Design of Multifunctional Nanostructure for Ultrafast Extraction and Purification of Aflatoxins in Foodstuffs

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ABSTRACT: Aflatoxins (AFs) are a class of carcinogens associated with liver cancers which are used to exist in foodstuffs. There are extremely low maximum limits of AFs in foodstuffs (0.025–20 μg kg$^{-1}$). To fast and sensitively detect such low concentration of AFs in foodstuffs is dominated by the efficiency and selectivity of AFs enrichment process, which is extremely challenging although substantial efforts have been made over the past decades. Here we designed and synthesized a multilayer nanoarchitecture composed of a broad-spectrum AFs monoclonal antibody shell, chitosan middle layer and magnetic bead core (denoted AFs-mAb/CTS/Fe$_3$O$_4$). The efficiency of AFs-mAb/CTS/Fe$_3$O$_4$ in extracting AFs has been found to be more than 60 times higher than both conventional immunoaffinity chromatography and solid-phase extraction. Furthermore, the nanocomposite displays excellent selectivity and good reusability apart from outstanding efficiency. Coupling to ultra-performance liquid chromatography–tandem quadrupole mass spectrometry, this new nanoarchitecture enables to probe six AFs as low as 0.003 μg kg$^{-1}$ in foodstuffs with free matrix effects, which is nearly 10 times smaller than the regulated maximum tolerated does. It is believed that the new nanoarchitecture would provide an efficient and fast pathway to detect AFs in foodstuffs to protect human being from some critical liver cancers.
Liver cancers are the third leading cause of cancer death worldwide, and the fifth commonest cancers in men and the eighth commonest in women.\textsuperscript{1,2} Aflatoxins (AFs) are associated with acute liver damage, liver cirrhosis, the induction of tumors, and teratogenic effects.\textsuperscript{3,4} An extremely tragic event associated with AFs-contaminated foods occurred in Kenya in 2004–2005, when more than 1000 people were poisoned, 120 of whom died.\textsuperscript{5} The most important AFs are AFB\textsubscript{1}, AFB\textsubscript{2}, AFG\textsubscript{1}, AFG\textsubscript{2}, AFM\textsubscript{1}, and AFM\textsubscript{2}.\textsuperscript{6} The International Agency for Research on Cancer (IARC) has classified AFB\textsubscript{1}, AFB\textsubscript{2}, AFG\textsubscript{1}, AFG\textsubscript{2}, and AFM\textsubscript{1} as Group 1 human carcinogens.\textsuperscript{7} Unfortunately, these AFs are regularly found in improperly stored staple commodities, including corn, peanuts, rice, tree nuts, wheat, etc.\textsuperscript{7–9} To safeguard human health, almost all countries have set extremely low maximum tolerated limits (MTLs) of AFs in food.\textsuperscript{10} The MTLs of AFB\textsubscript{1} and of total AFs in food are 5 and 10–20 μg kg\textsuperscript{-1}, respectively, in more than 75 countries around the world,\textsuperscript{11,12} whereas they are 2 and 4 μg kg\textsuperscript{-1}, respectively, in the European Union (EU).\textsuperscript{13} Stricter MTLs, as low as 0.1 μg kg\textsuperscript{-1} for AFB\textsubscript{1} and 0.025 μg kg\textsuperscript{-1} for AFM\textsubscript{1}, are set for infant formulae and infant foods in the EU.\textsuperscript{13} Considering the potential threat of AFs to human health, efficient approaches are urgently required for the rapid and sensitive analysis of those in foodstuffs.

At present, liquid chromatography coupled to triple quadrupole mass spectrometry (LC–MS/MS) is frequently used for the analysis of AFs in foods.\textsuperscript{14,15} Despite the universality, sensitivity, and selectivity of the LC–MS/MS method, overcoming the variable matrix effects of electrospray ionization remains a big challenge in this process.\textsuperscript{8} To eliminate signal suppression or enhancement, several typical purification techniques have been developed to compensate for matrix effects, such as liquid–liquid extraction (LLE),\textsuperscript{16} solid–phase extraction (SPE),\textsuperscript{17} and immunoaffinity chromatography (IAC).\textsuperscript{4} However, LLE and SLE usually require a lot of organic
solvent, and SPE techniques often involve nonspecific retention. Although IAC can generally produce clean extracts, with small variability between samples, and chromatograms free of matrix interference, the limited flow rate and possible congestion by larger sample particles often cause very long waiting time for the samples analysis.

Building on the discovery of functional nanoparticles, immunomagnetic techniques have been used successfully to separate tumor cells, bacteria, viruses, allergens, proteins, hormones, and AFB1 from food matrices, providing attractive alternatives for the rapid concentration and purification of AFB1. Xie et al. used indirect method to immobilize antibody on biotinylated magnetic beads for the pretreatment of AFB1 in soybean sauce with about 0.5 h of time consuming. Xiong et al. also designed a magnetic beads carrying poly (acrylic acid) brushes as “nanobody containers” for immunoaffinity purification of AFB1 from corn samples. Although high loading and adsorption capacity were available owing to the using of nanoparticles, one hour was needed to capture AFB1, which was comparative with the conventional IAC method. Chitosan (CTS) has abundant amino (−NH2) and hydroxyl (−OH) groups for chemical modifications and has been tried for the separation or purification of heavy metal ions, bovine serum albumin (BSA), and proteins. CTS thus has a potential to covalently immobilize magnetic beads and antibodies.

Herein we designed a multifunctional nanoarchitecture by integrating magnetic property of Fe3O4, functional groups of CTS and selective monoclonal antibody. The nanostructured CTS/Fe3O4 were prepared with a simple one-step method and characterized in detail. Further, a broad-spectrum monoclonal antibody directed against six AFs was immobilized on the nanocomposite through the amidocarbonylation reaction, generating the AFs-mAb/CTS/Fe3O4 adsorbent (Scheme 1). The multifunctional architecture has been validated with standard
reference materials for ultrafast (ca. 0.5 min) sample extraction and purification, demonstrating
its advances in the fast and efficient detection of ultratrace levels of chemicals in complex food
matrices.

**EXPERIMENTAL SECTION**

**Materials.** AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2 standards (purity ≥ 99%) were
purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The monoclonal antibody against
AFs (3D10) was prepared in our laboratory (see Supporting Information). FeCl3·6H2O, ethylene
glycol, sodium acetate, and CTS (≥95% deacetylation, with a viscosity average molecular weight
of 3.0 × 10^5 g mol⁻¹) were obtained from Beijing Chemical Reagent Co., Ltd (Beijing, China).

*N*-Hydroxysulfosuccinimide (sulfo-NHS), morpholinoethanesulfonic acid (MES),
*N*- (3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and bovin serum
albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Synthesis of CTS/Fe₃O₄ and Fe₃O₄.** The magnetic particles CTS/Fe₃O₄ and Fe₃O₄ were
synthesized and characterized based on the modified solvothermal reduction method. The
detailed procedures were given in the Supporting Information.

**Characterization.** TEM images of Fe₃O₄ and CTS/Fe₃O₄ were taken with a JEM-2100F
field-emission high resolution transmission electron microscope (JEOL Co., Japan). SEM images
were scanned by a Merlin Compact field-emission scanning electron microscope equipped with
an EDS unit (ZEISS, Germany). XRD patterns were recorded on a Rigaku Dmax/2400
diffractometer (Rigaku Co., Japan) with Cu Ka radiation at 40 kV and 200 mA. FTIR spectra
were recorded with a Nicolet™ iS™ 50 FTIR spectrometer (Thermo Fisher Scientific, Waltham,
MA, USA), with scanning waves ranging from 400 to 4000 cm⁻¹. TGA was performed with a
Q600 SDT thermogravimetric analyzer (TA Instruments, USA), under an air flow of 100 mL
min\(^{-1}\) at a heating rate of 20 °C min\(^{-1}\).

**Preparation of AFs-mAb/CTS/Fe\(_3\)O\(_4\).** CTS/Fe\(_3\)O\(_4\) nanoparticles (0.1 g) were added to 1.0 mL of MES buffer (0.05 mol L\(^{-1}\), pH 5.0) and mixed thoroughly. Then, 20 μL of EDC (0.5 mg mL\(^{-1}\)) and 20 μL of sulfo-NHS (0.5 mg mL\(^{-1}\)) were added to the mixture and shaken for 15 min. Anti-AFs mAb (1.0 mg) was mixed with the sulfo-NHS/EDC-treated CTS/Fe\(_3\)O\(_4\) solution for 30 min. Finally, 20 μL of 20% BSA was added for 10 min to block any excess activated sites. After magnetic separation, the aggregates were resuspended in 2 mL of phosphate buffer (0.05 mol L\(^{-1}\), pH 7.4) containing 0.02% (v/w) sodium azide, 0.5% BSA, and 1% Tween 20. The prepared magnetic immunoadsorbent was stored at 4°C before use. The maximum coupling amount of AFs-mAb on 0.1 g CTS/Fe\(_3\)O\(_4\) was monitored with the same method used to prepare AFs-mAb/CTS/Fe\(_3\)O\(_4\), except different concentration of AFs-mAb solution (5, 10, 15, 20, 25, 30 mg mL\(^{-1}\)) was used.

**Sample Extraction and Preconcentration.** Corn, rice, wheat, peanut, peanut oil, sunflower oil, olive oil products destined for human consumption were obtained from several supermarkets in Beijing (China). Cereal samples were finely milled using a knife mill Grindomix GM 200 (Retsch, Haan, Germany) and homogenized to achieve a representative samples and then dispensed into plastic bags. Vegetable oil samples were directly measured. All samples were stored under cool conditions and out of direct sunlight until the analysis. Samples (25 g) were weighed into 500 mL polypropylene bottles, to which 5 g NaCl was added, and the samples were extracted with 125 mL of methanol–deionized water (60:40, v/v) with vigorous shaking for 30 min. The extract was filtered and 20 mL of filtrate was diluted with 120 mL of water. The pH was adjusted to 5.5–6.5. A suspension of immunomagnetic beads (0.3 mL) was then added to 60 mL of diluted extract (equivalent to 2.0 g of sample), and shaken gently for 0.5 min to capture the
AFs. An external magnet was attached to the outside bottom of the vial and the AFs-mAb/CTS/Fe₃O₄ was gathered to the bottom of the tube. The supernatant was discarded. Water (20 mL) was added to the tube, which was shaken for 10 s. Methanol (1.0 mL) was added and the tube was vortexed for 5 s. The magnet was then attached to the outside bottom of the tube. The supernatant was collected and evaporated to near dryness under a gentle stream of nitrogen at 45 °C. The residue was reconstituted with 1.0 mL of the initial mobile phase used in the ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry (UPLC–MS/MS) analysis. The development and conditions of the UPLC–MS/MS method are given in the Supporting Information.

Applications. To compare the purification efficacy of the AFs-mAb/CTS/Fe₃O₄ immunomagnetic absorbent, SPE, and IAC, three certificated reference materials were purchased from Trilogy Analytical Laboratory (Washington, MO, USA). The procedures for SPE and IAC were provided in the Supporting Information.

■ RESULTS AND DISCUSSION

Synthesis and Characterization of CTS/Fe₃O₄. The powder X-ray diffraction (XRD) spectra for CTS/Fe₃O₄ are presented in Figure 1a. It has six diffraction peaks, with 2θ = 30.1°, 35.4°, 42.9°, 53.5°, 56.9°, and 62.6°, corresponding to (220), (311), (400), (422), (511), and (440) planes, respectively, which is consistent with the standard spectra for Fe₃O₄ with an inverse cubic spinel structure. The diffraction peaks are highly symmetric and sharp, indicating that the particles were well crystalized. Fe₃O₄ crystal shape remain unchanged during the solvothermal reaction with CTS while, a small peak appears in the range from 17° to 20°, indicating the presence of amorphous CTS. The morphology and structure of the CTS/Fe₃O₄ nanospheres were examined with field-emission scanning electron microscopy (SEM) and high-resolution
transmission electron microscopy (HR-TEM). SEM images suggested that the CTS/Fe₃O₄ nanospheres are spherical with a very narrow size distribution (average particle size of ~200 nm) (Figure 1b), consistent with HR-TEM observation (Figure 1c). The Fe₃O₄ particles of ~200 nm (black region) were successfully coated by the CTS layer with a thickness of ~10 nm (shadow region) (Figure 1c). The Fourier transform infrared (FTIR) spectrum (Figure 1d) of CTS was characterized by the following absorption bands: ν(C−H) of the backbone polymer, around 2871 and 2929 cm⁻¹; ν(C−O) of the primary alcoholic group, 1377 cm⁻¹; ν(C−O) of amide, 1060 cm⁻¹; and δ(N−H) of the primary amine, around 3356 cm⁻¹. The spectrum of CTS/Fe₃O₄ is a combination of both CTS and Fe₃O₄ FTIR spectrum, further indicating that CTS was successfully coated onto the surfaces of the Fe₃O₄ nanoparticles. Energy dispersive spectroscopy (EDS) (Figure S1) shows 8.65% (wt.) nitrogen, also implying that the CTS were successfully coated on Fe₃O₄ nanoparticles. The thermal stability of the materials was investigated with a thermogravimetric analysis (TGA) as shown in Figure S2, which indicated that the CTS content of CTS/Fe₃O₄ was about 19.6%.

**Preparation and Characterization of AFs-mAb/CTS/Fe₃O₄.** The conditions of the coupling reaction for preparing the immunomagnetic beads were optimized because these conditions may strongly influence CTS–monoclonal antibody (mAb) binding. According to the reported study,⁵５ BSA was used as a model protein in the optimization experiment before immobilizing antibody on the solid support material. The final optimized conditions were then applied to the preparation of AFs-mAb/CTS/Fe₃O₄. To optimize the coupling efficiency of the CTS/Fe₃O₄ nanoparticles with the AFs-mAb, different coupling methods were compared, including the glutaraldehyde (GA) method,⁶ the electrostatic adsorption (ESA) method,⁴⁰ and the EDC/sulfo-NHS method.⁴⁷ The coupling efficiency of the GA method was below 30% (Figure 2a), because the reaction solvent
used in this method is carbonate buffer (pH 9.0), in which both the CTS/Fe₃O₄ nanoparticles and BSA are negatively charged. The electrostatic adsorption BSA on the CTS/Fe₃O₄ nanoparticles was also investigated. The coupling efficiency of the ESA method was 90%. An explanation for this phenomenon might involve the surface charge on the CTS/Fe₃O₄ nanoparticles and BSA in weak acidic buffer. At the isoelectric point, BSA has a negative charge, whereas CTS/Fe₃O₄ nanoparticles have a positive charge, and the electrostatic interaction between them is one of the driving forces of the adsorption process. However, the electrostatic bonding is not very stable, especially when the pH changes or in the presence of other compounds in the solution. The highest coupling efficiency (Figure 2a) was obtained with the EDC/sulfo-NHS method, because it efficiently cross-linked the –COOH groups of BSA or IgG with the –NH₂ groups of CTS without leaving a spacer molecule. Therefore, BSA or IgG was conjugated to the CTS/Fe₃O₄ nanoparticles with a commonly used biochemical protocol based on EDC/sulfo-NHS, a highly efficient ‘zero-length’ cross-linking agent. The coupling of the CTS/Fe₃O₄ nanoparticles with BSA was also investigated in four coupling solutions of different ionic strength, using the selected EDC/sulfo-NHS method. As shown in Figure 2b, the amount of BSA bound on the CTS/Fe₃O₄ nanoparticles was better (98.3%) in 0.05 mol L⁻¹ MES solution than that in other three binding solutions. Therefore, 0.05 mol L⁻¹ MES was selected as the coupling solution. The effects of pH on the coupling reaction were next studied, as shown in Figure 2c. Various pHs were tested, ranging from 3.0 to 8.0. The pH had a remarkable effect on the coupling of BSA with the CTS/Fe₃O₄ nanoparticles. Under neutral or alkaline conditions, negligible BSA was bound to the CTS/Fe₃O₄ nanoparticles. The maximum adsorption of BSA occurred at approximately pH 5.0, which is very close to the isoelectric point of BSA (pI = 4.7). As the pH decreased from 5.0 to 3.0, the amount of BSA adsorbed onto the CTS/Fe₃O₄ nanoparticles varied slightly, which
can be explained by the low protonation of the –NH₂ groups at high pH. Therefore, pH 5.0 was selected as the optimal pH for the coupling reaction. A series of reaction times for the coupling process was also tested. As shown in Figure 2d, the coupling efficiency of BSA to CTS/Fe₃O₄ increased gradually at coupling times of less than 30 min, whereas after 30 min, the CTS/Fe₃O₄ was saturated with BSA. Therefore, 30 min was selected as the coupling time to ensure that the coupling with BSA was at saturation equilibrium. Under the optimized conditions, AFs-mAb/CTS/Fe₃O₄ nanoparticles were then fabricated. In order to test the maximum coupling amount of AFs-mAb on 0.1 g CTS/Fe₃O₄, a set of AFs-mAb solution (5, 10, 15, 20, 25, 30 mg mL⁻¹) was investigated. As illustrated in Figure S3, the maximum amount of AFs-mAb coupled on the magnetic beads was found to be 23.5 mg g⁻¹. When the the coupling amount was 10 mg g⁻¹, the maximum AFs-binding capacities were 337, 351, 306, 113, 462 and 389 ng/mL for AFB₁, AFB₂, AFG₁, AFG₂, AFM₁ and AFM₂ (Figure S4), respectively, which meets the detection requirements. Therefore, 10 mg g⁻¹ was selected as the optimum coupling amount and used in the following experiment.

**Sample Extraction and Preconcentration.** Negative samples (Corn, rice, wheat, peanut, peanut oil, sunflower oil, olive oil) fortified with each analyte (1.0 μg kg⁻¹) were used to investigate the extraction performance of the immunomagnetic beads under different conditions. Results for the extraction of processed corn are given in Figure 3, and slightly different results were obtained in other matrices. Considering all the results, the optimized results of corn sample were selected as the best conditions for every matrix. First, the effect of the amount of immunomagnetic absorbent was investigated in the range 0.1–0.6 mL. As shown in Figure 3a, the extraction recoveries of the targets gradually increased as the amount of AFs-mAb/CTS/Fe₃O₄ immunoadsorbent increased from 0.1 to 0.3 mL, and then remained almost constant at amounts
of absorbents > 0.3 mL. Therefore, 0.3 mL of AFs-mAb/CTS/Fe₃O₄ was selected. We then studied the effects of different proportions of methanol in the adsorption solution (0%, 5%, 10%, 15%, 20%, 25%, or 30%) during the adsorption step. As shown in Figure 3b, the best recoveries were obtained for all six AFs with 10% methanol. The adsorption time was also evaluated. The recoveries (Figure 3c) for all the analytes were satisfactory when the sample solution was mildly shaken for 0.5–5 min. A longer period significantly reduced the recoveries of the target analytes. This phenomenon is due to the fact that the organic solvents (methanol) and co-extracts may break antibody or antigen–antibody interaction with a longer time. Therefore, an adsorption time of 0.5 min was chose. The desorption conditions were also evaluated. The effect of the volume of desorption solution (Figure 3d) on the desorption efficiency of the analytes was determined. When 1 mL of methanol was used, the analytes were almost completely desorbed from the immunomagnetic adsorbent. The time for desorption was also investigated, ranging from 5 to 60 s, with vortexing. The desorption time had no obvious effect on the extraction efficiency. Therefore, a desorption time of 5 s was used. These results indicate that the desorption process is quick and efficient, which may be attributable to the fact that methanol is highly destructive of the antigen–antibody interaction. Finally, under the optimized conditions, the whole purification procedure developed here only took less than 1 min, whereas it takes more than 1 h to pass the 60 mL of loading solution and 20 mL of washing solution through an IAC/SPE column at a flow rate of 1 mL min⁻¹. Therefore, the new nanoarchitecture is a very time-efficient pretreatment process. The reusability of the immunomagnetic adsorbent was also evaluated with regeneration and cycling tests. After regeneration with PBS, the adsorption capacity of AFs-mAb/CTS/Fe₃O₄ was almost constant in 10 continuous adsorption cycles. Therefore, it is clear that the AFs-mAb/CTS/Fe₃O₄ immunosorbent can be reused, reflecting the excellent stability of the
antibodies on the surfaces of magnetic CTS beads. This outstanding reusability means that the new nanomaterial can be regarded as a potential alternative to the enrichment of AFs.

**Method Validation.** A matrix effect (signal suppression/enhancement) is commonly encountered in conventional approaches, eg. LC–MS analyses in the electrospray ionization mode. When multiple compounds coelute with the target analyte, typical matrix suppression effects and sometimes matrix enhancement effects can occur as a result of ionization competition. In this case, the target content may be underestimated or overestimated when a pure standard is used for quantification. Several measures have been proposed to minimize this interference, including matrix-matched calibration, isotopic dilution, immunoaffinity purification, and sample dilution. Immunoaffinity purification is the most popular technique because its adsorption is specific. It has also been shown that matrix effects can be minimized or eliminated using this selective extraction nanoarchitecture. In this study, the matrix effects based on the slope ratios (R_slope) between the matrix-matched curves and the solvent standard curves were investigated. The results indicate that little matrix suppression was observed for 6 AFs with R_slope ranging from 0.832 to 1.109, which has been considered as matrix effects free and solvent standard curves can be used for quantification. The mean recoveries for the six AF when samples of corn, rice, wheat, peanut, peanut oil, sunflower oil, and olive oil were spiked with three concentrations (1.0, 5.0 and 10.0 μg kg⁻¹) of AFs were in the range of 63%–118%. The repeatability (intra-day precision) was less than 10.7%, and the within-laboratory reproducibility (inter-day precision) did not exceed 16.3% (Table 1), which were expressed as relative standard deviations (RSDs). Typical chromatograms for corn fortified with the six AF compounds (1.0 μg kg⁻¹) are shown in Figure S5. The limits of detection (LODs) based on three times the signal-to-noise ratio were in the range of 0.003–0.007 μg kg⁻¹ and the limits of quantification
(LOQs) based on 10 times the signal-to-noise ratio were in the range of 0.009–0.023 μg kg$^{-1}$. Thus, the nanoarchitecture developed here is about 10-fold more sensitive than the IAC$^{43}$ and SPE methods.$^{44}$

**Applications.** Finally to evaluate the reliability of the developed nanoarchitecture, three certificated real foodstuffs were used to validate it: one brown rice and two corn powder samples. The results are shown in Table 2. The brown rice sample was contaminated with 7.4 μg kg$^{-1}$ AFB$_1$, 0.2 μg kg$^{-1}$ AFB$_2$, and 0.5 μg kg$^{-1}$ AFM$_1$. AFB$_1$, AFB$_2$, AFG$_1$, and AFM$_1$ were detected in the 1# corn sample at concentrations of 7.1, 0.7, 0.3, and 0.6 μg kg$^{-1}$, respectively. Five AFs were found in the 2# corn sample, at concentrations of 17.7, 1.5, 0.4, 1.8, and 0.1 μg kg$^{-1}$ for AFB$_1$, AFB$_2$, AFG$_1$, AFM$_1$, and AFM$_2$, respectively. No sample was contaminated with AFG$_2$. The results for AFB$_1$, AFB$_2$, and AFG$_2$ using immunomagnetic separation agreed well with the certified values$^{32}$ and those obtained with the IAC method.$^{33}$ The AFB$_2$ detected in the brown rice sample and the AFG$_1$ detected in the corn sample were below the LOD (0.5 μg kg$^{-1}$) of the Association of Official Analytical Chemists (AOAC) Official Method 994.08. However, there was no significant difference between the results for AFB$_2$ and AFG$_1$ when the IAC and AFs-mAb/CTS/Fe$_3$O$_4$ methods were used. Three samples were also contaminated with AFM$_1$ and AFM$_2$, with maximal contents of 1.8 and 0.2 μg kg$^{-1}$, respectively. Huang et al.$^6$ and Han et al.$^8$ also detected AFM$_1$ and AFM$_2$ in peanuts and traditional Chinese medical materials. It is noteworthy that AFM$_1$ and AFM$_2$ are not only regarded as metabolites occurring in foods of animal origin, but are also found in plant-derived foodstuffs. Taking into account all these factors, the current UPLC–MS/MS method based on the AFs-mAb/CTS/Fe$_3$O$_4$ extraction procedure developed in this study can be regarded as highly selective, sensitive, and effective for the detection of six AFs in food samples.
CONCLUSIONS

A multifunctional nanostructured architecture has been successfully fabricated by a facile approach. The nanocomposite is composed of a magnetic Fe$_3$O$_4$ core, a middle layer of CTS and the shell of AFs monoclonal antibody. Such design can fast and efficiently enrich extremely low-level AFs contaminants in different foodstuffs, which can cause liver cancers. Magnetic separation greatly improved the phase separation, while avoiding the time-consuming requirement in the conventional approaches, eg. passaging through an IAC or SPE cartridge. Because CTS/Fe$_3$O$_4$ nanoparticle have high adsorption capacities (23.5 mg g$^{-1}$) and high coupling rates (more than 90%), a small amount of immunomagnetic absorbents (0.3 mL) can be used and only half a minute is required to extract the analyte from the samples. Our results indicate that this new strategy developed here is very efficient and sensitive, with potential application to purification of trace contaminants in foodstuffs which are detrimental to human health.

ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of CTS/Fe$_3$O$_4$ and Fe$_3$O$_4$ ; SPE and IAC separation used in application procedure; UPLC–MS/MS detection; preparation of the broad-spectrum monoclonal antibody (mAb) against AFs; and additional references. The Supporting Information is available free of charge on the ACS Publications website.

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All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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(32) AOAC Official Method 994.08.
5169–5177.


Figure captions

Scheme 1. A schematic illustration of the procedure used to synthesize the AFs-mAb/CTS/Fe₃O₄ nanoarchitecture and its application.

Figure 1. (a) XRD curves for CTS/Fe₃O₄, Fe₃O₄, and CTS; (b) SEM image of CTS/Fe₃O₄; (c) TEM images of CTS/Fe₃O₄ and Fe₃O₄; (d) FTIR curves for CTS/Fe₃O₄, Fe₃O₄, and CTS.

Figure 2. Optimized preparation of the immunomagnetic absorbent (n = 3). (a) The coupling efficiency achieved with different coupling methods: GA, glutaraldehyde method; ESA, electrostatic adsorption method; EDC/sulfo-NHS, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysulfosuccinimide method. (b) The coupling efficiency with different concentrations of coupling solution. (c) The coupling efficiency achieved with coupling solution of different pHs. (d) Effect of reaction time on the coupling process.

Figure 3. Optimized extraction procedure of the immunomagnetic absorbent (n = 3) in corn samples spiked with AFs concentration of 1.0 μg kg⁻¹. (a) Recovery curve for AFs with different amounts of immunomagnetic beads. (b) Recovery curve for AFs with different ratios of methanol in the loading solution. (c) Effect of loading time on the recognition of AFs by immunomagnetic beads. (d) Recovery variation curve with different volumes of elution solution. Evaluations were performed as for other types of samples.
Scheme 1

Synthesis

\[ \text{Fe}^{3+} \]

\[ \text{CTS} \]

\[ \text{AFs-mAb} \]

\[ \text{AFs} \]

\[ & \& \text{Interferences} \]

\[ 195^\circ C \quad \text{Refluxing} \]

\[ \text{H}_2\text{N} \quad \text{NH}_2 \quad \text{H}_2\text{N} \quad \text{NH}_2 \]

\[ \text{pH 5.0} \quad \text{Coupling} \]

\[ \text{FeO}_4 \]

\[ \text{AFs-mAb/CTS/Fe}_3\text{O}_4 \]

\[ \text{Adsorption} \]

\[ \text{Desorption} \quad \text{Methanol} \]

\[ \text{Magnetic separation} \]

\[ \text{Magnet} \]

\[ \text{Magnet} \]
Figure 1

(a) Relative intensities (a.a.)

(b) SEM image

(c) TEM image

(d) Absorbance spectrum
Figure 2

(a) Coupling efficiency (%)

- GA
- ESA
- EDC/sulfo-NHS

The coupling method

(b) Coupling efficiency (%)

- Pure water
- 0.01 M MES
- 0.05 M MES
- 0.1 M MES

The type of coupling solution

(c) Coupling efficiency (%)

- pH of coupling solution

3-8

(d) Coupling efficiency (%)

- Coupling time (min)

10-60
Figure 3

(a) Recovery (%) vs. The amount of immunomagnetic absorbents (mL)

(b) Recovery (%) vs. The ratio of methanol in adsorption solution (%)

(c) Recovery (%) vs. The adsorption time (min)

(d) Recovery (%) vs. The volume of desorption solution (mL)
Table 1. LODs, LOQs, mean recoveries, repeatability and reproducibility of AFs (n = 3).

<table>
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<tr>
<th>Compounds</th>
<th>LOD</th>
<th>LOQ</th>
<th>Added</th>
<th>Mean recoveries (intra-/inter-, RSDs) (%)</th>
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<tr>
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<td>(µg kg⁻¹)</td>
<td>(µg kg⁻¹)</td>
<td>(µg kg⁻¹)</td>
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<td>AFB₁</td>
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<td>0.009</td>
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<td></td>
<td></td>
<td>10.0</td>
<td>87 (5.3/7.4)</td>
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<tr>
<td>AFB₂</td>
<td>0.003</td>
<td>0.012</td>
<td>1.0</td>
<td>78 (2.1/5.2)</td>
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<td></td>
<td>5.0</td>
<td>69 (7.2/7.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td>91 (9.3/10.4)</td>
</tr>
<tr>
<td>AFG₁</td>
<td>0.005</td>
<td>0.017</td>
<td>1.0</td>
<td>85 (6.6/8.9)</td>
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<td>5.0</td>
<td>97 (6.7/9.2)</td>
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<td>10.0</td>
<td>73 (8.2/12.8)</td>
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<tr>
<td>AFG₂</td>
<td>0.007</td>
<td>0.023</td>
<td>1.0</td>
<td>86 (7.5/9.6)</td>
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<td></td>
<td>5.0</td>
<td>87 (4.6/7.7)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>10.0</td>
<td>96 (4.2/6.4)</td>
</tr>
<tr>
<td>AFM₁</td>
<td>0.006</td>
<td>0.021</td>
<td>1.0</td>
<td>88 (6.2/7.8)</td>
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<td>5.0</td>
<td>103 (9.2/11.5)</td>
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<td>10.0</td>
<td>96 (5.5/8.6)</td>
</tr>
<tr>
<td>AFM₂</td>
<td>0.005</td>
<td>0.018</td>
<td>1.0</td>
<td>82 (4.5/6.5)</td>
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<td>5.0</td>
<td>81 (7.5/8.1)</td>
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<td></td>
<td>10.0</td>
<td>108 (5.9/8.5)</td>
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</tbody>
</table>
Table 2. Validated results for real-life specimens using SPE, IAC, and AFs-mAb/CTS/Fe₃O₄.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Methods</th>
<th>Concentrations (µg kg⁻¹)</th>
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<tr>
<td></td>
<td></td>
<td>AFB₁</td>
</tr>
<tr>
<td>Brown rice</td>
<td>SPE a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>IAC e)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>AFs-mAb/CTS/Fe₃O₄</td>
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<td>7.4</td>
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<tr>
<td>1# Corn</td>
<td>SPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>IAC</td>
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</tr>
<tr>
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<td>6.9</td>
</tr>
<tr>
<td></td>
<td>AFs-mAb/CTS/Fe₃O₄</td>
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<td>7.1</td>
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<tr>
<td>2# Corn</td>
<td>SPE</td>
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<tr>
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<td>IAC</td>
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<td>20.1</td>
</tr>
<tr>
<td></td>
<td>AFs-mAb/CTS/Fe₃O₄</td>
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<td>17.7</td>
</tr>
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a) The results were obtained from Trilogy Analytical Laboratory Inc. (Washington, MO, USA) with HPLC using AOAC Official Method 994.08, with modifications. The detection limits were 0.5 µg kg⁻¹ for AFB₁, AFB₂, AFG₁, AFG₂; b) Range of product incorporating uncertainty ranges for the total of AFB₁, AFB₂, AFG₁ and AFG₂; c) Data not given; d) ND= not detected; e) IAC method using the same pretreatment procedure as in our previous study, with some modifications; f) The total values for AFB₁, AFB₂, AFG₁, and AFG₂ determined with the IAC method; g) The total values for AFB₁, AFB₂, AFG₁, and AFG₂ determined with the nanoarchitecture developed in this study.
For TOC only