

## RECENT ADVANCES IN BIOSENSORS BASED ON MOLECULAR IMPRINTING POLYMERS

**Aziz Amine**

Faculty of Sciences and Techniques, Hassan II University of Casablanca, Morocco

[azizamine@yahoo.fr](mailto:azizamine@yahoo.fr); [aziz.amine@fstm.ac.ma](mailto:aziz.amine@fstm.ac.ma)

**XIV Convegno Nazionale “Ricerca e Innovazione per Ambiente, Salute ed Alimentazione”  
Dedicato alla memoria del Prof. Gustavo Mita**

# Bioremediation and biodeterioration of bisphenol A (BPA) in aqueous solutions.

**Author(s)** : [Mita, D. G.](#) ; [Attanasio, A.](#) ; [Diano, N.](#) ; [Grano, V.](#) ; [Bencivenga, U.](#) ; [Rossi, S.](#) ; [Canciglia, P.](#) ; [Mita, L.](#) ; [Portaccio, M.](#) ; [Arduini, F.](#) ; [Amine, A.](#) ; [Moscone, D.](#)

**Author Affiliation** : Department of Experimental Medicine, Faculty of Medicine and Surgery, Second University of Naples, Via S. Maria di Costantinopoli, 16, 80138 Naples, Italy.

**Author Email** : [mita@igb.cnr.it](mailto:mita@igb.cnr.it)

**Editors** : [Marino, M.](#); [Gustavo Mita, D.](#)

**Book chapter** : [The endocrine disruptors, 2007](#) 2007 pp.159-179 ref.24



Merzouga



Agadir



Marrakech

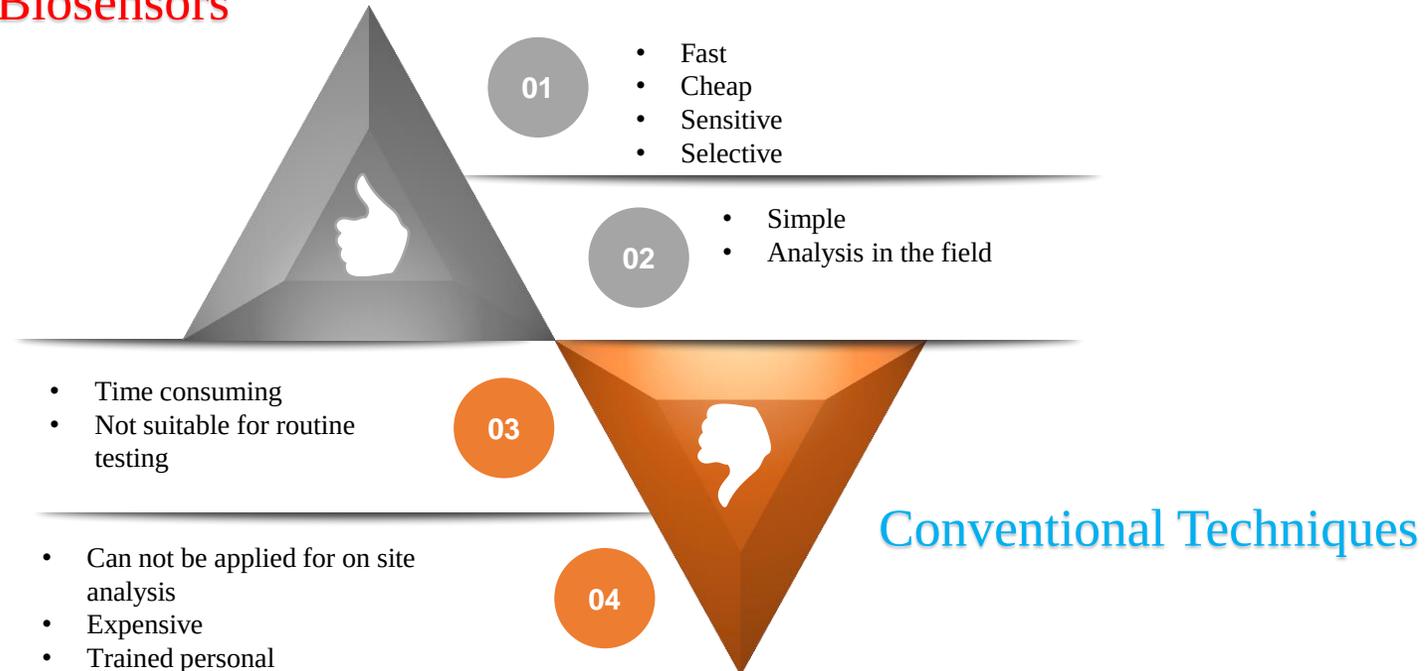


Ifrane



# Biosensors Advantages

## Biosensors



Paper-based analytical devices (PADs)





# *Outline*



*Generalities about molecularly imprinted polymers (MIPs)*

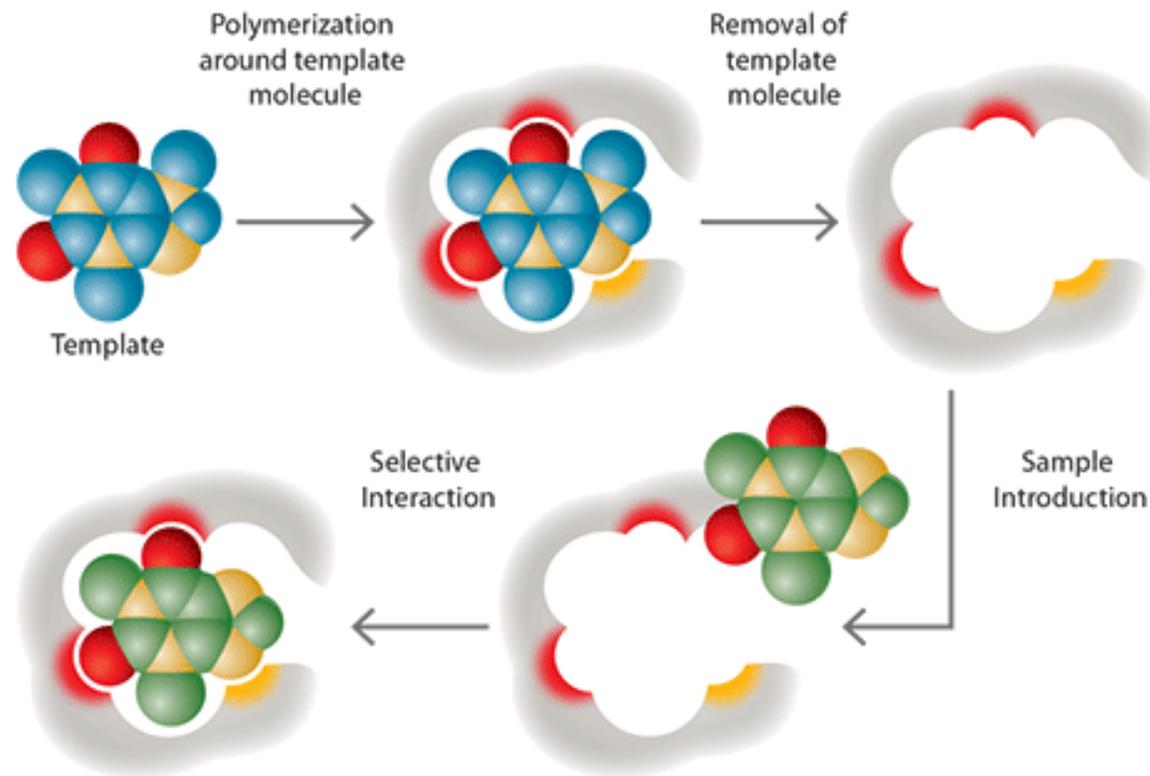
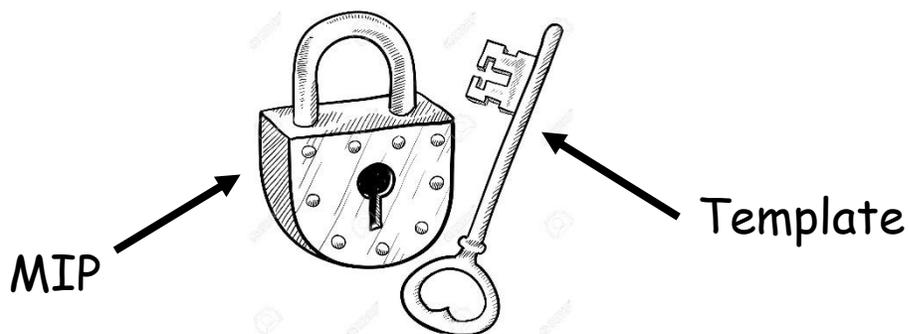


*Recent applications of sensors based on MIPs*

# Introduction

Molecularly imprinted polymers, **MIPs**, are best described as synthetic analogues to the natural, biological antibody-antigen systems. As such, they operate by a "lock and key" mechanism to selectively bind the molecule with which they were templated during production.

Chem. Rev. 2019, 119, 94–119



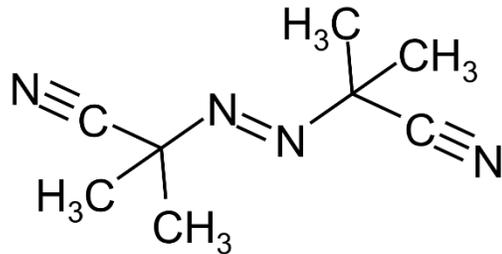
Components of MIPs

- Template (Analyte)
- Monomer
- Cross-linker
- Porogen solvent
- Initiator

# Generalities about MIPs

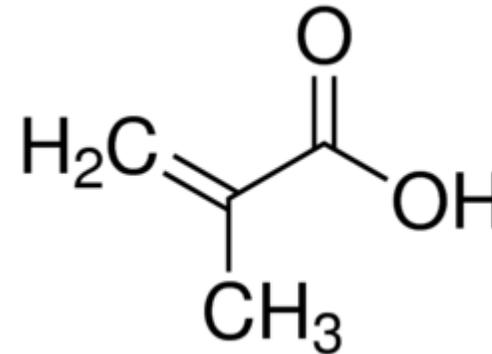
## Components of MIPs

**Initiator:** Initiate the polymerization



Azobisisobutyronitrile (AIBN)

**Functional monomer:** The choice of functional monomers will determine the structure of the recognition site and the concentration will influence the number of binding sites

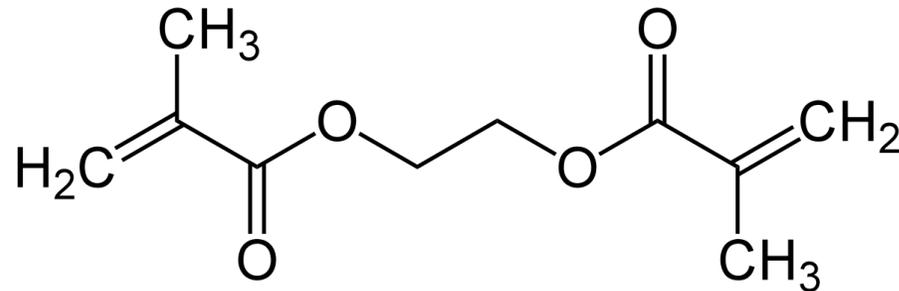


Methacrylic acid

# Generalities about MIPs

## Components of MIPs

**Cross-linker:** According to Cormack and Elorza [1] the cross-linker used to obtain a MIP has three main roles: (i) the cross-linker is important in controlling the morphology of the polymer matrix (gel-type, macroporous or a microgel), (ii) it serves to stabilize the imprinted binding site and (iii) it imparts mechanical stability to the polymer matrix.



Ethylene glycol dimethacrylate

[1] Cormack, P.A.G. and Elorza, A.Z., *Molecularly imprinted polymers: synthesis and characterisation*. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 2004. 804(1): p. 173-182 DOI: 10.1016/j.jchromb.2004.02.013.

# Generalities about MIPs

## Components of MIPs

**Porogen solvent:** The solvent is part of the medium where the polymerization is carried out.

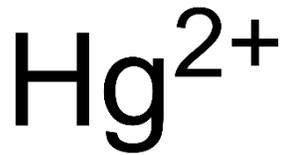
Because of the creation of pores in macroporous polymers, it is common to refer to the solvent as the "porogen"

Acetonitrile  
Toluene  
Chloroforme  
DMSO  
DMF  
Ethanol  
Methanol

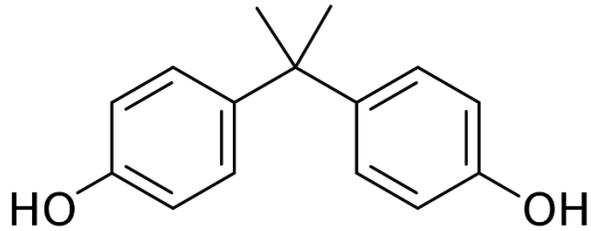
# Generalities about MIPs

## Components of MIPs

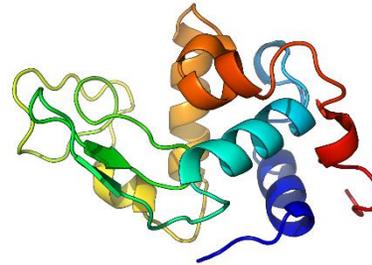
**Template:** The template should ideally be chemically inert under the polymerization conditions and stable under the synthesis conditions



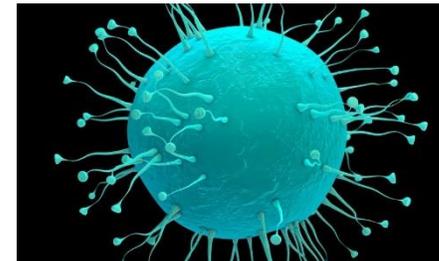
Ion



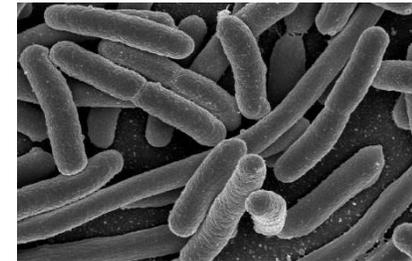
Organic Molecule



Protein



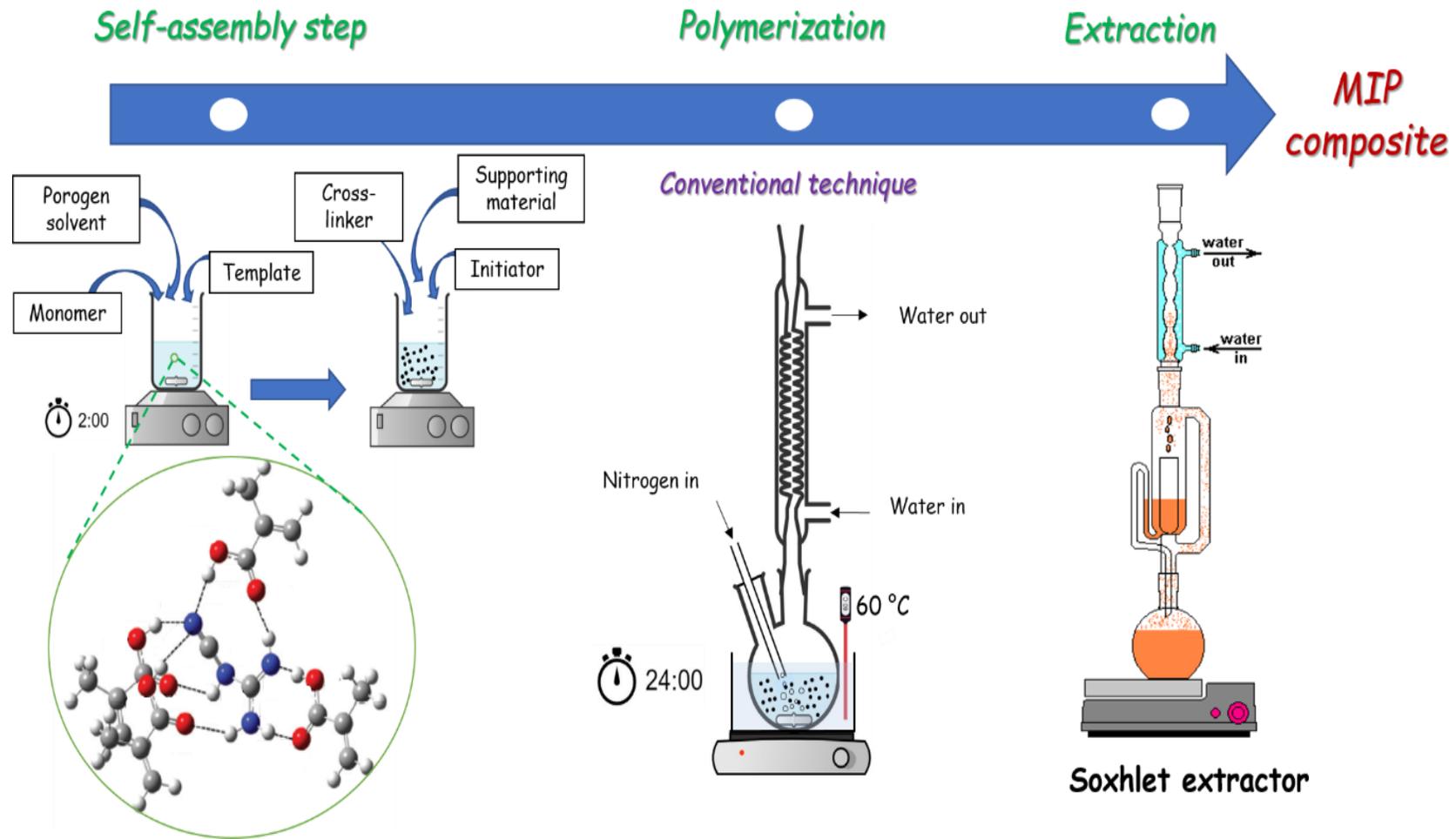
Virus



Bacteria

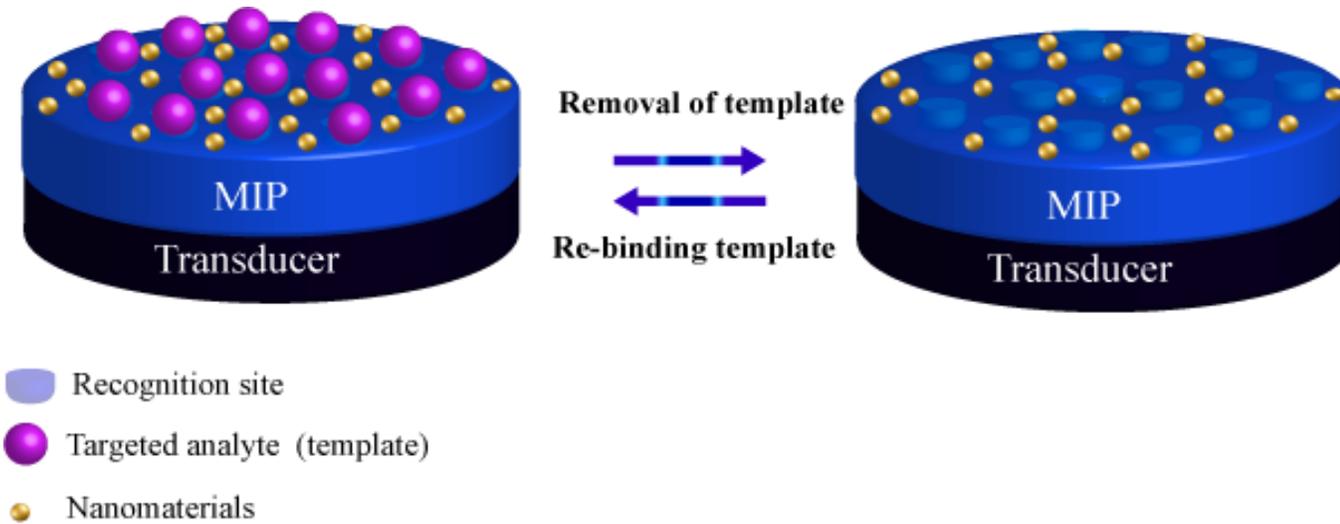
[Label-free electrochemical sensor based on spore-imprinted polymer for \*Bacillus cereus\* spore detection](#)

[Sensors and Actuators B: Chemical, 2018, 276, 114-120](#)



**General procedure of preparation of MIP composites using the conventional technique of thermal heating**

# Electropolymerization on surface of electrode



Schematic representation of the preparation of electrochemical sensors based on MIPs and nanomaterials

Electropolymerization can be achieved through a variety of electrochemical techniques, namely, voltammetric, potentiostatic, and galvanostatic.

# Generalities about MIPs

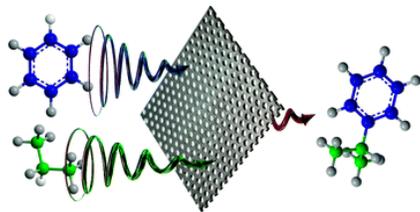
## Application of MIPs



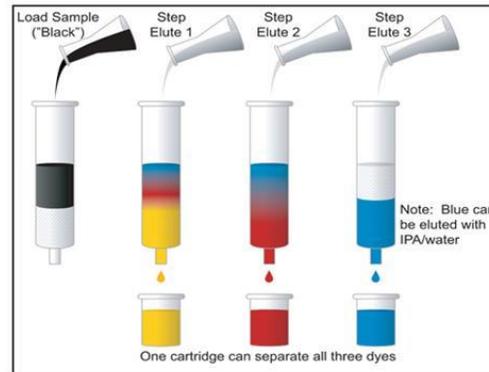
Drug delivery



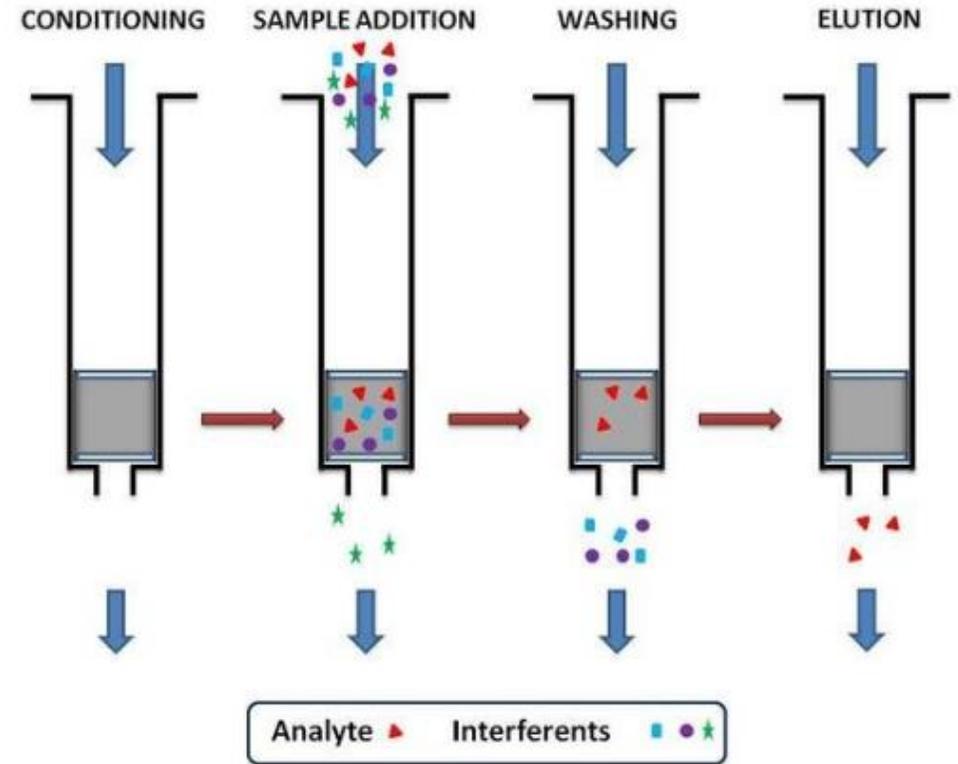
Chemical sensors



Catalysis



Separation and purification processes



Stages of solid phase extraction (SPE)

# Generalities about MIPs

## Advantages

- Higher physical robustness
- Not sourced from animals
- Resistance to elevated temperature and pressure
- Inertness towards acids, bases, metal ions and organic solvents
- Less expensive to be synthesized
- Long storage life of the polymers compared to natural receptors

## Drawbacks

- Long time required for development and optimization
- No mass production of sensors modified with MIPs is available !!!
- Require of non-negligible amount of the target analyte during the MIP preparation
- Deterioration of the recognition sites during the target analyte step removal.
- Regeneration of the sensors in some cases is still difficult.
- Residual analyte at trace level still captured to the MIP cavities during the preparation of imprinted polymer

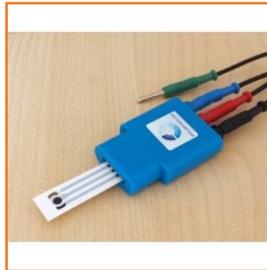
MIP: Molecularly Imprinted Polymer-Decorated  
Magnetite Nanoparticle for 17- $\beta$ -estradiol Detection



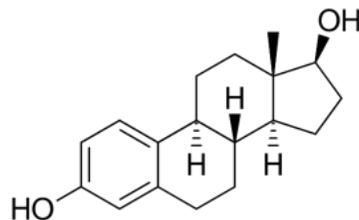
# MagMIP for 17- $\beta$ -estradiol



The synthesis and characterization of a magnetic molecularly imprinted polymer ( $\text{Fe}_3\text{O}_4$ -MIP) for 17- $\beta$ -estradiol determination



Development of an electrochemical sensor based on Magnetic MIPs for the detection of 17- $\beta$ -estradiol in water samples.

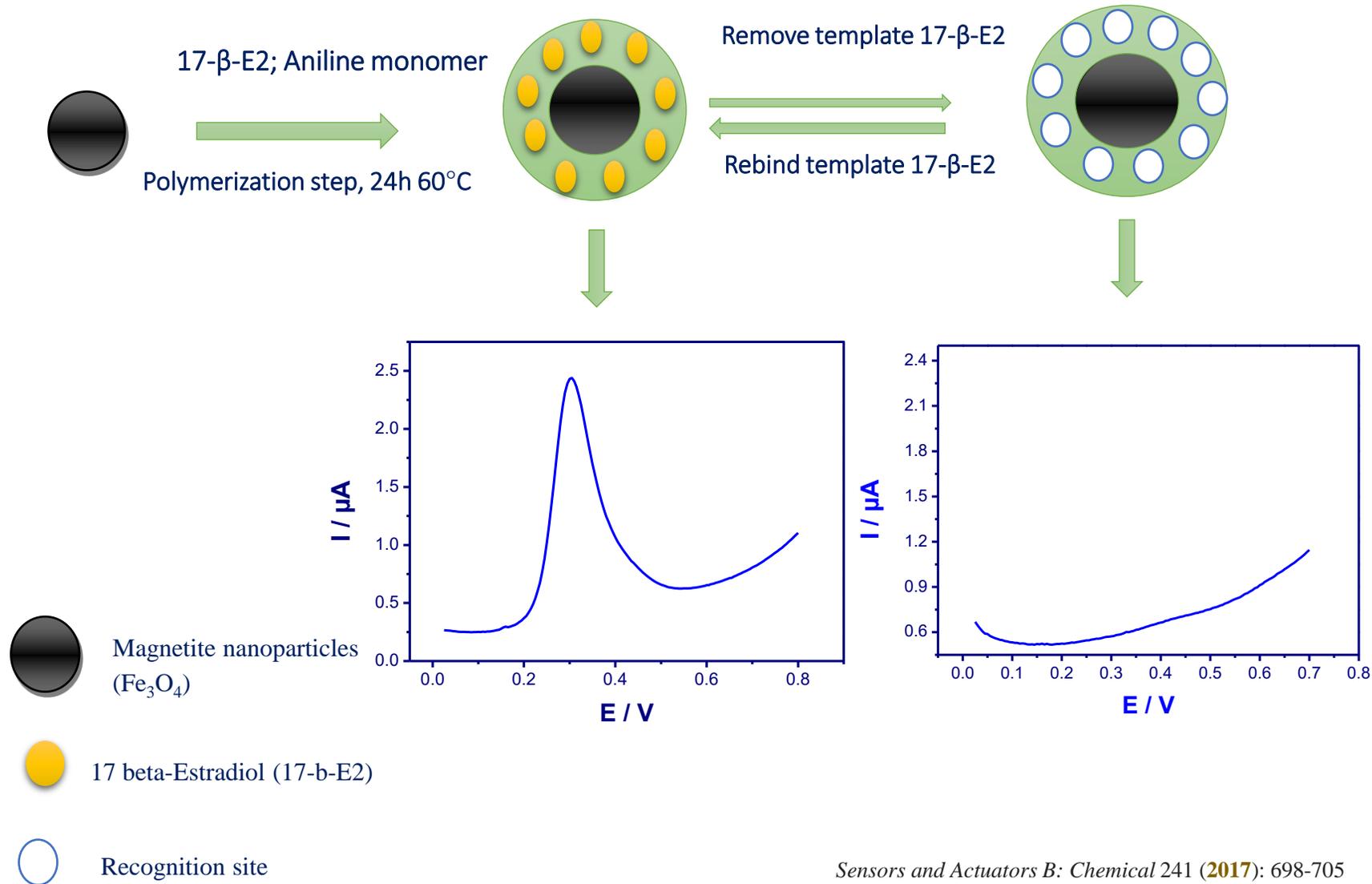


17- $\beta$ -estradiol

- Synthetic and naturally occurring hormones
- toxic,
- carcinogenic
- disturb the function of male reproductive system
- induce an abnormality of growth



# Synthesis of Fe<sub>3</sub>O<sub>4</sub>-MIP



# Synthesis of magnetite nanoparticles

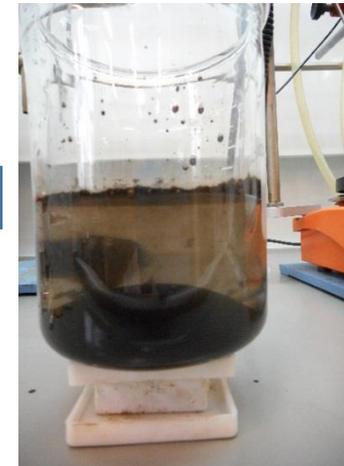


Mixture oxidation of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  under nitrogen conditions



Addition of  $\text{NH}_4\text{OH}$

40 min, 80°C



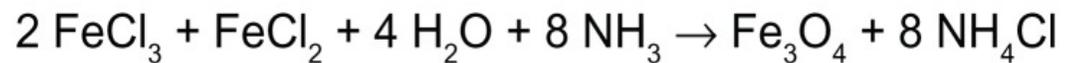
Separation of  $\text{Fe}_3\text{O}_4$  NPs by an external magnet



Dried at 45°C

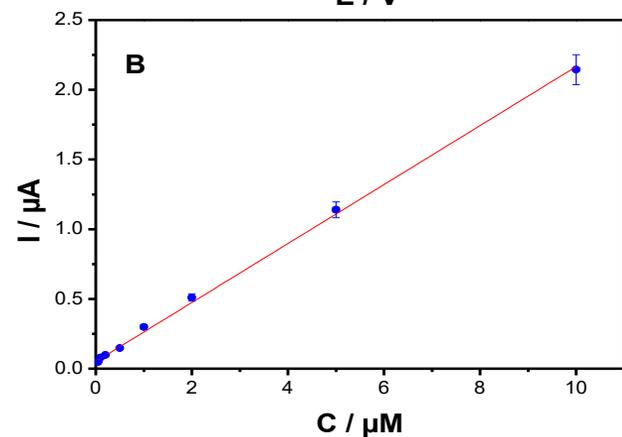
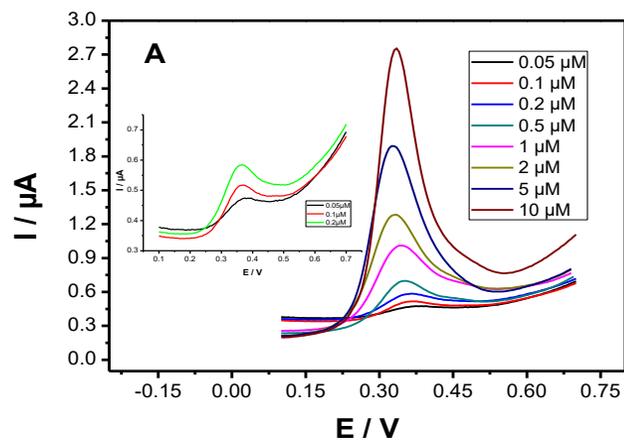


Final magnetite Product



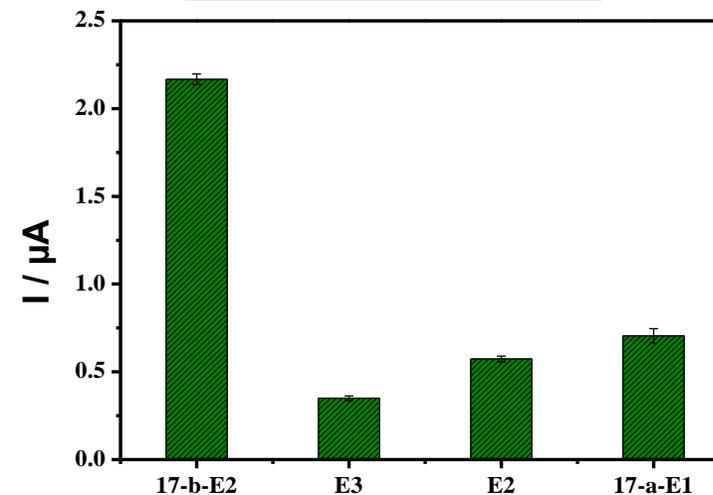
Anal. Chem. 2008, 80, 2250-2254  
J. Chromatogr., A 2009, 1216, 3710-3719

## Sensitivity



→ LOD = 20 nM

## Selectivity



17- $\alpha$ -ethinylestradiol (17- $\alpha$ -E1), Esterone(E1) and Esteriol (E3) are Structurally similar estrogens

## Analytical application in River water samples

17- $\beta$ -E2 spiked ( $\mu$ M)	Amount found ( $\mu$ M)	Recovery %	RSD %*
2	1.91	95.6	6.5
5	4.85	97	5.2

# Conclusions

A novel electrochemical sensor based on **Fe<sub>3</sub>O<sub>4</sub>-MIP/SPCE** was developed and used for the electrochemical detection of **17-β-estradiol**.

Wide linear range from 0.05 to 10 μM and a **high selectivity** and **sensitivity** towards 17-β-E2 and was successfully applied for its determination of in **river water samples** showing a satisfactory results



Sensors and Actuators B: Chemical

Volume 241, 31 March 2017, Pages 698–705



## Synthesis and electrochemical characterization of nanostructured magnetic molecularly imprinted polymers for 17-β-Estradiol determination

Abdellatif Ait Lahcen<sup>a, b</sup>, Abd Almonam Baleb<sup>a</sup>, Priscilia Baker<sup>a</sup>, Emmanuel Iwuoha<sup>a</sup>, Aziz Amine<sup>b</sup>  



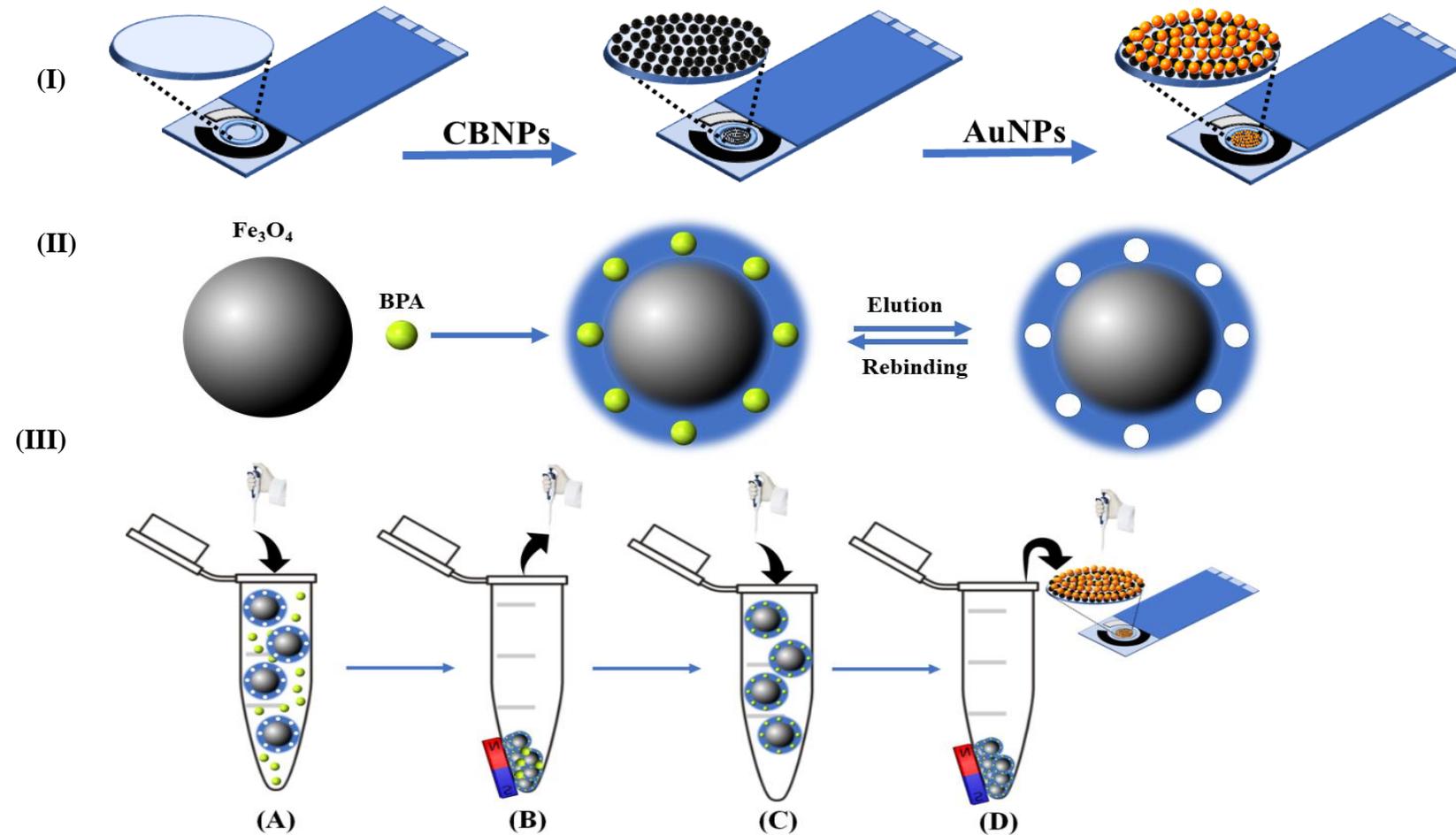
<sup>a</sup> Sensor Lab, Department of Chemistry, University of the Western Cape, Bellville, 7535, South Africa

<sup>b</sup> Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca, B.P. 146. Mohammedia, Morocco

Received 16 September 2016, Accepted 25 October 2016, Available online 29 October 2016

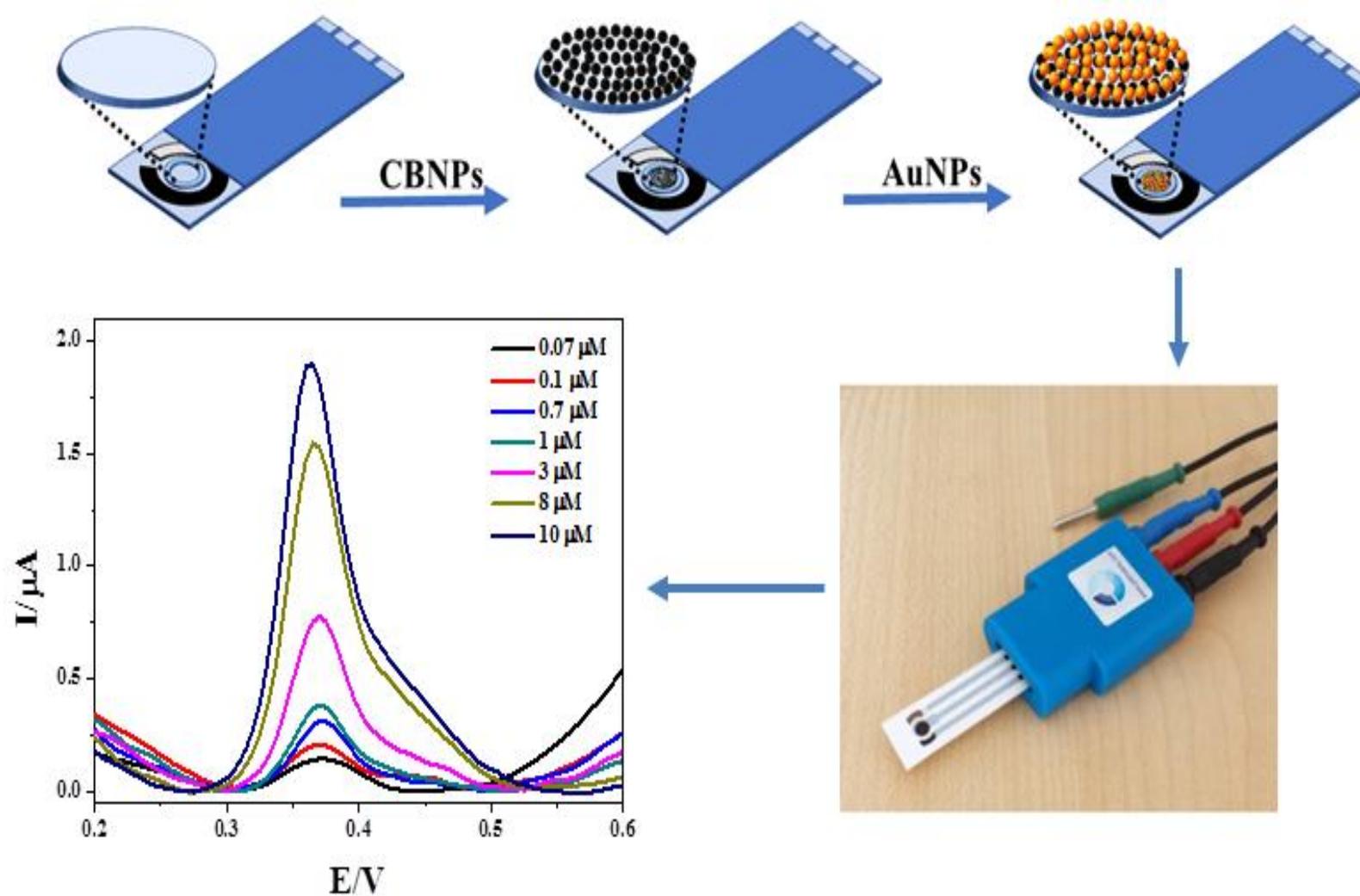
*MIP: Ultrasound Assisted Mag-MIP combined sensor for selective and sensitive detection of Bisphenol A*

# Basic principle of the proposed analytical method



Schematic illustration of the developed analytical procedure

# Ultrasound Assisted Mag-MIP combined sensor for selective and sensitive detection of Bisphenol A





## Ultrasound Assisted Mag-MIP combined sensor for selective and sensitive detection of Bisphenol A



**MIP synthesis in 24h**



**MIP synthesis in 2 h**



Contents lists available at ScienceDirect

## Sensors and Actuators B: Chemical

journal homepage: [www.elsevier.com/locate/snb](http://www.elsevier.com/locate/snb)



# Ultrasound assisted magnetic imprinted polymer combined sensor based on carbon black and gold nanoparticles for selective and sensitive electrochemical detection of Bisphenol A



Najib Ben Messaoud<sup>b</sup>, Abdellatif Ait Lahcen<sup>a</sup>, Chérif Dridi<sup>b,\*</sup>, Aziz Amine<sup>a,\*</sup>

<sup>a</sup> Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca, B.P. 146, Mohammedia, Morocco

<sup>b</sup> NANOMISENE Lab, LR16CRMN01, Centre for Research on Microelectronics and Nanotechnology CRMN of Technopark of Sousse B.P. 334, Sahloul, 4034, Sousse, Tunisia

### ARTICLE INFO

#### Keywords:

Bisphenol A

Electrochemical sensor

Ultrasound assisted magnetic molecularly imprinted polymer

Gold nanoparticles

Carbon black nanoparticles

### ABSTRACT

Sample preparation is considered as one of the most interesting applications of magnetic molecularly imprinted polymer (MagMIP) used as adsorbents for various compounds prior to their determination using the developed analytical techniques. In this context, we present in this paper a developed Ultrasound-assisted magnetic molecularly imprinted polymer (US-MagMIP) combined to an electrochemical sensor modified with a nano-composite of carbon black nanoparticles (CBNPs), and Gold nanoparticles (AuNPs) for highly selective and sensitive detection of Bisphenol A (BPA). The electrochemical characterization of the developed sensor was investigated by electrochemical impedance spectroscopy and cyclic voltammetry. Various parameters were optimized such as the effect of voltammetric mode, the effect of pH, the amount of US-MagMIP and the rebinding time. The electrochemical sensor exhibits a high sensitivity for BPA with a LOD of 8.8 nM with a wide linear range from 0.07  $\mu$ M to 10  $\mu$ M. Moreover, this sensor provides a high selectivity towards various likely interferences by combination with US-MagMIP prior to analysis. The developed analytical approach was applied

**MIP:** Fast route for the synthesis of decorated nanostructured magnetic MIPs using an ultrasound probe

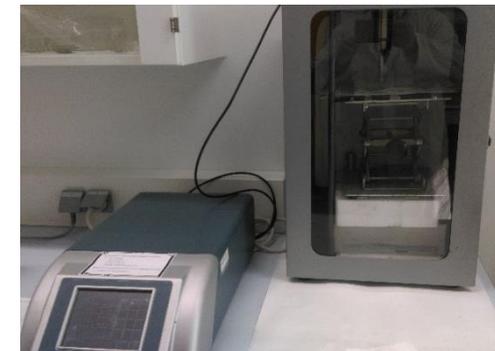
# Fast route for the synthesis of decorated nanostructured magnetic MIPs using an ultrasound probe



Thermal heating **24h**



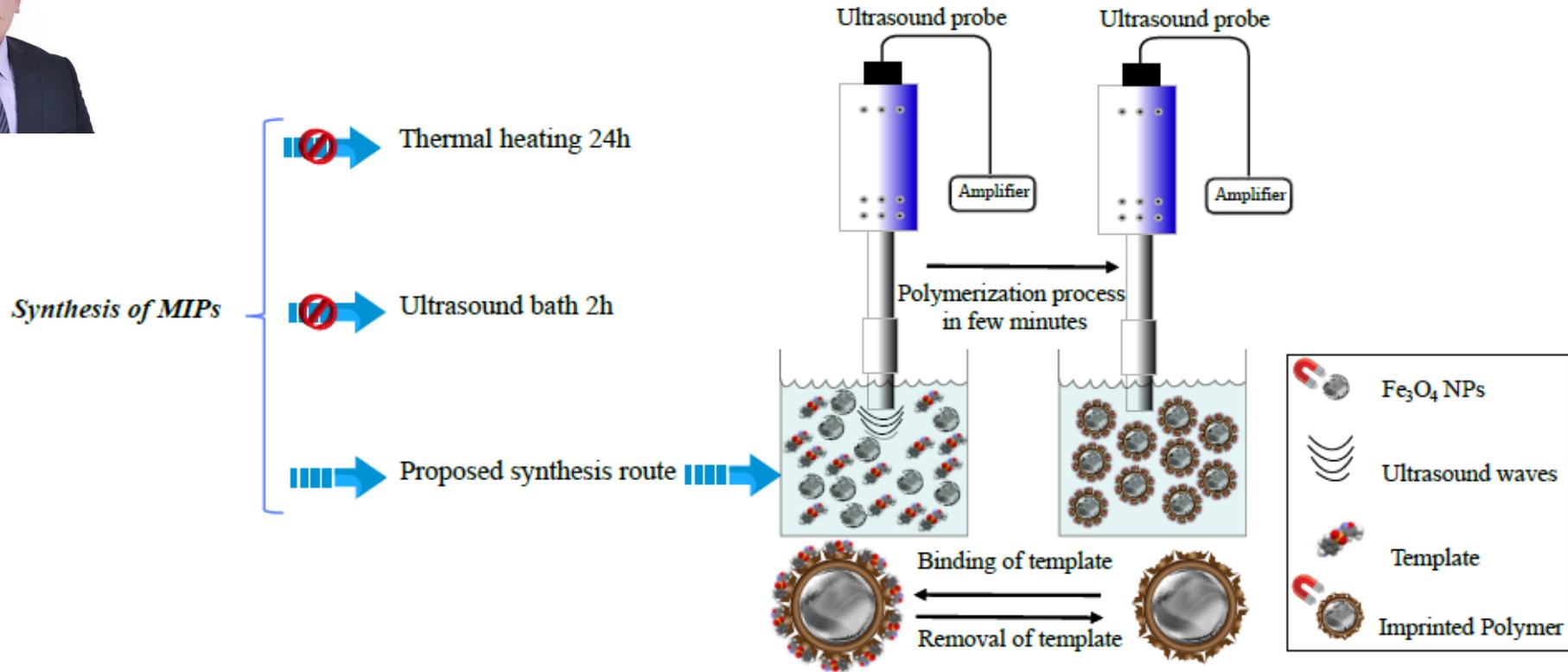
Ultrasound assisted in  
bath **2 h**



Ultrasound probe assisted  
**few minutes**

Decreasing MIP synthesis time

# Fast route for the synthesis of decorated nanostructured magnetic MIPs using an ultrasound probe





## Fast route for the synthesis of decorated nanostructured magnetic molecularly imprinted polymers using an ultrasound probe

Abdellatif Ait Lahcen<sup>a,b</sup>, Juan José García-Guzmán<sup>a</sup>, Jose Maria Palacios-Santander<sup>a,\*</sup>,  
Laura Cubillana-Aguilera<sup>a</sup>, Aziz Amine<sup>b,\*</sup>

<sup>a</sup> *Institute of Research on Electron Microscopy and Materials (IMEYMAT), Department of Analytical Chemistry, Faculty of Sciences, Campus de Excelencia Internacional del Mar (CEIMAR), University of Cadiz, Campus Universitario de Puerto Real, Polígono del Río San Pedro S/N, Puerto Real, Cádiz 11510, Spain*

<sup>b</sup> *Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca, B.P. 146. Mohammedia, Morocco*

### ARTICLE INFO

#### Keywords:

High-energy ultrasound probe  
Magnetic molecularly imprinted polymer  
Electrochemical detection  
Sulfonamides  
Magnetic nanoparticles

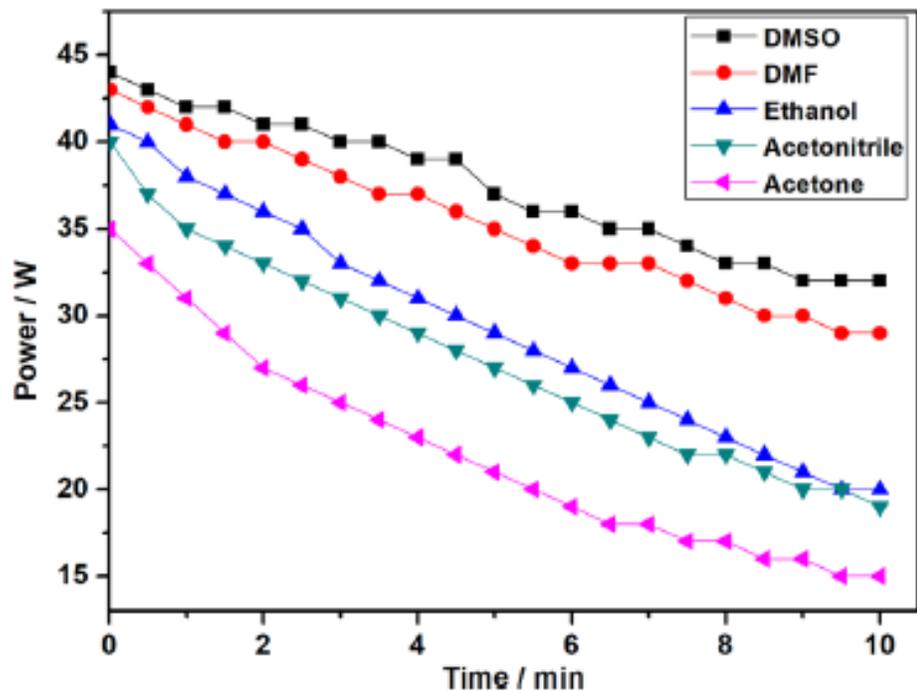
### ABSTRACT

In this paper, we report for the first time a novel, simple and fast method for the synthesis of magnetic molecularly imprinted polymers (Mag-MIPs) based on high-energy ultrasound probe. Sulfamethoxazole (SMX) was used as template molecule, methacrylic acid as functional monomer, ethylene glycole dimethacrylate as cross-linking agent and magnetic nanoparticles (NPs) as the supporting core. The effects of time (5, 7.5 and 10 min) and the applied amplitude (20, 30, 40, 50 and 60%) using the ultrasound probe for the synthesis of Mag-MIPs were studied and optimized. By applying the proposed synthesis method, the US-magMIPs synthesis time was satisfactorily reduced from several hours to a few minutes (7.5 min) in a simple way. For comparison purposes, the Mag-MIP and the non imprinted polymer (MagNIP) were also synthesized employing an ultrasound bath assisted approach (2 h, 65 °C).

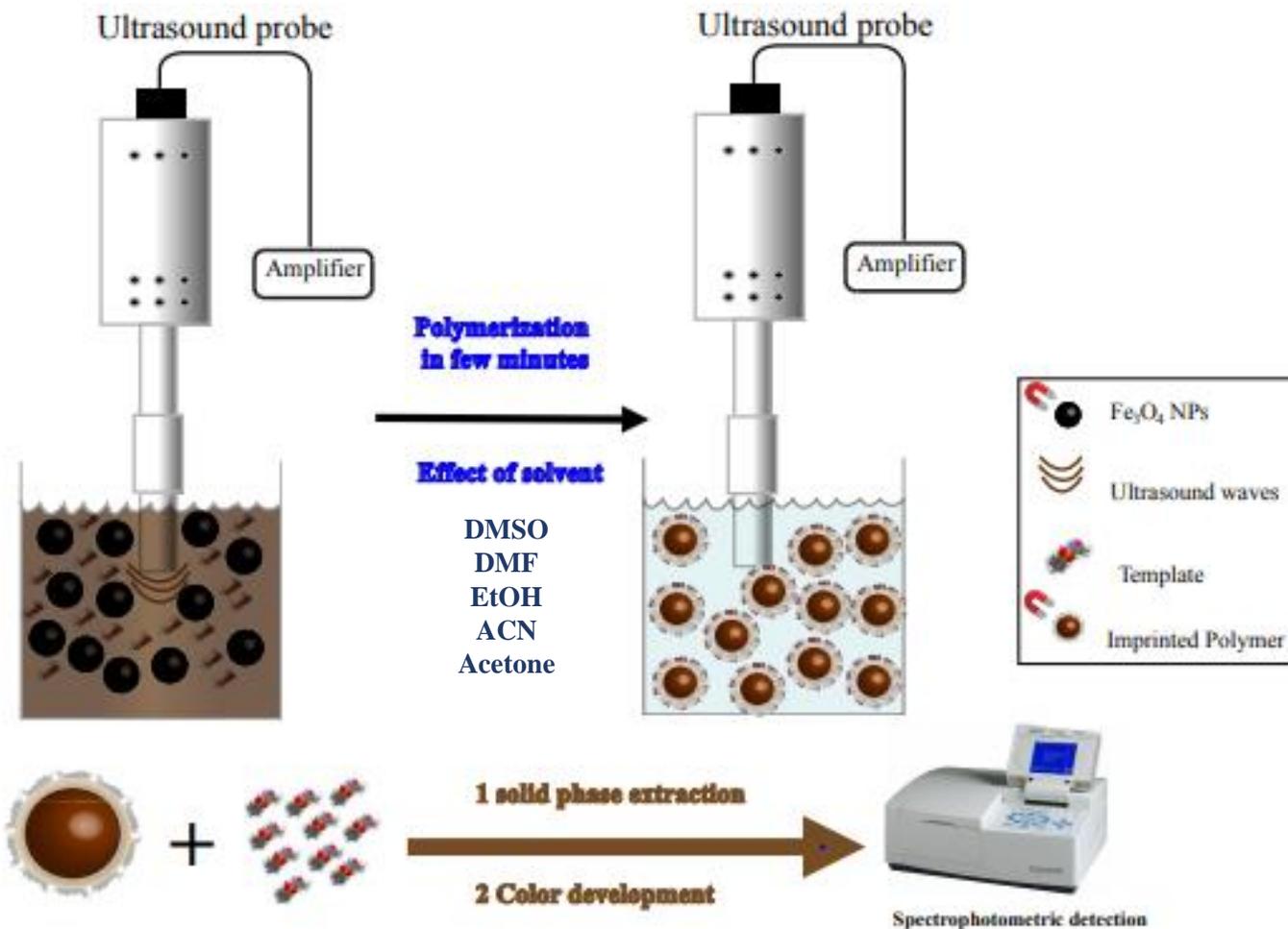
Magnetic NPs and US-magMIPs synthesized by both ways were investigated by means of several character-

**MIP:** Study of solvent effect on the synthesis of nanostructured magnetic MIPs based on ultrasound probe: application for sulfonamide detection

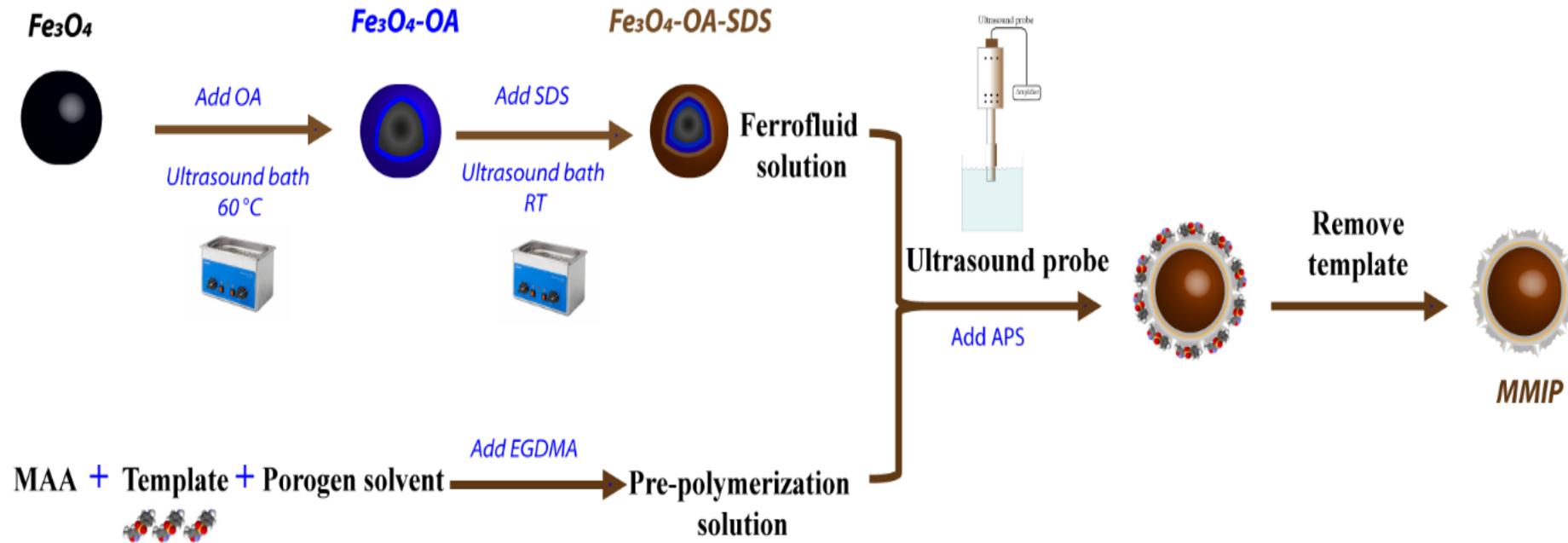
# Study of solvent effect on the synthesis of nanostructured magnetic MIPs based on ultrasound probe: application for sulfonamide detection



Power evolution as a function of time during the synthesis of MMIPs in different solvents.

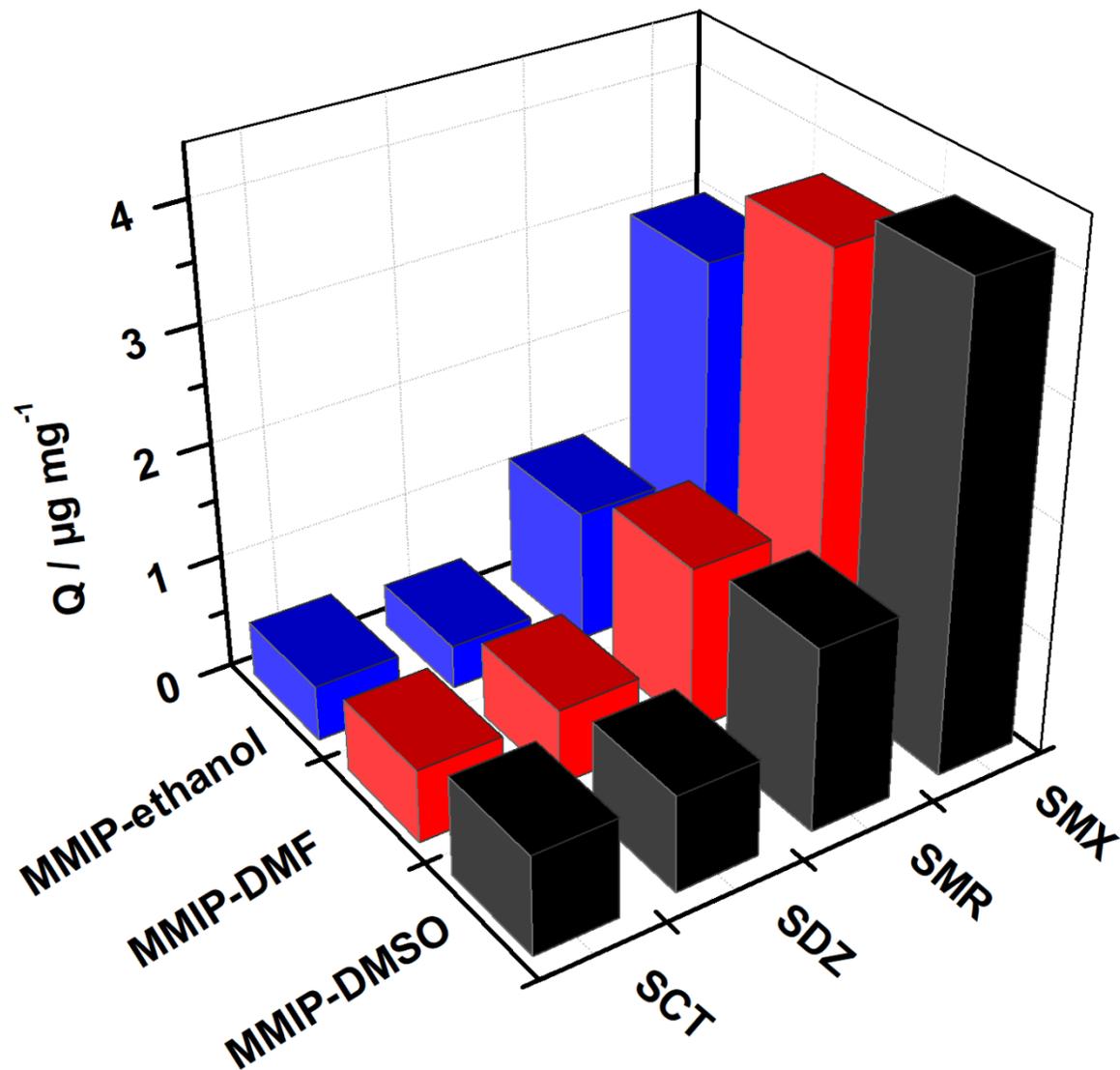


# Study of solvent effect on the synthesis of nanostructured magnetic MIPs based on ultrasound probe: application for sulfonamide detection



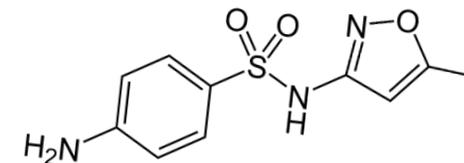
Synthesis of magnetic molecularly imprinted polymers based on ultrasound probe (MAA: Methacrylic acid, OA: Oleic acid, SDS: Sodium dodecyl sulfate, EGDMA: Ethylene glycol dimethacrylate, RT: Room temperature).

## Study of solvent effect on the synthesis of nanostructured magnetic MIPs based on ultrasound probe: application for sulfonamide detection

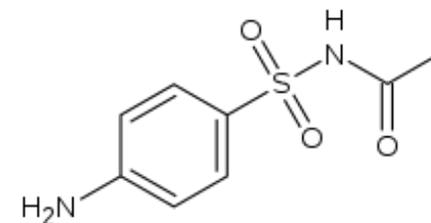


Selectivity of MMIP-DMSO, MMIP-DMF and MMIP-ethanol for different analytes.

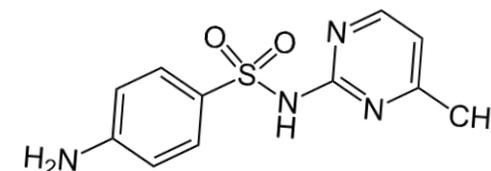
SMX



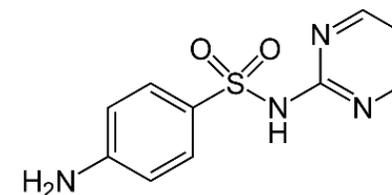
SMR



SDZ



SCT





Contents lists available at ScienceDirect

## Ultrasonics - Sonochemistry

journal homepage: [www.elsevier.com/locate/ultson](http://www.elsevier.com/locate/ultson)



### Study of solvent effect on the synthesis of magnetic molecularly imprinted polymers based on ultrasound probe: Application for sulfonamide detection



Abderrahman Lamaoui<sup>a,b</sup>, Abdellatif Ait Lahcen<sup>a,b</sup>, Juan José García-Guzmán<sup>a</sup>, José María Palacios-Santander<sup>a,\*</sup>, Laura Cubillana-Aguilera<sup>a</sup>, Aziz Amine<sup>b,\*</sup>

<sup>a</sup> *Institute of Research on Electron Microscopy and Materials (IMEYMAT), Department of Analytical Chemistry, Faculty of Sciences, Campus de Excelencia Internacional del Mar (CEIMAR), University of Cadiz, Campus Universitario de Puerto Real, Polígono del Río San Pedro S/N, 11510 Puerto Real, Cádiz, Spain*

<sup>b</sup> *Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca, B.P. 146. Mohammedia, Morocco*

#### ARTICLE INFO

##### Keywords:

High-power ultrasound probe  
Organic solvents  
Nanostructured magnetic molecularly imprinted polymer  
Solid-phase extraction  
Spectrophotometry  
Sulfonamide in tap and mineral water

#### ABSTRACT

In this work, a comparative study of the effect of various solvents on the synthesis of magnetic molecularly imprinted polymers (MMIPs) based on the use of high-power ultrasound probe is reported for the first time. Dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethanol, acetonitrile and acetone were studied as solvents for the synthesis of MMIPs. Several crucial experimental conditions such as the time of synthesis and the applied amplitude were investigated. DMSO, DMF and ethanol were successfully used for ultrasound-assisted synthesis of MMIPs. However, for the polymerization performed using acetonitrile and acetone, no significant conversion to product was observed. Under optimal conditions for each solvent tested, the synthesized MMIPs were characterized using several techniques such as Scanning/Transmission Electron Microscopy (SEM and STEM modes), X-Ray Diffraction, Fourier Transform Infra-Red Spectroscopy, Thermal Gravimetric Analysis and Vibrating Sample Magnetometer system. The study of adsorption time of MMIPs showed that fast adsorption occurred due to the presence of specific imprinted sites on the surface. Moreover, isotherm study showed that the experimental equilibrium data fitted well with Freundlich model. The results of selectivity study indicated that

**MIP: Spore-Imprinted Polymer based sensor for *Bacillus cereus* Spores Detection**

## Electrosynthesis of MIPs

- ❑ Application of a suitable potential or range of potentials to a solution containing the template with the monomer, originating a film formation on the surface of the electrode
- ❑ Adjusting the electrochemical conditions such as potential range, number of cycles and scan rate)
- ❑ No initiator is required, nor UV light or heat.

### *Bacillus*

***B. anthracis*: anthrax of the animals and humans.**

***B. cereus*: food poisoning; opportunistic infections.**

#### Morphology and Physiology

Aerobic or facultatively anaerobic.

Large gram-positive rods, have square ends, arranged in long chains.

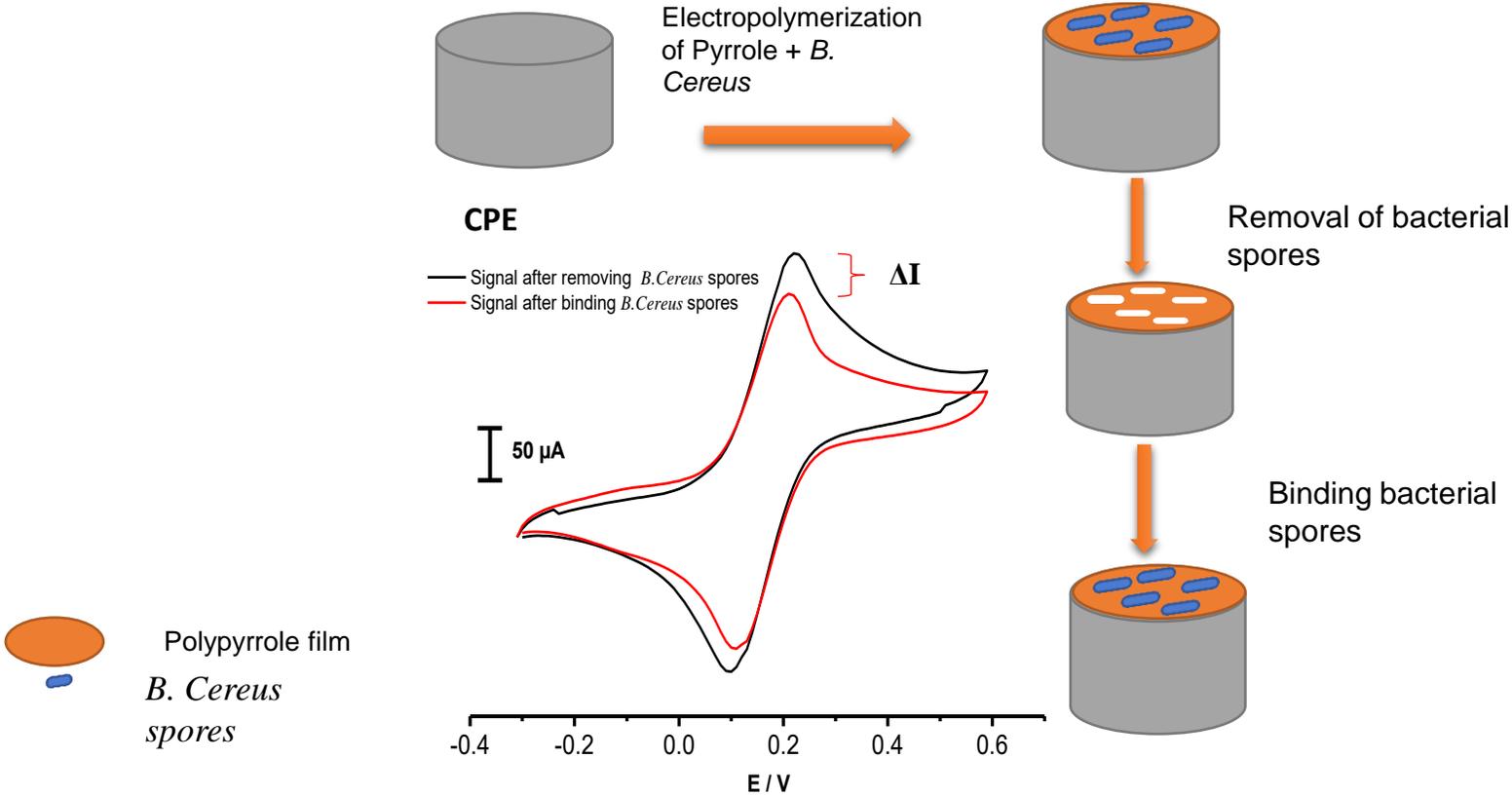
Spore is located in the center of the cell.

Most are saprophytic (soil, water, air, and on vegetation.)



# Spore-Imprinted Polymer based sensor for *Bacillus cereus* Spores Detection

## Schematic illustration



Preparation of the electrode modified with spore-imprinted polymer for the determination of *B. cereus* spores. The proposed sensing system operates via CV.



Contents lists available at ScienceDirect

## Sensors and Actuators B: Chemical

journal homepage: [www.elsevier.com/locate/snb](http://www.elsevier.com/locate/snb)



### Label-free electrochemical sensor based on spore-imprinted polymer for *Bacillus cereus* spore detection



Abdellatif Ait Lahcen<sup>a</sup>, Fabiana Arduini<sup>b,\*</sup>, Florigio Lista<sup>c</sup>, Aziz Amine<sup>a,\*</sup>

<sup>a</sup> Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca, B.P. 146, Mohammedia, Morocco

<sup>b</sup> Department of Chemical Science and Technologies, University of Rome Tor Vergata, Via della Ricerca Scientifica, Rome, 00133, Italy

<sup>c</sup> Army Medical Center, Scientific Department, Via S. Stefano Rotondo, 00184, Rome, Italy

#### ARTICLE INFO

##### Keywords:

Carbon paste electrode  
Polypyrrole  
Cyclic voltammetry  
*Bacillus anthracis* spores simulant  
Biological warfare agents

#### ABSTRACT

Herein, we report a simple, cost-effective, selective, and rapid electrochemical sensor for *Bacillus cereus* spore detection, used as simulant for *Bacillus anthracis* spores. The sensor was developed using carbon paste electrode functionalized with imprinted polymer, fabricated by pyrrole electropolymerization in presence of a known amount of spores as template onto the surface of working electrochemical sensor. The measurement encompasses the cyclic voltammetry as technique and  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  as redox probe for electrochemical measurements. The effect of ultrasonication and surfactants with positive, negative and neutral charge on template removal was investigated. Several parameters including the concentration of monomer, the number of scans, the type and the concentration of removal agent, and the incubation time were optimized. The carbon paste electrode modified with spore-imprinted polymer (CPE/SIP) exhibited a good affinity and selectivity towards

**MIP : Acetazolamide**

# Enzyme inhibition coupled to molecularly imprinted polymers for acetazolamide determination in biological samples

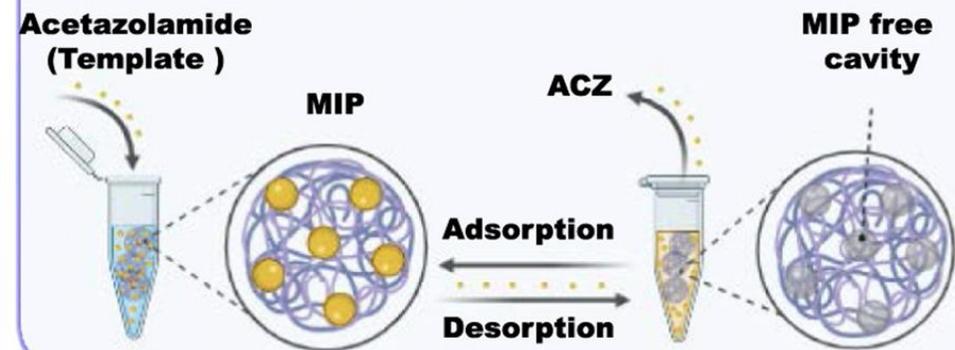
Dounia Elfadil <sup>a, b</sup>, Sara Palmieri <sup>a</sup>, Flavio Della Pelle <sup>a</sup>, Manuel Sergi <sup>a</sup>, Aziz Amine <sup>b</sup>  , Dario Compagnone <sup>a</sup>  

## Real samples



Complex matrix  
+  
Acetazolamide (ACZ)  
+  
Interferents

## Molecularly imprinted polymers



## Readout

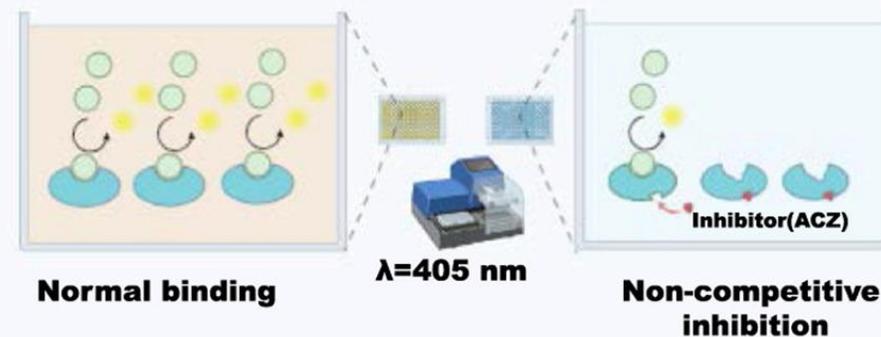


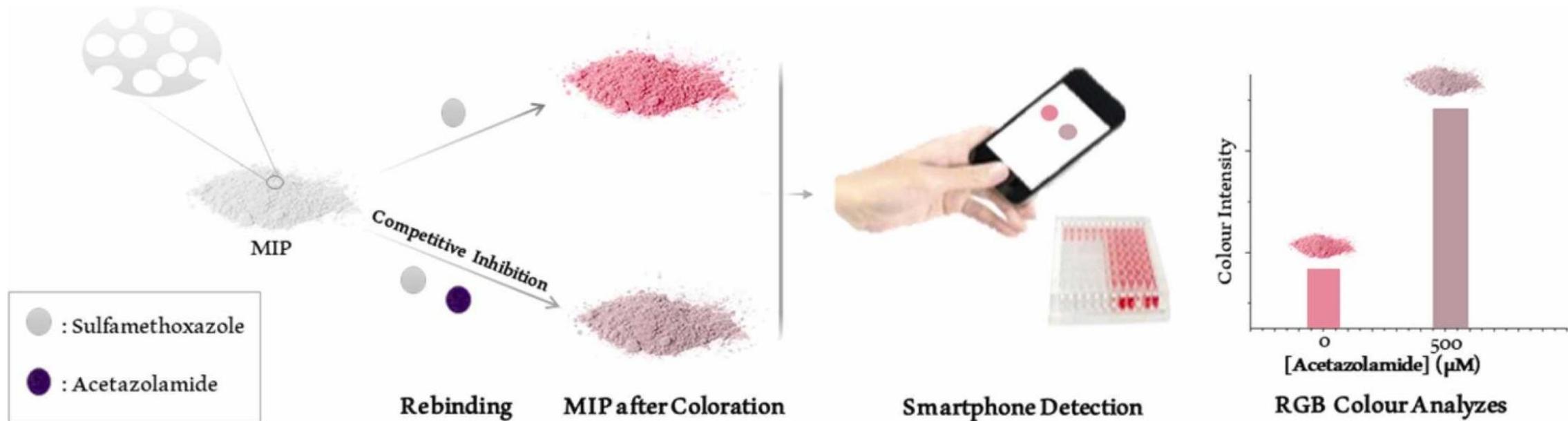
LC-MS/MS



Enzymatic assay

## Carbonic anhydrase inhibition



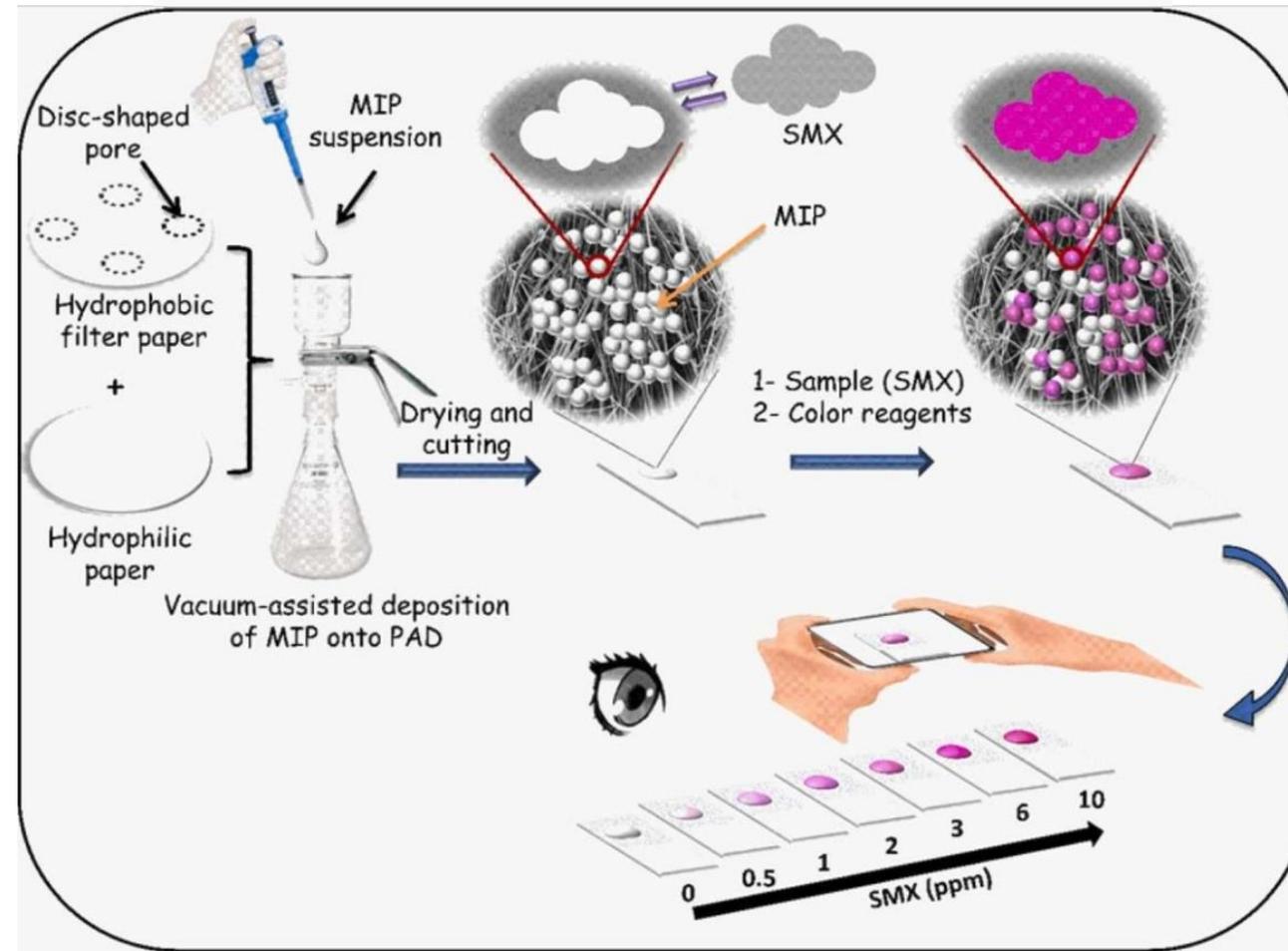


## Acetazolamide smartphone-based detection via its competition with sulfamethoxazole on molecularly imprinted polymer: A proof-of-concept

**PAD-MIP: SMX**

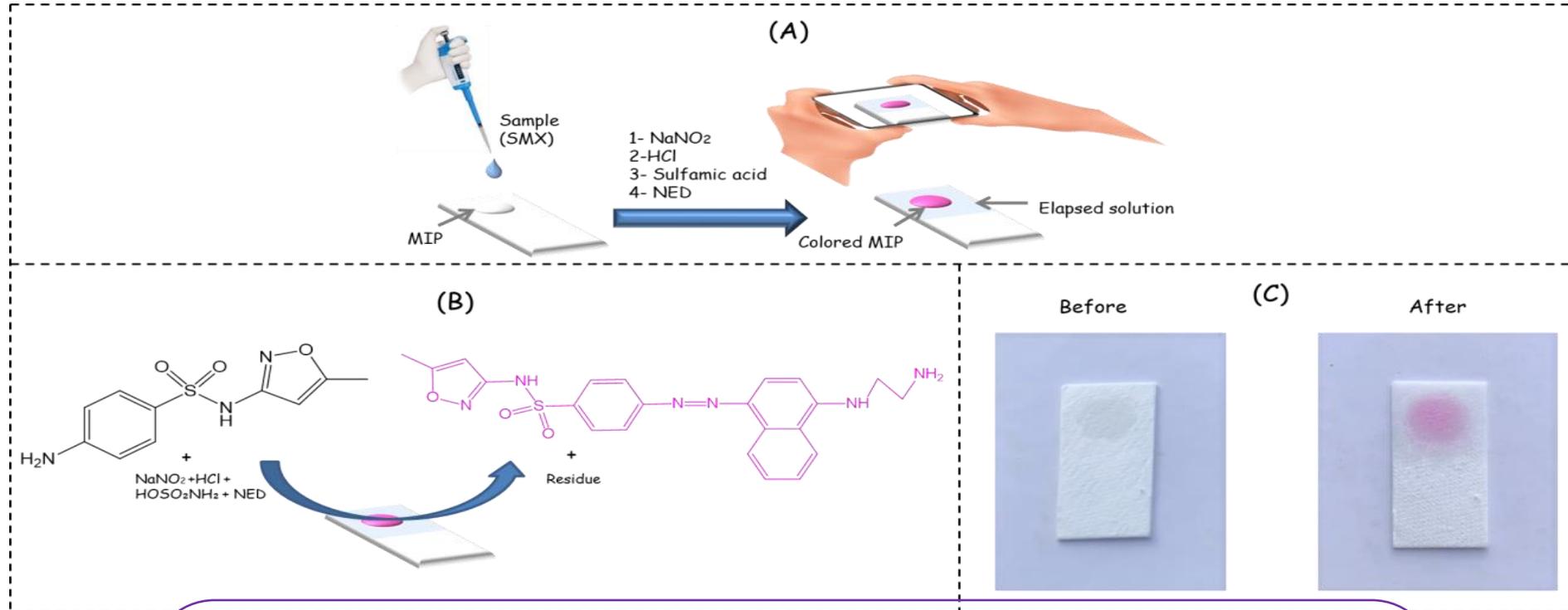
# Molecularly imprinted polymer integrated into paper-based analytical device for smartphone-based detection: Application for sulfamethoxazole

Abderrahman Lamaoui , Abdelhafid Karrat, Aziz Amine 

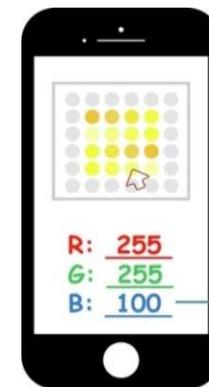


# Approach, results and discussion

## ► Colorimetric detection based on smartphone



Optical



# Approach, results and discussion

## ► Analytical performance and real-world application

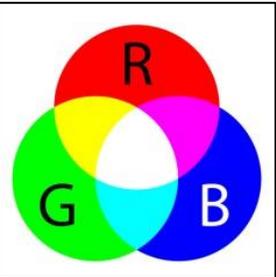


1

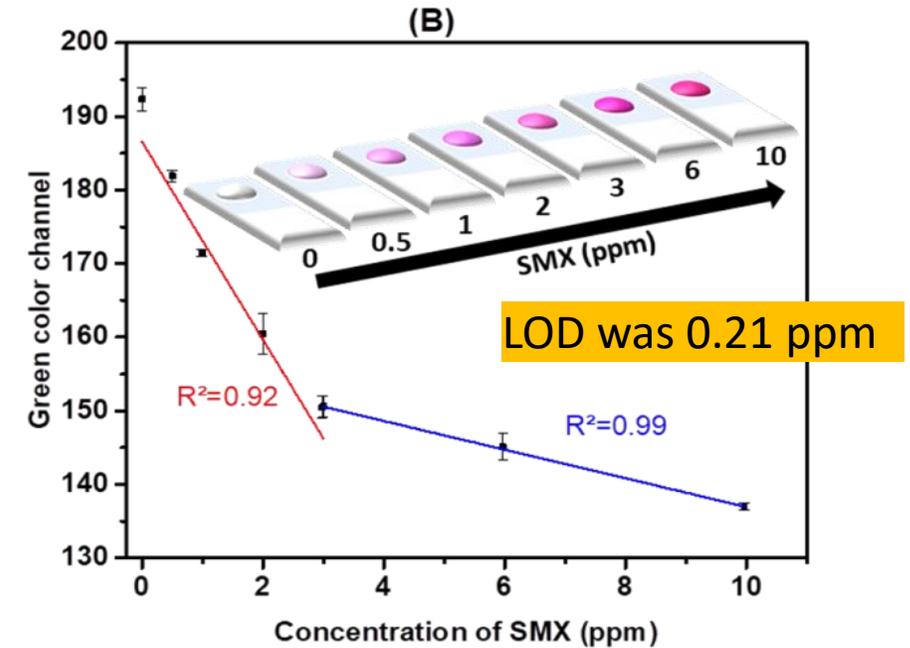
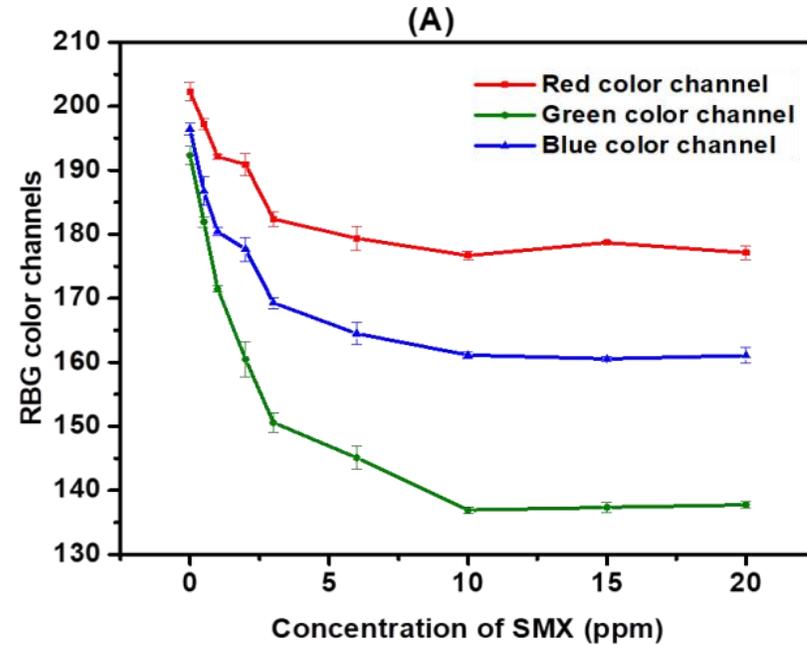


RGB Color Detector  
The programmer  
Contient des ann  
Achats via l'appli

2



3

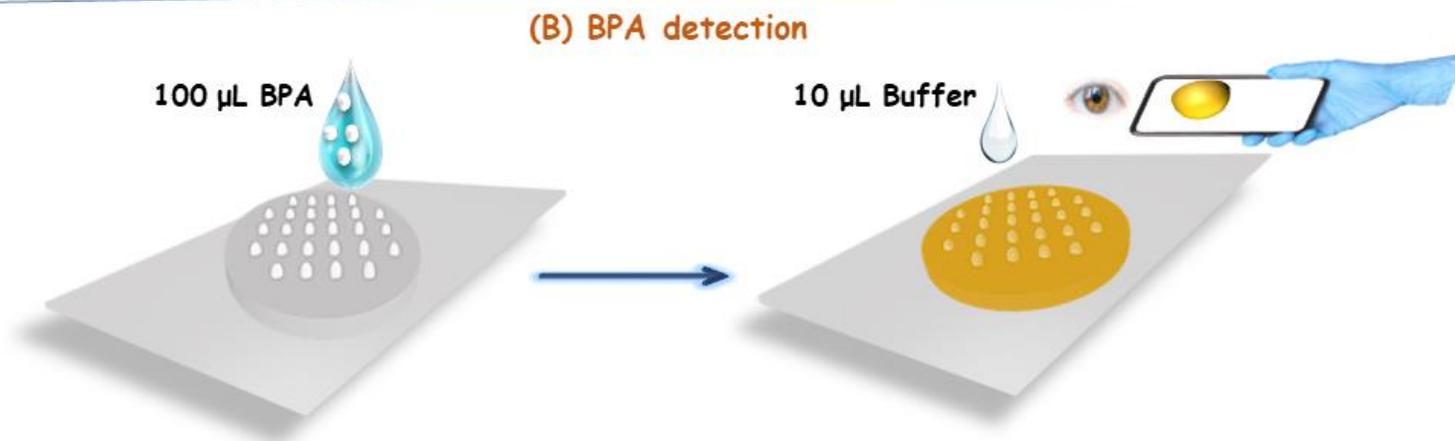


Water sample	Spiked SMX (ppm)	SMX found (ppm)	Recovery (%)	RSD (n=3)
Tap water	1	1.00	100.2	1.5
	5	5.07	101.4	3.3
River water	1	1.14	114.0	4.7
	5	5.60	112.0	3.6

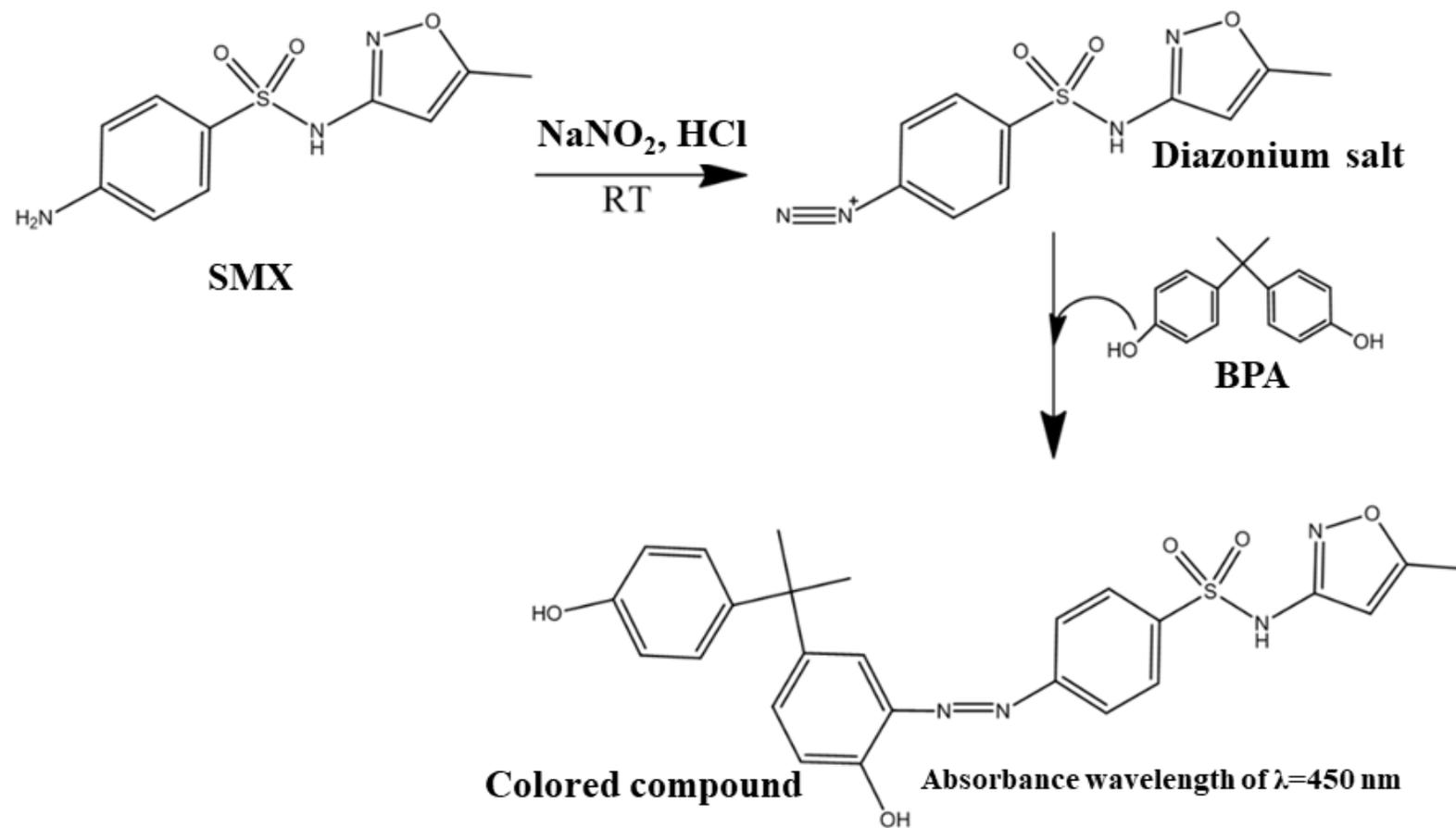


On-site analysis rapid.mp4

**PAD-MIP: Bisphenol A**



**Schematic illustration of MIP-PAD preparation and smartphone BPA detection**

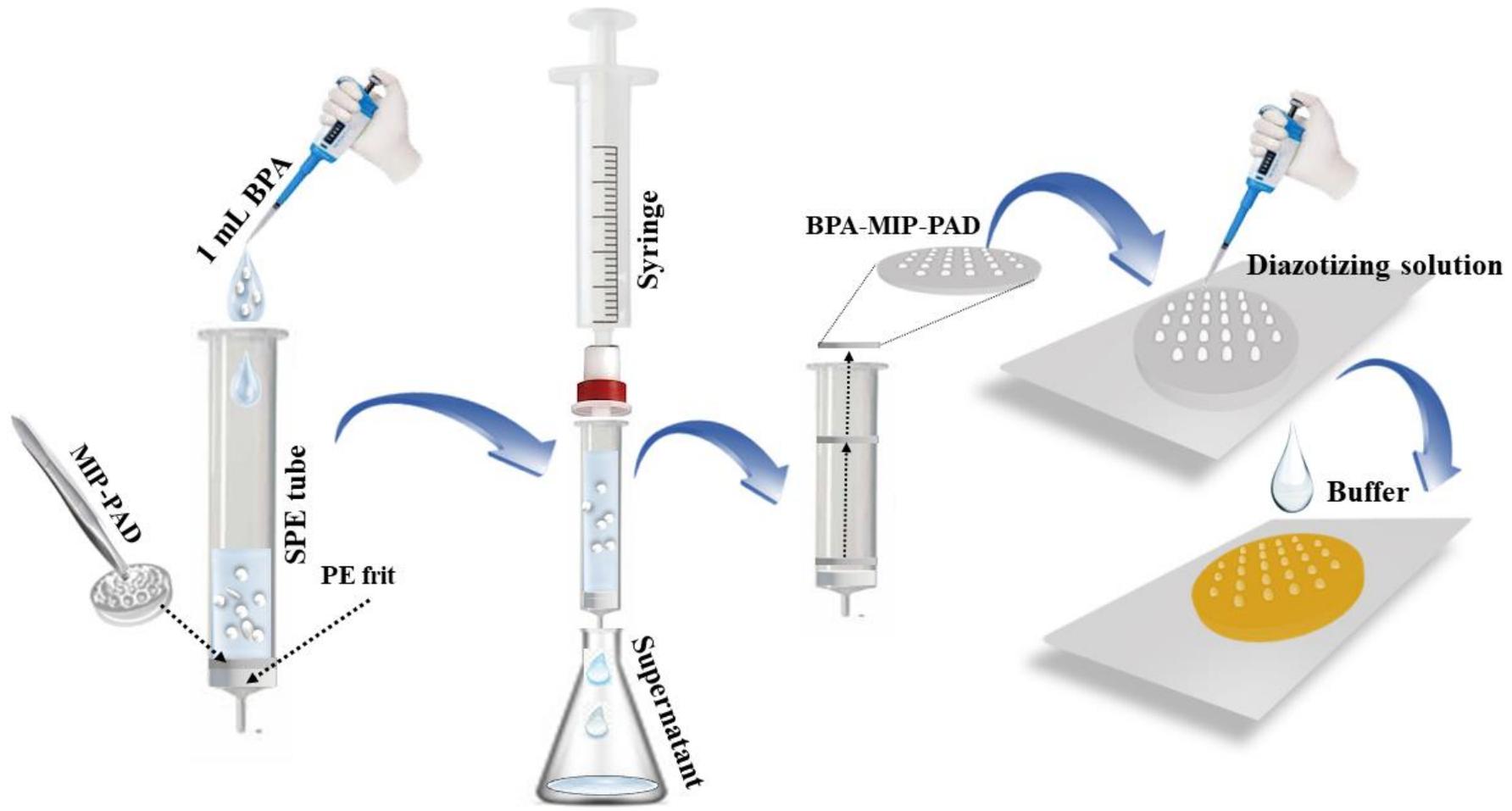


**Mechanism of the coupling reaction between BPA and the diazonium salt of sulfamethoxazole.**

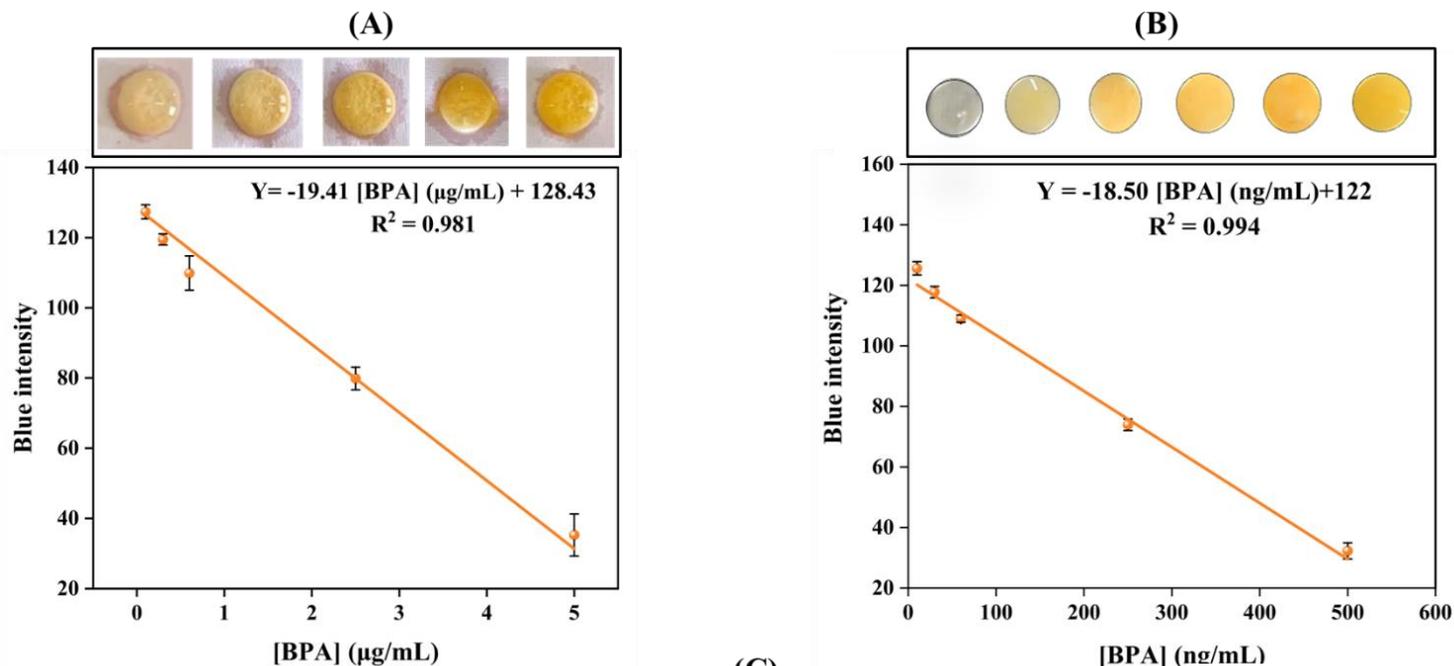


## Smartphone detection of BPA using MIP-PAD, NIP-PAD, and PAD

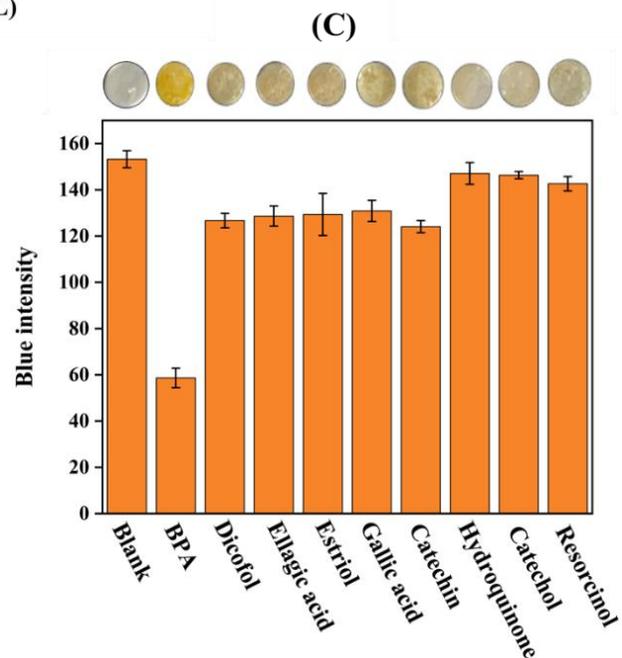
[BPA] ( $\mu\text{g/mL}$ )	0	0.1	5	8	10	30
<b>MIP-PAD</b>						
<b>NIP-PAD</b>						
<b>PAD</b>						



**Pre-concentration procedure for BPA trace determination.**



(A) Calibration curve of BPA according to the blue channel. (B) Calibration curve of BPA analysis by MIP-PAD using 1 mL loading volume. (C) Selectivity study testing 2  $\mu\text{g/mL}$  of BPA and other phenolic molecules.



Microchemical Journal

Available online 11 November 2022, 108157

In Press, Journal Pre-proof

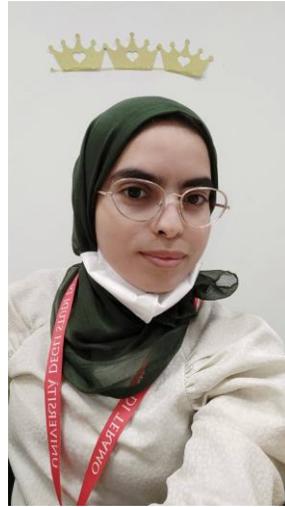


Advanced molecularly imprinted polymer-based paper analytical device for selective and sensitive detection of Bisphenol-A in water samples

Ouarda El Hani, Abdelhafid Karrat, Khalid Digua, Aziz Amine



**Ouarda El Hani**  
PhD student



**Dounia El Fadil**  
PhD student



**Hafid Karrat**  
PhD student



**Abderrahmane Lamaoui**  
PhD student



**Dr. Abdellatif**  
Ait Lahcen

