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Layer-by-layer assembled smectite-polymer nanocomposite film for rapid fluorometric detection of aflatoxin B_1

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ABSTRACT

Aflatoxin B_1 (AFB_1) is a potent biological toxin produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Current quantification methods for AFB_1 are mostly established on immunoaffinity columns which are both costly and labor intensive. Inspired by smectites' high AFB_1 adsorption capacity and affinity, we report the synthesis of a smectite-polyacrylamide (PAM) nanocomposite film for AFB_1 detection and prove the feasibility of such a film for AFB_1 quantification. A layer-by-layer assembly process was developed to achieve uniform morphology and thickness of the nanocomposite films on flat silicon substrates. Positive correlations between the peak fluorescence intensity of the adsorbed AFB_1 and its concentration in the test solutions were obtained. The smectite-PAM nanocomposite film has shown similar AFB_1 adsorption capabilities as the smectite, while exhibiting excellent structural stability in aqueous solutions. Our results suggest the feasibility of using the smectite-PAM nanocomposite film as a simple biosensor to achieve rapid fluorometric quantification of AFB_1.

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1. Introduction

Aflatoxins (especially aflatoxin B_1, AFB_1), as a major type of biological toxins, are harmful by-products of the fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). As they can cause acutely toxic and carcinogenic effects on human and animal health [1], their contaminations in agricultural commodities, human food and animal feed have become major concerns in the food and feed industries. Therefore, rapid, quantitative and low-cost detection methods are critical for the timely evaluation, monitoring and mitigation of the hazardous effects caused by aflatoxins. Currently, the "gold standard" for aflatoxins detection is the high-performance liquid chromatography (HPLC) followed by fluorometric or mass spectroscopic analysis [1,2], which is time-consuming and costly, and thus it is mainly limited to laboratory uses. In the past few years, a number of rapid detection methods based on immunoassays have been developed [3,4]. These methods utilize antibodies to selectively capture aflatoxins from the test solution [5–7]. In spite of their high adsorption selectivity and affinity, the antibodies are susceptible to denaturation and degradation, and therefore stringent testing conditions are necessary to ensure their performance. In addition, the production of antibodies requires live animals, which is a complex and expensive process.

During the last three decades, several bentonites (smectite-rich clays) have been used as adsorbent additives to detoxify aflatoxin-contaminated animal feeds [8]. Recent studies have shown that the divalent cations and transition cations in the interlayers of smectite can induce the substantial binding of AFB_1 to the smectite [9]. Unlike the antibodies, the smectite-AFB_1 binding is not drastically affected by adsorption conditions, e.g., temperature or pH value. In addition, a high adsorption capacity (e.g., 10–20% of the self weight of the smectite [10]) can also be obtained due to the large surface area (about 800 m^2/g) of the smectite. Because of its high adsorption selectivity and capacity for AFB_1, it is possible to use smectite as an inexpensive inorganic molecular recognition agent to replace the delicate and costly antibodies for AFB_1 detection. However, due to its small particle size and tendency to disperse in water or solvents, it is difficult to directly use smectite as the biosensor for AFB_1 detection.

In this paper, we report the synthesis of smectite-polyacrylamide (PAM) nanocomposite films on flat silicon substrates and application of these films to the quantification of AFB_1 in aqueous and corn extraction solutions. A layer-by-layer assembly approach was developed to provide good morphology and thickness control of the nanocomposite film. Different from previous fluorometric characterization methods (by measuring the fluorescence intensity of the eluent solutions [11–13]), the fluorescence emission spectra of AFB_1 adsorbed nanocomposite films were characterized. Strong correlations between the peak fluorescence intensity of the AFB_1 adsorbed to the nanocomposite film and the AFB_1 concentration in the test solution were

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identified. Base on our results, the smectite-PAM nanocomposite film is proven to be able to work as an effective sensing material in the direct fluorometric quantification of the AFB_1 (without the elution steps).

2. Materials and methods

2.1. Smectite preparation

Unless otherwise noted, the following preparation procedures were performed at the room temperature. A Greek calcium bentonite was obtained from the S&B Industrial Minerals S.A. (1934-now, Athens, Greece). Our X-ray diffraction (XRD) and infrared spectroscopy analysis (data not shown) indicated that its mineral composition was dominated by the smectite containing a small amount of calcite, quartz and feldspars. To extract relative pure smectite, 10 g of the bentonite was dispersed in a diluted (pH 10) Na_2CO_3 solution, and then the <2 μm clay fraction was separated by the sedimentation method. The collected clay particles were stored in a suspension solution.

2.2. Layer-by-layer assembly of the smectite-PAM nanocomposite films

The smectite-PAM nanocomposite film was synthesized on flat silicon substrates through a layer-by-layer assembling process. Since the smectite is negatively charged, a positively charged copolymer (PAM 494C obtained from Kemira Chemicals Inc., Atlanta, GA) was used in the layer-by-layer assembly. This copolymer contains 80% nonionic acrylamide units and 20% cationic N,N,N-trimethyl-aminoethyl acrylate units [14]. The multiple points of electrostatic attractions between the cationic functional groups of PAM chains and the negative charge sites of the smectite, combining with the high entropy gain from water replacement by the polymer, make the smetite–polymer complexes stable in both water and solvents [15]. The random arrangement of the smectite in the nanocomposite matrix also helps to increase its porosity and binding site accessibility for AFB_1 molecules [16].

A single-crystalline silicon wafer (4″ P (100) 0–100 Ω cm SSP 500 μm Test wafers), purchased from University Wafer Inc. (South Boston, MA), was used as the substrate for the layer-by-layer assembly of the smectite-PAM nanocomposite film. Single-crystalline silicon does not manifest optical fluorescence that will interfere with that from AFB_1 molecules. The pristine surface condition and high reflectivity of the single-crystalline silicon wafers also help the assembly, characterization and testing of the smectite-PAM nanocomposite film. The silicon wafer was diced into rectangular pieces (13 mm × 15 mm), such that they could be snuggly fit into the sample holder of a spectrofluorometer used in this study. It should be noted that the layer-by-layer assembly process is substrate-independent and can be performed on other substrates as well.

The layer-by-layer assembly was conducted using 1 g/L PAM aqueous solution and 1 g/L smectite aqueous dispersion as described below [17]: (1) a group of pre-cleaned silicon substrates were immersed into the PAM solution for 7 min and then rinsed in deionized (DI) water for 2 min to remove excessive PAM coating on the surface; (2) the silicon substrates were immersed in the smectite dispersion for 5 min and rinsed in DI water for 2 min; and (3) the above cycle was repeated ~30 times until the silicon substrate surface was fully covered with the nanocomposite film. The immersion times in steps (1) and (2) were determined based on a preliminary trial of the growth rate and quality of nine films after 5, 7, 9 min of immersion in the polymer and clay suspension.

2.3. Preparation of AFB_1 aqueous solution

Fifty mg AFB_1 from *A. flavus* (Sigma–Aldrich Inc., St. Luis, MO) was dissolved in 50 mL acetonitrile to obtain a 1000 ppm stock solution. An 8 ppm AFB_1 test solution was made by diluting the stocking solution with DI water. AFB_1 aqueous solutions at lower concentrations were prepared by further diluting the 8 ppm solution with DI water.

2.4. Smectite-PAM film characterization and AFB_1 quantification

The surface and the cross-section of the assembled smectite-PAM nanocomposite film were inspected under an FEI Quanta® 600 scanning electron microscope. The fluorescence emission spectra of AFB_1 adsorbed smectite suspension and smectite-PAM nanocomposite films were characterized using a PTI QuantaMaster® series spectrofluorometer with an ultraviolet (UV) excitation wavelength of 365 nm.

(1) Fluorescence intensity characterization of AFB_1 spiked smectite suspension.

A series of 1 mL of 1 g/L smectite suspensions were mixed with different amounts of 8 ppm AFB_1 solutions to achieve AFB_1 mass loadings of 0, 0.25%, 0.5%, 1.0% and 1.5% of the smectite, respectively. Since the maximal AFB_1 mass loading concentration (1.5%) was much lower than the typical absorption capacity (10–20%) of the smectite [10], we believe most of AFB_1 molecules would adsorb into the smectite. After letting the mixture react overnight, each of the mixed suspensions was injected into an ink-well device made of a poly(methyl methacrylate) (PMMA) substrate bonded with a micro-molded polydimethylsiloxane (PDMS) structure. Water was evaporated at 70 °C. In the ink-wells, the spread area of the mixture during the drying process was well-controlled. A uniform thickness of the resulting smectite film was obtained to ensure a consistent measurement of the fluorescence intensity. The reasons for using ink-wells are that the spectrofluorometer only samples and integrates the fluorescence signal from a certain area (about 5 mm × 5 mm) of the surface of the sample and the fluorescence intensity is affected by both the AFB_1 concentration and the film thickness of the sampled region.

(2) Fluorescence intensity characterization of AFB_1 adsorbed smectite-PAM nanocomposite films.

A total of 32 smectite-PAM nanocomposite films on silicon substrates were prepared, and tested in one control (DI water,

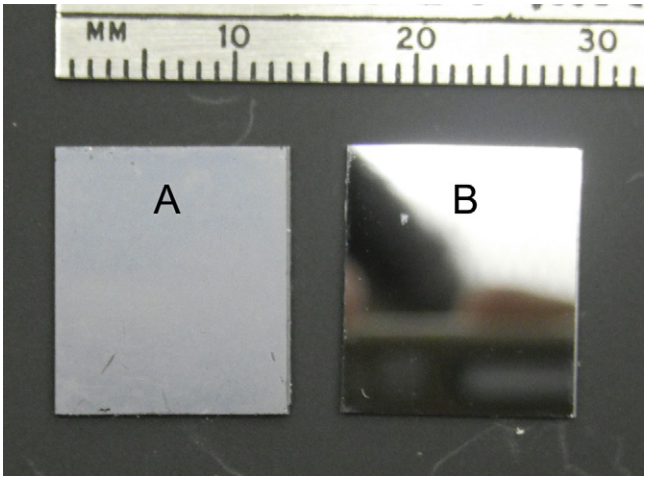

Fig. 1. Two single-crystalline silicon substrates: (A) coated with the smectite-PAM nanocomposite film; (B) uncoated.