Oxidant-dependent antioxidant activity of polydopamine films: the chemistry-morphology interplay

Salima El Yakhlifi[a], *Maria-Laura Alfieri*[b], *Youri Arntz* [a,c], *Matilde Eredia*[d], *Artur Ciesielski*[d],

Paolo Samorì[d], *Marco d'Ischia*[b], *Vincent Ball* [a,c,*].

[a]Université de Strasbourg, Faculté de Chirurgie dentaire, 8 rue Sainte Elisabeth, 67000 Strasbourg, France.

[b]Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, I-80126 Naples, Italy.

[c]Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche 1121, 11 rue Humann; 67085 Strasbourg Cedex, France.

[d]Université de Strasbourg, CNRS, ISIS, 8 allée Gaspard Monge, 67000 Strasbourg, France.

*corresponding author : vball@unistra.fr

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Abstract

Polydopamine (PDA) films allow to functionalize almost all materials with a conformal and chemically active coating. These coatings can react with reducible metallic cations and with all kinds of molecules carrying nucleophilic groups. Recently, our team extended PDA chemistry to a vast repertoire of oxidants and to acidic conditions. However, the influence of changes in the method of PDA deposition on the properties of the obtained coatings, in particular the antioxidant properties, have not been sufficiently explored. It is anticipated that the antioxidant properties should depend on the film preparation method. A combination of experimental techniques, atomic force microscopy, cyclic voltammetry and X ray photoelectron spectroscopy are used to relate the antioxidant properties of PDA films to their structural features and to their chemical composition. It is demonstrated that the antioxidant properties of PDA films are not only dependent on the type of the employed oxidant – which can be expected to affect a variable density of oxidizable groups on the surface of PDA - but also on the oxidant film morphology and roughness.

1. Introduction

The field of materials functionalization has undergone a spectacular evolution in the recent past years with the development of different approaches and techniques towards versatile coatings, which can adhere to the surface of all materials families. The chemistry used to deposit those coatings is mostly based on catecholamines [1-4] and on polyphenols [5-7] and it was largely bioinspired by the proteins of the mussels' byssus [8] and natural plant polyphenols [9], respectively. Polydopamine films, in particular, were produced by dopamine oxidation with oxygen dissolved in tris(hydroethyl)aminomethane (Tris) buffer [1], and those films could be easily post-functionalized with metallic nanoparticles [1], molecules carrying nucleophilic groups

[1, 10] and DNA [11]. Such coatings appeared particularly appealing for applications in materials science, for energy conversion processes and as biomaterials [12, 13]. Rapidly it appeared that the use of Tris buffer - containing free amino groups - interfered with the formation of PDA [14]. Additionally, the structure of possible polymeric [15, 16] or supramolecular assemblies [17, 18] of PDA was investigated and discussed. Hence, even if the structural knowledge of PDA lies far beyond the requirements needed by its application fields and their improvements, vast number of perspectives emerged from the discovery that the oxidant used to trigger PDA formation modifies the structure and the properties of the obtained coating in a significant manner [19, 20]. Among different oxidants used to produce PDA starting from dopamine, Tris-O_2 [1], sodium periodate ($NaIO_4$), [19, 20], ammonium peroxodisulfate $(NH_4)_2S_2O_8$) [19, 20], copper sulfate alone [21] or a mixture of copper sulfate with hydrogen peroxide ($CuSO_4$+H_2O_2) [22], vanadyl cations [23] or photogenerated radical species [24] have been used. It has to be noted that surface specific electrochemical oxidation from catecholamine containing solutions will also lead to films close to polydopamine but differing in their electrochemical properties compared to those of the films obtained by solution deposition in the presence of an oxidant different from dissolved oxygen [25-29]. Note that even if it is possible to obtain PDA films using O_2 dissolved in water, the addition of additional oxidants allows to speed up the deposition process [20] which is of major importance for practical applications and allows also to produce films over a broad range of pH values.

Despite these research efforts, one of the few certitudes concerning all the obtained PDAs is their structural analogy with eumelanins, the ubiquitous and heterogeneous black-brown pigments in mammals [30, 31]. Hence the possibility of fine tuning the properties of PDA films in relation to its oxidant dependent composition and structure requires more evidence.

Herein, we investigate the relationship between the oxidant used to produce PDA films and their surface roughness, thickness, electrochemical behaviour, composition and antioxidant properties. We compare PDA films produced by oxidizing dopamine in the presence of four different oxidants: Tris-O_2, sodium periodate ($NaIO_4$), ammonium persulfate ($(NH_4)_2S_2O_8$) and a ($CuSO_4$+H_2O_2) mixture. To provide a more accurate rationale on the possibility of fine-tuning of the structure and properties of PDA as well as to gain insight into the difference in morphology/roughness, electrochemical behavior and composition, we performed a multi-technique study by making use of atomic force microscopy (AFM), cyclic voltammetry (CV) and X-ray photoelectron spectroscopy (XPS), respectively. Finally, the antioxidant properties of those coatings have been compared and were related to their morphological/chemical attributes. The obtained films are herein referred to as PDA/oxidant-*x*h for materials produced in the presence of the cited oxidant for *x* hours of oxidation. Even if the antioxidant properties of melanin like materials [32] - playing a central role in the protection of the brain - and of PDA films [33, 34] are known, to our knowledge no investigation has been reported on the influence of the oxidant used to produce the PDA films on their anti-oxidant properties. Hence this article is aimed to complement our previous investigation in which we compared the PDA/O_2 and PDA/$NaIO_4$ films [20] with respect to their composition and a specific property, namely the films' hydrophilicity. Herein, we expand the library of used oxidants and we focus on the relationship between surface chemistry, surface morphology and the antioxidant activity. The aim is to design the PDA based film displaying the optimal antioxidant activity which is of fundamental importance for cells or tissues in contact with PDA films [32]. The rationale to reach such a goal has not yet been investigated to our knowledge.

2. Materials and Methods

2.1 Chemicals and solutions

All the chemicals used in this investigation were purchased and used without additional purification. Dopamine-hydrochloride (ref. H8502), sodium periodate ($NaIO_4$, ref. 311448), ammonium persulfate ($(NH_4)_2 S_2O_8$, ref. 1257273), anhydrous copper (II) sulfate ($CuSO_4$, ref. 61230), hydrogen peroxide (H_2O_2, in the form of a 35 % w/v solution, ref. H1009), anhydrous sodium acetate (ref. W302406) and 2,2-diphenyl-1-picrylhydrazyl (DPPH, ref. 257621), were all purchased from Sigma-Aldrich (L'isle d'Abeau Chesnes, France).

Tris(hydroxymethyl)aminomethane (Tris buffer) was from Euromedex (Schiltigheim, France). The buffer solutions were prepared in ultra-pure water (Milli Plus, Millipore, Billerica, MA, USA) having a resistivity of 18.2 MΩ.cm.

Two buffers were used: 50 mM sodium acetate buffer at pH 5.0, and 50 mM Tris buffer at pH 8.5. The pH of each buffer was adjusted with concentrated HCl, and checked with a calibrated Hannah 802 pH meter as explained in our previous article [20].

2.2 Synthesis of polydopamine films

Four oxidants were added to dopamine solutions in order to compare their effect on the obtained films. All the oxidation experiments were conducted at room temperature, 19±1 °C for 3 and 8h. In this investigation, we restricted to investigate the deposition of PDA in the presence of different oxidants based on our previous investigation [20] which allowed to show that for strong oxidants like $NaIO_4$, the film deposition process is finished after 8 h and almost complete after 3 h.

Regardless of the employed oxidant, 50 mg of dopamine were dissolved in the used buffer, either sodium acetate (for the experiments performed with an exogenous oxidant) at pH = 5.0 or Tris-hydrochloride (for the autooxidation experiments with dissolved O_2) at pH = 8.5. At time t=0 of the deposition experiments, 5 mL of the oxidant was added to 45 mL of dopamine solution. We freshly prepared each oxidant solution at a concentration of 0.1 mol L^{-1}, to reach a final oxidant concentration of 1.0×10^{-2} mol L^{-1}. In the case of $CuSO_4$-H_2O_2, the molar ratio of hydrogen peroxide to copper was equal to one. Hence, in all experiments the dopamine concentration was of 1 mg mL^{-1}, namely 5.3×10^{-3} mol L^{-1}, implying a constant oxidant / dopamine molar ratio of 1.9 with exception of Tris-O_2 where the oxygen concentration was that of water at about 19°C, namely about 4×10^{-4} mol.L^{-1}. However, since the synthesis was performed in an open flask, the O_2 can be refreshed continuously by diffusion. In case of the added oxidants, the oxidant/dopamine molar ratio is the same as the one used in our previous investigation [20]. In the case of autooxidation experiments in the presence of Tris buffer, using dissolved O_2 as the oxidant, the 50 mg of dopamine were directly added in 50 mL of buffer. All experiments were performed without permanent stirring: the dopamine solution and the oxidant solution were vigorously shaken after mixing and left at rest during the film deposition.

Whatever the used oxidant, plasma cleaned (PDC-32G plasma cleaner, Harrick Plasma, USA) glass slides (76 mm x 26 mm) were placed vertically in the dopamine solutions before the addition of the oxidant solutions prepared as described previously [20]. In these conditions and in the used flasks, the surface area of the glass slides in contact with the dopamine + oxidant solution was of 45 mm × 26 mm. The deposition process of PDA/oxidant films was stopped after 3 or 8h by removing the glass slides from the reaction medium and intensive rinse with distilled water. The same protocol was used for the glass slides employed in the AFM characterization experiments and for the antioxidant tests. For the XPS characterization, PDA films were deposited on a gold covered substrate to avoid the presence of silica in the penetration depth region of the X-ray beam. Indeed, the presence of oxygen containing silica would unavoidably modify the O/C ratio, which is a key information we aim to acquire in our PDA films.

2.3 Characterization of the PDA/oxidant films

Atomic force microscopy

The morphology and the thickness of the PDA films deposited on glass slides were analyzed by recording topography images in Intermittent Contact Mode by using a BioScope Catalyst AFM (Bruker Inc, Santa Barbara, USA) using the scan assist fluid method. To determine the film thickness, a scratch using a sterile syringe tip was made on each slide. The roughness and thickness were then calculated with the Gwyddion software as explained in our previous paper [20]. The average roughness of the films was determined on image sizes of 2×2 µm², 5×5 µm² and 10×10 µm².

X-ray photoelectron spectroscopy analysis

X-ray photoelectron spectroscopy on PDA/oxidant films was performed to compare their chemical composition. Au (111) substrates, which consist of a flame annealed layer of Au (200 nm) evaporated on mica, were previously treated by a plasma cleaner during 10 min. and were placed in each dopamine + oxidant solution (same protocol as for the glass slides). An X-ray photoelectron spectrometer (Thermo Scientific K-Alpha) equipped with an aluminum X-ray source (energy 1.4866 keV) under ultra-high vacuum (pressure of 10^{-8}–10^{-9} mbar in the main chamber) was used to determine the carbon, oxygen and nitrogen content of the PDA films. The X-ray spot size was set at 400 µm. 10 scans with a pass energy of 200.00 eV and a step size of 1 eV were performed to get the survey spectra of the PDA films. High-resolution spectra were also obtained as an average of 10 scans with a pass energy of 50.00 eV and a step size of 0.1 eV. Energy calibration was performed on the basis of the adventitious carbon present on the gold coated substrates.

Cyclic voltammetry (CV)

The cyclic voltammetry measurements were carried out with a CHI604B three electrode setup (CHI Instruments, Houston, Texas) in order to identify different redox moieties in the PDA/oxidant films. The PDA films were deposited from the dopamine/oxidant blends on a carbon working electrode. Just before the deposition step (8h), the carbon electrode was polished with aluminum oxide slurries (particles having 1.0 µm and at 0.1 µm in diameter). Then, each electrode was placed in a water containing beaker for ultrasound treatment for 20 minutes (repeated 2 times). To check the surface state of the working electrode, potassium hexacyanoferrate was used at 1 mM in the presence of either sodium acetate or Tris buffer. A CV cycle between -1.0 and +0.7V versus the reference electrode was performed at a potential sweep rate of 100 mV s^{-1}. The electrode was used for film deposition if the oxidation and reduction peak potentials were separated by less than 80 mV, the theoretical value for a one electron process being of 59 mV at 298 K. Otherwise, the electrode was polished once more. After having measured the CV capacitive curve of the polished electrode in presence of sodium acetate buffer or Tris buffer, the electrode was immersed in a dopamine and oxidant blended solution. After 8 hours of reaction, the electrode was rinsed with the same buffer as for the film deposition and voltammetry measurements were carried out again in the presence of buffer with no exogeneous oxidant at a potential sweep rate of 100 mV s^{-1}.

Anti-oxidant properties of the PDA/oxidant films

The antioxidant activity of the films (45 mm × 26 mm on both sides of the glass slides used as substrates) was investigated by the DPPH discoloration method. DPPH is a purple colored radical species. When it is reduced in the presence of an oxidant, it looses its free radical character, the electron delocalization is modified and the molecules undergoes a color change from purple to yellow, which can be easily followed by spectrophotometry. Hence, the change in absorbance of ethanol solubilized DPPH was measured with an mc² UV-Vis spectrophotometer (SAFAS, Monaco). The PDA/oxidant-3h and PDA/oxidant- 8h coated glass slides were placed in a beaker containing 50mL of DPPH (10^{-4} mol/L in pure ethanol), and allowed to react with DPPH for 2 hours in the absence of external light. Great care was taken to use the same surface area of the film and the same volume of DPPH solution in all experiments. Then, the absorbance of each DPPH solution in contact with the PDA/oxidant films was measured at 516 nm, the reference cuvette containing DPPH alone. This reference DPPH solution was prepared in the same condition as the DPPH solution in contact with the films to account for spontaneous discoloration of the dye in the presence of dissolved O_2. The radical scavenging efficiency was calculated according to:

$$Efficiency = \left(1 - \frac{A_f}{A_i}\right) x100 \qquad (1)$$

where A_i and A_f are the absorbance of the initially used DPPH solution and of the same solution exposed during 2h to the PDA/oxidant-xh films, respectively.

Each measurement, AFM, cyclic voltammetry, characterization by XPS and antioxidant activity was performed three times (each time on an individually prepared film) for each oxidant and each deposition time.

3. Results and discussion

3.1 Film morphology, thickness and roughness

Our first observation is that the morphology of the PDA films is markedly influenced by the oxidant used to produce them (Fig. 1A). In agreement with our previous report [20], the PDA/O_2-8h films are smoother than the highly granular PDA/$NaIO_4$ -8h films. The same trend, but less pronounced, is observed after 3h of deposition whatever the image size (Fig. S1, S2 and S3 in the Supplementary file). An intermediate scenario between the smooth PDA/O_2 films and the granular PDA/$NaIO_4$ films is observed when films are produced in the presence of $CuSO_4$-H_2O_2 and $(NH_4)_2S_2O_8$ (Fig. 1A and Fig.S1, S2, S3 in the Supplementary file). From a quantitative viewpoint, the film roughness increases following the order PDA/O_2 ~ PDA/$(NH_4)_2S_2O_8$ < PDA/$CuSO_4$-H_2O_2 ~ PDA/$NaIO_4$ (Fig. 1C) and depends markedly on the image size in the case of the very rough films. This finding is not unexpected for granular films, and it is consistent with the observation that the grain size of the particles on the PDA/$NaIO_4$-8h films is in the 500nm-1µm size range (Fig. 1A), a smaller grain size than for the films produced with the O_2 and $(NH_4)_2S_2O_8$ oxidants. However, the PDA/$CuSO_4$-H_2O_2 films are also very grainy and display an rms roughness similar to that of the PDA/$NaIO_4$ films.

We found that the film thickness, as determined by topographic AFM imaging, is higher for the PDA/$NaIO_4$ -8h films and for the PDA/$CuSO_4$-H_2O_2 -8h films compared to PDA/$(NH_4)_2S_2O_8$ -8h and PDA/Tris -8h films (Fig. 1B). The same trend holds true after 3 h of deposition (Fig. S4 in the Supplementary Information file) but in a less marked manner. In the literature, the PDA films produced with $NaIO_4$ (65 nm in thickness after 1 h in the presence of 20 mM $NaIO_4$ [20]) and with $CuSO_4$-H_2O_2 (43 nm in thickness after 1h [19]) have a thickness consistent with the values obtained in the present investigation. However, the PDA/O_2 films produced herein are much thinner than the values found in the literature-around 25-30 nm after 8h [1]- in probable relationship to the absence of stirring inducing a slow depletion of available O_2. Regardless the used oxidant, the film thickness is almost independent on the image size, indicating conformal coatings (Fig. 1B). The large standard deviations on the films' thickness and roughness are obviously increasing with the granular morphology of the films.

It is worth noting that the films morphology, thickness and roughness, are not a monotonous function of the oxidant's strength. This is not unexpected on the basis of kinetic effects as discussed in our previous article [20]: even if $(NH_4)_2S_2O_8$ is the strongest investigated oxidant (the standard redox potentials are listed in Table 1) the formation kinetics of the oxidizing sulfate through decomposition of peroxodisulfate is a much slower process than the direct dopamine oxidation using the two electron oxidant $NaIO_4$ [35]. It has to be noted also that the presence of $CuSO_4$ alone induces a slow film deposition mainly through Cu^{2+}- catechol coordination [21] and that H_2O_2 only induces a yellow coloration of the dopamine solution and no measurable film deposition in agreement with the known reaction mechanism of catecholamines with H_2O_2.[36]

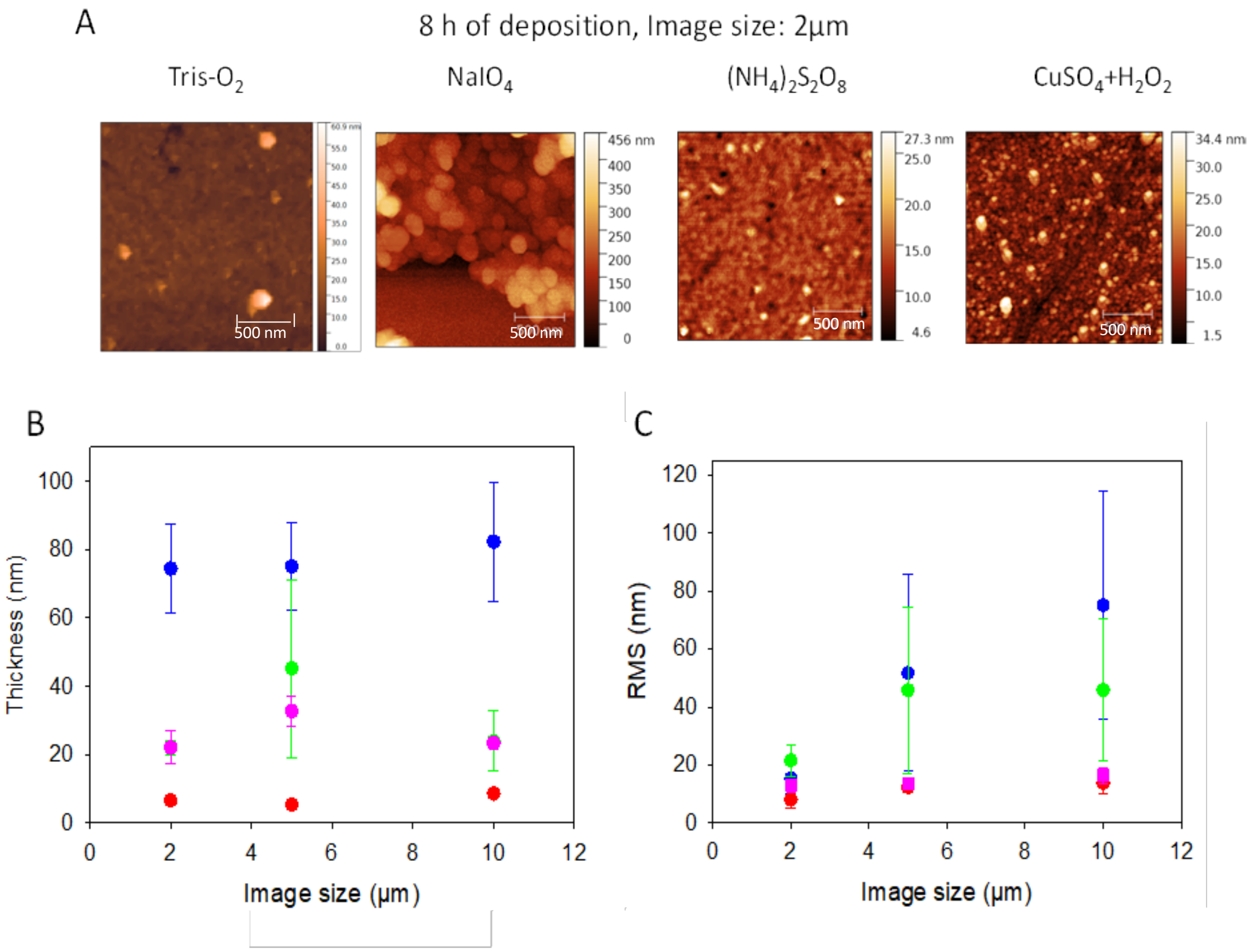


Fig. 1: A: 2 µm × 2 µm AFM surface topographies of PDA/oxidant-8h films. B: Film thickness of the PDA/oxidant-8h films as determined from topographical profiles determined in AFM images. C): dependence of the PDA/oxidant -8h film root-mean square roughness as a function of the image size. The data in part B and C correspond to 5 measurements ± one standard deviation.

(●): PDA/O_2, (●) PDA/$NaIO_4$, (●) PDA/$CuSO_4$-H_2O_2, (●) PDA/$(NH_4)_2S_2O_8$ films.

3.2 Characterization of the electrochemical activity of the PDA films

All the PDA/oxidant-8h films contain electroactive groups as revealed by measuring their CV curves in the absence of a redox probe added in the buffer solution put in the presence of the film (Fig. 2). The area under such curves represent the intrinsic electrochemical activity of the films which is related to their thickness and the surface area in contact with the solution which is modulated by the films' roughness. The area under the CV curves increases following the order of the PDA/O_2 < PDA/$(NH_4)_2S_2O_8$< PDA/$CuSO_4$-H_20_2 < PDA/$NaIO_4$ films (Fig. 2) and this ranking is in good qualitative agreement with the thickness of the deposits (Fig. 1B) thereby emphasizing again that the strongest oxidant (see Table 1) is not the most efficient in the deposition of PDA films. The most important information from the electrochemical experiments is however the clear evidence that the CV curves of the PDA films depend on the oxidant used to oxidize dopamine (Fig. 2). This is a new result from the present investigation. Films produced in the presence of $NaIO_4$ display not only a high oxidation current but also a broad distribution of oxidation peaks revealing a higher chemical heterogeneity of those films compared to those produced with other oxidants.

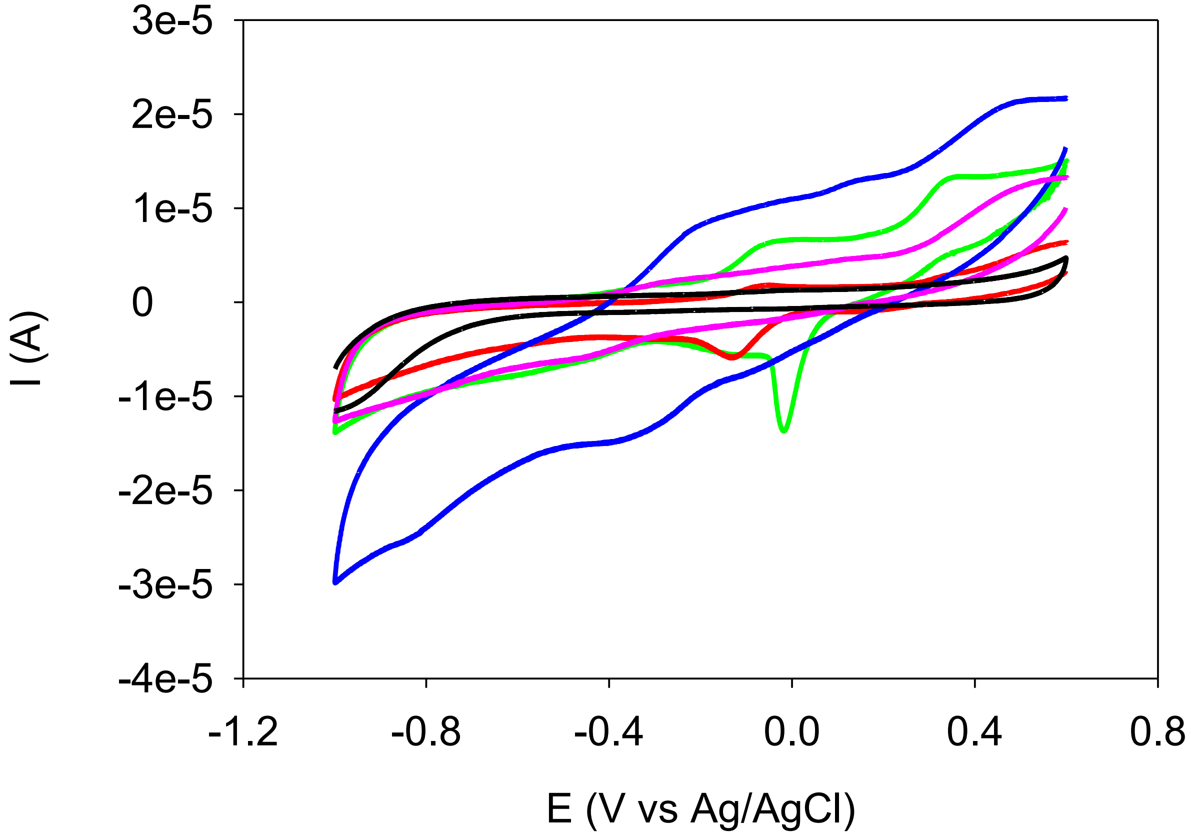


Fig. 2 : Cyclic voltammetry curves in the presence of the same buffers (sodium acetate or Tris) used for the film deposition : polished electrode (——, sodium acetate buffer), PDA/O_2-8h (——, Tris buffer), PDA/$NaIO_4$-8h (——, sodium acetate buffer), PDA/$CuSO_4$-H_2O_2-8h (——, sodium acetate buffer) and PDA/$(NH_4)_2S_2O_8$-8h (——, sodium acetate buffer).

The reduction peak observed at about -0.05 V vs. Ag/AgCl in the case of the PDA/$CuSO_4$-H_2O_2 -8h film may well be due to the presence of Cu^{2+} cations in the films, in accordance with XPS measurements made on PDA films using $CuSO_4$ alone as an oxidant [21].

In all cases, when successive oxidation-reduction cycles are performed, the area under the CV curve-hence the total charge- progressively decreases implying irreversible chemical processes taking place in the part of the films accessible to the conductive amorphous carbon electrode (Fig. S5 in the Supplementary file, in the particular case of PDA/$(NH_4)_2S_2O_8$ films. The same data were obtained for the films produced in the presence of the other oxidants, but not shown herein).

This observation is in very good agreement with previous results based on gate current measurements on aqueous suspensions of a synthetic eumelanin sample [37]. Indeed, it was found that repeated CV cycles on eumelanin deposited on a Pt working electrode induced an irreversible reduction in the measured gate current suggesting a non-equilibrium redox state of the deposited material.

In addition, the obtained films are conformal since the hexacyanoferrate redox probe is not oxidized -reduced anymore when put in presence of the PDA/oxidant films deposited on the amorphous carbon working electrode (Fig. S6 in the Supplementary file, in the particular case of PDA/$(NH_4)_2S_2O_8$-8h films. The same data were also obtained for the films produced in the presence of the other oxidants, but not shown herein).

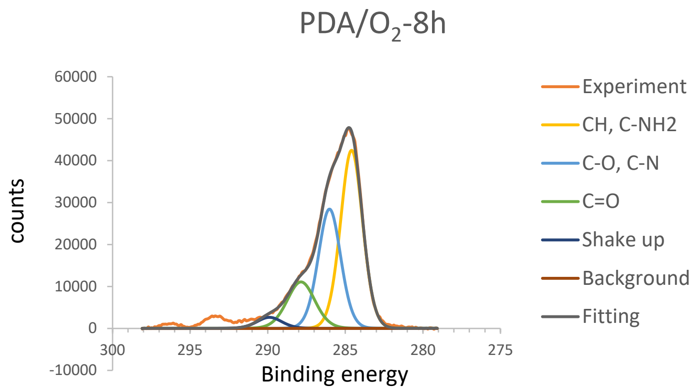
The obtained CV curves (Fig. 2) are an illustration of the complexity/heterogeneity of PDA films and cannot yet be interpreted with respect to the chemical moieties at the origin of the different oxidation peaks. Some model studies in which the oxidation/reduction waves of model compounds related to 5,6-dihydroxyindole and related molecules will be performed in the future in relation to numerical simulations of CV curves.

3.3 X-ray photoelectron spectroscopy

Anyway, these marked oxidant specific changes in the electrochemical behavior of the PDA/oxidant-8h films suggest that they are characterized by different compositions and structures, as evidenced by their high resolution C1s XPS spectra (Fig. 3, the O1S and N1S high resolution spectra for the PDA/oxidant-8h films are given in Fig. S7 and S8 of the Supplementary file respectively).

Those XPS spectra of the films were analyzed, taking the Scoffield sensitivity parameters into account, to determine the composition of the films (Table 1). It is interesting to note that gold was never detected, within the uncertainty of the data (± 1%), after 8h of deposition. This suggests, in agreement with the AFM (Fig. 1B) data and electrochemical data (Fig. S6 in the Supplementary file), that the obtained films are conformal without regions of the substrate accessible to the X-ray beam used in the XPS experiments. Indeed, in XPS only the photoelectrons emitted from a depth of a few nm inside a film are detected.

However, the PDA/$CuSO_4$ -H_2O_2-8h films contain about 2.7 % Cu (Table 1) in agreement with previous data [21] and with the CV curve (Fig. 2) of the corresponding film.


PDA/O$_2$-8h
counts
60000
50000
40000
30000
20000
10000
0
-10000
300
295
290
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280
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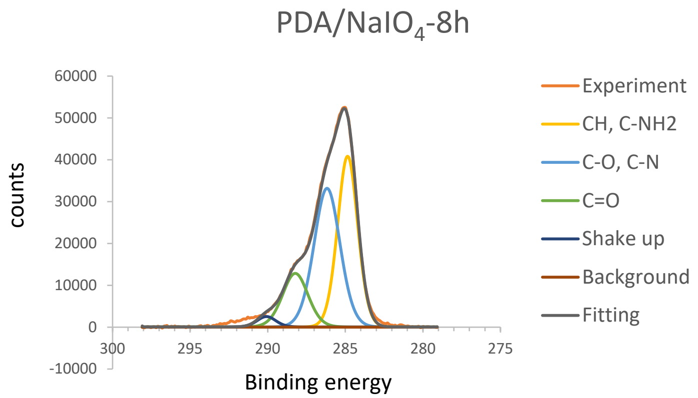
Binding energy
Experiment
CH, C-NH2
C-O, C-N
C=O
Shake up
Background
Fitting


PDA/NaIO$_4$-8h
counts
60000
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C-O, C-N
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Shake up
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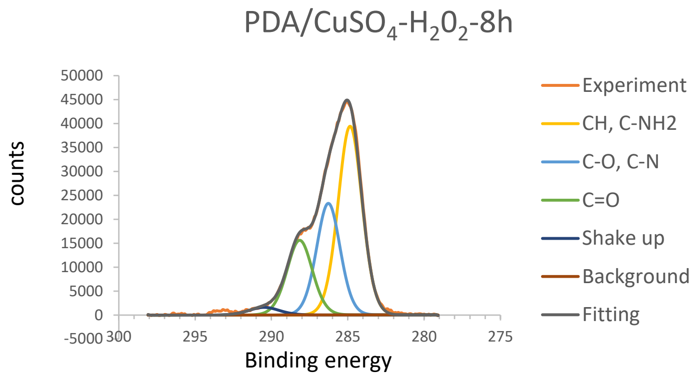
PDA/CuSO$_4$-H$_2$0$_2$-8h
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45000
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25000
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-5000
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Binding energy
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C-O, C-N
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Shake up
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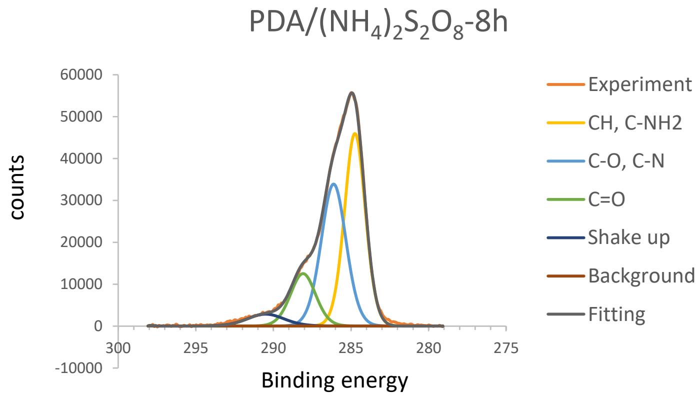
Fitting


PDA/(NH$_4$)$_2$S$_2$O$_8$-8h
counts
60000
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30000
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10000
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-10000
300
295
290
285
280
275
Binding energy
Experiment
CH, C-NH2
C-O, C-N
C=O
Shake up
Background
Fitting

Fig. 3: Representative XPS spectra in the C1s region of PDA/oxidant-8h films. The colored lines correspond to the spectral deconvolution with the corresponding chemical assignments on the right.

Table 1: Elemental composition (in % and average N/C and O/C ratios for the PDA/oxidant-8h films as obtained by XPS on *n* = 3 samples. All data are given ± one standard deviation.

Element or elemental ratio in %	O_2	$NaIO_4$ $E^{\circ}_{IO_4^-/IO_3^-} = 1.55\ V$	$CuSO_4$-H_2O_2 $E^{\circ}_{H_2O_2/H_20} = 1.78\ V, E^{\circ}_{Cu^{2+}/Cu} = 0.34\ V$	$(NH_4)_2S_2O_8$ $E^{\circ}_{S_2O_8^{2-}/HSO_4^-} = 2.12V$	Dopamine (calculated)
C	71.5±0.5	68.4±0.3	65.1±0.9	70.5 ± 0.3	62.7
N	8.2 ± 1.0	8.0±0.2	8.2±0.4	7.8 ± 0.2	9.1
O	20.3 ± 0.7	22.3±0.5	23.4±0.4	21.2 ± 0.3	20.9
Au	0.0	0.0	0.0	0.0	/
Cu	0.0	0.0	2.7±0.2	0.0	/
N/C	0.11±0.02	0.12±0.01	0.13±0.01	0.11±0.01	0.12

O/C	0.28±0.01	0.33±0.01	0.36±0.01	0.30 ± 0.01	0.25
Oxygenated units (%)*	24	64	88	40	-

The data in Table 1 show that PDA samples exhibit virtually similar N/C ratios resembling that of the monomer, suggesting relatively intact aliphatic amine chains with no detectable loss of amine group, but display variable degrees of structural modifications as apparent from the O/C ratios. The latter indicate that all PDA samples contain newly oxygenated units which may arise from various mechanisms depending on the oxidation process, including epoxidation (in the case of H_2O_2-containing media, pathway a in Fig. 4) and hydroxylation of the o-quinone ring (1 O atom per unit, pathway b in Fig. 4) and muconic-type fission of quinone rings (2O atoms per unit, pathway c in Fig. 4). It is thus reasonable to suggest that variable proportions of the aforementioned units account for the incorporation of oxygen via the above oxidant-dependent mechanisms, as illustrated in Fig 5. All these reaction pathways are discussed in the older literature describing the oxidation kinetics of catechols in solution in the presence of periodate [35]. We make the assumption, partially based on our XPS data, that the same pathways are possible for the strong oxidants used in this investigation. The occurrence of epoxidation of dopamine in the presence of hydrogen peroxide has been demonstrated in [36].

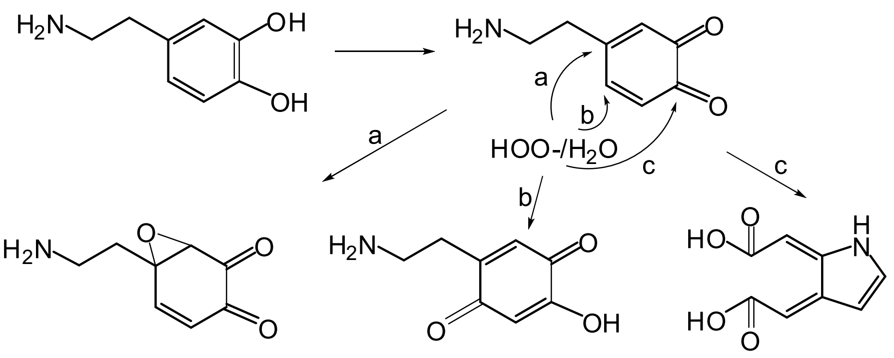

Fig. 4: Possible mechanisms of oxygenation of dopamine units during PDA formation and film deposition. The different pathways correspond to epoxidation (pathway a), hydroxylation (pathway b) and muconic cleavage (pathway c).

It can be concluded that all oxidants can induce oxidative degradation of the PDA films to a variable degree, depending both on their redox potential and their mechanism of action, i.e. via electron transfer ($(NH_4)_2S_2O_8$) or ester formation ($NaIO_4$) as the dominant mechanistic step, in agreement with previous observations in the case of PDA/$NaIO_4$ films [20]. Consistent with this view, milder oxidation conditions, i.e. PDA/O_2-8h films, produce minimal oxygenation/oxidative breakdown modifications, which can be estimated in the order of 24% and can be attributed to the generation of low levels of H_2O_2 by electron transfer from the catechol ring to molecular oxygen. $(NH_4)_2S_2O_8$, $NaIO_4$ and $CuSO_4/H_2O_2$ caused the most significant modification to the original level of oxygenation (ca. 40, 64 and 88 % of units displaying an additional oxygenated function, in that order). This observation is in line with the known formation of complexes between Cu^{2+} salts and catecholamines, which may evolve in an aerobic/oxidizing environment with partial electron transfer generating Cu(I) species capable of interacting with H_2O_2 to induce oxidative breakdown and oxygenation. Copper is a well-documented promoter of dopamine oxidation with concomitant formation of reactive species, such as $^{\bullet}O^{2-}$ and hydroxyl radicals via Fenton type chemistry [38].

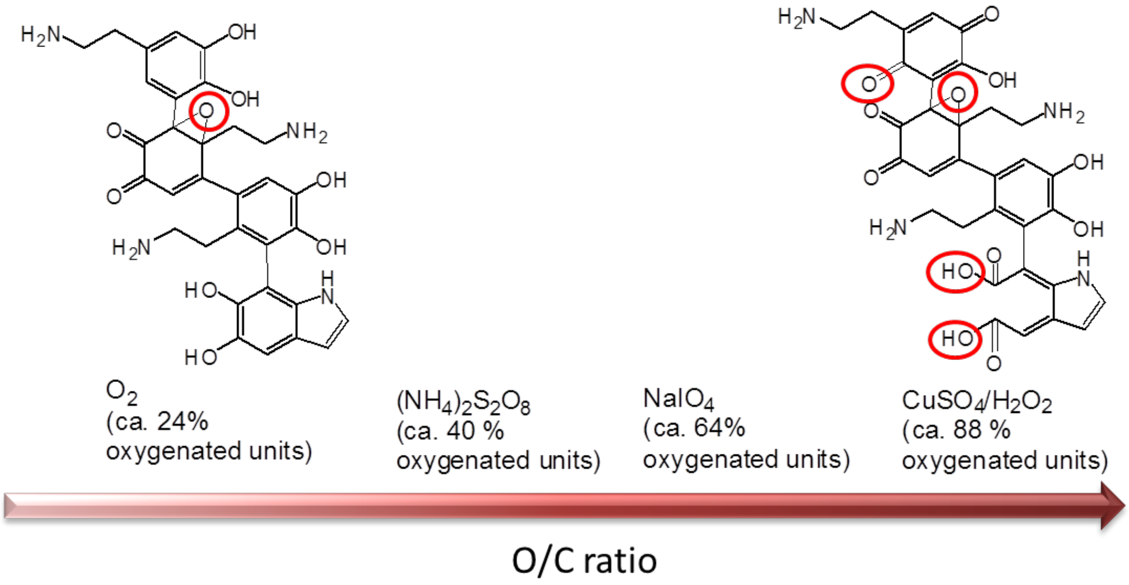

Fig. 5: Proposed structures for PDA samples incorporating low (on the left) to high (on the right) levels of additionally oxygenated units. Epoxide moieties can be regarded as markers of H_2O_2 nucleophilic reactivity.

We do not focus on the O/C and N/C ratios for the films obtained on gold coated mica substrates after 3 h of contact with dopamine + oxidant blends because most of them (with exception of the PDA/$NaIO_4$-3h films) are not conformal, indeed their XPS spectra still displayed the presence of a few % of Au atoms.

3.4 Antioxidant properties of the PDA/oxidant films

As expected [32-34], all the PDA/oxidant-*x*h films display some antioxidant activity as evidenced by the discoloration of DPPH. Surprisingly, owing of the strong oxidation of those films (Table

1), the PDA/$NaIO_4$ films display the most efficient effect (Fig. 6A). The DPPH scavenging efficiency-calculated using equation (1)- of the PDA/O_2, the PDA/$NaIO_4$ and the PDA/$CuSO_4$+H_2O_2 films slightly decreases with the deposition time (Fig. 6B) whereas the film thickness continues to increase (Fig. 1B and Fig. S4 of the Supplementary file). This is not the case for the PDA/$(NH_4)_2S_2O_8$ films which have a constant scavenging efficiency upon an only slight increase in film thickness from 3 to 8 h of deposition (Fig. 6B). Taken together, these data show that the antioxidant activity of the films is not an increasing function of the film thickness. This result is not surprising based on the fact that DPPH is a large pretty hydrophobic molecule which should not diffuse deeply in the hydrophilic PDA/oxidant coatings. We have shown that even a small hydrophilic redox probe like hexacyanoferrate is not able to cross the PDA/oxidant films (Fig. S6 in the Supplementary file).

The slight decrease in antioxidant activity of the PDA/O_2, the PDA/$NaIO_4$ and the PDA/$CuSO_4$+H_2O_2 films with the deposition time (Fig. 6B) may be attributed to a progressive oxidation of the surface catechol groups, even if this should also occur in the case of the PDA/$(NH_4)_2S_2O_8$ films. One would have expected the highest anti-oxidant effect for the films containing the highest fraction of catechol groups and displaying the lowest O/C ratio, i.e. the less oxidized films, namely those produced in the presence of O_2 dissolved in Tris buffer (Table 1). It has to be noticed also that, at first glance, the scavenging efficiency of the PDA/oxidant films appears pretty low-less than 25%. This is however due to the low surface area (45 mm × 26 mm on each side of the glass slide) to volume (50 mL) ratio in the performed experiments. Hence, the most important observation from Fig. 6B is the apparent higher scavenging efficiency of the PDA/$NaIO_4$ films with respect to the other films which appear similar from this point of view.

Interestingly, when the antioxidant activity is normalized by the film roughness (Fig. 1C) it appears that it is almost independent on the nature of the used oxidant (Fig. 7).

In addition, despite the chemical expectation of a loss of antioxidant functionalities, the films produced in the presence of strong oxidants are remarkably efficient with respect to this property (Fig. 6). Indeed, a reduction in the surface concentration of oxidizable functionalities is partially compensated by an increase in the surface area accessible to the solvent. This increase in surface area is estimated as the film roughness in this case (Fig. 1C). We recognize that the use of the rms roughness to estimate the increase in surface area of the film with respect to a planer film is somewhat arbitrary, owing to the other ways to quantify the roughness. But the incontestable increase in film roughness for the PDA/$NaIO_4$ films which is appreciable on the AFM topographies (Fig. 1 and Supplementary file) constitutes, for the moment, a reasonable explanation for the apparent higher antioxidant activity and higher oxidation currents measured by CV (Fig. 2).

Owing to the large standard deviation of the absorbance / roughness values displayed in Fig. 7, those values are the same regardless the used oxidant and the film deposition time, either 3 or 8h.

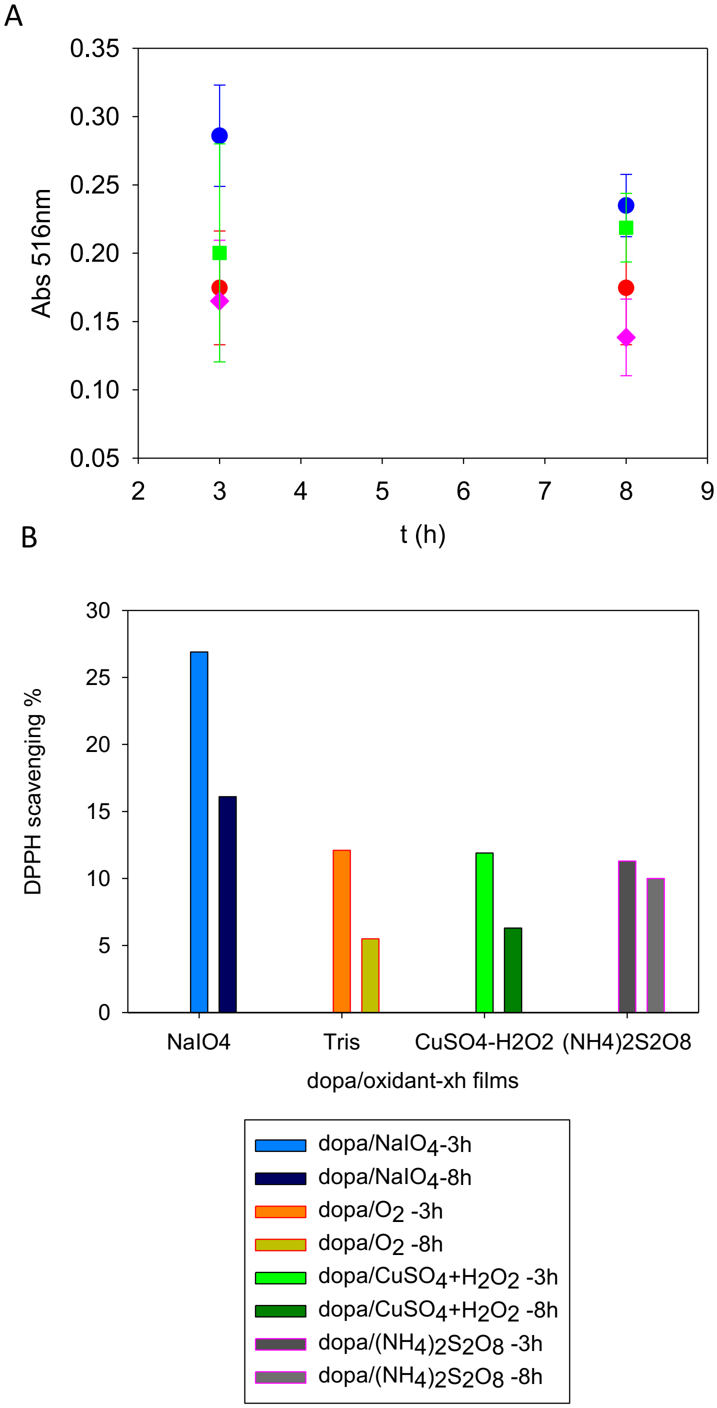

Fig. 6: A): Absorbance of the PDA/oxidant films after 3h and 8h deposition time on glass slides and after 2h immersion in a DPPH solution: (●) PDA/Tris, (●) PDA/$NaIO_4$, (■) dopa/$CuSO_4$-H_2O_2; (◆) PDA/$(NH_4)_2S_2O_8$.

B): DPPH scavenging efficiency of the PDA/oxidant-*x*h films as indicated in the inset. The scavenging efficiency is quantified as the reduction in absorbance at λ=516 nm divided by the initial absorbance of the DPPH solution (10^{-4} mol. L^{-1} in all experiments). The relative standard deviation of these data are smaller than 15 % for all oxidants (measurements performed on 3 independently prepared samples).

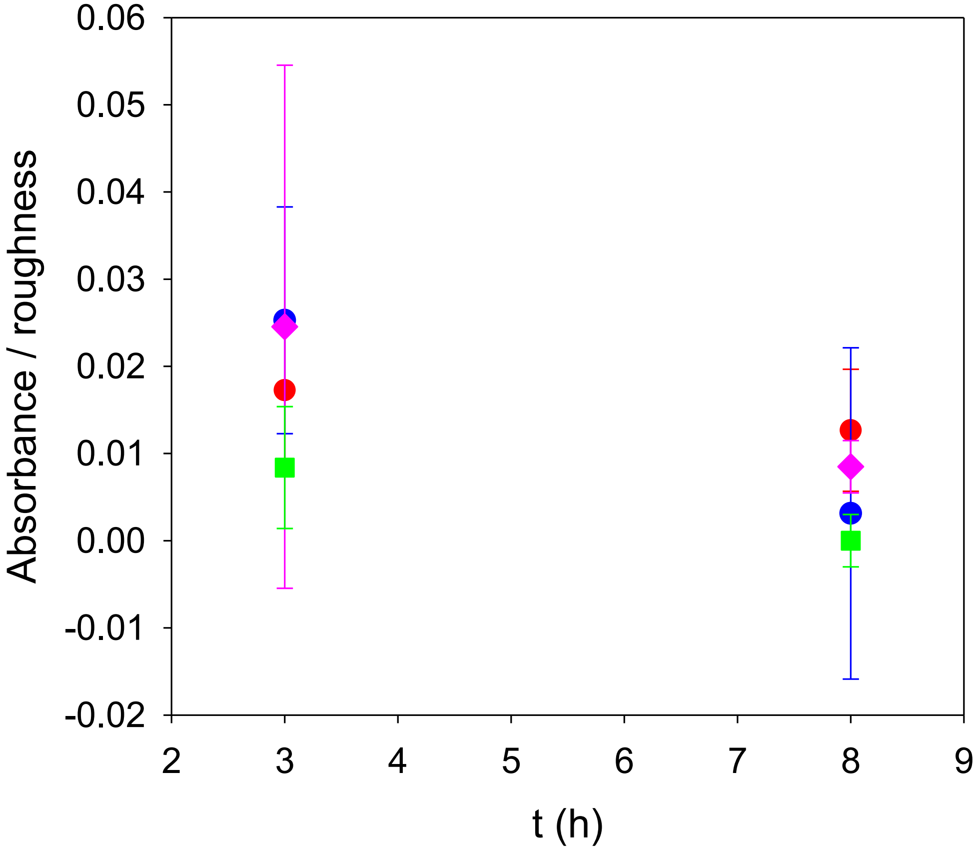

Fig. 7: Relative antioxidant activity, as quantified by the ratio of the absorbance peak at 516 nm by the film roughness (as determined on 2 µm x 2 µm AFM topographic images) for each PDA@ oxidant film as a function of the deposition time. The significance of the

colored symbols is: (🔴) PDA/Tris, (🔵) PDA/$NaIO_4$, (🟩) dopa/$CuSO_4$-H_2O_2; (◆) PDA/$(NH_4)_2S_2O_8$.

Hence, the PDA/$NaIO_4$ films feature numerous advantages with respect to the films prepared in the presence of the other investigated oxidants, not only due their higher hydrophilicity [20] and faster deposition kinetics (Fig. 1C) but also in term of conservation of antioxidant properties, which is due to a compensation of a lost in the catechol surface density (due to oxidation) by an increase in the film's roughness.

4. Conclusions

The morphology, electrochemical behavior and the composition of PDA films prepared by the oxidation of dopamine are markedly dependent on the used oxidant in agreement with previous findings [19, 22]. As expected [32, 34], all PDA films exhibit some antioxidant properties, reflecting the presence of unaffected catechol groups even when the deposition was performed in the presence of strong oxidants like $NaIO_4$ and $(NH_4)_2S_2O_8$. Surprisingly, the antioxidant activity seems the strongest on the PDA/$NaIO_4$ films which have undergone strong oxidation, but similar to that of the PDA@$CuSO_4$-H_2O_2 and PDA/$(NH_4)_2S_2O_8$ films. This result could be rationalized after normalization of the antioxidant activity by the roughness of the films yielding to an almost oxidant-independent behavior.

The comparison between the antioxidant properties of PDA films prepared using different oxidants was never made before, to our knowledge, and highlights that the films which are expected to display a lower antioxidant activity, owing to high degradation and post deposition oxidation, could indeed be extremely efficient from this point of view because of a high roughness and hence a higher specific surface area. This means that in the case of PDA films a control over the morphology could improve properties expected to be low on the basis of the chemical composition only.

The findings presented in this report highlight again the great diversity of PDA films in their morphology, structure and composition as well as in their properties. Such fundamental knowledge should pave the way to a rational control over polydopamine film properties for various applications. The action of strong oxidants on PDA/O_2 films should also be investigated to investigate the possibility of oxidant specific changes in the catechol/quinone balance as a function of the oxidation time in order to explain the decrease in the antioxidant activity of the PDA/0_2, the PDA/$NaIO_4$ and the PDA/$CuSO_4$+H_20_2 films with the deposition time in opposition to a constant activity of the PDA/$(NH_4)_2S_20_8$ films. The antioxidant activity of different kinds of PDA films should also be investigated in the presence of adhering cells.

CRediT authorship contribution statement

Salima El Yakhlifi: film deposition, anti-oxidant measurements, paper writing
Maria-Laura Alfieri: Film preparation, electrochemistry
Youri Arntz: AFM characterization of the PDA films
Matilde Eredia: XPS characterization of the PDA films
Artur Ciesielski: interpretation of the XPS spectra
Paolo Samori: general discussions, supervision
Marco d'Ischia: interpretation of the reaction mechanisms, specific role of each oxidant
Vincent Ball: coordination of the research, first draft, paper writing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel inspired surface chemistry for multifunctional coatings. Science 318 (2007) 426-430.

[2] S.M. Kang, J. Rho, I.S. Choi, P.B. Messersmith, H. Lee, Norepinephrine : material-independent, multifunctional surface modification reagent. J. Amer. Chem. Soc. 131 (2009) 13224-13225.

[3] S. Hong, J. Kim, Y.S. Na, J. Park, S. Kim, K. Singha, G.L. Im, D.K. Han, W.J. Kim, H. Lee, Poly(norepinephrine) : ultrasmooth material independent surface chemistry and nanodepot for nitric oxide. Angew. Chem. Int. Ed. 52 (2013) 9187-9191.

[4] M. Jaber, J.-F.Lambert, A new nanocomposite: L-DOPA/laponite. J. Phys. Chem. Lett. 1 (2010) 85-88.

[5] T.S. Sileika, D.G. Barrett, R. Zhang, K.H.A. Lau, P.B. Messersmith, Colorless multifunctional coatings inspired by polyphenols found in tea, chocolate, and wine. Angew. Chem . Int. Ed. 52 (2013) 10766-10770.

[6] D.G. Barret, T.S. Sileika, P.B. Messersmith, Molecular diversity in phenolic and polyphenolic precursors of tannin-inspired coatings. Chem. Comm. 50 (2014) 7265-7268.

[7] H. Ejima, J.J. Richardson, K. Liang, K.; J.P. Best, M.P. van Koeverden, G.K. Such, J. Cui, F. Caruso, One-step assembly of coordination complexes for versatile film and particle engineering. Science 341 (2013) 154-157.

[8] B.P. Lee, P.B. Messersmith, J.N. Israelachvili, J.H. Waite, Mussel inspired adhesives and coatings. Ann. Rev. Mater. Res. 41 (2011) 99-132.

[9] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységy, Plant polyphenols : chemical properties, biological activities, and synthesis. Angew. Chem. Int. Ed. 50 (2011) 586-621.

[10] H. Lee, J. Rho, P.B. Messersmith, Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings. Adv. Mater. 21 (2009) 431-434.

[11] H.Q. Ham, Z.Q. Liu, K.H.A. Lau, H. Lee, H.; P.B. Messersmith, Facile DNA immobilization of surfaces through a catecholamine polymer. Angew. Chem. Int. Ed. 50 (2011) 732-736.

[12] M.E. Lynge, R. van der Westen, A. Postma, B. Stadler, B. Polydopamine : a nature-inspired polymer coating for biomedical science. Nanoscale 3 (2011) 4916-4928.

[13] Y. Liu, K. Ai, L. Lu, Polydopamine and its derivative materials : synthesis and promising applications in energy environmental, and biomedical fields. Chem. Rev. 114 (2014) 5057-5115.

[14] N.F. Della Vecchia, A. Luchini, A. Napolitano, G. D'Errico, G. Vitiello, N. Szekely, M. d'Ischia, L. Paduano, L. Tris buffer modulates polydopamine growth, aggregation, and paramagnetic properties. Langmuir 30 (2014) 9811-9818.

[15] J. Liebscher, R. Mrówczyński, H.A. Scheidt, C. Filip, N.D. Hădade, R. Turcu, A. Bende, S. Beck, Structure of polydopamine : A never-ending story ? Langmuir 29 (2013) 10539-10548.

[16] P. Delparastan, K.G. Malollari, H. Lee, P.B. Messersmith, Direct Evidence for the Polymeric Nature of Polydopamine. Angew. Chem. Int. Ed. 58 (2019) 1077-1082.

[17] D.R. Dreyer, D.J. Miller, B.D. Freeman, D.R. Paul, C.W. Bielawski, Elucidating the structure of poly(dopamine). Langmuir 28 (2012) 6428-6435.

[18] S. Hong, Y. Wang, S.Y. Park, H. Lee, Progressive fuzzy cation-pi asssembly of biological catecholamines. Science Adv. 4 (2018) art. eaat7457.

[19] Q. Wei, F. Zhang, J. Li, B. Li, C. Zhao, Oxidant-induced dopamine polymerization for multifunctional coatings. Polym. Chem. 1 (2010) 1430-1433.

[20] F. Ponzio, J. Barthès, J. Bour, M. Michel, Ph. Bertani, J. Hemmerlé, M. d'Ischia, V. Ball, Oxidant Control of Polydopamine Surface Chemistry in Acids : A Mechanism-Based Entry to Superhydrophilic-Superoleophobic Coatings. Chem. Mater. 28 (2016) 4697-4705.

[21] F. Bernsmann, V. Ball, F. Addiego, A. Ponche, M. Michel, J.J. de Almeida Gracio, V. Toniazzo, D. Ruch, Dopamine-Melanin Film Deposition Depends on the Used Oxidant and Buffer Solution. Langmuir 27 (2011) 2819-2825.

[22] C. Zhang, Y. Ou, W.-X. Lei, L.-S. Wan, J. Ji, Z.K. Xu, $CuSO_4/H_20_2$-Induced Rapid Deposition of Polydopamine Coatings with High Uniformity and Enhanced Stability. Angew. Chem. Int. Ed. 55 (2016) 3054-3057.

[23] J.P. Park, L.T. Song, J. Lee, J.H. Ryu, Y. Lee, H. Lee, Vanadyl-Catecholamine Hydrogels Inspired by Ascidians and Mussels. Chem. Mater. 27 (2015) 105-111.

[24] X. Du, J. Li, J. Li, C. Yang, N. Frenkel, A. Welle, S. Heissler, A. Nefedov, M. Grunze, P.A. Levkin, P.A. UV-Triggered Dopamine Polymerization : Control of Poymerization, Surface Coating, and Photopatterning. Adv. Mater. 26 (2014) 8029-8033.

[25] Y. Li, M. Liu, C. Xiang, Q. Xie, S. Yao, Electrochemical quartz microbalance study on growth and property of the polymer deposit at gold electrodes during oxidation of dopamine in aqueous solutions, Thin Solid Films 497 (2006) 270-278.

[26] F. Bernsmann, J.-C. Voegel, V. Ball, Different Synthesis Methods Allow to Tune the Permeability and Permselectivity of Dopamine-Melanin Films to Electrochemichal Probes. Electrochim. Acta. 56, (2011) 3914-3919.

[27] B. Stöckle, D.Y.W. Ng, C. Meier, T. Paust, F. Bischoff, T. Diemant, R.J. Behm, K.E. Gottschalk, U. Ziener, T. Weil, Precise control of polydopamine film formation by electropolymerization, Macromol. Symp. 346 (2014) 73-81.

[28] J. Vatral, R. Boca, W. Linert, Oxidation properties of dopamine at and near physiological conditions, Monatshefte Chemie 146 (2015) 1799-1905.

[29] L.C. Almeida, J.P. Correia, A.S. Viana, Electrochemocal and optical characterization of thin polydopamine films on carbon surfaces for enzyamtic sensors. Electrochim. Acta 263 (2018) 480-489.

[30] M. d'Ischia, A. Napolitano, A. Pezzella, P. Meredith, T. Sarna, Chemical and structural diversity in eumelanins: unexplored bio-optoelectronic materials. Angew. Chem. Int. Ed. 48 (2009) 3914-3921.

[31] M. d'Ischia, A. Napolitano, V. Ball, C.-T. Chen, M.J. Buehler, Polydopamine and eumelanin : from structure-property relationships to a unified tailoring strategy. Acc. Chem. Res. 47 (2014) 3541-3550.

[32] Y. Liu, K. Ai, X. Ji, D. Askhathova, R. Du, L. Lu, J. Shi, Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury and ischemic stroke. J. Amer. Chem. Soc. 139 (2017) 856-862.

[33] K.Y. Ju, Y. Lee, S. Lee, S.B. Park, J.K. Lee, Bioinspired polymerization of dopamine to generate melanin like nanoparticles having an excellent free-radical scavenging property. Biomacromolecules 12 (2011) 625-632.

[34] H. Liu, X. Qu, H. Tan, J. Song, M. Lei, E. Kim, G.F. Payne, C. Liu, C. Role of polydopamine's redox activity on its pro-oxidant, radical scavenging and antimicrobial activities. Acta Biomater. 88 (2019) 181-196.

[35] S.W. Weidman, E.T. Kaiser, The mechanism of periodate oxidation of aromatic systems. III. A kinetic study of the periodate oxidation of catechol. J. Amer. Chem. Soc. 88 (1966) 5820-5827.

[36] P. Manini, L. Panzella, A. Napolitano, M. d'Ischia, M. A novel hydrogenperoxide-dependent oxidation pathway of dopamine via 6-hydroxydopamine. Tetrahedron. 59 (2003) 2215-2221.

[37] G. Tarabella, A. Pezzela, A. Romeo, P. D'Angelo, N. Coppedè, M. Calicchio, M. d'Ischia, R. Mosca, S. Iannotta, Irreversible evolution of eumelanin redox states detected by an organic electrochemical transistor: en route to bioelectronics and biosensing. J. Mater. Chem. B. 1 (2013)3843-3849.

[38] M. Bisaglia, L. Bubacco, Copper ions and Parkinson's disease: why is homeostasis so relevant. Biomolecules. 10 (2020) art. 195.

Figure captions

Fig. 1: A: 2 µm × 2 µm AFM surface topographies of PDA/oxidant-8h films. B: Film thickness of the PDA/oxidant-8h films as determined from topographical profiles determined in AFM images. C): dependence of the PDA/oxidant -8h film root-mean square roughness as a function of the image size. The data in part B and C correspond to 5 measurements ± one standard deviation.

(●): PDA/O_2, (●) PDA/$NaIO_4$, (●) PDA/$CuSO_4$-H_20_2, (●) PDA/$(NH_4)_2S_2O_8$ films.

Fig. 2 : Cyclic voltammetry curves in the presence of the same buffers (sodium acetate or Tris) used for the film deposition : polished electrode (——, sodium acetate buffer), PDA/O_2-8h (——, Tris buffer), PDA/$NaIO_4$-8h (——, sodium acetate buffer), PDA/$CuSO_4$-H_2O_2-8h (——, sodium acetate buffer) and PDA/$(NH_4)_2S_2O_8$-8h (——, sodium acetate buffer).

Fig. 3: Representative XPS spectra in the C1s region of PDA/oxidant-8h films. The colored lines correspond to the spectral deconvolution with the corresponding chemical assignments on the right.

Fig. 4: A): Absorbance of the PDA/oxidant films after 3h and 8h deposition time on glass slides and after 2h immersion in a DPPH solution: (●) PDA/Tris, (●) PDA/$NaIO_4$, (■) dopa/$CuSO_4$-H_2O_2; (◆) PDA/$(NH_4)_2S_2O_8$.

B): DPPH scavenging efficiency of the PDA/oxidant-*x*h films as indicated in the inset. The scavenging efficiency is quantified as the reduction in absorbance at λ=516 nm divided by the initial absorbance of the DPPH solution (10^{-4} mol. L^{-1} in all experiments).

Fig. 5: Relative anti-oxidant activity, as quantified by the ratio of the absorbance peak at 516 nm by the film roughness (as determined on 2 µm x 2 µm AFM topographic images) for each PDA@ oxidant film as a function of the deposition time. The significance of the colored symbols is: (●) PDA/Tris, (●) PDA/$NaIO_4$, (■) dopa/$CuSO_4$-H_2O_2 ; (◆) PDA/$(NH_4)_2S_2O_8$.

Table caption

Table 1: Elemental composition (in % and average N/C and O/C ratios for the PDA/oxidant-8h films as obtained by XPS on *n* = 3 samples. All data are given ± one standard deviation.