



Application of oxo-manganese complex immobilized on ion-exchange polymeric film as biomimetic sensor for nitrite ions



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ABSTRACT

A biomimetic sensor based on oxo-bridged dinuclear manganese–phenanthroline complex immobilized into an ion-exchange polymeric film deposited on glassy carbon electrode was applied to detection of nitrite ions and studied according to their kinetics parameters. The cyclic voltammetry at the modified electrode in universal buffer showed a two electron oxidation/reduction of the couple $\text{Mn}^{\text{IV}}(\mu\text{-O})_2\text{Mn}^{\text{IV}}/\text{Mn}^{\text{III}}(\mu\text{-O})_2\text{Mn}^{\text{III}}$ and electrocatalytic property toward nitrite oxidation with a decrease of the overpotential of 320 mV compared with the bare glassy carbon electrode. A plot of the anodic current vs. the nitrite ions concentration for potential fixed (+0.480) at the biomimetic sensor was linear in the 2.49×10^{-6} to 9.90×10^{-6} mol L⁻¹ concentration range with a detection limit of 6.50×10^{-6} mol L⁻¹. The kinetic mechanism was derived by Michaelis–Menten, then, kinetics parameters were calculated through four methods: Lineweaver–Burke, Eadie–Hofstee, Hanes–Woolf and Nonlinear curve fitting. The best results were Michaelis–Menten rate constant = $3.42 \mu\text{mol L}^{-1}$, catalytic rate constant = 0.0114 s^{-1} , catalytic efficiency = $3.3 \times 10^3 (\text{mol L})^{-1} \text{ s}^{-1}$ and heterogeneous electrochemical rate constant = $1.15 \times 10^{-5} \text{ cm s}^{-1}$.

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1. Introduction

Nitrite ions become important and extensively used because of their high reactivity and may act as an oxidizing, reducing or nitrosating agent, beyond the possibility of being converted to a variety of compounds, including nitrous acid, nitrogen oxides and nitrates, and the possibility to be used as a stabilizer and preservative in meats, canned foods, cheeses, among others food [1]. In contrast, there is evidence that ingestion of large amounts of nitrite may be linked to cancer of the stomach [2,3], bowel, leukemia and the appearance of brain tumors in children [4].

Then, due to its potential toxicity, a series of rules that restrict their level in drinking water and food products were implemented, such as, the World Health Organization (WHO/SDE/WSH/07.01/16) stipulates that maximum admissible levels of nitrite ions in drinking water at 3 ppm. In Brazil, the National Health Surveillance Agency (ANVISA) establishes for foods the maximum value of 0.015 g/100 g or 0.015 g/100 mL of nitrite ions (as sodium nitrite), as a stabilizer in color and conservative. In this way, different methods for detection and quantification of nitrite ions have been

developed, among them, electrophoresis [5,6], chemiluminescence [7], chromatography [8], colorimetry [9], fluorometry [10], gas chromatography–mass spectrometry [11], polarography [12], spectrophotometry [13–15], stopped-flow [16] and electrochemistry [17–19].

In the development of electrochemical sensors for nitrite ions highlights the use of biosensors and non-enzymatic sensors. For nitrite biosensing there are many reports using enzymes [20], as an amperometric biosensor for nitrite based on cytochrome *c* immobilized on a gold electrode that was modified with Nafion® and a Cu–Mg–Al layered double hydroxide, which exhibited bioelectrocatalytic activity for the oxidation of nitrite with a linear range from 0.75 to 123 $\mu\text{mol L}^{-1}$, detection limit of 0.20 $\mu\text{mol L}^{-1}$ and Michaelis–Menten constant of 0.08 mmol L⁻¹ [21]. In another way, several electrochemical sensors for nitrite ions based on nonenzymatic molecules have been developed (Table 1). Recently, Azad and Ganesan studied the electrocatalytic oxidation of nitrite ions based on $\text{Fe}(\text{bpy})_3^{2+}$ (where bpy = 2,2'-bipyridyl) immobilized on ion-exchanged polymer coated glassy carbon electrode with an efficient amperometric response for NO_2^- [22]. At the CG/Nf/ $\text{Fe}(\text{bpy})_3^{2+}$, the peak current increased linearly up with successive additions of nitrite from 0.2 to 20 mmol L⁻¹, reaching a detection limit of 0.03 mmol L⁻¹ and standard deviations less than 2.0% to six measurements of reproducibility.

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Table 1
A brief review of nonenzymatic sensors for nitrite ions.

Material	Ref.
Au nanoparticles/poly(3-methylthiophene)	[57]
Poly(1,3-diaminobenzene)	[58]
Poly(Nile-Blue)	[59]
Thionine/aligned carbon nanotubes	[60]
Au–Fe(III) nanoparticle	[44]
Chemically reduced graphene oxide	[61]
Poly(amidoamine)/carbon nanotubes	[62]
Poly(vanillin)/carbon nanotube	[63]
Oxovanadium(IV)-4-methyl salophen	[64]
Poly(diphenylamine)/Pt nanoparticles	[65]
Chitosan-carboxylated/multiwall carbon nanotube	[66]
Poly(ethylenimine)/graphene oxide/Au nanoparticles	[67]
Poly(vinylimidazole)	[68]
Tris(1,10-phenanthroline)iron(II)-bentonite	[69]
Fe ₂ O ₃ nanoparticles/reduced graphene oxide nanosheets	[70]
Titanium dioxide nanoparticles/ionic liquid	[71]

A novel advance in the construction of electrochemical sensors is the development and application of biomimetic elements [23], which imitate natural reactions and enzymatic processes, aiming to improve the chemical properties itself [24], beyond stability when compared to biosensors. A biomimetic sensor can be characterized as a device consisting of a recognition element closely linked to a transducer that has equal or greater sensitivity and stability as sensors which use biological components, such as antibodies and enzymes. Among these biomimetic molecules, several metallic complexes have been studied by mimic enzyme active sites, such as the polynuclear complexes of manganese with μ -oxo units, which are related to the oxygen-evolving complex (OEC) in photosystem II (PSII), by presenting electron-transfer process of multi-stage that result in good stability for high oxidation states and the ability to charge exchange between metal ions [25–27].

In this manuscript we studied application of the oxo-bridged dinuclear manganese–phenanthroline complex ($[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$) incorporated into a Nafion[®] film coated on the glassy carbon electrode as biomimetic sensor in the oxidation of nitrite ions and determined their kinetics parameters according to the Michaelis–Menten model.

2. Experimental

2.1. Reagents and solutions

All the chemicals were of analytical high-purity. A universal buffer solution (citric acid, monopotassium phosphate, boric acid and diethyl barbituric acid [28]) containing NaCl 0.5 mol L⁻¹ (pH = 5.00) was used as a supporting electrolyte, where the adjustment was made by adding HCl and/or NaOH concentrated. The analyte was prepared by the addition of sodium nitrite (1.0 mmol L⁻¹) to the solution of supporting electrolyte. The Nafion[®] solutions (tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid copolymer) used for the formation of ion-exchange polymeric film on the electrode surface, were prepared from the concentrated solution of the perfluorosulfonate polymer 5% (v/v) diluted in absolute ethanol.

2.2. Synthesis of biomimetic complex

The immobilization of the oxo-bridged dinuclear manganese–phenanthroline complex on Nafion[®] thin film was performed starting from the $[\text{Mn}^{\text{IV}}_3\text{O}_4(\text{phen})_4(\text{H}_2\text{O})_2]^{4+}$ complex. The synthesis of $[\text{Mn}_3\text{O}_4(\text{phen})_4(\text{H}_2\text{O})_2](\text{NO}_3)_4$ complex was performed according to procedure found in the literature [29]. It was added 10.16 mmol of the ligand phenanthroline to a solution

of 4.94 mmol of $\text{Mn}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ in dilute nitric acid (1.6 mol L⁻¹, 20 mL) and aqueous solution of $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (6.02 mmol), obtain a brown solution, which was filtered and kept at room temperature. The precipitate formed was filtered, washed with dilute nitric acid (0.2 mol L⁻¹) and dried at room temperature. The electronic absorption spectrum of complex in aqueous solution showed two maxima peaks in 623 nm and 784 nm (Supplementary material). The complex also characterized by cyclic voltammetry in aqueous solution showed three redox couples and an irreversible cathodic process (Supplementary material).

2.3. Construction of $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion[®]/GC modified electrode

The $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion[®]/GC modified electrode was constructed by depositing a rate of 5.0 μL of solution of Nafion[®] 5% (v/v) on the electrode surface by the casting method. Next, it was left on desiccators for 4 h to complete solvent evaporation. Thereafter, successive drops of 1.0 mmol L⁻¹ $[\text{Mn}_3\text{O}_4(\text{phen})_4(\text{H}_2\text{O})_2]^{4+}$ complex in NaNO₃ 0.5 mol L⁻¹ were added to the electrode surface coated with the polymeric membrane, for 4 h for immobilization of electroactive material, and finally, the modified electrode was washed with deionized water, dried at room temperature and submitted to electrochemical measurements.

2.4. Performance electrochemical

The cyclic voltammetry measurements were made using a potentiostat/galvanostat microAutolab Type III (Eco Chimie) connected to a microcomputer, in a thermostated electrochemical cell with three electrodes, being the platinum wire (Pt) as auxiliary electrode, saturated calomel electrode (SCE) as reference electrode and modified glassy carbon electrode ($[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion[®]/GC) as working electrode (area = 0.06 cm²). Before use, the working electrode was subjected to a polishing with alumina solution (0.5 mmol L⁻¹) and then with ethanol. All the electrochemical measurements were realized in the absence of dissolved oxygen.

2.5. Performance of the modified electrode as biomimetic sensor for nitrite ions

The nitrite ions determination was made by application of potential *via* linear voltammetry using a potential range from -0.9 to 0.9 V vs. SCE at a scan rate of 25 mV s⁻¹. The $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion[®]/GC was submitted to potential scans in 20 mL of universal buffer with 0.5 mol L⁻¹ NaCl at pH 5.0 (deoxygenated with high purity nitrogen). The evaluation of the electrochemical performance of sensor for nitrite ions was conducted by the addition of the analyte (1.0 mmol L⁻¹) in the electrochemical cell. All the electrochemical measurements of nitrite detection were realized in the absence of dissolved oxygen, because its presence may limit the determination of NO₂⁻ [30,31].

3. Results and discussion

3.1. Basic characteristics of $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion[®]/GC modified electrode

The electrochemical behavior of the oxo-bridged binuclear manganese–phenanthroline complex incorporated into tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid thin film coated on a glassy carbon electrode was studied by cyclic voltammetry in universal buffer (pH = 5.0) containing NaCl 0.5 mol L⁻¹. Cyclic voltammogram obtained for the modified

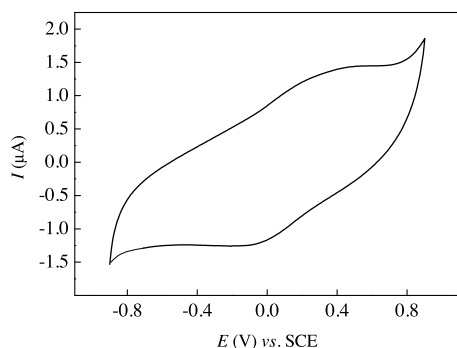
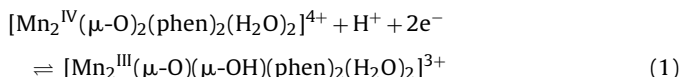


Fig. 1. Cyclic voltammogram for $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ electrode in universal buffer containing $\text{NaCl } 0.5 \text{ mol L}^{-1}$ solution ($\text{pH} = 5.0$) from -0.9 to 0.9 V vs. SCE potential range at 25 mV s^{-1} .

electrode (Fig. 1) revealed a quasi-reversible system with only one redox couple ($E_{\text{pa}} = 0.48 \text{ V}$ and $E_{\text{pc}} = -0.15 \text{ V}$ vs. SCE) which is attributed to the redox process of $\text{Mn}^{\text{IV}}/\text{Mn}^{\text{III}}$, peak-to-peak separation (ΔE_p) of 0.630 V vs. SCE and half potential ($E_{\text{p}/2}$) of 0.165 V vs. SCE.

The complex immobilization on the polymeric membrane of tetrafluoroethylene-perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid causes a change in the molecular structure and the number of metallic centers of the complex. This change has been widely discussed by the authors previously [32]. In this way, the electrochemical behavior observed is assigned only to the material $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ on the surface of the glassy carbon electrode, according to Scheme 1:



The effect of the potential scan rates (5 to 200 mV s^{-1}) on the voltammetric response for the modified electrode in universal buffer ($\text{pH } 5.0$) solution 0.1 mol L^{-1} containing $\text{NaCl } 0.5 \text{ mol L}^{-1}$ was investigated. The recorded cyclic voltammograms exhibit an increase of anodic peak current as the scan rate increases and a linearity between the anodic peak current with the square root of scan rates, suggesting that the redox process follows a diffusion-controlled mechanism (Supplementary material). This behavior indicates which the mobility of counter ions of the supporting electrolyte is required to maintain the electroneutrality of the electrode surface during the redox processes [33,34]. Thus, the surface concentration of electroactive species was estimated by background-corrected electric charge (Q) obtained in the anodic peaks process according with the theoretical relationship [35]:

$$\Gamma = \frac{Q}{nFA} \quad (2)$$

where Γ is the concentration of electroactive species (mol cm^{-2}), the number of electrons transferred (assuming ≈ 2), F the Faraday constant ($96,485 \text{ C/mole}^{-1}$) and A is the electrode area (0.06 cm^2). The Γ value was calculated for 5 mV s^{-1} under the surface adsorption-controlled electrochemical process [36–41] and the concentration of electroactive species was found to be $3.45 \text{ nmol cm}^{-2}$.

3.2. Application of $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ as biomimetic sensor for nitrite ions

The determination of nitrite ions in conventional electrodes is generally difficult, because they occur at high values of potential [42]. Recently, Kozub and collaborators reported the oxidation of nitrite ions in aqueous solution using a bare glassy carbon electrode

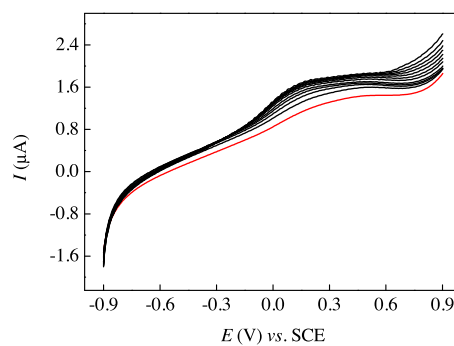
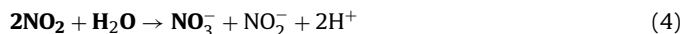


Fig. 2. Linear voltammograms for $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ electrode in universal buffer containing $\text{NaCl } 0.5 \text{ mol L}^{-1}$ solution ($\text{pH} = 5.0$) in absence (red line) and in presence of 2.49×10^{-6} ; 3.98×10^{-6} ; 5.47×10^{-6} ; 7.44×10^{-6} ; 9.90×10^{-6} ; 1.48×10^{-5} ; 2.44×10^{-5} ; 3.85×10^{-5} ; 5.21×10^{-5} ; $6.98 \times 10^{-5} \text{ mol L}^{-1}$ of nitrite ions (black lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

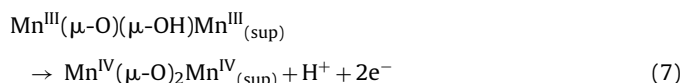
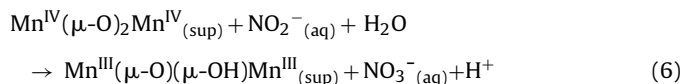
by three different techniques [43]. Using cyclic voltammetry, the anodic peak for nitrite oxidation increased at $+0.8 \text{ V}$ vs. SCE in linear range from 5.0×10^{-5} to $4.7 \times 10^{-4} \text{ mol L}^{-1}$ with a limit of detection 0.2 mmol L^{-1} and sensitivity of $3.3 \times 10^{-2} \text{ A L mol}^{-1}$. On the other hand, analyzing modified electrodes, a significant decrease in the values of the electrooxidation potential can be obtained, such as the Au-Fe(III) nanoparticle modified glassy carbon electrode developed by Liu et al. [44], which demonstrated the electrocatalytic oxidation of nitrite at $+0.73 \text{ V}$ vs. SCE (decrease of the overpotential of 130 mV compared to their unmodified electrode) with concentration range of 3.0×10^{-7} to $1.5 \times 10^{-4} \text{ mol L}^{-1}$ and detection limit of $2.0 \times 10^{-7} \text{ mol L}^{-1}$ in weak acid solution.

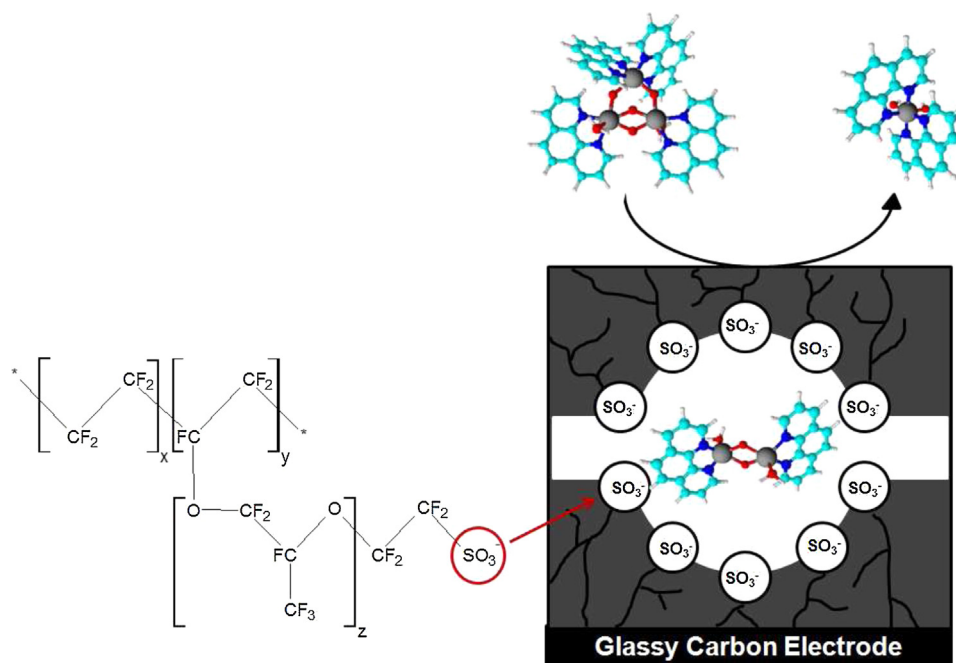
In the present paper, the cyclic voltammograms were obtained in the absence and in the presence of nitrite in universal buffer containing $\text{NaCl } 0.5 \text{ mol L}^{-1}$ ($\text{pH} = 5.0$), as showed in Fig. 2. The anodic peak current of the $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ electrode increase significantly with the addition of nitrite ions in solution, as well as a decrease in the overpotential of 320 mV compared with unmodified glassy carbon electrode [43].

The results obtained for the modified electrode show its electrocatalytic activity for oxidation of nitrite and its biomimetic properties. The mechanism of nitrite ions electrooxidation has been reported by Guidelli et al. [45]. According to this mechanism proposed, the nitrite ions are electrooxidized to nitrogen dioxide, followed by a disproportionation into nitrite and nitrate, thus regenerating the depolarizer, which constitutes the rate-determining step of the overall electrode process, as represented:



In this way, the oxidation mechanism at $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ occurs through the diffusion of the NO_2^- ions to the electrode surface and reduces the Mn^{IV} complex immobilized on the surface of glassy carbon (chemical step, Eq. (6)), followed by one electrooxidation process (electrochemical step, Eq. (7)):





Scheme 1. Formation of the oxo-bridged dinuclear manganese-phenanthroline complex on Nafion® polymeric membrane.

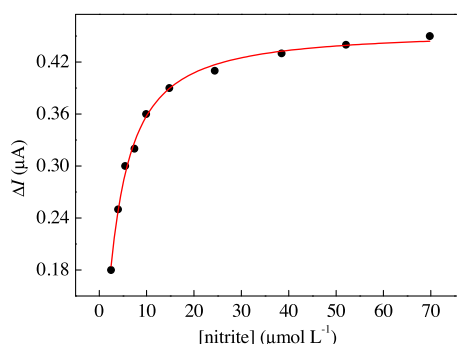


Fig. 3. Relationship between the current response of the biomimetic sensor with the nitrite ions concentration. Working potential = 0.480 V vs. SCE.

Successive additions of NO_2^- ions were made to evaluate the performance of the modified electrode as a sensor. The values of anodic peak current vary linearly in a concentration range from $2.49 \times 10^{-6} \text{ mol L}^{-1}$ to $9.09 \times 10^{-6} \text{ mol L}^{-1}$ with a detection limit of $6.50 \times 10^{-6} \text{ mol L}^{-1}$ and sensibility of $0.0228 \mu\text{A L mol}^{-1}$ (Fig. 3). At concentrations greater than $1.00 \times 10^{-5} \text{ mol L}^{-1}$, a saturation of the electrocatalytic sites was observed. This phenomenon can be explained by the adsorption of nitrite ions and subsequent saturation of the electroactive sites of Mn^{IV} on the electrode surface.

The detection performances of the $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion®/GC sensor in the oxidation of nitrite ions were compared with biosensors and non-enzymatic sensors in the literature (Table 2). It's important to emphasize that most work related to detection of nitrite ions by electrochemical (bio)sensors are based on its reduction [20], unlike the mechanism presented here. According to Table 2, it can be seen that the glassy carbon electrode coated with Nafion® film incorporated with the oxo-manganese-phenanthroline complex showed a detection limit lower than previously reported studies, proving to be a good device for the detection of nitrite ions and a potential sensor for monitoring of the concentration of nitrite ions in real samples.

The biomimetic property of the $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}$ /Nafion®/GC electrode can be observed through of the profile of anodic current vs. NO_2^- concentration, which exhibit similar characteristic to the Michaelis–Menten model of an enzymatic electrode. Therefore, the Michaelis–Menten equation applied to NO_2^- for the steady-state of catalytic current i_{SSC} can be expressed as [46–48]:

$$i_{\text{SSC}} = \frac{nFA\Gamma k_{\text{cat}}[\text{NO}_2^-]}{K_{\text{M}}^{\text{app}} + [\text{NO}_2^-]} = \frac{i_{\text{max}}[\text{NO}_2^-]}{K_{\text{M}}^{\text{app}} + [\text{NO}_2^-]} \quad (8)$$

where n is the number of electrons involved in the catalytic reaction, $K_{\text{M}}^{\text{app}}$ is the apparent Michaelis–Menten constant, A (cm^2) is the electrode area, Γ (mol cm^{-2}) is the electroactive concentration on the electrode surface, k_{cat} is the catalytic rate constant, i_{max} is the maximum catalytic current ($i_{\text{max}} = nFA\Gamma k_{\text{cat}}$) and $[\text{NO}_2^-]$ is the concentration of nitrite ions. Thus, the kinetic parameters were obtained from four ways through of rearrangement of the Michaelis–Menten equation:

By Lineweaver–Burke (LB) expression:

$$\frac{1}{i_{\text{pa}}} = \frac{K_{\text{M}}^{\text{app}}}{nFA\Gamma k_{\text{cat}}[\text{NO}_2^-]} + \frac{1}{nFA\Gamma k_{\text{cat}}} = \frac{S_{\text{LB}}}{\text{NO}_2^-} + I_{\text{LB}} \quad (9)$$

$$I_{\text{LB}} = \frac{1}{nFA\Gamma k_{\text{cat}}} \quad (10)$$

$$S_{\text{LB}} = \frac{K_{\text{M}}^{\text{app}}}{nFA\Gamma k_{\text{cat}}} \quad (11)$$

According to Eadie–Hofstee (EH):

$$\frac{i_{\text{pa}}}{[\text{NO}_2^-]} = \frac{nFA\Gamma k_{\text{cat}}}{K_{\text{M}}^{\text{app}}} - \frac{i_{\text{pa}}}{K_{\text{M}}^{\text{app}}} = I_{\text{EH}} + S_{\text{EH}}i_{\text{pa}} \quad (12)$$

$$S_{\text{EH}} = -\frac{1}{K_{\text{M}}^{\text{app}}} \quad (13)$$

$$I_{\text{EH}} = \frac{nFA\Gamma k_{\text{cat}}}{K_{\text{M}}^{\text{app}}} \quad (14)$$

Table 2
Analytical features of different sensors for nitrite determination.

Material	E_{pa}	L.D.	Ref.
Ni(II)TSPc film/GCE	0.94 V vs. Ag/AgCl	0.149 mM	[72]
ND	1.05 V vs. SCE	0.120 mM	[73]
Fe(bpy) ₃ ²⁺ /Nafion®/GCE	1.19 V vs. Ag/AgCl	0.030 mM	[22]
rGO-MWNTs/GCE	0.80 V vs. SCE	25.0 μmol L ⁻¹	[74]
[Mn ₂ ^{IV} O ₂ (phen) ₂ (H ₂ O) ₂] ⁴⁺ /Nafion®/GCE	0.48 V vs. SCE	6.50 μmol L ⁻¹	This work

E_{pa} = Oxidation potential peak of NO₂⁻; L.D. = limit of detection.

Also by Hanes–Woolf (HW):

$$\frac{[NO_2^-]}{i_{pa}} = \frac{[NO_2^-]}{nFA\Gamma k_{cat}} + \frac{K_M^{app}}{nFA\Gamma k_{cat}} = S_{HW} + [NO_2^-] + I_{HW} \quad (15)$$

$$S_{HW} = \frac{1}{nFA\Gamma k_{cat}} \quad (16)$$

$$I_{HW} = \frac{K_M^{app}}{nFA\Gamma k_{cat}} \quad (17)$$

and finally, through the curve fitting analysis using nonlinear least square regression program based on the Marquardt–Levenberg algorithm [49]. The incognita S and I denote the slope and intercept of the linearized equations, respectively. Still evaluating the kinetics parameters, the value of the specificity constant (which here we call of k_s) [50] and heterogeneous constant (k'_e) were calculated by Eqs. (15) and (16), respectively, for each method.

$$k_s = \frac{k_{cat}}{K_M^{app}} \quad (18)$$

$$k'_e = \frac{k_{cat}\Gamma}{K_M^{app}} \quad (19)$$

The I_{pa}^{-1} vs. $[NO_2^-]^{-1}$ for Lineweaver–Burke, $I_{pa}/[NO_2^-]$ vs. I_{pa} for Eadie–Hofstee and $[NO_2^-]/I_{pa}$ vs. $[NO_2^-]$ for Hanes–Woolf plots are presented in Fig. 4 and the kinetic results obtained are listed in Table 3.

As can be seen, the Michaelis–Menten kinetic parameters that calculated from four different methods are all in the same order. The Michaelis–Menten constant is related with the affinity of the enzyme (for biosensors) or of the biomimetic complex (as it is our case) for substrate, as well as its concentration required for effective catalysis occurs. Analyzing this parameter, the results reveal a slight difference ($\Delta = 0.66$) between the methods used and low values of Michaelis–Menten constant (order of μmol L⁻¹), which indicate that the [Mn₂^{IV}O₂(phen)₂(H₂O)₂]⁴⁺ complex immobilized on a Nafion® polymeric film had a high affinity to NO₂⁻ ions. Inclusive, the biomimetic sensor showed better affinity and K_M than the nitrite oxidase enzyme from *Nitrobacter agilis* [51], as also the biosensor which uses cytochrome *c* as a biological material [21]. The catalytic constant gives a direct measure of the catalytic production of product under optimum conditions (for saturated enzyme). Examining the [Mn₂^{IV}O₂(phen)₂(H₂O)₂]⁴⁺/Nafion®/GC, the k_{cat} can be thought of as the time required by a biomimetic complex to turn over one substrate molecule. In this way, the k_{cat} values obtained showed a great catalytic production in extremely fast times. The ratio k_{cat}/K_M^{app} could be used to analyze the relative rates of reaction of the substrate, when it is catalytically transformed by a material electroactive. This is because, if K_M^{app} is used on its own as the indicator of specificity, the effect of the substrate will be strongly manifested mainly at values of $[S]/K_M \ll 1$. As $[S]/K_M^{app}$ increases above this value, k_{cat} becomes the parameter that best describes the better catalytic efficiency of substrate [52]. In this manner, this dichotomy is resolved by using k_{cat}/K_M^{app} as specificity constant. Finally, the results indicate that the electrocatalytic mechanism for the biomimetic sensor is dependent on the concentration of

NO₂⁻ ions adsorbed on the electrode surface, enabling the calculation of heterogeneous constant, which are comparable to a system with an electron mediation mechanism [53–55].

Another parameter investigated was the reaction order for the oxidation of nitrite ions. Through the logarithm of the anodic current vs. the logarithm of the nitrite concentration (Supplementary material) at a constant potential there was obtained a slope of 0.5. The reaction order of 0.5 is a reaction with the rate-determining step occurs twice [56]. This implies that an intermediate species should be formed twice in one or two

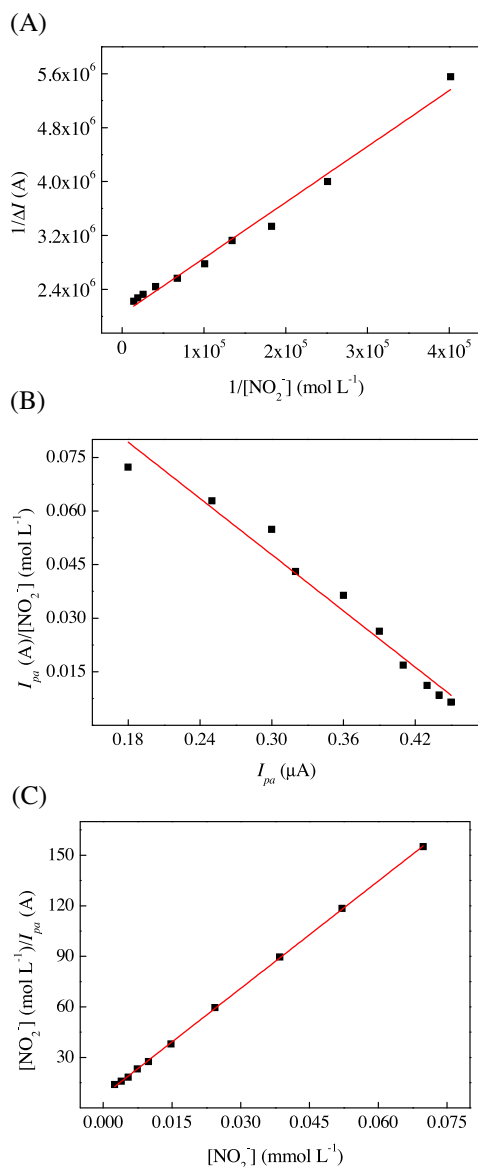


Fig. 4. Michaelis–Menten analysis in terms of Lineweaver–Burke (A), Eadie–Hofstee (B) and Hanes–Woolf (C) plots based on the data of Fig. 3.

Table 3
Kinetics parameters of Michaelis–Menten applied to the biomimetic sensor.

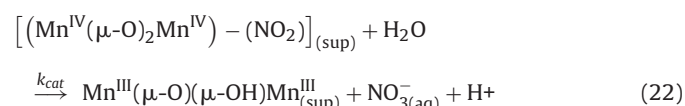
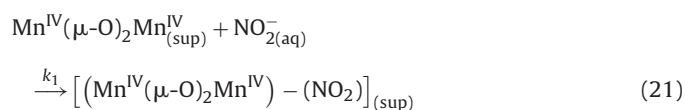
Method calculated	K_M^{app}	k_{cat}	k_s	k'_e
LB plot analysis	4.08	0.0123	3.02	1.04
EH plot analysis	3.81	0.0121	3.17	1.09
HW plot analysis	3.45	0.0118	3.42	1.18
Nonlinear curve fitting analysis	3.42	0.0114	3.33	1.15

LB = Lineweaver–Burke; EH = Eadie–Hofstee; HW = Hanes–Woolf; $K_M = \mu\text{mol L}^{-1}$; $k_{\text{cat}} = \text{s}^{-1}$; $k_s (10^3) = (\text{mol L})^{-1} \text{s}^{-1}$; $k'_e (10^{-5}) = \text{cm s}^{-1}$.

elementary chemical steps preceding the rate-determining step. On the basis of these results, the kinetic of nitrite oxidation on $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ sensor can be described as:

$$i_k = \frac{nFAk_{\text{obs}}\Gamma[\text{NO}_2^-]^{0.5}}{K_M^{\text{app}} + [\text{NO}_2^-]^{0.5}} \quad (20)$$

In this way, an alternative mechanism can be represented as:



where $[(\text{Mn}^{\text{IV}}(\mu\text{-O})_2\text{Mn}^{\text{IV}}) - (\text{NO}_2)]_{(\text{sup})}$ refers to the formation of an adduct between the metallic centers of the complex and the nitrite molecule.

4. Conclusion

This work described a biomimetic sensor for nitrite ions based on oxo-manganese complex immobilized on Nafion[®] ion-exchange polymeric film coated glassy carbon electrode. The $[\text{Mn}_2^{\text{IV}}\text{O}_2(\text{phen})_2(\text{H}_2\text{O})_2]^{4+}/\text{Nafion}^{\text{®}}/\text{GC}$ exhibited electrocatalytic activity in the nitrite oxidation and biomimetic properties, allowing the study of the kinetic parameters of Michaelis–Menten in different methods. Furthermore, the biomimetic sensor showed low detection, high sensibility, affinity and catalytic production in fast times for nitrite ions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2015.01.021>.

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