

PHASE EQUILIBRIUM AND PARTITION COEFFICIENT OF SELECTED
PESTICIDES AND NITROPHENOLS IN DOUBLE AND TRIPLE-PHASE
LIQUID-PHASE MICROEXTRACTION

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UNIVERSITI TEKNOLOGI MALAYSIA

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LIQUID-PHASE MICROEXTRACTION

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Dedicated to my wonderful mother, my beloved siblings, family, and friends....

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PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between December 2006 to December 2008 under supervision of Prof. Dr. Mohd Marsin Sanagi and Assoc. Prof. Dr. Wan Aini Wan Ibrahim. Part of my work described in this thesis has been reported in the following publications or presentations:

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ABSTRACT

A two-phase and three-phase hollow fiber-protected liquid-phase microextraction method (HF-LPME) has been developed and used for the determination of partition coefficient and analysis of selected pesticides and nitrophenols in water samples. For two-phase HF-LPME, the analysis was performed by gas chromatography-electron capture detector (GC-ECD). Extraction conditions of the selected pesticides were optimized. The following conditions have been selected as the optimum extraction conditions: toluene as organic solvent, 4 mL water samples, 20 min extraction time, 850 rpm stirring rate, 1.8 cm HF length, 1.5% (w/v) NaCl content, and pH 3. The analytes were extracted from 4 mL donor phase through 4 μ L of an organic solvent immobilized in the pores of a porous polypropylene hollow fiber and then into the acceptor phase present inside the hollow fiber. Correlation coefficients (r^2) of 0.996-0.997 and limits of detection (LOD) of 0.0026-0.0028 μ g/L were achieved under the optimized conditions. The proposed method provided good average enrichment factors of up to 350-fold and successfully determined the partition coefficient for the selected analytes that were found to be directly correlated to the enrichment factor. In the three-phase HF-LPME technique coupled with HPLC, nitrophenols were extracted from 2.5 mL aqueous solution with the adjustment of pH in the range of 3.0-5.0 (donor solution) into an organic phase (1-hexanol) immobilized in the pores of the hollow fiber and finally back-extracted into 5.0 μ L of the acceptor microdrop (pH 12.0) located at the end of the microsyringe needle. After a prescribed back-extraction time, the acceptor microdrop was withdrawn into the microsyringe and directly injected into the HPLC system under the optimum conditions (donor solution: 1.0 M H_3PO_4 , pH 3.0-5.0; organic solvent: 1-hexanol; acceptor solution: 5 μ L of 0.1 M NaOH, pH 8.0-12.0; agitation rate: 1050 rpm; extraction time: 15 min). The calibration curve for these analytes was linear in the range of 0.6-200 μ g/L with $r^2 > 0.9994$ was achieved under the optimized conditions. The enrichment factors of up to 500-fold were obtained. In this work, determination of analytes partition coefficients in three-phase HF-LPME has been successfully achieved. This study found that the partition coefficient ($K_{a/d}$) values were high for 2-nitrophenol and 3-nitrophenol and the individual partition coefficients ($K_{org/d}$ and $K_{a/org}$) promoted efficient simultaneous extraction from the donor through the organic phase and further into the acceptor phase. Both methods were successfully applied for the analysis of water samples.

ABSTRAK

Kaedah pengekstrakan mikro fasa cecair menggunakan gentian berongga (HF-LPME) yang terdiri daripada 2-fasa dan 3-fasa telah dibangunkan dan digunakan untuk menentukan pemalar partisi bagi pestisid dan nitrofenol di dalam sampel air. Bagi HF-LPME 2-fasa, analisis dijalankan menggunakan gas kromatografi-pengesan penangkapan elektron (GC-ECD). Parameter-parameter penting yang mempengaruhi pengekstrakan pestisid telah dioptimumkan: pelarut organik (toluena), isipadu sampel air (4 mL), masa pengekstrakan (20 min), kandungan NaCl (1.5% (w/v), dan pH (3). Analit yang diekstrak daripada 4 mL fasa penderma akan melalui 4 μ L pelarut organik yang terserap pada liang-liang rongga gentian ke dalam fasa penerima yang terdapat di dalam rongga gentian tersebut. Nilai pekali kolerasi (r^2) dan had pengesanan terendah (LOD) masing-masing ialah 0.996-0.997 dan 0.0026-0.0028 μ g/L. Teknik ini menghasilkan faktor pemekatan yang baik, iaitu melebihi 350-lipat dan dikenalpasti mempunyai hubungan dengan pemalar partisi bagi pestisid yang dianalisis. Bagi HF-LPME 3-fasa pula, analisis dijalankan menggunakan kromatografi cecair prestasi tinggi (HPLC). Nitrofenol diekstrak daripada 2.5 mL fasa penderma dengan mengubah pH di antara julat 3 – 5 melalui fasa organik (1-heksanol) yang terjerap pada liang-liang gentian ke dalam 5.0 μ L fasa penerima (pH 12.0) yang terletak pada hujung penyuntik mikro. Kemudian, titisan mikro tadi disuntik ke dalam HPLC dengan parameter yang telah dioptimumkan; pelarut sampel (1.0 M H_3PO_4), pH (3.0-5.0), pelarut organik (1-heksanol), 5 μ L larutan penerima (0.1 M NaOH, pH 8.0-12.0), kadar pengadukan (1050 rpm), dan masa pengekstrakan (15 min). Graf kalibrasi adalah linear di dalam julat 0.6-200 μ g/L dengan $r^2 > 0.9994$. Faktor pemekatan melebihi 500-lipat telah berjaya diperolehi. Penentuan pemalar partisi ($K_{a/d}$) bagi nitrofenol telah berjaya dikaji. Kajian mendapati nilai $K_{org/d}$ bagi 2-nitrofenol and 3-nitrofenol serta nilai partisi individu ($K_{org/d}$ dan $K_{a/org}$) yang tinggi telah menggalakkan proses pengekstrakan analit daripada fasa penderma ke dalam fasa organik dan seterusnya ke dalam fasa penerima. Kedua-dua teknik ini telah berjaya digunakan untuk menganalisis sampel air.

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LIST OF ABBREVIATIONS

EU	European Union
MRLs	Maximum Residue Levels

GC	Gas Chromatography
GC-ECD	Gas Chromatography-Electron Capture Detection
GC-MS	Gas Chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
CE	Capillary Electrophoresis
LLE	Liquid-liquid Extraction
SPE	Solid-phase Extraction
SPME	Solid-phase Microextraction
LPME	Liquid-phase Microextraction
HF-LPME	Hollow Fiber Liquid-phase Microextraction
EPA	Environmental Protection Agency
MDL	Method Detection Limit
$K_{org/d}$	Partition ratio at equilibrium between the organic phase and the donor solution
$K_{a/org}$	Partition ratio at equilibrium between the acceptor solution and the organic phase
AED	Atomic Emission Detector
FT-IR	Fourier transforms infrared spectrometry
TCD	Thermal Conductivity Detector
FID	Flame Ionization Detector
HAAs	Haloacetic acids
LLLME	Liquid-liquid-liquid microextraction
PAA	Phenylacetic acid
PPA	Phenylpropionic acid
AR	Androgen receptor
TMPAH	Trimethylphenylammonium hydroxide
TMSH	Trimethylsulfonium hydroxide
D	Distribution coefficient
SLM	Supported Liquid Membrane
LOD	Limit of Detection
RSD	Relative Standard Deviation
EF	Enrichment factor
R_s	Resolution
k	Retention factor

t_R	Retention time
α	Separation factor

CHAPTER 1

INTRODUCTION

1.1 Research Background

According to the status list of all active pesticide substances on the European Union (EU) market more than 1100 pesticides are currently registered (Hercegová *et al.*, 2007). The pesticides industry in Malaysia is made up of about 140 companies, both multinational and local companies that are involved in manufacturing, formulating or trading activities. The majority of pesticides are imported as technical materials, which are then blended, diluted or formulated. However, in recent years an increasing variety of pesticides are manufactured in Malaysia. Pesticides are widely used in Malaysia for agricultural activities due to their relatively low price and high effective ability to control pests, weeds, and diseases (Xiong and Hu, 2008). The increasing production of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground, and surface water which involves a serious risk to the environment and as well as human health due to either direct exposure or through residues in food and drinking water (Pan and Ho, 2004). The need for accurate determination of pesticides at the trace levels in the environmental samples is therefore obvious. With the improvement of self-safeguard consciousness and the development in analytical instruments, levels of pesticides in vegetables and fruits are currently regulated by international and national organizations and maximum residue levels (MRLs) have been established in many countries (Pan *et al.*, 2008).

Phenols and substituted phenols are important pollutants in water because of their wide use in many industrial processes such as the manufacture of plastics, dyes, drugs, antioxidants, and pesticides. Nitrophenols are formed photochemically in the atmosphere from vehicle exhaust. They are very toxic and have a diverse effect on the taste and odour of drinking water at low concentrations, so they are environmentally of particular interest and concern. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the most common analytical techniques used for the determination of phenols. However, in GC, derivatization is needed to analyze phenols in order to avoid peak tailing.

Sample preparation is normally required to isolate and concentrate compounds of interest from the sample matrix, before analysis (Xu *et al.*, 2007). Ultimately, the concentration of target compounds is enhanced (enrichment) and the presence of matrix components is reduced (sample clean up). In order to achieve a low detection limit, an enrichment step should be conducted prior to analysis (Liu *et al.*, 2007).

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are the classical techniques (Liu *et al.*, 2007) for sample pre-concentration and isolation in analytical chemistry. However, LLE and SPE are time-consuming, generally labour-intensive, and requires use of large amounts of expensive high-purity organic solvents, which are often hazardous. During the last 10 years, some interest has been focused on the miniaturizing of analytical LLE. The major idea behind this has been to facilitate automation, to speed up extractions, and to reduce the consumption of organic solvents.

An attractive alternative pre-treatment method to the traditional technique is solid-phase microextraction (SPME). SPME was developed by Pawliszyn and coworkers (Gallardo *et al.*, 2006; Lee *et al.*, 2007). SPME is a solvent-free extraction technique that incorporates sample pre-treatment, concentration, and sample introduction into a single procedure (Lambropoulou and Albanis, 2006; Beceiro-Gonzalez *et al.*, 2007). But the extraction fiber is expensive, fragile, and has a limited lifetime. Miniaturized LLE or liquid-phase microextraction (LPME), was first introduced in 1996 by Jeannot and Cantwell (Xu *et al.*, 2007), and was based on a droplet of organic solvent hanging at the end of a micro syringe needle. Although hanging drop LPME is very simple and efficient, and reduces the consumption of organic solvents per sample to a few μL , it is still used only in limited number of research laboratories. One reason for this may be the low stability of the hanging drop, which is easily lost into sample during extraction.

Alternatively, miniaturized LLE may be accomplished by hollow fiber protected LPME. In these systems, the small volume of extracting liquid is contained within the lumen of a porous hollow fiber. The major advantage of this is that the

extracting liquid is mechanically protected, and it is prevented from leaking into the sample during extraction. This is especially important since LPME is conducted with strong agitation of the sample to speed up the extractions. Hollow fiber LPME can be accomplished both in the two- and three-phase modes.

Compared with LLE and SPE, LPME gives a comparable and satisfactory sensitivity and much better enrichment of analytes, and the consumption of solvent is significantly reduced by up to several hundred or several thousand times (Xiao *et al.*, 2007). LPME is applicable to neutral compounds with the two-phase system, and the acidic and basic substances utilizing either the two- or three-phase concept.

In the present study, the determination of the partition coefficient of selected analytes using double-phase and triple-phase LPME in water samples was carried out. Two-phase LPME was applied combined with gas chromatography-electron captured detection (GC-ECD) for the extraction and preconcentration of pesticides, while three-phase LPME was applied combined with HPLC-UV for the extraction and preconcentration of nitrophenols. Different aspects of the extraction procedure such as the kinds of organic solvent for the immobilization, compositions of the acceptor and donor phase, the extraction time, the agitation rate, and the volume of acceptor phase were investigated. The feasibility of this methodology is also evaluated by determining the enrichment factor, linearity, detection limit and recovery.

1.2 Statement of Problem

The present work therefore focuses on the development, validation and application of a HF-LPME method prior to HPLC or GC for the analysis of pesticides in water samples. It is expected that, the developed method will eliminate problems related to carry-over effects because hollow fibers utilized were used only once for every experiment. This single use adds high demands on the reproducibility of the manufacturing of the hollow fiber. Conventional extraction methods such as

SPE and LLE need more time-consuming operation and using specialized apparatus. In contrast, HF-LPME is inexpensive and there is considerable freedom in selecting appropriate solvents for extraction of different analytes. Since very little solvent is used, there is minimal exposure to toxic organic solvent for the operator. The optimization of several parameters influencing the efficiency of pesticides extraction such as extraction solvent, agitation speed, ionic strength of the aqueous sample and exposure time are also explored.

1.3 Research Objectives

The objectives of this research are:

- i. To study two-phase (liquid-[membrane]-liquid) liquid membrane extraction mechanism based on phase equilibrium and analyte partition distribution using non-ionizable compounds.
- ii. To study triple-phase (liquid-liquid [membrane]-liquid) liquid membrane extraction mechanism based on equilibrium and analyte partition distribution using ionizable compounds.
- iii. To apply and optimize the critical parameters for efficient extraction and pre-concentration of pesticides and nitrophenols.

Figure 1.1 shows a flow chart of work involved in this research to achieve the objectives.

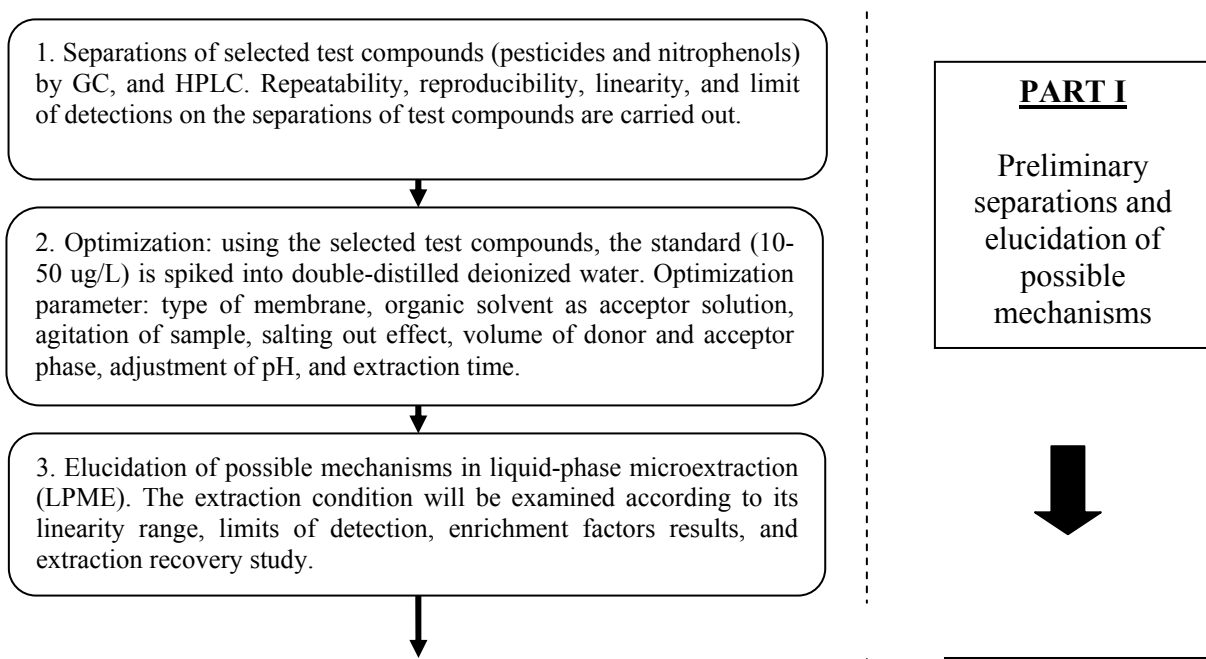


Figure 1.1: Flow chart of work involved in this study

1.4 Scope of Study

The study involves the use of hollow fiber liquid membrane in order to protect the extracting solvent, thus permitting extraction only on the surface of the solvent immobilized in the membrane pores. The research is divided into two main areas; to develop the HF-LPME using two-phase mode, (liquid-[membrane]-liquid) and three-phase mode, (liquid-liquid [membrane]-liquid).

For every mode of extraction, the condition of extraction will be optimized. The parameters of optimization consisted of type of membrane, organic solvent as acceptor solution, agitation of sample, salting out effect, volume of donor and acceptor phase, adjustment of pH, and extraction time.

1.5 Outline of the Thesis

This thesis consists of six chapters. Chapter 1 presents general introduction, research background, statement of problems, research objectives, and scope of study. Chapter 2 compiles the literature review and theoretical background on compounds studied, liquid-phase microextraction (LPME), analytical instruments for LPME, and application of LPME including double and triple-phase LPME. The procedures for LPME and chemicals used in this work are presented in Chapter 3. Chapter 4 and Chapter 5 report the results and discuss about the application of two-phase and three-phase LPME in water samples respectively. The concluding Chapter 6 summarizes this thesis by presenting the overall conclusions and suggestions for future study.

CHAPTER 2

LITERATURE REVIEW