

Impedimetric sensor for toxigenic *Penicillium sclerotigenum* detection in yam based on magnetite-poly(allylamine hydrochloride) composite

Gilcelia J.L. Silva^a, Cesar A.S. Andrade^{a,b}, Idjane S. Oliveira^c, Celso P. de Melo^d, Maria D.L. Oliveira^{a,*}

^a Programa de Pós-Graduação em Inovação Terapêutica, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil

^b Departamento de Bioquímica, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil

^c Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, 55608-680 Vitória de Santo Antão, PE, Brazil

^d Departamento de Física, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brazil

ARTICLE INFO

Article history:

Received 28 November 2012

Accepted 11 January 2013

Available online 8 February 2013

Keywords:

Impedance spectroscopy

Biosensor

Magnetic nanoparticles

Composite

Penicillium sclerotigenum

ABSTRACT

We describe a new DNA biosensor for the detection of toxigenic *Penicillium sclerotigenum* in pure culture or infected yams. The *P. sclerotigenum* detection takes place on a self-assembled monolayer of a (magnetite)/(poly(allylamine hydrochloride)) (Fe_3O_4 -PAH) composite that serves as an anchoring layer for the DNA hybridization interaction. Electrical impedance spectroscopy (EIS) was used to evaluate and quantify the hybridization degree. The Fe_3O_4 -PAH composite is a good platform for the immobilization of biomolecules, due to the presence of many possible binding sites for nucleotides and to its large surface-to-volume ratio and good biocompatibility. The biosensor was capable of not only qualitatively detecting the presence of the fungus genome at low concentrations, but also shown a good quantitative impedimetric response its electrical resistance was monitored along the time of exposure. A Fe_3O_4 -PAH-probe biosensor would require only small volumes and low concentrations of the analyte when used, for instance, in detecting *P. sclerotigenum* contamination of food, besides presenting many comparative advantages, such as selectivity, specificity and reproducibility, relative to alternative techniques.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Biosensors are analytical devices used for direct measurement of a given substance in complex biological samples. Electrical biosensors are important class of these sensors in which an electrode is used as the transduction element. For them, the principle of detection is based on the specific binding of the analyte to the biomolecule immobilized on a solid support [1], producing an electrical signal proportional to the concentration of the analyte.

At present, there is a keen interest in biosensor technology due to its large potential for applications in many fields, such as drug research, disease diagnosis, environmental control and food technology [2–5]. Genosensors are biosensors that combine a single-stranded DNA (ssDNA) – the DNA probe – with a transducer to detect a specific genome. DNA biosensors present several advantages such as low cost, rapid analysis, simplicity and possibility of miniaturization. Currently, when compared to conventional methods, DNA biosensor is a useful tool for the diagnosis of diseases and detection of toxigenic fungi in food [6,7].

Classical methods for detection and identification of fungi in food are based on either cultures in different media or microscopic and biochemical analysis. However, usually these classical meth-

ods not only have low specificity and lack reproducibility, but they are also time-consuming and require the involvement of experienced technicians [8]. Hence, there is a current trend in using molecular techniques, such as PCR and fluorescence *in situ* hybridization, to detect and recognize if fungi are present. Although such methods are fast and have good specificity, they still demand high technological support and qualified personnel [9]. DNA array technology, another promising method for detection of fungi is still expensive and time-consuming [10].

Among the many alternative techniques that have been used to the development of DNA biosensors, electrical impedance spectroscopy (EIS) stands out. As a simple, sensitive, low cost and useful method to monitor the interfacial properties of a modified surface, this technique is very well suited for the development of DNA biosensors [11,12]. The EIS operating principle is the analysis of the response of the sample of interest to an electrical stimulus selectively applied to it. The stimulus consists in submitting the system to a continuous sinusoidal potential of small amplitude [13–15]. The immobilization of the DNA at the electrode surface and corresponding recognition events result in changes in the capacitance and interfacial electron transfer resistance of the sample [16,17].

Penicillium sclerotigenum is a phytopathogenic and toxigenic fungi responsible for high losses in the production of yams for causing the postharvest green rot disease. Yam green rot is a com-

* Corresponding author. Fax: +55 81 2126 8547.

E-mail address: m_danielly@yahoo.com.br (M.D.L. Oliveira).

mon problem in Nigeria (Tropical Africa) and Brazil (South America), important yam export-producing countries. In addition, this fungus also produces the mycotoxin patulin [18,19]. Patulin (Ptn) is a mycotoxin that represents a problem in food industry and public health because of its mutagenic, neurotoxic and immunotoxic properties that include genotoxic effects. About 10 biochemical reactions associated to 15 enzymes and production of other co-metabolites are involved in the biosynthetic pathway of Ptn [20], where one of the key steps is the conversion of isopoxycon to phyllostine catalyzed by isopoxydon dehydrogenase (IDH). The *idh* gene encodes the enzyme isopoxydon dehydrogenase and the presence of this gene in fungi has been correlated to patulin production [21]. Therefore, the *idh* gene can be used in the screening of the presence of this toxigenic fungus and in the development of new devices for patulin detection.

In this paper we describe the development of an impedimetric biosensor based on magnetite/(poly(allylamine hydrochloride)) – hereafter Fe_3O_4 -PAH – composite for detection of *idh* as a probe of the presence of toxigenic *P. sclerotigenum* in yam (Fig. 1). While use of new materials can contribute to improve the electrical response of a given biosensor, due to their properties of biocompatibility and large surface-to-volume ratio [22], magnetite (Fe_3O_4) nanoparticles have been extensively used in immobilizing biomolecules. The use of superparamagnetic iron oxide nanoparticle (SPION) in a biosensor has the advantage of high magnetic susceptibility and good dispersibility (not form cluster as long as no magnetic field is applied) [23]. SPION has also been covered with gold (an external inert shell) in order to add new properties (inertness, protection of the magnetic core against oxidation), without modifying their superparamagnetic behavior [24]. Mirkin

group has developed a bio-bar code assay for detection of genomic DNA based on the use of magnetic particles for molecule isolation/purification and the oligonucleotide-functionalized gold nanoparticles as recognition agents [25]. Poly(allylamine hydrochloride) (PAH) is a cationic polyelectrolyte with many ionizable amine groups in its structure, which is fully protonated in neutral and acid solutions, but partially deprotonated in slightly basic solutions [26]. To the best of our knowledge, we have shown for the first time the development of an electrochemical biosensor for detecting toxigenic *P. sclerotigenum* in yam.

2. Experimental

2.1. Materials

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Synth, Brazil), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Reagen, Brazil) and NH_4OH (Quimex, Brazil) were the reagents used in the preparation of the magnetic particles. PAH was obtained from Sigma Chemical (St. Louis, USA). All chemicals and solvents were of analytical-grade and used as received, without further purification. High-purity water was obtained after a Milli-Q plus (Billerica, USA) treatment.

2.2. Extraction of DNA from *P. sclerotigenum* and Yam with green rot

The sensor layer of the detection system was based on *idh1* primer to recognize complementary target DNA of *P. sclerotigenum* and yams contaminated [21]. The primer sequence of *idh1* is 5'-CAATGTGTCGTACTGTGCCC-3'. Pure culture of one isolate of *P. sclerotigenum* was grown in liquid medium CY and after obtaining the

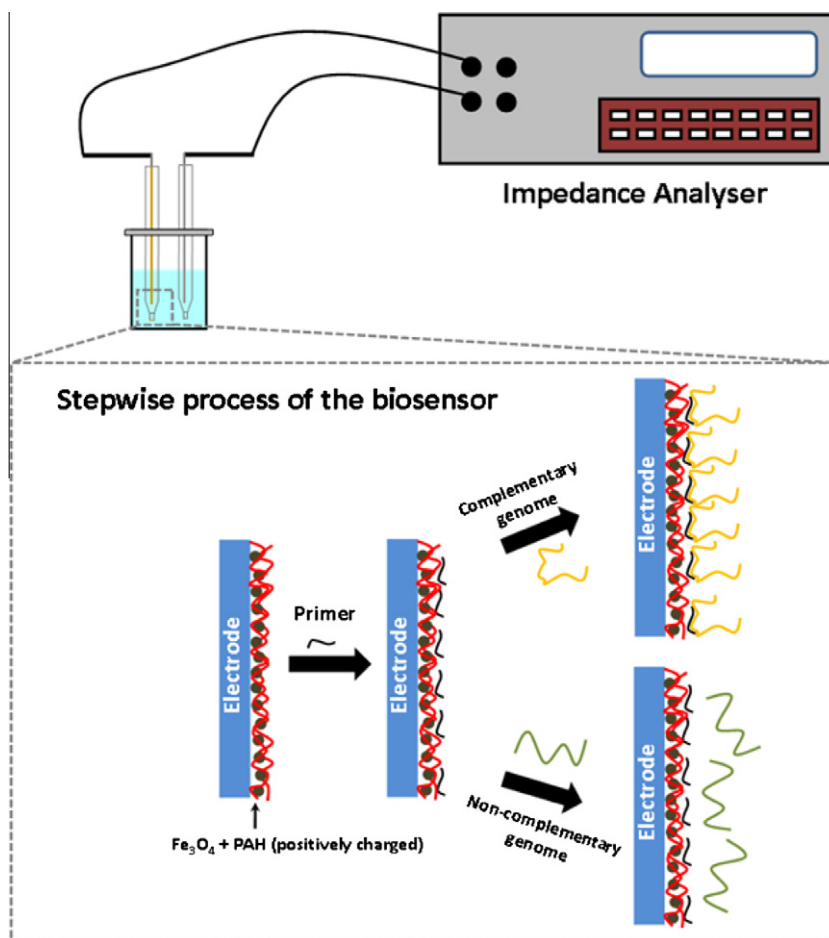


Fig. 1. Schematic representation of the biosensor fabrication.

mycelium, genomic DNA was extracted using the AxyPrep Multi-source Genomic DNA Miniprep Kit (Axygen, USA), from 0.1 g of the mycelium of the fungus. The same kit was used to extract genomic DNA from naturally contaminated yam with *P. sclerotigenum*, from 0.2 g of yam fragments with green rot, following the protocol established by the manufacturer. The samples were subjected to maceration in liquid nitrogen until resulting in a powder, prior to DNA extraction. The negative control sample of DNA was Yam experiments without contamination (non-complementary).

2.3. Preparation of magnetic nanoparticles

The magnetic nanoparticles were prepared via a previously reported co-precipitation method [27,28]. Equal volumes (200 mL) of aqueous solutions of 0.1 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.5 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were prepared with deionized water and transferred to a three-necked round-bottom flask, maintained under intense stirring in a nitrogen atmosphere for 30 min, and then 0.45 M HCl solution diluted in deoxygenated water was mixed in until a pH of 1.7 was reached. Thereafter, small quantities of a 1.5 M aqueous solution of NH_4OH were progressively added to the flask until the liquid turned blackish and the pH increased to 8. The system was then stirred for another 12 h, after which the resulting magnetic particles could be magnetically separated [28].

2.4. Preparation of Fe_3O_4 -HPA matrix and biosensing system

Initially, the bare gold electrode was cleaned using piranha solution. A sol-gel method was employed to modify the gold electrode surface [29]. A small volume (3 mL) of a 2% p/v PAH solution was mixed to 200 μL (corresponding to 20 mM) of an aqueous solution of the Fe_3O_4 nanoparticles. Finally, 2 μL of this mixture were deposited on the electrode surface and allowed to dry for 30 min.

For the oligonucleotide immobilization, 2 μL of the DNA probe (495 nM) were added to the Fe_3O_4 -PAH-modified gold electrode surface. The adsorption was then allowed to occur for 10 min at 20 °C, by means of the electrostatic interaction between Fe_3O_4 -PAH and the DNA probe. The hybridization experiments were carried out by the addition of 2 μL of different concentrations of complementary genome sequence (24, 33, 49, 99 and 196 $\text{pg}/\mu\text{L}$) on the Fe_3O_4 -PAH-probe-modified electrode surface for 10 min at 20 °C. Following the previous procedure, a small volume (2 μL) of different concentrations (50, 66, 100, 200 and 400 $\text{pg}/\mu\text{L}$) of yam's genome contaminated with *P. sclerotigenum* was also analyzed.

2.5. Dielectric measurements

The EIS measurements were performed with a 1260 impedance/gain-phase analyzer (Solartron, UK) by monitoring the variation of the real and imaginary parts of the impedance in the frequency range of 0.1– 10^5 Hz. The amplitude of the applied sine wave potential was 10 mV. We used a bare gold electrode ($\phi = 2$ mm) and a platinum wire as working and auxiliary electrodes, respectively. All experiments were carried out using deionized water. All measurements were performed in triplicate using at least three different sensors.

2.6. Atomic force microscopy characterization

The atomic force microscopy (AFM) measurements were performed with a commercial PicoPlus microscope (Molecular Imaging, USA). Cantilevers with a Cr-Au tip (NSC18, MikroMasch, $F_0 = 90\text{KHz}$, nominal spring constant = 5.5 N m^{-1}) were used for the tapping mode AFM in air at room temperature (approximately 25 °C). Scan areas varying from $1.0\text{ }\mu\text{m} \times 1.0\text{ }\mu\text{m}$ with a resolution

of 512×512 pixels were obtained. To reduce artifact errors, for each sample the images were obtained from at least two macroscopically separated areas.

3. Results and discussion

3.1. Morphological analyses

We performed an AFM analysis to elucidate the variations of the stepwise process influencing the surface topography and morphology of the biosensor. The surface morphology of Fe_3O_4 -PAH-probe-complementary sequence system immobilized on gold surface was studied by high-resolution *in situ* AFM imaging measurements. In Fig. 2a we show homogenous film of the PAH with some aggregates, while the PAH- Fe_3O_4 system can be seen in Fig. 2b; in this latter image we can observe some particles that demonstrate the presence of Fe_3O_4 particles. Also, it is possible observe some aggregates. In Fig. 2c we show a complete covering of the PAH- Fe_3O_4 surface due to the hybridization of the PAH- Fe_3O_4 -probe with *P. sclerotigenum* genome. In addition, differences in the film roughness were obtained. The typical heights of the different PAH, PAH- Fe_3O_4 and PAH- Fe_3O_4 -probe-complementary genome systems were 16.9 nm, 43.2 nm and 97.2 nm, respectively.

3.2. EIS analysis

EIS is a technique that allows the investigation of the interaction between different molecules on a given interface through the corresponding changes in its electrical properties, e.g., capacitance and resistance. For us, it became a useful tool in obtaining information about changes in the impedance of the modified electrode during the process of fabrication of the sensor.

In Fig. S1a (see Supporting information) we show the Nyquist plots after the progressive addition of PAH on the electrode surface at distinct times within a 5 min to 45 min interval. We observed a gradual increase of the resistance to the passage of electric current (R_e) on the electrode surface. The R_e is dependent on the time of exposure of the electrode to the PAH solution and, thus, this increase is proportional to the amount of adsorbed material. In this way, one can follow the corresponding kinetics adsorption by monitoring variation in the blockage of the passage of current at the electrode surface. From the data shown in Fig. S1b, one can see that after 30 min there is already saturation of the response measured on the gold electrode surface. Thus, 30 min was chosen as the time limit to perform the experiments of this study.

Several concentrations (10, 15, 20, 25 and 30 mM) of the Fe_3O_4 nanoparticles were tested. The results of the variation of the magnetic particles concentration for Fe_3O_4 -PAH system are shown in Fig. S2a. We observed a gradual increment of R_e for Fe_3O_4 -PAH, which is proportional to the increase of the Fe_3O_4 concentration (Fig. S2a). From the data presented in Fig. S2b, it can be observed that, after an initial R_e increase between 10 mM and 20 mM, there begins a trend to form a saturated plateau. The Fe_3O_4 concentration used for all experiments was 20 mM.

The DNA probe was immobilized for different intervals of time varying from 5 min to 20 min (Fig. S3a), when a corresponding increase of the R_e was observed. From the data shown in Fig. S3b (see Supporting information) it can be observed that the saturation behavior develops above 10 min. As a consequence, 10 min was the time limit chosen for the immobilization of the *P. sclerotigenum* probe on Fe_3O_4 /PAH modified electrode surface.

3.3. Specificity tests

As explained before, the highest R_e values were obtained for the Fe_3O_4 -PAH-probe-complementary sequence system, that are ex-

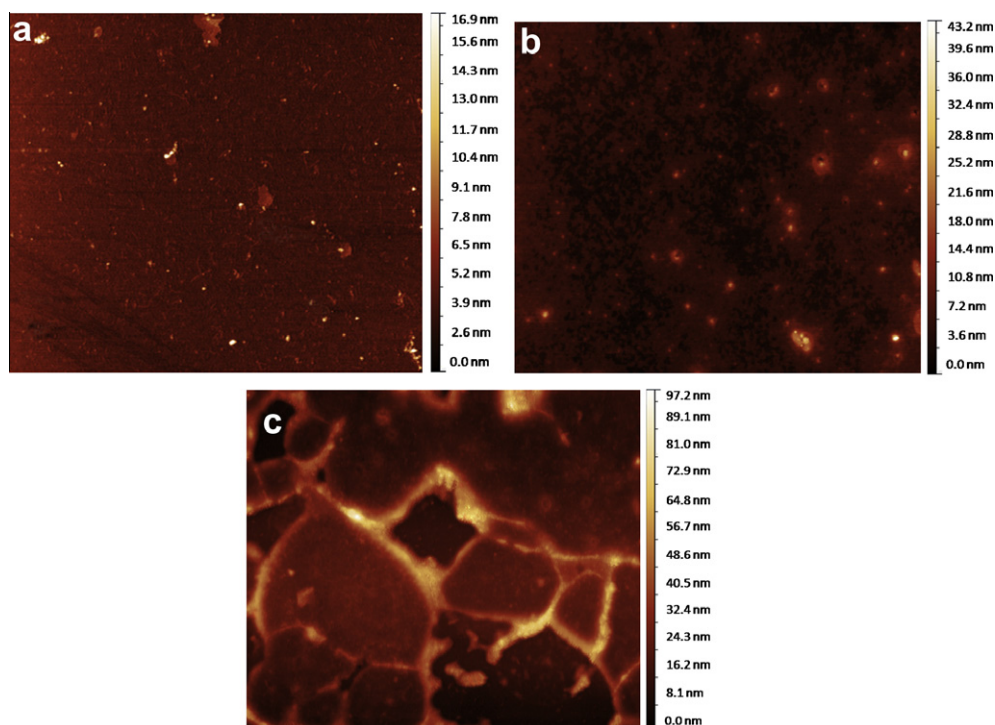


Fig. 2. An AFM topographic image of the electrode surface modified with PAH (a), PAH-Fe₃O₄ (b) and PAH-Fe₃O₄-probe-complementary (c). All scan areas were varied from 1.0 $\mu\text{m} \times 1.0 \mu\text{m}$ with a resolution of 512 $\times$ 512 pixels.

actly those associated to the blockage in the passage of current caused by the progressive addition of molecules to the electrode surface. This result is consistent with the hypothesis that the Fe₃O₄-PAH-probe system can effectively detect the hybridization process associated to the use of a complementary genome. In fact, lower R_e values were obtained for the case of Fe₃O₄-PAH-probe-non-complementary systems when compared to the dielectrical response of Fe₃O₄-PAH-probe-complementary ones, in a demonstration of the specificity of the biosensor (Fig. 3a). We consider that, when taken together, these results have demonstrated that our biosensor shows selectivity to specific DNA sequences.

Nyquist plots of the stepwise assembly of the sensor taken in the 1–10⁵ Hz a frequency range, and for a sine wave potential of amplitude equal to 10 mV, are shown in Fig 3a. The curve obtained for the pure gold electrode corresponds to a small semicircle, which can be associated to a small R_e and to the occurrence of a diffusional process. The assembly of the Fe₃O₄-PAH composite atop the gold electrode surface leads to an increase in the R_e value ($6.64 \times 10^6 \Omega$ as compared to that of the pure electrode ($3.63 \times 10^6 \Omega$). The immobilization of the DNA probe on the Fe₃O₄-PAH-modified gold electrode promotes a further increase in the R_e value ($8.90 \times 10^6 \Omega$). During the hybridization tests, when genome complementary sequence from *P. sclerotigenum* was used, a new increase in the value of R_e was observed. This continuous increase in the R_e values results from the progressive blockage of the passage of the electric current in the electrode/solution interface. Thus, the amount of adsorbed material on the electrode surface contributes directly to the total impedance of the system. In fact, a negligible contribution was observed for the dielectric response of the Fe₃O₄-PAH-probe-modified gold electrode after contact with a non-complementary DNA sequence.

In Fig 3b we can observe that while small changes can be noticed in the impedance values at high frequencies, at lower frequencies there is a clear distinction between the impedimetric responses of the different analyzed systems. After the hybridiza-

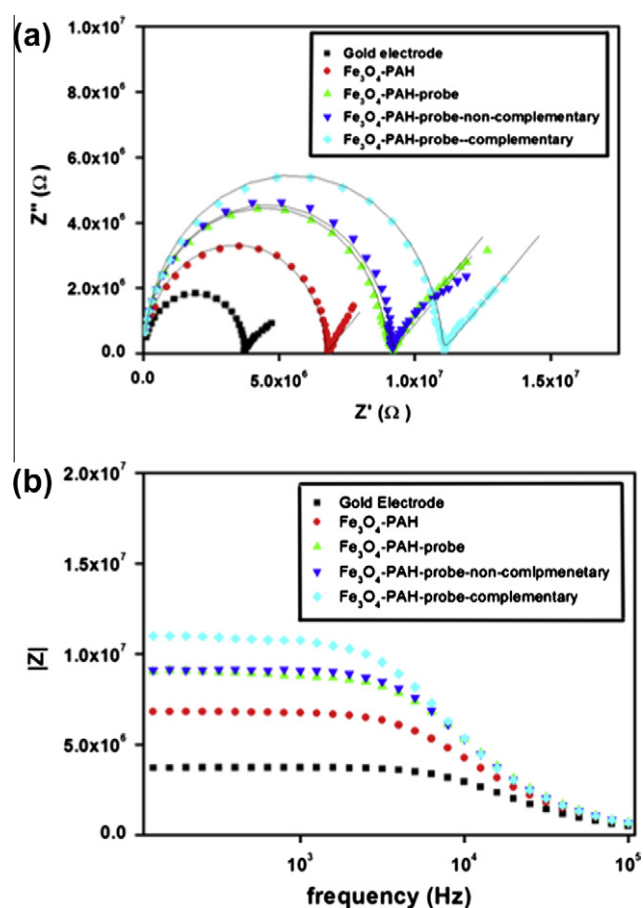


Fig. 3. Nyquist (a) and Bode (b) plots of the stepwise modification process: (■) Gold electrode, (○) Fe₃O₄-PAH, (▲) Fe₃O₄-PAH-probe, (×) Fe₃O₄-PAH-probe-non-complementary, (◆) Fe₃O₄-PAH-probe-complementary sequence.

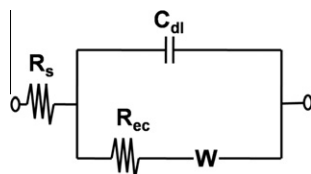


Fig. 4. Equivalent circuit adopted to fit the impedance data, where R_s is the ohmic resistance of the solution, C_{dl} the capacitance double layer, W the Warburg impedance and R_e the resistance to passage of electric current.

tion process, one can observe an increase in the impedance response that can be explained by the reduction in the density of water molecules. When of the adsorption of the genome on the Fe_3O_4 -PAH-probe-modified gold electrode, an additional barrier to the displacement of ions arises near of the surface and, as a consequence, noticeable changes occur in the electrical impedance [30].

3.4. Simulated curves

Detailed information about the impedimetric spectra can be obtained by analyzing the system through the use of equivalent circuits. In the present work, the Randles circuit was chosen to fit the experimental data (Fig. 4). The Randles model consists of the ohmic resistance of the solution (R_s), which represents the resistive effect associated to the ion migration in the solution, the Warburg impedance (W) resulting from the diffusional transport of electroactive species to an electrode surface, the resistance to the passage of electric current (R_e) and the capacitance of the double layer (C_{dl}).

The response curve (Fig. 3a) obtained for the equivalent circuit exhibits excellent agreement to the experimental impedance plots. The parameters calculated from the fitting for the stepwise formation of the sensor are presented in Table 1. As discussed before, the successive increase in the R_e values along the Fe_3O_4 -PAH, Fe_3O_4 -PAH-probe and Fe_3O_4 -PAH-probe-(complementary sequence) tests indicates the progressive formation of insulating layers on the gold electrode surface.

3.5. Sensitivity measurements

The sensitivity of the sensor for *P. sclerotigenum* was also investigated by exposing the system to distinct concentrations of complementary DNA sequence (Fig. 5). The impedance spectra indicate that the increase in the R_e was directly proportional to the concentration of complementary sequence.

The performance of the modified gold electrode for detection of *P. sclerotigenum* genome was evaluated through the relative variation of this parameter (ΔR_e), according to

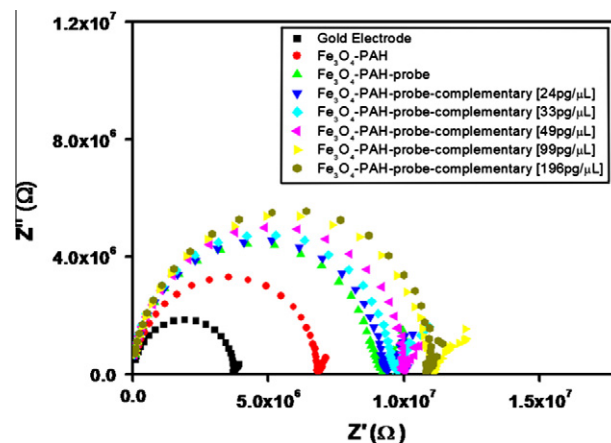


Fig. 5. Nyquist plots of solutions of genome *P. sclerotigenum* at different concentrations.

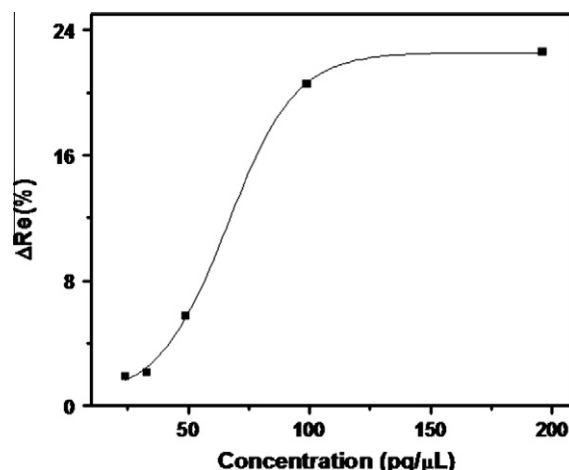


Fig. 6. ΔR_e of Fe_3O_4 -HPA-probe system after hybridization process with the *P. sclerotigenum* genome at different concentrations.

$$\Delta R_e(\%) = \left(\frac{R_e(H) - R_e(S)}{R_e(S)} \right) \times 100$$

Here, $R_e(S)$ is the value of the resistance to the passage of the electric current of the system (Fe_3O_4 -PAH-probe-modified gold electrode) and $R_e(H)$ is the value of the resistance to passage of electric current in the hybridization process of the Fe_3O_4 -HPA-

Table 1

Values of the equivalent circuit elements from fitted impedance results and relative to passage of electric current variation from impedance data.

Modified electrode	R_s (Ω) ($\times 10^3$)	R_e (Ω) ($\times 10^6$)	C_{dl} (F) ($\times 10^{-12}$)	W (Ω) ($\times 10^{-7}$)	ΔR_e (%)
Bare gold electrode	0.45	3.63	3.27	9.92	–
Fe_3O_4 -PAH	0.74	6.64	2.69	7.06	–
Fe_3O_4 -PAH-probe	0.30	8.90	2.35	2.50	–
Fe_3O_4 -PAH-probe-non-complementary	0.22	9.07	2.42	2.99	1.91
Fe_3O_4 -PAH-probe-complementary (24 pg/ μL)	0.26	9.07	2.38	2.57	1.91
Fe_3O_4 -PAH-probe-complementary (33 pg/ μL)	0.26	9.09	2.35	2.23	2.13
Fe_3O_4 -PAH-probe-complementary (49 pg/ μL)	0.37	9.41	2.42	2.99	5.73
Fe_3O_4 -PAH-probe-complementary (99 pg/ μL)	0.52	10.73	2.49	2.48	20.56
Fe_3O_4 -PAH-probe-complementary (196 pg/ μL)	0.52	10.91	2.35	4.01	22.58
Fe_3O_4 -PAH-probe-yam contaminated (50 pg/ μL)	0.26	9.77	2.17	6.96	9.77
Fe_3O_4 -PAH-probe-yam contaminated (66 pg/ μL)	0.38	9.93	2.19	4.04	11.57
Fe_3O_4 -PAH-probe-yam contaminated (100 pg/ μL)	0.46	10.22	2.21	4.79	14.83
Fe_3O_4 -PAH-probe-yam contaminated (200 pg/ μL)	0.37	10.34	2.30	8.79	16.17
Fe_3O_4 -PAH-probe-yam contaminated (400 pg/ μL)	0.26	10.40	2.14	4.38	16.85

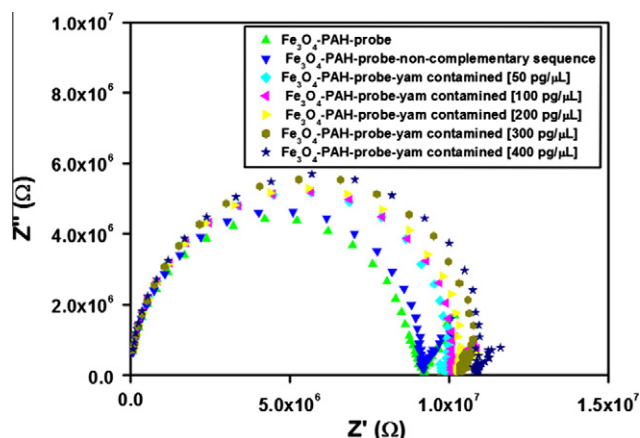


Fig. 7. Nyquist plots of solutions obtained from yam contaminated with *P. sclerotigenum* at different concentrations.

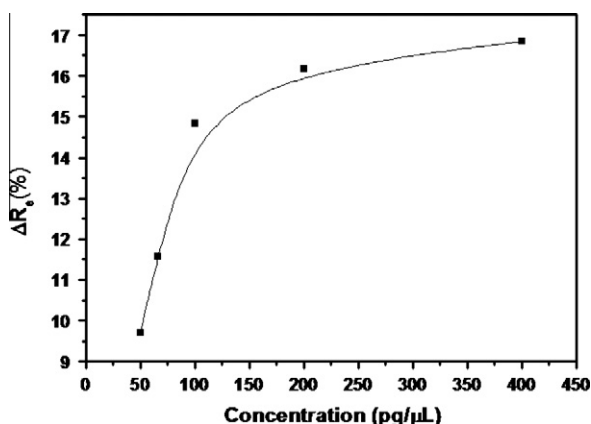


Fig. 8. ΔR_e of Fe_3O_4 -HPA-probe system after hybridization process with yam contaminated with *P. sclerotigenum* at different concentrations.

probe system, after exposing the electrode to solutions containing complementary and non-complementary genomic sequences.

The results corresponding to the use of different concentrations on the ΔR_e during the hybridization process between the Fe_3O_4 -PAH-probe system and complementary genome sequence are shown in Fig. 6. One can observe a systematic increase in the ΔR_e values between 24 pg/ μL and 99 pg/ μL followed by a plateau, indicating a non-linear relationship between ΔR_e and the DNA concentration. These results show that the hybridization can be assessed quantitatively using the Fe_3O_4 -PAH-probe biosystem. In addition, the high sensibility of the biosensor was demonstrated by using small volumes (2 μL) and low concentrations (pg/ μL) of the test samples.

Thereafter, we used real samples obtained from yam contaminated with *P. sclerotigenum*. From the data shown in Fig. 7, it can be observed an increase in R_e ($10.40 \times 10^6 \Omega$) after interaction between the Fe_3O_4 -PAH-probe-modified gold electrode and the yam's genome contaminated. In Fig. 8 we observed a gradual increase in the ΔR_e values (ranging from 50 to 200 pg/ μL). Therefore, our data indicates that Fe_3O_4 -PAH-probe system is capable of detect the presence of *P. sclerotigenum* in foods.

4. Conclusions

In the present work we developed a biosensor for the detection of the genome of fungus *P. sclerotigenum* based on Fe_3O_4 -PAH-

probe systems in yam. The Fe_3O_4 -PAH composite has a good biocompatibility, large surface-to-volume ratio and many binding sites for nucleotide immobilization, therefore confirming that it can be a good platform for the immobilization of biomolecules. This biosensor was capable to detect the fungus genome at low concentrations not only in a qualitative manner but also quantitatively, both in pure cultures of the fungus and in yam contaminated samples. This study is the first description of impedimetric sensor for detection of toxigenic strains of fungus in food contaminated with *P. sclerotigenum*, as a model system. EIS was successfully applied for detection of DNA sequences of toxigenic fungi. The sensor showed good specificity and sensitivity, representing a promising tool for the development of improved quality control of foods.

Acknowledgment

The authors are grateful for the support from the Rede de Nanobiotecnologia/CAPES and INCT_IF (Instituto Nacional de Ciência e Tecnologia para Inovação Farmacêutica). Da Silva would like to thank CAPES for a graduate scholarship. Andrade and Oliveira are also gratefully for CNPq (grant 310305/2012-8 and 310361/2012-5, respectively) and FACEPE financial support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcis.2013.01.023>.

References

- [1] M.N. Velasco-García, T. Mottram, Biosyst. Eng. 84 (2003) 1.
- [2] L.P. Lin, L.S. Huang, C.W. Lin, C.K. Lee, J.L. Chen, S.M. Hsu, S. Lin, Drug Targets Immune Endocr. Metabol. Disord. 5 (2005) 61.
- [3] H.P.O. Nascimento, M.D.L. Oliveira, C.P. de Melo, G.J.L. Silva, M.T.S. Cordeiro, C.A.S. Andrade, Colloid Surf. B 86 (2011) 414.
- [4] G. Bagni, D. Osella, E. Sturchio, M. Mascini, Anal. Chim. Acta 573 (2006) 81.
- [5] J. Spadavecchia, M.G. Manera, F. Quaranta, P. Siciliano, R. Rella, Biosens. Bioelectron. 21 (2005) 894.
- [6] F. Zezza, M. Pascale, G. Mule, A. Visconti, J. Microbiol. Methods 66 (2006) 529.
- [7] P. Snejavskova, L. Tison, I. Brozkova, J. Vytrasova, R. Metelka, K. Vytras, Electrochem. Commun. 12 (2010) 106.
- [8] N.L. Wengenack, M. Binnicker, Clin. Chest. Med. 30 (2009) 391.
- [9] C.K.M. Tsui, J. Woodhall, W. Chen, C.A. Lévesque, A. Lau, C.D. Schoen, C. Baschien, M.J. Najafzadeh, G.S. Hoog, IMA Fungus 2 (2011) 177.
- [10] A.W. Peterson, R.J. Heaton, R. Georgiadis, J. Am. Chem. Soc. 122 (2000) 7837.
- [11] S. Siddiquee, N.A. Yusof, A. Salleh, F. Abu Bakar, L.Y. Heng, Bioelectrochemistry 79 (2010) 31.
- [12] E. Katz, L. Willner, Eletroanalysis 15 (2003) 913.
- [13] J.R. Macdonald, Impedance Spectroscopy: Emphasizing Solid Materials and Systems, Wiley, New York, 1987.
- [14] M. Sluyters-Rehbech, Pure Appl. Chem. 66 (1994) 1831.
- [15] J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and applications, John Wiley, New York, 2001.
- [16] C.-Z. Li, Y. Liu, J.H.T. Luong, Anal. Chem. 77 (2005) 478.
- [17] A. Li, F. Yang, Y. Ma, X. Yang, Biosens. Bioelectron. 22 (2007) 1716.
- [18] I.S. Oliveira, E.D.M.N. Luz, R.M. Moura, L.C. Maia, Fitopatol. Bras. 32 (2007).
- [19] J.C. Frisvad, R.A. Samson, Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins, in: R.A. Samson, J.C. Frisvad (Eds.), *Penicillium* Subgenus *Penicillium*: New Taxonomic Schemes, Mycotoxins and their Extrolites. Studies in Mycology 2004.
- [20] O. Puel, I. Oswald, Toxins 2 (2010) 613.
- [21] R.R.M. Paterson, Food Control 17 (2006) 741.
- [22] S.P. Gubin, Y.A. Koksharov, G.B. Khomutov, G.Y. Yurkov, Chem. Rev. 74 (2005) 539.
- [23] P. Tartaj, M.P. Morales, S. Veintemillas-Verdaguer, T. Gonzalez-Carreno, C.J. Serna, J. Phys. D: Appl. Phys. 36 (2003) R182.
- [24] M. Pita, J.M. Abad, C. Vaz-Dominguez, C. Briones, E. Mateo-Martí, J.A. Martín-Gago, M.P. Morales, V.M. Fernández, J. Colloid Interf. Sci. 321 (2008) 484.
- [25] H.D. Hill, R.A. Vega, C.A. Mirkin, Anal. Chem. 79 (2007) 92183.
- [26] E.R. Sartori, H.H. Takeda, O. Fatibello-Filho, Electroanalysis 23 (2011) 2526.
- [27] L. Yang, X. Ren, F. Tang, L. Zhang, Biosens. Bioelectron. 25 (2009) 889.
- [28] H.P. de Oliveira, C.A.S. Andrade, C.P. de Melo, J. Colloid Interf. Sci. 319 (2008) 441.
- [29] M.D.L. Oliveira, M.T.S. Correia, L.C.B.B. Coelho, F.B. Diniz, Colloid Surf. B 66 (2008) 13.
- [30] M. Gheorghe, A. Guiseppi-Elie, Biosens. Bioelectron. 19 (2003) 95.