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Antioxidant activity of films inspired by prebiotic chemistry

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Abstract: Conformal films obtained through polymerization of aminomalononitrile in solution at pH = 8.6 display an irreversible electrochemical oxidation current and a thickness/morphology dependant antioxidant activity as measured by the quenching of 2,2-diphenyl-1-picrylhydrazyl. These findings illustrate that materials issued from molecules suspected to have played an important role in prebiotic chemical process offer application potentials in biomaterials science.

1. Introduction

Since Stanley Miller's experiment in 1953, it is known and highly discussed that building blocks of biomolecules can be produced in a primitive atmosphere -containing N_2 , CO , CH_4 and water in the presence of an energy supply [1]. Indeed, amino acids and nucleic acids have been isolated from such reactive mixtures. The most probable intermediates to amino acids and polymers are hydrogen cyanide oligomers [2-5]. Among those, the trimer of hydrogen cyanide, namely aminomalononitrile (AMN, inset in Fig. 1) is aimed to play a major role in initiating pathways for further polymerization. Recently it has been shown that when various kinds of substrates are immersed in slightly alkaline AMN solutions, a uniform [6,7] and biocompatible [8] coating can be deposited. AMN based coatings can also be obtained on conductive substrates via electrodeposition using cyclic voltammetry (CV) in acidic conditions at which no chemical transformation occurs in the AMN solution [9]. This electrochemical behaviour of AMN based films could deserve some interesting catalytic as well as biological or electrochemical properties. Herein it will be demonstrated, as a preliminary study, that as deposited AMN based films have electrochemical moieties affording antioxidant properties as measured by quenching of DPPH (2,2-diphenyl-1-picrylhydrazyl).

2. Materials and Methods

Aminomalononitrile-*para*-toluenesulfonate (AMN, ref. 221147), sodium hydrogen phosphate (ref. S-9638), potassium hexacyanoferrate (ref. P9387) and DPPH (ref. 257621) were purchased from Sigma-Aldrich and used without further purification. The AMN based films were produced as described in a previous work [7]. Briefly: AMN was dissolved in 50 mM sodium phosphate buffer at $pH = 7.5$ at a concentrations of $1g.L^{-1}$. The dissolution of AMN produced a significant pH decrease which stabilized the reactant. The reaction was triggered by adjusting the pH to (8.6 ± 0.1) with a concentrated sodium hydroxide solution. Immediately after pH adjustment, samples to be covered (Glass slides, Silicon wafers or polished glassy carbon electrodes) were immersed in the AMN solution and left therein for various durations up to 24 h. The glass slides (Knittel glass, Germany) and silicon wafers ((100) oriented, Siltronix, Archamps), cleaned for 5 min in an air plasma (PDC-32G-2, Harrick Scientific, USA), were hold vertically in the AMN solution to minimize the deposition of precipitate issued from the reaction in solution. Amorphous carbon working electrodes (CHI ref. 104 from CHI instruments, Austin, Texas) previously polished on a

silicon carbide (SiC) disk were further polished using alumina (Al_2O_3) slurries (Escil, France) with a grain size of 1.0 μm and 0.1 μm . The quality of this cleaning steps was checked in the presence of 50 mM sodium phosphate buffer containing 1mM of potassium hexacyanoferrate as described previously [9]. The working electrode covered with an AMN based deposit was mounted in a three electrode cell (CHI 604B) using an Ag/AgCl reference electrode and a platinum wire as the counter electrode and the potential was cycled between -0.6 and 1.0 V (versus Ag/AgCl) at a potential sweep rate of 100 $\text{mV}\cdot\text{s}^{-1}$.

The film thickness of the AMN based coatings deposited on silicon wafers was measured with ellipsometry (PZ2000, Horiba, Longjumeau, France) as explained elsewhere [7]. The morphology of the AMN based dry films deposited on glass slides was investigated with scanning electron microscopy (SEM). The AMN coated glass slides (after 4.5 and 21 h of deposition) were sputter-coated with gold-palladium (20%/80%) using a Hummer JR sputtering device (Technics, CA, USA). The films were subsequently analyzed using a Quanta 250 FEG scanning electron microscope (FEI Company, Eindhoven, The Netherlands) with an electron beam at 10 keV.

The antioxidant activity of the AMN based films deposited, for different time durations, on both faces of 2x4 cm glass slides was investigated by the DPPH quenching method. The change in absorbance of ethanol solubilized DPPH ($10^{-4}\text{mol}\cdot\text{L}^{-1}$ in pure ethanol) was measured with an mc^2 UV-Vis spectrophotometer (SAFAS, Monaco). The AMN coated glass slide (AMN deposition was performed for various durations up to 21 h) was rinsed with water and immersed in a given volume (50 mL) of DPPH solution and allowed to react for 30 min. in the absence of external light. Then, the absorbance of each of these DPPH solutions was measured at 517 nm [10], the reference cuvette containing DPPH alone. This reference DPPH solution was prepared in the same condition as the DPPH solution in contact with the AMN films to account for spontaneous discoloration in the presence of dissolved O_2 .

3. Results and discussion

The deposition of AMN based films from a phosphate buffer solution at $\text{pH} = 8.6$ containing an initial AMN concentration of $1\text{g}\cdot\text{L}^{-1}$ is a pretty slow process as shown in Fig.1. Indeed, the film deposition is preceded by a lag phase as shown previously [7] which strongly suggests a nucleation process before the film deposition. Indeed the morphology of the deposit undergoes a transition from clustered particles to a fiber like material (SEM images in the upper part of Fig. 1). The film obtained after long deposition times, made of micrometer

long fibers and about 100 nm in diameter, is clearly porous offering a large developed surface area which has to be quantified in the future.

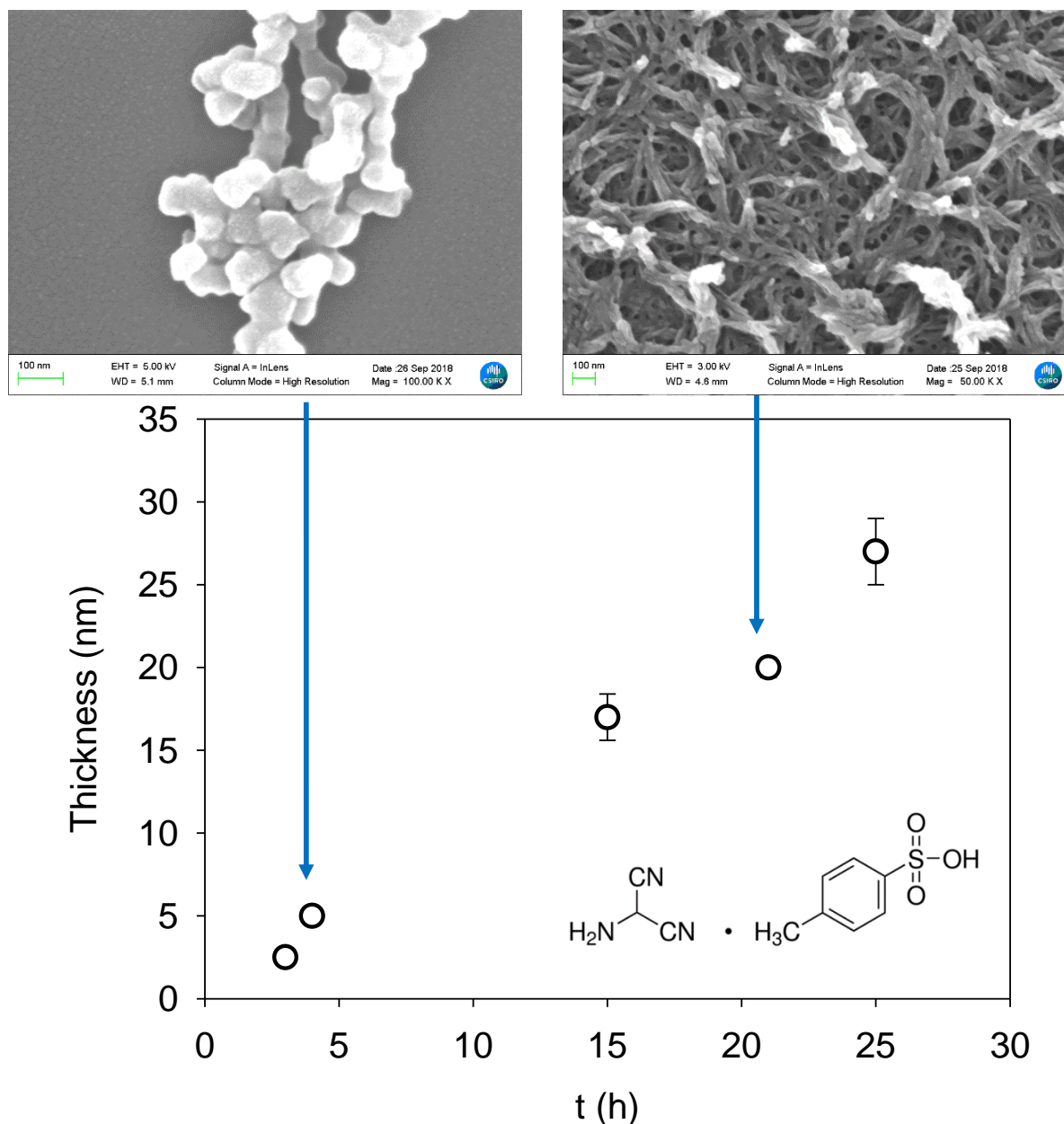


Fig. 1: Evolution of the AMN based film thickness as measured by means of single wavelength (632.8 nm) ellipsometry. The data correspond to the average \pm one SD over 5 measurements taken along the major axis of the silicon wafer. The inset displays the structure of used AMN salt. The film morphologies obtained by SEM after 4.5h and 21h of deposition are shown in the upper part. The green scale bar on the left corresponds to 100 nm in each micrograph. The full image size is about $1\mu\text{m} \times 1\mu\text{m}$.

From an electrochemical point of view, the AMN based films deposited on amorphous carbon working electrodes display an irreversible electrochemical activity in agreement with the presence of reducible moieties in the deposits (Fig. 2A). The CV curves obtained in the absence of an external redox probe, after deposition on the amorphous working electrode and buffer rinse, are markedly dependant on the deposition time: for longer reaction in the presence of the AMN solution, the oxidation peaks shift to more anodic potentials and the amplitude of the maximal oxidation current decreases (Fig. 2A). This reflects simultaneously a change in the composition of the films, in agreement with the X-ray photoelectron spectroscopy data obtained previously [7] and the formation of a more compact film in which fewer chemical groups are accessible by the electrode. This last assumption is in agreement with the marked change in film morphology from clustered particles to a fiber like material (upper part of Fig. 1). Note that the film morphology after 6h of deposition is intermediate between the clustered particles (SEM picture after 4.5h of deposition) and the fibers (SEM picture after 21h of deposition) displayed in Fig. 1. The CV of the deposited films does not change significantly after 6h of deposition (data not shown) reflecting hence a steady state from an electrochemical point of view. But it has not to be forgotten that the working electrode probes the electroactive groups in its close proximity, the film behaving overall as an insulator as shown by electrochemical impedance spectroscopy [9].

The assumed chemical reactions leading to a polymer, based on compositional analysis published elsewhere [7] is represented in Fig. 2B. The measured decrease in the O/C ratio and the simultaneous increase in the N/C ratio are attributed to a water elimination and could be at the origin of the anodic shifts observed by CV (Fig. 2A). Owing to the hypothetical structure of the AMN derived species (Fig. 2B), it might be that the morphological transition from clusters to fibers (Fig. 1) is related to a directional mode of interactions between the formed species, like cation- π interactions.

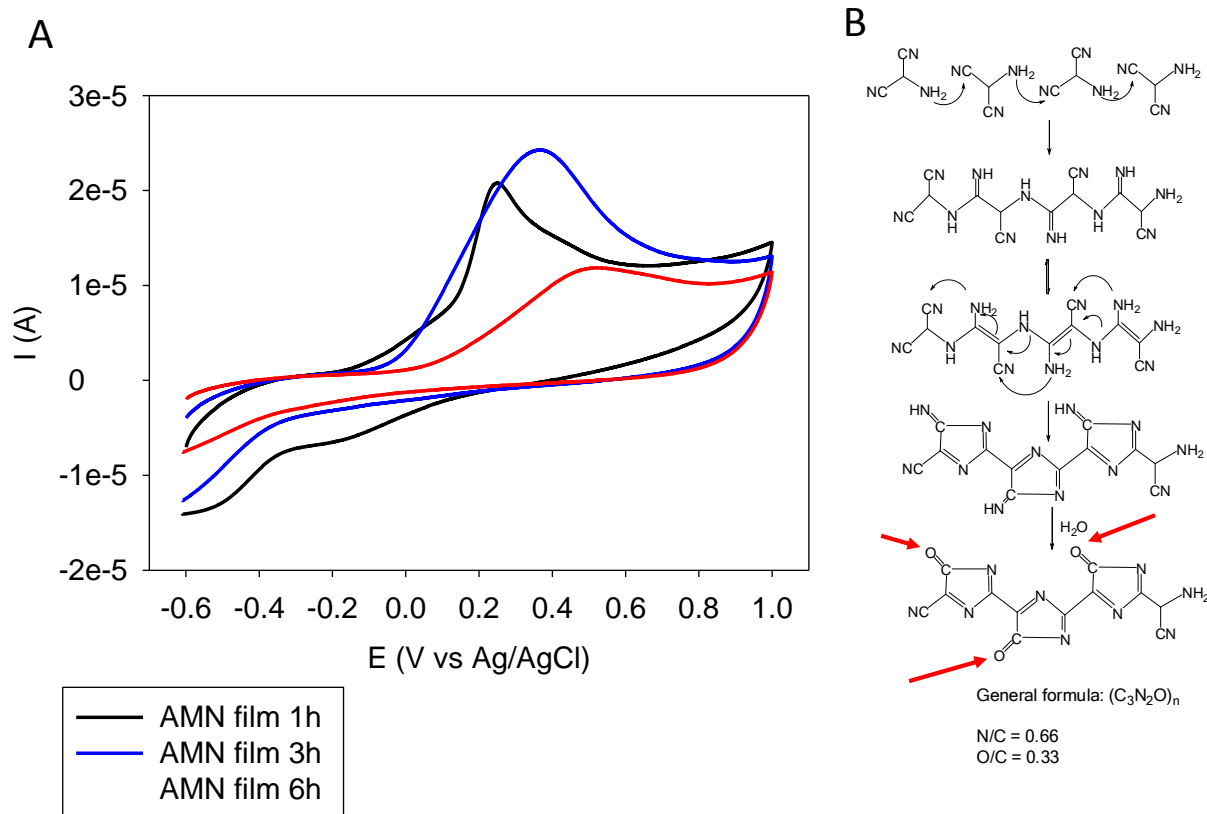
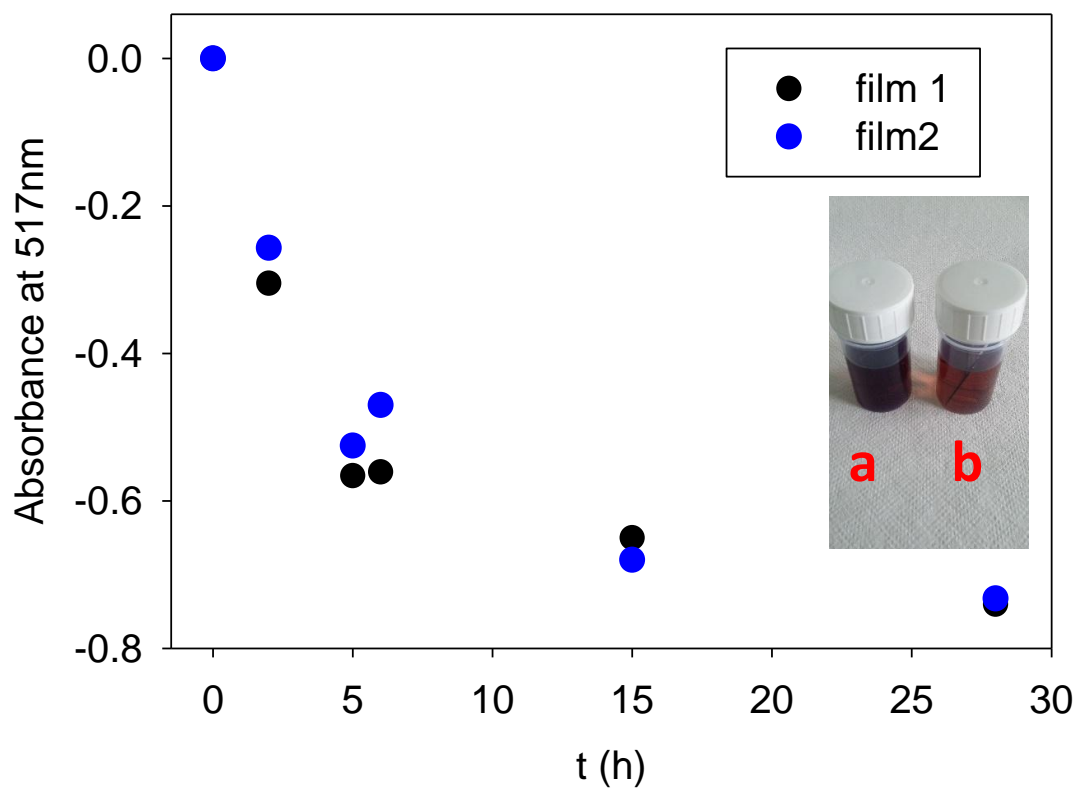


Fig. 2: CV scans (potential sweep rate: $100 \text{ mV}\cdot\text{s}^{-1}$) of AMN based films deposited on amorphous carbon electrodes for various durations (see inset). B: proposed reaction mechanism of an AMN based polymer as obtained from X-ray photoelectron spectroscopy in ref. [7]. The red arrows in the chemical structure pinpoint to C=O groups which are the oxidized form of C-OH groups suspected to be responsible for the antioxidant properties of the AMN based films.

The presence of redox active groups on AMN based films could afford many applications. For this reason, their antioxidant activity was investigated by measuring the discoloration of DPPH. It appears that longer deposition times lead to increased quenching of DPPH (the reaction was performed in the dark for 30 min.) up to reach a pseudo plateau after about 20h of film deposition (Fig. 3A). This evolution in the antioxidant potential of the AMN based films follows the slow deposition kinetics and the progressive change in film morphology (upper part of Fig. 1). After longer deposition times, even if the film thickness continues to increase, the antioxidant activity seems to saturate. This may be explained by a transition in the film morphology from aggregates made of globular particles to μm long fibers (Fig. 1) producing a progressive increase in the number of reactive groups up to a

critical stage where the DPPH radicals cannot have access anymore to all available surface sites. This interpretation is not in contradiction with the electrochemical data displayed in Fig.2, where a decrease in oxidation current is found for longer deposition times. Indeed, CV probes only the chemical groups which are accessible to the electrode. When the insulating film, as probed by electrochemical impedance spectroscopy [7] becomes more compact (as expected during the island to fiber morphological change) the number of available oxidizable groups should decrease. Anyway, the present findings show that AMN based films display an intrinsic antioxidant activity which can be tuned by playing on the deposition time and hence of the film thickness and morphology.

A



B

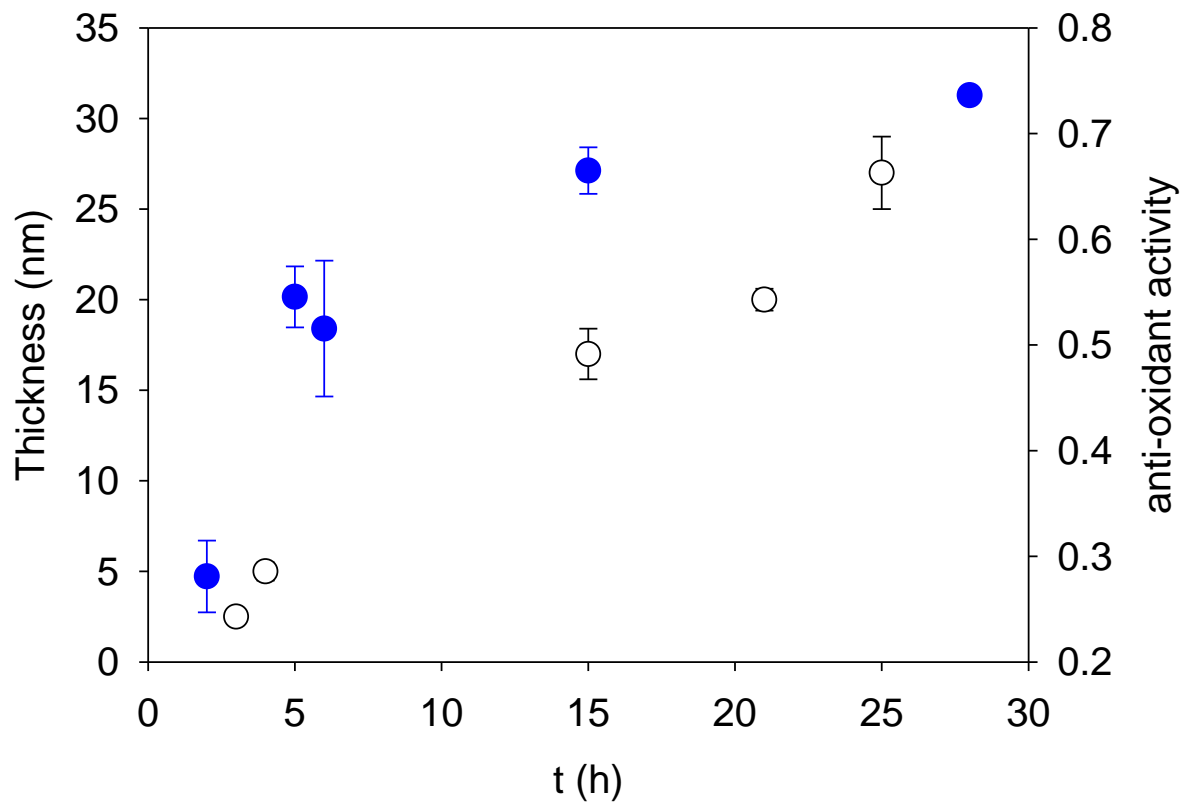


Fig. 3: A: Evolution of the absorbance at 517 nm of a DPPH solution (10^{-4} M in ethanol) put in contact for 30 min with an AMN film deposited on glass for the indicated duration. The picture corresponds to a DPPH solution kept in the dark (reference, picture a) and a DPPH solution kept in the dark but in contact with an AMN based film deposited for 5 h on a glass slide (picture b). 2 individual measurement series were performed (●, ●).

B: Evolution with time of the AMN based film thickness (the same data as in Fig. 1, left hand scale) and their anti-oxidant activity (taken as the average \pm one SD from the data in part A, right hand scale).

According to the proposed structure in Fig. 2B, which is consistent with the final O/C and N/C ratios obtained by XPS in ref. [7] we make the assumption that part of the C=O groups, which are the oxidized form of C-OH groups are still present in the reduced form and that those groups contribute to the films' antioxidant properties. Note that the O/C and N/C ratios are not affected upon oxidation reduction processes provided the material does not undergo some further chemical change.

In this investigation, AMN based films were deposited on different kinds of substrates (silicon covered with a thin silica layer, quartz slides, amorphous carbon) and we cannot exclude an influence of the nature of the substrate on the film morphology even if its composition seems to be almost substrate independent (Fig. 1 in ref.[6]). This last point deserves however high probability that all AMM based coatings display the electrochemical and antioxidant properties highlighted in the present research.

4. Conclusions

AMN based films display a slow growth kinetics at the glass/water interface and a morphological change from island like to fibrillar as well as a marked electrochemical activity providing them with a deposition time dependant antioxidant activity. In future studies, the nature of chemical moieties responsible for such a redox and antioxidant activity will be investigated in detail. Some assumptions, related to the presence of -C-OH groups in aromatic structures have been suggested herein.

Credit authorship contribution statement

V. Ball performed, analysed the experiments and wrote the manuscript.

Declaration of competing interest

The author certifies to have no competing interests with other research groups or persons on this topic.

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