

TESTING OF NANOPOROUS POLYMER MEMBRANES AS SMART DEVICES FOR MYCOTOXINS DETECTION

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Abstract

Mycotoxins are naturally toxic compounds produced by fungi. Their toxicity occurs at very low concentrations, therefore there is a need of sensitive and reliable methods for their detection. Along with conventional methods nanomaterials are able to be functionalized with various biomolecules such as enzymes, antibodies, nucleic acids, DNA/RNA aptamers that make them suitable for detection of various substances such as food toxins, bacteria, and other compounds important in food and feed analysis.

Track-etched polyethylene terephthalate (PET) membranes with pore size between $700 \pm 45\text{nm}$ and without being modified on pore surface were tested for fumonisins and aflatoxins detection. The detection principle was based on the diffusion process of mycotoxins in the analyte. The challenge was to see if the mycotoxins molecules will pass in the pores and catch to the non modified inner walls. The polymer membranes before and after immersing in the mycotoxin solution were characterized by Scanning Electron Microscopy (SEM) and Fourier-Transform Infrared Spectroscopy (FTIR). New experimental protocol allows to obtain an information about the membranes tested if they could play a role as smart detectors for mycotoxins without any surface immobilization.

Key words: *Fourier-Transform Infrared Spectroscopy, mycotoxins, nanoporous membranes, nanobiosensors, Scanning Electron Microscopy*

Introduction

Mycotoxins are low molecular weight secondary toxic metabolites produced by fungi. The most important genera are *Aspergillus*, *Fusarium*, *Penicillium* (Creppy et al., 2002). As natural contaminants, they occur in many feed matrices like wheat, barley, maize, beans, as well as oilseeds.

Guan et al. (2011) reported about 98% of the agricultural commodities, including corn, compound animal feeds, silage, cornmeal, puffed corn, wheat, bran, soybean, meal, rapeseed meal, cottonseed meal and whole cottonseed content various group of mycotoxins.

The presence of mycotoxins in feed has severe implications on animal health even at very low levels, due to their mutagenic, teratogenic, carcinogenic, nephrotoxic, and immunosuppression effects (Fernandez et al., 2017).

Currently the most used methods are analytical methods but they have some disadvantages as time consuming, expensive and gather adequate information on the levels of exposure to mycotoxins and to assess the relevant toxicological risk for humans and animals.

Consequently, sensitive and accurate methods of analysis are needed to detect even low concentrations of mycotoxins (Pascale and Visconti, 2008).

Comparing with the traditional analytical methods used for mycotoxins analysis, the main advantages of biosensors include the fast analysis time and rapid detection, high sensitivity, easy sample preparation, and low cost.

Nanomaterials such as gold, silver, metal oxides and quantum dots have been extensively employed for enhancing the detection capability of biosensors due to their remarkable optical, electronic, thermal, and mechanical properties, allowing the increase of their sensitivity, stability, and selectivity towards mycotoxins (Santos et al., 2019).

The development of biosensors for mycotoxins has risen sharply in the last decade with a large number of different bio-sensing technologies applications.

The aim of the present study was to test nanoporous PET membranes if without any surface modification they could be a future candidate for mycotoxins detection.

Materials and methods

The nanoporous templates used in this work were prepared by track-etched technology where the polymer membranes (thickness 12 microns) were irradiated with swift heavy ions at GANIL accelerator (Caen, France). The irradiated membranes were chemically etched in a way to create nanochannels (pores) along the ion-tracks using alkaline solutions. They were immersed in a 2N aqueous NaOH solution at 80°C for 20 min. Thereafter, were rinsed with DI water and dried in air in order to evaporate residual water from the pore channels. After etching, some of the samples were sputter-coated with a thin layer of gold and inspected by SEM (TESCAN/LYRA). In parallel, each irradiated and chemically etched sample was measured by FTIR.

The etched membranes contain randomly distributed cylindrical pores of uniform diameter (700 ± 45) nm based on SEM with a pore density of 5.0×10^8 pores/cm². Within experimental error, the number of etched pores (pore density) is equal to the fluence of heavy ions used initially to irradiate the polymer foils.

The developed nanoporous membranes were immersed directly in fumonisins standard solution (0,666 mg/l) and aflatoxins standard solution (5 µg/kg), R-Biopharm, Darmstadt, Germany. And as well as the same type etched membranes were tested in the anti-fumonisin antibodies solution (R-Biopharm, Darmstadt, Germany) and in anti-aflatoxins antibodies (R-Biopharm, Darmstadt, Germany). In all the solutions, they stay for 2 hours. After that, the membranes were examined by SEM and FTIR.

Results

The developed track-etched nanoporous membranes were tested whether they can play a role as nanobiosensors for mycotoxins detection by diffusion process and without any surface modification concerning immobilization of antibodies or other chemical structures. In Figure 1 (a and b) are shown SEM image and FTIR spectrum (4000-1000 cm^{-1}) of chemically etched PET.

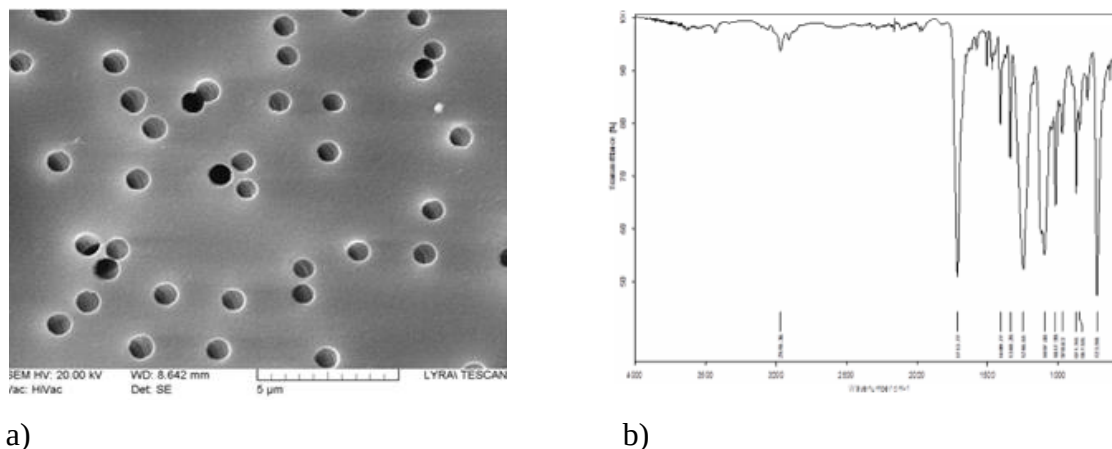


Figure 1. SEM image (a) and FTIR spectrum (b) of nanoporous PET membrane obtained by etching solution of 2N NaOH, 20 min, at 80°C.

The peaks in FTIR spectra give an information about molecular bonding structure or functional groups existing in the material. As it is known (Thomson and Maragos, 1996) to capture a mycotoxin molecule is necessary the presence of antibody or modification with other chemical agents. In our case, the nanoporous membranes were not chemically modified or coated with capture antibodies. The challenge here was to see if it is possible the molecule of mycotoxin to catch on the inner wall of the nanopore just by diffusion process.

In the first scenario, the membranes were immersed in aflatoxins and fumonisins standard solutions at concentrations 5 $\mu\text{g}/\text{kg}$ and 0,666 mg/l respectively. After two hours stay, the membranes were examined by SEM and IR spectroscopy. The cross section of SEM micrograph, Figure 2a, shows that any object did not pass and caught to the inner walls of the pores. This is confirmed by Infrared spectroscopy measurement (Figure 2b), which shows that there are no changes in the band intensity or a new band which corresponds to aflatoxin structure appears after immersing of the membrane in the mycotoxins solutions.

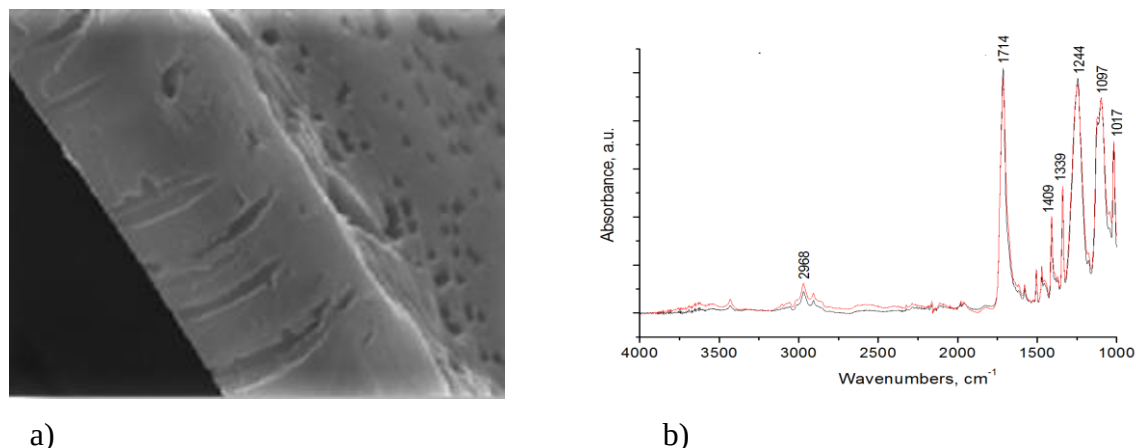


Figure 2. SEM micrograph (a) - cross section and FTIR spectrum (b) of non surface modified PET membrane tested for aflatoxin detection

This could be because the structure of mycotoxin's molecule makes it difficult to pass just by diffusion or because the membrane is not attractive without being immobilized with biorecognition objects as antibodies. The results for fumonisin standard solution were similar with those for aflatoxin standard solution, Figure 3a and b.

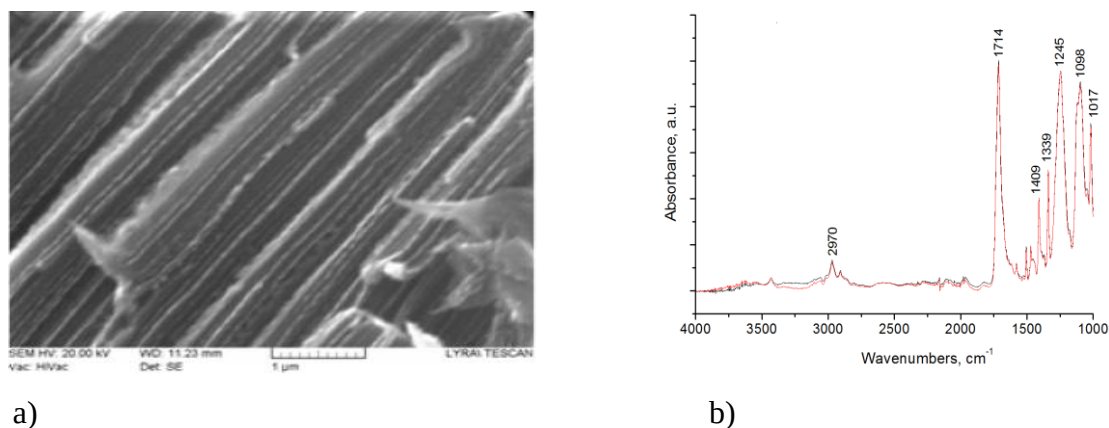


Figure 3. SEM micrograph (a) - cross section and FTIR spectrum (b) of non surface modified PET membrane tested for fumonisin detection

In order to continue with a test and to see if the membranes could play a role as nanotrappers for antibodies molecules, the membranes were immersed in anti-fumonisin antibodies and anti-aflatoxin antibodies for 2 hours. The results got by SEM and FTIR spectroscopy were similar to the previous one concerning the mycotoxin's diffusion. Actually, the micrographs images and Infrared spectroscopy can provide true information whether or not some objects are present in the pores.

Even that membranes stay a long time immersed there is no molecules hung on the inner walls of the nanopores. This scenario shows that besides the mycotoxin molecule did not enter in the nanopore and anti-mycotoxin antibody did not enter too. From these results, it becomes clear that a direct competitive assay will not be happened without antibody implementation in order to catch the mycotoxin molecule.

Along the classical methods for mycotoxins detection, the biosensors based on nanostructured materials are more hopeful because of less time consuming for analyses, rapid detection, and mobility.

In Table 1 are summarized the used methods for mycotoxins analyses with their advantages and disadvantages.

Table 1. Presentation of the most applicable methods for analysis of mycotoxins

Type of method	Pros	Cons
GC	Good sensitivity, simultaneous analysis of multiple mycotoxins Selective and sensitive detection	Derivatization required, expensive equipment, specialist expertise required
TLC	Less equipment are used, simple and sensitive method, not expensive	The sample stay in open place, poor precision and sensitivity
LC/MS	No derivatization required, simultaneous analysis of multiple mycotoxins, low detection limits	Specialist expertise required, expensive equipment
HPLC	Good sensitivity, automated by autosampler, short time analyses, good repeatability	Expensive equipment, derivatization required, specialist expertise required
ELISA	Rapid sample screening, limited use of organic solvents, inexpensive equipment, high sensitivity, simple sample preparation	Possible false positive/negative results, cross-reactivity with related mycotoxins
Nanobiosensors	No clean-up procedure, rapid analysis, detection of very small amounts of a chemical contaminant, virus, or bacteria in agricultural and food systems	Extract clean-up to improve sensitivity, cross-reactivity with related mycotoxins

Discussion

It is interesting to mention that the same type of nanoporous polymer membranes previously have been used as templates for nano objects synthesis by diffusion process (Sertova et al, 2006). However, when expected the same membranes to play a role as antigen trappers the principle is not the same without antibody immobilization.

In conformation of that is Thomson and Maragos (1996) research experiment. They developed fiber-optic immunosensor to measure the mycotoxin fumonisin B1 (FB1). Monoclonal antibodies produced against FB1 were covalently bound through a heterobifunctional silane to an etched 800 μm core optical fiber. An evanescent wave effect was utilized to excite fluorescein isothiocyanate labeled FB1 (FB1-FITC) molecules near the surface of the fiber. A direct competitive assay was used to measure FB1 concentrations with the following steps: saturation of antibody binding sites by FB1-FITC, competition of FB1 and FB1-FITC with displacement of the labeled toxin, and resaturation of binding sites by FB1-FITC. The signal generated in the assay was found to be inversely proportional to the FB1 concentration. This sensor exhibited a working range of 10–1000 ng of FB1/mL, and a limit of detection of 10 ng/mL.

Concerning the possibility of mycotoxins detection Kudr's et al. (2006) in their work reported about HT2 mycotoxin detection. They developed microelectrodes and used it as an immunoenzymatic biosensor for the detection of HT-2 mycotoxin based on carbodiimide linking of the microelectrode surface and HT-2 toxin antigen-binding fragment of antibody. This type biosensor showed detection limits of 1.6 ng mL^{-1} .

In this connection, it is interesting to mention that Rovina et al. (2019) in their work developed a third-generation enzymatic biosensor 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. A third-generation enzymatic biosensor to quantify STEh in corn samples spiked with the mycotoxin. The biosensor was based on 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 biosensor showed a linear response in the concentration range from 6.9×10^{-9} to $5.0 \times 10^{-7} \text{ mol L}^{-1}$. The limit of detection was $2.3 \times 10^{-9} \text{ mol L}^{-1}$.

The nanotechnology has a contribution in the construction of different types of biosensors, which helps of fast and sensitive analyses of mycotoxins. Upgraded, more sensitive, more accurate and rapid mycotoxins biosensors are still required.

In this connection, our attention is focused on polymer nanoporous membranes as new alternative devices for fast detectors of mycotoxins.

Conclusion

The results show that the tested nanoporous polymer membranes without any surface modification can not play a role as nanotrappers for mycotoxins.

Immobilization of biorecognition molecules as specific mycotoxins antibodies onto membrane surface is needed for antigen/antibody reaction to happen.

The obtained information will help us in our future research investigations. It has to keep in mind that there is a need to change the methodology. As well as to test other kind of nanoporous membranes in a way to improve their properties to analyze very small quantities of mycotoxins.

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