Molecularly Imprinted Polymers for the Detection of Food Toxins: A Minireview

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ABSTRACT

Food contamination from natural or anthropogenic sources poses severe risks to human health. It is now largely accepted that continuous exposure to low doses of food Toxins such as mycotoxins, phycotoxins can be related to several chronic diseases, including some type of cancer and serious hormonal dysfunctions. Contemporary analytical methods have the sensitivity required for contamination detection and quantification, but direct application of these methods on real samples can be rarely performed because of matrix complexity. Thus, selective analytical methods, relying on intelligent functional materials are needed. Recent years have seen the increasing use of molecular imprinted polymers in contaminant analysis in food because these materials seem to be particularly suitable for applications where analyte selectivity is essential. It offers several advantages to the agrofood industry in areas such as analysis, sensing, extraction, or preconcentration of components. It has the potential of becoming a tool for acquiring truly simple, rapid, and robust direct measurements.

Keywords: AFB1; AFB2; AFM1; WHO; EPA; SPE; HPLC

1. Introduction

Food contamination due to natural toxicants can represent a significant source of food-borne illness and it poses severe risks to human health. In fact, besides the well-known food contamination due to the presence of living bacterial cells (e.g. enterotoxins from certain strains of Escherichia coli or Staphylococcus aureus), several natural contaminants represented by low mass molecules of a non-proteinic nature are extremely potent acute toxins (e.g. T2 toxin) or are very strong carcinogens (e.g. aflatoxins) which are officially recognized by the World Health Organization (WHO) as biocontaminants representing a significant source of foodborne illnesses [1]. Dangerous substances in food may include natural toxicants such as mycotoxins, phycotoxins and phytotoxins [2,3], environmental contaminants such as polychlorinated dioxins [4] and polycyclic aromatic hydrocarbons [5], and chemicals such as pesticides and veterinary drugs deliberately used to increase the food supply, whose residues can be present in the processed food and potentially affect human health [6].

Contemporary analytical methods have the sensitivity required for contamination detection and quantification, but direct application of these methods on food samples can be rarely performed. Usually, contaminants are present in food at low concentration (ng/g) levels, dispersed in highly complex (thousand of different components) and morphologically structured matrices, with an elevated degree of sample-to-sample variability. Thus, such a type of matrix introduces severe disturbances, and analysis can be performed only after some clean-up and preconcentration steps [7-9].

The agricultural and food sector is in constant need of improved analytical techniques that can be used to control manufacturing processes and the safety and quality of the products [10,11].

Recent years have seen a significant increase of the “molecularly imprinted solid phase extraction” (MISPE) technique in the food contaminant analysis. In fact, this technique seems to be particularly suitable for extractive applications where analyte selectivity in the presence of very complex samples represents the main problem.

MIPs have been employed in fields where a certain degree of selectivity is required such as sensors [12], chromatography [13] and catalysis [14]. However, nowadays their use in solid-phase extraction, so-called molecularly imprinted solid-phase extraction (MISPE), is by far the most advanced technical application of MIPs. Current sample pre-treatment methods, mostly based on the solid phase extraction technique, are very fast and economical. As economical, rapid and selective clean-up methods (relying on “intelligent” materials) are needed,
solid phase extraction and clean-up methods based on molecularly imprinted polymers (molecularly imprinted solid phase extraction, MISPE) seem to represent natural candidates to circumvent the drawbacks typical of more traditional solid phase extraction techniques [15,16].

2. Molecularly Imprinted Solid-Phase Extraction

Three different approaches to prepare MIPs have been reported: covalent, non-covalent and semi-covalent approaches. The non-covalent approach is the most widely used for the preparation of MIPs in food analysis thanks to its versatility. However, in parallel, such versatility is also the origin of some of the drawbacks attributed to MIPs. In this sense, the necessity of using a high amount of functional monomer leads to the formation of non-selective binding sites. Besides, template bleeding, over-use of certain “standard” formulations and tedious synthesis procedures are other weak points associated to this area that need to be improved. Significant attempts made during past years to improve the performance of MIPs in solid-phase extraction. There are several variables, such as kind and amount of monomer or nature of cross-linker and solvent that affects the final characteristics of the obtained materials in terms of capacity, affinity and selectivity for the target analytes.

Thus, the obtainment of the optimum MIP to be used in solid-phase extraction might take several weeks of trial-and-error experiments using different formulations. This fact has provoked an overuse of certain standard formulations (i.e. the typical 1:4:20 template:monomer:cross-linker molar ratio) [17].

3. Applications to Food Samples

The modern food industry is a complex and highly organized business, manufacturing a wide range of food types, including minimally processed products, modified atmosphere-packaged foods, specialist dietary formulas, and products with few or no additives. The industry is increasingly aware of the demands from the consumer for wholesome manufactured foods and, indeed, must now ensure compliance with national and international legislation on ensuring food safety and quality for the consumer. With requirements such as these, the industry is aware of the risks posed by improper control of the product quality and the resulting financial consequences. The food industry is rapidly changing its practices of end product testing (quality control) to wider quality assurance and management systems.

Analytical testing for microbiological and non-microbiological analytes must be considered in the context of a properly evaluated and implemented quality assurance system, which can be specific, rapid, and on- or nearline to cope with modern just-in-time manufacturing. From the food safety perspective, the majority of testing carried out by the agrofood industry is for microorganisms (e.g., pathogens and spoilage organisms), microbial toxins (e.g., mycotoxins and bacterial toxins), antibiotic residues, pesticides, and artificial hormones (Table 1).

As mentioned above most of the MIPs used in food analysis using SPE were prepared via noncovalent imprinting by bulk polymerization, several MISPE applications have focused on the extraction of compounds in food samples (Table 2). De Prada et al. [18] developed an on-line MISPE procedure for the selective preconcentration and voltammetric determination of sulfamethazine in milk, spiked at low concentrations.

The analytes studied included the residues of herbicides or drugs in food [19], Chapuis et al. [20] investigated the retention mechanism of analytes in MISPE by means of molecular modeling and applied MISPE to the cleanup of grape juice and soil extracts, spiked with triazines.

Maier et al. [21] studied a new analytical method for the determination of the carcinogenic mycotoxin ochratoxin in red wines, involving two-dimensional SPE clean-up on C18 silica and a target-selective MIP. Zhou et al. [22] developed an on-line MISPE-PE method for the rapid screening of OTA in wheat extracts with fluorescence detection, in which each analysis process required less than 5 min to complete. Moreover, LC/MS can be combined with this MISPE-PE for the rapid identification and quantification of OTA.

Blahova et al. [23] reported a MISPE method for the selective extraction of (+)-catechin in green tea. Under optimal conditions, a very good recovery (95%) and selec-

Table 1. Advantages of MIPs in comparison with natural receptors.

<table>
<thead>
<tr>
<th>Advantage</th>
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<tbody>
<tr>
<td>MIPs can be prepared for practically any compound</td>
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<tr>
<td>MIPs can work in organic solvents</td>
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<tr>
<td>Polymers are compatible with microfabrication</td>
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<tr>
<td>MIPs have similar affinity as compared to natural biomolecules but often better specificity</td>
</tr>
<tr>
<td>MIPs are stable at low/high pHs, pressure and temperature</td>
</tr>
<tr>
<td>Polymers are inexpensive</td>
</tr>
</tbody>
</table>

Table 2. Food analysis.

<table>
<thead>
<tr>
<th>Analyte Class</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceuticals</td>
<td>Antibiotics, steroids</td>
</tr>
<tr>
<td>Additives</td>
<td>Preservatives, sweeteners, colorants, flavors</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Pathogenic bacteriae, microbial toxins</td>
</tr>
<tr>
<td>Herbicides, pesticides</td>
<td>Triazines</td>
</tr>
<tr>
<td>Minerals, trace metals</td>
<td>Heavy-metal ions</td>
</tr>
</tbody>
</table>
tivity were obtained by MISPE. Theodoridis and Manesiotis [24] presented the first application of caffeine MIP in SPE, allowing direct loading of aqueous samples, such as beverages and spiked human plasma.

Molinelli et al. [25] reported the first application of MISPE for complex beverage analysis, in which quercetin was selectively extracted from spiked red wine and determined by HPLC, demonstrating the potential of MISPE for rapid, selective, and cost-effective sample pretreatment. Puoci et al. [26] established a simple MISPE method for concentrating trace amounts of Sudan I from food matrices. Brambilla et al. [27] reported that the MISPE based on clenbuterol MIP was effective for the multi-residue purification of clenbuterol and aniline-like β2-agonists, and successfully applied it to the SPE of clenbuterol from feeds, urine, and liver.

MISPE were used as for the preconcentration of additives in foods and compounds originating from foods [21-31]. This new MISPE procedure was able to remove the matrix compounds almost completely, and could be consequently used to determine triazines at concentrations below the established maximum residue limits, making the procedure suitable for monitoring these analytes in vegetable samples. MISPEs in preconcentration of analytes in various food samples (Table 3).

A. K. Wihlborg et al. [32] evaluate the utility of molecular imprinted polymer SPE technology for the extraction of chloramphenicol from shrimp. Because selectivity is introduced during the development of the MIP phase itself, it allows for a binding site that is sterically and chemically complementary to the target analyte. For chloramphenicol, the SupelMIP SPE approach provided improved and significant increases in selectivity relative to a described conventional hydrophilic polymer SPE method. The SupelMIP Chloramphenicol method allowed high recoveries, above 90%, low levels of interfering contaminants and very low limits of detection in the low ppt range [33].

Foodborne pathogens and microbial toxins are generally recognized to be the biggest problem in the food industry. In conjunction with other methods on the market, MIPs have the potential to become an efficient recognition element in detecting food contaminants [34-39]. In accordance to several reviews and scientific databases [40-42], in the last 10 years more than 1600 papers concerning molecular imprinting have been published worldwide. Considering the number of papers/year, molecular imprinted solid phase extraction—dedicated reviews excluded—is one of the fastest growing applications, with 223 papers published. Extraction of contaminants from food and beverage samples has been reported in 30 papers.

Here, Listeria monocytogenes and Staphylococcus aureus were employed in a surface imprinting protocol by which recognition sites were created on the surface of polyamide microcapsules for later use in binding assays. Although somewhat preliminary in nature, inasmuch as the selectivities were rather poor, the study well supports the potential of using whole cells as target species. As mentioned above, imprinting protocols using proteins as target substances are less easily developed. Nonetheless, the studies that have been published demonstrate the

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>Template (T)/Monomer (M)</th>
<th>Detection Technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>Food sample</td>
<td>Caffeine</td>
<td>LC/UV</td>
<td>[28]</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Milk</td>
<td>DEAEM (M)</td>
<td>Square wave voltametry</td>
<td>[33]</td>
</tr>
<tr>
<td>Flavonol</td>
<td>Olive oil</td>
<td>MAA(M)</td>
<td>Fluorescence</td>
<td>[34]</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>Milk</td>
<td>MAA(M)</td>
<td>Square wave voltametry</td>
<td>[35]</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>Methanolextract of green tea</td>
<td>(+)-Catechin</td>
<td>LC/UV</td>
<td>[23]</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Red wine</td>
<td>Ochratoxin A</td>
<td>LC/fluorescence</td>
<td>[21]</td>
</tr>
<tr>
<td>Caffeine and theophylline</td>
<td>Green tea</td>
<td>Caffeine and theophylline</td>
<td>LC/UV</td>
<td>[30]</td>
</tr>
<tr>
<td>Sudan I</td>
<td>Red chili, powder spiked with Sudan I</td>
<td>Sudan I</td>
<td>LC/UV</td>
<td>[26]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Fish</td>
<td>MAA(M)</td>
<td>Chemiluminescence</td>
<td>[36]</td>
</tr>
<tr>
<td>Vanillin</td>
<td>Vanilla sugar</td>
<td>MAA(M)</td>
<td>Piezoelectric sensor</td>
<td>[37]</td>
</tr>
<tr>
<td>Benzoic acid derivatives</td>
<td>Melissa officinalis</td>
<td>Sulfamethazine</td>
<td>LC/UV</td>
<td>[29]</td>
</tr>
<tr>
<td>Atropine/scopolamine</td>
<td>Tablets</td>
<td>Atropine</td>
<td>LC</td>
<td>[38]</td>
</tr>
<tr>
<td>Harmine, harmaline</td>
<td>Seeds</td>
<td>Harman</td>
<td>LC</td>
<td>[39]</td>
</tr>
</tbody>
</table>

DEAEM: (diethylamino)ethyl methacrylic acid, MAA: methacrylic acid.

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potential of preparing efficient MIPs toward bacterial toxins and other protein contaminants.

The most commonly tested chemical analytes include antibiotics (e.g., ß-lactams and sulfonamides), mycotoxins (e.g., aflatoxins and ochratoxins), and pesticides (e.g., organophosphates and atrazine). There is also concern over the presence of degraded carrageenans (polysaccharide) in infant foods and bioavailability of vitamins. The current techniques for the analysis of polysaccharides [43], antibiotics [44], and vitamins [45] include GC, HPLC, electrophoresis, microbial assays, radioligand assays, and immunological tests. Each technique has its advantages and limitations [46]. The major limitations of the chromatographic techniques include the requirement for high technical skills, expensive equipment, and lengthy and cumbersome sample preparation, whereas microbiological assays are not always reliable, require technical expertise, and are time-consuming. ELISAs are technically simple, but the antibody reagents can show batch-to-batch variation; the assays are not wholly robust, and the commercial kits generally have limited shelf life.

A large number of studies have dealt with herbicides and pesticides, not only because these substances can be enriched in crops and cattle but also for environmental analyses. Thus, a number of studies have put forward the possibility of using the imprinted materials in, for example, sewage and wastewater analyses. In addition to basic recognition studies and imprinting protocol advancement [47-50], several applications have been developed. Thus, MIPs toward herbicides/pesticides have been used in radioligand binding assays [51-53] and in sensor devices [54].

A considerable number of studies have focused on metal ions as target species. In these cases, various metal coordination monomers have been arranged around a metal ion and subsequently been fixed during polymerization. By this method, highly selective matrices have been produced, capable of distinguishing closely related metal ions. In food analysis, the possibility of detection and quantitation of traces of contaminant heavy metals is of high value. Molecular imprinting offers a means of coupling robust and highly ion selective matrices to sensor devices. In this way, rapid analyses of trace metals may be performed.

4. Future Prospects

MIPs has proven to be useful as a tool in agricultural and food technology. Highly selective and robust recognition matrices produced in this way can be employed in various applications when the analysis of diverse food analytes is an issue. Given the advantages of molecularly imprinted materials such as high stability, endurance, and low cost of production, it is plausible that products based on MIPs will reach the market soon.

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