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Construction of Fluorescence Sensing Platform on the Basis of Molybdenum Disulfide Nanosheet for the Detection of AFB_1

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Abstract

Purpose: Aflatoxin B_1 is the most common mycotoxin in cereal crops; it is of stronger toxicity and has a carcinogenic effect. In recent years, a series of fluorescence sensors constructed on the basis of MoS_2NS fluorescence quenching property have become a research hotspot. Therefore, we can construct a fast and simple analysis method with high specificity to detect AFB_1 by utilizing MoS_2NS, which can be effectively applied to food safety monitoring and clinical diagnosis. **Method:** In the current research, a fluorescence biosensor is developed on the basis of a new type of two-dimensional nano-material namely MoS_2NS applied for the detection of AFB_1. The fluorescence of Apt@AFB_1 can be quickly quenched by MoS_2NS through the fluorescence resonance energy transfer (FRET). When the target molecule AFB_1 exists, after the specificity binding between AFB_1 and aptamer, the Apt@AFB_1 loses its single stranded structure and is away from MoS_2NS, and the fluorescence of Apt@AFB_1 cannot be quenched effectively. Such sensing signals can be used to achieve the sensitive detection of AFB_1. **Result:** With this new method, under the optimized conditions, the AFB_1 is analyzed in the MoS_2NS/Apt@AFB_1 sensing platform. Within the dynamic range of 0.2 - 25 ng/mL, the sensing platform expresses a good linear response to the level of AFB_1 with the $R^2 = 0.9964$ and LOD as 90 pg/mL. This method is applied to detect the actual serum samples and soybean milk with the recovery rate of 93.10% - 107.23% and 95.15% - 102.60% separately, and it can be used in the quantitative detection under the interference of other mycotoxins in a relatively accurate way. **Conclusion:** It is proved that this new detection method can be used as a potential biosensor platform for the detection of AFB_1. This detection method features several advantages such as specificity, rapidness and low costs, which can meet the requirement of trace detection in clinical detection and food safety.

Keywords

MoS_2NS, Aptamer, Aflatoxin B_1, Fluorescence Sensing Analysis, Food Monitoring

1. Introduction

Aflatoxin is a secondary metabolite derived from fungus such as *Aspergillus flavus* and parasitic *Aspergillus* under the stressful condition. Although the amount of toxin produced by mold is very low, but the Aflatoxin is the mycotoxin with the strongest toxicity and Carcinogenicity founded by far [1], and the Aflatoxin B_1 (AFB_1) is a subtype of the Aflatoxin with the strongest toxicity [2]. The normal sterilization method of heating process cannot destroy its structure [3]. When a human eats crops containing a large amount of toxin such as seriously moldy peanuts, corn and bean at a time, acute poisoning could be easily caused, leading to diseases such as acute hepatitis, hemorrhagic necrosis and hepatocyte steatosis. If a human eats moldy cereal for a long time, liver chronic injuries would be caused, leading to the carcinogenesis and teratogenicity [4] [5].

Soybean milk is a special drink in China made from soybeans, which is well received among the population. If the soybean milk is made from moldy soybeans, the Aflatoxin is hard to be degraded during the preparation of soybean milk [2] [3]. The long-term drinking of such soybean milk will easily cause chronic poisoning of the human body. Therefore, the inspection of AFB_1 content in soybean products is of great importance. Countries of the world implement strict controls on the safety content of Aflatoxin in foods. China regulates that the content of Aflatoxin in soybean and its products shall not exceed 5 μg/mL [6]. The European Commission regulates the maximum pollution limit of crops and crop products, which is 4 μg/kg [7] for Aflatoxin and 2 μg/kg [8] for AFB_1 separately. Clinically, the toxicology analysis of food poisoning is also of great importance in the etiological treatment. Therefore, detecting the content of Aflatoxin in a fast, responsive and specific way is of great significance to ensure food safety, improve people's quality of life and enhance the sustainable development of agriculture, which can also be applied effectively in the etiological diagnosis of clinical patients of acute food poisoning.

Molybdenum disulfide nanosheet (MoS_2NS) is a new type of two-dimensional nanomaterial, which is another hot research topic after graphene. It has an outstanding fluorescence-quenching feature and the absorbability of single-stranded nucleic acid [9] [10]. At present, a large number of studies have applied this feature to the detection of small molecules such as miRNA [11] and DNA [12] as well as the biological analysis of large molecules such as carcinoembryonic antigen, prostate specific antigen [13], and cardiac troponin T [14]. Such applications are conducive to the development of basic research in life science and the reform of medical clinical detection technology. In addition, the MoS_2NS fea-

tures good dispersibility in water solution without surface-active agent or additional modification [12] [15]. It can also be prepared massively, which is a good kind of material for fluorescence sensing. The current research constructs a simple and rapid functional nano-biosensor MoS_2NS/Apt@AFB_1 on the basis of MoS_2NS and the aptamer Apt@AFB_1. With MoS_2NS as the fluorescence quenching carrier, Apt@AFB_1 is absorbed on the surface of MoS_2NS through the deposition and combination of π-π chemical bonds. Due to the Fluorescence Resonance Energy Transfer (FRET), the FAM fluorescent dye modified on the aptamer is quenched. When the target molecule AFB_1 exists, it can specifically bind to the aptamer to change the spatial construction of the aptamer, causing the MoS_2NS unable to effectively absorb the compound structure of Apt@AFB_1 and AFB_1. The fluorescence quenching effect is lost and the fluorescent dye can release signals. Through the intensity of fluorescence sensing signals, the content of AFB_1 can be detected effectively. This method is of possibility to open up a new path to the ultra-sensitive analysis of other mycotoxin or microorganisms. The sensing method constructed by the material of MoS_2NS is conductive to the application in clinical detection and food safety management and control.

2. Materials and Methods

2.1. Material

Tris-HCl buffer (20 mM Tris, 5 mM KCl, 5 mM $MgCl_2$, 100 mM NaCl, pH 7.4) is purchased from Sangon Biotech (Shanghai) Co., Ltd.; Molybdenum disulfide nanosheet is purchased from Nanjing XFNANO Materials Tech Co., Ltd.; Fetal bovine serum (FBS) is purchased from gibco Company (Grand Island, USA); The soybean milk sample is purchased from the canteen of the university; The water used in the experiments is all ultra-purified water; the AFB_1 aptamer sequence marked by FAM fluorescein is 5'-FAM-GTT GGG CAC GTG TTG TCT CTC TGT GTC TCG TGC CCT TCG CTA GGC CCA CA-3', which is composed by Sangon Biotech (Shanghai) Co., Ltd. All samples are reacted under the temperature of 37˚C. The fluorescence spectrophotometer (RF-6000, Shimadzu) is used to conduct fluorescence detection.

2.2. Methods

2.2.1. Preparation of MoS_2NS

Process the purchased water dispersing solution of molybdenum disulfide nanosheet with ultrasonic treatment for 1 h, then conduct centrifugation with 15,000 rpm for 15 min. Dissolve the centrifugal precipitates again into the ultra-purified water to obtain the black suspension liquid of MoS_2NS. Dilute the MoS_2NS into a solution with the concentration of 1 mg/mL as the reserve solution and store it under the temperature of 4˚C for later use [16]. Before its usage, process it with ultrasonic treatment for 20 min to ensure its good dispersibility.

2.2.2. Optimization of Experimental Conditions

1) Optimization of MoS_2NS Concentration: in order to determine the best

fluorescence quenching effect of this sensing method, fix the concentration of the aptamer probe Apt@AFB_1 as 100 nM, then react the Apt@AFB_1 probe and the MoS_2NS with different concentration (0, 10, 20, 40, 60, 80, 100 and 120 μg/mL) at the temperature of 37˚C. Use fluorescence spectrophotometer to measure the fluorescence intensity and observe the fluorescence quenching conditions.

2) Optimization of Quenching Time: incubate 100 nM Apt@AFB_1 and the MoS_2NS with the final concentration of 80 μg/mL for a certain time (1, 2, 4, 6, 8, 10, 15, 30, 40, 60 and 120 min) under the temperature of 37˚C. After the incubation, use the fluorescence spectrophotometer to measure the fluorescence intensity and observe the fluorescence quenching conditions.

(3) Optimization of the Reaction Time of Apt@AFB_1 and AFB_1: incubate 100 nM Apt@AFB_1 probe with 10 ng/mL AFB_1 for different durations (0, 10, 20, 30, 40, 50 and 60 min) under the temperature of 37˚C. After the incubation, add the MoS_2NS with the concentration of 80 μg/mL into the reaction system and conduct incubation with fluctuation for 8 min, then use the fluorescence spectrophotometer to record the fluorescence intensity and observe the best reaction time of the sensing platform.

2.2.3. Fluorescence Analysis of the Sensing System

1) Feasibility analysis: the current research needs to discuss the response of fluorescence spectrometry with different experimental ingredients (Apt@AFB_1, MoS_2NS, AFB_1, MoS_2NS + Apt@AFB_1, and MoS_2NS + Apt@AFB_1 + AFB_1). After the incubation in accordance with the sensing scheme, record the emission spectrometry within the range of 505 - 650 nm at the excitation wavelength of 496 nm to analyze the detection feasibility of the MoS_2NS/Apt@AFB_1 sensing strategy.

2) Sensitivity analysis: Firstly, dissolve the AFB_1 powder into the methyl alcohol, and then dilute it with the Tris-HCl buffer into the AFB_1 solution with the concentration of 300 ng/mL as the reserve solution. Dilute the Apt@AFB_1 marked by FAM in the Tris-HCl buffer preliminarily to the solution with the concentration of 300 nM as the reserve solution, and store it in sub-package under the temperature of 4˚C. In order to evaluate the analysis and application capability of this sensing platform in actual samples, we conduct quantitative detection to the AFB_1. Under the best experimental conditions, prepare AFB_1 reserve solution with different volumes (2, 5, 10, 20, 40, 80, 140, 200, 250, 300, 400 and 600 μL) and mix them with 1 mL Apt@AFB_1 (300 nM) probe. Add Tris-HCl buffer to 2 mL and conduct fluctuation for 30 min under the temperature of 37˚C. After the fluctuation, add 0.24 mL MoS_2NS solution (1 mg/mL) and 0.76 mL Tris-HCl buffer, then continue the incubation of the reaction solution for 8 min. Finally, use the fluorescence spectrophotometer to measure the fluorescence intensity F, and record the fluorescence intensity of the samples without AFB_1 as F_0. Each concentration is measured in parallel for 3 times. Record the emission spectrometry within the range of 505 - 650 nm under the excitation wavelength of 496 nm. Use x coordinate to represent the concentration of AFB_1 and y coordinate to

represent the fluorescence intensity of the reaction system to draw a standard curve, then calculate and analyze the linear range of the sensing detection and the lower limit of detection to explore the detection sensitivity of this sensing method.

2.2.4. Specificity Detection

In order to verify the selectivity of the MoS_2NS/Apt@AFB_1 sensing platform, we choose AFB_2, AFM_1, OTA and FB_1 and compare them with AFB_1. Prepare the AFB_1 with the concentration of 10 ng/mL and the AFB_2, AFM_1, OTA and FB_1 with the concentration of 20 ng/mL as the samples to be tested. In addition, mix 10 ng/mL of AFB_1 with 20 ng/mL of AFB_2, AFM_1, OTA and FB_1 separately to prepare the mixed solution as the sample to be tested, and prepare the blank control group without any toxin. Add 1 mL of Apt@AFB_1 probe solution with concentration of 300 nM into 1 mL of sample to be tested separately and fully mix them, and then conduct incubation with fluctuation under the temperature of 37˚C for 30 min. After the incubation, add 0.24 mL MoS_2NS (1 mg/mL) solution and 0.76 mL Tris-HCl buffer for 8min incubation reaction. Use fluorescence spectrophotometer to measure the fluorescence intensity at the wavelength of 517 nm under the excitation wavelength of 496 nm. This is repeated for 3 times in parallel for each sample, then compare and analyze all detected fluorescence intensity data.

2.2.5. Inspection of Samples

This is in order to evaluate the analysis and application capability of this sensing platform in actual serum samples and soybean milk. The serum samples to be tested are the 10% serum samples prepared by diluting FBS for 10 times. Then we prepare the soybean milk sample. Firstly, add 70% methyl alcohol into the purchased soybean milk (the volume ratio between soybean milk and 70% methyl alcohol is 1:2). Mix it evenly through shaking at room temperature for 30 min, then process ultrasonic treatment for 5min and centrifugation at 10,000 rpm for 10 min. The supernatant was obtained and filtered with 0.22 μm organic filter membrane. After the filtering, dilute the soybean milk for 10 times as the soybean milk residual solution. Then the detection performance of the MoS_2NS/Apt@AFB_1 fluorescence sensing platform in the actual serum and soybean milk samples will be examined. Under the best conditions, add AFB_1 with three types of concentrations (0.2, 5 and 15 ng/mL) into the 10% FBS and mix them well to prepare the sample to be tested, then detect it in accordance with the sensing strategy and record the fluorescence intensity. The same operation is conducted to prepare the soybean milk sample. Add AFB_1 with different concentrations (0.2, 5 and 15 ng/mL) into the soybean milk residual solution and mix them well, then conduct detection in accordance with the sensing strategy, record the fluorescence intensity and calculate the recovery rate. For the samples with each concentration, 5 experiments in parallel shall be set. The blank sample without toxin shall be set to record its fluorescent signal.

3. Results

3.1. Experiment Principle

MoS_2NS is capable to absorb ssDNA effectively. The current research uses the identifying probe of Apt@AFB_1. Apt@AFB_1 is the single-stranded deoxynucleotidyl modified with FAM fluorescein at the 5' end. The detection principle of MoS_2NS/Apt@AFB_1 fluorescence sensing platform constructed by the current research is as shown in Figure 1. If the target molecule AFB_1 exists, after the specificity binding between Apt@AFB_1 and the target molecule, when the MoS_2NS is added, the aptamer undergoes conformational change due to the binding of Apt@AFB_1 and AFB_1, causing the Van der Waals force between MoS_2NS and Apt@AFB_1 weaken, making it unable to absorb and leading to the conjugate of Apt@AFB_1 and AFB_1 dissociated. The conjugate is away from the quenching agent MoS_2NS. Therefore, the fluorescence resonance energy transfer (FRET) is blocked, and the fluorophore on the Apt@AFB_1 probe emits fluorescence. When the target molecule does not exist, due to the π-π deposition, MoS_2NS is capable to absorb Apt@AFB_1. When the spatial distance between MoS_2NS and FAM is less than 10 nm, the FRET occurs to quench the fluorescent signal of the fluorescent dye FAM. The MoS_2NS/Apt@AFB_1 achieves the sensitive quantitative monitoring to AFB_1 through fluorescence sensing, which is expected to be developed as the new type of fluorescence sensors in the application of AFB_1 detection.

3.2. Feasibility Analysis

As shown in Figure 2, we evaluate the feasibility of MoS_2NS/Apt@AFB_1 as the fluorescence sensing platform to detect AFB_1. Apt@AFB_1 is the identifying probe

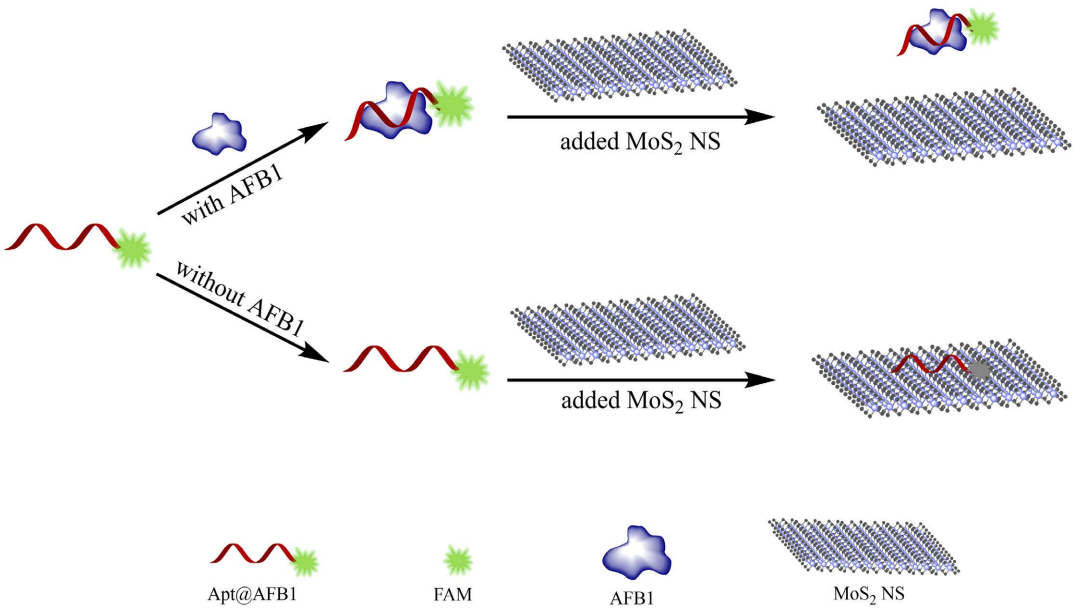

Figure 1. Schematic illustration of the principle of the method for AFB_1 detection based on fluorescence quenching of Molybdenum disulfide nanosheets.

modified with FAM. When the sample to be tested only contains the ingredient of AFB_1, the fluorescent detection result indicates that a strong fluorescent signal appears at the position of 517 nm. In order to detect whether there is autofluorescence for the ingredients of quenching agent MoS_2NS and target molecule AFB_1, we separately conduct fluorescence analysis by regarding them as the separate ingredients in the samples to be tested. The detection results of the MoS_2NS sample and the AFB_1 sample indicate that their fluorescent signal intensity at the position of 517 nm is almost zero, which means that it will not interfere the specificity fluorescent detection of the sensing platform on AFB_1, and the detection result of MoS_2NS + Apt@AFB1 sample indicates that MoS_2NS, as the fluorescence quenching agent, can effectively quench the fluorescence of the probe after its binding with Apt@AFB_1. The background signal of the constructed sensing platform is relatively low, which is conductive to improve the accuracy of the AFB_1 detection. The MoS_2NS + Apt@AFB_1 + AFB_1 sample refers to the detection of AFB_1 in the complete sensing system. The detection result indicates that when the molecular target AFB_1 exists, a strong fluorescence emission peak appears at the position of 517 nm. This result shows that the sensing strategy of MoS_2NS/Apt@AFB_1 can effectively detect AFB_1, and this method is feasible.

3.3. Optimization of Experimental Conditions

In order to determine the best detection performance of the MoS_2NS/Apt@AFB_1 sensing platform, the concentration of MoS_2NS, fluorescence quenching time and the reaction time of Apt@AFB_1 probe and the target object AFB_1 shall be optimized. With MoS_2NS as the fluorescence quenching agent, whether the fluorophore FAM can be effectively quenched by MoS_2NS is the key to the effectiveness of this sensing method. The result is as shown in **Figure 3(a)**. When the MoS_2NS with different concentrations exists, the fluorescence intensity of

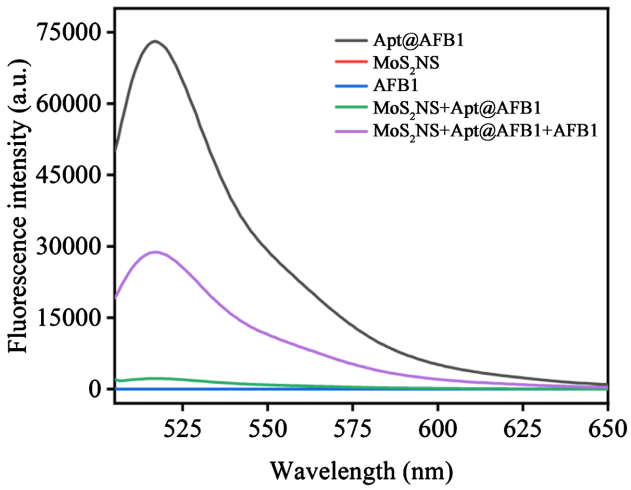

Figure 2. Fluorescence spectra under different conditions: Apt@AFB_1, MoS_2NS, AFB_1, MoS_2NS + Apt@AFB_1 and MoS_2NS + Apt@AFB_1 + AFB_1 (with the presence of 25 ng/mL AFB_1). The concentrations of Apt@AFB_1 and MoS_2NS were 100 nM and 80 μg/mL, respectively.

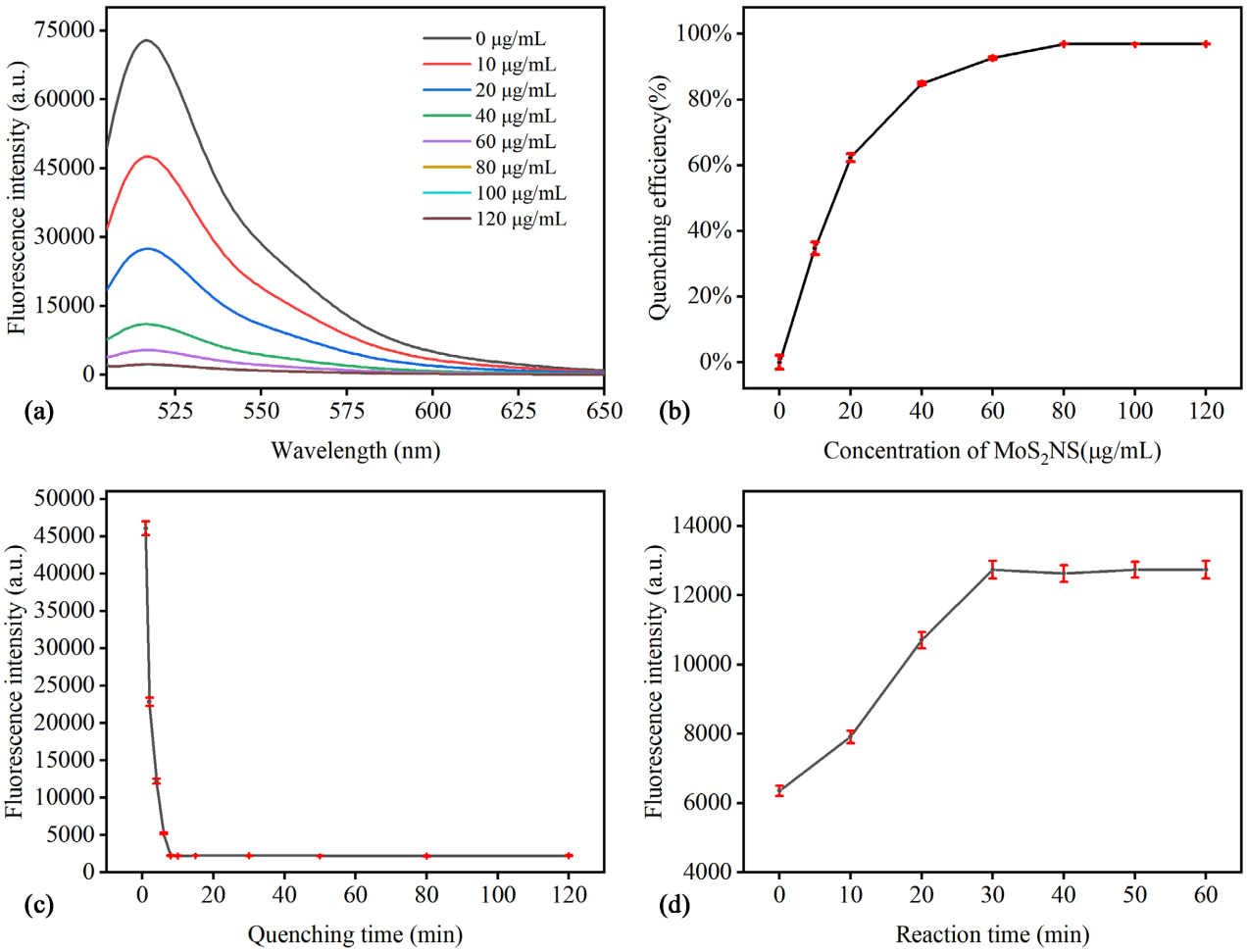

Figure 3. (a) The optimization of MoS_2NS concentration. The concentration of Apt@AFB_1 was 100 nM. (b) The quenching efficiency of MoS_2NS. (c) Fluorescence Quenching time. The concentrations of Apt@AFB_1 and MoS_2NS were 100 nM and 80 μg/mL, respectively. (d) Reaction time of 100 nM Apt@AFB_1 and 25 ng/mL AFB_1, and then added 80 μg/mL MoS_2NS to continue reaction for 8 min.

fluorescent signal expressed by the Apt@AFB_1 aptamer with a fixed concentration of 100 nM is constantly weakening as the MoS_2NS concentration increases. The fluorescence intensity reaches its minimum value when the MoS_2NS concentration reaches 80 μg/mL, as shown in **Figure 3(b)**. MoS_2NS quenches 96.9% of the fluorescent signals. The background signal is very low. This only existed background fluorescent signal is probably caused by the individual Apt@AFB_1 probes which cannot be absorbed on MoS_2NS effectively due to their voluntary changes on secondary structure. But such phenomenon is very rare, which will not produce obvious interference to the detection. In terms of the optimization of the fluorescence quenching reaction time, as shown in **Figure 3(c)**, the fluorescence of Apt@AFB_1 probe can be quenched by MoS_2NS rapidly. After 8 min, the fluorescence quenching effect reaches the lowest value and the quenching system tends to be stable until 120 min. The best reaction time of Apt@AFB_1 and AFB_1 is as shown in **Figure 3(d)**. When the reaction time reaches 30 min, the detected fluorescent signal intensity reaches the highest level and the fluores-

cence intensity remains unchanged until 60 min. In summary, the best reaction condition of this sensing platform is to use MoS_2NS with the concentration of 80 μg/mL with the quenching time of 8 min and the reaction time of probe and target material of 30 min. Such conditions will be applied to the subsequent experiments. Compared with the detection and analysis time of sensors with MoS_2NS as the quenching material reported by other references [11] [15] [16] [17], their quenching time is relatively close. This sensing platform even has a shorter quenching time and simple operation. In terms of the sensing system detection, MoS_2NS has a high quenching efficiency and a stable quenching performance. The fluorescent signals can be expressed stably, which meet the experimental requirement of signal detection and will not cause bias in detection results.

3.4. Sensitivity Analysis

Analyzing from the perspective of detection sensitivity, we research on the relations between the fluorescent signal intensity and the AFB_1 concentration in the reaction system under the best reaction conditions. The result is as shown in Figure 4. When the AFB_1 concentration is within the range of 0.2 - 25 ng/mL, the value of fluorescence intensity ΔF ($\Delta F = F - F_0$) is positively correlated with the concentration of AFB_1. The linear regression equation is $\Delta F = 1093.142X - 71.028$ and the correlation $R^2 = 0.9964$. As the increase of AFB_1 concentration, the fluorescent intensity of the sensors is significantly enhanced. The limit of detection (LOD) can be calculated by the ratio of the standard deviation of fluorescence intensity of three-time blank sample to the slope of the standard curve, which is 90 pg/mL. This MoS_2NS/Apt@AFB_1 sensing platform has sensitive effect in the detection of AFB_1, which can achieve the quantitative detection of AFB_1 molecules by monitoring the changes of fluorescent signal intensity. Compared with methods reported by related research, the electrochemical detection and surface-enhanced Raman scattering (SERS) has a low LOD, but the

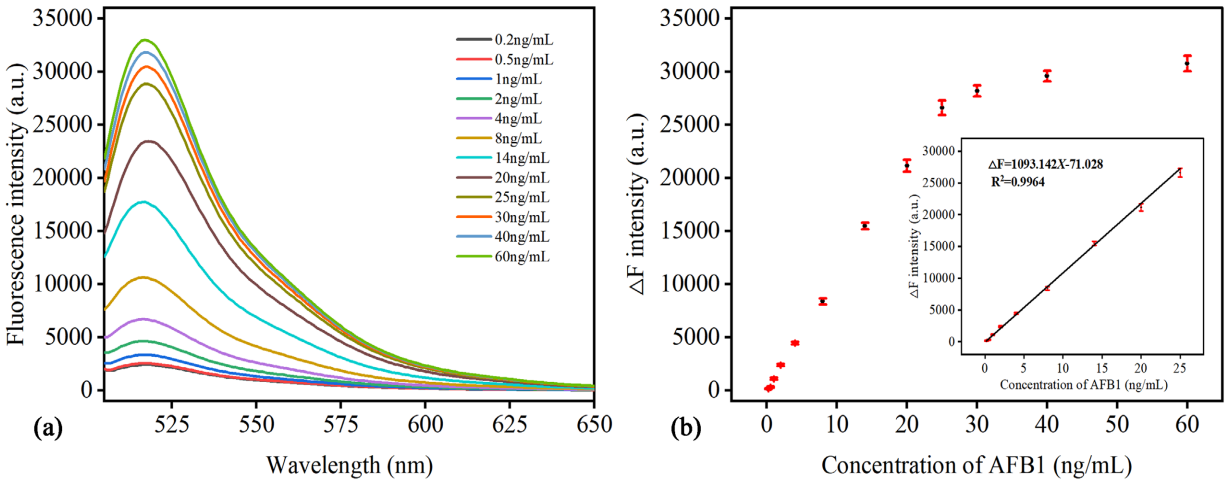

Figure 4. (a) Fluorescence intensity spectra of AFB_1 with different concentrations. (b) Standard curve of fluorescence intensities against the concentrations of AFB_1. The concentrations of Apt@AFB_1 and MoS_2NS were 100 nM and 80 μg/mL, respectively. Reaction on 37˚C for 30 min, and then added 80 μg/mL MoS_2NS to continue reaction for 8 min.

electrochemical detection method has a long pretreatment time and many steps [8] [18] [19]. The SERS experiment is time-consuming [20] [21]. While as the traditional direction method, the high-performance liquid chromatography (HPLC) has high costs, expensive daily maintenance costs and complicated pretreatment [7] [22]. The MoS_2NS/Apt@AFB_1 sensing platform constructed by the current research has an outstanding LOD on AFB_1. Comprehensively compared, this method has relatively high cost performance. In addition, the sensitivity of this method can meet the detection requirement of AFB_1.

3.5. Specificity Analysis

Analyzing in terms of the detection specificity, we examine the selectivity of the constructed MoS_2NS/Apt@AFB_1 sensing platform. As shown in **Figure 5**, when the target molecule AFB_1 exists, a strong fluorescent signal is detected by the sensing platform. Simultaneously, in terms of some common interfering substance existing in the nature, we identify and detect some other mycotoxins such as AFB_2, AFM_1, OTA and FB_1. When these mycotoxins exist, the sensing platform does not detect an obvious fluorescent signal response, while in the mixed solution (AFB_1, AFB_2, AFM_1, OTA and FB_1), the MoS_2NS/Apt@AFB_1 sensing platform can detect the content of existed AFB_1 accurately, indicating that the MoS_2NS/Apt@AFB_1 sensors can distinguish other impurities and conduct specificity detection to AFB_1 in complex samples. This result shows that this fluorescence sensing method has the potential possibility in the application of complex sample analysis.

3.6. Sensing Application in Actual Samples

Serum is the clinical detection sample with easy access. To detect the existence of AFB_1 is serum can assist the clinical etiological diagnosis and analysis on acute

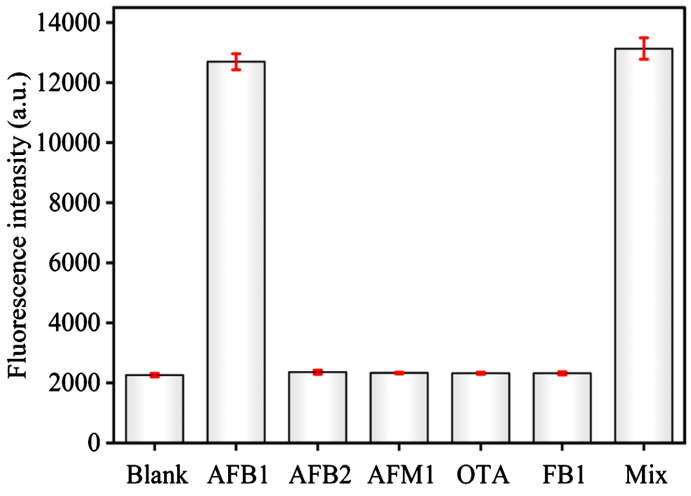

Figure 5. The selectivity of the Fluorescence assay. The concentration of AFB_1 was 10 ng/mL, nonspecific molecules were all 20 ng/mL and the concentration of mixed liquid: AFB_1 was 10 ng/mL, AFB_2,AFM_1,OTA and FB_1 were all 20 ng/mL. Blank sample was without AFB_1.

food poisoning for patients. *Aspergillus flavus* is easy to pollute bean crops and produce Aflatoxin through metabolism. If the soybean milk, a soybean product, has AFB_1, it is hard to degrade it through normal food processing. Therefore, we use the MoS_2NS/Apt@AFB_1 sensor to conduct AFB_1 standard addition recovery test on the serum samples and the food samples of soybean milk. Under the best experimental condition, add AFB_1 with the concentration of 0.2, 5 and 15 ng/mL into the serum and soybean milk samples and then conduct detection. The detection result of the sensor constructed in the current experiment is as shown in Table 1. The standard addition recovery rate of the serum sample is 93.10% - 107.23% and the relative standard deviation (RSD) is 2.15% - 4.42%. The AFB_1 standard addition recovery rate of the soybean milk sample is 95.15% - 102.60% and the relative standard deviation (RSD) is 1.78% - 3.98%. Therefore, it may be judged that this sensing method is applicable for the analysis of real biological sample with interference. This method has a good reproducibility, which can satisfy the requirement of actual application.

4. Conclusion

As a common contaminant in crops, AFB_1 is the toxin with great harm to the human body, which is listed by the International Agency for Research on Cancer (IARC) as a Group 1 Carcinogen [3]. Its main source is the metabolites derived from *Aspergillus flavus* after polluting grain crops with high starch contents, such as soybean, peanut and corn. If a patient has acute food poisoning symptoms or chronic liver diseases with suspected relations to the Aflatoxin, it is needed to conduct quick etiological diagnosis. At the same time, food safety supervision and management departments also need a simple and fast detection method to control the food safety and to prevent diseases from entering through the mouth. The current research constructed a biosensor on the basis of the new type of nano-material namely MoS_2NS. The detection strategy is as follows: With Apt@AFB_1 as the biological probe and MoS_2NS as the quenching receptor of fluorescent signals, the fluorescence signal is released by AFB_1 by being specifically bound to Apt@AFB_1 and moving away from MoS_2NS together leading to blocking FRET. When the AFB_1 is within the range of 0.2 - 25 ng/mL, the

Table 1. Determination results of AFB_1 in real sample analysis (n = 5)

Sample	AFB_1 spiked (ng/mL)	AFB_1 detected (ng/mL)	Recovery (%)	RSD (%)
Serum	0.2	0.214	107.23	2.15
	5	5.062	101.24	3.58
	15	13.965	93.10	4.42
Soybean Milk	0.2	0.190	95.15	1.78
	5	5.130	102.60	2.72
	15	15.209	101.39	3.98

fluorescent signal intensity detected by the MoS_2NS/Apt@AFB_1 sensor has good linear relations with the level of AFB_1, and the LOD is 90 pg/mL. The experimental recovery rate of serum samples and soybean milk samples with different concentrations of AFB_1 added is satisfactory. The current research achieves the rapid and sensitive quantitative detection of AFB_1 with specificity for serum and soybean milk. Aflatoxins are currently detected using thin-layer chromatography (LC) [23] [24], high performance liquid chromatography (HPLC) [25] [26] [27] [28], and enzyme linked immunosorbent assays (ELISA) [29] [30]. The above detection methods have several drawbacks, including cumbersome operation, expensive detection instruments and equipment, and time-consuming and labor-intensive issues. Compared with traditional methods, this sensing method has characteristics such as low costs and quick analysis time. Therefore, MoS_2NS/Apt@AFB_1 is a convenient, sensitive and stable sensing platform, featuring great potential of application in fields such as food safety inspection, environmental monitoring and clinical detection.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Al-Zoreky, N.S. and Saleh, F.A. (2019) Limited Survey on Aflatoxin Contamination in Rice. *Saudi Journal of Biological Sciences*, **26**, 225-231. https://doi.org/10.1016/j.sjbs.2017.05.010

[2] Cao, W., Yu, P., Yang, K. and Cao, D. (2022) Aflatoxin B_1: Metabolism, Toxicology, and Its Involvement in Oxidative Stress and Cancer Development. *Toxicology Mechanisms and Methods*, **32**, 395-419. https://doi.org/10.1080/15376516.2021.2021339

[3] Rushing, B.R. and Selim, M.I. (2019) Aflatoxin B_1: A Review on Metabolism, Toxicity, Occurrence in Food, Occupational Exposure, and Detoxification Methods. *Food and Chemical Toxicology*, **124**, 81-100. https://doi.org/10.1016/j.fct.2018.11.047

[4] Wang, L., Huang, Q., Wu, J., Wu, W., Jiang, J., Yan, H., Huang, J., Sun, Y. and Deng, Y. (2022) The Metabolism and Biotransformation of AFB_1: Key Enzymes and Pathways. *Biochemical Pharmacology*, **199**, Article ID: 115005. https://doi.org/10.1016/j.bcp.2022.115005

[5] Pickova, D., Ostry, V., Toman, J. and Malir, F. (2021) Aflatoxins: History, Significant Milestones, Recent Data on Their Toxicity and Ways to Mitigation. *Toxins*, **13**, Article No. 399. https://doi.org/10.3390/toxins13060399

[6] da Graça Pereira Nunes, M., Pizzutti, I.R., Brackmann, A., Reichert, B., Fontana, M.E.Z., dos Santos, I.D., Cuti, L.K., Jänisch, B.D., Panciera, M.P., Ludwig, V. and Cardoso, C.D. (2021) Multimycotoxin Determination in Grains: A Comprehensive Study on Method Validation and Assessment of Effectiveness of Controlled At-

mosphere Storage in Preventing Mycotoxin Contamination. *Journal of Agricultural and Food Chemistry*, **69**, 11440-11450. https://doi.org/10.1021/acs.jafc.1c03208

[7] Liu, H., Zhao, Y., Lu, A., Ye, J., Wang, J., Wang, S. and Luan, Y. (2020) An Aptamer Affinity Column for Purification and Enrichment of Aflatoxin B_1 and Aflatoxin B_2 in Agro-Products. *Analytical and Bioanalytical Chemistry*, **412**, 895-904. https://doi.org/10.1007/s00216-019-02300-4

[8] Hui, Y., Wang, B., Ren, R., Zhao, A., Zhang, F., Song, S. and He, Y. (2020) An Electrochemical Aptasensor Based on DNA-AuNPs-HRP Nanoprobes and Exonuclease-Assisted Signal Amplification for Detection of Aflatoxin B_1. *Food Control*, **109**, Article ID: 106902. https://doi.org/10.1016/j.foodcont.2019.106902

[9] Ma, X., Du, C., Zhang, J. Shang, M. and Song, W. (1966) A System Composed of Vanadium(IV) Disulfide Quantum Dots and Molybdenum(IV) Disulfide Nanosheets for Use in an Aptamer-Based Fluorometric Tetracycline Assay. *Mikrochimica Acta*, **186**, Article No. 837. https://doi.org/10.1007/s00604-019-3983-7

[10] Lu, J., Chen, M., Dong, L., Cai, L., Zhao, M., Wang, Q. and Li, J. (2020) Molybdenum Disulfide Nanosheets: From Exfoliation Preparation to Biosensing and Cancer Therapy Applications. *Colloids and Surfaces B: Biointerfaces*, **194**, Article ID: 111162. https://doi.org/10.1016/j.colsurfb.2020.111162

[11] Wei, J., He, S., Mao, Y., Wu, L., Liu, X., Effah, C.Y., Guo, H. and Wu, Y. (2021) A Simple "Signal off-on" Fluorescence Nanoplatform for the Label-Free Quantification of Exosome-Derived MicroRNA-21 in Lung Cancer Plasma. *Microchimica Acta*, **188**, Article No. 397. https://doi.org/10.1007/s00604-021-05051-1

[12] Kukkar, M., Mohanta, G.C., Tuteja, S.K., Kumar, P., Bhadwal, A.S., Samaddar, P., Kim, K.-H. and Deep, A. (2018) A Comprehensive Review on Nano-Molybdenum Disulfide/DNA Interfaces as Emerging Biosensing Platforms. *Biosensors and Bioelectronics*, **107**, 244-258. https://doi.org/10.1016/j.bios.2018.02.035

[13] Sun, Y., Fan, J., Cui, L., Ke, W., Zheng, F. and Zhao, Y. (2019) Fluorometric Nanoprobes for Simultaneous Aptamer-Based Detection of Carcinoembryonic Antigen and Prostate Specific Antigen. *Microchimica Acta*, **186**, Article No. 152. https://doi.org/10.1007/s00604-019-3281-4

[14] Gogoi, S. and Khan, R. (2018) Förster Resonance Energy Transfer (FRET) Between Carbon Dots/MoS_2 Based Fluorescence Immunosensor for Sensitive Detection of Cardiac Troponin T. *Physical Chemistry Chemical Physics*, **24**, 16501-16509. https://doi.org/10.1039/C8CP02433B

[15] Kong, L., Zhou, X., Shi, G. and Yu, Y. (2020) Molybdenum Disulfide Nanosheets-Based Fluorescent "off-to-on" Probe for Targeted Monitoring and Inhibition of Beta-Amyloid Oligomers. *Analyst*, **145**, 6369-6377. https://doi.org/10.1039/D0AN00019A

[16] Xu, Y., Kang, Q., Yang, B., Chen, B., He, M. and Hu, B. (2020) A Nanoprobe Based on Molybdenum Disulfide Nanosheets and Silver Nanoclusters for Imaging and Quantification of Intracellular Adenosine Triphosphate. *Analytica Chimica Acta*, **1134**, 75-83. https://doi.org/10.1016/j.aca.2020.08.011

[17] Wang, L., Dong, L., Liu, G., Shen, X., Wang, J., Zhu, C., Ding, M. and Wen, Y. (2019) Fluorometric Determination of HIV DNA Using Molybdenum Disulfide Nanosheets and Exonuclease III-Assisted Amplification. *Microchimica Acta*, **186**, Article No. 286. https://doi.org/10.1007/s00604-019-3368-y

[18] Wang, C., Liu, L. and Zhao, Q. (2020) Low Temperature Greatly Enhancing Responses of Aptamer Electrochemical Sensor for Aflatoxin B_1 Using Aptamer with Short Stem. *ACS Sensors*, **5**, 3246-3253. https://doi.org/10.1021/acssensors.0c01572

[19] Ramezani, M., Jalalian, S.H., Taghdisi, S.M., Abnous, K. and Alibolandi, M. (2022)

Optical and Electrochemical Aptasensors for Sensitive Detection of Aflatoxin B_1 and In: Ossandon, M.R., Baker, H. and Rasooly, A., Eds., *Biomedical Engineering Technologies. Methods in Molecular Biology*, Vol. 2393, Humana, New York, 417-436. https://doi.org/10.1007/978-1-0716-1803-5_21

[20] He, H., Sun, D. W., Pu, H. and Huang, L. (2020) Bridging Fe_3O_4@Au nanoflowers and Au@Ag Nanospheres with Aptamer for Ultrasensitive SERS Detection of Aflatoxin B_1. *Food Chemistry*, **324**, Article ID: 126832. https://doi.org/10.1016/j.foodchem.2020.126832

[21] Lin, B., Kannan, P., Qiu, B., Lin, Z. and Guo, L. (2020) On-Spot Surface Enhanced Raman Scattering Detection of Aflatoxin B_1 in Peanut Extracts Using Gold Nanobipyramids Evenly Trapped into the AAO Nanoholes. *Food Chemistry*, **307**, Article ID: 125528. https://doi.org/10.1016/j.foodchem.2019.125528

[22] Guo, Z., Lv, L., Cui, C., Wang, Y., Ji, S., Fang, J., Yuan, M. and Yu, H. (2020) Detection of Aflatoxin B_1 with a New Label-Free Fluorescent Aptasensor Based on Exonuclease I and SYBR Gold. *Analytical Methods*, **12**, 2928-2933. https://doi.org/10.1039/D0AY00967A

[23] Zhang, K. and Banerjee, K. (2020) A Review: Sample Preparation and Chromatographic Technologies for Detection of Aflatoxins in Foods. *Toxins*, **12**, Article No. 539. https://doi.org/10.3390/toxins12090539

[24] Debon, E., Rogeboz, P., Latado, H., Morlock, G.E., Meyer, D., Cottet-Fontannaz, C., Scholz, G., Schilter, B. and Marin-Kuan, M. (2022) Incorporation of Metabolic Activation in the HPTLC-SOS-Umu-C Bioassay to Detect Low Levels of Genotoxic Chemicals in Food Contact Materials. *Toxics*, **10**, Article No. 501. https://doi.org/10.3390/toxics10090501

[25] Du, X., Schrunk, D.E., Imerman, P.M., Smith, L., Francis, K., Tahara, J., Tkachenko, A., Reimschuessel, R. and Rumbeiha, W.K. (2019) Evaluation of a Diagnostic Method to Quantify Aflatoxins B_1 and M_1 in Animal Liver by High-Performance Liquid Chromatography with Fluorescence Detection. *Journal of AOAC International*, **102**, 1530-1534. https://doi.org/10.5740/jaoacint.18-0355

[26] Li, R., Meng, C., Wen, Y., Fu, W. and He, P. (2019) Fluorometric Lateral Flow Immunoassay for Simultaneous Determination of Three Mycotoxins (Aflatoxin B_1, Zearalenone and Deoxynivalenol) Using Quantum Dot Microbeads. *Mikrochimica Acta*, **186**, Article No. 748. https://doi.org/10.1007/s00604-019-3879-6

[27] Houissa, H., Lasram, S., Sulyok, M., Šarkanj, B., Fontana, A., Strub, C., Krska, R., Schorr-Galindo, S. and Ghorbel, A. (2019) Multimycotoxin LC-MS/MS Analysis in Pearl Millet (*Pennisetum glaucum*) from Tunisia. *Food Control*, **106**, Article ID: 106738. https://doi.org/10.1016/j.foodcont.2019.106738

[28] Li, S., Li, X., Liu, X., Zhang, Q., Fang, J., Li, X. and Yin, X. (2022) Stability Evaluation of Aflatoxin B_1 Solution Certified Reference Material via Ultra-High Performance Liquid Chromatography Coupled with High-Resolution Mass Spectrometry. *ACS Omega*, **7**, 40548-40557. https://doi.org/10.1021/acsomega.2c05829

[29] Lu, D., Wang, X., Su, R., Cheng, Y., Wang, H., Luo, L. and Xiao, Z. (2022) Preparation of an Immunoaffinity Column Based on Bispecific Monoclonal Antibody for Aflatoxin B_1 and Ochratoxin A Detection Combined with ic-ELISA. *Foods*, **11**, Article No. 335. https://doi.org/10.3390/foods11030335

[30] Dai, H., Huang, Z., Liu, X., Bi, J., Shu, Z., Xiao, A. and Wang, J. (2022) Colorimetric ELISA Based on Urease Catalysis Curcumin as a Ratiometric Indicator for the Sensitive Determination of Aflatoxin B_1 in Grain Products. *Talanta*, **246**, Article ID: 123495. https://doi.org/10.1016/j.talanta.2022.123495