

# Ciências Exatas e da Terra e a Dimensão Adquirida através da Evolução Tecnológica 3

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## APRESENTAÇÃO

A obra “*Ciências Exatas e da Terra e a Dimensão Adquirida através da Evolução Tecnológica vol. 3*” aborda uma publicação da Atena Editora, apresenta, em seus 23 capítulos, conhecimentos tecnológicos e aplicados as Ciências Exatas e da Terra.

Este volume dedicado à Ciência Exatas e da Terra traz uma variedade de artigos que mostram a evolução tecnológica que vem acontecendo nestas duas ciências, e como isso tem impactado a vários setores produtivos e de pesquisas. São abordados temas relacionados com a produção de conhecimento na área da matemática, química do solo, computação, geoprocessamento de dados, biodigestores, educação ambiental, manejo da água, entre outros temas. Estas aplicações visam contribuir no aumento do conhecimento gerado por instituições públicas e privadas no país.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos nas Ciências Exatas e da Terra, os agradecimentos dos Organizadores e da Atena Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias para a área da Física, Matemática, e na Agronomia e, assim, contribuir na procura de novas pesquisas e tecnologias que possam solucionar os problemas que enfrentamos no dia a dia.

Jorge González Aguilera  
Alan Mario Zuffo

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## ELECTROCHEMICAL SENSING OF OH RADICALS AND RADICAL SCAVENGERS BASED ON POLY(METHYLENE BLUE)-MODIFIED ELECTRODE

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**ABSTRACT:** A new modified electrode for indirect sensing of OH<sup>•</sup> 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  $1.11 \times 10^{-9}$  mol cm<sup>2</sup> was obtained, revealing a complete surface coverage of the polymeric film on the electrode surface. OH<sup>•</sup> 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.

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

**RESUMO:** Um novo eletrodo modificado para detecção indireta de OH<sup>•</sup> e sequestradores de radicais foi descrito. A polimerização eletroquímica do azul de metileno em soluções aquosas e as propriedades dos filmes resultantes em um eletrodo de carbono vítreo foram realizadas utilizando voltametria cíclica. Uma cobertura superficial de  $1,11 \times 10^{-9}$  mol cm<sup>-2</sup> foi obtida, revelando uma cobertura completa da superfície do filme polimérico na superfície do eletrodo. O radical OH foi capaz de destruir o filme de poli(azul de metileno) por exposição a uma solução de Fenton. A perda do sinal eletroquímico do filme polimérico residual ligado à superfície do eletrodo foi relacionada à extensão de sua dissolução. A aplicabilidade do sensor foi demonstrada através da avaliação do efeito de eliminação do radical OH em diferentes concentrações de ácido ascórbico. A capacidade de remoção de radicais obtida foi de 31,4%, 55,7%, 98,9% e 65,7% para as concentrações de ácido ascórbico de 5, 10, 30 e 50 mM, respectivamente.

**PALAVRAS-CHAVE:** sensor eletroquímico, solução de Fenton, eletrodo modificado, radical OH, poli(azul de metileno).

## 1 | 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<sup>•</sup>, 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, 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, Yu et al. 2012, Yu et al. 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, Gualandi et al. 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. (Karyakin et al. 1993)

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  $\text{OH}^\cdot$ , 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.

## 2 | MATERIAL AND METHODS

### 2.1 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  $0.02 \text{ mol L}^{-1}$  sodium tetraborate and  $0.1 \text{ mol L}^{-1}$  sodium nitrate (pH 9.2) (Karyakin et al. 1999). The monomer dissolved concentration in this solution was always  $0.76 \text{ mmol L}^{-1}$ .

### 2.2 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  $3.0 \text{ mol L}^{-1}$  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.

### 2.3 Preparation of PMB-modified Electrode

Prior to surface modification, the GC electrode was mechanically polished with  $0.5 \mu\text{m}$  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  $0.76 \text{ mmol L}^{-1}$  monomer, at a scan rate of  $0.05 \text{ V s}^{-1}$ . 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).

## 2.4 PMB Film Degradation by Fenton Attack

After the working electrode modification, a cyclic voltammogram was recorded in a 0.1 mol L<sup>-1</sup> sodium nitrate and 0.05 mol L<sup>-1</sup> acetate buffer solution (pH = 5.0), from -0.4 to +0.5 V at 0.05 V s<sup>-1</sup>. 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 H<sub>2</sub>O<sub>2</sub>, 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 Fe<sup>2+</sup>:H<sub>2</sub>O<sub>2</sub> was maintained at 1:1 (10 mmol L<sup>-1</sup>) 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.

## 2.5 Radical Scavenging Capacity

The radical scavenging capacity was achieved by plotting the logarithm of the normalized oxidation peak areas [ln (Q<sub>t</sub>/Q<sub>0</sub>)] 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  $k_{\text{sample}}$  and  $k_{\text{control}}$  are the kinetic constants obtained with and without ascorbic acid in the Fenton solution, respectively.

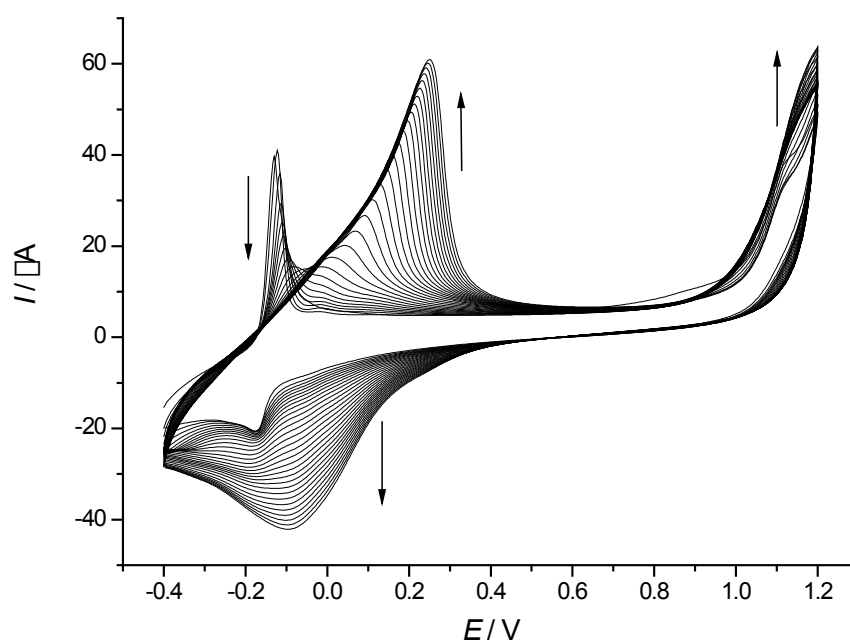
Blank experiments were also performed. PMB films were dipped in aqueous solutions of iron (II), H<sub>2</sub>O<sub>2</sub> and ascorbic acid in all the working concentration range for 5 minutes, without OH<sup>•</sup> generation.

### 3 | RESULTS AND DISCUSSION

#### 3.1 Electropolymerization of the MB and Electrochemical Characterization of the PMB-modified Electrode

The electropolymerization of the MB 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 1. 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 1. 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. 1993, Liu and Mu 1999).



**Figure 1:** Voltammetric profile of MB electropolymerization; [MB] = 0.76 mmol L<sup>-1</sup> in 0.02 mol L<sup>-1</sup> sodium tetraborate and 0.1 mol L<sup>-1</sup> NaNO<sub>3</sub> (pH 9.2); 30 cycles between 0.4 and +1.2 V at 0.05 V s<sup>-1</sup>.

The surface coverage of the PMB film on the GC electrode can be estimated from the surface coverage concentration ( $\Gamma$ ) of the polymer. The  $\Gamma$  value was calculated from the charge ( $Q$ ) associated with the electropolymerization process according to (Marinho et al. 2012, Yogeswaran and Chen 2008):

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

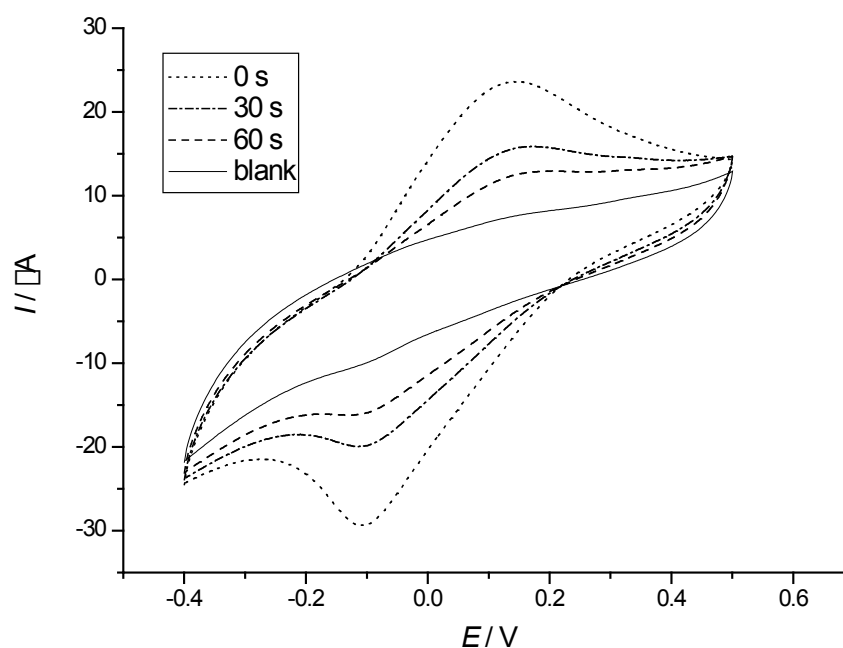
where  $A$  is the geometric area of the GCE ( $0.070 \text{ cm}^2$ ),  $n$  is the number of electrons involved in the redox process ( $n = 2$ ), and  $F$  is the Faraday constant ( $96,485 \text{ C mol}^{-1}$ ).

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

The obtained  $\Gamma$  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  $0.1 \text{ mol L}^{-1}$  sodium nitrate and  $0.05 \text{ mol L}^{-1}$  acetate buffer solution ( $\text{pH} = 5.0$ ), from  $-0.4$  to  $+0.5 \text{ V}$  at  $0.05 \text{ V s}^{-1}$ , 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  $\text{OH}^-$  and not by the dissolution of the polymeric film.

### 3.2 Quantification of the PMB Film Degradation by Fenton Attack

The effect of the electrode modification and the  $\text{OH}^-$  attack to the PMB film can be seen in Figure 2. Also, Figure 2 shows cyclic voltammograms of the PMB-modified electrode after 2 successive attacks of the polymeric film with  $\text{OH}^-$  produced in a Fenton solution for 30 s. As can be noticed, the electrochemical signal of the residual film decreases with extending the  $\text{OH}^-$  attack. Blank experiments showed that neither ascorbic acid, iron (II) or  $\text{H}_2\text{O}_2$  solutions were able to destroy the PMB film formed on GC electrode.

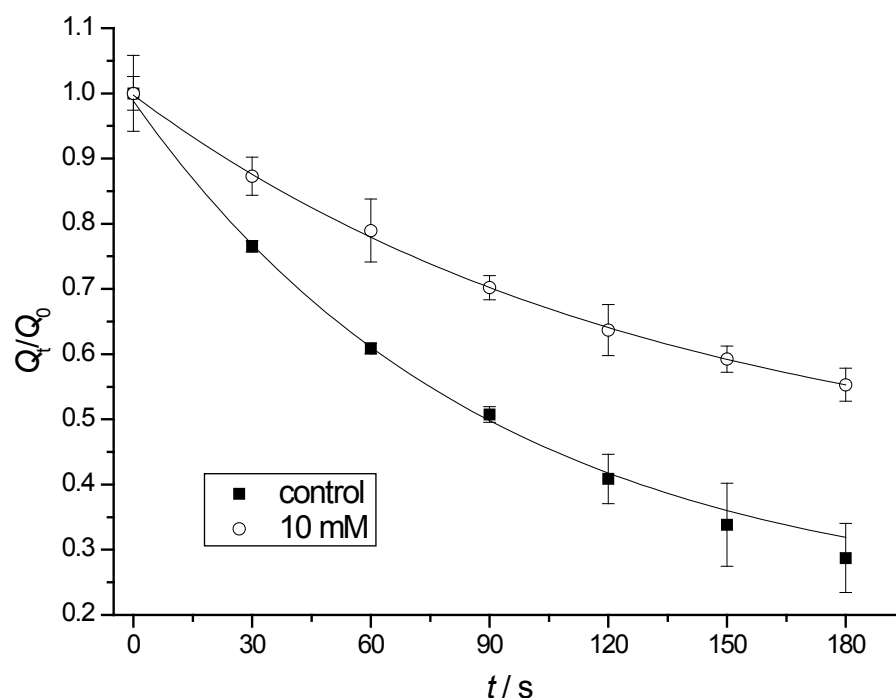


**Figure 2:** Cyclic voltammograms recorded at a PMB-modified electrode in 0.1 mol L<sup>-1</sup> NaNO<sub>3</sub> and 0.05 mol L<sup>-1</sup> acetate buffer solution (pH 5.0) before and after two successive Fenton attacks; scan rate = 0.05 V s<sup>-1</sup>.

In order to prove the applicability of the proposed modified electrode to sense OH<sup>-</sup> 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<sup>-</sup> (Gualandi and Tonelli 2013, Gualandi et al. 2014). Therefore, the addition of AA to the Fenton solution causes a decrease in OH<sup>-</sup> concentration. Such reduction is proportional to the amount of AA added. Now, since less OH<sup>-</sup> 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<sup>-</sup> radicals in the absence and in the presence of 10 mM ascorbic acid show a clear distinction between the two evaluated reaction conditions (Figure 3). 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<sup>-</sup> 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<sup>-</sup> to the PMB-film (3), and the other occurs when a radical scavenger (an antioxidant AH) is added to the Fenton solution (4):





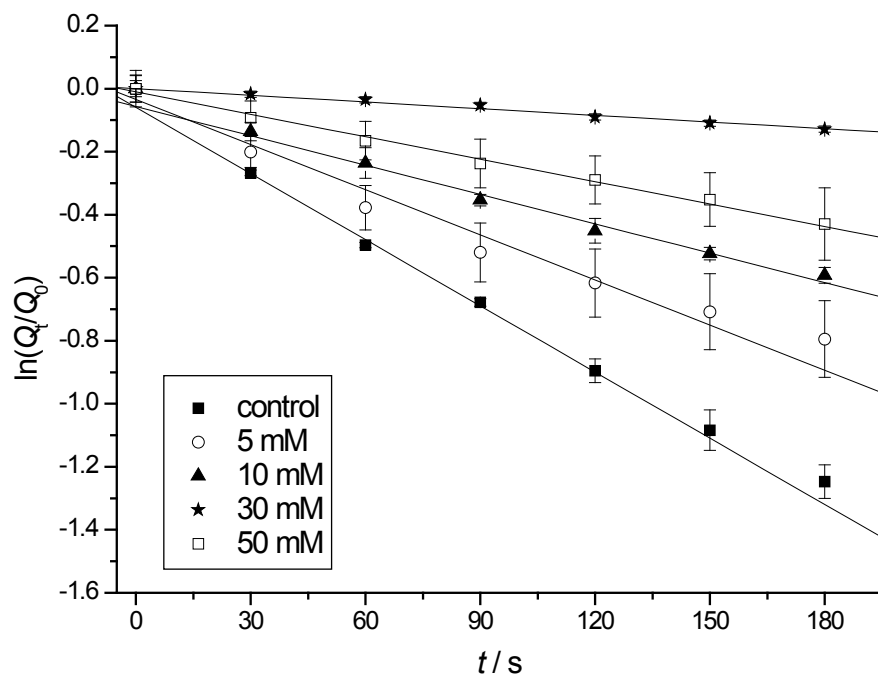
**Figure 3:** Exponential decay after  $\text{OH}^\bullet$  attack to the PMB-modified electrode in presence and absence of 10 mM ascorbic acid added to the Fenton solution. Error bars are related to triplicate experiments.

Since the  $\text{OH}^\bullet$  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  $\text{OH}^\bullet$  and the concentration of AH is sufficiently large, the observed rate constant  $k_{\text{obs}}$  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:

$$\ln(Q_t/Q_0) = -k_{\text{obs}} \cdot t \quad (6)$$

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



**Figure 4:** Logarithmic linearized plot of the variation of the anodic peak charge after  $\text{OH}^\cdot$  attack to the PMB-modified electrode *versus* time of reaction of the polymeric film with  $\text{OH}^\cdot$  radicals. Error bars are related to experiments in triplicate.

The slope of each straight line in Figure 4 corresponds to the observed kinetic constant, i.e., the reaction velocity between AH and  $\text{OH}^\cdot$ . 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  $\text{OH}^\cdot$  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.

### 3.3 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. Therefore, Table 1 presents also the kinetic constant observed for the reaction between PMB and  $\text{OH}^\cdot$  against AA concentration.

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

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

It can be observed that the minimum value for  $k_{\text{obs}}$  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<sup>•</sup> 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<sup>3+</sup> to Fe<sup>2+</sup>. These ferrous ions can promote further OH<sup>•</sup> formation via Fenton chemistry, if enough H<sub>2</sub>O<sub>2</sub> is still available. Thus, in these cases, low AA levels should be maintained. Such behavior could explain why a 100% OH radical scavenging was not reached in our previous work even when AA levels were systematically enhanced (Hilgemann et al. 2010). As aforementioned, the radical scavenging capacity is calculated based in a kinetic constant for a blank experiment ( $k_{\text{control}}$ ), where OH<sup>•</sup> is generated solely through the reaction between Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>. Therefore, the concentration of OH<sup>•</sup> 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<sup>•</sup> 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.

#### 4 | 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<sup>•</sup> 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<sup>•</sup> to biomolecules, such as proteins, vitamins and membranes.

## 5 | ACKNOWLEDGEMENTS

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