LIQUID MEM

MALATHION

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ABSTRACT

The application of liquid membrane tip extraction (LMTE) for the extraction of organophosphorus pecticide (OPP) which is malathion was investigated. The LMTE was performed in dip extraction mode. The extraction parameters that were optimized are the type of extraction solvent, time of extraction and agitation of extraction. Quantification of the malathion was carried out using High Performance Liquid Chromatography (HPLC). Optimization was carried out using water sample spiked with malathion. Under the optimum conditions which are acetonitrile as a solvent of extraction, 60 min and 60 rpm, the method showed good regression which is 0.9986 and showed acceptable reproducibility (RSD 7%), low limits of detection of 1.2 mg/L for malathion, and satisfactory relative recoveries (90%). Due to the low cost, the LMTE device was disposed after each run. The validated method was tested for the analysis of analytes in spiked tap water with good success. LMTE expected to be able to provide low cost, simple technique and rapid extraction.

ABSTRAK

Pengekstrakan cecair sokongan membran menggunakan tip (LMTE) untuk pengekstrakan pestisid organofosforus (OPP) iaitu malation telah dikaji. LMTE telah dijalankan dalam mod pengekstrakan celupan. Parameter-parameter pengekstrakan yang telah dioptimumkan ialah jenis pelarut pengekstrakan, masa pengekstrakan dan pengocakan pengekstrakan. Analisis malation telah dijalankan menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC). Pengoptimuman telah dijalankan menggunakan sampel air dicampur dengan malation. Di bawah syarat-syarat optimum yang mana ialah asetonitril sebagai pelarut pengekstrakan, 60 min dan 60 rpm, kaedah menunjukkan regresi yang baik iaitu 0.9986 dan menunjukkan kebolehulangan semula yang boleh diterima (RSD 7%), had-had rendah pengesanan 1.2 mg/L untuk malathion, dan pemerolehan semula hubungan yang memuaskan (90%). Disebabkan kos rendah, peralatan bagi LMTE dibuang selepas setiap analisis. Kaedah telah diuji untuk analisis malation di dalam air tidak berion menunjukkan pengestrakan yang berjaya. LMTE dijangka mampu memberi kos yang rendah, teknik yang mudah dan pengekstrakan yang pantas.

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μ	Micro
Ω	Ohm

LIST OF ABBREVIATIONS

OPP	Organophosphorus
LLE	Liquid-Liquid Extraction
SPE	Solid Phase Extarction
ME	Micro Extraction
LMTE	Liquid Membrane Tip Extraction
AP	Acceptor Phase
AF	Acceptor Film
MP	Membrane Phase
EF	Enrichment Factor
PTFE	Polytetrafluoroethylene
UV-VIS	Ultra Violet Visible
I.D.	Internal Diameter
LOD	Limit of Detection
RSD	Relative Standard Deviation
HCl	Hidrochloric Acid
CSM-LPME	Cone-Shaped Membrane Protected Liquid Phase Microextraction

CHAPTER 1

INTRODUCTION

1.1 Introduction

The increasing production and application 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. In the world, alarming levels of pesticides have been reported in air, water, soil, as well as in foods and biological materials. However, chemists are facing difficulties with pesticides analysis, since the pesticides belong to different groups of chemical substances, having a broad range of polarity and acidic characteristics.

1.2 Background of Study

In general, herbicides are deliberately sprayed onto crops or farmland to prevent competition to agricultural products from unwanted plants. Determining these compounds is important, as potential exposure to them has increased due to their persistence in the environment, from the crop itself via the food chain, or through contamination of the soil. In recent years, most conventional methods of removing herbicides from soil and sediment samples, such as Soxhlet extraction, liquid-liquid extraction, and ultrasonic extraction, have largely been based on solvent extraction (Gertenbanch, 2002). Numerous solvents, such as hexane, acetone, ethyl acetate, methanol and dichloromethane, are used in large quantities, resulting in high volumes of waste that requires further treatment. These organic solvents are not only flammable, but some are toxic, thus hazardous and environmentally unfriendly. In practice these extractions need a require amount of sample and long extraction times, which prone to the coextraction of many interfering substances, and ultimately lead to multiple clean-up steps and loss of analyte.

1.3 Objectives of Study

The objectives of this study are

i) To fabricate and construct the liquid membrane tip extraction for determination of malathion in deionized water.

ii) To optimize the extraction parameters which are type of solvent used, time of extraction and agitation of extraction.

At the end of this study, these objectives will help in validating method for palm oil for liquid membrane tip extraction.

1.4 Scope of study

In order to achieve the objectives, the scopes of the study are identified as follows:

i) The validated method will be tested for the analysis of malathion spiked with deionized water.

CHAPTER 2

LITERATURE REVIEW

2.1 Extraction of Pesticides

Pesticide concentrations in real samples (water, fruit and vegetables) are frequently very low and their direct determination is not possible; it is therefore necessary to perform enrichment and separation. To determine organophosphorus (OPPs) pesticides in aqueous samples, the procedures used differ according to the pesticide's volatility. In samples with volatile pesticides, dynamic extraction systems ("purge and trap" or "delayed injection preconcentration") or static techniques such as "head-space analysis" (Cole and Woolfendan, 1992), which is now highly recommended, are used. For less volatile pesticides, supercritical fluid extraction (Pershina *et al.*, 1989) can be performed, although the most common procedures are based on LLE (liquid-liquid extraction) and SPE (solid-phase extraction) (Pershina *et al.*, 1989).

SPE uses solid phase adsorbent loaded in cartridge. The most popular adsorbent for the OPPs are C_{18} (Manes *et al.*, 1989), C_8 (Johnson *et al*, 1999) or Florisil (Barcelo *et*

al., 1990), or two of them in series (Pershina *et al.*, 1989). Recently, however, other types of solid phases have been proposed, such as Carbopack B (Johnson *et al.*, 1991). The cartridge, with the retained pesticides, is dried by air flow to eliminate any residual water, and the compounds are generally eluted with an organic solvent, e.g. diethyl ether (Tomkins *et al.*, 1987), ethyl acetate (Manes *et al.*, 1989), hexane (Manes *et al.*, 1989), dichloromethane (Barcelo *et al.*, 1990) or with mixtures such as dichloromethane-acetonitrile-hexane (Johnson *et al.*, 1999). In recent years, great advances in the possibility of automating SPE systems have been made, and a system that performs all pre-treatment steps (SPE, nitrogen evaporation) automatically has been described (Barcelo *et al.*, 1990). The use of miniaturized systems, based on pre-concentration in the syringe used in the injection into the chromatograph, is an interesting alternative (Arthur *et al.*, 1992), but it still needs more sensitivity.

The most common procedure is LLE, which is carried out by subjecting the aqueous phase to consecutive extractions (normally two or three) with different proportions of the organic phase. The most useful solvents for performing the extraction are dichloromethane (Barcelo *et al.*, 1990), chloroform (Pershina *et al.*, 1989), hexane (Tsuchihashi *et al.*, 1988) or solvent mixtures such as chloroform-benzene; the use of solvents with a larger polarity, such as ethyl acetate (Mallet *et al.*, 1990), has also been proposed. Immediately after the extraction, the organic phase is dried with anhydrous sodium sulphate (Tomkins *et al.*, 1987). Currently, the interest are more towards the use of continuous extraction systems (in cross-current) to give complete automation of the process (Melcher *et al.*, 1990)

A very interesting alternative to the conventional LLE is microextraction (Cacho *et al.*, 1992). The aqueous solution must be saturated with inorganic salts in order to avoid high irreproducibility, which was observed when working with high phase-ratios (Murray, 1979). This technique is based on the physical-chemical principle that the solubility of organic compounds in water is due to their capacity to make hydrogen bonds. The addition of high concentrations of inorganic salts in the aqueous phase

reduces the capacity of these solvents to make hydrogen bonds with organic compounds; the solubility of these compounds and of the extraction solvent is thus decreased, which is advantageous for both quantitative extraction and separation of the phases.

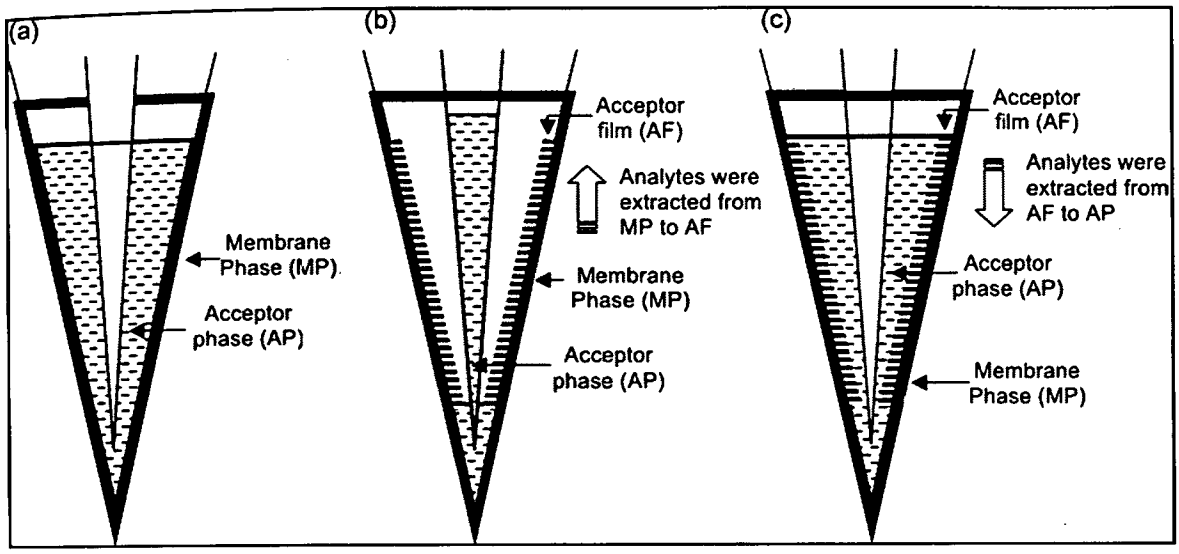
The procedure is based on the use of on organic phase (normally Kaltron) which is completely immiscible in water and an aqueous phase saturated with inorganic salts (monohydrated sodium dihydrogen phosphate and anhydrous ammonium sulphate). Up to now, this procedure has not been tested on OPPs, although with organochloride pesticides it generally produces good results (Cacho *et al.*, 1992). The use of this high ratio between the phases avoids the necessity of subjecting the organic extracts to further preconcentration processes by evaporation or gas flow [air (Kumar *et al.*, 1987) or nitrogen (Johnson *et al.*, 1991)], common to the methods that use conventional LLE or SPE, and gives a considerable saving of organic solvent.

Because of the small number of studies comparing the different extraction procedures (Kumar *et al.*, 1987), this paper includes a comparative study of the results obtained for the extraction of eight pesticides (chlorfenvinphos, diazinon, ethyl parathion, ethiofencarb, fenitrothion, malathion, metalaxyl, pirimicarb) by ME, conventional LLE and SPE. The determination procedure used was capillary gas chromatography with selective nitrogen-phosphorus detection (Sanz *et al.*, 1991).

2.2 Influence of Extraction Time

LMTE is a non-exhaustive extraction method and is generally based on phase equilibrium between donor, membrane and acceptor phase. Similar to other micro extraction methods, good accuracy of the results can be obtained easily with a spiked sample calibration for analytical quantification (Cacho *et al.*, 1992). For method optimization, it is therefore important to establish the extraction-time profiles of target analytes so as to configure the time after which equilibrium is attained in practice. Extractions were first performed to compare the static LMTE with the dynamic LMTE procedure. In order to generate comparable data for the static and dynamic procedures,

extractions were accomplished with the identical LMTE setup as well as the same extraction parameters and conditions. The only difference was the application of continuous withdrawal and discharge movements of acceptor solution in the dynamic LMTE. A description of the dynamic LMTE procedure is given in Figure 2.2.



Source: Sanagi M. S. *et al.*, (2008)

Figure 2.2: Expanded view of LMTE. (a) The AP resided in the membrane tip. (b) The AP is withdrawn and a thin layer of AF is left on the inner surface of membrane. (c) The AP is discharged back into the membrane and the analyte is transferred rapidly from AF into AP.

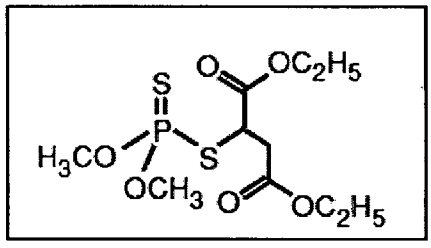
Extraction-time profiles using dynamic procedures were further investigated under different extraction times of 15 min, 30 min, 45 min, 60 min, 75, and 90 min. It can be observed that the enrichment factor (EF) increased radically on going from 15 min to 60 min and remained constant when longer extraction times (75 min and 90 min) were applied. It is apparent that the amount of extracted analyte increased with the prolonged extraction time and eventually reached maximum in the equilibrium stage. Based on the results obtained, an extraction time of 60 min was chosen as the optimum extraction time.

2.3 Malathion

Malathion is a man-made organophosphate insecticide that is commonly used to control mosquitoes and a variety of insects that attack fruits, vegetables, landscaping plants, and shrubs. It can also be found in other pesticide products used indoors and on pets to control ticks and insects, such as fleas and ants. Malathion is the active ingredient in mosquitocontrol products including Fyfanon and Atrapa. These products contain over 95% malathion and are often applied undiluted. However, they may be diluted with a petroleum solvent similar to kerosene before application, in which case petroleum solvent will make up most of the pesticide solution.

Table 2.3: Physical properties of malathion

Physical properties	Value
Molecular weight (g/mol)	330.35
Molecular formula	$C_{10}H_{19}O_6P_1S_2$
Log Ko/w	2.29
Boiling point (°C)	351.17
Melting point (°C)	-23.58
Vapor pressure at 25°C (mmHg)	$3.38e^{-6}$
Water solubility at 25°C (mg/L)	78.45



Source: Sanagi M. S. *et al.*, (2008)

Figure 2.3: Chemical structure of malathion

2.4 Liquid-Liquid Extraction (LLE)

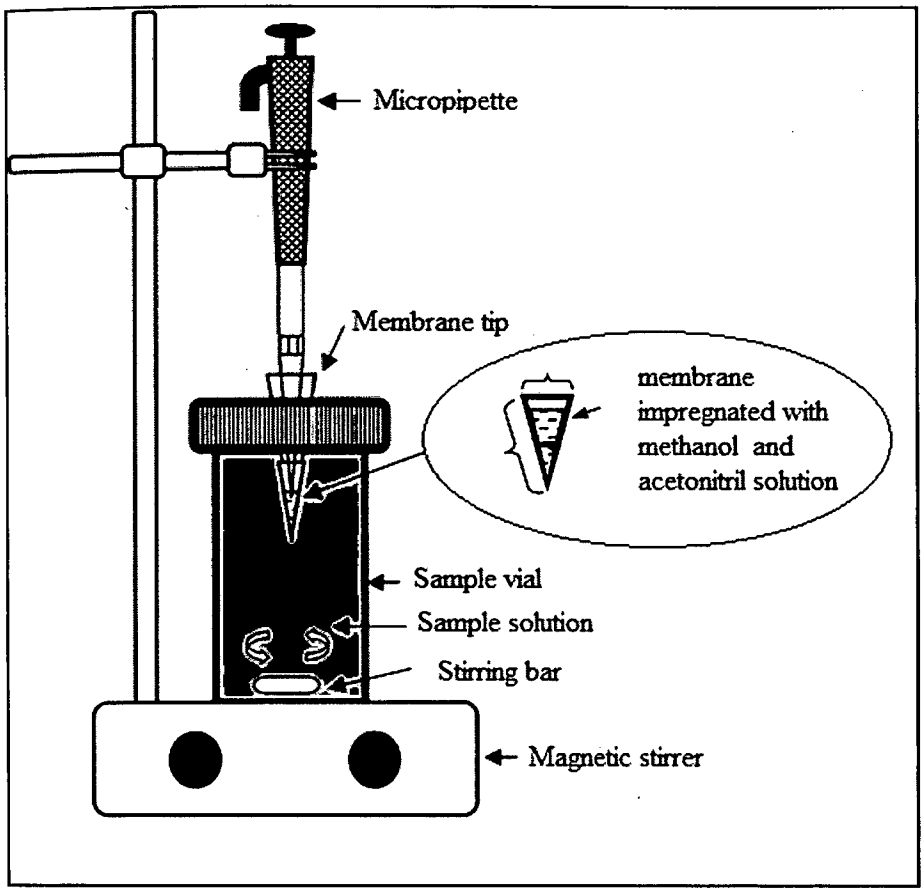
Sample preparation techniques such as ion-pair liquid–liquid extraction (LLE) (Eberbach, 1999) and ion-exchange solid phase extraction (SPE) methods (Coutinho *et al.*, 2008; Ibanez *et al.*, 2005) are commonly used to isolate glyphosate, glufosinate-ammonium and malathion from different matrices. The major drawback of these techniques is that they usually require high consumption of organic solvents, tedious procedures, and time consuming work while liquid membrane (LM) extraction is a versatile technique that provides high selectivity, flexibility, low operating cost and involves single-step operation (Khrolenko and Wieczorek, 2005; Dzygiel and Wieczorek, 2000). Generally, LM extraction involves the use of a porous polymeric hydrophobic membrane with organic solvent immobilized in its pores. The extraction mechanisms involve extraction of analyte from the donor phase into the organic membrane phase and re-extraction into the acceptor phase. When dealing with acidic and basic compounds, high enrichment in SLM extraction can be easily achieved by adjusting the donor phase and acceptor phase pH to appropriate values (Wieczorek *et al.*, 1997).

As for multicharged compounds, a cationic or anionic carrier incorporated in membrane organic phase should be used to transport the analyte of interest by forming ion-pair complexes and thus allowing diffusion through the organic liquid membrane into the acceptor stripping phase (Wieczorek *et al.*, 1997). Recently, SLM extraction by utilizing cationic and anionic carriers dissolved in the impregnation solvent, have been used for extraction of polar analytes such as organic compounds (Jonsson *et al.*, 1999), peptides (Drapala *et al.*, 2005), amino acids (Wieczorek *et al.*, 1997; Drapala *et al.*, 2005), antibiotics (Dzygiel *et al.*, 1998; Tao *et al.*, 2009), herbicides (Dzygiel *et al.*, 1998; Tao *et al.*, 2009), and heavy metals (Shariati *et al.*, 2009).

Solid phase extraction (SPE) is one of the widespread used methods that successfully redressed the limitations inherent in the classical LLE method. The SPE

technology is now keep growing especially in the research involving environmental sample preparation. The advancement of SPE technology has resulted in SPE membranes filters or membrane filter disks (Raynie *et al.*, 2006). The SPE membrane disks provide the opportunity to reduce solvent usage compared to classical LLE and traditional packed particle SPE. The applicability of fast sample flow rates as the mass transfer kinetics of the tightly packed particles allow recoveries that are independent of sample flow rate and significantly reduce the total extraction time (Westbom *et al.*, 2004; Tollback *et al.*, 2006).

The Liquid Membrane Tip Extraction device consisted of a home-made cone-shaped polypropylene membrane attached to a modified 1000 µL capacity pipette tip. A polypropylene sheet membrane will be cut into an isosceles triangle with sides of approximately 20 mm and base of 15 mm. The edge of the membrane will be folded to form a scalene triangle shape with sides of 20 mm, 18.5 mm and 7.5 mm. The edge of the longest flap will be then heat-sealed using a portable impulse heat sealer with seal width of 0.5 mm. The remaining open-end of membrane will be then cut to form a cone-shaped membrane with sides of 15 mm and width of 6 mm. A commercially available 1000 µL pipette tip will be slightly modified. The tip-end (~9 mm length) will be cut-off. The tip-end of the pipette tip will be inserted into the membrane's remaining open-end. The 1000 µL digital micropipette tip will be positioned into the membrane tip acceptor phase and the Liquid membrane Tip Extraction device will be exposed to the sample during the extraction as illustrated in Figure 2.4.



Source: M. S. Sanagi *et al.*, (2008)

Figure 2.4: Schematics of the Liquid Membrane Tip Extraction setup.

2.5 Membrane Filter

2.5.1 Nylon-66

Nylon-66, a polyamide derived from 1, 6-hexamethylene diamine and adipic acid, is a semicrystalline polymer, which possesses good thermal stability and mechanical strength, and is considered to be an important engineering thermoplastic (Tohgo *et al.*, 2001; Albano *et al.*, 2001; Murthy *et al.*, 2002). Porous polyamide membrane has been commercialized for many years and is nowadays widely used in fine-separation processes (Persson *et al.*, 2003; Castilho *et al.*, 2002). Microporous membranes are often manufactured by the so-called immersion–precipitation process (Mulder *et al.*, 1991), in which a polymer solution is cast on a substrate and then immersed in a nonsolvent bath to induce polymer precipitation by means of crystallization and/or liquid– liquid demixing. Unlike a nonporous Nylon-66 film, which has a water contact angle of ca. 60°, a skinless microporous Nylon-66 membrane is water wettable; i.e., water drops can penetrate into the membrane matrix within a few seconds. This property is associated with the porous morphology of the membrane.

Extractable compounds from nylon membrane filters generate significant background signals in UV absorption chromatograms at 214 nm, and are also detected by electrospray ionization mass spectrometry, with nominal *m/z* values of 453 and 679. It is shown that rinsing the nylon membranes before their use can reduce, but will not eliminate the extractable contaminants from the mobile phase and recommended that nylon membrane filters be avoided when conducting trace level analysis, particularly when conducting LC/MS experiments (Junk *et al.*, 1998).

2.5.2 Polytetrafluoroethylene (PTFE)

Polytetrafluoroethylene (PTFE) is a remarkable membrane material. PTFE membrane media for filtration is made of PTFE (polytetrafluorothylene), and were drawn 2-demension. It is micro-pore film. The PTFE membrane was laminated with

great variety of fabric and paper. They are new filter media. PTFE has been applied to extensive industries, including biochemistry, microelectronic, lab material and etc. Directly and indirectly related to pharmacy brewing, manufacture of pure water and special need water, beverage and dairy, chemical regent, biochemical regent, air filtration of fermentation tank in microelectronic, purification and filtration in microelectronic plants, filtration and separation of antibacterial fluid, production of medicine, air conditioning of hospitals and commercial buildings.

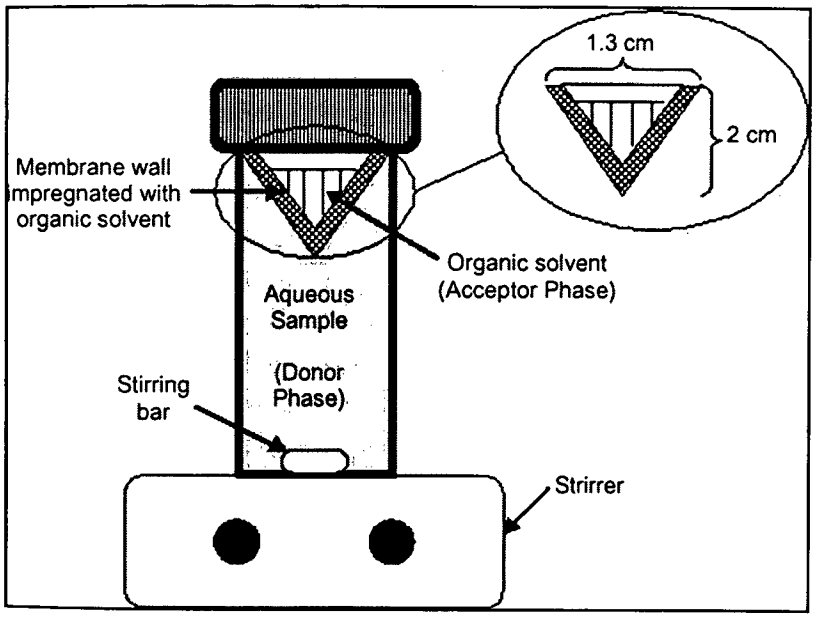
Table 2.5: Membrane filter overview

Membrane Media	Material	Pore Size (μm)	Diameter (mm)
Nylon-66 (μm)	Polymer (Hexamethylenediamine; Nylon-66)	0.2, 0.45, 0.8	13, 25, 47, 90
PTFE (μm)	Polytetrafluoroethylene	0.2, 0.5, 1.0	25, 47

2.6 Cone-Shaped Membrane Protected Liquid Phase Microextraction (CSM-LPME)

Round-shaped Nylon-66 membrane filters (200μm thickness and 0.2 μm pore size) were purchased from Whatman (USA). The filterwas cut into halves and each half was folded and sealed with flame into a cone-shaped membrane (CSM) with an open diameter of approximately 13 mm and height of approximately 20 mm to fit the vial I.D. (Figure 2.6). Before use, the CSM was ultrasonically cleaned in acetone for several minutes in order to remove any contaminants. Extraction was performed according to the following scheme. A 15 mL aliquot of sample solution was placed into a 15 mL sample vial. The membrane was immersed into organic solvent for approximately 10 s to allow the solvent to impregnate the pores of the membrane wall. After solvent

impregnation, the CSM was quickly positioned in the sample vial that already contained the aqueous sample, and a 200 μL aliquot of organic solvent was pipette into the membrane as shown in Figure 2.6. The sample was continuously stirred with a magnetic stirrer at room temperature (25 °C) to facilitate the mass transfer of the analytes between donor phase and acceptor phase. The agitation also significantly decreased the time required for the equilibrium to be established. After 20 min of extraction, the analyte-enriched solvent (100 μL) was withdrawn and transferred into a 1.5 mL cone shaped vial, the solvent was dried with gentle flow of nitrogen and redissolved with 100 μL of acetonitrile solution containing 1 ppm profenofos (I.S.). A 0.5 μL of solvent was withdrawn into a syringe and injected into micro-LC system for analysis.



Source: M. S. Sanagi *et al.*, (2008)

Figure 2.6: Schematic of CSM-LPME.