“Electrochemical Preparation of a Molecularly Imprinted Polypyrrole - modified Glassy Carbon Electrode for Determination of Isoproturon”

Imer SADRIU[1,2]

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Outline

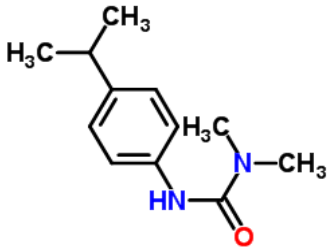
- Context
- How to detect selectively isoproturon in water?
- EXPERIMENTAL SECTION
- Experimental apparatus & Experimental Strategy
- Synthesis of the Molecularly Imprinted Polymers and optimization
- Electroanalytical method optimization
- Presentation of isoproturon detection results *(in milli Q & natural water samples)*
- Conclusion and perspectives

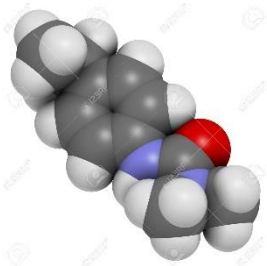
Context

The use of many pesticides in agriculture !

From the beginning of the century. XX up to the 1960s

Their degradation products and their metabolites are toxic toward living organisms !





Isoproturon – *a herbicide*

(3-(4-isopropylphenyl)-1,1-dimethylurea or 3-p-cumenyl-1,1-dimethylurea)

kill weeds in agricultural soils

Its harmful effects

The World Health Organization & The European Water Framework Directive (WFD) the maximum allowed concentration at *300 ng.L^{-1} (1.45 X 10^{-9}M)* for inland surface waters

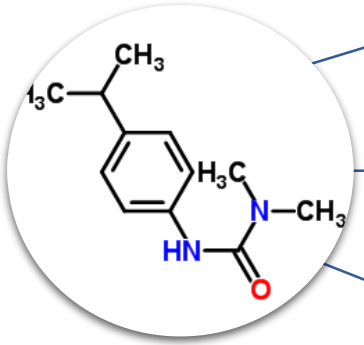
Each method which provides us the simplest way how to determine these pollutants in waters is welcomed !

European Water Framework Directive

M.M. Nemat Alla, N.M. Hassan, *Pesticide Biochemistry and Physiology*, 112 (2014) 56–62. doi:10.1016/j.pestbp.2014.04.012
World Health Organization, ed., *Guidelines for drinking-water quality*, 4th ed, World Health Organization, Geneva, 2011

Context

HPLC



Isoproturon

UV-Vis

GC analysis

LOD = ~10 and 100 ng L^{-1}

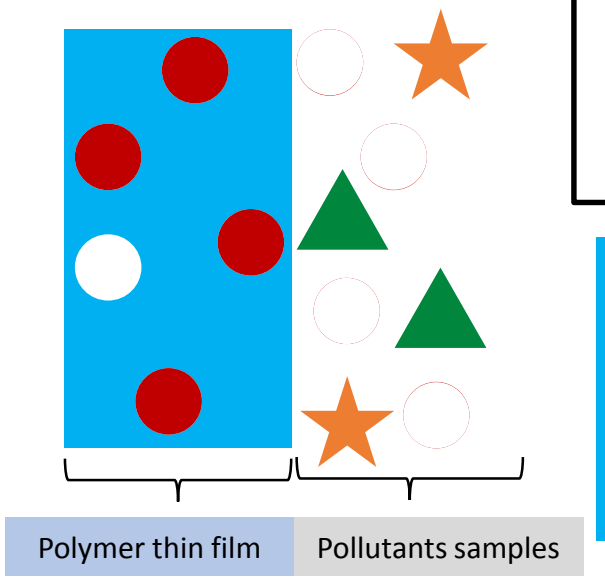
difficult to implement for on-site analysis

- Many pollutants can be found in this region of detection limits, including isoproturon and other pesticides
- Recently, numerous studies have been focused on modified electrodes for determination of pollutants in waters

➢ Our main goal is to develop rapid selectively and sensing devices for pesticides and especially for isoproturon detection on-site analysis in real time

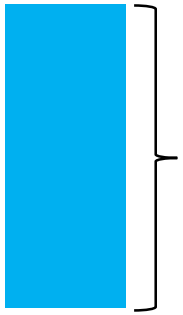
H.A.T. DIRECTIVE, DIRECTIVE *2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12* August 2013
L.M.L. Nollet, H.S. Rathore, eds., *Handbook of pesticides: methods of pesticide residues analysis*, CRC Press, Boca Raton, Fla., 2010

How to detect selectively isoproturon in water ?



What is a MIP sensor ?

A MIP sensor is a polymer thin film containing some cavities which can selectively recognize the template molecule !



NIP
(non imprinted polymer)

Why we use a MIP sensor for isoproturon ?

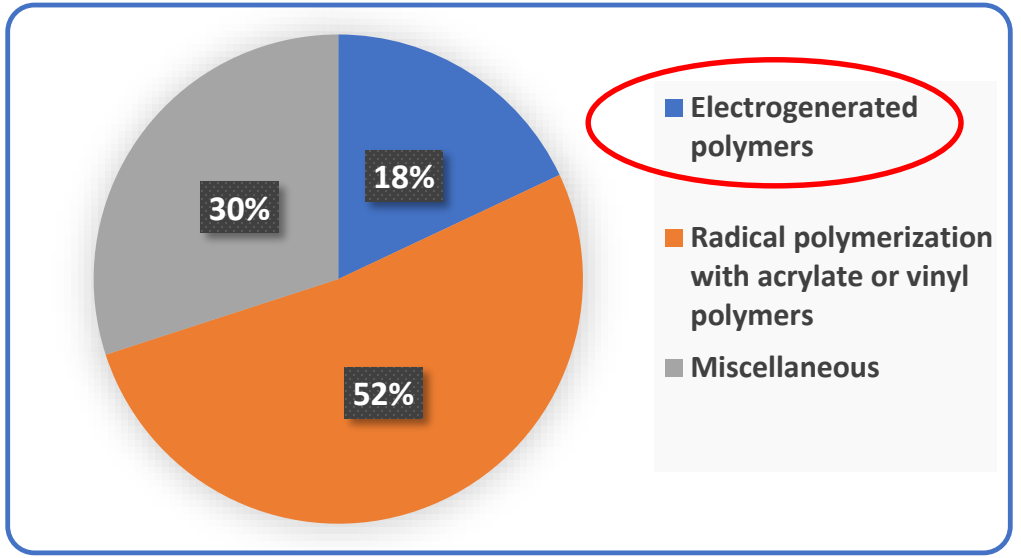
The molecular imprinting methods is a very suitable technique for determination of the micropollutants which are organic compounds !

A thin molecularly imprinted polymer film easily can be deposited onto working electrode during an electropolymerization process.

MIP Electrochemical Sensor

What is the strategy of these sensors ?

The deposition of a MIP thin film on the surface of the working electrode which plays a role of recognition unit resulting in an electrochemical sensor



Synthesis of MIP for electrochemical sensor application

M.C. Blanco-Lopez, M.J. Lobo-Castan˜ o´ n, A.J. Miranda-Ordieres, P. Tun˜ o´ n-Blanco, *Trends in Analytical Chemistry*, Vol. 23, No. 1, 2004
Piyush Sindhu Sharma a, Marcin Dabrowski a, Francis D'Souza b, Wlodzimierz Kutner a, *Trends in Analytical Chemistry*, 51 (2013) 146–157

Experimental section

Experimental apparatus

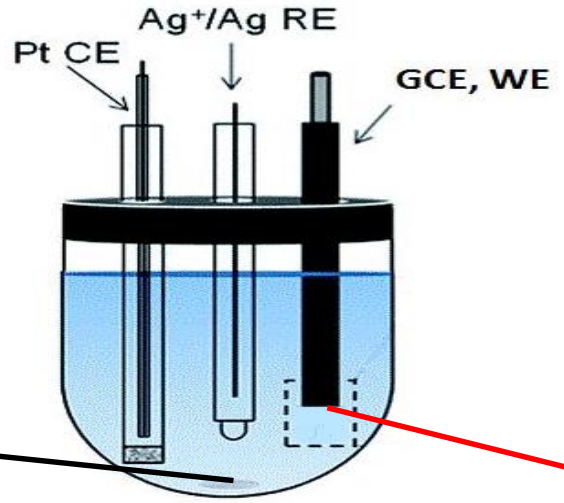
NIP – non imprinted polymer
MIP – moleclularly imprinted polymer

Electrochemical solution for NIP & MIP film preparation:

- Electrolyte ($LiClO_4$ & H_2SO_4)
- an ethanol/aqueous mixed solvent

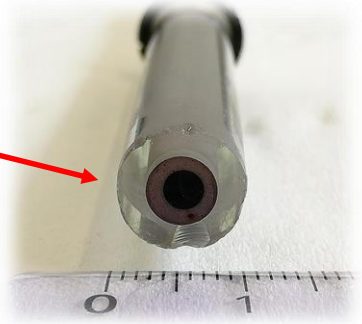
(a limited solubility of isoproturon in water)

- Dissolved pollutant (isoproturon 1mM)
- Dissolved monomer (pyrrole 10mM)



A three electrode cell

GCE
(A = 0.07cm²)
Well polished with an automatic polisher



Reference electrode (Ag/AgCl)

Counter electrode (curved Pt wire)

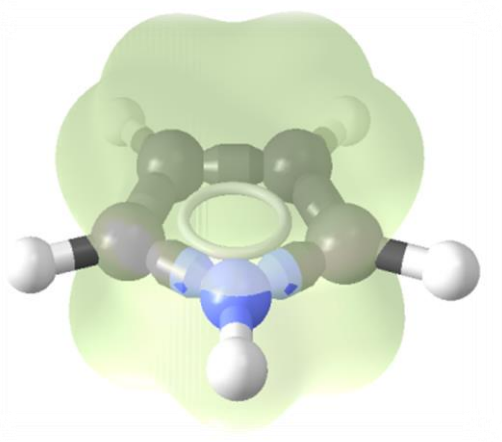
Working electrode (glassy carbon electrode)

AUTOLAB
PGSTAT Metrohm

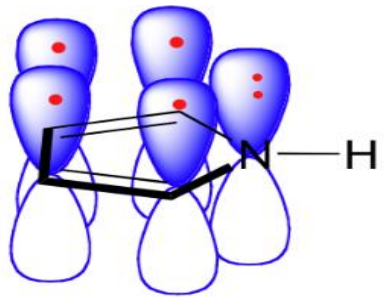
PC
NOVA 2.1.3 Software

All of the solutions were prepared in mili-Q water 18.2 MΩ cm and experiments were carried out under room temperature (25 °C)

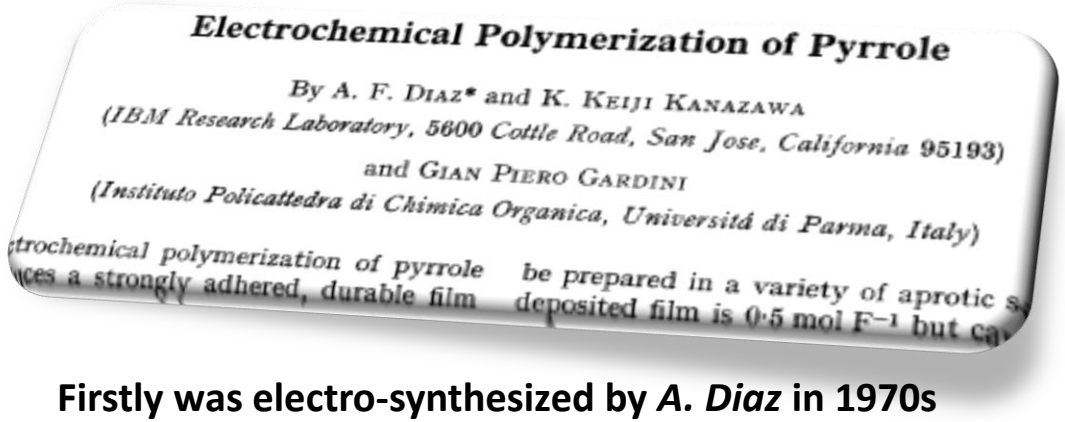
Synthesis of the Molecularly Imprinted Polymer and optimization



Pyrrole
(an aromatic heterocycle)
-chosen monomer



- A π-conjugated structure
- A conductive polymer

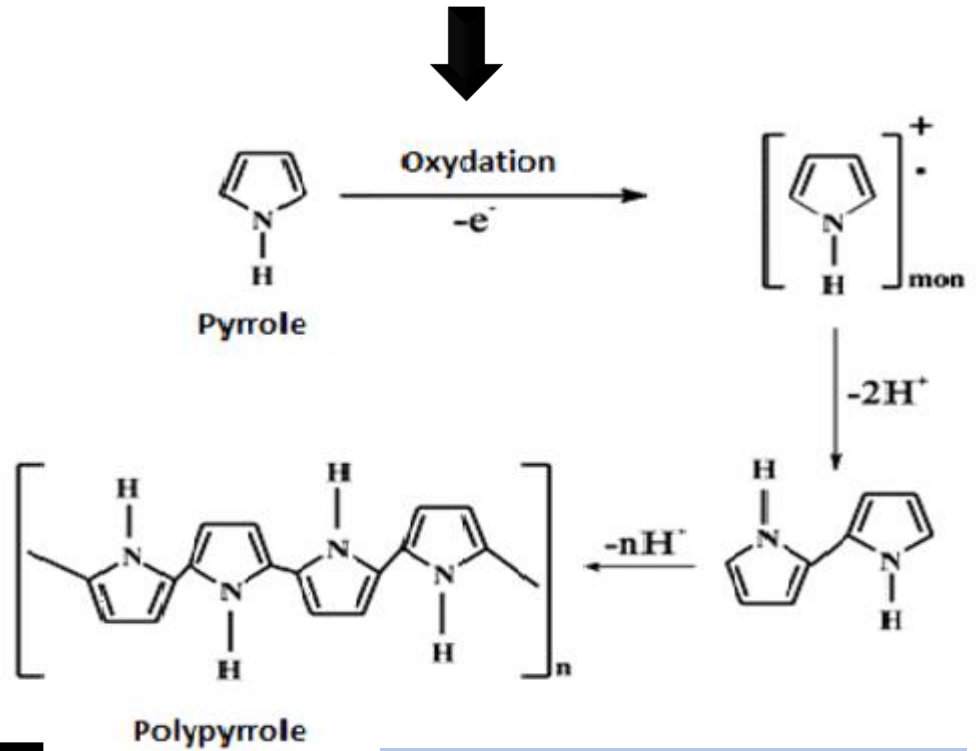


Electrochemical Polymerization of Pyrrole

By A. F. Diaz* and K. Keiji Kanazawa
(IBM Research Laboratory, 5600 Cottle Road, San Jose, California 95193)
and Gian Piero Gardini
(Instituto Policattedra di Chimica Organica, Universitá di Parma, Italy)

trochemical polymerization of pyrrole be prepared in a variety of aprotic s
ces a strongly adhered, durable film deposited film is 0·5 mol F⁻¹ but ca

Firstly was electro-synthesized by *A. Diaz* in 1970s



a radical coupling mechanism

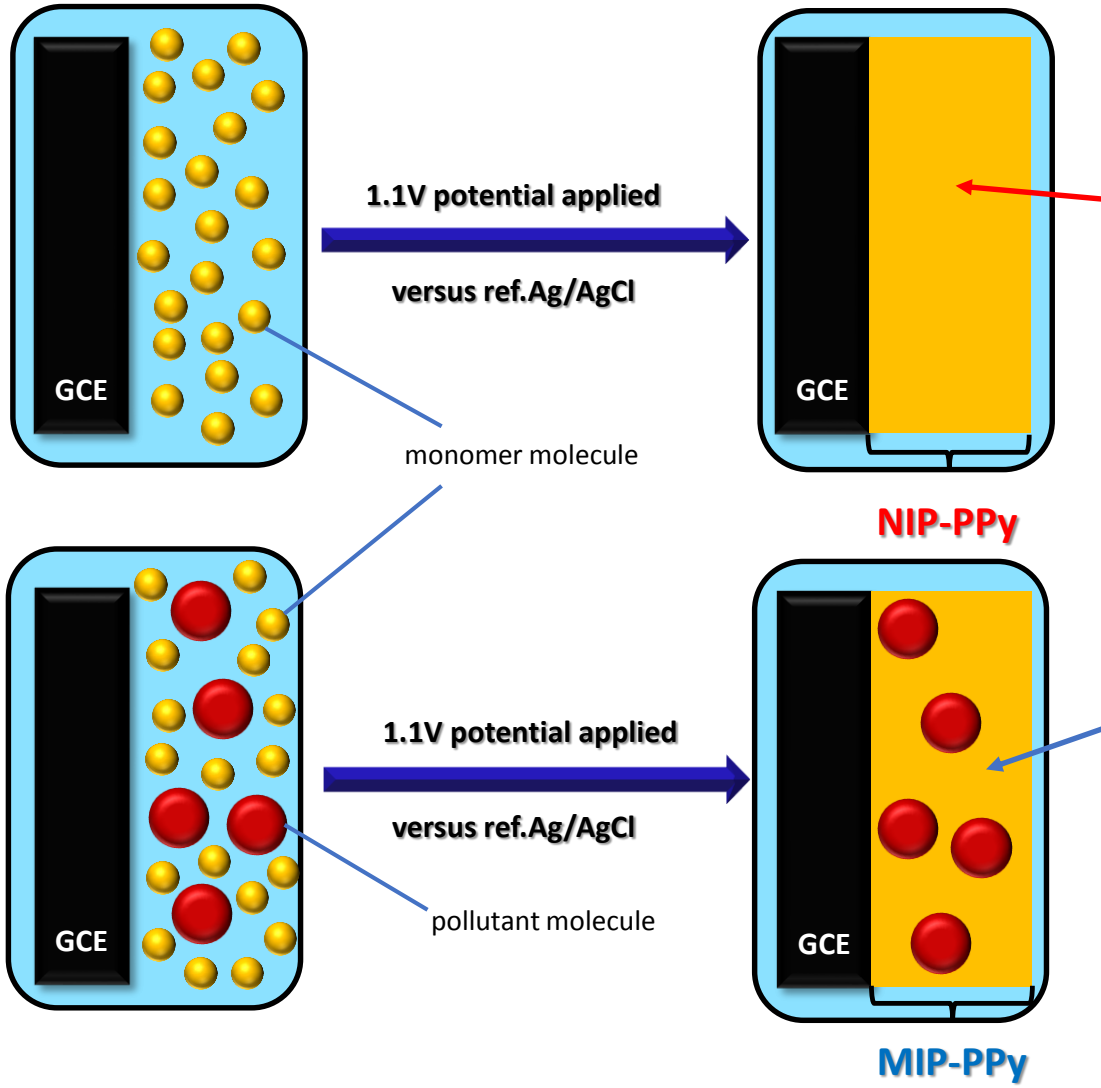
This method has many advantages:

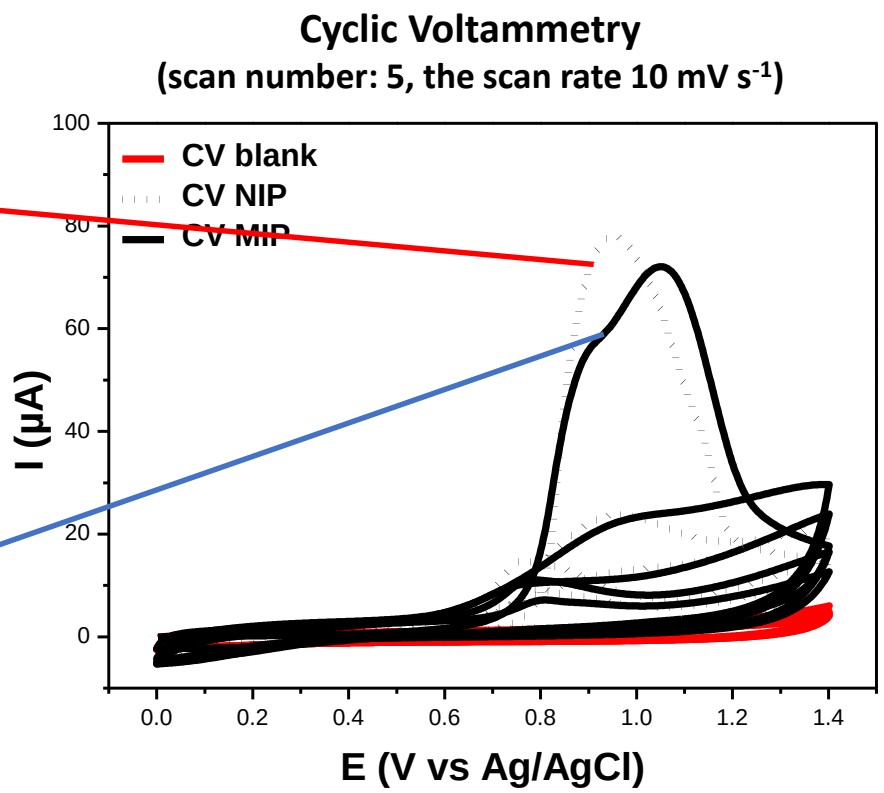
- ***Modification of different electrode substrates (C & metal substrate)***
- ***Mechanical stability***
- ***Relative environmental stability***
- ***Conductive polymer***

A. F. DIAZ, K. KEIJI KANAZAWA, *Electrochemical Polymerization of Pyrrole*, J.C.S. CHEM. COMM., 1979
M. Zhou; J. Heinze, *Electrochemical Study of Polypyrrole, J. Phys. Chem. B* 1999, *103,* 8451-8457

Preparation of a glassy carbon modified electrode by NIP and MIP

1st Step: Electropolymerization of Pyrrole



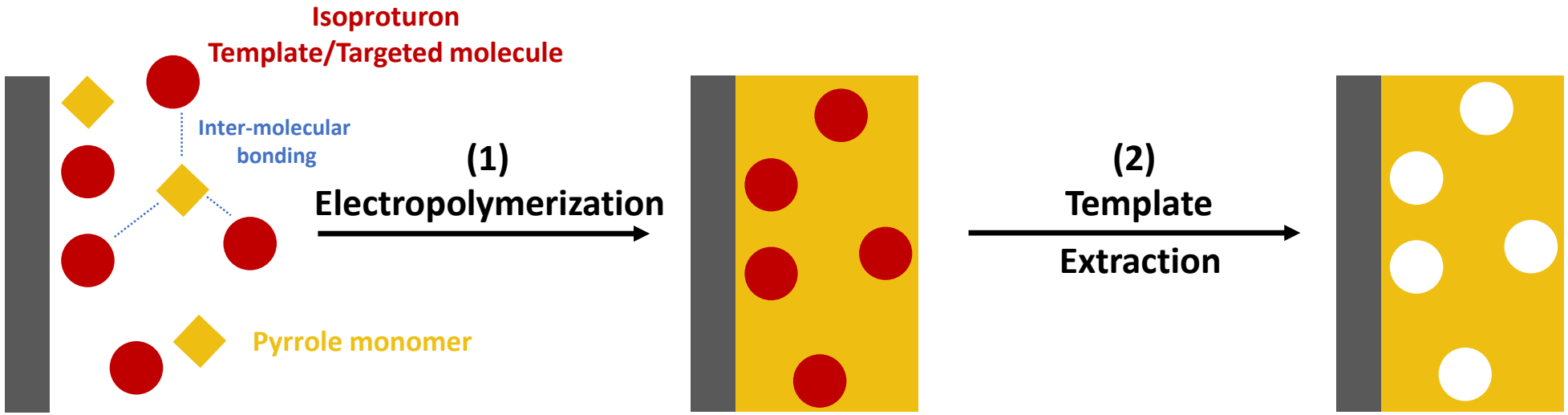


For NIP-PPy films: pyrrole (0.01M) + $LiClO_4$ 0.1M dissolved in an ethanol/aqueous solution (20:80 v/v). We perform NIP films in order to verify that it is the same process, but these films cannot detect isoproturon.

For MIP-PPy-Iso films: isoproturon (1mM), pyrrole (0.01M) + $LiClO_4$ 0.1M dissolved in an ethanol/aqueous solution (20:80 v/v), the ratio between the monomer and the pollutant concentration is 10 : 1

Experimental section

Experimental strategy for the fabrication of the NIP and MIP PPy thin films



- The volume ratio between water and ethanol
- The electropolymerization time

- Number of Cyclic Voltammetry scans

Synthesis of the Molecularly Imprinted Polymer and optimization

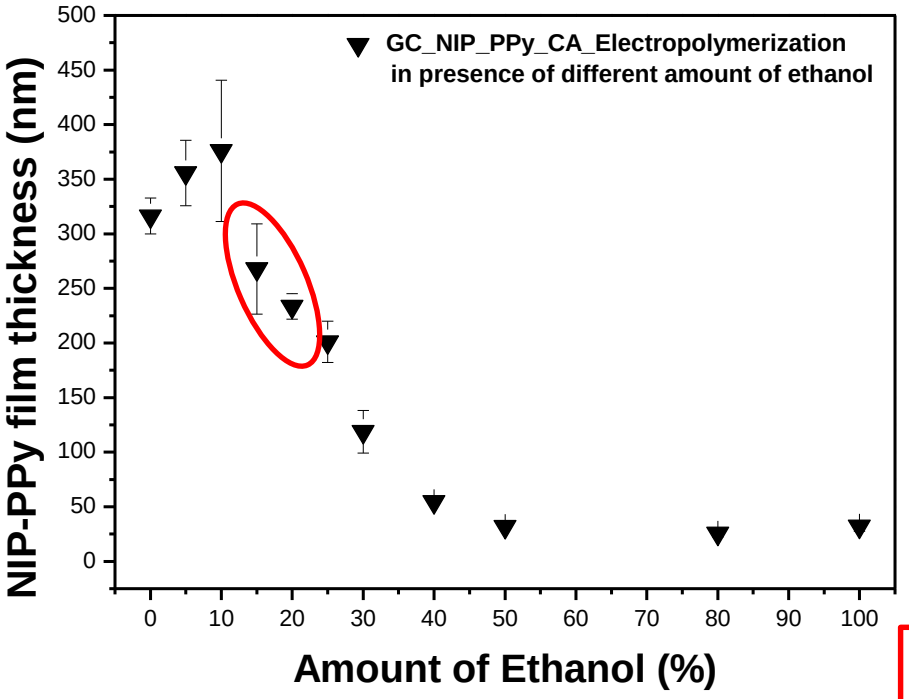
Faraday's Law used for the theoretical calculation of film thickness:

$$(PPy\ film\ thickness)\ x = \frac{q \cdot M}{\rho \cdot A \cdot z \cdot F}$$

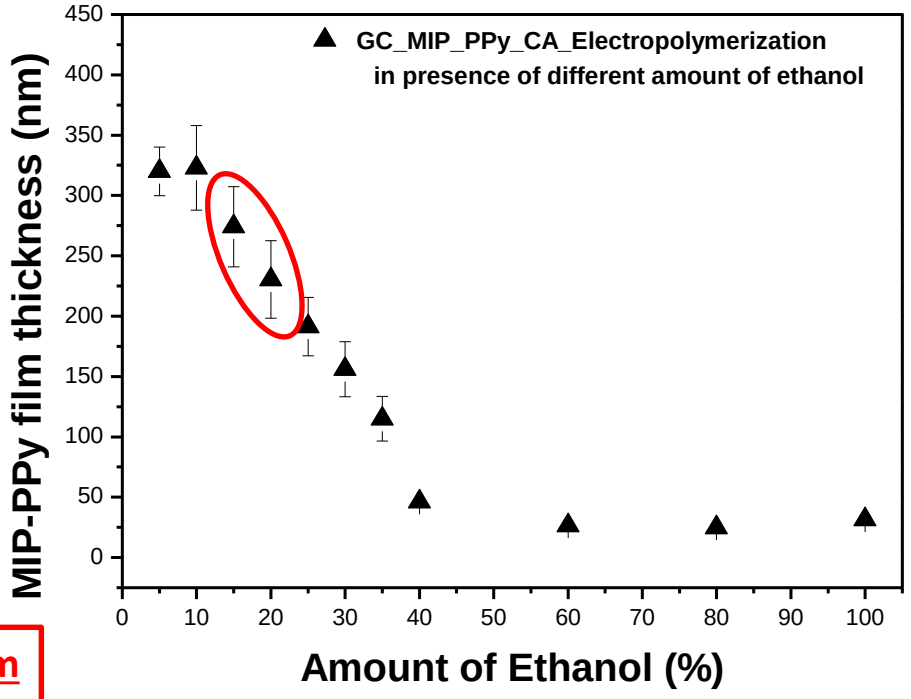
-where: q is number of charge in coulomb (C), M monomer molar mass, ρ is polypyrrole density (1.5 g cm^{-3}), A is electrode surface area (7mm^2), z is number of electrons (z = 2.25), F is Faraday constant F = 96 485.33 C mol^{-1}

The influence of the amount of ethanol present in the solution was optimized at different ratios water/ethanol (v/v):

Chronoamperometry Electropolymerization ***(potential applied 1.1V vs Ag/AgCl, duration time 600 sec)***
Pyrrole 10mM, $LiClO_4$ 0.1M for NIP + Isoproturon 1 mM for MIP in different ratio between ethanol and milli-Q water



NIP **MIP**



The average thickness 230 - 270 nm (15% & 20% Ethanol)

The evaluation of NIP-PPy film thickness with amount of ethanol present in solution

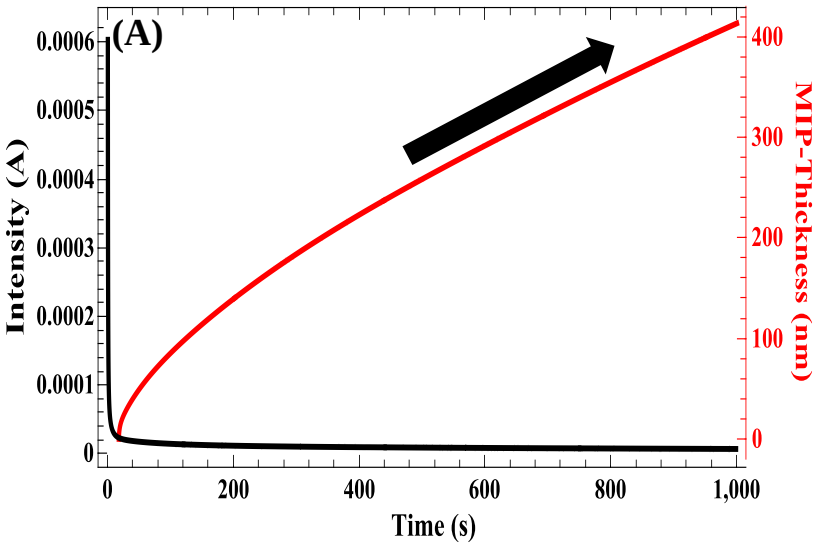
The evaluation of MIP-PPy film thickness with amount of ethanol present in solution

Jérôme Dejeu, Morphological and adhesive properties of polypyrrole films synthesized by sonoelectrochemical technique, Synthetic Metals 160 (2010) 2540–2545

Synthesis of the Molecularly Imprinted Polymer and optimization

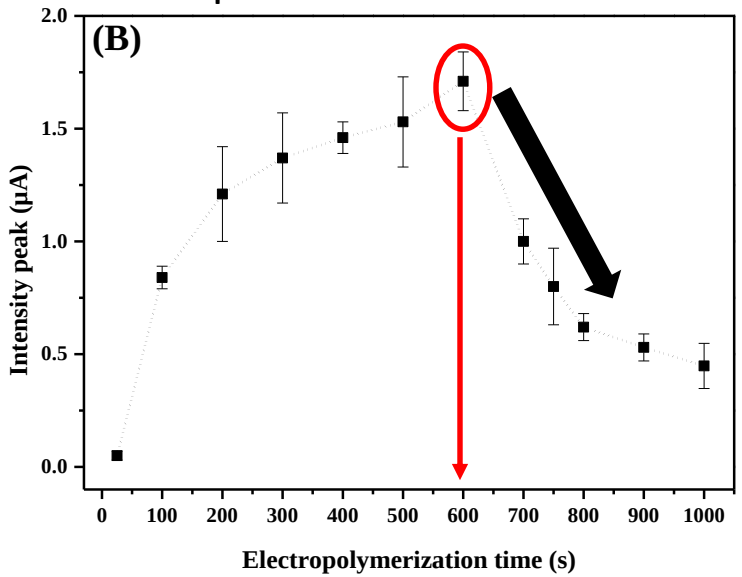
The influence of electropolymerization time has been studied *by chronoamperometry* (at different time duration):

Chronoamperometry Electropolymerization
(potential applied 1.1 V vs Ag/AgCl)



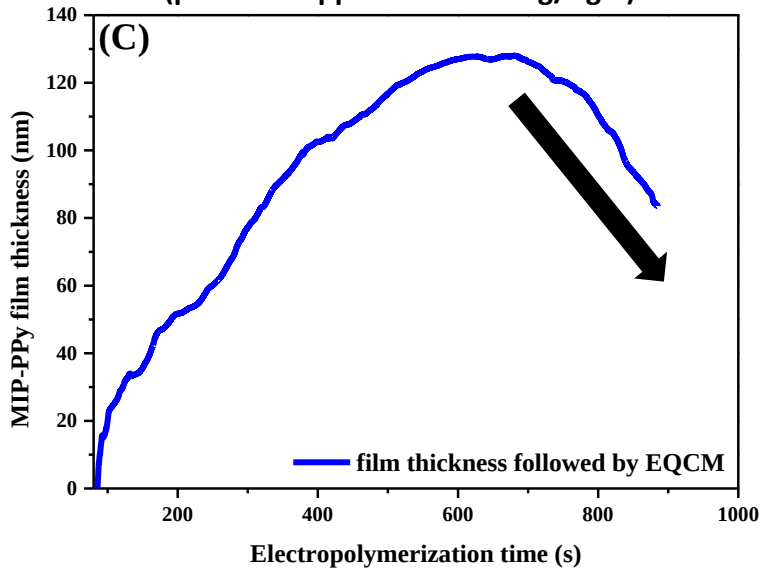
The polypyrrole film growing by electropolymerization duration times 25 sec up to 1000 sec (potential applied 1.1 V vs Ag/AgCl)

Square Wave Voltammetry analysis of isoproturon at Conc. Iso = 5 x 10^{-7} M



The square wave voltammograms obtained in an aqueous/ethanol solution (30:70) solution of sulfuric acid (pH ~1.0)

EQCM/Chronoamperometry Electropolymerization
(potential applied 1.1 V vs Ag/AgCl)



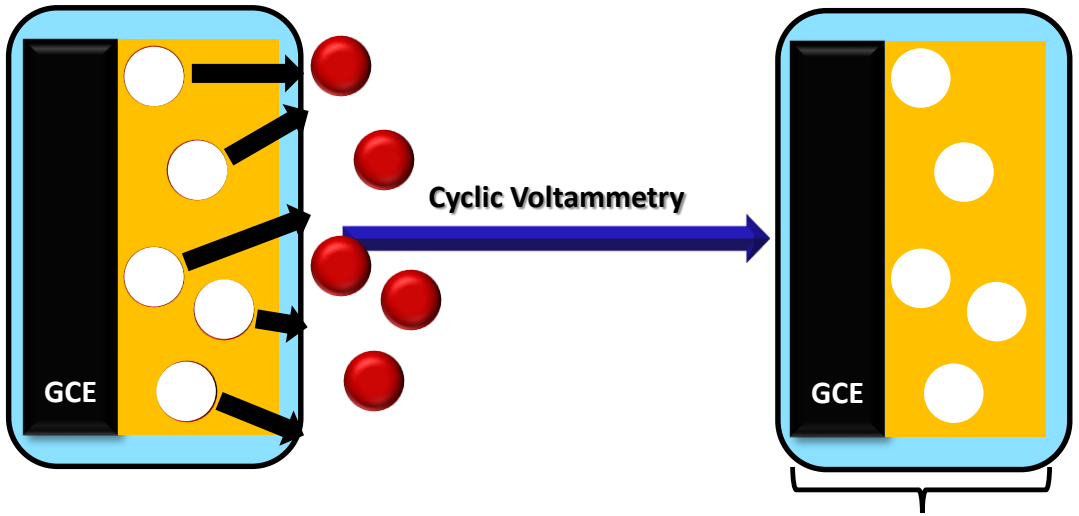
EQCM study of anodic polypyrrole film growth on gold quartz electrodes by chronoamerometry 1000 sec (potential applied 1.1 V vs Ag/AgCl)

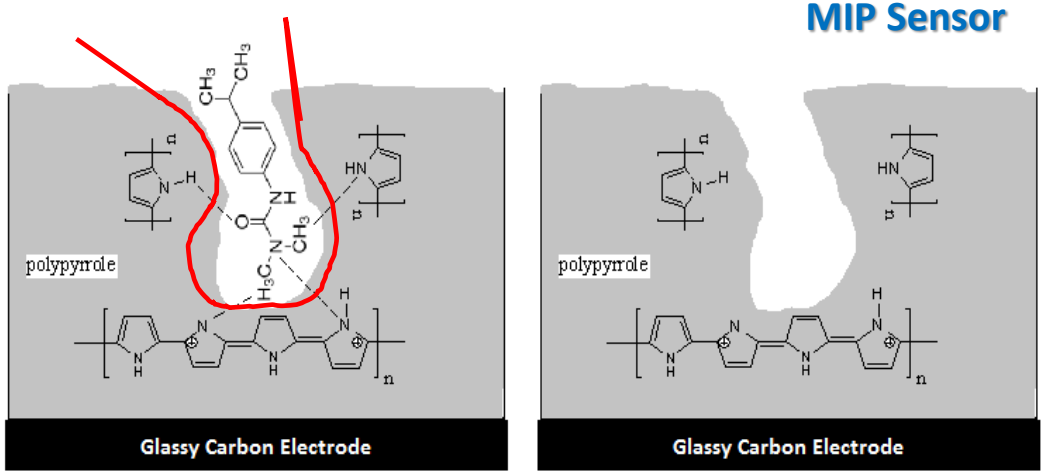
- Was showed that the peak current increased rapidly from 25 sec up to 600 sec
- Over 600 sec have shown an rapidly decreasing of the peak current,
- EQCM studies show that after a time ~600 s was obtained an immediately decrease in film mass

Electropolymerization time was optimized to be *600 sec*

Synthesis of the Molecularly Imprinted Polymer and optimization

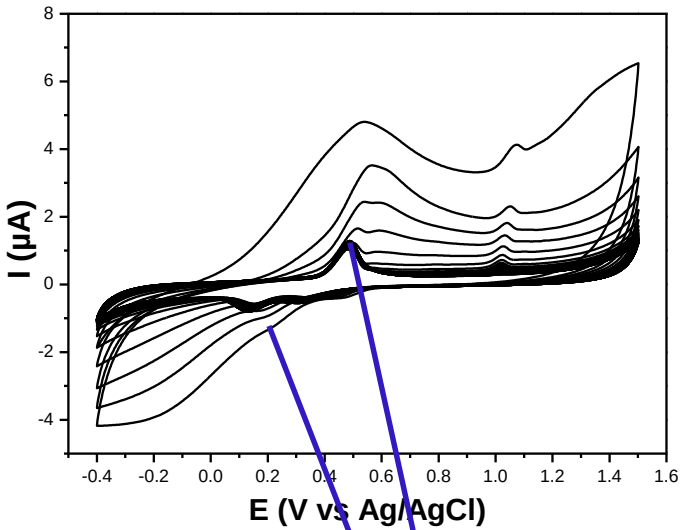
2nd Step: Template (isoproturon) extraction

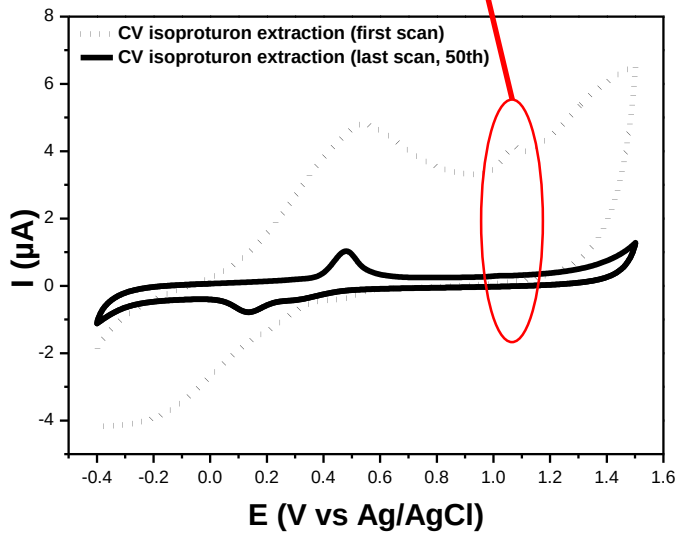




Between pollutant molecule and polymer exist "hydrogen bonds"

isoproturon oxidation peak (position 1.05 V vs ref.)





polypyrrole oxidation peak ~0 V vs Ag/AgCl

by scanning the potential between -0.4 and 1.5 V vs Ag/AgCl in an ethanol/water (70:30 v/v) solution of 0.1 M H_2SO_4 (pH ~1.0)

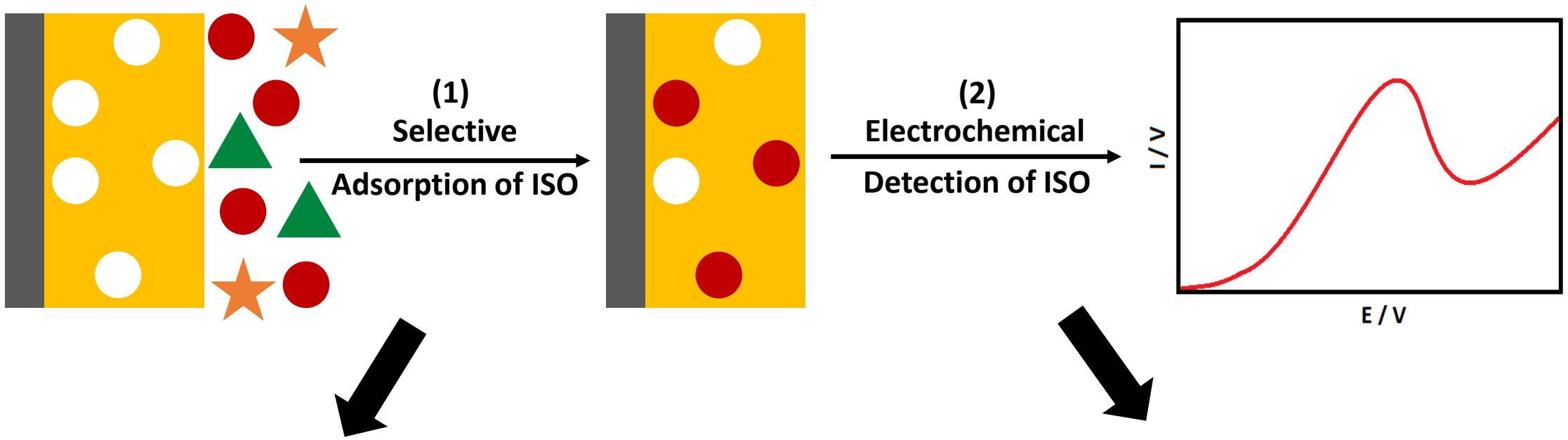
- **This method provides us to know exactly when the pollutant is removed in total form the polymer matrix, the polymer can be over oxidized at the end of the process and we avoid using of many chemical products.**

Levent Özcan, Mutlu Sahin and Yücel Sahin, *Sensors* 2008, *8*, 5792-5805;
Y. Li, Conducting Polymers, in: Y. Li (Ed.), Organic Optoelectronic Materials, Cham, 2015: pp. 23–50

Pollutant molecule Polymer thin film

Electroanalytical method optimization

Experimental strategy for the electroanalytical detection of isoproturon

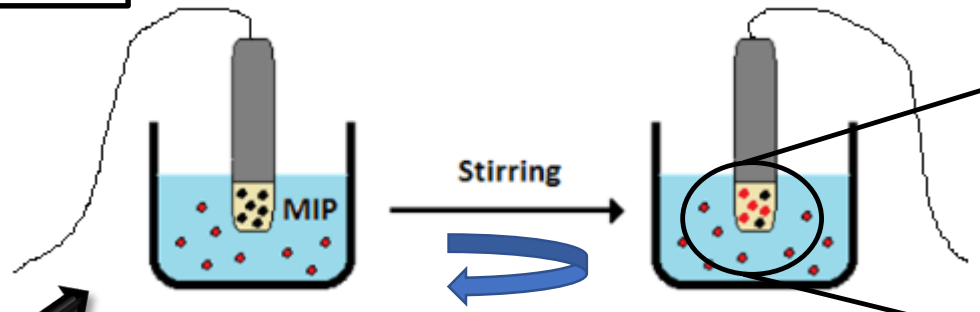


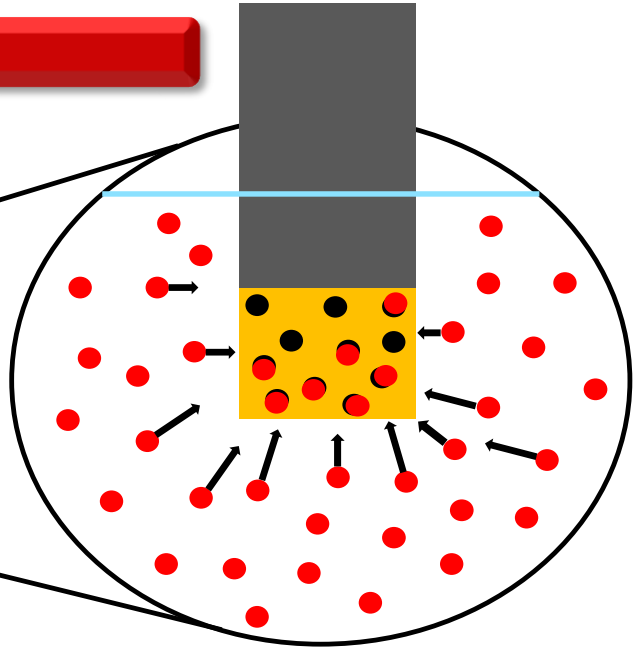
- The incubation (adsorption) time

- The calibration plot for isoproturon detection in milli Q water media
- The interference of the other pollutants
- The calibration plot for isoproturon detection in real natural water samples

Electroanalytical method optimization

3rd Step: Isoproturon detection

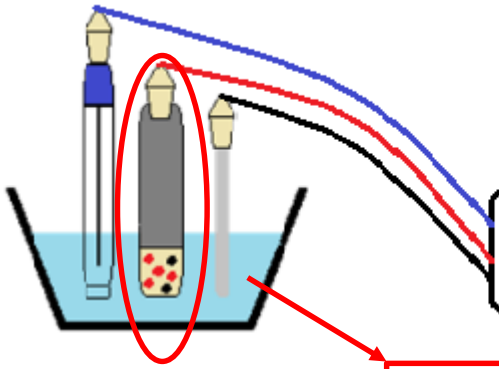




a) Incubation (rebinding)
isoproturon solution (low concentration)

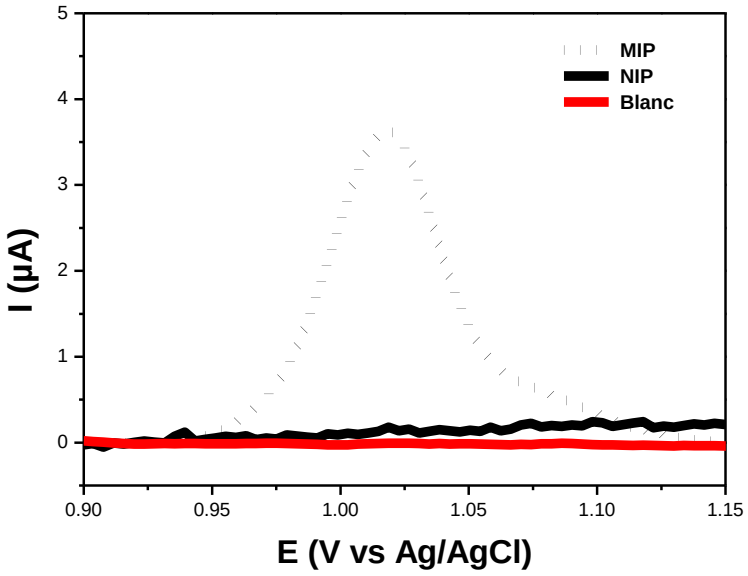
water samples

b) Electrochemical determination of isoproturon



Potentiostat
I / V

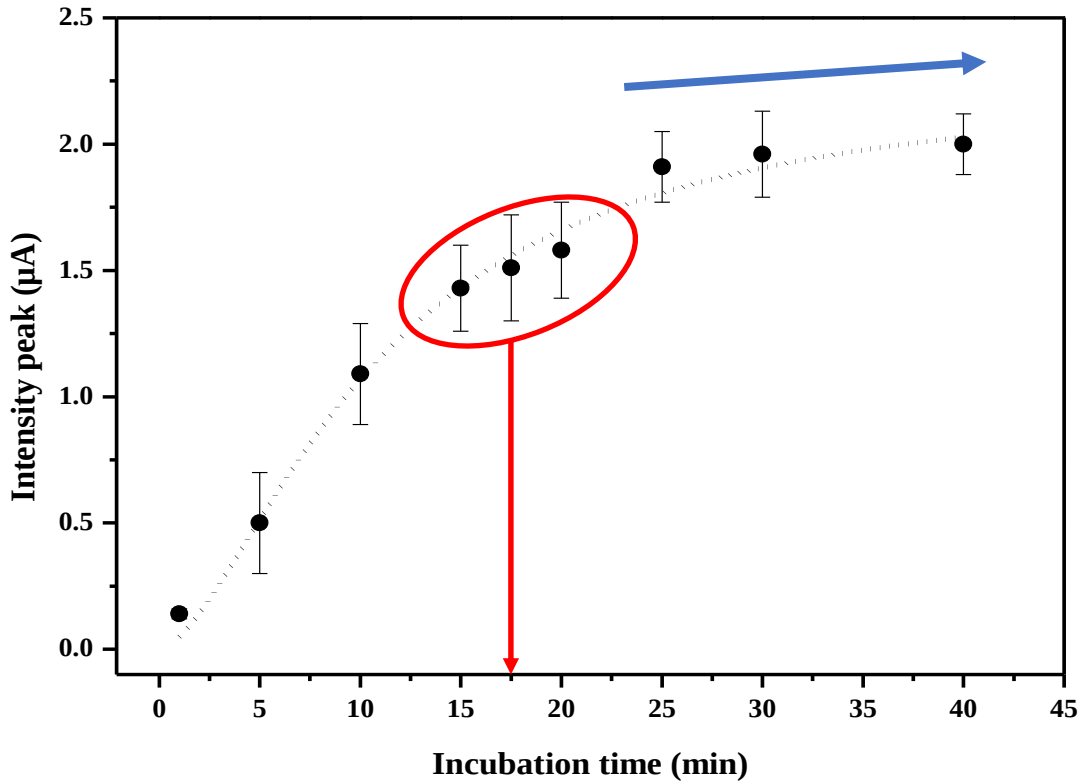
No pollutant in the electrochemical cell for isoproturon detection !



SWV detection of isoproturon

Electroanalytical method optimization

The incubation time is a very important factor



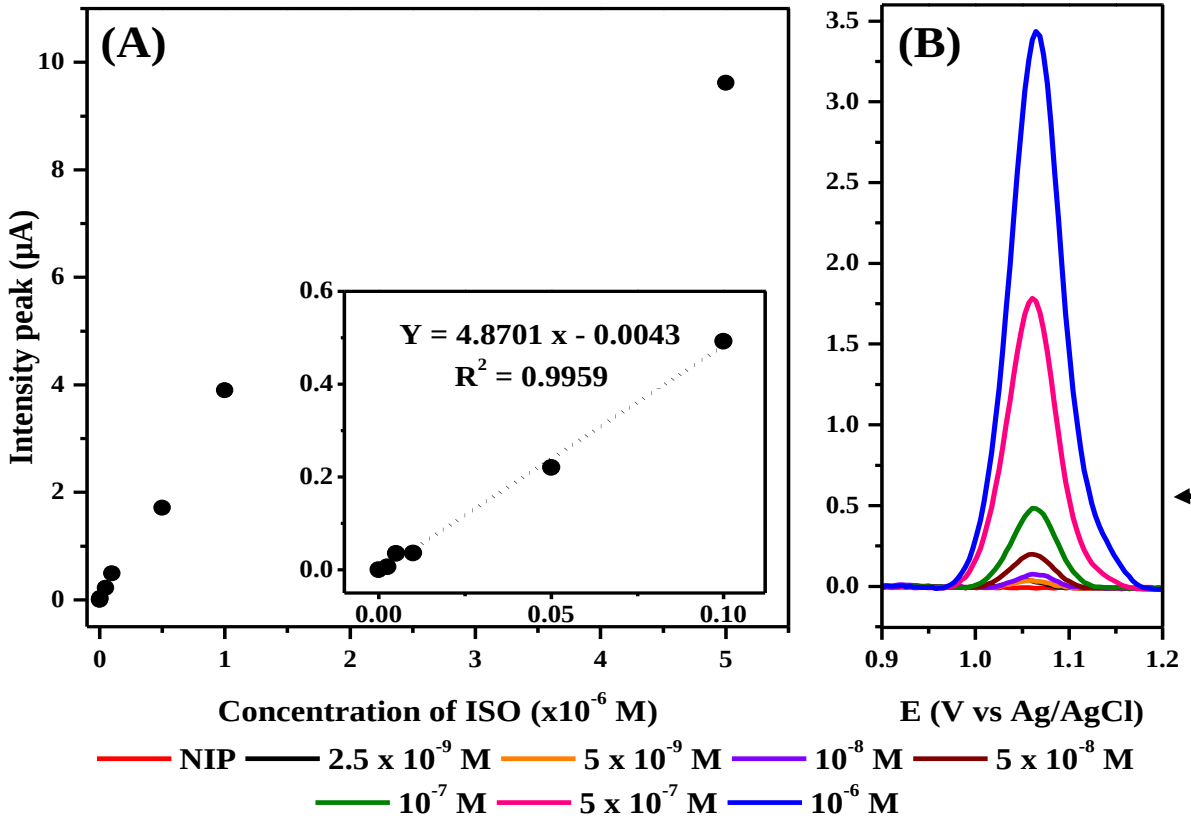
The adsorption time for the GC-MIP-PPy electrode was optimized to be *15 to 20 mins*

The response of the sensor over different incubation time (the sensor was incubated in a milli-Q solution containing isoproturon 5 x 10^{-7} M)

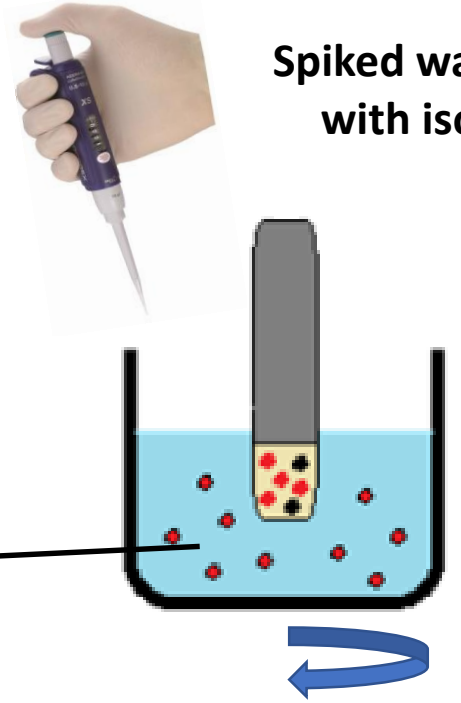
- **Was shown that the peak current increased rapidly from 5 to 25 min**
- **When the incubation time reached 25 min, the peak current is leveled off gradually**

Presentation of Isoproturon detection results – in milli Q water

The calibration plot is found to be linear between 0 and 10^{-7} M and obeys to the following relation:
$I\ (\mu A) = 4.8701\ [Isoproturon] - 0.0043\ (R^2 = 0.9959)$



by Square Wave Voltammetry in an ethanol/water solution (70:30 v/v) of H_2SO_4 0.1 M



Spiked water samples with isoproturon

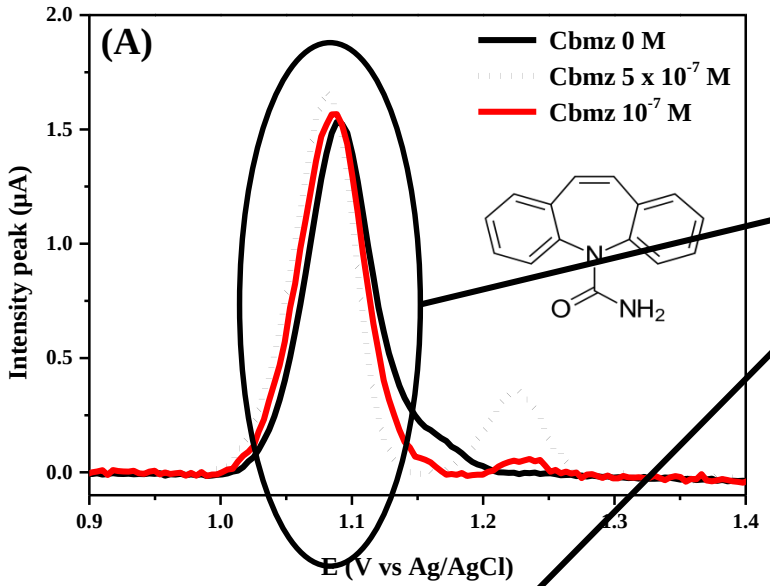
Rebinding of GC-MIP-PPy sensors in different isoproturon concentrations prepared in milli Q water *(18.2 MΩ.cm at 25°C)*

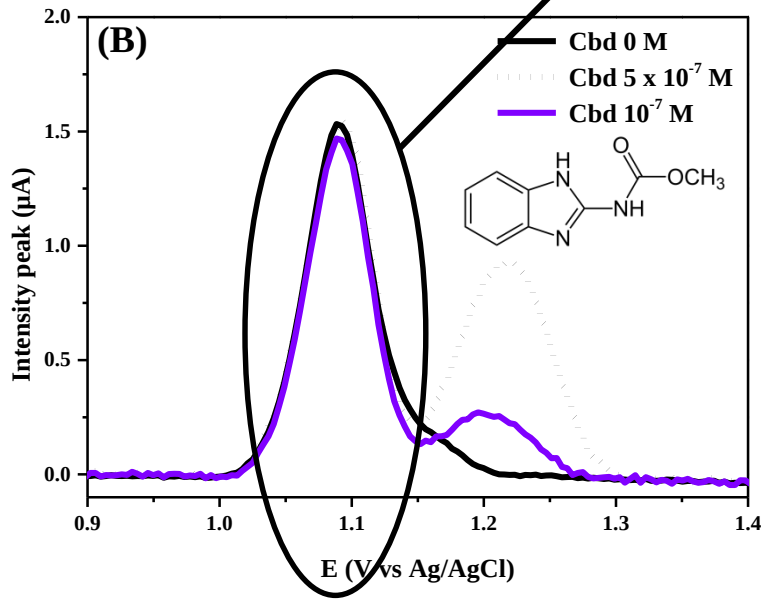
Limit of Detection (LOD) = 2.76 x 10^{-9} M (0.5 µg L^{-1})

Limit of Quantification (LOQ) = 9.2 x 10^{-9} M (1.9 µg L^{-1})

World Health Organization, ed., *Guidelines for drinking-water quality*, 4th ed, World Health Organization, Geneva, 2011

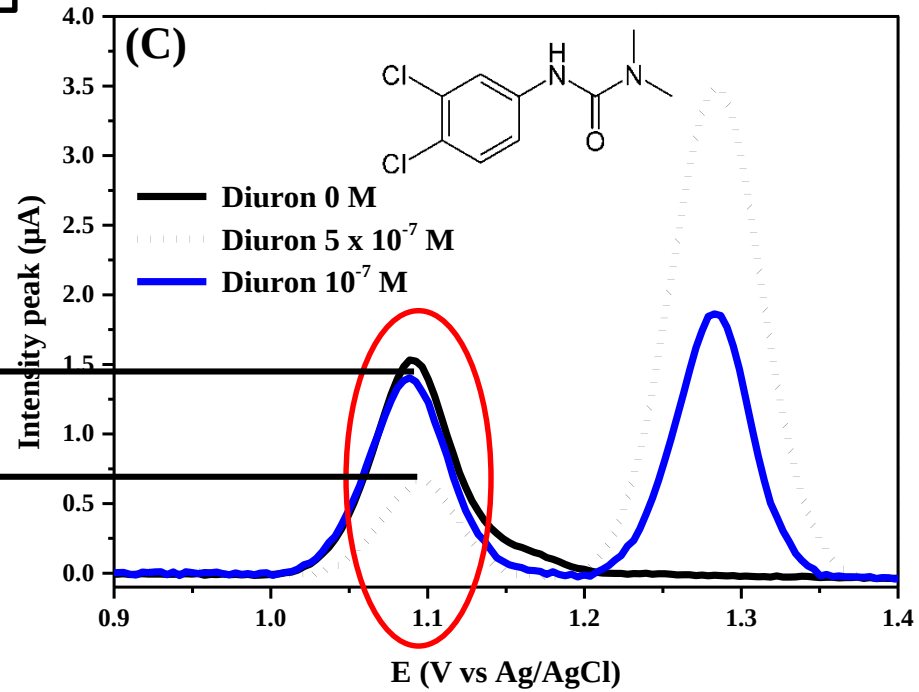
Intereference study





Isoproturon conc. 5 x 10^{-7} M

Isoproturon oxidation Peak (~1.09 V)

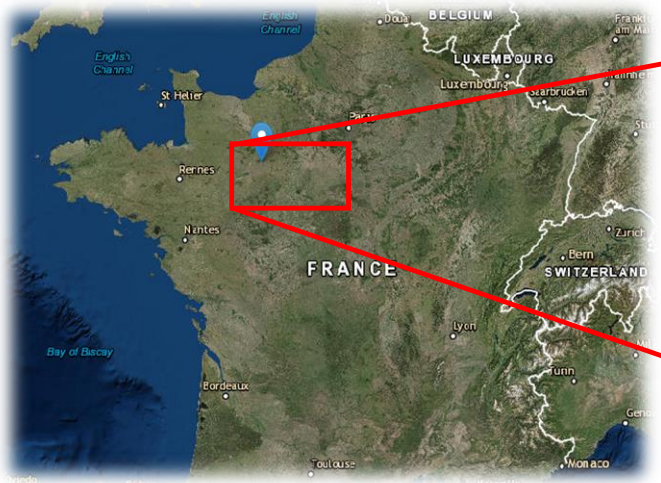


(A) Carbamazepine – *a medicament used against epileptic diseases*
(B) Carbendazim – *a benzimidazole used as a fungicide*
(C) Diuron – *an aryl-urea used as a herbicide*

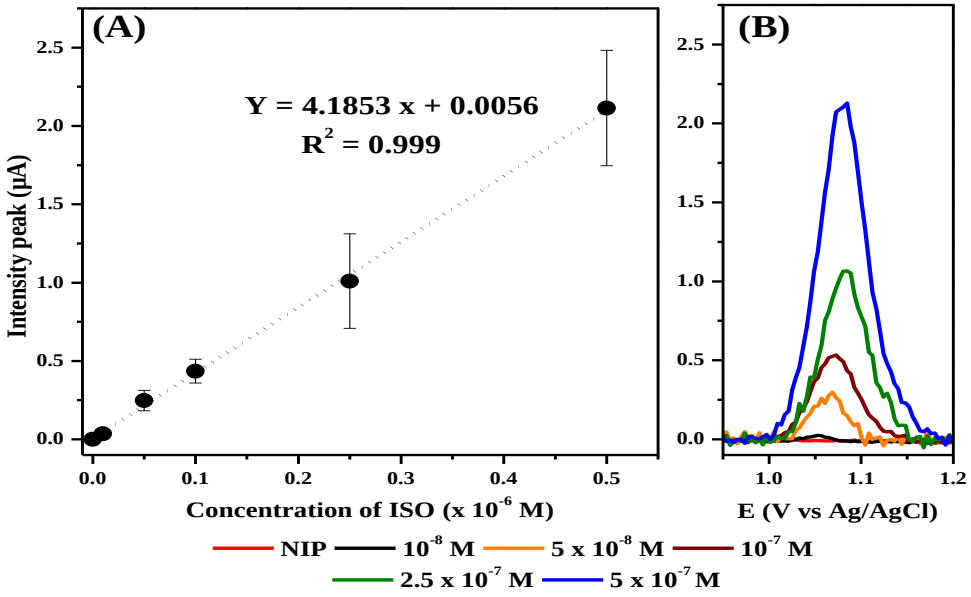
None of carbendazim (Cbd) and carbamazepine (Cbz) seems to affect the MIP-GC sensitivity toward isoproturon.
The presence of diuron at the same concentration than isoproturon lowers its signal by ***~50%***.

Agence de l'eau Loire -Bretagne, *Pesticides Water quality of "bassin Loire Bretagne" -report (2015)*

Presentation of Isoproturon detection results – in real water samples



Commune Saint-Rémy-Du-Val (72317) - Sarthe (72)
province of Pays-de-la-Loire region, an altitude of 117 m and a depth of investigation 23 m
(coordinates X, Y: 446125, 2372347 (Lambert II Etendu) / 497082, 6807017 (Lambert 93))



by Square Wave Voltammetry in an ethanol/water solution (70:30 v/v) of H_2SO_4 0.1 M

The calibration plot is found to be linear between 0 and 5 x 10^{-7} M and obeys to the following relation:
I (µA) = 4.1853 [Isoproturon] + 0.0056 (R^2 = 0.999)

Limit of Detection (LOD) = 1.1 x 10^{-8} M (2.2 µg L^{-1})

Limit of Quantification (LOQ) = 3.7 x 10^{-8} M (7.6 µg L^{-1})

Conclusion & perspectives

❑ Conclusions

- **The molecularly imprinted polypyrrole modified glassy carbon electrode was successfully employed as a sensor for fast determination of isoproturon *on-site* analysis in real time through electrochemical techniques.**

- **The advantages of a very simple instrumentation and a fast preparation of proposed sensor can make these sensors a very useful tools for determination of isoproturon.**

WHO max. allowed conc. Isoproturon	Limit of Detection in milli Q water	Limit of Detection in natural water samples
0.3 µg.L^{-1} (1.45 X 10^{-9}M)	0.5 µg L^{-1} (2.76 x 10^{-9} M)	2.2 µg L^{-1} (1.1 x 10^{-8} M)

❑ Perspectives

- **The ongoing research will be focused on the preparation of such sensors with even higher performances using *the functionalized graphene electrodes*, after seeing the excellent properties that this material possesses.**

Thank you so much for your attention !

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