



HAL
open science

Easy cleaning plus stable activation of glassy carbon electrode surface by oxygen plasma

Camille Colin, Pierre Levallois, Ugo Botsos-Margerit, Franck Clement, Dodzi Zigah, Stéphane Arbault

► To cite this version:

Camille Colin, Pierre Levallois, Ugo Botsos-Margerit, Franck Clement, Dodzi Zigah, et al.. Easy cleaning plus stable activation of glassy carbon electrode surface by oxygen plasma. *Bioelectrochemistry*, 2023, 154, pp.108551. <10.1016/j.bioelechem.2023.108551>. <hal-04467684>

HAL Id: hal-04467684

<https://hal.science/hal-04467684v1>

Submitted on 20 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Easy Cleaning Plus Stable Activation of Glassy Carbon Electrode Surface by Oxygen Plasma

Camille Colin^a, Pierre Levallois^a, Ugo Botsos-Margerit^a, Franck Clément^b, Dodzi Zigah^{a,c*},
Stéphane Arbault^{a,d*}

a. Univ. Bordeaux, Bordeaux INP, CNRS, ISM, UMR 5255, F-33400 Talence, France.

b. Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM UMR 5254, 64600 Anglet, France.

c. Institut de Chimie des Milieux et Matériaux de Poitiers (IC2MP), Université de Poitiers, CNRS, F-86073 Poitiers, France.

d. Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, F-33600 Pessac, France.

Corresponding Authors :

* Dr Stéphane ARBAULT, email : stephane.arbault@u-bordeaux.fr, Tel.: +33540006851 ;

* Pr Dodzi Zigah, e-mail: dodzi.zigah@univ-poitiers.fr, Tel: +33549453970.

ABSTRACT:

Glassy carbon (GC) electrodes are widely used in electroanalytical applications especially in bioelectrochemistry. Their use starts with an efficient surface cleaning and activation protocol, mostly based on surface polishing steps. We studied the use of an oxygen plasma exposure of GC electrodes to replace common polishing procedures. The cyclic voltammetry (CV) responses of ferrocyanide and ferrocene-dimethanol were used to compare brand new, surface-polished and plasma-treated GC electrodes. Plasma treatment induces CV responses with improved features, close to theoretical values, as compared to other methods. The plasma effects were quasi-stable over a week when electrodes were stored in water, this being explained by increased surface energy and hydrophilicity. Furthermore, when electroreduction of diazonium was performed on GC electrodes, the surface blockade could be removed by the plasma. Thus, a short oxygen plasma treatment is prone to replace polishing protocols, that display person-dependent efficiency, in most of the experiments with GC electrodes.

KEYWORDS : glassy carbon; oxygen plasma; ferrocyanide; electron transfer; surface chemistry; diazonium.

1. INTRODUCTION

Carbon materials are the most widely used electrode surface materials for electroanalytical and especially bioelectrochemical studies.[1-5] Carbon materials for electrochemistry include graphite, pyrolytic graphite, glassy carbon, nanotubes, graphene, etc.[4] Their physico-chemical properties are suitable to the experimental working conditions of studies in aqueous solutions (buffers) and on proteins, DNA, living cells, etc.[6-8] Indeed, the surface chemistry of carbon electrodes is often adequate or better than that of metal electrodes (Au, Pt) for good chemical interactions or adsorption of species to be detected in bioanalytical studies. In particular, favorable interactions with quinones allow their detection by oxidation at reasonable overpotentials (e.g. + 600 mV vs Ag/AgCl). Such feature allowed the development of neurotransmitter sensing by carbon electrodes under in vitro to in vivo conditions.[9-13]

Glassy carbon surfaces remain the gold standard among carbon electrodes used for electroanalysis because of their well-defined geometries (usually a disk, cylinder, etc.), their mechanical properties-robustness and reproducible responses, for instance in laboratory courses. Their use starts with their surface cleaning and activation.[14-18] While platinum and gold disk electrodes can be electrochemically pre-treated in acidic media (e.g. sulfuric acid), such procedure is hardly efficient for GC electrodes.[19] In addition, if electrochemical pre-treatments are performed in solvents or buffers, the ensuing presence of electrodeposited/adsorbed species must be checked. Hence, GC surface preparation is most likely performed via polishing protocols. Each laboratory or user has its own protocol, depending on its experiences and study objectives. Then, surface polishing may include one or more gridding on hard to soft sand papers, polishing with nanoparticles (aluminum oxide suspension in water) of decreasing size, plus solvent rinsing and sonication steps between polishing steps.[14, 15]

Besides, plasma treatments are increasingly used to clean and reproducibly prepare any type of electrode surface.[20-24] Low-pressure plasma setups with air or pure oxygen as a gas carrier are widely used as so-called plasma cleaners to eliminate organic or micro-nanoparticles surface contaminants prior to the use of electrodes. Highly reactive oxygen species - ROS - and reactive nitrogen species - RNS - (in presence of nitrogen) are generated in the plasma phase[25-27] leading to a strong oxidation of any organic matter adsorbed or bonded to carbon surfaces.[28-31] Surface cleaning is the first application of plasma in electrochemistry, although such high-energy physical processes can be used to modify the structure of carbon materials and generate an increased porosity and various geometries.[24, 32-35] Atmospheric plasma are also used to expose solutions and produce in situ ROS and RNS, detectable by electrochemical sensors[21, 36-38]. The interaction between ROS (RNS) and carbon surfaces can modify their surface chemistry depending on plasma parameters, including the plasma source power, the exposure duration... Common plasma treatments modify the very outer layer of carbon (nanometer range) that will be in interaction with the solution, by creating locally more oxidized chemical functions such as alcohol, carbonyl and carboxylic entities.[39-41] These functions improve the overall surface energy and thus its wettability for aqueous solutions (similar to glass or platinum surfaces). Such features have recently been used to maximize the surface area and electrochemical responses of e.g. screen printed carbon electrodes.[30, 39, 41]

In this work, we propose to assess if tedious and person dependent procedures of surface polishing for GC electrode preparation can be solely replaced by an oxygen plasma treatment. Thus, we studied by cyclic voltammetry (CV) and compared the electroanalytical responses of GC electrodes that were either brand new (never used before), surface polished according to a two-step procedure, or non-polished and exposed to a low pressure oxygen plasma. GC electrode responses were studied with several redox species, which differ in their mechanistic behavior (outer vs inner sphere electron transfer). The stability of the oxygen plasma effects was studied as function of the electrode conservation method. The plasma effects were shown to improve surface cleaning, electroactivity, geometric area and to remove electrografted organic layers very efficiently. Consequently, for a wide range of applications, oxygen plasma should advantageously replace any surface polishing procedure of GC electrodes.

2. MATERIALS AND METHODS

2.1. Cyclic Voltammetry experiments. The electrochemical measurements were carried out using a potentiostat (BioLogic inc. VSP-300, EC-Lab software) with a classical three electrodes-setup. A reference Ag/AgCl (NaCl 3 M) electrode and a large platinum wire used as a counter electrode were purchased from ALS (Japan). The working electrodes are glassy carbon (quoted as GC) disk macroelectrodes of 3 mm diameter (CH instruments, USA). All the CV were acquired at the scan rate of 100 mV s⁻¹. Electrochemical experiments were carried out in solutions of the studied redox compounds prepared in KNO₃ 0.3 M (aerated solutions).

2.2. Electrode preparation and treatments. GC electrodes were either used as new (never polished nor polarized) or after a polishing (not plasma-treated before) or after a plasma treatment (not polished before). Polishing procedure was based on manual omega movements of the electrode tip on a P2000 grading paper (Presi, France) and then on a polishing cloth covered with a suspension of Al₂O₃ particles (50 nm size; Presi, France). Following, the electrode was thoroughly clean with milli-Q water and dried before use. Oppositely, GC electrodes were rinsed, dried and exposed to an oxygen plasma in a low-pressure plasma cleaner (plasma-Flow PDC-FMG, Harrick Plasma Inc., USA). The exposure chamber was previously cleaned with acetone and isopropanol. After inserting the sample, chamber was subjected to vacuum in order to reach a 75 mTorr pressure *in situ*. 100 % oxygen flow was then injected in the cell to reach a pressure of 1250 mTorr. Exposure was performed at maximum power (30 mW) for a defined time varying from 1 to 60 min (caution, after such long exposure, the sample may be hot). Following, the electrode was removed and immediately placed either in a clean box under air or in pure milli-Q water (covered beaker) and stored up to one week; polished electrodes were similarly conserved in similar conditions for comparison.

The grafting of GC electrodes by phenyl groups was based on the use of phenyldiazonium cations. These were synthesized *in situ* by adding 4 mM of aniline to a solution containing 8 mM of sodium nitrite and 0.5 M of hydrochloride acid. Then, phenyldiazonium cations were reduced electrochemically directly on the GC

electrode by cyclic voltammetry, performed between 0.2 and -1.2V vs Ag/AgCl (3 M KCl) at 100 mV/s scan rate during ten cycles.

2.3. Reagents. Potassium ferrocyanide, ferrocenedimethanol, hydrochloric acid 37%, aniline, phosphate buffer tablets, potassium chloride species were purchased from Sigma-Aldrich (France). Potassium nitrate was obtained from Scharlau (Germany). Milli-Q grade water (Millipore, France) was used to prepare all the solutions.

2.4. Contact angle measurements. A drop shape analyzer (Krüss DSA 100), was used for the determination of contact angle. The size of the water droplet was 20 μ L. Each surface was tested three times with two measurements on one surface. Glassy carbon plates used for the contact angle measurement were from Sigradur.

2.5. Electrode surface state analyses. An Atomic Force Microscope (AFM; Veeco Instruments, model Multimode 8) was used to analyse the carbon surface topography in dry state before and after plasma exposures (10 or 20 min.). Mean values of the roughness over 2 μ m x 2 μ m area of the electrodes were calculated and compared. X-Ray photoelectron spectroscopy (XPS) analyses were performed (K-Alpha ThermoFisher Scientific spectrometer) to study the surface chemical compositions of the GC electrodes after polishing or after exposure to the plasma (10 or 20 min.). Just after each treatment, electrodes were dried with a pure N₂ flow and inserted in the spectrometer chamber so as to prevent their contamination by external contaminants. The samples were excited with amonochromatic Al Ka X-ray 400 μ m spot. A pass energy of 200 eV was used for survey spectra and 40 eV for high-resolution spectra. The peak fitting and quantifications were performed using OriginPro software.

3. Results and discussion

3.1. Effects of an oxygen plasma on GC electrode responses.

We used throughout this work a simple protocol to assess the effects of an oxygen plasma (low-pressure RF-generated plasma in 100 % pure O₂ gas) on the electrochemical activity of GC macroelectrodes. Analyses were performed under standard laboratory conditions, i.e. by cyclic voltammetry at a scan rate of 100 mV s⁻¹ in solutions of usual redox probes: Ferrocene-dimethanol, Fc(MeOH)₂, and ferrocyanide Fe(CN)₆⁴⁻ (potassium hexacyanoferrate (II)). These redox species differ in their electron transfer mechanism, namely an outer-sphere process for Fc(MeOH)₂ and an inner-sphere process for Fe(CN)₆⁴⁻. The second one depends on chemical interactions with the electrode surface (here carbon) as a first step before the charge transfer, while the first one is much less dependent on such interactions. CVs were thus measured in 1 mM solutions of Fc(MeOH)₂ or Fe(CN)₆⁴⁻ for GC electrodes that were either brand new, never used for electrochemical measurements before (not polished), in comparison with electrodes treated with an oxygen plasma for different durations, ranging from 1 to 60 min.. Figure 1 shows the CVs and the analysis of typical qualitative parameters (oxidation and reduction peak amplitudes, potential difference between peaks) detected for each redox species in the different conditions. Typical well-resolved, reversible and fast electron transfer CVs were detected for ferrocene-dimethanol for any condition (Fig.1A). The plasma treatment generated a slight

increase (10 % difference at most) of both anodic I_{pa} and cathodic I_{pc} peak amplitudes (Fig.1B), probably because of a cleaning effect provided by the plasma treatment, i.e. the desorption of organic molecules, air contaminants, etc. ΔE_p was close (Fig.1C) to the theoretical expected value : 59 mV for a one-electron transfer process. For the detection of ferrocyanide, stronger effects were observed after the plasma treatment. As expected, poorly resolved CVs were observed on the non-polished GC surface, (see Fig. 1D, raw surface). When the GC electrodes were pre-treated by the oxygen plasma, better resolved CVs were detected. The amplitudes of both I_{pa} and I_{pc} increased after only 1 min of exposure, and continued to increase up to 30-40 % for long-term exposures, i.e. $t > 30$ min. (Fig.1E). The effect may be attributed to a larger exposed, active electrode surface but not solely since the charge transfer kinetics were improved, as depicted by the evolution of ΔE_p (Fig.1F). ΔE_p values can reach 150 to 200 mV for surfaces not activated by any usual process; the oxygen plasma treatment leads to a clear drop of the potential difference that reached 72 mV for GC electrodes treated between 30 to 60 min. Even if such long treatment durations were the most effective, net effects were already observed after 10 min of plasma exposure. Besides, the exposure duration directly depends on the power of the RF source used to generate the plasma. Therefore, it should be shorter for more powerful setups (herein a 30 W source).

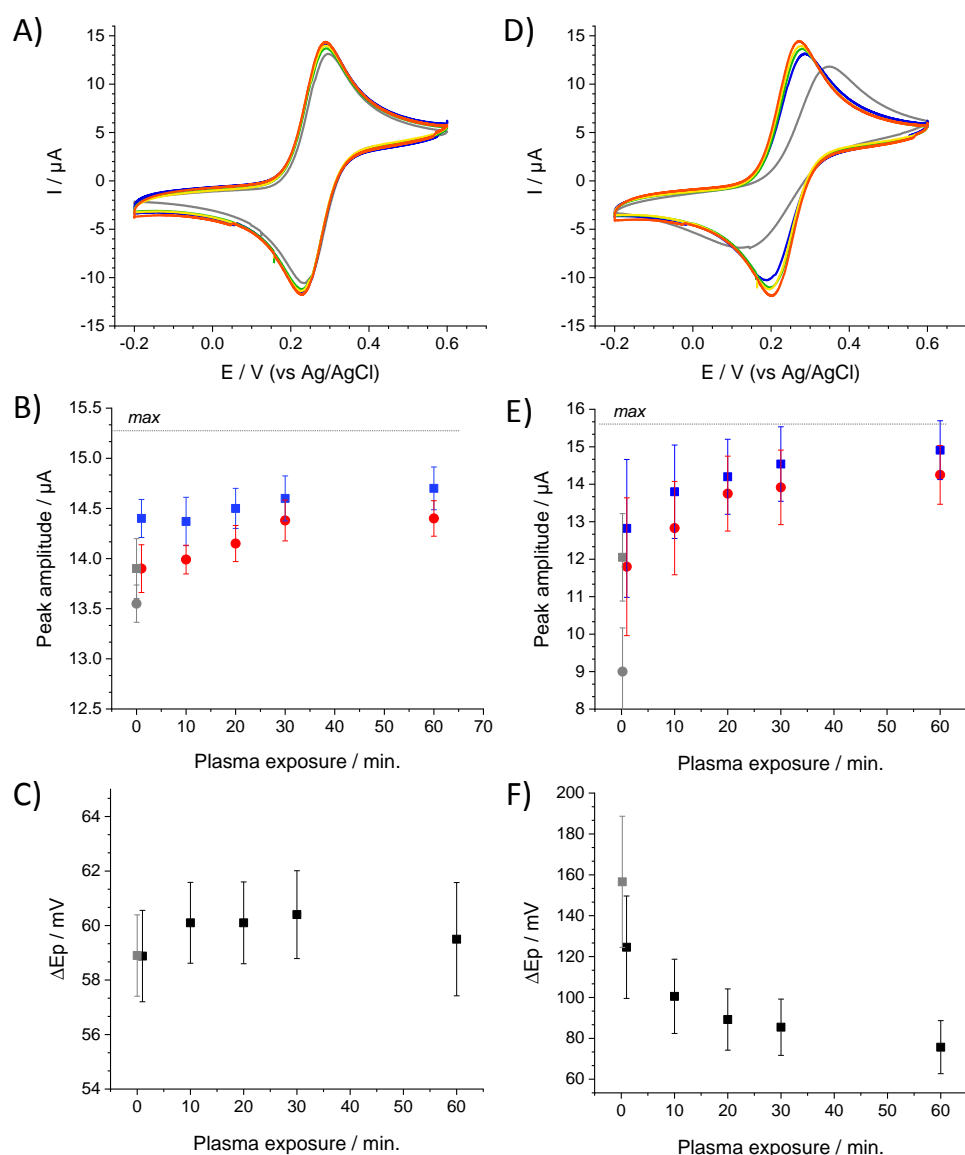


Figure 1. Effect of the oxygen plasma treatment on the voltammetric responses of glassy carbon (GC) electrodes for two redox probes: $\text{Fc}(\text{MeOH})_2$ (left column) and $\text{Fe}(\text{CN})_6^{4-}$ (right column). Electrodes were exposed to the oxygen plasma for 0 (raw; grey curves), 1 (blue), 10 (green), 20 (yellow), 30 (red) or 60 minutes prior to analyses in KNO_3 0.3 M electrolyte solutions containing either $\text{Fc}(\text{MeOH})_2$ or $\text{Fe}(\text{CN})_6^{4-}$ at 1 mM. On each CV (A, D; 100 mV s^{-1} scan rate), the oxidative (blue points) and reductive (red points) peak amplitudes (B, E) and the potential difference between peaks (C, F) were measured for $\text{Fc}(\text{MeOH})_2$ and $\text{Fe}(\text{CN})_6^{4-}$ responses, respectively. Values are the mean \pm SD data from experiments with at least 3 different treated GC electrodes.

Overall, these experiments show that a cleaning effect by the plasma on GC electrodes occurs and leads as well to an improvement of the charge transfer efficiency, as observed for ferrocyanide oxidation. On the one hand, the increases of CV currents show that the accessible active surface of the electrodes gets closer to the maximum geometrical surface area, since the calculated peak current for $\text{Fc}(\text{MeOH})_2$ and $\text{Fe}(\text{CN})_6^{4-}$ species in diluted aqueous solutions display diffusion coefficients calculated as $6.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $6.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, respectively; consequently, at 1 mM concentration detected with a 3 mm-diameter GC electrode, the corresponding peak currents calculated from the Randles Sevcik's equation should be at most $15.3 \mu\text{A}$ and

15.5 μA , respectively. Experimental currents tend toward these theoretical values as displayed on Fig.1B and Fig.1E. On the other hand, more kinetically-reversible CVs, i.e. displaying lower ΔE_p , were detected for plasma-treated electrodes, showing that the active surface interacts better with species involved in inner-sphere electron transfer mechanisms. To quantify such differences, CVs were acquired at several scan rates (between 10 to 1000 mV s^{-1}) for $\text{Fc}(\text{MeOH})_2$ and $\text{Fe}(\text{CN})_6^{4-}$ species and with pristine and 20 min. plasma-exposed GC electrodes. The electron transfer rate constants (k^0 , cm s^{-1}) with each redox species were calculated based on the method reported by Lavagnini I et al.[42] extended from the Nicholson theory. The correlation between the dimensionless parameter ψ and k^0 (Equation 1):

$$\psi = k_0 \left[\frac{\pi D n \nu F}{RT} \right]^{-1/2} \quad (1)$$

where, n is the number of electron transferred, i.e. $n = 1$, F is the Faraday constant, R is the gas constant, T is the absolute temperature), k^0 the slope of the graph, can be converted as function of the potential difference ΔE_p between oxidation and reduction peaks (for reversible and quasi-reversible systems) (Equation 2):

$$\psi = \frac{(-0.6288 + 0.0021\Delta E_p)}{(1 - 0.017\Delta E_p)} \quad (2)$$

Following this protocol, we found for $\text{Fc}(\text{MeOH})_2$, $k^0 = 6.1 \cdot 10^{-2} \text{ cm s}^{-1}$ at pristine electrodes and $k^0 = 7.5 \cdot 10^{-2} \text{ cm s}^{-1}$ at plasma treated electrodes, and in comparison for $\text{Fe}(\text{CN})_6^{4-}$, $k^0 = 2.3 \cdot 10^{-3} \text{ cm s}^{-1}$ at pristine electrodes and $k^0 = 8.1 \cdot 10^{-3} \text{ cm s}^{-1}$ at plasma treated electrodes. These values are in agreement with values reported elsewhere[43] by the same method and demonstrate that ferrocene-dimethanol is a fast electron transfer species (inner sphere) and lightly sensitive to the carbon surface changes induced by the plasma while ferrocyanide (outer sphere process) is more sensitive to the surface state.

3.2. Time evolution of the oxygen plasma effects.

Some other procedures commonly used to clean up and activate electrode surfaces may give similar performances to the oxygen plasma.[19] Although a plasma treatment appears to be simple and efficient, its stability over experiments and time had to be assessed. The first advantage of a plasma exposure is its high reproducibility (assuming that the plasma generator and exposing chamber are in good condition, e.g. not contaminated by any chemicals) while surface polishing is tedious, person-dependent and takes a multi-step process to get good results. Electrochemical treatments in acidic media (e.g. cycling in H_2SO_4) lead to partial surface activation of GC after only long cycling protocols and require cleaning of the electrode surfaces prior and after treatment before use for any electroanalysis.

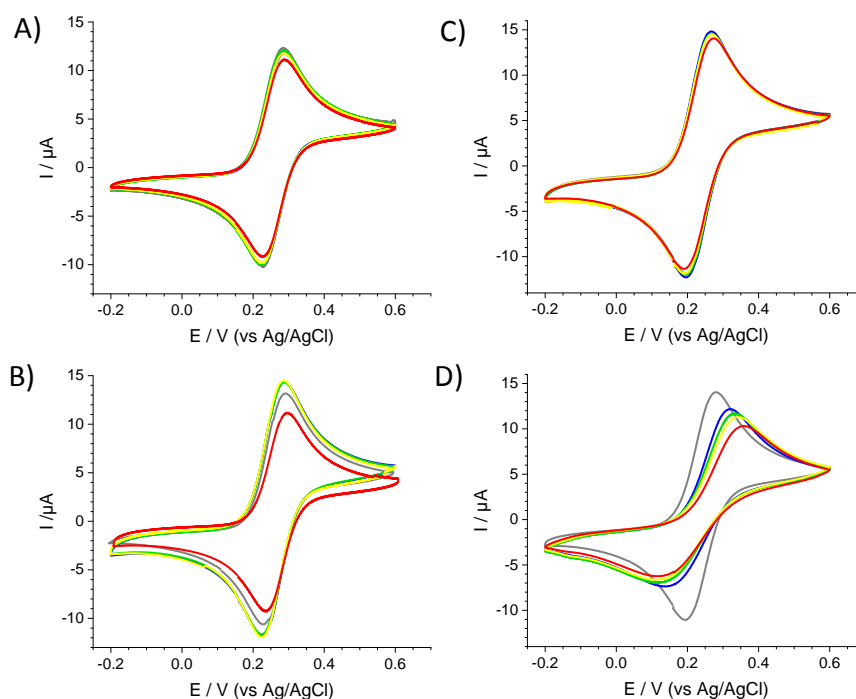


Figure 2. Stability of the plasma effect over one week (day 0 to day + 7) on the voltammetric responses of GC electrodes. CVs detected with GC electrodes exposed to the oxygen plasma for 20 min. at day 0 (D0; grey curve) were recorded every day (curves at D1 in blue, D2 in green, D3 in yellow and at D7 in red) in solutions of either $\text{Fc}(\text{MeOH})_2$ (left column) or $\text{Fe}(\text{CN})_6^{4-}$ (right column) at 1 mM concentration (KNO_3 0.3 M electrolyte). In between analyses, GC electrodes were either kept in MilliQ-grade water (A, C) or under air in a vial (B, D). CVs were obtained at a scan rate of 100 mV s^{-1} with a different GC electrode in each condition; the parameters of mean responses from several electrodes used in the same conditions are reported in Figure 3.

Then, the evolution of voltammetric responses from GC electrodes treated by the oxygen plasma (20 min.) versus the mechanical polishing protocol (a 3 step-polishing protocol, see experimental section), were analysed over two weeks. Within the first day after the electrode surface preparation, both approaches improved the CV features – amplitude, kinetics - as shown in Figure 1. These features evolved rapidly for the surface-polished electrodes with both ferrocyanide and ferrocene-dimethanol probes. With this technique any GC electrode should be polished again daily before use (as generally made in electrochemistry research labs). In comparison, the CVs evolved more slowly for plasma-treated GC electrodes (Figure 2); the decrease in response quality was clear after one week but limited to a 10 % current loss for $\text{Fe}(\text{CN})_6^{4-}$ after three days, even less for $\text{Fc}(\text{MeOH})_2$, and a slight progressive increase of ΔE_p . In conclusion, oxygen plasma-treated GC surfaces preserve their activation and surface area for at least several days.

In order to further investigate the stability of the plasma activation over time, we assessed two modes of conservation of the GC electrodes just after their surface treatments, either in deionized water (Fig.2A and 2C for $\text{Fc}(\text{MeOH})_2$ and $\text{Fe}(\text{CN})_6^{4-}$ species, respectively) or at ambient air in a vial (Fig.2B and 2D for $\text{Fc}(\text{MeOH})_2$ and $\text{Fe}(\text{CN})_6^{4-}$ species, respectively). Comparison of the CVs show that the conservation in water positively prolongates the response stability and thus the physico-chemical surface state of glassy carbon; a clear loss of activity is observed after only two weeks of storage in water. Besides, we did not observe any

clear difference between aerated and deaerated solutions for the quality of electrode conservation. A quantification of these evolutions over time is provided in Figure 3, where are compared the CV features for the polished and plasmas-treated electrodes as function of their storage mode, water vs air.

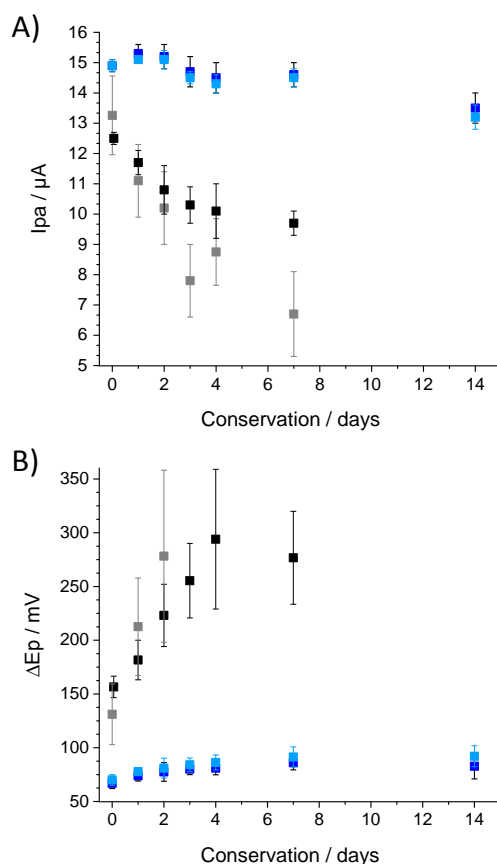


Figure 3. Evolution over the time of the CV parameters - oxidation peak current in A) and potential difference between peaks in B) - measured in $Fe(CN)_6^{4-}$ solutions (1 mM concentration in KNO_3 0.3 M electrolyte) with GC electrodes either solely polished (grey symbols) or exposed to an oxygen plasma for 20 min (blue symbols). Electrode responses were analyzed over two weeks, while in between measurements electrodes were kept either at air (light color symbols) or in deionized water (dark color symbols) in closed containers. Values are the mean \pm SD from at least 3 different experiments in each case.

The conclusions drawn from the results in Figures 2 and 3 are the following:

- first, plasma-treated electrodes provide better and more reproducible features than polished ones. Overall, faster charge transfer kinetics and higher currents (anodic and cathodic peaks) are observed on CVs;
- the surface chemical activation induced by the polishing, despite the combination of sand papers and alumina, is lost rapidly, over a few days, while the activation provided by the plasma exposure remains much longer; a slight decrease of the peak current occurs after one week;
- the GC surface activation is much better conserved when the electrodes are stored in water prior to use, as opposed to ambient conditions.

3.3. GC electrode surface modifications induced by an oxygen plasma.

Then, the question arises about the origin of the effects of oxygen plasma on GC electrode responses. As mentioned above, oxygen or air plasma is commonly used to clean surfaces (glass, silicon, metals, etc.) and remove adsorbed chemicals or solid particles present in the air. Such effect possibly uncovers a part of the electrode surface and thus potentially increases their electrochemically active area, leading to higher currents, as we observed. A carbon roughening by the plasma may as well increase the electrode active area. Though a low power-plasma cleaner was used for the present study, a device which a priori does not provide enough energy to etch glassy carbon, we performed surface roughness analyses by AFM of the plasma-exposed electrodes. We measured a decrease of surface roughness from 8.5 to 4.5 nm after 1 min plasma and a slight increase to 6 nm after a 20 min. plasma exposure. Consequently, values are all low and the oxygen plasma seems to rapidly etch material defects and clean up dust or particles adsorbed on it. Moreover, the comparison had to be performed with surfaces which were not mechanically polished (new surfaces) before the plasma, since such protocol always generate scratches and edges, which depths and profiles are in tens to hundreds of nanometer and thus modify the possible interpretations of the plasma effects.

Plasma treatment might as well modify the carbon chemistry and create some different chemical groups at the surface. This approach is often used to generate hydrophilic surfaces during microsystem developments (enabling surface bonding). In order to assess such physico-chemical change on GC surfaces, the surface contact angle with water of plasma-exposed electrodes was compared to that of new electrodes. As reported in Figure 4, the contact angle is high, about 60-70° for a non-treated (dirty) surface and improves slightly when kept in water (cleaning effect and water adsorption). Following the oxygen plasma treatment, the contact angle drops down to 10-20°, indicating that the GC surface has an excellent hydrophilicity. Moreover, the hydrophilic feature progressively decreases in intensity over several days, though a lower contact angle than for non-treated surfaces is kept after one week.

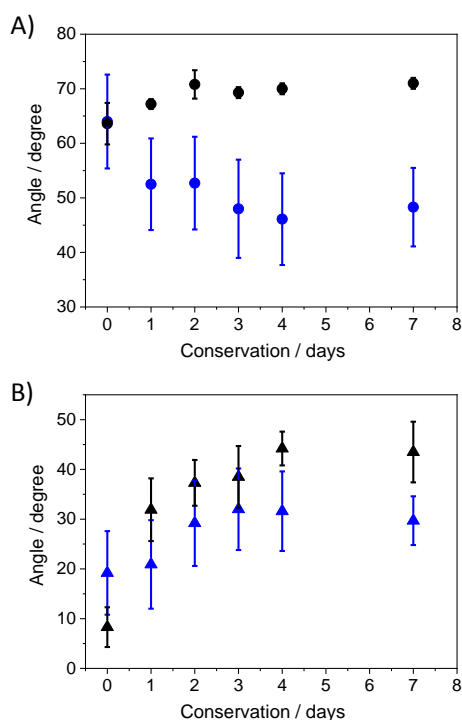


Figure 4. Evolution over 7 days of the contact angle of water drops on A) untreated GC surfaces, either stored in (●) water or at (●) air, and on B) GC surfaces exposed to the oxygen plasma (20 min.) and stored in (▲) water or at (▲) air.

In order to get more insight into the evolution of the carbon surface chemistry after plasma exposure, high resolution XPS analyses were performed. Spectra shown in Figure 5 and quantitative measurements reported in table1, based on C1s and O1s peaks, depict the rise of oxygenated functions of carbon versus its native C-C sp³ state. C-CO, C-O, C=O, COOR were found at substantial amounts on both polished and plasma-treated GC surfaces but with an increase of the overall O1s/C1s ratio and of the percentage of higher energy functions including C=O and COOR on plasma exposed ones. Aged surfaces, previously polished or plasma activated weeks before, displayed a very weak O1s peak, which was hard to deconvolute into sub-fractions. Moreover, surface etching and analysis in depth of the carbon chemistry showed that the pristine glassy carbon response (undetectable O1s) was found at few nanometres depth from the exposed surface. Consequently, we demonstrate at this stage that the soft oxygen plasma modifies the glassy carbon chemistry at its outer surface, by generating multiple oxygenated functions[41, 44] and a sufficiently higher surface energy, which lead to better hydrophilicity and wetting properties for aqueous solutions observed in Figure 4.

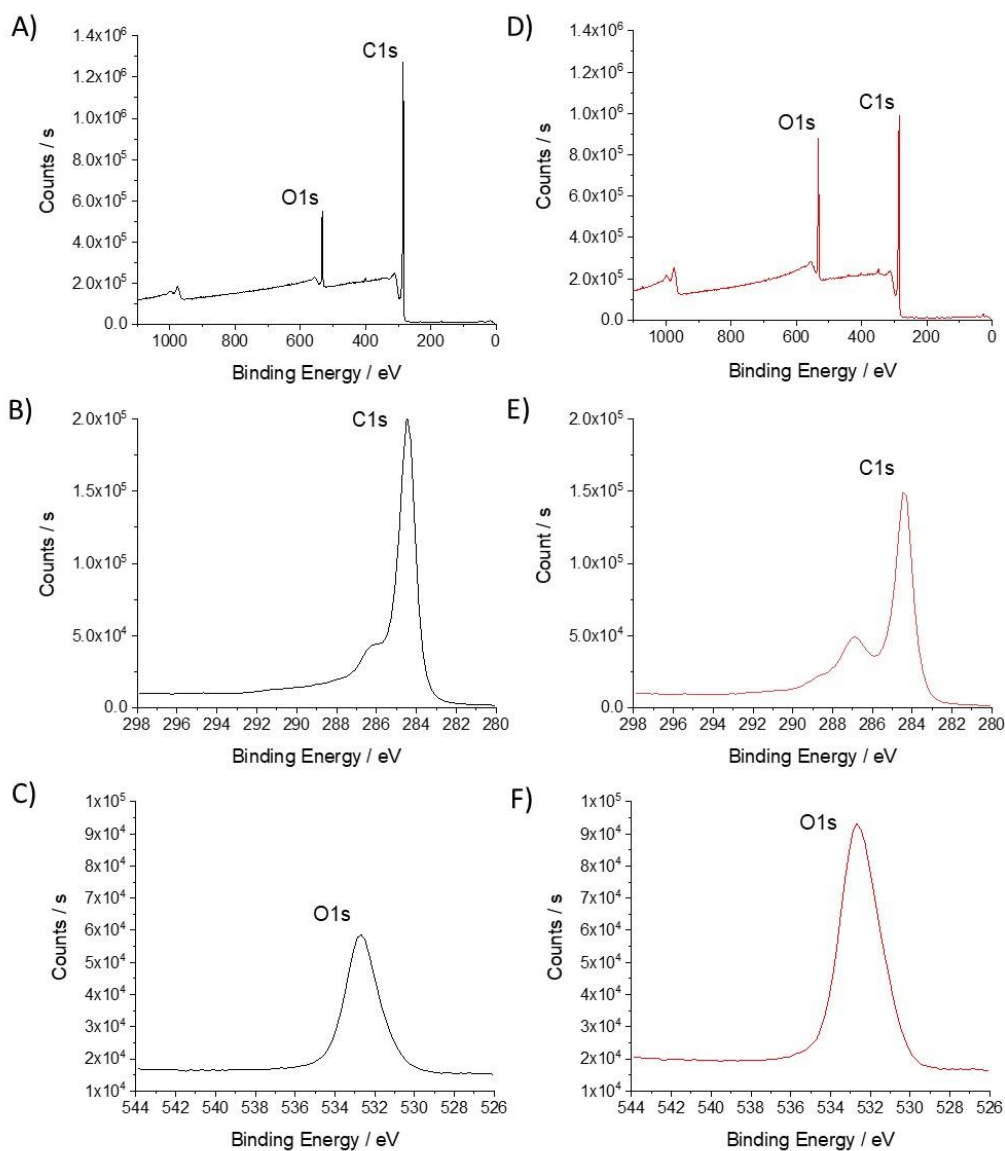


Figure 5. Comparison of high resolution XPS spectra detected on glassy carbon surfaces, either mechanically-polished (left column) or exposed to the oxygen plasma (20 min., right column); in (A) and (D) are compared the survey spectra, in (B) and (E) the peak corresponding to C1s near 284 eV and in (C) and (F) the peak corresponding to O1s near 531 eV. Quantitative analyses of these responses based on deconvolutions are reported in Table 1.

Table 1: Relative content (%) of C1s and O1s from the XPS spectra of mechanically-polished and plasma-treated GC surfaces, and their deconvolution into sub-components of oxygenated chemical functions of carbon.

Glassy carbon	C1s (284 eV)	O1s (532 eV)	C1s sp ³ (284,40 eV)	C-CO (285.53 eV)	C-O (286.65 eV)	C=O (287.30 eV)	COOR (288.61 eV)
polished	82.42	15.69	54.06	9.82	10.57	4.78	3.37
Plasma treated	77.37	20.37	47.86	5.89	10.72	7.58	4.63

3.4. GC electrode surface recovery by plasma after electrochemical grafting.

The high efficiency of the plasma treatment to clean up and activate GC electrodes is used daily to regenerate electrodes, fouled by proteins and lipids during electrochemical measurements in cell cultures, mitochondria suspensions or biological fluids. In the present work, we assessed a more challenging situation for plasma treatments, which is the chemical grafting of compact layers on GC surfaces. As reported in Figure 6A, a diazonium derivatives, benzenediazonium, was electrochemically reduced on a GC electrode. The characteristic peak ($E_{\text{peak}} = -0.75$ V vs Ag/AgCl) of the irreversible reduction reaction disappeared after the first cycle, indicating a blockage of the electron transfer and therefore the presence of an immobilized organic layer on the GC surface. The deposition cycles were repeated ten times to ensure the presence of a layer that should prevent charge transfers with a mediator present in the solution. In comparison with previous experiments, the reversible oxidation of ferrocyanide and ferrocene-dimethanol species were studied by cyclic voltammetry with such modified GC electrodes (Figures 6B and 6C). For both types of probes, an important loss of response was observed. For $\text{Fe}(\text{CN})_6^{4-}$, the faradaic contribution was no longer detected and a very weak capacitive current was observed on CVs (Figure 6B), while for FcDiMeOH, which is less sensitive to surface conditions, an undefined and low amplitude peak remained, the reversibility being also undetectable (Figure 6C). Consequently, the electrodeposited organic layer prevented from performing the usual electrochemical characterizations, a feature often used on purpose in diazonium grafting protocols.

Remarkably, these blocking properties were reversed after the plasma treatment of the GC electrodes. Recovery of peaks was observed after 1 min. exposure and was maximal after 10 min. At 10 min. plasma exposure, ΔE_p and I_p values were similar, at more than 90 %, to the ones obtained with non-modified GC electrodes (Figure 2). Consequently, an oxygen plasma treatment was fully efficient to remove chemically grafted organic layers on GC, a process that is difficult to achieve by simple hand polishing of electrodes (in

multiple steps, starting from hard sand papers to soft ones plus with nanoparticle powders). The plasma-cleaned and activated GC electrodes could be easily reused for novel experiments.

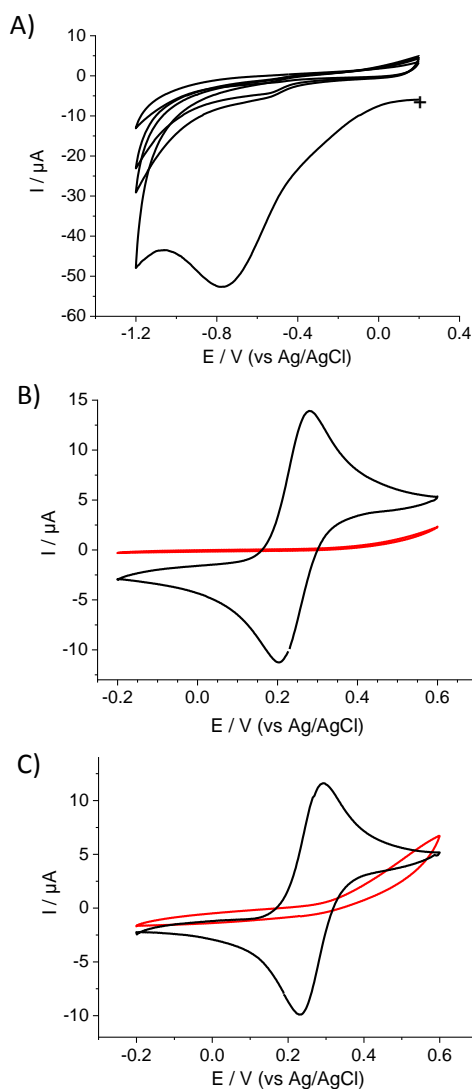


Figure 6. A) cyclic voltammograms for the reduction on a GC electrode of benzenediazonium at 4 mM in 0.5 M HCl; 4 cycles are displayed to show up the electrode surface coverage. CVs obtained with the GC electrode modified by phenyl layers, in B) 1 mM Fe(CN)₆⁴⁻ or in C) 1 mM FcDiMeOH solutions (KNO₃ 0.3 M), before (red line) and after (black line) 10 min exposure to the oxygen plasma. All CVs were performed at a scan rate of 100 mV s⁻¹.

Conclusion

In the present study, we have shown that the response of glassy carbon electrodes improves through oxygen plasma treatments when tested with different redox probes. The oxygen plasma treatment is simpler and superior to a standard polishing protocol for cleaning and activating GC electrodes. Treated surfaces get rid of organic contaminations, chemical coverage (adsorption, bonding), get improved surface energy and then hydrophilicity. Such effects are stable over one week when electrodes are stored in water. In most experimental cases, polishing is not necessary anymore and can be replaced by a plasma treatment. In future works, other types of plasma should be tested, including reductive plasma, in order to compare their

efficiency for the electroanalysis of other families of redox probes and detection protocols. More energetic plasma might also induce surface structural changes and thus some 3D structuration, which is another key feature for electrodes.

Credit authorship contribution statement

CC, FC, DZ and SA were involved in the design of the study. CC, PL and UBM performed the experimental works. CC and PL performed the data treatments. SA and DZ conducted the project scientific supervision and wrote the manuscript. SA conducted the project funding acquisition and administration. All authors have given approval to the final version of the manuscript.

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.

Acknowledgments

This work was financially supported by the French Agence nationale de la Recherche (ANR, project MITOCARD - ANR-17-CE11-0041-01). The authors thank our colleagues from the University of Bordeaux-France, at ISM-C2M group (Dir. Luc Vellutini) for contact angle measurements and at Placamat platform (Christine Labrugère) for high resolution XPS analyses.

References

- [1] G. Collins, P.R. Kasturi, R. Karthik, J.-J. Shim, R. Sukanya, C.B. Breslin, Mesoporous carbon-based materials and their applications as non-precious metal electrocatalysts in the oxygen reduction reaction, *Electrochimica Acta*, 439 (2023) 141678, [10.1016/j.electacta.2022.141678](https://doi.org/10.1016/j.electacta.2022.141678)
- [2] H. Gao, H. Duan, 2D and 3D graphene materials: Preparation and bioelectrochemical applications, *Biosens Bioelectron*, 65 (2015) 404-419, [10.1016/j.bios.2014.10.067](https://doi.org/10.1016/j.bios.2014.10.067)
- [3] M. Hersey, S.N. Berger, J. Holmes, A. West, P. Hashemi, Recent Developments in Carbon Sensors for At-Source Electroanalysis, *Anal Chem*, 91 (2019) 27-43, [10.1021/acs.analchem.8b05151](https://doi.org/10.1021/acs.analchem.8b05151)
- [4] R.L. McCreery, Advanced Carbon Electrode Materials for Molecular Electrochemistry, *Chemical Reviews*, 108 (2008) 2646–2687, [10.1021/cr068076m](https://doi.org/10.1021/cr068076m)
- [5] S. Michalkiewicz, A. Skorupa, M. Jakubczyk, Carbon Materials in Electroanalysis of Preservatives: A Review, *Materials (Basel)*, 14 (2021) 7630, [10.3390/ma14247630](https://doi.org/10.3390/ma14247630)
- [6] F. Arduini, L. Micheli, D. Moscone, G. Palleschi, S. Piermarini, F. Ricci, G. Volpe, Electrochemical biosensors based on nanomodified screen-printed electrodes: Recent applications in clinical analysis, *Trends in Analytical Chemistry*, 79 (2016) 114-126, [10.1016/j.trac.2016.01.032](https://doi.org/10.1016/j.trac.2016.01.032)
- [7] H. Karimi-Maleh, F. Karimi, M. Rezapour, M. Bijad, M. Farsi, A. Beheshti, S.A. Shahidi, Carbon Paste Modified Electrode as Powerful Sensor Approach Determination of Food Contaminants, Drug Ingredients, and Environmental Pollutants: A Review, *Current Analytical Chemistry*, 15 (2019) 410-422, [10.2174/1573411014666181026100037](https://doi.org/10.2174/1573411014666181026100037)

- [8] S. Tajik, H. Beitollahi, F.G. Nejad, M. Safaei, K.Q. Zhang, Q.V. Le, R.S. Varma, H.W. Jang, M. Shokouhimehr, Developments and applications of nanomaterial-based carbon paste electrodes, *Rsc Advances*, 10 (2020) 21561-21581, 10.1039/d0ra03672b
- [9] C. Amatore, S. Arbault, I. Bonifas, M. Guille, Quantitative investigations of amperometric spike feet suggest different controlling factors of the fusion pore in exocytosis at chromaffin cells, *Biophysical Chemistry*, 143 (2009) 124-131, 10.1016/j.bpc.2009.04.007
- [10] A.S. Cans, A.G. Ewing, Highlights of 20 years of electrochemical measurements of exocytosis at cells and artificial cells, *Journal of Solid State Electrochemistry*, 15 (2011) 1437-1450, 10.1007/s10008-011-1369-9
- [11] L. Jiang, G.W. Nelson, J. Abda, J.S. Foord, Novel Modifications to Carbon-Based Electrodes to Improve the Electrochemical Detection of Dopamine, *Acs Applied Materials & Interfaces*, 8 (2016) 28338-28348, 10.1021/acsami.6b03879
- [12] S. Sharma, N. Singh, V. Tomar, R. Chandra, A review on electrochemical detection of serotonin based on surface modified electrodes, *Biosensors & Bioelectronics*, 107 (2018) 76-93, 10.1016/j.bios.2018.02.013
- [13] X.W. Zhang, A. Hatamie, A.G. Ewing, Nanoelectrochemical analysis inside a single living cell, *Current Opinion in Electrochemistry*, 22 (2020) 94-101, 10.1016/j.coelec.2020.05.008
- [14] G. Cui, J.H. Yoo, J.S. Lee, J. Yoo, J.H. Uhm, G.S. Cha, H. Nam, Effect of pre-treatment on the surface and electrochemical properties of screen-printed carbon paste electrodes, *Analyst*, 126 (2001) 1399-1403, 10.1039/b102934g
- [15] F.J. Lopez-Garzon, M. Domingo-Garcia, M. Perez-Mendoza, P.M. Alvarez, V. Gomez-Serrano, Textural and chemical surface modifications produced by some oxidation treatments of a glassy carbon, *Langmuir*, 19 (2003) 2838-2844, 10.1021/la026848d
- [16] O. Dumanli, A.N. Onar, Activation of glassy carbon electrodes by photocatalytic pretreatment, *Electrochimica Acta*, 54 (2009) 6438-6444, 10.1016/j.electacta.2009.05.096
- [17] D. Volpe, E. Casado-Rivera, L. Alden, C. Lind, K. Hagerdon, C. Downie, C. Korzeniewski, F.J. DiSalvo, H.D. Abruna, Surface treatment effects on the electrocatalytic activity and characterization of intermetallic phases, *Journal of the Electrochemical Society*, 151 (2004) A971-A977, 10.1149/1.1756886
- [18] J.F. Zhou, D.O. Wipf, UV/ozone pretreatment of glassy carbon electrodes, *Journal of Electroanalytical Chemistry*, 499 (2001) 121-128, 10.1016/s0022-0728(00)00498-8
- [19] T. Bystron, E. Sramkova, F. Dvorak, K. Bouzek, Glassy carbon electrode activation – A way towards highly active, reproducible and stable electrode surface, *Electrochimica Acta*, 299 (2019) 963-970, 10.1016/j.electacta.2019.01.066
- [20] M. Domingo-Garcia, F.J. Lopez-Garzon, M. Perez-Mendoza, Modifications produced by O-2 plasma treatments on a mesoporous glassy carbon, *Carbon*, 38 (2000) 555-563, 10.1016/s0008-6223(99)00136-0
- [21] S. Ben-Amor, A. Devin, M. Rigoulet, N. Sojic, S. Arbault, Oxygen Plasma Treatment of Platinized Ultramicroelectrodes Increases Sensitivity for Hydrogen Peroxide Detection on Mitochondria, *Electroanalysis*, 25 (2013) 656-663, 10.1002/elan.201200409

- [22] V. Flexer, M. Marque, B.C. Donose, B. Viridis, J. Keller, Plasma treatment of electrodes significantly enhances the development of anodic electrochemically active biofilms, *Electrochimica Acta*, 108 (2013) 566-574, 10.1016/j.electacta.2013.06.145
- [23] Y.-C. Lin, C.-H. Chuang, L.-Y. Hsiao, M.-H. Yeh, K.-C. Ho, Oxygen Plasma Activation of Carbon Nanotubes-Interconnected Prussian Blue Analogue for Oxygen Evolution Reaction, *Acs Applied Materials & Interfaces*, 12 (2020) 42634-42643, 10.1021/acsami.0c07821
- [24] A.A. Kava, C.S. Henry, Exploring carbon particle type and plasma treatment to improve electrochemical properties of stencil-printed carbon electrodes, *Talanta*, 221 (2021), 121553, 10.1016/j.talanta.2020.121553
- [25] F. Girard, M. Peret, N. Dumont, V. Badets, S. Blanc, K. Gazeli, C. Noel, T. Belmonte, L. Marlin, J.-P. Cambus, G. Simon, N. Sojic, B. Held, S. Arbault, F. Clement, Correlations between gaseous and liquid phase chemistries induced by cold atmospheric plasmas in a physiological buffer, *Physical Chemistry Chemical Physics*, 20 (2018) 9198-9210, 10.1039/c8cp00264a
- [26] M. Lopez, T. Calvo, M. Prieto, R. Mugica-Vidal, I. Muro-Fraguas, F. Alba-Elias, A. Alvarez-Ordenez, A Review on Non-thermal Atmospheric Plasma for Food Preservation: Mode of Action, Determinants of Effectiveness, and Applications, *Frontiers in Microbiology*, 10 (2019) 1, 10.3389/fmicb.2019.00622
- [27] A. Privat-Maldonado, A. Schmidt, A. Lin, K.D. Weltmann, K. Wende, A. Bogaerts, S. Bekeschus, ROS from Physical Plasmas: Redox Chemistry for Biomedical Therapy, *Oxidative Medicine and Cellular Longevity*, 2019 (2019) 1-29, 10.1155/2019/9062098
- [28] H. Mortensen, Y. Kusano, P. Kingshott, B.F. Sorensen, B. Stenum, N. Rozlosnik, H. Bindslev, Atmospheric pressure plasma modification of the morphology and chemistry of glassy carbon surfaces, 2nd International Workshop on Cold Atmospheric Pressure Plasmas: Sources and Applications Bruges, BELGIUM, 2005, pp. 269-269.
- [29] Y. Kusano, H. Mortensen, B. Stenum, S. Goutianos, S. Mitra, A. Ghanbari-Siahkali, P. Kingshott, B.F. Sorensen, H. Bindslev, Atmospheric pressure plasma treatment of glassy carbon for adhesion improvement, *International Journal of Adhesion and Adhesives*, 27 (2007) 402-408, 10.1016/j.ijadhadh.2006.09.011
- [30] S.C. Wang, K.S. Chang, C.J. Yuan, Enhancement of electrochemical properties of screen-printed carbon electrodes by oxygen plasma treatment, *Electrochimica Acta*, 54 (2009) 4937-4943, 10.1016/j.electacta.2009.04.006
- [31] E. Adhamash, R. Pathak, K. Chen, M.T. Rahman, A. El-Magrous, Z. Gu, S. Lu, Q. Qiao, Y. Zhou, High-energy plasma activation of renewable carbon for enhanced capacitive performance of supercapacitor electrode, *Electrochimica Acta*, 362 (2020) 137148, 10.1016/j.electacta.2020.137148
- [32] H. Fredriksson, D. Chakarov, B. Kasemo, Patterning of highly oriented pyrolytic graphite and glassy carbon surfaces by nanolithography and oxygen plasma etching, *Carbon*, 47 (2009) 1335-1342, 10.1016/j.carbon.2009.01.018
- [33] W. Chen, X. Wang, M. Luo, P. Yang, X. Zhou, Fast one-pot microwave preparation and plasma modification of porous carbon from waste lignin for energy storage application, *Waste Management*, 89 (2019) 129-140, 10.1016/j.wasman.2019.03.056

- [34] P.K. Adusei, S. Gbordzoe, S.N. Kanakaraj, Y.-Y. Hsieh, N.T. Alvarez, Y. Fang, K. Johnson, C. McConnell, V. Shanov, Fabrication and study of supercapacitor electrodes based on oxygen plasma functionalized carbon nanotube fibers, *Journal of Energy Chemistry*, 40 (2020) 120-131, 10.1016/j.jechem.2019.03.005
- [35] S. Jayasinghe, D.P. Siriwardena, I. Munaweera, C. Perera, N. Kottegoda, Sustainable Synthesis of Highly Functionalized Activated Carbon using Plasma Technology, *Chempluschem*, 87 (2022) e202200202, 10.1002/cplu.202200202
- [36] V. Badets, J. Pandard, N. Sojic, S. Arbault, Deciphering the Platinized Surface Reactivity to Improve the Detection of Hydrogen Peroxide in Bioanalyses, *Chemelectrochem*, 3 (2016) 2288-2296, 10.1002/celec.201600558
- [37] P. Lefrancois, F. Girard-Sahun, V. Badets, F. Clement, S. Arbault, Electroactivity of Superoxide Anion in Aqueous Phosphate Buffers Analyzed with Platinized Microelectrodes, *Electroanalysis*, 33 (2021) 882–890, 10.1002/elan.202060456
- [38] F. Girard-Sahun, P. Lefrancois, V. Badets, S. Arbault, F. Clement, Direct Sensing of Superoxide and Its Relatives Reactive Oxygen and Nitrogen Species in Phosphate Buffers during Cold Atmospheric Plasmas Exposures, *Analytical Chemistry*, 94 (2022) 5555-5565, 10.1021/acs.analchem.1c04998
- [39] F. Ghamouss, E. Luais, C. Thobie-Gautier, P.Y. Tessier, M. Boujtita, Argon plasma treatment to enhance the electrochemical reactivity of screen-printed carbon surfaces, *Electrochimica Acta*, 54 (2009) 3026-3032, 10.1016/j.electacta.2008.12.011
- [40] C. Saka, O. Baytar, Y. Yardim, O. Sahin, Improvement of electrochemical double-layer capacitance by fast and clean oxygen plasma treatment on activated carbon as the electrode material from walnut shells, *Biomass & Bioenergy*, 143 (2020) 105848, 10.1016/j.biombioe.2020.105848
- [41] S. Cheunkar, S. Oaew, A. Parnsubsakul, P. Asanithi, Reactive argon-plasma activation of screen-printed carbon electrodes for highly selective dopamine determination, *Analytical Methods*, 14 (2022) 4193-4201, 10.1039/d2ay01154a
- [42] I. Lavagnini, R. Antiochia, F. Magno, An Extended Method for the Practical Evaluation of the Standard Rate Constant from Cyclic Voltammetric Data, *Electroanalysis*, 16 (2004) 505-506, 10.1002/elan.200302851
- [43] E.P. Randviir, A cross examination of electron transfer rate constants for carbon screen-printed electrodes using Electrochemical Impedance Spectroscopy and cyclic voltammetry, *Electrochimica Acta*, 286 (2018) 179-186, 10.1016/j.electacta.2018.08.021
- [44] G. Pankratova, J.Y. Pan, S.S. Keller, Impact of plasma-induced surface chemistry on electrochemical properties of microfabricated pyrolytic carbon electrodes, *Electrochimica Acta*, 410 (2022) 139987, 10.1016/j.electacta.2022.139987