DEVELOPMENT OF AMINO-FUNCTIONALIZED MAGNETIC SILICA NANOCOMPOSITE FOR THE MAGNETIC SOLID-PHASE EXTRACTION OF β-LACTAM ANTIBIOTICS IN MILK

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by

WAN IBRAHIM THANI BIN ABD HALIM

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LIST OF SYMBOLS

β	beta
cm	centimetre
cm^3g^{-1}	centimetre cube per gram
°	degree
°C	degree celcius
$g\ mol^{-1}$	gram per mol
K	kelvin
$emu\ g^{-1}$	mass magnetization
m^2g^{-1}	meter square per gram
$\mu g\ kg^{-1}$	microgram per kilogram
$\mu g\ L^{-1}$	microgram per litre
µl	microlitre
$mmol\ L^{-1}$	micromole per litre
mg	milligram
min	minute
M	molar
nm	nanometre
rpm	revolution per minute
s	second
θ	theta
w/v	weight per volume

LIST OF ABBREVIATIONS

µSPE	Micro solid-phase extraction
ACN	Acetonitrile
APTES	(3-aminopropyl)trimethoxysilane
APTES-MSN	amino-modified magnetic silica composite
BET	Brunauer-Emmett-Teller
BLAs	Beta-lactam antibiotics
CLO	Cloxacillin
DCM	dichloromethane
DIC	Dicloxacillin
DLLME	Dispersive liquid-liquid microextraction
DSPE	Dispersive solid-phase extraction
EtOH	Ethanol
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared
HFLPME	Hollow fiber liquid-phase microextraction
HPLC-DAD	High-performance liquid chromatography with a diode array detector
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limits of quantification
MeOH	Methanol
MEPS	Microextraction by packed syringe

MNP	Magnetic nanoparticles
MRL	Maximum residue limits
MSN	Magnetic silica nanocomposite
MSPD	matrix solid-phase dispersion
MSPE	Magnetic solid-phase extraction
NAF	Nafcillin
NPs	Nanoparticles
OXA	Oxacillin
SDME	Single drop microextraction
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TEM	Transmission electron microscope
TGA	Thermogravimetric analysis
VSM	Vibration sample magnetometer
XRD	X-ray diffractometer

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PEMBANGUNAN KOMPOSIT NANO SILIKA BERMAGNET TERFUNGSI AMINO DALAM PENGEKSTRAKAN FASA PEPEJAL BERMAGNET BAGI ANTIBIOTIK β-LAKTAM DI DALAM SUSU

ABSTRAK

Kajian ini menerangkan kaedah pengekstrakan fasa pepejal magnetik (MSPE) berdasarkan (3-aminopropil)triethoxysilane (APTES) nanokomposit magnetik silika nano (MSN) berfungsi untuk penentuan empat antibiotik β-laktam (BLA) iaitu oksasilin (OXA), kloksasilin (CLO), dikloksasilin (DIC), dan nafsilin (NAF) dalam sampel susu sebelum kromatografi cecair berprestasi tinggi dengan analisis pengesan tatasusunan diod (HPLC-DAD). Struktur kimia dan morfologi permukaan APTES-MSN telah dianalisis dan dicirikan menggunakan spektroskopi inframerah transformasi Fourier (FTIR), penganalisis kawasan permukaan Brunauer-Emmett-Teller (BET), pembelauan sinar-x (XRD), analisis termogravimetrik (TGA), penghantaran mikroskop elektron (TEM), mikroskop elektron pengimbasan pelepasan medan (FESEM), dan magnetometer sampel bergetar (VSM). APTES-MSNs ini digunakan sebagai penjerap untuk analisis empat antibiotik β-laktam (BLA) iaitu oksasilin (OXA), kloksasilin (CLO), dikloksasilin (DIC), dan nafsilin (NAF) dalam sampel susu menggunakan pengekstrakan fasa pepejal magnet (MSPE). Penentuan dan pengenalpastian BLA dilakukan oleh kromatografi cecair prestasi tinggi dengan pengesan tatasusunan diod (HPLC-DAD). MSPE telah dioptimumkan dari segi pH sampel, jumlah sorben, masa pengekstrakan, jenis pelarut nyahjerapan, masa nyahjerapan, isipadu pelarut

nyahjerapan, isipadu sampel, dan penambahan garam. Kaedah APTES-MSN-MSPE yang dibangunkan menyediakan julat kelinearan yang baik dari 5 hingga 500 μg L^{-1} untuk OXA, CLO, DIC dan NAF dengan pekali penentuan yang baik (R^2 = 0.9987 - 0.9998), had pengesanan yang rendah (0.26 - 1.00 μg L^{-1}), % RSD boleh diterima (n = 3) (1.59% – 5.00%) dan pemulihan relatif yang memuaskan (93.43% – 113.42%). APTES-MSN-MSPE digabungkan dengan HPLC-DAD telah berjaya digunakan untuk analisis BLA yang disasarkan dalam 15 sampel susu terpilih, dan kehadiran analit yang dikaji dikira dalam 4 sampel.

DEVELOPMENT OF AMINO-FUNCTIONALIZED MAGNETIC SILICA NANOCOMPOSITE FOR THE MAGNETIC SOLID-PHASE EXTRACTION OF β-LACTAM ANTIBIOTICS IN MILK

ABSTRACT

This study describes a magnetic solid-phase extraction (MSPE) method based on (3-aminopropyl)triethoxysilane (APTES) functionalized magnetic silica nanocomposites (MSN) for the determination of four β-lactam antibiotics (BLAs) namely oxacillin (OXA), cloxacillin (CLO), dicloxacillin (DIC), and nafcillin (NAF) in milk samples prior to high performance liquid chromatography with diode array detector (HPLC-DAD) analysis. The chemical structure and surface morphology of APTES-MSN were analyzed and characterized by Fourier transform infrared (FTIR) spectroscopy, Brunauer-Emmett-Teller (BET) surface area analyzer, x-ray diffraction (XRD), thermogravimetric analysis (TGA), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and vibrating sample magnetometer (VSM). Several essential parameters affecting the extraction efficiency were studied, such as sample pH, amount of sorbent, extraction time, type of desorption solvent, desorption time, volume of desorption solvent, volume of sample, and salt addition. The developed APTES-MSN-MSPE method provided good linearity range from 5 to 500 µg L^{-1} for OXA, CLO, DIC, and NAF with good coefficient of determination (R^2 = 0.9987 - 0.9998), low limits of detection (0.26 -1.00 µg L^{-1}), acceptable RSD% (n = 3) (1.59% – 5.00%) and satisfactory relative recoveries (93.43% – 113.42%). The APTES-MSN-

MSPE combined with HPLC-DAD was successfully employed for the analysis of targeted BLAs in 15 selected milk samples, and the presence of studied analytes was quantified in 4 samples.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The extensive usage of pharmaceuticals has seen them being classified as emerging contaminants due to the health risks associated with exposure of living organisms to these compounds. Antibiotics are used to effectively produce beef, milk, and eggs on farms while maintaining strict hygienic standards, one class of pharmaceutical chemicals frequently utilized in the dairy business. They are utilized as growth promoters at the low level. They are used to treat sick animals at high levels and to prevent diseases at the intermediate level (Samanidou *et al.,* 2007). β-lactam antibiotics have been extensively used to treat mastitis, pneumonia, bacterial diarrhea, and bacteria arthritis in livestock animals (Goto *et al.*, 2005). Mastitis, also known as inflammation of the mammary gland, is presently one of the most common illnesses afflicting dairy cattle. Mastitis used antimicrobials to prevent and cure dairy farms, accounting for 60–70% of all antimicrobials used. Mastitis dramatically reduces milk supply and farm profitability (Cobirka *et al.,* 2020).

The most serious concern regarding the antibiotic residues issue is due to the development of resistance, whereby a body has developed resistance to antibiotics that have been used to treat bacteria in the human body (Ghidini *et al.*, 2003). With rising demand for more highly nutritious animal-sourced food products (meat, poultry, dairy, etc.) in people's diets and the increasing intensification of food production, antibiotics will continue expanding in the years to come. Consequently, the growing use and misuse of antibiotics in the veterinary industry has resulted in antibiotic resistance in

microorganisms, making particular treatments against disease-causing organisms inefficient (Wang *et al.*, 2017). These antibiotics may result in the deposition of residues in meat, milk, and eggs, thus jeopardizing food safety for humans. The concern over antibiotic residues in food or livestock origin occurs in two conditions; one, which produce a potential threat to direct toxicity in human, second is whether the low levels of antibiotic exposure would result in alteration of microflora, cause disease, and the possible development of resistant strain which cause the failure of antibiotic therapy (Nisha, 2008).

β-lactam have been widely employed as antibacterial agents against a variety of pathogens. They prevent the synthesis of the peptidoglycan layer of the bacterial cell wall (Evaggelopoulou *et al.,* 2013). Based on their lipophilic properties, β-lactam can accumulate in milk. Milk has been acknowledged as one of the most stringently controlled foods due to its high consumption and the likelihood of antibiotic residues at low and minute amounts (FAO, 2016). The European Union Commission regulation has established maximum residue limits (MRLs) for BLAs in foods of animal origin at 30 $\mu g\ kg^{-1}$ (*Commission regulation (EU) No 37/2010*, 2010). From the analytical chemistry perspective, due to the milk matrix's complexity, which results from the presence of many interfering chemicals such as proteins and lipids, determining the experimental results is challenging. Monitoring trace BLAs in complicated matrices necessitates the development of simple, sensitive, quick, and effective analytical techniques. Improving productivity needs an excellent sample preparation method (Sahebi *et al.,* 2020).

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) were widely used as a method for sample preparation procedures (Xu *et al.*, 2016). However, these methods have some significant disadvantages. For instance, LLE consumes a large

amount of organic solvent, which becomes an environmental problem due to its waste and SPE is tedious and time-consuming (Vasconcelos *et al.,* 2017). In recent years, magnetic solid-phase extraction (MSPE), an improved model of old SPE, has been developed. It uses magnetic particles as SPE adsorbents without packing them into an SPE cartridge (Jeddi *et al.*, 2015). This technique requires only minute amounts of solvent and milligrams of adsorbent in the extraction. MSPE has an excellent adsorption efficiency and good removal from mixture solution without centrifugation (Xu *et al.*, 2016). Many adsorbents have been developed for the trace analysis of BLAs in various food matrices, but finding selective, green, and efficient adsorbents for extraction and preconcentration of BLAs continues to be a challenge.

Nano-sized adsorbent provides a high surface area to volume ratio, enhancing adsorption capacity and efficiency (Nor *et al.*, 2016). However, in MSPE, the first issue that needs to be resolved is the selection of magnetic adsorbent. Among the magnetic adsorbents, iron oxide nanoparticles are promising and have been popularly used in many research areas due to their nano-sized, superparamagnetic property, and biocompatibility (Wu *et al.,* 2008). However, it is difficult to control the size, shape, stability, and dispersibility of magnetic iron nanoparticles (NPs) in favour of solvent (Wu *et al.,* 2008). Moreover, naked iron oxide NPs tend to agglomerate and quickly oxidize by air due to their high chemical activity, losing their magnetism and dispersibility and limiting their potential (Qiao *et al.*, 2019). Thus, further protection strategies need to be applied to the surface of NP. Because of this, polymer, carbon, and silica have been used as passive materials that can be used as a surface coating for iron oxide nanoparticles, but silica is the most suitable material that effectively suppresses their aggregation and improves their chemical stability (Xie *et al.,* 2018). However, the

silica surface is hydrophilic and thus has a limited affinity for hydrophobic matrix (Shafqat *et al.*, 2019).

The silica surface modification with a selected functional group from organic solvent has been comprehensively investigated. An organic solvent can increase covalent bonding with the hydrophobic matrix and improve affinity between two phases (Kickelbick *et al.,* 2003). Organic compounds functionalized iron oxide NPs possess good biocompatibility and biodegradability of the functional organic materials (Wu *et al.,* 2008). They can provide various functional reactive groups such as aldehyde, hydroxyl, carboxyl, and amino groups (Wu *et al.,* 2008). The amino-functionalized groups have been used previously to improve the protonation under the acidic conditions, which subsequently enhance the stability of the β-lactam ring in acidic solution (Sun *et al.*, 2014).

1.2 Problem statement

Extensive usage of BLAs may raise the possibility of adverse effects of residues on the consumer, such as antibiotic resistance, hypersensitive reactions (Beyene *et al.,* 2015), and allergic reaction (Sachi *et al.*, 2019). Low concentration levels and the complexity of milk necessitate the employment of susceptible, selective methods for BLAs determination (Lara *et al.*, 2012). Sahebi *et al.,* 2020, reported that the challenge in interpreting the experimental results in milk is due to the complexity induced by the presence of numerous compounds such as proteins or fats.

SPE is one of the conventional methods that has been used widely in sample preparation (Nor *et al.*, 2016). Despite its advantages, most SPE procedures are tedious, laborious, and time-consuming and use a high amount of sorbent and organic solvent

(Nasir *et al.*, 2019). It also has a low capability for extraction (Zhang *et al.*, 2010). MSPE, which uses a magnetic material as a sorbent, has been introduced for improvement to overcome its weakness. In this method, the selection of adsorbent plays a vital role as it can determine the efficiency, anti-interference ability, and selectivity toward targeted analytes (Nor *et al.*, 2016). However, finding selective, sensitive, and efficient adsorbents for the extraction of BLAs is a very challenging task due to the presence of BLAs in complex milk matrices. In various applications, nano-sized particles are suitable due to their high surface size to volume ratio (Nor *et al.*, 2016). As reported by Nor *et al.*, 2016, much research has been published using iron oxide magnetic nanoparticles (MNP) as the adsorbent for MSPE. However, pristine MNP has high chemical activity and quickly oxidize in atmospheric air resulting in losing their magnetic properties and stability (Thangaraj *et al.*, 2016). It is noted that silica has excellent potential as a coating material known to have excellent biocompatibility, stability, nontoxicity, and easily furthered conjugation with various functional groups and known as magnetic silica nanocomposite (MSN) (Dindar *et al.*, 2010).

The MSN were thoroughly researched and designed to relate to magnetism's unique properties, which may be applied directly to particles and allow for further modification of particle interactions, resulting in a wide range of potential applications (Philipse *et al.,* 1994). However, the silica surface is hydrophilic and thus has a limited affinity for hydrophobic matrix (Shafqat *et al.*, 2019). Therefore, surface functionalization of silica with an organic group is typically efficient for increasing the compatibility between organic and inorganic phases and even forming covalent connections between two phases to generate unique properties. (Lu *et al.,* 2013). In

addition, the structure of silica consists of silanol groups that can easily react with organosilane compounds (Thangaraj *et al.,* 2016).

The process of modifying (3-aminopropyl)trimethoxysilane (APTES) into silica-coated magnetic nanoparticles has been documented in a previous study conducted by Hassan *et al.*, 2020. In the study, graphene oxide has been used as a magnetic nanoparticle with lower electrical and thermal conductivity and poor control of post-preparation functionalization (Catania *et al.*, 2021). This study employs iron oxide nanoparticles as a foundation, commonly used sorbents in magnetic solid phase extraction (MSPE) due to their inherent magnetism, minimal toxicity, uncomplicated synthesis, and substantial surface area-to-volume ratio (Huang *et al.*, 2016). The inclusion of APTES results in the facile protonation of NPs in acidic solutions and introduces an additional functional group of ($-NH_2$) that can function as an active site for analyte adsorption, as reported by Hassan *et al.*, 2020. The functionalization of MSN with APTES results in a surface modification that facilitates a swift and effective sample preparation process. This method allows for the treatment of a substantial quantity of samples, leading to a high extraction efficiency of BLAs in milk samples.

1.3 Objectives of study

The study aims to develop a new amino-functionalized magnetic silica nanocomposite (APTES-MSN) adsorbent in magnetic solid-phase extraction to determine BLAs. The specific objectives are to:

i- To synthesize amino-functionalized magnetic silica nanocomposite adsorbent.

ii- To characterize and compare APTES-MSN adsorbent with native adsorbent.

iii- To apply the newly synthesized APTES-MSN adsorbent in MSPE and optimize the MSPE parameters.

1.4 Scope of study

This study focuses on developing a new microextraction method based on MSPE incorporated with APTES-MSN adsorbent to determine four β-lactam antibiotics in commercial milk products. Four selected BLAs, including oxacillin (OXA), cloxacillin (CLO), dicloxacillin (DIC), and nafcillin (NAF), were used in this study. The extraction capability of the developed microextraction methods was determined using high-performance liquid chromatography with a diode array detector (HPLC-DAD). Characterization of the synthesized APTES-MSN was performed by Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and field emission scanning electron microscopy (FESEM) for the morphology, and Brunauer-Emmett-Teller (BET) for the surface area analysis. Several effective parameters on MSPE, such as the amount of sorbent, the effect of salt addition and pH, sample volume, extraction, and desorption time, were optimized thoroughly. Validation studies were performed for the MSPE method in terms of their linearity, limits of quantification (LOQs), detection limits (LODs), repeatability, and recovery. APTES-MSN-MSPE method was successfully applied to determine BLAs in commercial milk products.

1.5 Significance of study

In line with the UN Sustainable Development Goal 2 (SDG 2) to end hunger, achieve food security, improve nutrition and promote sustainable agriculture, concern for the safety of foods from animals' origin to be consumed by humans should not be ignored. The development of APTES-MSN-MSPE as a new sample preparation strategy is beneficial in terms of fast extraction time, minimum solvent requirement, inexpensive, ease of operation, and sensitive determination of BLAs compounds in milk matrices below the maximum permissible content. APTES-MSN-MSPE system indicated that this method has great potential as an alternative 'green' and environmentally friendly microextraction method over the most common conventional sample preparation methods.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter compiles and discusses the fundamentals of the selected BLAs, their analytical determination, development of conventional and microextraction methods, and functionalized magnetic sorbent applications in sample preparation.

2.2 β-lactam antibiotics

Antibiotics have been mainly used to treat or prevent human diseases since their first invention in the 1930s (Chen *et al.,* 2019). β-lactam are one of the groups of antibiotics considered the oldest group and the most employed among the group antibiotics (Ghidini *et al.*, 2003). Figure 2.1 shows the general structure of β-lactam antibiotics.

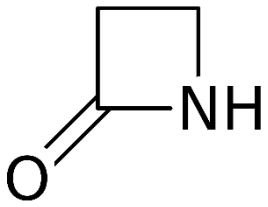


Figure 2.1 β-lactam group.

Environmental pollution has been a problem for many years due to toxic chemicals being discharged into the environment, and the contaminants now exceed permissible limits (Ng *et al.*, 2018). Since antibiotics have been found in practically all recorded aquatic habitats, exposure to an antibiotic-contaminated environment can also cause antibiotics to build up in marine products. The leading causes of antibiotic residue

in vegetables are contamination of irrigation water by antibiotics and antibiotic residues in manure used as organic fertilizer (Chen *et al.,* 2019).

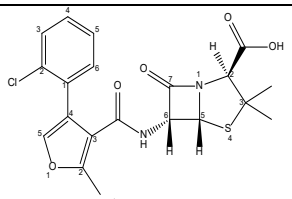
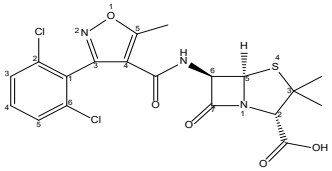
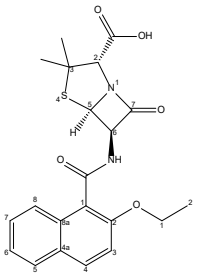
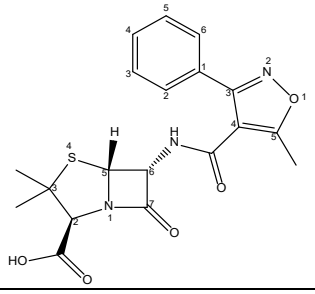
Nowadays, antibiotics have been expanded and frequently used in animal agriculture to treat and prevent mastitis and other microbial infection; However, the non-fulfilment of the antibiotics withdrawal period, which ensures that the drug has been wholly excreted for consumers, may lead to antibiotic residues (Cámara *et al.*, 2013a). Antibiotic residues can be determined primarily on animal source products such as milk.

Antibiotic residues can be harmful to human life. Antibiotics in food-origin animals have been a public concern recently due to the growing spread of antibiotic-resistant bacteria from humans to animals (Lara *et al.*, 2012). According to World Health Organization (WHO), over 25000 people die from infections caused by antibiotic-resistant each year. If this trend continues, in 35 years, 300 million people worldwide are expected to die prematurely due to antibiotic-resistant (Cristea *et al.,* 2017).

Therefore, EEC Regulation 2377/90 has been established in the European Union (EU) to control antibiotic residue in animal-producing products to set maximum residue limits (MRLs). Among antibiotic groups, the molecules belonging to the β-lactam group have the lowest tolerance. In milk, the MRLs for OXA, CLO, DIC, and NAF are 30 μg kg^{-1}. This shows the importance of determining the level of antibiotics in milk products. Because of the complexity of the milk matrix, direct analysis using instruments such as HPLC or LC-MS is nearly impossible. Therefore, sample preparation and extraction method are required (Adlnasab *et al.,* 2012).

Four β-lactam antibiotics have been selected for this study which are oxacillin (OXA), cloxacillin (CLO), dicloxacillin (DIC), nafcillin (NAF), and the chemical structure and characteristics of selected antibiotics are described in Table 2.1.

Table 2.1 Physical properties of some common β-lactam antibiotics.

Compound	**Structure**	**Molecular weight ($gmol^{-1}$)**	**pKa**	**Log P**
Cloxacillin $C_{19}H_{18}ClN_3O_5S$		435.879	2.78	2.48
Dicloxacillin $C_{19}H_{17}Cl_2N_3O_5S$		470.326	3.75	2.91
Nafcillin $C_{21}H_{22}N_2O_5S$		414.475	3.31	3.3
Oxacillin $C_{19}H_{19}N_3O_5S$		401.436	2.72	2.38

2.3 Conventional methods of extraction

Direct determination of the experimental finding is challenging due to the complexity of the matrices created by the occurrence of multiple compounds (Sahebi *et al.,* 2020). Consequently, analytical processes comprise five steps: sample collection, sample preparation, separation, detection, and data processing (Babic *et al.,* 2007). Over 80% of the analytical procedure is spent on sampling and sample preparation steps in these steps (Babic *et al.,* 2007).

Chromatographic is the most common detection method for antibiotics in any matrices (Pietschmann *et al.*, 2020). However, due to the complex matrices in food samples, a sample extraction procedure is compulsory to achieve a reliable result in the selectivity and specificity of analysis (Chen *et al.,* 2019). Several extraction techniques have been developed over the past decades, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). However, these techniques require an additional clean-up step and preliminary chromatographic analysis, and it is also tedious, time-consuming, and laboratory (Garrido *et al.*, 2010). Therefore, a new appropriate approach for new extraction methods is necessary because over 80% of the total analysis time is sample preparation and extraction step (Rossi *et al.*, 2017).

2.3.1 Liquid-liquid extraction (LLE)

LLE is one of the oldest and most widely used extraction techniques, traditionally used to clean up the sample. This technique separates compounds into different immiscible liquids based on relative solubilities, usually water and organic solvent (Rashmi *et al.,* 2019). The analytes should be soluble in the extraction solvent

and should exhibit high partition coefficients in the solvent (Kyle *et al.,* 2017). LLE has been broadly utilized as sample pretreatment for metal cations (Khoutoul *et al.*, 2016), biological (Jouyban *et al.*, 2016), beeswax (Yáñez *et al.*, 2013), and aqueous samples (Farajzadeh *et al.,* 2016).

Nevertheless, there are significant limitations of conventional LLE; it needs enormous amounts of organic solvent that is costly and hazardous to the environment. It is tedious due to evaporating step of a large volume of organic solvent. It also would be difficult to obtain good sample separation using LLE if there are a lot of target compounds with a slight difference in polarity in the sample (Rashmi Urkude *et al.,* 2019). Figure 2.2 shows the setup for LLE.

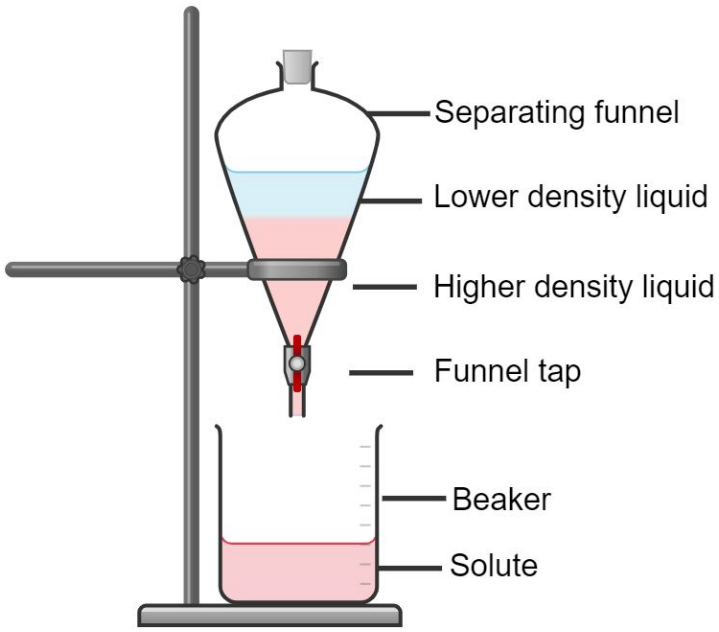


Figure 2.2 Liquid-liquid extraction.

2.3.2 Solid-phase extraction (SPE)

SPE is a sample preparation technique utilized in numerous fields due to its significant advantages over conventional methods. SPE was created as an alternative to

liquid/liquid extraction. It reduced the disadvantages, such as the necessity for a large volume of solvent, longer operating time or procedure stages, potential error sources, and high prices. (Ötles *et al.,* 2016). SPE can be conducted offline, the sample preparation isolated from subsequent chromatographic analysis, or online by direct connection to the chromatographic system (Hennion, 1999). The selectivity of the SPE method depends on the attractive forces between the analytes and the functional groups on the sorbent surface (Rossi *et al.*, 2017).

Standard SPE contains four steps, conditioning, loading, washing, and elution. Figure 2.3 shows the steps of SPE (Sosa-ferrera *et al.,* 2017). In SPE, disposable cartridges or columns packed with the proper sorbent are served as a solid surface for distributing the desired compound between the liquid sample and the solid phase until the equilibrium phase is achieved (Ötles *et al.,* 2016). Simplicity, flexible selection of sorbents, and low consumption of organic solvents are the significant advantages of the SPE technique (Gao *et al.*, 2011).

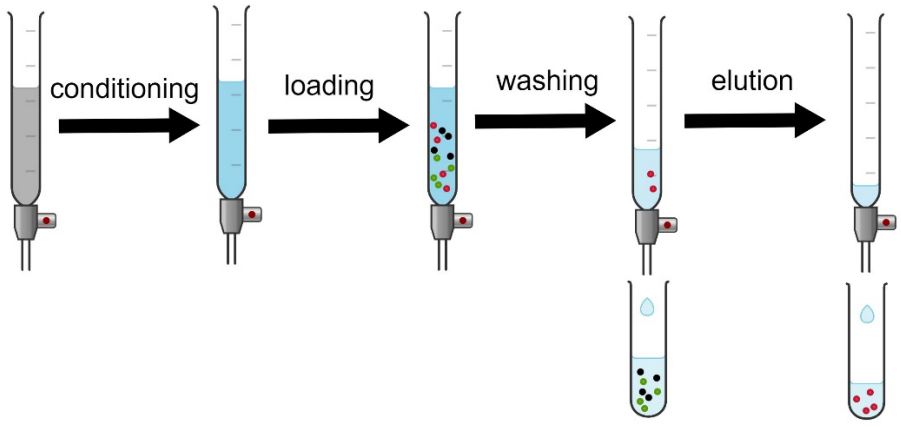


Figure 2.3 The procedures of SPE.

2.3.3 Supercritical fluid extraction (SFE)

Supercritical fluids have been used to isolate natural products since the end of the 1970s, but applications have been limited to just a few products for a very long time. Industries are becoming increasingly interested in supercritical techniques as the development of processes and equipment starts to pay off (Herrero *et al.*, 2010). SFE is an extraction method that employs a supercritical solvent. Higher diffusion coefficients and lower viscosity in this state give supercritical fluids improved mass transfer properties compared to liquids (Basheer *et al.*, 2012). A supercritical fluid is a strong choice for extraction because of its mixture of mass transfer characteristics like gas and solvating abilities resembling a liquid. Additionally, highly selective extractions can be accomplished by altering the density while varying the temperature and pressure (Smith, 2003).

A typical SFE system comprises a high-pressure pump that transfers the fluid and an extraction cell that contains the sample and is kept at the appropriate pressure and temperature. Adding an organic solvent (also known as the modifier) to the fluid can improve its solvating capabilities. The cell's temperature is elevated to surpass the fluid's critical value. After depressurization, the analytes are collected using a tiny volume of organic solvent or a solid adsorbent trap. Extraction can be carried out in static, dynamic, and recirculating modes (Fidalgo *et al.*, 2007). One of the primary benefits of SFE in sample preparation is the reduced usage of organic solvents (nil in many situations), allowing extractions to be performed using non-polluting, nontoxic supercritical fluids, such as carbon dioxide, the most commonly used extracting solvent.

Carbon dioxide is inexpensive and environmentally benign, and safe (Mendiola *et al.*, 2007).

Supercritical carbon dioxide is also desirable due to its high diffusivity and easily tenable solvent strength. Another advantage is that CO_2 is a gas at room temperature and pressure, simplifying analyte recovery and providing analytes without solvents (Plaza *et al.*, 2009). Essential for preparing food and natural product samples is the capability of SFE employing CO_2 to be operated at low temperatures with a non-oxidant medium, allowing the extraction of thermally labile or easily oxidized components (Zougagh *et al.,* 2004). However, SFE also have several drawbacks such as, high critical temperature and pressure for water; the high flammability of nitrous oxide, ethers and hydrocarbons; and the chemical reactivity and corrosiveness of ammonia (Mendiola *et al.*, 2007).

2.3.4 Dispersive solid-phase extraction (DSPE)

Due to constraints such as cartridge obstruction and the time required to analyze materials with considerable volumes in SPE, a new solid-based extraction technology known as dispersive solid-phase extraction (DSPE) was developed (Seidi *et al.,* 2017). In this method, the clean-up procedure is limited to shaking and centrifugation because the solid sorbent is applied straight to a sample solution without any sample manipulation steps like conditioning (Kumar *et al.*, 2019). The adsorbents in DSPE are dispersed throughout the sample solution and collected using centrifugation or an external magnetic field. The target analyte is then extracted and eluted with a suitable desorption solvent before being analyzed. DSPE has become one of the most common techniques for extracting/pre-enriching analytes from environmental samples due to its

ease of use, minimal usage of organic solvents, and quick separation (Huang *et al.*, 2021). Figure 2.4 shows the procedure of DSPE.

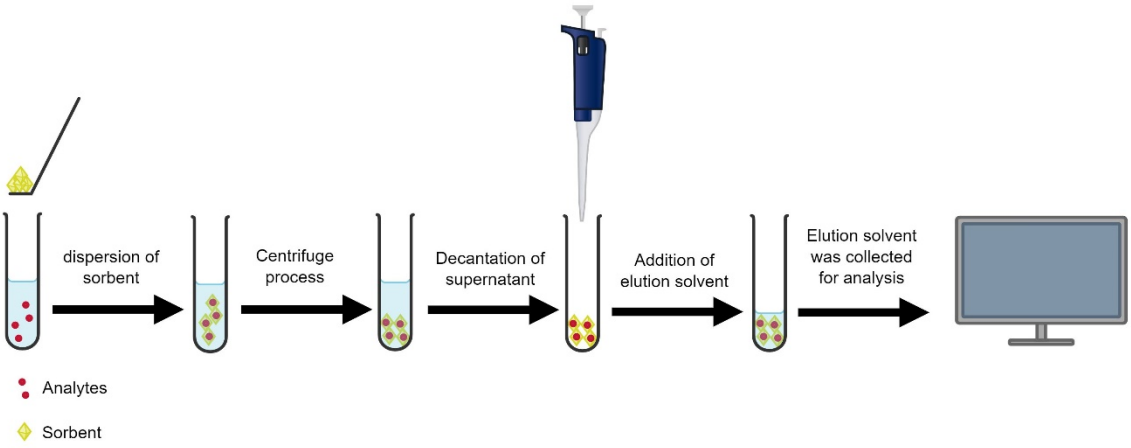


Figure 2.4 The procedure of DSPE.

2.4 Trends in solid based microextraction method for chemical analysis

Recently, creating a new microextraction method based on miniaturized methods tackles the drawbacks of traditional extraction techniques, such as significant solvent consumption, labor-intensive operations, and high cost in SPE (Ma *et al.*, 2018). Researchers have given them much attention since they are more straightforward, efficient, cost-effective, and environmentally benign because they use less toxic chemicals. Miniaturization in the development of sample preparation technologies is crucial to decrease environmental damage and move towards a green analytical chemistry process. The solid-based microextraction technique has become popular, as evident by the number of publications in Figure 2.5.

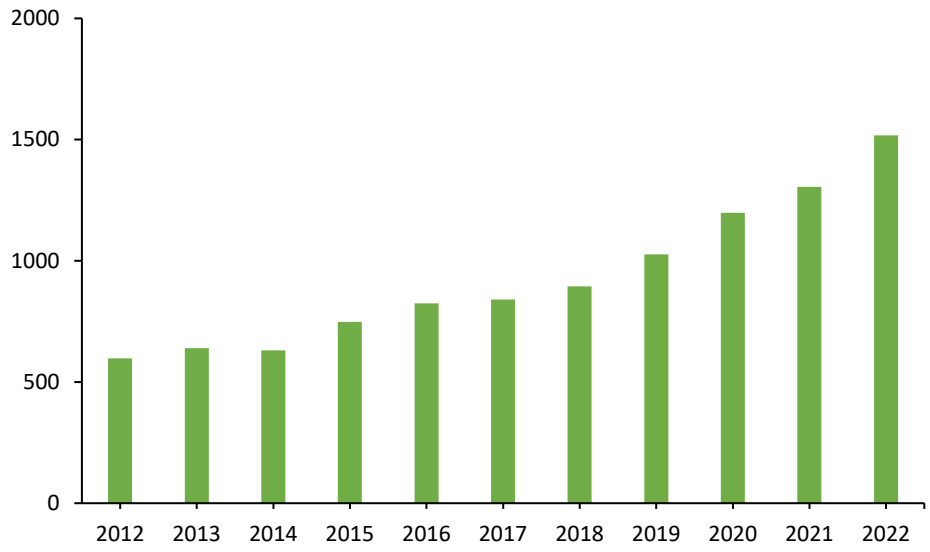


Figure 2.5 Number of publications in 2012-2022 regarding solid-based microextraction technique (https://www.sciencedirect.com/).

2.4.1 Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) was introduced in 1990 as chemically modified fused silica fibers with thermal desorption eliminates eliminate the problems associated with SPE while retaining the advantages (Arthur *et al.,* 1990). SPME method is based on a single equilibrium in which analytes are partitioned between a fiber coating and a matrix. The equilibrium constant and the equilibration duration are affected by a variety of parameters, including pH, temperature, salt content, and stirring (Abdel-Rehim *et al.,* 2004)

SPME is a solvent-free technique that can be easily applied to analyze organic or volatile compounds by merging sampling, extraction, concentration, and sample introduction into a single solvent-free phase, SPME. SPME is an analytical technique capable of meeting the abovementioned parameters (Pico *et al,* 2022). Fibers and capillary tubes coated with a suitable stationary phase are commonly used in SPME.

Fiber SPME is the most extensively used approach. An extraction device is a fiber-coated polymeric stationary phase, and analytes are absorbed or adsorb on the fiber's outer surface (Kataoka *et al,* 2011). Figure 2.6 shows the SPME setup.

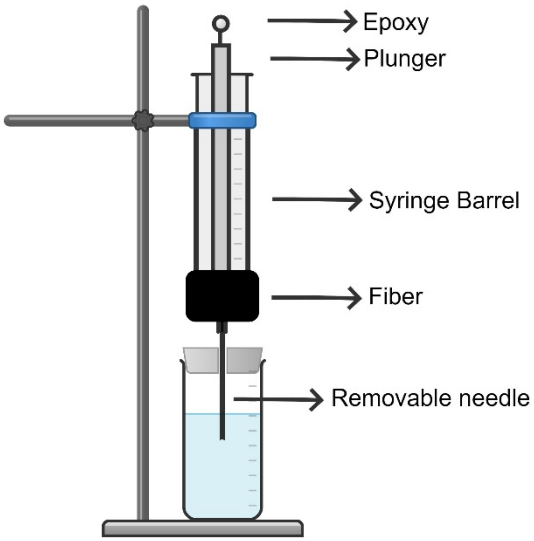


Figure 2.6 PME setup for extraction.

2.4.2 Micro solid-phase extraction (μ-SPE)

Another developing extraction method, μ-SPE, is frequently utilized for sample pretreatment because of its high enrichment factor, minimal solvent consumption, and ease of automation (Piri-Moghadam *et al,* 2016). The online μ-SPE pre-enrichment technology is ideal for determining trace inorganic and organic mercury because of its simplicity, flexibility, high sample throughput, and adaptability (Huang *et al.*, 2021).

2.4.3 Magnetic solid-phase extraction (MSPE)

Magnetic solid-phase extraction (MSPE), which uses magnetic particles as adsorbents and is based on the idea of solid-phase extraction (SPE), is an ideal sample pretreatment method with high extraction efficiency and ease of use. The magnetic core of the adsorbents is commonly Fe_3O_4 (Zhou *et al.*, 2020). MSPE is based on the ability of magnetic sorbents (in the mg or g range) to adsorb and desorb analytes under an external magnetic field without the need for time-consuming steps like centrifugation or filtration. In addition, sorbent particles can be easily isolated and collected, making sample pretreatment more convenient, time-saving, and cost-effective (Nasir *et al.*, 2019). Figure 2.7 demonstrates the procedure of MSPE.

Despite the benefits MSPE provides compared to other sample preparation processes, it has significant drawbacks. Depending on the magnet's strength, separating magnetic material from the liquid phase might be challenging with insufficient separation. In addition, a portion of the magnetic material might be wasted during the liquid phase, resulting in analyte loss and reduced recovery (Vasconcelos *et al.,* 2017).

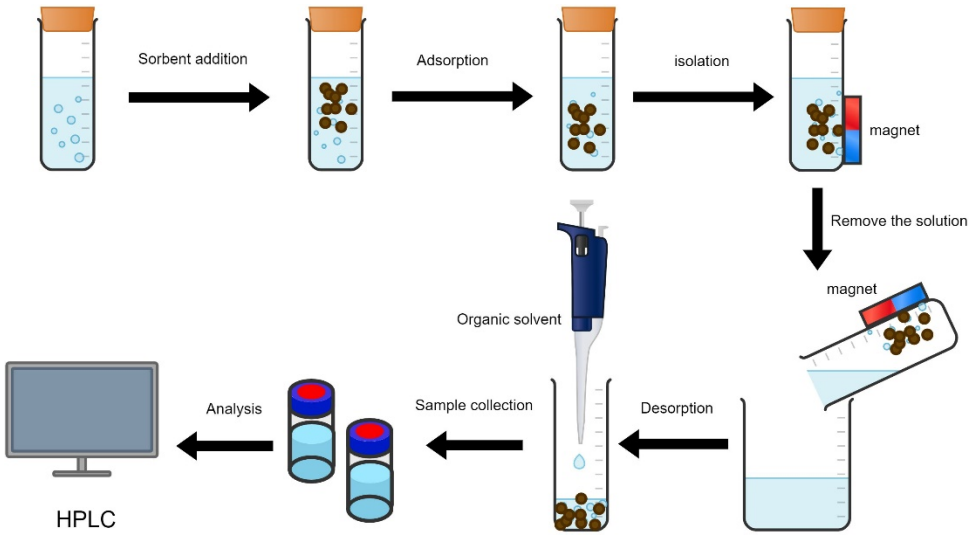


Figure 2.7 MSPE procedure.

2.4.4 Microextraction by packed syringe (MEPS)

Based on a paper published by Abdel-Rehim et al., 2004, in MEPS, 1 mg of the solid packing material is injected as a plug into a syringe (100–250 µl), as shown in the Figure 2.8. An autosampler (which pumps the sample up and down) draws the plasma sample (20–1000 µl) through the syringe. After the plasma passed through the solid support, the analytes were adsorbed by the solid phase. After that, the solid phase is washed once with water (50 µl) to remove the proteins and other interfering material. The analytes are eluted directly into the instrument's injector using an organic solvent such as methanol or the LC mobile phase (20–50 µl). The procedure is completely automated.

The packing in MEPS is integrated directly into the syringe, as opposed to being isolated into a separate column, as in commercial SPE. In addition, a packed syringe can be reused numerous times, up to 100 times, with plasma or urine samples, whereas a conventional SPE column can only be utilized once. MEPS is capable of handling both

small and large sample quantities (1000 µL). However, MEPS extraction steps are identical to those used in conventional SPE extraction, washing, and elution, and these must be optimized to achieve the highest analyte recovery. Additional steps for post-cleaning and reconditioning must be included in MEPS extraction procedures to enable multiple uses of the MEPS sorbent (Moein *et al.*, 2014).

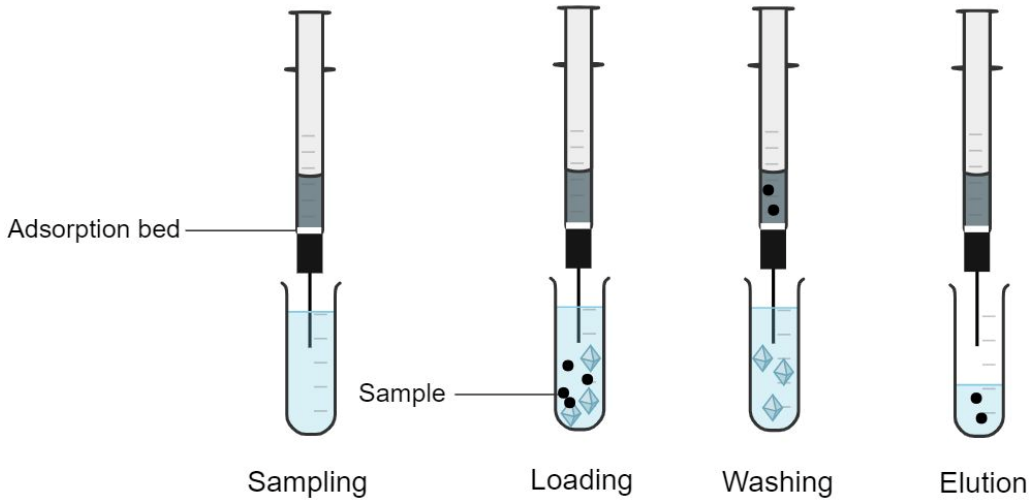


Figure 2.8 Microextraction by packed sorbent (MEPS).

2.5 Trends in liquid-based microextraction

A micro-scale liquid-liquid extraction termed liquid-phase microextraction (LPME) has been developed to reduce the consumption of organic solvents. Furthermore, various formal organic solvents with medium to high health impacts can be employed in LPME. Without a waste solvents management system, replacing or reducing the amount of the solvents used in the analytical procedure resulted in cost savings due to decreased supply of pure solvents (Moradi *et al.,* 2021). Since its introduction in 2003, liquid phase micro-extraction (LPME) has grown in popularity as a more efficient and environmentally friendly alternative to traditional LLE (Herce-Sesa *et*

al., 2021). The three majors, modalities of LPME have been widely studied: single drop micro-extraction (SDME), dispersive liquid-liquid micro-extraction (DLLME), and hollow fiber liquid phase micro-extraction (HFLPME). Figure 2.9 illustrates the trend of LPME publications.

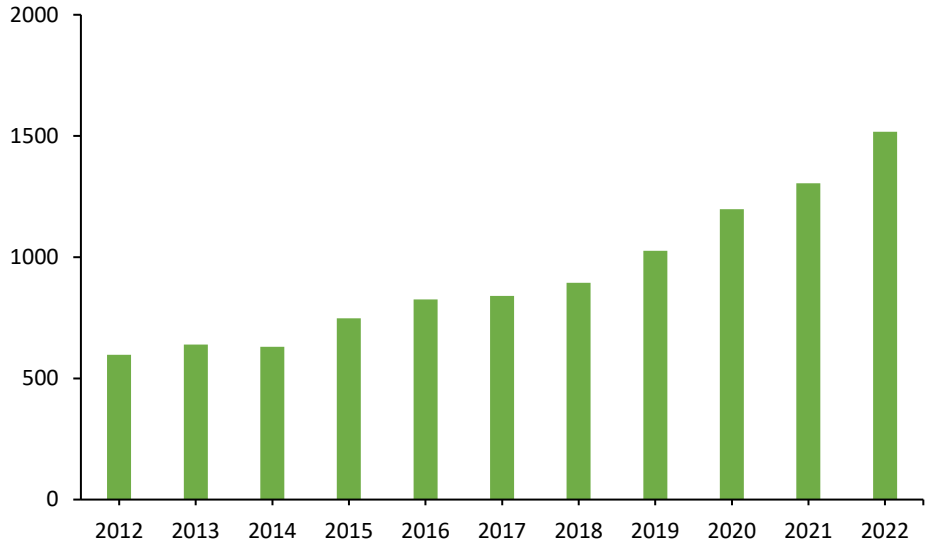


Figure 2.9 Number of publications in 2012-2022 regarding the liquid-based microextraction technique (https://www.sciencedirect.com/).

2.5.1 Hollow fiber liquid-phase microextraction (HFLPME)

The HF-LPME system is based on single, disposable, porous hollow fibers composed of polypropylene that support an organic solvent in the pores (liquid membrane) (Pedersen-Bjergaard *et al.,* 1997). According to Sharifi *et al.,* 2016, This approach is composed of four major components; Typically, the donor phase is an aqueous sample containing the desired analytes. Hollow polypropylene fiber with holes for the immobilization of organic solvents. An organic solvent that has been immobilized within the hollow fiber's pores. The acceptor phase is an organic, acidic, or

basic solution that fills the hollow fiber lumen. These components are illustrated in Figure 2.10.

Practically, analytes of interest are extracted from aqueous samples by passing them through a small layer of organic solvent (several microlitres) trapped inside the pores of a porous hollow fiber and into an acceptor solution within the hollow fiber's lumen. Organic acceptor phases result in a two-phase extraction arrangement that is immediately compatible with capillary gas chromatography. In addition, it can be an aqueous solution, resulting in a three-phase system that can be used with high-resolution liquid chromatography, capillary electrophoresis, or mass spectrometry. Movement in the donor and acceptor phases is pH-dependent in two and three-phase setups (Bello-López *et al.,* 2012).

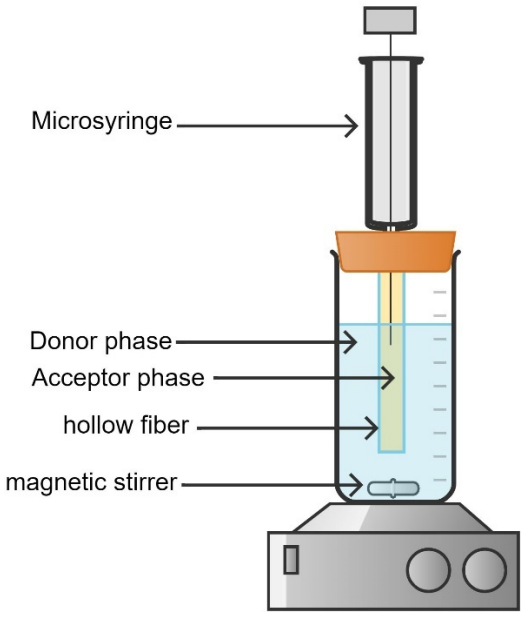


Figure 2.10 HFLPME components.