EFFICIENCY OF MESOPOROUS SILICA NANOPARTICLES ON AFLATOXIN B₁ ADSORPTION

G.D. Savi; E.T. Zanoni; K.C. Piacentini; B.G. Furtado; T. Bortolotto; E. Angioletto

1 - Iparque: Parque Científico e Tecnológico - Universidade do Extremo Sul Catarinense (UNESC) - Rod. Gov. Jorge Lacerda - 88806-000 - Santa Catarina - Brasil. Phone: (48) 99958-2419 - Fax: (48) 3444-3709 - Email: geovanasavi@gmail.com
2 - Departamento de Biotecnologia - Universidade de São Paulo (USP) - Av. Professor Lineu Prestes - 03178-200 - São Paulo - Brasil. Phone: (11) 94382-1104 - Email: karim.piacentini@usp.br
3 - Departamento de Ciências da Saúde - Universidade Federal de Santa Catarina (UFSC) - Jardim das Avenidas - Araranguá, 88906-072 - Santa Catarina - Brasil. Phone: (11) 99975-3792 - Email: tiago.bortolotto@gmail.com

ABSTRACT: Food contaminants such as mycotoxins represent a considerable risk to human and animal health. The development of methodologies that allow controlling the presence of these hazardous contaminants is of special relevance. In this sense, mesoporous silica nanoparticles (MSN) are important because of their interesting structural features and the potential applications, including adsorption process. Furthermore, the silica matrices are nontoxic and biocompatible for biomedical or food research. Considering that the most applied method for protecting animals against mycotoxicosis is the utilization of adsorbents, this study aims to prepare and characterize new MSN and evaluate their effectiveness on the adsorption of aflatoxin B₁ (AFB₁). The MSN was duly characterized by analytical techniques and they were significantly able to AFB₁ adsorption (89%) in the higher concentration (2 mg/mL). Due to strong effects on mycotoxin adsorption, could be studied as an effective agents for agriculture and food safety applications.

KEYWORDS: mesoporous silica; nanoparticles; fungi toxin; adsorption.

1. INTRODUCTION

Mycotoxins are poisonous chemical compounds produced by toxigenic fungi. Some of them are regularly found in foods and animal feedstuffs, such as grains and seeds. They are associated with disease crops, causing serious problem in many parts of the world, including significant economic losses (FAO, 2017). Mycotoxins have great significance in the health of humans and livestock have longer term chronic a cumulative effects on health. For example, aflatoxin B₁ (AFB₁) is the predominant and most potentially mutagenic, teratogenic and hepatocarcinogenic according to the International Agency for Research on Cancer (IARC, 1993). They are produced mainly by Aspergillus flavus, A. parasiticus and A. nomius strains and have been usually found in storage grains (Roigé et al., 2009; Riba et al., 2010).

Considering that food safety is an issue of special concern in the current society in which millions of people should be fed under adequate conditions, the development of methodologies that allow controlling the presence of these hazardous contaminants in food is of special relevance for the scientific community (Soeas-Rodríguez et al., 2017). In this sense nanotechnology and particularly the use of nanomaterials has emerged as an interesting via for achieving this purpose.

Nanomaterials with engineered features such as shape, size, composition and function, play an important role in diverse application areas (Vallet-Regí et al., 2007; Vallet-Regí and Balas, 2008;
Janatova et al., 2015; Sucas-Rodríguez et al., 2017). Among the different nanomaterials described in the literature, silica mesoporous materials have been used as inorganic scaffolds for the storage and release of drugs and organic molecules (Kresge et al., 1992; Janatova et al., 2015). These materials provide unique feature such as stability, biocompatibility, large load capacity (Munoz et al., 2003; Schmaljoham, 2006; Vallet-Regi et al., 2007). In addition, they are research biomedical purposes, since are excellent candidates for controlled drug-delivery systems (Vallet-Regí and Balas, 2008).

Silica nanomaterials have several important properties that make them a unique matrix for incorporating functional components. The high porosity of amorphous silica nanoparticles provides the three dimensional space required for the doping of functional components. Furthermore, the silica matrices are nontoxic and biocompatible for biomedical or food research (Frujtier-Pölloth, 2012; Bharti et al., 2015).

Current study shown the mesoporous silica nanoparticles (MSN) used to selective detection of mycotoxins in food (Ribes et al., 2017), however, its adsorption capacity on these toxic compounds yet has been not studied. Considering that the most applied method for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastrointestinal tract (Huwig et al., 2001), the MSN could be to studied with this proposes.

Therefore, this study aims to prepare and characterize MSN and evaluate their effectiveness on the adsorption of AFB1.

2. MATERIALS AND METHODS

2.1. Synthesis and Characterization of Mesoporous Silica Nanoparticles

Synthesis of MSN was performed by sol-gel process using an erlenmeyer flask. Briefly, it was mixed 1 g of cetylamonium bromide in 480 mL of water and 3.9 mL of NH4OH. The mixture was heated at 80°C and stirring by 2 h. Then, 5 mL of tetraethyl-orthosilicate was added dropwise. After filtration and washing with water and ethanol, the surfactant was removed by acid extraction (0.5 M HCl in ethanol), the product was filtered and washed by 3 times with 100 mL of water and with 50 mL of ethanol and dried at 100°C (Doadrio et al., 2017).

The MSN was characterized by the X-ray diffraction (XRD) patterns obtained using a X-Ray diffractor from Shimadzu (model XRD-6000, radiation Cu Kα λ=0.15405 nm), Fourier Transform Infrared Spectroscopy (FT-IR) from Shimadzu (model IR Prestige, KBr pellets)and transmission electron microscopy (TEM) from Jeol (model JEM-2100).

2.2. Adsorption Assays

Adsorption of AFB1 mycotoxin was assessed by high performance liquid chromatography (HPLC) using a modified method described by Nones et al. (2015). For this purpose, the samples contained only Mili-Q water or MSN in the concentrations of 0.6, 1 and 2 mg/mL. Then, it was added to AFB1 (previously extracted from the A. flavus) reaching out a final mycotoxin concentration of 0.05 µg/mL. Samples were stored overnight and then centrifuged (Eppendorf AG 22331) at 4000 rpm for 30 min. The assays were performed in triplicates. The amount of adsorbed AFB1 was determined in the supernatant, which was directly injected (20 µL) into a HPLC system (Nones et al., 2015).

The HPLC system (Shimadzu, Kyoto, Japan) is equipped with an isocratic pump (LC-20AT), column oven (CTO-20A), prominence communication bus module (CBM-20A), degasser (DGU-20A), autosampler (SIL-20A) and a fluorescence detector (FLD) (RF-2000). The HPLC/FLD system was set at an excitation wavelength of 360 nm and an emission wavelength of 440 nm for AFB1 detection. Chromatographic separations were performed on a C18 reversed-phase column (150 x 4.6 mm, 5µ), ACE (Scotland, UK, Europe). The mobile phase consisting of water:methanol:acetonitrile (600:200:200, v/v/v) was added to 119 mg potassium bromide and 47.6 µL nitric acid and delivered in a constant flow rate of 1 mL/min. The total analytical run time was 14 min for AFB1.

Considering linearity of the AFB1 standard, a five-point calibration curve was constructed with the following concentrations 0.005 to 0.500...
μg/mL, which showed a correlation $R^2$ of 0.998. Quantification of AFB$_1$ levels was performed by measurement of the peak area at AFB$_1$ retention time compared with the standard solutions used for the calibration curve.

2.3. Statistical Analysis

Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni post-test, using GraphPad Prism 5.0 software. The $p$-values $<0.05$ were considered statistically significant. The experiments were performed in triplicates and the results were calculated as a mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

MSN crystallinity analyzed by XRD display their amorphous nature observed by the wide band of the region of 18° to 30°, without crystalline phases of quartz (Figure 1). At low angle, MSN presents diffraction due to the mesoporous hexagonal organization (Mizoshita and Tanaka, 2017).

![Figure 1. X-ray diffraction plot of MSN.](image1)

The effects on the adsorption of AFB$_1$ by MSN can be seen in Figure 4. The MSN were significantly ($p<0.001$) able to AFB$_1$ adsorption in all concentrations.

Figure 2. FT-IR spectra of MSN.

The infrared spectrum of MSN (Figure 2) shows vibrations in the region of 3451 cm$^{-1}$ and 958 cm$^{-1}$ assigned to the asymmetric stretching vibration of the Si-OH bond, indicating large amounts of silanol groups. In 1645 cm$^{-1}$ is related the presence of adsorbed water on the nanoparticle surface and in 1089 cm$^{-1}$ Si-O-Si are associated asymmetric stretching vibrations, confirming formation of the silica matrix during the synthesis step (Musić et al., 2011; Zulfiqar et al., 2016).

![Figure 2. FT-IR spectra of MSN.](image2)

Figure 3. Morphology of MSN showing pores on its surface. Scale bar = 100 nm.

The MSN resulted in small particle size analyzed by TEM, as shown in Figure 3.
Figure 4. Aflatoxin B$_1$ adsorption capacity of MSN at 0.6, 1 and 2 mg/mL concentrations (data expressed as mean ± SD; statistically significant when compared to control group with ***p<0.001).

At 2.0 mg/mL concentration, MSN showed adsorption capacity for AFB$_1$ of 89%, whereas at 1 mg/mL and 0.6 mg/mL exhibited 76% and 62% of adsorption, respectively.

The MSN with entry channels wide enough to permit the diffusion of aflatoxin molecules (sizes about 5.18 Å) to the intracrystalline structure are capable of demonstrating a clear sequestering effect. According to knowledge to authors, there are not studies that evaluation the effects of MSN on mycotoxins adsorption. However, recent studies have evaluated the MSN as adsorption agents for other purposes. In study of Li et al. (2017), siliceous hydrophobic nanoparticles were fabricated within the mesoporous channels, in order to enhance the efficiency of adsorbing tobacco specific N-nitrosamines in aqueous solution. Kachbouri et al. (2017) revealed that synthesized silica sphere nanoparticles are a promising material for removing dye from aqueous solutions and suggest still that the adsorption of dyes onto synthesized silica sphere nanoparticles is due to the chemisorption process (Boparai et al., 2011).

Due its porous surface, they may incorporate functional components that add advantage to material, as controlled drug-delivery systems or antimicrobial agents. In this sense, new MSN functionalized with Schiff base and Ni$^{2+}$ and Cu$^{2+}$ complexes have been evaluation on pathogenic bacteria. The results of the growth curve of treated bacteria showed that MSN-SB-Ni had bactericidal effect against S. aureus while MSN and MSN-SB-Cu had only inhibitory effect and caused growth retardation of this bacterium. In case of E. coli, MSN, MSN-SB-Ni and MSN-SB-Cu were bacteriostatic and caused reduction of bacterial growth (Tahmasbi et al., 2018).

In our study, the MSN showed strong effects on mycotoxin adsorption, therefore, could be studied as an effective agent in adsorption for agriculture and food safety applications, as for example, feed additive.

4. CONCLUSION

Herein, MSN were examined in adsorption capacity of AFB$_1$. At concentrations from 0.6 mg/mL indicate strong potential of this material as new agents with adsorption capacity of fungal toxin.

5. REFERENCES


6. ACKNOWLEDGEMENTS
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