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Selective tools for the solid-phase extraction of Ochratoxin A from various complex samples: immunosorbents, oligosorbents and molecularly imprinted polymers

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Abstract

The evolution of the instrumentation in terms of separation and detection has allowed a real improvement of the sensitivity and the analysis time. However, the analysis of ultra-traces of toxins such as Ochratoxin A (OTA) from complex samples (foodstuff, biological fluids…) still requires a step of purification and of preconcentration before their chromatographic determination. In this context, extraction sorbents leading to a molecular recognition mechanism appear as powerful tools for the selective extraction of OTA and of its structural analogs in order to obtain more reliable and sensitive quantitative analyses of these compounds in complex media.
Indeed, immunosorbents and oligosorbents that are based on the use of immobilized antibodies and of aptamers respectively and that are specific to OTA allow its selective clean-up from complex samples with high enrichment factors. Similar molecular recognition mechanisms can also be obtained by developing molecularly imprinted polymers whose synthesis leads to the formation of cavities that are specific to OTA thus mimicking the recognition site of the biomolecules. Therefore, the principle, the advantages, the limits of these different types of extraction tools and their complementary behaviors will be presented. The introduction of these selective tools in miniaturized devices will also be discussed.

Key words : ochratoxin A; antibody; aptamer; molecularly imprinted polymer; immunosorbent; oligosorbent.

Introduction

Ochratoxin A (OTA) is the most common mycotoxin found in temperate regions and produced by several Aspergillus and Penicillium genera. As OTA is a naturally occurring mycotoxin, its widespread occurrence in food and animal feeds results in probable human exposure. To limit the consumption of OTA by ingestion, the best solution is prevention by monitoring OTA in foodstuffs. Consequently, there is an important need for fast, reliable, and low-cost analytical methods for its determination. The hydrophobic properties of OTA (log Ko/w=4.6) and its native fluorescence largely favored the use of liquid chromatography in reversed phase mode followed by a fluorescence detection for its analysis. Over the last few years, mass spectrometry has also been proposed as an alternative to fluorescence detection, this detection mode allowing the simultaneous determination of OTA with several other toxins that do not possess native fluorescence.
Despite the advances in the development of such highly sensitive analytical instrumentation for its determination in environmental samples, biological fluids, or foodstuff, a pre-treatment is usually necessary in order to extract and isolate OTA from complex matrices. Pre-treatment methods such as liquid-liquid extraction and solid-phase extraction on conventional sorbents (such as n-alkyl bonded silica) have shown their capabilities to extract OTA. However, these extraction procedures are based on hydrophobic interactions whose selectivity is often too low for the trace analysis of OTA due to co-extraction of matrix components.
A selective extraction of OTA can be achieved by using sorbents providing a molecular recognition mechanism of retention thus allowing to isolate OTA from matrix components. One of them is an immunosorbent (IS, also

called immunoaffinity cartridge IAC,) that is prepared by immobilizing antibodies onto a solid-phase [1]. These antibodies possess specific binding sites allowing the molecular recognition of OTA and some of its structural analogs thus enabling their selective extraction from complex samples. An alternative to ISs can be obtained by immobilizing aptamers on a solid-phase, to form an oligosorbent (OS) [2]. At last, a molecular recognition mechanism can also be obtained by using molecularly imprinted polymers (MIPs) whose synthesis leads to the formation of specific cavities mimicking the recognition sites of the antibodies [3]. Over the last years, these three types of selective sorbents have been developed for the selective extraction of OTA and of its structural analogs from complex samples. This review will be dedicated to the presentation of these sorbents. Their synthesis, their characterization in term of specificity and capacity and their potential for the selective extraction of OTA and its analogs from real samples will be presented.
These selective tools are also particularly useful when developing miniaturized devices because of the decrease of the resolution that results from the use of short length separation devices. In this context, totally miniaturized analytical systems involving these selective sorbents were also developed for the quantitative analysis of OTA in complex samples. Therefore, the principle, the advantages and the complementarities of these different types of sorbents at these different scales will also be presented.

1- Immunosorbents

1-1- Introduction

Immunosorbents are made of antibodies immobilized on a solid support. Thanks to the high affinity (with affinity constant in the range 10^4 to 10^{12} M^{-1}) and high specificity of the antigen-antibody interactions, ISs constitute effective tools that enable a selective extraction and the concentration of individual compounds or classes of compounds from liquid samples in one step or the sample purification of extracts from solid matrices.
ISs were first described in the biological field because of the availability of antibodies for large molecules such as proteins that possess immunogenic properties. Obtaining selective antibodies for small molecules is more difficult thus explaining that the development of immunochemical methods targeting low-molecular-mass analytes was more recent. Indeed, small-size molecules (<1000 Da), such as OTA, have to be coupled to an immunogenic protein. Coupling proteins include bovine serum albumin (BSA), keyhole limpet hemocyanin, β-lactoglobuline, horse radish peroxidase, polylysine, BSA being predominantly used. Concerning OTA, its free carboxylic group was commonly used for coupling because of easy chemistry. The production and the use of anti-OTA antibodies were first described by the group of Chu F.S. in 1976 who first developed a radioimmunoassay for OTA [4]. This pioneer work was followed by the development of numerous ELISAs. Despite the large use of antibodies specific to OTA in those numerous studies, their K_d values were not mentioned. Recently, a K_d value of 3nM was reported for OTA antibodies thus confirming the high affinity of these antibodies towards OTA [5]. At the beginning of the 90's, an IS for OTA was proposed by Biocode under the trade mark of Easy-Extract. The potential of this selective approach easily explains that other ISs have been commercialized (under the trade names Ochraprep and Ochratest) and largely applied to the selective extraction of OTA from complex samples for more than twenty years.

1-2- Preparation of the immunosorbents

The immunosorbents are produced by linking antibodies to a solid support that constitutes a critical parameter for their design. In addition to essential properties such as chemical and biochemical inertness, good mechanical stability and uniformity in particle size, the solid supports should be easily activated to allow antibody attachment, have large pore sizes because antibodies are large molecules, and should be hydrophilic in order to avoid any non-specific hydrophobic interactions in aqueous media [6]. Finally, the immobilization procedure should preserve the biospecific activity of antibodies [1]. No data related to the nature of the sorbent used are provided by the suppliers of OTA ISs, but agarose gel such as sepharose was used for most of the ISs developed and used as SPE sorbent in disposable cartridges for various chemicals. These agarose gels cannot be operated at high flow rates because of their limited mechanic stability, high pressures generating compacting and fouling.

Samples, washing solvents and other solutions implied in SPE procedure are then applied under gravity flow or very low flow rates.
Fig. 1 represents the three steps of an immunoextraction sequence that are very close to the sequence applied to a conventional SPE sorbent: (1) percolation of the sample after a conditioning step, (2) washing of the IS and (3) elution of the target analyte(s). One difference with conventional SPE sorbents is that the ISs are stored before use in a wet media, usually phosphate buffer saline (PBS) solution. To prevent microbial contamination, sodium azide, a bacteriostatic agent, is generally added in the storage buffer, this solution being removed by percolating pure water or by renewing the PBS solution during the conditioning step.

1-3- Control of the selectivity of the retention mechanism

Despite the high specificity of the antigen-antibody interactions, non-specific interactions can occur between the analyte and the sorbent chosen for the immobilization of antibodies or the constant region of antibodies. These non-specific interactions may also cause an undesirable retention of sample components on the ISs [6]. Most commonly, hydrophobic and ionic forces are responsible of undesirable adsorption of non-target compounds, which results in a decrease of the selectivity and of an increase of the limit of detection. Therefore, their control is particularly important when developing an IS for OTA because of its hydrophobic and acid-base properties. Silica- and agarose- based ISs have been shown to minimize the non-specific interactions generated by the solid support [1].
In order to investigate the retention mechanism of the analyte on the IS, it can be interesting to compare the retention of OTA on three different supports: a non-bonded sorbent, a sorbent bonded with non-specific antibodies and the sorbent bonded with the specific antibodies of interest. This kind of study gives an indication about the contribution of non-selective interactions in the retention mechanism[6]. Nevertheless, these studies reported by academic groups for the evaluation of the potential of numerous ISs, were not reported by OTA IS users who mainly follow the procedures described by OTA IS manufacturers. Nevertheless, to ensure a high selectivity by the removal of matrix components that can be also retained by non-specific interactions, most of them applied large volumes of water and/or aqueous buffer on the ISs during the washing step, from 10 ml [7–15] to 20 ml [16–23].

1-4- Application of immunosorbents to real samples

ISs have largely been reported for the purification of OTA from cereal extracts [9, 16–19, 22, 24–26] but also for the selective treatment of other types of samples such as coffee and related products [10, 14, 16, 21, 25, 27], milk [15], cocoa and related products [16, 23], wine [7, 8, 12, 28], beer [12], grape, grape wine fruit or winery products [20], meat or animal products [29], spices, oil, nuts or infant formula [16]. They were also applied to the extraction of OTA from biological fluids such as serum, plasma [15, 30, 31] and urine [11, 13].
In most of the cases, the main objective was to only extract OTA from real samples before its analysis by HPLC/Fluo [9, 10, 13, 14, 18, 20, 21, 27, 28, 31] or by ELISA [7]. Some works also reported the simultaneous extraction of OTA and aflatoxins [8, 16, 29, 32] by using a cartridge containing both anti-OTA and anti-aflatoxins antibodies or using two cartridges in tandem. The same approach was proposed for the simultaneous determination of OTA and zearalenone [24], five [17] or ten [22] other mycotoxins, the last approaches implying the use of LC/MS analysis for the simultaneous determination of the whole mycotoxins in the same samples.
In most of the cases, the immunosorbent particles are packed between two frits in a SPE disposable cartridge to be used off-line upstream to the separation methods. After the percolation of the sample, a washing solution, having a low elution strength towards OTA, is passed through the IS to remove undesirable sample components slightly retained by non-specific interactions. The elution step is then achieved using a solution allowing the disruption of the OTA-antibody interactions.
The volume and the nature of the sample that can be percolated without loss in recovery constitute the most important parameters [6]. Indeed, during the percolation of the sample, matrix components or sample additives may affect the affinity between antibodies and OTA thus lowering extraction recoveries as will be discussed

below. Moreover, extraction recoveries also depend on the amount of immobilized antibodies that is fixed by the suppliers of ISs.

To decrease the risk of matrix effect on the recoveries of OTA from aqueous samples that could be directly percolated through the IS, a dilution with a buffer solution was proposed before the percolation of the sample. This was done for the determination of OTA in a ready-to-drink coffee sample (dilution factor of about 11) [27] or for urine from human (factor 2) or rat (factor 24) [13]. This dilution allows to adjust the pH of the sample and to reduce its viscosity.

The extraction of OTA from solid samples such as cereals requires a mixture of water and methanol (up to 80%) [19, 22] or acetonitrile (up to 60%) [10, 18, 21, 25]. This high percentage of organic solvent may affect the OTA-antibody interactions and then the retention of OTA on the IS during the percolation step thus causing a decrease of the extraction recoveries. This organic content can be also lowered by the dilution of the extract, with water or with an aqueous buffer, to 7-10% methanol [19, 22] or to 2-6 % acetonitrile [10, 18, 21, 25]. This effect of the residual amount of solvent on the recoveries was studied for OTA but was particularly well illustrated in a study related to the extraction of another mycotoxin, deoxynivalenol (DON), on an anti-DON IS [22]. OTA was also extracted with toluene that was further totally removed by evaporation of the extract up to dryness [9]. Alcohol content in wine also explains that wine sample were diluted by a factor 2 [7, 8] or 12 [28] before being percolated through the IS.

Despite the possibility demonstrated by these works to percolate directly the sample through the IS after a simple dilution step, the use of a previous clean-up step by liquid-liquid extraction of a serum sample [31] or of a SPE step on C18 silica of a plasma sample [30] and using QuECHERS for solid food samples [16] was also reported.

Before the elution, a washing step using an aqueous buffer is recommended to remove compounds slightly retained by non-specific interactions with the antibodies or the sorbent used for their immobilization as previously mentioned.

Concerning the elution step, OTA was recovered using pure methanol [10–16, 20–22, 29, 31] or methanol acidified with 2% acetic acid [8, 9, 18, 19, 23, 27] as often described for the elution of small size molecules from ISs [1]. These conditions allow an efficient disruption of the antigen-antibody complex, acid-base properties of OTA explaining the effect of low pH on this disruption. The effect of alkaline solutions was also reported, with a decrease in the affinity of antibodies for OTA because of the open ring structure of OTA generated in these conditions [25]. Indeed, it was observed a decrease of recoveries of OTA for basic samples. This off-line mode also allows the use of high amounts of organic solvents thus providing an efficient disruption of the antigen-antibody interactions particularly for single-use cartridge, the irreversible denaturation of the antibodies being not a problem in this case [6].

As for conventional SPE procedure, the eluate is often evaporated up to dryness to be reconstituted in a solvent that is compatible with the mobile phase of separation method, mainly a mixture of acetonitrile and water for OTA analysis in RP-LC. The addition of water to the methanol fraction for the direct injection of the elution fraction in LC was also proposed [17, 28].

Apart from the common use of LC/Fluo to analyze OTA in the eluate, the selectivity brought by the IS that allows to isolate OTA in a final clean extract offered also the possibility to directly determine OTA by fluorescence detection without using LC [29]. It was also demonstrated that ISs allowed to remove false positives that occurred when using ELISA for the determination of OTA in wines [7] and to improve the sensitivity of LC/MS for the determination of OTA in cereals [16].

2. Oligosorbents

2.1. Introduction

As an alternative to ISs, aptamers can be immobilized on a solid support to form an oligosorbent (OS) (also called aptamer-affinity column, AAC). Aptamers are short single stranded oligonucleotides (DNA or RNA, typically 20–110 base pairs) able to bind a specific molecule with the same affinity as antibodies. They are identified *in vitro* by an iterative selection process called SELEX (Systematic Evolution of Ligands by Exponential enrichment) [33]. Most of the already identified sequences are directed against large molecules such

as peptides, proteins, or nucleic acids but also for a significant number of small molecules. In comparison with antibodies, aptamers offer several advantages as selective extraction tools. First, they can be prepared for toxic target as well as for targets that do not induce an immune response *in vivo*. Their production at large scales with little batch to batch variation in activity was demonstrated [34]. Aptamers can be regenerated within minutes, whereas antibodies need 1 or 2 days to recover their active conformation [35]. Moreover, modifications can be introduced during their chemical synthesis to improve their stability or to facilitate their immobilization [36].
Various analytical methods involving aptamers have been developed and recently reviewed, including enzyme-linked oligonucleotide assays (ELONAs, variant of ELISAs with aptamers instead of antibodies) [37], biosensors ("aptasensors") [38, 39] and separation methods [38, 40, 41] such as affinity chromatography, electrochromatography and affinity capillary electrophoresis. The immobilization of aptamers onto a solid phase, to use them as SPE sorbents is very recent but it appears to be a very promising approach [2, 42] and was particularly reported for OTA extraction [43] owing to the description of the aptamer OTA sequence by Cruz-Aguado in 2008 [44].

2.2. Preparation of the oligosorbents

2.2.1. Aptamer selection

Indeed, in 2008, an OTA aptamer sequence was described [44]. This study reported the very high affinity of this aptamer sequence towards OTA by estimating the dissociation constant in the nanomolar range. The feasibility of using aptamers for SPE was clearly demonstrated by the application of this aptamer to the determination of OTA in wheat grain extracts and then confirmed by numerous other developments.
During the selection process, different reagents such as structural analogs (warfarin, N-acetyl-L- phenylalanine, low amount of solvent –dimethylsulfoxyde- and aqueous wheat grain extract) were introduced. These conditions allowed the selection of a DNA strand that does not present any affinity towards these different reagents or that is not affected by the presence of these reagents thus improving the specificity of the sorbent and reducing the risk of matrix effects during SPE procedure [44].
For the most promising OTA aptamer sequence, a 36-merDNA oligo (5'-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3') a K_d value of 0.2 µM was measured (value evaluated in the selection buffer -10 mM HEPES, pH 7.0, 120 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$-).
It was shown that the binding of OTA to this DNA aptamer depends on the presence of divalent cations. Indeed, Cruz-Aguado *et al.* showed that including calcium in the buffer instead of magnesium resulted in a lower *K*d (49 nM when adding 20mM Ca^{2+}). This was explained by the fact that OTA forms a coordination complex with magnesium or calcium with the aid of both the carboxyl and the 8-hydroxyl groups, and this complex enhances its binding to the aptamer. A schematic diagram of this quadruplex structure is given on **Fig. 2**.
It was also noticed in this work that the OTA-aptamer affinity was not affected significantly by the concentration of sodium or potassium to the point that complete removal of these ions did not affect the affinity as long as magnesium or calcium were present. This indicates that the ionic strength of the solution had little effect on the interaction between OTA and its aptamer [44]. However, this was refuted by a recent study that demonstrated that the ionic strength of binding buffer solution had large effect on the binding affinity of OTA for the same sequence though Mg^{2+} or Ca^{2+} is present. The presence of 120 mM NaCl can improve the binding affinity of aptamer by a factor 18. The presence of 120 mM NaCl or KCl is important for the aptamer to maintain strong binding affinity to OTA (K_d close to 50 nM). The mere presence of Mg^{2+} or Ca^{2+} in binding buffer solution allows the aptamer to bind with OTA, but the binding affinity is weaker [45].

2.2.2. Immobilization of the aptamers

The oligosorbents are prepared by linking the aptamers to a solid support as for ISs. But, unlike ISs, the chemical synthesis of aptamers allows the introduction of modifications to the 5' or 3' end of the DNA sequence to facilitate its immobilization. This modification is chosen according to the nature of the activated function of the selected solid support (amino, thiol, carbonyl groups…). A spacer arm can also be introduced between this

chemical group and the DNA/RNA strand in order to maintain the binding properties of the aptamer when bound to a surface. It can be an n-alkyl chain in C6, in C12, in tri-, tetra-ethylene glycol, polyethylene glycol or in hexaethoxyglycol [2].
The aptamers can be immobilized using the well-known non covalent biotin-streptavidin or avidin bridge that is easy to perform on commercially available activated sorbents such as polystyrene, porous glass and magnetic beads. This type of ligand is known to efficiently maintain the aptamer binding ability. However, this non-covalent approach presents some drawbacks, in terms of reusability and sorbent life-time, particularly when organic modifiers are involved in the SPE procedure because they affect the biotin-streptavidin affinity. This was shown for the extraction of OTA from wine whose ethanol content caused a loss of biotinylated-aptamers, thus forbidding the reusability of the OS [46]. Concerning the covalent immobilization of aptamers, a few sorbents widely used for the covalent grafting of antibodies have been used for the immobilization of OTA aptamers such as CNBr-activated sepharose [46, 47] or NHS-activated sepharose [35]. The immobilization of OTA aptamers on diaminodipropylamine agarose was also described [44, 48]. These sorbents are particularly well adapted to develop extraction devices because they can be easily packed between two frits in disposable cartridges or columns. Moreover, the choice between them is difficult because they were not compared for OTA OSs. Nevertheless, it can be noticed that this type of comparison was done for anti-cocaine aptamers. They were covalently immobilized on several solid supports to prepare OSs: cyanogen-bromide activated sepharose, thiol-activated sepharose, glutaraldehyde-activated silica [49]. It was shown that the best sorbent in terms of cocaine retention on the resulting OS was the one obtained using cyanogen-bromide activated sepharose, the lowest retention of cocaine on the two other sorbents being explained by lower grafting yields of aptamers for these supports. For dispersive solid-phase extraction procedures that consists of the suspension of an OS in a given volume of sample, aptamers immobilized on magnetic NH_2-nanospheres were also reported [50].

2.3. Control of the selectivity of the retention mechanism

As for ISs, non-specific interactions can occur between OTA and the solid-phase used for the immobilization of aptamers but also with the nucleotide sequence. In order to investigate the retention mechanism of OTA on the OS and to evaluate the risk of nonspecific interactions it is interesting to compare its retention in parallel on a non-bonded sorbent [46, 47, 50, 51], on the sorbent bonded with non-specific aptamers [47] and on the sorbent bonded with a scrambled sequence that contains the same nucleotides than in the OTA aptamer but in a random position (thus preventing the formation of the specific complex with the target analyte) [50]. These types of experiment that are similar to those previously mentioned for ISs (part 1-3) give an indication whether non-selective interactions are mainly due to the solid-phase, to the nucleotides, to the spacer or to the coupling groups. These control sorbents are also helpful for optimizing the washing procedure and then obtaining the optimal selectivity [2]. As an example, the comparison of results corresponding to the use of nanospheres with or without OTA aptamers, and with the corresponding scrambled sequence shows that two washing steps were required for the removal of OTA from the control nanospheres while 90% OTA still remained on the nanospheres grafted with OTA aptamers [50].

2.4. Development of OS-SPE procedure for real samples

The use of OSs as selective SPE supports for OTA are summarized in Table 1. The mycotoxin was extracted from various solid samples such as wheat extracts [44, 46–48, 53], coffee extracts [50], ginger powder extracts [35] and traditional Chinese medicines [51] but also from red wines [46].
After the immobilization of aptamers to a solid support by covalent or non-covalent interactions, OS particles are generally packed between two frits into disposable cartridges or columns as conventional sorbents for SPE procedure. The binding of analytes to aptamers during the percolation of the sample results from a good spatial complementarity between OTA and its aptamer, i.e. from the sum of intermolecular interactions between the nucleotides and OTA. The nature and the strength of these interactions are defined during the selection of the aptamers by applying a buffer, i.e. selection buffer. Therefore to favor the retention of OTA during the percolation step, the sample composition must be as close as possible top the selection buffer composition.

Indeed, the lowest K_d value of the OTA aptamers in the presence of calcium (instead of magnesium used during the selection process) encouraged users to introduce this cation in the sample or in the buffer (also called binding buffer, BB) used for the dilution of real samples.
As for ISs, the nature of the percolation and washing solutions involved in the extraction procedure may affect the affinity between the aptamer and its target. It was shown by studying the pH of the sample, that the trapping of OTA by aptamers was favored by a pH close to 7.5 [51]. At least, to limit the effect of organic solvent used for the treatment of solid samples, a simple dilution of the extract using the BB to decrease the organic solvent content was applied as mentioned in Table 1. It was also reported that sample extracts containing 10% [50] to 15% methanol [44, 48] could be applied to the OTA OS without observing an effect on the trapping efficiency of the aptamers.
Effective elution solutions should ideally disrupt the OTA-aptamers interactions without adversely affecting the immobilized aptamers. As indicated in Table 1, different conditions were used and sometimes combined together: scavenging agents such as EDTA that is well-effective for this aptamer because of its sensitivity to the presence of Ca^{2+}, pH variations or water-organic modifier mixtures. These elution conditions must take into account the way of immobilization of aptamers. As an example, 40% ACN can be passed through an OS prepared by covalent immobilization of the aptamers while only pure water can be applied when bonding the same aptamers via non covalent biotin/streptavidin interactions [46].
To evaluate the performance of the OSs for the treatment of real samples, different approaches were used. First, the application of the OSs to the extraction of OTA from certified contaminated wheat sample extracts was often reported. These samples were treated using the OSs packed in SPE cartridge [44][47] or after incubation of aptamer based nanospheres in the sample [50] showing that the format does not affect the performance of the sorbent. Nevertheless, the most common way of evaluation consists in the comparison of chromatograms obtained after using the OS or a conventional extraction sorbents such as C18 silica as it was done for the extraction of OTA from wine [46] or from coffee extract [50]. Results obtained with OS were also compared to those obtained using a commercialized immunosorbent for red wine [46] or in ginger powder [35] samples. The comparison of the LC/Fluo chromatograms obtained before and after the purification on an OS of OTA from a contaminated matrix reference (B-myc0880) also highlighted the selectivity brought by the OS that allows the removal of a large amount of interfering compounds [47]. It was also noticed that the extraction recoveries of OTA from ginger powder were the same whatever the spiking level used [35]. For cereals products, wheat flour and coffee, these recoveries were in the range 67-91% with RSD values of 2 to 8% (n=5) [50].
In their pioneer work, Cruz-Aguado *et al.* detected OTA in the eluate by a simple fluorometric determination [44] showing that this simple device was sufficient for the determination of OTA in grain samples. Even so, like other selective sorbents, OSs were associated with liquid chromatography coupled to fluorescence detection [35, 47, 48, 50] or mass spectrometry [51] systems for a sensitive determination of OTA. The direct use of time-resolved fluorescence (TRF), implying an aptamer probe and terbium, was also proposed [53].

3. Molecularly Imprinted polymers

3-1- Introduction

Molecularly imprinted polymers (MIPs) are synthetic polymeric materials with specific recognition sites complementary in shape, size and functional groups to the template molecule, providing, as both previous sorbents, a retention mechanism based on molecular recognition. They present the advantage to be synthesized in a few days. Their stability, ease of preparation and low cost for most of the target analytes make them attractive for numerous applications [54]. Therefore, MIPs have already been successfully used as an alternative tool to antibodies or aptamers in several analytical fields such as enantiomeric separation in LC or capillary electrochromatography [55], binding assays [56, 57] and sensors [58–60]. In recent years, their use for the selective extraction or clean-up of different classes of compounds from various complex samples (environmental samples, foodstuff, biological fluids…) has been extensively reported [42, 54, 61, 62], SPE constituting the most advanced application area for the MIPs. Concerning more specifically the determination of OTA, numerous papers have already been reported as shown by the SPE developments and applications listed in Table 2 [63–74].

3-2-Synthesis of molecularly imprinted polymers

In most cases, the synthesis of MIPs involves first the complexation in solution of a template molecule with functional monomers, through non-covalent bonds, followed by polymerization of these monomers around the template using a cross-linker and an initiator. After the polymerization, the template molecule is removed from the polymer by extensive washing steps to disrupt the non-covalent interactions between the template and the monomers thus making available the binding sites, *i.e.* cavities, complementary to the template in size, shape, and position of the functional groups [54]. Therefore, the choice of the reagents involved in the MIP synthesis must be judicious in order to create highly specific cavities designed for the template molecule.

Concerning the choice of the template molecule, its structure and its functionalities define the subsequent properties of the binding sites. For the synthesis of MIP for OTA, the template was OTA [66–69] but, to avoid the risk of residual OTA leaking from the polymer that could cause erroneous results, the possibility to use a structural analog of OTA as template was proposed by several groups [63–65, 73, 74]. These studies showed that while selecting a mimic template, changes to the amino acidic sub-structure or the presence/absence of a chlorine atom in position 4 on the naphthalene ring system does not affect the molecular recognition of OTA by the resulting MIP. On the contrary, the presence of the bulky naphthalene ring system seems to be necessary to preserve the molecular recognition of OTA [75].

The nature and the strength of the non-covalent interactions between the template and the monomer during the synthesis is related to the physical and chemical characteristics of the porogen solvent. It has to be selected as a function of the nature of the interactions expected between the template and the monomers. As illustrated by the conditions of synthesis depicted in Table 2, despite the evaluation of various monomers, MAA was the most used monomers for the synthesis of OTA MIPs in association with ethylene dimethacrylate (EDMA) as cross-linker in a moderately polar and aprotic solvent, *i.e.* chloroform, thus favoring polar interactions such as hydrogen bonds with OTA.

Different methods of polymerization can be used for MIP synthesis such as suspension polymerization, seed polymerization and dispersion/precipitation polymerization [76]. Concerning OTA MIP, the bulk polymerization method was chosen certainly because of its simplicity of use. With this approach, the obtained monolith has to be crushed, ground and sieved to obtain particles adapted to extraction devices (particle diameters in the range 25 to 50 µm), the last step consisting in sedimentation to remove the finest particles. In most cases, the particles of interest are then packed between two frits into disposable cartridges like the two previous sorbents. The non-regular shape of the particles obtained by this method compared to others does not constitute a real limitation for off-line SPE applications but may cause broad asymmetric peaks in on-line coupling with LC. However, this direct on-line coupling of MIP particles with LC has not been yet described for OTA analysis. In return, the synthesis of MIP OTA by *in situ* electrochemical polymerization of pyrole (Py) onto a stainless-steel frit to couple the MIP on line with LC was described [68, 69]. MIP particles were also introduced between two sheets of membrane (*i.e.* envelope) to be immersed in samples [71].

3-3- Control of the selectivity of the retention mechanism

The selectivity of the MIP towards OTA results from the presence of specific cavities whose presence can be evaluated by different methods. They generally consist in studying the interactions between the template or the target analyte and the MIP in a solvent close to the one used during the synthesis in order to favor the interactions that took place during the polymerization step. Moreover, these studies are generally carried out in parallel on a non-imprinted polymer (NIP), also named control polymer, that is obtained by applying the same procedure of synthesis in the absence of the template. The NIP possesses the same chemical properties as the MIP but without possessing any specific cavities. Therefore, the nature of the interactions developed between the target analyte, i.e. OTA, and both sorbents are the same, but the strength of these interactions are different. If well-defined cavities were created during the polymerization step, the strength of these interactions is higher on the MIP than on the NIP because the target analyte can be retained by different points (sum of the interactions) due to the spatial complementarities between the target analyte and the cavities [54].

Particularly when the studies have focused on the screening of the synthesis conditions , the presence of cavities were first assessed by binding experiments [66] and chromatographic measurements [63, 64, 73] by carrying out the same experiments on the NIP in parallel.
Equilibrium batch rebinding experiments consisted in introducing a known amount of OTA in a vial with a given amount of MIP particles. Once the system has come to equilibrium, the amount of adsorbed OTA is deduced from the determination of OTA that remains in solution. The adsorbed amount is compared to the amount adsorbed on the NIP (after carrying out the same experiment in parallel), the number of cavities being correlated to the difference between the amounts adsorbed on both sorbents.
The selectivity of a MIP can also be evaluated by injecting OTA on two columns packed with MIP and NIP and by measuring its retention on each column. The ratio between retention factors obtained on MIP and on NIP allowing the calculation of the imprinting factor (IF) for a given mobile phase [63, 64]. The nature of this mobile phase governs the nature and the strength of the interactions involved in the retention process of OTA, thus rendering its choice is very important. Carrying out LC measurements in different conditions can also be helpful for determining the main factors (pH, nature of the solvent...) affecting the retention and the selectivity. As an example, the elution strength of acetic acid for a MIP prepared with methacrylic acid for which H bonds were expected was highlighted by this approach [63].

3-4- Development of a selective SPE-MIP extraction procedure

As for ISs and OSs, the principle of the extraction on a MIP is the same as when using conventional SPE sorbents. It still generally consists in a conditioning of the MIP, percolation of the sample, washing of the MIP to remove interfering compounds and desorption of OTA by percolating a solvent able to disrupt the interactions between the target analyte and the monomer residues in the cavities of the MIP.
An optimal selective extraction procedure applied to a real sample should allow OTA to specifically interact with the monomer residues located in the cavities. These interactions should not be affected by other sample components (ions, humic acids…). Moreover, the procedure of extraction should allow OTA to develop strong interactions with the monomer residues present in the cavities while at the same time limiting as much as possible the interactions between OTA and the monomer residues that cover the surface of the polymers (i.e. located outside of the cavities). Therefore, the procedure should be based on the use of a solvent for the percolation step and/or the washing step that possess an elution strength sufficiently high to disrupt the interactions that can take place with residual monomers at the surface of the polymer without affecting interactions that take place in the cavities. This means that the procedure has to be optimized in order to eliminate low energy interactions at the surface without damaging specific interactions taking place in the cavities and that are expected to be of stronger energy due to the spatial recognition. Testing the extraction procedure in parallel on the NIP constitutes an easy mean to evaluate the strength of the interaction caused by the residual monomers located at the surface of the polymers. This comparison can be done in pure media but also in real samples in order to evaluate the effect of the matrix on the retention process and to optimize the washing conditions [54]. As an example, **Fig. 3a** corresponds to the LC/Fluo analysis of the washing and elution fractions from the MIP after the treatment of a wheat extract and **Fig. 3b** allows the comparison of the chromatograms corresponding to the elution fraction obtained with the MIP and the NIP (with, in the insert, the amount of OTA determined in each fraction of the SPE procedure applied to the MIP and the NIP)[70]. These figures show that the washing steps allow both the removal of matrix components from the MIP while maintaining a high retention of OTA on the MIP that is mainly recovered in the elution fraction. They also show the complete removal of OTA from the NIP thus highlighting the high selectivity brought by the MIP and the associated extraction procedure. The possibility to optimize the washing conditions in the absence of NIP by studying the effect of different washing conditions on the removal of interfering compounds was also reported [72].
These works illustrated that, despite their easy and short-time synthesis, MIPs require the optimization of a SPE procedure that can be long and difficult, in order to reach an optimal selectivity, compare to the development of a SPE procedure on OSs and ISs. Indeed, the nature of the solvent to be passed through the MIP strongly depends on the nature of the interactions developed between the target, *i.e.* OTA (and matrix components to remove) and

the monomer residues while the use of aqueous media ensures a strong retention of OTA on OSs and ISs and an efficient washing step.

3-5- MIPs applied to real samples

It has been largely demonstrated for various molecules that MIPs offer the highest selectivity when samples are dissolved in the solvent used for the MIP synthesis [61]. Indeed, this solvent is able to recreate the interactions that took place during the polymerization between the template and the monomers. The synthesis conditions described in Table 2 show that most of the syntheses, when they were described, involved the use of acid monomers in an aprotic and moderately polar organic solvent thus favoring polar interactions such as H bonds. When using MIPs as SPE sorbents, the same interactions can be developed but it necessitates the use in the extraction procedure of non-polar or moderately polar solvents. However, in most of the cases, real liquid samples (wine, coffee, grape juice, beer) or solid sample extracts (cereals, coffee,..) are aqueous or contain a large content of water thus rendering difficult the direct establishment of polar selective interactions. Moreover, to favor the retention of OTA during the percolation step, the samples or extracts were acidified thus favoring hydrophobic interactions [70–72], a moderately polar solvent such as acetonitrile being used in a second step as washing solvent to favor the selective polar interactions with the cavities [70].
The first studies related to the development of MIPs for OTA concerned the screening of the conditions of synthesis [63, 64] or their use as SPE sorbent in pure media [66]. The first applications to real samples consisted in the use of a MIP for the purification of a wine extract after a first concentration step on C18 silica [65] or of a chloroform extract of a wheat sample previously treated on a polar sorbent [67]. Then, MIPs were directly used for the selective treatment of real liquid samples [68, 69, 71, 72, 74] and of extracts from solid samples [67, 70]. Wine samples were diluted with water to decrease the alcohol content and then to favor the retention of OTA [74]. The dilution with water was also proposed for wheat extract to decrease its acetonitrile content [70].

4. Comparison of the potential of these selective sorbents

4.1. Extraction of OTA analogs

The selective extraction of OTA from real complex samples was the primary objective of the development of such types of selective sorbents. The binding of analytes to antibodies is the result of a good spatial complementarity, which is a function of the sum of the intermolecular interactions and it was shown that OTA antibodies were also able to bind with other structural analogs such as the non-chlorinated analog OTB thus allowing the possibility to monitor the occurrence of OTA and OTB in wine samples [8] or cereals and coffee [25] with a unique IS. This cross-reactivity constitutes in this case a real advantage with the possibility to determine both molecules that are identified individually owing to the separation method used for analyzing the eluates of IS while it may be a drawback when using ELISA for their determination that will not allow to elucidate which form causes a signal.
The ability of a MIP synthesized using OTA as template to simultaneously extract OTB from spiked water samples was demonstrated, with recoveries of extraction lower for OTB than for OTA, thus indicating a lowest affinity of the MIP for OTB. These results are in good agreement with studies related to MIPs developed for other molecules and showing their ability to trap structural analogs of the template molecule [61].
Generally, oligosorbents largely differ from ISs or MIPs in their ability to retain target structural analogs. If antibodies or MIP cavities often present a broad cross-reactivity for small molecules, the ability of aptamers to retain structural analogs can be poor [2]. Indeed, it was reported that OTA aptamers present 100-fold less affinities for OTB than for OTA, while a difference of only 3-fold being observed for anti-OTA monoclonal antibodies [44] and that the hydroxyquinone metabolite of OTA was not retained by OTA OS [47]. These results highlight the possibility offers by of the SELEX process to determine a unique sequence of oligonucleotides that will adopt a conformation very specific for its target while removing oligonucleotides that could present an affinity for other structural analogs. In return, it is possible to adapt the SELEX procedure in

order to select aptamers showing an affinity for different targets by using them alternatively during the selection. These possibilities offered by the SELEX technology cannot be achieved to the same extent with antibodies [6].

4.2. Potential to remove matrix components

Even if the cost of these three types of selective sorbents may be higher than that of conventional sorbents, their main advantage is that extraction and cleanup are performed using a single cartridge. In addition, this cost should be compared with the cost resulting from the use of two cartridges or a combination of a liquid–liquid extraction and a SPE clean-up, both requiring the tedious development of two different extraction procedures. As previously mentioned, the improvement in selectivity was mainly demonstrated by comparing chromatograms obtained using the newly developed sorbents (IS, OS or MIP) with chromatograms resulting from conventional extraction procedures. Nevertheless, some authors have compared these selective sorbents among themselves. Moreover, IS being the first used and commercially available sorbent, the validation of the most recently developed sorbents (MIP and OS) was mainly achieved by comparing their performance with ISs.

Indeed, an MIP was recently compared with an IS through the determination of the amounts of OTA recovered when percolating 20 mL of pretreated wine samples on the MIP and by comparing the results with those obtained using an IS : a good agreement was found between the two extraction methods applied to 17 red wine samples (r^2_{adj} = 0.9817) [74]. The efficiency of a MIP to remove other matrix constituents by comparing chromatograms resulting from the use of MIPs and ISs has also been often reported, as for instance in the case of contaminated coffee [71], beer [72]or wheat [70] samples.

The same type of comparison was also proposed to illustrate the potential of oligosorbents. Indeed, an OS was compared with an IS for the clean-up of OTA from spiked ginger samples (**Fig. 4**). From chromatograms of ginger powder sample spiked with OTA based on IS and OS clean-up (**Fig. 4c** and **Fig. 4d**), it was shown that more interfering peaks were exposed after clean-up with OS than IS, whereas the occurrence of these peaks had no noticeable inference on the separation of OTA under the optimal chromatographic conditions. This was attributed to the largest washing volumes that the IS could tolerate without any loss of OTA. However, no significant difference was found in recoveries : the mean recoveries were 91.16% with RSD of 3.45% for OS and 94.45% with RSD 3.13% for IS, respectively [35]. Similar results in terms of removal of interfering compounds and recoveries were obtained for the analysis of a red wine sample [46].

4.3. Capacity

As for SPE on conventional sorbents, a frontal chromatography process takes place with these selective sorbent during the extraction step while a displacement chromatography process occurs during the desorption step. Therefore, a breakthrough volume that represents the maximal sample volume which can be percolated with a theoretical 100% recovery can be defined. This volume depends on the affinity between the analytes and the sorbent and on the capacity of the sorbent [77]. As mentioned before, the affinity strongly depends on the nature of the samples thus explaining the dilution step generally achieved on real samples.

Concerning the capacity of a sorbent, it is defined as the maximal amount of a molecule that can be retained by a sorbent during the percolation step. For these selective sorbents, it depends on the numbers of active antibodies or of aptamers immobilized on the solid sorbent or on the number of selective cavities of a MIP.

The capacity of an IS/OS depends of the bonding density and on the accessibility of the immobilized biomolecules of concern, i.e. antibodies and aptamers. The bonding density for ISs/OSs is theoretically defined as the total number of antibodies/aptamers linked to the surface of the solid support and is usually expressed in mg/ml of sorbent bed or mg/mg of sorbent. It depends on the specific surface area of the solid support accessible for the immobilization of the biomolecules[6]. Supports with small pore sizes have a high surface area, but a low accessibility for the large biomolecules. On the other hand, supports with large pore sizes have a good accessibility but a small surface area. The bonding density can be calculated experimentally by measuring by UV spectrophotometry the concentration of the antibodies/aptamers that remains in the binding solution. Nevertheless, this approach only allows the determination of the amount of grafted biomolecules including some of them that are not accessible for the target analyte. In the same way, the presence of cavities in a MIP can be

assessed by different methods such as binding experiments. However, as previously mentioned, the capacity value determined in a pure solvent can be different from the available capacity in real media, particularly when different solvents are applied during the MIP SPE procedure, the nature of the sample and of the solvents involved in the SPE procedure controlling the nature of the interactions that take place during the recognition mechanism.

Consequently, an easy way to determine the real capacity of a selective sorbent consists in measuring the amount of retained target analyte as a function of its concentration in the percolated sample when applying the optimized extraction procedure. The curve obtained when plotting the amount of target analyte retained by the sorbent and then recovered in the elution fraction as a function of its concentration in the percolated sample will show two different parts. First, it will present a linear part for a range of concentrations for which a constant recovery of extraction is obtained, this recovery being given by the slope of this linear part. Working in this range ensures the possibility to carry out quantitative analyses. In the second part of the curve, the amounts of target analyte retained by the sorbent tend to reach a plateau. For the corresponding concentration range, recoveries decrease when increasing the percolated amount of target analyte. This decrease in recovery is caused by the overloading of the sorbent capacity, *i.e.* the saturation of the antibodies/aptamers binding sites or of the MIP capacity [6, 54]. This approach was applied to determine the capacity of different newly developed selective sorbents for OTA, *i.e.* OS and MIP. Concerning commercialized OTA ISs, capacity values are not available but for some of them, it is indicated to users that ISs can be applied to sample contaminated with up to 100 ppb of OTA. As a comparison, for ISs developed for small molecules such as pesticides, values ranging from 4 to 93.6 nmol/g of sorbent were reported [78].

Concerning aptamers, their bonding density on sepharose and the resulting capacity values were studied by Hadj Ali *et al*. The grafting of 150, 450 and 600 µg of OTA aptamers on 35 mg of CNBr-activated sepharose results in capacity value of 24, 28 and 38 nmol/g respectively with a binding efficiency decreasing from 37 to 14% . These results illustrate the presence of a steric hindrance effect that leads to a maximal amount of aptamers that can be grafted to the solid phase with acceptable binding efficiencies [47]. Capacity values were in the range of 8.9 to 24 nmol/g of OS [46, 47] while binding efficiency values were between 22 and 37%. It was also reported that a higher capacity was obtained by using a C12 spacer arm instead of a C6 spacer arm when immobilizing an OTA aptamer on CNBr-activated sepharose. On the other hand, the immobilization of an aptamer through the 3'-end side and from the 5'-end side gave rise to similar capacity values [47].

If the capacity values of ISs and of OSs are very similar, MIP capacity values 30-fold higher than the capacity of the immunosorbent were mentioned when comparing sorbents developed for the same target [61]. MIPs and ISs for OTA were recently compared in terms of selectivity and capacity. The chromatograms corresponding to the analysis of cereal extracts resulting from a purification on a MIP or on a IS were similar (with a very clean baseline highlighting the removal of interfering compounds in both cases). On the other hand, the extraction recoveries were linear up to 5000 ng/g for the MIP, whereas for the commercialized IS when percolating an extract containing 900 ng or 4300 ng of OTA per g of wheat, only 650 ng were detected in the elution fraction of the IA thus indicating an overloading of the capacity of the IS [70]. This highest capacity of MIPs compared with ISs and of OSs highlights the advantage of MIPs for their use in miniaturized devices.

4.4. Regeneration and reusability

Ideally, as with other SPE sorbents, these selective sorbents should not be reused. However, when they are not commercially available, trends are for their reusability due to the cost of biomolecules or templates necessary to prepare the sorbents. Their reusability implies their possible regeneration, but also the chemical stability of the support. Despite their susceptibility to irreversible denaturation in some conditions, numerous studies have shown the possibility to reuse ISs after their storage at 4°C in a PBS solution, which is close to physiological conditions and in the presence of sodium azide as previously mentioned. In the same way, when not in use, the OS is stored at 4°C in a buffer solution whose composition is close to the binding buffer and containing also sodium azide. Their reusability can be explained by their high stability to low and high temperature, broad pH range and salt, these different parameters affecting the binding of the target without causing irreversible denaturation. This can be explained by the stability of the phosphodiester bond and by the possibility to improve

their stability by chemical modification as previously mentioned [34]. At last, despite their sensitivity to nuclease, OS can be reused even after the percolation of biological fluids as reported for cocaine aptamers [49]. It was also shown that an OS developed for another target than OTA can be stored 30 days [79] or for months before it is reused [80]. Of course, this stability depends on the number of applied samples. From five [48] to eight [35] food extracts were applied to the same OS without observing a decrease in the OTA extraction recoveries, the advantage of the use of an OS in these cases being also the fast aptamer regeneration time, *i.e.* a few minutes instead of more than 12 hours for antibodies [35].
Concerning OTA MIP, the use of a MIP for up to 6 samples of wine [68] and 14 samples of beer [72] without observing any loss of recoveries was also reported. For this polymeric sorbent, the reusability will depend on the possibility to find conditions of storage and of conditioning that ensure the possibility for the monomer residues in the cavities to interact with the same strength as for the first use with the analyte, the storage solvent often being identical to the solvent used for the synthesis of the MIP.

5- Miniaturization of the extraction sorbents

The miniaturization of analytical devices such as when working in nanochromatography (nanoLC) results in an increase in sensitivity of the whole analytical method, the improvement in sensitivity for a compound being in theory in inverse proportion to the square of the column diameter. However, to be optimal, miniaturized separation devices must be associated to miniaturization extraction devices. This will allow the analysis of target analytes in complex samples while limiting solvent consumption, reducing sample volume and decreasing the required amount of biomolecules or template thus reducing the cost of the device. Moreover, the miniaturization of the separation device is often associated to the reduction of the separation length thus causing a loss of resolution that can be counterbalanced by the use of selective sorbents during the sample pretreatment step in order to reduce the number of components in the analyzed extract.
For large formats, the packing of particles of IS, OS or MIP only necessitates frits to maintain the beads in the cartridge. For 75-100 µm i.d. capillaries or microchip channels, frits can be synthesized but their preparation is tedious and difficult. Therefore, the introduction of the selective sorbents in such miniaturized devices implies new strategies of synthesis.
One strategy may consist in the direct immobilization of antibodies [81] or aptamers [52] on capillary walls but this approach leads to very low capacity thus limiting the amount of trapped analytes. An emerging alternative to improve the density of biomolecules within the capillary is to proceed to the *in situ* synthesis of a monolithic support, linked to the capillary walls, and presenting at its surface the appropriate functions to immobilize antibodies. Two types of monoliths can be synthesized, organic and inorganic. Grafting of antibodies has largely been considered using the former ones. Glycidyl methacrylate (GMA), presenting an epoxy group, was used to immobilize OTA antibodies in capillaries (5 cm, 75 µm i.d.), this capillary being connected directly to LIF detection [82] or to CE-UV or –LIF [83]. A capacity of 0.6 pmol of OTA was reported with a bonding density of 18 µg/mg. This miniaturized OTA IS was not applied to real samples.
A miniaturized oligosorbent (mOS) was also recently prepared via the *in situ* sol-gel synthesis of a hybrid organic-inorganic monolith in 100 µm i.d. capillary columns followed by covalent binding of a OTA aptamer [84]. The mOS was coupled on-line to RP-nanoLC-LIF. Selective extraction of OTA on several mOSs was demonstrated with an average extraction recovery above 80 % when percolating spiked binding buffer and a low recovery on control monoliths grafted with a non-specific aptamer. Reproducibility of mOSs preparation was highlighted by comparing extraction yields. Due to the high specific surface area of the hybrid silica-based monolith, the coverage density of DNA aptamers covalently immobilized in the capillaries was very high and reached 6.27 nmol/µL, thus leading to a capacity above 5 ng of OTA (*i.e.* 12 pmol). This miniaturized device was then applied to the selective extraction of OTA from beer samples. Chromatograms presented on **Fig. 5** correspond to the percolation of a sample of beer simply diluted by a factor 2 with the binding buffer (BB) and of BB spiked with the same amount of OTA (75 pg, 250 nL) on an OTA mOS coupled on-line to nanoLC-LIF.
Their comparison shows that other beer components were removed during the percolation and washing steps on the mOS while OTA was on the contrary selectively retained on the mOS thus giving rise to a chromatogram very similar to the one corresponding to the spiked BB. Furthermore, OTA was not retained on the control

sorbent (that corresponds to the same monolith grafted with non-specific aptamers) even in complex samples thus proving the contribution of the OTA aptamers in the selective retention process.
More recently, a mOS was prepared by activating a silica monolith prepared in a 75 µm i.d. capillary with a vinyl spacer allowing the grafting of a SH-modified OTA aptamer by thiol-ene photoclick chemistry. The aptamer bonding density was estimated at 5pmol/cm. The mOS (1.5 cm length) was coupled in-line with CE-LIF to be applied to the extraction of OTA from wine and beer samples [85].
MIP monoliths have been first *in-situ* synthesized by Schweitz *et al.* for capillary electrochromatography (CEC) [86]. Recently, MIP monoliths have also been prepared for the development of miniaturized extraction devices such as pipet tips [87] and hollow fiber membrane [88] for off-line extraction but, until now, no development for OTA analysis has been reported. Nevertheless, it can be important to notice that the possibility to achieve the *in situ* synthesis of a MIP into a 100 µm i.d. capillary was already demonstrated for other mycotoxins, *i.e.* aflatoxins B1, B2 and G1. The MIP was connected on line to nanoLC and applied to the selective extraction of aflatoxins from a spiked pure water sample [89].

Conclusions

Because of its occurrence at the trace level in very complex food samples, OTA required the development of very powerful tools for the sample treatment before its LC/Fluo or LC/MS analysis. Immunosorbents involving immobilized antibodies are still the most largely used selective sorbents applied to the selective extraction of OTA from various types of samples. Despite the presence of solvent in food extract that may affect the antigen-antibody interaction, high enrichment factors can be obtained because of the high affinity of antibodies toward OTA. Nowadays, oligosorbents based on immobilized aptamers and molecularly imprinted polymers constitute an interesting alternative to antibodies because some of them present similar affinities and they can be produced at a lower cost than immunosorbents.
The interactions that take place between OTA and the monomers residues of the MIP cavities, the nucleotides involved in the aptamer binding sites or the amino acid sequence of the antibodies are different thus implying the necessity to adjust the extraction procedure to each sorbent. It also explains a more or less strong retention of analogs on these different sorbents.
The selectivity brought by these sorbents improved the reliability of LC/UV and LC/MS analysis by removing matrix components thus reducing matrix effect. This improvement in selectivity compared to conventional approaches that imply the use of several steps of sample pretreatment constitutes a key-point in the development of miniaturized analytical method by giving the possibility to couple them to less powerful analytical method in terms of resolution while maintaining sufficient analytical performances.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1: Principle of an immunoextraction sequence.

Fig. 2 : Schematic diagrams of the binding of OTA to aptamer (adapted from [35]).

Fig. 3: LC/Fluo analysis of the washing and elution fraction from the MIP after the treatment of the wheat extract (1 g of wheat extracted with ACN) (a) and comparison of the elution fraction obtained with the MIP and the NIP (b). P: percolation of the cereal extract spiked with 100 ng of OTA diluted, Washing steps with ACN (W1): ACN/MeOH (50/50, v/v, W2, W3), MeOH (W4) and elution with ACN/MeOH/acetic acid (45/45/10, v/v/v). In insert: corresponding elution profiles of OTA obtained on MIP and on NIP (adapted from [70]).

Figure 4. Typical UHPLC–Fluorescence chromatograms of (a) OTA standard solution (3 ng/mL), (b) blank ginger powder samples after clean up by OS, (c) blank ginger powder samples spiked with OTA at the level of 15 µg/kg after clean-up by IS, (d) and after clean up by OS (adapted from [35]).

Fig. 5: NanoLC chromatograms corresponding to the percolation of 250 nL of beer/ binding buffer (BB) (50:50) and BB spiked with 75 pg OTA on mOS and on a control sorbent (grafted with a nonspecific aptamer), each miniaturized sorbent being coupled on-line with nanoLC-LIF (adapted from [83]).

Table 1: Applications of OSs to the extraction of OTA from real samples.

Matrix	Sample treatment	Aptamer modification /Spacer	Sorbent	Elution	Analytical method	Ref.
Wheat extract	MeOH/Water 60/40 extraction, dilution x 4 with BB	-/-	DADPA (400µL)	MeOH/Tris Buffer, EDTA, pH=9 (2/8)	Fluorescence	[44]
Wheat extract	MeOH/Water 60/40, dilution x 4 with BB	-/-	DADPA (300µL)	MeOH/Tris Buffer, EDTA, pH=9 (2/8)	HPLC/Fluo	[48]
Wheat extract	ACN/Water 60/40 extraction, dilution x 20 with BB	-/-	OTA sense®		TRF spectroscopy	[53]
Red wine	pH adjustment (NaOH, pH=8.5)	NH_2, biotin/C6	CNBr-sepharose, Streptavidin-agarose (35 mg)	Water/ACN (6/4)	HPLC/Fluo	[46]
Wheat extract	ACN/Water 60/40 extraction, dilution x 10 with BB	NH_2/C6, C12	CNBr-sepharose (35 mg)	Water/ACN (6/4)	HPLC/Fluo	[47]
Coffee extract	Incubation of nanosphere in Methanol/water 1/1/ extract diluted x 5 with BB	NH_2/C6	NH_2-Magnetic nanosphere	ACN/Water (95/5)+ 1% acetic acid	HPLC/Fluo	[50]
Ginger powder	ACN/Water 60/40 extraction, dilution x 10 with BB	NH_2/C6	NHS-sepharose (200µL)	MeOH	UHPLC/Fluo	[35]
Traditional Chinese medicines	ACN/Water 60/40 extraction, dilution x 10 with BB	NH_2/C6	NHS-sepharose (200µL)	MeOH	UHPLC/MS, Fluo	[51]

BB: binding buffer (buffer close to the selection buffer); DADPA: diaminodipropylamine agarose; TRF: Time-resolved fluorescence.

Table 2: Development of MIPs for OTA.

Matrices	Template	Monomer/Cross-Linker/ solvent	Comments	ref
-	L-Phe-CHNA	MAA/EDMA/chloroform	HPLC evaluation	[63]
-	Several OTA mimics	Various home-made monomers /EDMA/Chloroform	HPLC evaluation	[64]
Wine	OTA mimic	Home-made monomers/EDMA/ chloroform	Purification on MIP of the C18 silica wine extract	[65]
-	OTA	MAA+acrylamide/EDMA/DMF	Binding experiments	[66]
Wheat	OTA	PAM/TRIM/ chloroform	Purification on MIP of assisted LLE extract wine extract – MIP directly connected to fluorescence detection	[67]
Wine	OTA	Pyrrole/EDMA/ACN	Coupling of a MIP via a loop with LC Fluo	[68]
Wine	OTA	CNTs,Pyrrole/EDMA/ACN	Coupling of a MIP via a loop with LC Fluo	[69]
Wheat	-	Commercialized MIP (AffiniMIP^{TM})	MIP-SPE applied to wheat extract	[70]
Coffee, grape juice, urine	-	Commercialized MIP (AffiniMIP^{TM})	MIP in a envelope (2 sheets of membrane)	[71]
Beer, red wine, grape juice	-	Commercialized MIP (AffiniMIP^{TM})	MIP-SPE applied to real samples	[72]
-	OTA mimic	MAA/EDMA/chloroform	HPLC evaluation	[73]
Wine	OTA mimic	MAA/EDMA/chloroform	MIP-SPE applied to real samples	[74]

CNTs: carbon nanotubes; EDMA: ethylene dimethacrylate; MAA: methacrylic acid, DMF: dimethylformamide; L-Phe-CHNA: N-(4-chloro-1-hydroxy-2-naphtoylamido)-(L)-phenylalanine; PAM: N-Phenylacrylamide; TRIM: trimethylolpropane trimethacrylate.

Figure 1

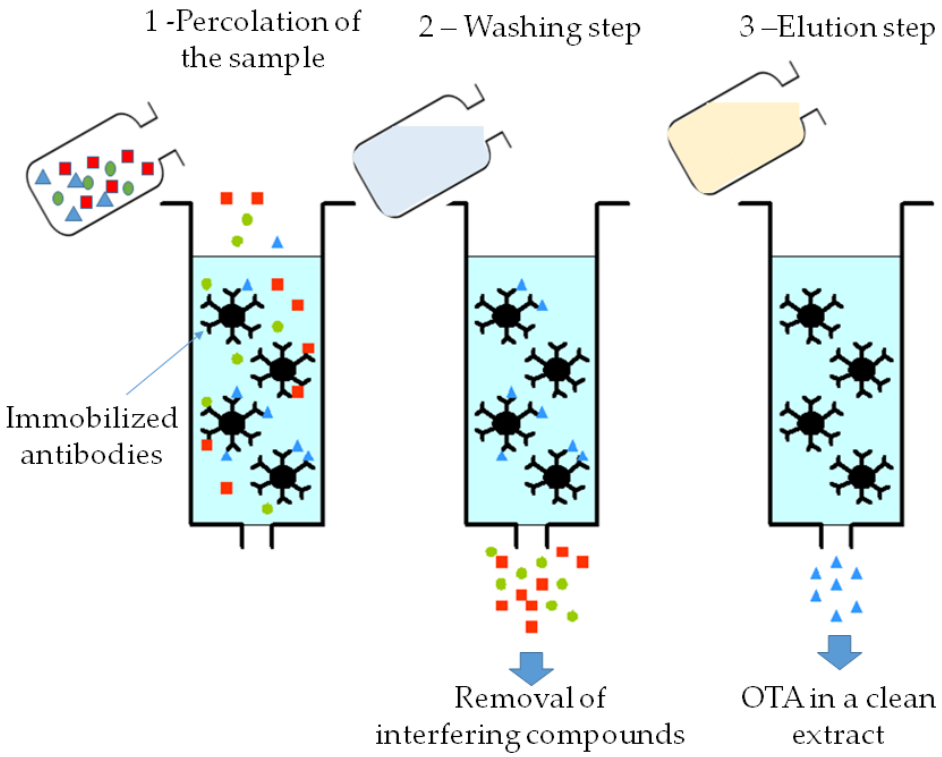


Figure 2

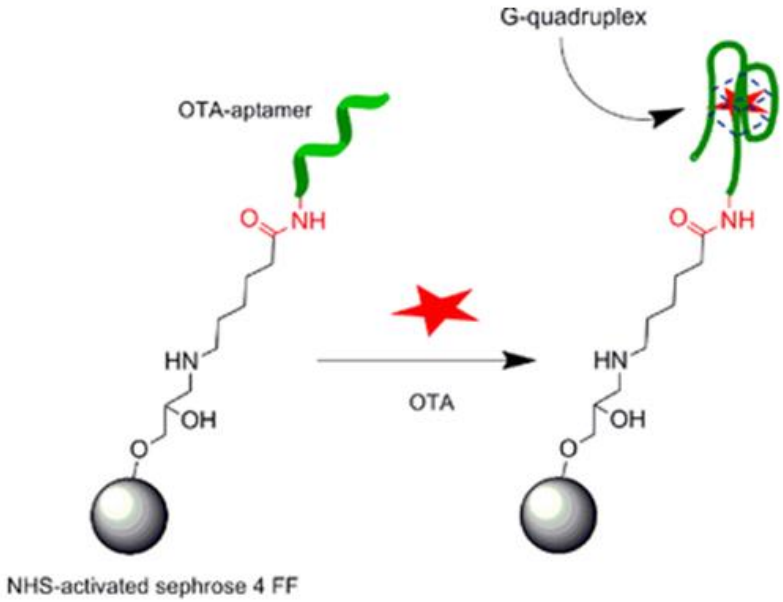


Figure 3

Figure 3:

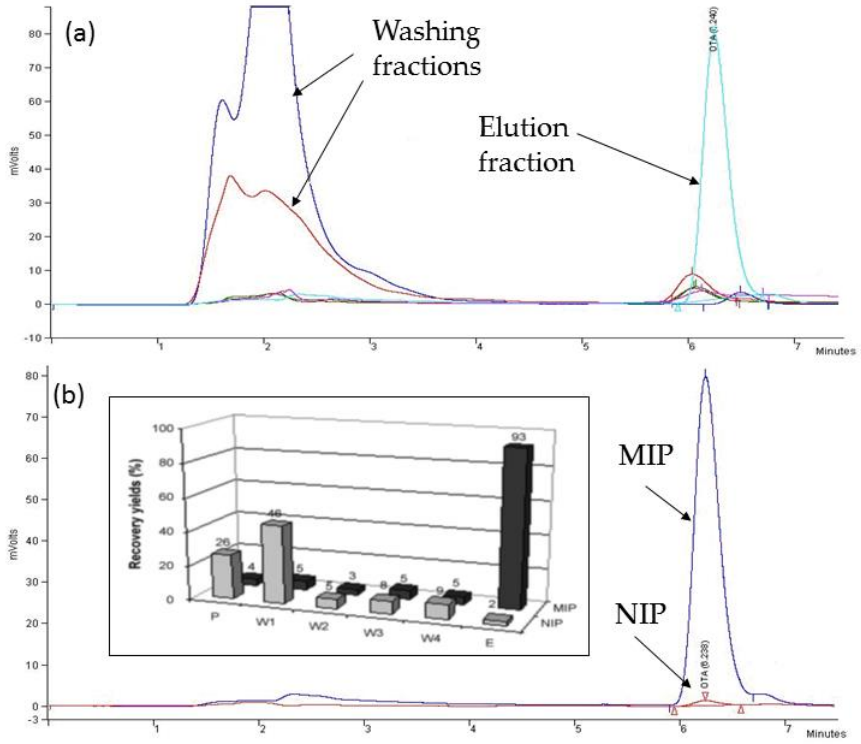


Figure 4:

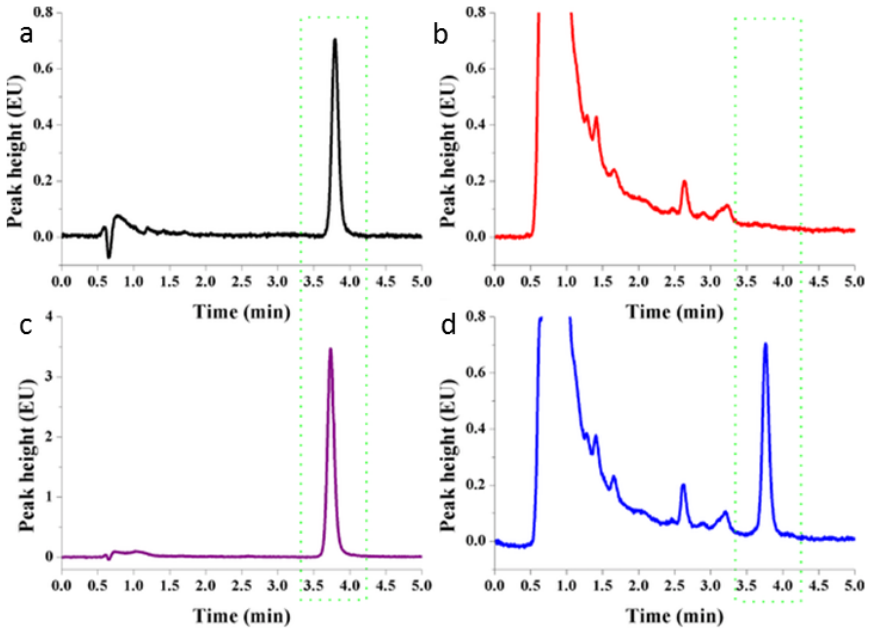
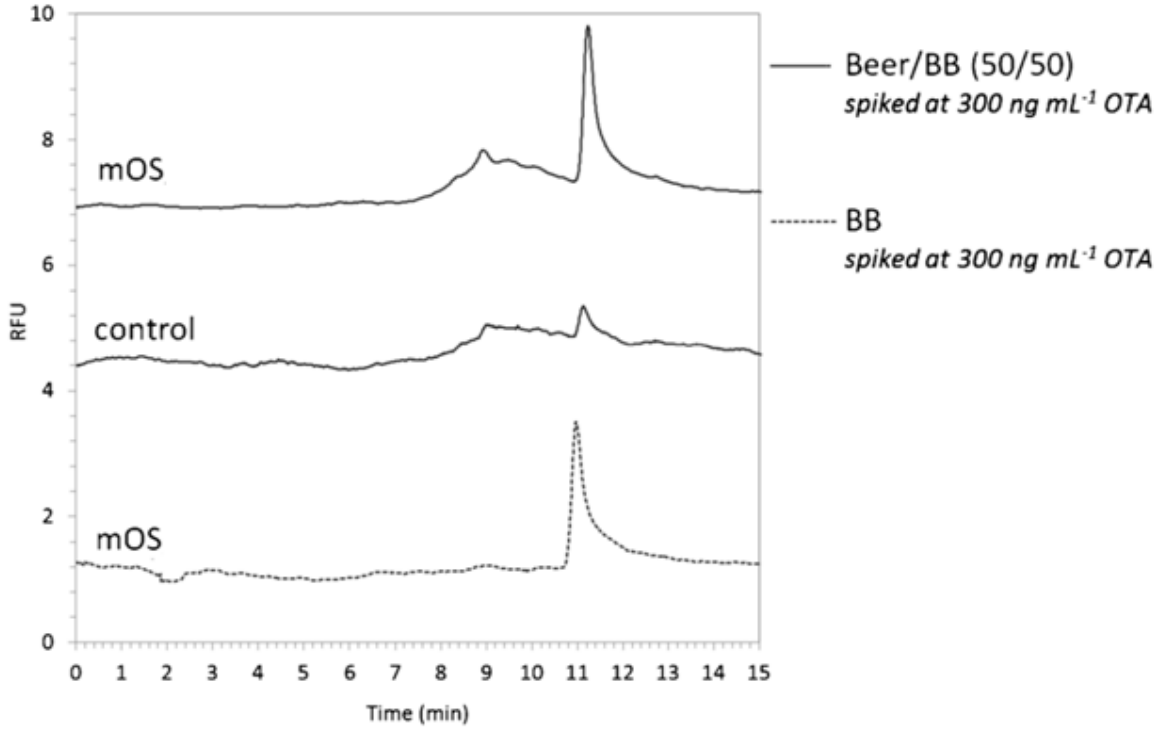


Figure 5: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Time (min)
Beer/BB (50/50)
spiked at 300 ng mL-1 OTA
BB
spiked at 300 ng mL-1 OTA