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Synthesis, characterization and application of a mesoporous nanomaterial integrated in a bioanalytical microsensor with electrochemical detection for the determination of mycotoxin T2 in samples of agri-food interest

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Abstract

An ordered mesoporous material (OMM) type SBA-16 was synthesized from sol gel type reactions and the self-assembly of surfactants. Once SBA-16 was obtained, it was functionalized with APTES (3-aminopropyltriethoxysilane) on the one hand and with MEA (monoethanolamine) on the other. Subsequently, the characterization of these nanomaterials was carried out using Scanning Electron Microscopy (SEM), Energy Disperse Spectroscopy (EDS), Infrared Spectroscopy (FTIR), N2 adsorption-desorption and X-ray Diffraction (XRD). In addition, a biosensor was developed where the central channel was modified with the material obtained SBA-16 (MEA) and a commercial ELISA Kit (enzymatic immunoassay) was used for a quantitative determination of the mycotoxin T-2 and compared with the analytical results of our generated biosensor.

Keywords: nanomaterial, biosensor, agroalimentario, contaminación, toxina T2

Introduction

In recent years, conventional analytical techniques have experienced a marked trend towards miniaturization [1]. The development of bioanalytical sensors since its inception has been focused primarily on the environmental, chemical, biochemical, pharmaceutical, agri-food fields, among others, because their use entails innumerable advantages such as: high sensitivity, selectivity, reproducibility, low cost for small volumes of sample and reagents required, short analysis times and, fundamentally, the detection limit can be reduced by modifying the sensor electrode with silica nanomaterials as a platform in order to amplify the analytical signal, this being a possible innovation in the development of sensors endowed with a larger effective electrochemical area. With which in the present work a new specific, sensitive and fast analytical methodology is proposed, which through the use of nanomaterials in bioanalytical sensors, allow the quantitative evaluation of an analyte of agri-food interest such as mycotoxin T2, which as a contaminant It produces serious complications for human health [2-4].

Materials and methods

The SBA-16 material was synthesized from the sol-gel process [5], once obtained we functionalized it with APTES (3-aminopropyltriethoxysilane) and MEA (monoethanolamine).

Functionalization of the SBA16 with APTES

The functionalization of the MMO SBA-16 with APTES (3-aminopropyltriethoxylan) is carried out by reflux where the SBA-16 obtained is dried for 24hs at 80°C, later we add 1g of SBA-16, 1ml of APTES, 100ml in a round bottom flask of toluene we leave it for 24hs under agitation at 90°C. After 24hs, the supernatant solid is washed with toluene and then dried for 24hs at 80°C in an oven.

Functionalization of the SBA16 with MEA

This functionalization process is carried out in a 50ml beaker where we add 10ml of methanol, 0.42ml of MEA (monoethanolamine), mix for 0.5h, then deposit 1g of SBA16 in the solution, mix for 60min, the solid obtained is washed with methanol and dried for 24hs in the oven at 80°C.

Characterization of the materials obtained

Scanning Electron Microscopy (SEM)

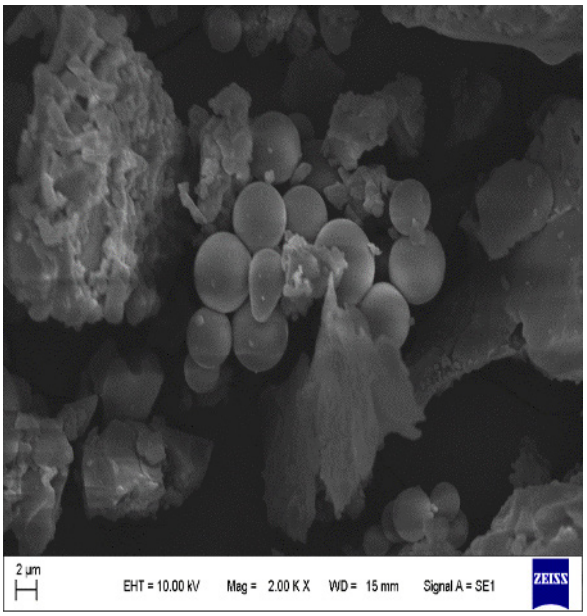

Figure 1. SEM micrographs of SBA-16 materials

In the figure we can see the SEM micrographs of the mesoporous silica SBA-16 where it can be seen that the particles of said material have spherical shapes of very uniform size and the rest of the material that does not have a spherical shape is part of the material that was not functionalized [7].

Energy Dispersive Spectroscopy (EDS)

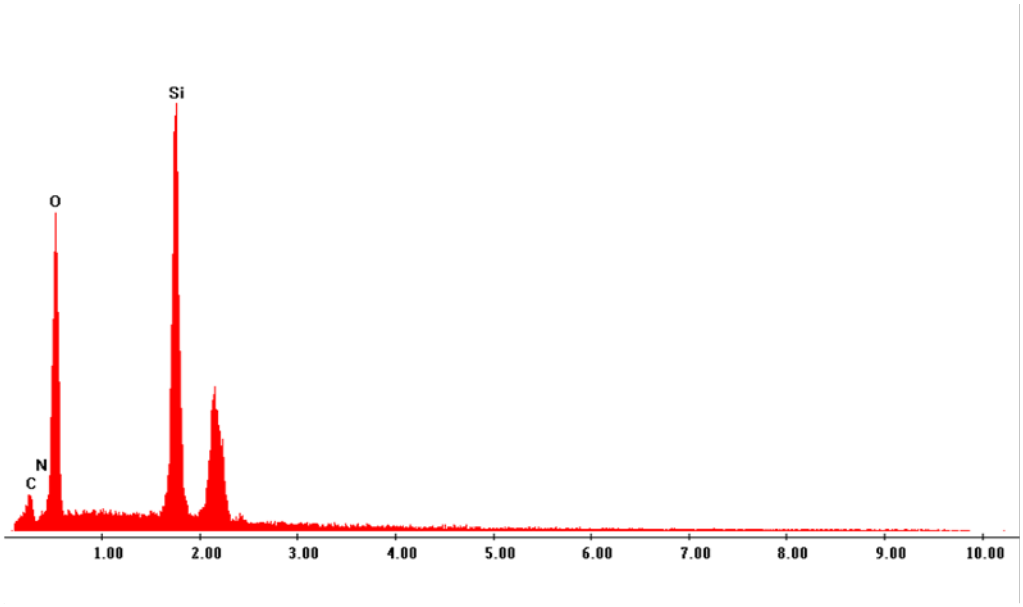

Figure 2. Chemical analysis by EDS of the material SBA-16

Figure 2 shows the spectrum of microanalysis carried out on the functionalized and impregnated SBA-16 material. As you can see, there is the presence of the chemical elements that are silicon and oxygen indicating that this material is SiO_2.

Adsorption-desorption of N_2 at 77K

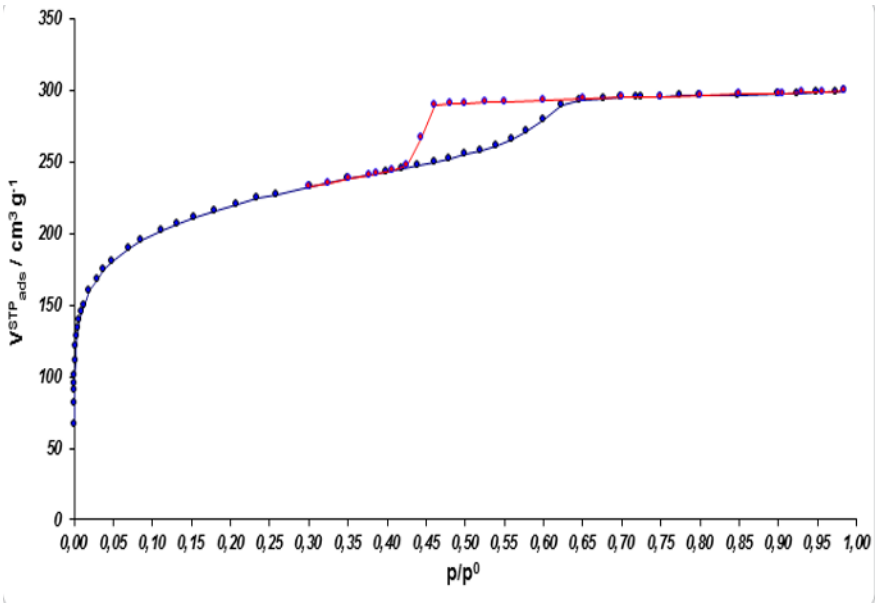

Figure 3. Isoterma de adsorción-desorción de N_2 de la SBA-16

Figure 3 shows the experimental N_2 adsorption-desorption isotherm at 77 K of the SBA-16 material under study. A type IV isotherm is observed, indicating that it is a mesoporous material. In addition, the presence of an H2 type hysteresis loop can be observed, indicating that this material presents an important degree of ordering of its pores, which are characteristic of this type of material [8].

Pore size distribution (PSD)

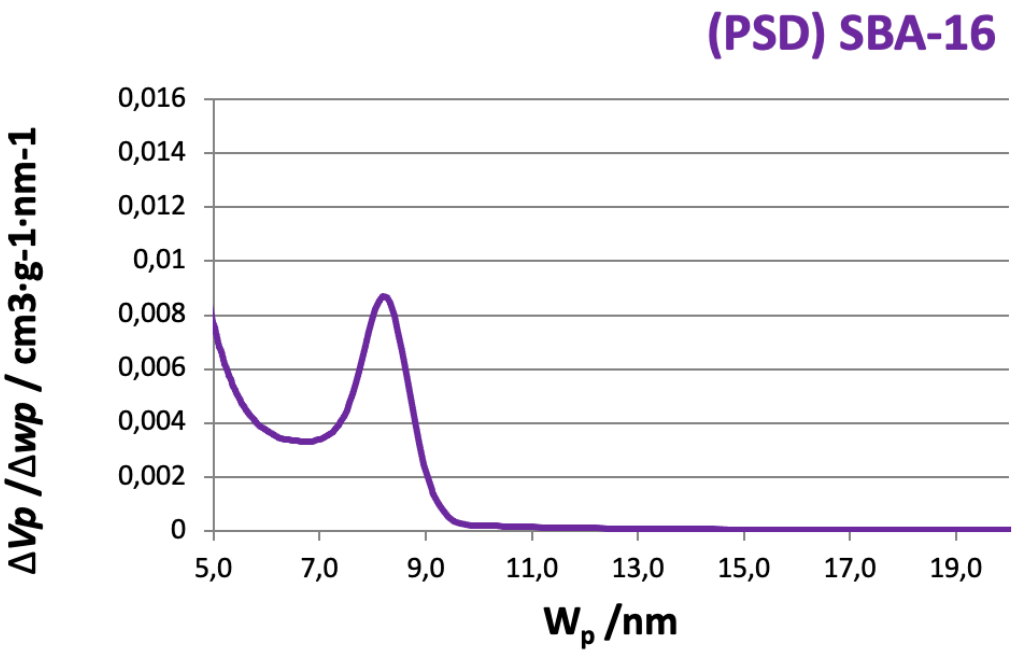

Figure 4. Pore size distribution of the SBA-16 material.

Figure 4 shows the pore size distribution of the MMO SBA-16 that was obtained using data from the adsorption branch. Said pore size distribution presents a uniform distribution of mesopore sizes, centered approximately at 8.1nm.

Infrared analysis by Fourier transform. (FTIR)

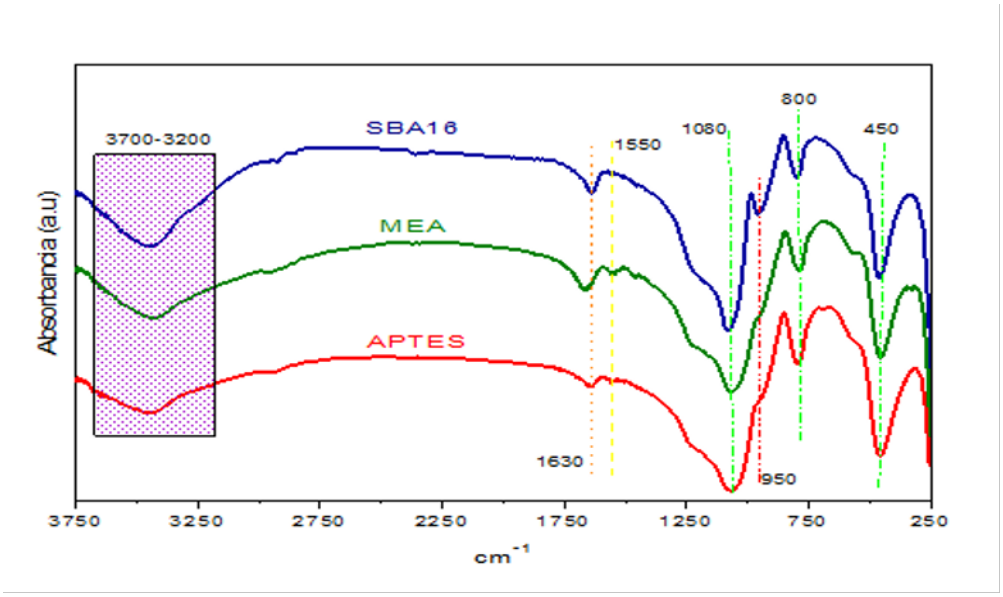

Figure 5. Infrared Spectrum of pure SBA-16 functionalized with MEA and APTES

Figure 5 shows the IR spectrum of the samples under study, where it can be seen that the stretching band of the bond of the Si-OH group (silanol group) located at 950cm-1 decreases after SBA-16 was functionalized with APTES and with MEA, indicating that in the functionalized materials the amino groups could be anchored in the –OH groups of the silanols.

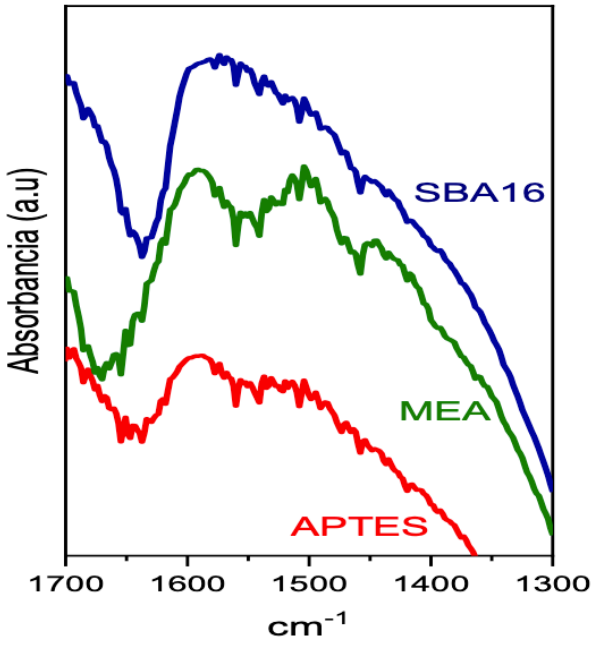

Figure 6. Representation of the band spectrum of the NH_2 amino groups.

The previously mentioned can also be observed in figure 6, since this spectrum (which is only from the region where the bands of the amino groups appear) we can observe that the functionalization of SBA-16 with MEA and APTES has been effective. due to the fact that in the functionalized materials the 1550cm-1 band appears, which is characteristic of amino groups $–NH_2$, where the one with the greatest intensity of said band is the SBA-16 functionalized with MEA, demonstrating a greater anchorage of amino groups with said reagent [6].

Modification of the surface of the central channel

In the bioanalytical stage, we developed a microfluidic immunosensor [5] in which the central channel (CC) was modified with the functionalized material SBA-16-MEA. This material was chosen as the nanoplatform since it presented a higher band intensity in FTIR and this confirms that more groups (NH2) were anchored in the SBA-16.

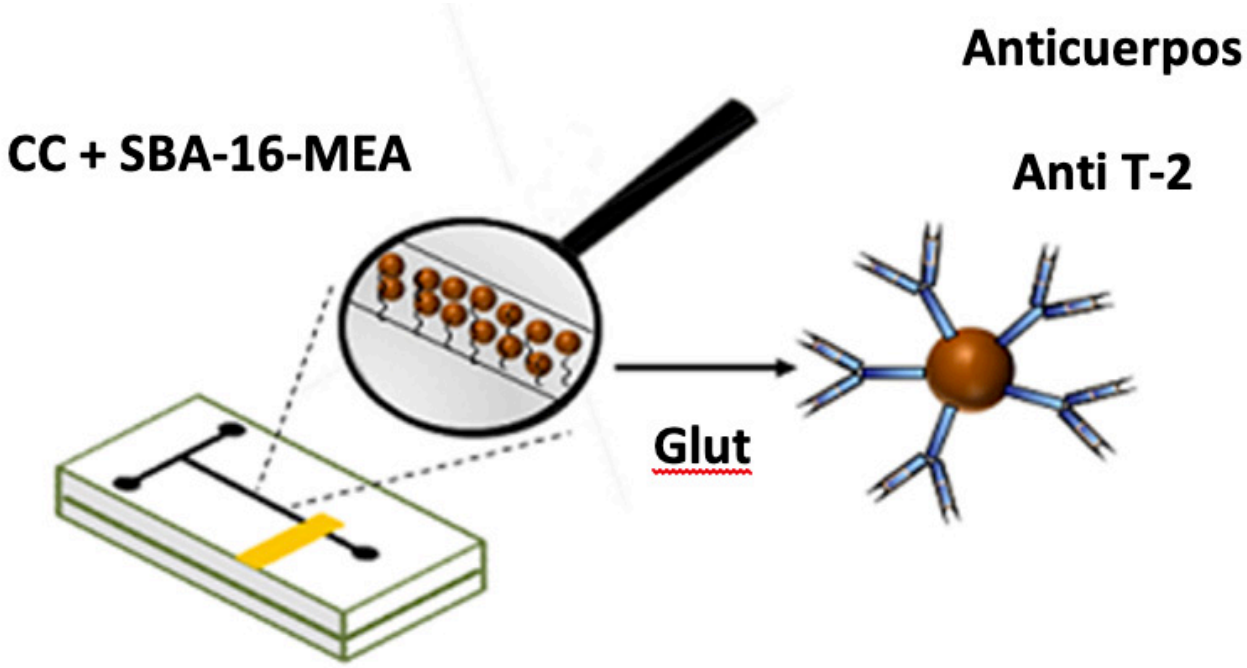

Figure 7. Central channel (CC) modification representation

Determination of mycotoxin T2 by commercial Elisa

A series of standards covering a concentration range from 0 to 400 µg kg-1, were provided by the RIDASCREEN®FAST ELISA Kit for T2 toxin. Thus, a calibration curve was built following the manufacturing protocol for spectrophotometry. The T2 toxin concentrations of the samples were determined using this commercial ELISA Kit and were detected spectrophotometrically by measuring the absorbance changes at 450 nm.

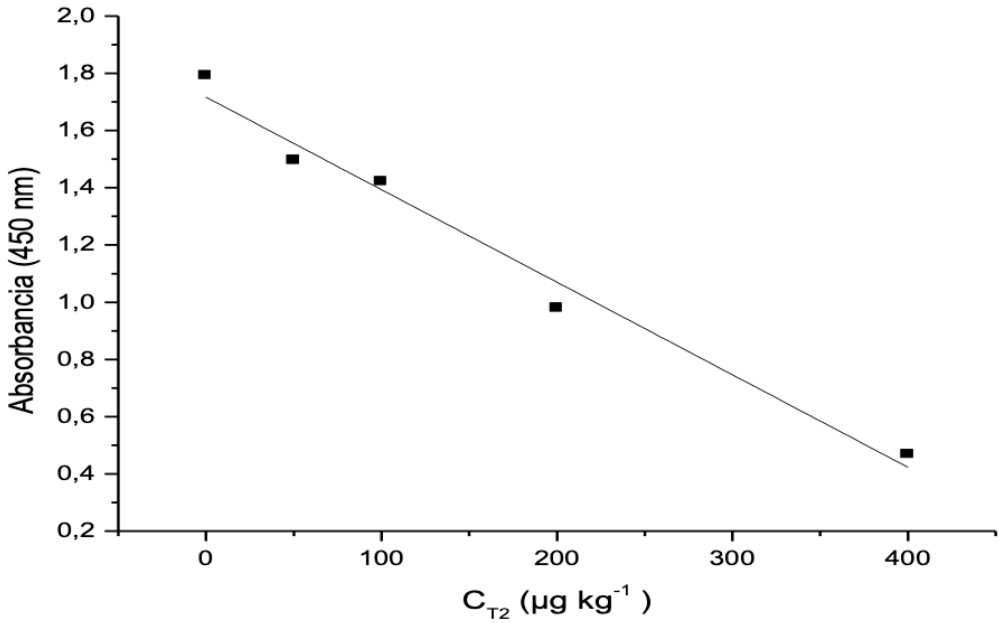

Figure 8. Calibration curve for the determination of T2 toxin by ELISA

In addition, the commercial ELISA was performed following the manufacturer's instructions. The absorbance changes versus the corresponding T2 toxin concentration were graphically represented in Figure 8. The linear regression equation was: A= 1.71 – 0.003* CT2 , with a linear regression coefficient r = 0.985 and a CV for the determination of 100 µg kg-1 of 6.78% T2 toxin (six replicates).

Determination of T2 toxin by amperometric analysis

A calibration curve was obtained to predict the concentration of T2 toxin present in the sample, which is linear in a concentration range of 0-400 µg kg-1. For its construction, the T2 toxin standards provided by the commercial ELISA Kit were used. The linear regression equation was: i = 214.50 – 0.46* CT2, with a linear regression coefficient r = 0.998, figure 9. The coefficient of variation (CV) for the determination of 100 µg kg-1 of T2 was less than 4 % (six replicates) with a Detection Limit of 0.05µg Kg-1. These results demonstrate that our microfluidic immunosensor can be used to quantify T2 toxin in unknown samples.

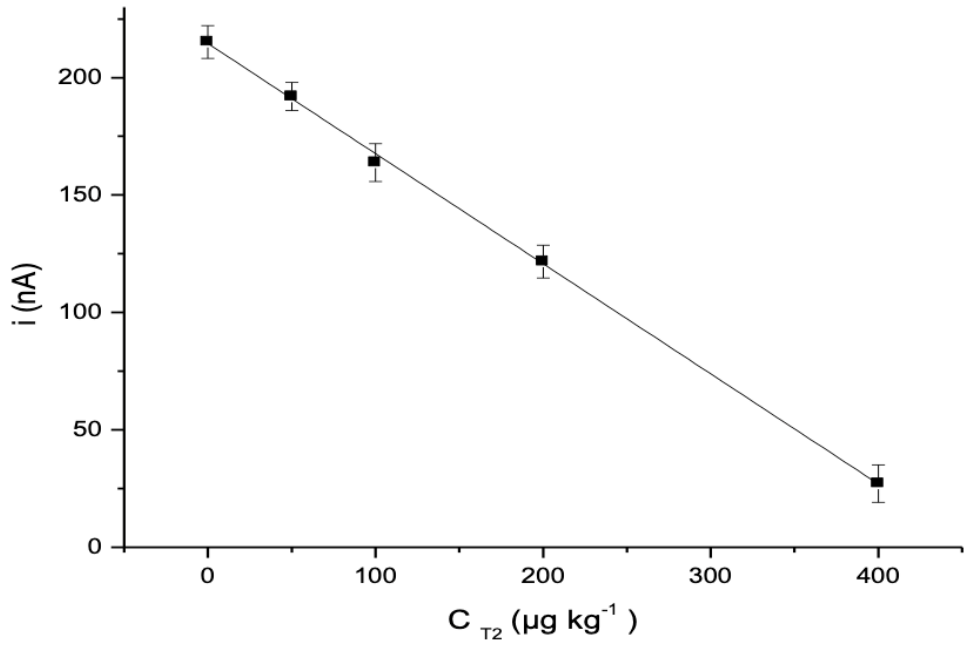

Figure 9. Curva de calibración para la determinación de T2 por el método amperométrico

Correlación con el método de ELISA comercial

The developed method was compared with the commercial spectrophotometric method for the quantification of T2 toxin in 20 samples of agri-food interest. The slope obtained was reasonably close to 1, indicating a good correlation between both methods Figure 10. Compared with the commercial ELISA, our method shows a significant increase in sensitivity, and this sensitivity is high enough to determine T2 toxin in unknown samples with very low levels of mycotoxin

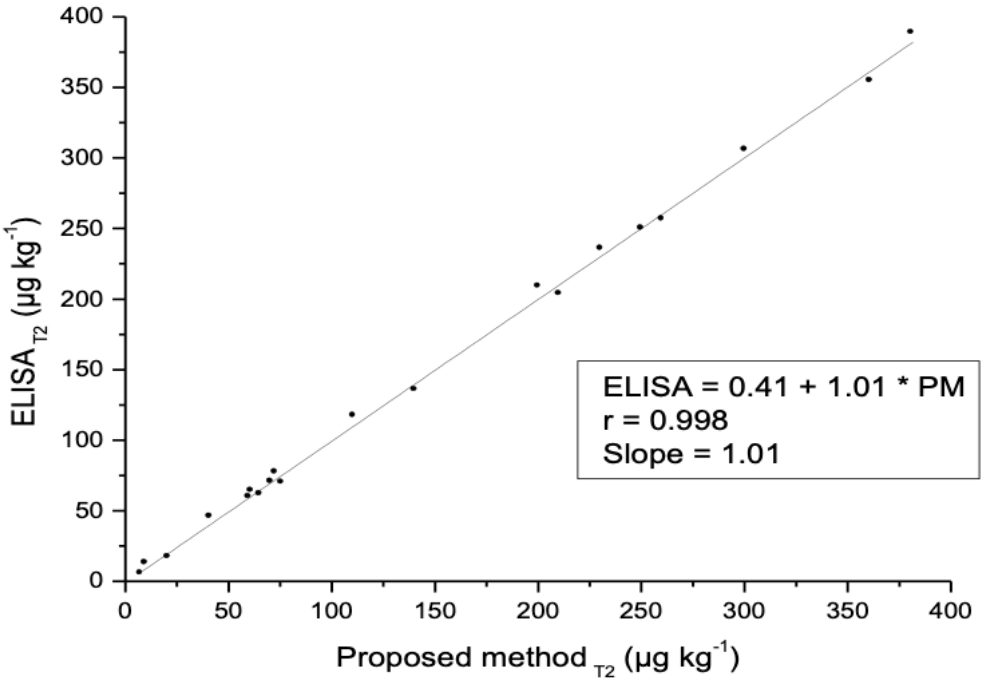

Figure 10. Correlación entre el método propuesto y el ensayo ELISA comercial

Conclusions

In this work, a microfluidic immunosensor coupled to a flow injection system with amperometric detection was developed to rapidly, sensitively and selectively quantify T2 toxin in samples of agri-food interest. The use of the selected nanoplatform modified with monoclonal anti-T2 antibodies allowed a significant increase in sensitivity without reducing selectivity, this being an important advantage. The increase in the reactive surface and the reduced diffusion distances of the present device allowed a total analysis time of 21 min, which was less than the time reported for the commercial ELISA Kit (40 min). In addition, this methodology, being a microfluidic-based device, minimized the cost of expensive reagents, showed physical and chemical stability, low background currents, a wide range of work potential and accuracy. Finally, we can propose this device as a possible analytical tool for its application in the agri-food industry in order to guarantee the safety and quality of these foods, as well as the health of consumers.

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Bios

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Alex Simioli carries out research in the field of volatile organic compounds (VOCs) and particulate matter, all related to atmospheric pollution and its determination using gas chromatography and mass spectrometry techniques. He has a master's degree in chemistry since 2022 from the National University of San Luis in which his final work was the determination of T2 toxin in samples of agri-food interest from a Biosensor. He has been a Chemical Analyst since 2015 from the National University of San Luis and since 2014 he has worked in the Environmental Measurements Laboratory at the Chemical Technology Research Institute (INTEQUI), which depends on the National University of San Luis and CONICET.