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Molecularly imprinted conducting polymer based electrochemical sensor for detection of atrazine

Elodie Pardieu[1], Helene Cheap[1], Christophe Vedrine, Mathieu Lazerges, Youssef Lattach, Francis Garnier, Samy Remita[*,2], Christine Pernelle

Chaire de Génie Analytique, EA 4131, Conservatoire National des Arts et Métiers, CNAM, 292 rue Saint-Martin, 75141 Paris Cedex 03, France

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ABSTRACT

An original electrochemical sensor based on molecularly imprinted conducting polymer (MICP) is developed, which enables the recognition of a small pesticide target molecule, atrazine. The conjugated MICP, poly(3,4-ethylenedioxythiophene-co-thiophene-acetic acid), has been electrochemically synthesized onto a platinum electrode following two steps: (i) polymerization of comonomers in the presence of atrazine, already associated to the acetic acid substituent through hydrogen bonding, and (ii) removal of atrazine from the resulting polymer, which leaves the acetic acid substituents open for association with atrazine. The obtained sensing MICP is highly specific towards newly added atrazine and the recognition can be quantitatively analyzed by the variation of the cyclic voltammogram of MICP. The developed sensor shows remarkable properties: selectivity towards triazinic family, large range of detection (10^{-9} mol L^{-1} to 1.5×10^{-2} mol L^{-1} in atrazine) and low detection threshold (10^{-7} mol L^{-1}).

1. Introduction

1.1. Conducting polymers (CPs) as transducers in electrochemical sensors

Conducting polymers (CPs) attracted attention not only for their numerous applications, *e.g.*, in batteries, electronics devices or ion-selective membranes but also as-sensitive layers in electrochemical sensors [1–5]. CPs are known to possess for some of them many interesting features. These polymers, which present π-conjugated structures, are characterized by a high electrical conductivity, and a good electrochemical reversibility, which justify their use as transducers in the fabrication of efficient electrochemical sensors. Moreover, CPs can be chemically functionalized with various chemical groups, which can be chosen as tags for their ability to recognize biological or chemical target molecules. These latter can thus be quantitatively analyzed. For instance, the functionalization of polypyrrole with oligonucleotides, ODNs, allowed the quantitative recognition of complementary ODNs [6].

Thin films of CPs can be easily synthesized by chemical or electrochemical means [7]. Their physico-chemical properties strongly depend on the electropolymerization conditions, *i.e.*, solvent, supporting electrolyte, electrode material, polymerization potential, and electropolymerization method. In CPs, *e.g.*, polypyrrole [8], polyaniline [9], polythiophene [10], the delocalization of charges along the polymer chains induces the formation of states in the gap, polarons and bipolarons, which are involved in charge transport. The formation of charge carriers on their conjugated backbone realized by oxidation (p-doping) or by reduction (n-doping) allows the appearance of a metal-like intrinsic conductivity. In the case of p-doping of polymers such as polypyrrole or polythiophene, the cationic charges carried by the polymer backbones, are counter balanced by negative charges carried by anions. Concerning the morphology of CPs, which is an important feature when considering sensing applications, it has been suggested that their porosity is dependent on the doping anion used, which controls the polymer network spacing, as proposed for ion-selective electrodes [11].

However, even if the use of CPs as transducers has largely been described in the literature, their associated sensing devices have been mainly limited to the detection of large bioactive species (ODNs, peptides), able to induce modifications at the surface of the CPs matrix. On the other hand, the detection of small (bio)active molecules still remains an open challenge, as their simple association to pendent sensing groups on the CP matrix is not sufficient to create the needed variation of the electrochemical signature. The key should be to build highly specific recognition sites in the

* Corresponding author. Tel.: +33 140 272695; fax: +33 140 272844.
E-mail address: samy.remita@cnam.fr (S. Remita).
[1] These authors equally contributed to the present work.
[2] Also invited Researcher at Institut des Nanosciences de Paris, INSP, CNRS-UMR 7588, Université Pierre et Marie Curie et Denis Diderot, Campus Boucicaut, 140 rue de Lourmel, 75015 Paris, France.

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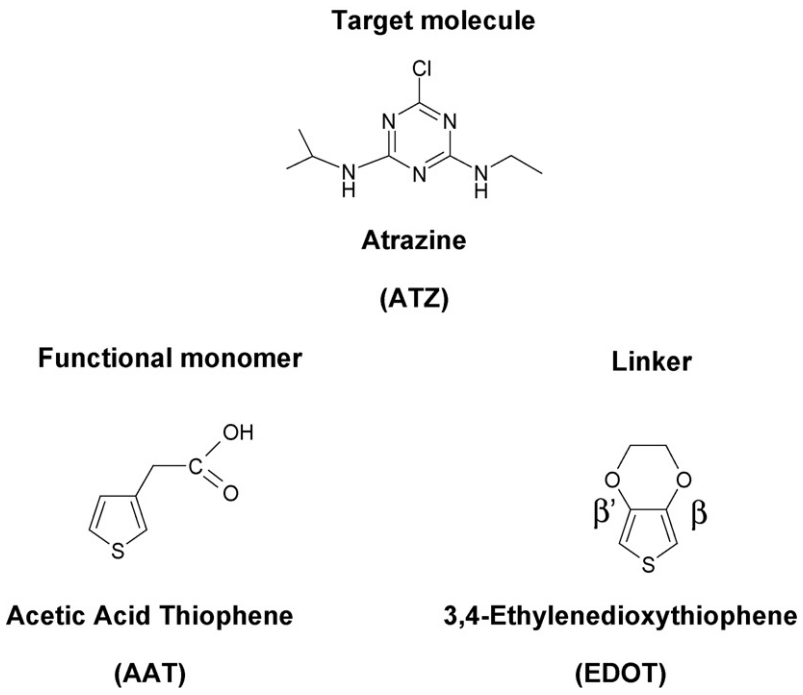


Scheme 1. Chemical structures of atrazine, ATZ, acetic acid thiophene, AAT, and 3,4-ethylenedioxythiophene linker, EDOT.

CPs, which will both (i) increase the selectivity and (ii) improve the sensitivity of the recognition process.

As specificity is concerned, a literature survey shows that molecular imprinting technology can be applied to the manufacture of synthetic polymers with pre-determined molecular recognition properties. Molecularly imprinted polymers (MIPs) are obtained through polymerization in the presence of a template molecule (the target) [12,13]. Hence, highly specific cavities are created into the polymeric matrix. After target removal, MIPs demonstrate interesting recognition properties towards the template (analyte), originating from shape and chemical functionality considerations in the cavities present in the polymer matrix.

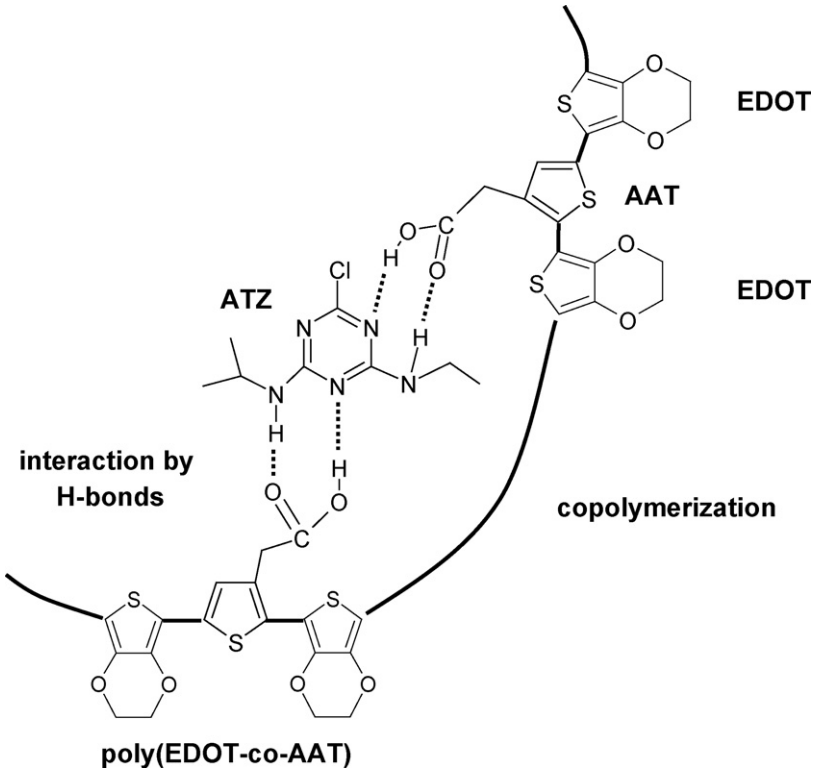
Thus, the objective of high specificity together with high sensitivity can be realized by combining the concept of MIPs with the use of CPs. In this context, we report, in the following, the design of molecularly imprinted conducting polymers (MICPs), used as tranducer for the selective and real time recognition of a small molecule, *e.g.*, atrazine. Although imprinting techniques were proposed for the preparation of artificial receptors, based on molecularly imprinted conducting polymers containing tailor-made sites [14], only few studies concerned the synthesis of such MICPs. For instance, poly(*o*-phenylenediamine) film was used for the detection of glucose [15] and polypyrrole-based MIP enabled the detection of glutamic acid as template molecule [16,17]. Amperometric morphine sensor [18] was also prepared using the concept of MIP. Besides, the detection of analytes binding with electropolymerized layers [19] of molecularly imprinted polymers was performed by different techniques. Electrical conductivity of receptor layers allowed the electrochemical detection of redox active analytes and was applied for detection of paracetamol bound to polypyrrole film [20]. Other detection techniques included a monitoring of electrical capacitance by impedance measurements [21] and resonance frequency of quartz microbalance [20]. Recently, MIPs were used as transducers based on quartz crystal microbalances [22], surface Plasmon resonance devices [23], conductimetric [24] or impedometric techniques [25]. Among these reports, the most relevant ones concern with the use of pulsed amperometric detection for MICPs-based sensors [26–28].

1.2. Use of conducting MICPs for the electrochemical detection of pesticides

Pesticides belong to an interesting class of analytes frequently probed by sensors measurements owing to their increasing presence as contaminant in water [29] or in agriculture products. Among pesticides, triazinic compounds, which include atrazine (Scheme 1), were intensively studied. Atrazine detection by MIPs-based sensors was previously studied by Sergeyeva et al. [29], Luo et al. [30], Piletsky et al. [31], and Matsui et al. [32]. However, these studies were based on the use of conventional transducers: Shoji et al. studied by electrochemistry the reduction behavior of atrazine using a classical non-conducting polymeric sensing layer [33]. To our knowledge, no work has yet been reported in the literature concerning the use of MICPs for the quantitative detection of pesticides.

Thus the aim of our work was the design and the development of an original electrochemical sensor based on intrinsically MICPs for the selective detection of atrazine. Two moieties are necessary for the building of such chemically functionalized, conducting polymer-based sensing layer, (i) a molecular sensing unit (functional monomer), together with (ii) a conjugated linker:

(i) Acetic acid thiophene (AAT) [34] was chosen as functional monomer (Scheme 1) owing to its ability to establish hydrogen-bonds with atrazine, forming a precursor complex according to Scheme 2. This assumption is comforted by the results obtained by Matsui et al., using NMR [37], who suggested that one atrazine molecule interacts with two acetic acid thiophene monomers. Indeed, the two side-chain amino groups and the two nitrogen hetero-atoms of the aromatic structure of atrazine can establish four hydrogen-bonds with the oxygen atoms of two acetic acid thiophene functional monomers (Scheme 2).
(ii) The polymer linker chosen in this work was a β,β'-substituted derivative of thiophene, 3,4-ethylenedioxythiophene (EDOT) (Scheme 1). EDOT monomers, contrary to AAT monomers, cannot interact with atrazine by hydrogen-bonds. However, due to the presence of dioxy groups, no (co)polymerization could



Scheme 2. Schematic representation of poly(EDOT-co-AAT) copolymerization after the establishment of H-bonds between AAT and ATZ in a prepolymerization complex.

occur at β and β' positions (Scheme 1). EDOT polymerization was intensively studied [35–38] even in aqueous medium [39,40], and EDOT has already been copolymerized with thiophene [41]. The obtained poly 3,4-ethylenedioxythiophene (PEDOT) have been shown to lead to stable and homogenous films. But the most important feature concerns the hydrophilic properties of PEDOT-based films, which are able to counter balance the hydrophobic character of acetic acid thiophene. As a matter of fact, sensing devices are required to operate in aqueous media and EDOT is one of the very few conjugated monomers which lead to hydrophilic conjugated films.

In the present paper, we report on the development of a new generation of electrochemical sensors based on poly(EDOT-co-AAT) MICPs. These kinds of sensors have been used for the selective and quantitative detection of a pesticide, atrazine.

2. Materials and methods

2.1. Chemical products

The 3-acetic acid thiophene functional monomer, AAT (Scheme 1), was purchased from Acros. 3,4-Ethylenedioxythiophene linker, EDOT (Scheme 1), was kindly provided by the Bayer Company. These chemicals were dissolved in dichloromethane solvent, CH_2Cl_2, obtained from VWR. Before use, dichloromethane was distilled at 50 °C and purged under argon for 30 min. Tetrabutylammonium trifluoromethane sulfonate, TBATFMS, used as supporting electrolyte for electrochemical measurements, was obtained from Aldrich. Pesticides used as target molecules (Table 1) were atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine) (ATZ), simazine (2-chloro-4,6-bis-(ethylamino)-1,3,5-triazine), terbutylazine (2-chloro-4-(ethylamino)-6-(tertiobutylamino)-1,3,5-triazine), and diuron (3-(3,4-dichlorophenyl)-1,1-dimethyl-urea). They were purchased from Sigma. These pesticides, except diuron, belong to the same herbicides family: triazinic compounds. Diuron belongs to another class of pesticides: halogenophenylurea family. Methanol (CH_3OH) and acetic acid (CH_3COOH) used as solvents for pesticides extraction were obtained from VWR and Acros, respectively and used without further purification.

2.2. Solutions preparation

Two kinds of conducting poly(EDOT-co-AAT) copolymers were electrosynthesized in this work. First, MICPs correspond to the copolymerization of AAT and EDOT in the presence of target molecules. Secondly, non-imprinted conducting polymers (NICPs) were prepared in the absence of pesticides.

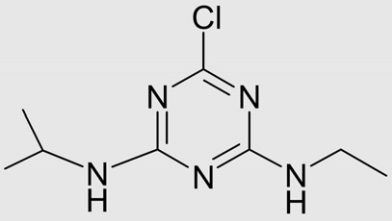
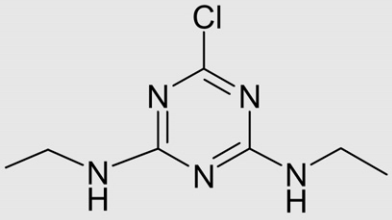
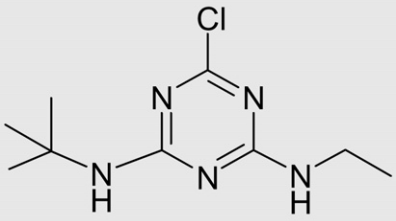
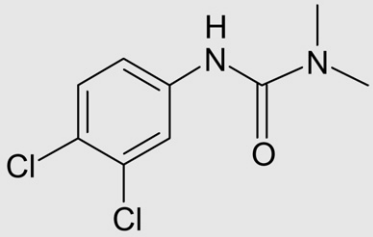
For MICPs preparation, AAT and ATZ (3×10^{-2} mol L^{-1} and 1.5×10^{-2} mol L^{-1}, respectively) were dissolved in 1.5 mL of CH_2Cl_2 in the presence of TBATFMS (0.1 mol L^{-1}) as supporting electrolyte. A time of 10 min was used for the association, through hydrogen-bonds, between two molecules of AAT and one of ATZ. Then, EDOT at a concentration of 7.5×10^{-3} mol L^{-1} was added before electrosynthesis (Scheme 2).

For NICPs preparation, AAT 3×10^{-2} mol L^{-1} and EDOT 7.5×10^{-3} mol L^{-1} were dissolved in CH_2Cl_2 solutions containing 0.1 mol L^{-1} TBATFMS as supporting electrolyte. In this case, pesticides were not present during electropolymerization.

2.3. Conducting MICPs and NICPs electrosynthesis

Platinum electrode (bioanalytical system) was used as substrate for electrosynthesis of poly(EDOT-co-AAT) conducting polymer. The area of electrode was 1 mm^2. Before use, the electrode surface was rinsed with distilled water then with ethanol solution. The electrode surface was polished and ultrasonically cleaned in distilled water for 5 min. It was finally rinsed with CH_2Cl_2 and immersed in

Table 1
Chemical structures of used pesticides: atrazine, simazine, terbutylazine, and diuron.

	Pesticides	Chemical structures
Triazinic family	Atrazine	
	Simazine	
	Terbuthylazine	
	Diuron	

one of the previously prepared solutions before use as substrate for MICPs and NICPs electrosynthesis.

Chonoamperometric and cyclic voltammetric measurements were performed by using a BAS Epsilon potentiostat and an EGG potentiostat. The electrochemical system was composed by a three-electrode cell, where platinum disc (previously described) acted as working electrode and a stainless steel wire as counter electrode. All potentials were measured *versus* a platinum wire. All measurements were performed at room temperature.

In order to study the electrochemical behavior of monomers and synthesized polymers, cyclic voltammograms were recorded from −0.5 V to 0.5 V (*versus* Pt) with a scan rate set at 25 mV s^{-1}. All cyclic voltammograms were obtained by subtracting the background current corresponding to supporting electrolyte (TBATFMS 0.1 mol L^{-1}) in CH_2Cl_2 solution from the current of the samples containing monomers and target molecules. Several preliminary experiments were performed in order to determinate oxidation potentials of EDOT and AAT monomers. These potentials were found to be at 1.10 V *versus* platinum (Pt) and 2.20 V *versus* Pt, respectively (data not shown). Chronoamperometry was used for electrosynthesis of (EDOT-co-AAT) MICPs and NICPs copolymers. Due to the values of oxidation potentials of the monomers, several electropolymerization potentials were applied between 0.85 V and 1.80 V *versus* Pt.

All chronoamperometric experiments were carried out at a constant oxidation potential of 1.45 V *versus* Pt. At this potential, the charge difference between MICPs and NICPs was optimal, revealing a difference of their redox behavior during the electrosynthesis process. All chronoamperometry-induced electropolymerizations were performed during 10 s leading to poly(EDOT-co-AAT) MICPs and NICPs, deposited at the surface of platinum electrode. Since we used a same electropolymerization charge for all MICP and NICP devices, we may assume that all the polymer films have roughly the same thickness. These polymeric films were also characterized by cyclic voltammetry between −0.5 V and 0.5 V *versus* Pt.

2.4. Electrochemical detection

Poly(EDOT-co-AAT) MICPs were synthesized in the presence of atrazine (Fig. 1, step 1) while NICPs were electropolymerized in the absence of pesticides. After electropolymerization and in order to remove atrazine from the MICPs matrix by destroying hydrogen-bonds between ATZ and AAT, a mixture of protic solvents, methanol/acetic acid solution (0.7:0.3 v/v), was used to wash the polymer-coated electrodes for 10 min according to reference [38]. This washing step allows preparation of conducting poly(EDOT-co-AAT) modified electrodes which serve as electrochemical sensors to quantify the presence of new additional pesticides (Fig. 1, step 2).

In order to check and to compare the ability of NICPs and washed MICPs to detect the presence of newly added pesticide targets (triazinic compounds and diuron, see Table 1), thanks to the establishment of new hydrogen-bonds between AAT and target molecules, the modified platinum electrodes were immersed in CH_2Cl_2 solutions containing both TBATFMS 0.1 mol L^{-1} and pesticides at concentrations ranging from 10^{-9} mol L^{-1} to 1.5×10^{-2} mol L^{-1}. The presence of added pesticide targets was then analyzed using an amperometric method (Fig. 1, step 3). Modification of electrochemical signature of polymers, which results from the establishment of new hydrogen-bonds, was recorded by the use of cyclic voltammetry. To quantify the current modifications when targets were added to the solution, the oxidation and reduction charges were calculated from cyclic voltammograms, by area integration under the curve of current *versus* time, this latter being related to the applied potential and to the scan rate.

3. Results and discussion

3.1. Atrazine electrochemical behavior

Experiments were first carried out to check the electrochemical behavior of atrazine on a platinum electrode in the absence of poly(EDOT-co-AAT) copolymers. This electrochemical study was performed by cyclic voltammetry at a scan rate of 25 mV s^{-1}. For this purpose, different CH_2Cl_2 solutions containing 0.1 mol L^{-1} TBATFMS and various concentrations of atrazine, up to 10^{-2} mol L^{-1}, were used. Fig. 2 displays the cathodic currents measured at platinum electrode in the absence of pesticides (Fig. 2a) and for increasing concentrations of atrazine (Fig. 2b–d).

As expected, measured current clearly increases with atrazine concentration. This result is in agreement with those obtained by Shoji et al., in the case of atrazine reduction on gold electrode [38]. Moreover, in all cyclic voltammograms obtained in the presence of atrazine (Fig. 2b–d), reduction peaks can be observed while the oxidation is embedded in a large wave with no clear oxidation peak. This observation is consistent with previous studies concerning atrazine reduction on gold and mercury electrodes [42,43] and implies that atrazine reduction, which is estimated to be a two-electron process, is an irreversible process. However, in our experimental conditions using platinum electrode, atrazine reduc-

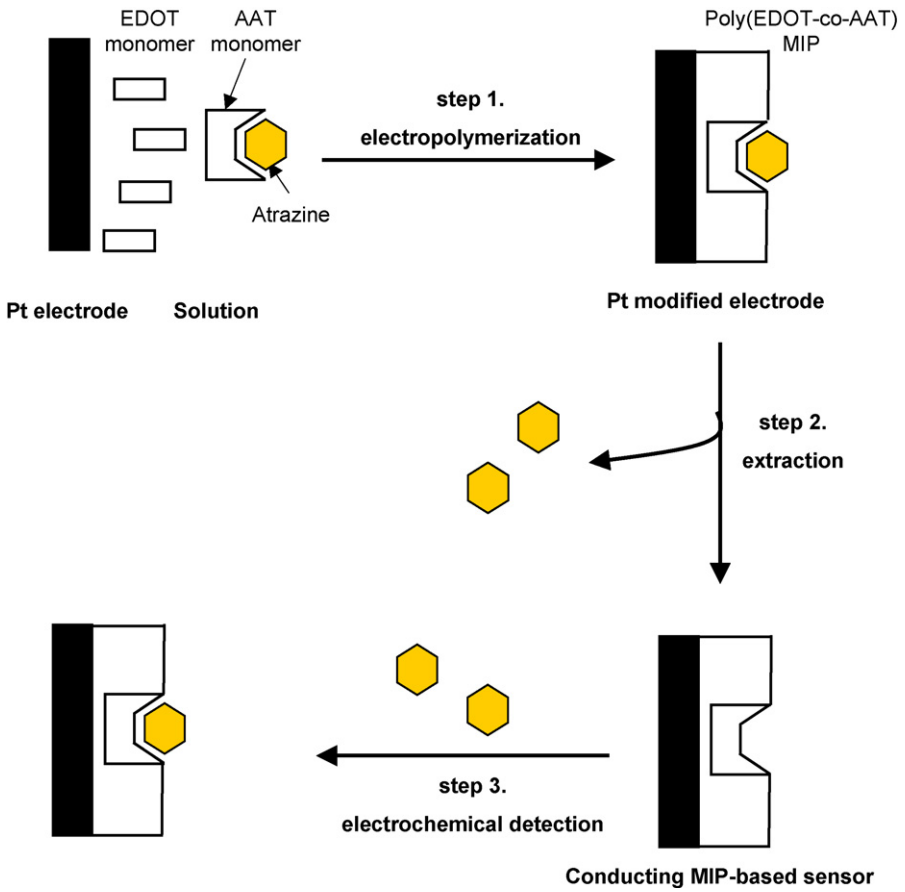


Fig. 1. Schematic representation of the preparation and the use of conducting MICPs-based electrochemical sensor.

tion occurs at about −0.5 V *versus* Pt. Finally, a negative reduction potential shift can be observed with an increase in atrazine concentration, which suggests an effective adsorption of atrazine onto the platinum electrode surface.

The above electrochemical study of atrazine allows now to understand the redox behavior of poly(EDOT-co-AAT) copolymers on platinum electrode.

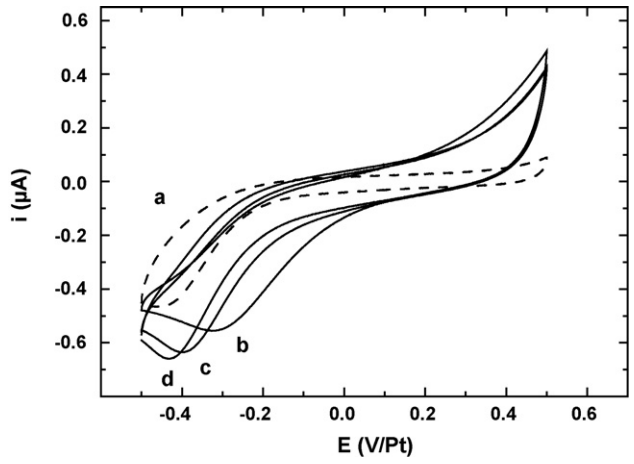


Fig. 2. Cyclic voltammograms of atrazine (*versus* Pt electrode) in CH_2Cl_2 solutions containing 0.1 mol L^{-1} of TBATFMS and increasing concentrations of ATZ: (a) 0 mol L^{-1}, (b) 10^{-3} mol L^{-1}, (c) 5.7×10^{-3} mol L^{-1} and (d) 10^{-2} mol L^{-1}. The scan rate was 25 mV s^{-1}. A platinum electrode and a stainless steel wire acted as working electrode and counter electrode respectively. All potentials were measured *versus* platinum electrode.

3.2. MICPs and NICPs electrochemical behavior

Two kinds of poly(EDOT-co-AAT) copolymers, MICPs, and NICPs, were electrosynthesized on the platinum electrode surface.

For MICPs synthesis, a CH_2Cl_2 solution containing TBATFMS, AAT, ATZ (1.5×10^{-2} mol L^{-1}) and EDOT was prepared. Then, chronoamperometry was used at a constant oxidation potential of 1.45 V *versus* Pt during 10 s leading to the electrosynthesis of a (EDOT-co-AAT) MICP copolymer, the cyclic voltammogram of which is displayed on Fig. 3a.

For NICPs synthesis, a CH_2Cl_2 solution containing TBATFMS, AAT, and EDOT was prepared in the absence of atrazine. Chronoamperometry, used in the same conditions as before led to the electrosynthesis of a (EDOT-co-AAT) NICP copolymer, characterized by the cyclic voltammogram displayed Fig. 3b.

MICPs and NICPs clearly exhibit two different voltammograms (Fig. 3). In particular, the NICP film exhibits higher currents than that of MICP. This result clearly shows the noticeable influence of the presence of atrazine, first on the polymerization step implied during the formation of imprinted and non-imprinted polymers, and also on the conformation and the conductivity of both electrosynthesized copolymers.

In the absence of atrazine, there is no conformational strain during the polymer electrosynthesis. This should lead to an easier polymerization and also to an increased yield in the electropolymerization in the case of poly(EDOT-co-AAT) NICP. This feature can explain the higher electroactivity of the NICP film, as observed in Fig. 3. On the contrary, the poly(EDOT-co-AAT) MICP film was built with atrazine-complexed AAT, which logically induces conformational strain in the film. In this case, lower electroactivity can be expected. Nevertheless, it leads to the desired creation of molecular imprinted

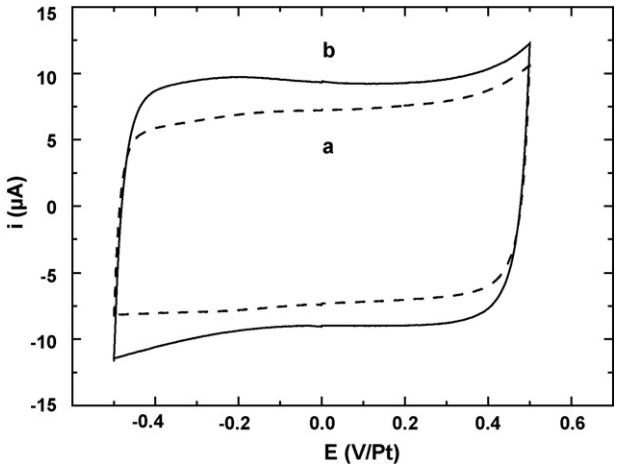


Fig. 3. Cyclic voltammograms of (a) conducting poly(EDOT-co-AAT) MICPs modified electrode and (b) conducting poly(EDOT-co-AAT) NICPs modified electrode in CH_2Cl_2 containing 0.1 mol L^{-1} of TBATFMS. MICPs and NICPs were electrosynthesized by chronoamperometry at 1.45 V during 10 s, respectively in the presence of 1.5×10^{-2} mol L^{-1} and the absence of atrazine. In addition to ATZ, the starting CH_2Cl_2 solution contained TBATFMS (0.1 mol L^{-1}), AAT (3×10^{-2} mol L^{-1}), EDOT (7.5×10^{-3} mol L^{-1}). The scan rate was 25 mV s^{-1}.

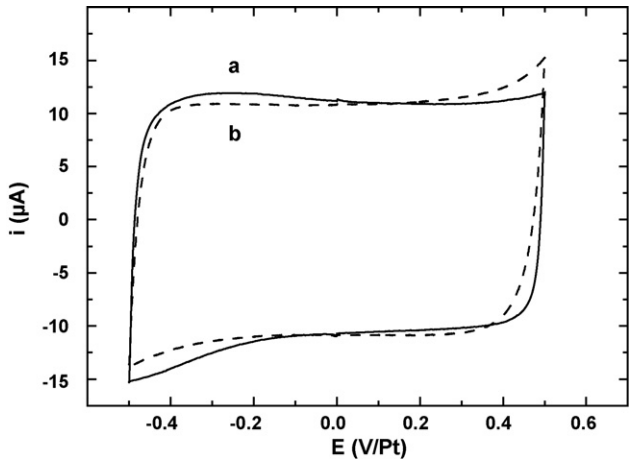


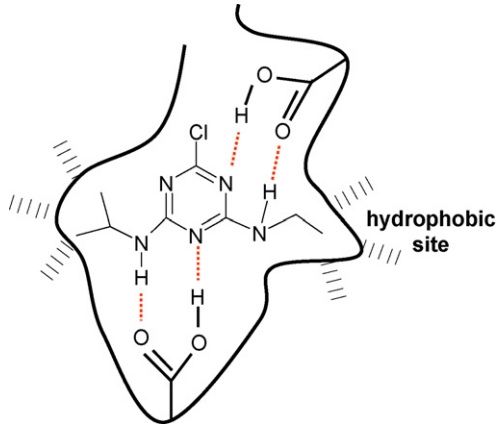
Fig. 4. Cyclic voltammograms of conducting poly(EDOT-co-AAT) MICPs modified electrode in a CH_2Cl_2 solution containing 0.1 mol L^{-1} in TBATFMS (a) before and (b) after extraction of 1.5×10^{-2} mol L^{-1} in ATZ. The extraction was performed using methanol/acetic acid solution (0.7:0.3 v/v). The scan rate was 25 mV s^{-1}.

cavities on the copolymer matrix, where pendent acetic acid groups are spatially distributed for matching precisely the presence of the nitrogen atoms of atrazine (Scheme 3). These imprinted cavities keep the memory of atrazine at the molecular level, which explains the selectivity which can be expected from these sensing structures.

3.3. Atrazine extraction from MICPs

After MICPs electrosynthesis, atrazine was still present at the surface of polymer-coated electrodes (cyclic voltammogram, Fig. 4a). Then the corresponding cyclic voltammogram of MICP after extraction of atrazine (as previously described) was recorded (Fig. 4b). One can observe a difference between voltammograms of poly(EDOT-co-AAT) MICP before and after the washing step. This modification of the electrochemical behavior of the MICP is attributed to the quantitative extraction of atrazine, which logically induces a conformational change of the copolymer.

After extraction, one can suppose that quantitative removal of atrazine enabled the formation of free specific cavities into the copolymer matrix. As a consequence, modified electrodes, based on washed poly(EDOT-co-AAT) MICP, could act as effective sensors towards atrazine.



Scheme 3. Schematic representation of the specific recognition of ATZ by a functionalized cavity of the MICP matrix. This interaction involves hydrogen-bonds and hydrophobic interactions.

3.4. Electrochemical detection of atrazine

The two kinds of conducting copolymers, NICPs and washed MICPs, are both free from atrazine but contain pendent AAT residues able to establish hydrogen-bonds with additional atrazine. However, only MICPs are supposed to contain specific cavities as "functional and geometrical memories" enabling the selective chemical recognition of added atrazine target molecules.

In order to check and to compare the ability of NICPs and of washed MICPs to detect the presence of additional atrazine, thanks to the establishment of new hydrogen-bonds between AAT residues and target molecules, the modified platinum electrodes were immersed in CH_2Cl_2 solutions containing 0.1 mol L^{-1} TBATFMS. Then, 10^{-3} mol L^{-1} of atrazine was added to the electrolytic solution. This quantity was lower than that used for MICPs electrosynthesis (1.5×10^{-2} mol L^{-1}). Cyclic voltammetry was used to check the modification of the voltammogram of the polymers, which could result from the establishment of new hydrogen-bonds, and to quantify the pesticide detection by MICPs and NICPs (Fig. 5A and B, respectively).

Fig. 5A displays the cyclic voltammograms corresponding to the poly(EDOT-co-AAT) MICP before (5a) and after (5b) atrazine addition. A significant decrease in current is observed when atrazine is added to the solution, which can be associated to the conformational variations occurring in the polymer during its redox activity. Two forms of polymer chains exist: the neutral state, which is polyaromatic, and the oxidized state, which is polyquinonic. In the neutral state, there is a free rotation around the simple exocyclic bonds between thiophene units. The polymer chain can twist for adapting to the steric strain imposed by the presence of atrazine linked to the pendent acetic acid groups. On the other hand, the electrooxidation of the polymer imposes the coplanarization of the polymer chains, owing to the required exocyclic double bonds between the thiophene units. In this case, the electrooxidation of the polymer chain will become much more difficult, owing to the steric strain brought by the presence of associated atrazine. When compared to atrazine-free MICP, the redox currents will decrease all the more as the quantity of complexed atrazine along the polymer chains will be higher. Thus, as already shown by Garnier et al. [44,45] in the case of oligonucleotide recognition by ODN functionalized polypyrroles, these considerations indicate that a quantitative characterization of atrazine can be obtained from the analysis of the redox currents (or the redox charges) as a function of the concentration of atrazine in the analyzed solution.

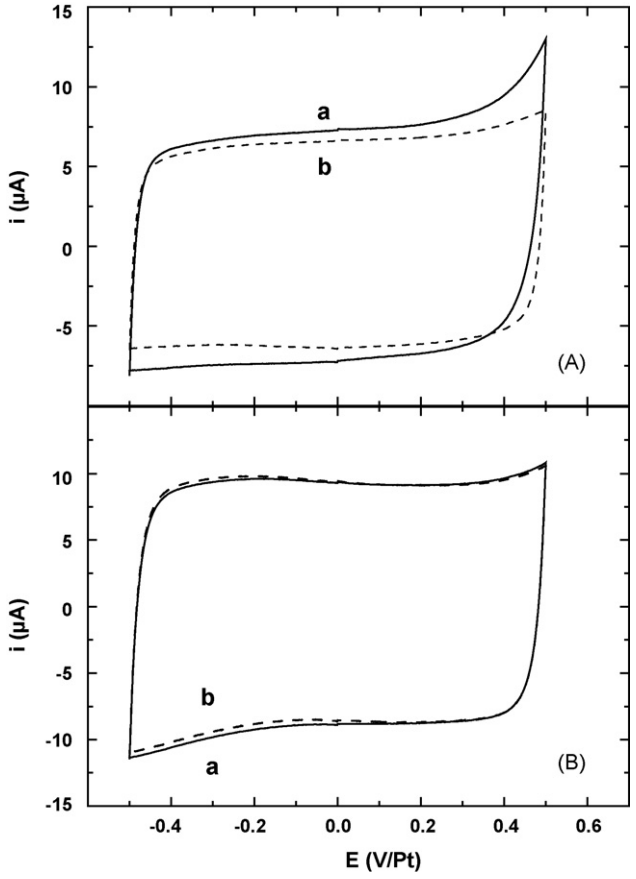


Fig. 5. Cyclic voltammograms of (A) MICPs modified electrode and (B) NICPs modified electrode in CH_2Cl_2 solutions containing TBATFMS (0.1 mol L^{-1}). (a) In the absence of ATZ after washing step, and (b) in the presence of additional ATZ (10^{-3} mol L^{-1}), the scan rate was 25 mV s^{-1}.

In the case of NICPs, in the absence of atrazine, no modification of the cyclic voltammogram should be observed upon addition of atrazine. This is clearly shown on Fig. 5B, which displays the cyclic voltammograms of poly(EDOT-co-AAT) NICP before (5a) and after (5b) addition of atrazine. This confirms the absence of any association of the polymer with atrazine. This result allows to conclude that NICPs, contrarily to MICPs, are not able to detect the presence of atrazine in the surrounding medium, pointing out the specificity of the molecular imprints for detecting these small molecules.

The difference observed between the ability of MICPs and NICPs to recognize atrazine target molecules proves that the presence of pendent AAT residues along the polymer chains or at the polymer matrix surface, able to establish hydrogen-bonds with additional atrazine, are not sufficient for the detection of this target molecule. The fact that only MICPs are able to interact, and then to detect atrazine, demonstrates the essential presence of pre-shaped cavities into the copolymer matrix (Scheme 3). Moreover, the absence of any detection of atrazine molecules in the case of NICPs indicates that the unspecific adsorption of target molecules as well as their interaction by "π–π stacking" with aromatic groups of the copolymers can be neglected for both NICPs and MICPs.

The poly(EDOT-co-AAT) MICP electrochemically synthesized on platinum electrode constitutes an electrochemical sensor able to detect additional atrazine molecules thanks to the presence of preformed functionalized cavities bearing the spatial, conformational and molecular memories of the target. In the following, the properties of this sensor will be specified in terms of selectivity, range of detection as well as detection threshold.

3.5. Range of atrazine detection by the MICP-based sensor

The sensitivity of an affinity-based sensor is primarily determined by the association constant between probe and target, *i.e.*, between AAT and ATZ and for a given probe, the quantity of associated ATZ targets will increase with the amount of AAT probes deposited on the sensor film. Atrazine concentration used for MICP electrosynthesis was 1.5×10^{-2} mol L^{-1}.

Ideally, this concentration corresponds to that of preformed functionalized cavities present into the MICP matrix after the washing step. Thus, one can expect the ability of the MICP-based sensor to detect atrazine targets at a concentration of 10^{-3} mol L^{-1}. However, if we suppose that all atrazine molecules do not serve as molds during electropolymerization, and if we assume that the washing step does not allow the release of all pesticides trapped into the MICP matrix, we can then consider that the concentration of preformed functionalized cavities can be much lower than 1.5×10^{-2} mol L^{-1}. Therefore, it was important to check whether the sensor is able to detect higher concentrations of atrazine. In addition, the characterization of our sensor in terms of detection range and detection threshold was essential.

In order to check the ability of washed poly(EDOT-co-AAT) MICPs to detect the presence of atrazine at different concentrations, thanks to the establishment of new hydrogen-bonds between AAT residues and target molecules, the modified platinum electrode was immersed in a CH_2Cl_2 solution containing 0.1 mol L^{-1} of TBATFMS. Then, increasing amounts of atrazine (from 10^{-9} mol L^{-1} to 1.5×10^{-2} mol L^{-1}) were successively added to the solution. After each addition, a cyclic voltammogram was recorded from −0.5 V to 0.5 V (*versus* Pt) with a scan rate set at 25 mV s^{-1} (data not shown).

In order to quantify the modification of the current at each step of the successive additions of target molecules, the electrooxidation charges, *Q*, were calculated from cyclic voltammograms by area integration under the curves of current *versus* time. A charge *Q* (0 M) was also calculated for the washed MICP in the absence of any atrazine molecules. After each addition of atrazine, a relative charge was calculated according to Eq. (1):

$$\frac{Q(0M) - Q}{Q(0M)} \quad (1)$$

The relative charge, calculated from the previous formula, was then plotted as a function of the atrazine concentration in solution (Fig. 6). Each point of the curve corresponds to a mean value obtained from three independent measurements. Besides, the uncertainty bars corresponding to each point were displayed on the graph.

The relative charge increases with atrazine concentration as can be seen in Fig. 6. This evolution can be understood in terms of variation in MICP electroactivity in the presence of increasing amounts of targets. However, the evolution of the relative charge is not linear. At low atrazine concentrations, the relative charge is close to 0. Its value becomes significative for atrazine concentrations higher than 10^{-7} mol L^{-1}. This latter concentration could then be considered as the detection threshold of our electrochemical sensor. Above 10^{-7} mol L^{-1}, a non-linear increase of the relative charge can be observed. This increase is maximal around 10^{-4} mol L^{-1} in atrazine. For higher concentrations, a very slight increase is observed still 1.5×10^{-2} mol L^{-1} in atrazine, which could be explained by saturation of sensor response, due to the limited number of preformed functionalized cavities.

Contrary to washed MICPs which contain specific cavities enabling the chemical recognition of pesticide target molecules, NICPs were shown to not detect atrazine, even at a concentration of 10^{-3} mol L^{-1}. In order to check whether the response of NICPs depends on atrazine concentration and in order to compare this electrochemical response with that of MICPs, the NICPs-based

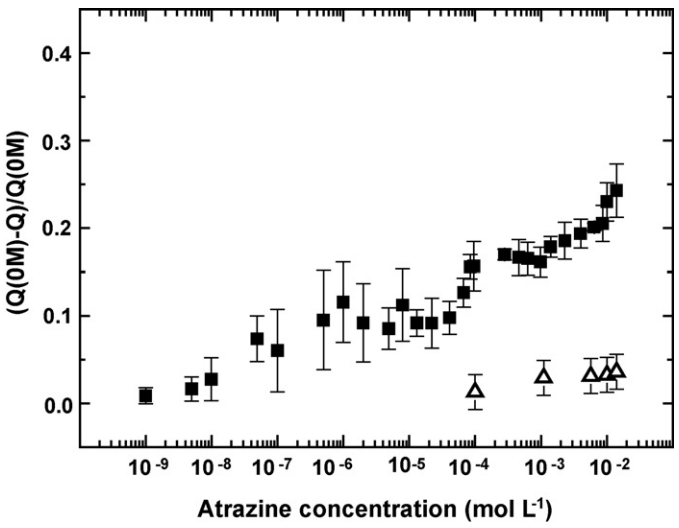


Fig. 6. Variation of the relative charge of poly(EDOT-co-AAT) MICPs (■) and poly(EDOT-co-AAT) NICPs (Δ) modified electrodes as a function of additional ATZ concentration (ranging from 10^{-9} mol L^{-1} to 1.5×10^{-2} mol L^{-1}). The relative charges were deduced from cyclic voltammograms. Each point of the curves, represented with its error bar, corresponds to the mean value obtained from three experiments.

modified platinum electrode was immersed in a CH_2Cl_2 solution containing 0.1 mol L^{-1} TBATFMS. Then, increasing amounts of atrazine were added to the solution. The successive cyclic voltammograms were recorded and the relative charges were calculated and plotted *versus* atrazine concentration on Fig. 6.

In the case of NICPs, all the values of relative charges remain low, even lower than the relative charge obtained in the case of MICPs for a concentration of 10^{-8} mol L^{-1} in atrazine. This can be explained by the absence, into NICPs matrix, of specific cavities enabling the chemical recognition of pesticide target molecules. However, a very slight increase of charge is observed when atrazine concentration increases. This result suggests that atrazine interacts, but very weakly, with poly(EDOT-co-AAT) NICPs. This weak interaction may result from the presence of AAT residues at NIP surface enabling the establishment of hydrogen-bonds with atrazine molecules, the equilibrium of H-bonds formation being favored by increasing amounts of pesticides.

The modification of relative charge is much more important for the poly(EDOT-co-AAT) MICP modified electrode than for the NICP-based modified electrode. This result highlights the behavior difference between the two modified electrodes towards atrazine detection.

3.6. Selectivity of the MICP-based sensor towards atrazine

The poly(EDOT-co-AAT) MICP constitutes an electrochemical sensor able to detect a large range of atrazine concentrations owing to the presence of pre-shaped functionalized cavities present into the MICP backbone. These cavities, which are obtained by atrazine removal during the washing step are supposed to constitute atrazine molecular imprints. Ideally, the size of the cavities, the chemical functionalization of their surface by carboxylic groups of AAT residues and finally the spatial distribution of these functionalities must enable the selective and even the specific recognition of the complementary atrazine molecules.

In order to check the specificity of recognition of the sensor towards atrazine, we chose, as target molecules, two triazinic molecules which belong to atrazine family: terbutylazine and simazine (Table 1). These two pesticides present the same chemical functionalities as atrazine: two side-chain amino groups and two nitrogen hetero-atoms of the aromatic structure, which may establish four hydrogen-bonds with the oxygen atoms of two AAT residues present at the surface of MICP cavities. However, atrazine, terbutylazine, and simazine differ by the nature of one of the two lateral alkylamino groups linked to the aromatic structure: isopropylamino, tertiobutylamino, and ethylamino radicals, respectively. In consequence, atrazine, terbutylazine, and simazine differ slightly by their molecular size and structure.

In order to check and to compare the ability of washed MICPs to detect the presence of these triazinic compounds, the MICPs-based platinum electrodes were immersed in CH_2Cl_2 solutions containing 0.1 mol L^{-1} TBATFMS and 10^{-3} mol L^{-1} of pesticide. As in the case of atrazine (Fig. 5A), cyclic voltammetry was used to quantify the detection of terbutylazine and simazine by MICPs-based modified electrodes.

Fig. 7A displays cyclic voltammograms corresponding to the poly(EDOT-co-AAT) MICP before (graph a) and after (graph b) terbutylazine addition, while Fig. 7B is related to cyclic voltammograms corresponding to the poly(EDOT-co-AAT) MICP before (graph a) and after (graph b) simazine addition. In both the cases, only a slight decrease in current is observed when pesticide molecules are added to the solution, indicating a weak recognition by the electrochemical sensor of terbutylazine and simazine in comparison with atrazine.

Even if terbutylazine and simazine are detected at a concentration of 10^{-3} mol L^{-1} by the electrochemical sensor, the relative charges deduced from the cyclic voltammograms of Fig. 7 are much lower than the relative charge corresponding to 10^{-3} mol L^{-1} in atrazine (Fig. 5). The weaker recognition of terbutylazine can be understood in terms of molecular size. Indeed it may be attributed to the substitution of isopropylamino radical present in atrazine by the bigger tertiobutylamino group existing in terbutylazine. However, the weak detection of simazine is more troublesome, when considering its smaller size in comparison with atrazine. Could the substitution of an isopropylamino radical by a smaller ethylamino group decrease the interaction ability of the pesticide with the complementary functionalized cavities of the MICPs? Certainly, the side-chain amino groups of pesticides play a role in the recognition process. The specific cavities created into the polymer backbone during MICP electrosynthesis could involve, in addition to hydrogen-bonds, van der Waals interactions and a hydrophobic effect between alkyl chains of the target molecules and apolar parts of the monomers involved during polymerization (EDOT and AAT). Hydrophobic sites, present in the functionalized cavities of washed MICPs, could then favor the detection of atrazine (Scheme 3).

The MICPs-based electrochemical sensor developed in this work enabled the quantitative detection of atrazine, since this molecule is the target in the presence of which the MICP had been prepared. Besides, the two other studied triazinic compounds, simazine and terbutylazine, were slightly detected. In order to check the selectivity of recognition of the sensor towards triazinic compounds, we chose a structurally different target molecule, diuron, which does not belong to triazinic family (Table 1). This molecule possesses functional carbonyl and amino groups, able to establish H-bonds with preformed functionalized cavities. However, its size, its structure and the spatial distribution of its polar groups are very different from those of triazinic compounds.

In order to check the ability of washed MICPs to detect the presence of diuron, the MICPs-based platinum electrodes were immersed in CH_2Cl_2 solutions containing 0.1 mol L^{-1} TBATFMS and 10^{-3} mol L^{-1} diuron. Cyclic voltammetry was used as previously to quantify the detection of these target molecules.

Fig. 7C displays cyclic voltammograms corresponding to the poly(EDOT-co-AAT) MICP before (graph a) and after (graph b) diuron addition. No current modification is observed in this case when diuron is added to the solution. Due to its fundamentally

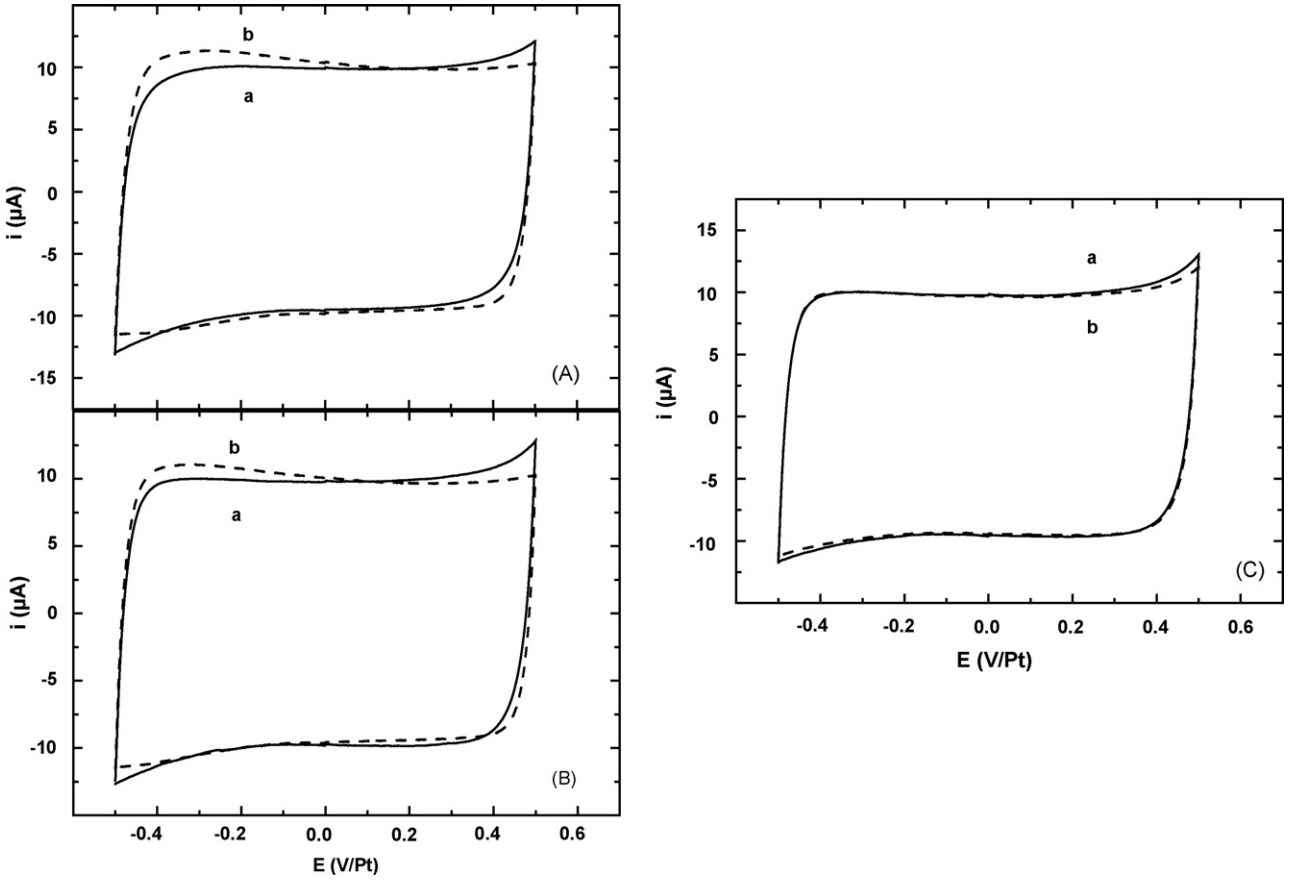


Fig. 7. Cyclic voltammograms at a scan rate of 25 mV s^{-1} of: (A) MICPs modified electrode in CH_2Cl_2 solutions containing 0.1 mol L^{-1} in TBATFMS (a) in the absence and (b) in the presence of terbutylazine (10^{-3} mol L^{-1}). (B) MICPs modified electrode in CH_2Cl_2 solutions containing 0.1 mol L^{-1} in TBATFMS (a) in the absence and (b) in the presence of simazine (10^{-3} mol L^{-1}). (C) MICPs modified electrode in CH_2Cl_2 solutions containing 0.1 mol L^{-1} in TBATFMS (a) in the absence and (b) in the presence of diuron (10^{-3} mol L^{-1}).

different structure, this pesticide does not match the functionalized cavities of poly(EDOT-co-AAT) MICP even if it can establish hydrogen-bonds.

As a conclusion, the developed electrochemical sensor displays a selective recognition towards triazinic family. The shape, the size and the spatial disposition of the functional cavities of the MICPs formed during the electropolymerization enable the more specific detection of atrazine (Scheme 3).

4. Conclusion

In conclusion, an electrochemical sensor is presented which combines the selectivity shown by molecularly imprinted polymers, with the sensitivity and the real time detection offered by the use of an electrochemical transduction. In the example shown, the pesticide atrazine has been chosen as the target. The conducting copolymer matrix associates (i) a sensing element, thiophene-acetic acid, known to bind with atrazine, with (ii) a highly hydrophilic linker, EDOT, for ensuring electroactivity in aqueous medium. The sensor is fabricated by the co-electropolymerization of EDOT and thiophene-acetic acid, already bound with atrazine. The further release of atrazine leaves empty, highly specific recognition sites in the polymer matrix. This memory effect of the polymer matrix is analogous to the one developed in MIPs. The voltammogram of the conducting polymer-based matrix is modified upon the specific bonding of atrazine, which allows the real time access to the atrazine recognition process and opens very promising studies of the intimate kinetics of the sensing site–target interaction.

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