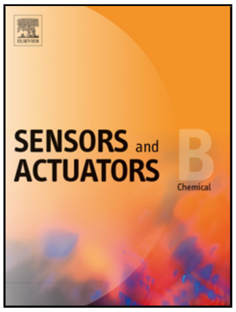

Title: Molecularly Imprinted Silica Films Prepared by Electroassisted Deposition for the Selective Detection of Dopamine

Author: María Porcel-Valenzuela Alfonso Salinas-Castillo
Emilia Morallón Francisco Montilla

PII: S0925-4005(15)30220-3
DOI: http://dx.doi.org/doi:10.1016/j.snb.2015.08.042
Reference: SNB 18897

To appear in: *Sensors and Actuators B*

Received date: 14-4-2015
Revised date: 17-7-2015
Accepted date: 10-8-2015

Please cite this article as: M. Porcel-Valenzuela, A. Salinas-Castillo, E. Morallón, F. Montilla, Molecularly Imprinted Silica Films Prepared by Electroassisted Deposition for the Selective Detection of Dopamine, *Sensors and Actuators B: Chemical* (2015), http://dx.doi.org/10.1016/j.snb.2015.08.042

Molecularly Imprinted Silica Films Prepared by Electroassisted Deposition for the Selective Detection of Dopamine

María Porcel-Valenzuela[1], Alfonso Salinas-Castillo[2], Emilia Morallón[1], Francisco Montilla[1,]*

[1]*Dept. Química Física e Instituto Universitario de Materiales, Universidad de Alicante, Ap. 99, E-03080, Alicante, Spain*

[2]*ECsens, Department of Analytical Chemistry, Campus Fuentenueva, University of Granada, 18071 Granada, Spain*

*Corresponding author: francisco.montilla@ua.es. Tel/fax + 34 9695909820

Abstract:

Dopamine (DA) can be detected by electrochemical oxidation in conventional electrodes. However, the presence of other oxidizable species (interferents) usually present in physiological fluids at high concentrations (like ascorbic acid) makes very difficult its electrochemical detection. In the present work, glassy carbon electrodes have been modified with Molecularly Imprinted Silica (MIS) films prepared by electroassisted deposition of sol-gel precursors. The production of MIS films was performed by adding the template molecule (DA) to the precursor sol. The molecular impression of silica was assessed showing a high coherency allowing a filtering capacity in the molecular scale. The MIS-modified electrodes present a high selectivity for the detection of DA in neutral or acidic solutions. The MIS-modified electrodes allow the amperometric determination of dopamine in solutions containing ascorbic acid with molar ratios lower than 1:50000.

Keywords: molecular imprinting; electrodeposited silica; dopamine, ascorbic acid; electrochemical sensor

1. Introduction

Molecularly imprinting is one of the best-known techniques to generate synthetic polymers with recognition sites for a given molecule. The production of novel molecularly imprinted polymers is through the synthesis of highly cross-linked polymers in presence of “printing” molecules (called templates). Thus, the subsequent removal of the template allows for using the polymer as selective medium for printed molecules or structurally related compounds[1]. However, more research needs to be done to increase the direct application of molecularly imprinted materials to the generation of new analytical sensing devices. [2–11]

Although, these materials are considered critical for the production of diverse chemical sensors, there are only few studies about the application of these systems on different transduction systems such as piezoelectric, electrochemical or luminescent devices[1,12–14]

Usually molecularly imprinted polymers for electrochemical applications are made of acrylic, vinyl or siloxanic networks deposited over suitable electrode substrates by standard film processing methods, such as, spin-coating, photografting, silanization, self-assembling, etc.[2,4–7,9,10,15]These films often suffer from the presence of pinholes because film does not grow uniformly over the electrode with standard techniques.[16] It drives to a lowering of the performance of the resulting electrochemical sensor. Then, the use of electrochemical deposition methods can improve the performance of the modified electrodes by obtaining films that are more homogeneous.

The detection and quantification of neurotransmitters in physiological fluids is a sticking point of electroanalytical science. Many catecholamine-based neurotransmitters can be detected by electrochemical oxidation in conventional electrodes[17–21]. However, the presence of other oxidizable species in physiological fluids at high concentrations strongly masks the oxidation of neurotransmitters. An archetypical approach, which reproduces the conditions of this detection method, is the electrochemical determination of dopamine (DA) in the presence of ascorbic acid (AA). DA is easily oxidized with conventional electrodes (such as GC, graphite, platinum, etc.)[22–28]. However, there are a number of problems associated to the electrochemical detection of DA due to the oxidative reaction of this compound. One of the primary problems is the presence in physiological fluids of other species that undergoes oxidation at similar potentials than DA, being the main interferents AA or uric acid. The major problem comes since concentration of dopamine in the extracellular fluids, is extremely low (0.01-1 μM) for a healthy individual while the concentrations of the main detection interferents are several orders of magnitude higher (30-90 μM for AA). Therefore the concentration ratio for DA:AA in blood plasma is around 1:10000 or lower.[29,30] In order to improve the selectivity of the electrodes for this application big efforts have been made to modify the electrode surfaces by different strategies, including graphene, nanoparticles, conducting polymers, self-assembled monolayers, etc. [31–34]These modified electrodes obtained good selectivity for ranges of DA:AA concentration ratio of 1:1000 in the best case.[33,35–37]

This work presents a novel method for the preparation of modified electrodes with Molecularly Imprinted Silica (MIS) for the selective electrochemical detection of dopamine. The MIS layers were prepared over suitable electrodes by electroassisted deposition method. The method for electroassisted deposition of silica has been extensively studied for the preparation of modified electrodes for several applications, including nanolithography, enzyme immobilization, ion-exchangers and electroanalysis, among others.[38–42]

The application of negative current for the reduction of the solvent containing a silica precursor in the presence of a molecular template produces a silica film with encapsulated template molecule. The removal of the template (also performed by electrochemical method in the present work) produced a molecularly imprinted silica film, with improved selectivity towards the selected analyte. The present work is focused on the electroassisted synthesis of molecularly imprinted silica sol-gel materials, using DA as model compound, but the procedure can be extended to other species of electroanalytical interest.

2. Experimental part

Tetraethyl orthosilicate (TEOS, Sigma Aldrich, reagent grade), ethanol (Sigma-Aldrich, p.a.), potassium chloride (Merck, p.a.), dopamine hydrochloride (Sigma-Aldrich), L-ascorbic acid (Sigma-Aldrich, reagent grade), hydrochloric acid (Merck, reagent grade), potassium dihydrogen phosphate (Merck), potassium phosphate dibasic (Sigma-Aldrich) and sulfuric acid (Merck, p.a.) were used as received. All solutions were prepared with purified water obtained from an ELGA Lab Water Purelab system (18.2 MΩ·cm).

Electrochemical experiments were performed in conventional electrochemical glass cells. The working electrode was a glassy carbon rod (geometric area=0.07 cm^2, GC, Carbone Lorraine, model V-25). The GC electrode was carefully polished with emery paper and subsequently rinsed with distilled water. A platinum wire was employed as counter electrode, and a reversible hydrogen electrode introduced in the same electrolyte solution placed in Luggin capillary was used as reference electrode.

The surface morphology of the modified glassy carbon electrodes was studied by scanning electron microscopy (SEM, JEOL JSM-840).

3. Results and discussion

3.1. Electroassisted deposition of Molecularly Imprinted Silica (MIS)

A sol-gel silica precursor solution was prepared by mixing 6 mL of TEOS, 8.2 mL EtOH and 5.8 mL of 0.01 M HCl+0.46 M KCl solution in a glass vial. KCl is necessary to provide conductivity to the solution in order to perform the electrodeposition of silica gel. This solution was stirred for 1 hour for the hydrolysis of the TEOS. For the electroassisted deposition of silica a polished GC electrode was immersed in the precursor solution. The application of reduction potentials to values more negative than –1.5 V induces the hydrogen evolution reaction. The concomitant increase of pH near the electrode accelerates the gelification of the silica colloids nearby the electrode, producing a silica deposit on it. This deposition method produces uniform and coherent silica films avoiding the formation of pinholes.[43,44]

When the deposition is performed in solutions in absence of the template molecule (dopamine) the films obtained are labelled as Non-Imprinted Silica (NIS). Different conditions for the galvanostatic step were checked with current densities ranging between –5 to –20 mA cm^{-2}. After the preparation of the NIS film, the electrode was rinsed with abundant ultrapure water and immersed in a 0.5 M H_2SO_4 blank solution.

The electrode is submitted to 10 voltammetric cycles in order to clean its surface. This electrochemical treatment is similar to that performed to MIS film as indicated below.

Figure 1 shows SEM images of NIS-modified GC electrode. The film deposited at –10 mA cm^{-2} (deposition charge 150 mC cm^{-2}) is composed by aggregated silica colloids with spherical shape and diameters ranging between 40-300 nm. Films formed at more negative currents (–20 mA cm^{-2}) resulted in more cracked surface due to the higher rate of hydrogen bubbling produced on the electrode surface upon reduction of proton. The colloids deposited at this current are more heterogeneous with aggregated silica particles ranging between 80 and 700 nm.

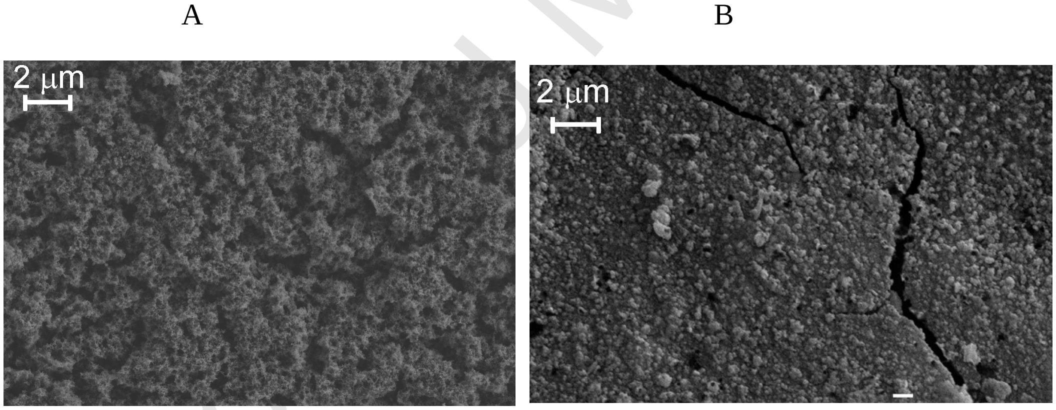

Figure 1: SEM micrographs of NIS-modified electrodes synthesized at different potentials. (A) NIS-prepared at –10 mA cm^{2}; (B) NIS-prepared at –20 mA cm^{2}.

Other set of silica films were deposited on GC electrode after the addition of 0.1M dopamine (DA, template molecule) in the silica precursor solution. Those films are labelled as MIS. The deposition of MIS films was performed galvanostatically in similar conditions than the NIS films. After the preparation of the MIS film, the electrode was rinsed with abundant ultrapure water. However, the deposited silica retains DA molecules within its pores as demonstrated by the cyclic voltammograms of the MIS-modified electrodes in a blank solution (see figure 2). The voltammogram during the first positive sweep shows an anodic current from 0.5V that corresponds to the oxidation of DA. During the negative going sweep, a reduction peak is observed at 0.88V.

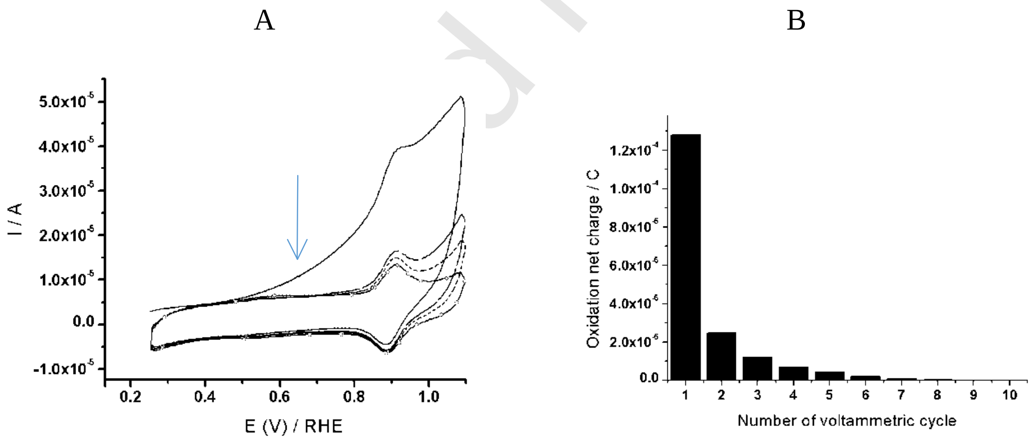

Figure 2: (A) Successive cyclic voltammograms of a MIS-modified electrode immersed in a solution 0.5 M H_2SO_4. Scan rate 100 mV s^{-1}. (B) The net oxidation charge as a function of the number of cycle is represented in the bar diagram.

The cyclic voltammograms after the first cycle of MIS-modified electrodes immersed in a 0.5 M H_2SO_4 solution still show anodic currents at potential more positive than +0.9 V corresponding to DA oxidation (figure 2.A). The corresponding reduction process is observed in the negative scan at +0.88 V. The anodic charge is always higher than the cathodic charge during the first 8 cycles, indicating a degradative oxidation of the DA species entrapped (fig. 2.B). The anodic current decreases and a stable redox processes is clearly observed at +0.9 V after nine potential cycles. This experiment can be used to leach out the DA from the entrapped site in the silica film. Thus, this electrochemical treatment is proposed to extract the template molecule from MIS film.

Figure 3 shows SEM images of DA-molecularly imprinted silica deposited on GC electrodes at –10 mA cm^{-2}. The presence of DA during gelification produces more heterogeneous aspect of the film and the shape of the colloids are less-defined, becoming more compact films than NIS.

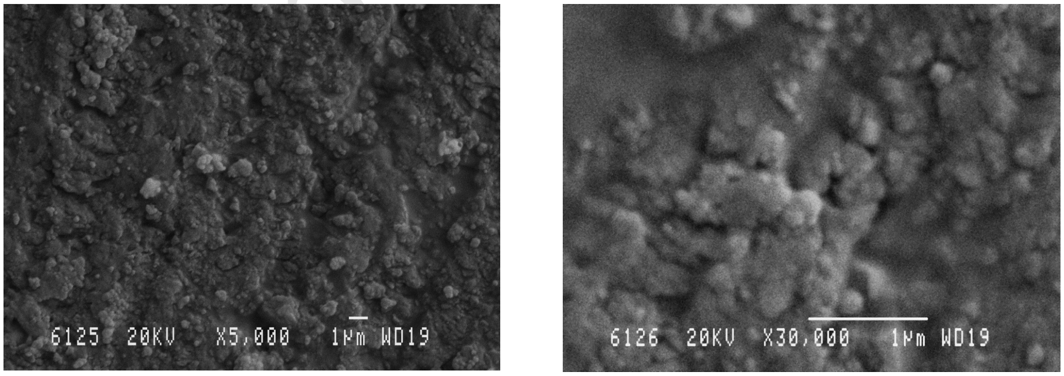

Figure 3: SEM micrographs of MIS-modified GC electrodes prepared at –10 mA cm^{-2}.

3.2. Electrochemical response of dopamine and ascorbic acid

Figure 4 shows the voltammograms obtained for bare GC and NIS-modified GC electrode in different solutions containing DA and AA in a molar ratio (DA:AA) of 1:100. Figure 4.A shows the cyclic voltammogram of a bare GC electrode in a solution containing both analytes in concentration 0.1 mM DA + 10 mM AA in phosphate buffer solution (pH 7). The scan to positive potentials shows an oxidation peak at +0.69 V corresponding to the irreversible oxidation of AA and an oxidation peak at +0.82 V due to the oxidation of DA to dopaminequinone. The reduction peak of dopaminequinone to DA is observed at +0.79 V during the reverse scan.

The oxidation of DA follows a typical ECE mechanism, initially DA is oxidised to dopaminequinone in the scan to positive potentials. The dopaminoquinone suffers a further chemical cyclation to leucodopaminechrome. The redox processes corresponding to the dopaminechrome/leucodopaminechrome transition appear at potentials lower than 0.4V (a potential lower than the selected lower limit, a complete voltammogram is shown in the Supporting information, fig. S1). The appearance of those processes has been related also with the polymerization of dopamine on the electrode surface that drives to the modification of the electrocatalytic performance of the GC surface towards dopamine oxidation. [45]

Figure 4.B shows the cyclic voltammogram of the bare GC electrode in a solution containing DA and AA in 0.5 M H_2SO_4 solution. In this case, the dopamine response is not complicated by the formation of leucodopaminechrome or polydopamine since the chemical step is much slower in acidic pH than in neutral medium.[16,23,46]

As observed, the scan towards positive potentials shows an oxidation peak at +0.63 V corresponding to the irreversible oxidation of AA, and the oxidation peak of DA appears at +0.81 V, overlapped to the diffusion tail of the AA oxidation. In the reverse scan dopaminechrome is reduced in a single peak at +0.79 V.

Figure 4.C shows the cyclic voltammogram of a non-imprinted silica-modified GC electrode (NIS) obtained at current density of –10 mA cm^{-2} immersed in the solution containing DA and AA in phosphate buffer solution (pH 7). The scan to positive potentials shows an oxidation peak at +0.57 V corresponding to the irreversible oxidation of AA and the oxidation peak of DA appears at +0.80 V and its reduction process at +0.76 V in the reverse scan. Then, the redox processes of DA appear poorly defined in this electrode, probably by the formation of polydopamine films that occludes the silica pores and therefore worsens the response of the modified electrode towards the detection of DA.

Figure 4.D presents the electrochemical response of a solution containing DA and AA in acidic solution of a NIS modified electrode obtained at current density of –10 mA cm^{-2}. The scan to positive potentials shows a peak at +0.57 V corresponding to the irreversible oxidation of AA, and at +0.80 V appears the oxidation peak of DA and the reduction counterprocess at +0.78 V during the negative sweep. The latter processes are very well defined and appear clearly separated of the AA oxidation.

The comparison of the voltammograms for bare glassy carbon electrode with those in the presence of silica shows that electrochemically deposited silica modifies the voltammetric response in both media. The oxidation peaks corresponding to ascorbic acid and dopamine present a higher peak-to-peak separation and better definition mainly in the case of the acid solution (Figure 4.D).

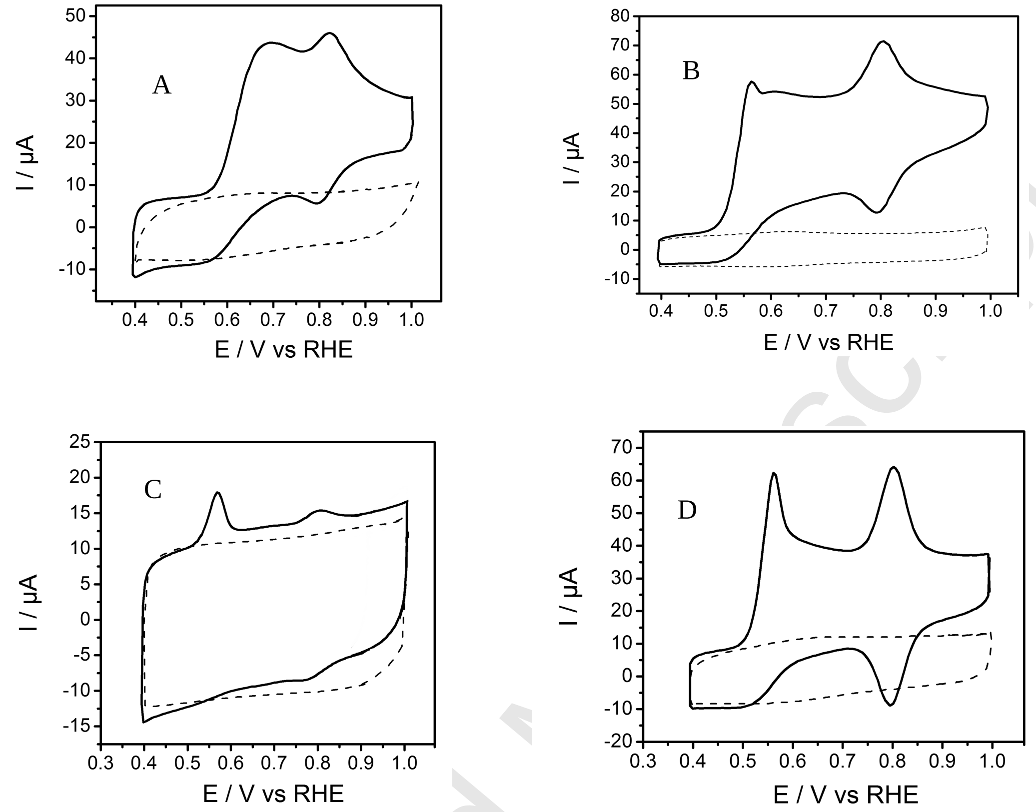

Figure 4: (A) and (B) Electrochemical response of bare GC electrodes inphosphate buffer solution, pH 7 and 0.5M H_2SO_4, respectively. (C) and (D) Electrochemical response of NIS-modified electrode (–10 mA cm^{-2}) in phosphate buffer solution, pH 7 and in 0.5 M H_2SO_4, respectively. Solid lines: solutions containing 0.1 mM DA + 10 mM AA. Dashed lines blank solutions. Scan rate 100 mV s^{-1}.

A Selectivity Parameter (*SP*) for the detection of DA in the presence of AA was defined from the voltammograms obtained, as the ratio of the oxidation faradic current for DA and for AA voltammetric peaks obtained in these conditions (DA:AA concentration ratio 1:100):

$$SP = \frac{I_{DA}}{I_{AA}} \qquad (1)$$

This *SP* has a value of 0.48 for NIS-modified electrode of figure 4.D. The *SP* was obtained for different molecularly imprinted silica films prepared at different reduction currents ranging between –5 to –20 mA cm^{-2}.

The effect of the concentration of molecular template was studied by adding dopamine template to the silica precursor solution at different concentration ranging from 10^{-3} to 0.2 M. The *SP* of the MIS film (SP_{MIS}) was compared with the *SP* of NIS film deposited in the same electrochemical conditions but in absence of template (SP_{NIS}) and an Imprinting Effect Parameter (*IEP*) is defined as:

$$IEP = \frac{SP_{MIS}}{SP_{NIS}} \qquad (2)$$

Therefore, *IEP* =1 indicates that, for electrodes prepared using the same electrochemical conditions, the Selectivity Parameter of the molecularly imprinted electrode is the same than for the electrode prepared in the absence of template molecule, NIS, and no imprinting effect is observed.

Figure 5 shows the contour plot for the value of *IEP* for different silica films prepared by the electrodeposition at a constant current of –10 mA cm^{-2}.

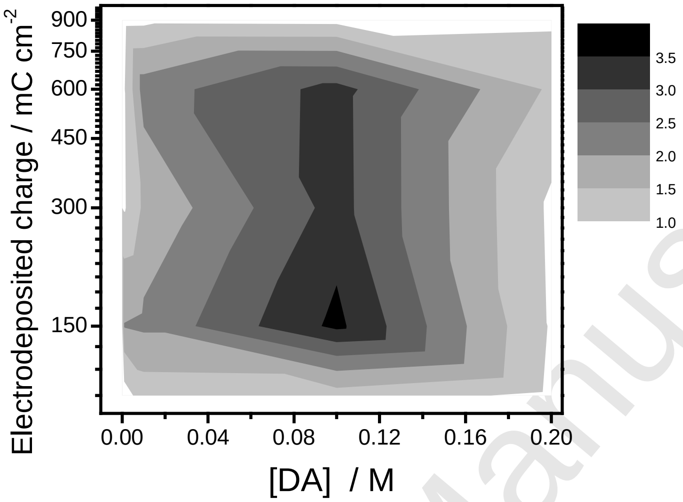

Figure 5: Contour plot for the Imprinting Effect Parameter (*IEP*) of silica films as function of the concentration of the template, dopamine [DA] and the absolute value of electrodeposition charge used (Electrodeposited charge). Deposition current –10 mA cm^{-2}.

The *IEP* is represented as a function of two variables: the dopamine concentration in the template (abscissa) and the amount of electrodeposited silica accounted as electrical charge density passed through the electrode (ordinate).

There are little *IEP* values for concentrations of the template lower than 0.04 M. For those films the *IEP* is always lower than 2, indicating that the behaviour of the MIS films is similar to NIS. The contour plot showing indicates the optimum values of *IEP* (and therefore of selectivity), are obtained for concentration of the template molecule around 0.1 M for all the deposited charges.

From figure 5, the maximum value of *IEP* is obtained for a deposited charge of –150 mC cm^{-2} but it remains high for deposited silica up to –600 mC cm^{-2}. Thicker films drive to a worsening of the selectivity. It can be due to a loss of the coherency of the films from a critical amount of deposited silica, since the film may present a limited adherence to the glassy carbon electrode.The cyclic voltammogram of a MIS-modified electrode prepared in the optimised conditions is presented in figure 6 (charge –150 mC cm^{-2} and concentration of the template 0.1 M) with an *IEP* of 3.62. The figure compares the voltammogram obtained in a solution containing 1 mM DA + 100 mM AA for a NIS-modified (dashed line) and a MIS-modified electrode (solid line) in the same optimal conditions.

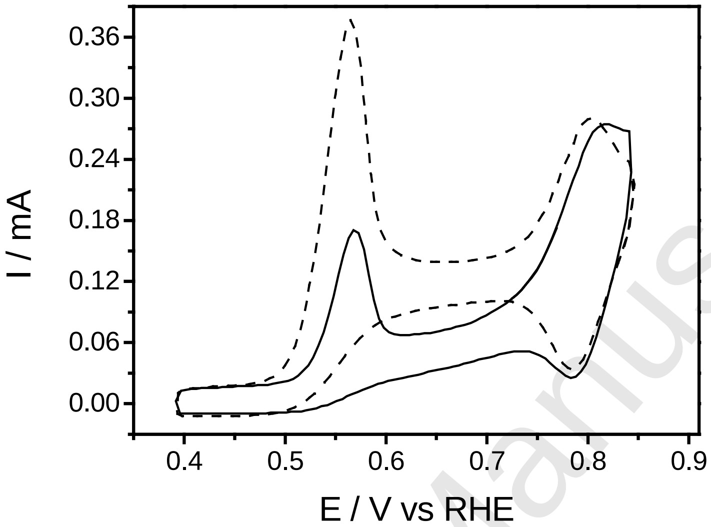

Figure 6: Steady state cyclic voltammogram of NIS-modified (deposition current –10 mA cm^{-2}; charge –150 mC cm^{-2}, dashed line) and MIS-modified electrode (deposition current –10 mA cm^{-2};charge –150 mC cm^{-2} and 0.1M [DA] template, solid line) in a solution containing 1 mM DA + 100 mM AA (concentration ratio 1:100) in 0.5 M H_2SO_4. Scan rate 100 mV s^{-1}.

The MIS-modified electrode presents a better selectivity for the detection of DA than NIS. While the oxidation current for DA is similar for both NIS and MIS-modified electrode, the most important difference is related with the decrease of the intensity for the oxidation peak of AA in the MIS-modified electrode, indicating that this layer acts as selective molecular filter for DA, excluding partially the pass of the AA molecules across the film.

The silica-modified electrodes can be used as a suitable voltammetric sensor for the determination of DA. Figure 7 shows the voltammogram of a MIS-modified electrode immersed after the dosage of different amounts of DA. It can be seen that an increase in dopamine concentration is accompanied by an increase in the oxidation current around +0.80 V.

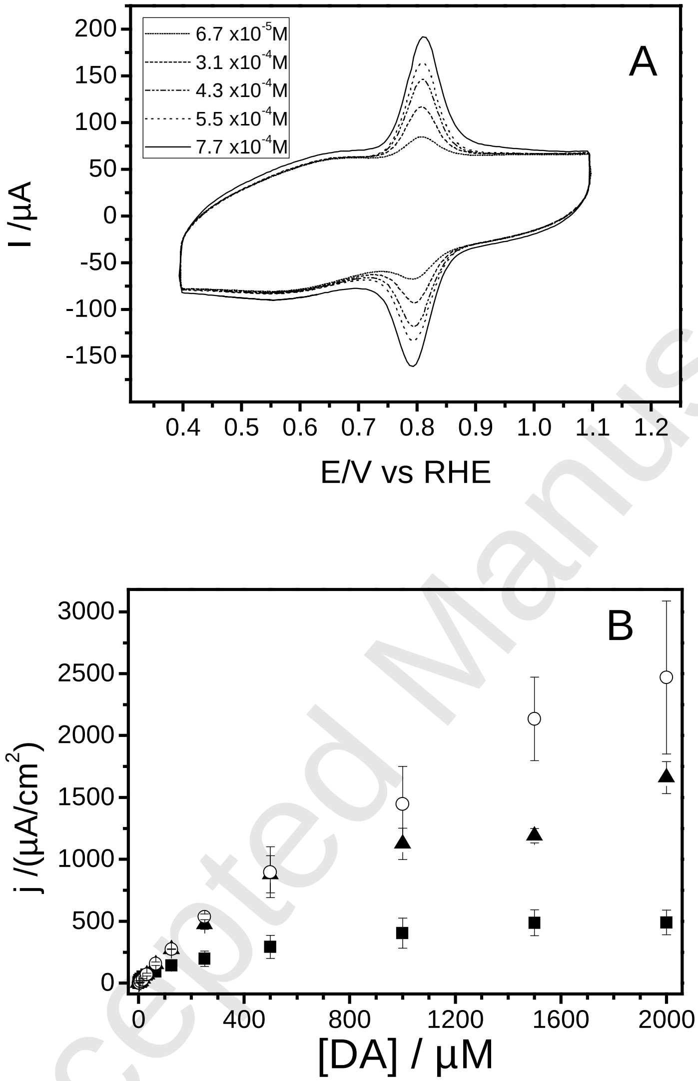

Figure 7: (A) Electrochemical response of the optimal MIS-modified electrode (deposition current –10 mA cm^{-2};charge –150 mC cm^{-2} and 0.1M template) in 0.5 M H_2SO_4 solution after the dosage of different amounts of dopamine. Scan rate 100 mV s^{-1}.(B) Calibration curves for dopamine quantification with a different modified electrodes: GC electrode (squares), NIS-modified electrode (deposition current –10 mA cm^{-2};charge –150 mC cm^{-2} , triangles) and MIS-modified electrode (deposition current –10 mA cm^{-2};charge –150 mC cm^{-2} and 0.1M template, open circles).

Figure 7.B shows the calibration curves obtained for a bare GC electrode and NIS-modified and MIS-modified electrodes. It is remarkable that the bare GC electrode presents a sensitive response against DA for concentration lower than 250 μM but this electrode becomes less sensitive at higher concentration of DA. The slope of the curve below 250 μM can be fitted to a linear response (R^2=0.999) and the sensitivity of the electrode amounts 0.83 μA μM^{-1}. As observed the presence of non-imprinted silica (NIS) over the GC electrode improves the sensitivity towards DA due to the affinity of the silica for this molecule[43] and extends the range of sensitivity up to 500 μM. The sensitivity of the NIS-modified electrode is increased to 2.21 μA μM^{-1}.

The electrodes modified with molecularly imprinted silica present an improved behaviour with respect the bare GC and NIS-modified electrode, as it is observed in figure 7.B, for a MIS-modified electrode obtained in optimised conditions (concentration of template 0.1M). While the sensitivity for concentrations lower than 250 μM slightly increases, with respect NIS-modified electrode (sensitivity 2.24 μA μM^{-1}) the MIS-modified electrode present good sensitivity at higher concentrations up to 2 mM. The molecular imprinting of silica has a positive effect in terms of sensitivity towards DA as observed for other MIS-modified electrodes prepared with different concentrations of DA template, 10^{-4} and 10^{-2} M films (see fig. S2 in the Supporting Information).

The detection limit (LOD) obtained for the MIS-modified electrode is 5.51 x 10^{-7} mol L^{-1} of DA in these conditions and it is calculated as the concentration, which produces an analytical signal 3 times the standard deviation of the blank signal, IUPAC criterion.[47]

Figure 8 shows the voltammogram of the MIS-modified electrode after further addition of AA up to concentrations of 0.1 M, keeping the dopamine concentration fixed at 10 µM. The addition of the amount of interfering agent (in a molar ratio DA:AA 1:10000) slightly affect the measured current of the dopamine.

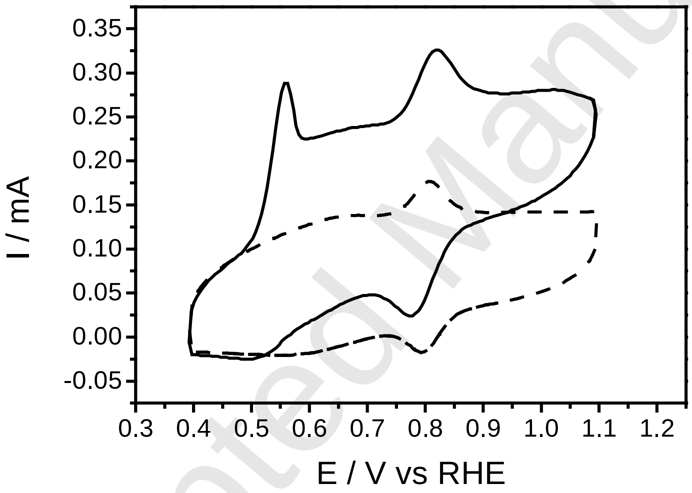

Figure 8: Steady state cyclic voltammogram of MIS-modified (deposition current –10 mA cm^{-2};charge –150 mC cm^{-2} and 0.1M template) in a solution of 10 µM DA (dashed line) and in a solution of 10 µM DA + 0.1M AA (solid line) in 0.5 M H_2SO_4. Scan rate 100 mVs^{-1}.

The MIS-modified electrodes can be used as amperometric sensor for the determination of DA in the presence of AA. Figure 9 shows the chronoamperometric response recorded with a MIP-modified electrode in a solution containing 50 mM AA. The potential of the electrode was fixed at +0.9V during the successive addition of different amounts of DA. After the dosage of the neurotransmitter, an increase of the current can observed, proportional to the concentration of DA.

Further addition of higher concentrations of AA (1 μM) does not affect the measured current during the experiment. The final addition of AA is three orders of magnitude greater than previous DA additions, but current is not significantly affected. We consider that the silica layer is effective as molecular filter for AA.We observe a linear response for a concentration range of DA between 100nM to 1 μM, in a solution containing 50 mM of interfering AA.

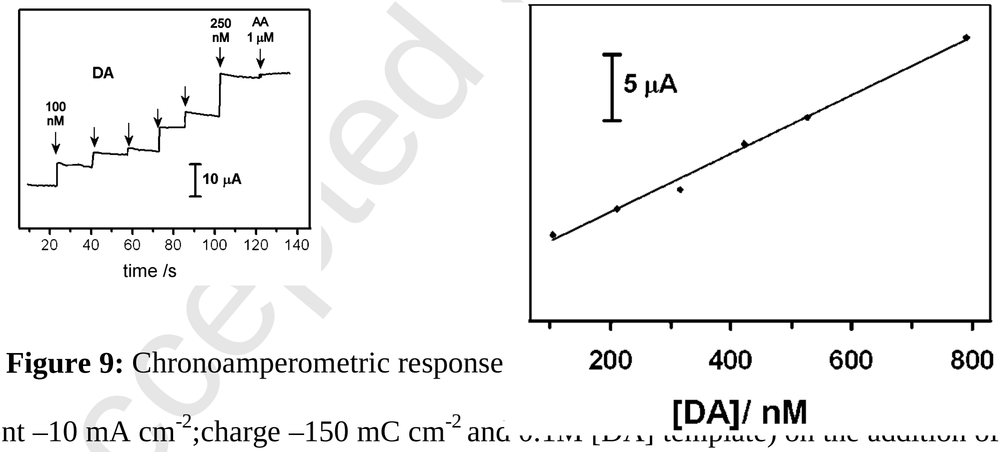

Figure 9: Chronoamperometric response

current –10 mA cm^{-2};charge –150 mC cm^{-2} and 0.1M [DA] template) on the addition of increasing amounts of dopamine. Volume of solution 0.1L. Detection potential +0.9V. Solution 50 mM AA in pH 7 phosphate buffer solution.

The linear regression yields a sensitivity of 22.3(μA/μM) (correlation coefficient of 0.994). The detection limit, calculated as the concentration which produces an analytical signal 3 times the standard deviation of the blank signal was 14 nM of DA in these conditions.

Similar chronoamperometric experiments were performed in acidic medium (0.5M H_2SO_4). A linear response was also obtained for a concentration range of DA between 100nM to 1 μM in the presence of AA. The linear regression yields a sensitivity of 21.0 (μA/μM) (correlation coefficient of 0.996). The detection limit was 57 nM of DA in these conditions.

Table 1 shows the analytical parameters obtained with different modified electrodes appeared in the recent literature applied to the determination of DA in the presence of AA. As observed, the proposed sensor appears to have a high sensitivity in conditions of high concentration of AA.

Further improvements of detection limit of MIS films could be obtained by the introduction of electrocatalytic species within the silica matrix, like carbon nanotubes and/or conducting polymers, following a similar methodology to that propose by us in previous papers.[44,48] Further work is under development in our group in that direction.

Table 1: Comparison of the electrochemical determination of DA in the presence of AA of different modified electrodes reported in the literature.

Electrode modifier	**Limit of detection µM**	**Sensitivity µA/µM**	**Molar ratio DA:AA**	**Ref.**
Carbon nanotube-polypyrrole	0.06	0.086	1:50	[49]
Au- Silica core shell	0.02	0.0417	-	[34]
Graphene oxide- silica	0.03	0.0047	-	[50]
Au nanoparticles-polymer	0.0078	0.2443	1:100	[51]
Graphene- polymer	0.11	0.025	-	[52]
Graphene	2.64	0.0659	1:250	[53]
β-cyclodextrin-graphene nanocomposite	0.005	-	1:1000	[54]
Amino-β-cyclodextrin-carbon Nanotubes	0.5	0.514	1:100	[55]
Poly-β-cyclodextrin	4.1	-	1:33	[56]
Graphene-Au,Ag	0.205	0.03	1:1000	[33]
Poly-*o*-aminophenol	0.00198	0.2369	1:1000	[37]
Cysteamine self-assembled monolayer	2.31	0.014	1:500	[57]
Carbon nanotube-ionic liquid gel	0.1	0.237	1:5	[58]
L-cysteine self-assembled monolayer	0.02	-	1:4	[32]
Poly-EDOT-co-(5-amino-2-naphthalenesulfonic acid)	0.5	1	1:500	[31]
Gold nanorods	0.0055	3.28	1:1000	[36]
Phytic acid-single walled carbon nanotube	0.08	-	1:250	[59]
Microelectrode array	0.454	5.21×10^{-4}	1:220	[60]
Poly(nicotinamide)/CuO	0.08	0.236	1:25	[61]
Poly-o-phenylenediamine	0.58	-	1:50	[62]
Polystyrene sulfonate - multiwalled carbon nanotubes	15.7	12.37	1:500	[63]
Silanized graphene	0.01	-	1:1000	[35]
Poly-methylthiophene-, Pd, Pt	0.008	1.44	1:100	[64]

Nanoporous gold	0.017	-	1:500	[65]
Electrodeposited Molecularly Imprinted Silica	0.014	22.3	1:50000	This work

Finally is necessary to indicate that silica-modified electrodesare stable during several days if the silica film is not dried. In this manner, the modified electrode can be reused after the recovery of the electrode sensitivity by applying a cleaning procedure similar to that shown in figure 2. In this manner, we used the same electrode up to 7 measurements with similar performance.

4. Conclusions

In the present work glassy carbon electrodes have been modified with silica films prepared by electroassisted deposition of sol-gel precursor. Dopamine molecularly imprinted silica films where prepared for the electrochemical detection of dopamine in the presence of ascorbic acid. These films present a high coherency allowing a filtering capacity in the molecular scale. The MIS-modified electrodes present a very high selectivity and allow the electrochemical determination of dopamine in solutions containing high concentration of ascorbic acid (DA:AA molar ratio lower than 1:50000). These levels of interference are similar to those found in physiological samples and therefore MIS-modified electrodes presented in this work are very promising for direct determination of dopamine. The fabrication method is also very versatile and can be applied to the determination of other molecules of interest simply modifying the template molecule.

5. Acknowledgements

We acknowledge the financial support from the *Fundación Ramón Areces* (CIVP16A1821), Spanish *Ministerio de Economía y Competitividad* (MAT2013-42007-P) and *Generalitat Valenciana* (PROMETEO2013/038).

6. References

[1] B. Sellergren, Molecularly Imprinted Polymers Shaping Enzyme Inhibitors, Nat. Chem. 2 (2010) 7–8.

[2] M.C. Blanco-Lopez, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Electrochemical sensors based on molecularly imprinted polymers, Trac-Trends Anal. Chem. 23 (2004) 36–48.

[3] M.C. Blanco-Lopez, S. Gutierrez-Fernandez, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Electrochemical sensing with electrodes modified with molecularly imprinted polymer films, Anal. Bioanal. Chem. 378 (2004) 1922–1928.

[4] M.E. Díaz-García, R.B. Laínño, Molecular Imprinting in Sol-Gel Materials: Recent Developments and Applications, Microchim Acta Microchimica Acta. 149 (2005) 19–36.

[5] E. Granot, R. Tel-Vered, O. Lioubashevski, I. Willner, Stereoselective and enantioselective electrochemical sensing of monosaccharides using imprinted boronic acid-functionalized polyphenol films, Adv. Funct. Mater. 18 (2008) 478–484.

[6] E.L. Holthoff, F. V Bright, Molecularly imprinted xerogels as platforms for sensing, Acc. Chem. Res. 40 (2007) 756–767.

[7] F.R.F. Leite, W.D.R. Santos, L.T. Kubota, Selective determination of caffeic acid in wines with electrochemical sensor based on molecularly imprinted siloxanes, Sensors Actuators B Chem. 193 (2014) 238–246.

[8] C. Malitesta, E. Mazzotta, R.A. Picca, A. Poma, I. Chianella, S.A. Piletsky, MIP sensors - the electrochemical approach, Anal Bioanal Chem Analytical Bioanal. Chem. 402 (2012) 1827–1846.

[9] S. Marx, A. Zaltsman, I. Turyan, D. Mandler, Parathion Sensor Based on Molecularly Imprinted Sol-Gel Films, Anal. Chem. 76 (2003) 120–126.

[10] R.Z. Ouyang, H.P. Lei, H.X. Ju, Y.D. Xue, A molecularly imprinted copolymer designed for enantioselective recognition of glutamic acid, Adv. Funct. Mater. 17 (2007) 3223–3230.

[11] A. Turco, S. Corvaglia, E. Mazzotta, Electrochemical sensor for sulfadimethoxine based on molecularly imprinted polypyrrole: Study of imprinting parameters, Biosens. Bioelectron. 63 (2015) 240–247.

[12] K. Haupt, K. Mosbach, Molecularly imprinted polymers and their use in biomimetic sensors, Chem. Rev. 100 (2000) 2495–2504.

[13] A. Salinas-Castillo, I. Sanchez-Barragan, J.M. Costa-Fernandez, R. Pereiro, A. Ballesteros, J.M. Gonzalez, et al., Iodinated molecularly imprinted polymer for room temperature phosphorescence optosensing of fluoranthene, Chem. Commun. (2005) 3224–3226.

[14] J.L. Urraca, A.J. Hall, M.C. Moreno-Bondi, B. Sellergren, A stoichiometric molecularly imprinted polymer for the class-selective recognition of antibiotics in aqueous media, Angew. Chemie-International Ed. 45 (2006) 5158–5161.

[15] R. Makote, M.M. Collinson, Template Recognition in Inorganic-Organic Hybrid Films Prepared by the Sol-Gel Process, Chem. Mater. 10 (1998) 2440–2445.

[16] P.S. Sharma, A. Pietrzyk-Le, F. D’Souza, W. Kutner, Electrochemically synthesized polymers in molecular imprinting for chemical sensing, Anal. Bioanal. Chem. 402 (2012) 3177–3204.

[17] S.-M. Chen, K.-T. Peng, The electrochemical properties of dopamine, epinephrine, norepinephrine, and their electrocatalytic reactions on cobalt(II) hexacyanoferrate films, J. Electroanal. Chem. 547 (2003) 179–189.

[18] A. Salimi, C.E. Banks, R.G. Compton, Abrasive immobilization of carbon nanotubes on a basal plane pyrolytic graphite electrode: application to the detection of epinephrine, Analyst. 129 (2004) 225.

[19] M.L.A. V Heien, M.A. Johnson, R.M. Wightman, Resolving neurotransmitters detected by fast-scan cyclic voltammetry., Anal. Chem. 76 (2004) 5697–704.

[20] C.B. Jacobs, M.J. Peairs, B.J. Venton, Review: Carbon nanotube based electrochemical sensors for biomolecules., Anal. Chim. Acta. 662 (2010) 105–27.

[21] N.F. Atta, A. Galal, R.A. Ahmed, Simultaneous Determination of Catecholamines and Serotonin on Poly(3,4-ethylene dioxythiophene) Modified Pt Electrode in Presence of Sodium Dodecyl Sulfate, J. Electrochem. Soc. 158 (2011) F52.

[22] P. Ramesh, G.. Suresh, S. Sampath, Selective determination of dopamine using unmodified, exfoliated graphite electrodes, J. Electroanal. Chem. 561 (2004) 173–180.

[23] D.C.S. Tse, R.L. McCreery, R.N. Adams, Potential Oxidative Pathways of Brain Catecholamines, J. Med. Chem. 19 (1976) 37–40.

[24] R.L. McCreery, Advanced carbon electrode materials for molecular electrochemistry, Chem. Rev. 108 (2008) 2646–2687.

[25] S. Chumillas, M.C. Figueiredo, V. Climent, J.M. Feliu, Study of dopamine reactivity on platinum single crystal electrode surfaces, Electrochim. Acta. 109 (2013) 577–586.

[26] M.. Cotarelo, F. Huerta, C. Quijada, F. Cases, J.. Vázquez, The electrocatalytic behaviour of poly(aniline-co-4adpa) thin films in weakly acidic medium, Synth. Met. 144 (2004) 207–211.

[27] H. Zhao, Y. Zhang, Z. Yuan, Study on the electrochemical behavior of dopamine with poly(sulfosalicylic acid) modified glassy carbon electrode, Anal. Chim. Acta. 441 (2001) 117–122.

[28] A. Gamero-Quijano, F. Huerta, D. Salinas-Torres, E. Morallón, F. Montilla, Electrochemical Behaviour of PSS-Functionalized Silica Films Prepared by Electroassisted Deposition of Sol–Gel Precursors, Electrocatalysis. (2014) 33–41.

[29] S.R. Ali, Y.F. Ma, R.R. Parajuli, Y. Balogun, W.Y.C. Lai, H.X. He, A nonoxidative sensor based on a self-doped polyaniline/carbon nanotube composite for sensitive and selective detection of the neurotransmitter dopamine, Anal. Chem. 79 (2007) 2583–2587.

[30] S.A. Piletsky, A.P.F. Turner, Electrochemical sensors based on molecularly imprinted polymers, Electroanalysis. 14 (2002) 317–323.

[31] A. Balamurugan, S.M. Chen, Poly(3,4-ethylenedioxythiophene-co-(5-amino-2-naphthalenesulfonic acid)) (PEDOT-PANS) film modified glassy carbon electrode for selective detection of dopamine in the presence of ascorbic acid and uric acid, Anal. Chim. Acta. 596 (2007) 92–98.

[32] G. Hu, Y. Liu, J. Zhao, S. Cui, Z. Yang, Y. Zhang, Selective response of dopamine in the presence of ascorbic acid on L-cysteine self-assembled gold electrode, Bioelectrochemistry. 69 (2006) 254–257.

[33] S. Pruneanu, A.R. Biris, F. Pogacean, C. Socaci, M. Coros, M.C. Rosu, et al., The influence of uric and ascorbic acid on the electrochemical detection of dopamine using graphene-modified electrodes, Electrochim. Acta. 154 (2015) 197–204.

[34] D. Yu, Y. Zeng, Y. Qi, T. Zhou, G. Shi, A novel electrochemical sensor for determination of dopamine based on AuNPs@SiO2 core-shell imprinted composite, Biosens. Bioelectron. 38 (2012) 270–277.

[35] S. Hou, M.L. Kasner, S. Su, K. Patel, R. Cuellari, Highly Sensitive and Selective Dopamine Biosensor Fabricated with Silanized Graphene, J. Phys. Chem. C. 114 (2010) 14915–14921.

[36] Z. Jia, J. Liu, Y. Shen, Fabrication of a template-synthesized gold nanorod-modified electrode for the detection of dopamine in the presence of ascorbic acid, Electrochem. Commun. 9 (2007) 2739–2743.

[37] J.P. Li, J. Zhao, X.P. Wei, A sensitive and selective sensor for dopamine determination based on a molecularly imprinted electropolymer of o-aminophenol, Sensors and Actuators B-Chemical. 140 (2009) 663–669.

[38] M.M. Collinson, Electrochemistry: An important tool to study and create new sol-gel-derived materials, Acc. Chem. Res. 40 (2007) 777–783.

[39] M.M. Collinson, D.A. Higgins, R. Kommidi, D. Campbell-Rance, Electrodeposited silicate films: Importance of supporting electrolyte, Anal. Chem. 80 (2008) 651–656.

[40] O. Nadzhafova, M. Etienne, A. Walcarius, Direct electrochemistry of hemoglobin and glucose oxidase in electrodeposited sol-gel silica thin films on glassy carbon, Electrochem. Commun. 9 (2007) 1189–1195.

[41] A. Walcarius, D. Mandler, J.A. Cox, M. Collinson, O. Lev, Exciting new directions in the intersection of functionalized sol-gel materials with electrochemistry, J. Mater. Chem. 15 (2005) 3663–3689.

[42] A. Walcarius, E. Sibottier, M. Etienne, J. Ghanbaja, Electrochemically assisted self-assembly of mesoporous silica thin films, Nat. Mater. 6 (2007) 602–608.

[43] A. Gamero-Quijano, F. Huerta, D. Salinas-Torres, E. Morallón, F. Montilla, Electrochemical Behaviour of PSS-Functionalized Silica Films Prepared by Electroassisted Deposition of Sol-Gel Precursors, Electrocatalysis. (2014) 1–9.

[44] D. Salinas-Torres, F. Montilla, F. Huerta, E. Morallon, E. Morallón, All electrochemical synthesis of polyaniline/silica sol-gel materials, in: Electrochim. Acta, 2011: pp. 3620–3625.

[45] M.D. Hawley, S. V Tatawawa, S. Peikarak, R.N. Adams, Electrochemical Studies of Oxidation Pathways of Catecholamines, J. Am. Chem. Soc. 89 (1967) 447–&.

[46] E. Mazario, J. Sanchez-Marcos, N. Menendez, P. Herrasti, M. Garcia-Hernandez, A. Munoz-Bonilla, One-pot electrochemical synthesis of polydopamine coated magnetite nanoparticles, Rsc Adv. 4 (2014) 48353–48361.

[47] G.L. Long, J.D. Winefordner, Limit of Detection A Closer Look at the IUPAC Definition, Anal. Chem. 55 (1983) 712A–724A.

[48] A. Gamero-Quijano, F. Huerta, D. Salinas-Torres, E. Morallon, F. Montilla, Enhancement of the Electrochemical Performance of SWCNT dispersed in a Silica Sol-gel Matrix by Reactive Insertion of a Conducting Polymer, Electrochim. Acta. 135 (2014) 114–120.

[49] X. Kan, H. Zhou, C. Li, A. Zhu, Z. Xing, Z. Zhao, Imprinted electrochemical sensor for dopamine recognition and determination based on a carbon nanotube/polypyrrole film, Electrochim. Acta. 63 (2012) 69–75.

[50] Y. Zeng, Y. Zhou, L. Kong, T. Zhou, G. Shi, A novel composite of SiO2-coated graphene oxide and molecularly imprinted polymers for electrochemical sensing dopamine, Biosens. Bioelectron. 45 (2013) 25–33.

[51] C. Xue, Q. Han, Y. Wang, J. Wu, T. Wen, R. Wang, et al., Amperometric detection of dopamine in human serumby electrochemical sensor based on gold nanoparticles doped molecularly imprinted polymers, Biosens. Bioelectron. 49 (2013) 199–203.

[52] Y. Mao, Y. Bao, S. Gan, F. Li, L. Niu, Electrochemical sensor for dopamine based on a novel graphene-molecular imprinted polymers composite recognition element, Biosens. Bioelectron. 28 (2011) 291–297.

[53] Y.R. Kim, S. Bong, Y.J. Kang, Y. Yang, R.K. Mahajan, J.S. Kim, et al., Electrochemical detection of dopamine in the presence of ascorbic acid using graphene modified electrodes, Biosens. Bioelectron. 25 (2010) 2366–2369.

[54] L. Tan, K.G. Zhou, Y.H. Zhang, H.X. Wang, X.D. Wang, Y.F. Guo, et al., Nanomolar detection of dopamine in the presence of ascorbic acid at b-cyclodextrin/graphene nanocomposite platform, Electrochem. Commun. 12 (2010) 557–560.

[55] A. Gaied, N. Jaballah, M. Tounsi, M. Braiek, N. Jaffrezic-Renault, M. Majdoub, Selective Detection of Dopamine in Presence of Ascorbic Acid by Use of Glassy-Carbon Electrode Modified with Amino-b¦-Cyclodextrin and Carbon Nanotubes, Electroanalysis. 26 (2014) 2747–2753.

[56] M. Palomar-Pardavé, S. Corona-Avendaño, M. Romero-Romo, G. Alarcón-Angeles, A. Merkoçi, T. Ramírez-Silva, Supramolecular interaction of dopamine with b-cyclodextrin: An experimental and theoretical electrochemical study, J. Electroanal. Chem. 717-718 (2014) 103–109.

[57] R.K. Shervedani, M. Bagherzadeh, S.A. Mozaffari, Determination of dopamine in the presence of high concentration of ascorbic acid by using gold cysteamine self-assembled monolayers as a nanosensor, Sensors Actuators B Chem. 115 (2006) 614–621.

[58] Y. Zhao, Y. Gao, D. Zhan, H. Liu, Q. Zhao, Y. Kou, et al., Selective detection of dopamine in the presence of ascorbic acid and uric acid by a carbon nanotubes-ionic liquid gel modified electrode, Talanta. 66 (2005) 51–57.

[59] S. Jo, H. Jeong, S.R. Bae, S. Jeon, Modified platinum electrode with phytic acid and single-walled carbon nanotube: Application to the selective determination of dopamine in the presence of ascorbic and uric acids, Microchem. J. 88 (2008) 1–6.

[60] A. Aggarwal, M. Hu, I. Fritsch, Detection of dopamine in the presence of excess ascorbic acid at physiological concentrations through redox cycling at an unmodified microelectrode array, Anal Bioanal Chem Anal. Bioanal. Chem. 405 (2013) 3859–3869.

[61] B. Li, Y. Zhou, W. Wu, M. Liu, S. Mei, Y. Zhou, et al., Highly selective and sensitive determination of dopamine by the novel molecularly imprinted poly(nicotinamide)/CuO nanoparticles modified electrode, Biosens. Bioelectron. 67 (2015) 121–128.

[62] D. Wu, H. Li, X. Xue, H. Fan, Q. Xin, Q. Wei, Sensitive and selective determination of dopamine by electrochemical sensor based on molecularly imprinted electropolymerization of o-phenylenediamine, Anal. Methods. 5 (2013) 1469–1473.

[63] R. Manjunatha, G.S. Suresh, J.S. Melo, S.F. D'Souza, T.V. Venkatesha, Simultaneous determination of ascorbic acid, dopamine and uric acid using polystyrene sulfonate wrapped multiwalled carbon nanotubes bound to graphite electrode through layer-by-layer technique, Sensors Actuators B Chem. 145 (2010) 643–650.

[64] N.F. Atta, M.F. El-Kady, Novel poly(3-methylthiophene)/Pd, Pt nanoparticle sensor: Synthesis, characterization and its application to the simultaneous analysis of dopamine and ascorbic acid in biological fluids, Sensors Actuators B Chem. 145 (2010) 299–310.

[65] H.J. Qiu, G.P. Zhou, G.L. Ji, Y. Zhang, X.R. Huang, Y. Ding, A novel nanoporous gold modified electrode for the selective determination of dopamine in the presence of ascorbic acid, Colloids Surfaces B Biointerfaces. 69 (2009) 105–108.

María Porcel-Valenzuela was born in 1986 in Granada, (Spain). She received the M.Sc. degree in Chemistry in 2010 from University of Granada, Spain. Now, she is Ph.D. student in the University of Alicante, Spain. Her research interest is the design of sensors based on molecularly imprinted silica.

Alfonso Salinas-Castillo was born in 1978 in Granada (Spain). He received the M.Sc. degree in chemistry (2001) and PhD in Analytical Chemistry (2005), both from the University of Granada. He is currently working as a researcher at the ECSens group, department of Analytical chemistry, University of Granada. His current research interest included optical sensor and biosensor, luminescent nanoparticle, colorimetric sensor.

Emilia Morallon Full Professor of Physical Chemistry at the University of Alicante, received his B.Sc. in Chemistry (1988) and Ph.D. in Chemistry (1993) from the University of Alicante (Spain). From 2008, she is leader of the Group of Electrocatalysis and Polymer

Electrochemistry at the University of Alicante. Her current research interests are the design, development and fabrication of new materials electrodes for different applications in sensors, energy generation and energy storage. Her work has produced nearly 145 peer-reviewed scientific papers, 11 book chapters and 4 patents.

Francisco Montilla received his B.Sc. in Chemistry (1997) and Ph.D. in Materials Chemistry (2003). From 2007 behaves to the Group of Electrocatalysis and Polymers and is tenured Professor of Physical Chemistry at the University of Alicante from 2011. His present work is focused in the development of hybrid organic-inorganic electrodes based on silica sol-gel and conducting polymers for applications in electrochemical sensors, biosensors and devices. He is the author of more than 40 peer reviewed papers, 3 book chapters and 1 patent.