *0.013*
Terbutryn	36	4470	4.3	22	8, *0.828*
Pirimicarb	39	50	4.4	3100	8, *0.685*
Prometryn	241.36	2190	9.95	33	8, *0.864*
Metribuzin	214.29	45	13	1165	7, *0.481*

Table 4.3(continued)

Pesticides	M_w (g/mol)	K_{ow} pH 7, 20 °C	pK_a	S_w (g/L), 20 °C	**Diameter,***deviation from plane*
Molinate	187.3	724	*na*	110	8, *0.610*
Benfuresate	⁓.3	257	*na*	261	8, *0.820*
Dichlobenil	72.01	501	*na*	21.2	5, *0.006*
EPTC	9.3	1580	*na*	370	7, *0.660*
Chlorpropham	56	5750	*na*	110	8, *0.747*
Benalaxyl	5.4	3470	*na*	28.6	8, *0.821*

The studies were conducted on a mixture of thirteen diverse pesticides whose structures and physicochemical properties are in Table 4.3. EF_{exp} and EF_{cal} of thirteen diverse pesticides were compared in Figure 4.3.Most of the pairs show large discrepancies between the predicted values to the experimental ones pointing that partition coefficient is not the only factor that influences the enrichment process. The concentration profile plot (Figure 4.4) indicates that most pesticides behave similarly except for dichlobenil, benalaxyl, pirimicarb, and metribuzin. Pirimicarb and metribuzin have very low K_{ow}value so it is not surprising that their enrichments are low. The situation of dichlobenil and benalaxyl are more mistified because $K_{ow}$of benalaxyl is very large but the enrichment obtained was poor. To the contrary, $K_{ow}$of dichlobenil is in the middle range but its enrichment was the highest. Figure 4.4 suggests that most pesticides reached equilibrium at 30 minutes; however, this was not true for benalaxyl. Benalaxyl is the largest pesticide in the group with large steric factor (0.821) that affectsits partition kinetics.According to Fick's First law, flux is directly proportional to the distribution coefficient (D) which is a function of molecular size (a).

$$J = -D\frac{dC}{dx} \qquad (4.2)$$

$$D = \frac{kT}{6\pi a\eta} \qquad (4.3)$$

where$J$ is flux, dC is the concentration gradient across the distant dx (membrane thickness), k is Boltzman's constant, T is absolute temperature, a is molecular radius, and $\eta$is the solution viscosity. Since benalaxyl has the largest molecular size, it has low distribution coefficient which affects its flux across the liquid membrane.
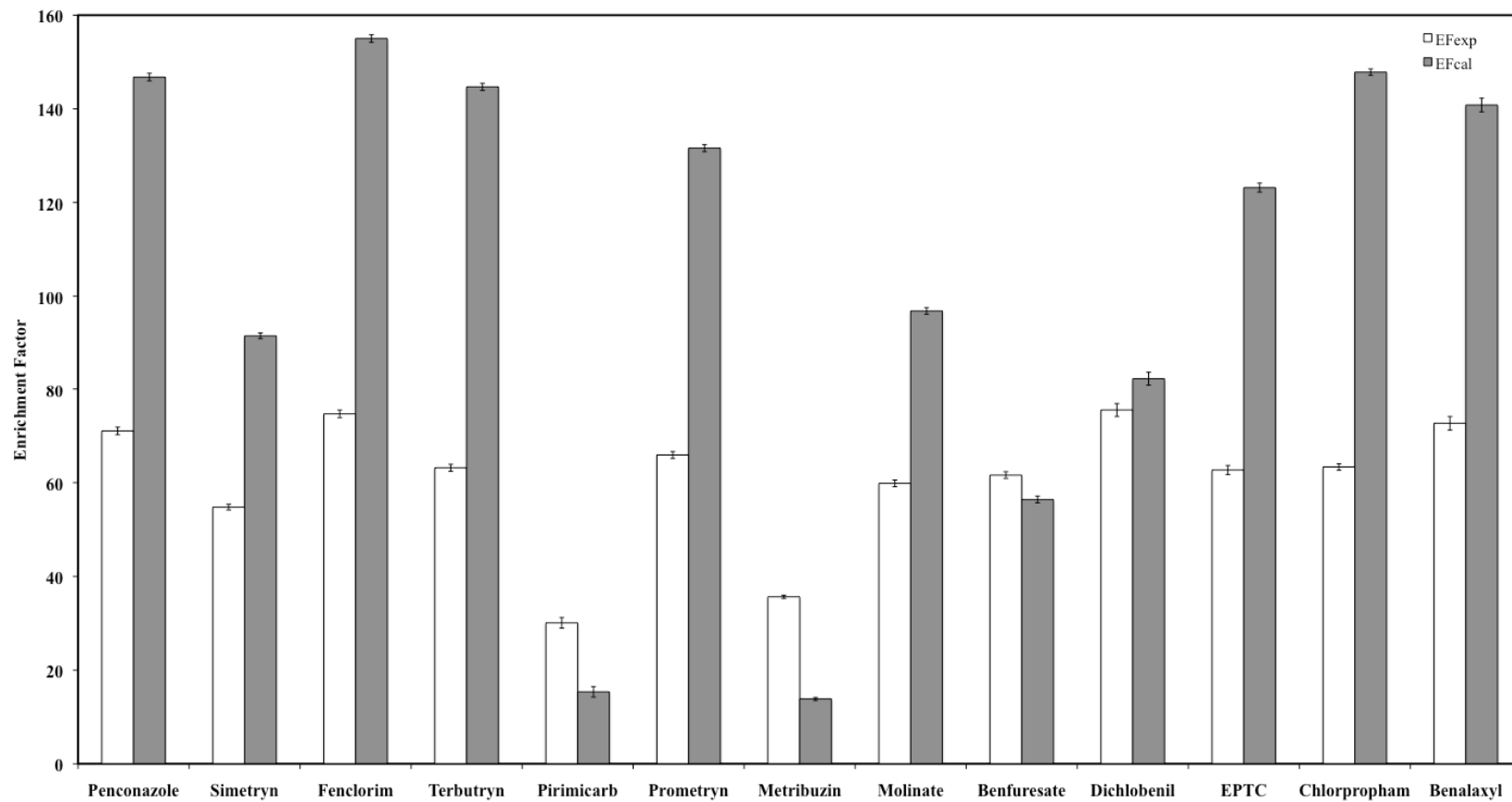
Figure 4.3 Comparison of predicted enrichment (EF_{cal}) vs. experimental values (EF_{exp}) obtained from two-phase LPME of thirteen diverse pesticides
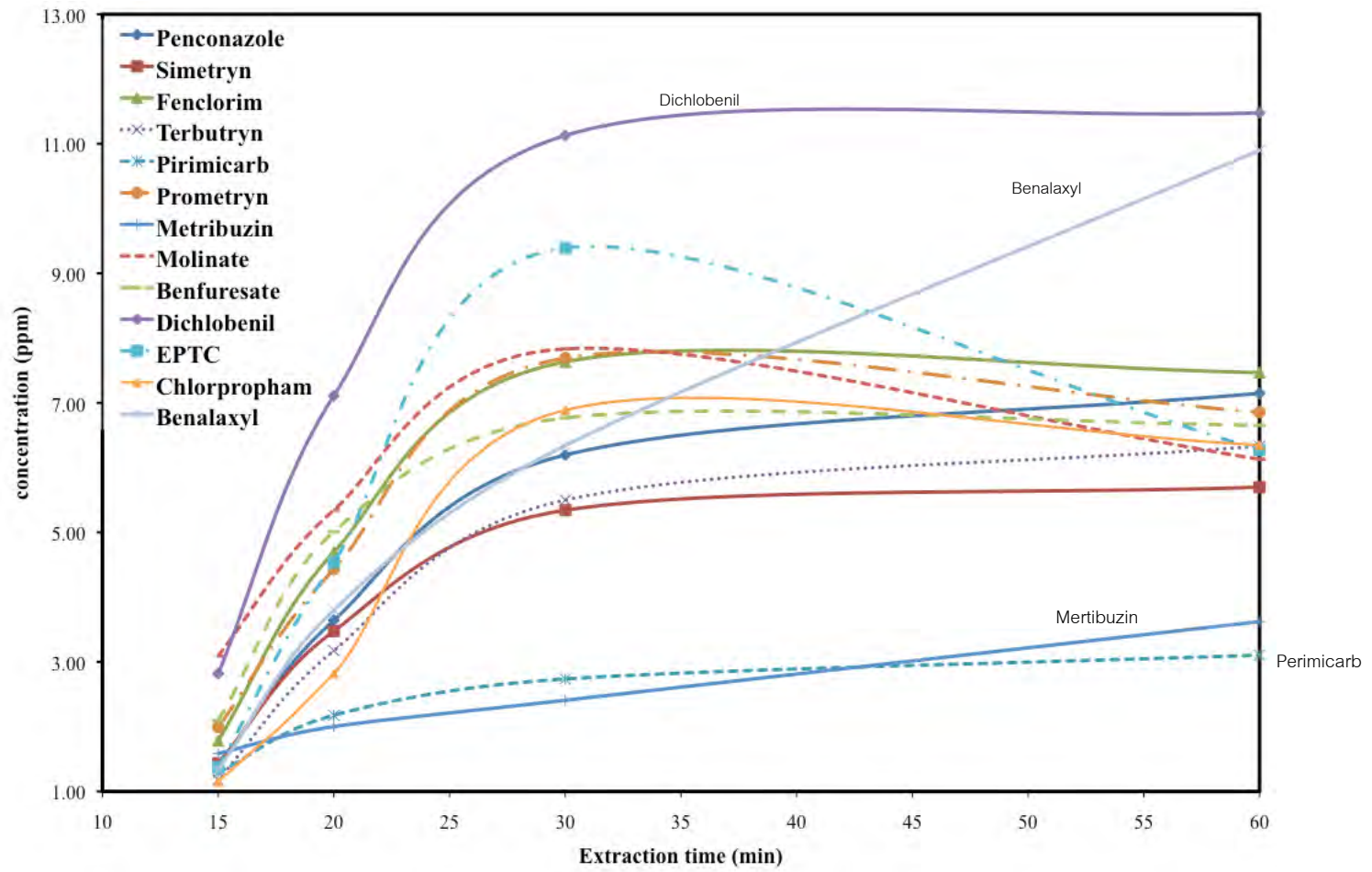
Figure 4.4 Acceptor concentration as a function of extraction time

Figure 4.5 compares EF_{exp} and EF_{cal} of six neutral pesticides--molinate, benfuresate, EPTC, dichlobenil, chlopropham, and benalaxyl. Chlorpropham, benalaxyl, EPTC, and molinate have high to very high K_{ow} values and should provide high enrichment. However, the experimental EFs were approximately half of the predicted values, this can be explained in the case of benalaxyl that takes more than 60 minutes to reach equilibrium. While the predicted EF of dichlobenil, a small planar molecule, was closed to the experimental one. The data suggested that analyte of high partition coefficient mightnot partition better into the organic phase as expected due to the influence of molecular structure, dimension, steric, and functional group of the molecule. These pesticides were selected based on their diversities to represent real world worst cases scenario, they belong to different classes and have very different structures and functional groups. In a homologous series, the magnitude of n-octanol/water partition coefficient generally increases with molecular weight. This phenomena were not observed here because these pesticides were not homologous.

To verify if we can use K_{ow} to estimate analyte K_D in other organic solvent, enrichment values were compared in the system of n-octanol and di-n-hexyl ether. Figure 4.6 compares experimental enrichments of thirteen pesticides in n-octanol and di-n-hexyl ether. No specific correlation was observed. Log K_{ow} values have been used to represent hydrophobicity of compounds. Chimuka et al. [99] demonstrated correlations between any pairs of aqueous-organic partition coefficient.

$$\log K_1 = a \log K_2 + b \quad (4.4)$$

whereK_1 and K_2 are organic-water partition coefficients, and a and b are constants. Equation (4.4) suggests that it is possible to use K_{ow} to predict analyte dissolution into an organic liquid but the constant a and$b$ of each analyte must be evaluated.At this point we can conclude that the simplified mathematical model using equations 2.14 and 2.20 is only limited to thermodynamics of ideal partitioning equilibrium and does not consider other influencing parameters affectingthe partition process such as molecular structure, size, and functional group.
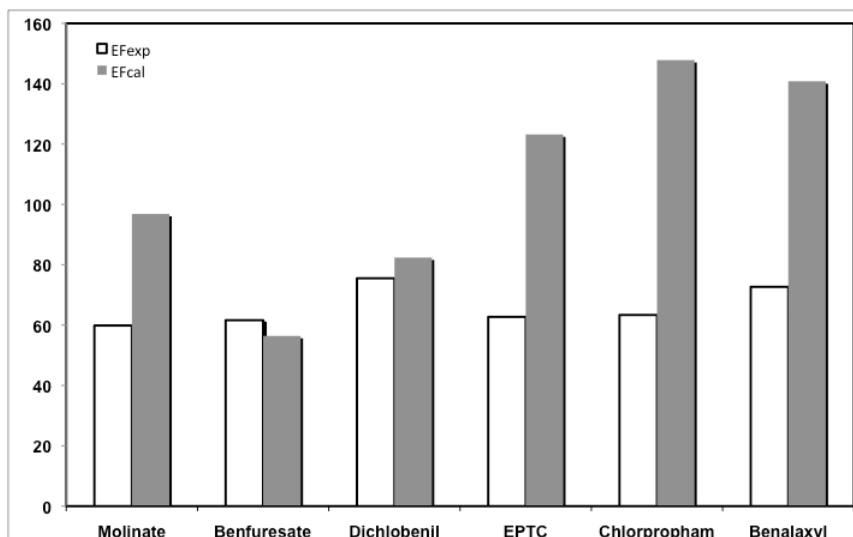
Figure 4.5 Effect of molecular size and dimension on predicted enrichment of neutral pesticides

Table 4.4 Physicochemical data of the neutral pesticides

Compound	Diameter (Å)	Diversion from plane(Å)	K_{ow}	EF_{exp}	EF_{cal}
Dichlobenil	5	0.006	501	75.51	85.39
EPTC	7	0.66	1580	62.71	123.19
Molinate	7	0.61	724	59.85	96.86
Benalaxyl	8	0.821	3470	72.67	140.82
Benfuresate	8	0.82	257	61.62	56.42
Chlorpropham	8	0.747	5750	63.35	147.83
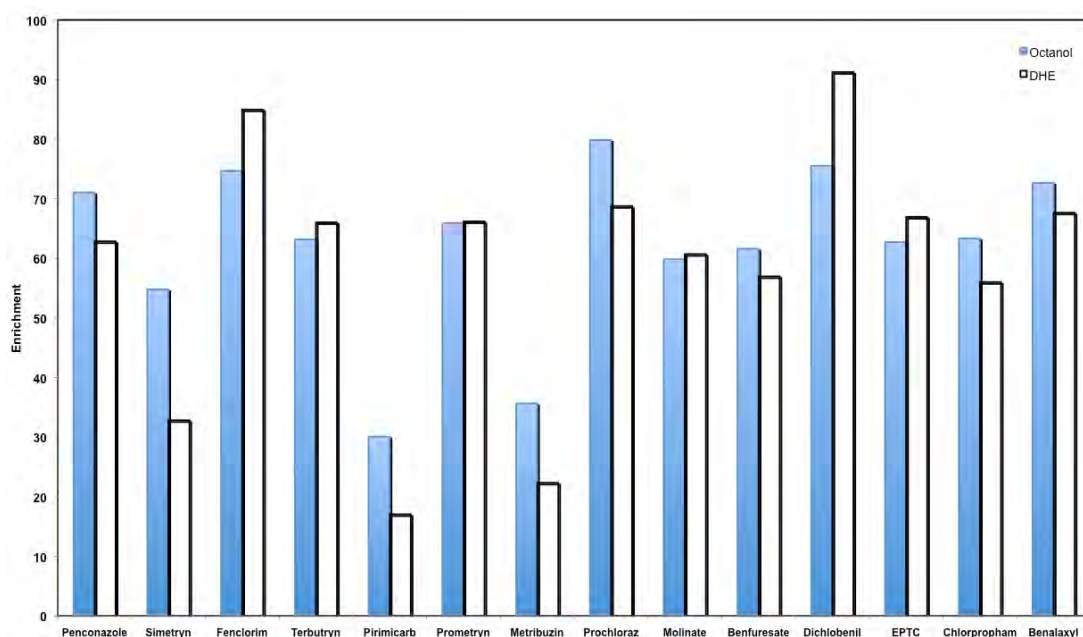
Figure 4.6 Comparison of experimental enrichments obtained from two-phase LPME using *n*-octanol and di-*n*-hexylether as liquid membranes

4.1.2 Influence of Solution pH and Analyte pK_a

The extraction of ionic compounds by liquid membrane is tricky but possible by employing three-phase LPME. The extraction system consists of three immiscible phases (aq/org/aq): (1) *donor*, which is the aqueous sample to be extracted, (2) *membrane*, which is an organic liquid held in the pores of a hydrophobic polymer, and (3) *acceptor*, which is an aqueous buffer. pH of the donor and acceptor can be modified to keep ionic compounds in extractable forms. For example, if the ionic compound is an acid, only the undissociated molecular form (HA) can be transported from the donor, through the membrane, and be trapped in the acceptor. The partition in the donor phase can be described as:

$$K_D = \frac{C_M}{\alpha_D C_D} \qquad (4.5)$$

whereC_M and C_D are equilibrium concentrations in the membrane and in the extracted sample, $\alpha_D$is the undissociated fraction in the donor. In a similar way, the process in the acceptor phase can be described as:

$$K_A = \frac{C_M}{\alpha_A C_A} \tag{4.6}$$

$K_A$and $K_D$are the partition coefficients of the analyte in the membrane/acceptor and donor/acceptor, respectively. $\alpha_A$is the undissociated fraction in the acceptor,$C_A$is the equilibrium concentration of analyte in the acceptor.

If we assume that the ionic strength of the donor and the acceptor are not significantly different and $K_A$and $K_D$are of the same order, we can derive a concentration gradientfrom equation (4.5) and (4.6):

$$\Delta C = \alpha_D C_D - \alpha_A C_A \tag{4.7}$$

The concentration gradient is driven by the undissociated fractions of analyte in the donor and the acceptor solutions. Initially, extraction condition is adjusted to maximize $\alpha_D (\alpha_D \approx 1)$,$\alpha_A$at the beginning is zero. As the extraction proceed, $\alpha_D$is getting smaller and smaller as $\alpha_A$approaches 1. To evaluate the enrichment of ionic compounds in three-phase LPME, enrichment factor can be derived from previous equations:

$$EF_{cal} = \frac{C_A}{C_D} = \frac{1}{\{(\alpha_A K_A)/(\alpha_D K_D) + (\alpha_A K_A V_M)/V_D + (V_A/V_D)\}} \tag{4.8}$$

If $\alpha_D \approx 1$ and $K_A \approx K_D$ then,

$$EF_{cal} = \frac{1}{\{(\alpha_A + (\alpha_A K_A V_M)/V_D + (V_A/V_D)\}} \tag{4.9}$$

Equation 4.9 predicts that high enrichment can be achieved when $\alpha_A$is very small and V_D is large, V_M is negligible in micro-scale membrane extraction.The undissociated

fraction can be calculated from

$$\alpha_A = \frac{1}{1+10^{(pH_A - pK_a)}}$$

(4.10)

where pK_a is the acid dissociation constant and pH_A is the pH in the acceptor.

If we assume $K_A = K_D$, [HA] in the sample can be determined based on the measured equilibrium concentration in the acceptor (C_A), the pK_a of the analyte, and the pH of the acceptor. α_D can be calculated in a similar way and we can derive C_D to be:

$$C_D = \frac{[HA]}{\alpha_D}$$

(4.11)

In equilibrium extraction, the enrichment factor is governed by the distribution coefficient (D) between the donor and the acceptor, and a characteristic of negligible depletion sampling is that the concentration in the sample is constant throughout the sampling process.

$$D = \frac{C_A}{C_D} = \frac{\alpha_D K_D}{\alpha_A K_A}$$

(4.12)

LPME experiments were conducted to evaluate to influence of pH on enrichment of ionic pesticides. Figure 4.7a shows the influence of pH on analyte enrichment. Penconazole ($pK_a = 1.51$) stayed in base form (A⁻) and cannot be enriched within this pH range. Optimum donor pH for penconazole should be at 2.2 pH units below its pK_a. The data indicated that simetryn ($pK_a = 4.00$), fenclorim ($pK_a = 4.23$), and terbutryn ($pK_a = 4.30$) could be enriched when the donor pH was 3.0 which were below their pK_a values. Suitable donor pH for pirimicarb ($pK_a = 4.40$) was determined to be 4.0. As for prometryn ($pK_a = 9.95$), it was determined that the optimum donor pH was 5.0. Enrichment of metribuzin ($pK_a = 13.00$) was constant throughout the range until the donor pH approached pH 12.0. The rest of the pesticides which are non-ionic showed no influence by the donor pH.

Once the optimum pH values were obtained, acceptor pH must be optimized to trap the analyte. According to equation 4.9, optimum enrichment (at certain donor pH) can be achieved when the acceptor pH is adjusted to keep α_A very low. Three-

phase LPME was employed to extract mixture of thirteen diverse pesticides. Donor pH was set at 5.49 and the acceptor pH was varied from 0.24 to 13.49 units. Figure 4.7b shows the influence of acceptor pH on the enrichment. According to the data, different conditions are required for the extraction of these diverse pesticides.Once again, no pH influence was observed on neutral pesticides.

The data indicated that pH values of the donor and the acceptor solutions can be used to control the extractable fraction of ionic compounds. Optimum pH values can be calculated using the equations displayed in this section prior to actual optimization to save time and to select the best conditions for select group of analyte. For the extraction of diverse compounds, it may be necessary to employ a few extraction conditions. Suggested LPME conditions for the extraction of thirteen diverse pesticides are summarized in Table 4.5. Ionic pesticides can be enriched with three-phase LPME by adjusting suitable pH value for the donor and the acceptor solutions. However, metribuzin cannot be extracted and will need carrier molecule for its extraction. pH has no effect on neutral pesticides and the suitable extraction method is two-phase LPME.

Table 4.5 Optimum condition for the extraction of thirteen diverse pesticides

| Pesticides | M_w (g/mol) | K_{ow} pH 7, 20 °C | pK_a | Three-phase LPME | | Remarks |
				donor pH	acceptor pH	
Penconazole	284.18	5250	1.51	<1.0	3.0	
Simetryn	213.3	631	4.00	3.0	6.0	
Fenclorim	225.07	14800	4.23	3.0	6.0	
Terbutryn	241.07	4470	4.30	3.0	6.0	
Pirimicarb	238.39	50	4.40	4.0	6.0	
Prometryn	241.36	2190	9.95	5.0	12.0	
Metribuzin	214.29	45	13.00	*na*	*na*	carrier-mediated transport
Molinate	187.3	724	*na*	*na*	*na*	two-phase LPME
Benfuresate	256.3	257	*na*	*na*	*na*	two-phase LPME
Dichlobenil	172.01	501	*na*	*na*	*na*	two-phase LPME
EPTC	189.3	1580	*na*	*na*	*na*	two-phase LPME
Chlorpropham	213.66	5750	*na*	*na*	*na*	two-phase LPME
Benalaxyl	325.4	3470	*na*	*na*	*na*	two-phase LPME

a)
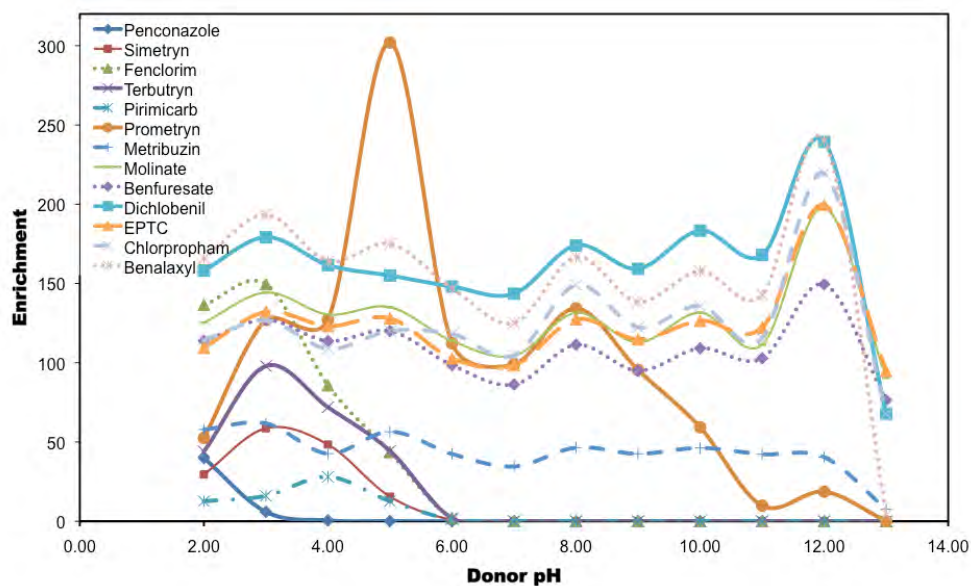
b)
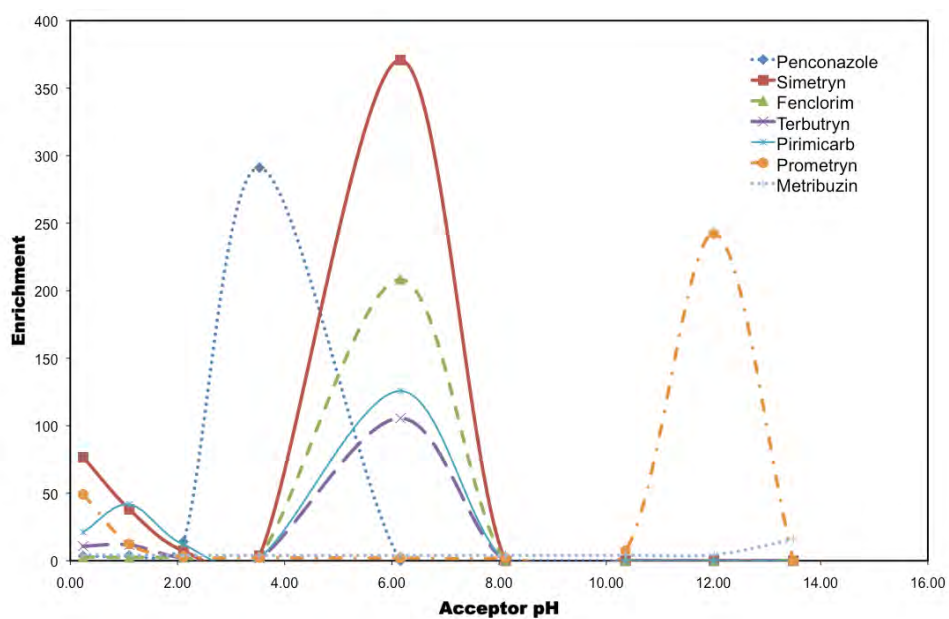
Figure 4.7 Enrichment of thirteen diverse pesticides a) as a function of donor pH; b) as a function of acceptor pH

4.1.3 Influence of System Volume Ratio

In equilibrium extraction, sufficient sample volume should be used to avoid analyte depletion in the donor phase. Therefore, maximum V_D should be determined. Figure 4.8 shows that *EF* stabilized after equilibriums were established. Once this volume was determined, it should be adopted for further study. Using a sample volume smaller than this value will affect enrichment reproducibility because an equilibrium could not be developed. The same trend is observed when varied V_A. Normally V_A is selected for the convenient of the operation. When V_A is too small, poor reproducibility and a drop in *EF* is observed due to loss of interfacial area where the partition occurs.

a)

b)
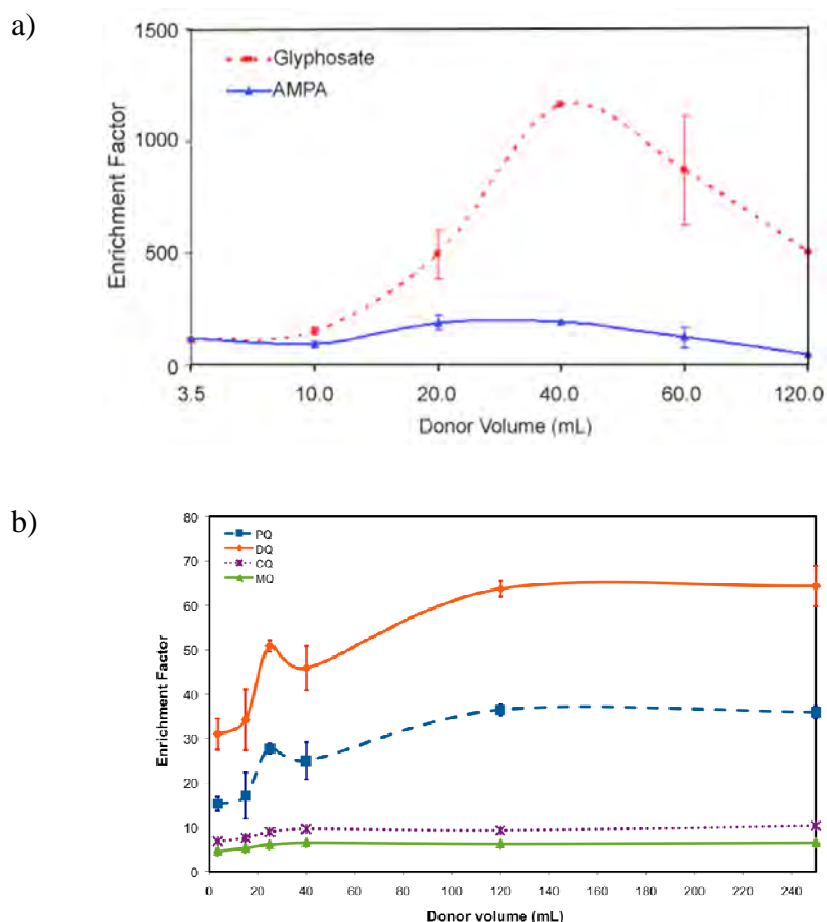
Figure 4.8 Effect of donor volume and acceptor volume on enrichment factor: a) LPME of glyphosate. Donor phase: 10μg/Lglyphosate andAMPA, pH9.0; acceptor phase: 1.0MKCl, V_a 20μL; membranesolvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time 45 min;

c)

d)
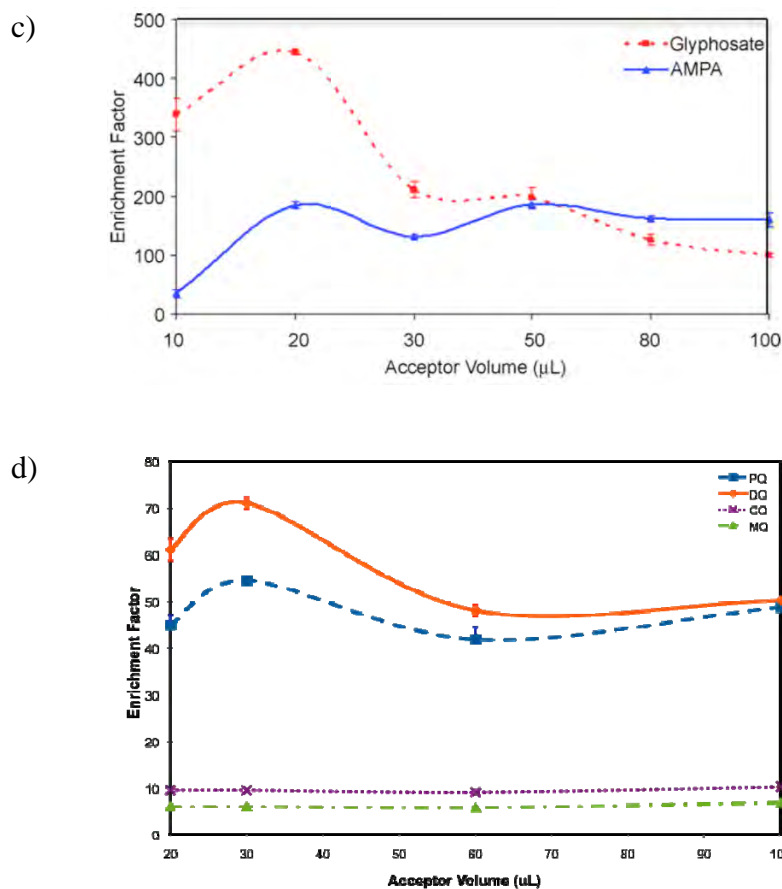
Figure 4.8 (continued) b) LPME of quaternary nitrogen herbicides.Donor phase: 100 μg/L of PQ, DQ, CQ, MQ inphosphate buffer pH 3.0;acceptor phase: 0.1M HCl, pH 0.5, V_a30μL; membranesolvent: DEHPA; extraction time 30 min;c) LPME of glyphosate. Donor phase: 10μg/Lglyphosate andAMPA,pH9.0,V_d 20 mL; acceptor phase: 1.0MKCl; membranesolvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time: 45 min; d) LPME of quaternary nitrogen herbicides. Donor phase: 100 μg/L of PQ, DQ, CQ, and MQ in 0.1 M phosphate buffer pH 5.0, V_d 120 mL; acceptor phase: 0.1 M HCl pH 0.5; membrane solvent: DEHPA; extraction time 45 min.

4.1.4 Influence of Equilibrium Time

To prevent bias of low measurements in equilibrium sampling, it is very important to allow the system to reach thermodynamic equilibrium. Figure 4.9 illustrated two cases of LPME extractions. Enrichment value increased with

extracting time to a maximum value and then decreased gradually. This is due to the loss of organic solvent from the pores of the hollow fiber. During strong agitation, microemulsion can be formed in the donor and acceptor solutions and breakthrough through the liquid membrane was gradually established. The breakthrough of liquid membrane resulted in the change in acceptor pH which directly affected the enrichment. Optimum enrichmentfactors were observed at 45 min. for the extraction of glyphosate, and at60 min. for the extraction of quaternary nitrogen herbicides. After this maximum point, reproducibilitywas slightly better but the gain was not worth the double extraction time. The equilibrium nature of non-exhaustiveextraction demands sufficient time for mass fluxto completely partition between the two phases through liquidmembrane. Equilibration time is extremely important forquantitative work.

To reduce equilibrium time, agitation such as shaking and stirring can be employed to increase the mass transfer flux by creating large concentrationgradients across the membrane. At higheragitation speed, reproducibility was poor and turbid solution was observed in both the extraction of glyphosate and quaternary nitrogen herbicides. Excessive turbulent flow ofthe donor solution destroyed the supported liquid membranesystem causing leakage of the liquid membrane and the carrier. Strong shaking should be avoided to prevent the formation of microemulsion of the liquid membrane at the interface of the donor and acceptor.

a)
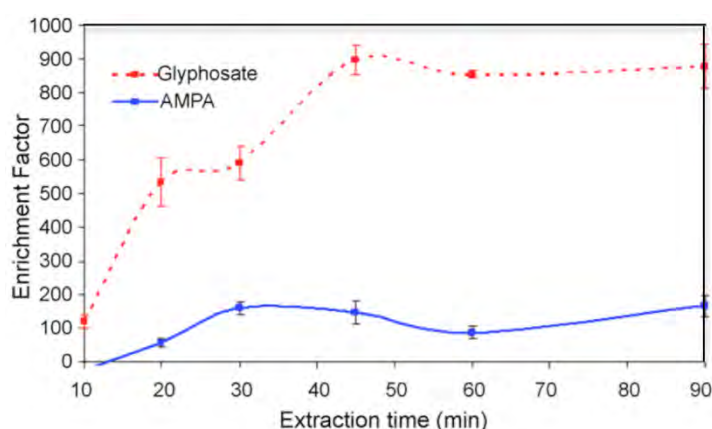
Figure 4.9 Effect of extraction time on enrichment factor. a) Donor phase: 10µg/Lglyphosate and AMPA, pH 9.0, V_d 20 mL; acceptor phase: 1.0MKCl, V_a 20µL;membrane solvent: 0.20M Aliquat-336 in di-n-hexyl ether.

b)
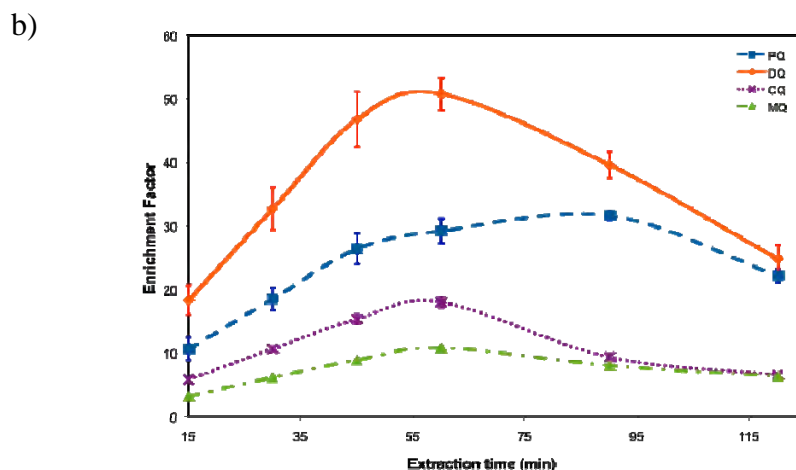
Figure 4.9 (continued) b) Donor phase: 100 μg/L of PQ, DQ, CQ, and MQ in 0.1 M phosphate buffer pH 5.0, V_d 20 mL; acceptor phase: 0.1 M HCl pH 0.5, V_a 30 μL; membrane solvent: 70% DEHPA in di-*n*-hexyl ether.

4.1.5 Other Optimization Parameters

4.1.5.1 OrganicLiquid Membrane

Selection of suitable membrane liquid is very important forhigh analyte enrichment. Membrane liquid should havelow solubility in both donor and acceptor solutions and be viscousenough to maintain a layer on the membrane surface to generatesufficient flux of analyte into the pores. Other requirementsof good membrane liquid are: low volatility, compatibility withthe membrane, can dissolve the carrier well, and compatibilityto the end analysis technique. Three most common solvents are undecane, dihexyl ether, and *n*-octanol. Solvent that offers the best reproducibility should be selected for the work.

a)

b)
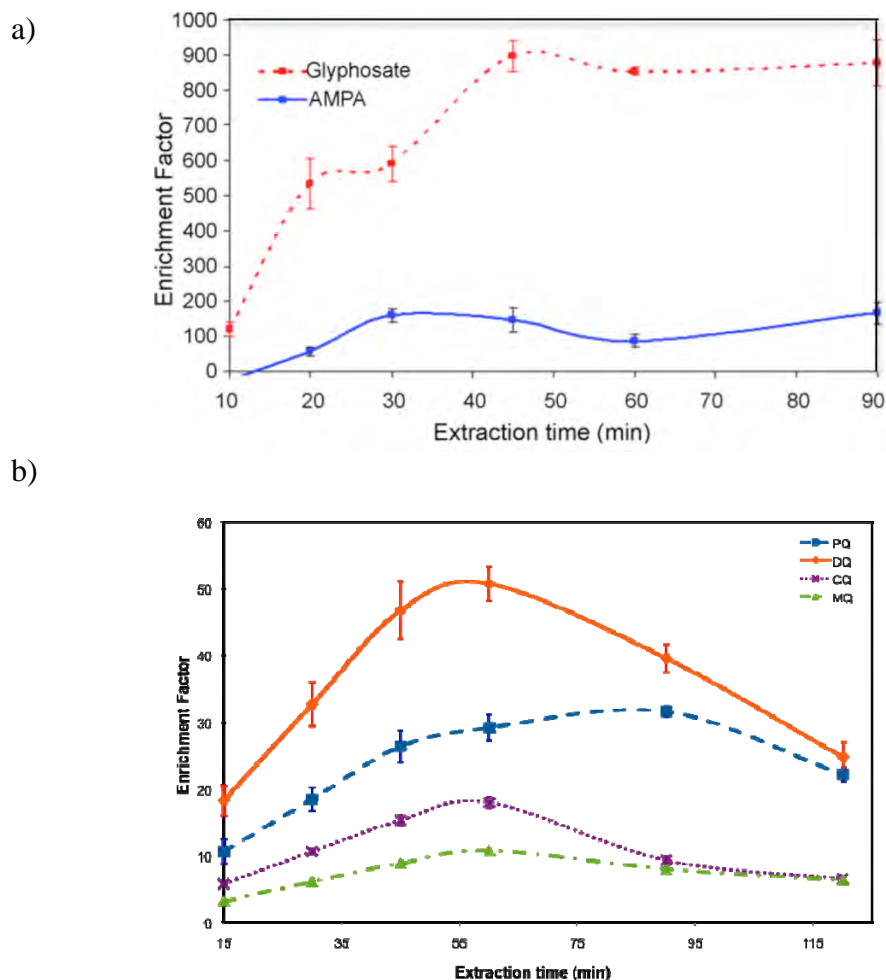
Figure 4.10 Enrichment factor as a function of extraction time. a) LPME of glyphosate. Donor phase: 10μg/Lglyphosate and AMPA, pH 9.0, V_d 20 mL; acceptor phase: 1.0MKCl, V_a 20μL;membrane solvent: 0.20M Aliquat-336 in di-*n*-hexyl ether. b) LPME of quaternary nitrogen herbicides. Donor phase: 100 μg/L of PQ, DQ, CQ, and MQ in 0.1 M phosphate buffer pH 5.0, V_d 20 mL; acceptor phase: 0.1 M HCl pH 0.5, V_a 30 μL; membrane solvent: 70% DEHPA in di-*n*-hexyl ether.

4.1.5.2 Carrier Molecules

Toenhance the extraction selectivity as well as to increase analyte enrichment, carrier molecule can be added to the liquid membrane system. The addition of a carrier molecule is highly beneficial for the extraction of ionic compounds that contain permanent molecular charges and are difficult to be extracted with organic solvent. Two examples of such systems were demonstrated. One is the extraction of

quaternary nitrogen herbicides whose structures shown in Table 2.2suggest that these compounds exist in cationic forms at neutral pH. DEHPA (common surfactant), LIX-84 and LIX-860 (hydroxyoxime extractants) were tested as the liquid membrane. These chelating agents have electron-rich functional groups that bind strongly with analytes with positive charge. In addition, their molecular structures also contain long hydrophobic chains which render the complex formed soluble in the apolar organic solvent. The enrichment factors obtained when employing these complexing agents were very similar to one another. Because the best precision was obtained with DEHPA, it was selected as the carrier in this work. Because DEHPA is very viscous and difficult to incorporate into the membrane, it was dissolved in organic solvents (*n*-decane, *n*-octanol, di-*n*-hexyl ether, and toluene were tested). The best results were observed when using di-*n*-hexyl ether (DHE). The mixture of DEHPA in DHE is insoluble in aqueous solution and offers suitable viscosity and low vapor pressure. These are important properties for the liquid membrane to be sufficiently stable throughout the extraction while providing a high diffusion coefficient for the target analyte. Because the carrier concentration is the key to obtaining high extraction efficiency, the optimum concentration was determined by varying its concentration at fixed extraction times (Figure 4.11).
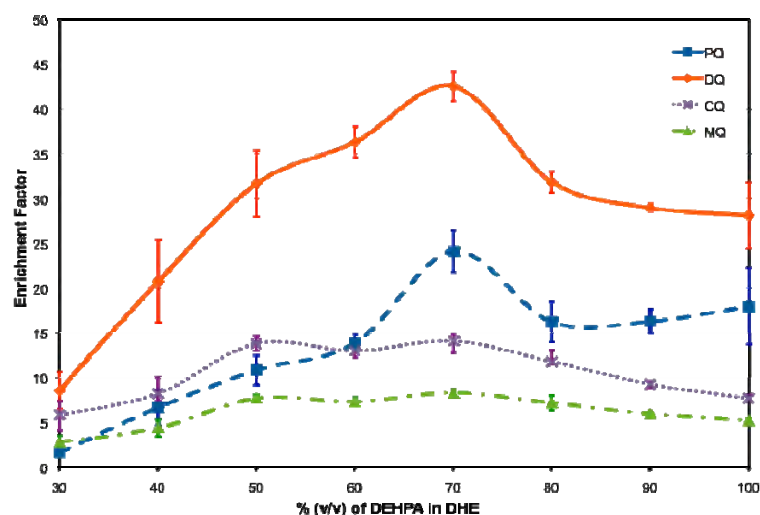
Figure 4.11 Enrichment factor as a function of carrier concentration: a) Donor phase: 100 μg/L of PQ, DQ, CQ, and MQ in 0.1 M phosphate buffer pH 5.0, V_d 20 mL; acceptor phase: 0.1 M HCl pH 0.5, V_a 30 μL; membrane solvent: DEHPA in di-*n*-hexyl ether; extraction time 45 min.

EF values increased when DEHPA concentration increased until an optimum value was observed at 70% (v/v) DEHPA. The graphs indicate the importance of carrier molecules in the extraction of polar analytes by organic liquid membranes. The carrier binds to target high polarity analytes and a neutral complex is formed. This neutral complex can be efficiently extracted by organic liquid membrane. Selective extractions can be tailored by choosing a suitable carrier molecule for the extraction. This selective extraction is powerful for matrix rich samples such as environmental and food matrices. The *EF*s peaked at 70% DEHPA after which the value dropped drastically. This is caused by an increase in solvent viscosity which, in turn, reduces the transport rate of the analyte within the membrane according to the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi a \eta} \qquad\qquad (4.3)$$

where *D* is the total flux, *k* is Boltzmann's constant, *T* is absolute temperature, *a* is the solute radius, and *η* is the solution viscosity.

The carrier (DEHPA) molecule is an essential component of the LPME system. When the membrane is immersed in the organic solvent, the solvent will automatically fill its pores by capillary action. Reported immersion times are in the range of 5 seconds to 30 minutes. To fine-tune our extraction conditions, immersion time was studied (Figure 4.12). It was observed that better precision was obtained when sufficient immersion time was allowed. For the system of DEHPA in DHE, a minimum of 3 hrs should be allowed for the membrane to be fully saturated. For improved consistency and ease of operation, we left our membrane soaking overnight.
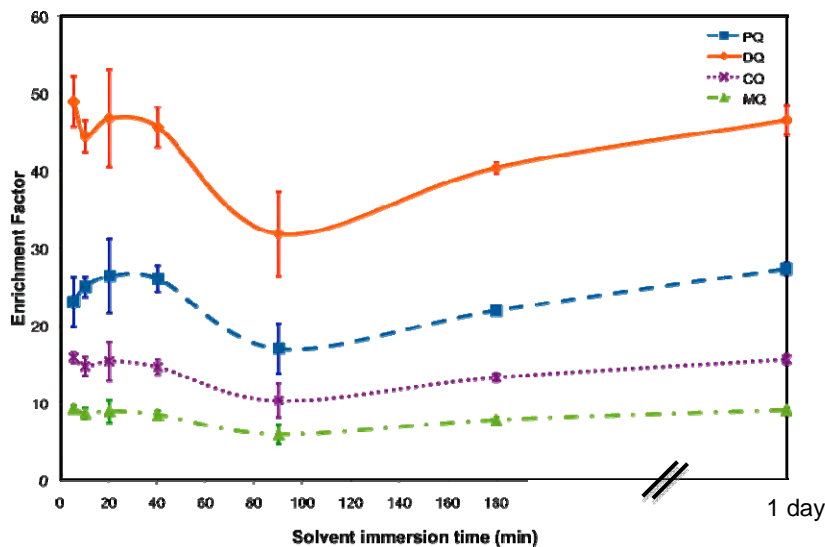
Figure 4.12 Influence of membrane immersion time. Donor phase: 100 μg/L of PQ, DQ, CQ, and MQ in 0.1 M phosphate buffer pH 5.0, V_d 20 mL; acceptor phase: 0.1 M HCl pH 0.5, V_a 30 μL; membrane solvent: 70% DEHPA in di-*n*-hexyl ether; extraction time 45 min.

4.2 Application of LPME in Environmental Analysis

4.2.1High Water Soluble Pesticides

Carrier-mediated transport LPME was selected for the analysis of glyphosate and its main metabolite AMPA. The pK_a values of glyphosate and AMPA in Table 2.3 suggest that both exist mostly as anions over a wide range of pH. The normal extraction scheme by dropping the pH of the sample down below pK_a will not work in this case. Another reason is that the both glyphosate and AMPA contain multiple charges that cannot be easily neutralized by adjusting the pH of the solution. Therefore, a carrier-mediated transport scheme is more suitable for their extraction.

4.2.1.1 Selection of the Membrane Liquid

Selection of suitable membrane liquid is very important forhigh analyte enrichment. Four different solvents were tested:dodecane, dodecane modified with dodecanol, kerosene, and di-*n*-hexyl ether. 1.0M HCl solution was selected as the acceptorphase at this stage. Solutions of 0.20M Aliquat-336 in differentsolvents were

prepared. The hollow fiber membrane was impregnatedwith each mixture and later used to extract the analytesfrom standard solutions at pH 11. Membrane liquid should havelow solubility in both donor and acceptor solutions and be viscousenough to maintain a layer on the membrane surface to generatesufficient flux of analyte into the pores. Other requirementsof good membrane liquid are: low volatility, compatibility withthe membrane, can dissolve the carrier well, and compatibilityto the end analysis technique. The best reproducibility wasobtained when employing di-*n*-hexyl ether which was selectedas the solvent of choice for this work.

4.2.1.2 The Carrier

Toenhance the extraction selectivity, Aliquat-336, a quaternaryammonium chloride salt was selected as a carrier and wasdissolved with the membrane liquid. The extraction mechanismsof glyphosate and AMPA in the presence of Aliquat-336occured via ion-exchange reactions between the zwitterionic functional groups of the analytes with chloride ions of the carrier.The formed complexes are transported across the membraneand undergo other ion-exchange processes to gain chloridesback at the membrane–acceptor interface [57]. Aliquat-336prefers to pair with amino acids or compounds of similarstructure to amino acid, therefore, it provides greater selectivityfor the extraction of glyphosate and AMPA [59]. Anotherunique feature of Aliquat-336 is that it stays in cationic formin all pH ranges and is therefore suitable for our extractioncondition.

In general, increasing the carrier concentration should enhance the enrichment factor. The data support a facilitated transport mechanism where the enrichment factorimproved when the carrier concentration increased until an optimal was reached at 0.20 M. After this point, the enrichment factor slightly decreased. The observation is in agreement with earlier works [57,100]. It was determined thatmembrane should be soaked in the carrier/membrane liquidsolution overnight prior to use. Shorter soaking period may notallow sufficient time for the solution to fully saturate the membranepores causing fluctuation in obtained enrichment factors.

4.2.1.3 The Donor

In carrier-mediated transport of negatively charged analyte, positively charged ligand binds to the analyte at donor–membrane interface forming a complex that is permeated across the membrane to the acceptor. To maximize the complex formation, pH of the donor should be adjusted to a value that keeps both analyte and ligand in charged forms. Figure 4.13 shows the enrichment factors of glyphosate and AMPA when the donor pH was varied from pH 1 to 12. High enrichment factors can be observed when the pH was above 8.0. The observations coincided with the dissociation profiles of glyphosate and AMPA that can be plotted using fractional composition equation of polyprotic acid (H_nA). Optimum enrichment factors were obtained at pH 10.0 for Glyphosate and at pH 9.0 for AMPA. At these pH values, the concentration of densely charged species dominating and binding to Aliquat-336 occurred with greater affinity resulting in high extraction efficiency values. The enrichment factors however decreased when the donor pH was higher and the donor solution around the membrane appeared to be turbid. Aliquat-336 is a water insoluble quaternary ammonium salt with permanent positive charge at all pH ranges. When the donor solution becomes highly basic and rich in hydroxide ions, the liquid membrane consisting of Aliquat-336 in di-*n*-hexyl ether collapsed. Because the solubility of both Aliquat-336 and di-*n*-hexyl ether are extremely low in aqueous media, cloudiness was observed. The optimum value of the donor pH is therefore selected to be 9.0 to compromise for maximum enrichment factors and reproducibility with minimum losses of the membrane liquid and the carrier.
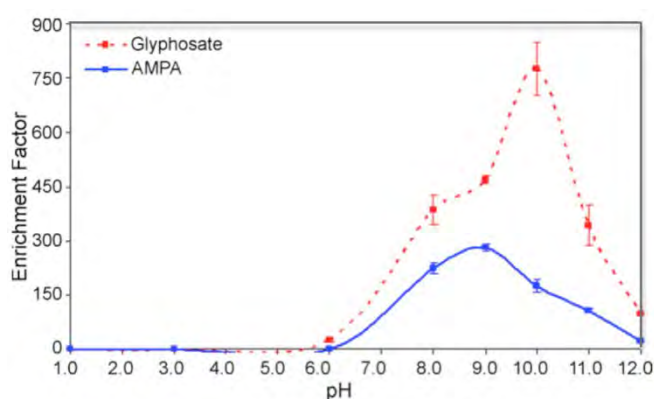
Figure 4.13 Effect of donor solution pH on enrichment factor. Donor phase: 10μg/L glyphosate and AMPA, V_d 20 mL; acceptor phase: 1.0M NaCl, V_a 30μL; membrane solvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time 45 min.

4.2.1.4The Acceptor

Once the analyte complex is transported to the membrane–acceptor interface, we want to transform this complex so that the analyte can be trapped in the acceptor phase and the carrier molecule releases back for reuse. For Aliquat-336 system, the analyte complex is exchanged for a chloride counter-ionvia an ion-exchange mechanism at the membrane–acceptor interface [57]. As a result, the analyte is released into the acceptor, and the carrier–chloride complex diffuses back to the membrane–donor interface where the chloride ion exchanges with another molecule of free analyte that complexes withAliquat-336 which is transported across the membrane, repeating the process. Sources of chloride ions in the acceptor were evaluated. The results confirmed the significance of chloride asthe counter-ion as almost no enrichment was observed in the system of formic acid when compared to other chloride enrichedsolutions (Figure 4.14). Chloride ion concentration is the main drivingforceof the enrichment until equilibrium is reached. The dataalso suggested that the enrichment process was greater whenusing chloride salts. When using HCl, the acceptor pH wasnear zero where glyphosate and AMPA existed in less densecharged forms resulting in lower solubility in aqueous solution(at membrane–acceptor interface) leading to lower enrichment.Optimum enrichment factor was obtained with 1.0M KCl.
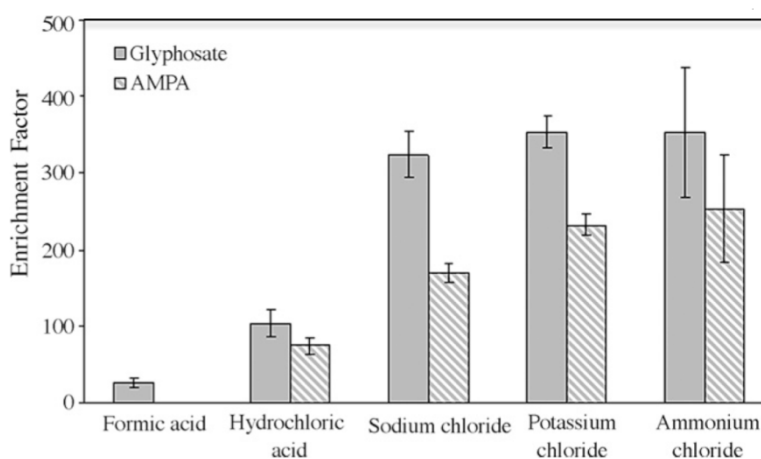
Figure 4.14 Effect of acceptor type on enrichment factor. Donor phase: 10µg/Lglyphosate and AMPA, pH 9.0, V_d 20 mL; acceptor phase: 1.0 M, V_a 30µL;membrane solvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time45 min.

4.2.1.5The Phase Ratio

Enrichment is directly proportionalto the phase ratio (V_d/V_a). Figure 4.15 and 4.16 showeddata of changing V_d and V_a at fixed extraction time. Enrichment should increase at larger $V_d$and lower V_a aslong as the equilibrium state could be maintained. According to Fick's first law, larger V_d and smaller V_a increase the concentration gradient which results in larger flux. When $V_d$was increasing at fixed V_a (20μL), concentration gradient wasgetting larger resulting in larger flux and higher enrichment.However, at volume above 40 mL, extraction time employed was insufficient to establish the equilibrium, resultingin dropping of enrichment factor and poor reproducibility. The opposite trendwas observed whenvarying V_a by using different lengths of hollow fiber to hold differentacceptor volumes. When V_a was increasing at constant $V_d$of 20 mL, the concentration gradient decreased which resultedin smaller flux and lower enrichment. The optimum value wasobserved when V_a equaled 20μL. Because mass flux is directlyrelated to diffusion surface area, shorter hollow fiber used tohold 10μL acceptor solution could not provide sufficient diffusionarea for the analyte and therefore equilibrium was notestablished resulting in lower enrichment factor.

Figure 4.15 Effect of donor volume on enrichment factor. Donor phase: 10μg/Lglyphosate andAMPA, pH9.0; acceptor phase: 1.0MKCl, V_a 20μL; membranesolvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time 45 min.
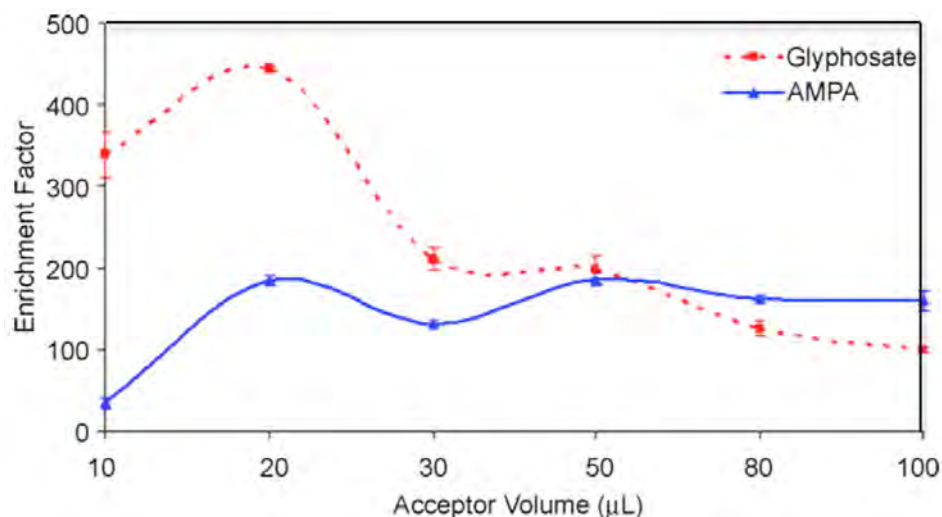
Figure 4.16 Effect of acceptor volume on enrichment factor. Donor phase: 10μg/Lglyphosate andAMPA,pH9.0,V_d 20 mL; acceptor phase: 1.0MKCl; membranesolvent: 0.20M Aliquat-336 in di-*n*-hexyl ether; extraction time: 45 min.

4.2.1.6Mass Transfer

To increase the mass transfer flux by creating large concentrationgradients across the membrane, agitation by vortex wasperformed at different speeds. The agitation was conductedat ambient temperature at arbitrary units that were increasedfrom 1 to 8. Optimum enrichment factor and good reproducibilitywas obtained when vortexed at 3 arbitrary units. At higheragitation speed, reproducibility was poor and turbid solution was observed. Excessive turbulent flow ofthe donor solution destroyed the supported liquid membranesystem causing leakage of the liquid membrane and the carrier.Consequently, the vortex unit set at 3 arbitrary units wasselected.

4.2.1.7Extraction Time

The last parameter to be evaluated was the extraction time.The hollow fiber filled with acceptor solution was dipped in theextraction bottle containing sample (donor) and vortexed for different lengths of time. Figure4.17 shows that the optimum enrichmentfactor was reached at 60 min. Above 60 min, reproducibilitywas slightly better but the gain was not worth the doubleextraction time. This is because the

equilibrium nature of nonexhaustiveextraction demands sufficient time for mass fluxto completely partition between the two phases through liquidmembrane. Equilibration time is extremely important forquantitative work.
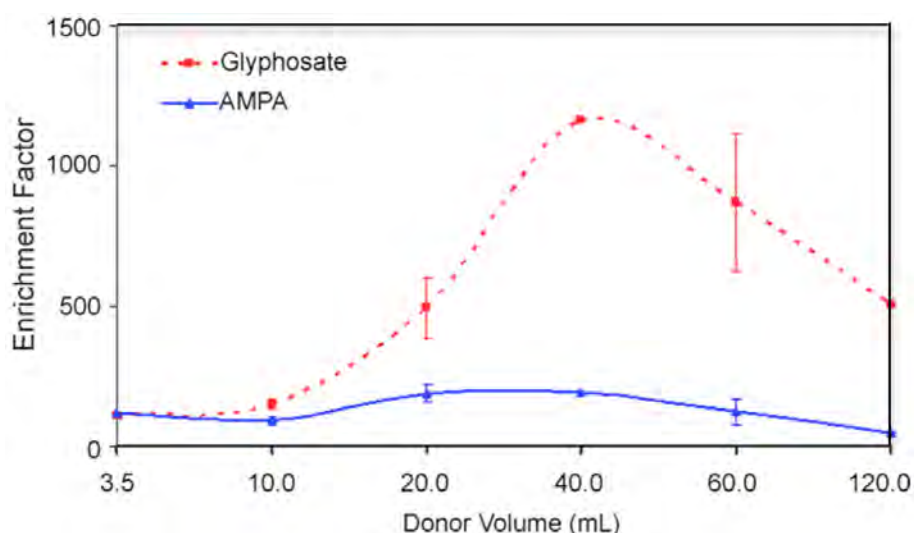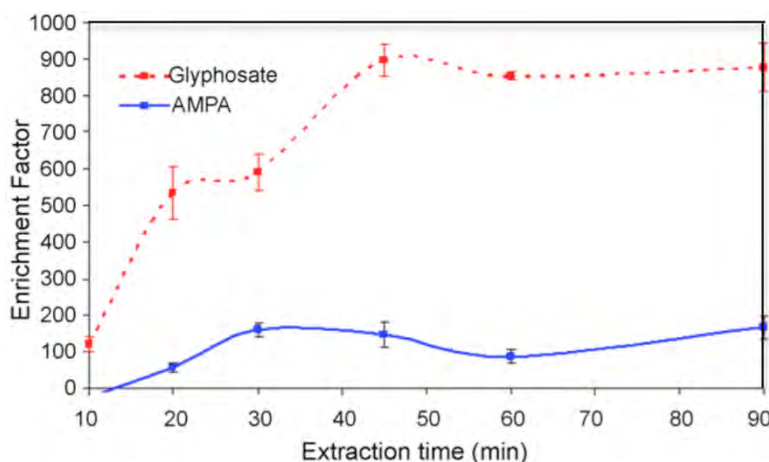

Figure 4.17 Effect of extraction time on enrichment factor. Donor phase: 10μg/Lglyphosate and AMPA, pH 9.0, V_d 20 mL; acceptor phase: 1.0MKCl, V_a 20μL;membrane solvent: 0.20M Aliquat-336 in di-*n*-hexyl ether.

4.2.1.8Analytical Method Performance

Linearity of the method was investigated using a series ofstandard solutions. The data in Table 3 show good linearitywith squared regression coefficients (R^2) > 0.9900 for bothglyphosate andAMPAover a large range. This implies that an externalstandard calibration method can be applied for quantitativepurposes. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the S/N values obtainedfrom repeat analyses (n = 10) of solution containing 1 μg/Lglyphosate and 5 μg/L of AMPA. LOD values were obtained at S/N = 3 and LOQ values were obtained at S/N = 10. The LOD ofthis method for glyphosate is 0.22 μg/L which is better than thepublished value of 6.00 μg/L by EPA method 547 [45]. The proposedLPME procedure was used for the extractions of spikedreagent water with glyphosate (3 μg/L) and AMPA (8μg/L). The percent extraction efficiencies (EEs) were obtained at twolevels. The obtained EEs were within the AOAC acceptableranges for trace analysis. Method precision was evaluatedby comparing the relative standard

deviations of repeat extractionsof spiked reagent water at two levels. The relative standarddeviations of the repeated analyses fall within the Horwitz trumpet range. The validation data imply that our proposedLPME procedure is sufficiently accurate for routine analysisof glyphosate and AMPA in water sample indicating that theproposed method has acceptable precision for routine analysis.

4.2.1.9 Method Application

The validated LPME procedure was tested on real samples.Samples of groundwater collected from agricultural sitesin Thailand were tested for glyphosate and AMPA. Because thesamples appeared to be clean after the first analysis, they were spiked with glyphosate at 1μg/L (six-times below the LOD ofEPA method 547) and AMPA at 5 μg/L due to its lower sensitivity.Data of duplicate analyses on different days showedenrichment factors of 530 and 811 for glyphosate and 117and 77 for AMPA. Percent extraction efficiency were 53±4.5and 81.1±3.9 for glyphosate; and 11.7±1.8 and 7.7±2.7 forAMPA. The results were within par of the validate data provingthat the LPME procedure are suitable for the analysis ofglyphosate and its metabolite AMPA in natural water samples.

Table 4.6 Performance and validation data of the LPME procedure coupled with HPLC post-column derivatization/fluorescence detection

	Extraction Efficiency (n=8)		Linearity		LOD (n=10) (µg/L)	LOQ (n=10) (µg/L)	Accuracy		Precision (%RSD) (n=7)	
	EF	RSD (%)	Range (µg/L)	R^2			Level 1[a]	Level 2[b]	Level 1[a]	Level 2[b]
Glyphosate	853	8.0 (35)[c]	1-1004	0.9962	0.22[d]	0.72	74.6 ± 4.8	86.4 ± 6.5	6.4 (45)[c]	7.5 (35)[c]
AMPA	136	5.2 (27)[c]	5-613	0.9943	3.40	11.34	11.1 ± 1.9	13.4 ± 0.5	6.1 (35)[c]	3.7 (27)[c]

[a]Level 1: glyphosate 1µg/L, AMPA 8µg/L.
[b]Level 2: glyphosate 5µg/L, AMPA 25µg/L.
[c]Value calculated from Horwitz equation (CV (%)$\approx 2^{(1-0.5\log C)}$) at each concentration level.
[d]EPA method 547 set MDL of reagent water at 6.00µg/L [45].

4.2.2 Water Disinfection Byproducts

The pK_a values of IAA, DBAA, TCAA, and TBAA suggested that they present mostly in acid forms at pH 1.0 or below (Table 2.7). In acid forms, all analytes are non-ionized and can partition into an organic solvent. To keep the analytes in extractable forms, the solution pH should be kept at least 1.5 units below pK_a values which is not very feasible and there will be some fractions of the analytes remaining in ionized forms. To promote partition into the supported liquid membrane, a carrier molecule was added to the organic liquid.

4.2.2.1 TheSupported Liquid Membrane

Selection of suitable membrane solvent is very important. The solvent must be compatible to the membrane, have suitable viscosity to stay within the pores throughout the vigorous extraction while not interfere with the analyte partition process. The solvent should have sufficient high boiling point to minimize loss during the extraction. Three common LPME solvents were evaluated (di-*n*-hexyl ether, dodecane and *n*-octanol). Di-*n*-hexyl ether provided several orders of magnitude higher in enrichment and was selected as the supported liquid membrane for the extraction of IAA, DBAA, TCAA, and TBAA (Figure 4.18).
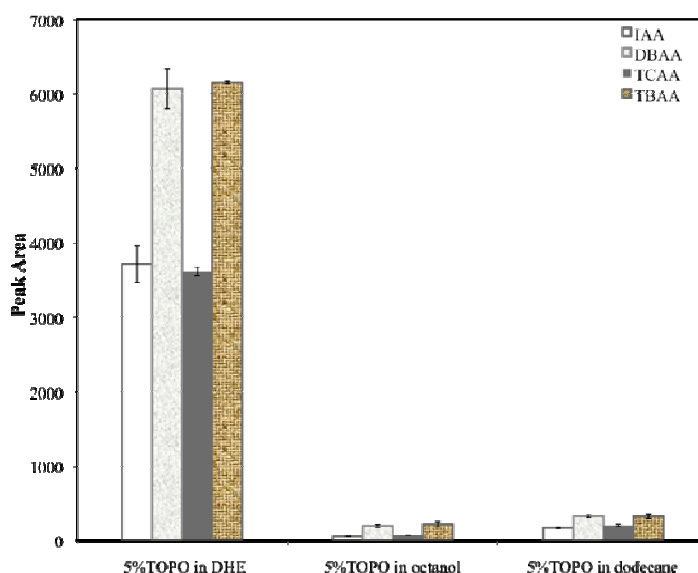
Figure 4.18 Enrichment factor as a function of type of organic solvent

Adding of carrier molecules into the supported liquid membrane can improve the enrichment of polar compounds. The idea is the same as using a complexing reagent to form the analyte neutral complex that is more compatible to the organic solvent. Trioctylphosphine oxide (TOPO) was successfully used for the extraction of HAAs [96], and therefore was evaluated in this work. The addition of TOPO significantly increased the enrichment (Figure 4.19). The best enrichment was obtained at 5% TOPO. When the concentration is above 5%, the concentration of OH⁻ in the acceptor solution was insufficient to break the H-bonding of TOPO and the analyte which results in less amount of analyte being released into the acceptor solution. For these reasons, the selected liquid membrane is 5%TOPO in di-*n*-hexyl ether.
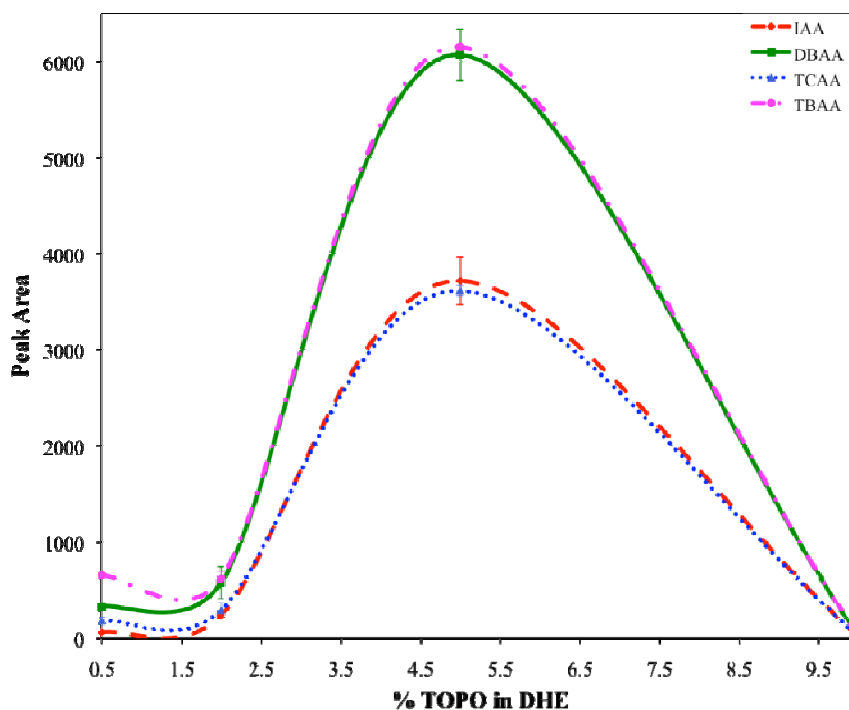
Figure 4.19 Enrichment factor as a function of carrier concentration

4.2.2.2 The Donor Solution

To promote the formation of neutral complexes, the optimum pH of both the donor solution and the acceptor solution were evaluated. According to the pK_a values, we can predict that our analytes exist in ionized forms in the sample (water). To have them in non-ionized forms, the pH of the solution must be kept below their

pK_a. At this low pH, good partitioning is expected. It was determined that the optimum donor pH is 1.0 (Figure 4.20).
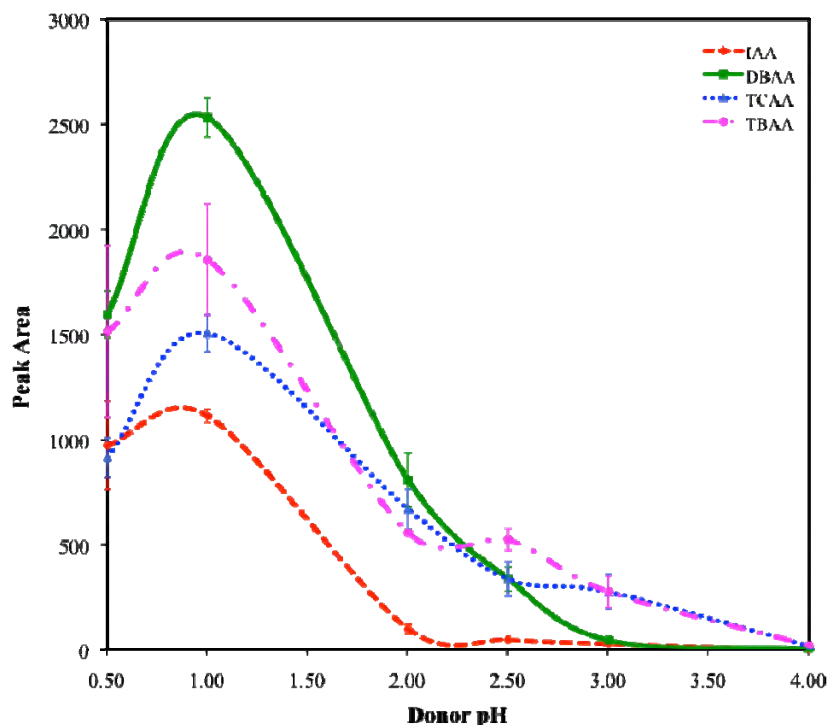
Figure 4.20 Enrichment factor as a function of the donor pH

4.2.2.3 The AcceptorSolution

Once inside the membrane, the analyte-TOPO complexes diffuse to the membrane-acceptor interface. To further enhance the extraction, the acceptor pH can be modified in such a way that the analyte is deprotonated [A⁻] and is trapped inside the acceptor solution. Figure 4.21 shows that when pH of the acceptor solution was above the analyte pK_a, increasing enrichment trend was observed. Maximum enrichment was observed at pH 12.70 which is more than $pK_a + 3.3$ units. This can be explained that TOPO binds to the analyte via H-bonding which takes much higher energy to break. Therefore, higher concentration of OH⁻ is required to break this bond.   At this pH, the analytes were stripped of the protons and moved into the acceptor solutions where they remain in charged forms and cannot be extracted back into the organic membrane. This trapping selectively promoted the analyte fluxes into the acceptor phase which further enhanced the enrichments.
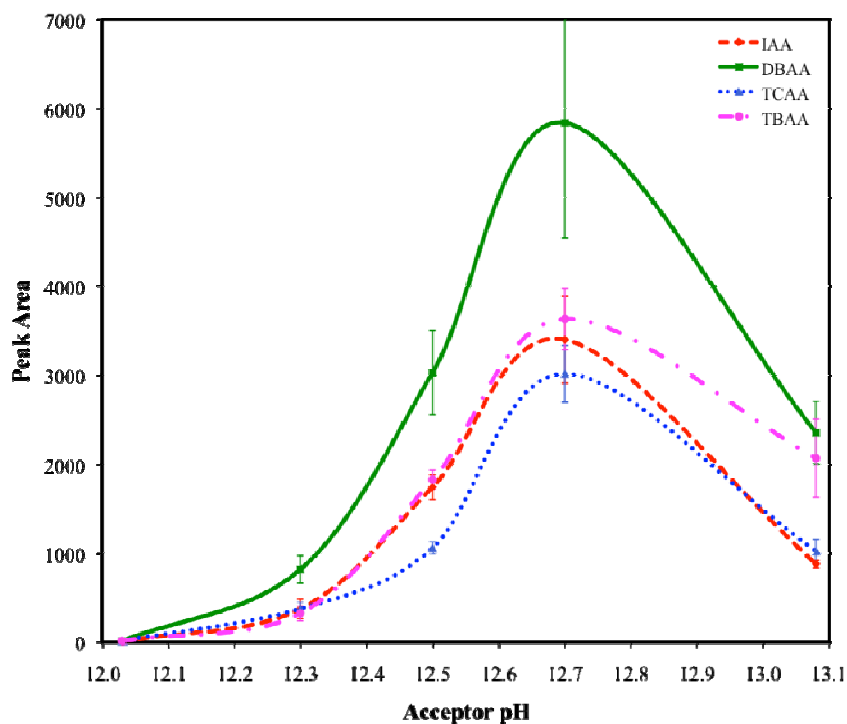
Figure 4.21 Enrichment factor as a function of acceptor pH

4.2.2.4 The Phase Ratio

The enrichment in anLPME process occurs when analyte moves from donor phase to acceptor phase while the aqueous is stripped off totally. The ratio of the change in volume from donor to acceptor (V_d/V_a) determines the level of enrichment. Figure 4.22 shows increasing trends of enrichments as donor volume increases. Because it is more difficult and more expensive to use a large sample, we selected our sample volume to be 28 mL. Optimum acceptor volume at this V_d is determined to be 20 µL. Larger enrichment value can be manipulated by optimizing the phase ratio. However, a good balance between sample throughput and large enrichment should always be under consideration.
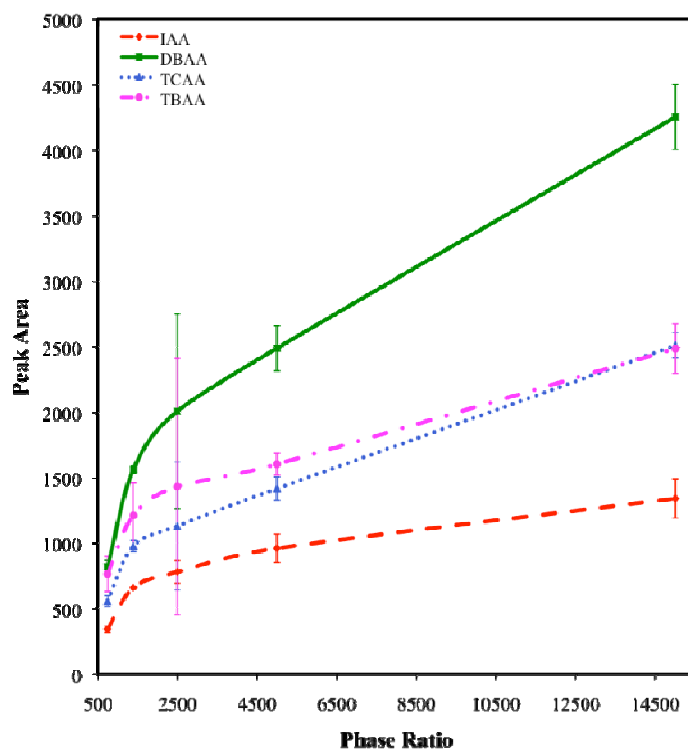
Figure 4.22 Enrichment factor as a function of phase ratio

4.2.2.5 Extraction Time

Because LPME is an equilibrium process, the equilibrium time must be determined to obtain maximum enrichment. Sufficient time is required for analyte to partition between the two liquid phases. Diffusion of the analytes through the pores also adds to the amount of time required. For quantitative analysis, it is very important to determine this equilibrium time otherwise method reproducibility will be sacrificed. Figure 4.23 shows increasing trends of enrichment which reached maxima at 60 minutes.

4.2.2.6 Stirring Speed

To generate sufficient flux of the analyte that can be extracted, solution is often stirred. The speed at which the sample is stirred during the extraction will affect the rate of equilibrium, and the physical stability of the organic solvent layer on the outside of the hollow fiber. It was determined that optimum enrichment was obtained

when stirred at 800 rpm. Stirring above 900 rpm destroyed the liquid membrane which led to the loss of the analytes and lower enrichment as observed in Figure 4.24.
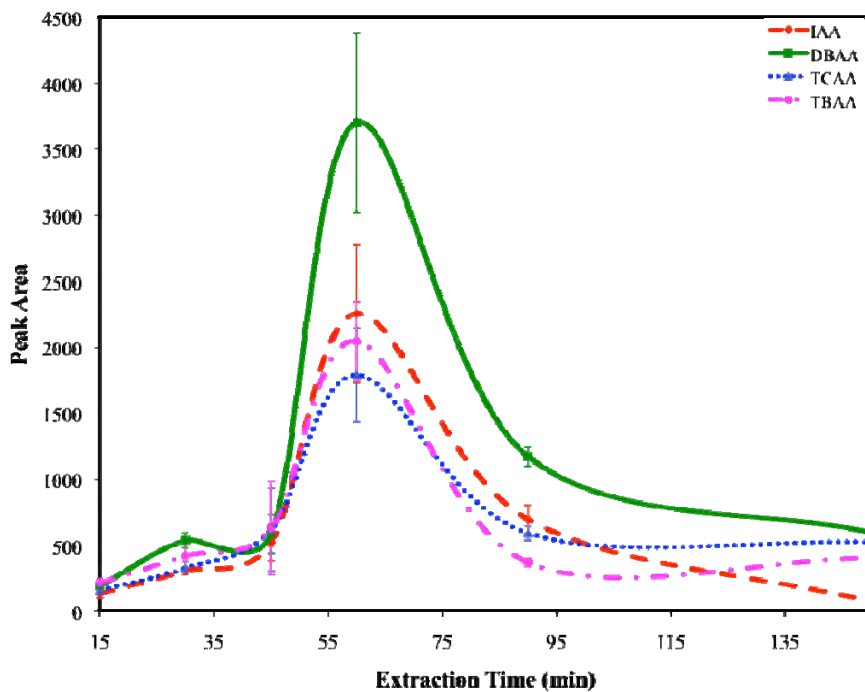
Figure 4.23 Enrichment factor as a function of extraction time
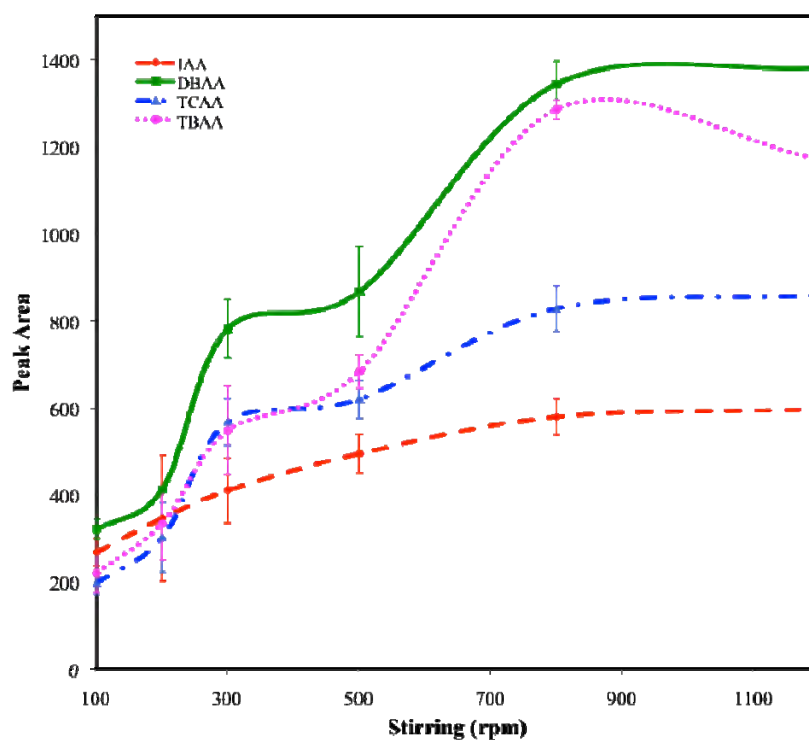
Figure 4.24 Enrichment factor as a function of stirring speed

4.2.2.7 Analytical Performance

The developed LPME procedure coupled to HPLC-UV was validated to test for method reliability and workable range. The combined analytical procedure can enrich the target analytes from 500 to 1200 fold. The validation data are in Table 4.7. The method is linear with exceptional R^2 values that are all better than 0.9900 from 2.6 to 62.6 μg/L. Limits of detection (LODs) are not better than the EPA standard method but can be improved easily by increasing the phase ratio. The EPA method employs a lengthy derivatization reaction to increase detection sensitivity of a very sensitive detector, an ECD, which is capable to detect at 10^{-15} M. Our method uses a UV detector that can detect at 10^{-8} or 10^{-9} M. The LODs can be improved by increasing the phase ratio from 1400 to larger numbers or by using a high sensitivity instrument such as an LC/MS. An increase in phase ratio will require larger sample volume and an analytical throughput will have to be sacrificed. Limits of quantitations (LOQs) range from 2.05 to 5.22 μg/L. The method was used for the analyses of spiked solutions for three consecutive days at LOQs. Both the within day and between days variances are all lower than the calculated Horwitz values. Percent recovery of spiked solutions also fell within the acceptable AOAC range for trace analysis. These data confirm the reliability of the developed LPME procedure coupled with HPLC-UV that suit for routine analysis of IAA, DBAA, TCAA, and TBAA in water.

Table 4.7 Analytical Performance of LPME procedure coupled to a HPLC/UV for the analysis of HAAs and IAA in reagent water

Analyte	EF	Linear range (µg/L)	R^2	[a]LOD (µg/L)	[a]LOQ (µg/L)	[b]EPA 553 (µg/L)	[c]Kou (µg/L)	Precision Day 1	Day 2	Day 3	[d]Horwitz	[e]Accuracy
IAA	415	5.22-62.64	0.9977	0.5220	5.2200	-	-	2.16	2.65	2.99	23.29	104.38
DBAA	888	2.595-41.52	0.9937	0.2595	2.5950	0.066	0.06	5.06	6.79	5.57	25.87	107.52
TCAA	1048	2.56-40.96	0.9951	0.2560	2.0480	0.079	0.05	16.40	9.82	11.06	26.81	92.68
TBAA	715	2.625-42.00	0.9930	0.2625	2.6250	0.820	0.03	11.50	7.48	16.72	25.83	79.30

[a]Phase ratio = 1400, sample volume 28 mL, this work
[b]Phase ratio = n/a, sample volume 40 mL, derivatization [98]
[c]Phase ratio = 8333, sample volume 250 mL [99]
[d]Calculated values from Horwitz equation, $\%RSD_R = 2^{(1-0.5\log C)}$
[e]Acceptable AOAC range 79-108%

4.2.2.8 The Analyses of Real Samples

The developed LPME-HPLC method was tested with real samples. Public tap water and bottled water samples were subjected to the extraction procedure and analysis described earlier. Sixty two percent of the samples were contaminated with some DBPs as showed in Table 4.8.

Table 4.8 Quantity of DBPs detected in some real world water samples by the developed LPME-HPLC method (n=3)

Sample	Description	DBP (mg/L)
1	Tap water 1	TBAA (0.002)
2	Tap water 2	TBAA (0.003)
3	Drinking fountain 1	TCAA (0.02)
4	Drinking fountain 2	-
5	Drinking fountain 3	-
6	Bottled water 1	IAA (0.002), TBAA (0.001)
7	Bottled water 2	IAA (0.003), TCAA (0.001), TBAA (0.001)
8	Bottled water 3	-

TBAA: not regulated
TCAA: WHO (0.3), Aus (0.1), US as total haloacetic acids (0.06)
IAA: not regulated, high priority DBPs by U.S. EPA

CHAPTER V
CONCLUSION AND SUGGESTION

5.1 Conclusion

Liquid-phase microextraction (LPME) combines the reduced organic solvent consumption and non-exhaustive natures of solid-phase microextraction (SPME) to the principles of liquid-liquid extraction (LLE). The first part of this work investigated the similarities and differences between LPME techniques and conventional LLE in the aspects of extraction efficiency. Prediction using simple calculations to compare extraction recovery and enrichment over a range of partition coefficients suggests that the exhaustive nature of LLE provides good recovery of the analyte but the technique enriches poorly. Two-phase LPME enriches the analyte very well regardless of poor recovery that roots from trapped analyte within the membrane pores. Three-phase LLE or LLE with back extraction does not enrich and the technique's recovery is driven by the partition coefficient of the back extraction step. Three-phase LPME provides high enrichment and selectivity and is very attractive in terms of providing greater flexibility. The mathematic descriptions pointed to many advantages of microscale extraction techniques over LLE in terms of analyte enrichment with very small volumes of organic solvent. This level of enrichment is difficult to achieve in LLE even when considering solvent evaporation and reconstitution.

The mathematical deductions implied that the partition coefficient (K_D) governs the extraction process in both two- and three-phase LPME. Since LPME extraction employs uncommon organic solvents, the research aimed to investigate if K_D of analytes in these solvents can be extrapolated by corresponding analytes' K_{ow} values that are widely available. Extraction data of thirteen diverse pesticides using n-octanol as liquid membrane were different from the calculated values. It was deduced that the simplified mathematical model is limited to thermodynamics of ideal partition equilibrium. Furthermore, the effect of variations in the degree of molecular hydrophobicity such as molecular structure, shape, size, and functional group must also be considered. The data from this part implied that K_{ow} values and

the simple mathematic equations commonly used for the determination of microscale extraction effectiveness cannot be used for precise enrichment prediction in LPME.

The acid-base character of the analyte and the solution pH were determined to have a strong effect on the enrichment of ionic compounds. The extraction for ionic compound is best done by adjusting the solution pH to force the compound in either the ionized state, or in the non-ionized state as completely as possible. The study showed that the optimum donor and acceptor pH of a three-phase LPME can be predicted when they accounted for the concentration of non-dissociated species at specific pH values. Enrichment of the extractable fraction at this pH can also be calculated and showed similar trends to the experimental data. In conclusion, for neutral and non-ionic compounds, degree of molecular hydrophobicity dictates the behavior of the extraction and a two-phase LPME is suitable for their extraction. For ionic compounds, enrichment is driven by the amount of non-dissociated fraction and a three-phase LPME can be systematically fine tuned by adjusting the system pH to maximize the non-dissociated fraction. Analyte selectivity can be enhanced in three-phase system by carrier molecules. Other influencing parameters are system volume ratio, equilibrium time, and agitation to promote mass transfer process.

To test the validity of the fundamentals, microscale membrane extraction was attempted on new world pollutants. The first method was developed for the extraction of glyphosate and its metabolite, aminomethyl phosphonic acid (AMPA), in water. To enhance the extraction of negatively charged analyte, a cationic carrier (Aliquat-336) was incorporated into the liquid membrane. The optimized procedure used 20 μL of 0.2 M Aliquat-336 in 1.0 M KCl to extract 20 mL of water sample adjusted to pH 9.0. Extraction time was 60 min. The procedure enriched glyphosate 853-fold and AMPA 136-fold with acceptable analytical performance. The developed LPME procedure coupled to a HPLC with OPA post-column derivatization provided improved analytical performance than many published and standard methods currently used for routine analysis of glyphosate.

The second method was developed for the extraction of quaternary nitrogen herbicides in water matrices. Quaternary herbicides have permanent positive charges and their analyses by conventional methods are very difficult. A carrier-mediated transport LPME using the anionic carrier, di(2-ethylhexyl) phosphoric acid (DEHPA), was developed for diquat, paraquat, mepiquat, and chlormequat. The procedure

extracted 120 mL of water sample (pH 5.0) with 30-µL of 70% (v/v) DEHPA in 0.1M HCl pH 0.5 in 60 minutes. The enrichment obtained were 56.30, 64.34, 11.30, and 8.85-fold for paraquat, diquat, chlormequat, and mepiquat, respectively.

And lastly, an LPME procedure was developed for emerging water disinfection byproducts: iodoacetic acid, dibromoacetic acid, tribromoactic acid, and trichloroacetic acid. Acidic compounds are difficult to extract because the donor solution must be kept at extremely low pH to keep them in extractable forms. The strategy used trioctylphosphine oxide (TOPO), a molecule of a very large dipole moment, to complex with iodoactic acid and haloacetic acids. The optimum procedure extracted 28 mL of water sample (pH 1.0) with 25 µL of 5% TOPO in dihexyl ether, pH 12.70. The extraction time was 60 minutes. The LPME procedure can enrich IAA and HAAs 500-1000 fold with acceptable analytical performance.

These examples serve as evidence of potential and flexibility of the hollow fiber based micro-scale membrane extraction for environmental sample preparation purposes. The procedures are very simple and can be optimized systematically based on fundamental principles.

5.2 Suggestion

This research touched on some fundamental aspects of membrane extraction with brevity to predict effective extraction scheme. The course of the research pointed to areas that need to be addressed before good acceptance of hollow fiber based micro-scale extraction for routine analysis can be achieved and develop into a commercial device like SPME.

1. The current LPME theory was developed from a concept of single drop microextraction in parallel to the principles of LLE which is an exhaustive technique. This model does not incorporate a partition at solution/membrane interfaces. To achieve quantitative transfer of the analyte in non-exhaustive system such as in a hollow fiber microscale extraction device, a large phase ratio and good design of system geometry is required. Parameters controlling the distribution constant at these interfaces must be carefully defined. Further study in this area will provide useful knowledge such as how to effectively control the extraction and in future designs of new devices.

2. One obvious problem of the non-exhaustive extraction technique is long extraction time that can double or triple the time of conventional exhaustive extraction. Improvement in this aspect can be achieved by the study of kinetic parameters.

3. The thermodynamics of the extraction between two immiscible phases alone can not fully apply to hollow fiber microextraction technique, a boundary layer model should be investigated for precise understanding of the extraction inside and outside the boundary layer of the membrane.

4. The optimization process of LPME is a challenge to an analytical chemist. A chemist need to understand some of the underlying knowledge that is not very common such as the principle of extraction, the equilibrium in multi-phase system, the mass transfer kinetics, and the boundary layer for successful operation of LPME. A new chapter in analytical chemistry that integrates these areas into practical sample preparation perspective would promote rapid evolution of this useful extraction technique.

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