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Aptamer-functionalized magnetic supports for sample preparation

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ABSTRACT

In the last few years, aptamer-functionalized materials have been used as promising affinity sorbents for sample preparation purposes. These selective materials have attracted much attention due to their excellent molecular recognition properties, high stability, and the possibility of incorporation onto the surface of different supporting materials, such as nanoparticles, polymers, etc. On the other hand, current trends in extraction techniques encompass the use of miniaturized devices integrated with advanced affinity-based sorbents. Within these sample preparation devices, magnetic nanoparticles have become excellent candidates for the preparation of new functional nanomaterials for the solid-phase extraction processes. In addition to this approach, magnetic elements can be integrated in extraction devices (e.g., stir bars, stir cakes, etc.) to provide efficient extraction/stirring integrated techniques able to simplify the extraction and improve the performance. This review gives an overview of the literature published regarding aptamer-based sorbents using as host supports magnetic nanoparticles and integrated stirred units. For this purpose, the most relevant developments and applications achieved in the last seven years (from 2017 to 2023) in sample preparation by the integration of these affinity-based materials in extraction techniques such as magnetic solid-phase extraction and stir bar sorptive extraction will be reviewed.

1. Introduction

Sample preparation can be considered as one of the most relevant steps in the whole analytical process, exerting influence on subsequent steps in the analytical workflow. Indeed, it may involve several operations such as mineralization, extraction, distillation and filtration, among others [1]. In fact, it would be advisable to circumvent this step by selecting direct analytical methods, wherein analytes are directly determined in the original sample. However, in many cases, isolation and preconcentration of analytes are required to reach adequate sensitivity and selectivity. Despite notable advancements in analytical instrumentation, especially in the integration of mass spectrometry and chromatographic separation, these techniques still lack the required sensitivity for the direct analysis of intricate matrices. Thus, sample preparation remains a key stage to extract and/or preconcentrate the analyte/s of interest from different types of complex samples such as biological fluids, foodstuffs or environmental samples [2,3].

Currently, microextraction techniques are established as reliable and environmentally friendly sample preparation procedures. The success of these techniques comes from their advantages that include simplicity, high reproducibility, good recoveries, lower consumption of organic solvents, reduced cost, adaptability to various organic or inorganic analytes

Abbreviations: 8-OHdG, 8-Hydroxy-20-deoxyguanosine; β-LG, β-Lactoglobulin; μSPE, Micro-solid-phase extraction; AFB_1, Aflatoxin B_1; AFB_2, Aflatoxin B_2; AFM_1, Aflatoxin M_1; Apt, Aptamer; BPA, Bisphenol A; CAP, Chloramphenicol; Con A, Concanavalin A; COF, Covalent organic framework; DMSPE, Dispersive micro solid-phase extraction; EDC, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; ELISA, Enzyme-linked immunosorbent assay; FAAS, Flame atomic absorption spectrometric; FF, Florphenicol; FLD, Fluorescence detection; GC, Gas chromatography; GMA, Glycidyl methacrylate; GO, Graphene oxide; HB, Hyperbranched; HPLC, High-performance liquid chromatography; Kana, Kanamycin; LDHM, Layered double hydroxide magnetic; LOD, Limit of detection; MALDI-TOF, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight; MAM, Magnetic agarose microspheres; MCE, Microchip electrophoresis; MEPS, Microextraction by packed sorbent; MIP, Molecularly imprinted polymer; MNP, Magnetic nanoparticle; MOF, Metal-organic framework; MS, Mass spectrometry; MS/MS, Mass spectrometry in tandem mode; MSPE, Magnetic solid-phase extraction; NHS, N-hydroxy-succinimide; NP, Nanoparticle; OH-PCB, Hydroxylated polychlorinated biphenyl; OTA, Ochratoxin A; PCB, Polychlorinated biphenyl; PTFE, Polytetrafluoroethylene; RDSE, Rotating disk sorbent extraction; SBSE, Stir bar sorptive extraction; SCSE, Stir cake sorptive extraction; SDM, Sulfadimethoxine; SELEX, Systematic Evolution of Ligands by EXponential enrichment; SPME, Solid-phase microextraction; SRSE, Stir-rod sorptive extraction; TAP, Thiamphenicol; TFME, Thin-film microextraction; UHPLC, Ultra high-performance liquid chromatography; UV, Ultraviolet.

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in different formats and high enrichment factor [4], among others. These techniques can be classified according to the type of extraction phase. In particular, special emphasis has been put on sample-preparation techniques based on solid phase [5]. In this sense, different alternatives (or approaches), primarily based on the type of extraction devices and the deposition of the solid phase, have been proposed such as solid-phase microextraction (SPME), thin-film microextraction (TFME), microextraction by packed sorbent (MEPS), micro-solid-phase extraction (μSPE), dispersive micro-solid-phase extraction (DMSPE) and magnetic solid-phase extraction (MSPE) [5,6], jointly with the so-called extraction/stirring integrated techniques such as stir bar sorptive extraction (SBSE), stir cake sorptive extraction (SCSE), stir-rod sorptive extraction (SRSE) and rotating disk sorbent extraction (RDSE) [7]. In these techniques, the target analytes can be retained and extracted from liquid samples or extracts using non-specific sorbents based on hydrophilic or hydrophobic interactions, before being subjected to their posterior analysis. However, one of their main disadvantages of these sorbents is their limited selectivity for trace analysis in many complex environmental, food, or biological samples owing to co-extraction of matrix components. To overcome this problem, several strategies that promote a selective retention mechanism based on molecular recognition have been described, including the use of antibodies, molecularly imprinted polymers (MIPs) and more recently the incorporation of aptamers (Apts) [3].

Apts are single-stranded RNA or DNA molecules, able of developing well-defined 3D structures, that exhibit high affinity and specificity for specific targets, ranging from individual molecules to entire organisms. They have many advantages such as high reproducibility and ease synthesis, higher stability and longer shelf life, no need of animals, relatively low cost, and tailorable chemical modification [8,9]. Apts can be generated via an in vitro selection procedure, known as Systematic Evolution of Ligands by EXponential enrichment (SELEX). This method consists of repeating several SELEX cycles until obtaining an oligonucleotide, from a random library, that binds specifically to the molecular target [10]. In addition to serving as a promising alternative to antibodies, they also present additional advantages over other artificial recognition systems, such as MIPs: where several challenges still remain, particularly in terms of mitigating non-specific binding and effectively preparing for large biomolecules, among other issues. On the other hand, Apts show certain limitations regarding their degradation in biological fluids caused by interactions with biomolecules (for example, nucleases with RNA Apts) and their tertiary structure's dependence on solution conditions. For instance, the use of high organic solvent in content can influence the Apt folding, which can affect its affinity for the target analyte(s) [3].

Taking into account the aforementioned advantages, the preparation of Apt-functionalized materials has attracted considerable interest in the last years, and a wide range of Apt applications can be found, where excellent reviews (with varying emphases) have been published [11,12]. Also, the integration of Apts combined with different supporting materials has been done to develop promising affinity sorbents in different microextraction techniques, and several reviews on this topic have been published in the last years [3,9,13]. However, in these works, the use of magnetic materials to perform magnetic SPE as sample preparation technique that is easy to perform has been briefly addressed. Additionally, the development of Apt-based affinity sorbents in stirred integrated techniques (such as SBSE) have received scarce attention, which is likely associated with the difficulties found in the preparation of durable coatings suitable for analyzing complex matrices.

In order to fill these gaps, the present review focuses on the most recent contributions (2017–2023) of aptamer-based miniaturized extraction approaches based on magnetic SPE and stirred integrated techniques (with special emphasis in SBSE). First, a brief explanation of these techniques is carried out jointly with the common binding protocols employed in the immobilization of Apts onto the surface of investigated supports. Then, the application of these affinity sorbents in environmental, food and biological samples will be discussed. Finally, challenges and future perspectives to develop Apt-based sorbents in these techniques and other sample preparation approaches is discussed.

2. Aptamer-based materials for magnetic SPE and SBSE

2.1. Aptamer-based materials for magnetic SPE

As mentioned above, several extraction modes have been described using Apt-based sorbents, being SPE the most popular extraction technique [3,8,9,13]. In the last years, magnetic SPE (MSPE) has consolidated as promising extraction technique due to their advantages such as enhanced contact surface area, efficient adsorption capabilities, easy-to-use operations, and the ability to adapt to various experimental conditions. This technique uses magnetic nanoparticles (MNPs) or magnetic beads (MBs) as miniaturized support, and these can be easily separated from the matrix sample by the application of an external magnet, thus avoiding filtration and centrifugation steps [14]. The use of nanostructured magnetic materials offers further advantages compared to traditional sorbents such as large surface-area-to-volume ratio, short diffusion route, high number of surface-active sites, and superparamagnetic features [15]. Other of their advantages include low organic solvents' consumption and reusability of the sorbents, thereby positioning them as environmentally friendly and cost-effective materials. Taking into account all these benefits and the good selectivity of Apts, the preparation of Apt-functionalized magnetic nanomaterials is highly desirable for extraction purposes.

Among the various types of magnetic nanomaterials used for extraction purposes, iron oxides such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticles (NPs) are the most commonly used. This is primarily attributed to their straightforward preparation, ease of functionalization and operation [16]. Once synthesized the MNPs, the magnetic core is usually coated with materials such as silica, styrene/acrylamide copolymer, or gold nanoparticles (AuNPs). Then, the resulting core-shell structure is modified with the Apt.

In order to prepare Apt-functionalized MNPs, the immobilization of modified Apts onto the core-shell support using appropriate linker groups is carried out. This approach aims to preserve the specific performance of Apts and prevent non-specific interactions of Apt-based materials. The most commonly used method involves the covalent attachment of NH_2-modified Apts to the support through the reaction of the free amino groups of the Apt with a platform containing reactive epoxide, aldehyde, carboxyl groups or alternatively moieties that can be activated using carbonyl diimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), or N-hydro-succinimide-groups, among others. Additionally, click chemistry has been employed for Apt immobilization [17]. These immobilization methods and their detailed protocols have been reviewed in recent literature [3].

2,2. Aptamer-based materials for SBSE

As mentioned above, another interesting approach is based on the integration of the agitation (e.g. inert magnetic stir bar) and extraction elements in the same device. This integration simplifies the workflow, minimizes analyte losses, and maximizes extraction yields, leading to more efficient and reliable analytical results. Within the extraction/stirring techniques, to our knowledge, SBSE has been the only technique used with these affinity ligands. SBSE is based on the same principles as SPME, however, the amount of sorptive phase on the stir bar is 50–250 times higher than that in SPME, leading to a significant enhancement in recovery and extraction capacity. Moreover, SBSE is also more robust and present greater sensitivity than SPME, since the bonding between the phase and the stir bar is strong, ensuring remarkable stability and reusability [18].

Concerning sorbent coating for SBSE, polydimethylsiloxane (PDMS) is still the most used phase for most SBSE applications. This coating has demonstrated to be suitable for non-polar analytes rather than those

Table 1
Applications of Apt-based magnetic sorbents used in MSPE for analyte extraction from different samples.

Analyte(s)	Sample	Sorbent	Technique	Recovery (%)	LOD ($ng \cdot mL^{-1}$)	Ref.
Mycotoxins						
AFM_1	Milk	Fe_3O_4@SiO_2@Apt	HPLC-FLD	97–116	$0.2 \cdot 10^{-3}$	23
AFB_1 and AFB_2	Maize	Fe_3O_4@SiO_2@Agarose@Apt	HPLC-FLD	83.6–97.8 (AFB_1) 77.5–86.5 (AFB_2)	0.025 (AFB_1) 0.010 (AFB_2)	24
AFB_1	Chinese condiment (*Pixian Douban*)	Fe_3O_4@SiO_2@Apt	ELISA and HPLC-MS/MS	80.19–113.92	0.17	25
OTA	Corn and peanuts	Fe_3O_4@MIL-101@Apt	UHPLC–MS/MS	82.8–108	$6.7 \cdot 10^{-5}$	26
Antibiotics						
CAP, TAP and FF	Milk	Fe_3O_4@SiO_2@Apt	HPLC-UV	78.6–95.9	0.12–0.17	27
CAP	Honey and milk	Fe_3O_4@GO@Apt	HPLC-UV	80.5–104.8	0.24	28
CAP	Serum, milk powders, fish and chicken	Mg/Al-LDHM mesoporous carbon@Apt	UHPLC–MS/MS	87.0–107	0.94 pmol L^{-1}	29
Tetracycline	Water and honey	Fe_3O_4@Apt	HPLC-UV	82.9–107.3	2.5	30
SDM	Milk	Fe_3O_4@GO@Apt	HPLC-UV	75.9–92.3	3.3	31
Pollutants						
BPA	Human serum and urine	Fe_3O_4@SiO_2@Apt	HPLC-FLD	90.8 (serum) 92.3 (urine)	2.0 (serum) 1.0 (urine)	32
BPA	Milk	Fe_3O_4@SiO_2@Apt	HPLC-FLD	78–113	0.05	14
OH-PCBs	Human serum	Fe_3O_4@SiO_2@COF@ Apt	HPLC-MS	> 90	$2.1 \cdot 10^{-3}$	33
Acetamiprid	Fruit juice and water	$CoFe_2O_4$@SiO_2@MIL-101(Cr)@ Apt	HPLC-UV	80.20–101.81	0.0018	34
Atrazine	Water	Fe_3O_4@SiO_2@Apt	HPLC-UV	85.5–106	0.005	17
Pb^{2+}	Tap and sea water	Fe_3O_4@Ag@Apt	FAAS	**96.8 (sea)** **102.8 (tap)**	10	35
Others						
Berberine (alkaloid)	*Cortex phellodendri* plant	Fe_3O_4@Apt	HPLC-UV	95.4–111.3	–	36
8-OHdG (biomarker)	Human urine	Fe_3O_4@Apt	HPLC-MS	82–116	0.01	37

with medium or strong polarity [18]. In order to address the needs of trace analysis for analytes of diverse polarities, the preparation of novel coated stir bar with high extraction efficiency, fast extraction kinetics and good mechanical stability has been a research hotspot in the last years. Unfortunately, most of the commercialized or homemade coatings showed a lack of selectivity. The improvement of sorption selectivity in SBSE has been achieved through various strategies, such as the incorporation of MIPs or Apts, being this later topic scarcely considered. The most salient Apt-based coatings and their applications will be presented in the Section 4. With regards to the immobilization of Apts onto stir bar, several strategies have been applied such as the use of hydrophilic polycations (such as poly (diallyldimethylammonium chloride, PDDA) to coat positively the substrate, followed by the immobilization of the negative DNA aptamer by charge adsorption [19]. Also, Apt SBSE coating has been prepared using AuNPs as intermediate platform [20]. In addition to these approaches, magnetic stir bars have been recently functionalized with aptamers via "thiol-ene" click chemistry [21,22].

3. Applications of aptamer-based materials for MSPE

In this section, several applications of Apt-based materials in MSPE will be discussed. For this purpose, the most recent contributions, are summarized in Table 1, being classified according to the type of target analyte extracted from different samples: mycotoxins [23,24,25,26], antibiotic residues [27,28,29,30,31], pollutants [14,17,32,33,34,35] and others [36,37]. These analytes have been usually determined by liquid chromatography (both HPLC and UHPLC) coupled to different detectors such as UV [17,27,28,30,31,34,36], FLD [14,23,24,32] and MS [25,26,29,33,37], although other techniques such as ELISA [25] and FAAS [35] have been also used.

Also, this table shows the magnetic materials used as host supports for the immobilization of the Apts. As you can see in this table, most studies of Apt-based MSPE rely on the use of silica coated MNPs [14,17,23,24,25,27,32], which contained proper ligands for posterior attachment of Apt. As an example, Khodadadi et al. [23] synthesized Fe_3O_4@SiO_2 MNPs modified with amino groups for the attachment of amino-ended Apt using glutaraldehyde as a linker. The authors used this magnetic oligosorbent for the extraction and enrichment of AFM1 in milk. It is relevant to highlight that the authors used sample extraction conditions (defatting step with hexane and methanol/water mixture (containing 2 mM NaCl) (8:2)) quite different from the binding buffer condition obtained in the SELEX process. Such situation is quite common when moving from standard solutions to matrix extracts, where interfering compounds are present. In this scenario, a balance between binding conditions that can preserve Apt affinity and selectivity and the extraction buffer that ensures and efficient recovery from complex samples is crucial. In any case, the developed Apt-MSPE method showed a satisfactory recovery (97–116%), low LOD (0.2 ng L^{-1}) and suitable extraction/analysis time (25 min); however, the oligosorbent cannot be reused.

In the last years, the hybridization of MNPs with other nanostructures such as graphene oxide (GO) [28,31] and AgNPs [35] has been done in order to develop affinity sorbents with high covering density of Apt taking into account their large surface area to volume ratio. Thus, Tu et al. [28] have developed a magnetic oligosorbent by the attachment of Apt to the Fe_3O_4@GO composite by EDC–N-hydroxysuccinimide (NHS) coupling (Fig. 1), which was used for the extraction of chloramphenicol (CAP) in honey and milk followed by HPLC with UV detection. After optimizing several parameters affecting extraction efficiency (such as solution pH, extraction time and temperature, elution solvent and time), the Apt-MSPE protocol was applied to the samples, which were previously treated to remove protein and fat, and dissolved in buffer at pH 7.0. The developed procedure gave good extraction efficiencies (86–105%) and a suitable LOD (0.24 ng mL^{-1}); however, it required of longer extraction times (5 h). Also, the synthesized Apt-based sorbent showed good selectivity (against other antibiotics) and an acceptable reusability (at least 6 cycles).

Other materials that have attracted significant attention in the preparation of hybrid materials based on Apt-functionalized MNPs are porous frameworks such as metal-organic frameworks (MOFs) [26,34], covalent organic frameworks (COFs) [33] and porous carbon materials from Mg/Al layered double hydroxide (Mg/Al-LDH) [29] due to their large surface area, good stability, and superior adsorption capacity. As an example, Ghiasi et al. [34] developed an Apt-functionalized magnetic MOF by attaching this affinity ligand to the surface of $CoFe_2O_4$@SiO_2-modified MIL-101(Cr)–NH_2. Fig. 2 depicts the preparation scheme of this magnetic oligosorbent. After synthesis of $CoFe_2O_4$@SiO_2, it was mixed with MOF precursors and subjected to solvothermal method. The resulting composite was treated with glutaraldehyde before immobilization of 5′–NH_2–Apt. The Apt-functionalized magnetic MOF was applied

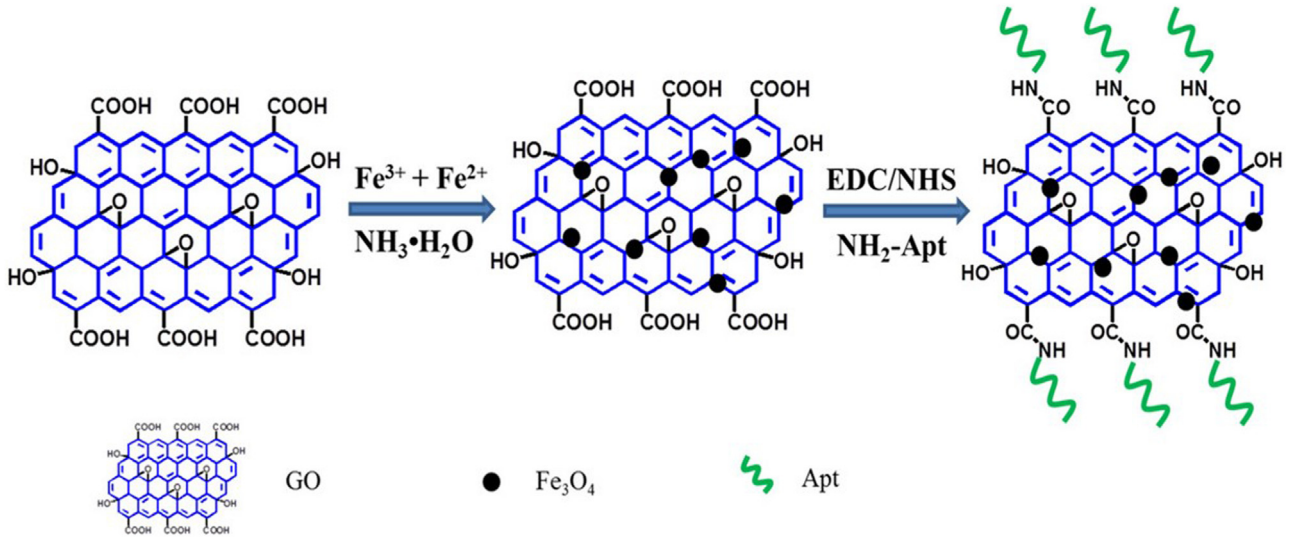

Fig. 1. Schematic illustration of the fabrication of Fe_3O_4@GO@Apt composite. Reproduced with permission from Ref. [28].

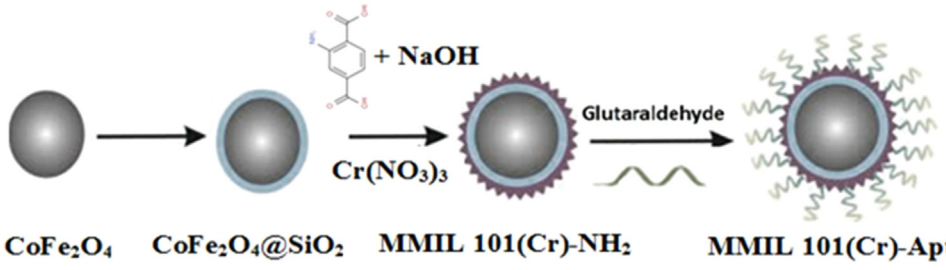

Fig. 2. Synthetic path for the $CoFe_2O_4$@SiO_2@MIL-101(Cr)@Apt composite. Reproduced with permission from Ref. [34].

to the selective extraction of acetamiprid present in water samples and fruit juices followed by HPLC with UV. The optimized Apt-MSPE protocol provided satisfactory recoveries (80.2–101.8%) and low LOD (0.01 ng·mL^{-1}).

The vast majority of reported studies devoted to the development of Apt-based MSPE protocols performed in off-line mode; however, the possibility of performing MSPE on-line with a chromatographic system would be highly advisable since it would simplify the analytical process, minimize the human errors, enhance the sensitivity, and enable the high throughput analysis. In this context, Gan et al. [37] developed an Apt-based magnetic sorbent for the selective extraction of a urinary metabolite (8-hydroxy-20-deoxyguanosine, 8-OHdG) using on-line MSPE-HPLC-MS. For this purpose, amino-functionalized-Fe_3O_4 NPs were reacted with glutaraldehyde, followed by a covalent linkage between the activated MNPs and the amine-modified Apt. Next, the resulting magnetic oligosorbent was fixed into a steel stainless tube using an external ring magnet and assembled on a six-port manual injection valve (see Fig. 3). After analyte loading, the retained target analyte was dynamically desorbed from the Apt-based sorbent (injection position), and then it was analyzed by HPLC-MS. Extraction conditions, including the amount of the sorbent, sample flow rate and volume were investigated. Under optimized conditions, good recovery values from urine samples (82–116%) and very low LOD (0.01 ng·mL^{-1}) were achieved. The synthesized sorbent also presented an excellent selectivity to 8-OHdG and good reusability (until 60 continuous runs with <20% loss of extraction efficiency). Besides, this online MSPE protocol produced a sensitivity enhancement of 800 times compared with offline MSPE mode. In summary, the proposed online MSPE-HPLC-MS approach has the advantages of rapid analysis (11 min, including extraction, desorption and separation steps) high accuracy, precision, selectivity, sensitivity and automation for determination of 8-OHdG traces in complex urine samples.

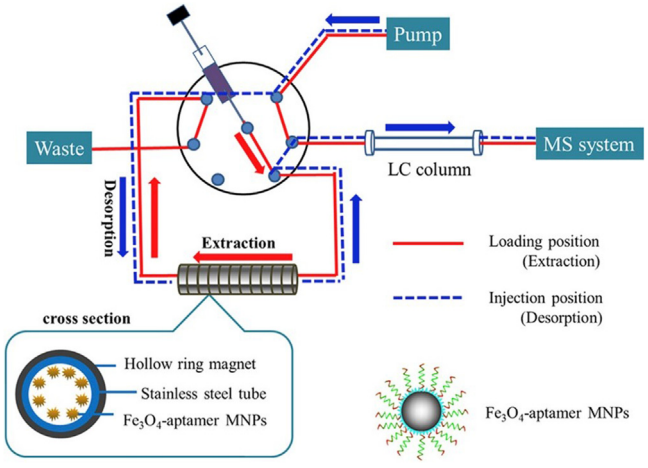

Fig. 3. Scheme of the online MSPE-HPLC-MS procedure developed for the extraction of 8-OHdG in urine samples. Reproduced with permission from Ref. [37].

4. Applications of aptamer-based materials for SBSE

In this section, some recent applications of Apt-based affinity sorbents in SBSE will be discussed. In these papers, included in Table 2, different analytes, such as antibiotics [38], pollutants [20], or proteins [21,22] have been extracted from in matrices such as soils [20] or foodstuffs [38,21,22].

Within the works focused on small organic compounds, Zeng et al. [20] developed a magnetic stir bar functionalized with an Apt selective for polychlorinated biphenyls (PCBs). To obtain the functionalized

Table 2
Applications of Apt-based materials in SBSE for analyte isolation from different samples.

Analyte(s)	Sample	Sorbent	Technique	Recovery (%)	LOD ($ng \cdot mL^{-1}$)	Ref.
Kana	Milk and fish	Apt-AuNPs functionalized stir bar	MCE	87.6–96.0 (fish) 95.4–106.0 (milk)	$0.29 \cdot 10^{-3}$	38
PCB72 and PCB106	Soil	Fe_3O_4@AuNPs@HB-Apt-functionalized stir bar	GC–MS	90.4–96.2 (PCB72) 91.3–96.4 (PCB106)	0.003–0.005 $ng \cdot g^{-1}$	20
Con A	White beans, chickpea, lentils and wheat flour	Apt-functionalized stir bar	MALDI-TOF-MS	81–97	500	21
***β*-LG**	Milk, dark chocolate and white bread	Apt-functionalized stir bar	MALDI-TOF-MS	84–93	80	22

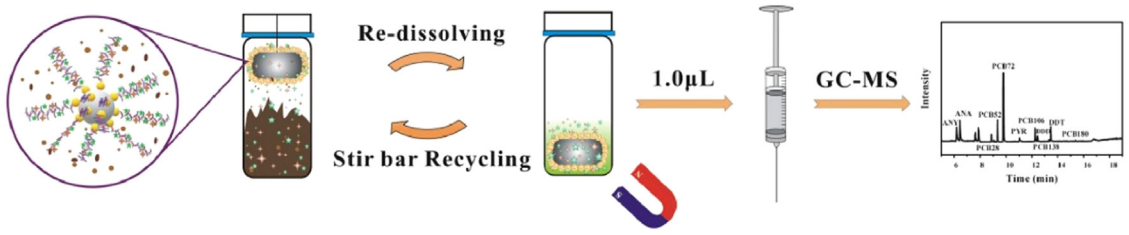

Fig. 4. Scheme of the extraction of PCBs in soil using AuMNPs@HB-Apt stir bar. Reproduced with permission from Ref. [20].

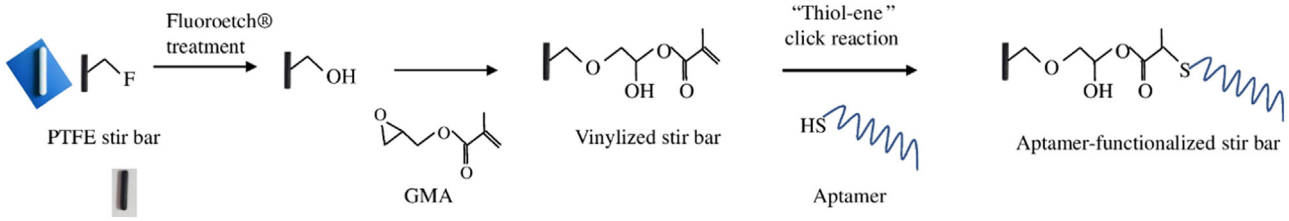

Fig. 5. Preparation scheme of the aptamer-functionalized PTFE stir bar for the extraction of Con A. Reproduced with permission from Ref. [21].

stir bar, gold magnetic NPs (AuMNPs) were firstly synthetized and used as substrate to immobilize an hyperbranched Apt (HB-Apt), which was obtained by a hybridization chain reaction (two types of complementary DNA hairpins assemble to obtain a long double-stranded DNA). The resulting composite sorbent (Fe_3O_4@AuNPs@HB-Apt) was fixed on a stir bar, and subsequently used to isolate PCBs from soil samples via headspace extraction (HS). After optimization of several parameters of HS-SBSE method, the sample was loaded in deionized water (adjusted at pH 7.0), extracted at 50 °C for 40 min, and then the retained PCBs were eluted from the stir bar using ethanol (1 mL for 20 min), and injected into the gas chromatography-mass spectrometry (GC–MS) system for analysis. A scheme of the extraction process is shown in Fig. 4. The developed method showed high recoveries (90.4–96.4%) in soil samples and very low LODs (0.003–0.005 ng g^{-1}). This Apt-based stir bar coating (AuMNPs@HB-Apt) also showed an excellent reusability (at least 60 times by keeping recoveries over 90%) and suitable device-to-device reproducibility (RSD < 8.1%). Despite these well-features, an improvement in the preparation process of coating, particularly in hybrid chain reaction step, would be desirable in order to make easier and accessible operation process.

Recently, interesting studies of Apt-based SBSE coatings dedicated to the isolation and enrichment of proteins from complex samples have been reported [21,22], constituting these works the first examples in this field. In particular, Vergara-Barberan et al. [21] have developed an Apt-functionalized SBSE coating for the selective isolation and preconcentration of an allergenic food protein, concanavalin A (Con A), followed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF.MS) determination. For this purpose, the surface of commercial polytetrafluoroethylene (PTFE) stir bar was firstly modified with an etchant reagent (Fluoroetch®) and subsequently modified with glycidyl methacrylate (GMA) in order to obtain a vinylized stir bar. Next, a thiol-modified-Apt was anchored to the modified stir bar via "thiol-ene" click chemistry reaction (see Fig. 5). Under the optimized extraction conditions, satisfactory recoveries (95–102%) in several food matrices, suitable LOD (0.5 μg mL^{-1}) and excellent selectivity compared to other lectins, were obtained. Also, the developed SBSE coating showed long-term stability (1 month), suitable reusability (10 and 5 cycles with standards and food samples, respectively) and good reproducibility (stir bars from different batches RSD < 7.6%). In summary, these developed Apt-affinity extraction devices represent an opportunity to prepare innovative and remarkably specific SBSE coatings for the selective extraction of proteins and peptides from intricate samples.

5. Conclusions and future perspectives

Along this review, the development and application of Apts as affinity sorbents in MSPE and stirring/extraction techniques (such as SBSE) to establish efficient and selective sample preparation protocols have been described.

Concerning MSPE, the use of Apt-functionalized materials in this extraction technique is a growing area of interest, where novel host materials such as reticular frameworks (for instance, MOFs or COFs) or carbon nanostructures can be used in combination with MNPs to provide novel affinity-based sorbents with high performance, stability, easy modification and very low non-specific adsorption. On the other hand, despite the advantages of MSPE, the current applications based on Apt-based sorbents are focused on few small molecules, being its extension to proteins and peptides a relatively unexplored field.

Regarding the development and application of Apt-based materials for SBSE, few works have been published on this topic, being still in their infancy, but their developments undoubtedly hold a promising future. Also, the extension of Apt-based sorbents to other extraction/stirring integrated techniques such as SCSE and RDSE could result in competitive and selective approaches in sample preparation.

Apart from these considerations, there are still several challenges in the development and effective application of these affinity ligands in these microextraction techniques and others, which can be summarized as follows.

i) The number of available Apts for different targets, including protein cells and tissues, should increase as well as the reduction of cost in the selection process and production, which is commonly carried out by SELEX. In order to improve selection efficiency of Apts, in the last years, computational tools and methodologies have demonstrated a substantial potential to assist in the identification of high-performance Apts. In this sense, artificial intelligence-based methods [39,40] have been proposed as enhanced and cost-effective strategies in the screening process of candidate Apts.
ii) In most studies, there is a deficiency in providing detailed information concerning crucial aspects regarding the optimal binding buffer conditions for matrix extracts. In this sense, further research and discussion about these aspects should be given in order to evaluate the performance of Apt-based materials and the feasibility of the protocol in real samples.
iii) The prospect of developing versatile extraction platforms consisting of multi-Apt-based materials capable of extracting efficiently and selectively two or more target analytes from complex samples is highly desirable.
iv) Also, advances towards the integration of affinity extraction devices in on/in-line modes to automate the sample preparation step would be beneficial and consistent with Green Analytical guidelines.

Regardless of the presence of these challenges, we believe that Apt-functionalized materials will constitute valuable tools in the coming years in the development and application of efficient and sustainable analytical methodologies in sample preparation techniques.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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