CHAPTER 1

INTRODUCTION

1. 1 Pesticides

The term "pesticide" is a composite term that includes all chemicals that are used to kill or control pests. In agriculture, this includes herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), and rodenticides (vertebrate poisons). Pesticides are used especially in agriculture and around areas where humans live. Some are harmful to humans, either from direct contact or as residue on food, or are harmful to the environment because of their high toxicity, such as DDT (which is now banned in many countries). Pesticides include fungicides, herbicides, insecticides, and rodenticide (The American Heritage Dictionary of the English Language, 2009; Stephenson and Solomon, 1993).

A fundamental contributor to the Green Revolution has been the development and application of pesticides for the control of a wide variety of insectivorous and herbaceous pests that would otherwise diminish the quantity and quality of food produce. The use of pesticides coincides with the "chemical age" which has transformed society since the 1950s. In areas where intensive monoculture is practised, pesticides were used as a standard method for pest control. Unfortunately, with the benefits of chemistry have also come disbenefits, some so serious that they now threaten the long-term survival of major ecosystems by disruption of predator-prey relationships and loss

of biodiversity. Also, pesticides can have significant human health consequences (Stephenson and Solomon, 1993).

1.2 Historical Development of Pesticides

The history of pesticide development and use is the key to understanding how and why pesticides have been an environmental threat to aquatic systems, and why this threat is diminishing in developed countries and remains a problem in many developing countries. Stephenson and Solomon (1993) outlined the chronology of development of pesticides and were presented in Table 1.1below.

Table 1.1: Chronology of Pesticides Development (Stephenson and Solomon, 1983)

Period	Examples	Sources	Characteristics
1800-1920s	Early organics, nitro-phenols, chlorophenols, creosote, naphthalene, petroleum oils.	Organic chemistry, by-products of coal gas production, etc.	Often lack specificity and were toxic to user or non-target organisms.
1945-1955	Chlorinated organic, DDT, HCCH, chlorinated cyclodienes.	Organic synthesis.	Persistent, good selectivity, good agricultural properties, good public health performance, resistance, harmful ecological effects.

1945-1970	Cholinesterase inhibitors, organophosphorus compounds, carbamates.	Organic synthesis, good use of structure-activity relationships.	Lower persistence, some user toxicity, some environmental problems.
1970-1985	Synthetic pyrethroids, avermectins, juvenile hormone mimics, biological pesticides.	Refinement of structure activity relationships, new target systems.	Some lack of selectivity, resistance, costs and variable persistence.
1985-present	Genetically engineered organisms.	Transfer of genes for biological pesticides to other organisms. Genetic alteration of plants to resist non-target effects of pesticides.	Possible problems with mutations and escapes, disruption of microbiological ecology, monopoly on products.

1.3 Pesticides Toxicity in Aquatic System

The toxicity of pesticides in aquatic systems is major significance of the pesticides uses. The ecological impacts of pesticides in water are determined by the following criteria which listed in Table 1.2 below (Munkittrick *et al.*, 1994; Calamari and Barg, 1993).

Table 1.2: Criteria of the Ecological Impact of Pesticides in Aquatic System (Munkittrick *et al.*, 1994; Calamari and Barg, 1993; OMAF, 1991).

Criteria	**Descriptions**
Toxicity	Mammalian and non-mammalian toxicity usually expressed as LD_{50} ("Lethal Dose": concentration of the pesticide which will kill half the test organisms over a specified test period). The lower the LD_{50}, the greater the toxicity; values of 0-10 are extremely toxic (OMAF, 1991). Drinking water and food guidelines are determined using a risk-based assessment. Generally, Risk = Exposure (amount and/or duration) × Toxicity. Toxic response (effect) can be acute (death) or chronic (an effect that does not cause death over the test period but which causes observable effects in the test organism such as cancers and tumours, reproductive failure, growth inhibition, teratogenic effects, etc.).
Persistence	Measured as half-life (time required for the ambient concentration to decrease by 50%). Persistence is determined by biotic and abiotic degradational processes. Biotic processes are biodegradation and metabolism; abiotic processes are mainly hydrolysis, photolysis, and oxidation (Calamari and Barg, 1993). Modern pesticides tend to have short half lives that reflect the period over which the pest needs to be controlled.

Depredates	The degradation process may lead to formation of "degradates" which may have greater, equal or lesser toxicity than the parent compound. As an example, DDT degrades to DDD and DDE.
Fate (Environmental)	The environmental fate (behaviour) of a pesticide is affected by the natural affinity of the chemical for one of four environmental compartments (Calamari and Barg, 1993): solid matter (mineral matter and particulate organic carbon), liquid (solubility in surface and soil water), gaseous form (volatilization), and biota. This behaviour is often referred to as "partitioning" and involves, respectively, the determination of: the soil sorption coefficient (K_{OC}); solubility; Henry's Constant (H); and the n-octanol/water partition coefficient (K_{OW}). These parameters are well known for pesticides and are used to predict the environmental fate of the pesticide.

1.4 Effect of Pesticides

1.4.1 Human Health

UNEP (1993) was linked the effects of pesticides to "the level of oncological (cancer), pulmonary and haematological morbidity, as well as on inborn deformities and immune system deficiencies". Human health effects are caused by the skin contact inhalation as well as ingestion. These were occurs maybe during the handling of pesticides products,

breathing of dusts or spray and pesticides was consumed as a contaminant on/in food or in water respectively.

Farm workers have special risks associated with inhalation and skin contact during preparation and application of pesticides to crops. However, for the majority of the population, a principal vector is through ingestion of food that is contaminated by pesticides. Degradation of water quality by pesticide runoff has two principal human health impacts. The first is the consumption of fish and shellfish that are contaminated by pesticides. This can be a particular problem for subsistence fish economies that lie downstream of major agricultural areas. The second is the direct consumption of pesticide-contaminated water. WHO (1993) has established drinking water guidelines for 33 pesticides. Many health and environmental protection agencies have established "acceptable daily intake" (ADI) values which indicate the maximum allowable daily ingestion over a person's lifetime without appreciable risk to the individual. As an example, in a recent paper reported by Wang and Lin (1995) studying substituted phenols, tetrachlorohydroquinone, a toxic metabolite of the biocide pentachlorophenol, was found to produce "significant and dose-dependent DNA damage".

1.4.2 Ecological Effect

Pesticides are included in a broad range of organic micro pollutants that have ecological impacts. Different categories of pesticides have different types of effects on living organisms; therefore generalization is difficult (WHO, 1993). Although terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is

that of water contaminated by pesticide runoff. The two principal mechanisms are bioconcentration and biomagnification.

Bioconcentration define as the movement of a chemical from the surrounding medium into an organism. The primary "sink" for some pesticides is fatty tissue ("lipids"). Some pesticides, such as DDT, are "lipophilic", meaning that they are soluble in, and accumulate in, fatty tissue such as edible fish tissue and human fatty tissue. Other pesticides such as glyphosate are metabolized and excreted (Jonsson *et al.*, 1990; Torstensson, 1990). Meanwhile, biomagnification is the term that describes the increasing concentration of a chemical as food energy is transformed within the food chain. As smaller organisms are eaten by larger organisms, the concentration of pesticides and other chemicals are increasingly magnified in tissue and other organs. Very high concentrations can be observed in top predators, including man (Jonsson *et al.*, 1990; Torstensson, 1990).

The ecological effects of pesticides are varied and are often inter-related. Effects at the organism or ecological level are usually considered to be an early warning indicator of potential human health impacts (Torstensson, 1990). The major types of effects are death of the organism, reproductive inhibition or failure, suppression of immune system, disruption of endocrine (hormonal) system, cellular and DNA damage, teratogenic effects (which is related to physical deformities such as hooked beaks on birds. In addition, cancers, tumours and lesions on fish and animals also were observed. Intergenerational effects (effects are not apparent until subsequent generations of the organism), poor fish health marked by low red to white blood cell ratio, excessive slime on fish scales and gills, and other physiological effects such as egg shell thinning were also observed as the major effects of pesticides (Torstensson, 1990).

The effects of pescticides will vary depending on the organism under investigation and the type of pesticide. Different pesticides have markedly different effects on aquatic life which makes generalization very difficult. The important point is that many of these effects are chronic (not lethal), are often not noticed by casual observers, yet have consequences for the entire food chain (Torstensson, 1990). These effects are not necessarily caused solely by exposure to pesticides or other organic contaminants, but may be associated with a combination of environmental stresses such as eutrophication and pathogens. These associated stresses need not be large to have a synergistic effect with organic micro pollutants (Torstensson, 1990).

Ecological effects of pesticides extend beyond individual organisms and can extend to ecosystems. Swedish work indicates that application of pesticides is thought to be one of the most significant factors affecting biodiversity. Jonsson *et al.* (1990) reported that the continued decline of the Swedish partridge population is linked to changes in land use and the use of chemical weed control. Chemical weed control has the effect of reducing habitat, decreasing the number of weed species, and of shifting the balance of species in the plant community. Swedish studies also show the impact of pesticides on soil fertility, including inhibition of nitrification with concomitant reduced uptake of nitrogen by plants (Torstensson, 1990). These studies also suggest that pesticides adversely affect soil micro-organisms which are responsible for microbial degradation of plant matter (and of some pesticides), and for soil structure (Stephenson and Solomon, 1993).

1.5 Pesticide Monitoring in Water

Monitoring data for pesticides are generally poor in much of the world and especially in developing countries. Key pesticides are included in the monitoring schedule of most western countries, however the cost of analysis and the necessity to sample at critical times of the year (linked to periods of pesticide use) often preclude development of an extensive data set. Many developing countries have difficulty carrying out organic chemical analysis due to problems of inadequate facilities, impure reagents, and financial constraints. New techniques using immunoassay procedures for presence or absence of specific pesticides may reduce costs and increase reliability. Immunoassay tests are available for triazines, acid amides, carbamates, 2,4-diphenoxy acid, paraquot and aldrin (Rickert, 1993).

Data on pesticide residues in fish for lipophilic compounds, and determination of exposure and/or impact of fish to lipophobic pesticides through liver and/or bile analysis is mainly restricted to research programmes. Hence, it is often difficult to determine the presence, pathways and fate of the range of pesticides that are now used in large parts of the world. In contrast, the ecosystemic impacts from older, organochlorine pesticides such as DDT became readily apparent and have resulted in the banning of these compounds in many parts of the world for agricultural purposes (Rickert, 1993).

Table 1.3 indicates why older pesticides, together with other hydrophobic carcinogens such as PAHs and PCBs, are poorly monitored when using water samples (Ongley *et al.,* 1992). As an example, the range of concentration of suspended solids in rivers is often between 100 and 1000 mg/l except during major runoff events when

concentrations can greatly exceed these values. Tropical rivers that are unimpacted by development have very low suspended sediment concentrations, but increasingly these are a rarity due to agricultural expansion and deforestation in tropical countries (Ongley *et al.*, 1992).

Table 1.3: Proportion of selected pesticides found in association with suspended sediment.

Pesticide	log K_{OW}	% of chemical load at different concentrations (mg/l) of suspended sediment			
		mg/l = 10	mg/l = 100	mg/l = 1000	mg/l = 10000
Aldrin	5.5	15	55	90	100
Atrazine	2.6	0	0	2	20
Chlordane	6.0	30	75	95	100
DDT	5.8	20	67	93	100
Dieldrin	5.5	15	55	90	100
Endrin	5.6	18	57	90	100
Endosulfan	3.6	0	0	21	57
Heptachlor	5.4	13	48	88	100
Lindane	3.9	0	2	30	80
Mirex	6.9	75	95	100	100
Toxaphene[1]	3.3	0	0	12	47
Trifluralin	5.3	12	45	87	100
2,4-D	2.0[2]	0	0	0	4

[1]Toxaphene mixture.

[2] Range is 1.5-2.5.

As an example, approximately 67% of DDT is transported in association with suspended matter at sediment concentrations as low as 100 mg/l, and increases to 93% at 1000 mg/l of suspended sediment (Ongley *et al.*, 1992). Given the analytical problems of inadequate detection levels and poor quality control in many laboratories of the developing countries, plus the fact that recovery rates (part of the analytical procedure) can vary from 50-150% for organic compounds, it follows that monitoring data from water samples are usually a poor indication of the level of pesticide pollution

for compounds that are primarily associated with the solid phase. The number of NDs (Not Detectable) in many databases is almost certainly an artifact of the wrong sampling medium (water) and, in some cases, inadequate analytical facilities and procedures. Clearly, this makes pesticide assessment in water difficult in large parts of the world. Experience suggests that sediment-associated pesticide levels are often much higher than recorded, and NDs are often quite misleading. Some water quality agencies now use multi-media (water + sediment + biota) sampling in order to more accurately characterize pesticides in the aquatic environment (Ongley *et al.,* 1992).

Another problem is that analytical detection levels in routine monitoring for certain pesticides may be too high to determine presence/absence for protection of human health. Gilliom (1984) noted that the US Geological Survey's Pesticide Monitoring Network in 1984 had a detection limit of 0.05 mg/l for DDT, yet the aquatic life criterion is 0.001 mg/l and the human health criterion is 0.0002 mg/l - both much less that the routine detection limit of the programme. NDs values, therefore, are not evidence that the chemical is not present in concentrations that may be injurious to aquatic life and to human health. That this analytical problem existed in the United States suggests that the problem of producing water quality data that can be used for human health protection from pesticides in developing countries must be extremely serious. Additionally, detection limits are only one of many analytical problems faced by environmental chemists when analysing for organic contaminants (Gilliom, 1984).

Pesticide monitoring requires highly flexible field and laboratory programmes that can respond to periods of pesticide application, which can sample the most appropriate medium (water, sediment, biota), are able to apply detection levels that have meaning for human health and ecosystem protection, and which can discriminate between those

pesticides which appear as artefacts' of historical use versus those that are in current use (Rickert, 1993; Stephenson and Solomon, 1993). For pesticides that are highly soluble in water, monitoring must be closely linked to periods of pesticide use. In the United States where there have been major studies of the behaviour of pesticide runoff, the triazines (atrazine and cyanazine) and alachlor (chlorinated acetamide) are amongst the most widely used herbicides. These are used mainly in the spring which in month of May. Studies conducted by Schottler *et al.* (1994) indicate that 55-80% of the pesticide runoff occurred in the month of June (Figure 1.1).

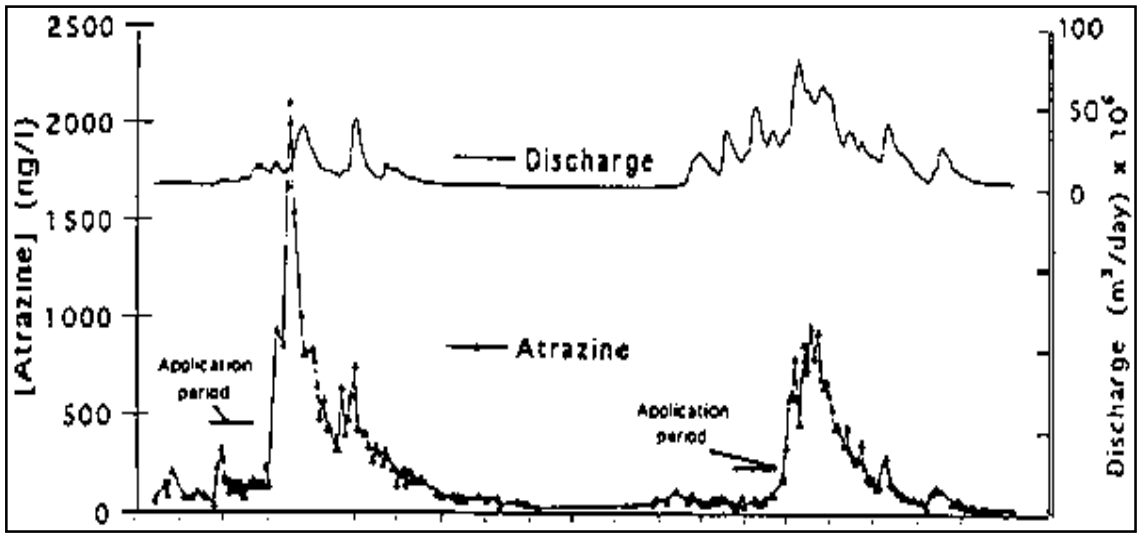

Figure 1.1: Occurrence of Atrazine, a widely used Herbicide in Surface Water.

The significance for monitoring is that many newer and soluble pesticides can only be detected shortly after application; therefore, monitoring programmes that are operated on a monthly or quarterly basis (typical of many countries) are unlikely to be able to quantify the presence or determine the significance of pesticides in surface waters (Rickert, 1993; Stephenson and Solomon, 1993). Pesticides which have limited application are even less likely to be detected in surface waters. The danger was lies in the presumption by authorities that NDs values imply that pesticides are absent. It may

well only mean that monitoring programmes failed to collect data at the appropriate times or analysed the wrong media (Stephenson and Solomon, 1993).

1.6 Sample Preparation

Sample preparation is a crucial step for its whole analysis and is often a bottleneck to rapidly obtain an accurate and sensitive result in an analysis. Traditional methods for sample preparation including liquid-liquid extraction, soxhlet extraction, chromatography, distillation, and absorption (Huang, 1994), usually suffer from the disadvantages of time-consuming and tedium, large amounts of toxic organic solvent to be used, and difficulty in automation to some extent. Therefore, a lot of research efforts in separation science and related fields have been focused on the development of new sample preparation techniques, which are less time-consuming, more effective, and require smaller amounts of organic solvents (Raynie, 2004; Falqui-cao *et al*., 2001; Wang *et al*., 2007; Hu *et al*., 2008).

In recent years, a lot of new sample preparation methods have been developed, such as solid-phase extraction (SPE) (Zhang and Zhu, 2000), molecular imprinting technique (MIT) (Vlatakis *et al*., 1993), solid-phase microextraction (SPME) (Arthur and Pawliszyn, 1990), single-drop microextraction (SDME)(Jeannot and Cantwell, 1996), and hollow fiber-based liquid-phase microextraction (HF-LPME). However, SPE is time-consuming and relatively expensive, sometimes shows a poor batch-to-batch reproducibility, and still needs a large amount of organic solvents.

For MIT, the recognition ability is greatly affected by solvent and the selectivity in aqueous solution is very poor. In the case of SPME, the fiber is quite expensive and

fragile, with limited lifetime, and sometimes encounters sample carry-over problems. Although SPME coupled with Gas Chromatography, GC is very effective for some applications, a special desorption apparatus is needed when it is used in coupling with HPLC. Single-drop micro extraction often requires careful manual operation to prevent drop dislodgment and is instable especially when high-speed stirring is used. In addition, an extra filtration step is usually needed for the sample solutions with complex matrixes, and its sensitivity and the precision still need further improvements (Psillakis and Kalogerakis, 2002).

1.6 .1 Dispersive Liquid-Liquid Microextraction (DLLME)

Recently, a novel microextraction technique, dispersive liquid-liquid microextraction (DLLME), has been reported (Rezaee *et al*., 2006). DLLME uses an extraction solvent mixture including a high-density non-polar water immiscible solvent (extraction solvent) and a polar water miscible solvent (disperser solvent). This method is based on a ternary component solvent system in which the extraction solvent and disperser solvent are rapidly injected into the aqueous sample by syringe. The mixture is then gently shaken and a cloudy solution (water/disperser solvent/extraction solvent) was formed in the test tube. After centrifugation, the fine particles of extraction solvent were sedimented in the bottom of the conical test tube. The resultant sedimented phase is taken with a microsyringe and injected into GC for further analysis (Rezaee *et al*., 2006).

DLLME involving two major steps which consist of injection of an appropriate mixture of the extraction and dispersive solvent into the aqueous samples which contain the

analytes. In this step, the extraction solvent is dispersed into the aqueous sample as very fine droplets and the analytes are enriched into it. Owing to the considerably large surface area between the extraction solvent and the aqueous sample, the extraction occurs very fast and consequently, the equilibrium state is reached quickly which is of great advantage. On the other hand, the second steps are involves is centrifugation of the cloudy solution is then carried out prior to the determination of the analytes in the sedimented phase by an instrumental analysis (Sorouraddin and Khoshmaram, 2010; Rezaee *et al*., 2006).

1.6.2 Principle of DLLME

Dispersive liquid–liquid microextraction is a miniaturized liquid-liquid extraction, LLE using microliter volumes of extraction solvent, which is based on the equilibrium distribution process of the target analytes between sample solution and extraction solvent. Distribution coefficient (*K*) is defined as the ratio between the analyte concentration in extraction solvent and sample solution. Dispersive liquid-liquid microextraction is only applicable for the analytes with high or moderate lipophilic property ($K > 500$) and not fit to those neutral analytes with high hydrophilic property. As for the acidic or alkaline analytes, distribution coefficient could be increased by controlling the pH value of sample solution, making the analytes existing in nonionic state. The enrichment factor and extraction recovery are calculated as follows (Rezaee *et al*., 2006).

$$F = C\text{sed}/C\text{o} \qquad \textbf{(1)}$$

$$R = (C\text{sed}\ V\text{sed})/(C\text{o}\ V\text{aq}) \qquad \textbf{(2)}$$

where, *F*, *C*sed and *C*o are the enrichment factor, the analyte concentration in the sediment, and the initial concentration of analyte in the aqueous sample, respectively; *R*, *V*sed and *V*aq are the extraction recovery, the volume of the sediment phase, and the volume of the aqueous sample, respectively. The extraction steps of DLLME are illustrated in Figure 1.2.

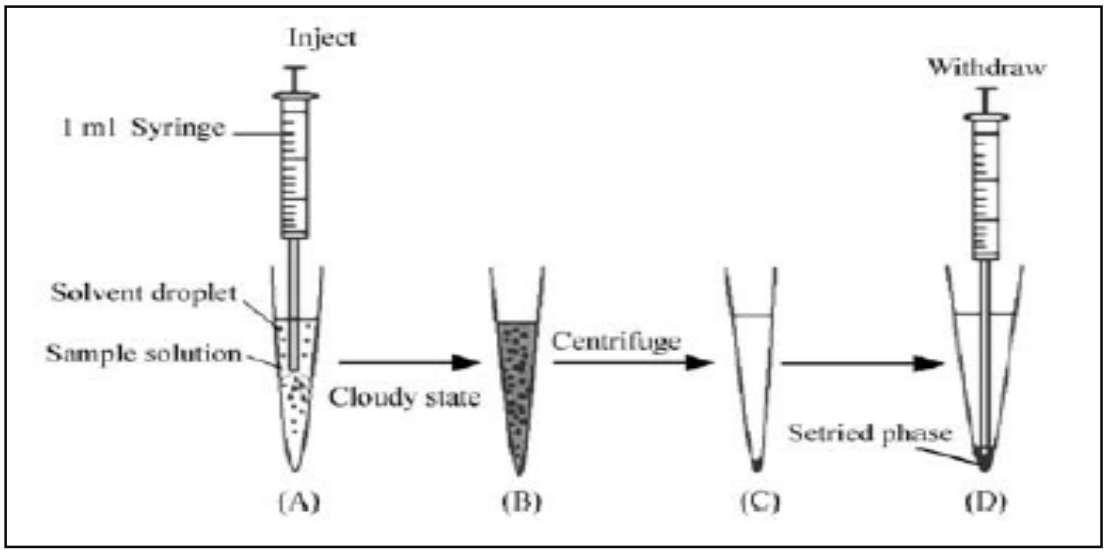

Figure 1.2: Dispersive Liquid-Liquid Microextraction Procedure.

A certain volume of sample solution is placed in a 10-mL screw cap glass test tube with conic bottom (A), followed by the rapid injection of disperser solvent containing extraction solvent into the sample solution with a syringe or pipette. Then, the mixture was gently shaken; thus, a cloudy solution (water/disperser solvent/extraction solvent) is formed in the test tube (B). After that, the surface area between extraction solvent and aqueous phase (sample) is infinitely large, thereby, transition of analyte from aqueous phase (sample) to extraction phase is fast. Subsequently, equilibrium state is achieved quickly, resulting in a very short extraction time, which is the remarkable advantage of DLLME compared with those of other techniques. Finally, the dispersed fine particles of extraction phase are sedimented in the bottom of conical test tube through

centrifugation (C). A certain volume of the sedimented phase is injected into chromatographic system using a micro syringe for further analysis (D).

1.6.3 Advantage and Disadvantages of DLLME

DLLME offer several advantages as per reported by scientists. Rapidity, high enrichment factor, operation simplicity and low cost as well as and environmental benignity are some of the advantages of the method (Sorouraddin and Khoshmaram, 2010; Rezaee *et al*., 2006).

However, the main disadvantage of the DLLME is that it is not a selective extraction technique and also fails if phases do not separate even after centrifugation (in the case of heavily contaminated extracts) Thus, in order to overcomethis problem it is necessary to include a clean-up stage after the analyte leaching from the sample and previous to DLLME technique (Rezaee *et al*., 2006).

1.6.4 Application of DLLME

DLLME have high preconcentration capabilities in a very short time. Because it is a simple and rapid method for extraction and preconcentration of organic compounds from water sample, DLLME has been used to determine of polycyclic aromatic hydrocarbons, PAHs (Rezaee *et al.,* 2006), triazine herbicides (Nagaraju and Huang, 2007), polychlorinated biphenyls, PCBs (Rezaei *et al.,* 2008), chlorophenols (Fattahi *et al.,* 2007) and organophosphorus flame retardants (García-López and Cela, 2007) in water samples.

As a novel sample preparation method, DLLME can be coupled with GC, HPLC, and AAS for application. It has been widely applied to the analyses of pesticide residues, heavy metals, and others. Table 1.4 below shows the typical application of DLLME (Zang *et al*., 2009).

Table 1.4: Application of DLLME Coupled with Different Analytical Instruments.

Analytes	**Analytical Method**	**Extraction Solvent**	**Disperser Solvent**	**LOD (μgL^{-1})**	**References**
PAH	GC-FID	C_2Cl_4	ACN	0.007-0.030	Rezaee *et al.*, 2009
Amitriptyline and Nortriptyline	GC-FID	CCL_4	MeOH	0.005- 0.01	Yazdi *et al.*, 2008
Phthalate esters	GC-MS	C_6H_5Cl	ACN	0.002-0.008	Farahani *et al.*, 2007
Fragrances, phthalate Esters and lindane	GC-MS	$CHCl_3$	$CHCl_3$	6.0-133	Regueiro *et al.*, 2008
Selenium	GC-ECD	C_6H_5Cl	EtOH	0.005	Bidar *et al.*, 2008
Chlorophenols	GC-ECD	C_6H_5Cl	ACN	0.010 –2.0	Fattahi *et al.*, 2004

1.7 Objective of This Study

This study was carried out to fulfil the following objectives:

i. To synthesis and modified the magnetic nanoparticles

ii. To study the DLLME method on water samples containing pesticides using GC-μECD

CHAPTER 2

METHODOLOGY

2.1 Introduction

Ferrous chloride tetrahydrate ($FeCl_2.4H_2O$ Merck > 99%), ferric chloride hexahydrate ($FeCl_3.6H_2O$ Merck > 99%), salts and ammonium hydroxide (NH_4OH Merck, 25% of ammonia) were used for the synthesis of iron oxide nanoparticles. All chemicals were of reagent grade and used without further purification.

A mixture of OCP standard solutions containing aldrin, chlorothalonyl, chloropyriphos, and profenofos was obtained from Supelco (USA). Carbon tetrachloride (CCl_4), tetrachloroethylene (C_2Cl_4), chlorobenzene (C_6H_5Cl), and chloroform ($CHCl_3$), 1-octanol, were analytical grade; these were purchased from Merck (Merck & Co., Inc., Germany) and were redistilled in glass apparatus at least three times before use. Other solvents used including methanol, acetone and acetonitrile, were pesticide grade and were obtained from Merck (Merck & Co., Inc., Germany). Deionized water was taken from a Milli-Q system (Millipore, USA). Each stock standard solution of OCPs was dissolved in 5 mL of methanol and then stored at 4 ^{o}C. The working solutions were prepared with suitable dilutions daily before use.

2.2 Synthesize and Derivatization of Magnetic Nanoparticles

2.2.1 Synthesize of Magnetic Nanoparticles

In this study, $FeCl_2$ and $FeCl_3$ were dissolved in 50 ml deionized water with molar ratio of 2/3. Total amount of iron ions in the solution was varied from 250 mmol to 12.5 mmol. 150 ml of ammonium hydroxide (25%) was added to 50 ml mixture of iron salts under vigorous mechanical stirring at 1500 rpm. The reaction was performed for 30 minutes at 20 ^{o}C in air medium. After the reaction, the precipitate was washed three times with distilled water (Karaagac *et al.,* 2010). To obtain the powder, the precipitate was dried in a freeze dryer for 24 hours.

2.2.2 Surface Modification of Magnetic Nanoparticles

The surface modification of nanoparticles was carried out by using 0.2 g of dried magnetic nanoparticles, 0.5 g of 3-chlrooctyl-triethoxysilane and 10 mL of anhydrous toluene were swirled for 10 min under nitrogen atmosphere. The mixture was transferred into autoclave to react at 110 ^{o}C for 10 hours. Then, the particles were washed with toluene and methanol in sequence and were subjected to dryness before further uses (Shi and Lee, 2010).

2.2.3 Characterization of Unmodified and Modified Magnetic Nanoparticles

2.2.3.1 FT-IR

For FT-IR analysis, 2 mg of modified and un modified magnetic nanoparticles were weighted. 200 mg of potassium bromide, KBr was used. The weighted sample and KBr is placed in an abate mortar and ground for at least 5 minutes. The sample is then placed in a suitable press evacuated to remove residual moisture and pressed for several minutes (~ 10 ton/s in.) thus was subjected to IR spectrometer

2.2.3.2 CHN Analysis

For CHN analysis, accurately 2 mg of mangnetic nanoparticles (modified and unmodified) was weighed. All microanalysis data were obtained using a Model 440 CHN/O/S analyser (Exeter Analytical, North Chelmsford, MA, USA).

2.3 Real Water Samples

Several real water samples for use in evaluating the performance of DLLME were collected. Treated waste water was collected from Selangor area while The tap water was obtained from University Malaya's laboratory and housing area located in Shah alam Selangor, while the deionised water was taken from a Milli-Q system in our laboratory. All the water samples were collected with the previously rinsed glassware without air bubbles and filtered through a 0.45-μm membrane (Whatman GF/F, USA) to

remove any particulates. All water samples were stored at 4 ^{o}C before further subjected to analysis.

2.4 Sample Preparation

A stock solution (containing 10 μg/mL of each analyte) was prepared by diluting pesticides standard (chloropyriphos, chlorothalonil, DDE and DDT) with methanol and were stored in refrigerator. Water samples were prepared by spiking deionized water with analytes at known concentrations (50 ng/mL) to study extraction performance under different conditions. Genuine water samples collected from a local river were processed and analyzed directly or after being spiked with pesticides standards at concentrations of 10 ng/mL. To address the potential adsorption of the analytes to the particulate phase, samples were not filtered prior to processing.

2.5 DLLME Procedures

For extraction procedures, 5 μL of 100 mg/L of stock solution was transferred into vial containing 10 mL of distill water. This procedure was resulted in the final concentration of $5x10^{-4}$ mg/L. Then vial was sealed and swirled on a vortex agitator (Scientific Industries, Bohemia, NY) at 3200 rpm for 5 minutes. After that, 10 mg of the derivatized magnetic nanoparticles were quickly added to the vial. The vortex was enabled for another 10 minutes. Subsequently, a magnet bar was held next to the bottom of the vial (Figure 1c) to attract and isolate the nanoparticles, and the sample solution was discarded simply by decanting it. Thereafter, the magnet was removed,

and 1 mL of methanol was introduced to the vial to desorb the 1-octanol as well as the pesticides from the nanoparticles by sonication for 12 min and resulted in final concentration of 0.5 mg/L. Finally, the magnet was again placed next to the vial, and the supernatant was collected into an Eppendorff tube by an automatic pipettor for analysis. The experimental setup of the extraction is illustrated in Figure 2.1 below (Shi and Lee, 2010).

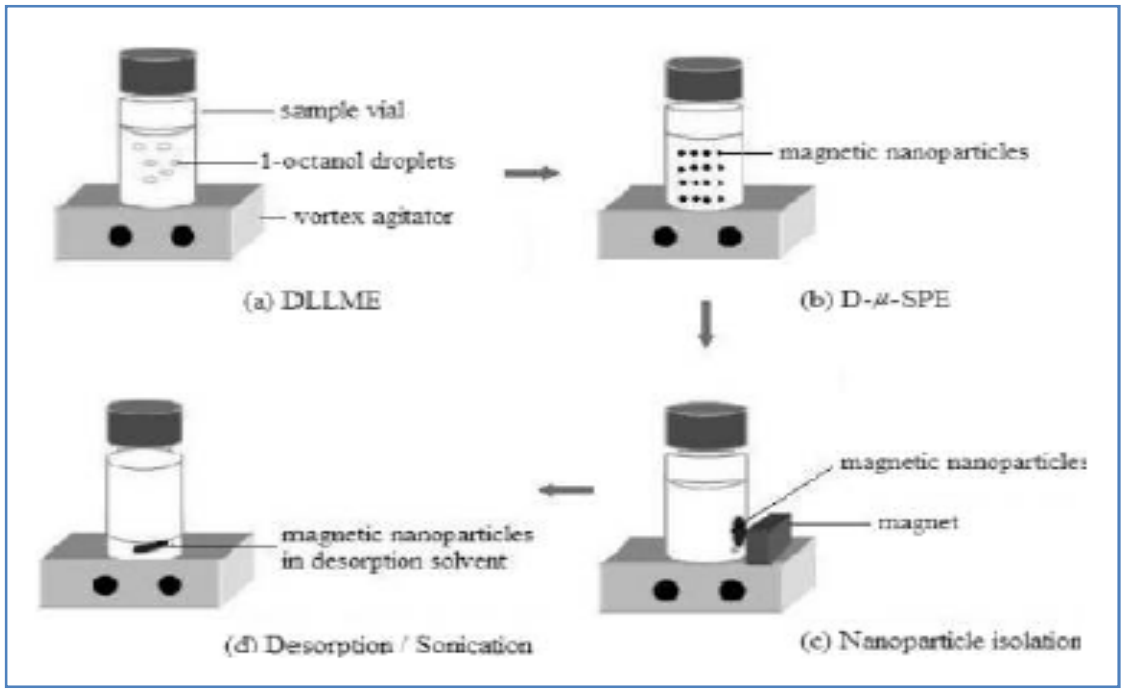

Figure 2.1: Experimental procedures of Two-step of DLLME

2.6 Instrumental Analysis

Concentrations of OCPs were quantitatively analyzed using an Agilent-7890 gas chromatograph (Agilent, USA), equipped with a μ-63Ni electron capture detector (GC-μECD) and an HP-5MS fused silica capillary column (30 m × 0.32 mm × 0.25 μm, J&W Scientific, Flosom, USA). Helium was the carrier gas, with a flow rate of 1.5 mL/min, while nitrogen was the make-up gas at the rate of 60 mL/min. The pressure

was set at 13.4 psi. The temperatures of the injector and detector were kept at 250 and 320 ^{o}C, respectively. The oven temperature was programmed from 60 to 170 ^{o}C (hold for 2 minutes) at the rate of 10 ^{o}C/min, to 280 ^{o}C and was hold for 3 minutes at the rate of 5 ^{o}C/min, and finally to 300 ^{o}C at the rate of 15 ^{o}C/min. The injection volume was 1.0 µL splitlessly.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Synthesize and Derivatization of Magnetic Nanoparticles

3.1.1 Synthesize of Magnetic Nanoparticles

Synthesized of magnetic nanoparticles were carried out by adjusting the amount of iron ion in the solution while keeping the other parameters constant. It was observed that the colour of the samples changed from black to reddish-brown as the amount of iron ion in the medium decreased from 250 to 12.5 mmol. This change may indicate the phase transform of magnetite to another iron oxide phase (maghemite, hematite) and/or iron oxyhydroxides since the colour of magnetite is black while it is reddish-brown for others.

In order to achieve a complete precipitation of iron oxide, the pH should be between 8 and 14 (Lauren *et aL*., 2008; Jolivet *et al*., 2004) according to the following reaction:

$$Fe^{2+} + 2Fe^{3+}\ OH^{-} \rightarrow Fe_3O_4 + 4H_2O$$

The final pH's are 11 for all reactions indicating that the reaction medium of all samples at the beginning was convenient to produce iron oxide nanoparticles but the structural and hence the magnetic properties of the product are different from each other. Thus,

the iron ion concentration has a significant effect on the synthesis of iron oxide nanoparticles by coprecipitation technique.

3.1.2 Surface Modification on Magnetic Nanoparticles

The dried magnetic nanoparticles then were subjected to surface modification. It was carried out by using 0.5 g of 3-chlrooctyl-triethoxysilane and 10 mL of anhydrous toluene. The mixtures were swirled for 20 minutes under nitrogen atmosphere. The mixture was transferred into autoclave to react at 110 ^{o}C for 24 hours. Then, the particles were washed with toluene and methanol in sequence and were subjected to dryness before further uses (Shi and Lee, 2010).

3.2 Characterization of Magnetic Nanoparticles

3.2.1 FT-IR Analysis

Both modified and unmodified magnetic nanoparticles were characterized by using FT-IR. FT-IR analysis was performed to confirm the formation of iron oxide nanoparticles. Iron oxide shows bands indicating the vibrations of 540–580 cm^{-1} where and correspond to the metal occupying tetrahedral and octahedral positions, respectively (Souza *et al.,* 2008). FT-IR spectra of the samples in the 1200–400 regions are shown in Table 3.1, Figure 3.1a and 3.1b and below.

Based on the IR spectrum of unmodified magnetic nanoparticles, which shown in Figure 3.1a, a broad band was detected at about 540–580 cm^{-1} which was related to the vibrations of Fe-O bond (Pavia *et al.,* 2010)**.** Absorption band at 3435.66 cm^{-1} was detected on IR spectrum of modified magnetic nanoparticles. The respected absorbance bands were indicates the present of O-H bonded in the molecular structure. Besides, the C-H alkane (stretch) and $-CH_2$ (bend) was detected at the absorption band of 2926.80 and 1408.98 cm^{-1} respectively. The presence of C-O, Fe-O and C-Cl bond were proved by the presence of absorption bands at 1052.03, 584.10 and 629.71 cm^{-1} respectively. The FT-IR spectrum of modified magnetic nanoparticles was shown in Figure 3.1b below.

Table 3.1: FT-IR Band Absorption of Modified Magnetic Nanoparticles (Pavia *et al.,* 2010).

Absorption Band (cm^{-1})	**Types of Vibration**	**Reference Vibration (cm^{-1})**
3435.66	OH, H-bonded	3650-3200
2960.45	C-H alkane (stretch)	3000-2850
2926.80	C-H alkane (stretch)	3000-2850
1408.98	$-CH_2$ bend	1465 - 1400
1052.03	C-O	1300-1000
629.71	C-Cl	785-540
584.10	Vibration of Fe-O	

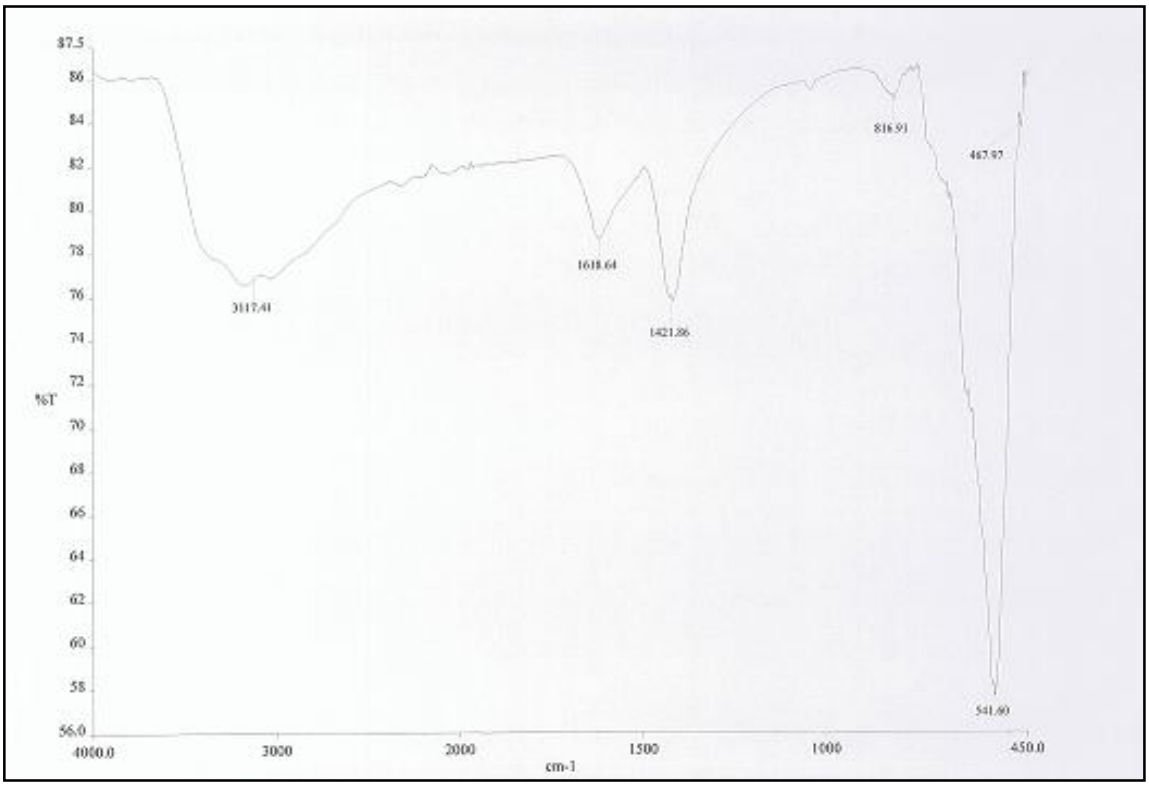

Figure 3.1a: FT-IR Spectrum of Unmodified Magnetic Nanoparticles

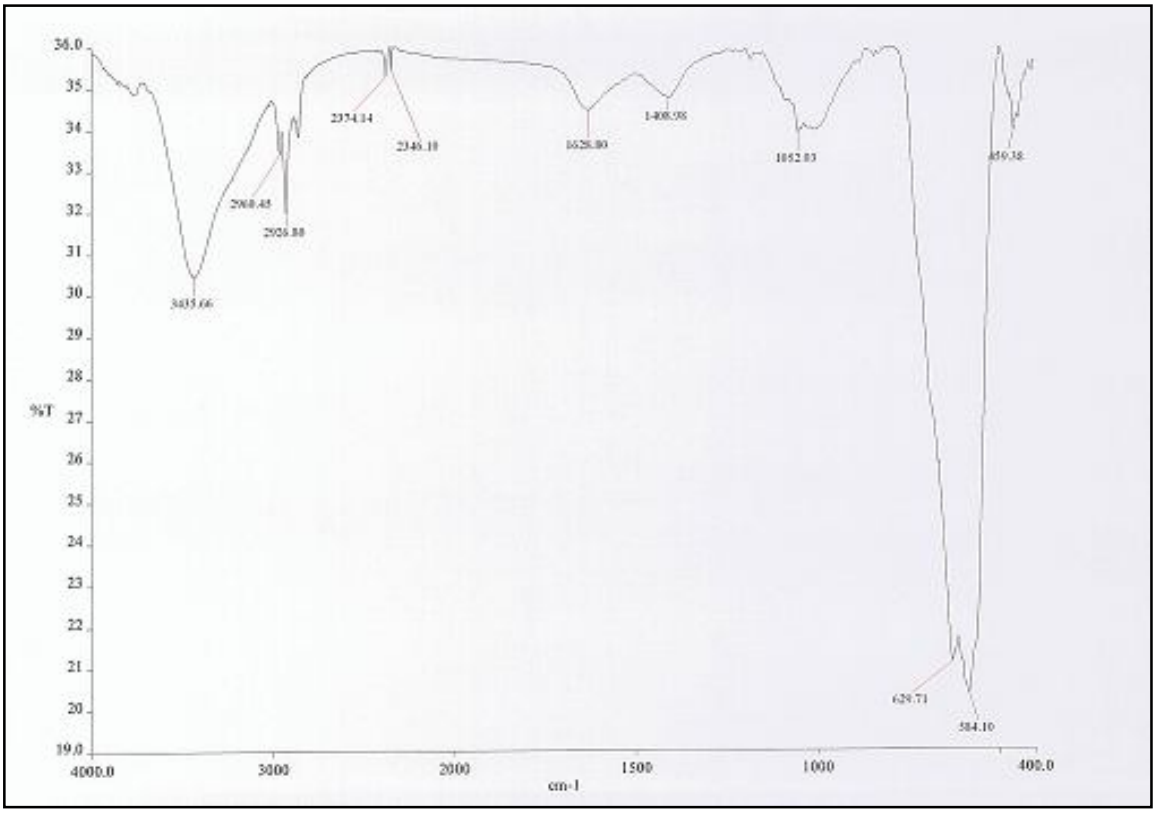

Figure 3.1b: FT-IR Spectrum of Modified Magnetic Nanoparticles

3.2.2 CHN Analysis

Carbon, hydrogen and nitrogen is one of the common elemental analysis in organic compounds containing carbon – carbon bonds. The elemental analysis of a compound enables one to determine the empirical formula of the compound. The empirical formula is the formula for a compound that contains the smallest set of integer ratios for the elements in the compound that gives the correct elemental composition by mass.

The technique provides the results as percentage amount of these atoms against the total weight. Most of the organic compounds are made up of these four elements and oxygen; hence after determining the former elements percentage weight of oxygen can be calculated. In this technique the substance under study is combusted under oxygen stream in a furnace at high temperatures. The end products of the combustion would be mostly the oxides of the concerned elements in the form of gases. These are then separated and carried to the detector using inert gases like helium or argon. It is one of the few analytical techniques that give a clear quantitative measurement of the carbon, hydrogen, nitrogen and sulphur.

For CHN analysis, 2,217 mg of magnetic nanoparticles was used and the results was shown in table 3.2 below

Table 3.2: CHN Analysis for Magnetic Nanoparticles

Elements	**Percentages (%) of Elements**
Carbon	3.550
Hydrogen	0.650
Nitrogen	-

3.3 Extraction Optimization

3.3.1 Organic Solvent Selection.

To achieve satisfactory LPME, several criteria on selecting the organic solvent phase or extractant should be met. First of all, the solvent should be immiscible with aqueous solution, except when the headspace mode is employed. Secondly, the target analytes should have good solubility in the selected solvent to ensure high extraction enrichment. Additionally, the solvent should have a low vapour pressure to prevent loss during agitation. According to these criteria, 1-octanol is a suitable extractant that has been widely used in many LPME applications. However, it is generally unsuitable for classical or conventional DLLME because of its low density relative to water.

In classical DLLME, only a few solvents, mainly halogenated hydrocarbons such as carbon tetrachloride (CCl_4), tetrachloroethylene (C_2Cl_4), and chlorobenzene (C_6H_5Cl) are suitable. These solvents were choosing since they easily separated from the aqueous matrix by centrifugation after extraction. However, these solvents are toxic, and moreover, they are not ideal extractants for many analytes. These drawbacks severely limit the wider applicability of DLLME.

In the present study, a simple method via hydrophobic adsorption was investigated to retrieve the low-density organic extractant from the aqueous sample solution after DLLME. Theoretically, any organic solvent immiscible with water can be recovered by this method. Therefore, the solvent choice for DLLME can be expanded depending on the target analytes, any specific organic solvent can be selected and utilized for this extraction method. Additionally, a special apparatus (such as conical-bottom test tubes)

and special solvent handling procedures associated with classical DLLME, as mentioned previously, are not necessary in the new procedure, which would be potentially suitable for automation (Rezaee *et al*., 2006; Shi and Lee, 2010).

In such a case, the extraction efficiency and efficacy as well as operational convenience can be enhanced. Since 1-octanol is one of the most widely utilized organic solvents in LPME, it was adopted in this study to investigate the feasibility of the present two-step procedure. 1-octanol can be dispersed as fine droplets much more efficiently, thus facilitating its contact with analytes. Additionally, the hydrophobic moiety of the 1-octanol conceivably has higher affinity for the pesticides than the chlorooctyl part of the nanoparticles. Thus, the extraction efficiency of the two-step method was much higher than that of the direct D-μ-SPE method (Shi and Lee, 2010)

An appropriate extraction solvent is a key point for achieving the higher recovery ratio of DLLME. The extraction solvent should meet several requirements including density higher than water, lower solubility in water, good recovery and higher enrichment factor for the target compounds, and also good chromatographic behaviour for further instrument analysis.

In the present study, 5 µL of 100mg/L of stock solution was transferred into the vials containing 10 mL of distil water and resulted in the final concentration of spiked stock solution of $5x10^{-4}$ mg/L. 10 mg of the derivatized magnetic nanoparticles were quickly added to the vial. A volume of 10 µL of 1-octanol as the disperser solvent were pour into the vials to determine the extraction efficiency. The vortex was enabled for another 10 minutes to ensure the mixtures were homogenously mixed. Subsequently, a magnet bar was held next to the bottom of the vial to attract and isolate the nanoparticles, and

the sample solution was discarded simply by decanting it. Then, they were freeze dried for 20 minutes. Thereafter, 1 mL of trichloromethane ($CHCl_3$), tetrachloromethane (CCl_4) and tetrachloroethene (C_2Cl_4) were introduced to the different vial respectively to desorb the 1-octanol as well as the pesticides from the nanoparticles and were sonicated for 5 min and yet was resulted in final concentration of 0.5 mg/L. Finally, the magnet was again placed next to the vial, and the supernatant was collected into an Eppendorff tube by an automatic pipettor for analysis.

The extraction recoveries of DLLME with different extraction solvents were tabulated in Table 3.3 below. Based on Table 3.1, CCl_4 was shown to have higher percentage recoveries which range from 41.20 to 98.30 % compared to $CHCl_3$ and C_2Cl_4, with the percentage recoveries of 14.91 to 75.53% and 35.71 and 81.53% respectively. Therefore, CCl_4 was selected as the extraction solvent for extracting pesticides compounds from water samples by using DLLME method.

Table 3.3: Extraction recoveries of different extraction solvents for analysis of OCPs in water samples using DLLME (mean extraction recovery (%) ± standard deviation, SD).

Pesticides	**Extraction Recovery (%) ± SD (*n*=3)**		
	$CHCl_3$	**CCl_4**	**C_2Cl_4**
Chloryphyros	59.94 ±0.83	97.59 ± 2.11	81.53 ± 2.23
Chlorothalonil	75.53 ± 0.15	98.31 ± 1.58	66.38 ± 0.51
DDT	53.63 ± 0.57	92.95 ± 3.21	42.59 ± 2.18
DDE	14.91 ± 0.19	41.20 ± 0.88	35.71 ± 0.03

3.3.2 Selection of Disperser Solvent.

Miscibility of dispersal solvent in organic phase (extraction solvent) and aqueous phase (sample solution) is the most important point for the selection of dispersal solvent. In addition, it should have good chromatography behaviour. 1-octanol, ACN and ETOH were choosing as dispersal solvent in DLLME method since they illustrated these feasibility and abilities as disperser solvents.

In this experiment, a series of sample solutions were studied by using 1.0 mL of each dispersal solvent containing 10 µL of 1-octanol, EtOH and ACN respectively as the solvent to concentrate pesticides from a series of water samples which have been spiked with pesticides standard solutions. The Results in Table 3.4 was demonstrated that the recoveries of individual's pesticides were in the range of 11.43 to 62.46 %, 27.44 to 71.90% and 29.02 to 81.39 % for ACN, EtOH and 1-octanol respectively. Based on the results obtained, 1-octanol was selected as dispersal solvent in the following experiments. It is due to the high extraction efficiency and it possesses less toxicity effects compared to others.

Table 3.4: Extraction recoveries of pesticides from waters samples using DLLME method with different dispersal solvents (mean extraction recovery (%) ± standard deviation, SD).

Pesticides	**Extraction Recovery (%) ± SD (*n*=3)**		
	Acetonitrile	**Ethanol**	**1-octanol**
Chloryphyros	11.43 ± 0.25	27.44 ± 0.58	29.02 ± 0.08
Chlorothalonil	56.10 ± 0.91	43.33 ± 0.67	61.27 ± 0.24
DDT	62.46 ± 0.32	71.90± 0.04	81.39 ± 0.18
DDE	29.64 ± 0.31	30.61 ± 0.19	49.86 ± 0.16

3.4 Evaluation of the Performance of DLLME with Real Water Analysis

Based on experiment and method optimization, CCl_4 and 1-octanol were selected as the extraction and dispersal solvent respectively as the optimum experimental condition. The proposed DLLME methodology was evaluated with a series of aqueous samples spiked with pesticides standard.

Several real water samples including treated waste water and tap water (laboratory and housing area) were also used to evaluate the applicability of DLLME method to determine pesticides in water samples. The samples were spiked with pesticides standard solution at the concentration level of 10 µg/L for each target compound to investigate the potential matrix effect on the extraction efficiency of DLLME under the optimum conditions.

Figure 3.2 shows the chromatogram of original treated waste water (i) and spiked treated waste water (ii) at the concentration levels of 10 µg/L for each pesticide standard. Blank analysis of original treated waste water without addition of pesticides standard solution showed that such samples were almost free of pesticides contaminations and was illustrated in Figure 3.2 (ii).

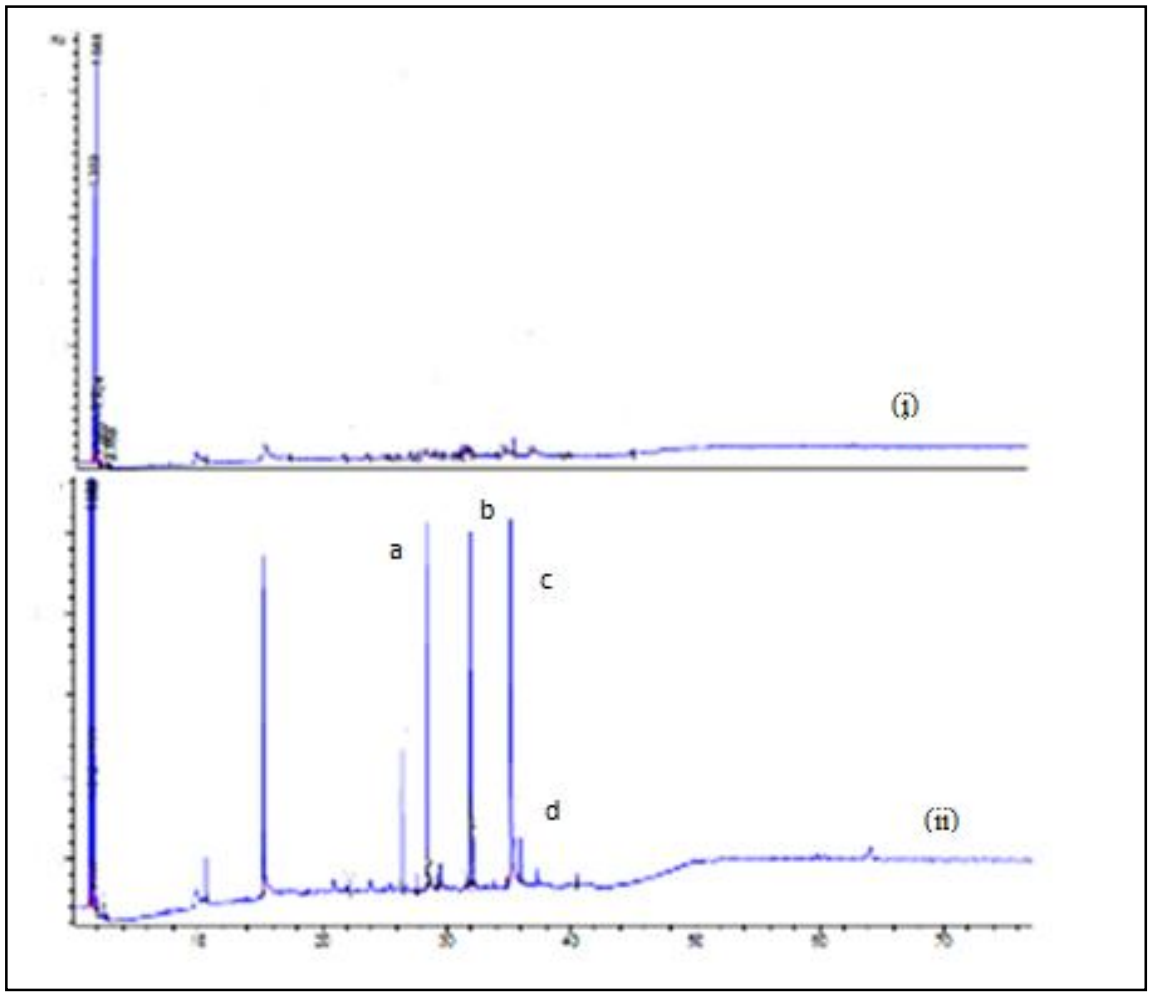

Figure 3.2: Chromatogram of original treated waste water (i) and spiked treated waste water (ii) at the concentration levels of 10 µg/L for each pesticides standard. Extraction condition: 5 mL sample volumes, 10 µL of 1-octanol and 1 mL of ethanol as dispersal and extraction solvent respectively. Peak identification: (a) Chloryphyros, (b) Chlorothalonil, (c) DDT and (d) DDE.

Based on the results obtained from the analysis of real water samples (Table 3.5), the percentage of relative recovery (RR) of pesticides which have been extracted in optimum DLLME method were tabulated. Chloropyriphos shows the highest percentage RR in three different water samples (treated waste water, tape water from laboratory and tap water from housing area) with ranges from 43.21 to 77.94 %.

Chlorothalonil in treated waste waters shows the highest content which corresponded to 0.6789 μgL^{-1} compared tap water in laboratory and hosing area, which lies in the values of 0.4781 and 0.4781 μgL^{-1} respectively. The presence of DDT residues in three different types of water were detected at the level of 0.1762 to 0.7173 μgL^{-1}. Treated waste water shows the higher percentage of DDT residues (71.73%) compared to tap water laboratory and housing area.

Meanwhile, DDE shows the lowest residues detected in treated waste water (0.2122 μgL^{-1}) compared to tap water which had been collected in housing area (0.1762 μgL^{-1}). However, there are no residues of DDE in tap water which collected in laboratory. The relative recovery of DDE was shown to have less than 50 % in three different water samples and they were indicates that, DDE was significantly have a lower extraction efficiently in DLLME method. It is particularly influence by the chemical behaviour and properties of DDE. As summary of real water analysis of three different water samples, Chloropyriphos shows the higher percentage recovery compared to the other pesticides residues. This result shows that, two step of DLLME method is most suitable method for trace and extraction procedures. The effective method of two step of DLLME in extraction of pesticides including organochloride and organophosphorues pesticides also were proved and reviewed. (Zhao *et al.*, 2011; Shi and Lee, 2010; Berijani *et al*., 2006).

Table 3.5: Relative recoveries (RR, %) of OCPs from spiked real water samples using DLLME method[a]

	Treated Waste Water		**Tap Water (Lab)**		**Tap water (Housing area)**	
Pesticides	Pesticides Content (Mean ± SD)[b]/μgL^{-1}	RR, %	Pesticides Content (Mean ± SD) [b] /μgL^{-1}	RR, %	Pesticides Content (Mean ± SD) [b] /μgL^{-1}	RR, %
Chloropyriphos	0.7794 ± 0.07	77.94	0.8679 ± 0.01	86.79	0.4321 ± 0.02	43.21
Chlorothalonil	0.6789 ± 0.01	67.89	0.4781 ± 0.18	47.81	0.1762 ± 0.01	17.62
DDT	0.7173 ± 0.01	71.73	0.68219 ± 0.11	68.219	0.1762 ± 0.05	12.54
DDE	0.212 ± 0.09	21.22	ND	0	0.1762 ± 0.01	21.87

a. Extraction Condition: 10 mL of water samples spiked with 10 μgL^{-1} of pesticides s standard solution; 10µL of 1-octanol as dispersive solvent; 1mL of CCl_4 as extraction solvent

b. Concentration of pesticides in spiked real water samples after being extracted using optimum DLLME method, mean content ± SD (*n*=3)

c. ND: not detected

CHAPTER 4

CONCLUSION

Magnetic iron oxide nanoparticles were synthesized and theoretically studied as Fe^{2+} + $2Fe^{3+}$ OH^- → Fe_3O_4 + $4H_2O$. The surface modification is purposely to change the polarities and the magnetic nanoparticles. The coated particles with octyl group on the surface ensure the attachment of the functional group hence improve the extraction process in two step of DLLME.

A CCl_4 was shows the higher percentage recoveries which range from 41.20 to 98.30 % compared to $CHCl_3$ and C_2Cl_4, with the percentage recoveries of 14.91 to 75.53% and 35.71 and 81.53% respectively. Therefore, CCl_4 was selected as the extraction solvent for extracting pesticides compounds from water samples by using DLLME method.

The recoveries of individual's pesticides were in the range of 11.43 to 62.46 %, 27.44 to 71.90% and 29.02 to 81.39 % for ACN, ETOH and 1-octanol respectively. Based on the results obtained, 1-octanol was selected as dispersal solvent in the following experiments. It is due to the high extraction efficiency and possesses less toxicity effects. Chloropyriphos shows the highest percentage recovery in three different water samples (treated waste water, tape water from laboratory and tap water from housing area) with ranges from 43.21 to 77.94 %.

Chlorothalonyl in treated waste waters shows the highest content which corresponded to 0.6789 μgL^{-1} compared tap water in laboratory and hosing area, which lies in the values

of 0.4781 and 0.4781 μgL^{-1} respectively. The presence of DDT residues in three different types of water were detected at the level of 0.1762 to 0.7173 μgL^{-1}. Treated waste water shows the higher percentage of DDT residues (71.73%) compared to the tap water laboratory and housing area.

Meanwhile, DDE shows the lowest residues detected in treated waste water (0.2122 μgL^{-1}) compared to tap water which had been collected in housing area (0.1762 μgL^{-1}). However, there are no residues of DDE in tap water which collected in laboratory. The relative recovery of DDE was shown to have less than 50% in three different water samples and they were indicates that, DDE was significantly have a lower extraction efficiently in DLLME method. It is particularly influence by the chemical behaviour and properties of DDE.

Real water analysis in three different water samples, Chloropyriphos shows the higher percentage recovery compared to the other pesticides residues. This result shows that, two step of DLLME method is most suitable method for trace and extraction procedures. The effective method of two step of DLLME in extraction of pesticides including organochloride and organophosphorues pesticides also were proved and reviewed. (Zhao *et al.*, 2011; Shi and Lee, 2010; Berijani *et al.*, 2006).

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