Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) is a polymer with a «memory» of the shape and the functional groups of a template molecule. This material is designed in order to recognize selectively the template molecule used in the imprinting process, even in the presence of compounds with structures and functionality similar to those of the template. Molecularly imprinted polymer then acts essentially as an antibody. High molecular recognition properties can be achieved with these MIPs for a variety of molecules

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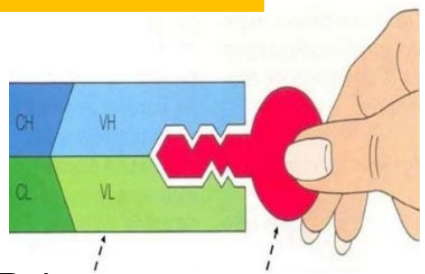
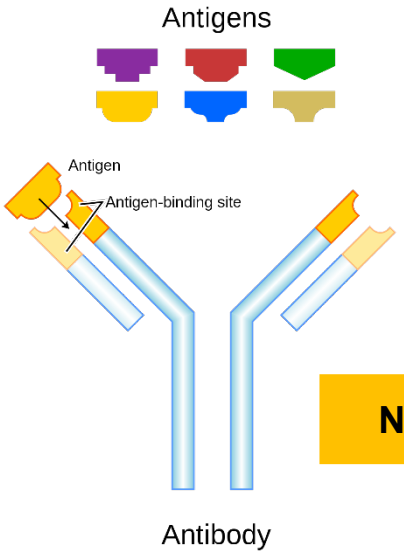
MIPs

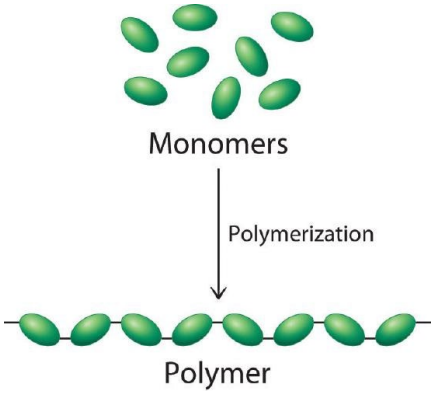




Natural Antibody

MIPs Synthesis

Components of MIP Mixture

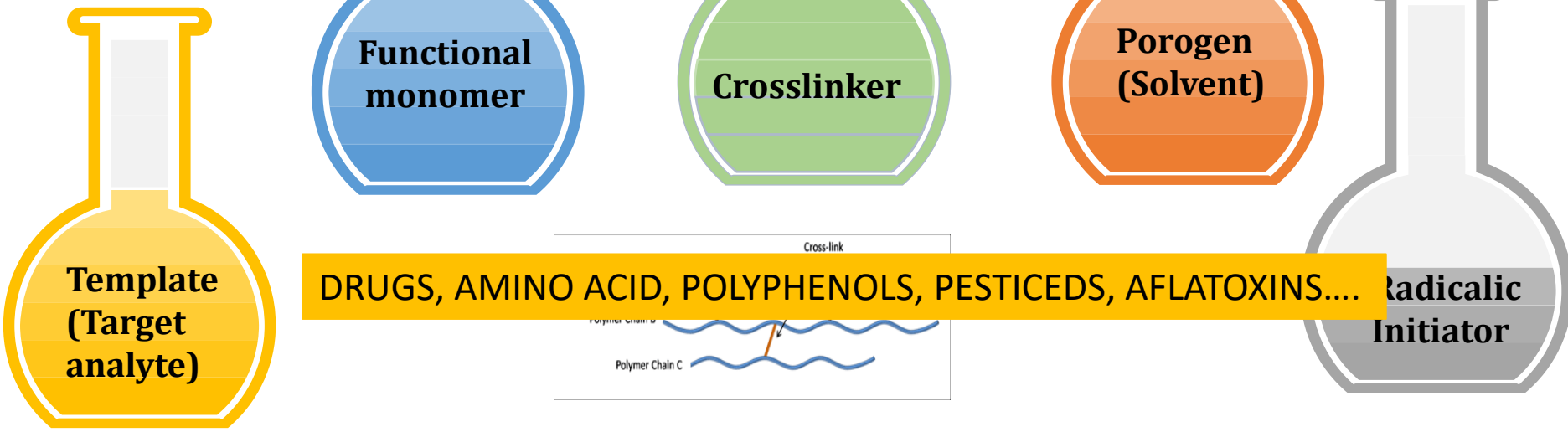


ETHYLENE GLYCOL DIMETHACRYLATE (EGDMA)

USED IN EXCESS COMPARED TO TEMPLATE (1:4), SUCH AS ACRILAMMIDE (AA), METHACRYL… METHYL METHACRYLATE (MMA),

TOLUENE, CHLOROFORM, DICHLOROMETANE, ACETONITRILE..

2,2' Azobis-Isobutyronitrile (AIBN)



DRUGS, AMINO ACID, POLYPHENOLS, PESTICEDS, AFLATOXINS….

The synthesis of polymers requires three steps:

1. Formation of a complex (covalent or non-covalent) between functional monomer and template molecule;

COVALENT MOLECULAR TEMPLATE

Advantages: The monomer-template complex is more stable, which makes the molding process more precise. Once the covalent bond has been established, different polymerization conditions can be employed (high temperatures, high or low pH, more polar solvents).

Disadvantages: the synthesis of the monomer-template complex is often problematic and not very economical. The number of available reversible covalent bonds is limited. It is difficult to remove the template from the polymer. The binding and release of target substances is slow (as it requires the formation and breaking of a covalent bond).

NON COVALENT MOLECULAR TEMPLATE

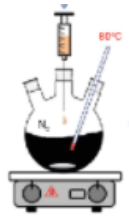
Advantages: It is not necessary to make a monomer-template complex before polymerization. It is easier to remove the template from the polymer since the monomer-template bond is quite weak. The binding of the target substances and their release is fast.

Disadvantages: The mold process is less precise. It is necessary to carefully evaluate the polymerization conditions to facilitate the formation of non-covalent interactions in the reaction mixture. Functional monomers present in large excess of the template often result in the formation of non-specific binding sites, causing a decrease in selectivity.

MIPs Polyimerization

Synthesis of MIPs

- Thermal heating :24h
- Ultrasound bath :2h
- Ultrasonic probe :Few min



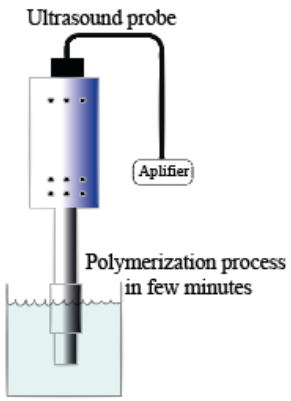
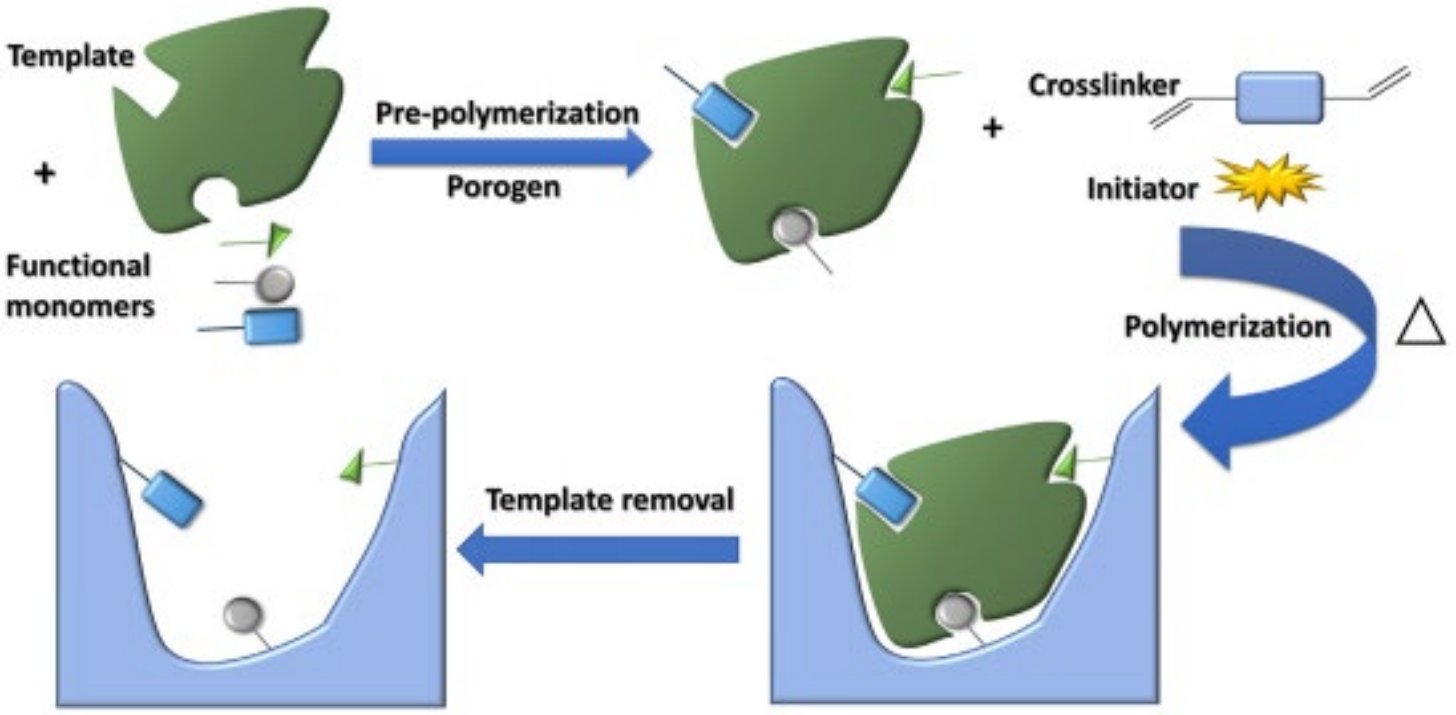


Figure : Synthesis of magnetic molecularly imprinted polymer

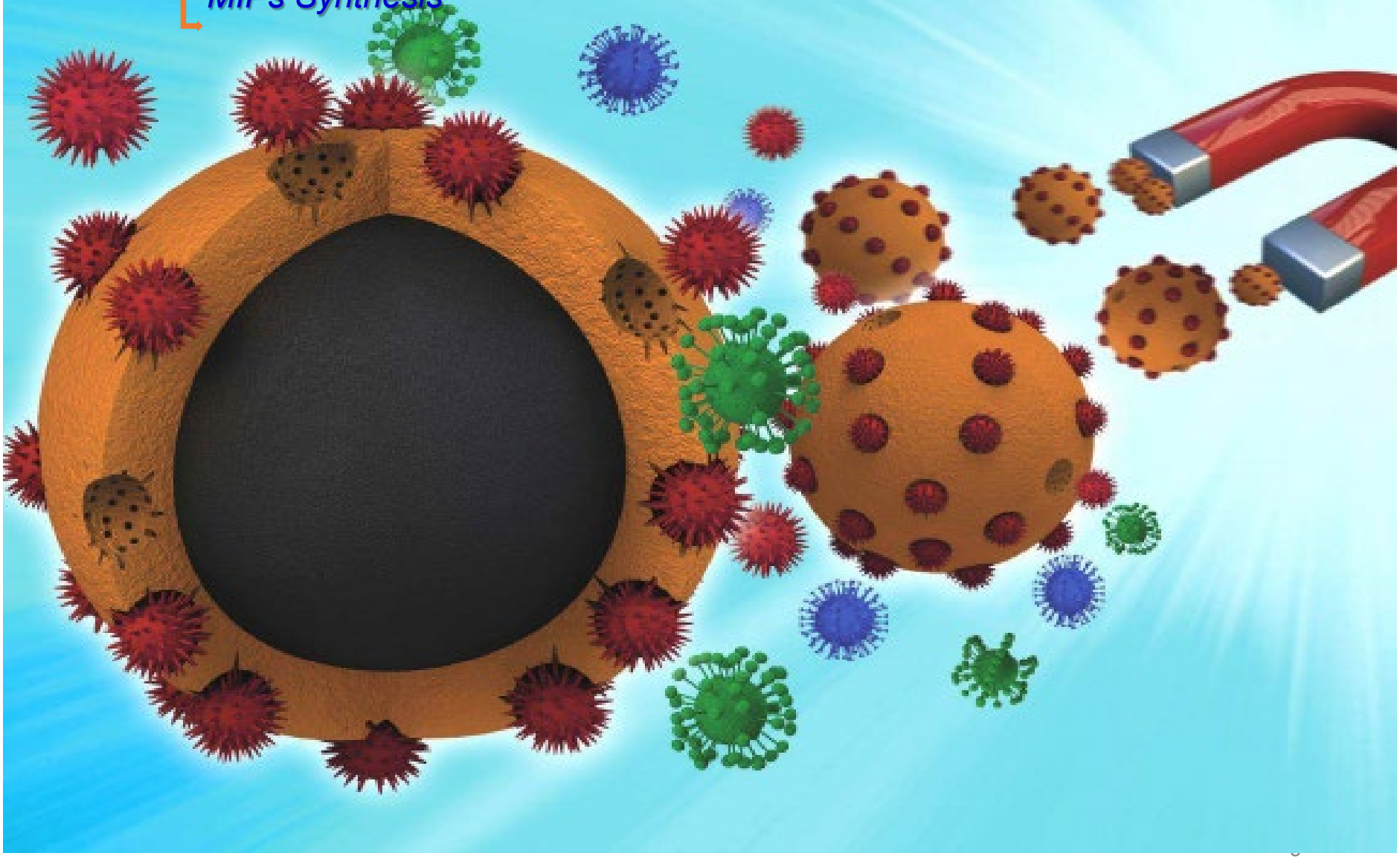
MIP synthesis

MIPs Synthesis



Synthesis of Magnetic Nanoparticles for MIP

MIPs Synthesis



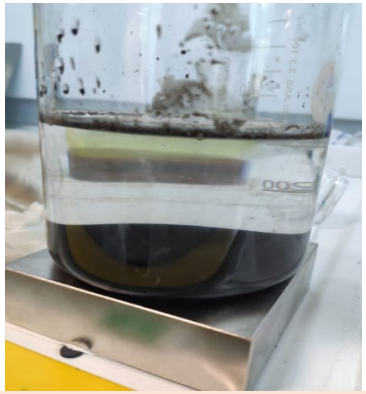
Synthesis of Fe3O4 magnetite Nanoparticles

Mixture oxidation of Fe^{2+}/Fe^{3+} under nitrogen conditions

50 min

Addition of NH4OH

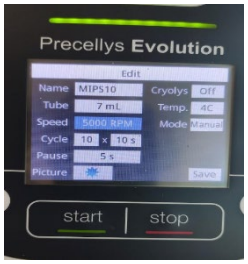
40 min



Separation of Fe3O4 NPs by an external magnet

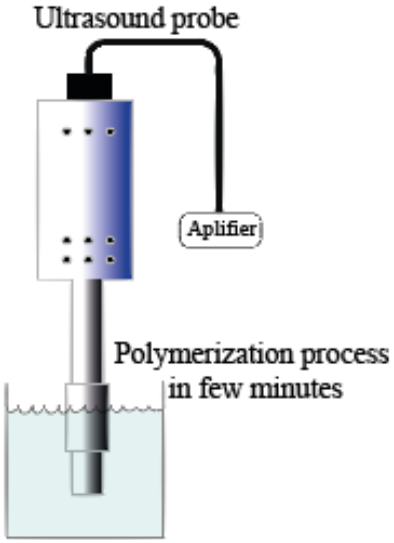
Dried at 60ºC under vacuum

$$2\ FeCl_3 + FeCl_2 + 4\ H_2O + 8\ NH_3 \rightarrow Fe_3O_4 + 8\ NH_4Cl$$



Final magnetite Product

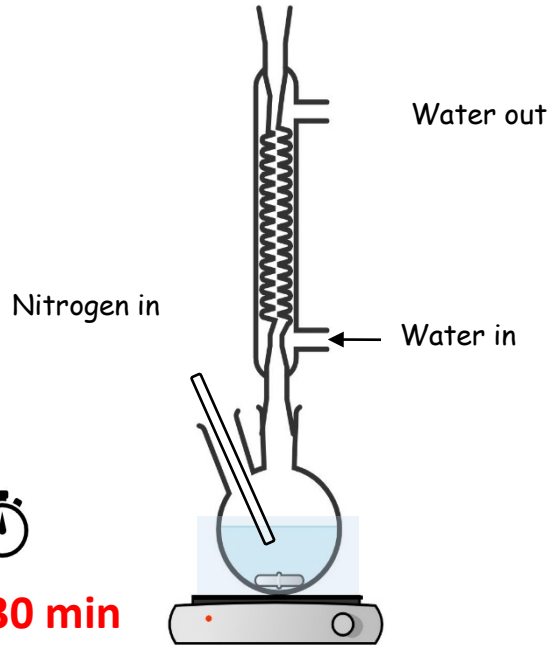
Synthesis of Fe3O4 magnetite Nanoparticles



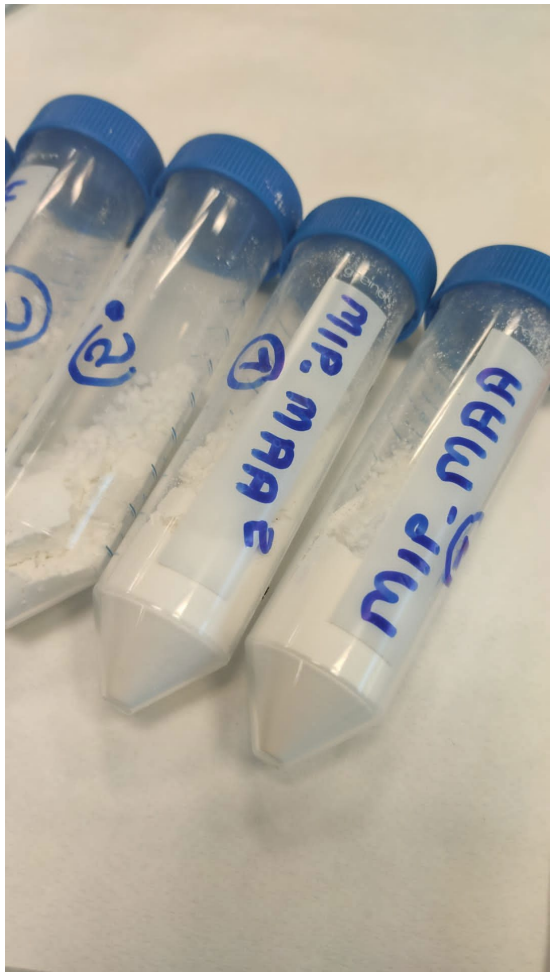
10 min

Fast **Long**

1h 30 min



$$2\ FeCl_3 + FeCl_2 + 4\ H_2O + 8\ NH_3 \rightarrow Fe_3O_4 + 8\ NH_4Cl$$

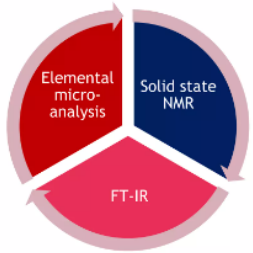


MORPHOLOGICAL CHARACTERIZATION:

I. Light microscopy - to verify natural integrity of polymer beads.
II. Scanning electron microscopy - to image polymer macro pores.
III. Nitrogen sorption porosimetry - determines the specific surface area, specific pore volume pore size distribution..etc
IV. Mercury intrusion porosimetry - suitable for large pores characterization

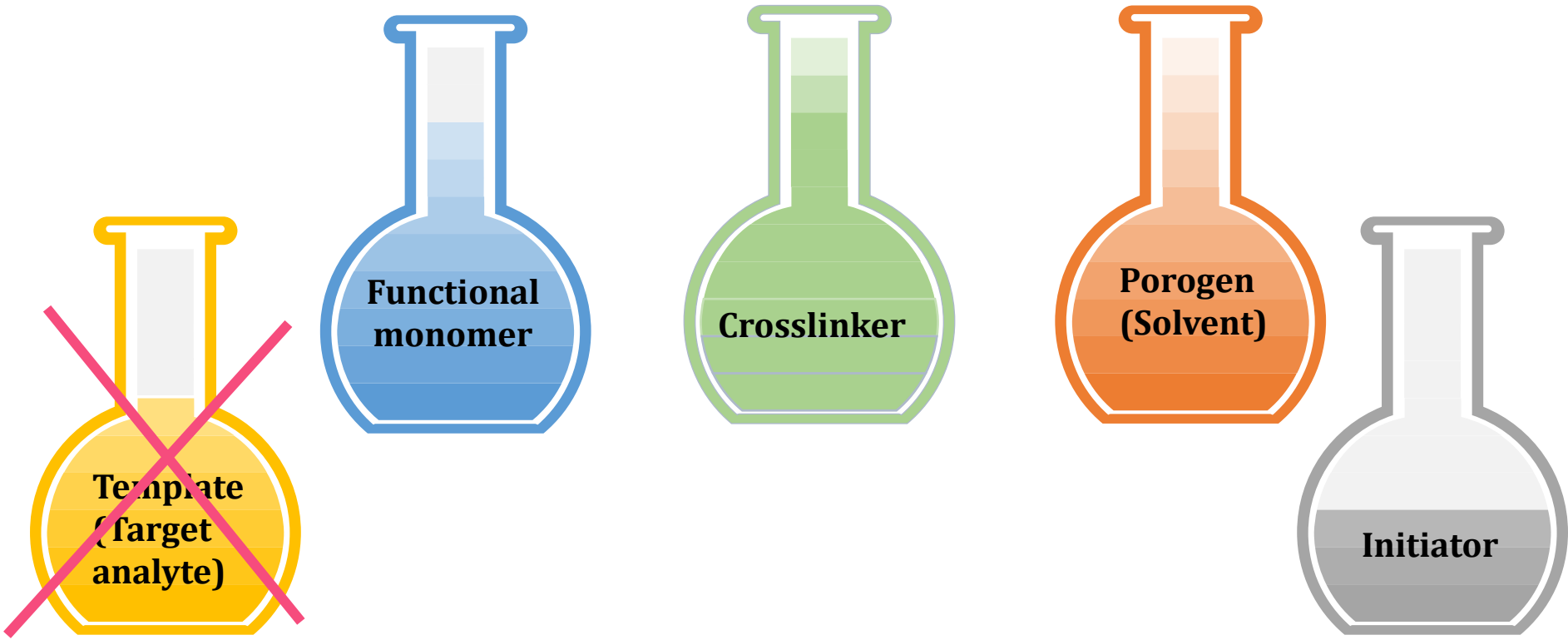
CHARACTERIZATION OF MIPS

1.CHEMICAL CHARACTERIZATION :



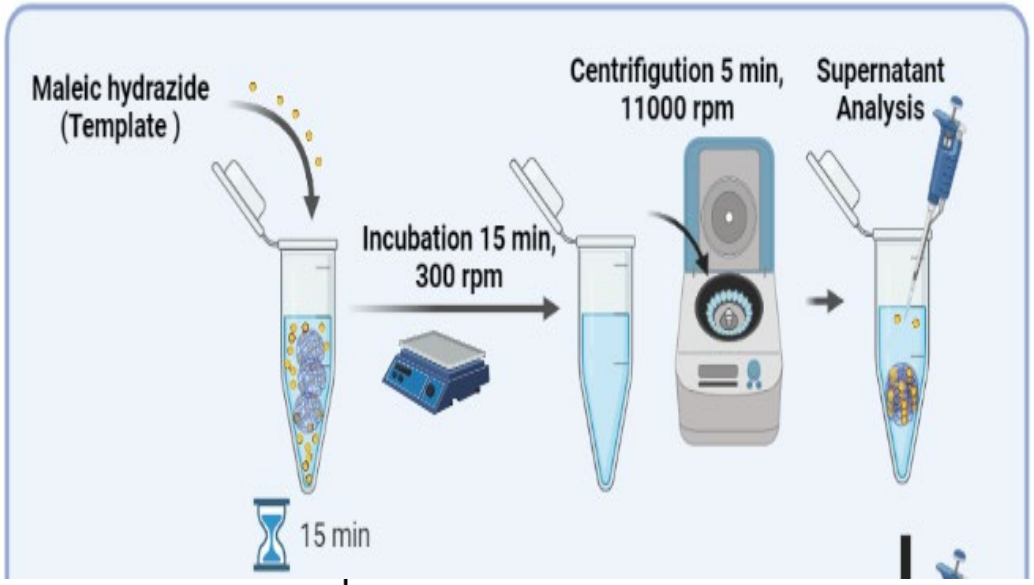
Not Imprinted Polymer (NIPs)

The NIP polymer is synthesized without the addition of any template molecule so that it can be used as a reference to compare the characteristics of the molded polymers for the target molecules.



Evaluation MIPs performance:

Binding experiment



$$Q = [(Ci-Ce)/m]V$$

$$IF = Q\ MIP/Q\ NIP$$

Figure . Adsorption isotherm of MIPs and NIPs for the template analyte.

MIP performance:

Selectivity test:

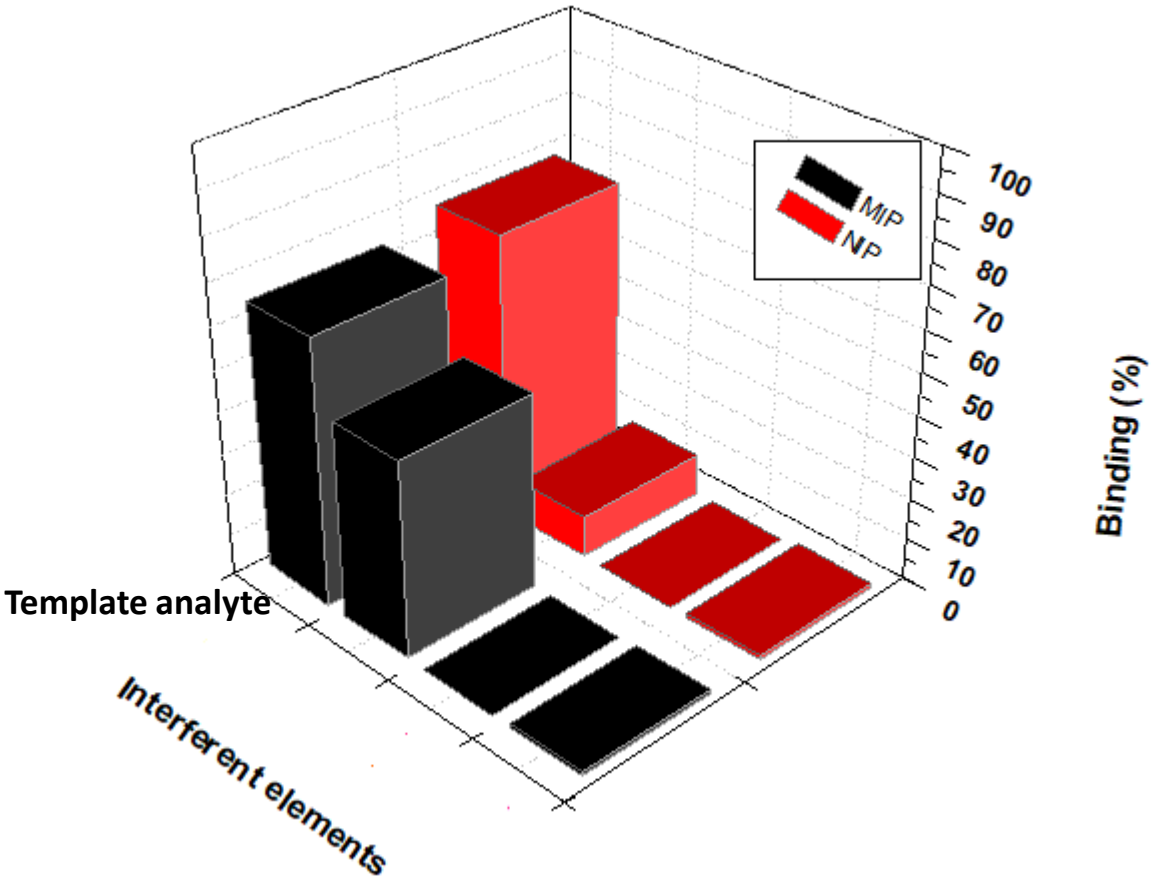
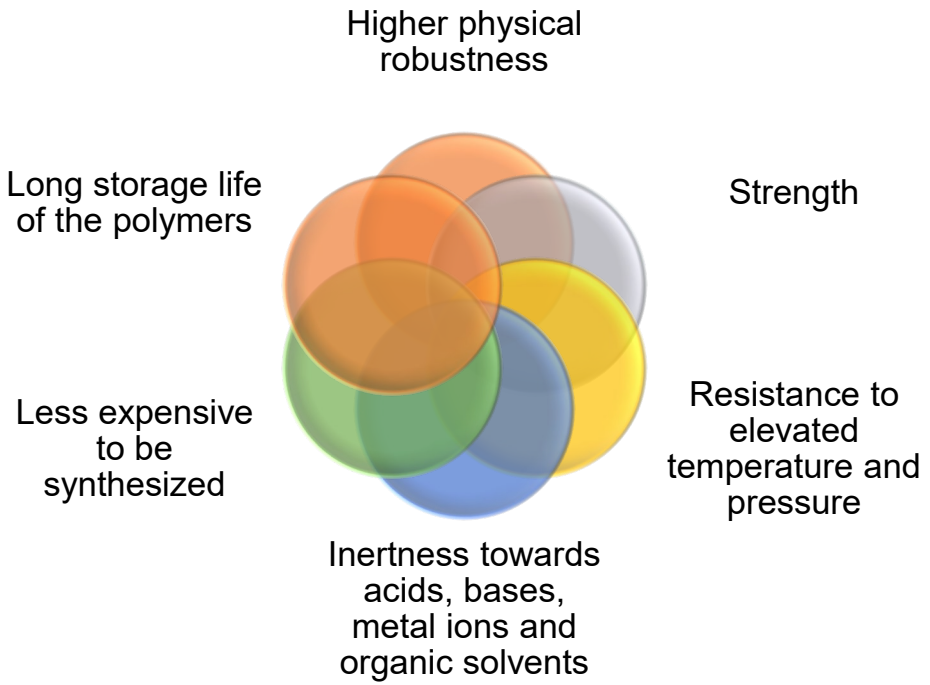


Figure . Binding adsorption of MIPs and NIPs for the template analyte and interferent.

Advantages of MIPs

- High **selectivity** and **affinity** for the **target molecule** used in the imprinting procedure.

Compared to biological systems such as proteins and nucleic acids MIP has:



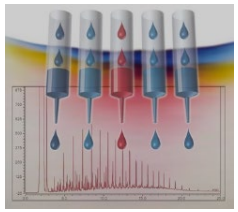
Int J Mol Sci. 2011; 12(9): 5908–5945
***Chem. Rev.* 2000, *100,* 2495-2504**

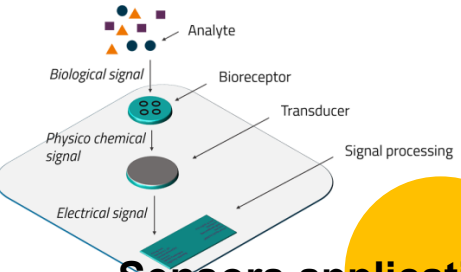
Applications of MIPs

MIPs are excellent materials with high selectivity and are widely used for:

Sample preparation in bio analytical methods

Chromatography

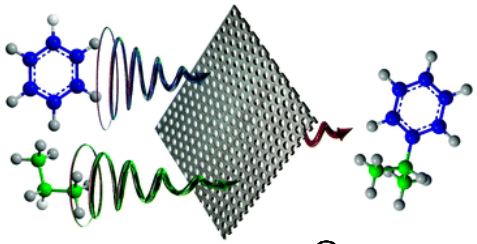




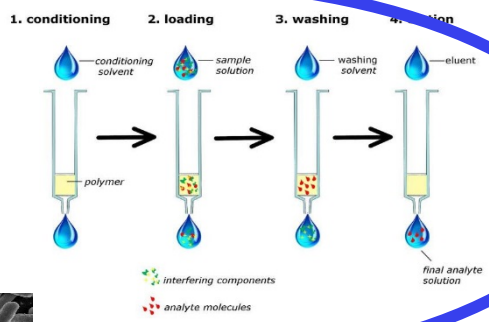
Sensors applications

Drug delivery

Catalysis

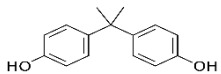


Solid phase extraction

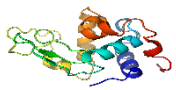


Hg^{2+}

Ion

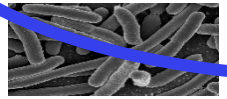


Molecule



Protein

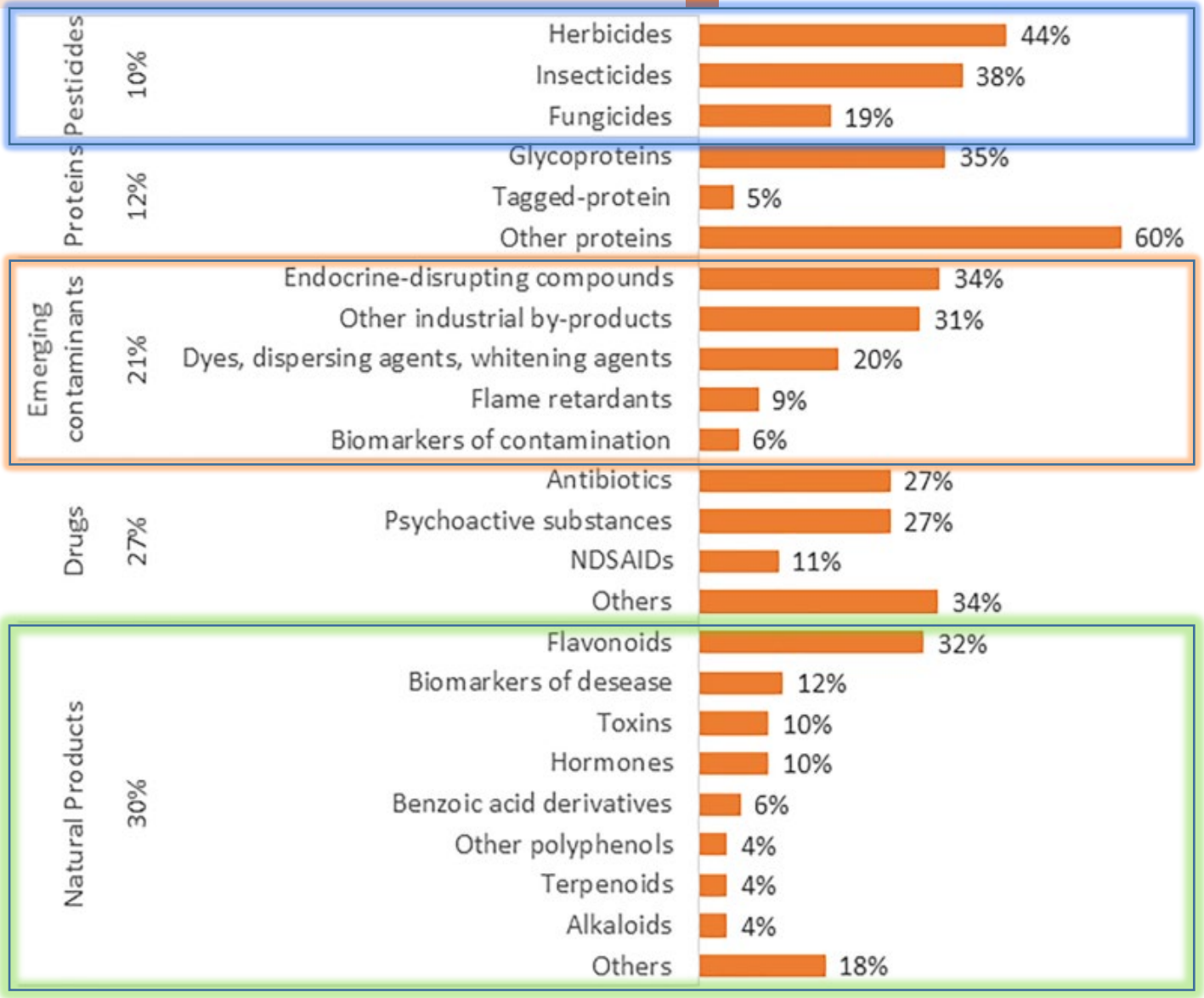
Virus



Bacterial cells

MIPs Applications

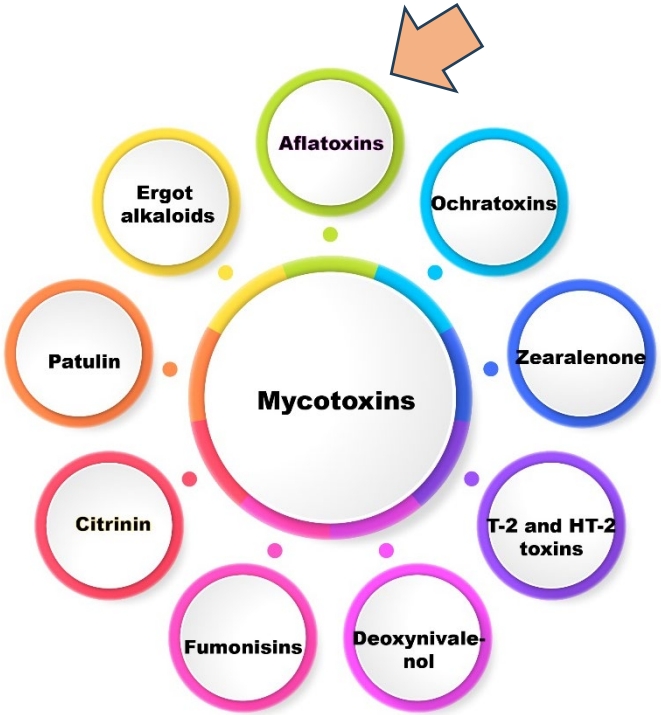
Groups of molecules for which MIPs have been recently developed

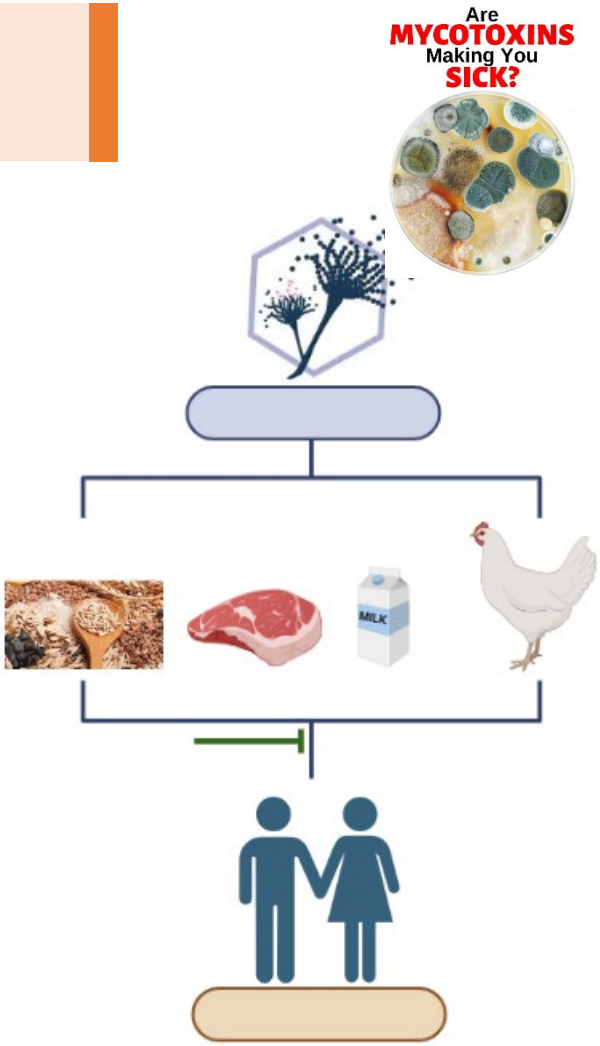


DOI: 10.1021/acs.analchem.9b04816

Anal. Chem. 2020, 92, 16-33

Case Report: MIP-Mycotoxins





Aflatoxins

molecules

MDPI

Article

Study on Molecularly Imprinted Polymers Obtained Sonochemically for the Determination of Aflatoxins in Food

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† These authors contributed equally to this work.

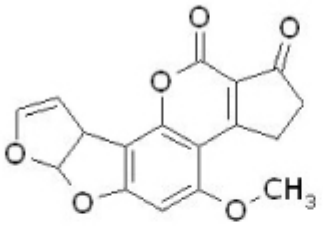
Abstract: Aflatoxins (AFs) are fungi secondary metabolites produced by the Aspergillus family. These compounds can enter the food chain through food contamination, representing a risk to human health. Commercial immunoaffinity columns are widely used for the extraction and cleanup of AFs from food samples; however, their high cost and large solvent consumption create a need for alternative strategies. In this work, an alternative strategy for producing molecularly imprinted polymers (MIPs) was proposed to extract aflatoxins AFB1, AFB2, AFG1, and AFG2 from complex food samples, using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). The MIPs were synthesized via a low-cost and rapid (5 min) sonochemical free-radical polymerization, using 1-hydroxy-2-naphthoic acid as a dummy template. MIPs-based solid phase extraction performance was tested on 17 dietary supplements (vegetables, fruits, and cereals), obtaining appreciable recovery rates (65–90%) and good reproducibility (RSD $\leq$ 6%, n = 3); the selectivity towards other mycotoxins was proved and the data obtained compared with commercial immunoaffinity columns. The proposed strategy can be considered an alternative affordable approach to the classical immunoaffinity columns, since it is more selective and better performing.

Citation: Palmieri, S.; Elfadil, D.; Fanti, F.; Della Pelle, F.; Sergi, M.;

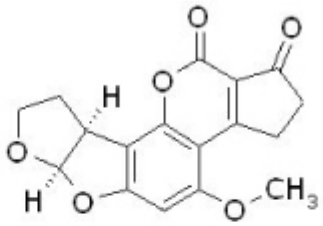
Application MIPs: Aflatoxins

Aflatoxins are produced by the secondary metabolism (i.e. the metabolism induced in a plant organism by external factors) of some species of filamentous microfungi such as, for example, Aspergillus flavus and Aspergillus parasiticus. They can develop during cultivation, harvesting and storage on numerous products of vegetable origin such as cereals (with particular reference to corn), oilseeds (such as peanuts), spices, grains, nuts and dried fruit.

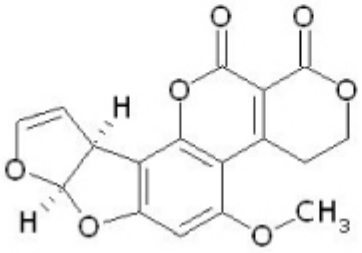
Aflatossina B1



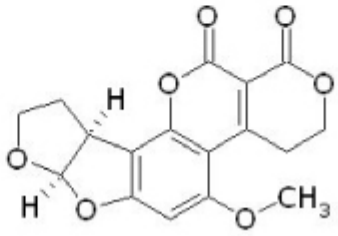
Aflatossina B2



Aflatossina G1



Aflatossina G2



Aflatoxins

MRL of AFG1, AFG2, AFB1, and AFB2 for processed food at **2 µg/kg**

Conventi
Extractio
method c
Aflatoxin

- Immunoaffinity colu
(IACs) are the standard
of choice for mycotoxir
preparation prior to HF
and LC-MS/MS. Monoc
antibodies selectively i
and concentrate the m
of interest, eliminating any interfering component from the sample. They are the ideal cleaning tool for analyzing complex or colored food and feed samples.

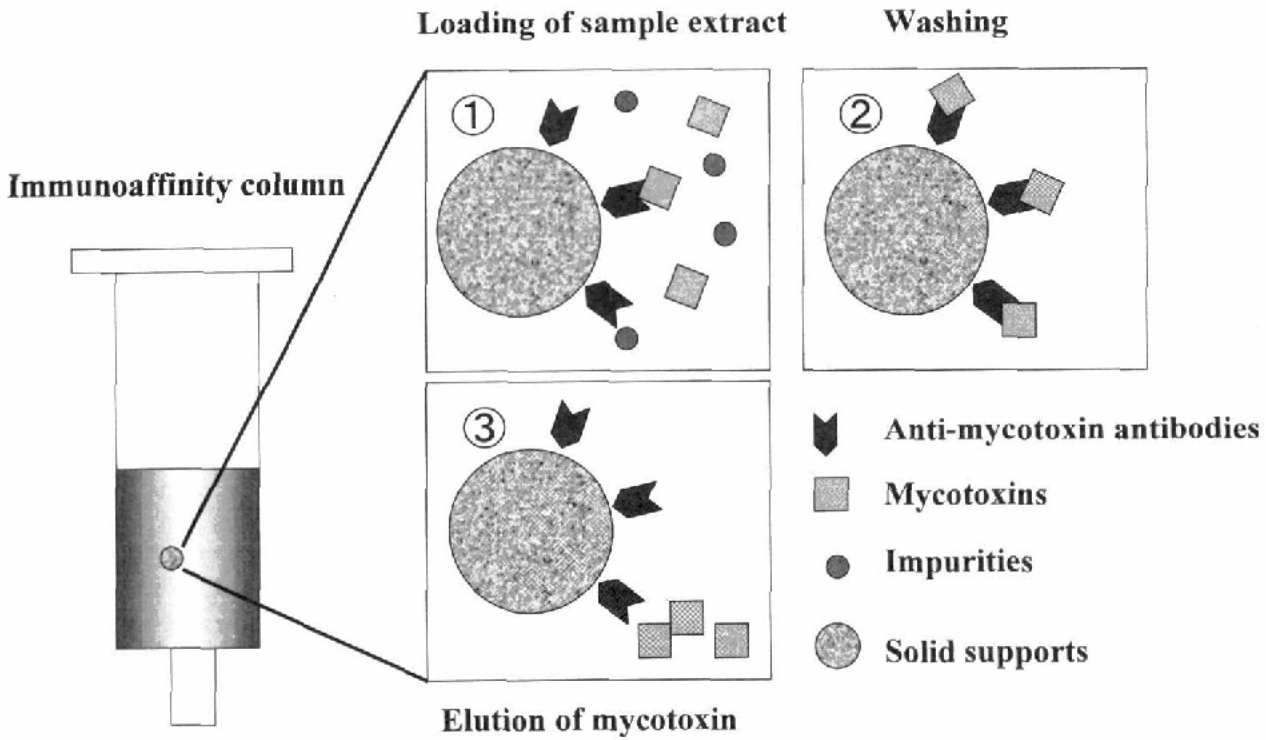
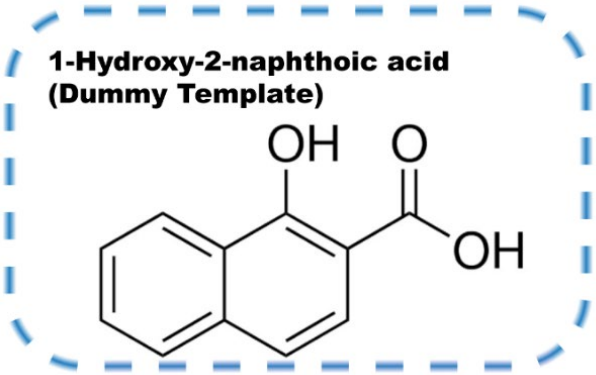


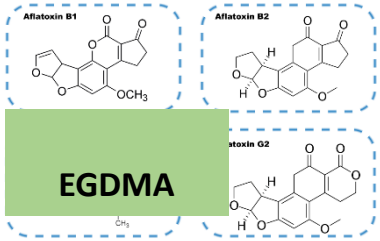
Fig. 1 Principle of immunoaffinity column.

Table 1 Commercial immunoaffinity columns.

MIPs Synthesis

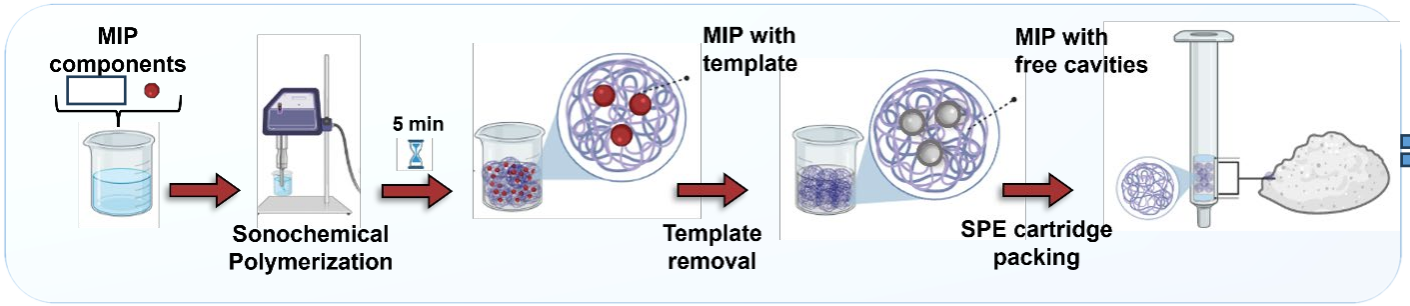


AA
MAA
MMA
MAA-VP



EGDMA

AIBN



MIP AA
MIP MAA
MIP MMA
MIP MAA-VP

N.B. the same procedure except of NIPs template was followed for the synthesis of NIPs

HPLC-MS/MS

HPLC Schimadzu Nexera
Fase A (Inorganic): 5mM Ammonium Formate
Fase B (Organic): 50_50 ACN/MeOH 5mM HCOOH
Colonna: Kinetex C18 2.6µ, 100A, 100x2.10mm

Column: ACE Excel 2 C18-PFP (10 cm x 2.1 mm id)

Sciex Qtrap 4500 mass spectrometer equipped with a V turbo source, which works in ESI positive mode.

Table. Lower limit of quantification (LLOQ), lower limit of detection L(LOD), calibration curve equation and determination coefficient obtained in analytical procedure validation.

Analyte	LOQ (ng*mL-1)	LOD (ng*mL-1)	Calibration Curve	R2
G1	0.02	0.005	y = 5696x - 0.0422	0.996
G2	0.09	0.027	y = 16831x - 3697.5	0.999
B1	0.02	0.007	y = 28753x - 6827.4	0.999
B2	0.03	0.009	y = 18892x - 15186	0.997

MIPs Characterization

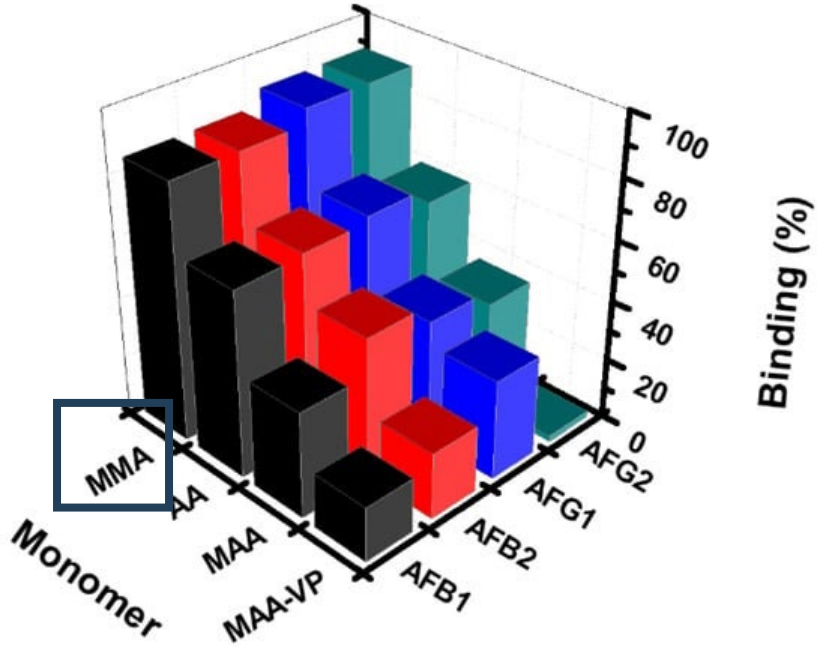
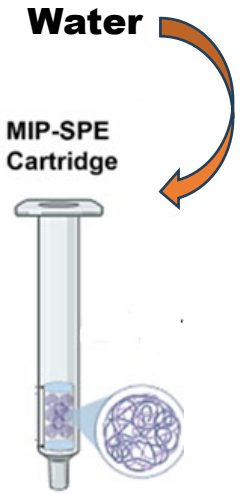


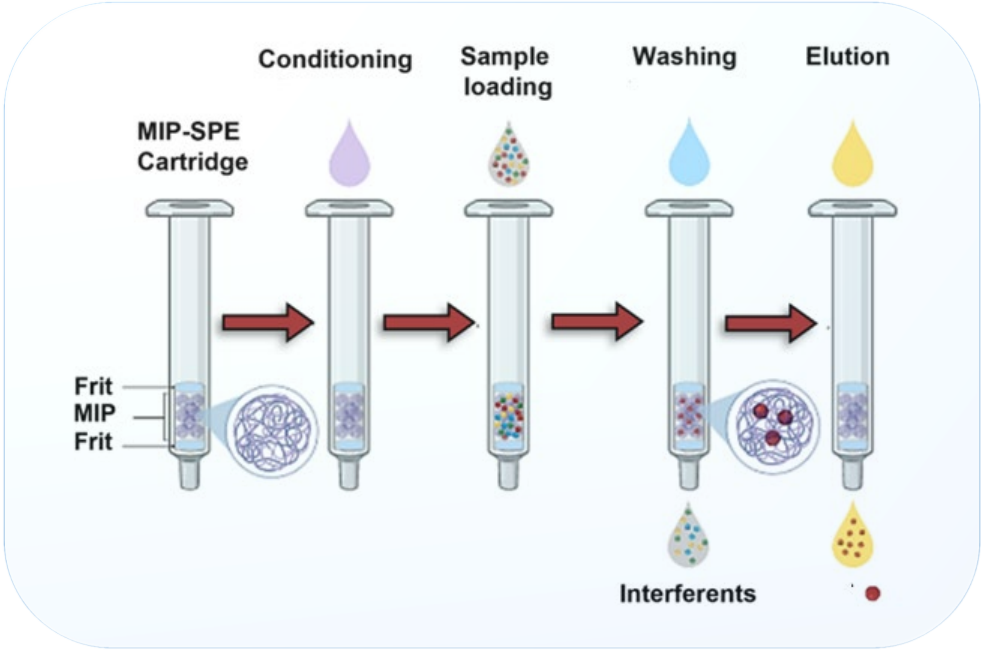
Figure. 3D histogram of % binding capacities of MIPs towards aflatoxin B1 (black), aflatoxin B2 (red), aflatoxin G1 (blue), aflatoxin G2 (green) at a concentration of 5 µg/mL. MIPs were synthetized with different monomers: methacrylamide (MMA), acrylamide (AA), methacrylic acid (MAA), and methacrylic acid +2-vinylpyridine (MAA-VP).

Optimization Solvent Adsorption



MIP-AFs based solid phase extraction (SPE)

Solid phase extraction



Amount of Polymer				
	AFB1 (%)	AFB2 (%)	AFG1 (%)	AFG2 (%)
2 mg	50	55	60	57
5 mg	**85**	**85**	**90**	**90**
10 mg	85	85	90	90
20 mg	85	85	90	90
Washing				
Water	8	10	7	5
(80:20) ACN:H20	70	70	48	50
0.5%ACN H20	**4**	**2**	**2**	**3**
5% ACN H20	40	31	39	42
1% ACN H20	25	20	17	27
Elution				
MeOH	63	64	65	64
2% acetic acid in MeoH	**85**	**85**	**90**	**90**

MIPs Characterization

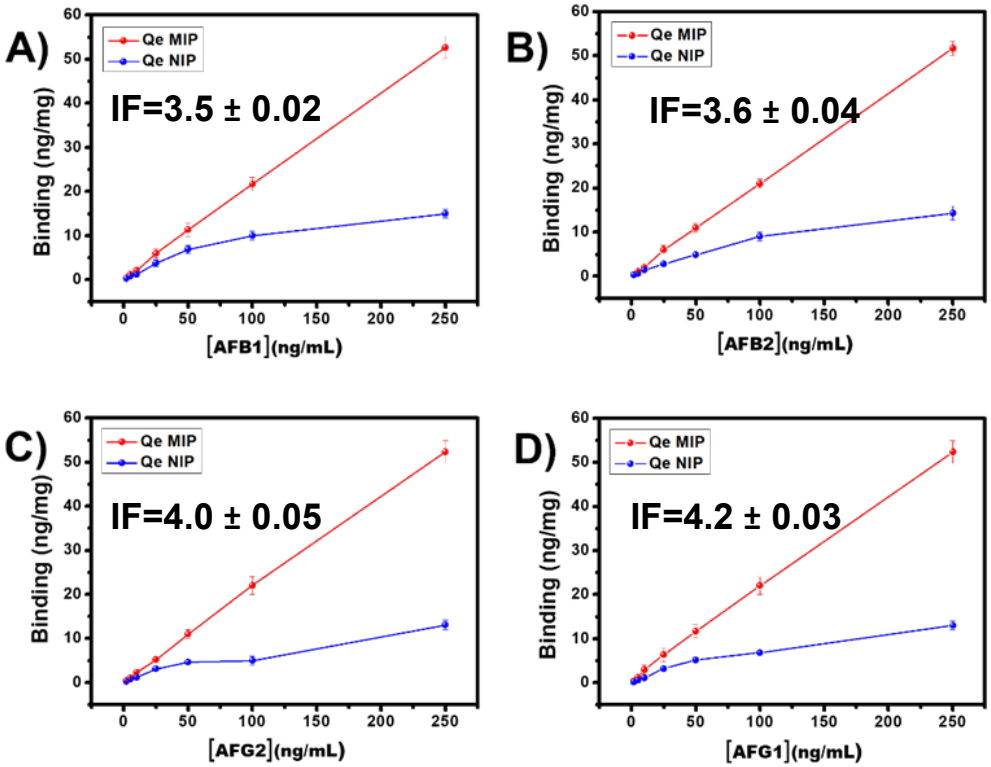


Figure. .Adsorption capacity (%) of the MIPs (red) and NIPs (blue) obtained analyzing different aflatoxin amounts (from 2 to 250 ng/mL). Graphs (A–D) correspond to aflatoxins B1, B2, G1, and G2, respectively.

Adsorption capacity % of MIPs and NIPs

MIPs Characterization

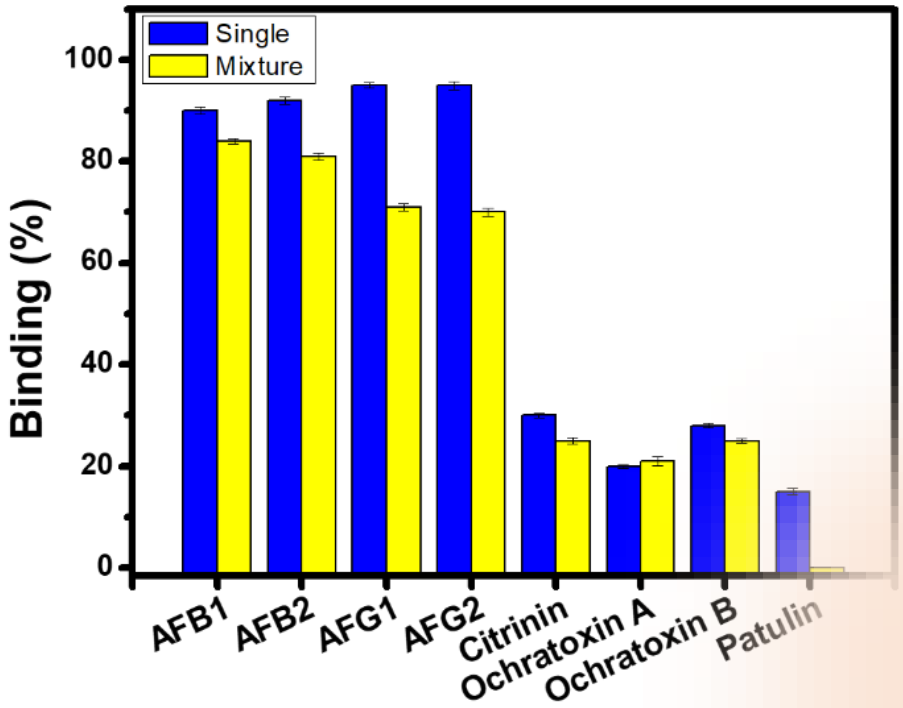
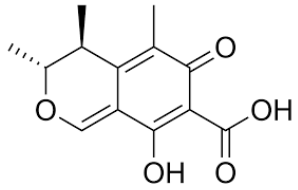
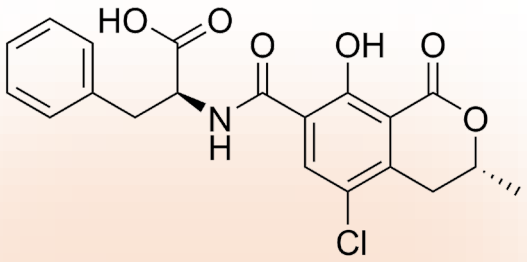


Figure. Selective adsorption of MIPs at a concentration of 5 ng/mL.

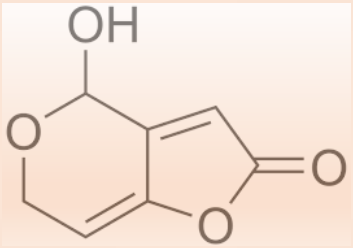
Selectivity Test



Citrinin



Ochratoxin A-B



Patulin

Reusability of MIPs

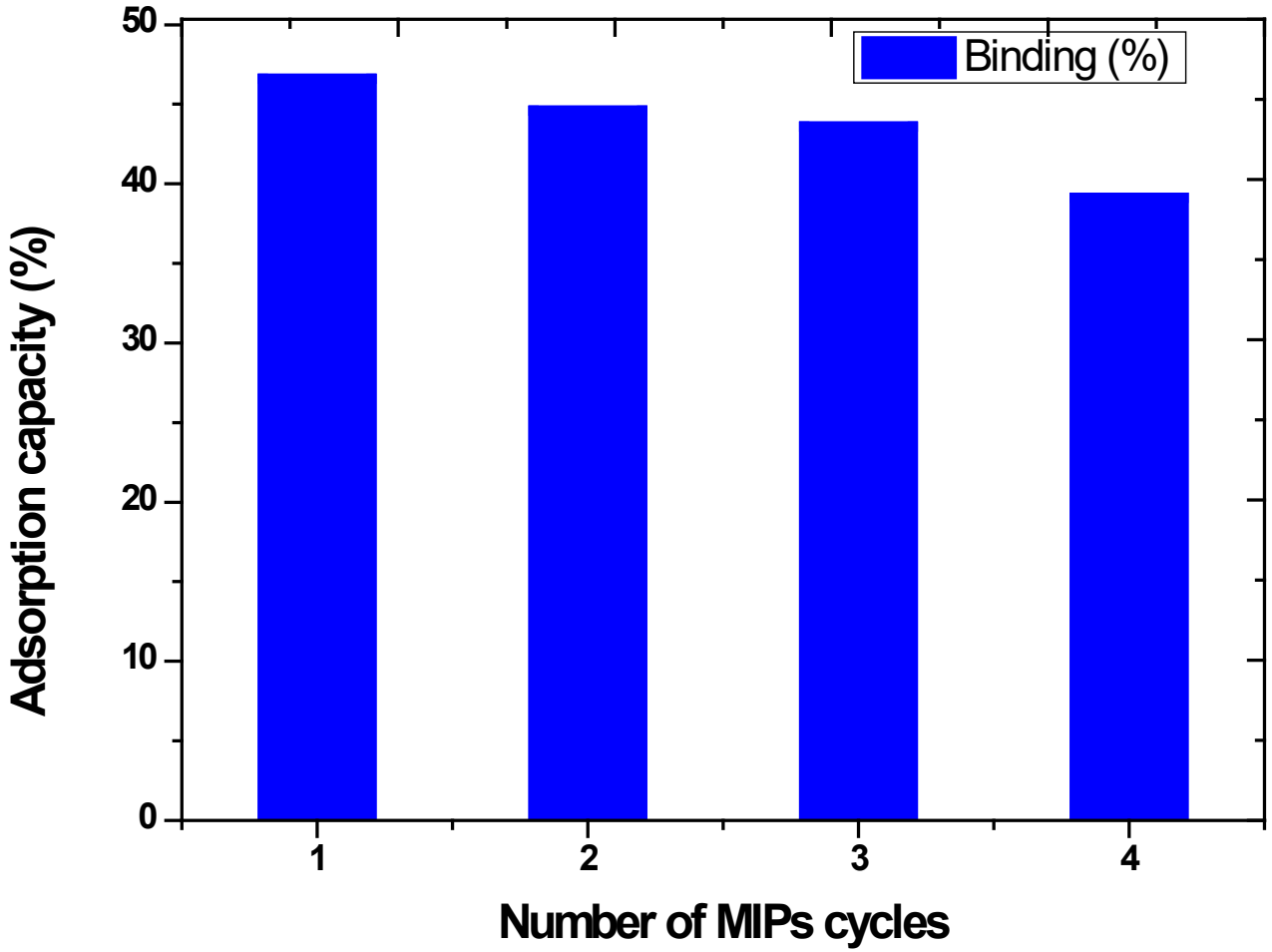


Figure . Adsorption of MIPs by Aflatoxins in four consecutive adsorption-desorption cycles.

MIPs Application

Sample analysis was carried out on 17 heterogeneous food supplements:
-Ginger,
-Echiancea purpurea,
-Ginseng,
-Hypericum,
-Red elm,
-Saffron,
-Mango,
-Red rice,
-Parsley,
-Red fruits,
-Grapefruit,
-Magnolia,
-Tilia Cordata,
-Root Salsopariglia,
-Hop,
-Verbene Officinalis,
-Galega Officinalis

MIPs Performance

Sample	AFG1				AFG2				AFB1				AFB2			
	MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)	
	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME
Ginger	60	+9	50	−30	63	−6	48	+28	64	−1	50	+25	81	−14	52	+30
Echinacea purpurea	83	+9	73	+15	66	+10	75	+15	90	−1	80	+10	60	−8	72	+12
Ginseng	75	+16	66	−26	60	+1	46	−23	90	+7	67	−28	78	+15	61	−29
Hypericum	50	+15	50	27	60	+15	38	+30	53	+11	52	+25	72	+1	50	+30
Red elm	77	+10	64	+5	70	+7	63	+15	90	−4	80	+10	74	−6	80	+8
Saffron	61	+16	60	+12	69	+11	62	+20	60	+15	60	+17	68	+9	64	+15
Mango	67	+10	65	+15	65	+7	60	+15	65	+10	70	+15	65	+10	68	+12
Red rice	76	+11	50	+25	65	+15	55	+20	89	+3	65	+20	70	+9	66	+20
Parsley	60	+9	50	+30	60	+10	40	+30	76	+1	45	+28	60	+4	43	+19
Red fruits	60	+7	55	+20	60	+10	56	+21	79	+6	60	+18	60	+10	58	+22
Grapefruit	61	+11	65	+15	69	+11	70	+12	68	+12	70	+15	71	+4	68	+16
Magnolia	61	−7	50	+30	64	+12	50	+20	64	+15	50	+18	77	+8	54	+20
Tilia cordata	60	+1	50	+20	62	+12	45	+18	62%	+11	50	+19	72	+2	50	+20
Salsapariglia root	62	+3	55	+20	67	+6	52	+18	72	+15	50	+20	69	+14	70	+17
Hop	60	+2	50	+20	72	+12	51	+20	74	+15	60	+17	57	+12	67	+18
Verbena officinalis	72	+5	56	+18	60	+15	55	+21	69	−8	67	+18	65	+13	66	+16
Galega officinalis	78	+4	64	+15	77	+17	65	+20	73	+14	65	+20	75	+3	74	+20

Appreciable recoveries (65–90%; RSD < 6%, n = 3)

low matrix effect (ME < 16%)

RC: Recovery
ME: Matrix effect
IAC: Commercial immunoaffinity column

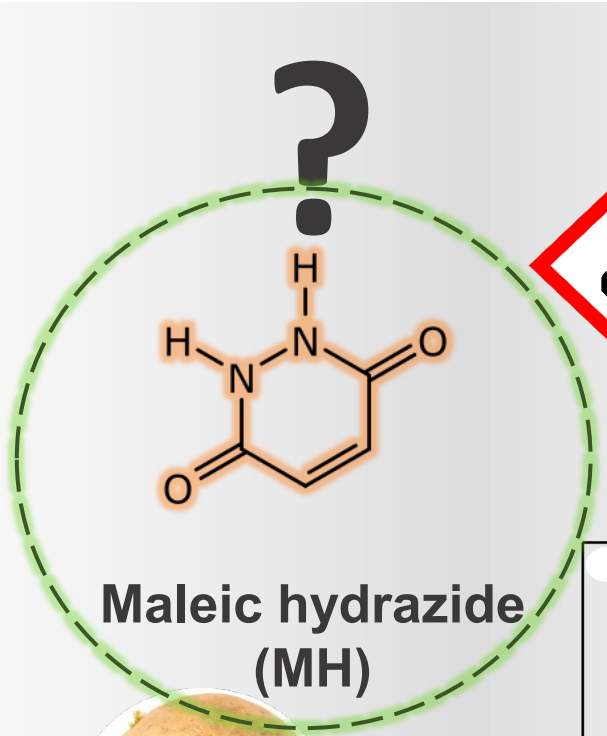
Conclusion

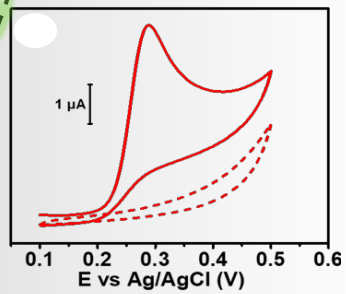
- A rapid and affordable method to synthesize MIPs to apply as sorbent phase in SPE for AFs extraction from different food matrices was successfully proposed;

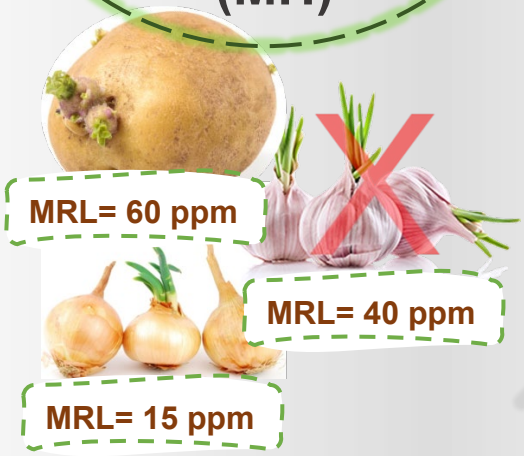
- The proposed MIPs-based SPE was applied to different food supplements showing appreciable recoveries (65%-90%; RSD <6%, n=3) and low matrix effect (ME <15%), resulting more performing compared to the immunoaffinity column-based commercial method;

- The proposed method is rapid, does not need organic solvents, and presents reduced cost with respect to commercial dedicated cartridges for AFS extraction.

MIP-Pesticides : Maleic hydrazide

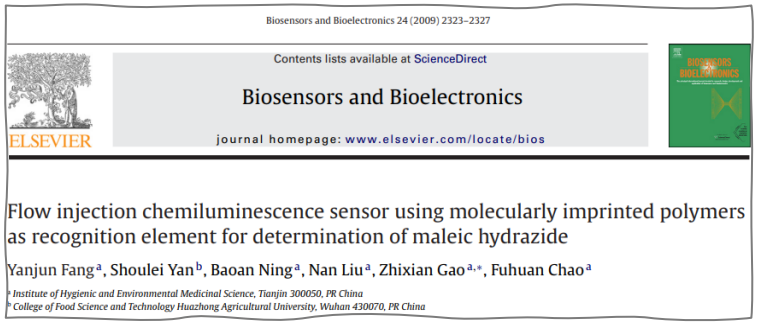






Development of a fast synthesis strategy based on a high-power ultrasound probe to synthesize effective MIPs for maleic hydrazide

▶ Sparking idea



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Flow injection chemiluminescence sensor using molecularly imprinted polymers as recognition element for determination of maleic hydrazide

Yanjun Fang[a], Shoulei Yan[b], Baoan Ning[a], Nan Liu[a], Zhixian Gao[a,*], Fuhuan Chao[a]

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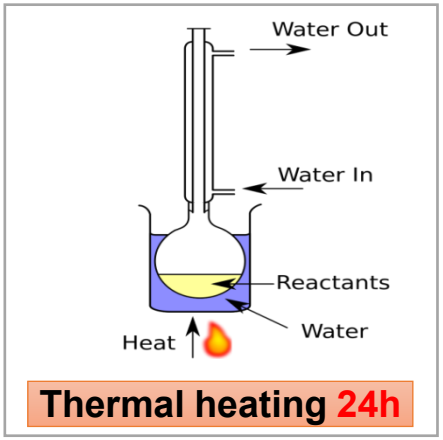
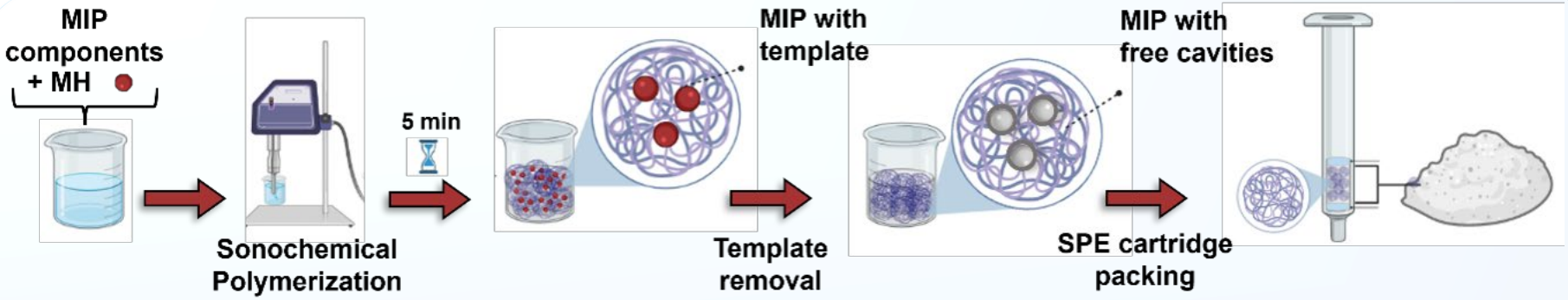
[b] College of Food Science and Technology Huazhong Agricultural University, Wuhan 430070, PR China



Long-time synthesis

MIP-Maleic hydrazide

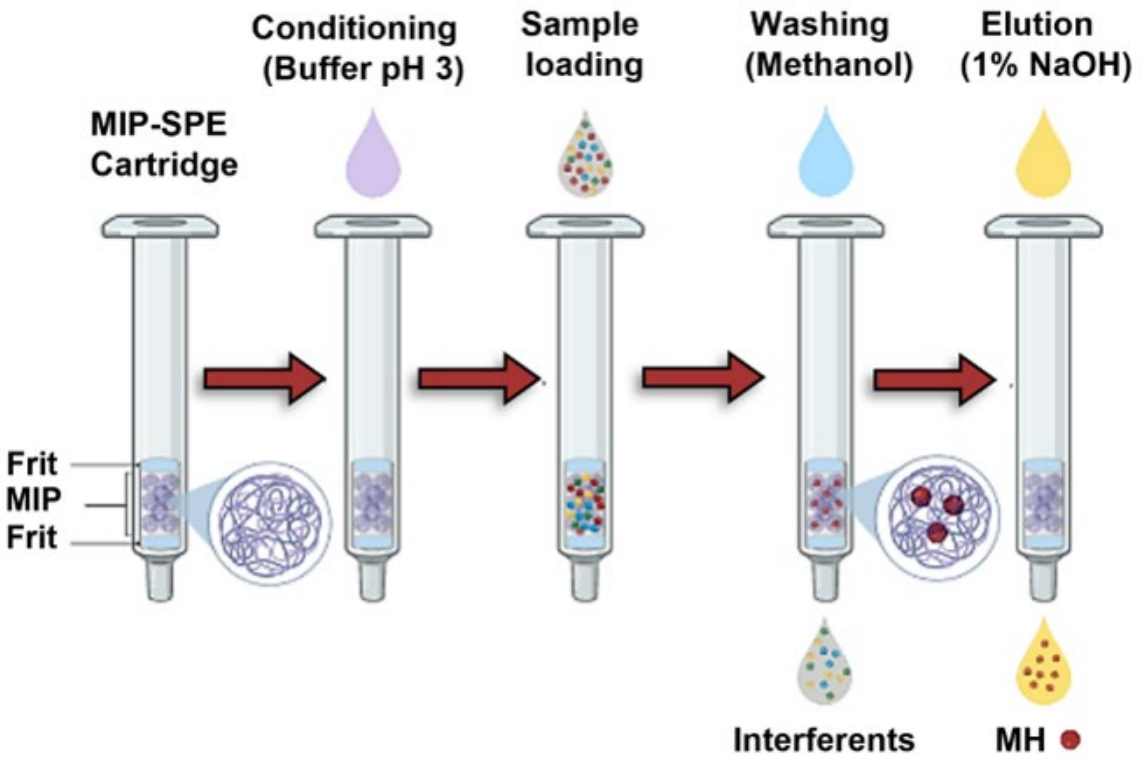
Sonochemical MIP synthesis



Scheme Graphical scheme of the ultrasound-probe-assisted MIP synthesis for MH, and its use as a solid-phase extraction cartridge.

MIP-MH based solid phase extraction

Solid phase extraction

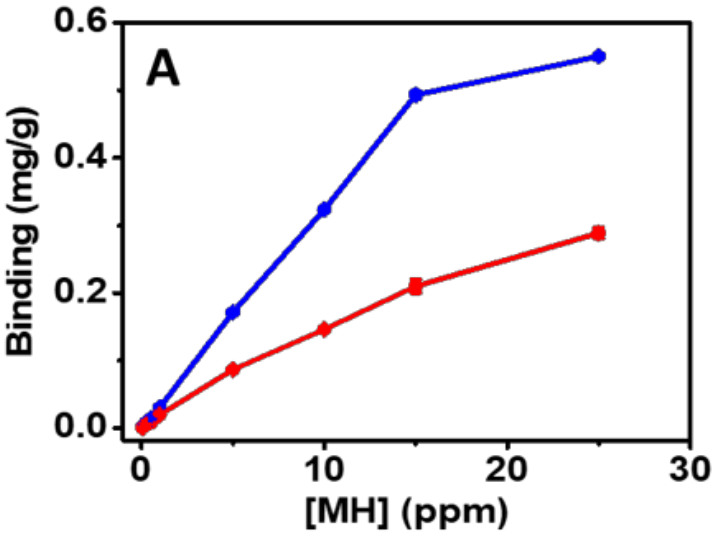


Parameters
A) Adsorption Solvent (1mL)
Water
Phosphate buffer pH 3
B) Amount of polymer (mg)
5
10
15
C) Desorption Solvent
NaOH 1 %
PBS (pH 7)
MeOH
H_20

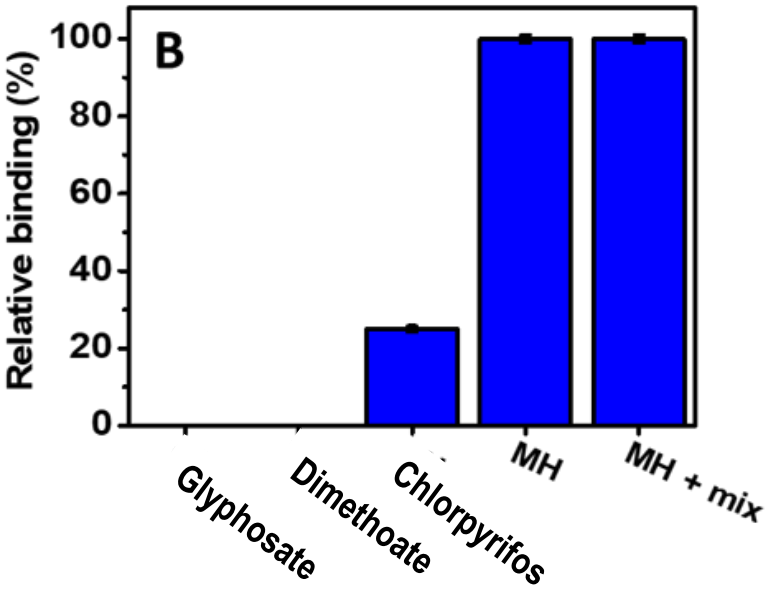
Scheme . Graphical scheme of the solid-phase extraction procedure based on the cartridge containing the MIP.

MIP-MH based solid phase extraction

Binding capacity



Selectivity



$$Q = \frac{(Ci - Cf)}{m} * V$$

$$IF = \frac{Q(MIP)}{Q(NIP)}$$

IF=2,30 ± 0.02 (n=3)

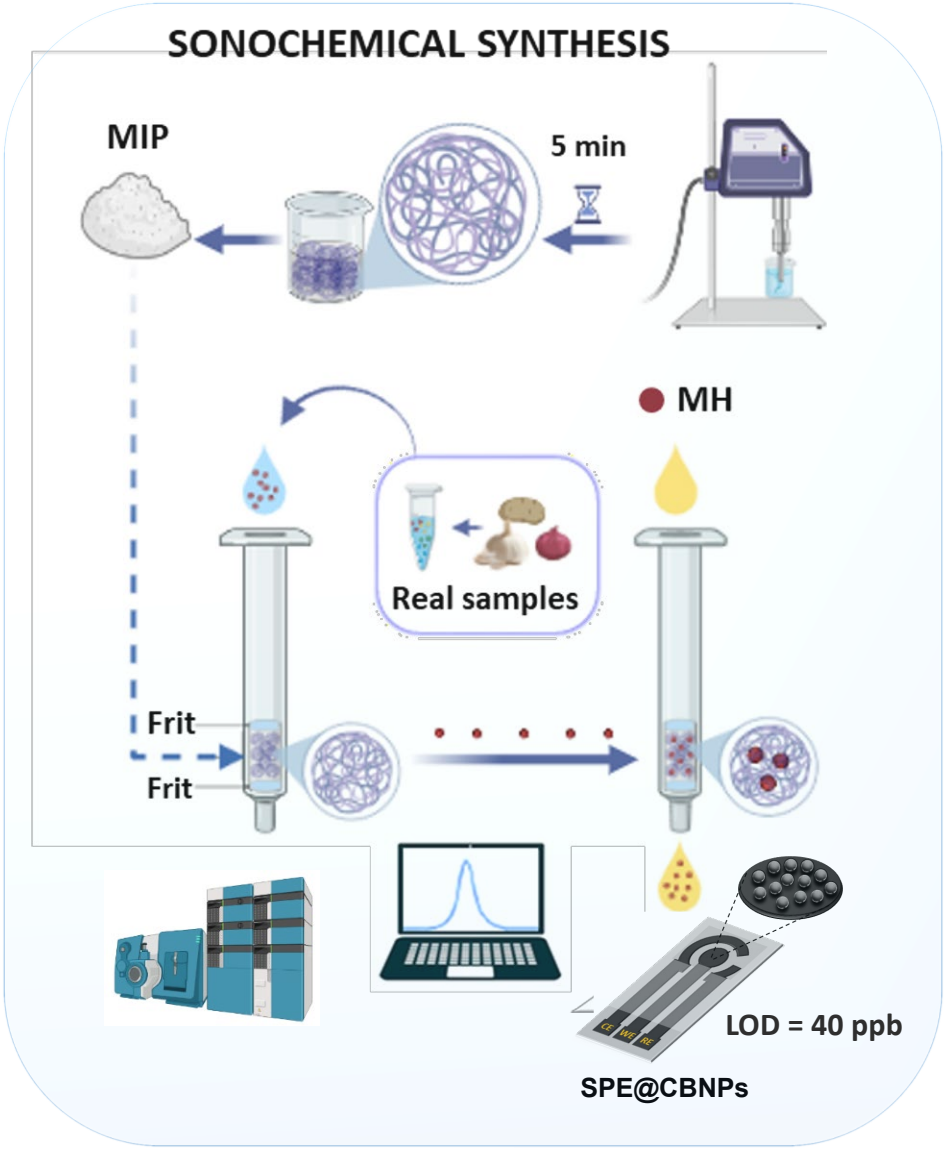
MIP-Maleic hydrazide

MIP-SPE and sample analysis

Table . Results of MH MIPs-SPE combined with CB-based electrochemical determination applied in food samples

MH added (ppm)	EC found (ppm)	Recovery (%)	LC-MS/MS found (ppm)	Relative Error (%)
		Onion		
5	4.6±0.2	92	4.8±0.02	-4.2
10	8.9±0.5	88.5	8.5±0.9	4.3
15	14.2±0.7	94.5	14.2±0.8	0.2
		Garlic		
5	4.1±0.1	82.2	4.5±0.4	-8.0
10	10.5±0.4	105.1	9.6±0.3	9.4
15	15.3±0.6	102.1	14.6±0.2	5.0
		Potato		
5	5.3±0.3	106	5.5±0.3	-3.6
10	9.7±0.3	97	10.0±0.3	-3.6
15	13±0.2	80.0	14.2±0.6	-8.5

In Europe, the maximum residue limits (MRLs) for MH in **potatoes**, **garlic**, and **onions** are **60**, **40**, and **15** **ppm**, respectively.



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Microchemical Journal

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Fast sonochemical molecularly imprinted polymer synthesis for selective electrochemical determination of maleic hydrazide

Dounia Elfadil [a, b, 1], Sara Palmieri [a, 1], Filippo Silveri [a], Flavio Della Pelle [a] ✉, Manuel Sergi [a], Michele Del Carlo [a], Aziz Amine [b] ✉, Dario Compagnone [a]

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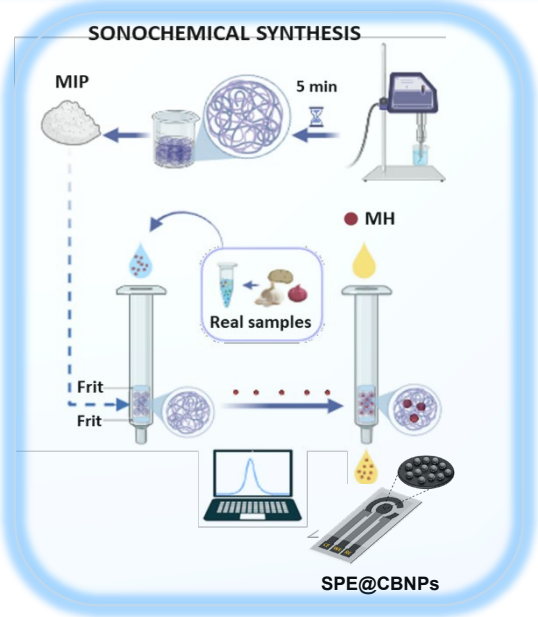

Application MIPs: Ochratoxin A

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Determination of Ochratoxin A in Italian Red Wines by Molecularly Imprinted Solid Phase Extraction and HPLC Analysis

Cristina Giovannoli,* Cinzia Passini, Fabio Di Nardo, Laura Anfossi, and Claudio Baggiani

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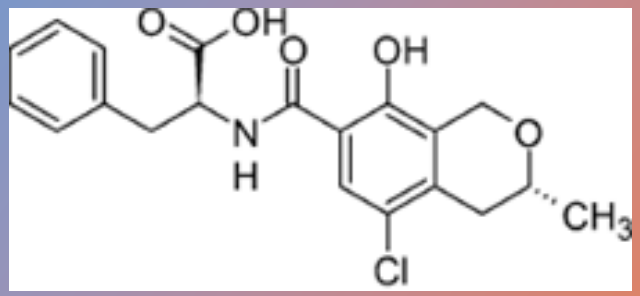
Supporting Information

ABSTRACT: An extraction method based on molecularly imprinted polymer prepared through a mimic template approach was used for the determination of ochratoxin A in 17 red wines from different geographical regions of Italy. Sample loading (wine sample diluted 1:1 with 1% v/v aqueous solution of PEG 8000), washing (2 mL water/acetonitrile 4:1 v/v), and elution (2 mL of acetonitrile/acetic acid 98:2 v/v) conditions allowed the optimization of the extraction method, capable of preconcentrating ochratoxin A below the maximum permitted level of 2 ng/mL. Under optimized conditions, recoveries of ochratoxin A from spiked samples ranged from 88 to 102% with sample volumes up to 20 mL. The HPLC determination by fluorescence detection allowed limits of detection and quantification, respectively, of 0.075 and 0.225 ng/mL. Sample extractions by an immunoaffinity protocol showed the method to be comparable, demonstrating the potential of the imprinting approach to substitute for the current immunoaffinity method

KEYWORDS: *mycotoxin analysis, wine analysis, ochratoxin A, molecularly imprinted solid phase extraction, molecularly imprinted polymer*



- Ochratoxin A (OTA), is a mycotoxin produced as a secondary metabolite by several toxigenic molds belonging to Aspergillus and Penicillum species provided with nephrotoxic, immunosuppressive, teratogenic, and carcinogenic properties. OTA is
- chemically stable; thus, it survives during storage and food processing and is not destroyed when cooked at high temperatures.3,6 As a consequence, OTA contamination affects many foods and beverages such as different kinds of cereals and derived products, beer, wine, grape juice, coffee beans, dry vine fruits, cocoa, nuts, and spices. The European Commission in regulation (EC) 123/2005 established a maximum level allowed in wine equal to 2 µg/L (ppb).

Synthesis of OTA A MIPs

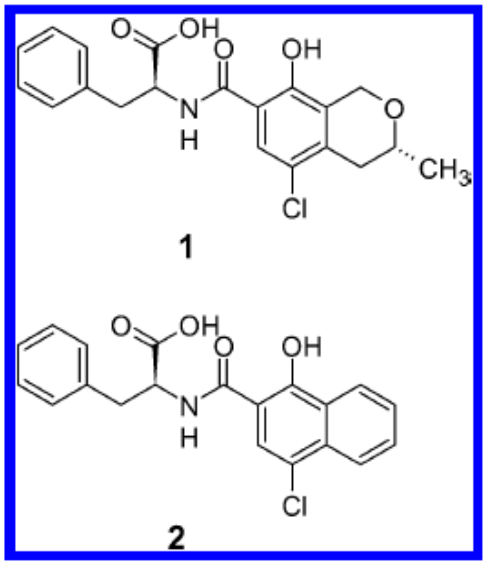
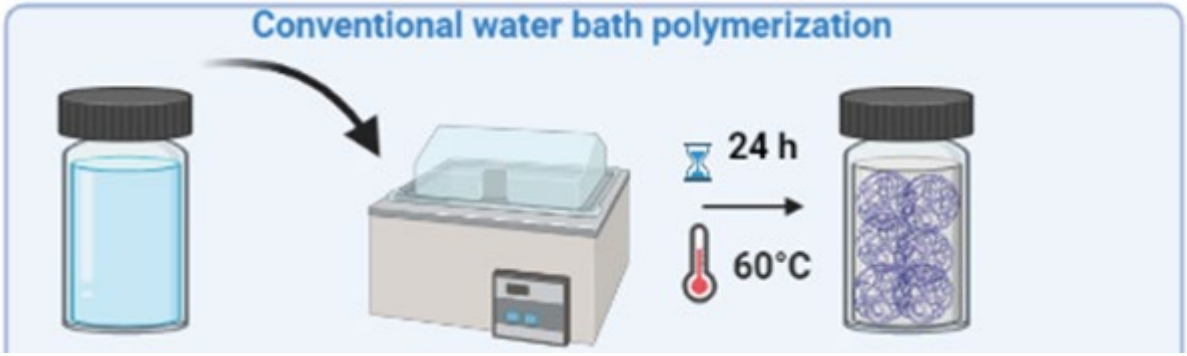
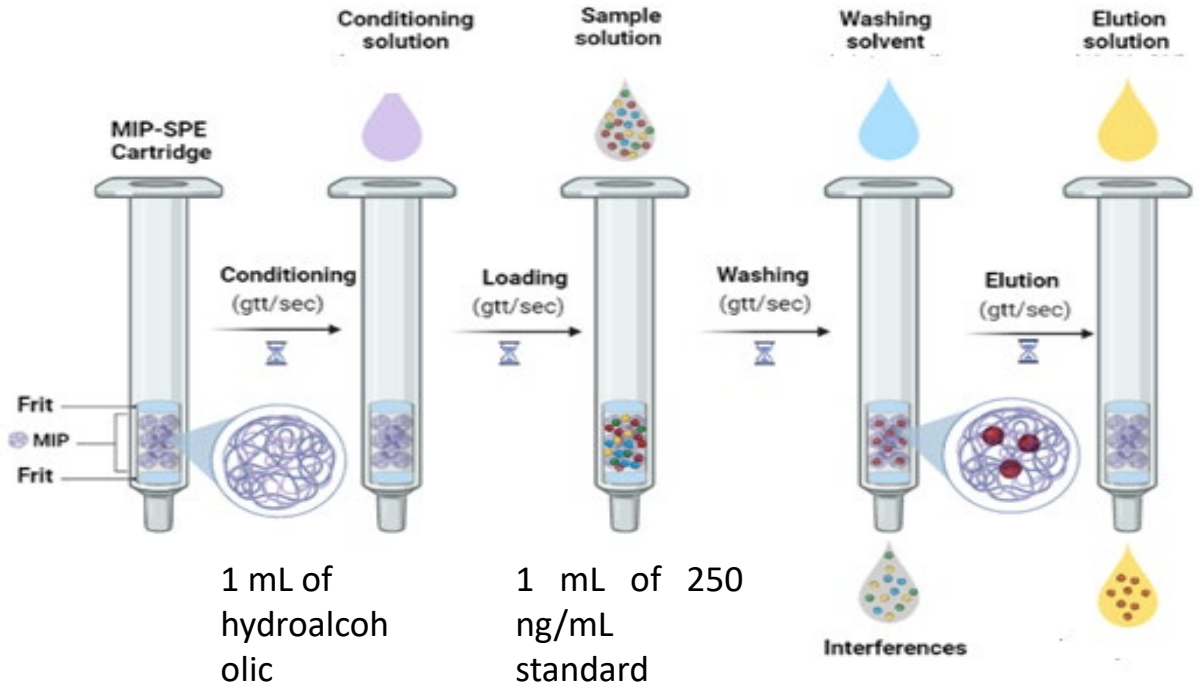


Figure. Molecular structures of ochratoxin A and its template mimic

The high selectivity toward OTA of a molecular imprinted polymer obtained by a thermal polymerization of **methacrylic acid** as functional monomer and **ethylene glycol dimethacrylate** as cross-linker in the presence of **N-(4-chloro-1-hydroxy-2- naphthoylamido)-L-phenylalanine** as mimic template



MIP-SPE



1 mL of hydroalcoholic citrate–tartrate buffer

1 mL of 250 ng/mL standard solution of OTA in hydroalcoholic citrate–tartrate buffer

acetonitrile + 1% v/v acetic acid

Performance of MIPs

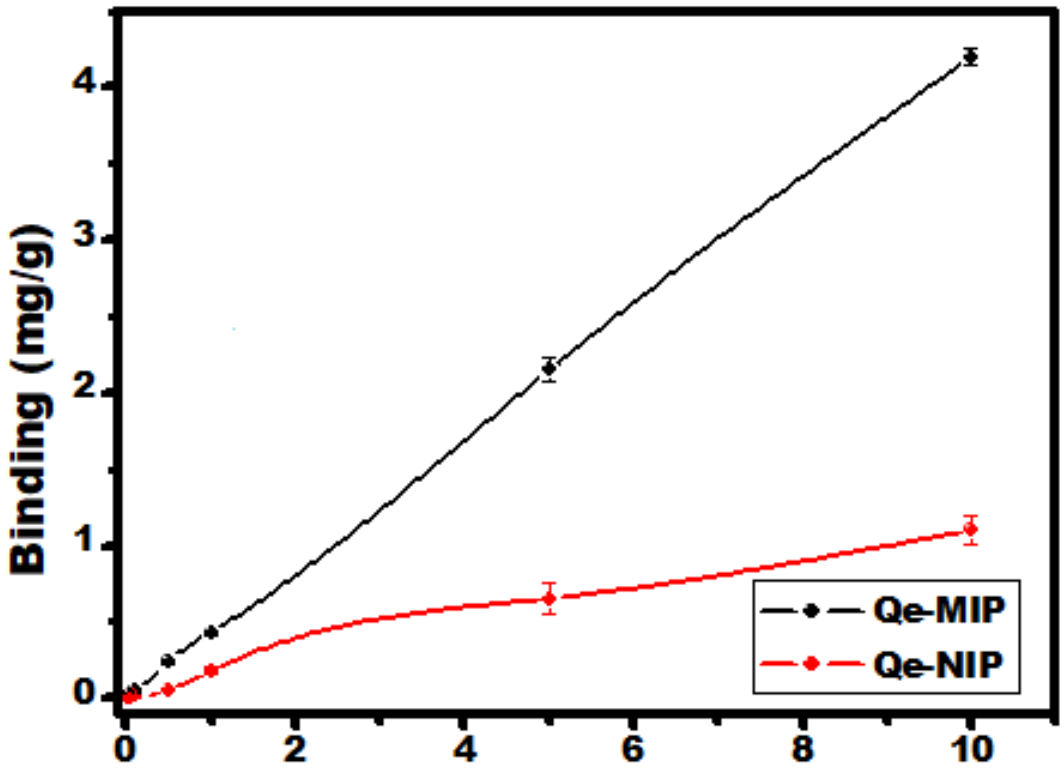
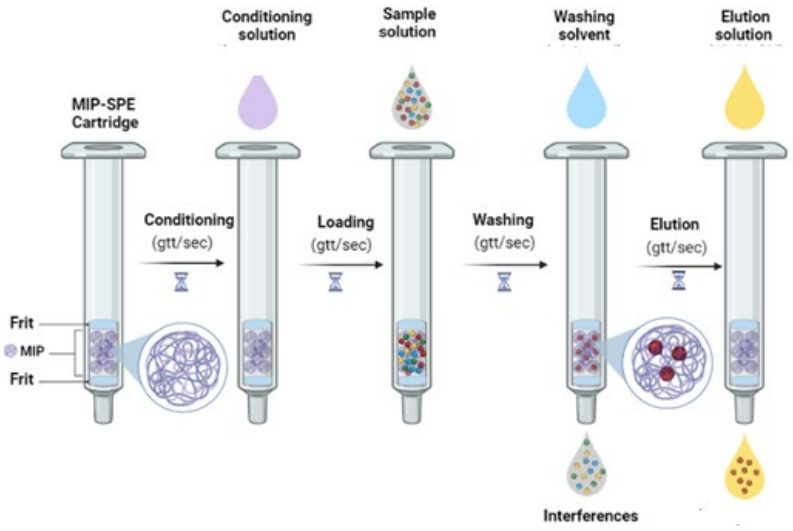


Figure . Adsorption isotherm of OTA (ppb) MIPs and NIPs for the template analyte.

Application in red wine

Preliminary wine treatment was performed in accordance with the literature. Tannins were precipitated by diluting the red wine samples 1:2 v/v with a 1% v/v aqueous solution of PEG 8000, incubated at 4 °C overnight, centrifuged at 8000 rpm for 15 min, and filtered on 0.22 µm polypropylene membranes.



Extraction of OTA from wine samples was performed by loading 2 mL of pretreated wine sample on the cartridge and applying a vacuum.

Afterward, the cartridge was washed with 1 mL water/acetone 4:1 v/v and the elution was then performed with 2 mL of acetonitrile/acetic acid 49:1 v/v, as optimized in the MISPE protocol with OTA standards.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