REMOVAL, PRE-CONCENTRATION AND DETERMINATION OF SELECTED PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

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UNIVERSITI SAINS MALAYSIA

2022

REMOVAL, PRE-CONCENTRATION AND DETERMINATION OF SELECTED PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

June 2022

DECLARATION

I declare that the content which is presented in this thesis is my work which was done at Universiti Sains Malaysia unless informed otherwise. The thesis has not been previously submitted for any other degree.

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ACKNOWLEDGEMENT

First and foremost, I would like to thank Almighty Allah for giving me the strength, knowledge, ability and opportunity to undertake this program. Without His blessings, this achievement would not have been possible.

I would like to sincerely thank my main supervisor, Dr. Mazidatulakmam Miskam for her guidance, understanding, patience and most importantly, she has provided positive encouragement and a warm spirit, enabling me to complete this research. It has been a great pleasure and honour to have her as my supervisor. I also owe many thanks to my co-supervisors Prof. Abdalla Ahmed Elbashir, Prof. Bahruddin Saad, Dr. Noorfatimah Yahaya and Dr. Jamilah Binti Karim for their guidance, kindness and advice throughout my project. I would also like to express my greatest gratitude to the Ministry of Higher Education, Malaysia for financial assistance through Fundamental Research Grant Scheme (FRGS/1/2020/STG04/USM/02/5).

My appreciation goes to Universiti Sains Malaysia for providing me an enabling environment to conduct my research. I also want to extend my earnest appreciation to Assoc. Prof. Dr. Melati Khairudean and Prof. Dr. Rohana Adnan for their kind support and guidance and endowment me the honour to join School of Chemical Science as graduate assistance. My thanks also to Open University of Sudan for their support. I thank you wholeheartedly.

I am also grateful and highly indebted to my dear friend Sara Abdelrahman Abuelmaali, for her moral support and all her uncountable contributions. May Allah reward her abundantly. I also-wish to express my profound appreciation to my caring kind aunt Dr. Fatima Ismail Adam for her support and courage. My deepest gratitude

also goes to all my extended family members, more so I would like to thank my dearest father, Edrees Adam and my mother Badria Mohamad Bahredin for their love, support and earnest prayers. Finally, I would like to extend my appreciation and acknowledge the efforts of all my lab mates, colleagues and friends too numerous to mention. May Allah shower His blessings on you all for contributing to the actualization of this study.

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AC	Acceptor phase
ARGs	Antibiotic resistance genes
ARB	Antibiotic-resistant bacteria
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CE	Capillary electrophoresis
ChCl	Choline chloride
DES	Deep eutectic solvent
DLLME	Dispersive liquid-liquid microextraction
DIC	Diclofenac
DP	Donor phase
ENR	Enrofloxacin
EPA	Environmental Protection Agency
ECs	Emerging contaminants ECs
EU	European Union
EMA	European Medicines Agency
EF	Enrichment factor
EG	Ethylene glycol
Fe_3O_4	Magnetic nanoparticles
FTIR	Fourier Transform infrared spectroscopy
FF	Ferrofluid
FF-LPME	Ferrofluid liquid phase microextraction

FDA	Food and Drug Administration
FQs	Fluroquinolones
Fe_3O_4	Magnetic iron particles
GEM	Gemifloxacin
GO@Fe_3O_4	Graphene oxide magnetite
GO@Fe_3O_4-DES FF	Graphene oxide magnetite deep eutectic ferrofluid
GC	Gas chromatography
GLY	Glycerol
HPLC	High-Performance Liquid Chromatography
HBD	Hydrogen bond donors
HBA	Hydrogen bond acceptors
IBU	Ibuprofen
KET	Ketoprofen
LPME	Liquid phase microextraction
LOD	Limit of detection
LOQ	Limit of quantification
LLE	Liquid-liquid extraction
Ms	Magnetic saturation
MRL	Maximum residue limit
MeOH	Methanol
MSPE	Magnetic solid phase extraction
NSAIDs	Nonsteroid anti-inflammatory drugs
NAP	Naproxen
OFL	Ofloxacin
Ppm	Part per million

P_{ZC}	Point zero charge
RSD	Relative standard deviation
SD	Standard deviation
SPE	Solid Phase Extraction
SEM	Scanning electron microscopy
SPR	Sparfloxacin
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
USEPA	US Environmental Protection Agency
VSM	Vibrating sample magnetometer
UV-Vis	Ultraviolet-visible
VALLME-BE	Vortex assist liquid liquid microextraction-back extraction
XRD	X-ray diffractometer

LIST OF SYMBOLS

pKa	Acid dissociation constant
q_e	Adsorption capacity (mg/g)
q_e cal	Calculated adsorption capacity (mg/g)
R^2	Coefficient of determination
-COOH	Carboxyl group
cm	Centimeter
ºC	Degree Celsius
Δ	Delta
R_L	Dimensional separation factor
Kd	Distribution coefficients
C_e	Equilibrium concentration of adsorbate (mg/L)
emu	Electromagnetic unit
ΔHº	Enthalpy
ΔSº	Entropy
q_e exp	Experimental adsorption capacity (mg/g)
g	Gram
Cf	Final concentration of adsorbate (mg/L)
KF	Freundlich constant
ΔG	Gibb's free energy
R	Gas constant (J/Kmol)
H^+	Hydrogen ion
-OH	Hydroxyl group
1/n	Heterogeneity Factor

C_o	Initial concentration of adsorbate (mg/L)
J	Joules
K	Kelvin
KJ	Kilojoules
L	Liter
µg	Microgram
mL	Mililiter
mm	Millimeter
mM	Millimolar
µL	Microliter
M	Molarity
q_m	Maximum adsorption capacity
mg	Milligram
N	Number of data points
nm	Nanometer
Δq (%)	Normalized standard deviation
Log P	Partition coefficient
π	Pi
θ	Theta
C	Thickness of boundary layer
V	Volume
λmax	Wavelength of maximum absorbance

LIST OF APPENDICES

PENYINGKIRAN, PRA-PEMEKATAN DAN PENENTUAN FARMASEUTIKAL TERPILIH DARIPADA SAMPEL AIR PERSEKITARAN

ABSTRAK

Farmaseutikal merupakan pencemar memuncul yang digunakan secara meluas dalam beberapa aplikasi termasuk ubatan manusia dan veterinar serta kegiatan pertanian. Analisis farmaseutikal bergantung pada pengkuatitan jitu farmaseutikal sasaran dari matriks kompleks, tetapi masih menjadi tugas yang sukar disebabkab oleh kepekatan yang rendah. Oleh itu, kajian ini memperlihatkan penggunaan kaedah penyediaan sampel yang berbeza untuk penentuan farmaseutikal terpilih di dalam sampel air. Teknik pengekstrakan mikro berdasarkan pengekstrakan mikro cecair-cecair berbantu vorteks dengan pengekstrakan belakang (VALLME-BE) telah dibangunkan untuk penentuan ubat anti-radang bukan steroid (NSAIDs) dinamakan, ketoprofen, naproxen, diclofenac dan ibuprofen. Prosedur ini dilakukan dengan menambahkan 225 µL butil asetat ke dalam 10 mL larutan piawai untuk prosedur VALLME, diikuti dengan pengekstrakan balik ke natrium hidroksida sebelum analisis kromatografi cecair berprestasi tinggi-UV-Vis (HPLC-UV-Vis). Di bawah keadaan optimum, kaedah yang dicadangkan memberikan kelinearan yang baik ($R^2 \geq 0.9809$), kebolehulangan (%RSD; 3.4 – 16.1%) dan nilai had pengesanan (LOD) dan had kuantifikasi (LOQ) yang baik (ketoprofen (0.134 dan 0.407 µg L^{-1}), naproxen (0.015 dan 0.047 µg L^{-1}), diclofenac (0.03 dan 0.091 µg L^{-1}) and ibuprofen (0.05 dan 0.152 µg L^{-1}) telah diperolehi. Selain itu, penjerap grafena oksida (GO), grafena oksida bermagnet (GO@Fe_3O_4) dan grafena oksida bermagnet-larutan eutektik terdalam cecair ferro (GO@Fe_3O_4-DES FF) telah disintesis dan dicirikan. Peratusan

penyingkiran fluorokuinolon (ofloxacin, naproxen, gemifloxacin dan sparfloxacin) telah dioptimumkan berdasarkan kaedah penjerapan kelompok menggunakan penjerap yang telah disintesis. Di bawah keadaan optimum, prestasi penjerapan GO@Fe_3O_4 dan GO@Fe_3O_4-DES FF telah dikaji. Data eksperimen yang diperolehi menunjukan semua analit sepadan dengan model kinetik tertib kedua pseudo dan model Freundlich. Kajian termodinamik menunjukkan yang proses penjerapan boleh terlaksana secara termodinamik, spontan dan eksotermik. Akhir sekali, kerana prestasi penjerapan GO@Fe_3O_4-DES FF adalah lebih baik berbanding GO@Fe_3O_4, ia telah dibangunkan lebih lanjut untuk diaplikasikan dalam GO@Fe_3O_4-DES FF- pengekstrakan mikro fasa cecair (LPME). Beberapa parameter termasuk jenis pelarut penyahjerap, isipadu cecair ferro, masa pengekstrakan, isipadu pelarut penyahjerap, masa penyahjerap, pH larutan dan isipadu sampel telah dioptimumkan dan dianalisa menggunakan HPLC-UV-Vis. Di bawah keadaan optimum, kelinearan yang baik telah dicapai dalam julat 1 – 1500 µg L^{-1} dengan nilai pekali penentuan, R^2 adalah 0.9921-0.9956. Nilai LOD dan LOQ telah direkodkan dalam julat masing-masing adalah 0.100 - 0.063 dan 0.190 - 0.303 µg L^{-1}. Kaedah GO@Fe_3O_4-DES FF-LPME yang dibangunkan telah digunakan untuk penentuan florokuinolon dalam sampel air dari Lembangan Sungai Langat, Selangor dan pengembalian sebanyak 70.3 – 120.3% diperolehi. Secara kesimpulannya, kaedah penyediaan sampel yang dibangunkan untuk penentuan farmaseutikal terpilih telah menunjukkan sensitiviti dan ketepatan yang baik dan mampu menjadi kaedah alternatif untuk pengekstrakan di dalam sampel air.

REMOVAL, PRE-CONCENTRATION AND DETERMINATION OF SELECTED PHARMACEUTICALS FROM ENVIRONMENTAL WATER SAMPLES

ABSTRACT

Pharmaceuticals are emerging contaminants that have been widely used in various applications, including human and veterinary medicine, as well as agricultural activities. The pharmaceutical analysis relies on accurate quantification of target pharmaceuticals from a complex matrix, yet this remains a difficult task due to their low concentrations. Therefore, this research demonstrated the use of different sample preparation methods for the determination of selected pharmaceuticals in water samples. A microextraction technique based on vortex-assisted liquid-liquid microextraction with back extraction (VALLME-BE) was developed for the determination of nonsteroidal anti-inflammatory drugs (NSAIDs) namely, ketoprofen, naproxen, diclofenac, and ibuprofen. The procedure was carried out by adding 225 µL of butyl acetate into 10 mL spiked working standard for VALLME procedure, followed by the back-extraction into sodium hydroxide prior to high performance liquid chromatography-UV-Vis (HPLC-UV-Vis) analysis. Under optimum conditions, the proposed technique provided good linearity ($R^2 \geq 0.9809$), repeatability (%RSD; 3.4 – 16.1), and excellent the limits of detection (LOD) and limit of quantification (LOQ) values (ketoprofen (0.134 and 0.407 µg L^{-1}), naproxen (0.015 and 0.047 µg L^{-1}), diclofenac (0.03 and 0.091 µg L^{-1}) and ibuprofen (0.05 and 0.152 µg L^{-1}) were obtained. On the other hand, graphene oxide (GO), graphene oxide magnetite (GO@Fe_3O_4), and graphene oxide magnetite-deep eutectic solvent ferrofluid (GO@Fe_3O_4-DES FF) adsorbents were successfully synthesized and characterized.

The removal percentage of fluoroquinolones (ofloxacin, naproxen, gemifloxacin, and sparfloxacin) were optimized based on the batch adsorption method using the synthesized adsorbents. Under the optimum conditions, the adsorption performances of GO@Fe_3O_4 and GO@Fe_3O_4-DES FF were investigated. The experimental data for all the analytes were fitted well with pseudo second-order kinetic model and Freundlich model. The thermodynamic studies showed that the adsorption process was thermodynamically feasible, spontaneous, and exothermic. Finally, as the adsorption performances of GO@Fe_3O_4-DES FF were better than GO@Fe_3O_4, it was further developed for the application of GO@Fe_3O_4-DES FF-liquid phase microextraction (LPME). Several parameters such as type of desorption solvent, ferrofluid volume, extraction time, desorption solvent volume, desorption time, solution pH, and sample volume were optimised and analysed using HPLC-UV-Vis. Under the optimized conditions, good linearity was achieved in the range of 1 – 1500 μg L^{-1} with a coefficient of determination, R^2 value of 0.9921-0.9956. The LOD and LOQ value recorded in the range of 0.100 - 0.063 and 0.190 - 0.303 μg L^{-1}, respectively. The developed GO@Fe_3O_4-DES FF LPME method was applied for the determination of fluoroquinolones in water samples from Langat River Basin, Selangor, and the recovery of 70.3 – 120.3% was obtained. In conclusion, the developed sample preparation techniques for the determination of selected pharmaceuticals showed excellent sensitivity and precision and may be an excellent candidate for the extraction of water samples.

CHAPTER 1
INTRODUCTION

1.1 Background of the study

Water is the most abundant and necessary source of life on the planet and it has been declared to be one of the human rights by the United Nations (UN), General Assembly, in 2010 (Onda et al., 2012; Unuabonah et al., 2019). For both personal and household use, everybody has the right to sufficient, constant, safe, appropriate, physically available, and affordable water. Likewise, the 6th Sustainable Development Goal (SDG 6) mandates the access to clean water and sanitation, as well as sound freshwater management. If this aim is not achieved, it will cause serious implications for the survival of life on earth (Unuabonah et al, 2019).

Pharmaceuticals are considered as emerging contaminants and they have been widely employed for a variety of uses, including human and veterinary medicine, and agricultural activities. Pharmaceutically active compounds (PhACs) such as anti-inflammatory drugs, antibiotics, analgesics, X-ray contrast media lipid regulators, estrogens, and beta-blockers have been used worldwide to enhance human and animal health and enhance life expectancy. The consumption of PhACs increased as a result of the world population's increment from 7.6 billion in 2017 to 10 billion in 2050 as expected (Gehrke et al., 2015). Direct discharge of untreated and treated wastewater is the principal source of these pollutants in aquatic environments (Krogh et al., 2017). While livestock farms runoff, agricultural regions, and aquaculture facilities provide a secondary source (Olasupo et al., 2021). In 1970, the first report on the presence of medicines in river water was released (Daughton, 2016). Since then, extensive study has been conducted on the monitoring of pharmaceuticals in aquatic environments.

Pharmaceutical analysis relies on accurate quantification of target pharmaceuticals from a complex matrix, yet this remains a difficult task. Furthermore, analytes are frequently present in the sample matrix at extremely low concentrations, ranging from mg L^{-1} to ng L^{-1}. As the result, increased attention has been placed on establishing sample preparation processes such as preconcentration and pre-treatment over the last few decades. Since it is one of the essential steps that consumes around 80% of the overall quantitative analysis time, using appropriate sample preparation techniques can help tremendously in the development of pharmaceutical analysis. (Daniel et al., 2017; Li et al., 2021).

Many researchers are intrigued by the miniaturization of analytical approaches. By reducing the dimension of conventional analytical methods and the solvents used and the quantity of reagents per analysis, the amount of waste generated is reduced. Therefore, innovative methods to simplify the extraction techniques have been introduced. Microextraction techniques either with solid or liquid extractants have been proposed. Some examples of liquid phase techniques are vortex assisted liquid-liquid microextraction (VALLME), dispersive liquid-liquid microextraction (DLLME) and vortex assist liquid-liquid extraction with back extraction (VALLME-BE) (Shalash et al., 2017). It is clear that the alternative sample pre-treatment that is simple and rapid but sensitive enough to detect low concentration of pharmaceuticals in water samples are required. Towards this end, the plethora of microextraction techniques, VALLME-BE seemed to be the best candidate for sample preparation for pharmaceutical as the extracts were in aqueous phase which able to be analysed using reversed phase HPLC directly without reconstitution step. In addition, the low volume of back extraction provided a high pre-concentration factor which enhanced the response in HPLC analysis.

As an alternative to extracting solvents (in the form of molecular liquids), deep eutectic solvent (DES), ionic liquids (IL), surfactant-based solvents, supercritical fluids, supramolecular solvents, and ferrofluid (FF) are now being considered in microextraction methods. FF or magnetic fluids (MF) are smart colloidal suspensions of single domain magnetic nanoparticles, such as iron oxide (Fe_3O_4), in a polar or non-polar liquid carrier, such as DES, IL, ester, or hydrocarbons (Farahani & Shemirani, 2013). The application of FF is widely seen in bioengineering, microelectronics, and material sciences (Mishra et al., 2014). Different modes of liquid phase microextraction based on FF have recently been discovered and are becoming increasingly popular due to advantages such as ease of use, rapid extraction, and reduced organic solvent use (Safari et al., 2016; Yang et al., 2018; Zohrabi et al., 2016) Furthermore, FF not only eliminates the need for centrifugation to retrieve organic solvents, but it also improves phase separation yield. This results in good extraction efficiency and reproducibility at pre-concentration factors that are acceptable. In general, the magnetic nanoparticles in FF are coated with a shell made of an appropriate material to prevent agglomeration. In this study, choline-chloride based DES was chosen as the carrier liquid. DESs have various advantages over ionic liquids since they are easier and less expensive to produce, as well as being more biodegradable and less hazardous to the environment. DESs are formed when hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) self-associate to produce eutectic mixtures having lower melting temperatures than the starting compounds (El-Deen & Shimizu, 2019; Sadeghi & Davami, 2019). The magnetic nanomaterial in DES aids in the accumulation of the target analytes due to its van der Waals forces, dipolar attractive interactions, and magnetostatic interactions (Zohrabi et al., 2016).

To form a stable FF, the MNPs must be coated to avoid agglomerations and leaching before being immersed in a carrier liquid. Graphene oxide (GO) has been widely used in sorbent based microextraction techniques. (GO)-based adsorbents have shown great promise to be used as candidates for adsorbents because of their distinct characteristics such as large surface area and small size (Amin et al., 2014; Daniel et al., 2017). However, due to the hydrophilic property of GO, it is difficult to be removed from the sample using traditional separation methods. Hence, it becomes a secondary pollutant and may increase the cost to retreat it. This problem can be solved by anchoring the magnetic properties (Hu et al., 2013). Graphene oxide magnetite (GO@Fe_3O_4) may increase the feasibility of GO with the help of external magnetic fields. This development implies the combination of benefits of adsorption and makes it easy to separate since it involves the use of external magnetic species.

The existing methods for the removal of pharmaceuticals are membrane separation, photochemical degradation, advanced oxidation processes, and adsorption treatments. Adsorption is well regarded as an efficient, effective, and cost-effective approach for removing various contaminants from aqueous solutions in water treatment applications (Dutta & Mala, 2020). Until now, a variety of adsorbents have been developed to reach high adsorption efficiency to remove pharmaceuticals from water and wastewater (Duan et al., 2020; Liu et al., 2018; Okaikue-Woodi et al., 2018; Wang et al., 2020; Yang et al., 2020).

This research demonstrated the development of liquid phase microextraction (LPME) techniques for the determination of pharmaceuticals in water samples, based on the above-mentioned considerations. The VALLME-BE method was developed and applied for the determination of nonsteroidal anti-inflammatory drugs (NSAIDs) as test

compounds. Several optimization parameters such as solvent type and volume, sample solution pH, and vortexing speed and time were studied. On the other hand, new GO based adsorbents were synthesized and characterized. The adsorption performances of the synthesized graphene-oxide magnetite (GO@Fe_3O_4) and graphene-oxide magnetite- deep eutectic solvent ferrofluid (GO@Fe_3O_4-DES FF) were evaluated to explore their potential in batch adsorption of fluoroquinolones (FQs). Finally, GO@Fe_3O_4-DES FF-liquid phase microextraction (GO@Fe_3O_4-DES FF-LPME) method was developed and validated prior to the application for the determination of FQs in environmental water samples collected along Langat River Basin, Selangor, Malaysia.

1.2 Problem statement

NSAIDs have made up more than 15% of all medications found in the environment and they were the most often reported of all classes. On the other hand, FQs are widely utilized not only by people but also in veterinary treatment, particularly in large-scale animal farming. About 50–90% of medicines that are frequently detected in wastewater and receiving surface water bodies were discharged in a mixture of parent and metabolite forms in faecal matter due to inadequate metabolism in people and animals. Owing to their high polarity and solubility in water, the extraction of pharmaceuticals from complex matrices faces significant challenges. Therefore, sample pre-treatment and preconcentration measures play a major role in enhancing the sensitivity and selectivity of the analytical procedure to reach the low limit of detections required for complex matrix analysis, such as aquatic environmental samples.

Thus, the removal of those pollutants and detection of their presence at low concentrations have attracted the attention of many researchers. Several methods such

as photocatalytic degradation, membrane separation, and adsorption have been established to eliminate them. However, these methods are expensive, non-selective, and require pre-treatment. Therefore, to overcome the constraints of conventional methods, numerous miniaturized sample preparation methodologies are widely explored including microextraction techniques. Many other benefits can be highlighted as possible advantages of miniaturization such as improved extraction performance and a widely reduced consumption of samples and chemicals per analysis (with the subsequent reduction in waste generation) is far from being the only driving force behind miniaturization. Instead, several desirable features can be identified for miniaturized methodologies, including Shrinking conventional analytical systems, integration of steps, simplification, enhanced portability, reduced human manipulation, and adequate performance (Pena-Pereira et al., 2021).

More innovative methods can be developed, to accelerate the removal, and extraction processes and to improve the separation of analytes. Nevertheless, a lot of progress is expected to be made with the use of new adsorbents which would make the whole sample preparation process simpler, faster, more economical, more efficient, and more environmental friendly. Hence, the adsorbent provides the surface area necessary to ensure a high extraction recovery and clean up.

In this study, the development of simple microextraction techniques named VALLME-BE for the determination of NSAIDs in environmental water samples was developed. Recent advances in the fabrication of new FF materials, on the other hand, have contributed significantly to the development of miniaturized methods. Given the advantages of GO@Fe_3O_4 adsorbent and DES, a new class of GO@Fe_3O_4-DES FF was

developed for GO@Fe_3O_4-DES FF-LPME of FQs in Langat River Basin, Selangor, Malaysia.

1.3 Objectives

This thesis was dedicated to the development of sample preparation techniques for the determination of selected pharmaceuticals in the environmental water samples. The specific objectives of this study were:

1. To develop, validate and apply VALLME-BE method for the determination of NSAIDs from environmental water samples.
2. To synthesize, characterize and study the adsorption performances of GO@Fe_3O_4 and GO@Fe_3O_4-DES FF for the removal of FQs using UV-Vis spectrophotometer.
3. To develop, validate and apply GO@Fe_3O_4-DES FF-LPME for the determination of FQs in water samples prior to HPLC-UV-Vis analysis.

1.4 Outline of the thesis

This thesis was divided into five chapters. **Chapter 1** is an introduction that explains the general context of the problem statements and research objectives. In **Chapter 2**, related literature was thoroughly reviewed. In **Chapter 3,** the methodology chapter highlights the chemicals and reagents, and procedures used in these studies. This chapter contains three main parts namely **Part I**, **Part II**, and **Part III**. **Part I** investigated the extraction of ibuprofen (IBU), diclofenac (DIC), ketoprofen (KET), and naproxen (NAP) using VALLME-BE. **Part II** illustrates the synthesis, characterization, and adsorption performances of GO@Fe_3O_4 and GO@Fe_3O_4-DES FF

adsorbents for the removal of FQs. Lastly, **Part III** encompasses the determination of ofloxacin (OFL), enrofloxacin (ENR), sparfloxacin (SPR), and gemifloxacin (GEM) using GO@Fe_3O_4-DES FF based liquid phase microextraction. In the following chapter, **Chapter 4** presents in detail the overall outcomes for results and discussions of the conducted project. Like Chapter Three, this chapter was also divided into three major parts, **Part I, Part II, and Part III**. Characterisation, optimisation, adsorption study, extraction study, method validation, real sample analysis, and reusability studies are all included in these sections, along with their relevant discussions. Finally, **Chapter 5** concludes the overall results as well as future research work recommendations.

CHAPTER 2
LITERATURE REVIEW

2.1 Pharmaceuticals

The advancement production of pharmaceuticals over the last century has resulted in greater disease prevention, increased life expectancy, mortality reduction, and health quality (Ebele et al., 2017). Pharmaceuticals have been identified as emerging contaminants (ECs), due to continuous discharge and prevalence of the trace quantities of the pharmaceutical components in the environment (Yu et al., 2011). Pharmaceuticals penetrate water bodies through multiple pathways, including human excretion, medications disposal, livestock husbandry, agricultural and pharmaceutical industries. In addition, inefficient elimination of pharmaceuticals during the treatment process in waste-water treatment plants (WWTPs) and water treatment plants (WTPs) also contributed to this issue (Boxall et al., 2012; Morales et al., 2016; Petrie et al., 2014).

The occurrence of certain pharmaceutical compounds in surface water bodies, even at low concentrations, can constitute a significant environmental risk. The removal of these compounds is a primary consideration in a variety of fields of research. Furthermore, as a result of population growth and high pharmaceutical usage, the concentration of pharmaceuticals in the aquatic environment is gradually growing. Several investigations have discovered several pharmaceuticals in surface water, wastewater treatment plant effluents, and groundwater (Chen et al., 2015; Golet et al., 2002; Sturini et al., 2012; Vázquez et al., 2012).

Unfortunately, even though pharmaceuticals are considered as ECs, no legislative maximum residue limits (MRLs) for aquatic ecosystems have been

established especially in Malaysia. Despite the fact that pharmaceutical-related industries must adhere to stringent legislation in the healthcare sector, with continuous monitoring from agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), environmental concerns have lagged behind. Only recently some global actions have reflected the increased concern of both the scientific community and legislative authorities about environmental pharmaceuticals. Agencies such as the US Food and Drugs Administration (US FDA) and legislature such as the European Union Water Framework Directive have established guidelines for the evaluation and use of certain pharmaceuticals. Furthermore, in 2009, the US Environmental Protection Agency (US EPA) classified several unregulated ECs comprising potential health hazards in the contaminant candidate List 3 for drinking water. Under the EU Water Framework Directive (WFD-2000/60/EC), pharmaceuticals such as diclofenac have been identified as a priority contaminant in surface water bodies (2013/39/EU, 2013; Stewart et al., 2014).

Pharmaceuticals are a large group of substances that are categorized into several categories. such as analgesics and antibiotics, anti-inflammatory drugs, anti-epileptics, beta-blockers, hormones, cytostatics, disinfectants, antidepressants, and antiseptics. Studies revealed that antibiotics and nonsteroid anti-inflammatories (NSAIDs) are the most typically found in water since these groups possessed the highest risk and are widely used pharmaceuticals to alleviate infections and inflammation (Taoufik et al., 2020).

2.1.1 Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to alleviate inflammatory, chronic, and acute pain conditions. NSAIDs have antipyretic and analgesic properties as well. This includes diclofenac, ibuprofen, ketoprofen, and

naproxen which are in the top ten persistent pollutants found in wastewater properties (Abd Wahib et al., 2018; Wong, 2019). The consumption of NSAIDs increasing rapidly since the first developed and marketing in 1899. This is parallel with the subsequent release of these pharmaceuticals in the water bodies (Thalla & Vannarath, 2020). NSAIDs have been identified as hazardous residues among pharmaceuticals, according to the International Council Directive 96/23/EC study (Tanwar et al., 2015). In particular, diclofenac was added to the list of priority substances of the European Commission. Prolonged use of these drugs, however, may result in substantial water contamination due to human excretion or inadequate disposal due to low degradation factors and polarity.

As a result of their well-known toxicity and negative effects, there is a lot of interest in monitoring and determining NSAIDs in environmental samples using sensitive, selective and repeatable analytical techniques. Several methods for determining NSAIDs have been reported such as solid-phase microextraction (SPME) (Sulej-Suchomska et al., 2016), single-drop microextraction (SDME) (Azzouz et al., 2010), dispersive liquid-liquid microextraction (DLLME) (Zgoła-Grześkowiak, 2010), and hollow fiber liquid-phase microextraction (LPME) (Es'haghi, 2009) as stated in Table 2.1.

Table 2.1 Sample preparation techniques for NSAIDs analysis.

Analyte	Matrix	Instrument	Method	Extraction solvent/volume	Extraction time (mins)	LOD (µg L^{-1})	Recovery (%)	EF	Ref
NAP KET IBU DIC	Biological fluids	GC-MS HPLC-DAD	DLLME-BE	200 µL n-dodecane/ TOPO (95:5)	11	0.10-1 0.1-6.0	86.8-1-5.2	-	(Ghambarian et al., 2020)
KET DIC	Urine	HPLC	DLLME-SFO	20 µL 1-undecanol dispersed in 100 µL ACN	9	5.20 – 4.70	95.7-115.6	_	(Shukri et al., 2015)
DIC IBU	Urine	GC-FID	USE-AALLME and LDS-DLLME	30 µL 1-octanol	10	0.10 - 1.00	94-103	115-135	(Barfi et al., 2015)
KET DIC IBU	Tap and river water	HPLC-UV/FP	Vortex-IL-DLLME	90 µL [BMIM] [PF_6] 210 µL methanol	6	17.00 – 95.00	89-103	49-57	(Toledo-neira & Álvarez-lueje, 2015)
IBU DIC	Human plasma and urine	HPLC	LDS-AALLME	n-octanol 65 µL	15	1.10 - 1.70	94-102	50-61	(Barfi et al., 2015)
KET NAP, DIC IBU	Wastewater	HPLC	DLLME-SFO	30 µL 1-undecanol 150 µL ACN	10	0.04 - 0.13	< 80	283-302	(Beldean-Galea et al., 2015)
NAP DIC IBU	Biological samples	HPLC	AALLME-BE	Chloroform 80 µL	10	0.20–0.52	78-94	390-470	(Farajzadeh et al., 2015b)

Table 2.1 (continued)

Analyte	Matrix	Instrument	Method	Extraction solvent/volume	Extraction time (mins)	LOD (µg L^{-1})	Recovery (%)	EF	Ref
NAP DIC IBU	Tap and drinking water	UHPSFC-PDA	US-IL-DLLME	85 mg $[C_8MIM][PF_6]$ 0.5 mL ACN	11	0.62 - 7.37	88-111	255-340	(Vázquez et al., 2013)
KET NAP	Human urine	HPLC	IL-dLPME	280 µL [BMIM PF_6] 720 µL methanol	5	55.00 – 70.00	99.6-107	42-36	(Cruz-vera et al., 2009)

KET; ketoprofen, NAP; naproxen, DIC; diclofenac, IBU; ibuprofen; US-DLLME; ultrasound-assisted dispersive liquid–liquid microextraction, DLL-SDME; dispersive liquid–liquid and single-drop microextraction, USE-AALLME; ultrasound-enhanced air-assisted liquid–liquid microextraction, LDS-DLLME; low-density solvent-based dispersive liquid–liquid microextraction, Vortex–IL–DLLME; ionic liquids dispersive liquid–liquid microextraction, LDS–AALLME; low density solvent-based air-assisted liquid–liquid microextraction, DLLME-SFO; dispersive liquid–liquid microextraction and solidification of floating organic droplets, AALLME–BE; air-assisted liquid–liquid microextraction - back extraction, US–IL-DLLME; ultrasound-assisted ionic liquid dispersive liquid–liquid, IL–dLPME; ionic liquid-based dispersive liquid–liquid microextraction, VALLME-BE; vortex assist liquid liquid microextraction-back extraction, ([BMIM][PF_6]); 1-butyl-3-methylimidazolium hexafluorophosphate, $[C_8MIM][PF_6]$; 1-octyl-3-methylimidazolium hexafluorophosphate, TOPO; potassium hydroxide, trioctylphos- phine oxide, EF; enrichment factor

2.1.2 Antibiotics

Antibiotics are now produced by chemical synthesis or chemical modification of natural substances (Kovalakova et al., 2020; Wang et al., 2019). They are biologically active compounds with antibacterial, antiparasitic, and antifungal properties that have been created as medications for human and animal illnesses, as well as food supplements for preventing disease in animal husbandry (Hu et al., 2018). The global consumption of antibiotics is rising in tandem with the world's growing human population. Furthermore, the rising demand for animal protein is intensifying food production, forcing the use of more growth promoters and antibiotics to keep the supply stable (Kovalakova et al., 2020; Wang et al., 2016)

The occurrence of antibiotics in environmental water may cause the occurrence of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB). ARB can release or transmit ARG in aquatic environments, which are rather persistent and can be acquired by other bacteria, resulting in an increase in ARB. Antibiotics also encouraged the proliferation of ARG in aquatic environments (Shao et al., 2018; Xu et al., 2019). ARGs can be transmitted to non-bacterial creatures, such as humans, to reduce their susceptibility to antibiotics. The proliferation and pollution of antibiotics and ARGs in environmental water systems have garnered widespread and growing attention as a serious hazard to human health. ARGs have been identified as emergent environmental pollutants because of their antibiotic resistance and threat to environmental safety and human health (Zhang et al., 2021). Besides, antibiotics also have toxic effects on aquatic species due to bioaccumulation and biomagnification (Wu et al., 2015).

Antibiotics including tetracycline, fluoroquinolone, sulfonamides, chloramphenicol, and macrolide are complex compounds that are difficult to analyse in environmental samples due to co-extracted components that are dependent on the analyte-sample combination and thus obstruct quantification, which is known as ion suppression effects. Fluoroquinolones (FQs) are a class of relatively new synthetic antibiotics. FQs are registered under the National Pharmaceutical Regulatory Agency (NPRA) of the Ministry of Health Malaysia and identified by WHO as critically important medicines for the human health and veterinary sector (WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), 2011). FQs are among the most widely found antibiotics in the environment. Despite their inherent risks, FQs have garnered less attention than other pollutants, thus the risk they pose in effluents is uncertain (Ding et al., 2020). FQs are generally partially immobilized within the human and animal body, with 20 to 80 % being released into the environment in pharmacologic active forms (Dutta and Mala, 2020). FQs were found in wastewater effluents and sewage sludge (Golet et al., 2002), soils (Salvia et al., 2015), surface waters (Sturini et al., 2012), and groundwater (Vázquez et al., 2012).

Researchers are increasingly concerned about these harmful antibiotics from the aquatic environment. Membrane separation, ozonation, advanced oxidation processes (AOPs), photochemical degradation, and adsorption treatments are some of the methods used to remove antibiotics. Adsorption is becoming increasingly vital for the removal of FQs due to its ease of operation, lack of by-products high efficiency, low cost, and availability of many adsorbents. Adsorption techniques have been used in numerous studies to remove FQs from environmental water using a plethora of adsorbent materials, including clay minerals (Peng et al., 2018; Speltini et al., 2017; Xiang et al., 2020). Table 2.2 describes different adsorbents used for the adsorption of FQs.

Table 2.2 Removal of FQs using different adsorbents.

Adsorbent	**Analytes**	**$Q_{e,exp}$ (mg g^{-1})**	**Ref**
Nanoscale zero-valent iron (NZVI)	OFL	48.9	(Zhao et al., 2020)
Chitosan- polyacrylic acid	ENR	387.7	(Wang et al., 2019)
Humic acid coated magnetic biochar	ENR	1.7	(Zhao et al., 2019)
Fe_3O_4/GO/citrus peel-derived magnetic bio-char nano- composite (mGOCP)	SPR	502.4	(Zhou et al., 2019)
Polypyrrole modified *Calotropis gigantea* fiber (PPy-O-CGF)	ENR	78.3	(Duan et al., 2019)
Metal-organic framework with polar – SO_3H (MIL-101(Cr)–SO_3H)	ENR	408.2	(Guo et al., 2019)
Lignocellulosic substrate	ENR	91.5	(Sayen et al., 2018)
Bamboo biochar	ENR OFL	45.9	(Wang et al., 2015)
TiO_2-modified zeolites	ENR	3.0	(Maraschi et al., 2014)
Polydopamine-coated graphene oxide/Fe_3O_4 (PDA@ GO/Fe_3O_4) imprinted nanoparticles	SPR	70.9	(Tan et al., 2017)
Graphene oxide (GO)	OFL	0.048 mmol/g	(Yadav et al., 2018)
Magnetic carboxylated cellulose nanocrystals with molecularly imprinted polymer (M-CCNs@MIP)	OFL	45.6	(Hu et al., 2018)
Layered double hydroxides (LDHs) onto cotton fiber	ENR	20.5	(Wang et al., 2018)
Magnetic biochar-based manganese oxide composite (MMB)	ENR	7.2	(Li et al., 2018)

OFL; ofloxacin, ENR; enrofloxacin, GEM; gemifloxacin, SPR; sparfloxacin.

Despite the benefits of nanoadsorbents for FQs adsorption, manipulating them from solution takes time since they have a high back-pressure in dynamic mode and require centrifugation and filtering in static batch mode. Hence, the analysis of FQs using liquid phase microextraction (LPME) was explored for different samples including food samples, biological samples (Moema et al., 2012), and water samples. Table 2.3 summarizes the FQs sample preparation based on LPME.

2.2 Sample preparation

The importance of sample preparation in the chemical analysis workflow has been recognized. As a result, substantial efforts have been undertaken in recent years to improve the overall sample preparation method. Without a doubt, sample preparation is among the most critical steps in the analytical process. Overall, it is the most time-consuming aspect of developing an analytical process and is regarded as a significant source of errors in analysis (Owczarek & Guardia, 2017). Sample preparation is expected to account for about 80% of the workload, time, and expense. The primary goals of sample preparation are to remove possible interferences, preconcentrate the analyte and transform the analyte (if necessary) into a more suitable form for detection or separation (Płotka-wasylka & Szczepan, 2015). The choice of a preparation method is determined by: (1) the analyte(s) concentration level(s), (2) the instrumental measurement technique, (3) the sample matrix, and (4) the appropriate sample size. Furthermore, sample preparation allows for the preconcentration and/or isolation of analytes, enhancing the selectivity and sensitivity of determination procedures (Tartaglia et al., 2019).

Table 2.3 FQs sample preparation based on LPME.

Analyte	Method	Sample preparation	Matrix	Extraction solvent	LOD	Recovery (%)	Ref
FLE, OFL, NOR, CIP and ENR	HPLC	ILSDME	Swin feed	$[C_8MIM][PF_6]$	0.07–0.61 μg kg^{-1}	90.6–103.2	(Wang et al., 2016)
NOR, CIP, DAN, ENR, SAR and DIF	HPLC	DLLME	Chicken liver	MeCN	5 - 19 μg kg^{-1}	62–106	(Moema et al., 2012)
OFL, NOR, CIP and ENR	HPLC	in situ hDES-SA-LLME	Surface water	hydrophobic deep eutectic solvents	3.0 ng mL^{-1}	-	(Li et al., 2020)
OFL, CIP and ENR	HPLC	HF-LPME	Milk	[OMim][BF4]	0.05 - 0.01 mg mL^{-1}	58-78	(Han et al., 2012)
OFL and CIP	HPLC	HF-LPME	Plasma and tap water	1-octanol	0.5 μg L^{-1}	88 – 108	(Esrafili et al., 2012)
DAN, NOR, ENR and CIP	HPLC-UV	MIP-HFM	Water and urine	Toluene	0.1–10 μg L^{-1}	-	(Barahona et al., 2019)

Fleroxacin (FLE), ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP), enrofloxacin (ENR), norfloxacin (NOR), sarafloxacin hydrochloride (SAR), danofloxacin (DAN), difloxacin hydrochloride (DIF). ionic-liquid-based, salt-induced, dual microextraction (ILSDME), 1-butyl-3-methylimi-dazolium tetrafluoroborate [OMim][BF4], Molecularly imprinted polymer- hollow fiber microextraction (MIP-HFM), in situ formation of hDES coupled with shaker-assisted LLME (in situ hDES-SA-LLME), liquid phase microextraction using a hollow fibre (HF-LPME), 1-octyl-3-methylimidazolium hexafluorophosphate ([C8mim]PF6)

The appropriate sample preparation procedure should meet the following criteria: (i) fast and simple implantation in any laboratory, (ii) eliminating or minimizing matrix interferences to minimize their impact on the instrument and create a clean extract, (iii) enhancing analyte sensitivity or selectivity (iv) low-cost materials and reagents, (v) environmentally friendly solvents and reagents, (vi) giving optimistic forms for analytes' instrumental analysis and ensuring a repeatable analysis procedure, (vii) sufficient to several analytes in fewer steps, and (ix) low energy consumption (Smith, 2003).

For the extraction of pharmaceuticals, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are two widely utilized and well-established sample preparation techniques. Traditional techniques, on the other hand, have a number of fundamental limitations, including costly and time-consuming procedures, the need for a considerable volume of organic solvents, and material and automation challenges. (Manousi et al., 2017; Samanidou, 2018). Recent sample preparation developments have concentrated on the replacement of procedures with nanosorbents, miniaturized, and environmentally friendly techniques.

As a result, new sorts of microextraction procedures, such as liquid liquid microextraction (LLME), have been developed, dispersive liquid liquid microextraction (DLLME) and vortex assisted liquid liquid microextraction (VALLME) as replacements for the classical LLE (Burato et al., 2020). Microextraction methods based on eliminating or reducing organic solvents and reagents for sorbent conditioning and elution, on the other hand, can be used to miniaturize SPE methods. Various miniaturized SPE methodologies have been developed in this regard, including solid-phase microextraction (SPME), micro-solid phase extraction (μ-SPE), stir-bar sorptive

extraction (SBSE), microextraction in a packed syringe (MEPS) and dispersive-micro solid phase extraction (D-μ-SPE) (Ali et al., 2020; Płotka-wasylka et al., 2015). Depending on the extraction phase used, a sample preparation process can be characterized as solvent-based or solid-based extraction. Figure 2.1 illustrates major extraction techniques that are commonly used by researchers.

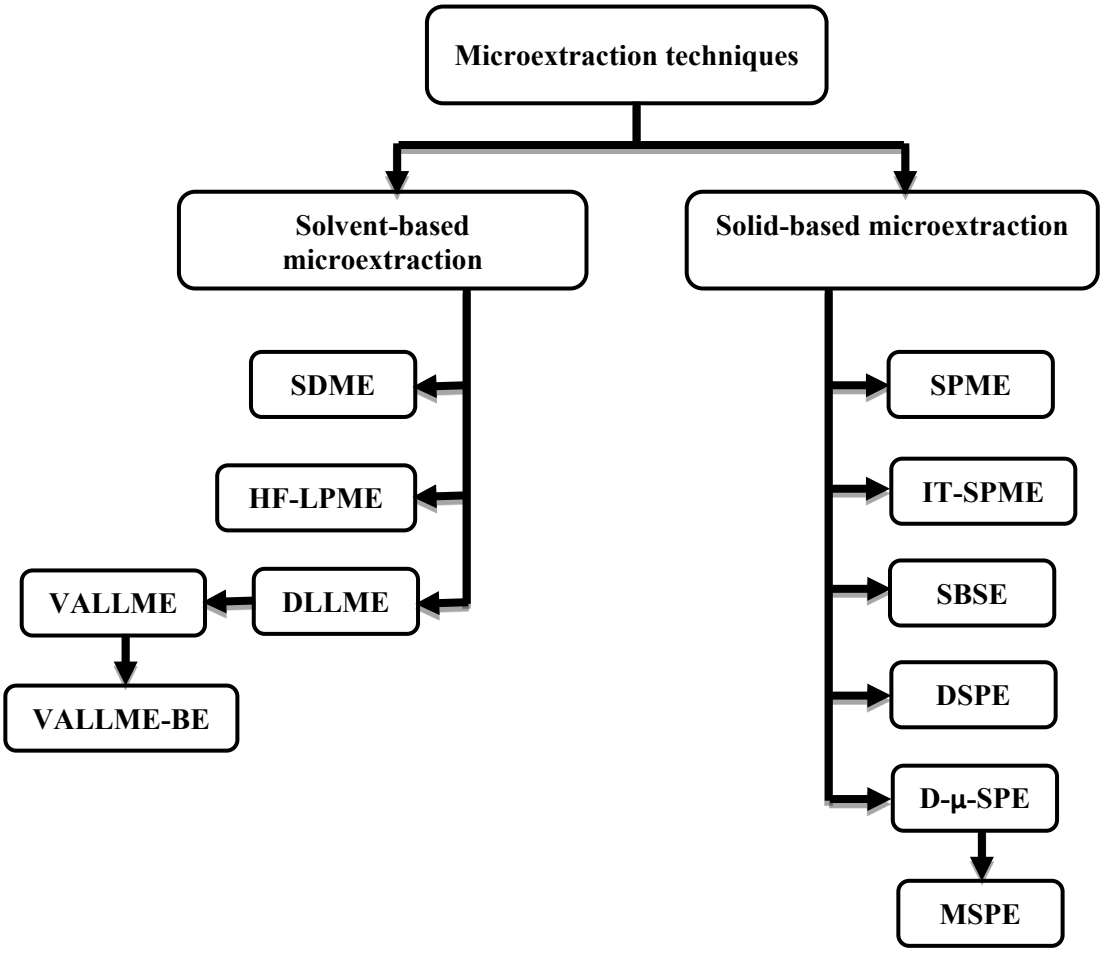

Figure 2.1 Major microextraction techniques

2.2.1 Solvent-based microextraction

Due to their high toxicity, costly disposal necessitates, and impact on future pollution problems, liquid-liquid extraction, and its various approaches have been under intense scrutiny for the past decade, enabling the development of liquid phase microextraction (LPME) (Burato et al., 2020; An et al., 2020). A number of approaches evolved in a short period of time, all with the objective of decreasing the quantity of solvent needed in the sample preparation procedure. In solvent microextraction, the analyte was extracted into a small volume of immiscible solvent (acceptor phase) from the sample solution (donor phase) (Soares da Silva Burato et al., 2020) (Burato et al., 2020). In 1995, the use of solvent-based microextraction was first documented. The first droplet-based analytic approach was developed by Liu and Dasgupta (1995). Since then, a number of approaches have been developed, all of which have a high sample-to-acceptor phase ratio as a common feature (Ma et al., 2011).

Table 2.4 summarizes major modifications of solvent-based microextraction techniques. Solvent base extraction has several benefits, including the need for a low volume of organic solvent, rapid, simple, low cost, and the ability to combine extraction, clean up, and preconcentration in one step. Numerous investigations on enhancing solvent microextractions have shown that the bulk of the suggested methods are insufficient and have yet to be regarded as a viable alternative to LLE.

To preserve the suspended drop, the SDME method was replaced by the HF-LPME (acceptor phase, AP). The two-phase and three-phase HF-LPME have been developed, with the latter being particularly important due to its direct application for HPLC and capillary electrophoresis (CE) studies. Although the electromembrane method (Bello-López et al., 2012) has decreased the lengthy extraction time (30–40

min), the limited repeatability has prompted scientists to look for other non-fiber microextraction strategies (Ojeda & Rojas, 2018). The DLLME, which was initially released in 2006, has attracted a lot of attention (Rezaee et al., 2006).

The DLLME method uses a dispersive solvent to enhance dispersion and thus increase the contact area between the organic phase (AP) and the aqueous material (donor phase, DP), which is important for rapid extraction (Rezaee et al., 2010). The use of dispersive solvents, on the other hand, may reduce the analytes' mass transfer and fractionation into the extraction solvent, lowering the extraction efficiency (Yiantzi et al., 2010). Another drawback of this approach is the use of high volume and hazardous organic solvents (Leng et al., 2012; Rezaee et al., 2006). An alternative dispersive technique known as VALLME has been designed to tackle the shortcomings of the DLLME technique (Yiantzi et al., 2010).

Significant progress has been made in the production of liquid magnetic materials in recent years, with ferrofluids (FFs) being a prominent example. (Clark et al., 2016; Sajid, 2019). Magnetic nanoparticles (MNPs) are dispersed in a carrier liquid to form FFs. A FF can be composed of three parts: magnetic particles, a coating, and a carrier liquid. To form a stable ferrofluid, the carrier liquid should have adequate interactions with the MNPs. To improve the distribution performance of the extractant, it must be insoluble in the aqueous medium and have a low vapour pressure to avoid any degradation during the microextraction method (Kokosa, 2019). As a result, researchers have experimented with various solvents for ferrofluid synthesis the most common carrier such as, organic solvents, ionic liquid, DES, and supramolecular solvents.

Table 2.4 Major modification on solvent microextraction techniques

Technique	Modification	Brief description and remarks	Ref
SDME	Continue flow microextraction (CFME)	Continuous flow of sample solution through extracting drop that trapped in a glass chamber. Remarks: high EF, limit to non-polar analytes, and need additional equipment.	(Werner et al., 2018)
	Solidified floating organic drop microextraction (SFODME)	Freely rotating organic solvent drop that separated from sample upon solidification by cooling an ice bath. Remarks: smaller drop led to higher EF, and limited choices of solvent according to melting point and solubility	(Thongsaw et al., 2017)
	Three phase mode	An aqueous drop immersed in the organic drop. The analytes extracted from sample to the organic drop then back extracted to the aqueous drop. Remarks: Ideal for both acidic and basic analytes.	(Garc, 2016)
	Drop to drop SDME (DD-SDME)	The sample is a drop (in microliters) Remarks: shorter extraction time, and suitable for low sample volume	(Shrivas & Kumar, 2011)
	Dynamic mode SDME	Repeated cycle (30-90) of the drop in and out the sample solution Remarks: efficiency improved	(Wang et al., 2014)
	Solid matrix support microextraction (SSME)	The suspended drop supported by the solid matrix Remarks: Improve drop stability	(Gao et al., 2012)

Table 2.4 (continued)

Technique	Modification	Brief description and remarks	Ref
HF-LPME	Electro membrane extraction (EME)	Electrokinetic migration of the analyte caused by electric filed across the hollow fiber membrane. Remarks: less than 5 mins extraction time.	(Han & Row, 2012)
DLLE	Hollow fiber solid-liquid phase microextraction (HF-SLPME)	A solid phase sorbent immobilized in the pores of the hollow fiber and used after impregnating in an organic solvent. Remarks: Faster extraction since it takes place in the pores	(Hamedi, 2017)
	Solvent bar microextraction (SBME)	HF piece is freely rotated in the sample. The two ends of the hollow fiber are sealed after filling with the extraction solvent. Remarks: Higher extraction efficiency due to more exposure	(Herce-Sesa, 2018)
	Ultrasound-assisted emulsification-microextraction (USAEME)	Use of ultrasound irradiation instead of dispersing solvent. Remarks: Less solvent used	(Sereshti et al., 2019)
	Vortex assisted liquid liquid microextraction (VALLME)	Use of vortexing instead of dispersing solvent to increase contact surface. Remarks: Less solvent used	(Makahleh et al., 2015)
	Air-assisted liquid-liquid microextraction (AALLME)	Fine droplets of the microextraction solvent formed due to pumping of a mixture of DP/AP in and out of a syringe. Disperse solvent free technique. Remarks: Less solvent used	(Barfi, et al., 2015)