Electrochemical sensing of ecstasy with electropolymerized molecularly imprinted poly(o-phenylenediamine) polymer on the surface of disposable screen-printed carbon electrodes

Rosa A.S. Couto[a], Séfora S. Costa[a], Bassim Mounssef Jr.[b], João G. Pacheco[c], Eduarda Fernandes[a],
Félix Carvalho[d], Cecília M.P. Rodrigues[e], Cristina Delerue-Matos[c], Ataualpa A.C. Braga[b],
Luís Moreira Gonçalves[b,*], M. Beatriz Quinaz[a,*]

[a] REQUIMTE, LAQV, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[b] Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo (USP), São Paulo, SP, Brazil
[c] REQUIMTE, LAQV, Instituto Superior de Engenharia do Porto (ISEP), Politécnico do Porto, Porto, Portugal
[d] REQUIMTE, UCIBIO, Laboratory of Toxicology, Department of Biological Sciences, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[e] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

ABSTRACT

This study demonstrates the ability of an electrochemical sensor based on molecularly imprinted polymers (MIPs) to selectively quantify 3,4-methylenedioxymethamphetamine (MDMA), also known as ecstasy, in biological samples. The device was constructed using *ortho*-phenylenediamine (*o*-PD) as the MIP's building monomer at the surface of a screen-printed carbon electrode (SPCE). The step-by-step construction of the SPCE-MIP sensor was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Density functional theory (DFT) calculations and modelling were performed not only to understand template-monomer interaction but also to comprehend which possible polymer structure - linear or ramified poly(*o*-PD) – indeed interacts with the analyte. The prepared sensor worked by directly measuring the MDMA oxidation signal through square-wave voltammetry (SWV) after an incubation period of 10 min. Several parameters were optimized, such as the monomer/template ratio, the number of electropolymerization scanning cycles, and the incubation period, to obtain the best sensing efficiency. Optimized sensors exhibited suitable selectivity, repeatability (2.6%), reproducibility (7.7%) and up to one month of stable response. A linear range up to 0.2 mmol L^{-1} was found with an r^2 of 0.9990 and a limit of detection (LOD) and quantification (LOQ) of 0.79 and 2.6 µmol L^{-1} (0.15 and 0.51 µg mL^{-1}), respectively. The proposed sensor was successfully applied to human blood serum and urine samples, showing its potential for application in medicine and in forensic sciences.

1. Introduction

Ecstasy is a term coined by a Californian drug-dealer to boost his sales of 3,4-methylenedioxymethamphetamine (MDMA) in the early 1980s [1]. It was at that decade when this compound started to be sold as street-drug that could generate novel sensations that are not easily describable: "MDMA represents a novel class of compounds, which are characterized by a unique set of effects that are neither hallucinogenic nor stimulant but a combination of both" [2,3]. However, its original synthesis can be traced back to 1912, by a German chemist named Anton Köllisch, who would probably continue to produce fine chemical work had not he died young in the midst of the first world war [1]. Up

to the moment, there seems not to be any recognized medical application of MDMA, but many voices continue to advocate a greater investment in its research as a therapeutic agent [4]. However, its detrimental health effects (which include enhancing mental disorders like depression and anxiety, promoting panic attacks, creating adverse health effects like seizures, arrhythmia, or hyperthermia, producing permanent damage in brain function, liver, kidney and heart toxicity; and even causing death [5–9]), along with an increase in its recreational consumption by some groups [10], generate fear around its handling, even if to the best intended purposes.

The metabolism of MDMA is mainly mediated by hepatic CYP450 enzymes, originating 3,4-methylenedioxyamphetamine (MDA) by *N*-

demethylation. Both MDMA and MDA are then *O*-demethylenated to the catecholic compounds *N*-methyl-α-methyldopamine (*N*-Me-α-MeDA) and α-methyldopamine (α-MeDA), respectively, which can undergo oxidation to the correspondent redox-active *ortho*-quinones. These *ortho*-quinones may be conjugated with reduced glutathione (GSH), originating glutathionyl adducts. The systemic formation of 5-(glutathion-S-yl)-*N*-methyl-α-methyldopamine [5-(GSH)-*N*-Me-α-MeDA] and 5-(glutathion-S-yl)-α-methyldopamine [5-(GSH)-α-MeDA] may be followed to further metabolism into *N*-acetyl-cysteine (NAC) conjugates [11]. From these compounds, MDA, *N*-Me-α-MeDA and α-MeDA can be also present, however MDMA levels in plasma and urine are normally higher than these compounds. Not surprisingly, since the quantification of this drug in biological fluids in an easy and accurate way would be of obvious great interest for health and forensics sciences, there is a large number of publications concerning the analytical determination of MDMA, in a wide variety of matrices – both biological fluids and tissues (such as plasma, urine, saliva, sweat, and hair), as well as the source materials (tablets, salts, and crystals) [2,12,13] – making use of a wide variety methods. Capillary electrophoresis (CE) with diode array detection (DAD) [14], gas chromatography (GC) with detection by flame ionization detection (FID) [15] or by mass spectrometry (MS) [16], liquid chromatography (LC) with detection by spectrophotometry [17], fluorescence [18] or MS [19], and even direct MS [20], among others [2,12,13,21–24] have been used to determine MDMA. One can also find scarce electroanalytical methodologies in literature, (more about these methods will be debated in the section Results and discussion) [25–28].

Considering the huge relevance of sample preparation in analytical chemistry [29], research efforts have also been dedicated to this matter including the use of classical techniques like liquid-liquid extraction (applied in the analysis of oral fluid by ion mobility spectrometry and infrared spectroscopy [22]) or supported liquid extraction (applied in the analysis of ecstasy tablets by GC–MS [30]) and less classical techniques like suspended droplet microextraction (applied in the analysis of human hair by LC-UV [17]). Molecularly imprinted polymers (MIPs) are 'chemical tissues' with selective recognition lacunas for analytes, somehow imitating antibodies [31–34]. These chemical fabrics are obtained by polymerization, a process in which the building blocks (monomers) are entangled around a target template (normally, the aimed analyte). Following polymerization, the template molecule is removed, and the MIP is manufactured, it now possesses spots complementary in shape, size, and functionality to the imprinted molecule. Electrochemical MIPs are a special kind of MIPs, which synergistically combine the MIPs' advantages (in particular their selectivity) with the inherent gains of electrochemical sensors, like simplicity, sensitivity, portability, speed of analysis and user-friendliness, these are important key features in forensics [35–37]. Electropolymerization has been the correct choice especially in the preparation of MIPs with electroanalytical sensing, it enables a simple way of controlling the polymers thickness and morphology, it is reproducible and permits polymerization in an aqueous media [38–41].

In the present study, a novel electrochemical sensor for MDMA was constructed based on the electrochemical polymerization of *o*-phenylenediamine (*o*-PD), in the presence of the analyte MDMA, thus forming a MIP, on the surface of a portable screen-printed carbon electrode (SPCE). Subsequently to optimization and analytical validation, it was applied in the analysis of biological samples, namely human blood serum and urine. Although one can find in literature a few manuscripts developing MIPs for MDMA [42–44] (MIPs were developed by thermopolymerization, using methacrylic acid, ethyleneglycoldimethacrylate and 2,2'-azobis(isobutyronitrile) as the building monomer, crosslinker and radical initiator, respectively), to the best of the authors' knowledge, none was developed by electropolymerization.

2. Experimental

2.1. Chemicals

All commercial reagents were of analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water with resistivity not less than $18.2\,M\Omega\,cm$ at 298 K. Potassium hexacyanoferrate (III), potassium hexacyanoferrate (II) trihydrate, *o*-PD, methamphetamine, epinephrine, dopamine, caffeine and tyramine were purchased from Sigma-Aldrich, noradrenaline was purchased from Fluka and amphetamine from Tocris Bioscience. Acetate buffer $0.1\,mol\,L^{-1}$, pH 5.0 was prepared with sodium acetate and acetic acid both purchased from Sigma-Aldrich.

2.2. Equipment

Voltammetric measurements, such as cyclic voltammetry (CV) and square wave voltammetry (SWV), were carried out using an Autolab PGSTAT10 potentiostat/galvanostat controlled by GPES software. Electrochemical impedance spectroscopy (EIS) studies were performed using an Autolab PGSTAT204 potentiostat/galvanostat expanded with a FRA32M EIS module (Metrohm) and the NOVA 1.10.1.9 software for data acquisition. Screen-printed carbon electrodes (SPCE, Metrohm, ref. 110) with carbon working (d = 4.0 mm) and auxiliary electrodes and a silver (Ag) pseudo-reference electrode were used.

Magnetic resonance spectroscopy (NMR) spectra were recorded with a Bruker Avance 300 spectrometer (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz; the internal standard was TMS.

Positive-ion Electrospray ionization (ESI) mass spectra were acquired with a QTOF 2 instrument. Nitrogen was used as nebuliser gas and argon as collision gas; the needle voltage was set at 3000 V, with the ion source at 80 °C and the desolvation temperature at 150 °C; the cone voltage was 35 V.

2.3. MDMA purification and characterization

MDMA (hydrochloride salt) was extracted and purified from high purity MDMA tablets kindly provided by the Portuguese Criminal Police Department. Briefly, tablets were crushed in a porcelain mortar, dissolved in 10% hydrochloric acid and filtered through G4. The filtered solution was neutralized with 20% sodium hydroxide and extracted 3 times with dichloromethane. The organic phase was then washed three times with water and dehydrated with anhydrous sodium sulfate. Dichloromethane was evaporated in a rotary evaporator to yield an oil, which was dissolved in methanol. The methanol solution was acidified with 37% hydrochloric acid and MDMA hydrochloride crystals were obtained by stepwise addition of ethyl ether, following by storage at 4 °C overnight. At the end, crystals were filtered and washed with cold ethyl ether. The obtained salt was pure and fully characterized by NMR and mass spectrometry (MS) methodologies (NMR and MS spectra are shown in the Supporting information). Obtained results are in accordance with literature [19,45–48].

2.4. Preparation of the SPCE-MIP

Prior to modification, the bare SPCE were rinsed with water and dried under a N_2 flow. The MIP-sensor was obtained by electropolymerization of a solution containing $0.5\,mmol\,L^{-1}$ of the monomer *o*-PD and $2.0\,mmol\,L^{-1}$ of the template and target molecule MDMA, in acetate buffer pH 5.0. A 20 μL drop of this solution was released at the surface of the SPCE and then the electropolymerization was achieved, in this solution, by applying 5 CV scans in the potential range between $-0.0\,V$ and $+1.2\,V$, at a scan rate of $100\,mV\,s^{-1}$. The non-imprinted polymer (NIP) sensor was prepared using the same polymerization conditions without the template molecule. After electropolymerization,

electrodes were washed with deionized water and subjected to template extraction by submersion in a methanol/deionized water (50:50, v/v) solution, under stirring, for 10 min. Electrodes were finally washed with deionized water and dried using a N_2 flow.

2.5. Theoretical studies

The following commercial software were used in the theoretical studies: Spartan'16 from Wavefunction and Gaussian09 package [49] from Gaussian. Spartan's systematic conformational analysis algorithm, mixing Monte Carlo and Molecular Dynamics simulations for the sampling of conformers with energies in accordance with the Boltzmann energy distribution at room temperature[83]. Merck Molecular Force Field (MMFF) was the force field used.

Subsequent geometry optimizations on the most stable conformations, considered good candidates to model the MIP and its interaction with the MDMA substrate, were performed with Gaussian09 using first the Hartree-Fock method with the 6-31G(p) basis set [50,51]. Following, B3LYP-D3 dispersion-corrected functional was used, based on Density Functional Theory (DFT) [52–54] with the 6-31G(d,p) basis set. Finally, the final geometries for the best candidates were evaluated as true minimum performing frequency calculations, accepting only optimized geometries with all positive eigenvalues in the Hessian matrix.

2.6. Electrochemical measurements

Electrochemical measurements were performed by SWV after incubation of the SPCE-MIP directly on 50 μL of MDMA solutions with different concentrations for 10 min, (non-labelled measurements, i.e. no redox probes were used), supporting electrolyte was acetate buffer, $0.1 \, \text{mol L}^{-1}$. SWV was performed in the potential range of 0.0 to +1.6 V, at a frequency of 25 Hz, with the pulse amplitude of 19.95 mV and step potential of 5.1 mV.

Additionally, the step-by-step construction of the modified electrodes was characterized by CV and EIS. CV measurements were performed between −0.5 and 1.0 V at a scan rate of $50 \, \text{mV s}^{-1}$ using a $2.0 \, \text{mmol L}^{-1}$ of $[\text{Fe(CN)}_6)]^{3-/4-}$ with $0.1 \, \text{mol L}^{-1}$ KCl in acetate buffer solution pH 5 as redox probe. EIS experiments were conducted using the same probe, a single sine wave type with amplitude of 0.01 V and applied potential of 0.16 V, in a frequency range from 0.1 Hz to 100 kHz.

All measurements were performed at room temperature (ca. 25 °C).

2.7. Biological samples

Drug-free blood plasma and urine samples were collected from a healthy adult. Both biological samples were spiked with different amounts of the stock solution of MDMA and the standard addition method was used for the quantification of MDMA.

3. Results and discussion

3.1. Sensor development and characterization

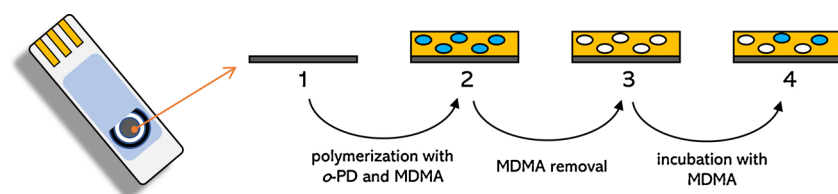The overall development process of the SPCE-MIP sensor is depicted in Fig. 1. During each step of the construction, the SPCE-MIP sensor was characterized by CV and EIS (Fig. 2) using $2.0 \, \text{mmol L}^{-1}$ $[\text{Fe(CN)}_6)]^{3-/4-}$ and 0.1 KCl solution. The CV characterization showed that the bare SPCE allow charge transfer with the typical pair of diffusional reversible peaks in CV [55,56]. When the MIP is formed on the electrode surface, the current decreases in CV and the charge transfer resistance (R_{ct}) in EIS clearly increases (the semicircle diameter of Nyquist Plot increased from $43.8 \, \Omega$ to $179.3 \, \text{K}\Omega$), thus both the SPCE-MIP before the removal of the template, and the SPCE-NIP ($167.3 \, \text{K}\Omega$) work as insulators. It is remarkably noticeable the difference between the SPCE-MIP before and after removing the template ($308.0 \, \Omega$), where the MDMA molecules left the cavities and the redox probe obtained easier access to the electrode surface [57], this being partially reverted when incubating with MDMA ($364.1 \, \Omega$). The removal of the template can also be visualized by SWV (Fig. S1 shown in the Supporting information), since there is not any peak after the template extraction.

Several information can be obtained from the CVs of the electropolymerization process (Fig. S2 shown in the Supporting information) [57,58]. Firstly, the lack of any peak is the reverse scan shows that the process is irreversible. Then, the current decreases substantially with each cycle showing that the process is quite efficient. The current quickly tends to zero showing that the newly formed poly(o-PD) is covering the electrode´s surface with a non-conductive film [58]. Several parameters in the preparation of the SPCE-MIP sensor were optimized, namely: monomer concentration, template concentration and number of cycles in the electropolymerization as well as the time of incubation with the analyte prior to analysis (Fig. 2). As showed in Fig. 3A and B, the best analytical signal was found with $0.50 \, \text{mmol L}^{-1}$ o-PD and $2.0 \, \text{mmol L}^{-1}$ MDMA, which means a monomer:template ratio of 1:4. The amount of polymer in the electrode surface greatly influences the obtained signal, an excessively thick MIP layer exhibits low binding capacity, poor site accessibility, and slow absorption kinetics. The MIP thickness can be adjusted by controlling the number of cycles of electropolymerization [59], the optimal sensitivity to MDMA was obtained with 5 scanning cycles (Fig. 3C). Fig. 3D shows the evolution of peak current with incubation time at the given conditions, 10 min was found to be the optimal analyte-MIP interacting time.

3.2. Theoretical studies

The preparation of electro-polymerized MIPs with o-PD has been applied in a variety of analytes ranging from human cardiac troponin T [60], paracetamol (also known as acetaminophen) [61], dopamine [62] to perfluorooctane sulfonate [58]. The produced films are rather advantageous since they can be thin, stable and can be created in-situ on several types of substrates [58]. o-PD is one of the most common monomers used in electropolymerization, it has good chemical stability and the easy polymerization creates a non-conducting compact (rigid) film with hydrophilic and hydrophobic recognition sites. Although o-PD can easily react using both amine groups simultaneously [63–67] and it is possible to obtain a fully 'linear' polymer [68], however that occurs at very low potentials [69]. Apparently, with this electropolymerization a polymer structure with free amine groups is obtained, which strongly facilitates monomer-analyte interaction [66].

In order to further our investigation of the interaction between the o-PD monomers and the MDMA substrate, computational studies of the interaction between two types of o-PD tetramers and the MDMA
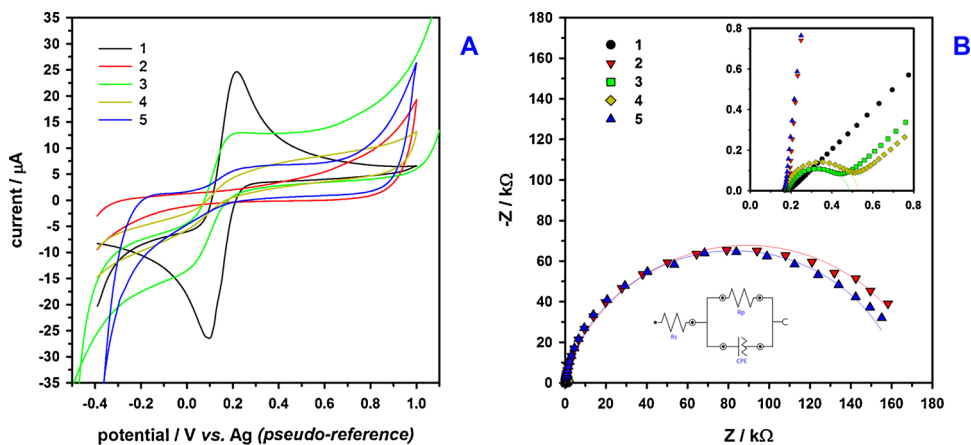

Fig. 1. Schematic illustration of the construction of the MIP-SPCE sensor.

polymerization with o-PD and MDMA   MDMA removal   incubation with MDMA

Fig. 2. A) characterization of the step by step construction of the sensor, measurements performed over a 2.0 mmol L^{-1} $[Fe(CN)_6)]^{3-/4-}$: A - CV measurements and B - EIS Nyquist diagrams with suitable fitting (the circuit used is shown within the figure). 1) bare SPCE; 2) SPCE-MIP after polymerization; 3) SPCE-MIP after template extraction; 4) SPCE-MIP after incubation; 5) SPCE-NIP.

substrate to find the strongest interaction were carried out, which would be a strong indication of the kind of polymer synthesized and responsible for the detector's selectivity. Two isomers, representative of the *o*-PD polymers were chosen (Fig. 4); their essential difference is

their "closed" or "open" character – depending on whether both terminal nitrogens of the monomers bond to the ring carbon on the next monomer during polymerization, or only one of them. This basic difference affects not only their stability – preserving monomer
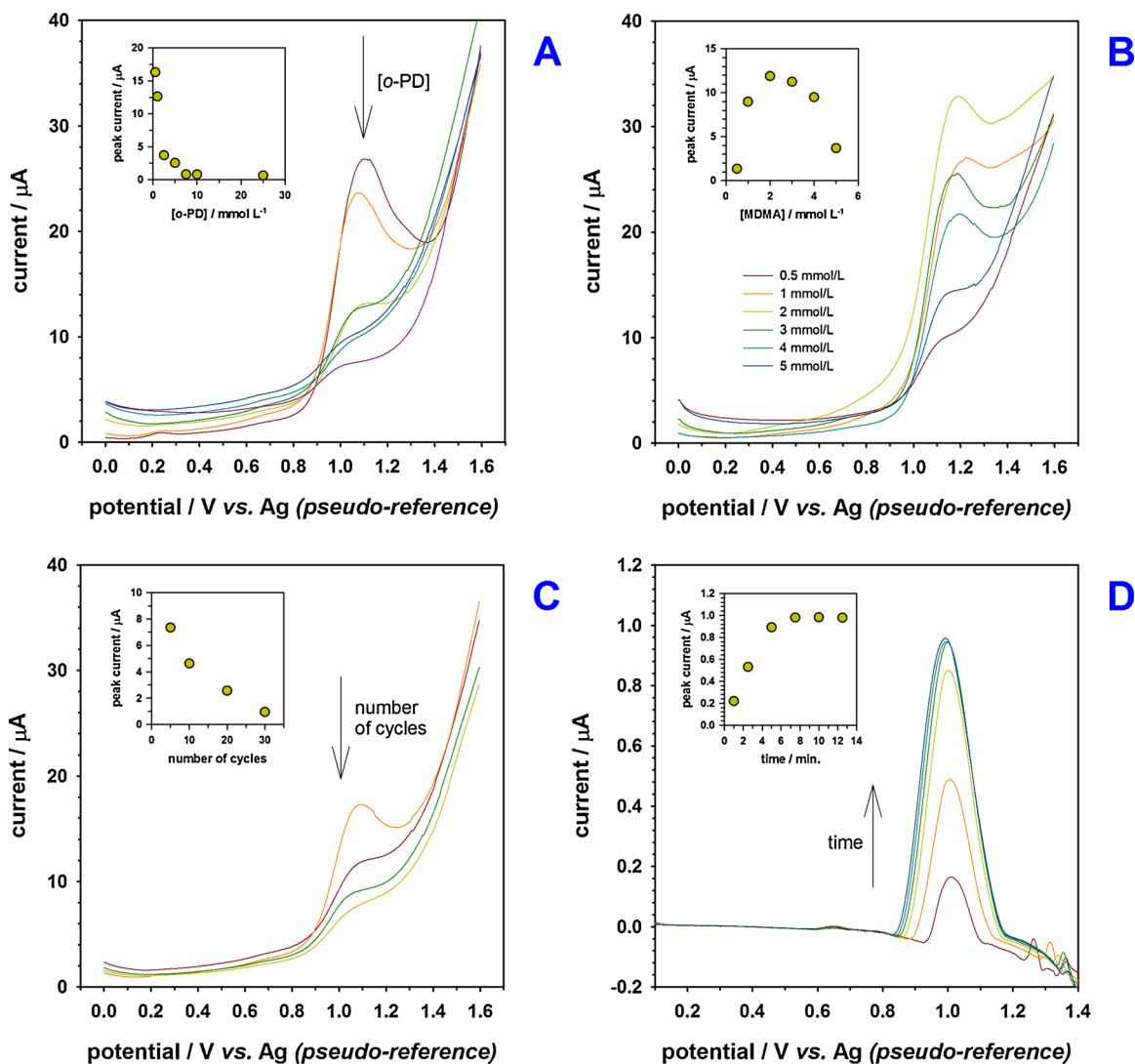

Fig. 3. A) Optimization studies, peak current obtained by SWV of an MDMA solution, 0.1 mmol L^{-1}, at pH = 5.0: A_1) with different *o*-PD concentration (polymerization conditions: 20 cycles, 2.0 mmol L^{-1} MDMA and 10 min of incubation); B) with different MDMA (template) concentration (polymerization conditions: 20 cycles, 0.50 mmol L^{-1} *o*-PD and 10 min of incubation); C) with different number of cycles of polymerization (polymerization conditions: 0.50 mmol L^{-1} *o*-PD, 2.0 mmol L^{-1} MDMA and 10 min of incubation); D) with different incubation time (polymerization conditions: 0.50 mmol L^{-1} *o*-PD, 2.0 mmol L^{-1} MDMA and 20 cycles), the CVs had their baseline corrected by the 'moving average' feature present in the GPES software.
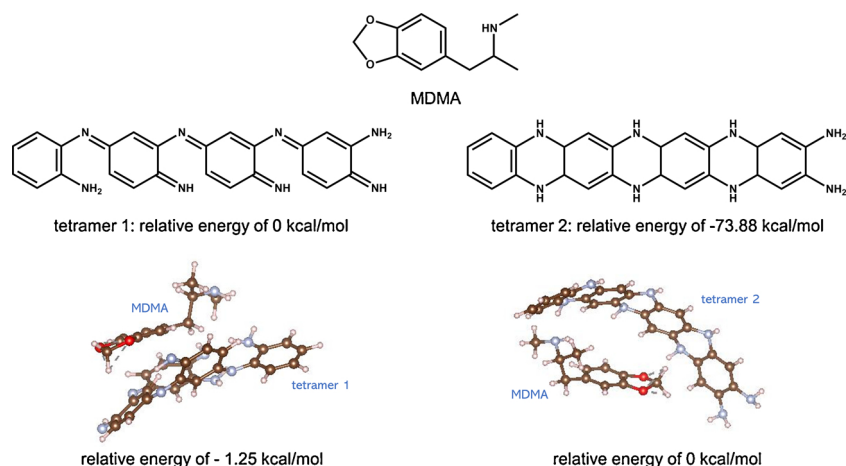
Fig. 4. Chemical structures of MDMA, ramified and linear tetramers of *o*-PD (tetramer 1 and 2, respectively). Relative energies and model for the most stable MDMA-tetramer conformer.

aromaticity in the first case but not in the second – but also their torsional abilities and the availability of terminal nitrogens able to non-covalently interact with the substrate, and thus contribute to the "lock and key" selectivity to MDMA.

Our computational study consisted of, initially, the systematic prospection of the potential energy surface of the many possible configurations of non-covalently interacting pairs specified below, *o*-PD tetramer 1 and MDMA and tetramer 2 and MDMA, and then geometry optimizations of the most stable conformers with Quantum Mechanical methods, in order to find the most favourable interaction in each case. The computational strategy used would be described as a "ladder" of successive calculation methods, approximating more precisely at each level the correct quantum-mechanical description of the total and interaction energy of the studied compounds. Such use of successive approximations is a standard computational protocol necessary to keep the vast number of calculations necessary for a systematic study of the wide range of possible conformers at feasible computational cost. As stated earlier, for initial conformer search and geometry optimizations, Spartan's systematic conformational analysis algorithm, mixing Monte Carlo and Molecular Dynamics was used, and in both cases, ten thousand conformers were searched; all with energies low enough to exist at a room-temperature Boltzmann distribution were kept, resulting in 5000 and 1100 conformers for the tetramers 1 and 2, respectively.

All of them were then geometry-optimized using Spartan's implementation of the PM6 semi-empirical method [70]. The PM6 method offers a more precise description than the pure molecular mechanics description used in the first "rung" of our investigation, the systematic conformer search employing the MMFF. In our second "rung", the geometry optimizations using PM6, we were able to investigate with higher precision the relative energy of the thousands of acceptable conformers, in order to select the most stable among them for further investigations.

As previously exposed in less detail, the twenty most stable conformers for each case were then geometry-optimized using the Gaussian09 package [49] with a yet more precise level of theory, a third "rung" in our ladder, the Hartree-Fock method. For this third step, the 6-31G(p) basis set [50,51] was used, alongside the PCM continuum model, for the simulation of the dielectric constant of the solvent used in the synthesis environment, water. Subsequently, the 10 most stable candidates for each case (the twenty conformers and respective energies are shown in Supporting information) were geometry-optimized in Gaussian09 using the fourth and highest-precision "rung" in our ladder: the B3LYP exchange-correlation functional with Grimme's D3 correction for dispersion interactions [52–54], a bigger 6-31G(d,p) basis set with polarization functions on hydrogen atoms to capture hydrogen bonding [50,51], and again the PCM model for water. Finally,

bonding and reaction energy for the most stable configuration in each case was calculated by geometry-optimizing the interacting molecules separately with the same methods, of the last step, that is, of our fourth "rung", and subtracting their separate energies from the total energy of the interacting molecules. Two such calculations were performed. The former involves the reaction energy for each tetramer formation. As the tetramer formation involves the condensation of four *o*-PD monomers with loss of six hydrogen molecules, both were optimized separately. The later was the separate calculation of both tetramers 1 and 2 in their final conformation in the interaction with the MDMA substrate.

For all the "fourth rung", B3LYP-D3 calculations, the stability of the molecule, that is, the potential energy surface (PES) minimum character of the resulting geometry, was confirmed by frequency calculations – that is, calculation of the Hessian matrix for the resulting forces in each geometry.

We can see that the so-called ramified tetramer, which we named tetramer 1, has the strongest interaction with the substrate (Fig. 4). The $1.25 \, kcal \, mol^{-1}$ difference in interaction energies is enough to strongly indicate that the majority of the MDMA molecules is interacting with the ramified tetramer, as indicated by previous literature [66]. However, the vast difference in formation energy for both tetramers heavily favours the formation of the so-called linear tetramer, which we named tetramer 2. This indicates that the tetramer 2 has a much larger population.

We should highlight that a $1.25 \, kcal \, mol^{-1}$ difference, when understood from the standpoint of Boltzmann populations, which follow an exponential trend, indicate a clear selectivity in the MDMA-MIP interaction favoring the ramified tetramer. While no specific interactions – e.g., strong hydrogen bonds – seem responsible for such a selectivity, it stands out that the ramified tetramer is able to fold using the MDMA molecule as a template. We are led to ascribe the MDMA-MIP stability to a sum total of non-covalent weak interactions able to shape the MDMA-MIP complex (see Fig. 4). The linear tetramer is less able to provide such a stabilization, as we note from the optimized geometries in Fig. 4. However, it is clearly influenced by the presence of the MDMA molecule, which breaks the tetramer's linearity even if that means apparently straining it, and despite its aromaticity. In other words, the interaction of the MDMA with the linear tetramer is also strong, to the point of compensating a disturbance of the latter's aromatic stabilization. This brings us to a second point of relevance in our results: the intrinsic stability of the linear tetramer, which is much larger than that of the ramified one, suggests that it is present in a much larger population. If we take into account that its interaction with MDMA is also strong, even if not the most favorable between the two isomers, we are pushed to consider that the linear tetramer also plays an important role in the electrode's detection ability. Most significantly, however, is the

discrepancy between intrinsic stability of the tetramers and their capacity to bind MDMA, which follow separate trends. Such a discrepancy could not be captured by an analysis of the interaction of monomers alone with MDMA, which is the most used protocol in theoretical modelling of MIPs. The interaction of monomers alone, which does not worry to look at the intrinsic stability of the polymer, may leave the greatest energetic contributions to the system out of the model, and be the source of important misevaluations. This would be even more the case in the present study, in which o-PD monomers are their own cross-linkers, and the stability of the system as a whole is greatly influenced by the intrinsic stability of the polymer chain.

Although this simple model is able to differentiate the interaction energy between the MDMA and the two tetramers, it is not sufficient to investigate the synthesis process, as it is not considering the transition states, and the associated activation energy necessary to build a complete energy profile of the reaction mechanism. As such, it cannot inform the rational design of one or another polymerization process, but offers important insights into the relative weights of the intrinsic stability of the polymer and the strength of its interaction with the analyte. It also throws further light into the nature of o-PD polymers, which had so far been inconclusive from experimental studies.

Finally, a more complete model, involving the prospection of a potential energy surface for the conformation of o-PD monomers around MDMA and other tested substrates and the reaction mechanism for the tetramer formation is under development, and will potentially provide further insight into the process of MIP synthesis and selectivity.

3.3. Analytical performance and sample analysis

Under optimized conditions, the sensor was tested with different concentrations of MDMA by SWV (Fig. 5). SWV was the electroanalytical technique applied due to the better sensibility it often allows [71–77]. The corresponding calibration curve (n = 11) had the following analytical parameters: r^2 of 0.9990; peak current (μA) = (89.0 ± 1.6) × 10^{-3} [MDMA] (μmol L^{-1}) + (61 ± 20) × 10^{-3}; limit of detection (LOD) and quantification (LOQ) of 0.79 and 2.6 μmol L^{-1} (0.15 and 0.51 mg L^{-1}), respectively; linear range up to 200 μmol L^{-1} (0.39 g L^{-1}). LOD and LOQ were calculated as three and ten times the

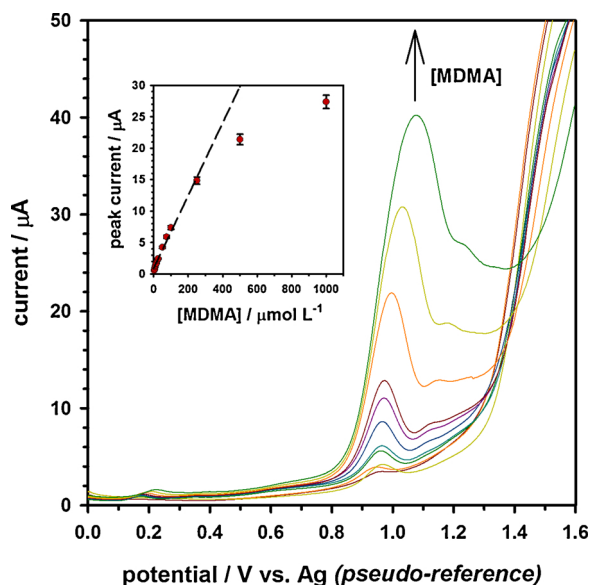**Fig. 5.** The SWV curves of the sensor after 10 min of incubation with 50 μL MDMA solution at different concentrations: 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100 and 200 μmol L^{-1}. SWV was performed in the potential range of 0.0 to +1.6 V, at a frequency of 25 Hz. Polymerization conditions: 0.50 mmol L^{-1} o-PD, 2.0 mmol L^{-1} MDMA and 5 scanning cycles. Inlay: the corresponding calibration curve.

Table 1
Selectivity experiments, all compounds were analysed with a concentration of 100 μmol L^{-1}.

compound	structure	peak current /μA	α
MDMA		7.38	–
dopamine		1.31	5.6
epinephrine		< LOD	–
norepinephrine		< LOD	–
tyramine		2.64	2.8
amphetamine		< LOD	–
metamphetamine		< LOD	–
caffeine		< LOD	–

standard deviation of the intercept/slope, respectively.

The selectivity of the developed MIP was studied in a comparison with the endogenous molecules epinephrine, norepinephrine and dopamine as well as tyramine, amphetamine, methamphetamine and caffeine, which are structurally similar to MDMA. The results are summarized in Table 1 and the SWVs are shown as Fig. S3 in the Supporting information. For each compound, the selectivity factor (α) was calculated by dividing the MDMA peak current by the other compound's peak current. It can be observed that only tyramine and dopamine could potentially give a positive result out of the tested compounds. However, tyramine oxidizes at a lower potential, thus being eletroanalytically distinguishable, and, besides, in both cases their concentration in body fluids is normally substantially lower than MDMA's [78,79], so in practice they are not expected to meaningfully interfere.

The produced SPCE-MIP sensors were studied in terms of repeatability, reproducibility and stability. Repeatability was obtained by intra-day analysis of a MDMA solution using the same electrode, which was subjected to the extraction step between each measurement. Reproducibility was determined by inter-day measuring a MDMA solution using newly obtained electrodes. The stability study was conducted testing a MDMA solution using the same electrode over several days. All studies were performed using a 100 μmol L^{-1} MDMA solution; values 2.6% (n = 5), 7.7% (n = 4) and a variation of less than 4.5% along 28 days, were found to repeatability, reproducibility and stability, respectively.

MDMA is an electroactive compound and one can find a few works concerning its electroanalysis, specifically using a glassy carbon electrode (GCE) [26], a GCE modified with cucurbit[6]uril [25], and screen printed graphite electrodes [28]. Literature suggests that MDMA voltammetric oxidation in solution, around +0.9 V at pH 7 [28], occurs at the aromatic forming a cation radical that dimerizes [26]; radical dimerization preceding further molecule oxidation or reduction is a common way of annulling radicals [80]. The LODs in the corresponding analysed matrices can be found in Table 2, however the selectivity of this methodology, due to the presence of MIPs, is a clear advantage.

Table 2
Electroanalytical studies aimed at MDMA determination.

technique	LOD/$\mu mol\,L^{-1}$	LOQ/$\mu mol\,L^{-1}$	matrix	reference
SWV, using a SPCE-MIP	0.79	2.6	supporting electrolyte	This work
	6.4	21	urine	
	3.9	13	serum	
CV, using a GCE modified with cucurbit[6]uril	2.7	9.1	supporting electrolyte	[25]
SWV, using a GCE	1,2	3.7	supporting electrolyte	[26]
	2.4	8.3	serum	
SWV, using a gold electrode	30.9[a]	–	urine	[27]
DPV, using a SPCE	0.21	–	supporting electrolyte	[28]

[a] The LOD was not calculated, the value is based in the lower limit of the calibration curve.

The proposed MIP-SPCE sensor was applied to the quantification of MDMA in biological matrices, namely human blood serum and urine samples (calibration curves are shown as Fig. S4 in the Supporting information). Recovery tests experiment was performed by spiking the samples with MDMA, followed by dilution with acetate buffer solution pH 5, a recovery of (81.0 $\pm$ 2.4) % was obtained with diluted urine (1:10) and (91.4 $\pm$ 1.1) % with diluted serum (1:20). Samples were not subjected to any additional pre-treatment steps. The calibration curves (n = 5) had the following analytical parameters: a) urine - r^2 of 0.998, peak current (μA) = (100.9 $\pm$ 2.7) $\times 10^{-3}$ [MDMA] ($\mu mol\,L^{-1}$) + (0.16 $\pm$ 0.17), LOD and LOQ of 6.4 and 21 $\mu mol\,L^{-1}$ (1.2 and 4.1 mg L^{-1}), respectively, with a linear range of up to 100 $\mu mol\,L^{-1}$ (0.19 g L^{-1}); b) blood serum – r^2 of 0.9997, peak current (μA) = (42.37 $\pm$ 0.95) $\times 10^{-3}$ [MDMA] ($\mu mol\,L^{-1}$) − (73 $\pm$ 69) $\times$ 10^{-3}, LOD and LOQ of 3.9 and 13 $\mu mol\,L^{-1}$ (0.75 and 2.5 mg L^{-1}), respectively, with a linear range of up to 100 $\mu mol\,L^{-1}$ (0.19 g L^{-1}). Both LODs and LOQs were calculated as three and ten times the standard deviation of the intercept/slope, respectively.

In fatalities associated with MDMA, levels between 0.6 and 4.3 mg L^{-1}, in admission, and post-mortem levels between 0.5 and 28.4 mg L^{-1} were found [81]. Another publication attributes death to the toxic effects of MDMA alone with a range of 0.5–54 mg L^{-1} (13 cases) [82].

4. Conclusions

In this work, it was successfully shown that it is possible to obtain a selective electrochemical sensor for MDMA using a SCPE-MIP. A poly(o-PD) film was generated at the surface of a SPCE by electro-polymerization of the monomer o-PD as a MDMA recognition element. To the best of our knowledge, electrochemical determination of MDMA based on an electropolymerized selective sensor has not been reported before. Experimental parameters that affected the performance of the SPCE-MIP sensor were studied and optimized, confirming electro-analytical properties such as linearity, selectivity (in comparison with endogenous and non-endogenous compounds with similar chemical structures), stability, repeatability and reproducibility. Furthermore, the sensor was applied in the analysis of MDMA in biological samples, namely human blood serum and urine.

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Rosa A.S. Couto, PhD student in Pharmaceutical Sciences in the Faculty of Pharmacy of the University of Porto through the PhD Program in Medicines and Pharmaceutical Innovation (i3DU). PharmD from the University of Porto, in 2014. Her research interests comprise the study of the electrochemical behaviour of pharmaceuticals and the development of electrochemical portable sensors for clinical and forensic purposes.

Séfora S. Costa, MSc student in Pharmaceutical Sciences in the Faculty of Pharmacy of the University of Porto. Her research interests include the study and development of portable sensors with several medical applications based in electrochemistry.

Bassim Mounssef Júnior is a PhD student at the Chemistry Institute of the University of São Paulo (USP) since 2017, studying, from a computational standpoint, zeolite-supported catalysis. He has graduated as a bachelor in Chemistry in 2016 and worked for two years as an undergraduate intern at the Applied Physics Department of USP's Physics Institute, working with techniques for the characterization of skutterudites, such as x-ray diffraction and x-ray absorption spectroscopy.

João G. Pacheco obtained his PhD in Chemistry in 2010 (Faculty of Sciences, University of Porto, Portugal). Currently he is a postdoctoral research in REQUIMTE and his present research interests include the development of analytical (electrochemical) methodologies based on the use of molecularly imprinted polymers for pharmaceutical, clinical, and food analysis.

Eduarda Fernandes, PharmD; PhD, is Associate Professor with Aggregation, at the Faculty of Pharmacy, University of Porto, and coordinates the Free Radicals and Antioxidants Unit at LAQV, REQUIMTE. Her research interests include the mechanistic toxicology of drugs of abuse and the development of methodologies for the study of pro-oxidant and pro-inflammatory processes, and related diseases. She is co-author of more than 170 publications in international peer journals (ca. 5500 citations, h-index 39) (https://sigarra.up.pt/ffup/en/FUNC_GERAL.FORMVIEW?p_codigo=238253).

Félix Carvalho, PharmD, PhD, is Full Professor in the Faculty of Pharmacy, University of Porto. He is presently Vice-President of the Portuguese Society of Pharmacology, President-Elect of EUROTOX, and Member of the Committee for Evaluation of Medicines at the National Authority of Medicines and Health Products (INFARMED). His main area of research is Toxicology, with a special interest in drugs of abuse. During the past 25 years, he has published about 300 scientific articles/book chapters (ca. 12,700 citations, h-index 58), and 7 patents (https://sigarra.up.pt/ffup/en/FUNC_GERAL.FORMVIEW?p_codigo=213232).

Cecília M.P. Rodrigues is Director of the Research Institute for Medicines (iMed.ULisboa, http://imed.ulisboa.pt/), Faculty of Pharmacy, University of Lisbon, Portugal. She received a PhD in Pharmacy from the University of Lisbon in 1996, after training at the University of Cincinnati, OH, followed by a postdoc at the University of Minnesota, MN, USA (1996–99). She is currently Full Professor and Group Leader. Her research interests aim at identifying novel mechanism-based molecular targets for therapeutic intervention, involved in cell death, differentiation and proliferation. Rodrigues received several prizes, including the University of Lisbon Scientific Prize 2016, and published 8 book chapters, ca. 200 papers, and 4 global coverage patents (h-index: 58; citations: ca. 19,000) (http://imed.ulisboa.pt/cv/cecilia-maria-pereira-rodrigues/).

Cristina Delerue-Matos obtained her PhD in Chemical-Physics, specialty in electrochemistry, in 1990. At the moment, she is principal coordinator professor at the School of Engineering of the Polytechnic Institute of Porto (ISEP-IPP, Portugal) and also coordinates the REQUIMTE/ISEP research group (www.graq.isep.ipp.pt). Her research interests include the development of analytical methodologies for environmental, food, pharmaceutical, biochemical and industrial control. She is co-author of more than 325 publications in international peer journals.

Ataualpa A.C. Braga obtained his PhD in Chemistry from Universidade Estadual de Campinas (UNICAMP), in 2004. In 2005, he joined the Feliu Maseras group at the Institute of Chemical Research of Catalonia (ICIQ), Tarragona, Spain. He returned to UNICAMP in 2011, obtaining a permanent position as professor at Universidade de São Paulo (USP) in 2012. His main field of interest is the application of methods of computational chemistry to the theoretical study of homogeneous catalysis. He has published around 60 articles (ca. 2600 citations, h-index 20).

Luís Moreira Gonçalves, BSc and PhD in chemistry (both from the University of Porto, in 2007 and 2011, respectively), and MD (University of Minho in 2015). In 2018, he became a professor in the University of São Paulo (USP). He has several research interests including the development of biosensors for medical applications. He has around 70 published papers (more than 1000 citations, h-index 17) and 4 patents, he has won several prizes including the Young Researcher in Electrochemistry attributed by the Portuguese Electrochemical Society in 2012 (http://www.iq.usp.br/lmgoncalves).

M. Beatriz Quinaz, lecturer in the Faculty of Pharmacy of the University of Porto. BSc and PhD in pharmaceutical sciences (both from the University of Porto, in 1984 and 1996, respectively). Her main research topics are the development of voltamperommetric techniques based in flow systems and the design and construction of biosensors for medical purposes. She has published around 20 papers (ca. 324 citations, h-index 10).