

# Organically Modified Clays for Uptake of Mycotoxins

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## INTRODUCTION.

Mycotoxins are toxic compounds produced by many species of fungi after infesting grain and other food crops. These toxins can be carcinogenic, mutagenic, teratogenic, oestrogenic, neurotoxic, or immunotoxic. Although mycotoxins threaten food supplies, these compounds are strongly retained by soil materials. Taking this into account, the ability of clay minerals added to animal feed has been tested to reduce the detrimental effects of mycotoxins. Among mycotoxins, aflatoxins produced by many species of *Aspergillus*, mainly *Aspergillus flavus* and *Aspergillus parasiticus*, are toxic and considered among the most carcinogenic substances known, being aflatoxin B<sub>1</sub> the most potent hepatocarcinogen.

Clays are usually employed as additives to pelletize animal feeds or to improve their flow characteristics, due to their non-toxicity and biocompatibility, together with rheological behaviour. Many research studies have demonstrated that clay minerals such as montmorillonite, palygorskite and sepiolite, can adsorb aflatoxin and effectively reduce its toxicity to animals.

In the present work, different non-modified and organically modified clays have been evaluated to find the most effective adsorbent for aflatoxin removal. For this purpose, surfactants such as cetyltrimethylammonium (CTA) and naturally occurring biocompatible compounds such as phosphatidylcholine (PC) have been tested as organic modifiers of sepiolite and montmorillonite.

## MATERIALS AND METHODS.

The studied clays were Wyoming montmorillonite (SWy-1, County Creek, Wyoming, USA) furnished by the Source

Clay Repository of the Clay Minerals Society (Columbia, Missouri, USA) and prepared as sodium montmorillonite, and sepiolite, a microfibrillar clay, delivered by TOLSA S.A. with the commercial name Pange<sup>®</sup> S9. Before use, sepiolite was dried at 120°C overnight. Phosphatidylcholine (PC, from egg yolk with a purity of 60% TLC) and cetyltrimethylammonium bromide (CTAB), both from Sigma-Aldrich, were employed as clay modifying organic compounds. As model toxin served aflatoxin B<sub>1</sub> (Fig. 1) from *Aspergillus flavus*, also obtained from Sigma-Aldrich.

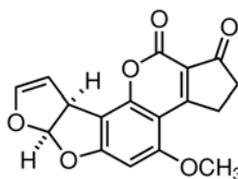


fig 1. Chemical structure of Aflatoxin B<sub>1</sub>.

The adsorbent materials were prepared from ethanolic and methanolic clay suspensions mixed with PC solutions in the concentration ranges 0.1 - 10 mM and 0.02 - 15 mM for sepiolite and montmorillonite, respectively. In each case, the mixtures were agitated on a magnetic stirrer for 48 h at ambient temperature. Then, the resulting hybrid materials were recovered by centrifuging with 4000 rpm for 10 min and finally dried under ambient conditions. For the preparation of CTA-sepiolite (SEP-CTA) and CTA-montmorillonite (MONT-CTA) adsorbents were followed the procedures indicated by Aranda *et al.* and by Letaief *et al.*, respectively. For the aflatoxin adsorption experiments a stock solution containing 500 µg AFB<sub>1</sub>/mL in methanol was prepared and aliquots of this stock solution were diluted in water. Typical formulations comprised 0.3 - 10 µg/mL AFB<sub>1</sub> in 5 mL aqueous solution and 5 mg of the clays and hybrids studied as adsorbents. These mixtures were shaken for 18 h at

ambient temperature and then centrifuged (8,000 rpm, 10 min).

Samples were characterized by means of chemical analysis (PerkinElmer 2400 Series II CHNS/O Elemental Analyzer), X-ray diffraction (Bruker D8, with Cu anode and Ni filter) and structural information was provided from Fast-Fourier Infra-Red spectroscopy (FTIR) (NICOLET 20SX Spectrometer). UV-visible spectroscopy (SHIMADZU UV-2401) was applied to measure the AFB<sub>1</sub> equilibrium concentration in the supernatant. The obtained spectra were baseline corrected by subtraction of a blank. The AFB<sub>1</sub> concentration could be determined from the AFB<sub>1</sub> absorbance band at 360 nm by comparing to a calibration curve.

## RESULTS AND DISCUSSION.

Adsorption of PC on montmorillonite takes place from methanol solutions following a typical Langmuir isotherm. PC is intercalated producing a 0.76 nm expansion of the layers, which is compatible with a bilayer disposition of flat biomolecules in the interlayer region. Washing of the intercalated compounds with methanol produces a partial leaching of PC. Measuring UV-Vis of the AFB<sub>1</sub> adsorbed on unwashed MONT-PC did not provide reliable data due to disturbance of the leached PC absorbance. Work is in progress to optimise the MONT-PC systems for aflatoxin adsorption applications.

PC is adsorbed on the external surface of sepiolite from both methanol and ethanol solutions following typical Langmuir isotherms. Calculated  $\Delta G$  values for the adsorption process indicate a higher tendency to be adsorbed from ethanol and stronger adsorption on sepiolite than on montmorillonite. Prior to application, the chemical composition of SEP-PC and SEP-CTA was determined by CHN analysis. A PC content of 5.35 wt.% and

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**key words:** clay minerals, aflatoxin, phosphatidylcholine, cetyltrimethylammonium

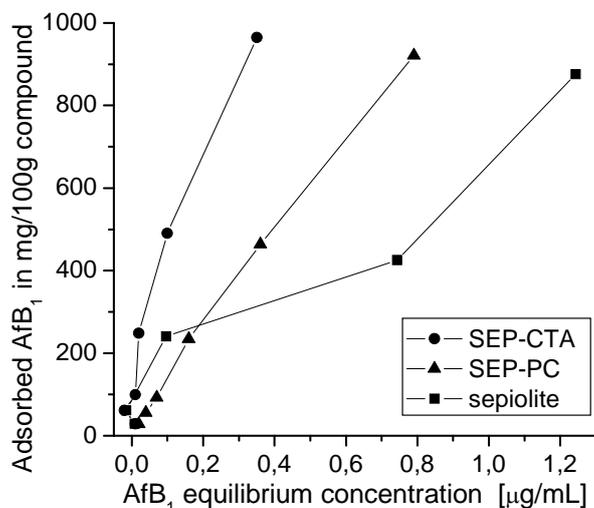


fig.2: AfB<sub>1</sub> adsorption from aqueous dispersions on pure and organically modified sepiolite, SEP-PC and SEP-CTA.

12.48 wt.% of CTA, respectively, were measured. The binding of PC to sepiolite was investigated by FTIR. The results indicated hydrogen bonding of PC to silanol groups of the sepiolite. This was concluded from the disappearance of the silanol OH stretching band at 3718 cm<sup>-1</sup>.

The adsorption of AfB<sub>1</sub> on pristine clays and hybrid materials with increasing starting concentration was followed by UV-Vis spectroscopy. From the obtained data adsorption isotherms for pure sepiolite, SEP-PC and SEP-CTA could be plotted (fig.2). Both SEP-CTA and SEP-PC adsorption isotherms resemble a Langmuir-type. The curve shape suggests that the maximum uptake at the investigated equilibrium concentration was still not reach. AfB<sub>1</sub> has the highest affinity to SEP-CTA where as AfB<sub>1</sub> adsorption was less pronounced on SEP-PC and least on pure sepiolite. The difference of adsorption capability between the two organically modified clays may be due the higher content in amphiphilic units in the SEP-CTA system as compared to SEP-PC. As pure sepiolite is having the lowest AfB<sub>1</sub> adsorption, a beneficial effect of the organic modification can be concluded.

From the equilibrium concentration the percentage uptake of AfB<sub>1</sub> by the studied clays was calculated (Tab 1). For a suspension with an initial AfB<sub>1</sub> concentration of 10 µg/mL, the uptake was highest for the CTA compound and

lowest for pure sepiolite. These results exceed AfB<sub>1</sub> retention on montmorillonite, as investigated by other researchers.

SEP	SEP-PC	SEP-CTAB
87.6	92.1	96.5

Table 1: Uptake of AfB<sub>1</sub> from a 10 µg/mL dispersion by SEP, SEP-PC and SEP-CTA in percentage.

## CONCLUSIONS.

The modification of clay minerals with organic compounds such as phosphatidylcholine and cetyltrimethylammonium enhances their effectiveness in the adsorption of mycotoxins. In this study, the resulting hybrid materials have shown ability in the uptake of aflatoxin B<sub>1</sub>. Although better results were obtained for some of the CTA-based organoclays, PC-modified clays seem to be better candidates for toxins removal in animal feed due to the biocompatible character of this biomolecule, and therefore the PC-SEP biohybrid material.

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