

## In Vitro Evaluation of the Efficacy of Sodium Humate as an Aflatoxin B1 Adsorbent

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**Abstract:** In vitro adsorption of aflatoxin B1 (AFB1) on sodium humate was investigated by using Freundlich and Langmuir isotherms and the Freundlich isotherm fitted the data better than the Langmuir isotherm. At the same time, effects of PH, interaction time, adsorbent amount and initial concentration of AFB1 were also studied and they all had a certain influence on AFB1 adsorption, especially the PH value. The coexistence of fresh feed (AFB1 < 1 ng/g) showed no significant influence on AFB1 adsorption, indicating that sodium humate have a high affinity to AFB1 and don't adsorb other nutrients. The AFB1-adsorbent complex was stable in phosphate buffer solution at PH 3.0 and PH 8.0. These results suggest that sodium humate have the potential as an aflatoxin B1 adsorbent to prevent the adsorption of AFB1 from the animal gastrointestinal tract.

**Key Words:** Aflatoxin B1; Sodium humate; ELISA; Adsorbent

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### INTRODUCTION

Aflatoxins, a class of mycotoxins produced by fungal species of *Aspergillus* (*A. flavus* and *A. parasiticus*), are found in various crops and forages in field or during storage, transportation, and processing. Among the aflatoxins, aflatoxin B1 (AFB1) is the predominant form, presents the highest toxic potential and much concerned due to carcinogenicity, mutagenicity, and teratogenicity (Ismail and Rustom, 1997; Mishra and Das, 2003; Smela *et al.*, 2001). Contamination of aflatoxin in poultry and animal feedstuffs is quite common in many countries and cause great economic losses in terms of growth retardation or meat production and the residues of aflatoxins in liver and eggs and other edible tissues (Bintvihok *et al.*, 2002; Charmley and Thienholm, 1995). Moreover, accumulation of aflatoxins in animal tissues may indirectly result in health risk to humans.

Consequently, large-scale, practical, and cost-effective methods for detoxifying aflatoxin-contaminated feedstuffs currently are in great demand.

A number of methods, including physical, chemical and biological techniques have been used to protect animals from the toxic effects of AFB1, but most of these methods are costly, time-consuming, and only partially effective (Doyle *et al.*, 1982; Phillips *et al.*, 1990; Phillips *et al.*, 1990). One of the most practical approaches is the use of nonnutritive adsorbents, which bind the mycotoxins and inhibit their adsorption from the gastrointestinal tract (Ramos *et al.*, 1996), but not all adsorbents are equally effective and several adsorbents have been shown to impair nutrients utilization (Kubena *et al.*, 1993; Scheideler, 1993). According to Ramos and Hernandez (1996), as an ideal adsorbent, it should have a high affinity to aflatoxin, resulting in the formation of a strong complex with little risk of dissociation. It also should have a high binding capacity to prevent saturation.

Sodium humate is a kind of macromolecular organic compounds, which contains many active groups, such as hydroxyl, phenolic hydroxyl, methoxy, carboxyl *et al.*, and has large specific surface area. So it has strong adsorption, ion exchange, complexation, chelation capacity and has been introduced into human medicine to reduce the adsorption and systemic availability of bacterial endotoxins (Klavins *et al.*, 2006), but it has never been evaluated previously as a mycotoxin adsorbent in animal feed.

The objective of this study was to evaluate the efficacy of sodium humate as an aflatoxin adsorbent by in vitro. The binding capability of AFB1 by sodium humate was also tested, and the Langmuir and Freundlich adsorption isotherms were determined for AFB1 to describe surface adsorption.

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## MATERIALS AND METHODS

### **Materials:**

Sodium humate was purchased from Liaoning Putian Tongle Fertilize Co.,Ltd. (China).Solid AFB1 was purchased from Fermentek. Ltd.(Jerusalem. Israel), standard purity>99%, and the purity was assayed by HPLC. ELISA-kit for AFB1 was purchased from Bioxl Diagnostic Systems. Lnc.(USA).All solvents and chemicals were analytical grade. Water was distilled in glass and demineralized prior to use.

### **AFB1 Adsorption/desorption Study:**

#### **Experiment 1:**

The binding of sodium humate to AFB1 was determined under the following conditions, and a study of the Langmuir and Freundlich adsorption isotherms was carried out. Effects of PH3.0 7.08.0adsorbent amount(4.0, 10.0, 20.0mg), interaction time(30, 45, 60, 75, 90min) and initial concentration of AFB1(20, 40, 60, 80, 100ng/ml) were investigated with sodium humate( 2.5mg/ml) added to 4ml phosphate buffered (PH7.0) containing AFB1 at a concentration of 100ng/ml. Samples were placed on a rotator shaker(120rpm, ) for 37 1h,centrifuged for 10min at 6000rpm, and the supernatant was decanted carefully into a clean tube and then measured by enzyme-linked immunosorbent assay(ELISA). A control treatment without adsorbent was prepared for each experiment in case of any possible nonspecific binding of AFB1, and each sample carried out in triple. The adsorptive amount of AFB1 was calculated from the concentration of unbound AFB1 remaining in the supernatant after incubation.

#### **Experiment 2:**

In order to investigate sodium humate whether or not adsorbed other nutrients, adsorbent mixed with some fresh feed(AFB1<1ng/g). If sodium humate was bound by the feed, it could inhibit the adsorption of AFB1. Sodium humate and feed were weighed into tubes at a concentration of 2.5mg of adsorbent/g of feed 4ml phosphate buffer(PH7.0 or 8.0) containing AFB1 at a concentration of 100ng/ml was added to the tubes. The tubes were placed on a rotator shaker(120rpm, 37) for 1h,centrifuged for 10min at 6000rpm, and the supernatant was decanted carefully into a clean tube and measured by ELISA, as previously described. All samples were run in triple.

#### **Experiment 3:**

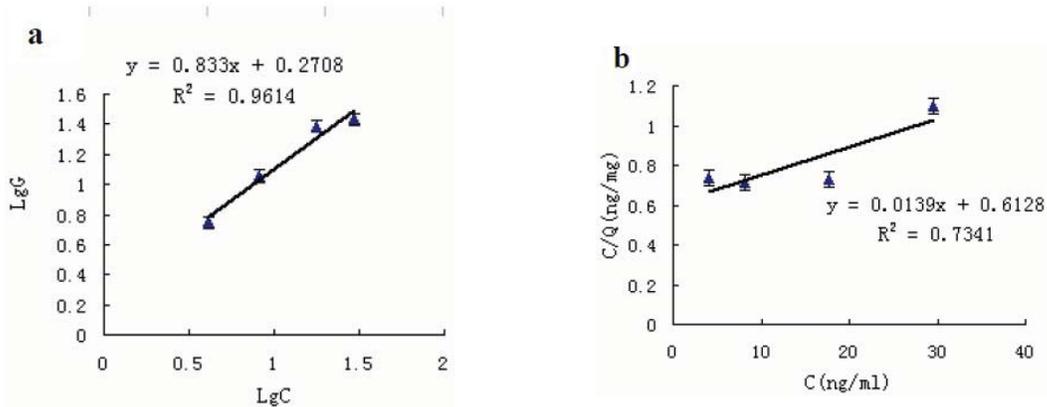
The effects of washes on desorption rate of AFB1 from sodium humate was determined following the experiment 2. After centrifugation, the supernatant was decanted carefully into a clean tube and leavings were added in 2ml phosphate buffered (PH3.0,8.0) and incubated for 10min at 37, centrifuged for 1 0min at 6000rpm, and the supernatant was removed to a clean tube for determining the concentration of AFB1. This washing step was repeated three times. The control groups also were washed, incubated, centrifuged and determined, and the total percentage of desorption of AFB1 was determined for each sample.

## RESULTS AND DISCUSSION

### **Adsorption Isotherm:**

The use of isotherms is one of the most efficient mathematical approaches to describe surface adsorption, in which the amount of compound adsorbed per unit of weight of in the external phase, under equilibrium conditions (Ramos and Hernandez, 1996). Multiple isotherm equations have been proposed for the modeling of the adsorption of compounds in aqueous solutions to the surfaces of solids (Kinniburgh, 1986), of which the Langmuir and Freundlich isotherms are most extensively used (Ramos and Hernandez, 1996). The Langmuir equation is most applicable to a single ligand adsorbed to a single type of site on a particular adsorbent (Langmuir, 1916), whereas the Freundlich equation relates to a heterogeneous adsorbent surface, or the coexistence of different adsorption mechanisms (Ramos and Hernandez, 1996).

In this study, the Langmuir and Freundlich isotherms of AFB1 adsorbed by sodium humate at and 3 PH7.0 were presented in Fig.1. The results showed that the Fredundlich adsorption isotherm(Fig. 1 a) fitted the data better than the Langmuir isotherm(Fig. 1b), as demonstrated by the higher coefficients of determination values ( $R^2$ )obainted. According to Ramos and Hernandez (1996), this might indicate that the presence of the adsorption centers within the sodium humate with different affinities for AFB1, resulting in a heterogenous adsorbent surface or the coexistence of different adsorption mechanisms.



**Fig. 1:** Isotherms of AFB1 adsorbed on sodium humate at 37 and PH 7.0, (a) Freundlich isotherm; (b) Langmuir isotherm.

**Effect of pH:**

The adsorption of AFB1 on sodium humate at 3 and at different PH levels were presented in Fig.2a. Each value was the average of 2 replicates compared with the control groups without added adsorbent. These data showed that adsorption of AFB1 on sodium humate was a continuous increase right from PH 3.0 to 8.0 (Fig.2a). It also showed that the amount of AFB1 adsorbed by sodium humate on unit mass increased slowly up to PH 7.0 after which there was a rapid rise, indicating that the active groups of sodium humate, such as hydroxyls, phenolic hydroxyls, methoxy, carboxyl, et al. and the physical and chemical properties of AFB1 may play a more important role than the large specific surface area. The variations in AFB1 adsorption between PH 3.0-7.0 and PH 7.0-8.0 can be explained that: i) at low PH, the concentrations of H<sup>+</sup> in the solution was very high, enhanced electrostatic pull between H<sup>+</sup> of solution and sodium humate surface, resulting in the competition for adsorption sites between H<sup>+</sup> and AFB1 ;ii) after PH 7.0, H<sup>+</sup> of active groups can be easily dissociated and provided more adsorption sites.

**Effect of Adsorbent Amount:**

It has been reported that between adsorbent and adsorbate existed the dose-response relationship (Grant *et al* 1998). Fig.2b showed the effects of adsorbent amount on the adsorption of AFB1. Sodium humate achieved the maximum adsorption amount of AFB1 when added to 2.5mg/ml and then declined with higher adsorbent amount. However, the percentage of AFB1 adsorption kept rapidly increasing when the adsorbent amount increased. The reasons were as follows: i) with the adsorbent amount increasing, more unsaturated adsorption sites were provided; ii) with the adsorption sites increasing, the competition for adsorption sites significantly enhanced, leading to the amount of adsorption decreased considerably.

**Effect of Interaction Time:**

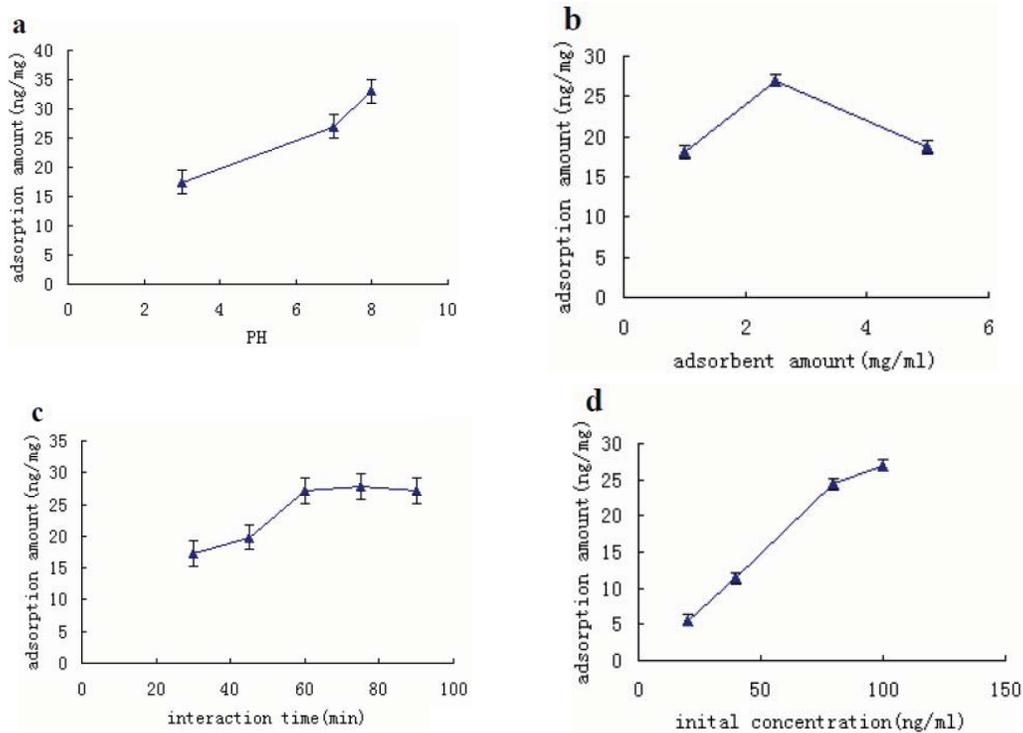
Fig.2c. showed the amount of AFB1 adsorption increased rapidly within 60min and then maintained unchanged; indicating that this was a fast adsorption process and the adsorption approached equilibrium in nearly 60min under the given experimental conditions.

**Effect of Initial Concentration:**

Fig.2d. described that the amount of AFB1 adsorption enhanced gradually with the initial concentration of AFB1 increasing. We can see that at low concentration of AFB1, the ratio of the number of AFB1 to the number of available adsorption sites was small, so the adsorption was independent of initial concentration; but as the concentration of AFB1 increasing, the competition among adsorption sites was significantly intensified. As a result, the adsorption ratio of AFB1 decreased considerably, but the total amount of AFB1 adsorption rose.

**Effect of Fresh Feed:**

In this experiment, sodium humate bound 76.36% of AFB1 at PH 7.0 and 88.12% at PH 8.0, whereas 77.01% and 90.83% respectively in buffer alone. The results showed that adsorbent mixed with fresh feed had no significant influence on the ratio of AFB1 adsorption as in buffer alone, suggesting that sodium humate have a high affinity to AFB1 and don't adsorb other nutrients.



**Fig. 2:** Effect of the factors on AFB1 adsorption amount(ng/mg) on sodium humate, (a) PH; (b) adsorbent amount(mg);(c) interaction time(min);(d)initial concentration of AFB1 (ng/ml).

#### **Desorption Study:**

In order to investigate the stability of AFB1-adsorbent complex, the complex washed by different PH phosphate buffer. Less than 10% of the AFB1 adsorbed by sodium humate was extracted at PH 3.0 and 6.5% at PH 8.0; which indicated that the complex was very stable in phosphate buffer at PH 3.0 and PH 8.0, and the mainly adsorptive mechanism was due to the chemical bonds involved in their interaction.

#### **Conclusions:**

Results reported in this paper demonstrate that sodium humate has the potential as an aflatoxin B1 adsorbent to prevent the adsorption of AFB1 from buffer solutions. Sodium humate has several advantages on AFB1 adsorption: i) higher affinity to AFB1; ii) not adsorb other nutrients; iii) the complex was very stable in different PH phosphate buffer. However, like other *in vitro* assays, the present study can't completely simulate the adsorptive conditions of the animal gastrointestinal tract, so *in vivo* studies are required to assess the efficacy of AFB1 adsorption under practical conditions.

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