

Target-Induced Stimuli-Responsive Liquid Crystal for Colorimetric Detection of Aflatoxin B1 via MnO₂ Nanosheet- Mediated Catalysis

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Abstract

Aflatoxin B1 (AFB1) poses a severe threat to global food safety, necessitating the development of rapid, label-free, and simple detection strategies. This study presents a novel colorimetric biosensing platform for AFB1 detection based on target-induced stimuli-responsive liquid crystal (LC) emulsions and MnO₂ nanosheet (MnO₂ NSs)-mediated catalysis. Within this system, glutathione (GSH) is encapsulated in sodium dodecyl sulfate (SDS)-stabilized LC droplets to serve as a responsive gate. In the absence of AFB1, a specific aptamer electrostatically sequesters the cationic surfactant hexadecyltrimethylammonium bromide (CTAB), preserving the integrity of the LC droplets and allowing the intact MnO₂ NSs to efficiently catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) into a yellow chromophore. Conversely, the presence of AFB1 triggers specific aptamer-target binding, which releases CTAB to interact with the LC interface, causing the rapid ejection of the encapsulated GSH. The released GSH subsequently reduces and decomposes the MnO₂ NSs, preventing TMB oxidation and generating a distinct "color-off" signal. Under optimized conditions, this sensor exhibited a linear dynamic range from 0.2 to 1.0 ng/mL with a calculated limit of detection (LOD) of 0.73 ng/mL. Furthermore, the platform demonstrated exceptional specificity against other common mycotoxins and achieved satisfactory recovery rates ranging from 92.18% to 108.23% in diverse real food matrices, including sunflower seeds, soy milk powder, and milk. Validated by high-performance liquid chromatography (HPLC), this simple, cost-effective, and highly responsive strategy offers a robust visual tool for the real-time monitoring of AFB1 in practical food applications.

Introduction

Aflatoxin B1 (AFB1), a potent secondary metabolite primarily produced by *Aspergillus flavus*, is recognized as one of the most hazardous mycotoxins and a Group 1 carcinogen (Xie et al. 2025; Liu et al. 2025). Due to its exceptional thermal stability and high prevalence in agricultural products such as wheat and edible oils, AFB1 remains a persistent and formidable challenge for global food safety monitoring (Shen et al. 2026; Hu et al. 2026). The contamination of food supplies not only poses severe threats to human health, including hepatotoxicity and immunosuppression, but also leads to substantial economic losses worldwide (Wang et al. 2026a; Lu et al. 2021). The traditional analytical methods for AFB1, such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS), offer high precision and sensitivity, their reliance on sophisticated infrastructure, expensive reagents, and professional expertise limits their utility for on-site, rapid screening in field or resource-limited settings (Wang et al. 2026b; Chen et al. 2026; Castro et al. 2026). Among the emerging detection strategies for AFB1, colorimetric biosensing assays are particularly promising due to their low cost, rapid response, portability, and ease of use (Moshirian-Farahi et al. 2026; Lu et al. 2025). Despite the rapid advancement of colorimetric biosensors, their practical application is often hindered by complex probe labeling and tedious operational procedures (Majdinasab et al. 2021; Lertsri et al. 2020). Hence, the establishment of a label-free, rapid visual sensing strategy for the detection of AFB1 that

merges ease of operation with robust accuracy is imperative for facilitating real-time food safety assessment and proactive intervention.

The realization of such a streamlined sensing format, which is characterized by the absence of complex probe labeling and minimized operational steps, could be effectively supported by the integration of biomimetic catalytic systems (Gao et al. 2007; Wu et al. 2019). To this end, researchers have increasingly turned to functional nanomaterials with intrinsic enzyme-like activities as robust alternatives to traditional protein-based enzymes (Gao et al. 2024; Guo et al. 2026; Gu et al. 2025). Among these, two-dimensional (2D) manganese dioxide (MnO_2) nanosheets are particularly attractive due to their high surface-to-volume ratio and robust oxidase-like activity, which enables the direct and rapid oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) without the need for biological enzymes or external oxidants (Mei et al. 2024; Liu et al. 2024). While this autonomous catalytic capability significantly simplifies the sensing architecture, it also leads to an “always-on” signal profile that is difficult to regulate in a single-step assay (Mei et al. 2024; Yang et al. 2020). Consequently, a persistent hurdle in creating a rapid, “one-pot” colorimetric system is the effective control of signal initiation and background suppression. In many redox-based sensors, the premature mixing or unintended interaction of catalytic components and inhibitory reagents/substrate often leads to unstable background signals and reduced detection reliability (Wei et al. 2024; Li et al. 2026). To maintain a truly label-free architecture while ensuring a sharp and distinct signal response, a highly sensitive “molecular gate” is required, which acts as a mechanism to sequester reactive species and instantly translate a specific recognition event into a color change without the need for complex chemical modification of the target or probe.

Liquid crystals (LCs), characterized by their unique interfacial sensitivity and long-range molecular ordering, offer a compelling solution to this requirement by acting as stimuli-responsive micro-reservoirs (Chen et al. 2022; Mehrzad et al. 2024; Khoshbin et al. 2022). These soft-matter droplets can effectively encapsulate signaling molecules within a water-in-LC architecture, forming a stable physical barrier that isolates the internal cargo from the external catalytic system (Li et al. 2024; Wu et al. 2022). Unlike traditional solid-phase sensors that often require lengthy incubation, centrifugation, or washing steps, LC droplets exhibit an extraordinary ability to undergo rapid “orientational transitions” or “cargo ejection” upon subtle interfacial perturbations (Li et al. 2024). Crucially, the “gatekeeping” function of the LC interface can be precisely modulated by the conformational and electrostatic states of aptamers (Mostajabodavati et al. 2024). As polyanionic oligonucleotides, aptamers can sequester cationic surfactants through strong electrostatic attraction, effectively “masking” the interfacial activity of these triggers and maintaining the integrity of the LC micro-reservoirs (Khoshbin et al. 2024; Ryu et al. 2024). The specific binding between an aptamer and its target induces an immediate competitive displacement, releasing the sequestered triggers to the LC interface. This molecular event triggers a rapid interfacial perturbation, leading to the efficient “cargo ejection” of encapsulated molecules (Li et al. 2024; Wu et al. 2022). By utilizing the aptamer as a programmable molecular switch to govern the LC interface, this sensing platform bypasses the need for complex covalent labeling while providing a nearly instantaneous signal readout upon target introduction.

In this work, we developed a novel colorimetric biosensing platform for AFB1 based on target-induced stimuli-responsive LC and MnO₂ NSs-mediated catalysis. GSH is encapsulated within LC droplets stabilized by sodium dodecyl sulfate (SDS). The sensing mechanism relies on the competitive interaction between AFB1, its specific aptamer, and the cationic surfactant hexadecyltrimethylammonium bromide (CTAB). In the presence of AFB1, the aptamer specifically binds to the target, leaving CTAB free to interact with the LC interface. This interfacial charge interaction triggers the “ejection” of GSH from the LC into the aqueous phase. The released GSH subsequently decomposes MnO₂ NSs, preventing the oxidation of TMB and resulting in a “color-off” signal. Conversely, in the absence of AFB1, the aptamer sequesters CTAB through electrostatic binding, maintaining the integrity of the LC droplets and allowing MnO₂ NSs to catalyze the TMB color reaction. This strategy combines the high specificity of aptamers, the sensitive gating of LC, and the powerful amplification of MnO₂ catalysis, providing a robust tool for the visual and sensitive monitoring of AFB1 in complex samples.

Material and methods

Chemicals and apparatus

4-Cyano-4'-pentylbiphenyl (5CB), potassium permanganate (KMnO₄) and anhydrous ethanol were procured from Macklin Biochemical Co., Ltd (Shanghai, China). 3,3',5,5'-tetramethyl benzidine (TMB) was purchased from Sigma-Aldrich (Shanghai, China). AFB1 was provided by Meizheng Biotechnology Co., Ltd (Rizhao, Shandong, China). Glutathione (GSH), cetyltrimethylammonium bromide, sodium dodecyl sulfate (SDS), sulfuric acid (H₂SO₄), sodium dodecylbenzenesulfonate (SDBS), and cetyltrimethylammonium bromide (CTAB) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Zearalenone (F-2), fumonisin B1 (FB1), trichothecene (T-2), aflatoxin B2 (AFB2), patulin (PAT), and ochratoxin (OTA) were acquired from Pribolab Biological Engineering Co., Ltd (Qingdao, Shandong, China). The oligonucleotide sequences are as follows: AFB1 Apt: 5'-CACGTGTTGTCTCTCTGTGTCTCGTG-3'. All solutions were prepared by using Milli-Q water (18.2 MΩ cm). All solutions were prepared with Milli-Q water (18.2 MΩ·cm⁻¹) obtained from the Milli-Q purification system.

Fluorescence spectra were measured with an automatic microplate reader (SPARK 20 M, TECAN, Switzerland). Transmission electron microscopy (TEM) images were collected on a JEM-F200 microscope (JEOL, Japan). Scanning electron microscopy (SEM) was conducted on a Sigma 300 microscope (ZEISS, Germany). UV-vis spectra were obtained by a T700 Spectrophotometer (Persee, China). The electrophoresis equipment was obtained from WIX (Shanghai, China). Garose gel electrophoresis was performed on a multifunctional gel imager (ChemiDocTM MP, BIO-RAD, USA). Dynamic light scattering (DLS) analysis was carried out on a Zetasizer Nano-ZS90 instrument (Malvern Instruments Ltd., UK).

Preparation of MnO NSs and LC Emulsion

Preparation of MnO₂ NSs

An aqueous solution (46 mL) containing CTAB (0.5 M) and H₂SO₄ (0.1 M) was mixed with ethanol (3.2 mL). The mixture was then heated to 90 °C and maintained for 15 min. Subsequently, 0.8 mL of KMnO₄ solution (0.05 M) was added, and the reaction was allowed to proceed for 30 min. Upon completion of the reaction, the mixture was cooled to room temperature, yielding a dark brown solution. The resulting dark brown solution was purified by centrifugation three times to obtain a pure MnO₂ NSs.

Preparation of LC Emulsion

First, 5CB was heated to 40°C. Next, 0.5 mL of SDS solution (18 mM) and 0.5 mL of GSH solution (200 μM) were thoroughly mixed. Then, 10 μL of this mixture was added into 90 μL of liquid 5CB, followed by vortexing for 3 min and sonication in an ultrasonic bath for 5 min. Finally, the system was vigorously emulsified by vortex mixing 3 min, followed by ultrasonication using a probe sonicator (5% amplitude, 12 s total duration). Following this procedure, a uniformly dispersed LC emulsion was obtained.

Feasibility experiment

To verify the feasibility of the colorimetric LC sensor based on MnO₂ NSs for AFB1-Apt detection, a series of control experiments were designed and performed in 0.5 mL centrifuge tubes at room temperature (25 °C), with all assays conducted in triplicate. First, to validate the detection capability of the sensor for the target AFB1, the results of introducing 50 μL CTAB (1 μM) + Apt (10 μM) or CTAB + Apt+AFB1 (0.1 ng/mL) into a standard detection system (comprising 20 μL of LC, 50 μL of MnO₂ NSs, 80 μL of TMB (1 mM), and 20 μL of H₂SO₄ (1 M)) were compared. Second, to determine whether the release of guest microdroplets from the LC emulsion was driven by interfacial charge interactions between surfactants, 50 μL of CTAB and 50 μL of H₂O were separately added to the aforementioned target-free system for comparison. Furthermore, to verify if MnO₂ NSs were the exclusive factor responsible for TMB oxidation, controlled experiments were conducted by separately adding 50 μL of MnO₂ NSs or H₂O to a GSH-free LC system (20 μL). Finally, to systematically evaluate the effect of varying GSH concentrations on the oxidase-like activity of the MnO₂ NSs, 20 μL of GSH at different concentrations (0, 10, 100, 1000, and 10000 μM) was mixed with 50 μL of H₂O, 50 μL of MnO₂ NSs, 80 μL of TMB, and 20 μL of H₂SO₄.

Optimization tests

To achieve optimal detection performance of the sensor, the reaction time, temperature, and concentrations of the core reagents (CTAB and Apt) were systematically optimized in this study. The specific procedures are as follows:

Optimization of time and temperature

Using the standard detection system (comprising 20 μL of LC emulsion, 50 μL of CTAB, 50 μL of MnO_2 NSs, 80 μL of TMB, and 20 μL of H_2SO_4), the effects of varying reaction times (0, 5, 10, 15, 20, 25, and 30 min) and temperatures (15, 20, 25, 30, and 35 $^\circ\text{C}$) on the absorbance of the system were investigated.

Optimization of CTAB concentration

To explore the optimal conditions for interfacial charge interactions, 30 μL of CTAB at varying concentrations (0, 0.1, 1, 10, and 100 μM) was mixed and reacted with 20 μL of H_2O , 20 μL of LC emulsion, 50 μL of MnO_2 NSs, 80 μL of TMB (1 mM), and 20 μL of H_2SO_4 (1 M). The absorbance values at each concentration were subsequently measured.

Optimization of AFB1-Apt concentration

Under the optimal conditions determined above, 30 μL of CTAB (10 μM), 10 μL of AFB1-Apt at varying concentrations (0, 0.2, 0.6, 1.0, 1.4, and 1.8 μM), 10 μL of AFB1 (0.1 ng/mL), 20 μL of LC emulsion, 50 μL of MnO_2 NSs, 80 μL of TMB (1 mM), and 20 μL of H_2SO_4 (1 M) were sequentially mixed. Following the reaction, the absorption spectra were measured to determine the optimal working concentration of the aptamer.

Colorimetric Detection of AFB1 Based on MnO NSs and LC Emulsion

10 μL of AFB1 standard solutions at various concentrations (0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ng/mL) were respectively mixed thoroughly with 30 μL of CTAB (10 mM) and 10 μL of AFB1-Apt (1 μM), followed by incubation for 10 min. Subsequently, 20 μL of the LC emulsion and the aforementioned incubated mixture were sequentially added into a centrifuge tube, and the reaction was allowed to proceed in a water bath at 35 $^\circ\text{C}$ for 10 min. After completion of the reaction, 50 μL of MnO_2 NSs and 80 μL of TMB solution (1 mM) were sequentially introduced. The system was allowed to react for an additional 5 min, after which 20 μL of H_2SO_4 (1 M) was added to terminate the reaction. Finally, 180 μL of the supernatant was accurately pipetted into a 96-well plate, and the absorbance of each well was measured and recorded using a microplate reader. To evaluate the selectivity of the developed sensor, six common mycotoxins (F-2, FB1, T-2, AFB2, PAT, and OTA) were investigated as potential interferents. Additionally, a mixture containing all the aforementioned mycotoxins was tested. All assays were conducted in accordance with the previously described protocol. The concentration of each individual interferent was set at 1.0 ng/mL. For the mixed interference assay, the final concentration of each non-target toxin was 10 ng/mL, whereas the target AFB1 was maintained at 1.0 ng/mL.

Real sample analysis

To evaluate the practical applicability of the proposed method, food samples with different matrices were purchased from a local supermarket for real sample analysis. The detection procedures were identical to those described in Section 2.5. The specific sample pretreatment methods were as follows:

Sunflower seeds and rice

the samples were fully pulverized, and 5 g of the powder was accurately weighed into a 50 mL flask. Subsequently, 25 mL of 60% methanol solution was added. The mixture was shaken for 10 min, centrifuged at 4000 r/min for 5 min, and then filtered. A 1 mL aliquot of the filtrate was collected and mixed thoroughly with 4 mL of deionized water for further use.

Soy milk powder

An aliquot of 5 g of soy milk powder was accurately weighed into a 50 mL Erlenmeyer flask, followed by the addition of 25 mL of 60% methanol solution. The mixture was shaken for 10 min, centrifuged at 4000 r/min for 5 min, and filtered. Then, 1 mL of the filtrate was mixed thoroughly with 4 mL of deionized water for subsequent analysis.

Milk

A 1 mL volume of milk sample was accurately transferred into a 7 mL polystyrene centrifuge tube. Then, 5 mL of deionized water was added, and the mixture was vortexed for 5 s before use.

Rapeseed oil

2 mL sample was mixed with 25 mL of 60% methanol solution, vortexed for 10 min, centrifuged at 4000 rpm for 5 min, and the supernatant was collected.

Subsequently, AFB1 standard solutions were spiked into the samples at concentrations of 200, 400, and 600 pg/mL. The recovery rate was determined. The AFB1 concentrations in the moldy rice and rapeseed oil samples were determined by our proposed assay as well as using the HPLC method specified in the Chinese National Standard GB 5009.22–2016 for comparison.

Results and discussion

Detection principle

The sensing mechanism of the LC emulsion-coupled MnO_2 NSs-based colorimetric biosensor toward AFB1 is schematically depicted in Fig. 1, which can be divided into three sequential operational stages. During the preliminary preparation step, GSH is encapsulated inside SDS-stabilized LC emulsion by dispersing the GSH-SDS mixture within the LC matrix to construct GSH@SDS LC emulsions. The second stage corresponds to target recognition and interfacial chemical reactions, wherein a pre-incubated ternary mixture of CTAB, Apt, and AFB1 is introduced into the as-prepared LC emulsion. For the target-containing system (Path A), the specific molecular recognition between AFB1 and its aptamer liberates positively charged free CTAB. The dissociated CTAB further triggers intense electrostatic interfacial interactions with LC emulsion, destabilizing the emulsified architecture and triggering the outward leakage of encapsulated GSH into the aqueous continuous phase. Entering the third colorimetric

quantification stage, the reductant GSH released from microdroplets fully decomposes MnO_2 NSs into Mn^{2+} ions and abolishes the intrinsic oxidase-mimicking catalytic activity of MnO_2 NSs. As a result, chromogenic substrate TMB fails to undergo oxidative conversion, and the whole system remains colorless after H_2SO_4 is supplemented to quench the chromogenic reaction. In contrast, in the absence of AFB1 (Path B), electrostatic attraction drives tight complexation between cationic CTAB and negatively charged aptamers, which sequesters CTAB and inhibits its binding to the LC droplet interface, thus completely suppressing GSH efflux. Under such circumstances, intact MnO_2 NSs preserve robust oxidase-like activity and efficiently catalyze the oxidation of colorless TMB to oxidized TMB (ox-TMB), subsequent acidification with H_2SO_4 converts ox-TMB into a characteristic yellow chromophore. Taken together, distinct visual chromatic contrasts (colorless vs. bright yellow) generated after H_2SO_4 treatment enable facile naked-eye qualitative detection of trace AFB1.

Preparation and characterizations of MnO_2 NSs and LC Emulsion

As illustrated in Fig. 2A, the UV-vis absorption spectrum of as-synthesized MnO_2 NSs exhibits a characteristic absorption peak at 380 nm. The high-resolution TEM image (Fig. 2B) reveals that the as-prepared products possess a typical two-dimensional layered structure with partial stacking and overlapping of nanosheets. These optical and micromorphological properties are consistent with those of two-dimensional layered MnO_2 NSs reported in previous literatures (Li et al, 2025; Ma et al, 2026), which verifies the successful fabrication of MnO_2 NSs. Meanwhile, the LC emulsion system was characterized by optical microscopy and DLS. The bright-field optical micrograph (Fig. 2C) demonstrates that the encapsulated aqueous microdroplets inside the emulsion feature uniform size and favorable dispersion. Particle size measurement results (Fig. 2D) indicate that the average diameter of microdroplets is approximately 0.8 μm . Collectively, the above characterization results confirm that the liquid crystal emulsion can stably and efficiently encapsulate aqueous microdroplets.

Feasible verification

The oxidase-like catalytic activity of MnO_2 NSs toward TMB oxidation is a fundamental prerequisite for the construction of this colorimetric sensing system, and the catalytic performance of MnO_2 NSs was first verified via UV-vis spectroscopy (Fig. 3A). The results showed that the reaction system with MnO_2 NSs displayed a distinct characteristic absorption peak at 450 nm, while no obvious absorption signal was detected in the blank group without MnO_2 NSs, verifying the intrinsic oxidase-like activity of MnO_2 NSs. On this basis, the reductive degradation effect of GSH on MnO_2 NSs was further explored (Fig. 3B). In the presence of CTAB, CTAB interacted with SDS in LC to trigger the release of GSH, and the released GSH with strong reducibility could degrade MnO_2 NSs into Mn^{2+} , leading to the complete disappearance of the 450 nm absorption peak. Conversely, GSH remained encapsulated in LCs without CTAB treatment,

which preserved the catalytic activity of MnO_2 NSs and thus produced a prominent absorption signal at 450 nm. Quantitative investigation with gradient GSH concentrations further clarified the interaction between GSH and MnO_2 NSs (Fig. 3C), where the absorbance intensity at 450 nm decreased continuously with the increase in GSH concentration, confirming a positive correlation between GSH concentration and the degradation degree of MnO_2 NSs and enabling tunable colorimetric responses by regulating LC-released GSH content. Finally, the feasibility of the proposed sensing platform for AFB1 detection was validated (Fig. 3D), and spectral comparison results revealed that the system containing AFB1 only exhibited weak baseline absorbance at 450 nm, whereas a significant characteristic absorption peak was observed in the AFB1-free system. Collectively, these spectral results demonstrate that the fabricated LC-based colorimetric biosensor is capable of specific detection of AFB1.

Optimization of parameters

To boost the sensing performance of the LC-based colorimetric assay for AFB1, single-factor optimization of four key experimental variables, namely reaction duration, incubation temperature, CTAB concentration and Apt concentration, was systematically performed in the present study. As depicted in Fig. 4A, the absorbance signal at 450 nm progressively decreased as the reaction proceeded and plateaued at 10 min; at this equilibrium state, GSH liberated from LC emulsions thoroughly decomposed MnO_2 NSs and inhibited the chromogenic oxidation of TMB, which justified the selection of 10 min as the optimal reaction time. Temperature-dependent assays (Fig. 4B) demonstrated that thermal elevation expedited the leakage of LC microdroplets and lowered the absorbance at 450 nm. Nevertheless, 5CB LC possesses a critical phase transition temperature of 35.5 °C (Dhara et al, 2019), and overheating would irreversibly break the switchable sensing readout, such that 35 °C was chosen as the optimal incubation temperature. Serving as a cationic surfactant, CTAB governs the interfacial electrostatic potential of LCs and dictates the microdroplet release rate. The UV-vis spectra in Fig. 4C indicated that higher CTAB concentrations accelerated microdroplet leakage, and the absorbance at 450 nm nearly reached the baseline at 10 μM , which was therefore designated as the optimal CTAB concentration. Aptamers are capable of electrostatically sequestering CTAB and diminishing its ability to initiate microdroplet efflux from LCs. As reflected in Fig. 4D, the absorbance at 450 nm gradually elevated with increasing Apt concentration and levelled off at 1 μM , indicative of saturated electrostatic complexation between Apt and CTAB; accordingly, 1 μM was confirmed as the optimal aptamer working concentration.

Sensitivity and specificity of the colorimetric strategy

Under the optimized experimental conditions, the analytical performance of the LC-based colorimetric aptasensor was evaluated using a series of AFB1 standard solutions with concentrations ranging from 0 to 1.2 ng/mL. As illustrated in Fig. 5A, the absorbance value at 450 nm decreased progressively with the elevation of AFB1 concentration. The corresponding sensing mechanism is elaborated as follows: AFB1 specifically binds to free aptamers in the reaction system, which attenuates the electrostatic shielding effect of aptamers on CTAB and further induces the release of GSH from liquid crystal emulsions. The

released GSH can reduce and decompose MnO_2 NSs into Mn^{2+} , thereby suppressing the formation of TMB chromogenic oxidation products and diminishing the characteristic absorbance signal at 450 nm. Notably, a favorable linear relationship between the 450 nm absorbance and AFB1 concentration was achieved in the range of 0.2-1.0 ng/mL (Fig. 5B). The linear regression equation was determined as $A = 0.2552 - 0.0020C_{\text{AFB1}}$ with a high coefficient of determination ($R^2 = 0.9977$), and the limit of detection (LOD) was calculated to be 0.73 ng/mL according to the $3\sigma/S$ method. Furthermore, the specificity and anti-interference ability of the developed sensor were investigated by selecting multiple common mycotoxins, including F-2, FB1, T-2, AFB2, PAT, and OTA, as interfering substances, with all interferents prepared at a 10-fold higher concentration than AFB1. As demonstrated in Fig. 5C, only the groups containing AFB₁ exhibited a significant decrease in absorbance, and the mixed toxin sample showed a negligible signal difference compared with the pure AFB1 group, while individual interfering toxins maintained stable high absorbance baselines. These results confirm that the proposed colorimetric strategy exhibits excellent specificity and prominent anti-interference performance for the selective detection of AFB1.

Practicability of the novel colorimetric strategy based on the LC emulsion and MnO_2 NSs

To assess the practical feasibility of the developed sensing strategy, spike-and-recovery assays were conducted by fortifying three typical food matrices (sunflower seeds, soybean milk powder, and milk) with AFB1 standard solutions at gradient concentrations of 200, 400, and 600 pg/mL (Table 1). The obtained results revealed that the recoveries of the proposed method varied from 92.18% to 108.23%, with relative standard deviations (RSDs) of 1.81%-3.27%. All analytical parameters fell within the acceptable error range, verifying the favorable stability and accuracy of this detection system. To further validate its practical reliability, HPLC was adopted as a benchmark method for comparative detection of AFB1 in moldy edible oil and moldy rice samples (Table 2). The HPLC measurements demonstrated that the AFB₁ content in moldy edible oil was 10.5 $\mu\text{g/kg}$, whereas AFB₁ in moldy rice was undetectable due to its concentration below the HPLC limit of detection (0.1 $\mu\text{g/kg}$). Notably, the developed sensor yielded an AFB1 content of 10.31 $\mu\text{g/kg}$ for moldy edible oil with a standard deviation of 0.032, exhibiting great agreement with the HPLC data. Collectively, these results prove that the established method is capable of precise and reliable quantitative detection of AFB1 in complex real food samples.

Table 1
The detection recoveries of AFB1 in real samples using the standard addition method.

Samples	Spiked level (pg/mL)	Detection (pg/mL)	Recovery rate (%)	RSD (% , n = 3)
Sunflower seed	200	195.28	97.64	2.19
	400	368.72	92.18	2.59
	600	574.38	95.73	2.41
Soymilk powder	200	212.98	106.49	1.81
	400	404.92	101.23	1.99
	600	584.16	97.36	3.27
Milk	200	216.46	108.23	2.53
	400	369.56	92.39	1.87
	600	638.88	106.48	3.07

Table 2
Detection results of AFB1 in moldy rice and oil samples by the proposed assay and HPLC method.

Method	Sample	Detected concentration (ug/kg)	RSD
HPLC	Moldy oil-1	10.5	/
	Moldy wheat-1	undetectable	/
This work	Moldy oil-1	10.31	0.032
	Moldy wheat-1	undetectable	/

Conclusions

In summary, a highly specific and label-free colorimetric aptasensor was successfully developed for the visual detection of AFB1 by integrating stimuli-responsive LC emulsions with the oxidase-like catalytic activity of MnO₂ NSs. The sensing mechanism leverages the competitive binding between the AFB1 target, its specific aptamer, and the interfacial trigger CTAB. This dynamic interaction effectively controls the phase state of the LC micro-reservoirs, dictating the release of encapsulated GSH and the subsequent modulation of the MnO₂ NSs-TMB colorimetric reaction. The developed strategy exhibited excellent analytical performance, featuring a linear detection range of 0.2–1.0 ng/mL, a low LOD of 0.73 ng/mL, and prominent anti-interference capability against multiple mycotoxin analogues. The practical reliability and accuracy of the sensor were further confirmed through the successful quantification of AFB1 in complex agricultural and food products, yielding results that were highly consistent with the standard HPLC method. By bypassing complex probe labeling and tedious operational procedures, this proposed assay prioritizes user-friendliness, cost-effectiveness, and rapid response. Ultimately, this work

provides a versatile and intuitive analytical platform for on-site food safety assessment and introduces a promising new paradigm for designing smart-material-based biosensors

Declarations

Conflict of Interest

The authors declare no competing interests.

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Author Contribution

Yi Wei: Conceptualization, Methodology, Formal analysis, Writing—original draft. Xinhui Xie: Methodology, Formal analysis, Writing – original draft. Jiajie Chen: Methodology, Formal analysis. Huilong Chen: Methodology, Formal analysis. Zehui Zhang: Formal analysis, Writing – original draft. Dai Lu: Conceptualization, Methodology, Funding acquisition, Project administration, Writing - review & editing, Supervision. Qian Zhao: Formal analysis, Methodology, Writing - review & editing.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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Figures

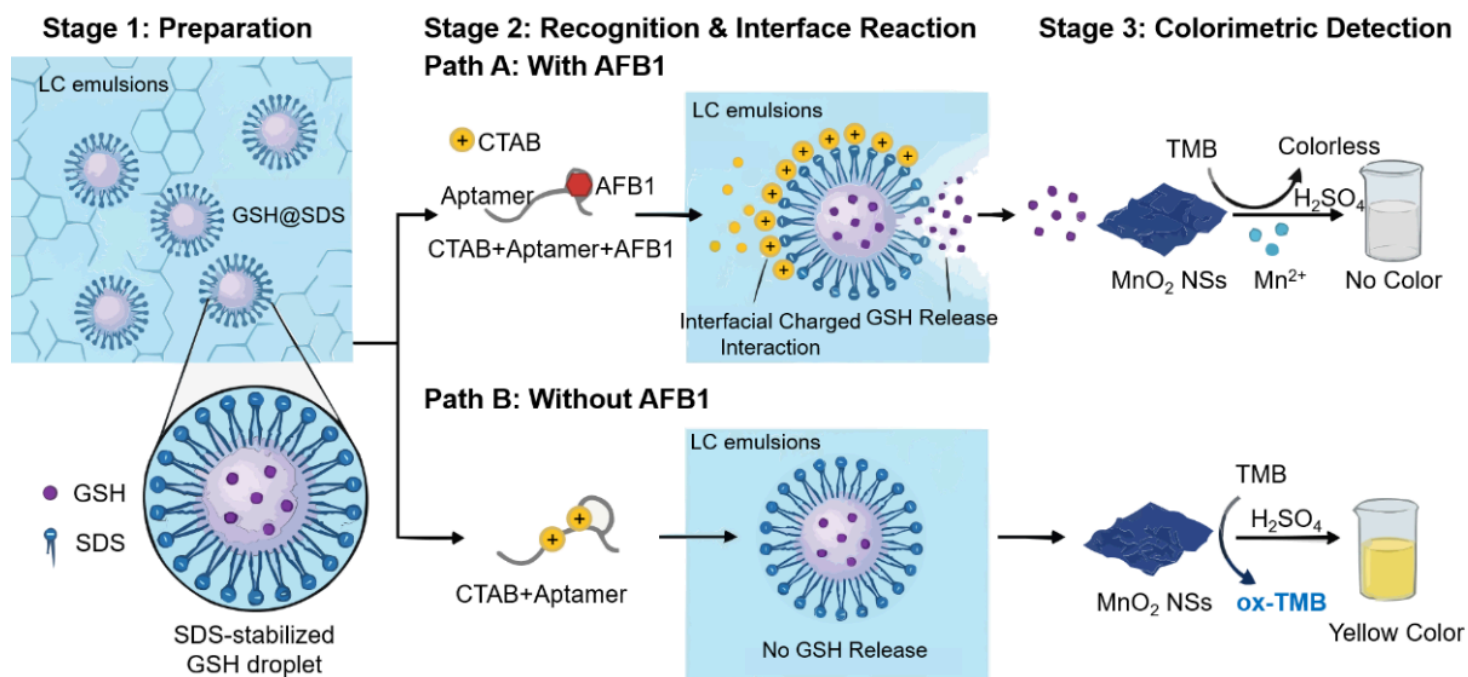


Figure 1

Schematic diagram of the principle of the colorimetric biosensor for aflatoxin B1 based on MnO₂ NSs and LC emulsions.

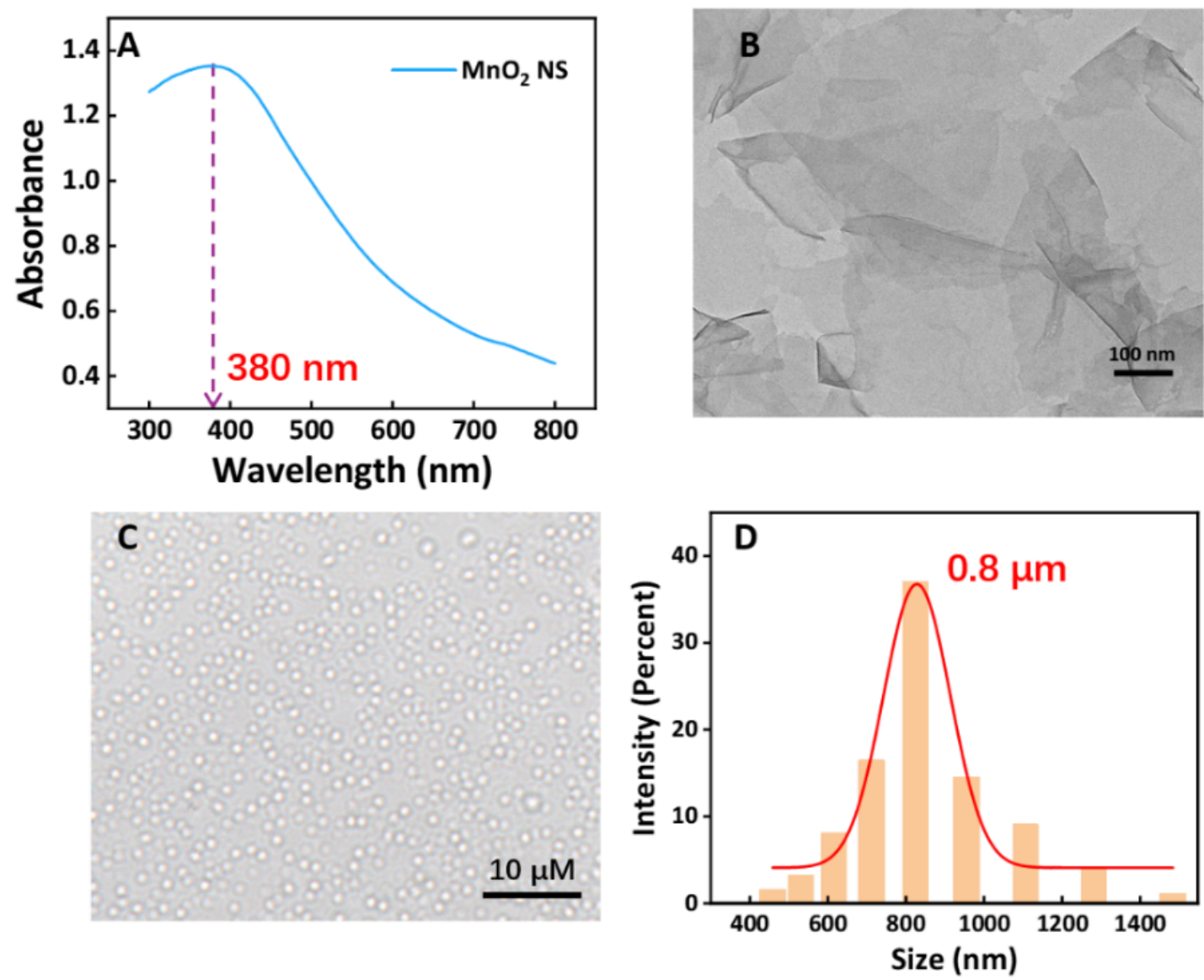


Figure 2

Synthesis and Characterization of MnO₂ NSs and LC Emulsion. (A) UV-vis absorption spectrum of MnO₂ NSs. (B) TEM image of MnO₂ NSs. (C) Bright-field microscopy image of LC emulsion. (D) Hydrodynamic diameter plot of LC emulsion.

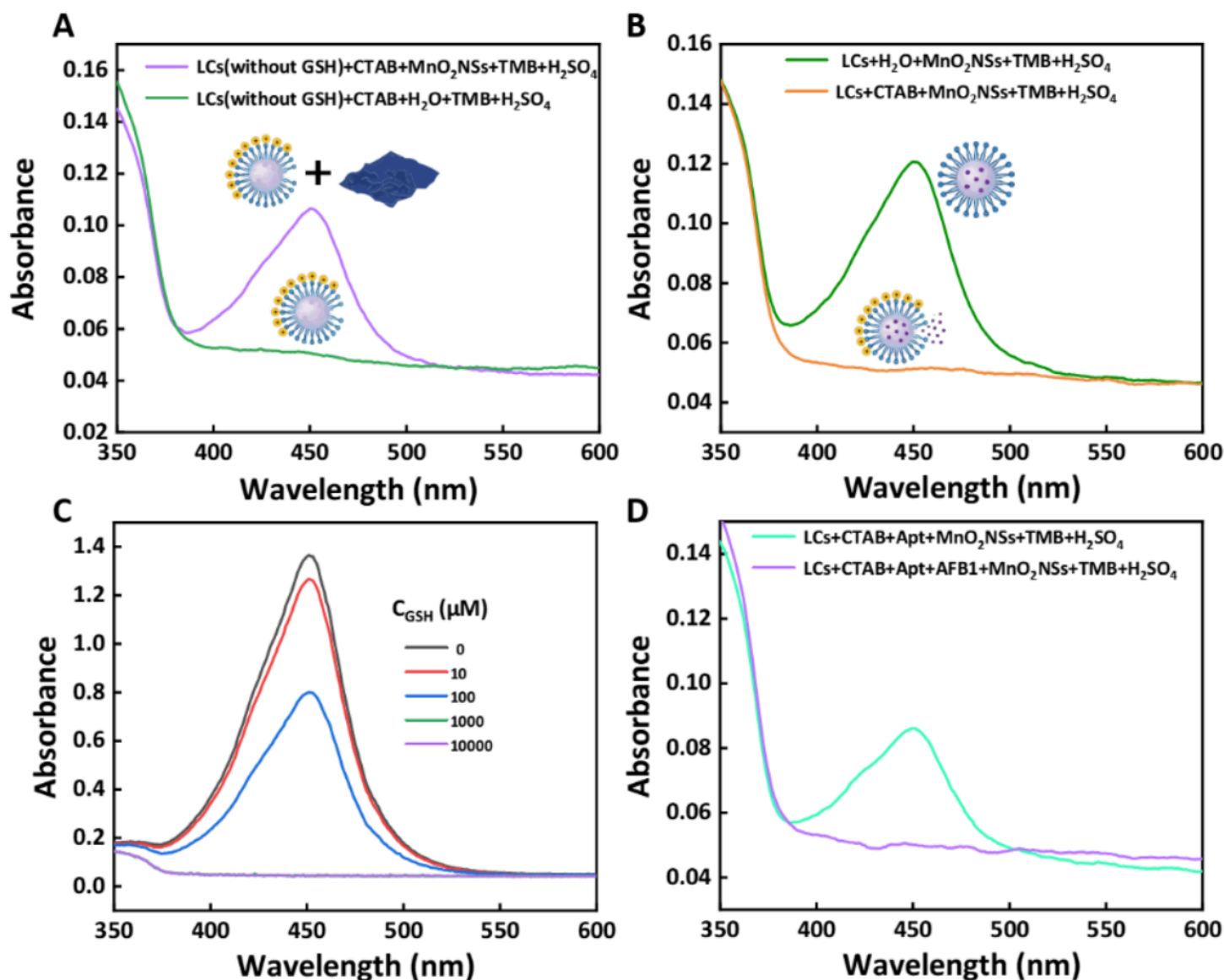


Figure 3

Feasibility analysis experiments. (A) Verification of the oxidase activity of MnO₂ NSs. (B) UV-vis absorption spectra demonstrating the GSH release effect induced by CTAB disruption of liquid crystal emulsion interface. (C) UV-vis absorption spectra illustrating the concentration-dependent inhibitory effect of exogenous GSH on MnO₂ NSs catalytic activity. (D) UV-vis absorption spectra confirming the target-responsive signal switching of the sensing strategy.

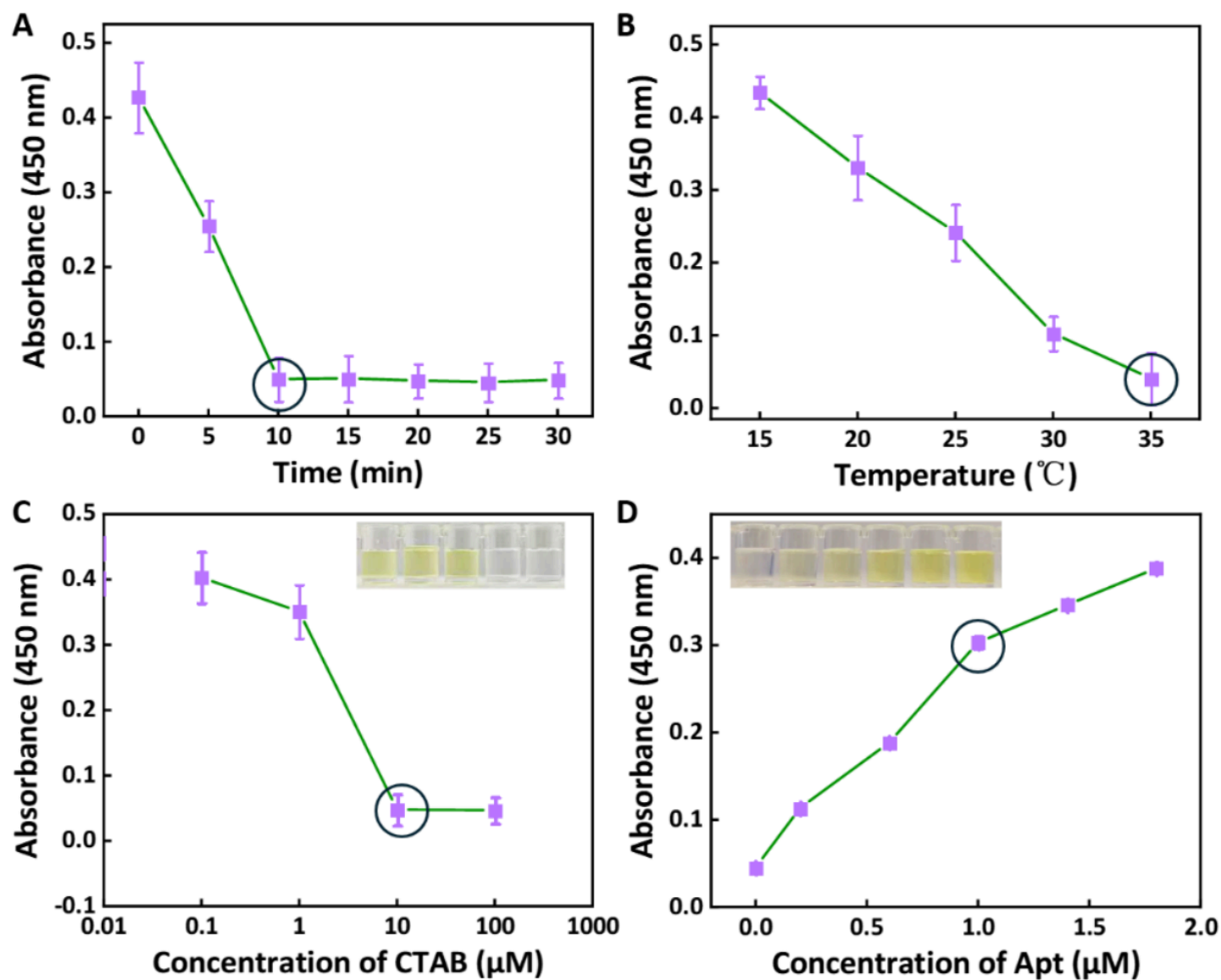


Figure 4

Optimization of experimental conditions. Optimization of (A) reaction time between CTAB and LC emulsion, (B) reaction temperature, (C) CTAB concentration and Apt concentration.

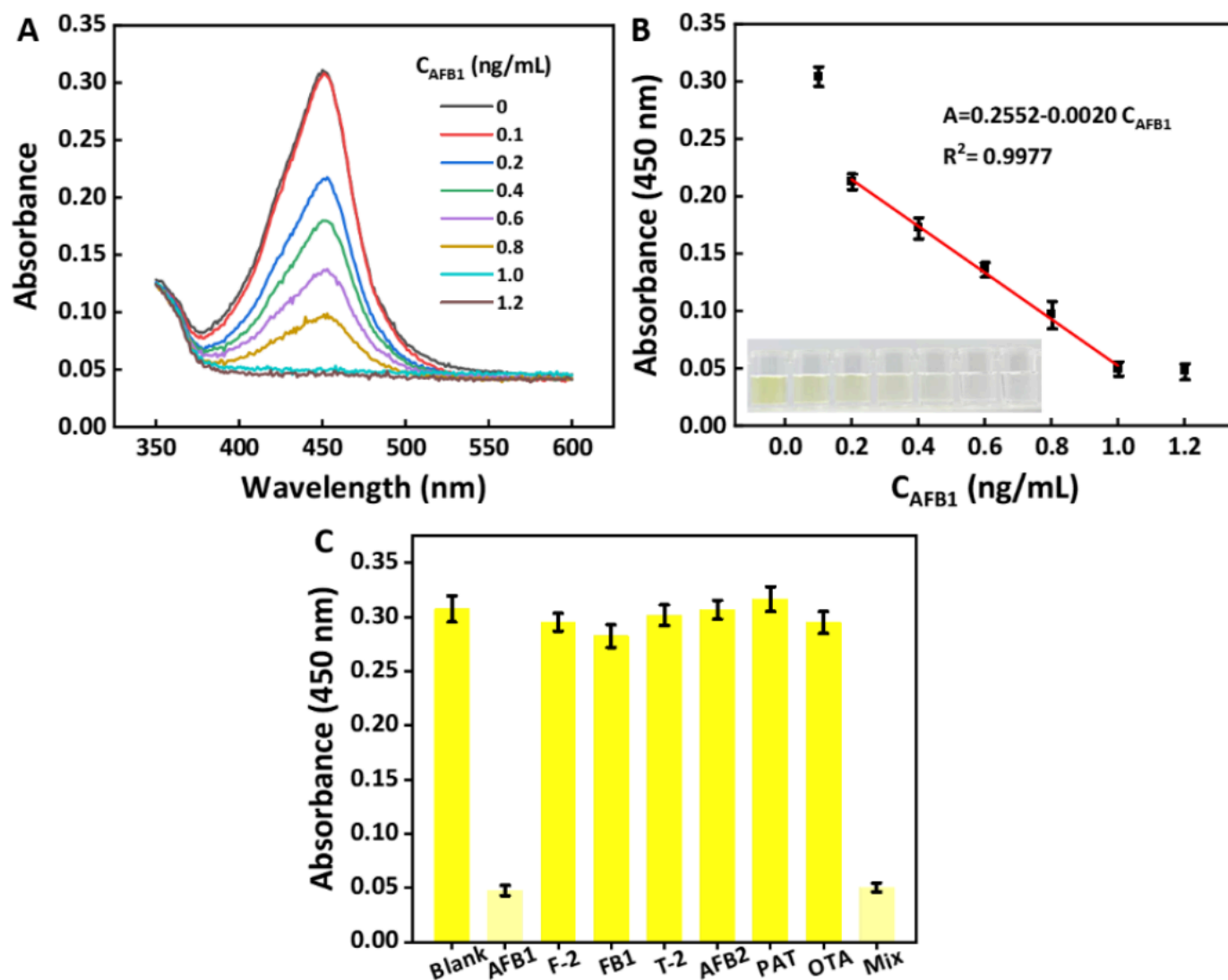


Figure 5

(A) UV-vis absorption spectra of the sensing system incubated with a series of gradient AFB1 concentrations. (B) Linear calibration curve between absorbance at 450 nm and AFB1 concentration in the range of 0.2 - 1.0 ng/mL. (C) Specificity evaluation of the sensing strategy toward AFB1 and various interfering mycotoxins, including F-2, FB1, T-2, AFB2, PAT, OTA, and a mixture of all interferent.