DEVELOPMENT OF FATTY ACID-BASED DEEP EUTECTIC SOLVENTS IN LIQUID PHASE MICROEXTRACTION WITH BACK EXTRACTION FOR DETERMINATION OF SELECTED ORGANIC POLLUTANTS

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TABLE OF CONTENTS

CHAPTER 4 RESULTS AND DISCUSSION 103

APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percent
µg mL^{-1}	Microgram per mililitre
µL	Microlitre
ºC	Degree of Celcius
m	Metre
µm	Micrometre
ng L^{-1}	Nanogram per litre
mg L^{-1}	Milligram per litre
mg kg^{-1}	Milligram per kilogram
<	Less than
>	Greater than
g	Gram
mL	Mililitre
kWh^{-1}	Kilowatt per hour
cm^{-1}	Per centimetre
mL min^{-1}	Mililitre per minute
ºC min^{-1}	Celcius per minute
nm	Nanometre
mm	Milimetre
R^2	Correlation of determination
C_0	Initial concentration
C_f	Final concentration
mg	Miligram
M	Molarity
rpm	Rotation per minute

pI	Polarity index
LD_{50}	Lethal dose

LIST OF ABBREVIATIONS

[HMIm][PF6]	1-hexyl-3-methylimidazolium hexafluorophosphate
[C8MIM][PF6]	1-octyl-3-methylimidazolium hexafluorophosphate
2,4-D	2,4-dichlorophenoxy)acetic acid
ACE	Acetone
ACN	Acetonitrile
ARfD	Acute reference dose
AA-LPME-GC-FID	Air-assisted liquid phase microextraction-gas chromatography-flame ionization detector
AGREE	Analytical Greenness Calculator
BBD	Box-Behnken design
CE	Capillary electrophoresis
CCD	Central composite design
ChCl	Choline chloride
CHR	Chrysene
CYP	Cytochrome P50
DES	Deep eutectic solvent
DOE	Design of experiment
DCM	Dichloromethane
DI-SDME	Direct immersion single drop microextraction
DSDME	Direct suspended droplet microextraction
DLLME	Dispersive liquid-liquid microextraction
d-SPE	Dispersive solid phase extraction
DD	Doehlert design
ELLME-BE	Emulsification liquid-liquid microextraction with back extraction
ELLME-HPLC	Emulsification liquid-liquid microextraction-high performance liquid chromatography
EF	Enrichment factor
EE	Extraction efficiency
FA	Fatty acid
FF	Ferrofluids
FTIR	Fourier transform infrared
GC-ECD	Gas chromatography-electron capture detector
GC-FID	Gas chromatography-flame ionisation detector

GCMS	Gas chromatography-mass spectrometry
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
GAC	Green Analytical Chemistry
GAPI	Green Analytical Procedure Index
GC	Green Chemistry
HS-SDME	Headspace single drop microextraction
HS-SPME	Headspace solid phase microextraction
Hex	Hexane
HPLC-UV	High performance liquid chromatography – UV/Vis
HPLC-DAD	High performance liquid chromatography-diode array detector
HF LPME	Hollow fiber liquid phase microextraction
HLLME-DLLME-GC-MS	Homogeneous liquid-liquid microextraction-dispersive liquid-liquid microextraction-gas chromatography-mass spectrometry
HMW	High molecular weight
HCl	Hydrochloric acid
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
IT-SPME	In tube solid phase microextraction
ICP-MS	Inductively coupled plasma mass spectrometry
IARC	International Agency for Research on Cancer
IL	Ionic liquid
IL-DLLME	Ionic liquid dispersive liquid-liquid microextraction
IPA	Isopropyl alcohol
LDH	Layered double hydroxide
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid phase microextraction
LPME-BE	Liquid phase microextraction-back extraction
LPME-FDES-HPLC	Liquid phase microextraction-freezing deep eutectic solvent-high performance liquid chromatography
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microextraction
LLME-LC	Liquid-liquid microextraction-liquid chromatography
LMW	Low molecular weight
MCPA	(4-chloro-2-methylphenoxy)acetic acid

MNPTEOS	Magnetic nanoparticle tetraethyl orthosilicate
MNP	Magnetic nanoparticles
MNPDES	Magnetic nanoparticles-deep eutectic solvent
MNP@CN/IL	Magnetic nanoparticles with cyano-ionic liquid
MeOH	Methanol
MMT	Montmorillonite nanoclay
NEMI	National Environmental Methods Index
NADES	Natural deep eutectic solvent
NSAID	Nonsteroidal anti-inflammatory drug.
NMR	Nuclear magnetic resonance
OFAT	One-factor-at-time
OPE	Organophosphate ester
H_3PO_4	Orthophosphoric acid
PP	Penalty point
POP	Persistent organic pollutants
π-π	Pi to pi
PAH	Polycyclic aromatic hydrocarbons
PDMS	Polydimethylsiloxane
QuEChERS	Quick. Easy, cheap, effective rugged, and safe
RSD	Relative standard deviation
RSM	Response surface methodology
SEM	Scanning electron microscopy
SDME	Single drop microextraction
NaCl	Sodium chloride
NaOH	Sodium hydroxide
SPE	Solid phase extraction
SPME	Solid phase microextraction
SFO	Solidification of floating organic drop
SD	Standard deviation
SBSE	Stir bar sorptve extraction
TEOS	Tetraethyl orthosilicate
THF	Tetrahydrofuran
TBaBr	Tetra-n-butylammonium bromide
TGA	Thermogravimetric analysis

UAE	Ultrasound assisted extraction
VSM	Vibrating sample magnetometer

LIST OF APPENDICES

PEMBANGUNAN PELARUT EUTEKTIK TERDALAM BERASASKAN ASID LEMAK DALAM PENGESTRAKAN MIKRO FASA CECAIR DENGAN PENGESTRAKAN BERBALIK UNTUK PENENTUAN BAHAN PENCEMAR ORGANIK TERPILIH

ABSTRAK

Pencemaran alam sekitar menjadi isu global utama sejak akhir-akhir ini. Aktiviti perindustrian, pencemaran laut, aktiviti pertanian, dan pembuangan kumbahan dan air buangan merupakan sumber utama pencemaran. Bahan pencemar organik, seperti racun perosak, racun herba, dan hidrokarbon aromatik polisiklik (PAHs) adalah antara bahan pencemar alam sekitar yang paling membimbangkan kerana kadar toksik yang tinggi di samping mampu berada dalam alam sekitar dalam jangka masa yang panjang. Bahan-bahan pencemar ini memberi kesan mudarat terhadap kesihatan manusia dan alam sekitar. Disebabkan kadar toksik yang tinggi dan kewujudan mereka memberi kesan mudarat terhadap alam sekitar, pembangunan kaedah pengekstrakan yang berkesan adalah penting. Pelarut eutektik terdalam dianggap sebagai alternatif hijau menggantikan pelarut konvensional yang biasa digunakan dalam kebanyakan kaedah pengekstrakan memandangkan peningkatan permintaan teknik pengekstarakan hijau. Pengekstrakan mikro yang berasaskan pelarut eutektik terdalam seiring dengan konsep Kimia Analitik Hijau (GAC), dengan keupayaan masa penyediaan sampel yang singkat, kadar penggunaan pelarut organik yang rendah dan prosedur yang mudah. Oleh itu, dalam penyelidikan ini, pengekstrakan mikro yang mesra alam telah dibangunkan menggunakan pelarut eutektik terdalam bersifat hidrofobik berasaskan asid lemak daripada gabungan asid laurik yang bertindak sebagai penerima ikatan hydrogen (HBA) dan asid pelagornik sebagai penderma ikatan hydrogen (HBD) untuk menentukan campuran racun herba dalam kaedah pengekstrakan mikro berbantukan pengemulsian dengan pengeskstrakan berbalik (ELLME-BE). Di samping itu, bendalir fero telah dibangunkan dengan menggabungkan pelarut eutektik terdalam yang terdiri daripada asid laurik (HBA) dan asid kaprilik (HBD) dengan zarah nano magnetik (MNP) untuk menentukan hidrokarbon aromatik polisiklik (PAHs) dalam kaedah pengestrakan mikro fasa cecair dengan pengestrakan berbalik (LPME-BE). Teknik-teknik seperti spektroskopi transformasi infra merah (FTIR), analisis termagravitimetrik (TGA), magnetometer sampel gegaran (VSM), dan microskopi elektron pengimbas dengan sinaran–x penyebaran tenaga (SEM–EDX) telah digunakan untuk menganalisa ciri-ciri fizikokimia pelarut eutektik terdalam dan bendalir fero berasaskan pelarut eutektik terdalam. Parameter pengekstrakan utama telah dioptimumkan. Kaedah ELLME–BE mempamerkan had pengesahan dan kuantifikasi yang rendah masing-masing berada dalam lingkungan 0.00001 hingga 0.0025 µg mL^{-1} dan 0.00003 hingga 0.0083 µg mL^{-1}, dan nilai RSD% kurang daripada 20%. Sebagai perbandingan, kaedah LPME-BE mencapai had pengesahan dan kuantifikasi masing-masing dalam lingkungan 0.0004 hingga 0.0017 µg mL^{-1} dan 0.0013 hingga 0.0057 µg mL^{-1}, dengan RSD% kurang daripada 20%. Pengembalian pengekstrakan sampel yang dimasukkan analit ialah 72.1 – 103.3% dengan ketepatan kurang daripada 20% untuk ELLME-BE, manakala pengembalian untuk LPME-BE ialah 75.78 – 118.65% dengan ketepatan kurang daripada 20%. Penilaian kehijauan menggunakan Skala Eko Analitik dan Sistem Pendekatan Metrik Kehijauan Analitik (AGREE) mempamerkan nilai keputusan sebanyak 84 % dan 0.61 untuk ELLME-BE. Manakala bagi kaedah LPME-BE, Skala Eko Analitikal dan AGREE menunjukkan 92% dan 0.64. Kesimpulannya, kedua-dua pengekstrakan mikro berasaskan pelarut eutektik terlarut asid lemak boleh dianggap sebagai kaedah yang berkos rendah dan

mesra alam, seterusnya mempamerkan keupayaan yang sangat menjaminkan untuk pengekstrakan racun herba dan hidrokarbon aromatik polisiklik daripada pelbagai sampel alam sekitar.

DEVELOPMENT OF FATTY ACID-BASED DEEP EUTECTIC SOLVENTS IN LIQUID PHASE MICROEXTRACTION WITH BACK EXTRACTION FOR DETERMINATION OF SELECTED ORGANIC POLLUTANTS

ABSTRACT

Environmental pollution has become a significant global issue in recent times. The primary sources of this pollution are human activities such as industrial processes, marine dumping, agricultural practices, and wastewater discharges. Some of the most concerning environmental pollutants are pesticides, herbicides, and polycyclic aromatic hydrocarbons (PAHs) due to their high toxicity and their persistence in the environment. These pollutants have harmful effects on human health and the ecosystem. Due to their high toxicity and trace amount in environment, developing an effective extraction method is essential. Deep eutectic solvent (DES) is a possible green alternative to the conventional solvent utilised in most extraction procedures in light of the increased demand for developing green extraction techniques. Microextraction-based DES fits favourably with the Green Analytical Chemistry (GAC) concept because of its rapid sample preparation time, low organic solvent usage, and straightforward procedure. In this study, an eco-friendly microextraction utilising hydrophobic deep eutectic solvents from fatty acids derived from the combination of lauric acid, which acted as a hydrogen bond acceptor (HBA), and pelargonic acid, which acted as a hydrogen bond donor (HBD), was prepared to determine herbicide mixtures using emulsification liquid-liquid microextraction with back extraction (ELLME-BE) method. In addition, a ferrofluid was developed by incorporating DES, a combination of lauric acid (HBA) and caprylic acid (HBD) with magnetic nanoparticles (MNP) to determine polycyclic aromatic hydrocarbons

(PAHs) using liquid phase microextraction with back extraction (LPME-BE) method. Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), vibrating sample magnetometer (VSM), and scanning electron microscopy with energy-dispersive x-ray (SEM–EDX) were used to analyse the physicochemical properties of the synthesised DES and fatty acid DES-based ferrofluids. The most crucial extraction parameters have been optimised. The ELLME-BE method has low detection (LOD) and quantification limits (LOQ) ranging from 0.00001 to 0.0025 µg mL^{-1} and 0.00003 to 0.0083 µg mL^{-1}, respectively, with RSD% below 20%. In comparison, the LPME-BE method achieved LODs and LOQs between 0.0004 and 0.0017 µg mL^{-1} and 0.0013 and 0.0057 µg mL^{-1}, respectively, with RSD% below 20%. The extraction recoveries of spiked samples for ELLME-BE range from 72.1 to 103.3% with RSD below 20%, whereas the recoveries for LPME-BE range from 75.78 to 118.65% with RSD below 20%. The Analytical Eco-scale and Analytical GREENness Metric Approach System (AGREE) for ELLME-BE are 84% and 0.61, respectively. As for LPME-BE, the Analytical Eco-Scale and AGREE measured 92% and 0.64, respectively. In conclusion, both fatty acid-DES-based microextractions are cost-effective and environmentally friendly, suggesting a highly promising strategy for extracting herbicides and PAHs from various environmental samples.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Pharmaceutical residues, pesticides, herbicides, phenols, and polycyclic aromatic hydrocarbons (PAHs) are emerging pollutants that have become a major focus of concern for Environmental Protection Agencies around the world (da Costa Filho et al., 2022; Maldaner & Jardim, 2012; Manousi & Zachariadis, 2020; Tadeo et al., 2000). These substances are constantly discharged into the environment. They can be identified at very low concentrations (µg L^{-1} or ng L^{-1}), yet they can significantly impact water quality and potentially harm human health, drinking water, and aquatic ecosystems.

Significant concerns about the presence of organic pollutants such as pesticides, herbicides, and PAHs in the environment, which offer unknown risks of chronic long-term exposure to people at measurable amounts (Cazorla-Reyes et al., 2011; Lu et al., 2020). Untreated industrial waste water discharges, domestic sewage discharges, and runoff are primary sources of water pollution due to the abundance of organic pollutants were released into surface water and groundwater that serve as the principal destinations (Jalili et al., 2020; Manousi & Zachariadis, 2020). To protect crops in agriculture, gardening, and soil treatment, organic pollutants such as pesticides and herbicides are commonly utilised. However, extensive use of pesticides and herbicides, coupled with poor treatment of these effluents, leads to their accumulation in environment matrices (water, soil, air) to the extent that the potential for environmental threat is substantial (Kaczyński et al., 2016; Weinberg & Teodosiwyu, 2012; Wu et al., 2009). Air pollution was mainly contributed by the release of harmful gases from various sources such as forest fires, industrial operations, and road transportation into the atmosphere. According to Manousi & Zachariadis (2020) and Poster et al. (2006), PAHs are regularly monitored for air quality assessment in the atmosphere due to incomplete combustion of natural and anthropogenic processes. In addition, due to their collection, mitigation, and environmental transformation, these organic pollutants can reach the food chain, posing severe health concerns to humans and other creatures. To preserve the health and sustainability of humans and other ecosystems, ongoing monitoring of these environmental contaminants is necessary (Sajid et al., 2021).

Highly selective sample preparation and sensitive detection methods are required for the extraction and preconcentration of these organic contaminants in environmental samples. The primary purpose of sample preparation is to eliminate interferences and matrices to establish a reliable method and achieve greater sensitivity, precision, and recovery (Burato et al., 2020). In this step, a hazardous organic solvent is utilised primarily to pre-concentrate the analyte, typically present in a very low concentration. In addition, chromatographic procedures, the most prevalent technique for identifying contaminants, necessitate a stringent cleaning and extraction procedure before instrumental analysis.

Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are commonly extraction procedures used to pre-concentrate the analyte and isolate the matrix. Conventional LLE and SPE require a large volume of toxic organic solvent to extract the targeted analyte from its original matrix into a solvent, thereby enabling a more selective and sensitive subsequent analysis. However, these processes are laborious, time-consuming, and require many organic solvents (Manousi & Zachariadis, 2020). In the sample preparation step of conventional LLE, a substantial amount of organic solvent is used, which is incompatible with sustainable development. Due to the use of a large quantity of harmful organic solvent during the extraction process, the generation of chemical waste and improper disposal of waste into the environment have the potential to negatively impact the environment (Aly & Górecki, 2020). Furthermore, a multitude of matrix constituents, apart from the intended analytes, adhered to the sorbent, may impeding the effective retrieval of the compounds of interest resulting in low recovery (Rawa-Adkonis et al., 2006). The currently available sorbents in the commercial market are insufficient for extraction purposes when dealing with analytes that possess diverse physicochemical properties (Manousi et al., 2022; Suseela et al., 2023). Obtaining reliable results might be problematic due to the disadvantages associated with LLE and SPE methods.

Green analytical chemistry (GAC) concepts are currently the most critical factor in sample preparation method development. By considering the 12 GAC principles, the GAC concept emphasises using a green approach in sample preparation (Kokosa & Przyjazny, 2022). Consequently, the GAC urges analysts to limit their use of organic solvents, reduce their energy consumption and waste output, and enhance human safety, all while adhering economic and analytical standards. The use of miniaturised extraction technologies and environmentally friendly solvents should be addressed while designing green sample preparation.

Miniaturised sample preparation techniques such as liquid phase microextraction (LPME) and solid phase microextraction (SPME) have been developed as alternatives to conventional LLE and SPE (Yuvali et al., 2021). The development of miniaturised sample preparation methods not only reduces the impact of chemical activities involving hazardous organic solvents, but offers more efficient and cost-effective extraction approaches (Kokosa & Przyjazny, 2022; Sajid et al., 2021). Numerous organic pollutants, including PAHs (Jalili et al., 2020), pesticides

(Pirsaheb & Fattahi, 2018), and phenolic compounds (Jalili et al., 2020; Yang et al., 2020), were extracted by LPME. Instead of toxic organic solvents (dichloromethane, hexane, toluene, carbon tetrachloride), new-generation solvents, such as ionic liquid (IL), deep eutectic solvent (DES), and supramolecular solvent, have proven to be the superior option in LPME methods. Using DESs, comparable to IL, as a replacement for toxic solvents in the sample preparation step has generated considerable interest in LPME. DESs combine two or more components, hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), formed from the starting materials, to produce a low melting point eutectic mixture. DESs are gaining recognition for their attributes including low vapor pressure, non-combustibility, biodegradability, less or no toxicity, ease of production, and low cost (Florindo et al., 2018; Zhang et al., 2020). However, most early DESs were water-soluble, limiting their application to water-based samples. This is due to the hydrophilic nature of DES, which restricts its use in the extraction of hydrophobic substances, such as PAHs, resulting in inefficient extraction (Kachangoon et al., 2020)). To combat the problem, the development of hydrophobic DES has attracted considerable interest.

Recent researchers produced hydrophobic DES-based quaternary ammonium halides and neutral chemicals, such as menthol and long alkyl chain fatty acid, to investigate their potential as a green alternative extraction solvent in LPME (Arcon & Franco, 2020; Makoś et al., 2020; Van Osch et al., 2015). The improved physicochemical features of hydrophobic DES over IL and hydrophilic DES include outstanding chemical stability, high tunability, and high melting temperature. Hydrophobic DES derived from fatty acid has been largely explored as green solvent in sample preparation step due to their good chemical stability, lower volume change during phase transition, and high latent heat of fusion and melting temperature in the desired operating range. The performance of fatty acids DES can be easily adjusted by mixing fatty acids with different alkyl chain lengths in suitable proportions while retaining the non-reactivity, recyclability, non-corrosiveness, low cost, and non-toxicity of fatty acids (Arcon & Franco, 2020; Florindo et al., 2018; Zhang et al., 2019).

Further development of LPME techniques by combining DES with magnetic nanoparticles (MNP) to create ferrofluid. The utilisation of magnetic materials with DES in LPME has attracted significant interest due to their simple separation, reusability, and they can be functionalised with various materials, resulting in high selectivity in the extraction process (Sajid et al., 2021). Ferrofluids are stable, homogeneous, solid-liquid composite materials composed of MNP suspended in fluids (Zarei et al., 2018). A suitable coating material for the MNP is necessary to stabilise the ferrofluid. DES are among the substances used to cover the MNP required to create ferrofluid (Jouyban et al., 2020; Majidi & Hadjmohammadi, 2019). Zarei et al. (2018) demonstrated that ferrofluid-based DES are stable in aqueous extraction solutions with high sorbing capabilities, low viscosity, and a lower density than water. Using an external magnet, ferrofluid is easily separable. Ferrofluid exhibits superior phase separation and magnetic performance for a quick extraction, obviating the need for instrumental separation techniques such as centrifugation. Given these characteristics, LPME using ferrofluids can minimise the environmental threats by replacing the toxic solvent or sorbent used in conventional LLE and SPE (Nayebi & Shemirani, 2021). Recently, ferrofluids have been extensively employed to preconcentrate various organic pollutants, including PAHs (Yih Hui et al., 2020), NSAID (Dil et al., 2020), and nitroaromatic contaminants (Zarei et al., 2018).

This research focuses on developing fatty acid DES and fatty acid-based DES ferrofluid as green approaches to determine selected organic pollutants, including herbicides and PAHs in environmental samples. The synthesis and characterisation are meticulously performed, and several significant extraction-influencing factors are studied and validated. For the recovery investigations, real sample analysis employing agricultural wastewater, vegetables, and leaves is performed. Both developed methods are assessed for their greenness using Analytical Greenness Calculator (AGREE) and Analytical Eco-scale.

1.2 Problem statement

In agriculture, herbicides are extensively employed to inhibit the development of weeds in crops (Quintana et al., 2007). They are inexpensive and highly potent, even at extremely low concentrations. The high chemical stability of these herbicides facilitates their easy release into the environment after application. Although they are beneficial for agricultural purposes, these substances are detrimental to the environment and have the potential to harm human health (Araujo et al., 2011; Hou et al., 2018). Numerous studies have explored various herbicides in samples from occupationally and non-occupationally exposed individuals (Othman & Jafari, 2014; Sulaiman et al., 2019; Sun et al., 2022). Simultaneous analysis of these pesticides presents a challenge due to their differing polarities and basic-acid properties (Cazorla-Reyes et al., 2011; Yang et al., 2013). The selection of a single solvent or a combination of solvents for efficient extraction of all herbicides while maintaining selectivity is challenging due to their different characteristics. This variation leads to poor recovery for herbicides with diverse polarities. It is crucial to develop a quick and sensitive method that can extract herbicides with different polarities and basic-acid properties simultaneously while utilizing a more environmentally friendly approach.

PAHs are widely recognised as byproducts of incomplete combustion, which are not limited to industrial facilities but also encompass routine activities like residential open burning and vehicle emissions. Since they are predominantly found in the atmosphere, they can penetrate the human body simply by breathing in the surrounding air. PAHs may also be introduced into the environment, including soil, via atmospheric deposition (Abdel-Shafy & Mansour, 2016; Manousi & Zachariadis, 2020). Similar to herbicides, PAHs are recognised for their carcinogenic and toxic characteristics, both of which detrimentally affect human health. Simultaneously analysing low molecular weight (LMW) and high molecular weight (HMW) PAHs is challenging due to their different levels of volatility and polarity (Ambade et al., 2021). LMW PAHs are more soluble in polar solvents and can be easily separated using gas chromatography (GC) analysis. On the other hand, HMW PAHs require high-performance liquid chromatography (HPLC) due to their low volatility (Patel et al., 2020). Additionally, complex compositions in environmental and biological samples can interfere with the analysis of both LMW and HMW PAHs, making it challenging to extract desired substances and reducing the method's sensitivity.

The occurrence of herbicides and PAHs in the environment raised alarming concerns regarding the health safety of human and animal (Wan Ibrahim et al., 2015). Therefore, these pollutants are particularly monitored in environmental samples according to the environmental quality assessments. However, their persistence in the environment for extended time and extremely low concentrations render their extraction from environmental samples to be challenging.

A proper sample preparation method to isolate and preconcentrate both pollutants for more precise and sensitive results incur substantial labour and financial expenditures. LLE and SPE are frequently employed in environmental analysis sample preparation due to their straightforwardness and precision of results. However, these approaches are labour-intensive and time-consuming, and they also necessitate a substantial quantity of toxic solvent or sorbent usage, contrary to sustainable development objectives.

As a result, analysts have found minituarised extraction to be a favourable alternative to conventional extraction, owing to its comparable methodology but reduced sample volume and adherence to GAC principles (Pena-Pereira et al., 2020; Sajid & Płotka-Wasylka, 2022). An alternative approach entails substituting hazardous solvents with environmentally friendly alternatives, including supramolecular solvents, DESs, and IL (Aydin et al., 2018; Tobiszewski et al., 2009). In addition, the incorporation of DES with MNP forming ferrofluid in LPME for extraction and preconcentration are presumed to be more environmentally friendly due to ease of phase separation and energy saver (Sajid et al., 2021).

This study presents the development of simple microextraction methods, namely emulsification liquid-liquid microextraction with back extraction (ELLME-BE) employing fatty acid-based DES to determine acid-basic herbicides in environmental and vegetables samples. Additionally, the present study utilised a fatty acid-based DES ferrofluid in conjunction with liquid phase microextraction with back extraction (LPME-BE) for the extraction of PAHs from environmental samples.

1.3 Research objectives

This study aims to develop green microextraction methods based on DES from fatty acids for the extraction of selected water pollutants (herbicides and polycyclic aromatic hydrocarbons) from environmental samples. The particular objectives of this work are:

i. To synthesise and characterise the fatty acid-based deep eutectic solvents (DES) and fatty acid-based deep eutectic solvent ferrofluid (MNPDES).

ii. To develop, optimise, and validate the synthesised fatty acid-based DES in emulsification liquid-liquid microextraction with back extraction (ELLME-BE) combined with high performance liquid chromatography method for the determination of acidic-basic herbicides from environmental and vegetables samples.

iii. To develop, optimise, and validate the synthesised fatty acid DES-based ferrofluid in liquid phase microextraction with back extraction (LPME-BE) combine with gas chromatography method for the determination of PAHs from environmental samples.

iv. To evaluate the greenness of both developed methods using Analytical Eco-scale and Analytical Greenness Calculator (AGREE).

1.4 Outline of the thesis

This thesis is divided into five chapters. Chapter 1 is an introduction that explains the general context of the problem statements and research objectives. A review of related literature is thoroughly summarised in Chapter 2. In Chapter 3, the methodology chapter highlights the chemicals, reagents, and procedures used in these studies. This chapter contains two main parts namely Part 1 and Part 2. Part 1 discusses the synthesis, characterisation, and extraction performance of DES-based fatty acids for the extraction of acidic-basic herbicides from environmental and vegetables samples. Part 2 emphasises the synthesis, characterisation, extraction performance of fatty acid DES-based ferrofluid for the extraction of PAHs from environmental samples. Chapter 4 discusses in detail the findings and outcomes of the of the conducted study. This chapter is also divided into two main parts similarly as Chapter 3. Synthesis, characterisation, optimisation, extraction study, method validation, greenness assessment, real sample analysis are all included in these sections, along with relevant discussion. The overall conclusions and further recommendations are presented in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Environmental pollutants

Pollution in various environmental compartments such as water, soil, and air resulting from industrialisation and human activities is one of the most critical matters humans must address or at least contain. Different pollutants with wide range of chemical properties and can be roughly categorised as organic, inorganic, or metal species pollutants (Bruzzoniti et al., 2000). The pervasive presence of organic contaminants such as pesticides, herbicides, phenols, and PAHs in the environmental water has become a significant concern. Organic pollutants in untreated industrial waste water discharges, domestic sewage, and runoff were released into surface water and groundwater that serve as the primary destinations (Bruzzoniti et al., 2000; Quintana et al., 2007). These compounds are continuously discharged into the environment and found at trace concentrations (μg L^{-1} or ng L^{-1}). Yet, they degrade water quality and adversely affect drinking water, aquatic ecosystems, and human health. The presence of priority pollutants such as pesticides, endocrine disruptors, and PAHs in the environment is an issue of concern due to its potential risks to human health. It is important to acknowledge that the European Protection Agency of the United States has designated phenoxy acid herbicides such as 2,4-D and MCPA, and PAHs as priority pollutants as posing a threat (Bonansea et al., 2013; Keith, 2015; Weinberg & Teodosiu, 2012). Hence, it is crucial to address this problem constructively to protect human health.

Owing to their trace concentrations in the environment (water, soil, air), their detection using the most commonly employed analytical techniques is quite challenging. Only recent advancements in these particular methods allow for their detection. Thus, developing analytical methodologies that allow investigation of these pollutants in various environmental matrices is of great interest (Nödler et al., 2010; Quintana et al., 2007).

2.1.1 Herbicides

Herbicides are synthetic chemicals used to combat environmental pests and boost agricultural productivity. As toxic chemicals with long-lasting bioaccumulation effects, these substances are frequently dangerous to live organisms and resistant. Herbicides are categorised based on chemical structures (Tadeo et al., 2000). Acidic herbicides protect plants and boost crop output by killing weeds (Ghoniem et al., 2017). In the post-emergence phase, phenoxy acids such as 4-chloro-2-methylphenoxy acetic acid (MCPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) are typically administered as esters and salts as selective herbicides to protect crops such as wheat and barley (Kaczyński et al., 2016) and rice fields (Dilipkumar et al., 2017; Mispan et al., 2019). In agriculture, citrus fruits are typically treated with herbicides containing uracil, such as bromacil. Bromacil possess numerous functional groups, including bromide, secondary amine, ketone, and tertiary amine (Kaliyan & Tamilselvan, 2018). The presence of bromide and amine disrupts the photosynthetic pathway of plants. This herbicide can be sprayed or dry-applied to plants. It is highly soluble in water and can remain longer in the soil (Roselló-Márquez et al., 2019). In addition, triazine herbicides are generated from s-triazine, a nitrogen-containing heterocycle having three nitrogen atoms within the ring system. The type of functional group connected to the heterocycle determines the physical properties of these triazine herbicides, including solubility, acidity, and thermodynamic properties. The acidity of triazine herbicides declines in the sequence -OCH_3 > -SCH > -Cl. In acidic circumstances, they are highly soluble in water (Rodríguez et al., 2013). Chlorothalonil is a 2,4,5,6-tetrachloro-1,3-phthalonitrile, a non-systemic fungicide with low toxicity commonly used to combat fungal diseases in agriculture, turf, and ornamental crops (Chaves et al., 2008). Chlorothalonil is a substituted benzene organochlorine insecticide that is non-ionic, hydrophobic and has a benzene ring structure. It is utilised extensively to prevent and manage illnesses in vegetables, fruits, rice, wheat, beans, tea, and other crops (Gao et al., 2019).

The agriculture sector has contributed significantly to Malaysia's economy over the years. However, it is essential to note that herbicide usage for crop protection was estimated to be high (at 83%) in 2014 (Dilipkumar et al., 2017). This data highlights the need for a more sustainable approach to agriculture by considering the potential consequences of herbicide usage on the environment and human health. The application of herbicides typically involves spraying them over the leaves, ground, and bodies of water. Unfortunately, this can indirectly contaminate surface water through runoff or leachate. Such pollution can harm aquatic ecosystems and water bodies. Herbicides can contaminate aquatic environments through direct application to environmental waters, migration from crops and soil via runoff and spray drift, excessive use in agriculture and accumulation in marine habitats (Abbas et al., 2015). The herbicide has chemical and photochemical stability under mild conditions, which hinders its degradation by physicochemical and biological processes in wastewater treatment facilities. It builds up in the soil and has long-lasting negative impacts on soil organisms for many years. The extensive application of herbicide can result in the exposure of unintended plants, animals, and humans, leading to significant impacts on the functioning of ecosystems in the environment.

Mostly herbicides are not environmentally safe and pose risks to human health and other ecosystems, despite their usefulness in weed control and crop productivity. The intense and frequent application of these herbicides may constitute a significant environmental risk to non-target organisms and has resulted in the persistence of herbicides in soil due to insufficient degradation (Weinberg & Teodosiu, 2012). The most significant concern regarding the exposure of humans to herbicides is their presence in the environment due to their continuous usage in agriculture and inappropriate treatment of wastewater, resulting in their dispersion in the environment, thus, entering the food chain (Tadeo et al., 2000; Weinberg & Teodosiu, 2012). In 2014, the Department of Occupational Safety and Health (DOSH) under the Ministry of Human Resources of Malaysia reported 2,648 cases of occupational poisoning and diseases caused by excessive herbicide exposure during farming. Despite their effectiveness in combating weeds, farmers continued to spray herbicides, leading to an increase in such cases from 2,588 in 2013. This indicates a growing trend of occupational diseases caused by inadequate working conditions and the absence of effective control measures in Malaysia (Ho, 2023; Othman & Jafari, 2014).

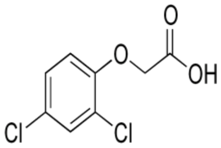
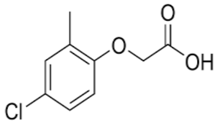
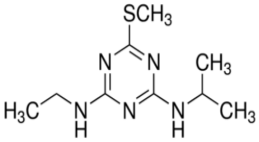
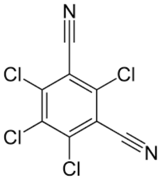
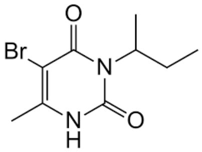
These herbicides exert their effects at low concentrations and are frequently absorbed by plants and roots for transfer to other plant organs. To ensure safe levels of herbicides in surface and groundwater, it is imperative to monitor their concentrations. According to Environmental Protection Agency (2018) and Pfeuffer & Beach (2015) have set maximum allowable concentrations for various herbicides, including phenoxy acid herbicides such as 2,4-D and MCPA, with limits of 0.1 and 0.4 mg L^{-1}, respectively.

Ivanova & Spiteller (2014) suggested the safe limit for uracil herbicides in fruit juices and drinking water is below 0.1 mg L^{-1}. The maximum acceptable concentration for triazine herbicides and chlorothalonil were set below 0.01 mg L^{-1} (Sun et al., 2022; Yu et al., 2021). It is essential to adhere to these limits to ensure our environment's and health's safety.

Limited evidence exists regarding acute toxicity in animals and people. For instance, the acute reference dose, ARfD for MCPA is 0.15 mg kg body weight^{-1} day, and LD_{50} is 926 mg kg^{-1} (Kaczyński et al., 2016). It is notable that the direct toxicity effect on humans is not specific. Nevertheless, the most frequently reported symptoms of pesticide poisoning are muscle weakness and inflammation in peripheral nerves. These herbicides' active ingredient and excessive dosages may produce teratogenic consequences in animals. Due to their high-water solubility, low volatility, and heavy agricultural use, the widespread of herbicides caused serious environmental pollutions and negatively affected the other living organisms through food chain (Köck-Schulmeyer et al., 2013; Tamer et al., 2020; Tang et al., 2021; Woudneh et al., 2007). In addition, phenoxy acid herbicides such as MCPA and 2,4-D have been put on the European list of priority pollutants (EPA, 2018) and 2,4-D is considered an endocrine disruptive agent (Woudneh et al., 2007).

A list of selected herbicides used and their properties are presented in Table 2.1.

Table 2.1 Selected herbicides used and their properties.

Herbicides	**Name**	**Group**	**pKa**
	2,4-dichlorophenoxy acetic acid (2,4-D)	Phenoxy acid	2.73
	4-chloro-2-methylphenoxy acetic acid (MCPA)	Phenoxy acid	3.13
	Ametryn	Triazine	4.10
	Chlorothalonil	Halogenated fungicide	N/A
	Bromacil	Uracil	9.30

Notes: N/A: Not Applicable

2.1.2 Polycyclic aromatic hydrocarbons

The major environmental concerns in urban and industrial areas are polycyclic aromatic hydrocarbons (PAHs). Environmental pollution caused by PAHs has been widely reported due to rapid growth of industrialisation and increased number of road vehicles. PAHs are a large class of chemical compounds with two or more fused benzene rings. They are hydrophobic chemicals with low water solubility, as their molecular weight increases, their solubility in water and volatility decreases. There are two main types of PAHs; low molecular weight (LMW) PAHs have fewer than four rings such as naphthalene, and high molecular weight (HMW) PAHs, characterised by more than four rings such as pyrene and chrysene. HMW PAHs are more stable and hazardous than LMW PAHs (Manousi & Zachariadis, 2020).

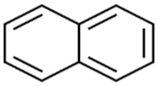
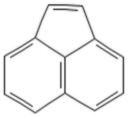
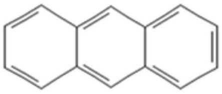
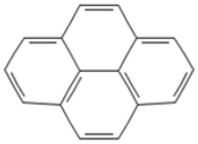
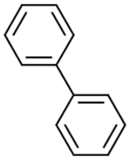
PAHs are byproducts of thermal degradation resulting from the incomplete combustion of organic materials and the geochemical production of fossil fuels. Power plants, residential heating, trash incineration, industrial activities, and, most significantly, automobile exhaust emissions are among the primary causes of anthropogenic pollution (Manousi & Zachariadis, 2020; Poster et al., 2006). Therefore, PAHs can easily enter into the environments (soil, air, water) through various routes. The direct release of PAH into atmosphere was caused by the incomplete burning, exhaust emission, and emission from industrial activities (Coelho & Almeida, 2008). PAHs contamination in water sources mostly caused by accidental oil spillage and effluent from industrial activities (Honda & Suzuki, 2020).

Most PAHs are introduced into the environment as atmospheric pollution due to the emissions from vehicles and factories. They can easily enter into the human body through inhalation. HMW PAHs usually exist bound to particulate matter, while LMW

PAHs remain in atmosphere as vapours (Abdel-Shafy & Mansour, 2016). A study by Hisamuddin et al. (2022) on the association of motor vehicles contribute to PAHs pollution in urban area. The study reveals a heightened lifetime cancer risk (LCR) among children residing in areas with heavy traffic as opposed to those in low-traffic areas, suggesting that exposure to indoor PAHs is the primary determinant of lifetime cancer risk. Another study by Abd Manan et al. (2021) on PAHs pollution in Sungai Perak, it was observed that the carcinogenic risk associated with the presence of PAHs in both raw and processed water surpassed the established threshold of permissibility. The calculated lifetime cancer risk (LCR) value, which equates to 1 in 1,000,000 individuals, was 1.0×10^{-6}, corresponding to a concentration of 0.0028 $\mu g\ L^{-1}$. According to Abd Manan et al. (2021), the LCR should be within the range of 1×10^{-4} to 1.0×10^{-6} for an individual PAH. Furthermore, it is recommended that the concentration of PAHs in water should be maintained below the levels observed in uncontaminated groundwater, which typically range from 0 to 0.005 mg L^{-1} (WHO, 2003).

PAHs are considered persistent organic pollutants (POPs) due to their slow breakdown rates, toxicity, and propensity for long-distance transport, and bioaccumulation in living organisms (Foan et al., 2015). The prevalence of PAHs in the environment is a significant concern owing to their inherent toxicity, mutagenesis properties, and ability to act as carcinogens. The toxicity of these substances is contingent upon various factors, including the specific species, molecular composition, and route of exposure (Ambade et al., 2021; Honda & Suzuki, 2020). Briefly, PAHs are transported into cells because of their hydrophobicity and induce gene expression of the cytochrome P450 (CYP) enzyme group. Expressed CYP enzymes metabolise PAHs into additional metabolites. It is important to note that several intermediates in this metabolic pathway can bind to DNA and become mutagenic and carcinogenic. Because of their carcinogenicity, the US-EPA has listed 16 PAHs including naphthalene, anthracene, acenaphthylene, and pyrene as priority pollutants (EPA, 2014). Individual PAHs are generally non-reactive, but most adverse health risks can be attributed to their metabolically modified derivatives. For instance, there is a significant association between 2-naphthol (naphthalene metabolite) with increased obesity among children (Bright et al., 2023). Table 2.2 shows the list of selected PAHs with their properties.

Table 2.2 Selected PAHs with their properties.

PAH	**Name**	**Boiling point (°C)**	**Log P**	**Log K**
	Naphthalene	217.9	3.3	5.19
	Acenaphthylene	280.0	3.94	3.93
	Anthracene	341.3	4.45	4.45
	Pyrene	394	4.88	4.88
	Biphenyl	256.1	4.01	6.15

2.2 Green Chemistry

2.2.1 Principles of Green Chemistry

The environmental impact of hazardous solvents has received considerable attention in recent years. Numerous innovations have been implemented to mitigate this damage and safeguard the environment. The ultimate objective of sustainable development is to improve the quality of life, even if this comes at the expense of imposing certain constraints on human behaviours. Technologies that primarily involve chemical activities at both the industrial and laboratory scales are regarded as significant sustainability factors. Irresponsible disposal of chemical residue can have a substantial environmental impact, even for small-scale chemist operations such as laboratory research that employs substantial quantities of hazardous chemicals (Aly & Górecki, 2020).

Due to the factors mentioned above, consideration should be given to the chemical activities and analyses, and a new, more environmentally friendly analytical method should be developed and implemented. Developing green analytical methods is likely the most active topic of green chemistry, as a result, Paul Anastas and John Warner established the 12 principles of Green Chemistry (Justyna Płotka-Wasylka et al., 2017) provided in Table 2.3, which focus on the minimisation or elimination of harmful solvents in chemical processes and analyses and the elimination of wastes generated by these processes (de Marco et al., 2019; Gałuszka et al., 2012; Keith et al., 2007). These concepts promote ecologically beneficial practices from the product's conception to its synthesis, processing, and disposal after usage. The primary purpose is to minimise industrial activity's inherent environmental and occupational risks. Thus, the current analytical procedure has become the most challenging task to improve, necessitating the introduction and development of new technologies, particularly a new generation of sample preparation techniques. Sample preparation is the chemical analysis workflow's most critical and time-consuming phase (Namieśnik et al., 2015). In addition, this process ensures the accurate separation of the target analytes, preventing contamination and providing matrix isolation for the development of dependable techniques and the subsequent attainment of greater sensitivity, precision, accuracy, and recovery (Burato et al., 2020).

Table 2.3 12 principles of Green Chemistry (de Marco et al., 2019).

Number	Principle	Description of principle
1	Prevention	It concerns the prevention of waste generation. It is better to avoid generating waste than to treat it after its generation.
2	Atomic economy	Synthetic methods should be planned so that the final product incorporates as many of the reagents used during the process as possible. Thus, waste generation will be minimised.
3	Safer chemical synthesis	Synthetic methods should be designed to synthesise and generate substances with low or no occupational and environmental toxicity. Thus, replacing toxic solvents with low or no toxicity solvents is highly recommended.
4	Safer chemical design	Great importance should be given to the design toxicity of the designed chemicals. They should fulfill their functions but should also present the lowest possible toxicity.
5	Safer, solvents and auxiliaries	The use of solvents and other reagents and auxiliaries should be avoided where possible. When it is not possible, these substances should be innocuous.

Table 2.3 (continued).

Number	Principle	Description of principle
6	Design for energy efficiency	Energy requirements of chemical processes should be recognised for their environmental and economic impacts and minimised. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7	Renewable raw materials	Whenever it is economically and materials technically feasible, renewable raw materials should be used instead of non-renewable.
8	Reduction of derivatives	Unnecessary derivatisation processes derivatives should be avoided or minimised as they require the additional use of reagents and, therefore, generate waste.
9	Catalysis	Catalytic reagents (as selective as possible) are better than stoichiometric reagents.
10	Degradation products design	Chemicals should be designed so that at the end of their function, they decompose into harmless degradation products and do not persist in the environment.
11	Real-time analysis for pollution prevention	Analytical methods should be monitored for pollution prevention in real-time to avoid the formation of hazardous substances.
12	Accidents prevention	Both the substances and the way they are used in a chemical process should be chosen considering the minimisation of potential accidents, such as leaks, explosions, and fires, aiming at more occupational and environmental safety.

2.2.2 Green analytical chemistry

Many analytical procedures have implemented the concept of green chemistry. Four fundamental processes comprise the analytical procedure: sample collection, sample preparation, final analysis, and result interpretation (Gałuszka et al., 2012; Tobiszewski et al., 2009). Tobiszewski et al. (2009) remarked that sample preparation is the most crucial step in the analytical procedure due to the extensive usage of organic solvents. During sample preparation, extraction, distillation, filtering, and other significant operations are required to avoid direct determination without isolation and preconcentration prior to analysis. Most analytical methods need the isolation and concentration of the sample. Consequently, the application of conventional sample preparations such as LLE and SPE exhibited deficiencies in terms of the use of toxic organic solvents leading to significant pollution by generating waste released into the environment, and these methods are labour intensive and time-consuming, which is inconvenient for the operator.

It is indeed impossible to reduce reagent usage. Therefore, green analytical chemistry provides chemists with a framework for incorporating this sustainable development into their procedures. Applying green analytical chemistry concepts improves the environmental impact of analyses. Hazardous reagents should be replaced with safer alternatives, and the threat caused by analytical wastes should be decreased. This is achievable via online waste decontamination (recycling, degradation, and passivation). The number of stages in an analytical technique is heavily influenced by the properties of the sample and the analytical method. Typically, the greater the number of steps in an analytical method, the less ecologically friendly it is (due to increased energy use and larger volume of waste). Thus, lowering the number of stages in a given analytical procedure would be the most environmentally friendly method (Gałuszka et al., 2012).

Nevertheless, specific steps are inevitable. Green analytical chemistry aims to develop more environmentally friendly analytical techniques. Table 2.4 shows the 12 Principles of Green Analytical Chemistry (de Marco et al., 2019; Gałuszka et al., 2013).

Table 2.4 The 12 Principles of Green Analytical Chemistry.

Number	**Principle and description**
1	Direct analytical techniques should be applied to avoid the sample treatment step.
2	The size and quantity of samples should be as small as possible.
3	In situ analyses should be carried out.
4	Integration of analytical processes and operations must be performed, as it promotes energy savings and reduces the use of reagents.
5	Automated and miniaturised methods must be selected.
6	Derivatisations should be avoided as they require additional reagents and generate waste.
7	The generation of large volumes of analytical waste must be avoided, and the correct handling of this waste must be provided
8	Multi-analyte methods should be preferred over methods that analyse one analyte simultaneously.
9	The use of electric energy should be minimised.
10	Reagents obtained from renewable sources should be preferred.
11	Hazardous reagents should be discarded.
12	The safety of operators should be increased.