ADSORPTION OF OCHRATOXIN A BY SURFACTANT MODIFIED PHILLIPSITE

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ABSTRACT

Two different surfactants, cetylpyridinum chloride (CP) and hexadecyltrimethylammonium bromide (HDTMA) were used to modify the surface of a natural zeolite - phillipsite (PHI) and adsorption of ochratoxin A (OCHRA) was investigated. Adsorption experiments were done with different amounts of adsorbents and with different initial OCHRA concentrations at pH 3 and pH 7. Results showed that modification of phillipsite surface with CP and HDTMA significantly improved adsorption of OCHRA in comparison to unmodified phillipsite. CP modified phillipsite showed similar adsorption behavior as HDTMA modified phillipsite. The highest adsorption capacity for OCHRA was obtained with CP modified phillipsite at pH 3.

Keywords: mycotoxins, ochratoxin A, adsorption, zeolite, surfactants.

INTRODUCTION

Ochratoxin A (OCHRA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi. It is a worldwide contaminant of various food and feed sources. It can have several toxicological effects including nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity[1]. The chemical structure of OCHRA is presented in Figure 1.

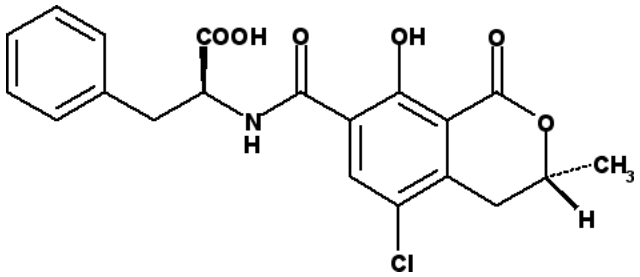

Figure 1. Chemical structure of ochratoxin A.

The known strategies for the detoxification of mycotoxin contaminated food and feed include chemical, biological and physical methods. Among these approaches, the addition of non-nutrition adsorbents into food and feed is regarded as the most effective and economical procedure to reduce the bioaccessibility of mycotoxins in the gastrointestinal tract. The preferred adsorbents are aluminosilicates (natural zeolites and clay minerals). The natural form of these adsorbents is effective in binding aflatoxin, but less effective in binding other mycotoxins which are more hydrophobic such as OCHRA. In order to resolve this problem, minerals have been modified with long chain organic cations resulting in an increased

hydrophobicity of the surface and improved adsorption for the majority of the mycotoxins [2]. Dakovic *et al.* [3] reported that modification of the natural zeolite clinoptilolite with different levels of surfactant – octadecyldimethylbenzyl ammonium (ODMBA) chloride increased adsorption of OCHRA. They found that adsorption of OCHRA on unmodified zeolite was moderate and that adsorption increased with increasing amounts of surfactant at the zeolitic surface. Besides clinoptilolite other natural zeolites which could be used as animal feed additive are chabazite or phillipsite [4].

In this study the natural zeolite phillipsite was modified with two surfactants, cetylpyridinum chloride (CP) and hexadecyltrimethylammonium bromide (HDTMA), and *in vitro* adsorption of OCHRA at pH 3 and 7 was studied. The aim of this research was to examine if phillipsite modified with surfactants is effective in the adsorption of OCHRA.

EXPERIMENTAL

The zeolite-rich rock from Neapolitan Yellow Tuff deposit (Campania, Italy), containing primarily phillipsite (69.6 wt.%) was used as the starting material. The cation exchange capacity (CEC) of the zeolitic tuff was 247 meq/100g, while the external cation exchange capacity (ECEC) was 13 meq/100g [5].

The surfactants, cetylpyridinum chloride (CP) and hexadecyltrimethylammonium bromide (HDTMA), were purchased from Sigma-Aldrich Co. The organozeolites were prepared by treating phillipsite (PHI) with CP or HDTMA equivalent to 100% of its ECEC. The natural zeolite (10 g) was mixed with 100 mL of each surfactant solution, stirred at 5000 rpm for 10 min, and afterwards filtered and dried at 60°C. The CP organozeolite was denoted as PCP-100, while the HDTMA sample was denoted as PHB-100.

In vitro OCHRA adsorption experiments were performed using the following procedure: duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3 or 7) containing 2 ppm OCHRA in solution (10 mL) were added to 15 mL Falcon polypropylene tubes to which had been added 20, 10, 5 or 2 mg of each adsorbent. Adsorption isotherms were obtained using 5 mg of each organozeolite and 10 mL of phosphate buffer containing from 1.0 to 4.5 ppm OCHRA solution. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.1 M phosphate buffer (pH 3 or 7) plus 10 mg adsorbent to Falcon tubes. The Falcon tubes were placed on a rotator shaker for 30 min at room temperature. The suspensions were centrifuged at 13000 rpm for 5 min and 2 mL of the aqueous supernatant was removed for HPLC analysis. An aliquot of the original buffered OCHRA test solution was used as the HPLC standard. OCHRA adsorbed amounts were calculated from the difference between the initial and final concentration in the aqueous supernatant after equilibrium.

RESULTS AND DISCUSSION

The organozeolites, PCP-100 and PHB-100, were obtained by cation exchange with the surfactants CP and HDTMA.

Ochratoxin A is a hydrophobic molecule (Figure 1) which possesses carboxylic and phenolic functional groups. Based on the dissociation constants of OCHRA, pK_{a1} = 3.5 (carboxylic group) and pK_{a2} = 7 (phenolic group), OCHRA is present in solution mainly in the anionic form at pH 3 and completely in the anionic form at pH 7 [6,7].

The adsorption of OCHRA for different amounts of adsorbents at constant initial mycotoxin concentration (2 mg/L) and at different pH values is presented in Table 1. At both pH values, the percentage of OCHRA adsorption by unmodified and modified phillipsite increased with increasing the amount of each adsorbent in suspension. This can be interpreted with increasing

number and availability of adsorption sites at the zeolitic surface. Results showed that adsorption of OCHRA by unmodified phillipsite was low at pH 3 (less than 10%), and even lower at pH 7. Modification of phillipsite with organic cations CP and HDTMA significantly increased OCHRA adsorption. For the highest adsorbent dose (20 mg/10 mL), adsorption of 9.9% for unmodified phillipsite increased to 94.5% for PCP-100 and 95.5% for PHB-100 at pH 3, and from 3.3% for PHI to 96.7% for PCP-100 and 97.1% for PHB-100 at pH 7.

Table 1. Adsorption of OCHRA by unmodified and modified phillipsite at pH 3 and pH 7.

	OCHRA adsorption indexes (%)	
	pH 3	pH 7
PHI (mg/mL)		
2	9.9	3.3
1	9.1	3.2
0.5	8.6	2.4
0.2	5.2	1.9
PCP-100 (mg/mL)		
2	94.5	96.7
1	93.2	92.2
0.5	71.9	73.6
0.2	37.5	29.1
PHB-100 (mg/mL)		
2	95.5	97.1
1	92.4	93.3
0.5	65.8	69.1
0.2	32.7	27.9

Adsorption isotherms for organozeolites PCP-100 and PHB-100 at pH 3 and 7 are presented in Figure 2.

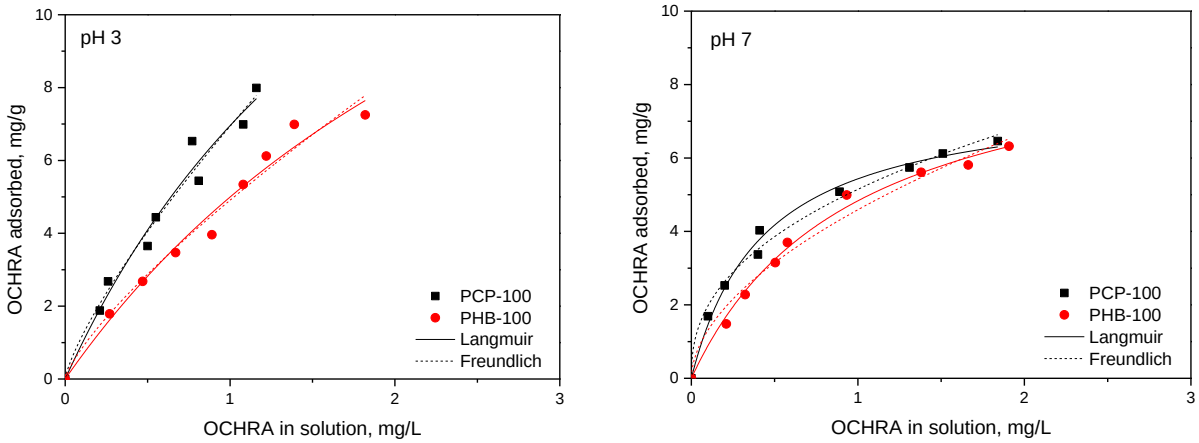

Figure 2. OCHRA adsorption isotherms for PCP-100 and PHB-100 at pH 3 and 7.

As can be seen, for both adsorbents PCP-100 and PHB-100, adsorption of OCHRA increased with increasing the initial concentration of OCHRA in solution. Experimental data were fitted to Langmuir and Freundlich adsorption isotherm models. High values of correlation coefficients (R^2 > 0.97) for both applied models demonstrate that Langmuir as well as Freundlich isotherm can describe adsorption of OCHRA on organozeolites. From the Figure 2 it can be seen that CP modified phillipsite shows similar behavior as HDTMA modified phillipsite, but slightly better adsorption was achieved with PCP-100 at pH 3 and 7. The maximum adsorbed amount of OCHRA, under applied experimental conditions was 7.99 mg/g for PCP-100 and 7.25 mg/g for PHB-100 at pH 3, while at pH 7 the maximum OCHRA adsorbed was 6.46 mg/g for PCP-100 and 6.32 mg/g for PHB-100. For PCP-100 as well as for PHB-100 higher adsorption of OCHRA was achieved at pH 3 where OCHRA is only partially in the anionic form in comparison to pH 7 where OCHRA is completely in the anionic form. Results showed that hydrophobic interactions between CP or HDTMA alkyl chains and OCHRA molecule are probably mainly responsible for its adsorption.

CONCLUSION

The organozeolites were prepared by treating the natural zeolite phillipsite with two different surfactants: cetylpyridinum chloride (CP) and hexadecyltrimethylammonium bromide (HDTMA), in amounts equivalent to its ECEC. *In vitro* adsorption of OCHRA by these organozeolites was studied at pH 3 and 7. Both organozeolites showed significantly higher adsorption of OCHRA in comparison to unmodified phillipsite confirming that CP and HDTMA present at the zeolitic surface are responsible for OCHRA adsorption. Similar adsorption isotherms obtained for the adsorption of OCHRA by organozeolites containing CP and HDTMA ions indicate that its adsorption was not dependent of the type of surfactant.

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