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***N*-halamine coatings formed via the electroreduction
of *in situ* generated diazonium cations: toward antimicrobial surfaces**

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ABSTRACT

Nowadays, antimicrobial *N*-halamine polymers and coatings are extensively studied thanks to their qualities such as effectiveness toward a broad spectrum of microorganisms and regenerability. An *N*-halamine organic polymer contains nitrogen-halogen covalent bonds. Its antimicrobial properties are due to the oxidizing power of the halide atoms which are at the oxidation state +I in chloramine (>N-Cl) or bromamine (>N-Br) groups. The aim of the present work was to generate a new *N*-halamine coating in a two-step route. First, we have synthesized by electroreduction of *in situ* generated aminophenyl diazonium cations polyaminophenyl films because these films contain numerous amino functions. Electrochemical quartz crystal microbalance (EQCM) allowed us to determine the mass of the film formed by cyclic voltammetry and to estimate its thickness, the highest one is about 1 micrometer. Then, the second step of the process was the formation of haloamine functions into the polyaminophenyl film by substitution of H atoms of the amine functions by Br or Cl atoms. The films were observed by SEM and their composition was determined by EDS. Finally the EQCM experiments allowed us to estimate that the *N*-halamine films that we have prepared contain 1 Cl atom or 1 Br atom for 4 or 4.5 phenyl groups, respectively.

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Introduction

Recently, antimicrobial *N*-halamine polymers and coatings have been extensively studied thanks to their qualities such as effectiveness toward a broad spectrum of microorganisms and regenerability.^{[1] [2] [3] [4]} An *N*-halamine organic polymer contains nitrogen-halogen covalent bonds. Its antimicrobial properties are due to the oxidation state +I of halide atoms in chloramine (>N-Cl) or bromamine (>N-Br) groups. In order to prepare an *N*-halamine coating a two-step route can be used, first the immobilization on the material surface of amino groups followed by a treatment with a NaOCl or a NaOBr solution in order to form haloamine functions, the reaction is the following (substitution reaction ; *X* being Cl or Br):



Therefore in order to obtain an *N*-halamine coating, the surface of the material should be covered with a polymer containing many primary or secondary amine functions (or primary imine). One way for having such organic polymers could be the reduction of aryl diazonium cations bearing amino groups *e.g.* aminophenyl diazonium ($\text{H}_2\text{N-C}_6\text{H}_4\text{-N}_2^+$). This compound can be *in situ* generated in acidic sodium nitrite aqueous solution in the presence of *p*-phenylenediamine molecules (scheme 1A, first reaction).^{[5] [6]}

Actually the method based on the (electro)chemical reduction of diazonium salts has been developed during the last decades.^{[7] [8] [9]} It leads to the covalent bonding of organic molecules through the formation of an aryl radical.^[10] Scheme 1A resumes the two-step process of this reaction. Depending on the nature of the substituent of the phenyl radical and the experimental conditions, the electrochemical or chemical (in the presence of hypophosphorous acid (H_3PO_2) or Fe^0 ^[11]) reduction of the diazonium function allows the formation of an organic monolayer or

multilayer. Notice that the process could take place even in the absence of an applied potential or of a reducing agent for example in the case of aminobenzene diazonium cation.^[12]

These films could have various applications, for some of them it is better to have monolayer notably amine-terminated monolayer which could be obtained using protected *p*-aminobenzene diazonium salt^[13] or compact thick layer (anticorrosion coating) or porous thick layer depending also on the post-functionalization treatment.^[14] Notably if the layer contains primary amino groups which can undergo a diazotisation in order to graft on it chemical compounds or solid compounds^[11] such as chitosan^[15] or silicon nanoparticules^[16].

A structure of the polyaminophenylene film obtained by electroreduction of aminophenyl diazonium ions on a gold electrode has been proposed by Lyskawa and Belanger (see scheme 1B).^[17] From this scheme one can notice that the structure could contain azo bonds (N=N) included in polyphenylene chains or at the electrode-film interface. The presence of such bonds in polyphenylene chains has notably been evidenced by XPS and TOF-SIMS.^[18]

The aim of the present work was to prepare thick organic films containing numerous haloamine groups in order to obtain antimicrobial coatings. For this purpose first the electrogeneration of thick organic film was done, its synthesis being followed by electrochemical quartz crystal microbalance (EQCM) and their characterization done by SEM and EDS analyses. The modification of the film by immersion in NaOBr or NaOCl aqueous solution (Reaction (1)) in order to immobilize Cl(+I) or Br(+I) oxidizing species in the polymer was also studied. To the best of our knowledge only a few works on electroreduction of diazonium salts have been conducted by EQCM. For example, Laforgue *et al.* have studied the reduction of three 4-

substituted phenyldiazonium cations in anhydrous acetonitrile media. EQCM allows them to show that on a gold electrode the film deposition follows a two-step process.^[19, 20]

By electrochemical way, two methods can be used either potentiostatic or potentiodynamic. Preliminary experiments allowed us to determine that the method which leads to the thickest films and then to the higher amount of N atoms in order to immobilize numerous Cl(+I) or Br(+I) is the potentiodynamic one.

Experimental

p-phenylenediamine (PDP), sodium nitrite and sodium bromide were purchased from Aldrich and used as received. Sodium hypochlorite solution was a commercial one (2.7 wt%), its exact concentration was determined by optical absorption (at 290 nm). All the solutions were prepared with bi-distilled water.

The aminophenyl diazonium cations were formed by addition of a volume of a 10 mM solution of NaNO₂ to the same volume of a 10 mM acid solution (0.1 M HCl) of PDP.

The NaOCl solution was used at a concentration of 25 mM and the NaOBr one at a concentration of 20 mM. The NaOBr solution was prepared by oxidation of bromide ions by hypochlorite ions:



After modification the substrates were thoroughly rinsed with bi-distilled water.

The electrochemical and electrogravimetric measurements were performed in a classical three-electrode electrochemical cell. A platinum grid was used as counter electrode and a saturated calomel electrode (SCE) as the reference one. For EQCM experiments, the working electrode was a 9 MHz AT-cut quartz crystal with a mass-sensitive surface of 0.2 cm² (Temex, France).

Each face of the crystal was coated with a keyhole designed gold layer.

The film electrochemical synthesis and the haloamine function formation were monitored by a lab-made QCM device providing the electrodeposited mass to be determined by frequency variation of the quartz crystal resonator based on the Sauerbrey equation.

The SEM observations were done with a field emission gun scanning electron microscope (FEG-SEM) coupled with EDS analyses, operating at 15 kV, Ultra55 Zeiss.

The results concerning the synthesis of three films are presented herein. All these films were obtained by cyclic voltammetry, the potential limits being 0 and -0.45 V/SCE.

Film ①: 20 cycles, $v = 5 \text{ mV s}^{-1}$; Film ②: 40 cycles, $v = 2 \text{ mV s}^{-1}$; Film ③: 70 cycles, 5 mV s^{-1} .

Results and discussion

1- Polyaminophenylene film formation

In each case the cathodic limit was -0.45 V/SCE, which is slightly lower than the peak potential of the reduction wave of aminophenyl diazonium ions which is observed at -0.35 V/SCE.

Figure 1 shows the current-time and mass-time curves recorded during the polarization of the gold electrode. The mass increase is slightly higher for the first cycle then it is constant (see Fig. 1B), the relationship between the electrode mass increase and the number of potential cycle is linear, with a slope of 612 ng per cycle, $3 \mu\text{g cm}^{-2}$ per cycle (Fig. 1C). After 20 potential cycles the mass of the polymer generated onto the electrode is 12.5 μg .

The electrode mass increases as the cathodic current appears (see Fig. 1A) in good agreement with the mechanism proposed in scheme 1 *i.e.* electroreduction and grafting of the *in situ* generated diazonium cations.

The cathodic limiting current, at -0.45 V/SCE, increases after each cycle until a more or less constant value, from 350 μA to 850 μA . Since the mass increase is constant from one cycle to another we can assume that the yield (cathodic charge/mass deposited) decreases.

The SEM micrograph presented in Fig. 2A shows that the electrogenerated film is porous, this porosity could be due to the evolution of N_2 during the process (see scheme 1A). The EDS spectrum shown in Fig. 2B confirms the formation of an organic film which contains N atoms.

Another example of film synthesis is shown in Fig. 3. It presents the same characteristics as the previous one but the mass of the deposited film is more important, notably because of the increase of the number of potential scans (40 instead of 20). Fig. 3A indicates that the mass of Film ② is 27.3 μg which correspond to 0.296 μmol of $\text{C}_6\text{H}_4\text{NH}_2$ ($M = 92 \text{ g mol}^{-1}$), without taking into account the presence of azo bonds into the layer. As for Film ①, the cathodic current increases from one potential scan to another until a threshold about 700 μA . This variation could be explained by a decrease of the interfacial pH when protons are released due to the formation of $\text{C}_{\text{aryl}}\text{-C}_{\text{aryl}}$ bonds of the multilayer structure.



In order to confirm this assumption, the influence of the pH is under study.

An estimation of the film thickness can be done. The mass of the film is 27.3 μg and the electrode surface 0.2 cm^2 therefore the normalized mass variation of the electrode is 136 $\mu\text{g cm}^{-2}$. This mass leads to a surface concentration of 14783 $10^{-10} \text{ mol cm}^{-2}$ ($136 \cdot 10^{-6} / 92$). By assuming that a monolayer is $10 \cdot 10^{-10} \text{ mol cm}^{-2}$ [20], the film is composed of an equivalent of about 1500 monolayers. Finally, taking into account that the thickness of a polyaminophenyl monolayer is 0.6 nm^[13], the thickness of the electrogenerated polymer is about 900 nm (1500 x 0.6).

This film was modified in NaOCl aqueous solution at open circuit potential (see below).

The last film which was studied (Film^③) has a mass of 19.5 μg (Fig. 4A). It was modified in NaOBr aqueous solution at open circuit potential.

2- Haloamine group formation

Figure 3C presents the mass variation of Film ^② in 25 mM NaOCl aqueous solution. After contact with the NaOCl solution the mass increase of this film is 2.6 μg which indicates that 0.075 μmol of Cl are immobilized into the polyaminophenylene film ($\Delta M = M_{\text{Cl}} - M_{\text{H}} = 34.45 \text{ g mol}^{-1}$). As previously mentioned this film is composed of 0.296 μmol of amino aryl groups. From these results we can conclude that into the film there is 1 atom of chlorine for 4 aryl groups.

Same kind of experiment was done with a NaOBr solution using Film ^③. The mass of this film is 19.3 μg which correspond to 0.21 μmol (Fig. 4A). After immersion of this film in the 20 mM NaOBr solution the mass increase is 3.7 μg (Fig. 4B). This mass variation indicates that 0.047 μmol of Br are linked to N atoms of the film. Therefore this film contains 1 atom of bromine for 4.5 aryl groups.

The EDS spectrum depicted in Fig. 4C highlights the presence of bromine in Film ^③ and the absence of Na. Thus the bromine atoms are linked to the organic polymer. This finding confirms

the formation of bromamine functions into the polyaminophenylene film.

Finally, from the comparison of Figs. 5A and 5B and the zones with and without the gold layer, it is clear that the organic film electrogenerated (Film ③) in the presence of aminophenyl diazonium cations in our experimental conditions is very thick, in agreement with the EQCM results.

From all these results, one can assume that some of the nitrogen atoms contained into the polyphenylene films are not engaged in amino functions but maybe rather in azo groups (see scheme 1B). Further experiments such as XPS analyses and/or IR characterizations^[18] have to be done to confirm this assumption.

Conclusions

The strategy to form *N*-halamine coatings from the chemical modification of electrogenerated aminophenyl polymeric films is validated by the present results. This preliminary study is being carried on in order to better understand the electrochemical processes involved in the film growth and to get more information about the structure of the films according to the conditions of synthesis.

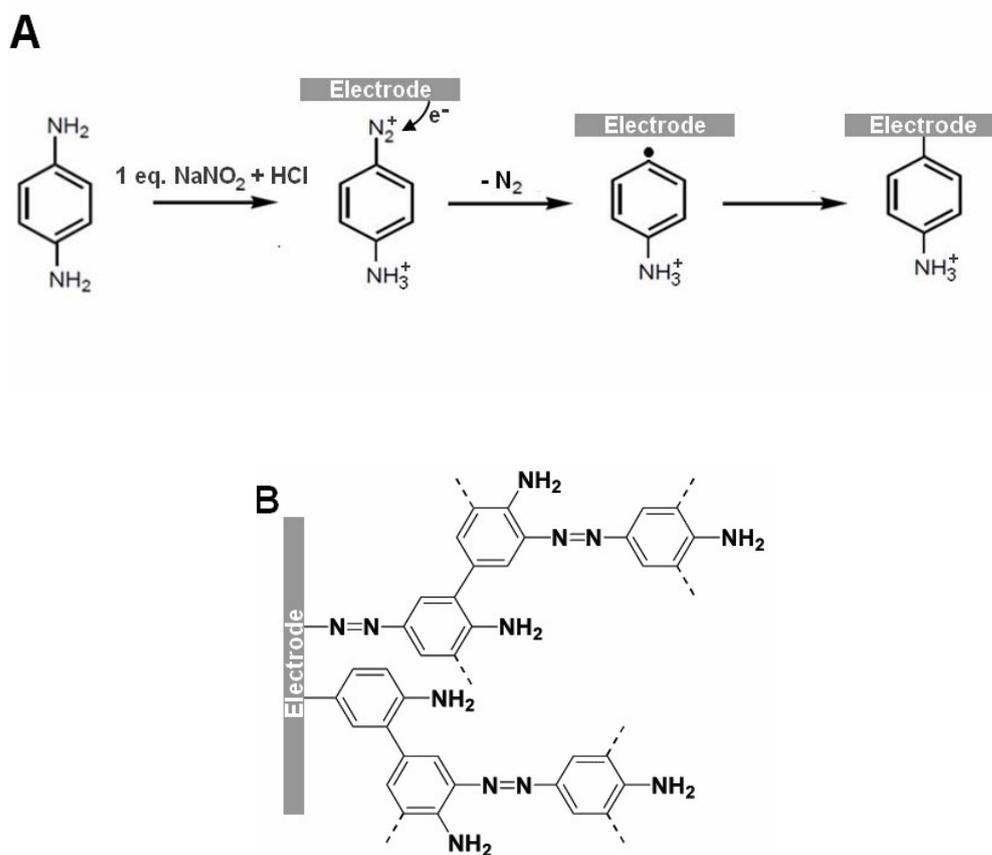
Acknowledgments

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Scheme 1: (A) Electrochemical reduction of *in situ* generated aminophenyl diazonium cation and its grafting on the electrode surface. (B) Structure proposed by Lyskawa and Bélanger of the organic film obtained by the electrochemical reduction of *in situ* generated aminophenyl diazonium cations.^[17]



FIGURES

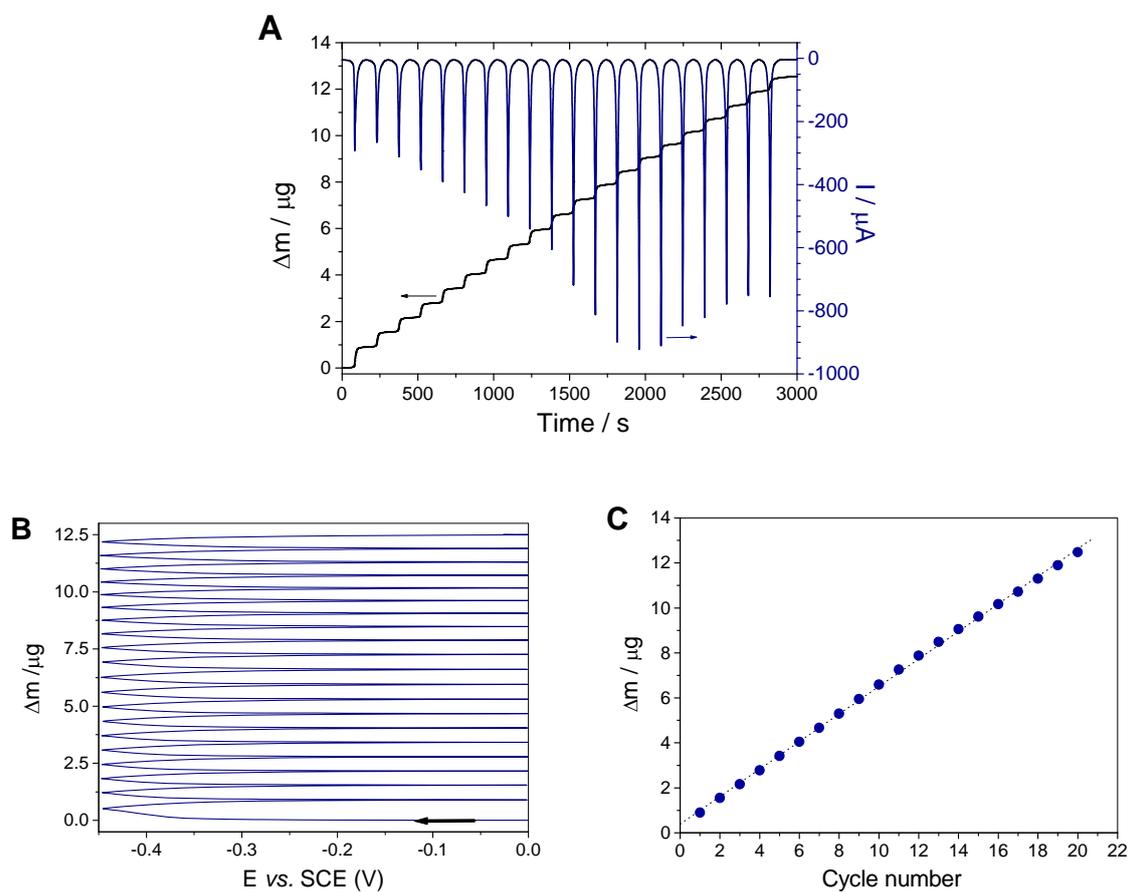


Figure 1 : Film ①. A) Mass variation (left) and current (right) vs. time of a gold electrode polarized between 0 and -0.45 V/SCE at 5 mV s^{-1} for 20 cycles in 5 mM PDP + 5 mM NaNO_2 + HCl 0.1 M aqueous solution. B) Mass variation in function of the applied potential. C) Mass variation vs. the cycle number (data from graph B).

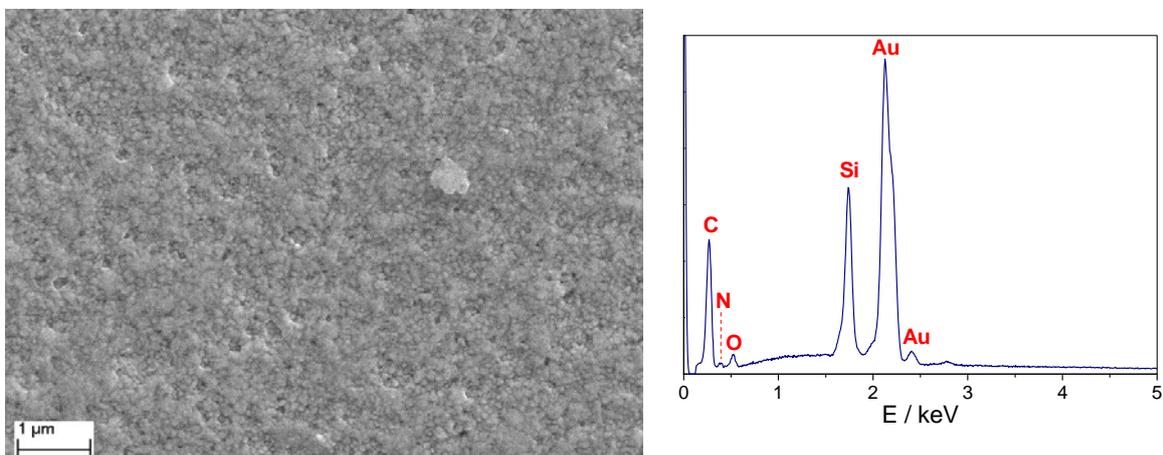


Figure 2: Film ①. FEG-SEM micrograph of a polyaminophenylene film electrogenerated at a gold electrode polarized between 0 and -0.45 V/SCE at 5 mV s^{-1} for 20 cycles, in 5 mM PDP + 5 mM NaNO_2 + HCl 0.1 M aqueous solution, scale bar: 1 μm . EDS spectrum of the film.

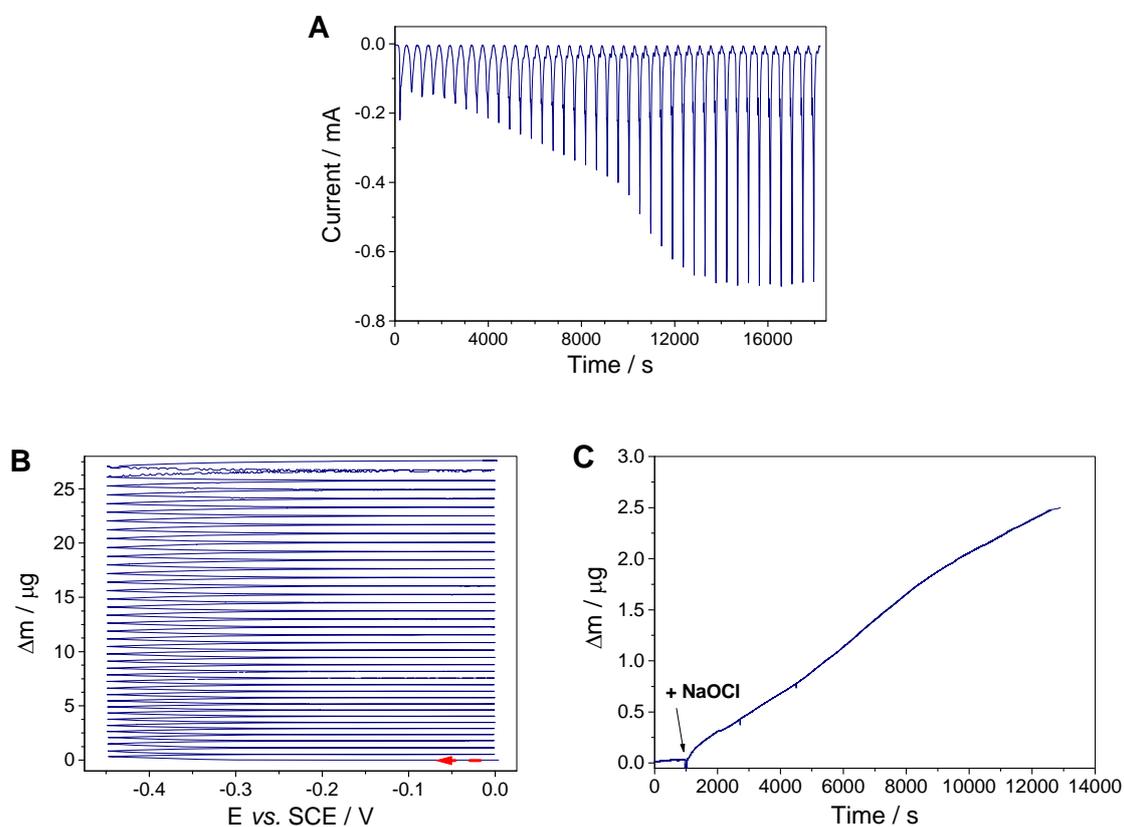


Figure 3: Film ②. A) Current vs. time at a gold electrode polarized between 0 and -0.45 V/SCE at 2 mV s^{-1} for 40 cycles, in 5 mM PDP + 5 mM NaNO_2 + HCl 0.1 M aqueous solution. B) Mass variation in function of the applied potential. C) Mass variation of the film immersed in water and after addition of NaOCl (at $t = 1000 \text{ s}$), $\Delta m = 27.3 \mu\text{g}$ ($S = 0.2 \text{ cm}^2$).

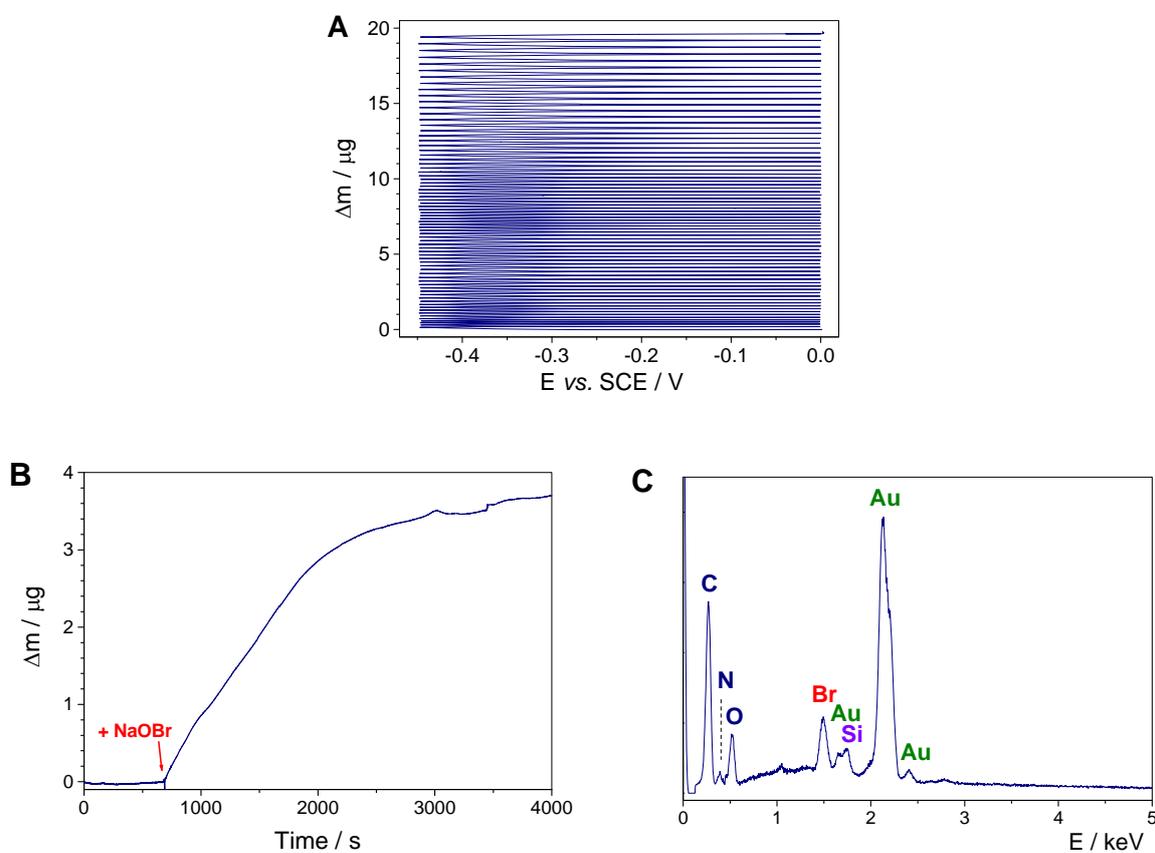


Figure 4 : Film ③. A) Mass variation in function of the applied potential at a gold electrode in 5 mM PDP + 5 mM NaNO_2 + HCl 0.1 M aqueous solution for 70 cycles, $v = 5 \text{ mV s}^{-1}$. B) Mass variation vs. time of the coated Au electrode immersed in water and after addition of NaOBr. C) EDS spectrum of the resulting film.

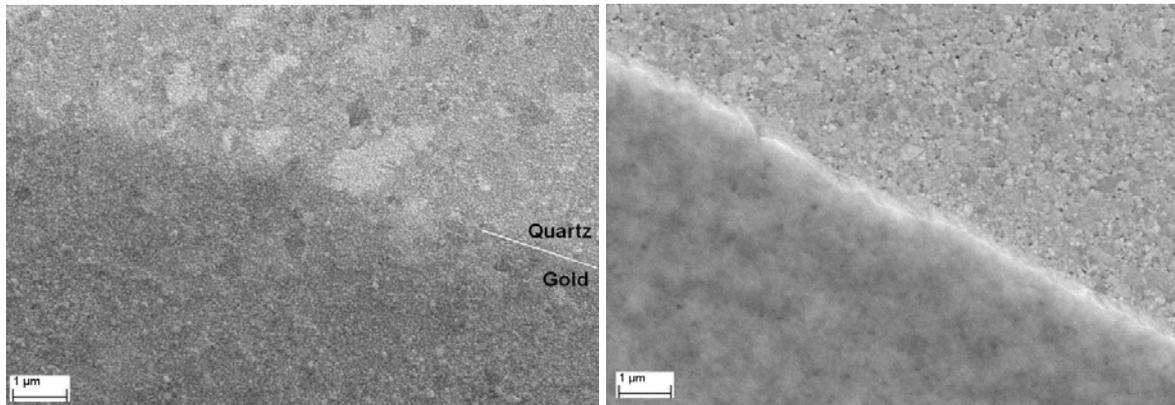


Figure 5: FEG-SEM micrographs before (left) and after (right) Film ③ electrogeneration and NaOBr treatment (Fig. 4).