DEVELOPMENT OF GREEN MICROEXTRACTION-BASED DEEP EUTECTIC SOLVENTS FOR THE EXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN MILK SAMPLES

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

$\bar{x}$	Mean
μgkg^{-1}	Microgram per kilogram
µL	Microliter
A	Number of PLS or PCA components in the model
a	Number of the PLS or PCA component
b	Langmuir energy of sorption
c	Intercept
C_e	Final concentration
C_o	Initial concentration
E	Polanyi potential related to the equilibrium concentration
K	Intraparticle diffusion rate constant
K	Kelvin
k_1	Rate constant of pseudo-first order model of kinetic
k_2	Rate constant of pseudo-second order model of kinetic
K_d	Thermodynamic equilibrium constant
k_{ext}	External diffusion rate constant
K_F	isothermal constant capacity
K_H	Halsey isotherm constant
kj mol^{-1}	Kilojoules per mol
mgg^{-1}	Milligram per gram
N	Number of data points
n_f	Freundlich isotherm constant
^oC	Degree celsius
q_e	Adsorption capacity
q_m	Langmuir constants related to the adsorption capacity

q_t	Adsorption capacity at any time
R	Universal gas constant
R_L	Dimensionless constant separation factor in Langmuir model
t	time
V	volume
α	Initial sorption rate
β	Activation energy
Δq	Normalized standard deviation value

LIST OF ABBREVIATIONS

AGREE	Analytical Greenness Evaluation
AGREEprep	Analytical Greenness Evaluation for Sample Preparation
ANOVA	Analysis of variance
BET	Brunauer-Emmett-Teller analysis
ComplexGAPI	Complementary Green Analytical Procedure Index
D-µ-SPE	Dispersive-micro-solid-phase extraction
DES	Deep eutectic solvents
D-SPE	Dispersive-solid-phase extraction
FDA	United States of America Food and Drug Administration
Fe_3O_4	Magnetic nanoparticles
Fe_3O_4@HDES	Magnetic hydrophobic deep eutectic solvents
FT-IR	Fourier-transform infrared spectroscopy
GAC	Green analytical chemistry
GAPI	Green Analytical Performance Index
GC-MS	Gas chromatography-mass spectrometry
GSP	Green sample preparation
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HDES	Hydrophobic deep eutectic solvents
HPLC-DAD	High performance liquid chromatography-diode array detector
HPLC-PDA	High performance liquid chromatography photo diode array detector
HPLC-UV	High performance liquid chromatography-ultraviolet detector
IL	Ionic liquids
LC-MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection

LOQ	Limit of quantification
MRLs	Maximum residue limits
NMR	Nuclear magnetic resonance spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Orthogonal array
OVAT	One-variable-at-one-time
RR	Relative recovery
RSD	Relative standard deviation
S/N ratio	Signal-to-noise ratio
SD	Standard deviation
SEM	Scanning electron microscopy
SFC	Sulfachloropyridazine
SFM	Sulfamethoxine
SPE	Solid-phase extraction
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
UHPLC-DAD	Ultra-performance liquid chromatography-diode array detector
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry
VSM	Vibrating sample magnetometer
XRD	X-ray diffractometer

LIST OF APPENDICES

PEMBANGUNAN PENGEKSTRAKAN MIKRO-HIJAU BERASASKAN PELARUT EUTEKTIK TERDALAM UNTUK PENGEKSTRAKAN UBAT ANTI-RADANG BUKAN STEROID DALAM SAMPEL SUSU

ABSTRAK

Penggunaan meluas ubat anti-radang bukan steroid (NSAIDs) dalam perubatan manusia dan veterinar, walaupun berkesan dalam menguruskan keradangan dan kesakitan, telah menimbulkan kebimbangan yang ketara terhadap keselamatan alam sekitar dan makanan. NSAIDs sering mengalami penyerapan yang tidak lengkap dalam sistem pencernaan manusia dan haiwan, mengakibatkan kehadirannya yang berterusan dalam air, susu, dan produk haiwan lain. Pengumpulan sisa NSAIDs ini memberi ancaman besar kepada integriti ekologi dan kesihatan manusia. Kaedah sedia ada untuk mengekstrak dan mengesan sisa NSAIDs seringkali kurang sensitif dan tidak mesra alam. Memandangkan keperluan mendesak untuk kaedah pengekstrakan yang lebih maju dan mampan, kajian ini memperkenalkan pelarut eutektik dalam bersifat hidrofobik magnetik (Fe_3O_4@HDES-2) sebagai penjerab untuk mengekstrak NSAIDs dari sampel susu. Fe_3O_4@HDES-2 telah berjaya disintesis dan dicirikan menggunakan pelbagai teknik pencirian, termasuk FT-IR, SEM, TEM, BET, VSM, TGA, analisis zeta potential, analisis NMR, dan pengukuran sudut sentuh serbuk. Kajian analisis tapak penjerapan menggunakan Material Studio menyokong pemilihan HDES-2 sebagai agen berfungsi, mencadangkan mekanisme penjerapan yang sesuai yang selari dengan penemuan empirik. Kajian penjerapan telah dilakukan untuk menjelaskan mekanisme penjerapan antara Fe_3O_4@HDES-2 dan model NSAIDs yang dipilih. Dalam keadaan penyingkiran penjerapan yang optimum, penjerapan model NSAIDs yang dipilih oleh Fe_3O_4@HDES-2 dikenal pasti sebagai proses kemisorpsi

yang dicirikan oleh kebolehlaksanaan, spontan, dan proses penjerapan eksotermik. Berdasarkan penemuan mekanisme penjerapan, penjerab Fe_3O_4@HDES-2 menunjukkan kapasiti penjerapan tertinggi dan seterusnya digunakan untuk pengekstrakan mikro-pepejal-diserakkan magnetik (magnetic D-µ-SPE) bersama HPLC-DAD untuk penentuan tepat NSAIDs dalam matriks kompleks seperti susu. Pendekatan inovatif ini menunjukkan kepekaan luar biasa, mencapai had pengesanan antara 0.003 µg kg^{-1} dan 0.88 µg kg^{-1} serta julat pemulihan antara 82.2 – 118.0%. Bagi memastikan pematuhan terhadap prinsip kimia analisis hijau dan penyediaan sampel hijau, kaedah pengekstrakan yang dibangunkan menggunakan Fe_3O_4@HDES-2 telah menjalani penilaian ketoksikan dan metrik kehijauan yang ketat. Kajian ini berjaya meneroka potensi Fe_3O_4@HDES-2 sebagai penjerab untuk teknik magnetik D-µ-SPE yang digabungkan dengan analisis HPLC-DAD dalam penentuan sisa NSAIDs (ketoprofen, naproxen, fenoprofen, diclofenac, dan ibuprofen) dalam matriks kompleks. Kaedah yang dibangunkan ini menawarkan penyelesaian yang menjanjikan untuk mengurangkan risiko alam sekitar dan keselamatan makanan yang berkaitan dengan pencemaran NSAIDs sambil mematuhi prinsip kimia hijau.

DEVELOPMENT OF GREEN MICROEXTRACTION-BASED DEEP EUTECTIC SOLVENTS FOR THE EXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN MILK SAMPLES

ABSTRACT

The pervasive use of non-steroidal anti-inflammatory drugs (NSAIDs) in human and veterinary medicine, despite their efficacy in managing inflammation and pain, has led to substantial environmental and food safety concerns. NSAIDs often undergo incomplete absorption in human and animal digestive systems, resulting in their persistent presence in water, milk, and other animal-derived products. This residue accumulation poses significant threats to both ecological integrity and human health. Existing methods for extracting and detecting NSAIDs residues often lack sufficient sensitivity and environmental compatibility. Given the pressing need for advanced, environmentally sustainable extraction methods, this study introduces magnetic hydrophobic deep eutectic solvents (Fe_3O_4@HDES-2) as a novel sorbent to extract NSAIDs from milk samples. The Fe_3O_4@HDES-2 was successfully synthesized and characterized using a variety of characterisation techniques, including FT-IR, SEM, TEM, BET, VSM, TGA, point of zero analysis, NMR analysis, and powder contact angle measurements. Adsorption site analysis study using Material Studio for substantiated the selection of HDES-2 as a functionalised agent, suggesting a favourable adsorption mechanism that harmonised with empirical findings. Adsorption studies were undertaken to elucidate the adsorption mechanism between Fe_3O_4@HDES-2 and selected model of NSAIDs. Under optimal adsorptive removal conditions, selected model of NSAIDs adsorption by Fe_3O_4@HDES-2 was ascertained to be a chemisorption process characterized by feasibility, spontaneity, and exothermic

adsorption process. Based on the adsorption mechanism findings, the Fe_3O_4@HDES-2 adsorbent exhibited the highest adsorption capacity and was consequently utilized for magnetic dispersive-micro-solid-phase extraction (magnetic D-µ-SPE) in conjunction with HPLC-DAD for the precise determination of NSAIDs residue in complex matrices like milk. This innovative approach demonstrated exceptional sensitivity, achieving a limit of detection between 0.003 µg kg^{-1} and 0.88 µg kg^{-1} and a recovery range of 82.2 – 118.0%. To ensure compliance with green analytical chemistry and green sample preparation principles, the developed extraction method using Fe_3O_4@HDES-2 underwent rigorous toxicity assessment and greenness metric evaluation. This study successfully explored the potential of Fe_3O_4@HDES-2 as a sorbent for magnetic D-µ-SPE combined with HPLC-DAD analysis in the determination of NSAIDs residue (ketoprofen, naproxen, fenoprofen, diclofenac, and ibuprofen) in complex matrices. The developed method offers a promising solution for mitigating the environmental and food safety risks associated with NSAIDs contamination while adhering to green chemistry principles.

CHAPTER 1

INTRODUCTION

1.1 General background

The widespread use of non-steroidal anti-inflammatory drugs (NSAIDs) in both human and veterinary medicine, while effective for managing inflammation and pain, has raised significant concerns regarding their environmental and food safety implications (Rodríguez-Saldaña et al., 2023, Rastogi et al., 2021). Due to incomplete absorption in the digestive systems of both humans and animals, where a portion of the NSAIDs is not fully metabolised, these drugs frequently persist in water, milk, and other animal-derived products, leading to the accumulation of residues (Lin et al., 2023, Izadi et al., 2020). This accumulation poses a substantial threat to the ecological integrity of aquatic environments and the safety of human consumption (Huynh et al., 2023, Radovic et al., 2023).

Even in trace amounts, NSAIDs can cause significant harm where prolonged exposure to low levels of these drugs can lead to chronic health issues such as gastrointestinal damage, kidney dysfunction, and cardiovascular problems in humans (Bindu et al., 2020). Residue of NSAIDs can contaminate water bodies through various pathways, including wastewater discharge, agricultural runoff, and the disposal of animal waste (Salahshoori et al., 2024, Hussain et al., 2023). Hospital sewage and municipal waste treatment plants are also significant contributors to NSAIDs pollution (Mussa et al., 2022, Parida et al., 2022). Overuse of NSAIDs, both through prescription and over-the-counter medication, can lead to increased concentrations of these drugs in wastewater treatment plants (Baratta et al., 2022). If these plants are not equipped to effectively remove NSAIDs, they can be released into nearby rivers or lakes, contaminating aquatic environments (Silva et al., 2022). Additionally, hospital

wastewater often contains higher concentrations of NSAIDs due to their frequent use in medical settings (Ortúzar et al., 2022). Wastewater discharge is the main source of NSAIDs contamination due to its constant and widespread release, unlike the more limited and seasonal impact of agricultural runoff and animal waste (Mejía-García et al., 2020, Tyumina et al., 2020).

Once released into aquatic environments, NSAIDs can disrupt biological functions in aquatic organisms. For instance, ibuprofen has been shown to alter brain lipid metabolism in adult zebrafish, affecting membrane composition, inflammatory responses, and potentially contributing to cardiovascular and cerebrovascular disorders. Notably, the two enantiomers of Ibuprofen exhibit different toxicological effects, indicating stereoselective toxicity. These findings underscore the complex and potentially hazardous impact of pharmaceutical pollutants on aquatic ecosystems (Petrie and Camacho-Muñoz, 2021, Zhang et al., 2020). These effects highlight the ecological risks posed by even low concentrations of NSAIDs in aquatic environments (Mejía-García et al., 2020). Additionally, the persistence of NSAIDs in the environment can lead to long-term exposure and health risks for humans (Hernández-Tenorio et al., 2022, Kaya et al., 2022).

To safeguard public health, the World Health Organization (WHO) has designated NSAIDs as emerging environmental contaminants (World Health Organization, 2022). This classification has prompted numerous countries and international organisations to establish maximum residue limits (MRLs) for NSAIDs in animal-derived products and the environment. For instance, the European Union has set MRLs for NSAIDs at 0.1 $\mu g\ kg^{-1}$ for bovine milk and 5.0 $\mu g\ kg^{-1}$ for bovine muscle and liver (Shishov et al., 2019). Similarly, the U.S. Food and Drug Administration (FDA) has established an MRL of 1.0 $\mu g\ kg^{-1}$ for NSAIDs in aquatic environments

(FDA, 2024, Li et al., 2024b). However, in Malaysia, specific MRLs for NSAIDs in environmental waters have not yet been established. The Department of Environment's National Water Quality Standards focus primarily on conventional pollutants such as heavy metals, nutrients, and pathogens, with pharmaceuticals like NSAIDs currently excluded from routine monitoring (Ministry of Natural Resources and Environmental Sustainability, 2025). These regulatory measures, or their absence, underscore the importance of regular NSAIDs monitoring in environmental water and food sources. By tracking NSAIDs levels, authorities can identify potential contamination sources, implement effective remediation strategies, and ensure the safety of public water supplies and food products.

Sample preparation is a critical preprocessing step in analytical chemistry that significantly influences the accuracy, precision, and reliability of analytical results (El Hosry et al., 2023). It addresses various challenges, including solubility, matrix interference, concentration, physical state, and preservation (Schulze et al., 2020). By effectively transforming samples into a suitable form for analysis, sample preparation ensures that the analyte of interest can be isolated and later can be analysed and quantified using analytical instruments such as high performance liquid chromatography-ultraviolet detector (HPLC-UV) or high performance liquid chromatography-diode array detector (HPLC-DAD) (Melissaropoulou et al., 2024, Minh et al., 2024).

Solid-phase extraction (SPE) is a commonly used method for organic pollutants extraction from complex matrices (Jiang et al., 2023, Rodríguez-Saldaña et al., 2023). It involves passing the sample through a solid sorbent material that selectively adsorbs the NSAIDs. By using appropriate sorbents and elution conditions, SPE can effectively concentrate pollutants from complex matrices, such as

wastewater, soil, and food products (Zhou et al., 2024). There are previous studies which utilised SPE as a method to isolate trace NSAIDs from various samples(Zhou et al., 2021, Hu et al., 2024b, Weixia et al., 2024).

SPE is a versatile sample preparation technique in analytical chemistry, but it can have significant environmental implications. The use of organic solvents, the generation of waste, and the selection of non-green sorbents can contribute to pollution and resource depletion (Sheldon, 2019, Godage and Gionfriddo, 2020). Thus, the domain of SPE has undergone a transformative evolution in recent years, driven by the principles of green analytical chemistry (GAC) and green sample preparation (GSP) (Esteve-Turrillas et al., 2024, Jatkowska, 2024). These green analytical approaches advocate for minimising the use of hazardous solvents, reducing waste, and enhancing efficiency (López-Lorente et al., 2022, Yin et al., 2024). In response, SPE techniques have been refined to incorporate greener practices. Miniaturisation of SPE, exemplified by microextraction and dispersive-SPE (D-SPE), has resulted in a substantial reduction in solvent usage and a concomitant enhancement in extraction efficiency (Peris-Pastor et al., 2024, Płotka-Wasylka et al., 2023). Magnetic SPE or magnetic D-SPE, a miniaturised version of D-SPE that employs functionalised magnetic particles, has further advanced the greening of SPE by streamlining sample handling and eliminating the need for complex apparatus (Corps Ricardo et al., 2020, Yin et al., 2021).

Magnetic nanoparticles, often used in SPE, require surface modification or functionalisation to enhance their stability and biocompatibility. The functionalisation material should be environmentally benign to mitigate any potential adverse effects on the environment (Keçili et al., 2021, Araújo et al., 2024). Deep eutectic solvents (DES), a class of green solvents, are formed by combining two components; a

hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). This combination results in a mixture with a lower melting point than either individual component, creating a unique solvent with properties that can be tailored for various applications (Bomfim Bahia et al., 2024, Gao et al., 2024).

One promising approach to enhancing the versatility and efficiency of DES is by functionalising them with magnetic nanoparticles. These nanoparticles offer several advantages, including easy separation from the target analytes or reaction mixture using a magnet (magnetic recovery), improved dispersion in aqueous or organic media, and enhanced adsorption capacity for specific analytes (Aguirre and Canals, 2022, Zainal-Abidin et al., 2023). While the majority of studies have focused on hydrophilic DES, which are compatible with aqueous environments, they have limitations in certain applications, such as the extraction of hydrophobic analytes. Hydrophobic DES, on the other hand, offer several advantages. They can effectively extract nonpolar and hydrophobic compounds, are more compatible with organic matrices, and have lower water solubility, which can be beneficial in applications of target analytes from complex matrices is a concern (Farooq et al., 2022, Hu et al., 2024a).

While previous research has investigated the use of hydrophobic deep eutectic solvents (HDES) in extracting nonsteroidal anti-inflammatory drugs (NSAIDs) from milk samples (Qiao et al., 2021), the application of magnetic HDES (Fe_3O_4@HDES) has primarily been focused on wastewater extraction, leaving a gap in the application for more complex food matrices such as milk (Yang et al., 2023b, Yıldırım et al., 2024). This study represents the first comprehensive attempt to utilise Fe_3O_4@HDES-2, which has high affinity towards NSAIDs as an extraction sorbent in magnetic dispersive-micro-solid-phase extraction (magnetic D-μ-SPE) for the extraction of

NSAIDs from milk matrices. By employing Fe_3O_4@HDES-2, this study not only introduces a novel sorbent for use in food safety applications but also pushes the frontier of green sample preparation techniques by integrating magnetic recovery and hydrophobic solvent properties into a single approach.

To evaluate the greenness of the proposed method, Analytical Eco-scale, Complementary Green Analytical Procedure Index (ComplexGAPI), and Analytical Greenness Metric for Sample Preparation (AGREEprep) were employed. These metrics assess the environmental impact of the method based on GAC and GSP principles. Notably, this study represents the first to systematically combine magnetic hydrophobic deep eutectic solvents (Fe_3O_4@HDES-2) with these green chemistry principles, setting a new benchmark for environmentally sustainable extraction methods. In conclusion, this research presents a pioneering application of magnetic HDES-based magnetic D-μ-SPE for the extraction of NSAIDs from milk. The combination of magnetic nanoparticles and hydrophobic deep eutectic solvents offers a promising solution for the sensitive and efficient analysis of trace contaminants in food products, ultimately contributing to food safety and public health.

1.2 Problem statements

The presence of trace NSAID residues in milk poses a significant threat to food safety, necessitating the development of sensitive, sustainable, and reliable analytical methods for their detection in complex food matrices. Conventional techniques such as solid-phase extraction (SPE), while widely used, suffer from several limitations including high cost, extensive solvent use, time-consuming procedures, and limited selectivity and reusability of sorbents. These drawbacks highlight the urgent need for greener, more cost-effective, and efficient extraction alternatives. In this context,

hydrophobic deep eutectic solvents (HDES) have emerged as promising candidates due to their eco-friendly nature, low toxicity, biodegradability, and strong hydrophobic interactions. Their potential for functionalizing magnetic nanoparticles offers a novel approach to enhance sorbent performance. However, the application of magnetic HDES (Fe_3O_4@HDES) has primarily been focused on wastewater extraction, leaving a gap in their use for more complex food matrices such as milk. Therefore, this study aims to develop a magnetic green sorbent based on HDES for use in magnetic dispersive micro-solid-phase extraction (magnetic D-µ-SPE), coupled with HPLC-DAD, to provide a simple, efficient, and environmentally sustainable method for analyzing NSAID residues in milk.

1.3 Scope of the study

This research aimed to investigate the performance of a novel material as a sorbent in extraction techniques for milk samples. Initially, hydrophobic deep eutectic solvents (HDES) were developed and synthesised using decanoic acid as the hydrogen bond acceptor and various classes of alcohols as hydrogen bond donors. Magnetic HDES (Fe_3O_4@HDES-2) were subsequently prepared by incorporating Fe_3O_4 nanoparticles into the selected HDES-2.

To confirm the functional groups and modifications of the synthesised sorbent, a comprehensive set of characterisation tools were employed. These included Fourier-transform infrared spectroscopy (FT-IR) to identify chemical bonds, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for morphological analysis, X-ray diffractometer (XRD) for crystallinity studies, and vibrating sample magnetometer (VSM) for magnetic property assessment. Additional characterisation techniques such as Brunauer-Emmett-Teller (BET) analysis for surface area and

porosity, thermogravimetric analysis (TGA) for thermal stability, zeta potential for surface charge, nuclear magnetic resonance spectroscopy (NMR) for molecular structure analysis, and contact angle measurements to evaluate surface hydrophobicity were also utilised to thoroughly investigate the physicochemical properties of the synthesised sorbents.

The synthesised Fe_3O_4@HDES-2 was subsequently evaluated for its adsorption capacity in batch mode studies involving two selected NSAIDs, diclofenac and ibuprofen are among the most commonly used NSAIDs in veterinary medicine, particularly for treating inflammation and pain in livestock such as cattle. The adsorption performance of Fe_3O_4@HDES-2 for these NSAIDs was assessed using an ultraviolet-visible (UV-Vis) spectrophotometer, which allowed for the determination of adsorption capacity, and the fitting of adsorption isotherms and kinetic models. These models were applied to both the unmodified and modified adsorbents to evaluate the interaction mechanisms and adsorption mechanisms between Fe_3O_4@HDES-2 and the NSAIDs. The adsorption studies were conducted to understand the interaction between the novel Fe_3O_4@HDES-2 adsorbent and the NSAIDs, providing insight into its adsorption behaviour and efficiency before proceeding to the extraction study. Following optimisation, the Fe_3O_4@HDES-2 was employed in the magnetic D-µ-SPE technique for the extraction of five NSAIDs (ketoprofen, naproxen, fenoprofen, diclofenac, and ibuprofen) from complex matrices, specifically milk samples. Additionally, the versatility of Fe_3O_4@HDES-2 was demonstrated by applying the same extraction method to other pharmaceutical contaminants; sulfonamides and estrogens, in milk samples. This study highlights the potential of Fe_3O_4@HDES-2 as a highly effective sorbent for a broad range of pharmaceutical compounds in complex sample matrices.

To further validate the sorbent greenness, the toxicity of the synthesised materials was assessed to ensure their safety for potential applications. Specifically, the biocompatibility and cytotoxicity of HDES-2, Fe_3O_4, and Fe_3O_4@HDES-2 were evaluated using two normal cell lines to determine their safety profiles. Meanwhile, the magnetic D-µ-SPE method was subjected to various greenness metric tools, in line with the principles of green analytical chemistry and green sample preparation, to evaluate its environmental sustainability and eco-friendliness. These assessments aimed to minimise the use of hazardous substances, reduce waste, and improve overall process efficiency. The data obtained from these evaluations underscore both the safety of the synthesised materials and the greenness of the extraction method, highlighting its potential as an environmentally responsible and safe analytical approach.

1.4 Research objective

1.4.1 General objective:

To explore the potential of novel magnetic nanoparticles modified with deep eutectic solvents (Fe_3O_4@HDES-2) sorbents in magnetic dispersive-micro-solid-phase extraction (magnetic D-µ-SPE) method, combined with HPLC-DAD analysis, for determination of five NSAIDs in complex matrices.

1.4.2 Specific objectives

1. To optimise hydrogen bond acceptor and hydrogen bond donor of hydrophobic deep eutectic solvents (HDES) modified with magnetic nanoparticles (Fe_3O_4).
2. To characterise the synthesised Fe_3O_4 and Fe_3O_4@HDES-2 sorbents to ensure their physical and chemical properties of the developed materials.

3. To investigate the effects of adsorption parameters and adsorption mechanism (kinetic, isotherm and thermodynamic) of Fe_3O_4@HDES-2 towards model analytes (diclofenac and ibuprofen) in water samples, using UV-Vis spectrophotometry.
4. To evaluate and validate the extraction performance of the magnetic D-μ-SPE method using Fe_3O_4@HDES sorbents, coupled with HPLC-DAD for NSAIDs determination in complex matrices.
5. To assess the greenness chemistry aspects of the developed magnetic D-μ-SPE method, using Fe_3O_4@HDES sorbents combined with HPLC-DAD.

1.5 Outline of the thesis

This thesis is structured into five main chapters. Chapter 1 provides context for the study, defines the research problem, objectives and significances, and outlines of the thesis structure. Chapter 2 summarises key theories, previous studies, and gaps in the literature, leading to the conceptual framework of the research. Chapter 3 describes the research design which includes the developing materials, Fe_3O_4@HDES-2 as an alternative sorbent in magnetic dispersive-micro-solid-phase extraction and analysis method. Chapter 4 details the findings in relation to the existing literature, discusses the highlighting key findings and their statistical significance. Finally, Chapter 5 offers a general conclusion of the research, along with recommendations for future research directions.

CHAPTER 2

LITERATURE REVIEW

2.1 Non-steroidal anti-inflammatory drugs and other pharmaceuticals

Pharmaceuticals, while essential for human health, have become a growing environmental concern due to their widespread use and persistence in the environment (Osuoha et al., 2023, Nguyen et al., 2024). They can enter water bodies through various pathways, posing risks to both aquatic ecosystems and human health (Samal et al., 2022, Nguyen et al., 2023). Examples of pharmaceuticals that have been identified as emerging pollutants include antibiotics, analgesics, anti-inflammatory drugs, and hormones (Al Falahi et al., 2022, Kock et al., 2023).

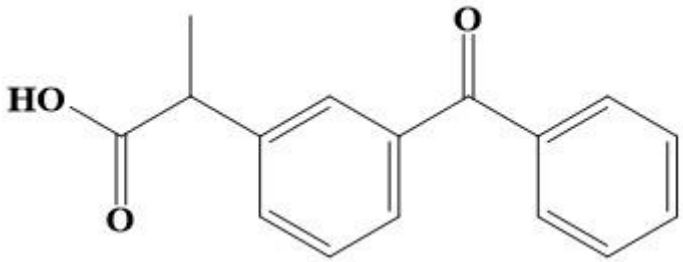
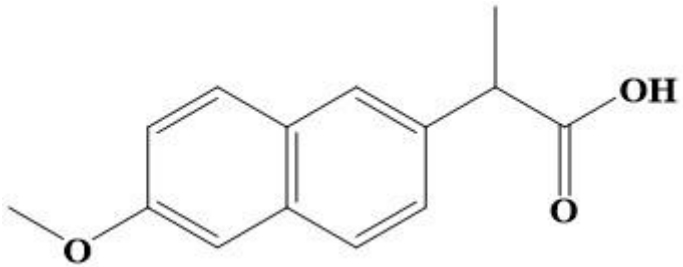
Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of medications commonly used to manage pain, fever, and inflammation (Huynh et al., 2023, Panchal and Prince Sabina, 2023). NSAIDs work by inhibiting the production of prostaglandins, which are substances in the body that cause pain and inflammation (Silva et al., 2022, Parikh et al., 2024). NSAIDs can enter the environment through various pathways, including wastewater discharge, agricultural runoff, and improper disposal (Lin et al., 2023, Rodríguez-Saldaña et al., 2023). Agricultural runoff contributes to NSAID contamination when livestock treated with these drugs excrete unmetabolized residues, which can leach into nearby water bodies, especially during rainfall or irrigation events (Dolu and Nas, 2023). Another significant source of NSAIDs contamination is hospital sewage (Lin et al., 2023, Placova et al., 2023). Hospitals often use large quantities of NSAIDs to treat patients, and these drugs can end up in wastewater treatment plants. If these plants are not equipped to effectively remove NSAIDs, they can be released into nearby waterways, contaminating aquatic environments (Chakraborty et al., 2023, Wydro et al., 2024). In Malaysia, current wastewater treatment technologies are not specifically

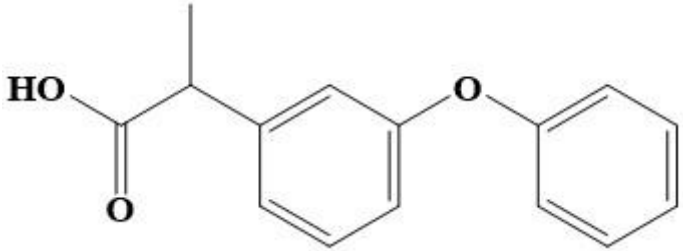
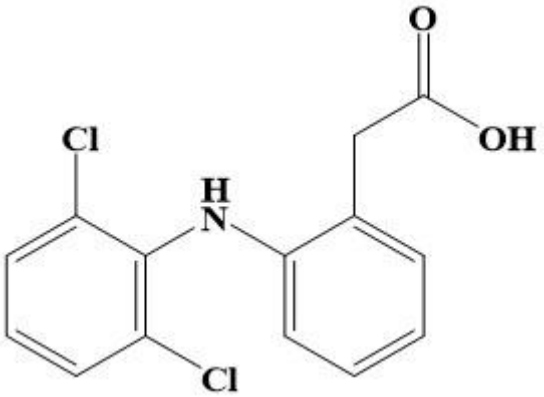
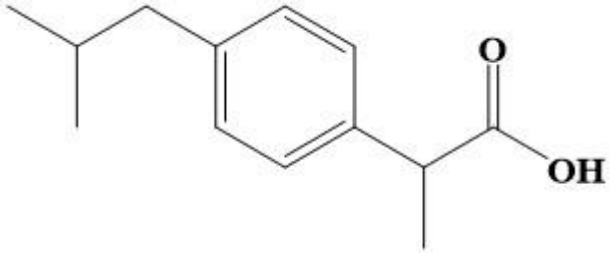
designed to remove pharmaceutical compounds like NSAIDs as study by Hanafiah et al. (2024) have shown that these compounds can persist through treatment processes and be discharged into waterways, posing ecological risks (Hanafiah et al., 2024). Additionally, municipal waste plants can also contribute to NSAIDs pollution, especially when there is excessive use of NSAIDs in the community. This can occur due to over-the-counter medication or the prescription of NSAIDs for a variety of conditions (Abdu et al., 2020, Wirth et al., 2024).

Once in the environment, these drugs can persist for extended periods, contaminating water bodies, soil, and biota (Izadi et al., 2020, Zhou et al., 2023). This persistence poses ecological and human health risks, even at trace concentrations. In livestock, NSAIDs administered for therapeutic purposes can be excreted unmetabolized, contaminating the environment and re-entering the food chain. Residues may accumulate in edible animal products such as milk, meat, and eggs, particularly when withdrawal periods are not adequately observed. Consequently, dietary exposure to NSAIDs represents a potential route of human intake (Liang et al., 2020, Li et al., 2024b).

The chemical structure of NSAIDs plays a crucial role in their persistence and accumulation in environmental water and food matrices. Most NSAIDs are organic compounds containing a carboxylic acid group and a hydrophobic aromatic ring (Ji et al., 2023, Fatih Dilekoglu and Yapici, 2023, Madikizela and Ncube, 2021) (Table 2.1). This structure allows them to interact with both hydrophilic and hydrophobic components of the environment, enhancing their solubility and mobility (Shamsudin et al., 2022, Chandel et al., 2024).

Table 2.1 Physicochemical properties of the studied NSAIDs.

Name	Chemical structure	Molecular formula	Molecular weight (g mol^{-1})	pKa
Ketoprofen		$C_{16}H_{14}O_3$	254.3	4.45
Naproxen		$C_{14}H_{14}O_3$	230.3	4.15

Fenoprofen		$C_{15}H_{14}O_3$	242.3	4.50
Diclofenac		$C_{14}H_{11}Cl_2NO_2$	296.1	4.90
Ibuprofen		$C_{13}H_{18}O_2$	206.3	4.02

2.2 Regulatory Standards and Monitoring of NSAID Residues in Food and the Environment

Maximum Residue Limit (MRL) is a scientifically established threshold defining the highest permissible concentration of a veterinary drug residue in animal-derived products. This regulatory measure serves as a safeguard for public health by mitigating the potential risks associated with excessive human exposure to drug residues. By adhering to the MRL guidelines, international trades can be facilitated while maintaining consumer confidence in the safety of food products (Chicoine et al., 2020). Given the significant environmental and health risks posed by pharmaceuticals, it is imperative to establish maximum residue limits (MRLs) for NSAIDs compounds in environmental and food matrices. Table 2.2 provides examples of MRLs for NSAIDs in bovine and environmental water. By setting MRLs for pharmaceuticals, governments can ensure that the levels of these substances in the environment and food are kept below safe limits, protecting public health. Besides, to effectively monitor and enforce MRLs, it is essential to develop sensitive and reliable detection methods for pharmaceuticals in environmental water and food matrices. Table 2.3 shows study of NSAIDs determination conducted in several countries.

Table 2.2 Regulation of NSAIDs MRLs in bovine and water by several agencies.

Agency	Pharmaceutical	Maximum residue limit (μg kg^{-1})					Reference
		Bovine				Water	
		Milk	Muscle and liver	Fat tissue	Kidney		
European Medicines Agency	Diclofenac	0.1	5.0	1.0	10.0	100.0	(European Medicines, 2009)
Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies	Ibuprofen	–	–	–	–	28	(Department of Climate Change, 2008)
	Ketoprofen	–	–	–	–	0.38	
	Naproxen	–	–	–	–	0.57	

Table 2.3 NSAIDs found in environmental water and food matrices around the globe.

Pharmaceuticals	Extraction method/ instrument	Matrix	Concentration detected ($\mu g\ kg^{-1}$)	Location	Reference
NSAIDs (phenacetin, meloxicam, naproxen, diclofenac sodium, and carprofen)	Metal-organic framework@graphene aerogel based-solid phase extraction/ UPLC-MS/MS	Bottled drinking water, tap water, river water, lake water, and wastewater	0.04 – 0.50	Shenyang, China	(Zhou et al., 2021)
NSAIDs (ibuprofen, salicylic acid, acetylsalicylic acid, naproxen, ketoprofen and diclofenac	Solid phase extraction/ UHPLC-DAD	Wastewater from treatment plants	0.68 – 25.91	Słupsk, Poland	(Stec and Astel, 2024)

NSAIDs (ibuprofen, diclofenac, ketoprofen, and naproxen	Solid phase extraction/ GC-MS	Wastewater from urban catchments	0.07 – 0.28	Selangor, Malaysia	(Mohd Hanafiah et al., 2022)
NSAIDs (ketoprofen, flurbiprofen, naproxen and diclofenac)	Cationic microporous organic network based-solid phase extraction/ HPLC-UV	River and lake water, and bovine milk	ND	Jinan City, China	(Hu et al., 2024b)
NSAIDs (ketoprofen, flurbiprofen, ibuprofen, naproxen and diclofenac sodium	Metal-organic frameworks based-solid phase extraction/ UPLC-DAD	Sheep muscle, chicken wing, and bovine milk	ND	Xi'an, China	(Zhang et al., 2021)

a: limit of quantification; ND: not detected

2.3 Magnetic dispersive-micro-solid-phase extraction (magnetic-D-μ-SPE)

Solid phase extraction (SPE), first introduced by Braus et al. in 1951, is a fundamental technique in analytical chemistry used to separate analytes from liquid samples by employing a solid sorbent (Castell et al., 2024, Kaykhaii et al., 2024). However, traditional SPE methods are often criticised for being time-consuming, solvent-intensive, and environmentally damaging (Suseela et al., 2023). To address these issues, green analytical chemistry has emerged, focusing on minimising waste and reducing solvent use (Mahdavi et al., 2024).

Dispersive solid phase extraction (D-SPE) is an innovative approach that aligns with the principles of green chemistry. Unlike traditional SPE, which involves complex and time-consuming procedures, D-SPE simplifies the process by dispersing a sorbent directly into the sample matrix. This method facilitates analyte adsorption with the application of external energy and then isolates the sorbent-analyte complex through centrifugation or filtration. D-SPE is considered greener than traditional SPE because it typically requires less solvent and generates less waste. The efficiency of D-SPE in terms of time and resource usage reflects its contribution to more sustainable analytical practices (Sun et al., 2022, Bedair et al., 2024).

Building on D-SPE, dispersive micro-solid-phase extraction (D-μ-SPE) represents a miniaturised version of this approach. D-μ-SPE offers several advantages, including reduced sample and reagent consumption, accelerated analysis times, enhanced sensitivity, and improved portability. By minimising the amounts of both sample and solvent needed, D-μ-SPE further advances the green chemistry principles, reducing the environmental footprint associated with traditional SPE (Sajid et al., 2021, Nasrollahi et al., 2023).

Among the notable advancements in D-μ-SPE is magnetic dispersive micro-solid phase extraction (magnetic D-μ-SPE). This technique employing magnetic nanoparticles

(MNPs) coated with a suitable sorbent material. The magnetic properties of these nanoparticles enable rapid extraction using an external magnet, significantly cutting down sample preparation time and minimising solvent use (Bunkoed et al., 2024). Magnetic D-μ-SPE stands out for its numerous benefits compared to conventional SPE techniques. The rapid dispersion and extraction of magnetic particles facilitated by an external magnet streamline the process, making it more efficient (Suliman et al., 2023). Additionally, magnetic D-μ-SPE requires smaller volumes of solvents, further supporting green chemistry objectives by reducing environmental impact (Nasrollahi et al., 2023). The selection of appropriate sorbent materials and optimised extraction conditions in magnetic D-μ-SPE can achieve high selectivity for target analytes. Furthermore, the use of MNPs enables the development of compact, portable devices that are suitable for small sample volumes and remote analysis (Keçili et al., 2021).

In summary, magnetic D-μ-SPE exemplifies a significant advancement in green analytical techniques, demonstrating improved efficiency, reduced environmental impact, and enhanced versatility compared to traditional SPE methods. Its ability to streamline sample preparation while minimising waste underscores its potential as a valuable tool in a wide range of analytical applications. Table 2.4 provides some studies which applied magnetic D-μ-SPE in extraction of NSAIDs in various complex matrices.

Table 2.4 Magnetic D-μ-SPE in extraction of NSAIDs from complex matrices.

Analytes	Sorbent/ instrument	Matrix	LOD/LOQ (μg kg^{-1})	Relative Recovery (%)	Reference
NSAIDs (ketoprofen, naproxen, and diclofenac)	Sea urchin shaped polyaniline-modified magnetic microporous organic network/ HPLC-UV	Chicken breast, beef, and pork	0.07 – 1.7/ 0.2 – 5.0	88.3 – 110.0	(Li et al., 2024b)
NSAIDs (indomethacin, mefenamic acid, diclofenac sodium, flurbiprofen, and naproxen)	Porphyrin-based magnetic porous organic polymer/ HPLC-UV	River and lake water	0.02 – 0.07/ 0.08 – 0.2	60.0 – 124.0	(Xu et al., 2023)
NSAIDs (diclofenac, fenbufen, aspirin, naproxen, and ketoprofen)	Magnetic ionic liquid hypercrosslinked polymer composite/ HPLC-DAD	Tap, river, mad lake water and urine	0.1 – 0.3/ 0.4 -1.0	92.8 – 109.0	(Han et al., 2023)

NSAIDs (diclofenac)	Magnetic covalent organic frameworks/ HPLC-MS	Bovine milk	10.0/ 25.0	87.0 – 118.0	(Huang et al., 2021)

2.4 Development of magnetic hydrophobic deep eutectic solvents (Fe_3O_4@HDES) as adsorbents/sorbents in sample preparation

Magnetic nanoparticles (MNPs), renowned for their unique properties such as small size, large surface area, and magnetic responsiveness, have emerged as versatile materials in various fields, including sample preparation. MNPs, particularly magnetite (Fe_3O_4), nano zero-valent iron (nZVI), and maghemite (γ-Fe_2O_3), have emerged as prominent materials in the field of environmental analysis (El-Deen and Hussain, 2023). Chemical co-precipitation is often used to synthesise MNPs due to its simplicity, scalability, and control over particle properties. Chemical co-precipitation is a particularly efficient and economical method for synthesising magnetic nanoparticles with a core/shell structure. This technique involves the simultaneous precipitation of two or more metal salts, resulting in a composite material with a distinct core and shell morphology (Binandeh, 2022, Díez et al., 2022). MNPs' ability to be easily manipulated and controlled using external magnetic fields makes them particularly attractive for applications like extraction and separation of analytes from complex samples (Jabbar et al., 2022, Rasheed, 2022). While MNPs exhibit numerous advantageous properties, they are not without drawbacks. Agglomeration and stability issues can hinder their performance, particularly in aqueous environments. Moreover, the high chemical reactivity of bare metallic nanoparticles can lead to oxidation, resulting in a decrease or even loss of their magnetic properties (Keçili et al., 2021).

To mitigate these limitations and promote sustainable sample preparation practices, researchers have turned to deep eutectic solvents (DES). In 2003, Abbott et al created the first DES, where a mixture consisting of urea and choline chloride in a 2:1 ratio, and their melting point was 12 °C. They noted that this eutectic mixture is

formed due to the hydrogen bonding between various compounds, which can accept or donate protons or electrons. They also noted that the mixture exhibited the same properties as ionic liquids (IL) (Abbott et al., 2003). However, in 2004, they used the term DES to differentiate it from IL as unlike ionic liquids, eutectic solvents can be created in a pure state. They are also easy to prepare and are nonreactive. DES can be divided into four classes, Class I (quaternary ammonium salts + metal chloride), Class II (quaternary ammonium salts + metal chloride hydrate), Class III (quaternary ammonium salts + hydrogen bond donor) and Class IV (metal chloride hydrate + hydrogen bond donor) (Abbott et al., 2004). In their study, Abranches and his colleagues proposed a new kind of DES known as Type V DES which composed of non-ionic species. Most of the time, the DES in Class I to IV is dominated by one ionic species, which leads to the formation of a strong hydrophilic mixture. The absence of ionic species makes Type V DES particularly convenient. This provides them with suitable physical properties, such as low viscosity and hydrophobicity, which can be used in many field applications (Abranches et al., 2019).

Functionalised magnetic nanoparticles, which are magnetic nanoparticles modified with specific functional groups, can be combined with DES to create novel materials with enhanced properties. These hybrid materials offer promising applications in sample preparation, leveraging the unique characteristics of both magnetic nanoparticles and DES. Hydrophilic DES, compatible with aqueous environments, has been widely used in sample preparation due to their ability to extract and separate analytes from aqueous samples. Hydrophilic DES had been used to functionalised MNPs and being used in extracting different types of analytes from aqueous samples. However, the hydrophilic nature of early-synthesised DES limited their use in various fields. Therefore, the interest for the preparation of hydrophobic DES (HDES) enabling