Full Paper Wiley Online Library ELECTROANALYSIS

DOI: 10.1002/elan.201300468

Electrochemical Detection of Nitrite in Meat and Water Samples Using a Mesoporous Carbon Ceramic SiO_2/C Electrode Modified with In Situ Generated Manganese(II) Phthalocyanine

Abdur Rahim,[a] Lucas S. S. Santos,[a] Sergio B. A. Barros,[a] Lauro T. Kubota,[a] Richard Landers,[b] and Yoshitaka Gushikem*[a]

Abstract: Mesoporous carbon ceramic SiO_2/50 wt % C ($S_{BET} = 170\ m^2 g^{-1}$), where C is graphite, were prepared by the sol-gel method. The materials were characterized using N_2 sorption isotherms, scanning electron microscopy, and conductivity measurements. The matrix was used as support for the in situ immobilization of Mn(II) phthalocyanine (MnPc) on their surface. XPS was used to determine the Mn/Si atomic ratios of the MnPc-modified materials. Pressed disk electrodes were prepared with the MnPc-modified matrix, and tested as an electrochemical sensor for nitrite oxidation. The linear response range, sensitivity, detection limit and quantification limit were 0.79–15.74 $\mu mol\ L^{-1}$, 17.31 $\mu A\ L\ \mu mol^{-1}$, 0.02 $\mu mol\ L^{-1}$ and 0.79 $\mu mol\ L^{-1}$, respectively, obtained using cyclic voltammetry. The repeatability of the proposed sensor, evaluated in terms of relative standard deviation was 1.7 % for 10 measurements of a solution of 12.63 $\mu mol\ L^{-1}$ nitrite. The sensor employed to determine nitrite in sausage meat, river and lake water samples showed to be a promising tool for this purpose.

Keywords: Carbon ceramic material · Sol-gel · Mn(II) phthalocyanine · Nitrite oxidation · Electrochemical sensor

1 Introduction

Nitrite is ubiquitous within natural environment and food because it is commonly used as food preservative [1]. Nitrite ions can have detrimental effects via two mechanisms. They can combine with blood pigments producing methahemoglobin in which oxygen is no longer available to the tissues. In addition, they may interact in the stomach with amines and amides forming highly carcinogenic N-nitrosamine compounds [2]. Because of its potential toxicity, its determination is important for public health and environmental security. Therefore, it is essential to develop a reliable and sensitive sensor to detect nitrite in food, drinking water and environmental samples. Several techniques, including spectrophotometry [3], chemiluminescence [4], chromatography [5] and capillary zone electrophoresis [6] have been developed. However, it must be pointed out that most of the above methods involve time-consuming, expensive instrumentation, and tedious procedures. Owing to the rapid response, cheaper, safer and simple use, electrochemical methods have often been employed for the detection of nitrite [7–11].

The electrochemical oxidation of nitrite usually involves a large overpotential at the surfaces of the unmodified carbon electrodes, and therefore, the determination of nitrite tends to suffering interferences from other more oxidizable compounds. To overcome these problems, some electrochemically modified electrodes based on porphyrin [12], Pt nanoparticles [13], Nafion/lead-ruthenatepyrochlore [14] and metallophthalocyanine [15] have been explored to diminish the operating potentials for nitrite oxidation.

Owing to their interesting redox properties, the transition metal phthalocyanine (MPc) complexes have been used as sensing materials for the detection of some biologically and environmentally important compounds, such as H_2O_2 [16], dissolved O_2 [17,18], oxalic acid [19], thiols [20,21], dopamine [22], thiocyanate [23] and epinephrine [24].

The important features of carbon ceramic electrodes (CCE) include physical rigidity and tunable porosity that lead to significant advantages in the design and development of electrochemical sensors [25,26]. In terms of electrode design, sol-gel approach distinguishes from other methods especially by its simplicity and versatility, for example, either external surface or bulk-modified CCE can be prepared in different dimensions and geometrical configurations (like monolithic rods, disks, micro electrodes). Moreover, the active section area of a bulk-modified electrode can be refreshed by a simple polishing step extending the useful life of the electrode [27,28].

---

[a] A. Rahim, L. S. S. Santos, S. B. A. Barros, L. T. Kubota, Y. Gushikem
Institute of Chemistry, State University of Campinas, P. O. Box 6154, 13084-971 Campinas, SP, Brazil
tel: +55 19 3521 3053; fax: +55 19 3521 3023
*e-mail: gushikem@iqm.unicamp.br

[b] R. Landers
Institute of Physics Gleb Wataghin, State University of Campinas, 13083-859 Campinas, SP, Brazil

The silica backbone, itself is electrochemically inactive, not only provides rigidity to the material, but the electroactive species can attach through physical entrapment or covalent and non-covalent binding, wherein the charge transfer occurs preferably by electron hopping [29,30].

In this study, a new CCE based on SiO_2/C-graphite was employed as an electrically conductive support to the in situ generation of manganese(II) phthalocyanine (MnPc) in the pores of a matrix SiO_2/C. The material obtained, SiO_2/C/MnPc, pressed in a disk format was used to fabricate an electrode, whose potential usefulness was tested by the electrochemical oxidation of nitrite.

2 Experimental

2.1 Synthesis of SiO_2/C by Sol-Gel

The material SiO_2/C was prepared following a procedure described elsewhere [19]. The SiO_2/C 50 % composite material was prepared by the sol-gel method: initially, tetraethylorthosilicate (TEOS, Sigma-Aldrich 99 %) was pre-hydrolyzed in an ethanol solution catalyzed by HNO_3 at 70 °C for 3 hours. After that, graphite (Aldrich 99.99 %, $S_{BET} = 9\ m^2\,g^{-1}$), deionized water and HF (catalyst) were added to the pre-hydrolyzed TEOS solution. The mixture was sonicated until gelation of the material and then, allowed to rest. The obtained xerogel was dried at room temperature, ground to fine powder and washed thoroughly, initially with deionized water and then with ethanol in a soxhlet extractor for 3 hours. Finally, the product was dried under vacuum at 393 K for 4 hours.

2.2 In Situ Synthesis of Mn(II) Phthalocyanine in the Pores of the Matrix

MnPc was generated in situ on the SiO_2/C powder matrix as described recently [19]. In brief, 1.0 g of SiO_2/C was immersed in 10 mL of 0.01 $mol\,L^{-1}$ manganese acetate solution and the mixture was heated in a water bath at 343 K until complete evaporation of the solvent. The dry solid SiO_2/C/Mn(II), was mixed with 0.22 g of phthalonitrile (Fluka 98 %) and heated in sealed ampoule at 493 K for 3 h to form the MnPc complex. The resulting material was washed in a soxhlet extractor with ethanol for 2.5 h to remove excess of manganese phthalocyanine and unreacted phthalonitrile. Then the solid was heated at 398 K under vacuum to evaporate all the remaining solvent in the material SiO_2/C/MnPc.

2.3 Fabrication of the Electrode

The working electrode, a disk with 0.5 cm diameter and an approximately ~0.01 cm thickness, was prepared by pressing 25 mg of SiO_2/C/MnPc under 3 tons of pressure. The disk was immersed in pure fused paraffin at 343 K under vacuum (10^{-3} Torr pressure), until all adsorbed gas in the matrix pores was eliminated. The resulting self-supported disk was polished with emery paper to remove the paraffin from the disk surface, and then glued to a glass tube with gel glue and was maintained in a vertical position facing downward and allowed to air-dry at room temperature for 24 h. The electrical contact was made by a copper wire inserted inside the glass tube. In order to improve the connection between the wire and the disk surface, pure graphite powder was added to the glass tube.

2.3 Characterization of the Materials

a) Specific surface area (S_{BET}) was determined by the BET multipoint technique and the average pore diameters were obtained from the sorption–desorption isotherms by the BJH method. The measurements were carried outwithan Autosorb 1 Quantachrome instrument with the materials previously degassed at 423 K for 19 h.

b) X-ray photoelectron spectra (XPS) were obtained from powdered samples pressed into a thin film pellet and fixed on a stainless steel holder with conducting double-faced adhesive tape. A VSW HA 100 hemispherical electron analyzer and an Al anode X-ray source were used to record the spectra. The analyzer was used in the fixed transmission mode with 44 eV pass energy and the X-ray source was operated at 12 keV and 15 mA. The binding energies were referenced to the Si2p line from silica, set at 103.4 eV. Prior to decomposing the spectra using Gaussian, a Shirley background was subtracted [31].

c) The electrical resistance (R) of the samples was obtained using the four points probe method on a National Instruments NI PXI-1033 equipment. Measurements were preformed for a disk of finely powdered SiO_2/C with 0.5 cm diameter and approximately 0.01 cm thickness (w) pressed under a pressure of 4.5 tons. The conductivities (σ) were calculated by applying the Equation 1.

$$\sigma = (RwF_2F_4)^{-1} \tag{1}$$

where R is the electrical resistance, w is the thickness of the disk, and F_2 and F_4 are correction factors taken from the literature as 0.50 and 0.98, respectively [32].

d) Diffuse reflectance spectra (UV–vis DRS) of (SiO_2/C/MnPc), plotted as the Kubelka–Munk function $F(R\infty)$, was recorded on a CARY 5G UV–Vis spectrophotometer. Barium sulfate was used as a white reference sample.

e) Electrochemical measurements were performed using a PGSTAT-20 Autolab potentiostat. All the experiments were carried out in a conventional three-electrode system, which was composed of a working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE). Measurements were carried out in an electrochemical cell containing 25 mL of 1 $mol\,L^{-1}$KCl supporting electrolyte solution, at pH 4. Adjustment of the pH of the electrolyte solution was carried out 0.1 $mol\,L^{-1}$ phosphate buffer of pH 4.

2.4 Assay of the Nitrite Content in Food Samples

Samples of sausage were purchased at local stores. The pretreatment was performed as follows: first, 5 g of the sausage sample was crushed into mash and mixed with 12.5 mL saturated borax solution. Then, 300 mL of 70 °C water were added and the mixture was heated at boiling for 15 min. To precipitate the proteins, 5 mL of 20 % zinc acetate was added. After being cooled to room temperature, the mixture was diluted to 500 mL with water and then filtered. The resulting sample solution was stored at 4 °C in a refrigerator [33]. The nitrite content in samples was determined according to the standard addition method. Standard nitrite solutions were added after measurement of the sample solution. Thus, the concentration of nitrite in the real sample could be determined by cyclic voltammetry.

3 Results and Discussion

3.1 Characterization of the SiO_2/C-Graphite Matrix

The N_2 adsorption-desorption isotherm of the matrix SiO_2/C (data not shown) exhibit the presence of hysteresis, typical of mesoporous materials. The material shows a mesopore distribution region (data not shown) with maximum at 15.8 nm diameter, according to the IUPAC classification [34]. The specific surface area, S_{BET}, and the pore volume, p_v, obtained from the isotherms of the material used to fabricate the electrode are: $S_{BET}=170\ m^2 g^{-1}$ and $p_v=0.90\ cm^3 g^{-1}$.

The conductivity obtained for SiO_2/C was $0.4\ S cm^{-1}$. The good conductivity of the matrix is provided both by the larger amount of graphite and by the well dispersed and interconnected graphite particles in the silica network. It is an important parameter for electroanalytical applications.

3.2 In Situ Synthesis of Manganese Phthalocyanine (MnPc)

The in situ synthesis of MnPc can be described by two reaction steps [19]. In the first step, Manganese acetate reacts with Brønsted acid –OH groups present on the silica surface (Equation 2), making the metal chemically adsorbed on the silica surface by a reaction with the formation of a Si–O–Mn bond. In the second step the adsorbed Mn(II) served as the template for phthalocyanine complex formation inside the silica pores (Equation 3).

$$\equiv \text{Si–OH} + \text{Mn(AcO)}_2 \rightarrow (\equiv \text{SiO})_2\text{Mn} + 2\text{HOAc} \qquad (2)$$

$$(\equiv \text{SiO})_2\text{Mn} + \text{phthalonitrile} \rightarrow 2\ \text{SiOH/MnPc} \qquad (3)$$

The solid state diffuse reflectance spectrum of the immobilized MnPc on SiO_2/C material shows two electronic transition peaks observed at 624 and 712 nm (Figure 1),and the standard solid state MnPc also shows these two peaks at 647 and 713 nm. These two transitions are assigned as Q bands for Mn(II) under D_{4h} symmetry and the weak band around in 550 nm corresponds to the metal to ligand charge transfer (MLCT) spectra [35,36].

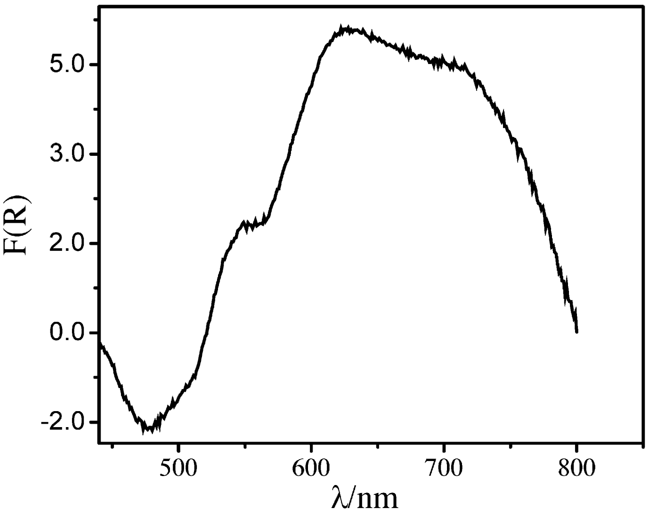

Fig. 1. UV–Vis diffuse reflectance spectra of SiO_2/C/MnPc, expressed in Kubelka–Munk units.

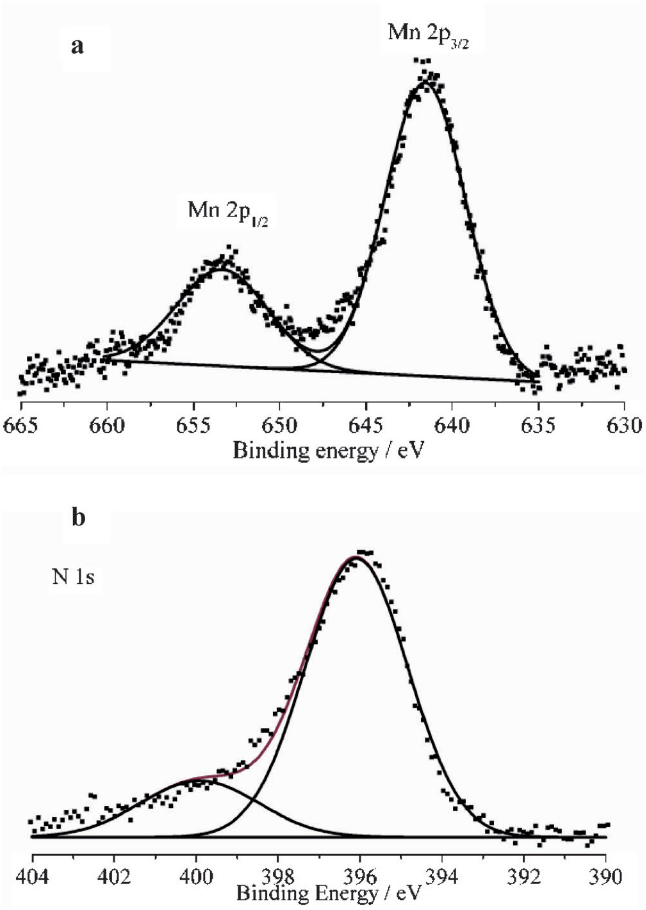

Fig. 2. XPS spectra for Manganese (a) and N (b).

The XPS spectra for Mn and N for the material SiO_2/C/MnPc are shown in Figures 2a and 2b, respectively. In

Table 1. Peak intensities and atoms % for SiO_2/C/MnPc calculated from XPS data.

SiO_2/C/MnPc	Peak intensity	Atoms (%)
N1s	3.19	1.4
Si2p	56.0	25.4
O1s	83.45	37.8
C1s	77.12	34.9
$Mn2p_{3/2}$	0.99	0.5

SiO_2/C/MnPc, the Mn $2p_{3/2}$ and $2p_{1/2}$ BE peaks are shown in 641.6 and 653.3 eV, respectively [37]. Other peaks, centered at 396.1 and 400 eV with the N 1 s BE, assigned to the pyrrolic and meso-nitrogen atoms in MnPc [38].

Table 1 shows the peak intensities and the atoms % obtained by XPS for SiO_2/C/MnPc. In particular the peak of interest, $Mn2p_{3/2}$ BE, allowed to estimate the amount of 0.5 Mn atom % at the depth probed by the technique.

3.3 Electrooxidation of Nitrite on MnPc Modified SiO_2/C

Figure 3 shows the cyclic voltammograms for the bare SiO_2/C electrode and SiO_2/C/MnPc electrode in presence of 15.74 μmol L^{-1} nitrite in 0.1 M phosphate buffer and solution pH 4.

As can be seen from the Figure 3, a well defined oxidation peak of nitrite was obtained on the surface of SiO_2/C/MnPc electrode, when compared with on the bare SiO_2/C electrodes where no oxidation peak of nitrite is observed. It is also evident from this figure that phthalocyanine plays an important role in the oxygen reduction due to their macro cyclic nature including extended π-systems. Phthalocyanines are capable of undergoing fast redox processes, with minimal reorganizational energies and can act as mediators in electron transfer processes involving a great variety of molecules [39].

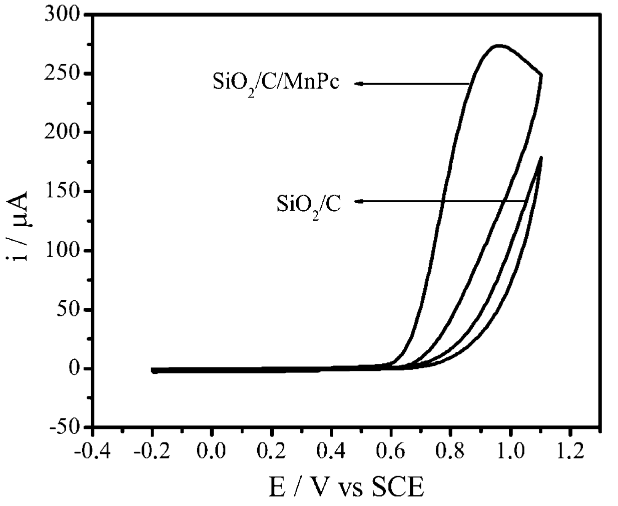

Fig. 3. Cyclic voltammograms obtained for: bare SiO_2/C electrode and SiO_2/C/MnPc electrode in the presence of 15.74 μmol L^{-1} nitrite. Experimental conditions: 1 M KCl solution, 0.1 M phosphate buffer (pH 4) and scan rate of 20 mV s^{-1}.

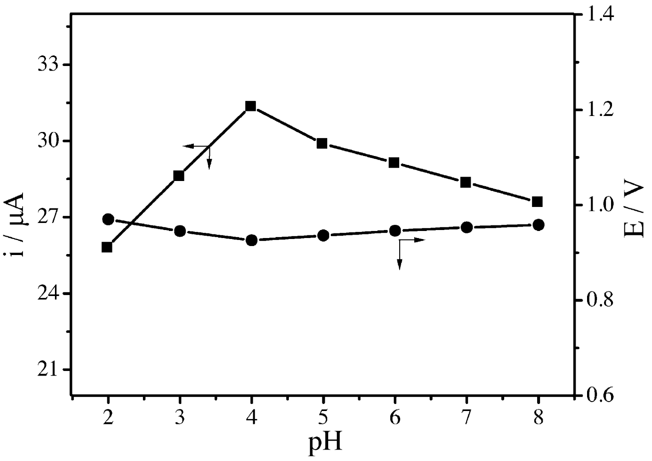

Fig. 4. Dependence of the oxidation peak current on pH values in 12.63 μmol L^{-1} nitrite, 0.1 mol L^{-1} phosphate buffer solution.

3.4 Influence of the Solution pH Value

The effect of solution pH on the electrochemical response of nitrite was investigated in the pH range from 2.0 up to 8.0 in 0.1 M phosphate buffer solution. Figure 4 shows the effect of pH values on the oxidation peak current of 12.6 μmol L^{-1}nitrite. It can be seen that the peak current increases with pH value from 2.0 to 4.0, and then a decrease in the current is observed for pH higher than 4.0. At pH 4.0, the peak current reaches a maximum. Thus, the optimum pH for further studies was set in 4.0. In addition, this study showed that the peak potential for nitrite oxidation is not affected by the solution pH (data not shown). This feature has been also verified by other authors [40,41] and it can be attributed to a kinetically controlled oxidation process, i.e. a proton independent catalytic step.

3.5 Analytical Characterization

Under optimized conditions, in order to obtain an analytical curve for the sensor, cyclic voltammograms for oxidation of nitrite were carried out at different concentrations in 0.1 mol L^{-1} phosphate buffer solution at pH 4.0 (Figure 5a). The plot of peak currents against the concentration of nitrite, inset Figure 5b, shows a linear relationship, in the concentration range between 0.79–15.74 μmol L^{-1} represented by Equation 4:

$$i\ (\mathrm{mA}) = 3.5\ (\pm 1.5) + 17.3\ (\pm 0.2)\ [\mathrm{NO_2}^{-}]\ (\mathrm{mmol\,L^{-1}}) \tag{4}$$

with a correlation coefficient of 0.999 (for $n=12$) and sensitivity of 17.3 μA L μmol^{-1}. Such good sensitivity can be attributed to the efficiency of the electron-transfer between the modified electrode and nitrite due to the catalytic effect and low charge transfer resistance of MnPc. The limit of detection (*LOD*) of 0.02 μmol L^{-1} was deter-

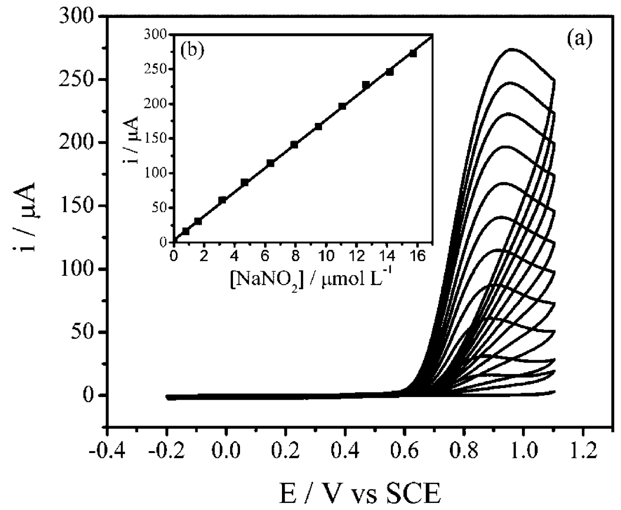

Fig. 5. (a) Cyclic voltammograms for SiO$_2$/C/MnPc electrode in different concentrations of [NO$_2^-$]/µmol L^{-1} : from 0, 0.79, 1.59, 3.19, 4.65, 6.36, 7.93, 9.51, 11.07, 12.63, 14.19 up to 15.74 µmol L^{-1} in 1 M KCl solution, 0.1 mol L^{-1} phosphate buffer (pH 4), scan rate: 20 mV s^{-1}. (b) Inset: Plot of peak current against the nitrite concentrations.

mined using a 3σ/slope ratio and limit of quantification (*LOQ*) was 0.07 µmol L^{-1} using 10σ /slope, where σ is the standard deviation (*SD*) of the mean value for 10 voltammograms of the blank, determined according to the IUPAC recommendations [42]. However, the first point in the analytical curve is 0.79 µmol L^{-1}, which it should be the quantification limit.

3.6 Studies on the Surface Confined Redox Process Behavior of MnPc

The adsorptive or surface confined redox process behavior of MnPc was confirmed by registering cyclic voltammograms at various potential scan rates from 10 up to 180 mV s^{-1}. A plot of the anodic peak current (i$_{pa}$) against the square root of scan rate (v$^{1/2}$) shows a linear relationship (Figure 6), adjusted by the equation: i_{pa} (µA) = $-17.4(\pm1.2)+15.1(\pm0.1)$ $v^{1/2}$(mV s^{-1})$^{1/2}$ and $r=0.999$. This linearity indicates that the oxidation process of nitrite is controlled by diffusion process [43]. Since MnPc is strongly confined in the pores of the matrix surface, the analyte must diffuse into the surface–solution interface.

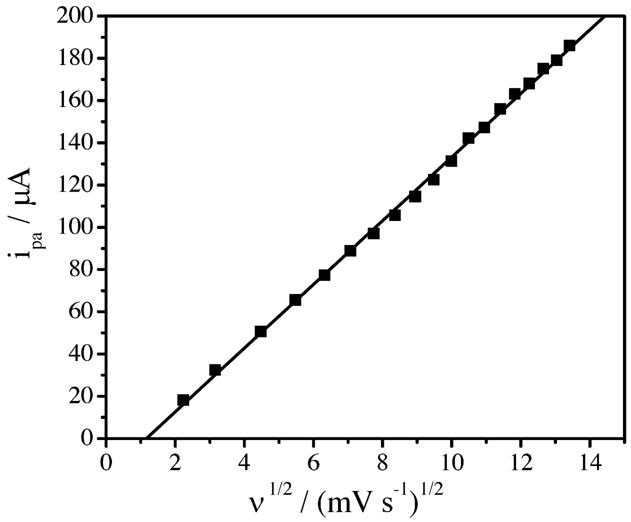

Fig. 6. Variation of the anodic peak current I_{pa} vs. square root of the potential scan rate $\nu^{1/2}$ for the SiO$_2$/C/MnPc electrode in 0.1 mol L^{-1} phosphate buffer solution (pH 4) containing 12.63 µmol L^{-1} nitrite. Scan rate 5–180 mV s^{-1}.

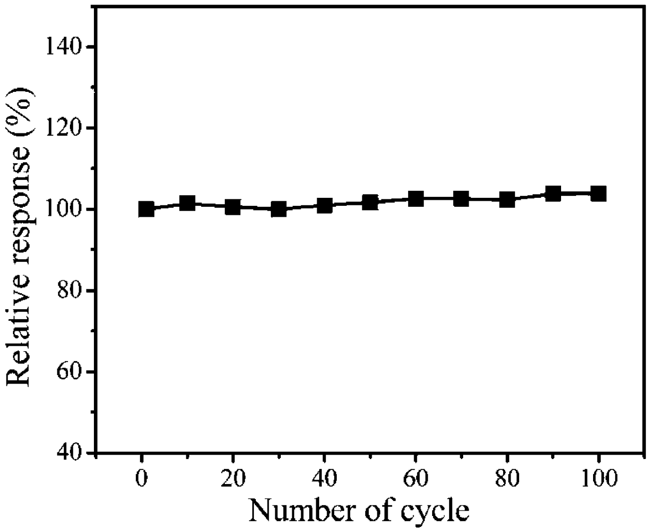

Fig. 7. Relative response (%) at a function of the number of determinations. Applied scan rate = 20 mV s^{-1}, in 0.1 mol L^{-1} phosphate buffer (pH 4.0), containing 12.63 µmol L^{-1} of nitrite.

3.7 Stability of MnPc on the SiO$_2$/C Electrode

The stability of the SiO$_2$/C/MnPc electrode was checked by recording successive cyclic voltammograms. After 100 cycles no significant changes in the currents response was observed (Figure 7). Furthermore, when the electrode was stored at room temperature no significant change in the response was observed for more than 24 months, just by polishing with emery paper to renew the surface to obtain the same response. This electrode showed good repeatability for nitrite determination. The relative standard deviation of the anodic peak for ten determinations of 12.63 µmol L^{-1} nitrite was 1.7 %. Similarly, a series of 5 sensors prepared in the same procedure and tested in the same experimental condition containing 16.2 µmol L^{-1} nitrite gave responses with a relative standard deviation of 4.6 %. These experiments indicated that the SiO$_2$/C/MnPc have good stability (data not shown) and repeatability, probably due to the strong adsorption of MnPc in the SiO$_2$ matrix.

3.8 Determination of Nitrite in Water and Sausage Meat Samples

In order to evaluate the practical utility of the method, nitrite was determined in samples using the standard ad-

Table 2. Determination of nitrite in water sample and sausage meat samples ($n=3$).

Samples	Detected [a] ($mg\,kg^{-1}$)	Added ($mg\,kg^{-1}$)	Found [a] ($mg\,kg^{-1}$)	Recovery [a] (%)
Lake water	6.78 ± (0.01)	7.00	13.80 ± (0.01)	99.85 ± (0.02)
River water	47.23 ± (0.03)	7.00	52.38 ± (0.02)	103.53 ± (0.01)
Sausage 1	14.13 ± (0.02)	9.00	24.01 ± (0.01)	103.80 ± (0.01)
Sausage 2	12.02 ± (0.03)	9.00	20.3 ± (0.02)	99.37 ± (0.03)

[a] Values in parenthesis are the standard deviation of three measures.

Table 3. Comparison of different nitrite sensors for the nitrite determination.

Electrode	Detection limit ($\mu mol\,L^{-1}$)	Sensitivity ($\mu A\,L\,\mu mol^{-1}$)	Reference
CPE-Mn-complex	0.8	1.27	[44]
CuTSPc/PLL/GCE	0.036	0.83	[45]
PdCu/GCE	0.3	0.056	[46]
Pt/Ch/GCE	0.4	0.088	[47]
Thionine/ACNTs	1.12	0.0023	[48]
Hb-Au-CPE	0.06	0.071	[49]
p-NiTAPc/GCE	0.9	0.18	[50]
PNB/GCE	0.1	0.047	[51]
MC/GCE	0.10	0.117	[52]
SWNTs/ssDNA/ GCE	0.15	0.216	[53]
SiO_2/C/MnPc	0.02	17.3	This work

dition method. Water samples were collected from lake and river, and sausage meat were purchased from local market. After filtration of the water to remove the suspension solid substances, and chemical treatment of sausage meat, the concentration values of nitrite in the samples were determined by the proposed method. The nitrite amount and the recoveries were displayed in Table 2, and in all cases, the RSD for each sample was less than 4%. The recoveries for the method were investigated and the values changed from 99.4 to 103.8%. These experimental data indicate that the determination of nitrite using the SiO_2/C/MnPc electrode was effective and sensitive. The results show that the proposed methods could be efficiently used for the nitrite determination in environmental and food samples.

In addition, applying a paired *t*-Student test to compare these results, it was possible to observe that, at 95% confidence level, there was no statistical difference. This good agreement indicates the reliability of the present electrochemical sensor for nitrite determination samples.

Based on the experimental data, it was clear that this SiO_2/C/MnPc sensor possesses high sensitivity and low limit of detection for sensing nitrite in samples. As shown in Table 3, the present sensor shows low limit of detections and high sensitivity when compared to the sensor reported in the literature [44,53]. This excellent performance may be attributed to the mesoporous nature of the matrix prepared by the sol-gel method and strong adsorption of MnPc in the matrix. In this process, C particles are highly and homogeneously dispersed throughout the bulk matrix phase, improving the connectivity of C particles and then increasing the conductivity of the material.

4 Conclusions

In this work, SiO_2/C/MnPc pressed disk electrode with solid paraffin filled pores considerably decreased the electrical resistance and current capacity. The C particles homogeneously dispersed throughout the bulk matrix phase, improving the connectivity of C particles and then increasing the conductivity of the material. Pressed disk electrode is a good alternative when compared with other electrodes based on SiO_2 to provide structural rigidity and chemical stability. The proposed sensor presented an excellent response for oxidation of nitrite. This sensor showed good repeatability for both the measurement and electrode preparation, evaluated in term of relative standard deviation, allied with a simple and easy preparation. Furthermore, its sensitivity, repeatability and stability are excellent. This sensor is the highest sensitive among other sensors reported in the literature. The proposed sensor is highly selective for nitrite determination; it does not show any interference with other ions present in the water. Indeed, this sensor was successful employed for determination of nitrite in sausage meat and water sample.

Acknowledgements

YG and LTK are indebted to *FAPESP*, *CNPq* and *INCTBio* for financial support, SBAB to *CAPES* and LSSS to *CNPq* for Doctoral fellowship, and AR to *CNPq*/*TWAS* for Doctoral fellowship.

References

[1] G.-R. Xu, G. Xu, M.-L. Xu, Z. Zhang, Y. Tian, H. N. Choi, W.-Y. Lee, *Bull. Korean Chem. Soc.* **2012**, *33*, 415–419.
[2] J. Davis, R. G. Compton, *Anal. Chim. Acta* **2000**, *404*, 241–247.
[3] R. A. Al-Okab, A. A. Syed, *Talanta* **2007**, *72*, 1239–1247.
[4] H. Kodamatani, S. Yamazaki, K. Saito, T. Tomiyasu, Y. Komatsu, *J. Chromatogr. A* **2009**, *1216*, 3163–3167.
[5] I. M. P. L. V. O. Ferreira, S. Silva, *Talanta* **2008**, *74*, 1598–1602.

[6] M. J. A. Shiddiky, M. S. Won, Y. B. Shim, *Electrophoresis* **2006**, *27*, 4545–4554.
[7] X. W. Chen, F. Wang, Z. L. Chen, *Anal. Chim. Acta* **2008**, *623*, 213–220.
[8] V. Biagiotti, F. Valentini, E. Tamburri, M. L. Terranova, D. Moscone G Palleschi, *Sens. Actuators B* **2007**, *122*, 236–242.
[9] M. Ammam, B. Keita, L. Nadjo, J. Fransaer, *Talanta* **2010**, *80*, 2132–2140.
[10] T. S. Liu, T. F. Kang, L. P. Lu, Y. Zhang, S. Y. Cheng, *J. Electroanal. Chem.* **2009**, *632*, 197–200.
[11] F. Wang, S. Hu, *Microchim. Acta* **2009**, *165*, 1–22.
[12] W. J. R. Santos, A. L. Sousa, R. C. S. Luz, F. S. Damos, L. T. Kubota, A. A. Tanaka, S. M. C. N. Tanaka, *Talanta* **2006**, *70*, 588–594.
[13] S. Wang, Y. Yin, X. Lin, *Electrochem. Commun.* **2004**, *6*, 259–262.
[14] J.-M. Zen, A. S. Kumar, H.-F. Wang, *Analyst* **2000**, *125*, 2169–2172 .
[15] P. Tau, T. Nyokong, *Electrochim. Acta* **2007**, *52*, 4547–4553.
[16] J. Pillay, K. I. Ozoemena, *Electrochim. Acta* **2009**, *54*, 5053–5059.
[17] L. S. S. Santos, R. Landers, Y. Gushikem, *Talanta* **2011**, *85*, 1213–1216.
[18] J. Arguello, H. A. Magosso, R. Landers, Y. Gushikem, *J. Electroanal. Chem.* **2008**, *617*, 45–52.
[19] A. Rahim, S. B. A. Barros, L. T. Arenas, Y. Gushikem, *Electrochim. Acta* **2011**, *56*, 1256–1261.
[20] J. Pillay, K. I. Ozoemena, *Electrochim. Acta* **2007**, *52*, 3630–3640.
[21] J. H. Zagal, S. Griveau, K. I. Ozoemena, T. Nyokong, F. Bedioui, *J. Nanosci. Nanotechnol.* **2009**, *9*, 2201–2214.
[22] A. Rahim, S. B. A. Barros, L. T. Kubota, Y. Gushikem, *Electrochim. Acta* **2011**, *56*, 10116–10121.
[23] K. I. Ozoemena, D. Nkosi, J. Pillay, *Electrochim. Acta* **2008**, *53*, 2844–2851.
[24] K. I. Ozoemena, T. Nyokong, *J. Chem. Soc. Dalton Trans.* **2002**, 1806–1811.
[25] B. Haghighi, A. Rahmati-Panah, S. Shleev, L. Gorton, *Electroanalysis* **2007**, *19*, 907–917.
[26] J. Arguello, H. A. Magosso, R. Landers, V. L. Pimentel, Y. Gushikem, *Electrochim. Acta* **2010**, *56*, 340–345.
[27] A. Salimi, S. Pourbeyram, *Talanta* **2003**, *60*, 205–214.
[28] Y. Yuan, P. Wang, G. Zhu, *Anal. Bioanal.Chem.* **2002**, *372*, 712–717.
[29] D. F. Rohlfing, J. Rathousky, Y. Rohlfing, O. Bartels, M. Wark, *Langmuir* **2005**, *21*, 11320–11329.
[30] K. Thenmozhi, S. S. Narayanan, *Biosens. Bioelectron.* **2007**, *23*, 606–612.
[31] D. A. Shirley, *Phys. Rev. B* **1972**, *5*, 4709–4714.
[32] E. M. Girotto, I. A. Santos, *Quim. Nova* **2002**, *25*, 639–647.
[33] W. J.R. Santos, P. R. Lima, A. A. Tanaka, S. M. C. N. Tanaka, L. T. Kubota, *Food Chem.* **2009**, *113*, 1206–1211.
[34] K. S. W. Sing, D. H. Everett, R. A. W. Haul, L. Moscou, R. A. Pierotti, J. Rouquerol, T. Siemieniewska, *Pure Appl. Chem.* **1985**, *57*, 603–619.
[35] A. B.P. Lever, S. R. Pickens, P. C. Minor, S. Licoccia, B. S. Ramaswamy, K. Magnell, *J. Am. Chem. Soc.* **1981**, *103*, 6800–6806.
[36] N. Sehlotho, T. Nyokong, *J. Electroanal. Chem.* **2006**, *595*, 161–167.
[37] Z.-L. Yang, H.-Z. Chen, L. Cao, H.-Y. Li, M. Wang, *Mater. Sci. Eng. B* **2004**, *106*, 73–78.
[38] J. Ahlund, K. Nilson, J. Schiessling, L. Kjeldgaard, S. Berner, N. Martensson, C. Puglia, B. Brena, M. Nyberg, Y. Luo, *J. Chem. Phys.* **2006**, *125*, 034709-1-034709-7.
[39] J. H. Zagal, S. Griveau, J. F. Silva, T. Nyokong, F. Bedioui, *Coord. Chem. Rev.* **2010**, *254*, 2755–2791.
[40] M. H. Pournaghi-Azar, H. Dastangoo, *J. Electroanal. Chem.* **2004**, *567*, 211–218.
[41] J. M. Zen, A. S. Kumar, H. W. Chen, *Electroanalysis* **2001**, *13*, 1171–1178.
[42] Analytical methods committee, "Recommendations for the definition, estimation and use of the detection limit", *Analyst* **1987**, *112*, 199–204.
[43] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., Wiley, New York **2001**.
[44] M. A. Kamyabi, Z. Asgari, H. H. Monfared, *J. Solid State Electrochem.* **2010**, *14*, 1547–1553.
[45] A. L. Sousa, W. J.R. Santos, R. C.S. Luz, F. S. Damos, L. T. Kubota, A. A. Tanaka, S. M.C. N. Tanaka, *Talanta* **2008**, *75*, 333–338.
[46] X. Zhu, G. Kang, X. Lin, *Microchim. Acta* **2007**, 159, 141–148.
[47] P. Wang, F. Li, X. Huang, Y. Li, L. Wang, *Electrochem. Commun.* **2008**, *10*, 195–199.
[48] K. Zhao, H. Song, S. Zhuang, L. Dai, P. He, Y. Fang, *Electrochem. Commun.* **2007**, *9*, 65–70.
[49] S. Liu, H. Ju, *Analyst* **2003**, *128*, 1420–1424.
[50] Z.-H. Wen, T.-F. Kang, *Talanta* **2004**, *62*, 351–355.
[51] X. Chen, F. Wang, Z. Chen, *Anal. Chim. Acta* **2008**, *623*, 213–220.
[52] L. Jiang, R. Wang, X. Li, L. Jiang, G. Lu, *Electrochem. Commun.* **2005**, *7*, 597–601.
[53] H. Xian, P. Wang, Y. Zhou, Q. Lu, S. Wu, Y. Li, L. Wang, *Microchim. Acta* **2010**, *171*, 63–69.

Received: September 30, 2013
Accepted: December 12, 2013