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ADVANCED ADSORBENTS FOR THE EXTRACTION AND PRECONCENTRATION OF PENICILLIN ANTIBIOTICS: AN UPDATED REVIEW

(Penjerap Lanjutan Bagi Pengekstrakan Dan Prapemekatan Antibiotik Penisilin: Satu Tinjauan Terkini)

Wan Ibrahim Thani Abd Halim[1], Siti Munirah Ishak[1], Salwani Md Saad[1], Nadhiratul-Farihin Semail[1], Ahmad Husaini Mohamed[1,2], Mohd Yusmaidie Aziz[1], Azam Taufik Mohd Din[3], Nur Nadhirah Mohamad Zain[1], Nik Nur Syazni Nik Mohamed Kamal[1], Sazlinda Kamaruzaman[4], Noorfatimah Yahaya[1]*

[1]Integrative Medicine Cluster, Advanced Medical and Dental Institute (AMDI),
Universiti Sains Malaysia, 13200 Bertam Kepala Batas, Penang, Malaysia
[2]Universiti Teknologi MARA Negeri Sembilan,
Kampus Kuala Pilah, Pekan Parit Tinggi, 72000 Kuala Pilah, Negeri Sembilan, Malaysia
[3]School of Chemical Engineering, Engineering Campus,
Universiti Sains Malaysia, 14300, Nibong Tebal, Penang, Malaysia
[4]Department of Chemistry, Faculty of Science,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

**Corresponding author: noorfatimah@usm.my*

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Abstract

Widespread use of penicillins (PENs) has brought about the pollution of wastewater and contamination of food, leading to antibiotic resistance which is detrimental to human life. PEN residues are being constantly identified in different matrices at low concentration. Several studies have been performed to extract these residues from environmental and food matrices. Sample preparation approaches like solid phase extraction (SPE) and liquid phase extraction (LLE) have been developed for their quantitation. SPE, in particular, shows great demand as an essential step to raise both practical efficiency and analytical sensitivity. Conventional SPE methods used for the extraction of antibiotics are relatively expensive, tedious and generally require long analysis time. In this review, recent miniaturized SPE methods and their applications for extraction of PENs from various matrices will be discussed. The methods represent fast, modern and efficient approaches to trace analytes. Selected examples will illustrate the various features of miniaturized SPE methods and their applications in food, biological, and environmental areas. With that, this paper presents a review of previous studies pertaining to miniaturized SPE methods that focused on the analysis of PENS in variety of matrices from the year 2015 to 2020.

Keywords: penicillins, sample preparation, solid phase extraction, advanced solid phase extraction, food security

Abstrak

Penggunaan penisilin (PEN) secara meluas telah menyebabkan pencemaran air kumbahan dan pencemaran makanan, yang berpotensi mewujudkan kerintangan antibiotik yang berbahaya terhadap kehidupan manusia. Sisa PEN kerap kali dikenal pasti dalam matriks berbeza pada kepekatan rendah. Beberapa kajian telah dilakukan untuk mengekstrak sisa ini dari matriks persekitaran dan makanan. Pendekatan penyediaan sampel seperti pengekstrakan fasa pepejal (SPE) dan pengekstrakan fasa cecair (LLE) telah dibangunkan untuk penyukatan secara kuantitatif. SPE, terutamanya, menunjukkan permintaan yang besar sebagai langkah penting untuk meningkatkan kecekapan praktikal dan kepekaan analisis. Kaedah SPE konvensional yang digunakan untuk pengekstrakan antibiotik agak mahal, susah dan biasanya memerlukan masa analisis yang panjang. Dalam tinjauan ini, kaedah SPE yang terkini dan aplikasi mereka untuk pengekstrakan PEN dari pelbagai matriks akan dibincangkan. Kaedah-kaedah ini mewakili pendekatan pantas, moden dan cekap untuk mengesan bahan analit. Contoh-contoh yang terpilih akan memberi gambaran mengenai ciri-ciri SPE lanjutan yang pelbagai dan aplikasi mereka dalam bidang makanan, biologi dan persekitaran. Dengan itu, manuskrip ini memberi tinjauan bagi kajian yang melibatkan kaedah SPE lanjutan yang mengfokuskan kepada analisa PENS di dalam pelbagai matriks dari tahun 2015 sehingga 2020.

Kata kunci: penisilin, penyediaan sampel, pengekstrakan fasa pepejal, pengekstrakan fasa pepejal lanjutan, kawalan makanan

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Introduction

Veterinary antibiotics are necessary for efficient production of meat, milk, and eggs in the frame of hygienic management of farms. Antibiotics have multiple uses depending on concentration level. At low levels they act as growth promoters, at intermediate levels they are to prevent diseases, while at high levels they are used as treatment for infected animals [1]. Among the antibiotics, β-lactam antibiotics (BLAs) constitute one of the most widely used drugs in veterinary medicine, especially to treat and prevent bacterial infections of dairy cattle [2]. BLAs can be classified into several subgroups according to their structural characteristics: penicillins (PENs), cephalosporins, and, more recently, carbapenems [3]. The penicillins subgroup is one of the most valuable drugs in primary care [4].

A penicillin's basic structure (as shown in Figure 1) consists of four parts: a thiazolidine ring, a β-lactam ring attached to it which forms 6-aminopenicillanic acid, a side chain R1 in the 6-position, and another side chain R2 which usually holds hydrogen except for the case of esters of some members. R1 determines the stability and the antimicrobial activity of the different derivatives [1]. Several common PENs are shown in Figure 2.

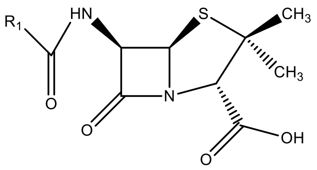
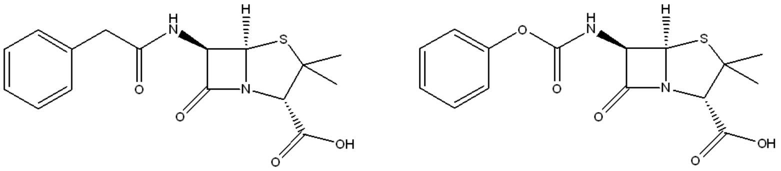
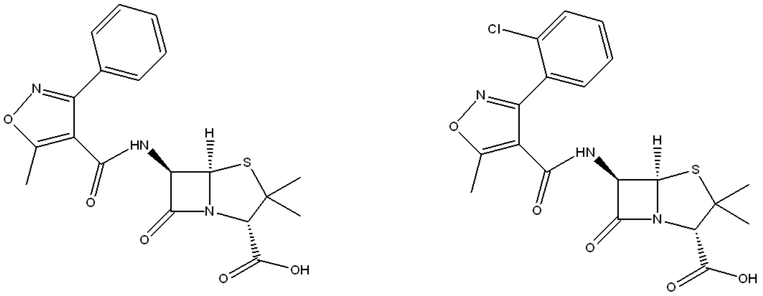


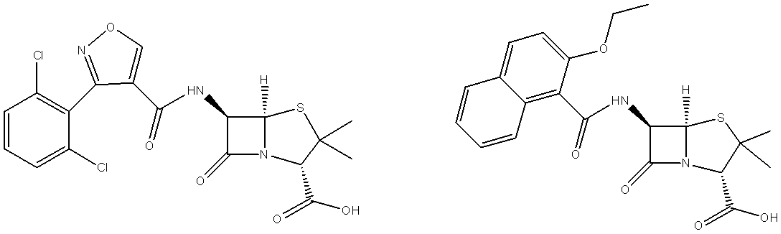
Figure 1. General structure of PENs (R = lateral amino chain)



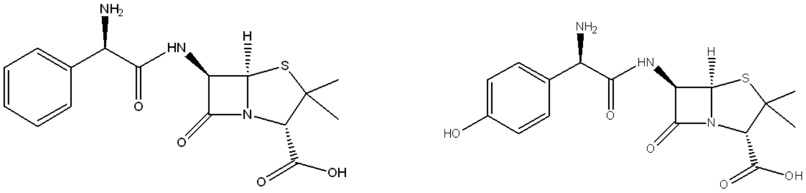
Penicillin G Penicillin V



Oxacillin Cloxacillin



Dicloxacillin Nafcillin



Ampicillin Amoxicillin

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Figure 2. Chemical structure of selected penicillins

PENs are the most frequently used antibiotics in poultry production [5]. In dairy industry, PENs have been used for mastitis treatment [2]. The use of PENs in both treatment and prevention of infection has resulted in antibiotic residues in animal edible tissue [6] and over time, the residues can lead to health problems in human and increase the incidence of microbial resistance against antimicrobial compounds [7]. In the past few years, due to heightened public concern regarding the overuse of antibiotics in food-producing animals and the risk of transmission of antibiotic-resistant bacteria to man, the control of antibiotic residues in edible animal tissues has been made mandatory [8]. To protect consumers from potential health risks, the EU has defined safe maximum residue limits (MRL) for PENs through Commission Regulation (EU) number 37/2010. The limits implemented in foodstuffs for PENs antibiotics are shown in Table 1.

Excessive use of veterinary antibiotics is one of the major sources of antibiotic residues in environment [9]. Since these compounds are poorly absorbed and metabolized by the animals, approximately 30-90% of them are excreted in the original form while the rest are excreted in the form of metabolites [10]. The use of animal manure as fertilizers on agricultural lands could lead to antibiotic pollution on the environment. Without proper treatment and waste management, antibiotic residues from farms, hospitals, pharmaceuticals and medical wastes would end up contaminating natural environments [11]. The antibiotic residues could enter other environmental media like soil, river and sea or end up in the food chain, further contributing to the appearance and transfer of antibiotic-resistant bacteria to human [8, 11].

To date, high performance liquid chromatography (HPLC) has been the most used technique for analyzing PENs and other BLAs in different matrices, even more so when compared to gas chromatography (GC) method [12]. However, the determination of the antibiotics is a difficult task due to the complexity of matrices like food and biological fluid, caused by the existence of various biochemical compounds such as proteins or fats [13]. Hence, further efficiency requires an improvement in the sample treatment step [14].

Numerous sample treatment methods have been developed to determine veterinary drugs in various samples, which include protein precipitation, liquid-liquid extraction (LLE), and solid phase extraction (SPE) [15]. Among them, SPE is one of the most popular techniques for pre-concentration of analytes in different samples due to its high enrichment factors, low reagent consumption, simplicity, speediness and convenience [16]. For optimization, adsorbent selection plays a key role in SPE procedures [17]. A new method of SPE called solid phase microextraction (SPME) technique has been on the rise since its introduction in the early twentieth century. SPME, which is a sorption-based, is considered to be a miniaturized version of SPE [18]. Following its success, SPME has been widely exploited by optimizing advanced materials which are more selective and have higher adsorption capacity in order to expand the availability of inexpensive, more easily synthesized adsorbents [19]. Other methods such as magnetic solid phase extraction (MSPE), dispersive solid phase extraction (DSPE) and stir bar sorptive extraction (SBSE) also received considerable attention as alternative means to sample preparation. MSPE, which is based on magnetic adsorbent, became an attractive approach in sample preparation due to its excellent adsorption efficiency and facile isolation from the mixture solution without reiterative centrifugation [20, 21].

Meanwhile, in DSPE method, the solid adsorbent is added directly to the sample solution without manipulation processes. Therefore, the extraction process relies only on shaking and centrifugation. The dispersion phenomenon allows the adsorbent to interact with all target rapidly and uniformly [22]. Another novel approach to sample enrichment in aqueous solution is SBSE, where the analyte is extracted by magnetically stirring a stir bar covered with a thick layer of polydimethylsiloxane (PDMS) in the sample solution [23]. In this technique, the phase, similar to GC stationary phases, is coated and bonded onto a magnetic stir bar. The stir bar is then immersed into the liquid sample for extraction [24]. The major advantages of this technique are its ease of use, improved sensitivity, high accuracy of analysis and reduced risk of contamination [25].

Despite its widespread use, conventional SPE extraction processes using the commercially available SPE cartridges are time-consuming and tedious [25]. Design of the column as well as the packed adsorbent are made to restrict flow rates in order to avoid channeling phenomenon, resulting in lengthened extraction process. Another main disadvantage of SPE based on cartridge format is the blockade of frits within passage of dirty samples [26]. On the other hand, in the newly developed miniaturized SPE methods, a number of adsorbent materials including carbon, silica, molecularly imprinted polymers, metal organic frameworks and

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ionic liquid modified nanoparticles have been developed with particularly useful applications in analytical chemistry [27]. The common advantages of these materials are their sensitivity, rapidity and efficiency.

In this review, recent development of different types of adsorbent materials and their applications in sample preparation for determination of penicillin are summarized and evaluated. The miniaturized SPE methods have been reviewed previously. The present review primarily addressed the development, from the year 2015 to 2020, of sample preparation method, the materials used, method of extraction, the sensitivity or limit of detection (LOD) and recovery of the compound.

Table 1. MRLs established by Commission Regulation (EU) number 37/2010 for PENs residues in foodstuffs

Analyte	MRL (µg kg^{-1})	
	Milk	Bovine
Oxacillin	30	300
Cloxacillin	30	300
Dicloxacillin	30	300
Nafcillin	30	300
Penicillin G	4	50
Penicillin V	4	50
Ampicillin	4	50
Amoxicillin	4	50

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Adsorbent-based Sample Preparation Technique

Nanoparticles

Over the past few decades, nanoparticles have been extensively used worldwide in many fields such as material sciences, physics, medicine, electronic and chemistry [28]. The shapes and types of nanomaterials such as nanotubes, nanohorns and nanocages can be prepared from organics and inorganics materials [29]. Nanoparticles differ from classical solid particles by their size which is smaller by several orders of magnitude, and by their specific properties due to their high surface area over volume ratio [30]. Nanoparticles are almost zero-dimensional which means all dimensions are in the nanoscale (1-100nm) [31]. Due to the sheer small size, particles in nanoscale often have different physical, chemical and biological properties compared to macro and micro scale [32]. Nanoparticles can be used for further enhancement of SPE methods as they have high surface area-to-volume ratio and provide a surface functionalization which can be tailored depending on applications.

Carbon-based adsorbents

Carbon-based materials are made up carbon atoms in the form of cages, sheets or tubes. Carbon nanotubes (CNTs) are one of such materials in the form of tubes. Due to their structure and adsorption properties, CNTs are considered promising adsorbents for SPE. This allotrope of carbon was first discovered in 1991 by accident during experimental synthesis of fullerene in an electric arc. CNTs are made up of graphene sheets rolled into cylindrical structure, with diameter in the range of nanometer and length up to several micrometers. There are two types of CNTs, single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs). SWCNTs are produced from the rolling of a single graphene sheet, while MWCNTs are composed of multiple SWCNTs arranged compactly together by van der Waals force around an axis. Properties of CNTs like lightness, high tensile strength, high resistance to mechanical damage, high thermal stability and conductivity and excellent electric conductor make them versatile materials for various applications including extraction. As adsorbents for SPE, CNTs provide large surface area for adsorption, and allow for fast and rapid extraction rate. Their selectivity towards target analytes and sensitivity in various matrices can be enhanced by further functionalization of carbon [33].

In 2016, Ahmadi et al. crown-functionalized a magnetic MWCNTs (MMWCNTs) adsorbent using a supramolecular compound 4,4'-diamindibenzo-18-crown-6 (DADB18C6), for spectrophotometric quantification of amoxicillin [34]. DADB18C6, which was synthesized according to Keypour et al. [35], has excellent host-guest molecular recognition ability. In addition to its high selectivity, the host, with its large cavities, showed better incorporation of guest molecules [35]. In the study, the sensitivity of amoxicillin detection was enhanced spectrophotometrically by treating the amoxicillin containing solution with aryldiazonium ion. This ion reacts with nucleophilic amoxicillin to produce a colored azo derivative of amoxicillin, which is more sensitive towards spectrophotometric detection than pure amoxicillin. The optimized method was validated by analyzing real life samples, amoxicillin capsules and human urine. The method succeeded in detecting amoxicillin in the concentration range of 5.0–1000.0 ng mL^{-1} with the LOD of 3.0 ng mL^{-1} [34].

Following this study, in 2018, Jia et al. developed a disposable SPE membrane for rapid removal and sensitive surface-enhanced Raman scattering (SERS) to detect antibiotics in water samples [37]. The membrane was prepared by trapping the activated carbon modified with silver nanoparticles (Ag NPs/AC) solution on the commercially available SPE column. The activated carbon showed high adsorption ability due to its porous structure and high surface area. In addition, the resulting "hotspots" due to embedded silver nanoparticles amplified Raman signals and thus improving SERS detection by Raman spectrometer. After optimizing the method, penicillin G sodium and ampicillin were analyzed for method validation. Penicillin G sodium was detected in the concentration range of 6.0×10^{-9} to 4.0×10^{-5} M with the LOD of 2.5×10^{-9} M, while ampicillin was detected in the concentration range of 8.0×10^{-9} to 8.0×10^{-5} M with the LOD of 3.2×10^{-9} M. The low relative standard deviation (RSD), which is less than 15% indicated that this disposable SPE membrane is highly reproducible [37].

The most recent study involving PENs detection using carbon-based adsorbents was by Golzari Aqda et al. [38]. They performed SPME using a graphene-oxide-starch-based nanocomposite for the determination of three antibiotic residues (amoxicillin, ampicillin and cloxacillin) in 114 milk samples. The pre-concentrated samples were further analyzed using high-performance liquid chromatography-ultraviolet detection (HPLC-UV). In this method, the synthesis of graphene oxide was initiated based on Hummers method. Then, following the procedure by Pourjava et al. [39], four types of hydrogels were synthesized: graphene oxide-starch-based nanocomposite without calcium carbonate ($CaCO_3$), graphene oxide-starch-based nanocomposite in the presence $CaCO_3$, porous graphene oxide-starch-based nanocomposite after removing $CaCO_3$, and starch-graft-polyacrylamide without $CaCO_3$ and graphene oxide. From the four hydrogels, hydrogels of porous graphene oxide-starch-based nanocomposite after removing $CaCO_3$ showed the highest extraction efficiency compared to the other three hydrogels. This was contributed by the use of graphene oxide and $CaCO_3$ that has increased the surface area of the adsorbent. The subsequent removal of $CaCO_3$ after its entrapment inside the nanocomposite structure resulted in increase in porosity and surface area. The analytes were adsorbed on the porous adsorbent by hydrogen bond interactions. After optimization, the method managed to detect the selected antibiotics in the concentration range of 2.7–5.0 μg kg^{-1} with the LOD of 0.8–1.5μg kg^{-1} [38]. The results of the studies are tabulated in Table 2.

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Table 2. Recent applications of miniaturized SPE for the determination of penicillin antibiotics in various matrices (2015-2020)

Type of SPE	Adsorbent Material	Analytes	Matrices	Detection method	Amount detected (μg L^{-1} or μg kg^{-1})	Sensitivity (LODs) (μg L^{-1} or μg kg^{-1})	Recovery (%)	Ref
MSPE	Crown-MMWCNTs	Amoxicillin	Amoxicillin capsules, human urine	UV-Vis	19.8 – 49.7 48.1 – 510.5	3.0	98.0 – 98.5 96.2 –102.1	[34]
Disposable, highly reproducible SPE membrane	Activated carbon nanocomposite modified with silver nanoparticles	Penicillin G sodium, ampicillin	Standard solution	Raman spectrometer	not recorded	0.9	not recorded	[37]
SPME	Graphene-oxide-starch-based nanocomposite	Amoxicillin, ampicillin, cloxacillin	Cow milk	HPLC-UV	13.8 – 242.9	0.8 – 1.5	83.0 – 105.0	[38]
MISPE	Silica@MIP	Oxacillin, cloxacillin, dicloxacillin	Milk	HPLC	7.5 – 480	1.60 – 1.90	92.0 – 102.0	[49]
SPME	silica@chitosan-glutaraldehyde	Penicillin G	Tap, river, lake, well water, wastewater	HPLC	1 – 300	0.49	91.0 – 123.3	[17]
MISPE	NAFC- MAA-EGDMA-ACN-AIMN	Ampicillin, amoxicillin, oxacillin, penicillin G, penicillin V, cloxacillin, dicloxacillin, nafcillin	Powdered infant formulas	HPLC-MS/MS	Not recorded	0.7 – 23.6	60.0 – 91.0	[3]
MISPE	Sulfaguanidine-imprinted polymer	Penicillin G/procaine	Wellspring water, wastewater	HPTLC	Not detected	5.0	95.0 – 108.0	[54]
SMISPE	Ampicillin-surface-molecularly-imprinted polymer	Ampicillin	Commercial milk, rat blood	HPLC-UV	Not detected Detected, not recorded	50.0 150.0	63.8 – 82.5 67.3 – 79.1	[11]
MISPE	Ampicillin-imprinted polymer	Ampicillin	Cow milk	HPLC-UV	Not recorded	35.8	> 95.0	[55]

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Table 2 (cont'd). Recent applications of miniaturized SPE for the determination of penicillin antibiotics in various matrices (2015-2020)

Type of SPE	**Adsorbent Material**	**Analytes**	**Matrices**	**Detection method**	**Amount detected (µg L^{-1} or µg kg^{-1})**	**Sensitivity (LODs) (µg L^{-1} or µg kg^{-1})**	**Recovery (%)**	**Ref**
SPME	Aluminium-based MOF polymer monoliths	Penicillin G, penicillin V, oxacillin, cloxacillin, dicloxacillin, nafcillin	River water	UPLC-UV	Not detected	0.06 – 0.26	81.0 – 100.7	[57]
UAMSPE	Copper-based magnetic MOF	Ampicillin	Cow milk	HPLC-UV	Not detected	0.29	95.0 – 100.8	[59]

**Abbreviations:*
MSPE – Magnetic solid phase extraction
SPME – Solid phase microextraction
MISPE – Molecularly imprinted solid-phase extraction
SPME – Solid phase microextraction
SMIPSE – Surface molecularly imprinted solid-phase extraction
SPME– Solid phase microextraction
UAMSPE – Ultrasonic-assisted magnetic solid phase extraction

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Silica-based nanoparticle

As a relatively inexpensive, chemically inert, thermally stable, and biocompatible material, silica is very appealing for analytical applications. Over the past two decades, silica nanoparticle (SiNP) has gained much attention on their application in catalysis, separation, biosensors, and adsorption [29] because SiNP is easy to be synthesized and modified by altering the functional groups on their surface [40]. According to Wang et al. [41], there are several physical properties that makes silica an attractive adsorbent, one of them being particle sizes can be tuned from 50 to 300 nm with stable and rigid frame that provides resistance to mechanical stress and degradation. Another desirable property is pore diameters can be tuned between 2 and 10 nm permitting different chemicals or analytes loading. Also, SiNP has a high surface area (> 700 m^2/g) and large pore volume (> 0.9 cm^3/g) allowing high loadings of chemicals. Silica nanoparticles were produced though different methods such as reverse micro emulsion, Stöber process, flame synthesis and widely utilized sol-gel method, in addition to several other novel methods later on [42]. The chemically synthesized silica nanoparticles are more widely used, because the chemical route is easy to comprehend and offers more options for modification of the different parameters of the silica nanoparticle [42].

In recent years, hybrid and composite materials have been extensively studied due to their diverse properties [43]. Their properties combine the advantages of both inorganic and organic materials [44]. In this respect, organically modified silica nanoparticles are a typical class of hybrid materials [45]. Due to the challenges in the detection of penicillin in complex samples such as food, it is crucial to develop sampling and sample preparation procedures for efficient analysis [46]. MSPE with magnetic nanoparticles (MNPs) has generated great interest in the analytical community [47]. However, pure MNPs suffer from an inherent limitation as they tend to agglomerate, which alters their magnetic properties in complex matrices. Therefore, the modification of these MNPs is essential to overcome such limitation. Among the different materials, silica nanoparticles have been proven to have the proper ability to enhance mechanical and interfacial properties [17]. At present, the Stöber process, sol-gel process, and aerosol pyrolysis seem promising for coating iron oxide nanoparticles with silica [48].

In 2016, Urraca et al. has developed selective molecularly imprinted polymers in the form of microspheres, using porous silica beads (40-75µm) for the analysis of beta-lactamase-resistant penicillins [49]. Molecularly imprinted polymers (MIPs) have been shown to be a useful alternative to immune-affinity columns and other commercially available SPE cartridges for sample clean-up and selective pre-concentration of different analytes in complex matrices, as the so-called molecularly imprinted solid-phase extraction (MISPE) [50]. In the study, the analytical method was validated according to EU guideline 2002/657/EC. The limits of quantification (S/N=10) were in the 5.3-6.3 µg kg^{-1} range and well below the maximum residue limits (MRLs). Interday mean recoveries were in the acceptable range of 99–102% with RSDs below 9% for the analysis of penicillins in milk samples.

Recently, Hassan et al. has synthesized silica@chitosan-glutaraldehyde (Si@Cs-G) for extraction of penicillin G (PG) from the synthetic and real samples followed by HPLC determination [17]. Chitosan (Cs) is a polysaccharide polymer which has bifunctional groups and as a consequence, possesses unique physiological activity and physiochemical properties. Hydrophilicity, harmlessness, biodegradability, low cost, abundance and ease of chemical derivatization result in Cs being regarded as a useful and auspicious material for preparing the adsorbent [51]. However, Cs is easily oxidized or destructed when exposed to high concentration of pollution and low pH, making Cs unstable and unreliable [52]. Therefore, modification with silica is necessary because silica nanoparticles have been proven to have the proper ability to enhance Cs mechanical and interfacial properties [51]. In the study, the following conclusions were observed: the optimized conditions for PG extraction by Si@Cs-G is at pH 6 with contact time 50 minutes and adsorbent dosage 25mg at room temperature. The best desorption rate was associated to acetonitrile solvent at volume of 0.75 mL under 40 minutes. Extraction recovery in synthetic samples was 99.0%, with LOD = 0.49 µg L^{-1}, limit of quantification (LOQ) = 1.64 µg L^{-1} and RSD < 1.95%. The method was successfully applied for determination of PG in real water samples (tap, river, lake and well water) and wastewater samples (hospital effluent). The obtained relative recoveries were in the range of 91.31-123.27% with RSD less than 6.34% for all the real samples. Table 2 shows the summary of silica nanoparticle-based techniques for detection of penicillins in various matrices in the years 2015-2020.

Molecularly imprinted polymers as adsorbents

Molecularly imprinted polymers (MIPs) are macromolecules synthetically produced in the presence of target molecules called template. After removing the template, the imprinted polymer would have binding sites with specific shape and size similar to target molecules. MIPs have higher selectivity and affinity towards target molecules and their analogues due to their prearranged structures and better molecular recognition ability which encourage both covalent and non-covalent bindings. In addition to having good stability, MIPs are easy to prepare and can be used repeatedly without significant loss of activity [53], making them an increasingly popular approach to selective sample preparation.

In January 2015, various MIPs adsorbent with different templates and cross-linkers have been tested for the determination of eight PENs in milk powder formulas for infants [3]. The eight PENs were ampicillin, amoxicillin, oxacillin, penicillin G, penicillin V, cloxacillin, dicloxacillin, and nafcillin. After pre-concentration step, the samples were analyzed with high-performance liquid chromatography coupled to tandem mass spectrometer (HPLC-MS/MS). In the study, oxacillin, amoxicillin, and nafcillin have been selected as template candidates. They were crosslinked to methacrylic acid and either to divinylbenzene 80%, ethylene glycol dimethacrylate or trimethylolpropane trimethacrylate. The loading and washing solvents were chosen between methanol, acetonitrile and

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acetonitrile/toluene. After optimization, nafcillin-methylacrylic acid-ethylene glycol dimethacrylate-acetonitrile was decided as the most suitable SPE adsorbent as they exhibit the highest retention capacity of the eight PENs. The PENs were detected in the concentration range of 2.4-78.4 µg kg^{-1} with the LOD of 0.7-23.6 µg kg^{-1} [3].

A study on preparation and application of sulfaguanidine-imprinted polymer was performed in 2015 to monitor eight pharmaceuticals including penicillin G/procaine from wellspring water and wastewater samples [54]. In this study, sulfaguanidine was used as the template molecule, methacrylic acid and 2-hydroxyethyl methacrylate were the functional monomers, ethylene glycol dimethacrylate acted as cross-linker, and 2,2'-azobis-isobutyronitrile served as the initiator. Unlike enzyme, which is completely specific, MIPs can retain more than one molecule. In case of sulfaguanidine-imprinted polymers, they were expected to retain eight other molecules of similar structure to sulfaguanidine, among them was penicillin G/procaine. The optimized method detected penicillin G/procaine from water samples using high-performance thin-layer chromatography (HPTLC) with the LOQ and LOD of 10 µg L^{-1} and 5 µg L^{-1}, respectively [54].

Later in 2016, Wu et al. studied a novel surface MIP (SMIP) for the selective determination of ampicillin sodium from milk and blood samples [11]. Like MIP, it uses silica gels as support. However, instead of using conventional bulk polymerization which resulted in irregular materials shape, poor site accessibility, slow mass transfer, incomplete templates removal, low binding capacity and serious templates leakage, SMIP is synthesized using surface molecular imprinting technique. This technique improves recognition site, binding capacity and mass transfer rate as well as contributing to more uniformly sized morphology. In this study, following the activation of silica gels, the silica gels were amino-functionalized before being surface-molecularly imprinted with ampicillin sodium. After method optimization, the samples were analyzed using HPLC-UV. The optimized method succeeded in detecting ampicillin in the concentration range of 5-200 µg mL^{-1}, with the LOD of 0.2 µg mL^{-1} and 0.5 µg mL^{-1} for milk and blood samples, respectively [11].

The most recent study on MIPs adsorbent for selective pre-concentration of PEN antibiotics was in 2017, where Soledad-Rodríguez et al. prepared MIPs with ampicillin as template for determination of ampicillin drugs in cow milk samples [55]. Through bulk polymerization, the procedure utilized methacrylic acid or methyl methacrylate as functional monomers, ethylene glycol dimethacrylate as crosslinking monomer, acetonitrile as porogen and hydrogen peroxide as initiator. In the study, the ratio of functional monomer to cross-linker was manipulated to achieve the highest binding capacity towards ampicillin drugs. Ratio 1:5 was selected as the most suitable ratio to achieve intended results. The cow milk samples went through quantitative analysis using HPLC-UV. The optimized method's LOQ and LOD were 10.7 µg L^{-1} and 35.8 µg L^{-1}, respectively [55]. Table 2 shows the results of selected studies on MIPs adsorbents.

Metal-organic frameworks as adsorbents

Metal-organic frameworks (MOFs) are crystalline network formed *via* coordination bonds between inorganic metals and multidentate organic ligands. MOFs are synthesized by self-assembly in a one-pot reaction. Varying metals and ligands and reaction conditions allows the pore size of MOFs to be manipulated to suit any specific application. The most common synthetic methods are solvothermal synthesis and hydrothermal synthesis. In both methods, high temperature and pressure result in increased rate of reaction. However, mild reaction conditions are preferred to obtain perfect crystalline formation. For SPE, four types of MOFs are commonly used: isoreticular MOF (IRMOF) series, materials of Institute Lavoisier (MIL) series, zeolitic imidazolate framework (ZIF) series, and University of Oslo (UiO) series [56].

In 2016, a study by Lirio et al. highlighted the application of MIL-53 as aluminium-based MOF (Al-MOF) polymer monolith adsorbents in solid phase microextraction (SPME) of selected penicillins [57]. In this study, MIL-53 was first synthesized according to Loiseau et al. [58] under hydrothermal conditions using

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aluminum nitrate nonahydrate as the metal and 1,4-benzenedicarboxylic acid as the ligand. Then, MIL-53 was mixed with butyl methacrylate and ethylene dimethacrylate monomers for co-polymerization, with azobisisobutyronitrile as the initiator. The resulting mixture was pretreated before being sealed into SPE column and underwent microwave-assisted polymerization for five minutes. The optimized method was utilized to analyze river samples for the determination of penicillin G, penicillin V, oxacillin, cloxacillin, dicloxacillin, and nafcillin using ultra-performance liquid chromatography-ultraviolet detection (UPLC-UV). The method managed to detect PENs with the LOQ of 0.20–0.87 μg L^{-1} and LOD of 0.06–0.26 μg L^{-1} [57].

Following that study, in 2020, Bagheri and Ghaedi developed copper-based magnetic MOFs (Cu-MMOF) as adsorbents for ultrasonic-assisted magnetic SPE (UAMSPE) of ampicillin from cow milk samples prior to HPLC-UV [59]. In addition to enhancing the adsorption capacity by MOF, the magnetic properties of the adsorbents decreased extraction time by eliminating the need for filtration, centrifugation and precipitation processes. Firstly, magnetic nanoparticles were synthesized according to their previous work [60]. Then, copper (II) nitrate trihydrate as metal and terepthalic acid as ligand were reacted in a one-pot solvothermal synthesis for 12 hours to obtain the Cu-MMOF adsorbents. After optimization, this method achieved detection in the concentration range of 1–5000 μg L^{-1} with the LOD of 0.29 μg L^{-1} [59]. Results for related studies were shown in Table 2.

Conclusion

Numerous numbers of nanomaterials with chemical composition and properties have been found to be excellent absorbents in sample preparation. In addition, the surface functionalization or modification of different nanomaterials aid the development of versatile approaches for efficient analysis of various analytes. So far, all the methods used have achieved the MRL detection with low LOD between 0.05-150.0 μg L^{-1}. This review paper has established that miniaturized SPE can be a powerful method for sample preparation today. Based on recent studies, miniaturized SPE methods like MSPE and SPME have much potential to trim down preparation time, laborious effort, cost and high chemical usage commonly associated with sample preparation. Miniaturized SPE adsorbent materials like magnetic adsorbents can be recovered and used repeatedly; and they eliminate the problems that come with conventional SPE cartridges. Optimizing the choice of adsorbents and maximizing their adsorption efficiency are the correct approach to solving the difficulties in extracting analytes from complex matrices. However, still much effort is required to improve application of advanced adsorbents so that they can extract analytes with higher selectivity, lower LOD and higher extraction recoveries. All in all, the future of advanced adsorbents as alternative for extraction of antibiotics including PENs is very promising. With collaboration between research institutes and governmental bodies, a facile, robust and economical sample preparation procedure using advanced adsorbents could be established to monitor MRL of antibiotics in our environmental water and food in the future.

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