DEVELOPMENT OF FUNCTIONALIZED OIL PALM EMPTY FRUIT BUNCH-BASED MAGNETIC-CARBOXYMETHYL CELLULOSE NANOFIBERS COMPOSITE FOR THE MAGNETIC SOLID-PHASE EXTRACTION OF ORGANOPHOSPHORUS PESTICIDES

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by

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LIST OF SYMBOLS

%	Percent
°	Degree
θ	Theta
Bar	Unit of pressure
°C	Degree Celsius
cm	Centimeter
cm^{-1}	Per centimeter
$cm^3\ g^{-1}$	Cubic centimeter per gram
d	Diameter
$emu\ g^{-1}$	Unit of magnetic moment
g	Gram
min	Minute
mL	Milliliter
mg	Milligram
mm	Millimeter
min^{-1}	Per minute
m^2g^{-1}	Square meter per gram
$mg\ L^{-1}$	Milligram per liter
$mg\ kg^{-1}$	Milligram per kilogram
M	Molar
$mL\ min^{-1}$	Millimeter per minute
MPa	Megapascal
$ng\ L^{-1}$	Nanogram per liter
$ng\ mL^{-1}$	Nanogram per milliliter
nm	Nanometer

μL	Microliter
μm	Micrometer
$\mu g\ kg^{-1}$	Microgram per kilogram
rpm	Revolution per minute
r^2	Correlative of determination
T_{max}	Maximum temperature
Log K_{ow}	*n*-octanol/water partition coefficient

LIST OF ABBREVIATIONS

AcHE	Acetylcholinesterase
ACN	Acetonitrile
APTES	(3-Aminopropyl)triethoxysilane
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CMC	Carboxymethyl cellulose
CNF	Cellulose nanofiber
CNC	Cellulose nanocrystal
CNW	Cellulose nanowhisker
CNT	Carbon nanotube
CPO	Crude palm oil
CrI	Crystallinity index
DAC	Dialdehyde cellulose
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DLLME	Dispersive liquid-liquid microextraction
DMAC	Dimethylacetamide
DSPE	Dispersive solid-phase extraction
EF	Enrichment factor
EU	European Union
EDX	Energy Dispersive X-Ray
FAO	Food and Agriculture Organization of the United Nations
Fe_3O_4-cmCNF	Magnetic-carboxymethyl cellulose nanofiber
Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)	Magnetic-carboxymethyl cellulose nanofibers grafted poly(1-vinyl-3-phenyl imidazolium bromide)
FT-IR	Fourier transform infrared
GC-µECD	Gas chromatography micro-electron capture detector
GC-FID	Gas chromatography flame ionization detector
GC-NPD	Gas chromatography nitrogen/phosphorus detector
GC-MS	Gas chromatography–mass spectrometry
GC-FPD	Gas chromatography-flame photometric detector
H_2O_2	Hydrogen peroxide

HPLC-PDA	High performance liquid chromatography photometric diode array
IL	Ionic liquid
KOH	Potassium hydroxide
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LiCl	Lithium chloride
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MCA	Monochloroacetic acid
MNP	Magnetic nanoparticle
MSPE	Magnetic solid-phase extraction
MRLs	Maximum residual limits
NaOH	Sodium hydroxide
NaCl	Sodium chloride
NaClO	Sodium hypochlorite
NC	Nanocellulose
OH	Hydroxyl
OPPs	Organophosphorus pesticides
OPEFB	Oil palm empty fruit bunch
OPM	Oil palm mesocarp
OPF	Oil palm frond
OPL	Oil palm leave
OVAT	One-variable-at-time
PIL	Poly(ionic liquid)
PPF	Palm pressed fiber
QuEChERS	Quick Easy Cheap Effective Rugged Safe
RSDs	Relative standard deviations
SDME	Single drop microextraction
SEM	Scanning electron microscope
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TGA	Thermogravimetric analyzer
TG	Thermogravimetric

TEM	Transmission electron microscope
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl, 2,2,6,6-tetramethyl-1-piperidinyloxy
VSM	Vibrating sample magnetometer
WHO	World Health Organization
XRD	X-ray diffraction

LIST OF APPENDICES

PEMBANGUNAN KOMPOSIT NANOGENTIAN SELULOSA KARBOKSIMETIL BERMAGNET TERFUNGSI BERASASKAN TANDAN KOSONG KELAPA SAWIT DALAM PENGEKSTRAKAN FASA PEPEJAL BERMAGNET BAGI RACUN PEROSAK ORGANOFOSFORUS

ABSTRAK

Penyalahgunaan bahan kimia racun perosak organofosforus (OPP) telah menyebabkan isu kesihatan awam yang serius dan boleh menyebabkan pencemaran alam sekitar. Tahap residu OPP yang pada umumnya rendah dan kompleksiti matriks, seperti air alam sekitar dan sayur-sayuran, adalah kebimbangan yang mesti ditangani oleh penyelidik. Sebagai respons kepada ini, adalah penting untuk membangunkan penyediaan sampel dan kaedah analisis yang sensitif, selektif dan cekap untuk menentukan kepekatan OPP dalam matriks kompleks. Pelbagai Teknik penyediaan sampel telah digabungkan dengan instrumentasi yang mana boleh mengatasi kelemahan yang dialami oleh kaedah klasik dengan mewujudkan jangka hayat yang panjang, prosedur yang mudah, pengekstrakan selektif yang sangat baik, dan mesra alam. Oleh yang demikian, komposit nanogentian selulosa karboksimetil bermagnet terfungsi baharu berasaskan tandan kosong kelapa sawit telah berjaya disintesis untuk digunakan dalam dalam pengekstrakan fasa pepejal bermagnet bagi analisis OPP terpilih di dalam sampel air alam sekitar, sayur salad, dan sayur saleri. Komposit ini dihasilkan daripada penggabungan nanogentian selulosa karboksimetil yang diekstrak daripada tandan kosong kelapa sawit (OPEFB), zarah nano bermagnet (Fe_3O_4 MNPs), serta imidazolium berasaskan cecair ionik melalui tiga proses (karboksimetilasi, sol-gel, dan pengkopolimeran cangkuk). Sisa biojisim OPEFB dimulakan dengan pra-rawatan diikuti dengan pengabungan protokol rawatan kimia (natrium hidroksida (NaOH) alkali) dan mekanikal (homogenisasi dan ultrasonik) untuk menghasilkan nanogentian selulosa (CNF). Penyediaan nanogentian selulosa karboksimetil bermagnet (Fe_3O_4-cmCNF) dan nanogentian selulosa karboksimetil bermagnet bercangkuk PIL (Fe_3O_4-cmCNF-g-poli(3Ph1VImBr)) telah dicirikan oleh analisis mikroskopi elektron pengimbas (SEM), mikroskopi elektron penghantaran (TEM), spektroskopi inframerah fourier transformasi (FT-IR), magnetometer Sampel Bergetar (VSM), analisis Brunauer–Emmett–Teller (BET), pembelauan sinar-X (XRD), dan analisis termogravimetrik (TGA). Penentuan dan kuantifikasi diperoleh menggunakan kromatografi gas-pengesanan penangkap mikro elektron (GC-μECD). Kaedah MSPE berdasarkan Fe_3O_4-cmCNF telah dibangunkan untuk menentukan kepekatan surih diazinon, fenitrotion, dan malathon di dalam air alam sekitar. Di bawah keadaan optimum, julat kelinearan dicapai antara 0.1 hingga 200 ng mL^{-1} dengan koefisien penentuan yang baik ($r^2 > 0.9962$). Kaedah yang dicadangkan ini membolehkan penentuan OPP dengan had pengesanan (LOD) pada 0.03 ng mL^{-1} dan memerlukan masa pengekstrakan yang singkat (3 min) serta penggunaan pelarut organik yang rendah (0.2 mL). Pengekstrakan telah berjaya dibuktikan dengan julat perolehan semula relatif yang baik iaitu 81.8–119.6 % dengan kepersisan (RSD) antara 1.7 hingga 14.0 %. Seterusnya, Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)-MSPE yang dioptimumkan telah berjaya digunakan untuk menentukan enam OPP terpilih (diklorvos, diazinon, tolklofos-metil, fenitrotion, malation, dan klopirifos) dalam sampel sayur salad dan sayur saleri. Kaedah ini menunjukkan lengkung kelinearan yang dipadankan dengan matriks pada julat kepekatan 0.1 hingga 250 μg kg^{-1} ($r^2 > 0.9954$), LOD rendah (0.01-0.07 μg kg^{-1}), dan perolehan semula relatif yang baik dalam julat 81-117 % (RSD ≤ 14 %). Teknik MSPE yang telah dibangunkan dan digabungkan kromatografi gas telah terbukti bermanfaat dan menawarkan proses penyediaan sampel yang sensitif, mudah, kurang penggunaan pelarut organik bertoksik, mesra alam dan sesuai untuk penentuan OPP dalam matriks sampel sebenar.

DEVELOPMENT OF FUNCTIONALIZED OIL PALM EMPTY FRUIT BUNCH-BASED MAGNETIC-CARBOXYMETHYL CELLULOSE NANOFIBERS COMPOSITE FOR THE MAGNETIC SOLID-PHASE EXTRACTION OF ORGANOPHOSPHORUS PESTICIDES

ABSTRACT

The abusive use of organophosphorus pesticides (OPPs) chemical has caused serious public health issues and can lead to contamination of the environment. The generally low levels of OPP residues and often the complexity of the matrix, such as environmental waters and vegetables, are the concerns that researchers must deal with. In response to this, it is vital to develop a sensitive, selective, and efficient sample preparation and analytical method to determine the concentration of OPPs in complex matrices. Various sample preparation techniques have been integrated with instrumentation which can overcome drawbacks experienced by the classical methods by establishing longer longevity, simple procedure, excellent extraction selectivity, and environmental friendly. For that reason, a new functionalized oil palm empty fruit bunch-based magnetic-carboxymethyl cellulose nanofibers composite was synthesized to be used in magnetic-solid phase extraction (MSPE) for analysis of selected OPPs in environmental water, lettuce, and celery vegetable samples. This composite was fabricated from the incorporation of carboxymethyl cellulose nanofibers extracted from oil palm empty fruit bunch (OPEFB), magnetic nanoparticles (Fe_3O_4 MNPs), and imidazolium-based ionic liquid (IL) via three steps processes (carboxymethylation, sol-gel, and grafting copolymerization). The OPEFB biomass residue was started with pre-treatment followed by combining chemical (sodium hydroxide (NaOH) alkaline) and mechanical (homogenization and ultrasonication) treatment protocols producing cellulose nanofibers (CNF). The prepared magnetic-carboxymethyl cellulose nanofibers (Fe_3O_4-cmCNF) and magnetic-carboxymethyl cellulose nanofibers grafted PIL (Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)) have been characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), vibrating sample magnetometer (VSM), Brunauer-Emmett-Teller (BET) analysis, X-ray diffraction (XRD), and thermogravimetric analysis (TGA). Determination and quantification of OPPs were obtained using a gas chromatography-micro electron capture detector (GC-µECD). An MSPE method based on Fe_3O_4-cmCNF was developed to determine the trace concentration of diazinon, fenitrothion, and malathion in environmental water. Under optimum conditions, the linearity range was achieved between 0.1 to 200 ng mL^{-1} with good determination coefficient ($r^2 > 0.9962$). The proposed method allows the determination of OPPs with limits of detection (LODs) at 0.03 ng mL^{-1} and required a short extraction time (3 min) as well as low organic solvent consumption (0.2 mL). The successful extraction was proved with a good relative recoveries range of 81.8–119.6 % with precision (RSDs) between 1.7 to 14.0 %. Next, the optimized Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)-MSPE was successfully applied to determine six selected OPPs (dichlorvos, diazinon, tolclofos-methyl, fenitrothion, malathion, and chlorpyrifos) in lettuce and celery samples. The method displayed matrix-matched linearity curves over a concentration range of 0.1 to 250 µg kg^{-1} ($r^2 > 0.9954$), low LODs (0.01-0.07 µg kg^{-1}), and good relative recoveries in the range of 81-117% (RSDs ≤ 14%). The established MSPE techniques hyphenated with gas chromatography have proved to be beneficial and offer a sensitive, simple sample preparation process, less consumption of toxic organic solvent, environmentally friendly, and appropriate for the determination of OPPs in real sample matrices.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The increase in global crop production to compensate the world's population demand between 2000 and 2019 has upsurge in global pesticide usage by 36 % (4.2 million tonnes in 2019), with the highest contributions coming from Asia, including Malaysia (FAO, 2021). The term pesticide can be defined as a chemical agent used to prevent, destroy, repel, or mitigate any pest. Pesticides can be classified into several categories: insecticides, fungicides, herbicides, rodenticides, molluscicides, and others, depending on their active ingredient and functionalities (Chawla *et al*., 2018). In the 1970s, organophosphorus pesticides (OPPs) have been developed to replace common pesticides such as 1,1,1-trichloro-2,2-bis[4-chlorophenyl]-ethane (DDT), organochlorine pesticides (OCPs), and pyrethroids pesticides (PYRs) which have been banned by various countries and discontinued their production. Generally, the OPPs chemical structure was designed by double-bonded central phosphorous (P) atom to either oxygen (O) or sulphur (S) atom, and single bonded with either two methoxy ($–OCH_3$) or ethoxy ($–OCH_3CH_3$) groups (Mohamed *et al*., 2021). In addition, the most widely used OPPs around the world come from phosphate, pyrophosphate, and thioester pesticides derivative (Gao *et al*., 2022).

Considering the presence of OPPs in trace level and complex matrix effect. Thus, suitable sample pre-treatment steps is necessary to provide sensitive, selective, high enrichment, and effective separation of targeted analytes from interfering components prior to instrumental analysis (Li *et al*., 2021). In recent years, efforts have been made by the researchers to develop diverse extraction techniques to determine the presence of interest OPPs from various real environmental matrices samples, including solid-phase extraction (SPE) (Amiri *et al.*, 2020; Sanagi *et al.*, 2013), cloud-point extraction (Liyuan Zhang *et al.*, 2022), supercritical fluid extraction (SFE) (Hopper, 1999), Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) method (Yu *et al.*, 2020), dispersive liquid-liquid microextraction (DLLME) (Wang *et al.*, 2016), single drop microextraction (SDME) (Salemi *et al.*, 2013), solid-phase microextraction (SPME) (Amini et al., 2021; Yavir and Kloskowski, 2021), dispersive solid-phase extraction (Amiri *et al.*, 2019; Mao *et al.*, 2021), and magnetic solid-phase extraction (MSPE) (Hassan *et al.*, 2021; Wan *et al.*, 2021).

Among these techniques introduced for various environmental matrices, MSPE is a better alternative approach that offers facile procedure and effective pre-treatment, including improved adsorption efficiency by reducing the mass transfer equilibrium time. In addition, the adsorbent can be separated without the intervention of centrifugation or filtration, enriches target analytes, reduces sample matrix interference, and improves the accuracy of results (Jiang *et al.*, 2019).

The first “MSPE” term was introduced by Safarikova and Safarik in 1999 (Safarikova and Safarik, 1999), and later the incorporation of this high surface area superparamagnetism components such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and cobalt ferrite ($CoFe_2O_4$) have avoided drawbacks experienced by several SPE-based techniques, for example, packing bed blockage and high-pressure involvement during sorbent packing, recyclability, potential contamination of fiber, and limiting the speed of sample pre-treatment (Mohamed *et al.*, 2022). Principally, MSPE operates by dispersing magnetic-based materials into an aqueous sample containing analytes. The adsorption mechanism takes place via physical or chemical adsorption over time, followed by separation using an external magnet. Finally, the analytes are then desorbed using appropriate solvents, and magnetic-based materials are separated using an external magnet (without the intervention of centrifugation or filtration). During the execution of the MSPE procedure, the most important parameters need to be considered is the types of adsorbents and its surface structural features. Generally, the adsorbents used in the MSPE are Fe_3O_4-based materials with different functional groups, which are suitable for various analytes. Nevertheless, the surface modification of Fe_3O_4 was usually complicated and laborious. Therefore, the key function to the superior capabilities of MSPE method is very much dependent on the performance of the adsorbent.

The production of nanomaterials from renewable and abundant biomass, such as nanocellulose (NC) obtained from native cellulose, has been drawing significant attention in the last decade. Isolation of nanocellulose from various sources and utilization of various techniques have enabled its multifunction application in various fields (Wang *et al*., 2022). Study on preparation of nanofibrillated cellulose or also known as cellulose nanofibers (CNF) via different isolation methods and viable raw materials has been extensively sought after its excellent characteristic in physicochemical, mechanical, and optical properties. Isolation of CNF from biomass waste source such as sorghum (Pennells *et al*., 2021), rice husk (You *et al*., 2021), pineapple fiber (Faria *et al*., 2020), corn (Ibrahim *et al*., 2019), husk of millet (Dominic *et al*., 2022), soybean residue (Wang *et al*., 2021), cotton textiles (Xia *et al*., 2021) and oil palm (Fahma *et al*., 2010; Lamaming *et al*., 2017; Okahisa *et al*., 2020) have benefited significantly for agricultural needs.

In Malaysia, oil palm has become one of the major contributions for economic growth with production up to 10.8 million ton annually (Noor *et al*., 2020). The increment of oil palm tree production has resulted in large amount of lignocellulosic bio-fiber waste which is rich in cellulose. Due to its limited application as organic fertilizers and combustion to generate electricity, the excessive leftovers of these materials have triggered significant environmental concerns due to decomposition issues. Oil palm empty fruit bunch (OPEFB) is one of the wastes produced after extraction of palm oil. The total mass of OPEFB contains approximately 23.7-65.0% cellulose content, followed by hemicellulose and lignin content with about 20.6-33.5% and 14.1-30.5%, respectively (Lamaming *et al.*, 2015; Yiin *et al.*, 2019). Therefore, this makes the OPEFB suitable candidate to be used as viable raw material for the preparation of CNF adsorbent. Notably, the adsorption performance of an adsorbent material mainly relates to the availability of the functional groups that are attached to its surface which further improves selectivity as well providing active sites for interaction to occur (Qi *et al*., 2022).

Ionic liquids (ILs) are liquid electrolytes made from the unique combination of the broad potential of organic cations (such as imidazolium, pyridinium, alkylammonium, alkylphosphonium, pyrrolidinium, piperidinium, and guanidinium) with inorganic (such as halides, mineral acid anions, polyatomic anions, and polyoxometalates) or organic (hydrophilic or hydrophobic) counter anions (Mohamed *et al.*, 2022). Besides their behavior as liquid below 100 °C, the combination of these organic cations with inorganic or organic counter anions has also produced distinguished tunable properties such as high thermal stability, tunable solvation capabilities, providing multi-interaction depending on fine-tuning the physicochemical properties such as type and size/length of cation-anion pairs, types of backbone structure attached to the organic cation, polarity, hydrophilicity, and structure stability.

Polymeric ILs (PILs) or also known as poly(ionic liquids) is a subclass of polyelectrolytes that combine unique properties of ILs in the polymeric backbone to form a macromolecular architecture that amplifies the functions and performance that ILs cannot deliver (Yuan *et al*., 2013). Although numerous pairings of cation-anion are available for the formation of PILs, the imidazolium-based PILs has attracted scientists and researchers due to its intriguing molecular structure which possessed imidazolium cation ring that has two nitrogen atoms (N1-amino nitrogen donor and N3-imino nitrogen acceptor) interconnected with a methylene group. During quaternization of the imidazole ring with various substituents such as alkyl and aryl groups, a permanent positive charge is produced which allows the nitrogen heteroatom ring to contribute its amphoteric behaviors (donate and accept protons) throughout the substitution reaction. Much of the inspiration for the preparation of such imidazolium-based PILs has involved three possible polymerization techniques such as direct polymerization (polycondensation), post-polymerization (chemical modification of polymeric precursors), or step-growth polymerization which depending on the types and functionality of the polymer produced (Yuan *et al*., 2013).

The exploration imidazolium-based PILs as modifiers or surface functional groups during chemical modifications of polysaccharide biopolymers such as cellulose-based materials enhanced conducive adsorption sites and adsorption capacity within the polysaccharide biopolymer structure (Qian *et al*., 2019; Zhang *et al*., 2022). Moreover, the compatibility and good affinity between the cellulose and synthetization polymers such as imidazolium-based PILs have been studied in various applications (Kerche *et al*., 2022; Murakami *et al*., 2007; Suo *et al*., 2020). In order to prepare a cellulose-based adsorbent with high selectivity and extraction efficiency towards OPPs, in this work, a novel PIL termed poly(1-vinyl-3-phenyl imidazolium bromide), was synthesized and used for the modification of magnetic-carboxymethyl cellulose nanofibers. To achieve this goal, magnetic-carboxymethyl cellulose nanofibers grafted poly(1-vinyl-3-phenyl imidazolium bromide) (Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)) was prepared via grafting polymerization (Abou-Zeid *et al*., 2021) and its performance as a dispersive solid adsorbent in MPSE technique against OPPs as model compounds in environmental water and vegetable samples were investigated. The experimental parameters affecting the extraction efficiency including the type and amount of adsorbent, extraction time, pH level, type of elution solvent, and ionic strength, were optimized using a one-variable-at-a-time (OVAT) approach. The main merit of this technique is using the adsorbent which is bio-renewable material, cost effective, biocompatible, handy magnetic driven approach, and posed surface chemistry which provide multiple adsorption sites during extraction process.

The first part of the study focuses on the synthesis and characterization of the synthesized adsorbents, namely native CNF, cmCNF, Fe_3O_4-cmCNF, and Fe_3O_4-cmCNF-g-poly(3Ph1VImBr). Next, the second part of the study discussed the development of Fe_3O_4-cmCNF composite as an adsorbent for magnetic solid-phase extraction coupled with GC-µECD for the analysis of three selected OPPs in environmental water samples. Finally, the last part of the study elaborated on the development of Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) as an adsorbent for magnetic solid-phase extraction coupled with GC-µECD for six selected OPPs in vegetable samples.

1.2 Problem statement

Although OPPs are feasible and effective as a tool to protect crops from pests and weeds invasion, only one percent was used, and the rest enter environment which causes deleterious effects on the ecological ecosystem. Notably, OPPs contamination in water bodies was particularly via various routes such as through runoff of irrigation water and precipitation, stormwater, as well as discharged from agricultural land sites (Derbalah *et al.*, 2019). Meanwhile, in food crops such as vegetables, OPPs have been reported to be applied repeatedly during the whole period of growth and this has been adsorbed by the crops and turned to be noxious when consumed by humans. Studies have revealed that OPPs are mutagenic, carcinogenic, cytotoxic, genotoxic, teratogenic and immunotoxic, and frequent exposure to OPPs leads to inhibition of acetylcholinesterase enzyme (AChE) at the nerve ending and subsequently results in disruption of nerve functioning causing paralysis or even casualty (Mohamed *et al.*, 2021; Sharma *et al.*, 2010). Triggered by public concerns to control and reduce human exposure to pesticides, international organizations such as the European Union (EU), World Health Organization (WHO), and Food and Agricultural Organization (FAO) have recommended the maximum residual limits (MRLs) in food and water samples at 0.01-0.5 mg kg^{-1} and 0.5 ng mL^{-1}, respectively (European Environment Agency, 2018; FAO and WHO, 2016). In Malaysia, OPPs' MRLs is following the guidelines provided by Sixteenth Schedule, Malaysia Food Act 1983 and adopted CODEX Alimentarius with maximum permitted proportion below than 1 mg kg^{-1} for specified food including lettuce (romaine lettuce) and celery (Chinese celery) (Malaysia Government, 2020). Due to low concentration, complexity, and high interference in diversity of matrices including environmental water and vegetables, direct analysis using instruments is rather impossible and required high-end instrumentation (Kiguchi *et al.*, 2014). Thus, the sample preparation step is crucial to remove major interferences and to enrich the target OPPs analytes. In addition, the detection and quantification procedures of OPPs in environmental water and vegetable samples is of significance to environmental monitoring and human health risk evaluation.

The classical liquid-liquid extraction (LLE) and SPE methods suffer from several major disadvantages such as large amounts of hazardous organic solvents usage, involve length steps of operation, laborious process, and limited reusability characteristics. Recently, MSPE is a dynamic and miniaturized version of dispersive solid-phase extraction (DSPE) which improves and simplifies LLE and SPE steps by applying magnetic component producing superparamagnetic responsiveness adsorbent, which allows for magnetic retrieval during the extraction and desorption process. Nevertheless, most of the conventional solid phase adsorbents developed lack of selectivity, and exhibit low extraction capacity, leading to low sensitivity. Furthermore, suppression or enhancement of analyte signal, due to strong matrix effects, is also encountered (Behpour *et al*., 2023).

Thus, to overcome these shortcomings, the CNF isolated from oil palm empty fruit bunch (OPEFB) via alkaline treatment was undergone first surface modification using carboxymethylation procedure followed by incorporation of iron (II, III) oxide (Fe_3O_4) MNPs through sol-gel technique. Next, the surface modification of Fe_3O_4-cmCNF further undergoes grafting polymerization with 3-phenyl-1-vinylimidazolium bromide (3P1VimBr) monomers. The synthesized Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) composite adsorbent is a novel class of poly(ionic liquid)-based adsorbent materials that associates the properties of IL and polymer. Thus, this hybrid composite structure is expected to have high and stable dispersion by providing electrostatic forces repulsion between the same charges contributed by imidazolium ion as well as good durability and long lifetime. Furthermore, the synergies hybrid of CNF with poly(3Ph1VImBr) is expected to demonstrate enhance surface properties, particle homogeneity, better colloidal dispersions, as well as outstanding sensitivity, selectivity, and adsorption capability, as MSPE adsorbent towards target OPPs. The surface modification forming

Fe_3O_4-cmCNF and Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) adsorbents derived from the abundant source of biomass employed in MSPE would provide additional benefits other than method simplicity and sensitivity, as well as low-cost of adsorbent preparation and environmentally friendly.

1.3 Objectives of the study

The aim of this study was to introduce alternative sample preparation methods to be used with GC-µECD for the determination of selected OPPs in environmental water and vegetable samples. Owing to the low levels of OPPs in water samples and due to the complexity of vegetable matrices, suitable sample pretreatment methods were explored. Thus, the specific aim was accomplished through the following objectives:

i. To synthesize and characterize magnetic-carboxymethyl cellulose nanofibers (Fe_3O_4-cmCNF) and magnetic-carboxymethyl cellulose nanofibers-g-poly(3-phenyl-1-vinylimidozolium) bromide (Fe_3O_4-cmCNF-g-poly(3Ph1VImBr)) from oil palm empty fruit bunch using different analytical instruments.

ii. To apply Fe_3O_4-cmCNF as an adsorbent for MSPE coupled with GC-µECD for the analysis of selected organophosphorus pesticides in water samples.

iii. To apply newly synthesized Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) as an adsorbent for MSPE coupled with GC-µECD for the analysis of selected organophosphorus pesticides in vegetable samples.

The selected OPPs compounds used as a model analyte in this study are dichlorvos, diazinon, tolclofos-methyl, fenitrothion, malathion, and chlorpyrifos. These five types of OPPs were selected as model analytes in water and vegetable samples due to the currently available and commonly used in the agriculture sector in Malaysia. Moreover, the respective OPPs are currently regulated and gazette in the Sixteenth Schedule, Malaysia Food Act 1983. In terms of structure and properties, the selected OPPs (diazinon, tolclofos-methyl, fenitrothion, malathion, and chlorpyrifos) are from the group of organothiophosphate (contain P=S, thion) except for dichlorvos from organophosphate (contain P=O, oxon). All these OPPs have been reported extensively to assess pesticide residue contamination in environments and food.

1.4 Significant of the study

In this research, two magnetic-based adsorbents derived from functionalized oil palm empty fruit bunch-based cellulose nanofiber will be synthesized and thoroughly characterized. These adsorbents, namely Fe_3O_4-cmCNF and Fe_3O_4-cmCNF-g-poly(3Ph1VImBr), were prepared for their application as selective adsorbents in the MSPE method, specifically designed for extracting OPPs compounds from complex matrices such as environmental water and vegetables.

The introduction of MSPE into the process of separating and enriching OPPs from challenging complex matrices involves adding adsorbents, performing magnetic separation, and desorbing the target substances in an extraction solution. The incorporation of magnetic nanoparticles (MNPs) with cost-effective organic materials derived from the conversion of oil palm empty fruit bunch (OPEFB) biomass into value-added nanocellulose-based biopolymeric adsorbent materials simplifies sample preparation by eliminating the need for centrifugation or filtration. This approach also requires a minimal volume of sample and solvents for extraction and desorption.

OPEFB residues have been explored as a potential source of nanocellulose due to their high cellulose content (ranging from 23.7% to 65.0%). These residues have previously been recognized as excellent adsorbents for heavy metals. Furthermore, surface functionalization with carboxymethyl groups ($-CH_2COO^-$) followed by grafting with imidazolium-based polymeric ionic liquids (PIL) significantly enhances the properties of the nanocellulose-based adsorbent. This modification introduces various potential active sites, including electrostatic, hydrogen bonding, hydrophobic, and π-π stacking interactions with the targeted OPPs. As a result, it is expected to considerably improve the adsorbent's capacity for adsorption and provide excellent selectivity, all while remaining an environmentally friendly adsorbent material.

Ultimately, the methods developed in this study are valuable tools for the analytical separation and determination of trace-level OPPs in environmental water and vegetable matrices.

1.5 Scope of the study

This study focuses on the development of new microextraction strategy termed MSPE using the newly synthesized adsorbents namely Fe_3O_4-cmCNF and Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) for the determination of selected OPPs in environmental water and vegetable samples, respectively. Several important spectroscopic techniques such as Fourier transform infrared spectroscopy (FT-IR), vibrating sample magnetometer (VSM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and

Brunauer–Emmett–Teller (BET) analysis was used for the characterization of the adsorbent materials. For MSPE, the important extraction parameters such as the type and amount of adsorbent, extraction time, pH of the solution, the type of the elution solvent, and ionic strength were optimized comprehensively in this study. Under the optimum extraction and instrument conditions, the developed MSPE methods incorporated with the synthesized adsorbents were validated in terms of linearity, repeatability or precision, and relative recovery or accuracy. The applicability of the adsorbents to the extraction of OPPs was evaluated by the application of the developed methods to the quantification of OPPs compounds in environmental water and vegetable samples. Figure 1.1 show the overall research flow for this study.

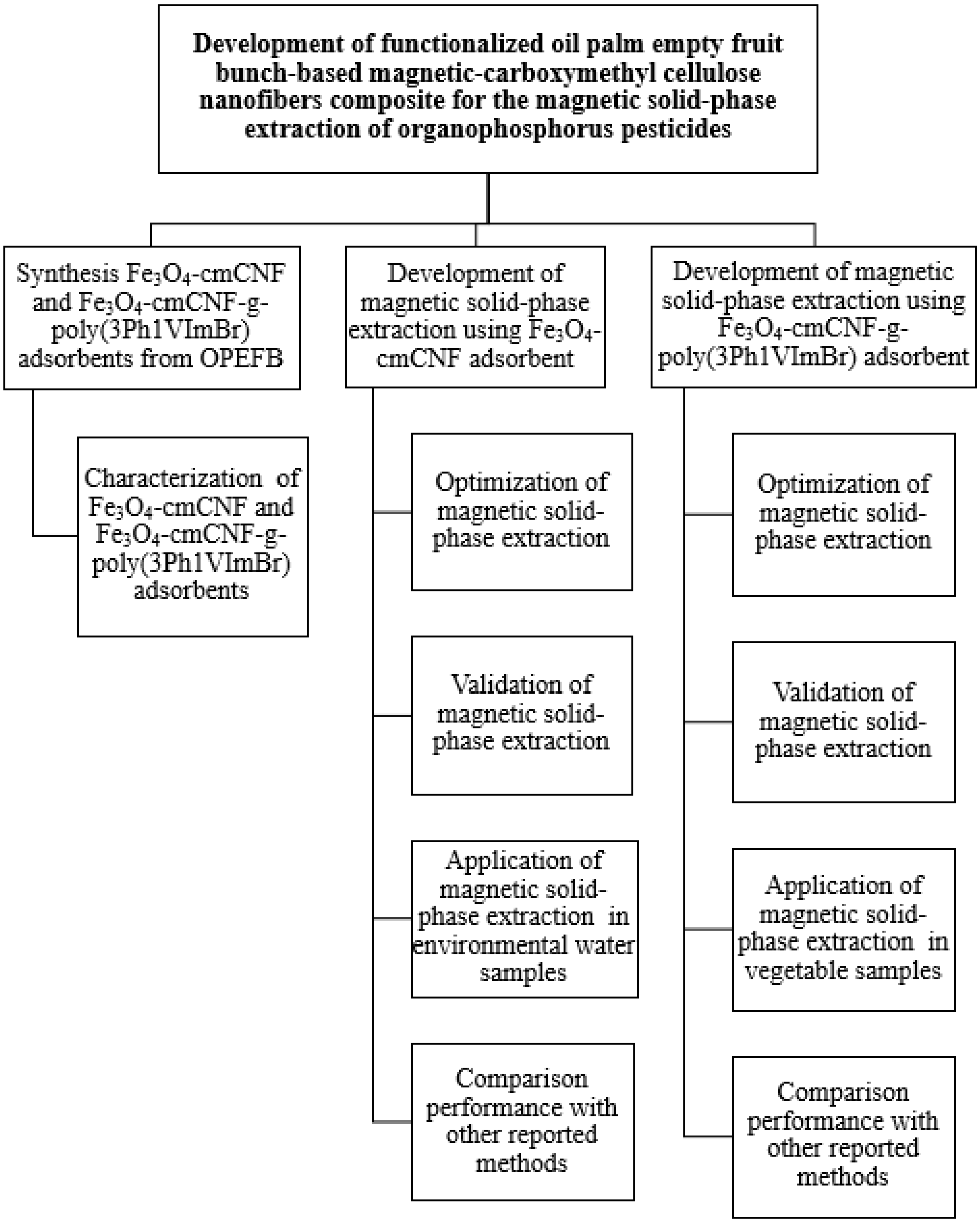


Figure 1.1 Overall research flow.

1.6 Outline of thesis

The present thesis is organized into six chapters. Chapter 1 provides a brief introduction on the background of the research, problem statement, objectives of the study, significance of the study, and scope of the study. A comprehensive literature review related to this research is presented in Chapter 2. Chapter 3 comprises the synthesis and characterization data of the synthesized adsorbents namely Fe_3O_4-cmCNF and Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) and the comparison to the native adsorbent materials. Chapter 4 describes the optimization and validation study of the MSPE method using Fe_3O_4-cmCNF for the determination of OPPs in environmental water samples. Chapter 5 discusses the development of the enhanced performance of the MSPE method using Fe_3O_4-cmCNF-g-poly(3Ph1VImBr) adsorbent for the determination of OPPs in more complex vegetable matrices. Finally, the overall conclusion with future recommendations is provided in Chapter 6 of the thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 Organophosphorus pesticides in environmental waters and vegetables

2.1.1 Overview

For more than 60 years, OPPs have been used to protect crops from pests and weed invasion which is essential to improve quality of crop yield as well as support the global food supply-demand. OPPs can be classified into three main groups, namely organophosphate (contain P=O, oxon), organothiophosphate (contain P=S, thion), and organophosphonate. Chemically, the OPPs toxicity level depends on the valency of phosphorus, the amount of sulfur present as well as functional groups which share similar properties with the nerve agents such as Sarin or Tabun (Ajiboye *et al*., 2022; Yang *et al*., 2022). Even though OPPs is commonly applied as plant protector due to their capabilities as high-performance spectral pesticide and low-price, the uncontrolled and excessive used of OPPs has cast negative side effects which damage, polluted and imbalance the ecological environment including human (Yang *et al*., 2022). In addition, the ability of OPPs to bioaccumulate, translocate and relatively persistent under acidic conditions (including reduced light and lower temperature) in environments has exposed human population unintentionally in various routes such as consumption food containing pesticides, ingestion, inhalation, swallowing or absorption via dermal contact (Guo *et al*., 2022).

Triggered by this life-threatening situation (such as inhibition of acetylcholinesterase (AChE) nervous system enzyme activity resulting in serious poisoning or even death due to respiratory failure), possible health hazards risks, and public concerns associates with the use of OPPs, various countries including regional/international organization (World Health Organization (WHO), Association of Southeast Asian Nations (ASEAN), European Union (EU), and Food and Agriculture Organization (FAO)) has established and practice guideline procedure and maximum residue limits (MRLs) to withstand safety consumption by human along with to sustain environment (Table 2.1) (Mohamed *et al.*, 2021). Table 2.2 shows the most common OPPs groups used and their physicochemical properties such as basic structure, molecular weight, solubility in water based on *n*-octanol/water partition coefficient (Log k_{ow}) value, as well as health and environment risks.

Table 2.1 Maximum residue limits (MRLs) for OPPs in different media.

Organization	**MRLs**	**Remarks**
FAO-WHO	0.3 mg kg^{-1}	Human consumption
EU	0.01-05 mg kg^{-1}	Food samples
	0.5 ng mL^{-1}	Water samples
ASEAN	0.01-0.05 mg kg^{-1}	Crops
International Food Act and Regulation 1985	< 1 mg kg^{-1}	Food samples
Codex Alimentarius Commission	< 1 mg kg^{-1}	Food samples

Source: Mohamed *et al*., (2021).

Table 2.2 Some of the OPPs used as model analytes and their properties.

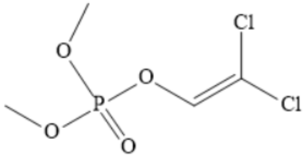
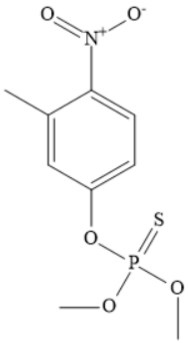
Analyte	Structure	Molecular weight	Log k_{ow}	Health and environmental risks
Dichlorvos		220.97	1.43	Reduced in AChE enzyme activity, chest tightening, wheezing, and muscle fasciculation.
Fenitrothion		277.24	3.30	Causes cholinesterase activity depression and irritating to the eyes.

Table 2.2 *Continued.*

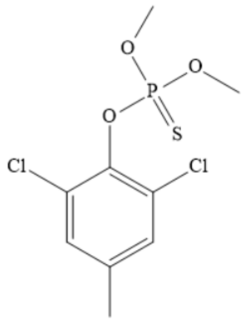
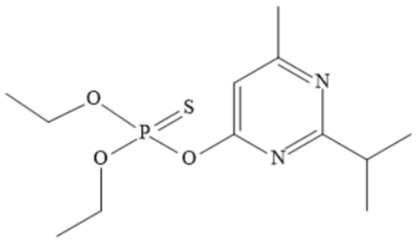
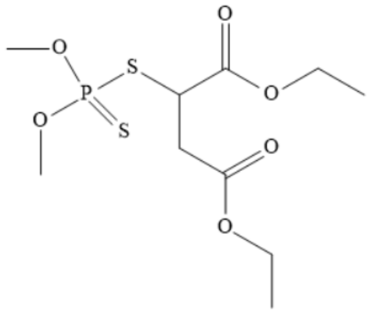
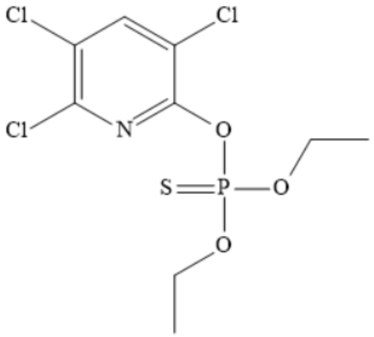
Analyte	**Structure**	**Molecular weight**	**Log k_{ow}**	**Health and environmental risks**
Tolclofos-methyl		301.13	4.56	Minimally irritating to eyes, decreased spontaneous motor activity, dyspnea, piloerection, urinary incontinence, and ataxia.
Diazinon		304.35	3.81	Eye and skin irritant and cause gastrointestinal symptoms.

Table 2.2 *Continued.*

Analyte	**Structure**	**Molecular weight**	**Log k_{ow}**	**Health and environmental risks**
Malathion		330.40	2.36	Inhibition of AChE enzyme activity affects central nervous and respiratory systems, and eye, nose, skin irritant.
Chlorpyrifos		350.60	4.96	Cholinesterase inhibition, fatigue dizziness, blurred vision, and muscle twitching.

2.1.2 Fate, migration routes, and toxicity impacts of residual OPPs

2.1.2(a) Environmental water

Despite OPPs numerous merits such as capable to control vector borne diseases, pests and weeds, their elicits deleterious effects has threatened human health when exposure via ingestion, dermal and inhalation (Elfikrie *et al.*, 2020). Typically, all pesticides discharge and contaminate environment water through spraying activities, soil leaching, effluent/runoff of precipitation, stormwater discharges from agricultural land sites (Derbalah *et al*., 2019; Tankiewicz *et al*., 2010). Once OPPs enter water bodies or aquatic environments, their bioaccumulation, persistency, and long migration flow lead to severe toxicity impacts and substantially lower the quality of the water. Nasiri *et al*. (2020) in their review illustrate sources, migration channel and fate of pesticides in the aquatic environment (Figure 2.1).

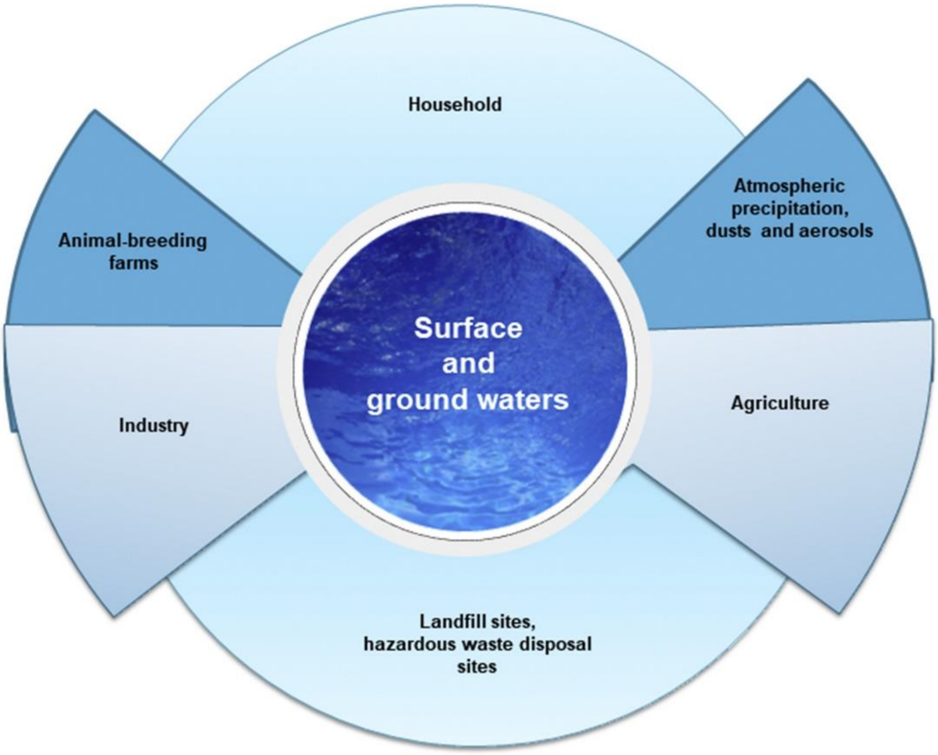


Figure 2.1 Sources, migration channel and fate of pesticides in the aquatic environment (Nasiri *et al*., 2020).

To date, OPPs residues have been detected in numerous water bodies (Chen *et al*., 2022; Derbalah *et al*., 2019; Elfikrie *et al*., 2020; Montuori *et al*., 2015; Wang *et al*., 2022; Wang *et al*., 2018; Wei *et al*., 2022). The fate of OPPs in water bodies is subjected to various processes such as physical (accumulation, deposition, and diffusion), chemical (hydrolysis and oxidation), photochemical (photolysis and photodegradation), and biochemical (biodegradation and bioaccumulation) which subsequently improve persistency and stability of the OPPs structure, resulting in rising the concentrations and even greater the toxicity when accumulated in water (Tankiewicz *et al*., 2010). Zhang *et al*. (2022) examined the presence of 81 OPPs in drinking water and identified that the total OPPs concentrations were from 15.1 to 41.6 ng L^{-1} and 16.3 to 30.2 ng L^{-1} in tap drinking water and bottled drinking water samples, respectively collected from Yangtze River Delta urban agglomeration, China. It was noted that the degradation of OPPs resulted in the formation of either primary or secondary metabolites, including derivatives of acephate. (1.64 to 2.99 ng L^{-1}) has been detected to be higher in concentrations compared to its parent methamidophos (1.17 to 1.39 ng L^{-1}) indicating increment of risk to human.

2.1.2(b) Vegetable

In today's scenarios of modernization of agricultural techniques, the application of pesticides such as OPPs is all-time rise for high crop productivity. Vegetables are often considered a significant reservoir for the accumulation of pesticides, and this accumulation depends primarily on factors such as location, duration, the lipid-solubility of pesticides, and the presence of OPPs in either gaseous or particulate form in the atmosphere. According to Farina *et al*., (2018), the dynamic uptakes of pesticides such as OPPs in vegetable samples were due to the morphology of the plant (such as waxy leafy surface can interact with an airborne volatile pesticide with lipophilic components as well as its surface area) or translocate via vegetable's phloem. Moreover, they also reported that the edible tissues in vegetables have been responsible for the formation of interactions with the pesticides, and the leafy vegetables such as spinach, celery, mustard, cabbage, and lettuce accounted in high concentrations as a result of large surface area which are prone to be more exposed to atmospheric contact. The finding was also in agreement with the studies by Yu *et al*., (2016) and Yuan *et al*., (2014) reporting that the residue of chlorpyrifos in leafy vegetables was more easily contaminated owing to larger surface areas than root and fruit vegetables. The reason that fruit vegetables such as cucumber, tomatoes, and eggplant and root vegetables such as radish have the lowest concentration of OPPs is due to the high water content contained in fruit vegetables which can enhance pesticide degradation process, while the translocation activity in root vegetables can desorbed back the OPPs to the soils (Sapbamrer and Hongsibsong, 2014).

Even though the concept of organic farming in the agricultural sectors has been introduced to reduce the utilization of OPPs, the fact that the existing soils has been contaminated and still bearing OPPs residues due to persistence was longer as well as strong adsorption by the soil particles (Ngan *et al*., 2005). Sharma *et al*. (2010) stated that the occurrence of OPPs residues in vegetables is not dependent on how much sprayed amount is anticipated during vegetable's period of growth or fruiting stage, but their content is measured based on presence in soil and water used for irrigation.

2.2 Application of magnetic solid-phase extraction to OPPs residue analysis

Just like in other solid-phase extraction methods, MSPE has been used as an alternative method for extraction and pre-concentration at the sample pre-treatment stage. Interestingly, MSPE offers many advantages by enhancing the contact surface between adsorbent and target analytes, simplifying the separation process by collecting the captured target analytes using an external magnetic field, and omitting the centrifugation and filtration steps during extraction and desorption stages employed in other SPE-based procedure, lowered consumption of adsorbent and toxic organic solvents as well as shorten the analysis time (Yin *et al*., 2021).

During the execution of the MSPE method, one must ensure that surface structural features of the developed adsorbent must contain numerous and diverse active adsorption sites for proper interaction with the target analytes along with the presence of superparamagnetism particles. In the case of OPPs, various materials have been developed and applied as selective adsorbents with particularly attractive feature-designability to allow distinctive designs and combinations for interaction with the desired OPPs.

2.2.1 MSPE approach in the sample preparation of OPPs

As an emerging sample pre-treatment technique which has received widespread attention among researchers, MSPE is one of the reliable alternative methods for trace level detection analytes such as OPPs in different complex matrices. Combining magnetic particles with single or multiple layers of inorganic or organic materials to create magnetic adsorbents reduces the need for large sample and solvent volumes during extraction and desorption. This results in an efficient separation and enrichment procedure involving a trace amount of concentration. The MSPE application that uses magnetic adsorbents has improved the extraction performance due to larger surface area as well as reduces several shortcomings and cumbersome during pre-treatment of OPPs experienced by conventional sample pre-treatment methods (Du *et al*., 2019).

Inspired by the performance of MSPE, Nasiri *et al*. (2021) synthesized magnetic graphene oxide coated with polyvinyl alcohol (PVA@MGO) and utilized as adsorbent for MSPE before GC-MS determination of OPPs residues in apple juices and environmental water (Figure 2.2). Based on the characteristics of PVA@MGO such as porous structure, large surface area of MGO, and increasing active sites for π-π interactions between the aromatic ring in OPPs and polar groups in PVA@MGO has become the main motivation for higher extraction efficiency achieved. Moreover, the superparamagnetic features with large saturation magnetization around 40 emu g^{-1} as indicated by VSM has provide sufficient and rapid magnetic separation during collection of OPPs anchored to PVA@MGO by external magnet. The proposed method demonstrated good linearity with determination coefficient (r^2) ranging between 0.9909 to 0.9994 and achieved high sensitivity with LODs within the range 20 and 80 pg mL^{-1} as well as satisfactory accuracy between 3.3 and 8.2 %. Under optimum conditions, it was noted that the PVA@MGO adsorbent also preconcentrated other common pesticide analytes which indicating that the selectivity is not very good even though better extraction efficiency can be obtained for polar OPPs with no possible interference affected the real samples analysis.