



Poly(methylene blue)-modified electrode for indirect electrochemical sensing of OH radicals and radical scavengers

WALTER A. BRAUN¹, BRUNA C. HORN¹, LUCÉLIA HOEHNE¹, SIMONE STÜLP¹, MARCELO B. DA ROSA² and MAURÍCIO HILGEMANN¹

¹Centro de Ciências Exatas e Tecnológicas, Centro Universitário UNIVATES,
Avenida Avelino Talini, 171, 95914-014 Lajeado, RS, Brazil

²Universidade Federal de Santa Maria, Departamento de Química, Avenida Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

Manuscript received on November 30, 2016; accepted for publication on March 24, 2017

ABSTRACT

A new modified electrode for indirect sensing of OH⁻ and radical scavengers was described. The electrochemical polymerization of methylene blue in aqueous solutions and the properties of the resulting films on a glassy carbon electrode were carried out using cyclic voltammetry. A surface coverage of 1.11×10^{-9} mol cm² was obtained, revealing a complete surface coverage of the polymeric film on the electrode surface. OH⁻ was able to destroy the poly(methylene blue) film by exposure to a Fenton solution. The loss of the electrochemical signal of the residual polymeric film attached to the electrode surface was related to the extent of its dissolution. The applicability of the sensor was demonstrated by evaluating the OH radical scavenging effect on different concentrations of ascorbic acid. The obtained radical scavenging capacity were 31.4%, 55.7%, 98.9% and 65.7% for the ascorbic acid concentrations of 5, 10, 30 and 50 mM, respectively.

Key words: electrochemical sensor, Fenton solution, modified electrode, OH radical, poly(methylene blue).

INTRODUCTION

Antioxidants attract great attention because of their importance on preventing the cell damaging action. They are essential substances to the human body, inactivating reactive oxygen species (ROS) by the endogenous and exogenous antioxidant systems, protecting against oxidative stress. This could lead to a number of problems such as lipid peroxidation and damage on tissue proteins, membranes,

enzymes, carbohydrates and DNA (Halliwell and Gutteridge 2007, Wang et al. 2014, Li et al. 2015).

Due to the great importance of free radicals and radical scavengers, several methodologies have been developed aiming the detection and/or quantification of these two groups of compounds in different matrices. The proposed assays focus on the interactions between radical scavengers and stable free radicals, as DPPH, or on the protection effect caused by an antioxidant to a target molecule, which is oxidized by ROS. Traditional techniques include spectrophotometry (Thaipong et al. 2006,

Correspondence to: Mauricio Hilgemann
E-mail: mauricio.hilgemann@univates.br

Al-Fatimi et al. 2007), chromatography (Borges et al. 2010, de Carvalho et al. 2013); fluorescence (Amado et al. 2009), and chemiluminescence (Guo et al. 2008). However, such techniques are being replaced by other innovative methods in recent years, such as redox-responsive fluorescent probes (Yu et al. 2011, 2012, 2013, Lou et al. 2015) and electrochemical approaches. In this sense, electrochemical assays are promising tools: suitable for fast analyses, based on inexpensive instrumentation and with simple operation protocols (Prieto-Simón et al. 2008). Additionally, studies propose the use of chemically modified electrodes (CMEs) as a simple electrochemical approach to sense ROS and radical scavengers, with DNA, self-assembled monolayers of thiols, polyphenol films, and conducting polymers (CPs) (Scholz et al. 2007, Liu et al. 2005, Mello et al. 2006, Hilgemann et al. 2010, Wu et al. 2012, Gualandi and Tonelli 2013, Gualandi et al. 2014, 2015) as electrode modifiers. The ROS may attack the CME and the extent of the modifier destruction can be evaluated directly (in this case, when an electroactive layer is monitored) or using an electrochemical probe in solution (when a non-electroactive layer is used).

Besides acting as radical probes (Gualandi et al. 2014), conducting polymers (CPs) can be used as a component in biosensors in order to detect ROS (Rajesh et al. 2010, Mahmoudian et al. 2013). In addition to that, the use of CPs in sensor technologies involves employing the conducting polymers as an electrode modification in order to improve sensitivity, to impart selectivity, to suppress interference and to provide a support matrix for sensor molecules (Inzelt 2008). Surface-modified electrodes based on the electropolymerization of several phenazines, phenoxazines and phenothiazines have been described in the literature (Karyakin et al. 1999). In this sense, electropolymerization is considered one of the most common and useful techniques used to obtain polymer dye thin films. Among CP, poly

(methylene blue) (PMB) has been widely studied due its electrocatalytic activity in the presence of biomolecules and different inorganic compounds. Karyakin et al. (1993a, b) were the first to report the electrochemical polymerization of such compound. The polymer film was used for a biomaterial due to its bioelectrochemical activity. The main advantage of electropolymerization of methylene blue (MB) relies on the long-term stability of the resulting polymer, which is synthesized from an inexpensive monomer in aqueous solution (Kaplan et al. 2010). However, so far PMB was never used for sensing radicals and radical scavengers.

Therefore, we investigated the electrochemical polymerization of MB and evaluated the stability properties of the PMB films produced when attacked by OH⁻, in order to develop a CME for indirect sensing of ROS and radical scavengers. Then, PMB films were prepared on the surface of a GC electrode and were very stable under ordinary conditions. However, when such polymeric film was exposed to the Fenton solution, ROS destroyed the film and the loss of the electrochemical signal of the residual PMB attached to the electrode surface was related to the extent of its dissolution.

MATERIALS AND METHODS

REAGENTS AND SUPPORTING ELECTROLYTE SOLUTIONS

All reagents used in this work were of analytical grade, and all solutions were prepared in purified water. The MB monomer was purchased from Quimex. The supporting electrolyte solutions used for electropolymerization of MB consisted of 0.02 mol L⁻¹ sodium tetraborate and 0.1 mol L⁻¹ sodium nitrate (pH 9.2) (Karyakin et al. 1999). The monomer dissolved concentration in this solution was always 0.76 mmol L⁻¹.

APPARATUS

The electrochemical measurements were carried out on a computer-controlled potentiostat/galvanostat PGSTAT 128N (AUTOLAB) with GPES software. The working electrode consisted of a conventional three-electrode cell assembly with a 3 mm glassy carbon electrode (GCE) or GCE modified with the PMB film, a Ag/AgCl in 3.0 mol L⁻¹ KCl reference electrode and a platinum wire as a counter electrode. In order to minimize electrical interferences, the electrochemical cell was enclosed in a grounded Faraday cage.

PREPARATION OF PMB-MODIFIED ELECTRODE

Prior to surface modification, the GC electrode was mechanically polished with 0.5 μm alumina to a mirror finish. Thereafter, the electrode was washed with ethanol to remove residual alumina particles that could be trapped in the surface. Finally, the GC electrode was sonicated in a water/ethanol solution (1:1) during 5 min, and then washed with ethanol and water. The surface modification of GC electrode was performed by cyclic voltammetry (CV) from the supporting electrolyte solution described above, containing 0.76 mmol L⁻¹ monomer, at a scan rate of 0.05 V s⁻¹. The potential was cycled between -0.4 and +1.2 V versus Ag/AgCl for 30 cycles. After this step the polymer-modified electrode was left aging overnight in electrolyte solution at 4 °C to allow unpolymerized monomer retained within the film to escape and lead to a more stable response (Brett et al. 1999, Marinho et al. 2012).

PMB FILM DEGRADATION BY FENTON ATTACK

After the working electrode modification, a cyclic voltammogram was recorded in a 0.1 mol L⁻¹ sodium nitrate and 0.05 mol L⁻¹ acetate buffer solution (pH = 5.0), from -0.4 to +0.5 V at 0.05 V s⁻¹. The electrode was then washed with water and introduced in an iron (II) solution in the presence or absence of ascorbic acid. The Fenton reaction

was started by addition of H₂O₂, and the polymeric film attached to the electrode was exposed to this solution for 30 seconds. The Fenton solution was always freshly prepared from iron (II) sulphate (Synth) and hydrogen peroxide 30% (v/v, Merck). The molar ratio Fe²⁺:H₂O₂ was maintained at 1:1 (10 mmol L⁻¹) until interruption of the reaction between the Fenton solution and the PMB-modified electrode by removing the electrode and washing it with water. Finally, the Fenton attack result was evaluated by measuring the charge decrease under the anodic peak of the PMB electropolymerized on the electrode. This whole procedure was repeated five more times with the same PMB film, for a total of 3 minutes of Fenton attack, in order to obtain the extent of the PMB destruction for each ascorbic acid concentration. After this process, the electrode surface was regenerated by mechanical polishing, washing and sonication conditioning. All measurements were performed in triplicate.

RADICAL SCAVENGING CAPACITY

The radical scavenging capacity was achieved by plotting the logarithm of the normalized oxidation peak areas [$\ln(Q_i/Q_0)$] versus the time of Fenton attack, since the respective decay constant refers directly to the given radical scavenging capacity of the corresponding ascorbic acid concentration. The OH radical scavenging capacities were calculated according to the equation (1) (Hilgemann et al. 2010):

$$\% \text{ radical scavenging} = \left(\frac{k_{\text{control}} - k_{\text{sample}}}{k_{\text{control}}} \right) \times 100\% \quad (1)$$

where k_{sample} and k_{control} are the kinetic constants obtained with and without ascorbic acid in the Fenton solution, respectively.

Blank experiments were also performed. PMB films were dipped in aqueous solutions of iron (II), H₂O₂ and ascorbic acid in all the working

concentration range for 5 minutes, without OH⁻ generation.

RESULTS AND DISCUSSION

ELECTROPOLYMERIZATION OF THE MB AND ELECTROCHEMICAL CHARACTERIZATION OF THE PMB-MODIFIED ELECTRODE

The electropolymerization of the MB (Figure 1) reaches higher growth rates in basic solutions (Karyakin et al. 1999, Kaplan et al. 2010, Brett et al. 1999, Marinho et al. 2012, Liu and Mu 1999).

Taking this into account, the electrochemical polymerization of MB for 30 cycles in pH 9.2 is shown in Figure 2. The voltammetric profile of the formation of a polymeric film shows two redox peaks additionally to a third peak, where only an oxidation process occurs. The first anodic peak at -0.12 V is related to the oxidation of the MB monomer. As electropolymerization takes place, the anodic peak decreases and a new set of peaks appears in cyclic voltammograms. Both peak potentials (anodic and cathodic) shift to more positive values with the increase of number of cycles. Such response indicates a polymer type redox activity, which is shifted to more positive potentials compared with the monomer. The oxidation peak observed in the potential region around 1.2 V is related to the formation of cation-radical species (Karyakin et al. 1999, Marinho et al. 2012). The peak system related to the polymer formation presented a quasi-reversible behavior, as can be seen in Figure 2. With the increment of number of cycles, the voltammetric profile becomes more irreversible, with no significant increase in the peak current. The exact mechanism of the electropolymerization of MB is a rather complicated question. However, a path of its polymerization was already suggested (Karyakin et al. 1993a, Liu and Mu 1999).

The surface coverage of the PMB film on the GC electrode can be estimated from the surface coverage concentration (Γ) of the polymer. The

Γ value was calculated from the charge (Q) associated with the electropolymerization process according to (Marinho et al. 2012, Yogeswaran and Chen 2008):

$$\Gamma = Q / (n \cdot F \cdot A) \quad (2)$$

where A is the geometric area of the GCE (0.070 cm²), n is the number of electrons involved in the redox process ($n = 2$), and F is the Faraday constant (96,485 C mol⁻¹).

The Q value was determined by integration of the voltammetric oxidation peak of PMB obtained with a scan rate of 0.05 V s⁻¹ ($Q = 15.0 \mu\text{C} \pm 1.33$). Therefore, the Γ is equal to $1.11 \times 10^9 \text{ mol cm}^{-2}$.

The obtained Γ value revealed a complete surface coverage of the PMB film on the surface of the GC electrode. Then, it is possible to assume good physical and chemical stability during the electrochemical measurements. In fact, the polymeric film attached to the electrode is very stable over time. Cyclic voltammograms of the PMB-modified electrode were recorded in a 0.1 mol L⁻¹ sodium nitrate and 0.05 mol L⁻¹ acetate buffer solution (pH = 5.0), from -0.4 to +0.5 V at 0.05 V s⁻¹, within a 30 minute interval to study the stability of the film formed over time. Seven voltammograms were obtained at 5-minute intervals between each measurement. Under these conditions, the results showed that the difference between Q values for the first and last voltammograms differ less than 6%, as shown in our previous work (Braun et al. 2016). This means that the disappearance of redox peaks resulted from the destruction of film caused by OH⁻ and not by the dissolution of the polymeric film.

QUANTIFICATION OF THE PMB FILM DEGRADATION BY FENTON ATTACK

The effect of the electrode modification and the OH⁻ attack to the PMB film can be seen in Figure

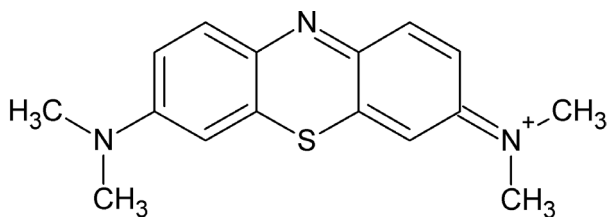


Figure 1 - Chemical structure of methylene blue.

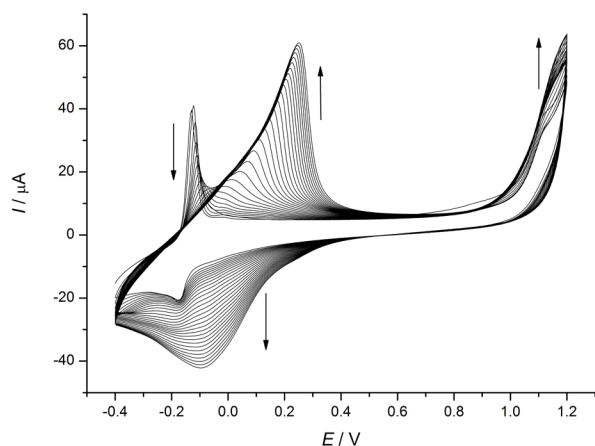


Figure 2 - Voltammetric profile of MB electropolymerization; [MB] = 0.76 mmol L⁻¹ in 0.02 mol L⁻¹ sodium tetraborate and 0.1 mol L⁻¹ NaNO₃ (pH 9.2); 30 cycles between 0.4 and +1.2 V at 0.05 V s⁻¹.

3. Also, Figure 3 shows cyclic voltammograms of the PMB-modified electrode after 2 successive attacks of the polymeric film with OH[•] produced in a Fenton solution for 30 s. As can be noticed, the electrochemical signal of the residual film decreases with extending the OH[•] attack. Blank experiments showed that neither ascorbic acid, iron (II) or H₂O₂ solutions were able to destroy the PMB film formed on GC electrode.

In order to prove the applicability of the proposed modified electrode to sense OH[•] radical scavenging capacity of antioxidant compounds, the dissolution of the polymeric film was studied in the presence of ascorbic acid (AA) at different concentrations. It is known that AA reacts very fast with OH[•] (Gualandi and Tonelli 2013, Gualandi et al. 2014). Therefore, the addition of AA to the Fenton solution causes a decrease in OH[•] concentration. Such reduction is proportional to

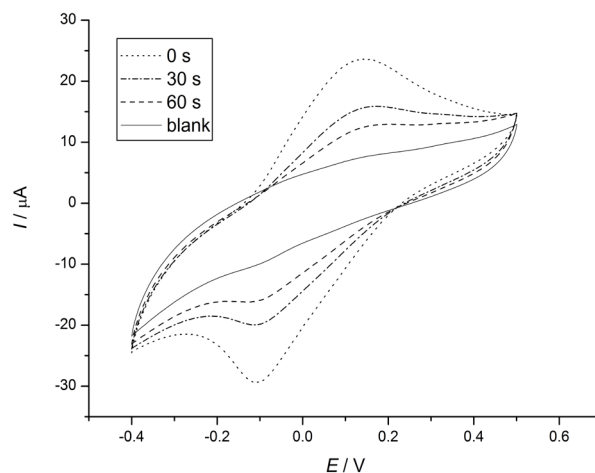
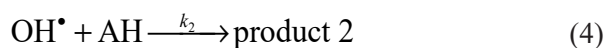


Figure 3 - Cyclic voltammograms recorded at a PMB-modified electrode in 0.1 mol L⁻¹ NaNO₃ and 0.05 mol L⁻¹ acetate buffer solution (pH 5.0) before and after two successive Fenton attacks; scan rate = 0.05 V s⁻¹.

the amount of AA added. Now, since less OH[•] is available to attack the PMB-modified electrode, a slower drop of the analytical signal (degradation of the polymeric film) is expected.

Exponential decays of the voltammetric signal (Q_t/Q_0) with the reaction time between PMB film and OH[•] radicals in the absence and in the presence of 10 mM ascorbic acid show a clear distinction between the two evaluated reaction conditions (Figure S1 - Supplementary Material). As stated above, it is possible to see a decrease in the polymeric film dissolution with the addition of AA, since part of the generated OH[•] in the Fenton solution reacts with the AA added. Therefore, a smaller drop of the analytical signal is observed when 10 mM of ascorbic acid is added to the Fenton solution. In this system, two simultaneous bimolecular reactions compete. The first one is the attack of OH[•] to the PMB-film (3), and the other occurs when a radical scavenger (an antioxidant AH) is added to the Fenton solution (4):





Since the OH^\bullet causes the dissolution of the polymeric film, the electrochemical signal decrease indicates the extension of this dissolution, and the addition of an antioxidant AH to the Fenton solution slows down this destruction. Ideally, if PMB and AH compete for OH^\bullet and the concentration of AH is sufficiently large, the observed rate constant k_{obs} would be equal to k_1 , and independent of the AH concentration (Zheng et al. 2015). Instead, due to multiple-step reactions, the study concerning Fenton reaction kinetics becomes more difficult, since each step is characterized by a different constant rate (Neyens and Baeyens 2003, Bautista et al. 2008, Mitsika et al. 2013).

The reaction rate can be graphically calculated by plotting the diagram $[\ln(Q_t/Q_0)]$ versus time of reaction (Figure 4). By this procedure, a straight line is formed, indicating a pseudo-first order rate constant, according to:

$$\ln(Q_t/Q_0) = -k_{\text{obs}} \cdot t \quad (5)$$

where Q_0 is the initial charge of the PMB film, Q_t is the charge of the PMB film at time t , and k_{obs} is the observed pseudo-first order rate constant.

The slope of each straight line in Figure 4 corresponds to the observed kinetic constant, i.e., the reaction velocity between AH and OH^\bullet . Therefore, the addition of a radical scavenger to the Fenton solution inhibits the destruction of the PMB film, acting as an indirect measurement of the interaction between OH^\bullet and radical scavenger compound.

As shown in Figure 4, the degradation of the polymeric film proceeds to a lower extent when the ascorbic acid concentration is enhanced. However, this protective effect is not proportional to the ascorbic acid concentration in the entire tested range. From 5 to 30 mM of AA, the polymeric

film dissolution slows down with increasing AA concentration. In fact, at 30 mM, almost no degradation was observed during Fenton attack. Nevertheless, by increasing the AA concentration to 50 mM, although an antioxidative effect is observed compared to the control group, a degradation of 35% is observed, indicating an oxidative effect caused by high amounts of ascorbic acid.

DETERMINATION OF RADICAL SCAVENGING CAPACITY

The concentration effect of AA in the Fenton solution was further studied. The radical scavenging capacity was calculated according to equation 1, and the results obtained were 31.4%, 55.7%, 98.9% and 65.7% for the ascorbic acid concentrations of 5, 10, 30 and 50 mM, respectively (Table I).

Figure 5 shows the kinetic constant observed for the reaction between PMB and OH^\bullet against AA concentration. It can be observed that the minimum value for k_{obs} is of 30 mM, indicating the ascorbic acid ability to promote oxidation in concentrations above 30 mM. This pro-oxidative capacity of ascorbic acid is in accordance with previous results reported in literature (Berger et al. 1997, Carr and Frei 1999, Valko et al. 2005, Wang et al. 2013). However, in the few studies related to chemically modified electrodes (CMEs) for OH^\bullet sensing (Gualandi et al. 2014, 2015, Gualandi and Tonelli 2013, Scholz et al. 2007, Hilgemann et al. 2010), it is the first time that such behavior is observed concerning this pro-oxidative effect of AA when it is used as an antioxidant. In aqueous solution, ascorbic acid is present mainly as ascorbate, which can reduce Fe^{3+} to Fe^{2+} . These ferrous ions can promote further OH^\bullet formation via Fenton chemistry, if enough H_2O_2 is still available. Thus, in these cases, low AA levels should be maintained. Such behavior could explain why a 100% OH^\bullet radical scavenging was not reached in our previous work even when AA levels were systematically enhanced (Hilgemann et al. 2010).

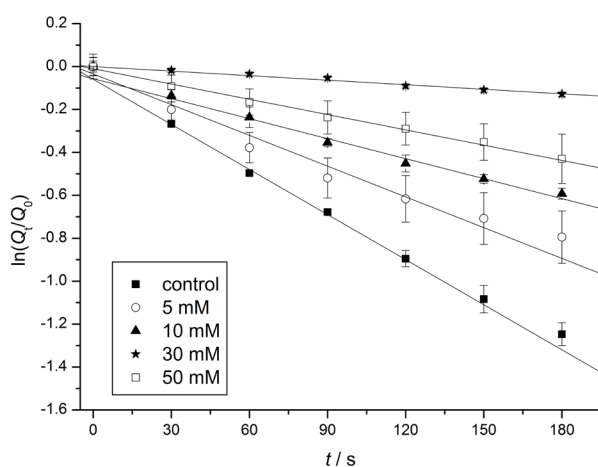


Figure 4 - Logarithmic linearized plot of the variation of the anodic peak charge after OH[•] attack to the PMB-modified electrode *versus* time of reaction of the polymeric film with OH radicals. Error bars are related to experiments in triplicate.

TABLE I
Pseudo-first order rate constant and radical scavenging capacities for different concentrations of ascorbic acid.

Ascorbic acid concentration / mM	$k_{\text{obs}} / 10^{-3} \text{ s}^{-1}$	Radical scavenging / %
0 (control)	7.00 ± 0.45	0
5	4.80 ± 0.58	31.40 ± 3.83
10	3.10 ± 0.12	55.70 ± 2.18
30	0.08 ± 0.01	98.90 ± 0.99
50	2.40 ± 0.20	65.70 ± 5.56

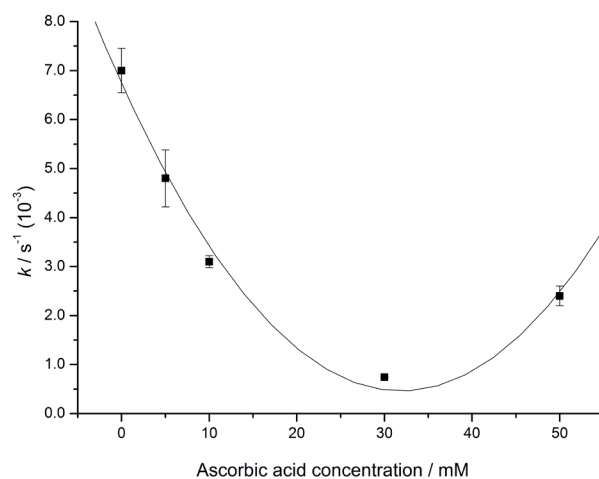


Figure 5 - Influence of ascorbic acid concentrations on the pseudo-first order kinetic constants. Error bars are related to experiments in triplicate.

As aforementioned, the radical scavenging capacity is calculated based in a kinetic constant for a blank experiment (k_{control}), where OH[•] is generated solely through the reaction between Fe²⁺ and H₂O₂. Therefore, the concentration of OH[•] in this experiment reaches a certain maximum value, with no contribution of any pro-oxidative effect from a radical scavenger compound. Considering the pro-oxidative effect added by ascorbate, the maximum OH[•] concentration reached in the Fenton solution is now higher than in the blank experiment. Consequently, a 100% of OH[•] radical scavenging effect cannot be reached. Thus, again, low AA levels should be maintained.

As can be noticed, the ascorbic acid concentration plays a key role in the radical scavenging capacity. However, the rate constants of the kinetic reactions for each concentration would also enable the comparison of radical scavenging capacity among different compounds at the same concentration.

CONCLUSIONS

The PMB modified electrode exhibited good performance for the indirect electrochemical sensing of ROS and radical scavengers, as ascorbic acid. Additionally, PMB showed excellent stability and reproducibility. The optimal concentration of ascorbic acid against the OH[•] generated in a Fenton solution was 30 mM. Above this concentration, ascorbic acid played a pro-oxidative role, although an antioxidative effect is observed compared to the control group. With this methodology, we try now to detect damages caused by OH[•] to biomolecules, such as proteins, vitamins and membranes.

ACKNOWLEDGMENTS

The authors thank Dr. Heike Kahlert (University of Greifswald) for her suggestions and proof reading the manuscript and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the financial support (process number 16/2551-0000214-2).

REFERENCES

- AL-FATIMI M, WURSTER M, SCHRÖDER G AND LINDEQUIST U. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol* 111: 657-666.
- AMADO LL, GARCIA ML, RAMOS PB, FREITAS RF, ZAFALON B, FERREIRA JLR, YUNES JS AND MONSERRAT JM. 2009. A method to measure total antioxidant capacity against peroxy radicals in aquatic organisms: Application to evaluate microcystins toxicity. *Sci Total Environ* 407: 2115-2123.
- BAUTISTA P, MOHEDANO AF, CASAS JA, ZAZO JA AND RODRIGUEZ JJ. 2008. An overview of the application of Fenton oxidation to industrial wastewaters treatment. *J Chem Technol Biotechnol* 83: 1323-1338.
- BERGER TM, POLIDORI MC, DABBAGH A, EVANS PJ, HALLIWELL B, MORROW JD, ROBERTS LJ AND FREI B. 1997. Antioxidant Activity of Vitamin C in Iron-overloaded Human Plasma. *J Biol Chem* 272: 15656-15660.
- BORGES G, DEGENEVEA, MULLEN W AND CROZIER A. 2010. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *J Agric Food Chem* 58: 3901-3909.
- BRAUN WA, KUSSLER RC, BAGATINI M AND HILGEMANN M. 2016. Eletropolimerização de azul de metileno sobre carbono vítreo e estabilidade do filme de poli(azul de metileno). *Sci Plena* 12: 097201.
- BRETT CMA, INZELT G AND KERTESZ V. 1999. Poly(methylene blue) modified electrode sensor for haemoglobin. *Anal Chim Acta* 385: 119-123.
- CARR A AND FREI B. 1999. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J* 13: 1007-1024.
- DE CARVALHO LM, MONSERRAT JM, SCHOLZ F, LIMA FO, DAROSA MB, HILGEMANN M, MÜLLER LS, FACCIN H, RAMOS PB AND VIANA C. 2013. Substrate-free Determination of the Radical Scavenging Activity of Phenolic Compounds by Photochemical Generation of Hydroxyl Radicals and HPLC-UV Detection. *Sep Sci Technol* 48: 1123-1131.
- GUALANDI I, FERRARO L, MATTEUCCI P AND TONELLI D. 2015. Assessment of the Antioxidant Capacity of Standard Compounds and Fruit Juices by a Newly Developed Electrochemical Method: Comparative Study with Results from Other Analytical Methods. *Electroanalysis* 27: 1906-1914.
- GUALANDI I, GUADAGNINI L, ZAPPOLI S AND TONELLI D. 2014. A Polypyrrole Based Sensor for the Electrochemical Detection of OH Radicals. *Electroanalysis* 26: 1544-1550.
- GUALANDI I AND TONELLI D. 2013. A new electrochemical sensor for OH radicals detection. *Talanta* 115: 779-786.
- GUO JZ, CUI H, ZHOU W AND WANG W. 2008. Ag nanoparticle-catalyzed chemiluminescent reaction between luminol and hydrogen peroxide. *J Photochem Photobiol A Chem* 193: 89-96.
- HALLIWELL B AND GUTTERIDGE JMC. 2007. Free radicals in biology and medicine, 4th ed., USA: Oxford University Press, 704 p.
- HILGEMANN M, SCHOLZ F, KAHLERT H, DE CARVALHO LM, DA ROSA MB, LINDEQUIST U, WURSTER M, DO NASCIMENTO PC AND BOHRER D. 2010. Electrochemical assay to quantify the hydroxyl radical scavenging activity of medicinal plant extracts. *Electroanalysis* 22: 406-412.
- INZELT G. 2008. Conducting Polymers, Berlin, Heidelberg: Springer, p. 282.
- KAPLAN İH, DAĞCI K AND ALANYALIOĞLU M. 2010. Nucleation and Growth Mechanism of Electropolymerization of Methylene Blue: The Effect of Preparation Potential on Poly(methylene blue) Structure. *Electroanalysis* 22: 2694-2701.
- KARYAKIN AA, KARYAKINA EE AND SCHMIDT H-L. 1999. Electropolymerized Azines: A New Group of Electroactive Polymers. *Electroanalysis* 11: 149-155.
- KARYAKIN AA, STRAKHOVA AK, KARYAKINA EE, VARFOLOMEYEV SD AND YATSIMIRSKY AK. 1993a. The electrochemical polymerization of methylene blue and bioelectrochemical activity of the resulting film. *Bioelectrochem Bioenerg* 32: 35-43.
- KARYAKIN AA, STRAKHOVA AK, KARYAKINA EE, VARFOLOMEYEV SD AND YATSLMIRSKY AK. 1993b. The electrochemical polymerization of Methylene Blue and bioelectrochemical activity of the resulting film. *Synth Met* 60: 289-292.
- LI Y, LI W, ZHOU H, WANG F, CHEN Y, WANG Y AND YU C. 2015. A facile method for the sensing of antioxidants based on the redox transformation of polyaniline. *Sens Actuators B Chem* 208: 30-35.
- LIU J AND MU S. 1999. The electrochemical polymerization of methylene blue and properties of polymethylene blue. *Synth Met* 107: 159-165.
- LIU J, ROUSSEL C, LAGGER G, TACCHINI P AND GIRAULT HH. 2005. Antioxidant Sensors Based on DNA-Modified Electrodes. *Anal Chem* 77: 7687-7694.
- LOU Z, LI P AND HAN K. 2015. Redox-responsive fluorescent probes with different design strategies. *Acc Chem Res* 48: 1358-1368.

- MAHMOUDIAN MR, ALIAS Y, BASIRUN WJ, AM GOLSHEIKH AND JAMALI-SHEINI F. 2013. Synthesis of polypyrrole coated manganese nanowires and their application in hydrogen peroxide detection. *Mater Chem Phys* 141: 298-303.
- MARINHO MIC, CABRAL MF AND MAZO LH. 2012. Is the poly (methylene blue)-modified glassy carbon electrode an adequate electrode for the simple detection of thiols and amino acid-based molecules? *J Electroanal Chem* 685: 8-14.
- MELLO LD, HERNANDEZ S, MARRAZZA G, MASCINI M AND KUBOTA LT. 2006. Investigations of the antioxidant properties of plant extracts using a DNA-electrochemical biosensor. *Biosens Bioelectron* 21: 1374-1382.
- MITSIKA EE, CHRISTOPHORIDIS C AND FYTIANOS K. 2013. Fenton and Fenton-like oxidation of pesticide acetamiprid in water samples: Kinetic study of the degradation and optimization using response surface methodology. *Chemosphere* 93: 1818-1825.
- NEYENS E AND BAEYENS J. 2003. A review of classic Fenton's peroxidation as an advanced oxidation technique. *J Hazard Mater* 98: 33-50.
- PRIETO-SIMÓN B, CORTINA M, CAMPÀS M AND CALAS-BLANCHARD C. 2008. Electrochemical biosensors as a tool for antioxidant capacity assessment. *Sens Actuators B Chem* 129: 459-466.
- RAJESH S, KANUGULA AK, BHARGAVA K, ILAVAZHAGAN G, KOTAMRAJU S AND KARUNAKARAN C. 2010. Simultaneous electrochemical determination of superoxide anion radical and nitrite using Cu, ZnSOD immobilized on carbon nanotube in polypyrrole matrix. *Biosens Bioelectron* 26: 689-695.
- SCHOLZ F, GONZÁLEZ GLDL, DE CARVALHO LM, HILGEMANN M, BRAININA KZ, KAHLERT H, JACKRS AND MINHDT. 2007. Indirect electrochemical sensing of radicals and radical scavengers in biological matrices. *Angew Chem Int Ed* 46: 8079-8081.
- THAIPONG K, BOONPRAKOB U, CROSBY K, CISNEROS-ZEVALLOS L AND BYRNE DH. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compos Anal* 19: 669-675.
- VALKO M, MORRIS H AND CRONIN MT. 2005. Metals, Toxicity and Oxidative Stress. *Curr Med Chem* 12: 1161-1208.
- WANG X, JIAO C AND YU Z. 2014. Electrochemical biosensor for assessment of the total antioxidant capacity of orange juice beverage based on the immobilizing DNA on a poly l-glutamic acid doped silver hybridized membrane. *Sens Actuators B Chem* 192: 628-633.
- WANG Y, XIONG H, ZHANG X, GU H AND WANG S. 2013. An electrochemical biosensor for rapid detection of bovine serum albumin damage induced by hydroxyl radicals in room temperature ionic liquid. *Sens Actuators B Chem* 188: 741-746.
- WU L, YANG Y, ZHANG H, ZHU G, ZHANG X AND CHEN J. 2012. Sensitive electrochemical detection of hydroxyl radical with biobarcode amplification. *Anal Chim Acta* 756: 1-6.
- YOGESWARAN U AND CHEN SM. 2008. Multi-walled carbon nanotubes with poly(methylene blue) composite film for the enhancement and separation of electroanalytical responses of catecholamine and ascorbic acid. *Sens Actuators B Chem* 130: 739-749.
- YU F, LI P, LI G, ZHAO G, CHU T AND HAN K. 2011. A near-IR reversible fluorescent probe modulated by selenium for monitoring peroxynitrite and imaging in living cells. *J Am Chem Soc* 133: 11030-11033.
- YU F, LI P, SONG P, WANG B, ZHAO J AND HAN K. 2012. Facilitative functionalization of cyanine dye by an on-off-on fluorescent switch for imaging of H₂O₂ oxidative stress and thiols reducing repair in cells and tissues. *Chem Commun* 48: 4980-4982.
- YU F, LI P, WANG B AND HAN K. 2013. Reversible near-infrared fluorescent probe introducing tellurium to mimetic glutathione peroxidase for monitoring the redox cycles between peroxynitrite and glutathione *in vivo*. *J Am Chem Soc* 135: 7674-7680.
- ZHENG X, BI C, LI Z, PODARIU M AND HAGE DS. 2015. Analytical methods for kinetic studies of biological interactions: A review. *J Pharm Biomed Anal* 113: 163-180.

SUPPLEMENTARY MATERIAL

Figure S1 - Exponential decay after OH[•] attack to the PMB-modified electrode in presence and absence of 10 mM ascorbic acid added to the Fenton solution. Error bars are related to triplicate experiments.