

**APPLICATION OF GREEN SILICONE
SURFACTANT IN MICROEXTRACTION OF
ORGANOPHOSPHORUS PESTICIDES IN FOOD
SAMPLES**

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UNIVERSITI SAINS MALAYSIA

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SAMPLES**

by

VASAGEE A/P ELENCOVAN

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LIST OF SYMBOLS

cm^3	Cubic centimeter
R^2	Coefficient of determination
$^{\circ}\text{C}$	Degree celsius
Fe_3O_4	Iron magnetite
L	Litre
μg	Microgram
μL	Microlitre
mg	Miligram
m^2	Square meter
m^2/g	Surface area
NaCl	Sodium chloride
Na_2SO_4	Sodium sulfate
Na_2CO_3	Sodium carbonate
nm	Nanometer
v/v	Volume per volume
w/v	Weight per volume

LIST OF ABBREVIATIONS

BET	Brunauer-Emmet-Teller
CAC	Codex Alimentarius Commission
ChAc	Choline acetate
ChCl	Choline chloride
CMC	Critical micelle concentration
CPE	Cloud point extraction
CPT	Cloud point temperature
CTAB	Cetyltrimethylammonium bromide
DeAc	Decanoic acid
DES	Deep eutectic solvent
DLLME	Dispersive liquid-liquid microextraction
DoAc	Dodecanoic acid
EU	European Union
FT-IR	Fourier transform infrared spectroscopy
GC-FID	Gas chromatography-flame ionization detector
GC-FPD	Gas chromatography-flame photometric detector
GC-MS	Gas chromatography-mass spectrometry
GC-NPD	Gas chromatography-nitrogen phosphorus detector
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HCl	Hydrochloric acid
HeAc	Hexanoic acid
HF-LPME	Hollow fiber-liquid phase microextraction
HPLC	High performance liquid chromatography
ILs	Ionic liquids
LLE	Liquid-liquid extraction
LPME	Liquid phase microextraction
LOD	Limit of detection
Log K_{ow}	Octanol/water (o/w) partition coefficient
LOQ	Limit of quantification
MCF-7	Human breast cancer cell lines

MCF-10A	Non-tumorigenic epithelial cell lines
MCCLLE	Miniaturized counter-counter liquid-liquid extraction
MIP-SPE	Molecularly imprinted polymer-solid phase extraction
MSPE	Magnetic solid phase extraction
MRL	Maximum residue limit
n.a	Not applicable
NaOH	Sodium hydroxide
Nd	Not detected
NMR	Nuclear magnetic resonance
OcAc	Octanoic acid
OCPs	Organochlorine pesticides
OPPs	Organophosphorus pesticides
PAHs	Polycyclic aromatic hydrocarbons
PEG	Polyethylene glycol
SA-DLLME	Surfactant assisted dispersive liquid-liquid extraction
SDME	Single drop microextraction
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SPE	Solid phase extraction
SPME	Solid phase microextraction
SRP	Surfactant rich phase
SS	Silicone surfactant
TEM	Transmission electron microscope
UAEME	Ultrasound assisted emulsification microextraction
UASEME	Ultrasound assisted surfactant enhanced emulsification microextraction
VALLME	Vortex assisted liquid-liquid microextraction
VSLLME	Vortex assisted surfactant enhanced liquid-liquid microextraction
VSM	Vibrating sample magnetometer
XRD	X-ray diffraction

LIST OF APPENDICES

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**APLIKASI SURFAKTAN SILIKON HIJAU UNTUK
PENGEKSTRAKAN MIKRO PESTISID ORGANOFOSFORUS DI DALAM
SAMPEL MAKANAN**

ABSTRAK

Racun perosak organofosforus (OPPs) adalah bahan kimia fosfat yang digunakan secara meluas dalam tanaman ladang untuk menyingkirkan haiwan perosak dan serangga. Penggunaan berulang OPPs dalam pertanian, membawa kepada kehadirannya pada kuantiti surih dalam produk makanan dan dengan itu, terdapat permintaan yang semakin tinggi untuk pengenalpastian dan kuantifikasi OPPs. Oleh itu, kajian ini bertujuan untuk meneroka aplikasi surfaktan silikon bukan ionik yang hijau (SS) dalam pembangunan pelbagai teknik pengekstrakan untuk penentuan OPPs dalam sampel makanan. Pertama, SS telah diteroka sebagai pengemulsi dalam pengekstrakan mikro fasa cecair dibantu vorteks (SS-VALLME) untuk penentuan OPPs dalam sampel makanan dengan penggunaan kromatografi gas-spektrometri jisim (GC-MS). SS memudahkan pengemulsian pelarut pengekstrakan ke dalam larutan sampel matriks, sekaligus meningkatkan pemisahan OPPs ke dalam pelarut pengekstrakan. Dalam keadaan optimum, had pengesanan (LODs) untuk kaedah yang dicadangkan telah dicapai antara $0.008 - 0.1 \mu\text{gL}^{-1}$ sementara, nilai pekali penentuan (R^2) yang baik antara 0.9989 hingga 0.9994 telah diperolehi. Aplikasi kaedah yang dikembangkan pada sampel makanan memberikan nilai perolehan semula yang dapat diterima (80 – 118%) untuk OPPs sasaran. Seterusnya, potensi penggunaan SS diterokai lebih lanjut dengan menggabungkan asid dodekanoik (DoAc) untuk menghasilkan pelarut eutektik terdalam (DES) di mana SS bertindak sebagai penerima ikatan hidrogen (HBA) sementara DoAc bertindak sebagai penderma ikatan hidrogen

(HBD). DES berasaskan SS:DoAc baharu yang disintesis digunakan dalam VALLME sebagai pengemulsi. Peranan DES berasaskan SS:DoAc sebagai pengemulsi, mendorong penyebaran pelarut pengestrakan sebagai titisan halus ke dalam larutan sampel berair, sehingga memudahkan pemindahan jisim OPPs sasaran ke dalam pelarut pengestrakan. Teknik SS:DoAc-VALLME yang dicadangkan menunjukkan nilai $R^2 > 0.9989$ sementara LODs dicapai antara $0.01 - 0.09 \mu\text{gL}^{-1}$ dan perolehan semula berada dalam lingkungan $80 - 118\%$. Seterusnya, potensi penggunaan DES berasaskan SS:DoAc diterokai lebih lanjut dengan menggabungkan SS:DoAc dengan zarah Fe_3O_4 untuk menghasilkan penjerap SS:DoAc@ Fe_3O_4 . Penjerap baharu yang disintesis berjaya dicirikan dan digunakan dalam teknik pengestrakan fasa pepejal magnetik (MSPE) untuk pemantauan OPPs dalam sampel sayur-sayuran. Berbanding dengan zarah Fe_3O_4 asli, OPPs sasaran dapat diserap ke atas permukaan SS:DoAc@ Fe_3O_4 dengan lebih mudah kerana pembentukan interaksi hidrofobik yang kuat antara OPPs dan penjerap sehingga meningkatkan kecekapan pengestrakan. Dalam keadaan optimum, nilai R^2 yang didapati adalah antara $0.9970 - 0.9999$ sementara, LODs dan perolehan semula dicapai antara $0.03 - 0.1 \mu\text{gL}^{-1}$ dan $80 - 199\%$ masing-masing untuk semua OPPs yang dikaji. Selain itu, kajian sitotoksiti menunjukkan bahawa SS dan DES berasaskan SS:DoAc adalah sebatian hijau kerana tidak memudaratkan garis sel normal dan selamat untuk pendedahan manusia. Secara keseluruhannya, dapat disimpulkan bahawa kaedah-kaedah dikembangkan, SS-VALLME, SS:DoAc-VALLME dan MSPE berasaskan SS:DoAc@ Fe_3O_4 adalah pendekatan yang hijau, mudah, murah dan efisien untuk pengestrakan OPPs dalam sampel makanan.

**APPLICATION OF GREEN SILICONE SURFACTANT IN
MICROEXTRACTION OF ORGANOPHOSPHORUS PESTICIDES IN
FOOD SAMPLES**

ABSTRACT

Organophosphorus pesticides (OPPs) are phosphate chemicals that have been extensively applied in field crops to get rid of pests and insects. The repeated consumption of OPPs in agriculture, lead to their presence at trace quantity in food products and thus, there is a growing demand for the identification and quantification of OPPs. Therefore, this study aims to explore the application of a novel green non-ionic silicone surfactant (SS) in the development of various extraction techniques for the determination of OPPs in food samples. Firstly, the SS has been explored as an emulsifier in vortex assisted liquid-liquid microextraction (SS-VALLME) for the determination of OPPs in food samples with the aid of gas chromatography-mass spectrometry (GC-MS). SS facilitates the emulsification of extraction solvent into the aqueous matrix solution thus enhancing the partitioning of target OPPs into the extraction phase. Under the optimum conditions, the limit of detection (LODs) of the proposed method were in the range of $0.008 - 0.1 \mu\text{gL}^{-1}$ while good determination coefficients (R^2) varied from 0.9989 to 0.9994 were obtained. Application of the developed method gave acceptable relative recovery of OPPs (80 – 118%) from food samples. Next, the green SS was further explored by combining with dodecanoic acid (DoAc) to produce deep eutectic solvent (DES) in which the SS acts as a hydrogen bond acceptor (HBA) while the DoAc acts as a hydrogen bond donor (HBD). The newly synthesized SS:DoAc based DES was applied in VALLME as an emulsifier. The role of SS:DoAc based DES as an emulsifier, induced the dispersion of extraction

solvent as fine droplets into the aqueous sample solution, thereby promoting the mass transfer of target OPPs into the extraction solvent. The newly proposed SS:DoAc-VALLME approach demonstrated excellent figure of merits, with $R^2 > 0.9989$, LODs ranged from $0.01 - 0.09 \mu\text{gL}^{-1}$ and the relative recoveries ranged $80 - 118\%$. The potential application of green SS:DoAc based DES was further investigated by combining the DES with Fe_3O_4 particles to create a new SS:DoAc@ Fe_3O_4 adsorbent. The newly synthesized adsorbent was successfully characterized and applied in magnetic solid phase extraction (MSPE) technique for the extraction of OPPs in vegetables samples. In contrast to pure Fe_3O_4 particles, the target OPPs can easily adsorbed onto the SS:DoAc@ Fe_3O_4 surface due to the formation of strong hydrophobic interaction between the OPPs and the adsorbents thus amplifying the extraction efficiency. Under the optimized conditions, R^2 values were in the range of $0.9970 - 0.9999$ while the LODs and relative recovery ranged $0.03 - 0.1 \mu\text{gL}^{-1}$ and $80 - 119\%$ respectively for all the studied OPPs. The cytotoxicity study confirmed that the SS and SS:DoAc based DES has no negative effects on normal cell lines, implying that both SS and SS:DoAc based DES are environmentally benign and safe for human exposure. Overall, it can be deduced that the newly developed SS-VALLME, SS:DoAc-VALLME and SS:DoAc@ Fe_3O_4 based MSPE approaches are green, facile, economical and efficient for the extraction of OPPs in food samples.

CHAPTER 1

INTRODUCTION

1.1 General background

Pesticides are extensively applied in agriculture to provide a bountiful and low-cost supply of fine-quality fruits and vegetables. Basically, these pesticides protect crops by destructing the pests, insects and bacteria thereby assuring food quality. In this context, organophosphorus pesticides (OPPs) are the most widely utilized insecticides throughout the world in cultivating plants owing to their effectiveness, low-cost and short environmental persistence. OPPs are carcinogenic phosphate chemicals with the general formula $O=P(OR)^3$ that could pose a major threat to human health (Zhao et al., 2021). The toxicity of OPPs lies in the selective inhibition of acetylcholinesterase enzyme, resulting in acetylcholine build-up and neurotoxic disorder development (Matsushita et al., 2020). Excess application of OPPs may result in accumulation of OPPs residues in the environment, agricultural crops, ground and water surfaces (Yuan et al., 2021). These traces of OPPs may ultimately reach human and animals through food chain, harming their organs, blood, reproductive and immune systems besides causing acute poisoning in them (Gilbert-lopez et al., 2009).

Since, living organisms are susceptible to pesticides exposure, the monitoring of OPPs residues in food products is indispensable to warrant safety consumption of food by consumers. Therefore, the practice of maximum residue limit (MRL) has been enforced in many countries to prevent the overconsumption of pesticides. Food industries and stakeholders are legally required to adhere to these MRL guidelines to avert pesticides posing a harm to human health and the environment (Zikankuba et al., 2019). Considering the importance of food security and the requirements of trade

barriers, these legislative limits are being amended stringent than ever, fuelling the development of more sensitive and reliable analytical methods for the determination of pesticides in various matrices (LeDoux, 2011).

The identification of pesticides traces in foodstuffs requires sample pre-treatment and instrumental detection. The extraction of pesticides before subjecting to instrumental determination is a crucial part of the study. This technique will reduce the effect of matrix interferences thereby isolating and enriching the target analyte to achieve high sensitivity (Farajzadeh et al., 2015). Over the last few decades, various approaches for the extraction and enrichment of pesticides have been developed. Among them, conventional liquid-liquid extraction (LLE) and solid phase extraction (SPE) are the most widely used protocols for the monitoring of pesticides. LLE requires large amount of extraction solvent, laborious, environmentally unfriendly and has lengthy steps (Abdollahzadeh et al., 2012). SPE, on the other hand suffers from drawbacks such as tedious, time-consuming steps and requires costly materials. Other methods like solid phase microextraction (SPME) is a quick, simple and solvent-free approach, however its fibre is fragile and has a short lifetime and sample carryover is a hindrance, limiting its usage (Jiang et al., 2013).

Lately, extraction approaches based on the notion of green chemistry have attracted considerable attention. The primary goal of green technology is to eliminate the use of harmful organic solvents and chemicals (Kokilambigai & Lakshmi, 2021). Replacing the hazardous organic solvents with green alternatives will reduce its deleterious effect on the environment besides ensuring human safety (Abdel-moety et al., 2021). Green alternatives would comprise practices such as minimal application of chemicals, consumption of low-toxic substances and recyclable reagents besides conserving energy and minimizing wastage (Nowak et al., 2021). In tandem with the

principle of green analytical chemistry, many eco-friendly compounds and solvents such as surfactants, ionic liquids (ILs), deep eutectic solvent (DES) and supramolecular solvent are being developed and incorporated to extraction techniques as a substitution for noxious organic solvents (Yamini et al., 2020).

Surfactants are an emerging class of green compounds that have been widely explored in micellar based extraction methods such as cloud point extraction (CPE) due to their excellent ability to solubilize solutes of varying polarity and nature, allowing the solubilisation of non-water soluble compounds (Wang et al., 2016). On the other hand, major drawback in conventional dispersive liquid-liquid microextraction (DLLME) is the use of toxic water miscible dispersive solvent. Therefore, this traditional DLLME method was modified to be greener by incorporating surfactant as a replacement for organic dispersive solvent (Saraji & Bidgoli, 2010). The newly developed method is called surfactant assisted dispersive liquid-liquid microextraction (SA-DLLME). Briefly, this technique involves quick injection of an aqueous solution containing surfactant and extraction solvent into the sample matrix forming an emulsion, which is then centrifuged to separate the organic and aqueous phases. Following DLLME, other types of liquid phase extraction techniques such as ultrasound assisted emulsification microextraction (UAEME) and vortex assisted liquid-liquid microextraction (VALLME) have also been modified with the addition of surfactant due to its eco-friendliness.

Many analysts have devoted their attention to the discovery and application of green solvents as a result of the advancement of green technology. In the past decades, ILs have been established as a type of green solvent because of their unique physical and chemical features. Nonetheless, the present literature has documented the disadvantages of ILs such as toxicity, low biodegradability and expensive raw

materials (Tang et al., 2015). To prevent these drawbacks, DES has emerged as an outstanding class of green solvent and is often preferred over ILs due to their facile synthesis method, low cost of preparation, biodegradability and low toxicity (Pal et al., 2018). DES has been a popular choice of extraction solvent in various types of liquid phase extraction techniques. Recently, several researchers have explored the use of DES as a disperser solvent that aids in the emulsification of extractant into the aqueous sample solution (El-deen & Shimizu, 2019; Bian et al., 2021).

Magnetic solid-phase extraction (MSPE) is a type of dispersive SPE in which a magnetic adsorbent is used to isolate the target molecules in an aqueous matrix solution. The adsorbent can easily be retrieved using an external magnet (Giakisikli & Anthemidis, 2013). In this regard, magnetic iron particles (Fe_3O_4 particles) have often been incorporated as adsorbents of interest in MSPE for the removal and extraction of various contaminants. Fe_3O_4 particles are known as nano adsorbents with average particle size of approximately 100 nm mainly synthesized from iron (II) chloride tetrahydrate and iron (III) chloride hexahydrate (Faraji et al., 2010). Fe_3O_4 particles possess unique characteristics such as distinctive magnetic property, high total surface area to volume ratio, the ability to be easily modified, biocompatible and environmentally benign (Bagheri et al., 2019). However, Fe_3O_4 particles are not stable, easily oxidized and tend to agglomerate and form big aggregates resulting in poor adsorption of target molecules. Therefore, Fe_3O_4 particles often require modifications.

This research focused to explore the potential application of a novel green non-ionic silicone surfactant (SS) in the development of various extraction techniques for the determination of OPPs in food samples. In part I of the study, a SS was employed as an emulsifier in vortex assisted liquid-liquid microextraction namely as SS-

VALLME to extract OPPs from honey and fruit samples. Based on the good interaction findings of SS towards OPPs, the SS has been employed as a hydrogen bond acceptor (HBA) with dodecanoic acid (DoAc) as a hydrogen bond donor (HBD) to form a novel DES. Subsequently, this SS:DoAc based DES was employed as an emulsifier in Part II of the study, where another sample pre-treatment technique called SS:DoAc-VALLME was developed. Finally, this new SS:DoAc based DES was used as a functional group to embed magnetic iron particles (Fe_3O_4) to generate a novel SS:DoAc@ Fe_3O_4 magnetic adsorbent. Subsequently, this green adsorbent was employed in Part III of the study, where a new sample preparation technique called SS:DoAc@ Fe_3O_4 adsorbent based MSPE was developed for the extraction of OPPs in vegetable samples.

1.2 Scope of the study

This study explored the potential application of green SS in liquid and solid phase extraction methods for the determination of OPPs in food matrices. Firstly, the green SS has been explored as an emulsifier in VALLME namely as SS-VALLME. Then, the green SS was combined with DoAc to prepare a novel SS:DoAc based DES which was characterized using attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FT-IR) and nuclear magnetic resonance (NMR) analysis. This novel SS:DoAc based DES was incorporated to VALLME technique as an emulsifier to explore the potential of novel DES consist of SS (HBA) and DoAc (HBD). Based on the performance of SS:DoAc based DES in VALLME, the new SS:DoAc based DES has been explored as a functional group on magnetic materials namely as SS:DoAc@ Fe_3O_4 adsorbent. This novel adsorbent was characterized using FT-IR, X-ray power diffraction (XRD), scanning electron microscope (SEM) and transmission electron microscope (TEM), Brunauer-Emmett-Teller (BET) analysis and vibrating

sample magnetometer (VSM). The newly synthesized SS:DoAc@Fe₃O₄ adsorbent was applied in MSPE technique. All the methods have been optimized and validated for the analysis of OPPs in food samples.

1.3 Research objectives

Main objective:

Exploring the potential application of green silicone surfactant (SS) in liquid and solid phase extraction methods to determine OPPs in food samples using gas chromatography-mass spectrometry (GC-MS).

Specific objectives:

1. To investigate and validate the extraction performance of a green silicone surfactant as an emulsifier in vortex assisted liquid-liquid microextraction (SS-VALLME) for the determination of OPPs in honey and fruit samples.
2. To investigate, characterize and validate the extraction performance of a green silicone surfactant-based DES as an emulsifier in vortex assisted liquid-liquid microextraction (SS:DoAc-VALLME) for the determination of OPPs in honey and fruit samples.
3. To investigate, characterize and validate the extraction performance of a new adsorbent of silicone surfactant-based DES modified magnetic nanoparticles (SS:DoAc@Fe₃O₄) in magnetic solid phase extraction (MSPE) for the determination of OPPs in vegetable samples.

1.4 Outline of the thesis

This thesis is divided into five chapters. Chapter 1 introduced the general background and research objectives. In Chapter 2, the literature review of the research

is discussed and organized in detailed. Chapter 3 describes the methodology of the entire research, which is further divided into Part I, Part II and Part III. Part I and II describes about the methodology of VALLME technique employing an emulsifier which were silicone surfactant (SS-VALLME) and silicone surfactant-based DES (SS:DoAc-VALLME) combined with GC-MS for the determination of OPPs in food samples, respectively, while Part III explains the procedure of MSPE technique using silicone surfactant-based DES modified Fe_3O_4 particles (SS:DoAc@ Fe_3O_4) as a sorbent combined with GC-MS for the determination of OPPs in food samples. In Chapter 4, the data for optimization and validation of SS-VALLME technique for the determination of OPPs in food samples are discussed in Part I. In Part II, characterization data of the newly synthesized SS:DoAc based DES are presented followed by the optimization and validation of SS:DoAc-VALLME technique for the determination of OPPs in food samples. Next, Part III describes characterization results for the novel SS:DoAc@ Fe_3O_4 adsorbent followed by the optimization and validation of MSPE technique using SS:DoAc@ Fe_3O_4 adsorbent for the determination of OPPs in food samples. Lastly, Chapter 5 highlights general conclusion of the study as well as the future research recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides

Owing to the growing global population, pesticides are extensively consumed in farming to fulfil the food supply requirement by preventing and controlling harmful pests, insects, fungi and weed growth (Xiong et al., 2012). The use of pesticides improves the quality of food and agricultural products, ensure crop yield and safeguard human health (Wang et al., 2014). Pesticides are large class of organic pollutants that can be categorized into several groups such as insecticides, herbicides and fungicides (Filik & Demirci, 2011). Insecticides such as organophosphorus (OPPs), organochlorine (OCPs) and carbamate pesticides involve in the destruction of insects and arthropods. Besides, triazine, phenylurea and sulfonylurea belong to herbicides that kill weeds and unwanted plants (Sherwani et al., 2015). Fungicides such as imidazole and triazole are used to kill fungi such as moulds, yeasts and mildews (Brauer et al., 2019).

2.1.1 Organophosphorus pesticides (OPPs)

Among the pesticides, OPPs are the most widely used insecticides due to their excellent effectiveness, low cost and short persistence in environment (Li et al., 2013). OPPs are esters of phosphoric acids that are composed of at least one carbon-phosphorus bond. There are mainly three derivatives of OPPs which differ in terms of chemical structure such as aliphatic derivatives with a straight carbon chain structure i.e., sulfotep and ethion, phenyl derivatives with an aromatic ring attached with the phosphorus moiety i.e., leptophos while heterocyclic derivatives are made up of a ring structure with oxygen, sulfur or nitrogen atoms i.e., diazinon and chlorpyrifos. Most

of the OPPs are highly lipophilic and poorly soluble in water (Rezg et al., 2010). Table 2.1 illustrates the chemical structure and properties of the studied OPPs in this work. OPPs with benzene ring such as diazinon, chlorpyrifos, quinalphos and triazophos are more stable and persistent than aliphatic OPPs such as sulfotep and ethion. Moreover, OPPs with halogen substituted aryl group such as chlorpyrifos tend to have high value of Log K_{ow} : octanol/water (o/w) partition coefficient.

Despite of the major role of OPPs in agriculture, their harmful residues cause environmental pollution which could pose a risk to human health. The toxicity of OPPs primarily depends on inhibiting the activity of acetylcholinesterase enzyme that regulates the nervous system functions resulting in paralysis and death (Sharma et al., 2010). Moreover, OPPs are also proven to possess carcinogenic, cytotoxic, teratogenic and mutagenic effects (Aladaghlo et al., 2020). Additionally, exposure to OPPs is also associated to cancer development besides exerting deleterious effect on male reproductive system causing testicular degeneration, low sperm count and infertility (Yu et al., 2013).

Table 2.1: Physicochemical properties of the studied OPPs (Sapahin et al., 2014).

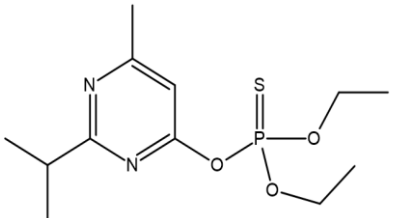
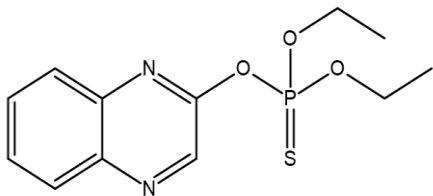
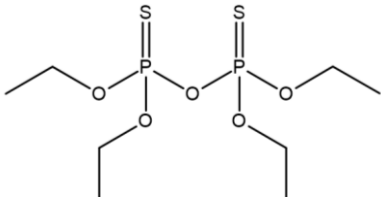
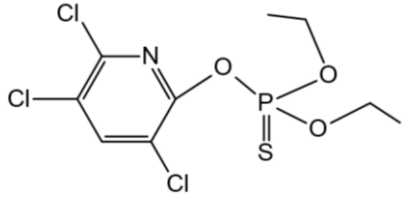
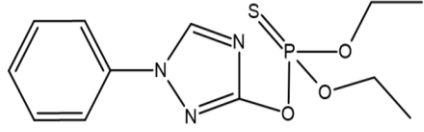
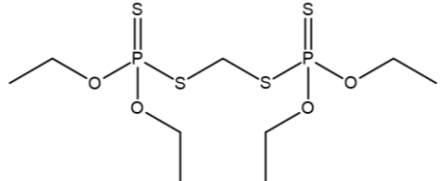
Name	Chemical structure	Molecular formula	Molecular weight (g/mol)	Solubility in water (mg/L)	Log K_{ow}
Diazinon		$C_{12}H_{21}N_2O_3PS$	304.35	60	3.81
Quinalphos		$C_{12}H_{15}N_2O_3PS$	298.3	17.8	4.44
Sulfotep		$C_8H_{20}O_5P_2S_2$	322.3	10	3.99

Table 2.1: "Continued"

Name	Chemical structure	Molecular formula	Molecular weight (g/mol)	Solubility in water (mg/L)	Log K_{ow}
Chlorpyrifos		$C_9H_{11}Cl_3NO_3PS$	350.6	1.05	4.96
Triazophos		$C_{12}H_{16}N_3O_3PS$	313.31	39	3.34
Ethion		$C_9H_{22}O_4P_2S_4$	384.5	2	5.07

Log K_{ow} : the octanol/water (o/w) partition coefficient

2.2 Fate of OPPs in environment

OPPs are applied to agricultural crops predominantly in the form of liquid or as dust. Upon released to the environment, OPPs may undergo either fast or slow rate of degradation. The relative rate of degradation is the determining factor for the environmental fate of OPPs. The degradation of OPPs is affected by microbial composition, pH, temperature and light exposure (Ragnarsdottir, 2000). A variety of microorganisms such as soil fungus, *Pseudomonas fluorescens* and *Thiobacillus thiooxidans* are known to hydrolyze OPPs residues. Furthermore, the light exposure causes oxidation or reduction of OPPs, converting them into metabolites such as oxons that are less or highly stable than the parent compound (Caceres et al., 2010). For instance, parathion is converted to paraoxon which is highly stable and more toxic to living things than the parent molecule, parathion. On the other hand, OPPs are prone to undergo rapid degradation under high pH conditions. It was postulated that the degradation of OPPs under genuine environmental conditions (5°C with pH 5) such as soil and groundwater were slower than that of laboratory conditions (25°C with pH 7) which were proven to degrade the OPPs rapidly (Ragnarsdottir, 2000). This solidifies the persistence of OPPs in environment for a long period of time. Shortly, it can be said that excessive use of OPPs tends to result in the accumulation of chemical residues that resists degradation and persists longer years in environment, contaminating the food chain and posing threat to living organisms.

2.3 Regulations of OPPs in food samples

Despite the fact that pesticides have many advantages on plant health in terms of disease and pest control, good productivity and better crop storage, their excessive use jeopardizes food safety (Zikankuba et al., 2019). Pesticides tend to retain and accumulate in fruits, vegetables and plants during growth and post-harvest treatment (Hayward et al., 2013). Besides, apiculture industry is also greatly affected by the extensive consumption of OPPs as it could pollute the honey hives. The use of pesticides in agriculture could contaminate bees when they collect nectar and pollen from flowers leading to the accumulation of toxic substances in honey which ended up degrading its quality (El-Nahhal, 2020).

Therefore, to prevent overconsumption of pesticides and to ensure food safety, strict regulations in terms of maximum residue limits (MRL) have been stipulated by national and international agencies, such as Codex Alimentarius Commission (CAC) and the European Union (EU) (Wanwimolruk et al., 2015). MRL is a standard that determines the maximum amount of pesticide residues permissible in a foodstuffs (MacLachlan & Hamilton, 2010). As stipulated by EU, the MRL values for fruits and vegetables lie in the scale of 0.01 – 0.03 mgkg⁻¹ (Mahpishanian et al., 2015) whereas for honey the value has been determined to be 0.01 mgkg⁻¹ (Fontana et al., 2010). Complying with this MRL values has become a requirement to facilitate international trade and to safeguard public health. Therefore, to serve this purpose there is a growing need for an efficient analytical method for qualitative and quantitative screening of these carcinogenic OPPs in foods (Seebunrueng et al., 2015).

2.4 Analytical determination of OPPs

Numerous analytical instruments have been employed to determine the presence of OPPs in various samples, including gas chromatography-mass spectrometry (GC-MS) (Khademi et al., 2021), gas chromatography-flame photometric detector (GC-FPD) (Sapahin et al., 2015), gas chromatography-nitrogen phosphorus detector (GC-NPD) (Salemi et al., 2013) and high performance liquid chromatography (HPLC) (Sanagi et al., 2011). Although these methods are developing rapidly, an appropriate sample pre-treatment technique prior to instrumental analysis is absolutely crucial to overcome the potential interferences in complex sample matrices thereby enabling the detection of OPPs at trace level (Zhang et al., 2012).

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) and are the most widely employed extraction techniques for the determination of OPPs in food samples. However, those methods are severely disadvantaged such that LLE requires large quantity of hazardous organic solvent besides having lengthy and tedious steps (Farajzadeh & Feriduni, 2016). SPE was proposed to diminish the drawbacks of LLE in terms of simplicity, good selectivity and analyte recovery with little usage of organic solvent (Fotouhi et al., 2017). However, conventional SPE technique is performed in specific column which diminish the effective interaction between the adsorbent and target compounds (Mahpishanian et al., 2015). Even though, solid phase microextraction (SPME) is a fast, simple and solventless technique, its fibre is fragile, unstable, requires low operative temperature and often results in needle bending and stripping of coatings (Azenha et al., 2006). Table 2.2 summarizes various sample preparation techniques and instrumental detection for the determination of OPPs in various samples. Recently, the trends in analytical chemistry have been focusing on the development of sample pre-

treatment technique involving small sample sizes, safe and eco-friendly materials with facile and expeditious steps aiming at high target analytes recovery and good precision.

Table 2.2: Summary of contemporary methodologies for the determination of OPPs.

Target OPPs	Sample	Method of extraction	Instrument	LODs (µg/L)	LOQs (µg/L)	Reference
Methamidophos, parathion and phoxim	fruit juice	MSPE ^a	HPLC	0.06–0.13	0.21–0.44	(Du et al., 2019)
Chlorpyrifos, chlorpyrifos-oxon, diazinon and diazinon-oxon	water samples	MIP-SPE ^b	HPLC	0.017–0.12	0.23–0.41	(Arias et al., 2020)
Malathion, triazophos and quinalphos	vegetable	SPE	HPLC	0.01–0.14	0.04–0.45	(Shakourian et al., 2020)
Trichlorfon, chlorpyrifos, phoxim, fenthion and diazinon	coarse cereal	DLLME ^c	HPLC	2.5–5.5	8.1–18.3	(He et al., 2009)
Diazinon and malathion	water samples	MCCLLE ^d	GC-FID ^e	0.1	n.a	(Hassan & Sarkouhi, 2016)

Table 2.2: “Continued”

Targeted OPPs	Sample	Method of extraction	Instrument	LODs (µg/L)	LOQs (µg/L)	Reference
Diazinon, malathion and chlorpyrifos	water samples and fruit juice	DLLME	GC-FID	0.65–1.3	2.2–4.5	(Farajzadeh et al., 2016)
Phorate, diazinon, disolfotane, methyl parathion, sumithion, chloropyrifos, malathion, fenthion, profenphose, ethion, phosalone and azinphose-methyl	tomato	DLLME	GC-FPD	0.1–0.5	n.a	(Bidari et al., 2011)

n.a: not applicable

^a MSPE: Magnetic solid phase extraction

^b MIP-SPE: Molecularly imprinted polymer-solid phase extraction

^c DLLME: Dispersive liquid-liquid microextraction

^d MCCLLE: Miniaturized counter-counter liquid-liquid extraction

^e GC-FID: Gas chromatography-flame ionization detector

2.5 Evolution of liquid-liquid extraction

LLE is a type of classical sample preparation method that involves the transfer of target analyte from aqueous matrix into water-immiscible organic solvent (Rezaee et al., 2010). The practice of LLE is less popular nowadays due to the requirement of high volume of organic solvent. Acetone, acetonitrile, ethyl acetate and hexane are the commonly used organic solvents for the preconcentration of pesticides. To minimize the shortcomings of LLE, a miniaturized sample pre-treatment technique called single drop microextraction (SDME) was developed in the mid-to-late 1990s (Jeannot & Cantwell, 1996). In this regard, Jeannot and co-workers classified SDME as miniaturized LLE or liquid phase microextraction (LPME). In contrast to LLE that needs large volume of organic solvent, SDME only requires a small microliter of organic solvent to isolate the target analytes. Briefly, SDME involves the transfer of target analyte from aqueous phase into the organic phase which is then back-extracted into a microdroplet of the acceptor phase floating in the organic layer (Li et al., 2018). Even though, SDME only requires a few microlitre of organic solvent, excessive stirring prone to break the organic droplet and it is quite challenging to attain equilibrium.

Therefore, another type of LPME called hollow-fibre liquid phase microextraction (HF-LPME) was introduced to overcome the limitations of SDME (Pedersen-Bjergaard & Rasmussen 1999). HF-LPME system involves the application of hollow fibre made up of polypropylene impregnated with an extraction solvent, which is then immersed into an aqueous matrix solution to isolate the target analytes (Khan et al., 2020). Even though, HF-LPME successfully overcomes the limitations of SDME by stabilizing the organic droplet, HF-LPME stills requires a long extraction time which impedes its efficiency (Quigley et al., 2016). In 2006, Rezaee et al. developed a new sample treatment method,

called dispersive liquid-liquid microextraction (DLLME) (Rezaee et al., 2006). In this method, a mixture of high-density extraction solvent and dispersive solvent will be rapidly injected into aqueous sample to aid the formation of emulsion. The extract containing the target analyte will eventually be sedimented at the bottom of the extraction vial via centrifugation (Zhang & Lee, 2013). The limitations and evolution of DLLME technique are discussed in Section 2.7.1.

2.6 Surfactant

Surfactants are “surface active agents” (De et al., 2015) which are also known as amphiphilic organic molecules that contain both hydrophobic and hydrophilic moieties; hence the surfactants are soluble in both water and organic solvent. A surfactant molecule typically has an R-X structure, where R is a hydrocarbon chain (linear, branched or containing aromatic rings) while X is the polar or ionic head group. Surfactants are classified according to their hydrophilic group (Filik & Demirci, 2011). Basically, there are four types of surfactants namely non-ionic, anionic, cationic, zwitterionic or amphoteric. A non-ionic surfactant has no net charge in its head, such as Triton X-100 and Tween 80. In contrast, anionic surfactant such as sodium dodecyl sulfate (SDS) carries a negative charge while cationic surfactant like cetyltrimethylammonium bromide (CTAB) has a positive charge. If a surfactant has a head with both positive and negative charges, it is termed as zwitterionic and its examples are sulfobetaines and amino acids (Yamini et al., 2020).

Surfactants form micelle beyond its critical micelle concentration (CMC) that occurs as a result of the self-assembly of surfactant molecule amphiphiles in various shapes and sizes depending on the nature of the surfactant (Samal et al., 2017). The

colloidal sized cluster called micelle is formed with the hydrophilic tails pointing inwards while the hydrophobic heads are drenched in the adjacent liquid. CMC of a surfactant is based on many factors including its molecular weight and experimental parameters such as temperature, counterions and ionic strength (Filik & Demirci, 2011). It is ought to be noted that upon reaching its CMC, the surfactant in aqueous solution undergoes cloud point phenomenon where the solution turns cloudy spontaneously due to reduced solubility of the surfactant in the aqueous phase. This cloud point phenomenon is typically induced by a specific temperature called cloud point temperature (CPT) (Mohd et al., 2018b). Each type of surfactant possesses its own unique CPT. The clouding phenomena results in the formation of a small portion of surfactant rich phase (SRP) and a bulky aqueous phase. The SRP contains the target analyte entrapped by micelle aggregates (Mohd et al., 2018a). One of the most prominent properties of surfactant is their excellent ability to solubilize solutes of varying polarity and nature. These solutes may interact with each other electrostatically, hydrophobically or by a combination of both (Yazdi & Es'haghi, 2005). The ability of the surfactants to solubilize different solutes has been exploited to improve relative recovery of organic compounds in many methodologies. To date, wide range of organic compounds such as pesticides (Santalad et al., 2009), drugs (Kori et al., 2018), phthalate esters (Li et al., 2016), phenolic compounds (Kiai et al., 2018), polycyclic aromatic hydrocarbons (PAHs) (Mateos et al., 2019), etc., have been recovered by surfactant based extraction techniques.

Silicone surfactants are a type of amphiphilic compounds that contain both water and a silicone soluble component in a single molecule. Lately, non-ionic silicone surfactant composed of polyethylene glycol (PEG) garnered much attention when it was widely experimented in micellar based cloud point extraction (CPE). The distinguished

ability of silicone surfactants to isolate wide range of organic compounds has been demonstrated in many CPE studies (Noorashikin et al., 2013; Zain et al., 2016). Silicone surfactants are thermally stable, biodegradable and environmentally benign with high surface activity and molecular weight mainly due to the unique properties of siloxane chain (Yin et al., 2019). The polysiloxane chain of silicone surfactant is flexible and contains methyl groups with low cohesive energy, resulting in low surface tension. The majority of commercial silicone surfactants have a linear backbone with functionalized pendant polyalkylene oxide moiety (Laubie et al., 2013). Furthermore, they have been a significant and evolving class of resources employed in cosmetics, food and pharmaceutical industries because of their excellent biocompatibility and non-toxicity (Annunziata et al., 2002; Yao et al., 2007). Moreover, silicone surfactants have been demonstrated to have a better stability, wettability, surface activity and effective spreading properties (Sheng et al., 2016).

2.7 Emulsification of organic solvent by surfactant

Based on CPE literature, surfactants have been proven to be an excellent candidate for isolating wide range of polar and non-polar organic compounds. Following CPE, many researchers have explored the application of surfactant as an emulsifier in different types of LPME techniques due to their ability to be soluble in both aqueous and water-immiscible phases. Section 2.7.1, 2.7.2 and 2.7.3 describe various LPME techniques that attempted the emulsification of organic solvent by employing surfactant.

2.7.1 Surfactant assisted dispersive liquid-liquid microextraction

DLLME is more advantageous compared to other LPME techniques in terms of rapidity, simplicity of operation, cost effective, good recovery and high enrichment factor. However, DLLME suffers from some disadvantages such as (i) inefficient to extract polar compounds, (ii) consumption of hazardous organic solvent, (iii) dispersive solvent decreases the partition coefficient of target analyte into the extraction solvent (Molaei et al., 2015). To overcome these limitations, surfactant is applied as a disperser solvent in DLLME as a green approach to extract analyte in an environmentally friendly way. The new technique is known as surfactant assisted dispersive liquid-liquid microextraction (SA-DLLME) (Saraji & Bidgoli, 2010). In SA-DLLME, surfactant acts as an emulsifier that promotes emulsification of organic solvent into aqueous sample. Furthermore, surfactant could increase the contact area between the aqueous and organic phases by reducing the surface tension among the two phases, eventually leads to high recovery of target analyte into the organic solvent (Vichapong et al., 2015).

2.7.2 Ultrasound assisted surfactant enhanced emulsification microextraction

The application of disperser solvent such as acetonitrile and methanol in DLLME reduces the partition coefficient of analytes into the organic solvent. To overcome this drawback, ultrasound assisted emulsification microextraction (UAEME) is introduced. In this method, ultrasound energy is used to disperse organic extraction solvent into the sample solution without the use of disperser solvent (Regueiro et al., 2008). However, UAEME possesses longer extraction time and prone to undergo analyte degradation due to ultrasound irradiation (Asadi et al., 2015). Therefore, a novel method called ultrasound assisted surfactant enhanced emulsification microextraction (UASEME) is proposed where surfactant is used as disperser solvent. Surfactant as an emulsifier in UASEME can facilitate the formation of fine droplets of extraction solvent into the aqueous phase with the use of ultrasound energy and accelerate the mass transfer of analytes from sample solution into the organic phase, thus reducing the extraction time (Liang et al., 2014).

2.7.3 Vortex assisted surfactant enhanced emulsification liquid-liquid microextraction (VALLME)

Following DLLME and UAEME, a new protocol called VALLME is initiated. In VALLME, dispersion of organic solvent into the aqueous sample is achieved by vortex mixing, a mild emulsification procedure (Bosch Ojeda & Sanchez Rojas, 2014). VALLME tackles the main problems of DLLME requiring the dispersive solvent and the degradation of analyte under UAEME and USAEME. However, longer extraction time is required for VALLME (Moreno-Gonzalez et al., 2015). Therefore, a new method termed

vortex assisted surfactant enhanced emulsification liquid-liquid microextraction (VSLLE) is proposed in which surfactant as an emulsifier could intensify extraction efficiency besides reducing the extraction time. Surfactant serves to reduce the interfacial tension between organic and aqueous phase thereby, facilitating the dispersion of extraction solvent into the sample (Donthuan et al., 2014).

2.8 Deep eutectic solvent

The philosophy of green chemistry encourages the development and application of methods that minimize and eliminate the use or production of toxic substances (Mustafa & Turner, 2011). Accordingly, to develop extraction methods that are environmentally friendly, noxious organic solvent is eliminated while miniaturized and automated analytical technique is used. In the quest for eco-friendly solvents, deep eutectic solvent (DES) has attracted considerable attention because of their benefits including ease of preparation and cheap raw materials. In 2003, Abbot and his co-workers initiated the synthesis of DES as a subclass of ionic liquids (ILs) (Abbott et al., 2003). Briefly, DES is synthesized by combining two different chemical compounds called hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) at a specific molar ratio (Ge et al., 2019). In other words, DES is a system arises through the mixing of Lewis or Bronsted acids and bases which is made up of a variety of anionic and/or cationic species (Smith et al., 2014). DES can also be prepared through freeze drying method where the HBA and HBD will be freeze dried to generate clear homogenous liquid of DES (Gutierrez et al., 2009).

Quaternary ammonium salts such as choline chloride (ChCl) and choline acetate (ChAc) are generally used as HBA while HBD are usually amides, amines, alcohols and

organic acids. The formation of DES is mainly due to the hydrogen bond interaction between the HBA and HBD (Musarurwa & Tavengwa, 2020). Unlike ILs, which has been reported to be poorly biodegradable and expensive (Kareem et al., 2010), DES possesses superior properties such as low toxicity, inexpensive and highly biodegradable (Li et al., 2016). DES is an emerging green solvent that have some remarkable characteristics such as low conductivity, minimal vapor pressure and good thermal stability (Tang & Row, 2013). Moreover, there are boundless possibilities to synthesis various DES due to high flexibility in choosing their HBA and HBD constituents. Besides, the physicochemical properties of the eutectic mixture can be easily tweaked in terms of pH, conductivity and freezing point by altering the constituents of HBA and HBD (Zhang et al., 2012).

2.9 Magnetic iron particles

Magnetic iron particles (Fe_3O_4) have been used as a potential sorbent in magnetic solid phase extraction (MSPE) for many years in the removal and extraction of various contaminants. They have many superior properties such as large surface area, highly active surface sites and a small diffusion path (Sharifabadi et al., 2014). Furthermore, their excellent magnetic properties enable them to be retrieved with ease by applying external magnetic field (Zhou et al., 2009). Moreover, these adsorbents can be seamlessly recycled and reused. Till to date, many popular approaches have been reported to synthesize Fe_3O_4 particles which are co-precipitation (Kandpal et al., 2014), sol-gel (Xu et al., 2007) and sonochemical reaction (Islam et al., 2011). Among other techniques, the co-precipitation method is well-known for its excellent time-saving properties, high yield of Fe_3O_4 particles, non-harmful solvent and low chemical consumption (Kaur et al., 2014). Co-precipitation process involves the synthesis of Fe_3O_4 particles using ferric and ferrous ions