Deciphering the Platinized Surface Reactivity to Improve the Detection of Hydrogen Peroxide in Bioanalyses

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Abstract: The reactivity of platinized electrode surface (Pt-black) was deciphered towards the oxidation of hydrogen peroxide in the most common physiological buffer, namely phosphate buffer saline, used in the development of biosensors. It is shown here that the oxidation of hydrogen peroxide by Pt-black involves first the adsorption of phosphate anions, which facilitates the oxidation of hydrogen peroxide at lower potentials when compared to classic polycrystalline platinum. This gives insight to the excellent analytical features of platinized electrodes for the detection of H₂O₂, even at nanomolar concentrations. The reactivity of platinized electrodes can be further improved by an oxygen plasma treatment. We demonstrate herein that such treatment creates an outermost layer of PtO₂ oxides on the deposit, which improves thermodynamically and kinetically the oxidation of hydrogen peroxide, as well as of hydroquinone and superoxide radical anion.

Introduction

The Reactive Oxygen Species (ROS), including at least hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•−}), hydroxyl radical (OH[•]), singlet oxygen (¹O₂) play an essential role in multiple pathways of aerobic cell metabolism. Their monitoring is thus crucial to gain understanding of ROS consequences on both physiological and pathological processes, such as respiration, apoptosis, protein synthesis and lipid peroxidation. Since a majority of the ROS and RNS (reactive nitrogen species) are electroactive, they have thus become the target of electrochemical sensors development for biomedical applications. Apart from the numerous biosensors that have been reported, such as the peroxidase- or cytochrome c - modified electrodes,^[1-7] many research efforts have been devoted to structured metallic electrode surfaces. In particular, it is known for more than a century that platinum is an excellent catalyst for

the decomposition of hydrogen peroxide. It has thus been used under multiple forms and structures (polycrystalline, monocrystalline, nanoscopic, mesoscopic, etc.) for the electroanalysis of H₂O₂^[8-10], O₂^{•−}^[11-13], as well as of their mother species, di-oxygen (³O₂)^[14-16].

Among these surfaces, the nanostructured platinum deposits, quoted as platinum black (Pt-black) because of their light absorbance property, have raised much interest over the last three decades. In particular, Amatore *et al.* have shown that platinum black could be deposited on flat carbon or platinum electrodes of various size (millimetric to micrometric) and geometry (disk, band)^[17]. Interestingly, they have shown that platinized microelectrodes are very efficient electroanalytical tools for the selective detection of different ROS-RNS in aqueous buffers and biological media: primarily H₂O₂, but also nitric oxide (NO[•]), nitrite (NO₂[−]), nitroxyl (NO[•]) and peroxynitrite (ONOO[•]) anions^[17-21]. Moreover, it was shown that platinized ultramicroelectrodes provide the required analytical features (size, response-time, linear response over a large concentration domain) to monitor successfully the dynamic of ROS and RNS production by living systems at multiple scales: tissue, cell population, single cell or organelles^[18-20]. The good compatibility between platinum black surfaces and biological systems has been illustrated by the incorporation of this material in the construction of implantable electrodes^[22-24] and in the fabrication of numerous biosensors, among which glucose biosensors occupy an important part^[25-29]. In general, the great sensitivity achieved by these biosensors is explained by both the roughness, and consequently high surface-area of the platinum black deposits, and the catalytic activity of this material toward the electro-oxidation of H₂O₂^[30-32]. Surprisingly, though the mechanism of this reaction has been extensively studied on polycrystalline platinum electrodes^[33-39], little is known about what happens exactly on platinum black and which features provide specific activity to this surface.

A recent study has shown that, in the case of electro-oxidation of high concentrations of hydrogen peroxide (about hundreds of millimolar), the local decrease of pH inhibits the active sites of platinum black, shifts the oxidation wave to higher potentials and leads to additional oxidation waves^[40]. Additionally, our group has shown that the electroanalysis of low H₂O₂ concentrations (nano- to micromolar range) could be improved owing to a physical treatment by oxygen plasma of the platinized surfaces^[41]. Lower capacitive currents as well as faster electron transfer rates for hydrogen peroxide oxidation were observed by modulating the oxidation state of platinum black electrode surface. The control of

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platinum oxidation state is of significant importance for industrial catalytic processes such as CO oxidation, NO_x reduction, oxidation of volatile organic compounds, etc. It is demonstrated that platinum oxides affect the thermodynamics and kinetics of various anodic reactions through multiple pathways including i) the change of electronic properties of the surface; ii) the creation of a charge transfer barrier; iii) the interaction with adsorbed reactants, intermediates or final products via a site-blocking effect. The oxidation of platinum is predominantly achieved via electrochemical anodization under highly acidic conditions [42-45] or via reactive sputtering [46, 47] but more rarely via plasma treatments [48, 49].

In this context, this paper is aimed at understanding further the properties of platinum black surfaces and particularly the mechanism of oxidation of hydrogen peroxide in phosphate buffer at physiological pH (7.4), which is a key process for many types of biosensors. The role of the medium for analyses, namely of phosphate ion adsorption in the overall oxidation mechanism was evidenced; the hypothesis was reinforced by comparing the oxidation waves for H₂O₂ with responses of hydroquinone and superoxide anion solutions at platinum black electrodes. Finally, the importance of platinum oxides in these electro-oxidation processes was studied under modulation by an oxygen plasma treatment and reduction-oxidation potential scanning of the platinum black surfaces.

Results and Discussion

Deciphering the electro-oxidation mechanism of H₂O₂ at platinum black electrodes

Platinum black-modified electrodes were obtained by a potentiostatic electrodeposition in a hexachloroplatinic acid bath prepared in phosphate buffer (PBS, pH 7.4) according to a well-known method [18-20, 41]. SEM imaging (Figure 1) shows the typical "cauliflower" structure of Pt-black deposits that presents both micrometric and nanometric roughnesses. At the lower scale, we observe that the surface is developing from the growth of Pt needles of 100 nm mean dimension. We further analyzed this crystalline structure of Pt-black surfaces by XRD spectroscopy (Figure 2). We characterized a ratio between platinum crystallographic planes, (111):(210):(110), that is very different from the one of polycrystalline metallic platinum on which the deposit was grown: 1/0.3/0.25 and 1/0.3/7, respectively. This may explain some of the specific catalytic properties of Pt-black reported so far and herein (see below).

Figure 1. SEM imaging at two different magnifications of a platinum black deposit on a Pt microelectrode.

Figure 2. XRD spectra of a polycrystalline platinum microelectrode (25 μm diameter) before and after platinization.

Then, platinized microelectrodes were employed for the electro-oxidation of H₂O₂ in bioanalytical conditions, i.e. at pH 7.4 in phosphate buffer (10 mM phosphate concentration). In comparison with polycrystalline platinum microelectrodes, which provide a single oxidation wave in voltammetry (Figure 3a), peaking around 0.6 V vs. Ag/AgCl and a slow transfer kinetic profile^[41], a wave is observed at platinum black modified electrode with much faster kinetic reaching a plateau current at about 0.3 V vs Ag/AgCl (Figure 3b). Therefore, a shift of about 300 mV is gained, which improves very significantly the selectivity of biological measurements. However, we also observed two complementary facts; first, H₂O₂ solutions at concentrations higher than 2 mM exhibit a second oxidation wave on Pt black electrodes (Figure 4, full line) with a plateau potential close to 0.65 V vs Ag/AgCl. The presence of two oxidation waves at pH 7.4 is somewhat unexpected. Even if two electrons-two protons are involved in the oxidation process, these are commonly transferred in a single wave on polycrystalline Pt^[50]. Secondly, when hydrogen peroxide oxidation is analyzed in an electrolyte solution of 0.1 M NaClO₄ (pH 7.4 unbuffered), a single oxidation wave is observed at the same potential as the second wave recorded in PBS (Figure 4, dash line). Therefore, it is evidenced here that phosphate anions play an important role in the mechanism, especially in the presence of the first oxidation wave. This mechanism is similar to the one proposed by Hall et al^[33-37]

regarding the oxidation of hydrogen peroxide on polycrystalline platinum electrode and phosphate buffer (Scheme 1).

Scheme 1. Oxidation mechanism of several species (denoted with M) on platinum black electrode and in phosphate buffer solutions.

The first step is the adsorption of phosphate at a pre-binding site (PS) on the platinum black surface, leading to the formation of a binding site (BS; equation 1 in scheme 1). Secondly, hydrogen peroxide adsorbs on this site facilitating energetically its oxidation (equation 2 and 3 in scheme 1). If the phosphate species are not present, hydrogen peroxide reactivity is apparently different. It may adsorb directly on the platinum electrode, prior to its oxidation, possibly on a different reactive site at the surface. The key step of this mechanism is the adsorption of phosphate species (H_2PO_4^- and HPO_4^{2-}). This phenomenon is well documented on platinum single crystal and polycrystalline electrodes [51-54]; it is clear from present data that phosphate adsorption on black platinum electrodes occurs as well. As shown in Figure 5a, this adsorption gives rise in PBS to a well-defined anodic peak, with an onset potential at 0.23 V and peak potential at 0.37 V, and a less defined cathodic reverse wave. These peak and wave disappear in sodium perchlorate electrolyte (pH 7.4, unbuffered).

Further, the competition between phosphate adsorption and formation of "Pt-O" surfaces oxides was taken into consideration [52]. When comparing Pt-black and polycrystalline Pt surface responses, the two phenomena are not clearly separated on polycrystalline platinum since they both display an onset potential at 0.23 V vs. Ag/AgCl, while on platinum black phosphate adsorption appears at a lower potential (0.23 V) than the onset of platinum oxides at 0.63 V (Figure 5b). Hence, the oxidation wave for hydrogen peroxide, reported at potentials above 0.6 V at polycrystalline electrode, can be explained by the fact that phosphate adsorption (essential for the adsorption of hydrogenated species) is hindered by the formation of surface oxides. On platinum black surface, the phosphate adsorption is clearly separated from the formation of surface oxides and thus the oxidation waves of these species appear at lower potentials, i.e. between 0.3 and 0.6 V. Other differences between platinized and polycrystalline platinum are observed in the $\text{H}_{\text{adsorption}}/\text{H}_{\text{desorption}}$ fingerprints and for the reduction of surface oxides. Specifically, $\text{H}_{\text{desorption}}$ appears at a more positive potential and the reduction peak at a more negative potential on platinized platinum compared to polycrystalline electrode (Figure 5b). These differences can be explained, in part, by the difference in the crystalline structure between black and polycrystalline platinum (Figure 2), but also by the high roughness factor of black Pt

compared to polycrystalline Pt, as reported elsewhere [24], and by the presence of chlorine in the PBS buffer. It is important to note here that the same differences between the two electrodes were observed in an electrolyte containing only 0.1 M NaClO_4 at pH 7, but they were not observed in electrolyte containing only 0.5 M H_2SO_4 (data not shown). They are also an indication about the differences that are encountered for the oxidation of several species at black platinum when compared with polycrystalline platinum.

Figure 3. Hydrogen peroxide oxidation at polycrystalline and Pt-black electrodes. Experimental conditions: 1mM H_2O_2 in air equilibrated PBS pH 7.4; platinization charge, 800 μC ; scan rate, 20 $\text{mV}\cdot\text{s}^{-1}$.

Figure 4. H_2O_2 oxidation at Pt black electrodes in two different electrolyte solutions. Experimental conditions: H_2O_2 20 mM in air equilibrated PBS pH 7.4 or air equilibrated 0.1 M NaClO_4 pH 7.4; platinization charge, 800 μC ; scan rate, 20 $\text{mV}\cdot\text{s}^{-1}$.

Figure 5. a) Voltammetric responses of platinized electrodes in phosphate buffer saline (PBS) and sodium perchlorate; b) Comparison of voltammetric

profile of polycrystalline platinum and platinum black electrodes in phosphate buffer saline (PBS). Experimental conditions: electrolyte, N_2 saturated PBS pH 7.4 or 0.1 M NaClO_4 pH 7.4; platinization charge, 800 μC ; scan rate, 20 $\text{mV}\cdot\text{s}^{-1}$ for platinum electrode and 100 $\text{mV}\cdot\text{s}^{-1}$ for platinized platinum electrode.

The second step of the mechanism involves the adsorption of H_2O_2 on the binding site created by the adsorption of phosphate. This favors energetically the electron transfer and gives rise to the first oxidation wave detected by cyclic voltammetry. Interestingly, we observed that another hydrogenated species similar to H_2O_2 , that is hydroquinone, follows the same trend and provides also an oxidative wave at low potentials in PBS (Figure 6) on the platinized electrodes. Moreover, both species, hydrogen peroxide and hydroquinone, are detected also at higher potentials, providing a second wave (close to 0.6 V vs. Ag/AgCl) on Pt-black electrodes. This is observed only when concentration of hydrogen peroxide or of hydroquinone rises above few millimolars.

Figure 6. Voltammetry of hydroquinone oxidation at a platinized electrode. Experimental conditions: the hydroquinone solution is at 5 mM in an air equilibrated PBS pH 7.4; platinization charge, 800 μC ; scan rate, 20 $\text{mV}\cdot\text{s}^{-1}$.

Consequently, we hypothesized that the first oxidation process detected below 0.4 V, which depends on adsorption should reach a limit when all adsorption sites would be occupied. In order to check this hypothesis, the influence of the species concentration on both waves has been studied (Figure 7). For hydrogen peroxide, the current intensity of both waves varies linearly up to a concentration of about 10 mM, with a better sensitivity being observed for the first wave (Figure 7a). For higher concentrations, a change in the slope is observed, the sensitivity of the first wave becomes low (about 50-fold lower) while the second wave current increases linearly. These results are in agreement with the ones of Kicela et al., who reported two waves for the oxidation of hydrogen peroxide at black platinum electrodes, and a dependence on the roughness factor of electrode surface and the strength of the phosphate buffer [40]. The appearance of the second wave was noticed at 100 mM for a phosphate buffer of 0.2 M while in the present work, the appearance of the second wave is observed at 2 mM for an usual

phosphate buffer of 0.01 M. For hydroquinone, the first wave offers a high sensitivity for concentrations lower than 10 mM, reaching further a plateau-limit, while the second wave presents a single fairly linear evolution for the entire domain of concentrations (up to 50 mM) (Figure 7b). Both species present thus a very similar electrochemical behavior showing the importance of the phosphate-dependent interaction with the Pt-Black surface. Nevertheless, from an analytical point of view, we confirm herein that a much higher sensitivity is obtained at low potentials, based on detecting the first oxidation wave. This is a major advantage of the platinized surface for the development of biosensors and other electro-analytical tools where nano to micromolar concentrations in phosphate buffer, mainly of H_2O_2 , must be measured.

Figure 7. Influence of the concentration of species (hydrogen peroxide and hydroquinone) in PBS on their first and second oxidation wave detected by cyclic voltammetry at platinized microelectrodes (experimental conditions of Figures 4 and 6).

Oxygen plasma treatment of platinum black electrodes

In the process of improving the analytical properties and understanding the reactivity of platinized microelectrodes, we have recently shown [41] that an oxygen plasma treatment reduces markedly their capacitive current in cyclic voltammetry and the corresponding root-mean-square noise in chronoamperometry measurements. These effects allowed to improve the sensitivity (3-fold) of hydrogen peroxide detection (10 nM Limit of Detection) and to monitor a flux of few hundreds picomoles $\text{H}_2\text{O}_2/\text{min}$ released during the respiration of mitochondria [41]. All effects were observed in the potential window of the first oxidation wave for H_2O_2 analyzed above. Consequently, a better understanding of the influence of oxygen plasma treatment on the oxidation mechanism of H_2O_2 , and hydroquinone, became necessary. Platinized microelectrodes were subjected to an oxygen plasma treatment (20 min, 750 mTorr of 100 % oxygen). The creation of platinum surface oxides was studied by XPS. Figure 8 shows the comparison of XPS spectra in the Pt 4f region obtained before and after treatment of the same microelectrode. The new peak appearing at 77.4 eV and corresponding to $\text{Pt 4f}_{5/2}$ is a conclusive evidence of the formation of PtO_2 -type platinum oxides. This result is consistent with previously reported formation of PtO_2 prepared by either anodization [42, 55] or plasma treatment [56]. Compared with oxygen plasma treatment of Pt thin films or nanoparticles [49], PtO -type oxides could not be detected on the treated platinized electrodes. This could be explained by the low power plasma device (18 W compared to 100 W [56]) that was used in the present work. XPS spectra were also collected in depth (0.2 – 80 nm) and the presence of PtO_2 is confirmed only at the extreme surface of the Pt-black deposit.

Figure 8. X-ray photoelectron spectra of Pt4f taken before and after the oxygen plasma treatment of platinized microelectrodes. Experimental conditions of the oxygen plasma treatment: 100 % O_2 at 750 mTorr; plasma power, 18 W; treatment time of 20 min.

The presence of PtO_2 was also evidenced by electrochemical experiments. Figure 9 presents the cyclic voltammograms obtained before and after the oxygen plasma treatment in a degassed sulfuric acid electrolyte. Before the

treatment, the well-known reduction peak at 0.43 V of 2D surface platinum oxides [57] is observed. After plasma treatment, the electrode potential was directly cycled in the reduction direction and two observations could be made: i) the characteristic reduction peak is displaced to a lower potential (0.34 V) and ii) a new reduction shoulder appears at 0.29 V. Both of these observations point out the existence of surface oxides with a higher oxidation state, such as Pt^{IV} . These results are very similar to the ones reported for the formation of PtO_2 -3D oxides obtained by high potential anodization of platinum electrodes [42, 57, 58]. The displacement of the 2D oxides reduction peak to lower potentials after the creation of 3D oxides is also well-known for the platinum electrodes [58]. In this later case, the higher the potential at which the anodic oxide film is formed, the lower (less positive) is the potential required for the reduction of this film. This irreversible behavior, that is opposite to thermodynamic expectations, is explained by the fact that the reduction process is not microscopically the reverse of the oxidation reaction (surface hysteresis) [58]. The complete reduction of PtO_2 created by the oxygen plasma is observed during the second cycle (Figure 9) where the characteristic reduction peak returns to its normal position (0.43 V), and neither other shoulder nor additional peak is observed. The disappearance of PtO_2 oxides after the electrochemical reduction was confirmed also by XPS spectra in which only the Pt 4f peaks characteristic of metallic platinum were observed (see supplementary Figure S1).

Figure 9. Cyclic voltammetry responses of a platinumized microelectrode before and after an oxygen plasma treatment. Experimental conditions: N_2 saturated 0.5 M H_2SO_4 ; scan rate, 20 mV s^{-1} ; E_{start} = open circuit potential. The arrow shows the scan direction.

As expected, the oxygen plasma treatment did not change the crystallographic structure of platinum black surfaces, since the temperature (room T°) of the oxygen plasma is not high enough to induce annealing of the surface. In addition, the presence of PtO_2 could not be evidenced in the XRD spectra because these oxides are encountered only at the extreme surface (as shown by depth profile of XPS spectra) and they don't form a new bulk phase in the rough platinumized platinum. Finally, the oxygen plasma treatment did not induce any changes in the morphology of Pt-black deposits, at least at the scale of high resolution SEM images.

Influence of the oxygen plasma treatment on the detection of several species

The effects of the oxygen plasma treatment on the oxidation waves of hydrogen peroxide and hydroquinone in PBS were studied (Figures 10a and 10b). A first observation was that in the condition, i.e. a large potential window, allowing the observation by cyclic voltammetry of two oxidation waves in PBS for both species, no clear and efficient effect of the plasma treatment was observed; transfer kinetics and thermodynamics were not improved. When focusing the study, by limiting the potential window, on the first oxidation wave, we observed variations of interest for mechanistic studies. As depicted in Figure 10 and detailed in Table 1, hydrogen peroxide and hydroquinone oxidation waves were displaced at lower half-wave potentials, typically a 50 mV-difference, and showed faster transfer kinetics with better defined voltammetric responses.

The difference of results between the two sets of experiments, as function of the potential window, is in agreement with the results of figure 5b showing the role of the upper potential limit on surface oxide creation and modification. As demonstrated in Figure S2, when the electrode potential reaches at least 0.6V vs. Ag/AgCl in PBS, the response of the surface changes, corresponding to a modification of the nature or distribution of platinum oxides. Indeed, Pt-O oxides can be formed at higher potentials. Such an electrochemical annealing effect is well documented on polycrystalline platinum electrode [59, 60], and our measurements show that the process is also possible for platinum black surface in phosphate buffer. Hence, the catalytic effect of the oxygen plasma treatment is observed only for platinumized electrodes that did not suffer a surface chemistry reconstruction at high potentials ($E > 0.6$ V), via the formation of Pt-O oxides. Since the first oxidation waves of hydrogen peroxide and hydroquinone are detected at potentials lower than 0.4 V, valuable effects of the oxygen plasma treatment on platinum black can be observed. This shows eventually that the PtO_2 surface oxides play a key role in the catalytic effects described above. Moreover, when storing the oxygen plasma treated platinumized microelectrodes in MilliQ-grade water, these oxides were stable for at least one week since the electrode responses were stable in terms of the oxidation waves features.

Table 1. Half-wave potential ($E_{1/2}$ vs (Ag/AgCl)/V) for the studied species before and after the oxygen plasma treatment

Species	Before plasma treatment		After plasma treatment	
	1 st wave ^[a]	1 st wave ^[b]	1 st wave ^[a]	1 st wave ^[b]
Hydroquinone	0.100	0.080	0.090	0.025
H_2O_2	0.200	0.275	0.230	0.240
O_2^-	-0.020	-	-0.060	-

[a] with surface reconstruction. [b] without surface reconstruction

Finally, we extended the above study to the detection of another species of bioanalytical interest, superoxide radical anion. Superoxide is a major ROS, the mother species of at least H_2O_2 and ONOO^- . It is involved in the physiological metabolism of all aerobic cells, but also in derived oxidative stress mechanisms. Although many electrochemical sensors have been developed for the detection of superoxide [11–13], the use of platinum black electrodes was not reported so far. We detected by cyclic voltammetry a clear single oxidation wave centered near 0 V vs. Ag/AgCl for superoxide in basic phosphate buffer solutions. It is worth to mention that the mechanism of superoxide oxidation at platinum black electrodes cannot be included in the general mechanism described above because this species is stable only in basic solution (pH 12). At this pH, the adsorption of phosphate (key step of the proposed mechanism) is severely hindered by the adsorption of hydroxyl anion. Nevertheless, we observed similarly that the oxygen plasma treatment could improve the detection of superoxide anion at platinum black electrodes, as the oxidation wave was displaced at even lower potentials (-0.05 V), with a lower background capacitive current after treatment (Figure 10c).

Figure 10. Cyclic voltammograms for the first wave of studied species before and after oxygen plasma treatment of the platinized microelectrode, without surface reconstruction. Experimental conditions: air equilibrated PBS pH 7.4; platinization charge, 800 μC for all the species, except for O_2^- for which a platinization charge of 150 μC was used.

Conclusion

Amatore *et al.* have reported at many instances the analytical interests of a special-structured platinum surface, the platinum black one. This surface can be electrochemically deposited under excellent control on electrodes of various geometry and dimension, even on a single carbon nanotube (unpublished results). It was reported earlier that the Pt-black surface provides a very efficient detection of the major redox product of oxidases, hydrogen peroxide. However, the mechanism of its detection was poorly understood until now. In this framework, we showed herein that two oxidation processes can occur on the same Pt-black surface; the first one at low potentials, below the OCP, involves the adsorption of phosphates and the following adsorption of H_2O_2 before its fast oxidation. In addition, the thermodynamic and kinetic of this overall process can be improved under an oxygen plasma treatment. The locally generated surface oxides, PtO_2 , favor the catalysis of the process. Oppositely, they do not affect the second oxidation wave, which behaves as a pure-diffusion wave and provides much less sensitivity for H_2O_2 detection. These studies pave the way for further surface treatments of Pt black and deciphering of corresponding electrochemical mechanisms. The advantage of such surfaces over Pt nanoparticle-modified electrodes is the full control of surface roughness and area by electrodeposition, providing high sensitivity for measurements of biological responses on cells, tissue or organelles.

Experimental Section

Microelectrodes used in this study are denoted as such with respect to the platinum disk diameter that is in micrometer range (25 μm) and not to the final body of the sealed Pt disk that was about 2 mm in diameter. The procedure for obtaining disk shaped microelectrodes consists in introducing the 25 μm diameter platinum wire (Goodfellow, Pt 99.99%) into

the tapered end of a glass tube (soft glass), then heat it and seal it with a heating loop. The non-sealed end of the wire was connected to a millimetric steel wire using silver paste. The flat surface of the microdisk was obtained after polishing with a coarse and a fine grade polishing papers. A polishing step using Al_2O_3 particle suspension was not employed in this study.

Platinization of the microelectrodes was achieved by reducing 115 mM hydrogen hexachloroplatinate (prepared in PBS buffer, see below) in the presence of 0.76 mM lead acetate at -60 mV versus Ag/AgCl/NaCl 3 M (this reference electrode has a potential of 0.209 V vs SHE). The electrodeposition was stopped when the total reduction charge reached 150 μC or 800 μC . All chemicals were from Sigma-Aldrich. Superoxide solution was prepared using ice-cold 0.03 M NaOH and the obtained concentration was calculated following the protocol reported elsewhere [61] (Caution! potassium superoxide reacts violently with water). Decomposition of this species is neglected as the cyclic voltammetry measurements were taken as quickly as possible after the solution preparation.

Platinized microelectrodes (also called black platinum electrodes) were exposed to oxygen plasma within so-called plasma cleaner (Harrick Plasma inc., plasma-flow PDCFMG). The exposure chamber was previously cleaned with acetone. After inserting the sample, the chamber was subjected to vacuum in order to reach a 100 mTorr pressure. 100% oxygen flow was then injected in the chamber in order to reach a final pressure of 750 mTorr. The plasma created in the chamber should be white coloured. After 20 min exposure, the chamber was opened and the sample was immediately soaked in MilliQ water.

The electrochemical measurements were carried out using a potentiostat (BioLogic inc. VSP-300, EC-Lab software, with a built-in impedance spectrometer) and a standard single compartment electrochemical cell. An Ag/AgCl/NaCl 3 M electrode and a large platinum wire were used as reference and respectively counter electrodes. Electrochemical experiments were carried out in PBS buffer (10 mM phosphates, 138 mM NaCl, 2.7 mM KCl, pH 7.4). All the solutions of the studied compounds were prepared using this buffer. Cyclic voltammograms (CVs) were conducted at 20 mV s^{-1} scan rate over different potential windows and cycle 3 is represented in the Figures, if not mentioned otherwise (see Figures). Electrochemical impedance spectroscopy was recorded in the 50 kHz - 100 MHz range, with a sinusoidal ac potential perturbation of 10 mV superimposed to the open circuit potential. Readings were taken at 15 discrete frequencies per decade.

A Thermo Fisher Scientific K-ALPHA spectrometer was used for surface analysis with a monochromatized Al K α source ($h\nu = 1486.6 \text{ eV}$) and a 200 micrometer-size spot. The full spectra (0-1100 eV) were obtained with constant pass energy of 200 eV and the high resolution spectra (C1s, O1s, Pt4f) at constant pass energy of 40 eV. Charge neutralisation was applied during analysis. Sputtering was performed with Ar^+ ions at 500 eV. High resolution spectra were fitted and quantified (Scofield sensitivity factors used for quantification) using the AVANTAGE software provided by Thermo Fisher Scientific. A SEM (FEG JEOL JSM 6700F) with an integrated EDX system was used for sample imaging before and after oxygen plasma treatment. X-ray diffraction (XRD) patterns were collected on a PANalytical X'pert MPD-PRO Bragg-Brentano θ - θ geometry diffractometer equipped with a secondary monochromator, a 3X15 positions sample changer and an X'celerator detector over an angular range of $2\theta = 8$ - 80° . Each acquisition lasted for 3 hours. The Cu-K α radiation was generated at 45 KV and 40 mA ($\lambda = 0.15418 \text{ nm}$).

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Keywords: platinization • platinum black • oxygen plasma • hydrogen peroxide • hydroquinone • surface annealing

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Layout 1:

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V. Badets, J. Pandard, N. Sojic and S. Arbault*

Page No. – Page No.

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