

Article

Rapid Detection of Fumonisin B1 Using a Fluorescent Aptasensor with Plasmon-Modified Graphene Oxide as a Quencher

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Abstract

Fumonisin B1 (FB1) is a secondary metabolite produced by *Fusarium* species, exhibiting strong toxicity and classified as a Group 2B carcinogen by the International Agency for Research on Cancer. It poses a significant threat to both human and animal health. Therefore, developing a simple and reliable method for FB1 detection and analysis is imperative. In this study, a biosensor based on nucleic acid aptamers was developed, utilizing plasma-modified graphene oxide (mGO) as a fluorescence quencher for FB1 detection. This system leverages the interaction between mGO and FAM-APT (a nucleic acid aptamer labeled with 5-carboxyfluorescein, FAM), achieving fluorescence quenching through fluorescence resonance energy transfer (FRET) under excitation at 490 nm and emission at 520 nm. In the presence of FB1, FAM-APT specifically binds to FB1 and dissociates from the mGO surface, resulting in fluorescence recovery. Quantitative detection of FB1 was achieved by measuring the differential fluorescence intensity. The biosensor demonstrated excellent linearity over a concentration range of 10 to 5×10^6 ng/L, with a detection limit (LOD) as low as 0.16 µg/L. Additionally, the sensor exhibited high specificity for FB1 among six common mycotoxins. In practical sample analysis, recovery rates ranged from 95.8% to 104.7% in corn samples and from 89.3% to 94.5% in rice samples. This aptamer-based biosensor features a simple structure, high sensitivity, and a wide detection range, providing important technical support for advancing mycotoxin research.

Keywords: fumonisin B1; graphene oxide; aptamer sensor; rapid detection; cold plasma



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1. Introduction

Fumonisin was initially identified in 1988 [1]. This group of structurally related mycotoxins is synthesized as secondary metabolites by various species within the *Fusarium* genus [2]. To date, six distinct fumonisin variants have been characterized, with fumonisin B1 (FB1) being the most prevalent and toxic [3,4]. FB1 is commonly detected in cereal grains, particularly maize. Prolonged exposure to FB1 has been associated with hepatic and renal toxicity, immunosuppression, and the induction of malignancies such as esophageal and liver cancers [5]. Consequently, the International Agency for Research on Cancer (IARC) has classified FB1 as a Group 2B carcinogen. In response to the health risks posed by

FB1, the European Commission has established a maximum permissible concentration of 2 milligrams per kilogram in maize and maize-derived products.

Currently, conventional techniques for the detection of FB1 encompass liquid chromatography–mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC). These methodologies are characterized by high sensitivity and selectivity [6–8]. Nevertheless, these laboratory-based approaches are often time-intensive, laborious, and expensive, thereby limiting their applicability for rapid, on-site analysis [9]. Additional commonly employed mycotoxin screening methods include enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), lateral flow immunochips (LFIs), and electronic-nose technologies [8]. Although ELISA is extensively utilized, it is susceptible to cross-reactivity and false positive or negative outcomes [8], and it necessitates supplementary reagents to preserve the stability of antibody coatings on microplates [10]. SPR sensors exhibit sensitivity to temperature fluctuations, whereas LFI and electronic-nose detection methods demonstrate comparatively lower sensitivity. Furthermore, immunochip technologies are hindered by complex labeling protocols and the requirement for specialized technical expertise [8]. In addition, aptamer sensors are now available for the detection of FB1. Zhao et al. developed an aptamer-based sensor for FB1 detection utilizing the principle of fluorescence resonance energy transfer, achieving a detection range of 0–3000 ng/mL and a detection limit of 14.42 ng/mL [11]. Guo et al. employed a fluorescent strip sensor incorporating europium nanospheres to detect FB1, with a detection range of 13.81–1000 µg/kg and a detection limit of 8.26 µg/kg [12]. However, the currently developed sensors suffer from high detection limits and complex operation. Therefore, it is crucial to develop a reliable and efficient sensor-based detection method to facilitate the identification of FB1 contamination in food and animal feed.

Graphene oxide (GO) contains a higher concentration of active functional groups—such as -C-O, -C=O, and carboxyl groups—due to the increased presence of oxygen-containing groups formed during the oxidation process, compared to graphene [13]. Among materials with lattice-like nanostructures, GO offers distinct advantages for biosensing applications because of its excellent ability to directly interact with biomolecules, its heterogeneous chemical and electronic structures, its processability in solution, and its tunable properties that range from insulator to semiconductor or semimetal [14–18]. Unlike graphene, GO provides greater flexibility in tailoring its material properties by manipulating oxygen-containing functional groups (OFGs) [4]. Due to the characteristics of GO, it can more easily adsorb aptamers and has better fluorescence quenching ability for aptamers. In this study, plasma treatment was used to modify graphene oxide (mGO), which significantly enhanced the adsorption performance and fluorescence quenching ability of the treated material. Therefore, GO was chosen as the superior option.

Aptamers are functional RNA oligonucleotides obtained through in vitro selection, independently developed by two research teams. Their name is derived from the Latin word “aptus,” meaning “fit” [19–23]. Aptamers exhibit high affinity for their targets and, upon binding, can fold to incorporate small molecules into larger molecular structures. These unique properties make them superior molecular tools compared to antibodies [24]. Aptamers are selected through the Systematic Evolution of Ligands by EXponential enrichment (SELEX) process, which can be customized to produce molecules with high specificity for particular targets while effectively minimizing interference [25]. The range of aptamer targets is broad, encompassing various types from small molecules to whole cells [26]. Their ability to form diverse and stable complex secondary structures makes them commonly used in the construction of biosensors [27,28]. Additionally, aptamers possess high chemical stability, ease of modification, simple synthesis, and good biocompatibility, making them especially suitable for applications in environmental monitoring [29].

The aptamer sequence selected in this study is based on the work of Chen et al. [30]. Chen and colleagues evaluated the affinity of various aptamers for FB1 using an 80-nucleotide (nt) sequence composed of a random 40-nucleotide region flanked by 20 nucleotides on each side. Their results showed that this aptamer sequence exhibited the highest affinity for FB1 compared to other sequences tested. Additionally, the 80 nt aptamer demonstrated greater affinity for FB1 than a 96 nt aptamer sequence. Therefore, this aptamer sequence was chosen for the present study.

Fluorescence resonance energy transfer (FRET) constitutes a non-radiative energy transfer process mediated by long-range dipole–dipole interactions within donor–acceptor pairs. Specifically, when the acceptor molecule is optically excited, electronic excitation energy is transferred from the donor in its excited state to the acceptor in its ground state [31]. Within the domain of fluorescent sensors, devices based on the Förster resonance energy transfer mechanism—facilitating detection through modulation of electronic interactions between donor and acceptor molecules—exhibit significant potential for diverse applications [32,33].

In this study, the 5′ terminus of the FB1-specific aptamer was conjugated with a 5-carboxyfluorescein (FAM) fluorescent moiety, while GO was dispersed through ultrasonic treatment. Utilizing the mechanism of FRET, the fluorescently labeled aptamer functioned as the fluorescence donor, and the modified GO served as the fluorescence acceptor, thereby facilitating the quantitative detection of FB1.

2. Materials and Methods

2.1. Materials

Fumonisin B1 (GR) was procured from Qingdao Pribolab Biotech Co., Ltd. (Qingdao, China). Graphene oxide was supplied by Shanghai HanLang New Material Technology Co., Ltd. (Shanghai, China). Phosphate-buffered saline was obtained from Shanghai Chuanqiu Biotechnology Co., Ltd. (Shanghai, China). Acetonitrile was purchased from Qingdao Pribolab Biotechnology Co., Ltd. (Qingdao, China). The FAM-APT reagent utilized in this study was produced by Sangon Biotech Co., Ltd. (Shanghai, China). The relevant sequence information is as follows: FAM-APT (5′-FAM-AGCAGCACAGAGGTCAGATGCGATCTGGATATTATTTTGATACCCCTTTGGGGAG-ACATCCTATGCGTGCTACCGTGAA-3′) [30].

2.2. Plasma-Modified GO

First, accurately weigh 12.5 mg of graphene oxide powder and dissolve it in ultrapure water. Then, dilute the resulting solution to a final volume of 50 milliliters to prepare a solution with a concentration of 250 mg/L. Next, transfer 10 mL of the graphene oxide solution into a beaker. Use nitrogen gas as the discharge gas and employ a 1.5 mm 304 stainless steel electrode as the discharge electrode, maintaining a 3.0 cm gap between the electrode and the graphene oxide aqueous solution. After turning on the high-voltage power supply, carry out plasma discharge treatment for 10 min.

2.3. Material Characterization

The crystal structure of mGO was characterized using a D8 ADVANCE X-ray diffractometer (Bruker, Berlin, Germany). Morphological and structural analyses were performed with a TM4000PLUS scanning electron microscope (HITACHI, Tokyo, Japan) and a Park NX12 atomic force microscope (Park Systems, Suwon-si, Republic of Korea). Hydrophilicity was assessed using an SDC-100 contact angle goniometer (SINDIN, Dongguan, China). X-ray photoelectron spectroscopy measurements were conducted with a monochromatic Al K α source on a Thermo Fisher Scientific Escalab 250Xi spectrometer (Thermo Fisher,

Waltham, MA, USA). Infrared spectra were obtained using a Nicolet iS5 Fourier transform infrared spectrometer (Thermo Scientific, Waltham, MA, USA). Ultraviolet–visible absorption–reflectance spectra in the 200–800 nm range were recorded with a Shimadzu UV-3600plus UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan).

2.4. Testing of FB1 Standard Solution

Mix the optimal concentrations of FAM-APT and GO in a 1.5 mL centrifuge tube. Vortex and shake the mixture of mGO and FAM-APT for 1 min to quench the fluorescence. Next, add FB1 standard solutions at concentrations of 10 ng/L, 100 ng/L, 1000 ng/L, 10,000 ng/L, 100,000 ng/L, 1×10^6 ng/L and 5×10^6 ng/L, respectively. Each solution is diluted with phosphate buffer to a final volume of 1 mL, vortexed for 1 min, and incubated at 37 °C for 1 h. Measure the fluorescence intensity of all samples using a fluorescence spectrophotometer (HITACHI, Tokyo, Japan) with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. Construct a standard curve by plotting the logarithm of FB1 concentration on the x-axis against the fluorescence intensity difference ($F - F_0$) on the y-axis. From sample preparation to detection completion, the total time required for the sensor is 40 min, whereas ELISA takes approximately 2 h. Therefore, the method used in this study is more time-efficient. Finally, calculate the detection limit using the following formula [34]:

$$LOD = 3.3 \times \frac{\sigma}{S} \quad (1)$$

where σ represents the standard deviation of the blank group, while S denotes the slope of the calibration curve.

2.5. Assessment of Specificity

First, add the optimal concentrations of FAM-APT and mGO into centrifuge tubes, then vortex the mixtures for 1 min. Next, add Ochratoxin A (OTA), Zearalenone (ZEN), Deoxynivalenol (DON), Aflatoxin B1 (AFB1), and Aflatoxin B2 (AFB2) each at a concentration of 10 µg/L, along with mixed-toxin solutions containing FB1 and pure FB1 solutions, into their respective tubes. Adjust the volume of each tube to 1 mL with phosphate buffer, then vortex again for 1 min. Incubate the samples at 37 °C for 1 h. Finally, measure the fluorescence using a spectrofluorometer with an excitation wavelength of 490 nm, and record the fluorescence intensity at an emission wavelength of 520 nm for all samples.

2.6. Effect of Storage Time

The impact of storage duration on the fluorescence quenching performance of mGO solution was examined. Both GO and mGO solutions were prepared, with the GO solution serving as the control. These solutions were stored at 4 °C for 1, 2, 3, and 4 weeks. Each week, two 1.5 mL centrifuge tubes were filled with GO and mGO solutions, respectively, and FAM-APT was added to achieve a final concentration of 80 nM. PBS was then added to bring the total volume to 1 mL, followed by vortexing for 1 min. Fluorescence measurements were conducted using a fluorometer with an excitation wavelength of 490 nm, and fluorescence intensity was recorded at 520 nm for both solutions. The fluorescence intensities of GO and mGO solutions stored for 2, 3, and 4 weeks were measured using the same procedure.

2.7. Preprocessing of Actual Samples

Accurately weigh 5 g each of corn flour and rice flour into eight 50 mL centrifuge tubes. Add FB1 standard solution at a concentration of 100 µg/L to six of the tubes to achieve spiking levels of 1 µg/kg, 10 µg/kg, and 100 µg/kg, respectively. Allow the mixtures to stand for 30 min. Then, add 20 mL of an acetonitrile–water solution (acetonitrile-to-water volume ratio of 84:16) to each tube. The two tubes without added FB1 standard serve

as blank controls. Vortex each sample for 1 min to ensure thorough mixing, followed by ultrasonic extraction at room temperature at 100% power for 30 min to extract FB1 from the samples. Next, centrifuge the tubes at 5000 rpm and 4 °C for 10 min. Filter the supernatant three times through a 0.22 µm membrane filter, labeling the samples as corn blank, low, medium, and high, and rice blank, low, medium, and high, respectively. Store all prepared samples at 4 °C for subsequent analysis.

3. Results and Discussion

3.1. Working Principle

As illustrated in Figure 1, the oxygen-containing functional groups and conjugated structure on the surface of mGO enable it to adsorb FAM-APT through hydrogen bonding and π - π stacking interactions. This adsorption leads to fluorescence quenching of FAM-APT by mGO via the FRET mechanism. However, when FB1 is introduced into the system, FAM-APT specifically binds to FB1 and detaches from the GO surface, interrupting the FRET process and restoring the fluorescence of FAM-APT [35,36].

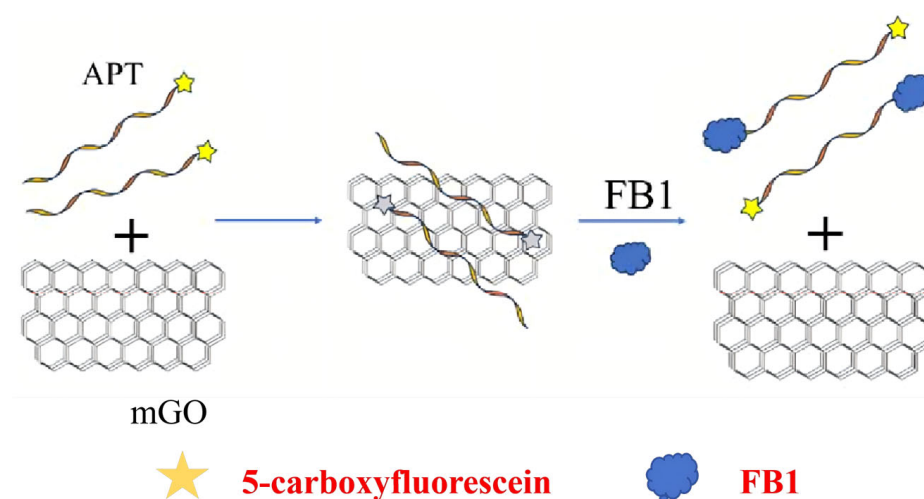


Figure 1. Schematic diagram represents the working principle of the developed aptasensor.

3.2. Materials Characterization Results

3.2.1. FT-IR Analysis

Figure 2 presents the FT-IR spectra of GO and mGO. The characteristic peaks of GO, as reported by Xu [37] and others, include a broad and intense O-H peak around 3400 cm^{-1} , a C=O peak near 1740 cm^{-1} , a C-OH stretching peak close to 1250 cm^{-1} , a C-O stretching peak near 1070 cm^{-1} , and a peak around 1620 cm^{-1} corresponding to the unoxidized graphite skeleton structure or adsorbed water molecules. In contrast, nGO exhibits only two prominent peaks: the O-H peak near 3400 cm^{-1} and the peak near 1620 cm^{-1} associated with the unoxidized graphite skeleton or adsorbed water. This occurs because the oxygen atoms in hydroxyl and carboxyl groups possess lone pairs that can form coordination bonds for adsorption, and these groups can also form hydrogen bonds with water molecules. The reduction in the number of absorption peaks suggests that the surface of mGO is smoother, which in turn implies a greater number of sites available for adsorbing other substances [38,39].

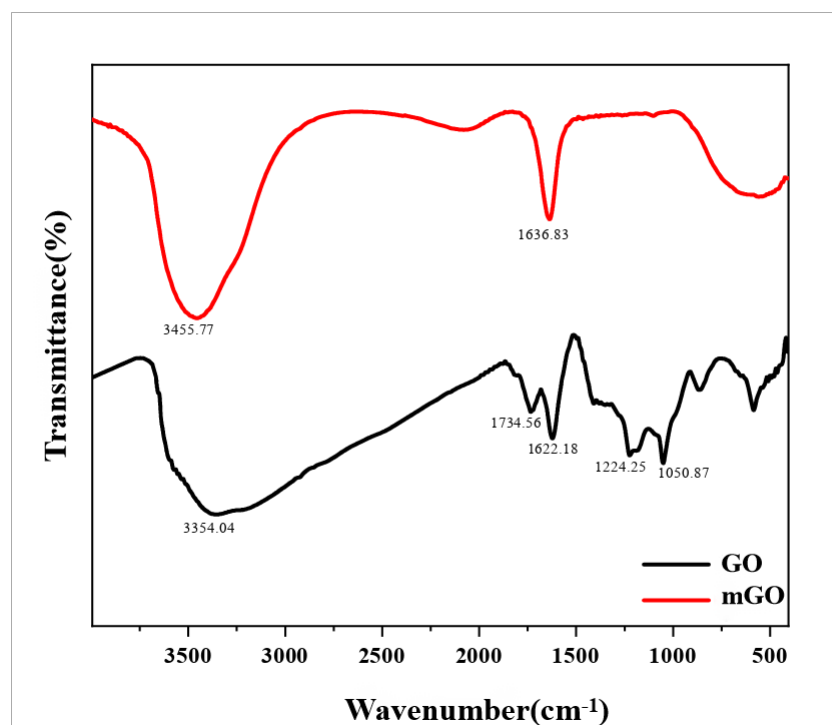


Figure 2. FT–IR spectra of GO and mGO.

3.2.2. SEM Analysis

Figure 3 presents the SEM results of GO and mGO. The number of GO layers can be roughly estimated by observing the color and surface wrinkles in the SEM images. Single-layer GO displays distinct wrinkles with noticeable thickness. To minimize surface energy, its morphology spontaneously shifts from two-dimensional to three-dimensional, making the surface wrinkles of single-layer GO significantly more pronounced than those of bilayer GO. Additionally, as the number of graphite layers increases, the degree of wrinkling decreases [40]. In Figure 3A, the clearly visible wrinkles on the GO surface indicate that most GO exists in a single-layer form. In contrast, Figure 3B shows fewer wrinkles and a smoother surface on mGO, suggesting an increased number of layers. This smoother surface and greater layer count provide more spatial sites for adsorbing other substances.

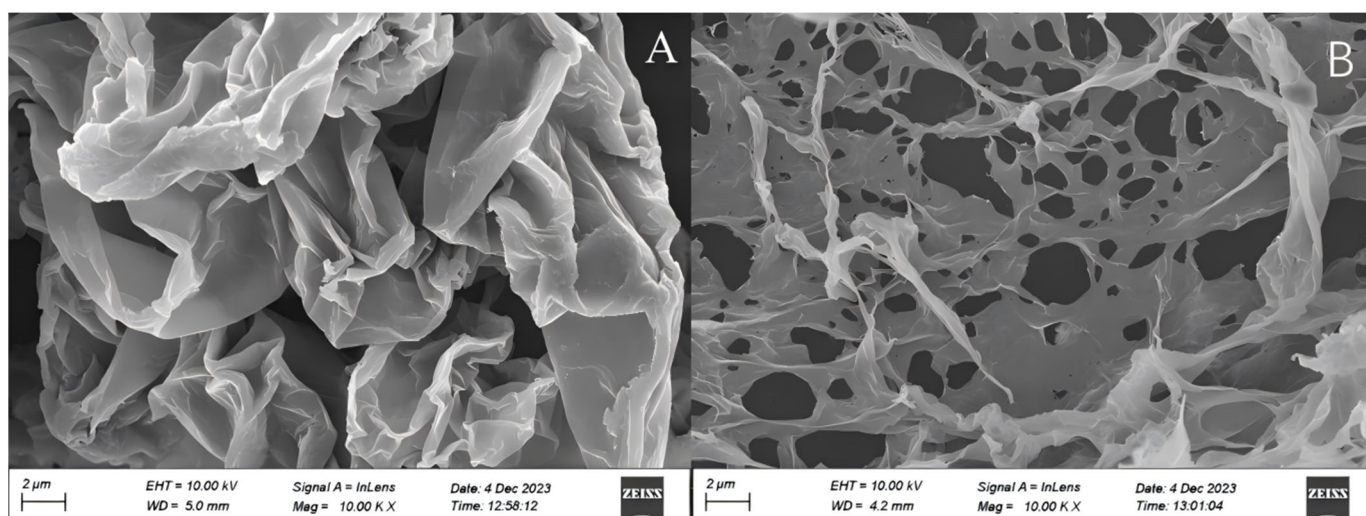


Figure 3. (A) SEM chart of GO. (B) SEM chart of mGO.

3.2.3. AFM Analysis

The AFM results for GO and mGO are presented in Figure 4. Figure 4A reveals that the surface morphology of GO is irregular, with a roughness of 1.370 nm. In contrast, Figure 4B shows that the surface morphology of mGO is more uniform, with a roughness of 0.674 nm. Figure 4C indicates that the thickness of GO is 6.003 nm, whereas Figure 4D shows that mGO has a thickness of 1.302 nm. These experimental findings demonstrate that the reduced thickness, increased surface regularity, and lower roughness of mGO contribute to its enhanced adsorption performance.

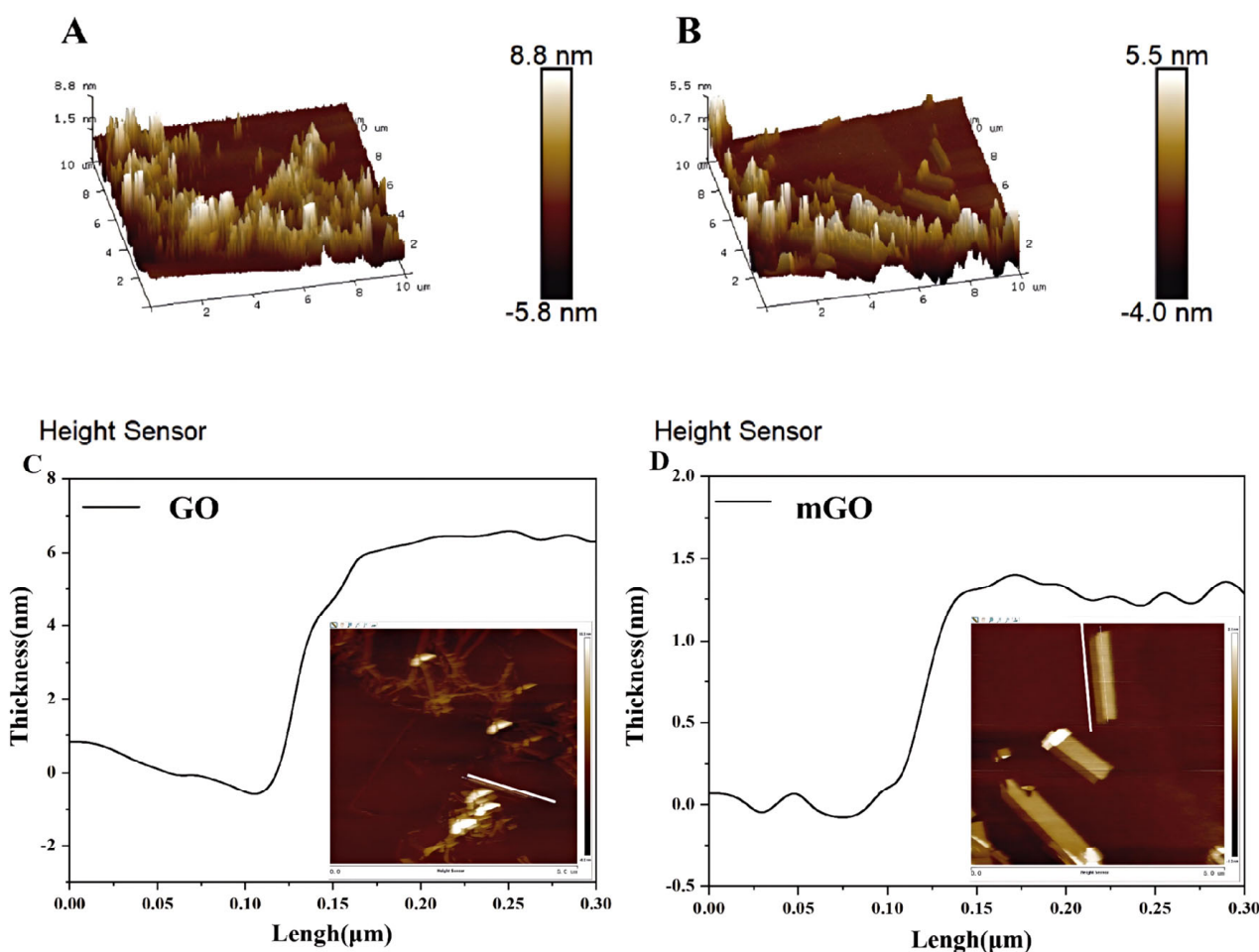


Figure 4. (A) Three-dimensional AFM images of GO. (B) Three-dimensional AFM images of mGO. (C) Two-dimensional AFM images of GO. (D) Two-dimensional AFM images of mGO.

3.2.4. XRD and XPS Analysis

To investigate the chemical structure and crystalline phase composition of GO and mGO, X-ray diffraction (XRD) was used to determine the phase and crystal structure of GO and mGO. High-resolution XPS spectral analysis was performed to identify the chemical states of mGO. All obtained spectra were calibrated using the C 1s electron peak at 284.6 eV as the reference. The results are shown in Figure 5.

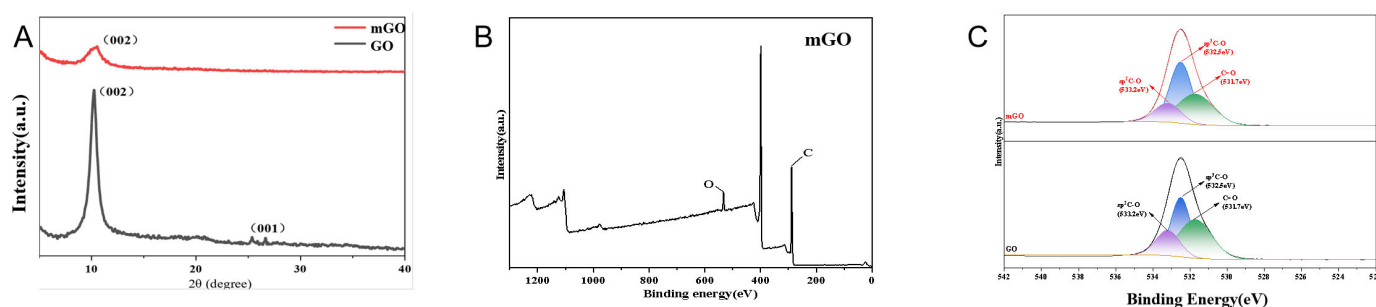


Figure 5. (A) XRD characterization of GO and nGO. (B) The wide scan spectrum of mGO. (C) XPS characterization of GO and mGO.

The XRD patterns of GO and mGO are shown in Figure 5A. The C(001) peak of GO appears at 10.41° , from which the lattice spacing is estimated to be approximately 0.850 nm [41]. After plasma modification, the C(001) peak of GO gradually weakens and shifts to 10.58° , confirming the successful preparation of mGO under specific conditions. During plasma treatment, oxygen-containing functional groups are reduced, and the crystal surface becomes increasingly refined [42]. No significant diffraction peak is observed for mGO at the C(002) plane, indicating that severe stacking did not occur during plasma modification. This also suggests that the grain size of mGO decreased, its thickness became thinner, and surface roughness was reduced, consistent with the results observed by AFM.

Figure 5B presents the full-range XPS spectrum of the mGO sample. The sample contains carbon and oxygen elements, with distinct photoelectron peaks observed at binding energies of 286 eV (C 1s) and 531 eV (O 1s), respectively. These C and O peaks are derived from the original graphene oxide sample.

To further verify the difference in the concentration of oxygen-containing functional groups on the surfaces of GO and mGO, XPS analysis was performed on both materials. Figure 5C presents the high-resolution O 1s spectrum. By fitting this spectrum, we identified characteristic peaks corresponding to C=O (531.7 eV), sp³ C–O (532.5 eV), and sp² C–O (533.2 eV) [43], which represent carbonyl, hydroxyl, and epoxy groups, respectively, as well as oxygen atoms in phenolic hydroxyl groups [44]. In the mGO, these characteristic peaks shifted: the peak areas for C=O and sp² C–O decreased, while the peak area for sp³ C–O increased. This peak analysis suggests that most functional groups in mGO are associated with sp³-type C–O bonds.

XPS analysis reveals that mGO has a higher content of oxygen-containing functional groups. An increased concentration of these groups enhances its hydrophilicity and adsorption capabilities [45,46].

3.2.5. Contact Angle Analysis

The contact angle is defined as the angle between the tangent line at the gas–liquid interface and the solid–liquid junction line at the point where the gas, liquid, and solid phases meet. It is an important parameter for assessing the wettability of a liquid on a solid surface. A contact angle of 90° represents a critical state: angles between 0° and 90° indicate wetting, with smaller angles corresponding to better wettability; angles between 90° and 180° indicate non-wetting. The contact angle measurements for GO and mGO are shown in Figure 6. Both GO and mGO have contact angles less than 90° , with mGO exhibiting a smaller contact angle than GO. This suggests that the modification enhances the material's ability to adsorb other substances [47,48].

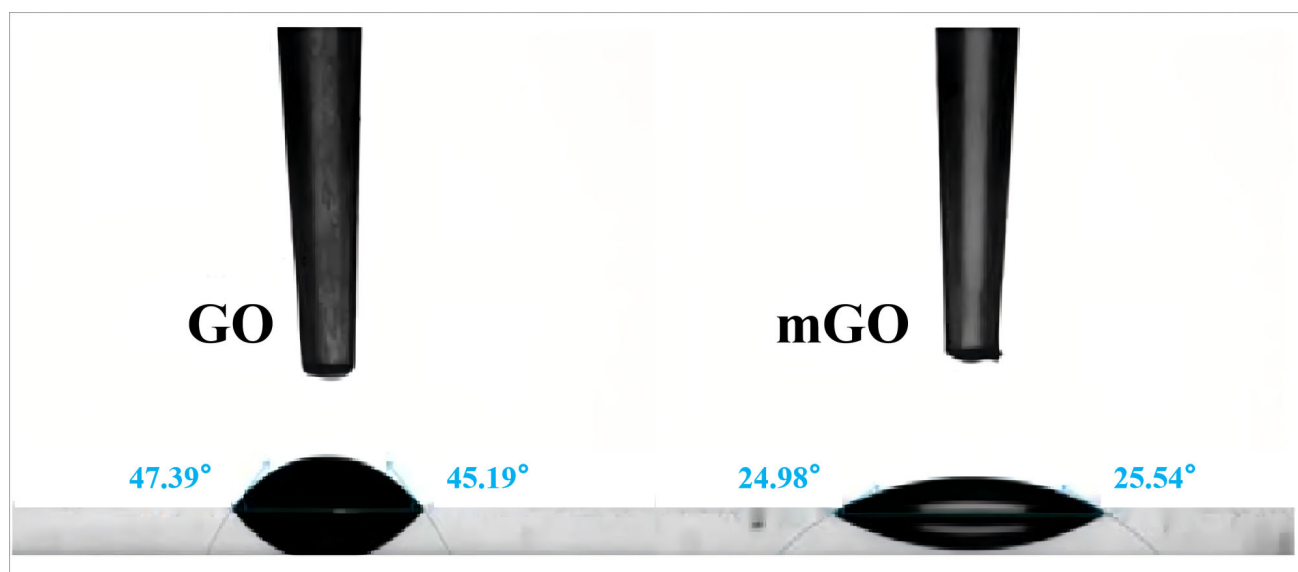


Figure 6. Contact angles of GO and mGO.

3.3. STX Standard Solution Assay

3.3.1. Feasibility Confirmation Studies

As illustrated in Figure 7, the experimental feasibility was validated using an on-off-on mode. Initially, the fluorescence intensity of FAM-APT was measured. Upon adding mGO, the fluorescence intensity decreased. Subsequently, the addition of the FB1 standard solution restored the fluorescence intensity. These results align with the experimental principle, confirming the experiment's feasibility.

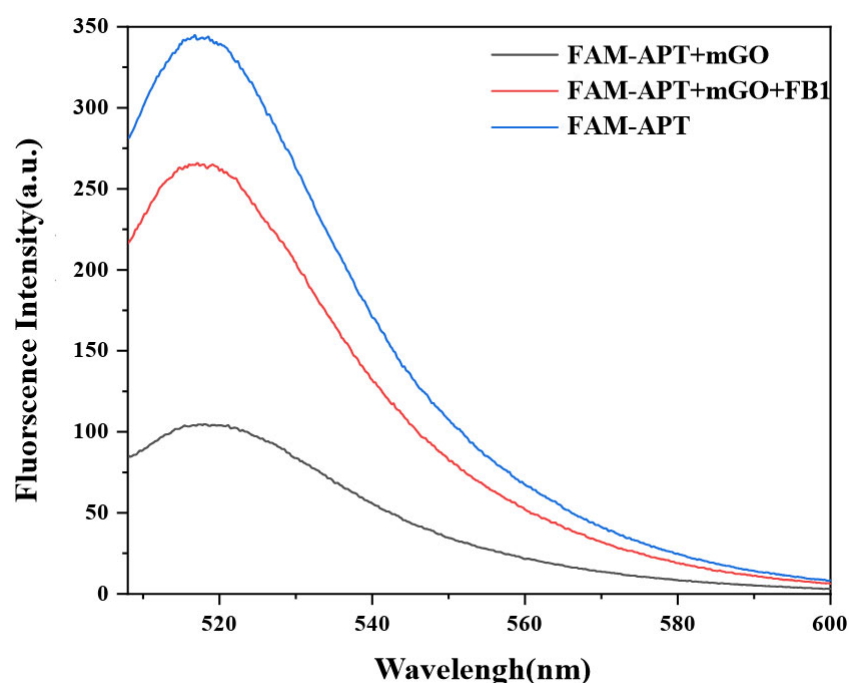


Figure 7. Schematic diagram of feasibility of verification experiment.

3.3.2. Optimization of Experimental Conditions

To ensure the robust performance of the nucleic acid aptamer sensor, we comprehensively optimized several parameters, including the buffer type, the concentrations of

FAM-APT and mGO, and the incubation time. The experimental results are presented in Figure 8A–D.

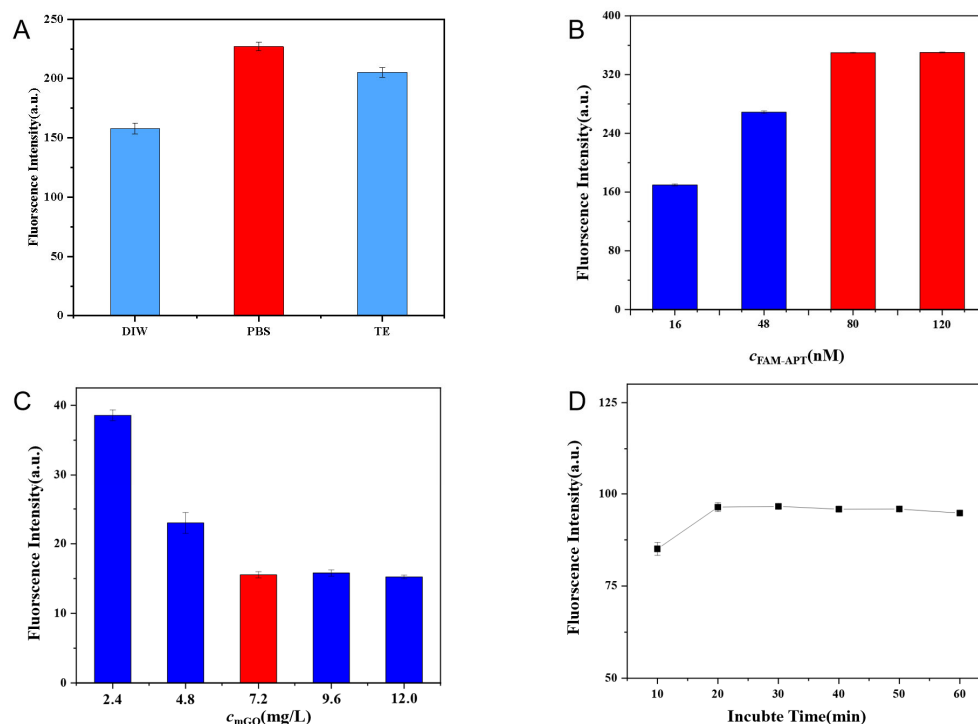


Figure 8. (A) Effect of buffer types on fluorescence intensity (the experiment was repeated three times, and the error bars represent the standard deviation). (B) Effect of FAM-APT concentration on fluorescence intensity (the experiment was repeated three times, and the error bars represent the standard deviation). (C) Selection of mGO concentration (the experiment was repeated three times, and the error bars represent the standard deviation). (D) Effect of incubation time on fluorescence intensity (the experiment was repeated three times, and the error bars represent the standard deviation).

Specifically, Figure 8A presents the results of selecting the buffer type. It shows that when phosphate is used as the buffer, the fluorescence intensity is highest; therefore, phosphate buffer was chosen. Figure 8B shows the optimization of FAM-APT concentration. The maximum fluorescence intensity at 520 nm was observed at a final FAM-APT concentration of 80 nM. When the concentration increased to 120 nM, the fluorescence intensity plateaued, indicating that in a 1 mL system, further increases in FAM-APT concentration did not enhance fluorescence. Therefore, 80 nM was chosen as the optimal final concentration of FAM-APT.

High concentrations of mGO cause FAM-APT to lose its specific binding ability to FB1, preventing fluorescence restoration. Conversely, low concentrations of mGO cannot fully quench FAM-APT, resulting in higher initial fluorescence levels in the system. As shown in Figure 8C, when the final concentration of mGO exceeds 7.2 mg/L, the quenching effect on FAM-APT is optimal; however, fluorescence cannot be restored after adding FB1. This is due to nonspecific adsorption of the fluorescent groups by the high concentration of mGO. At a final concentration of 7.2 mg/L, mGO effectively quenches FAM-APT, and fluorescence can be restored upon adding STX. Therefore, 7.2 mg/L was chosen as the optimal final concentration of mGO. Figure 8D presents the results of incubation time optimization. During the first 0–20 min, fluorescence intensity increases, indicating that FAM-APT has not yet fully and specifically bound to FB1. After 20 min, the fluorescence stabilizes, signifying complete and specific binding. Consequently, the incubation time for

this experiment was set to 20 min, The sample preparation time for ELISA is typically one hour; however, the method used in this study requires less time.

3.3.3. The Effect of Metal Ion Concentration on Fluorescence Signal

Since the concentration of metal ions can influence the fluorescence signal during detection, various ions were selected for study. Na^+ ions are present in the phosphate-buffered solution; Mg^{2+} , an alkaline earth metal ion, can affect the fluorescence signal; and Cu^{2+} , Cd^{2+} , and Co^{2+} are transition metal ions known to quench fluorescence. Therefore, these metal ions were chosen to investigate how variations in their concentrations impact the fluorescence signal, as shown in Figure 9.

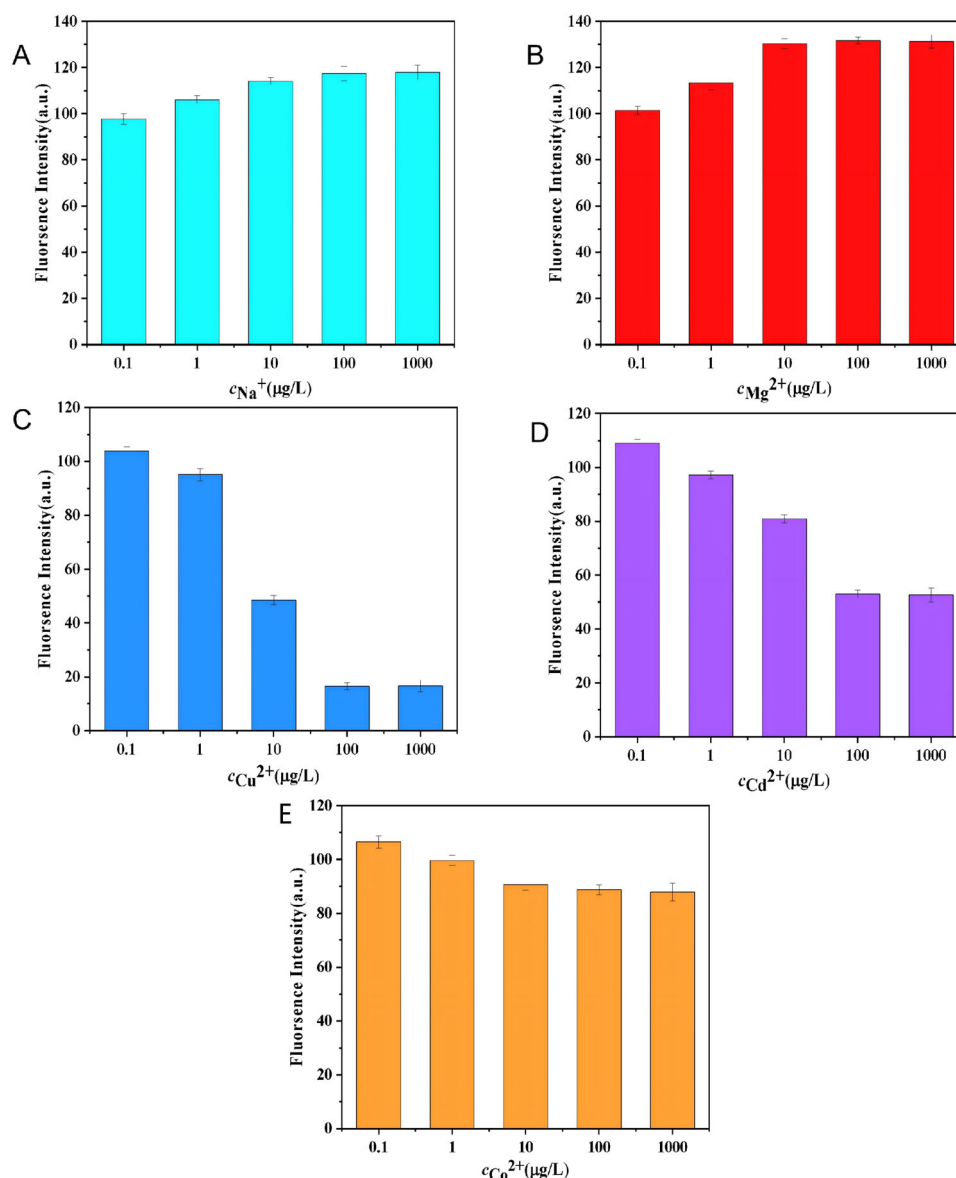


Figure 9. (A) Effect of different concentrations of sodium ions on the fluorescence signal. (B) Effect of different concentrations of magnesium ions on the fluorescence signal. (C) Effect of different concentrations of copper ions on the fluorescence signal. (D) Effect of different concentrations of cadmium ions on the fluorescence signal. (E) Effect of different concentrations of cobalt ions on the fluorescence signal (each experiment was repeated three times, and the error bars represent the standard deviations).

Figure 9A illustrates the effect of varying Na^+ concentrations on the fluorescence signal. As shown, the fluorescence intensity increases as the Na^+ concentration rises from 0.1 to 100 $\mu\text{g/L}$ and plateaus above 100 $\mu\text{g/L}$. Figure 9B shows the effect of different Mg^{2+} concentrations on the fluorescence signal. Similarly, the fluorescence intensity increases as the Mg^{2+} concentration rises from 0.1 to 10 $\mu\text{g/L}$ and stabilizes above 10 $\mu\text{g/L}$. Therefore, the presence of Na^+ and Mg^{2+} ions in the phosphate buffer slightly enhances the fluorescence signal.

Figure 9C,D illustrate the effects of varying concentrations of Cu^{2+} on the fluorescence signal. As shown, the fluorescence signal gradually decreases with increasing metal ion concentration. When the concentration exceeds 100 $\mu\text{g/L}$, the fluorescence signal stabilizes and no longer decreases. Therefore, exposure to Cu^{2+} should be minimized during the detection process to avoid interference with the results. Figure 9D,E depict the effects of different concentrations of Cd^{2+} and Co^{2+} on the fluorescence signal. The fluorescence signal decreases as the concentrations of Cd^{2+} and Co^{2+} increase but levels off after exceeding 100 $\mu\text{g/L}$. Although their quenching effect is not as strong as that of Cu^{2+} , exposure to Cd^{2+} and Co^{2+} should also be minimized during detection.

3.3.4. Detection of STX Using the Developed Aptamer Sensor

Different concentrations of FB1 were detected under optimal conditions, with the results presented in Figure 10A. The standard curve is described by the equation $y = 16.21x + 78.43$, exhibiting a strong correlation coefficient of $R^2 = 0.9974$. Within the concentration range of 10 ng/L to 5×10^6 ng/L, a clear linear relationship between FB1 concentration and fluorescence intensity was observed. The limit of detection (LOD) was determined to be 0.16 $\mu\text{g/L}$. Compared to the sensor developed by Zhao et al. using GO, this method exhibits a broader linear range [35]. Furthermore, as shown in Table 1, the LOD of the aptamer sensor developed in this study is lower than that of most previously reported FB1 detection methods, while also offering a relatively wide linear detection range.

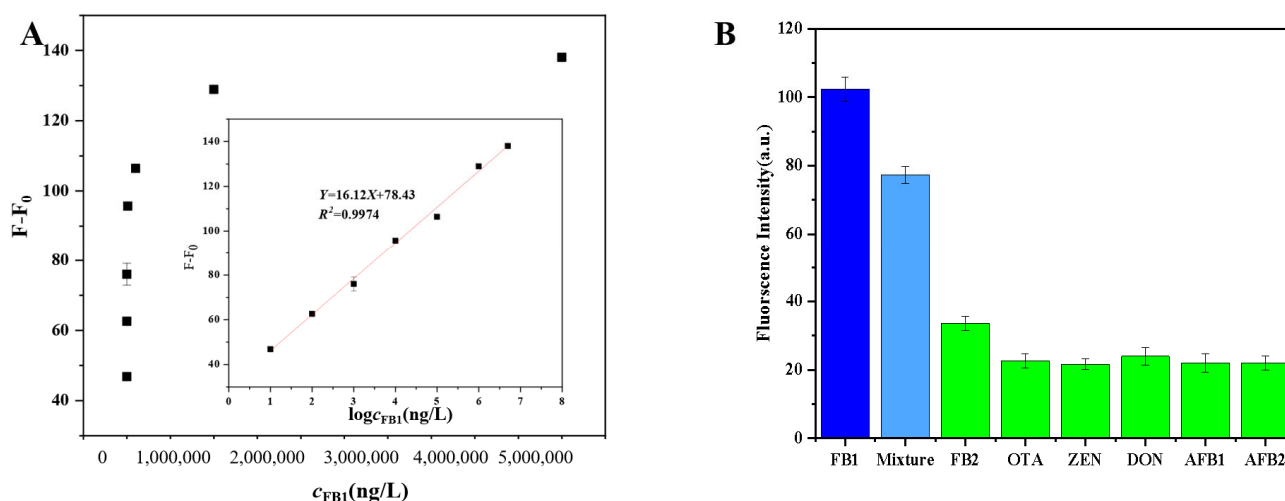


Figure 10. (A) Fluorescence intensity at 520 nm as a function of concentration of FB1. (B) Selectivity of the method with FB1, FB2, OTA, ZEN, DON, AFB1, AFB2, and the mixture of marine toxins (each experiment was repeated three times, and the error bars represent the standard deviations).

Table 1. Comparison of the aptasensor and the other reported methods.

Aptasensor	Linear Detection Range	LOD	Literature Resources
Multi-channel electrochemical sensor	0–54 µg/L	0.58 µg/L	[49]
Colorimetric sensor	3–200 µg/L	1.73 µg/L	[50]
Fluorescent aptasensor	13.81–1000 µg/L	8.26 µg/L	[11]
LSPR aptasensor	0.8–200 µg/L	0.17 ng/mL	[51]
Fluorescent aptasensor	0–3000 ng/mL	14.42 ng/mL	[12]
Fluorescent aptasensor (GO)	10–100,000 ng/L	0.26 µg/L	This Work
Fluorescent aptasensor (mGO)	10–5 × 10 ⁶ ng/L	0.16 µg/L	This work

3.3.5. Specific Detection Results

AFB1 and AFB2 were selected due to their high toxicity and frequent co-occurrence with FB1. DON and ZEN were chosen because they are produced by the same genus of fungi as FB1. OTA is commonly found in grains and may also coexist with FB1. Additionally, FB2 was selected because it belongs to the same fumonisin group as FB1. Therefore, these toxins were specifically targeted for detection.

The results presented in Figure 10B show that the addition of toxins such as FB2, OTA, ZEN, DON, AFB1, and AFB2 does not influence the quenching effect of mGO on FAM-APT, as the fluorescence intensity remains at its lowest level. In the mixed-toxin group containing FB1, some FAM-APT specifically binds to FB1 and detaches from the mGO surface, leading to a fluorescence intensity higher than that observed with the other toxins. When only FB1 is present in the system, the fluorescence intensity reaches its highest level. These findings demonstrate that the nucleic acid aptamer sensor, using mGO as the quencher, maintains strong selectivity and specificity for FB1 compared to other toxins.

3.3.6. The Effects of Preservation Time

The storage conditions of the GO solution significantly affect its stability and dispersibility, influenced by factors such as temperature [52], light exposure [53], pH value [54], appropriate solvents [55], and prolonged exposure to air [56]. To prevent experimental errors, the GO solution should be freshly prepared before each new experiment, as extended storage can cause solute precipitation, which diminishes its adsorption capacity for FAM-APT and reduces fluorescence quenching efficiency. As illustrated in Figure 11A, the quenching ability of GO on FAM-APT decreases over time, whereas the fluorescence quenching performance of mGO on FAM-APT remains nearly constant. Figure 11B demonstrates that after four weeks of storage, no solute precipitation is observed in the mGO solution, while noticeable precipitation occurs in the GO solution. This indicates that mGO exhibits superior hydrophilicity and fluorescence quenching performance compared to GO.

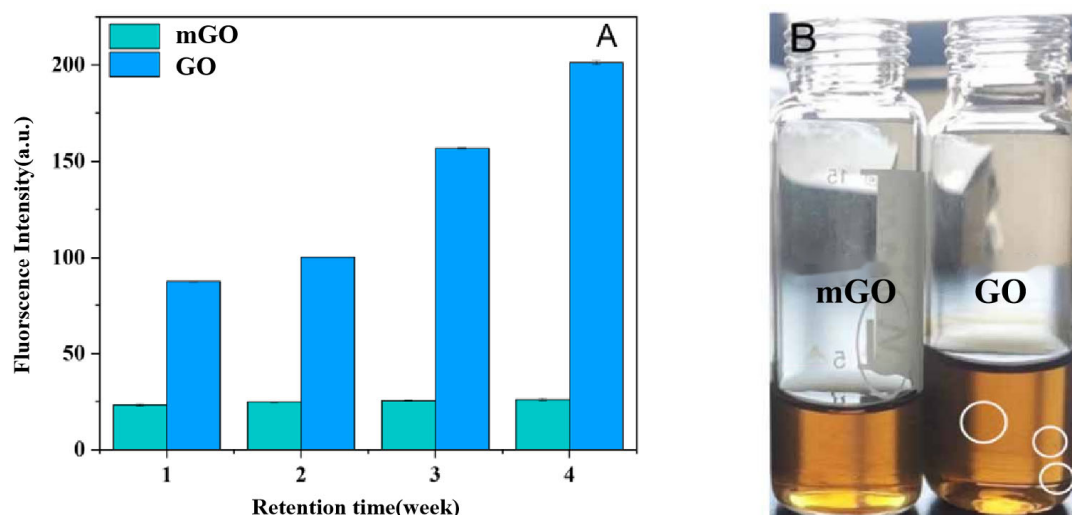


Figure 11. The effect of storage times on the quenching performance of mGO (A) and the preserved solution's status after 4 weeks (B) (each experiment was repeated three times, and the error bars represent the standard deviations).

3.3.7. Reproducible Results

Repeatability is a crucial criterion for evaluating whether a sensor can consistently perform experiments. Under identical conditions, five identical sensors were prepared by different personnel, and their repeatability was tested. As shown in Figure 12, the relative standard deviation (RSD) among the five sensors was 3.17%. These results demonstrate that the constructed fluorescent sensor has excellent reproducibility.

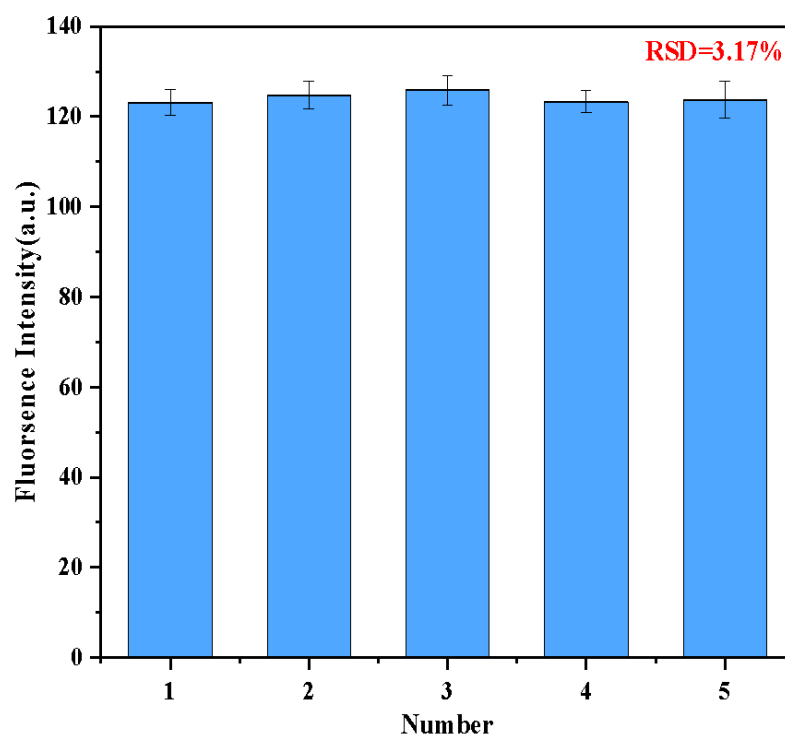


Figure 12. Reproducibility was evaluated by independently detecting 100 $\mu\text{g/L}$ of FB1 using five biosensor arrays (each experiment was repeated three times, and the error bars represent the standard deviations).

3.3.8. Actual Sample Test Results

To assess the capability of the nucleic acid aptamer sensor in detecting FB1 in real samples, this study evaluated corn and rice samples. The results, presented in Table 2, show recovery rates ranging from 95.8% to 104.7% for corn and from 89.3% to 94.5% for rice. These findings demonstrate that the sensor can effectively detect FB1 in real samples.

Table 2. The developed aptamer sensor is used to determine FB1 (ng/mL) in spiked corn and rice samples.

Specimen	Addition Level (µg/kg)	Recovery (% , Mean)
Corn	1	95.8
	10	99.6
	100	104.7
Rice	1	92.2
	10	89.3
	100	94.5

4. Conclusions

In this study, we developed a nucleic acid aptamer-based biosensor employing FRET technology for the rapid detection of FB1. Our results showed that mGO effectively quenched the fluorescence of the FB1-specific aptamer FAM-APT. An initial mGO concentration of 7.2 mg/L provided the optimal fluorescence quenching effect, while an incubation time of 20 min was found to be ideal. This method achieved a detection limit of 0.16 µg/L and demonstrated a strong linear correlation ($R^2 = 0.9974$) across a concentration range of 10 to 5×10^6 ng/L. In analyses of spiked real samples, the sensor accurately quantified FB1, with recovery rates between 89.31% and 104.7%. Furthermore, the biosensor exhibited high selectivity and specificity for FB1, even in the presence of potential interfering substances. Overall, compared to existing ELISA detection techniques, it is faster, more cost-effective, and relatively easier to operate. This detection strategy meets the requirements for reliable FB1 monitoring and offers a valuable framework for the rapid detection of other small-molecule toxins and analytes.

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