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Development of a third-generation biosensor to determine sterigmatocystin mycotoxin: an early warning system to detect aflatoxin B_1

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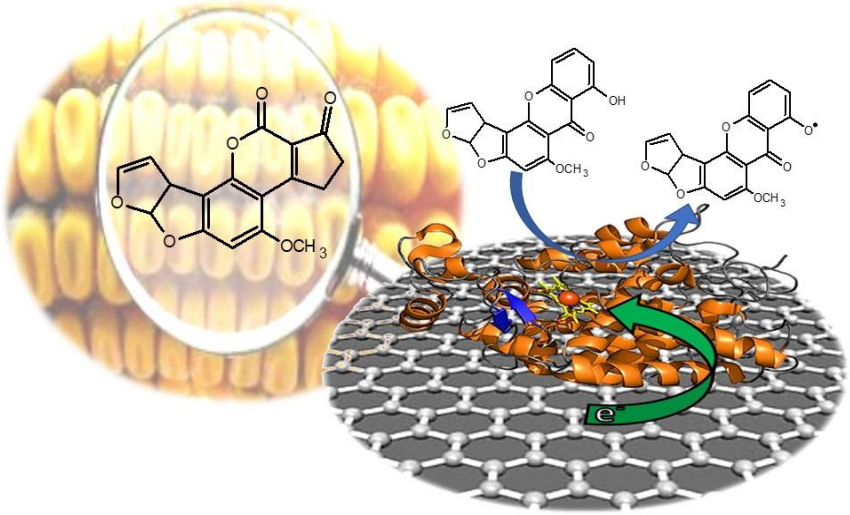
ABSTRACT

A third-generation enzymatic biosensor was developed to quantify sterigmatocystin (STEH). It was based on a glassy carbon electrode modified with a composite of the soybean peroxidase enzyme (SPE) and chemically reduced graphene oxide. The optimal conditions to construct the biosensor were obtained through an experimental design based on the response surfaces methodology. The experiments were performed in 0.1 mol L^{-1} phosphate buffer solution, pH 5. Amperometric measurements were carried out at -0.09 V vs Ag/AgCl (3 mol L^{-1} NaCl). The biosensor showed a lineal response in the concentration range from 6.9 x 10^{-9} to 5.0 x 10^{-7} mol L^{-1}. The limit of detection was 2.3 x 10^{-9} mol L^{-1} for a signal: noise ratio of 3: 1. Values of the apparent Michaellis-Menten constant, K_M^{app}, obtained by using both Lineweaver-Burk and Eadi-Hofstee methods were (1.5±0.2) x 10^{-6} and (1.2±0.2) x 10^{-6} mol L^{-1}, respectively. STEH was analyzed in corn samples spiked with STEH, with an average recovery of 96.5 %. The biosensor was also used to determine STEH in corn samples inoculated with the *Aspergillus flavus* fungus, which is an aflatoxins producer. Considering that STEH is a precursor of aflatoxin B_1 (AFB_1) in its biological transformation, its decrease over time was related to the production of AFB_1. The STEH concentration determined using the biosensor was in very good agreement with that determined by HPLC.

Graphical abstract



Keywords

Biosensor; Soybean peroxidase enzyme; Chemically reduced graphene oxide; Sterigmatocystin; Aflatoxin B_1.

1. Introduction

Climate changes are responsible for the production of mycotoxins in grains and their derived products. Aflatoxin B_1 (AFB_1) (Fig. 1) is a mycotoxin of particular interest [1]. It is a secondary metabolite produced mainly by strains of *Aspergillus flavus* and *Aspergillus parasiticus* [2]. AFB_1 is the best known and most intensively studied mycotoxin in the world because it is the most potent liver carcinogen [2]. IARC classifies AFB_1 within Group 1A [3,4].

AFB_1 can be found in different commodities such as peanut, nut trees, cotton, etc. AFB_1 is highly regulated; the maximum concentration of aflatoxins in agricultural food, feed products and their commodities is regulated worldwide, with specific restrictions in Europe [5]. AFB_1´s attention in Argentina is focused on corn (Zea mays L.) because it is one of the main crops and widely used in the human and animal diet [6]. This crop can be infected by a

variety of toxigenic fungal species during pre and postharvest periods, being *Aspergillus flavus* the dominant species [7].

Conventional methods to detect AFB_1 include high-performance liquid chromatography (HPLC), thin layer chromatography (TLC) and enzyme-linked immunosorbent assays (ELISA) [8,9]. Moreover, commercial kits are available for a qualitative detection of AFB_1. Although these methods provide good sensitivity, it is necessary to have well-trained operators and expensive equipment's. Because AFB_1 is generated in corn from the fourth day [10], the most AFB_1 detection methods are not useful for detecting the mycotoxin during the first days of contamination. Therefore, AFB_1 will not be detected and people will consume foods contaminated with the mycotoxin. The development of rapid and sensitive analytical methods is necessary to ensure food safety. An interesting alternative method is the determination of any precursor of AFB_1 in its biological transformation, which would favor a potential indirect detection of AFB_1. This precursor can be sterigmatocystin (STEH) mycotoxin (Fig. 1) [3]. If STEH is present, there is a possibility that AFB_1 could be generated in the following days.

STEH is classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC) [3,4]. It has been shown to affect several species of experimental animals [11-13]. STEH can be produced by fungal species phylogenetically and phenotypically different [3,14] such as *A. flavus*, *A flavus var. parvisclerotigenus*, *A. parasiticus*, *A. toxicarius*, *A. nomius*, *A. pseudotamarii*, *A. zhaoqingensis*, *A. bombycis* and from the ascomycete genus *Petromyces* (*Aspergillus*, section Flavi), *E. astellata* and *E. venezuelensis* and from the ascomycete genus *Emericella* (*Aspergillus*, section nidulantes) and *A. ochraceoroseus*. It is well known that STEH is a precursor of AFB_1 in its biological transformation for those fungi which are aflatoxins producer [15,16].

The main analytical methods used to determine STEH are chromatography's, ELISA immunoassays and chemical sensors [15]. The use of enzymatic biosensors to determinate STEH is an interesting proposal due to its simplicity and high sensitivity [17-18]. The soybean peroxidase enzyme (SPE) is a good biological element in the development of biosensors due to some intrinsic characteristics, i.e., it can maintain its bioactivity over a wide pH range and, it has higher thermal stability than other peroxidases [19-21]. The SPE as other peroxidases catalyze the oxidation of a phenolic substrate (in this case, STEH) to a radical product ($STE^{\bullet}$) by hydrogen peroxide (H_2O_2) in a three-stage mechanism with two well-characterized intermediates, Compound I (Cp I) and Compound II (Cp II) [22] (eqs. 1-3), being k_1, k_2 and k_3 the rate constants of the chemical three stages involved in the peroxidase catalytic cycle.

$$\mathbf{SPE(Fe^{3+}) + H_2O_2 \xrightarrow{k_1} Cp\ I\ [(Fe^{4+} = O) - R^{\cdot +}] + H_2O} \quad (1)$$

$$\mathbf{Cp\ I\ [(Fe^{4+} = O) - R^{\cdot +}] + STEH/e^{-} \xrightarrow{k_2/k_{het,1}} Cp\ II\ [Fe^{4+} = O] + STE^{\bullet}} \quad (2)$$

$$\mathbf{Cp\ II\ [(Fe^{4+} = O)] + STEH/e^{-} \xrightarrow{k_3/k_{het,2}} SPE(Fe^{3+}) + STE^{\bullet}} \quad (3)$$

On the other hand, when SPE is adsorbed at the electrode surface, Cp I and Cp II can also be electrochemically reduced, being $k_{het,1}$ and $k_{het,2}$ the electron transfer rate constants of the Cp I/Cp II and Cp II/ SPE redox couples, respectively [23]. Under these conditions, the substrate of SPE is an electron from the electrode surface and this process is known as a direct electron transfer [24], and the bio-electrode is denominated as a third-generation biosensor [25]. When STEH is present in solution, the direct electron transfer process can occur simultaneously with the oxidation of STEH [22] and the third-generation biosensor can be used to determinate STEH in food samples.

In this paper, we developed a third-generation enzymatic biosensor to quantify STEH in corn samples spiked with the mycotoxin. The biosensor was based on a glassy carbon (GC) electrode modified with a composite of SPE and chemically reduced graphene oxide (CRGO).

The biosensor was also used to determine STEH in corn samples inoculated with *Aspergillus flavus*, which is an aflatoxins fungus producer. The decrease of its concentration over time was correlated with the production of AFB_1, which was determined by HPLC. Therefore, an indirect potential method to detect AFB_1 is proposed. The same third-generation biosensor, GC/SPE/CRGO, was previously used to determine H_2O_2 in real samples [26], where it was demonstrated that the presence of CRGO in the composite is essential for obtaining the third-generation biosensor.

2. Materials and methods

2.1. Reagents

STEH and AFB_1 were purchased from Sigma-Aldrich, USA. For security reasons and considering the toxicity of AFB_1 and STEH, all solutions and experimental measurements were carried out using latex gloves and a facial mask. The residues were discarded in special bottles properly labelled. They were periodically collected by a company responsible for their final destination.

The 0.1 mol L^{-1} phosphate buffer solutions (PBS) were prepared by mixing equal concentrations of K_2HPO_4 and KH_2PO_4 (Merck p.a.). The pH was adjusted at pH 5 by adding 0.1 mol L^{-1} HCl (Merck p.a.). Hydrazine sulfate and 5% w/w nafion (in methanol) were purchased from Sigma-Aldrich. SPE was kindly provided by Dr G. J. Levin from Centro de Investigaciones y Transferencia de Entre Ríos, Gualeguachú, Entre Ríos, Argentina. The enzyme concentration was determined through uv-vis absorption measurements at λ = 403 nm (ε_{Enz}^{403} = 9 x 10^4 mol^{-1} L cm^{-1}) [20].

H_2O_2 (Merck p.a.) solutions were prepared daily. Their concentrations were determined through uv-vis absorption measurements at λ = 240 nm ($\varepsilon_{H_2O_2}^{240}$ = 43.6 mol^{-1} L cm^{-1}) [27]. All solutions were prepared with bi-distilled water. All reagents were used as received.

2.2. Apparatus

Amperometric measurements were performed using a BAS Epsilon potentiostat, with electrochemical software incorporated. A conventional three electrode cell was used in all electrochemical measurements. The working electrode was a glassy carbon (GC) disk (BAS, dia. = 3 mm). The reference and counter electrodes were Ag/AgCl (3 mol L^{-1} NaCl) and a large area Pt sheet, respectively. The volume of the electrochemical cell was 2 mL.

An uv-vis spectrophotometer Hewlett Packard, model HP8453 was used for spectrophotometric measurements. The cells had an optical path of 1 cm.

A chromatograph Hewlett Packard (Serie 1100), with uv-visible detector was used to validate the electrochemical measurements. An Hypersil C18 column (150 x 4,2 mm, 5 µm; Phenomenex) and an ODS-Hypersil pre-column (20 x 2,1 mm, 5 µm; Phenomenex) were used. An isocratic mode with a binary mixture of acetonitrile:water (70:30) was used, with a flow rate of 1 mL/min. The injection volume was 50 µL and the column temperature was 25 °C. The wavelength to detect STEH was 325 nm.

A chromatograph Waters 2696 separation module (Waters, Milford, USA) was used to detect AFB_1. A stainless steel C18 reverse phase column (150-4.6 mm, 5 µm, Phenomenex) was used. A water: methanol: acetonitrile (66.6: 16.7: 16.7) mixture was used as mobile phase at a flow rate of 1.5 mL/min. A Waters 2475 module was used for fluorescence detection (λ_{exc} 360 nm; λ_{em} 440 nm).

2.3. Extraction from the sample

STEH extraction was performed as previously described with minor modifications [28]. Twenty-five grams of homogenized corn sample was extracted with 100 mL of acetonitrile-water (84:16) during 30 min using a horizontal shaker. After filtering through a filter paper (Whatman Nº 4 of high retention), 10 mL of extract was diluted with 20 mL of

water and purified using Strata X (500 mg) solid phase extraction column (Phenomenex, Torrance, CA, USA). The column was conditioned with 4 mL of methanol, followed by 4 mL of water prior to use. Then, 30 mL of diluted extract was injected into the column, and washed with 40% acetonitrile in water and, then with 40% methanol in water. Finally, STEH was eluted with 4 mL of 100% acetonitrile. This solution was evaporated to dryness under argon atmosphere at 60 ºC and re-dissolved in 400 µL of acetonitrile.

2.4. Recovery studies

Spiked samples of corn grains were prepared by adding aliquots of a STEH standard solution to 25 g of sample in a 250 mL flask to obtain a final STEH concentration of 15, 40 and 70 µg/mL. The blank and spiked samples were allowed to stand overnight at room temperature in the dark to attempt to simulate a natural contamination. Three replicates for each concentration level were prepared. The samples were extracted as previously described.

2.5. Artificially pre-treated corn sample

An *Aspergillus flavus* strain (RCP08108) isolated from corn grain was sub-cultured on malt extract agar (MEA) plates and incubated at 25 ºC during 7 days to enable significant sporulation. After incubation, a sterile inoculation loop was used to remove the conidia from the surface of MEA plates and suspended in 5 mL of peptone aqueous solution (0.1%). After homogenization, suspension was adjusted using a Neubauer counting chamber to achieve final concentrations of 1-5×10^4 spores/mL [29].

Corn was previously ground and subsamples of 100 g were weighed into flasks and sterilized by autoclaving at 120 °C during 20 min twice. Corn milled was rehydrated to the required water activity (0.98 a_w) by the addition of sterile distilled water using a moisture absorption curve, and flasks were stored at 4 ºC during 72 h with periodic hand-shaking during this time to modify the milled grain to the required a_w. The amount of water necessary

to reach a_w level was determined by using a calibration curve (water activity-mL vs. water to be added/g substrate) according to Garcia et al. [30]. Therefore, 480 µL/g were added to reach the value of 0.98 a_w and checked with the Aqua Lab Water Activity Meter 4TE (Decagon Devices, Inc.). The flasks containing the rehydrated milled corn were inoculated with 1×10^4 spores of *A. flavus* / g of corn, mixed by hand and incubated at 25 ºC. Flasks were then placed in sealed environmental chambers at 25 ºC containing beakers of glycerol-water solutions at the same a_w as the treatment, in order to maintain the correct equilibrium relative humidity. Samples were analyzed daily during 13 consecutive days. Milled corn without *A. flavus* spores was used as the blank sample. The assays were made by triplicate.

The STEH analysis was performed with the GC/CRGO/SPE biosensor. The extracts were dried and dissolved in 200 µL of acetonitrile: water (9:1) for performing the AFB_1 detection. They were then derivatized with 700 µL of trifluoroacetic acid: acetic acid: water (20: 10: 70) at 65 ºC during 8.5 min. Then, 100 µL of the derivatized solutions were injected in the HPLC chromatograph.

3. Results and discussion

3.1. Optimization of factors involved in the construction of GC/CRGO/SPE biosensor

It is necessary to find out the best experimental conditions to obtain the highest sensitivity in the determination of STEH. Factors which may be involved in the construction of GC/CRGO/SPE biosensor are the volume of both SPE and CRGO to be mixed (I), the volume of composite deposited at the electrode surface (II), drying temperature of electrode (III), pH (IV), potential at which the amperometric measurements are performed (V), and $c^*_{H_2O_2}$ (VI). An Ishikawa diagram (or cause-effect diagram) was used to select the relevant factors to be studied [31]. The optimization was carried out using a composite central design. The variable that was optimized was the slope of the ΔI_{ss} vs c^*_{STEH} calibration curve (see below). The optimization process was carried out to obtain the highest value of the slope of

the calibration curve. The optimal conditions to detect STEH obtained from the experimental design were: I) 20:80 (is the amount of SPE and CRGO expressed in µL); II) 5 µL; III) 35 ºC; IV) 5; V) -0,090 V and VI) 50 µmol L^{-1}.

3.2. Determination of STEH

The biosensor was first introduced into the electrochemical cell and the current response was allowed to stabilize at E = - 0.090 V. Then, an aliquot of H_2O_2 was added, which starts the first stage of the catalytic cycle of SPE adsorbed at the electrode surface. After reaching a steady state reduction current (I_{ss}), different aliquots of STEH were added and new I_{ss} were obtained at a time less than 3 min (Fig. 2a). Figure 2b shows the corresponding calibration curve, where ΔI_{ss} represents the difference between the steady state currents obtained for a given aliquot of STEH and that of H_2O_2. The linear range of the calibration curve was from 6.9 x 10^{-9} to 5.0 x 10^{-7} mol L^{-1} (insert Fig. 2b), which satisfies the Linearity Test, i.e., $F_{experimental} < F_{theoretical}$ [32]. The sensitivity of the calibration curve was (0.082 ± 0.004) A mol^{-1} L and its analytical sensitivity (γ) was 120.36 µmol^{-1} L (1/ γ = 0.008 µmol L^{-1}), where 1/ γ indicate the lowest concentration difference that can be observed over the range of application of the analytical technique [33].

The limit of detection (LOD) was 2.3 x 10^{-9} mol L^{-1} for a signal: noise ratio of 3:1. In Table 1 are compared the LOD obtained with GC/CRGO/SPE biosensor with those obtained using other analytical methods to determine STEH. Therefore, the LOD is less than chromatographic methods with MS detection and the different chemical sensors (fluorescent sensor and biosensors) and it is slightly higher than chromatographic method with uv-visible detection. The advantage of the biosensor compared to chromatographic methods is based on

its low cost and shorter analysis time. Another advantage of this biosensor is that SPE is extracted from soybean, which is a very abundant grain in Argentina.

The Michaelis-Menten apparent constant (K_M^{app}) was determined through both the Lineweaver-Burk (eq. 4) and Eadi-Hofstee (eq. 5) methods [39].

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max}} c_{STEH} \quad (4)$$

$$I_{ss} = - K_M^{app} \frac{I_{ss}}{c_{STEH}} + I_{max} \quad (5)$$

where I_{max} is the current obtained at the maximum reaction rate, under saturation conditions. Values of K_M^{app} of (1.5±0.2) x 10^{-6} and (1.2±0.5) x 10^{-6} mol L^{-1} were obtained using the Lineweaver-Burk and Eadi-Hofstee methods, respectively.

3.2.1. Statistical parameters of the biosensor

Reproducibility was studied by preparing four different biosensors. From slopes of the corresponding calibration curves a percentage variation coefficient (% VC) of 2% was obtained. Repeatability was determined by measuring the slopes of four calibration curves obtained with the same biosensor. A % VC of 1% was calculated. The stability of the biosensor stored at 4 °C was 5 days. Then, the response of the biosensor falls below 80%. However, the SPE/CRGO composite was stable during three months and the construction of the biosensor takes only about 5 min. Besides, the biosensor can be used to analyze approximately twenty samples per day.

3.2.2. Recovery studies

Table 2 shows the recoveries of STEH from spiked corn samples, which were in the range from 88 to 114%. The average recovery, $\overline{R}$, was 96.5 %. A statistical test was applied to assess whether $\overline{R}$ is significantly different from 100% [33], which involves two hypotheses: Ho: $\overline{R}_{exp}$ = 100% and H_1: $\overline{R}_{exp} \neq 100\%$. The Ho is rejected at significance level α if t_{exp} (eq. 6) exceeds the critical value at level α, t(α,N-1), where N is the number of tested samples, and s_R is the standard deviation of recoveries.

$$t_{exp} = \frac{|100 - \overline{R}_{exp}|\sqrt{N}}{s_R} \quad (6)$$

Therefore, considering a significance level of 95 % and N = 6, t_{exp} = 0.65 and t_{theor} = 2.015 [40]. Given that $t_{exp} < t_{theor}$, it is concluded that the average recovery does not differ significantly from 100%.

3.2.3. Validation of the biosensor

After developing a new analytical method, it is necessary to validate it with a reference method. Then, the new developed analytical method could be suggested as a reliable method for routine analyses [33]. HPLC-DAD was used as the reference method to validate results obtained with GC/CRGO/SPE biosensor. Figure 3a shows a HPLC chromatogram of a STEH standard solution. Only one chromatographic peak was observed, with a retention time of 3.20 min, which corresponds to the elution of STEH. Chromatograms obtained for a corn sample without and with STEH are shown in Figs. 3b and 3c, respectively. As can be observed, there are no matrix peaks that interfere in the determination of STEH, which shows that the

cleaning method used was very effective. The peaks with a retention time < 2 min correspond to substances present in the corn sample that do not interfere with the STEH elution peak.

The comparison of results obtained with the GC/CRGO/SPE biosensor and HPLC-DAD was performed using the elliptical joint confidence region (EJRC) [33], which is of special relevance when two analytical methods are compared considering that it reveals the existence of significant differences in the estimation at different concentration levels [33]. The EJRC obtained is shown in Fig. 4. It contains the point (1,0) showing that there is no statistically significant difference between the values found by both methods at a confidence level of 95%.

3.3. Determination of STEH in corn samples inoculated with *A. flavus*

If STEH is initially present in corn and AFB_1 is absent, it is very likely that AFB_1 will be generated in the following days by transformation of STEH [8]. Figure 5a shows the corn sample contaminated with *A. flavus*, which is completely covered by the fungus in the seventh day after incubation. STEH and AFB_1 were determined during the first thirteen days of incubation using the GC/CRGO/SPE biosensor and HPLC-fluorescence detection, respectively. Figure 5b shows the biosynthesis curve of STEH (red line) and AFB_1 (black line) by *A. flavus* in ng/g of corn. The detection of STEH started from the first day of incubation, reaching a maximum value on the third day. In the following days, the STEH amount decreased obtaining a minimum on the tenth day. This decrease is due to the fact that STEH is consumed to produce AFB_1.

From day 7 to 13 of incubation, AFB_1 showed a maximum production on day 7. Then it decreased until it reached a minimum of production on day 10. On the 12th, it was observed a significant increase in the production of AFB_1 and then again decreased. García et al. [10] also found that the maximum production of AFB_1 occurs on the seventh day of incubation followed by a decrease on the eighth day. Mellon et al. [41] studied the production of AFB_1 by the fungus *A. flavus* in a synthetic medium simulating corn kernels. These authors also found that after reaching a maximum in the production of AFB_1, a decrease in its concentration was observed. The decrease in the amount of AFB_1 may be due to a decrease in the rate of growth [41], or to degradation produced by the fungus itself as an alternative source of carbon [42].

Thus, analysis of parallel results for the simultaneous determination of STEH and AFB_1 by the biosensor and HPLC, respectively, encourage following studies to develop a method for the early detection of AFB_1 in corn samples based on the STEH biosensor developed in this paper.

4. Conclusions

The GC/CRGO/SPE biosensor was successfully used to determine STEH in samples of corn both spiked with the mycotoxin and inoculated with the *A. flavus* fungus. Recovery percentages obtained does not statistically differ from 100%. In addition, results obtained using the biosensor were validated by HPLC-DAD, showing that there are no significant statistically differences between both methods. In spite of the LOD obtained with the biosensor is slightly higher than that obtained with HPLC-UV detection, the biosensor is simple to construct, very fast to assemble, and the analysis time is shorter. In addition, it

shows good reproducibility and repeatability. Besides, results found indicate the potential use of the STEH biosensor for the early detection of AFB_1 in corn samples.

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Fig. 1. Chemical structures of AFB_1 and STEH.

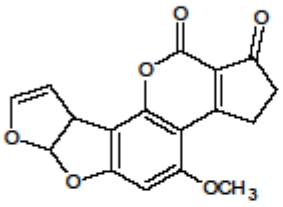
Fig. 2. a) I vs t plot obtained for the GC/CRGO/SPE biosensor when: (1) an aliquot of 50 μmol L^{-1} H_2O_2 was added and (2) different STEH aliquots of 5 μL (100 μmol L^{-1}) were added at pH 5.0. b) The corresponding ΔI_{ss} vs c^*_{STEH} plot. The insert shows the linear range of the calibration curve.

Fig. 3. a) HPLC chromatogram of a STEH standard solution, c^*_{STEH} = 231 μmol L^{-1}. Chromatograms recorded for a corn sample without STEH (b) and with STEH (c). c^*_{STEH} = 214 μmol L^{-1}.

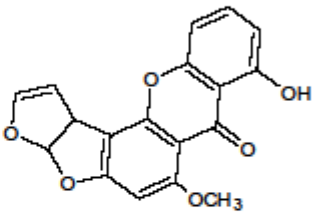
Fig. 4. Elliptical joint region (at a 95% confidence level) for the comparison of results obtained with HPLC-DAD and the GC/CRGO/SPE biosensor. The red star point indicates the theoretical (0,1) point.

Fig. 5. a) A corn sample contaminated with *A. flavus*on the seventh day of incubation. b) the biosynthesis curve of STHE (red line) and AFB1 (black line) by *A. flavus* expressed in ng/g of corn.

Figure 1

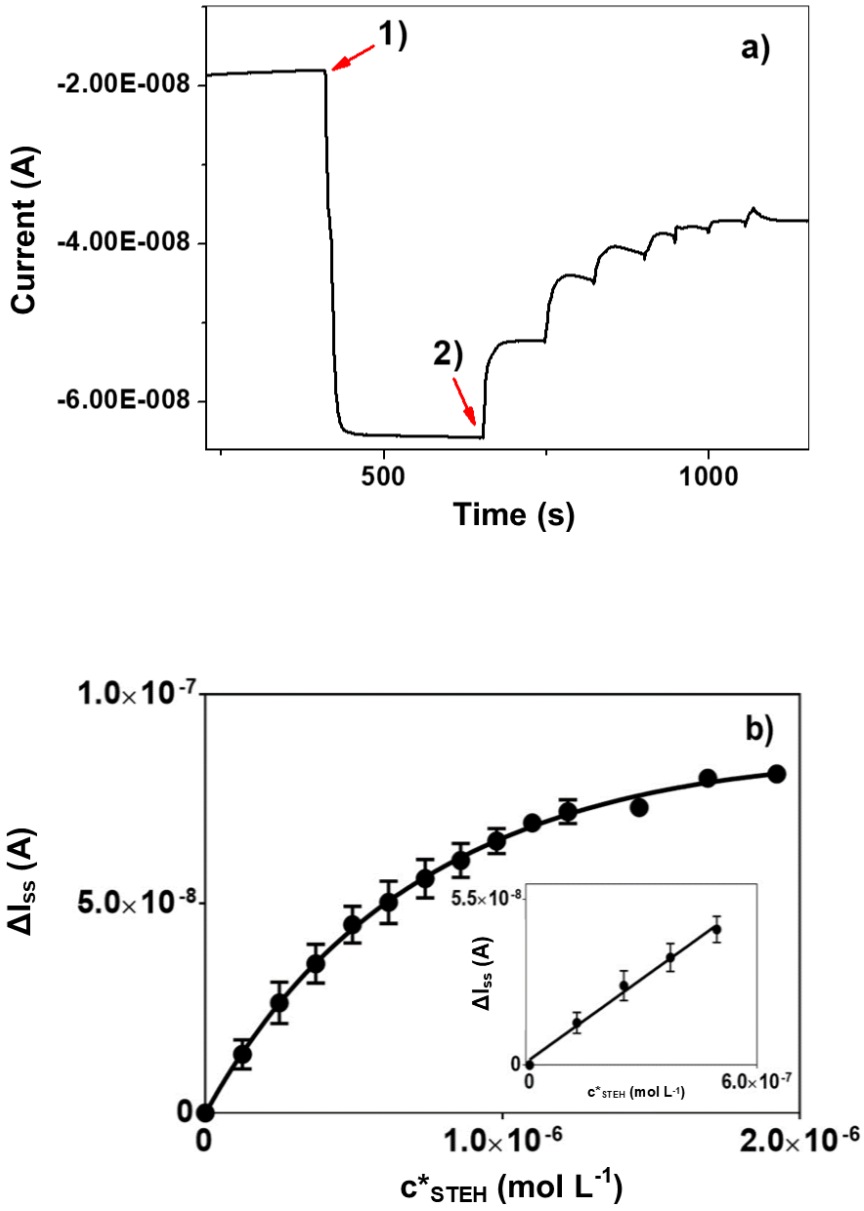


AFB_1



STEH

Figure 2


a)
1)
2)
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-4.00E-008
-6.00E-008
Current (A)
500
1000
Time (s)
b)
1.0×10-7
5.0×10-8
0
ΔIss (A)
0
1.0×10-6
2.0×10-6
c*STEH (mol L-1)
5.5×10-8
ΔIss (A)
0
0
6.0×10-7
c*STEH (mol L-1)

Figure 3

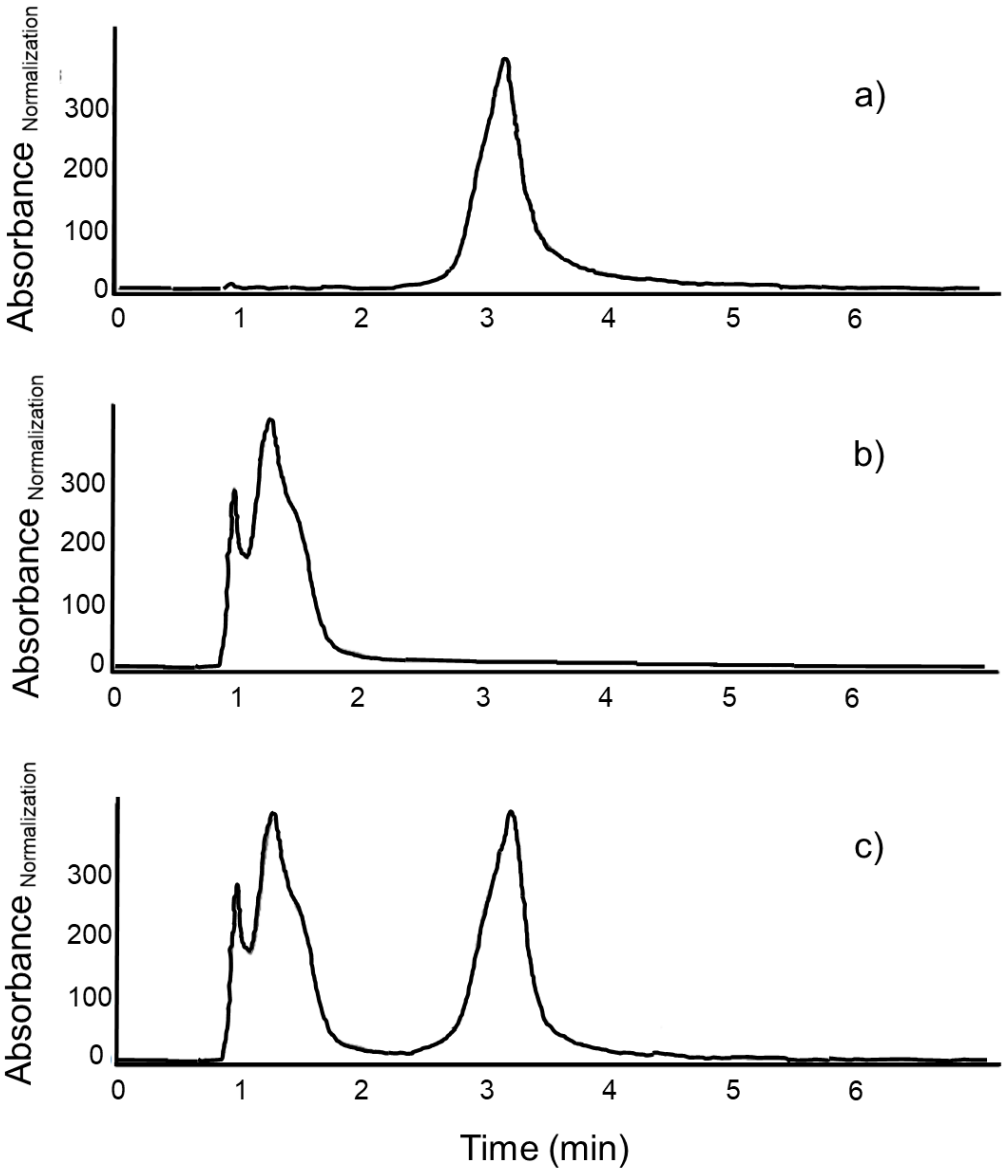

Absorbance Normalization
300
200
100
0
0
1
2
3
4
5
6
a)
b)
c)
Time (min)

Figure 4

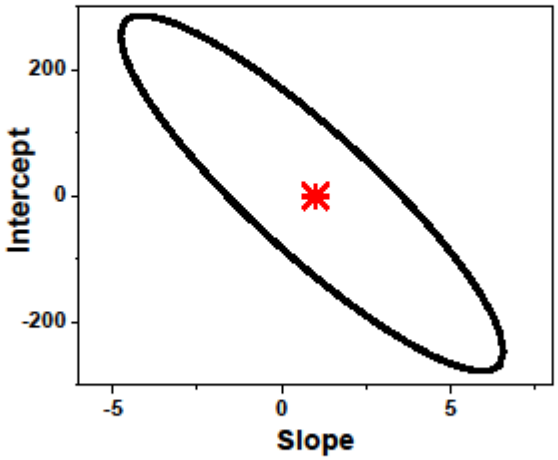
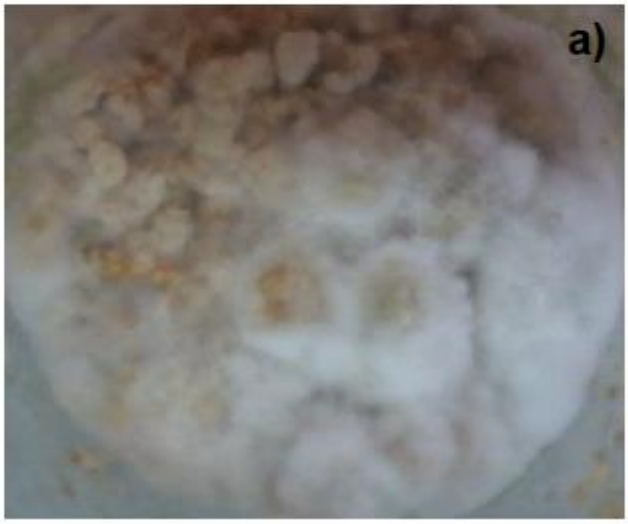


Figure 5



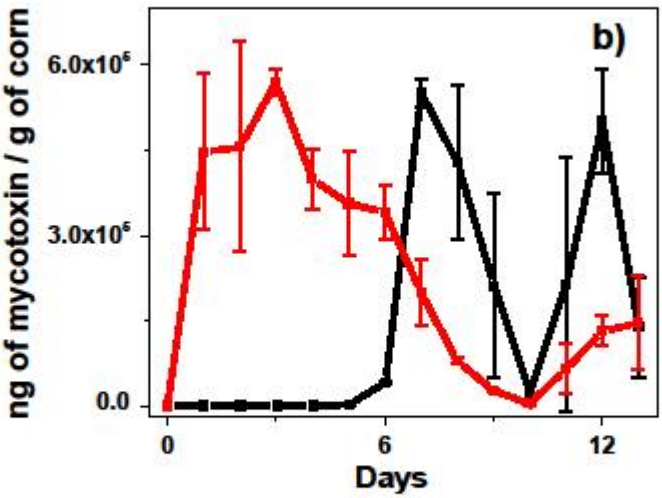


Table 1. Limits of detection obtained from different analytical methods to determine STEH.

Method	LOD (μg L^{-1})	Ref.
HPLC-MS/MS	1.5	[28]
HPLC-MS	3.3	[34]
HPLC-UV	0.26	[35]
Fluorescent Sensor	19	[36]
Biosensor: Au/ SWCNTs-AFO-CS*	3.0	[37]
Biosensor: Au/MWCNTs/ADTZ**	42	[38]
Biosensor: GC/CRGO/SPE	0.80	This work

* Single walled carbon nanotubes (SWCNTs), aflatoxin-oxidase enzyme (AFO), chitosan (CS) ** Multi walled carbon nanotubes (MWCNTs), aflatoxin–detoxifizyem enzyme (ADTZ).

Table 2. Percentage recovery (%R) obtained for six samples.

Samples	Nominal (μg mL^{-1})	Found (μg mL^{-1})	%R
1	15.0	14.4	96
2	15.0	13.5	89
3	43.5	43.5	100
4	43.5	49.6	114
5	70.0	64.4	92
6	70.0	61.6	88
Average recovery, %$\bar{R}$			96.5 + 9.7

- A third-generation biosensor was developed to determine sterigmatocystin.
- The biosensor shows a high affinity toward sterigmatocystin.
- The biosensor was used to determine sterigmatocystin in corn samples.
- Electrochemical results were in good agreement with those obtained by HPLC.
- The biosensor could be a potential sensor for AFB_1.