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Facile preparation of stable PEG-functionalized quantum dots with glycine-enhanced photoluminescence and their application for screening of aflatoxin B_1 in herbs

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ABSTRACT

Rapid and sensitive analytical methods of measuring mycotoxin contamination levels in medicinal herbs are urgently needed. This paper proposes a novel immunoassay utilizing PEG-modified CdSe/CdS quantum dots (QDs) with signal enhancement by glycine for aflatoxin B_1 (AFB_1) detection in medicinal herbs. Special attention was paid to the stability and photoluminescence (PL) properties of these QDs in multi-compound solutions. In this work, the dual ligands of 3-mercaptopropionic acid (MPA) and thiol-terminated methoxy PEG (mPEG-HS) were used collectively to overcome baffling coagulation and allowed for the successful transfer of hydrophobic QDs to aqueous solution. To maintain excellent PL intensity of the QDs, the ingenious solution of adding glycine was suggested, and the final PL intensity increased approximately 2 times compared to the baseline. The proposed surface modification significantly improved the colloidal stability and PL intensity of the QDs, as well as simplified the procedure compared to commonly used approaches. Furthermore, this is the first report of using these modified QDs as sensitive label for rapidly screening of AFB_1 in different medicinal herbs, with IC_{50}s ranging from 0.17 to 0.35 ng mL^{-1}. The QD labeling technique could potentially serve as a novel means of performing fast trace-detection in complex matrices.

1. Introduction

Since time immemorial, medicinal herbs have been important means of preventing and treating ailments. Recently, herbal remedies have become increasingly popular as dietary supplements and complementary medicines. However, as similar with some farm products, medicinal herbal raw materials may be exposed to various toxigenic fungi contamination during pre- and post-harvest that can cause spoilage and mycotoxins producing [1,2]. Previous studies have shown that medicinal herbs are frequently contaminated with the aflatoxins in a number of regions around the world [3–6], including the African countries (Egypt and Nigeria), the Asian countries (India, China, Malaysia, Oman, Indonesia, and South Korea), and the European countries (Spain and Turkey). Aflatoxin B_1 (AFB_1) is the most toxic member of the aflatoxins, and was designated a Group 1 carcinogen to humans by the International Agency for Research on Cancer (IARC) in 1993 [7]. Therefore, many countries and organizations have set strict limits on AFB_1 levels in food, feed, herbs, and spices.

In recent years, a number of methods of analyzing and detecting AFB_1 in food and feed have been proposed. Immunoassays are a powerful bioanalysis tool that can detect AFB_1, and are based on biospecific reactions between an antigen and a selective antibody. There have been recent reports [8–11] describing the development of more rapid immunoassay-based methods to detect AFB_1 in food and feed, including colorimetric assays, fluorescence immunoassay, electrochemical biosensors, and optical immunoassays. However, there are only a few rapid methods available for AFB_1 screening of medicinal herbs, such as conventional enzyme-linked immunosorbent assay (ELISA) [6,12] and immunochromatographic strips [13]. Moreover, the immunochromatographic strip test is typically employed as a qualitative method and does not offer an accurate quantification of the level of contamination in samples. ELISA requires complex and tedious multistep

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sample pretreatment to reduce interference from the complicated matrix in herbs. Hence, rapid, simple, and sensitive analytical methods for monitoring AFB_1 contamination and measuring its levels in medicinal herbs are badly needed.

Quantum dots (QDs) are popular labels that have been widely used in immunoassays [8,9,14,15]. The basic requirements for fluorescence biolabels are stability in aqueous solutions and a high quantum yield (QY). Often, when comparisons have been made directly in water, QDs synthesized in organic solvents have been shown to possess a wide emission and excellent fluorescence QY. However, it is extremely challenging to transfer high-quality hydrophobic QDs into the water phase and maintain stability during bioconjugation. There are three well-known approaches for realizing water solubilization of hydrophobic QDs: 1) ligand exchange [16], where hydrophobic ligands of QDs are replaced with water-soluble bifunctional molecules; 2) micelle-forming [17,18], where QDs are encapsulated through hydrophobic interactions with amphiphilic polymers; and 3) silica encapsulation [19], where nanocrystals covered in a silica shell are created. Of these methods, ligand exchange is a relatively simple, convenient, and low-cost method suitable for large-scale production that does not significantly change particle size. However, water-soluble QDs obtained by ligand exchange are predisposed to aggregate and precipitate when in solution [20], which affects further application. For this reason, the actual use of this phase transfer method to obtain hydrophobic QDs was limited in practice.

Polyethylene glycol (PEG) has been widely used to modify molecules to increase the stability of water-soluble QDs and reduce nonspecific binding. Many studies [21–23] have shown that when QDs are coated with silica and then PEG fragments are incorporated onto the silica surface, the instability of the modified QD@SiO_2 in water is abrogated, especially in buffer solutions. Reports by Speranskaya et al. [24], Susumu et al. [25], and Bentzen et al. [26] indicate that PEG-conjugated amphiphilic molecules improve colloidal stability of QDs over a wide range of pH values and reduce non-specific binding of biomolecules. In addition, Zhang et al. [27] reported on PEGylated polymer-caged QDs, and showed this modification of QDs enhanced the colloidal and pH stability, and decreased cell toxicity and non-specific binding. However, the process of PEGylation referenced above is complex and relatively time-consuming.

When attempting to achieve satisfactory robustness of QDs under tough conditions while maintaining their excellent photoluminescence (PL) properties in immunoassays, the ligands and adsorbates present during and after nanocrystal synthesis play an important role in determining the QY [28,29]. Binding of small molecules to the surface of QDs has emerged as an attractive approach to enhance PL efficiency, and there have been some notable successes using this approach. An early report showed that ligands containing amines can increase the QY of CdSe QDs from 2 to 10-fold through the strong electron donating ability of the amine nitrogen atom [30]. Furthermore, the affinity of some amino acids for the QD surface was investigated and it was found the photophysics of amino acids allow binding to the surface of a (CdSe)ZnS QD through a thiol group (cysteine), an amine (lysine), and an imidazole group (histidine) [31]. In addition, using a combination of two amino acids, histidine and N-acetyl-cysteine, to replace the original organic capping groups of (CdSe)ZnS QDs, water-soluble and highly luminescent QDs have been successfully prepared at pH 8 [32]. These studies revealed that the PL intensity of QDs could be increased by some small molecules, but there was no further report about practical use of QDs with signal enhancement.

In this study, novel PEG-functionalized QDs were generated and a glycine-enhanced fluoroimmunoassay was used to detect hazardous AFB_1 in medicinal herbs. On the basis of duly utilized ligands and adsorbates present after nanocrystal synthesis, a facile strategy for preparing water-soluble QDs with high stability and PL intensity was developed. First, the initial hydrophobic CdSe-based core–shell QDs were prepared in an organic solvent, and then transferred to an aqueous solution using 3-mercaptopropionic acid (MPA). In order to improve the colloidal stability, MPA-QDs were PEGylated with thiol-terminated methoxy PEG (mPEG-HS), and PEG fragments were coated onto the surface via coordination between mercapto group and Cd atom. The dual-ligand QDs with carboxyl functional groups and PEG molecules that were obtained had favorable colloidal stability, but a decrease in PL intensity. Therefore, the ingenious resolution of adding glycine was suggested to maintain the excellent PL intensity of QDs. Then, the PEG-functionalized QDs were coupled to antibodies for fluorescent biolabeling, and a rapid fluorescent immunoassay (FLISA) to detect AFB_1 was developed. This proposed method was highly sensitive with an IC_{50} three times lower than ELISAs. The QD-based FLISA was successfully applied to determine the natural levels of AFB_1 contamination in different complex medicinal herb matrices, including lotus seed, pilose asiabell root, liquorice root, and germinated barley.

2. Material and methods

2.1. Reagents and materials

Details of the reagents and materials can be found in the supporting information (S1).

2.2. Synthesis of hydrophobic core-shell QDs

CdSe/CdS nanocrystals were synthesized according to a protocol adapted from Peng's group [33] and Chu et al. [34]. Briefly, 1.0 mmol $Cd(St)_2$ and 0.5 mmol Se powder were dissloved into 20 mL of 1-octadecene (ODE), purged with nitrogen, and heated to 240 °C to produce CdSe cores with a size approximately 3 nm. Then, CdSe cores were purified at 50 °C with the mixture of tributylphosphine (1.0 mL), octylamine (1.0 mL), hexane (15 mL), and methanol (30 mL). $Cd(DDTC)_2$ was synthesized beforehand by the literature method [33]. To grow epitaxial CdS shell on CdSe core, a 0.1 mmol/mL $Cd(DDTC)_2$ precursor solution was prepared in a mixture of paraffin (1.5 mL), oleylamine (0.45 mL), and octylamine (1.05 mL). A mixture containing paraffin (8 mL), octylamine (8.4 mL), and oleylamine (3.6 mL) was heated to 60 °C under nitrogen flow, and then 2 mL CdSe core solution was added into the mixture. After the addition of $Cd(DDTC)_2$ solution, the mixture was heated to 140 °C for 20 min, and cooled down to 80 °C when the second injection of $Cd(DDTC)_2$ precursor solution was performed. In this work, the reaction cycle was accomplished after six continuous injections of the $Cd(DDTC)_2$ precursor solution, and the amount of precursor solution was 0.36, 0.48, 0.64, 0.80, 1.00, and 1.24 mL, respectively. UV–vis absorption spectra of the QDs were measured by Agilent Cary 100 UV–vis spectrophotometer (Agilent, USA). Photoluminescence measurements were performed with Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Japan). Relative quantum yield (QY) of the QDs nanoparticles was estimated by comparison with Rhodamine 6G in ethanol [35]. The Transmission electron microscopy (TEM) images were captured on a Hitachi 7700 transmission electron microscope with an acceleration voltage of 100 kV using copper grids (400 mesh) coated with a pure carbon-support film. The average diameter of the core–shell QDs (CdSe/6CdS) was calculated using Nano Measurer 1.2 software.

2.3. Water-solubilization and surface modification of QDs

MPA capped QDs were obtained by ligand exchange procedure. First, the oleylamine-capped CdSe/6CdS QDs were dispersed in 500 μL trichloromethane and an equal volume of water was added.

Then, a solution (400 μL) consisting of MPA and NaOH (pH~5) was gradually added with ultrasonicating until the QDs were transferred into the inverse phase. The trichloromethane layer was discarded. Then the particles were separated by centrifugation, and washed with absolute ethyl alcohol three times. The residue of the absolute ethyl alcohol was removed using nitrogen.

QDs capped with MPA were then modified by methoxy polyethylene glycol thiol (mPEG-SH, molecular weight of 2000): the QDs particles were dissolved in 500 μL water containing 1 mM mPEG-SH, and then the pH was adjusted to 8.0 using appropriate amount of NaOH (1 M). This mixture was gently mixed for 30 min at room temperature and then 500 μL of water was added. In this study, we used $V_{(mPEG\text{-}SH)}/V_{(CdSe/6CdS)}$ ~300 molar ratio. Redispersion of the QDs and then filtering through a 0.22 μm nylon membrane yielded a clear solution.

2.4. Covalent conjugation of QDs to IgG

For QD labeled IgG preparation, a technique described by Beloglazova et al. [36] and Sukhanova et al. [37] was adopted. A ratio of N-Hydroxysulfosuccinimide sodium salt (NHS)/N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/QDs/IgG equal to 200:200:1:10 was used. EDC (15.4 mg) and NHS (17.4 mg,) were dissolved and diluted to obtain a concentration of 800 μM, and 35 μL of the EDC-NHS mixture was added into QDs solution (100 μL, 1:3 diluted in 50 mM MES, pH 5.0, approximately 1.4×10^{-4} μmol). After gently shaking for 30 min, the QD solution was added dropwise into IgG diluted in PBS (210 μL, 1 mg/mL). This reaction mixture was continually shaked at room temperature for 3 h and then incubated overnight at 4 °C. After centrifugation at 30,000*g* for 5 min at 4 °C, the IgG–QD conjugates were obtained by clearing the supernatants and reconstituted to a final volume of 1 mL with 0.01 M PBS buffer containing 1% bovine serum albumin (BSA), and then stored at 4 °C.

2.5. FLISA using fluorescence enhancement with glycine

A description of the FLISA procedure can be found in the supporting information (S2).

2.6. ELISA procedure

A description of the ELISA immunoassay can be found in the supporting information (S3).

2.7. Sample preparation

The medicinal herb samples were ground through a high speed multi-purpose disintegrator (Henan Shuoman Machinery Equipment Company Limited, Henan, China) and passed through a 24-mesh sieve. Approximately 1 g of the sample was extracted with 5 mL of methanol/water (60:40, v/v) by ultrasonicating for 15 min. After centrifuging at 10,000 rpm for 5 min, the supernatant was saved for analysis. For immunoassays, the sample extracts were diluted 1:4 with PBS and then filtered through a 0.22 μm nylon membrane.

3. Results and discussion

3.1. Synthesis and characterization of the QDs

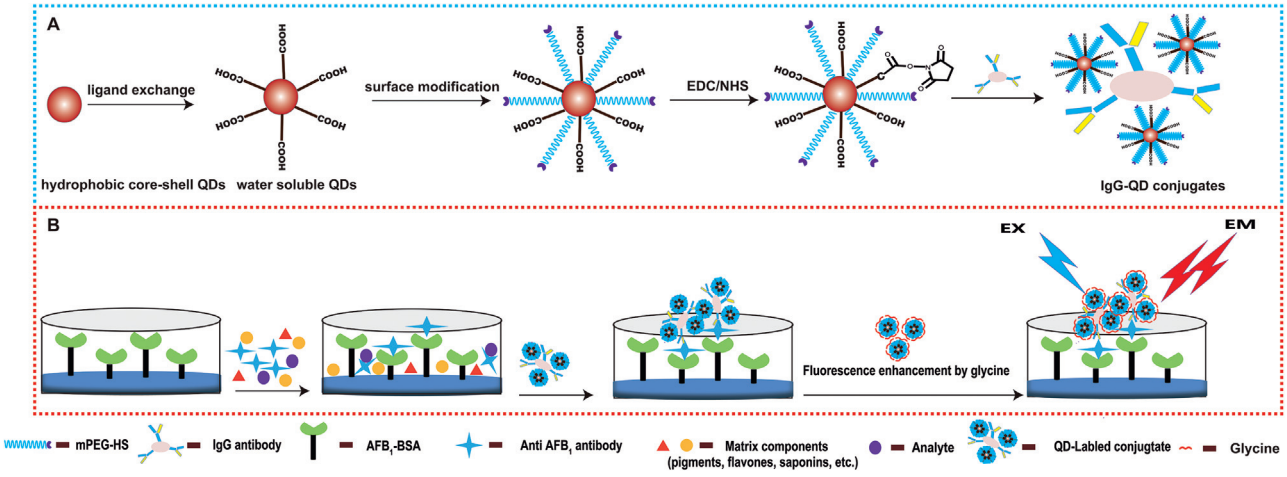
CdSe cores with initial sizes of d ~ 3.4 nm (λ_{fl} = 581 nm) were used to prepare core–shell CdSe/6CdS QDs (λ_{fl} = 632 nm, d ~ 7.2 nm). The TEM images of the original CdSe cores and core–shell QDs are presented in Fig. S2. After CdSe cores were successively overcoated with CdS shells, it showed a noticeable red-shift in both the absorption and fluorescence spectra (Fig. S1).The relative fluorescence QY of the CdSe cores did not exceed 7%, and increased to 41% after passivation of a wider band-gap semiconductor.

3.2. Water-solubilization of QDs and conjugation of QDs with IgG

A schematic of the procedure followed is presented as Scheme 1A. A successful phase-transfer of QDs needs to be confirmed prior to subsequent applications. Therefore, QD water-solubilization was optimized based on the photophysical properties and solution stability when the QDs were coupled with IgG.

After ligand exchange using MPA, the relative fluorescence QY of the QDs decreased slightly from 41 to 38%, similarly to a recently published report by Lin et al. [38]. While ligand exchange with MPA is not technically challenging, it was found that MPA-QDs in water-based solutions were more susceptible to aggregation and precipitation in the subsequent conjugation with reagent EDC/NHS (Fig. 1A). This negatively affected the coupling efficiency and use in further applications.

In an effort to increase the stability of QDs at different pHs and ionic strengths, PEG chains were introduced into the QDs ligand layer [21,24,39]. To do this, MPA-coated QDs were incubated with mPEG-HS. It has been speculated that mPEG-HS attaches onto



Scheme 1. (A) Schematic of generation of QD-based fluorescent label and (B) working principle behind AFB_1 detection by fluorescence immunoassay.

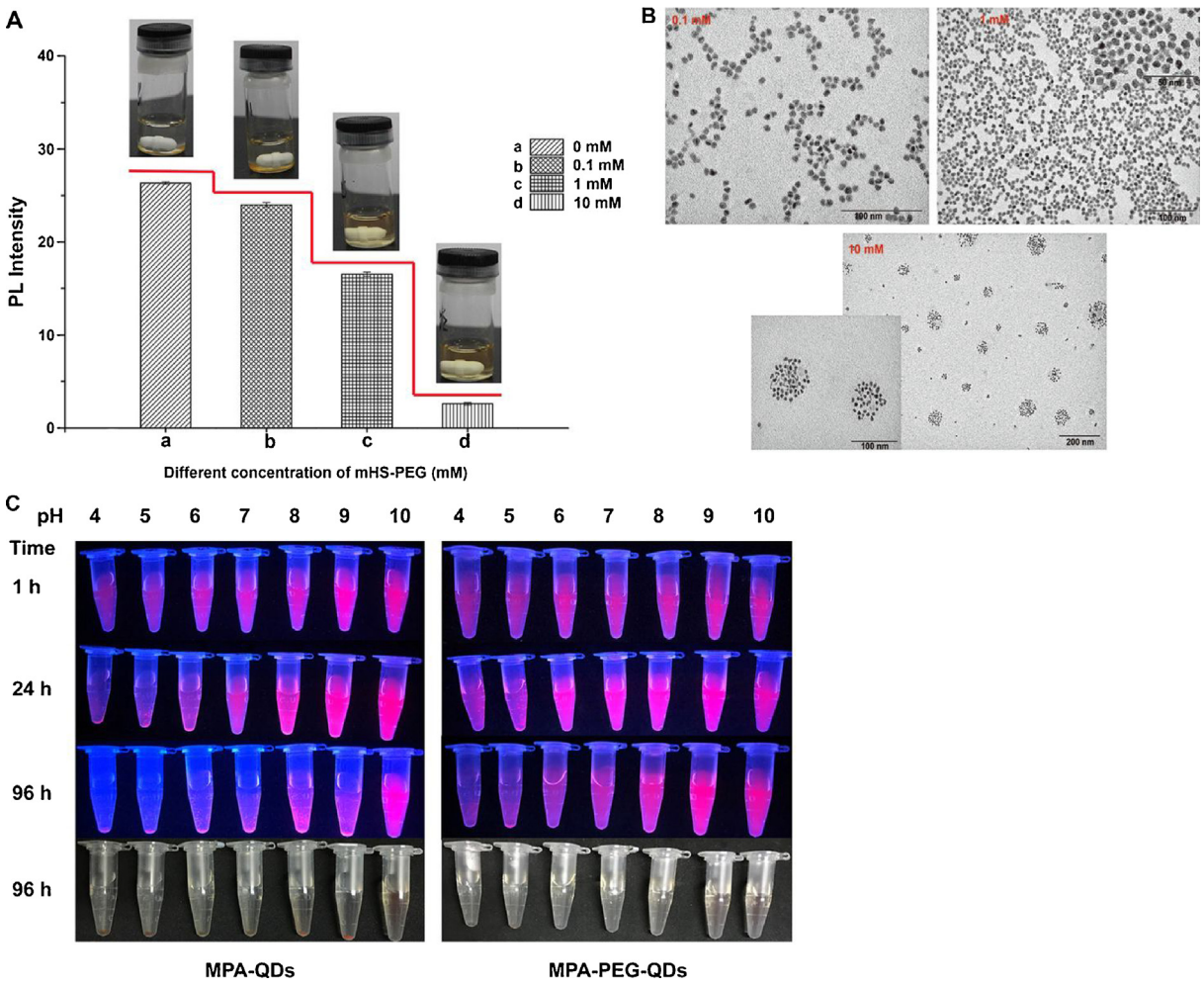


Fig 1. (A) Photoluminescent (PL) intensity of QDs with different mPEG-HS concentrations of 0, 0.1, 1, and 10 mM. Error bars refer to standard deviation (n = 3), which is displayed as a histogram at the bottom of figure, and their stability in reaction medium when coupled with IgG, which is displayed in the vial at the top of figure. (B) TEM images of PEGylated QDs. (C) Comprison of pH stability between MPA-QDs and MPA-PEG-QDs (the images were observed under UV 365 nm, except the last line which was observed under day light).

the surface of QDs via mercapto group binding to a Cd atom [34]. This prediction was further supported by comparing mPEG-HS with HS-PEG-COOH and mPEG-NH_2, where using HS-PEG-COOH also produced stable MPA-PEG-QDs, but NH_2-PEG did not (Fig. S3). However, the interactions between the thiols and Cd atoms were not strong enough for an exchange with the original ligand of hydrophobic QDs to occur. Therefore, mPEG-HS cannot be used in place of MPA to complete the phase transfer of QDs, and these reagents must be used in combination.

It has been reported that thiol ligands influence the photophysical properties of CdSe in a concentration-dependent manner [40]. In this work, different concentrations of mPEG-HS of 0.1 mM, 1 mM, and 10 mM were investigated. It was found mPEG-SH influenced the PL in a highly concentration-dependent manner. The highest mPEG-HS concentration (10 mM) dramatically decreased the PL while the lower concentration (0.1 mM) did not improve the stability of the MPA-QDs, which were prone to aggregating and precipitating when coupled to the IgG protein (Fig. 1A). In addition, as seen from the TEM images of the QDs after PEGylation in three different concentrations (Fig. 1B), the monodispersion of the QDs was observably affected by higher concentrations of mPEG-HS. Therefore, 1 mM of mPEG-HS was considered the optimal concentration and, after water-solubilization and surface-modification, no shift was seen in the fluorescence spectra (Fig. S4).

Compared to the often used covalent binding or silica-coated modifications, the novel mPEG-SH modified method proposed for water-solubilization of hydrophobic QDs was simple, easy to perform, and maintained the shape and size of MPA-PEG-QDs (Fig. 1B). In addition, to assess the stability of QDs at different pH environments, seven frequently-used buffers were utilized for a wide range of pH values: 50 mM MES buffer (pH 4 and pH 5), 10 mM phosphate buffer (pH 6 and pH 7), and 10 mM Tris-HCl buffer (pH 8, pH 9 and pH 10). As shown in Fig. 1C, the MPA-QDs without PEG fragments precipitated from the buffer solutions over time. It is obviously observed that the precipitation had occurred in the solutions with pH from 4 to 6 after 24 h, and the similar phenomenon also appeared in solutions with pH from 7 to 9 after 96 h. In contrast, the majority of MPA-PEG-QDs nanoparticles still main their optical properties and colloidal stability even after 96 h storage (Fig. 1C). Hence, the effectivity of the facile strategy for preparing the pegylated QDs by combining ligand of MPA and mPEG-HS is further confirmed.

3.3. Confirmation of MPA-PEG-QDs coupling with IgG

To verify the MPA-PEG-QDs successfully coupled with IgG, we adopted a method of lateral flow strip for rapid detection [41]. Briefly, anti-AFB_1 monoclonal antibody (0.5 mg/mL) was immobi-

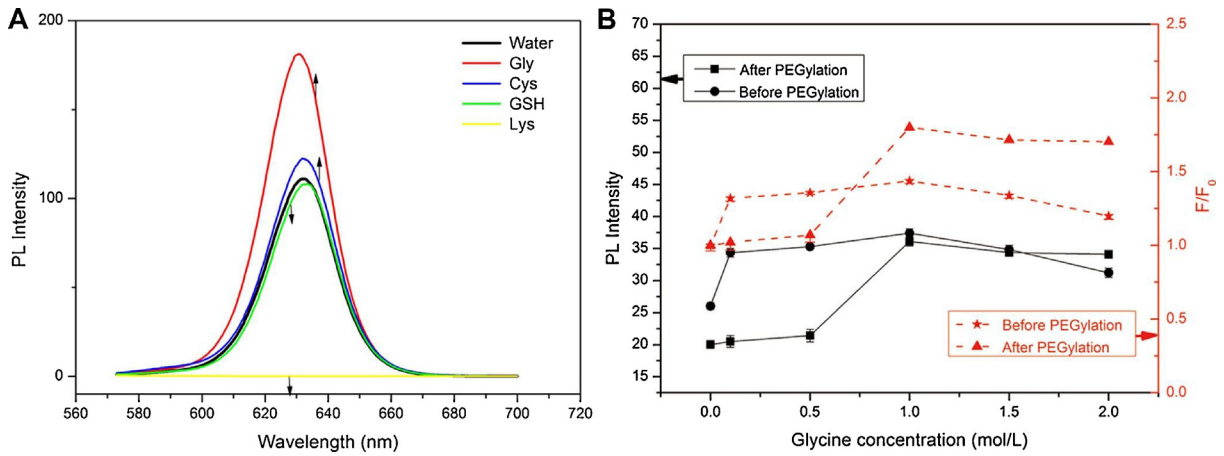


Fig. 2. (A) Photoluminescent (PL) intensity of QDs after adding solutions containing different amino acids. (B) Absolute and relative PL intensity of QDs before and after PEGylation in glycine concentrations of 0.1, 0.5, 1.0, 1.5, and 2.0 M. Error bars refer to standard deviation (n = 3).

lized on the nitrocellulose (NC) membrane as the test line, and dried at 37 °C for 12 h. Then the MPA-PEG-QDs-IgG solution (40 μL) was placed onto the sample pad, incubated for 15 min, and the strip was tested under a UV lamp (wavelength: 365 nm). A red light would be visible in the test line if the QDs had effectively conjugated to the IgG and maintained their biological activities (Fig. S5). The MPA-PEG-QDs-IgG conjugates maintained the biological activity for one month when stored at 4 °C. The main advantage of using the lateral flow strip is that it simultaneously confirms the coupling and biological activity of QD conjugates without requiring special instruments, unlike the more conventionally used methods (gel electrophoresis and dynamic light scattering).

3.4. PL enhancement of QDs by glycine

After PEGylation, the PL intensity of the nanocrystals decreased, which could influence the sensitivity of subsequent applications. Direct binding of small molecules to the surface of the QDs is an attractive approach to enhance the PL efficiency. The use of amine-capped ligands often increases the PL intensity of CdSe QDs [30–32]. First, in this study, the effect of yielding of the luminescence properties based on several amino acids, including glycine, cysteine, lysine, and glutathione (a condensation compound of glutamic acid, glycine, and cysteine), was evaluated. It was found glycine significantly enhanced the PL of the MPA-PEG-QDs, cysteine and glutathione had little effect, and significant fluorescence quenching was observed when lysine was added (Fig. 2A).

Dannhauser et al. [42] reported a strong enhancement of the trap emission from CdS colloids upon addition of amines. In addition, Bullen et al. [30] have proposed that Cd surface vacancies function as non-radiative recombination centers, where the adsorption of a Lewis base to the QD increases the surface vacancy energy to near or above the conduction band edge and eliminates electron capture by the surface vacancies. The same effect may also explain the higher PL yield of MPA-PEG-QDs after glycine addition.

To determine whether the increase in PL from glycine was concentration-dependent, 0.1 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M concentrations of glycine aqueous solution were tested, and MPA-QDs and MPA-PEG-QDs enhancement was compared. For both types of QDs, it was found that when the glycine concentration was less than 1.0 M, the enhancement increased with the concentration, and then steadily plateaued with a slight decline (Fig. 2B). Interestingly, the relative rate of increase of the MPA-PEG-QDs was higher than the MPA-QDs, which results in the approximate absolute PL value of the two types of QDs in the 1.0 M glycine (Fig. 2B). As a result, inhibition of PL after the mPEG-HS modification to create MPA-PEG-QDs could be eliminated (Fig. S6). In order to avoid interference from coupling of QDs and biomolecules, the addition of glycine would be performed in subsequent applications.

3.5. Application of QDs-PEG-IgG conjugates in immunoassays

The principle behind QDs-based immunoassays is shown in Scheme 1B. Compared to the traditional ELISA protocol, the FLISA had fewer steps and, therefore, had a shorter time required for analysis. PL intensity was immediately observable once the glycine solution added. An analytical parameters comparison between FLISA and ELISA is shown in Fig. 3A. The proposed competitive immunosorbent assays based on MPA-PEG-QDs-IgG conjugates resulted in an obvious increase in the sensitivity where the LOD and IC_{50} for FLISA were 0.05 ng mL^{-1} and 0.18 ng mL^{-1}, and decreased ~3-fold while for the ELISA to 0.13 ng mL^{-1} and 0.48 ng mL^{-1}, respectively. This makes it possible to apply QDs for trace-contaminants analysis or the detection in complex medicinal herb matrices.

In order to validate the applicability of the QD-FLISA, four representative medicinal herb matrices that are widely used and susceptible to mold growth, i.e. lotus seed, pilose asiabell root, liquorice root, and germinated barley, were tested. To correct for the matrix effect, calibration curves (Fig. 3B) were set up using standards prepared from blank sample extracts, where the absence of AFB_1 had been confirmed by LC–MS/MS. In order to simplify sample preparation to achieve rapid detection, the sample extract was directly diluted in PBS after a simple extraction without any additional clean up. This left complicated medicinal herb components behind that could possibly decrease the sensitivity of the assay. As expected, the assay sensitivity decreased 1.3-1.9 times for the tested matrices, with the excepting of the germinated barley matrix, where the IC_{50} values were 0.27, 0.35, 0.23, and 0.17 ng mL^{-1} for AFB_1 for the lotus seed, pilose asiabell root, liquorice root, and germinated barley extracts, respectively (Table 1). After taking into account the extraction, dilution procedure, and the maximum residue level of AFB_1 (5.0 μg kg^{-1}) proposed by the European Union, the sensitivity of the assay is approximated to be 0.25 ng mL^{-1}. Based on these experimental results, the FLISA developed in this study can be used for determination of naturally contaminated herbs samples.

The accuracy of this method was estimated using recovery experiments with spiked mycotoxin-free (blank) samples. Taking into consideration the legal maximum residue levels (5.0 μg kg^{-1}), as well as the linear range and IC_{50} (Table 1) for all four of the test matrices, 5.0 μg kg^{-1} was chosen as the spiked concentration, and 6

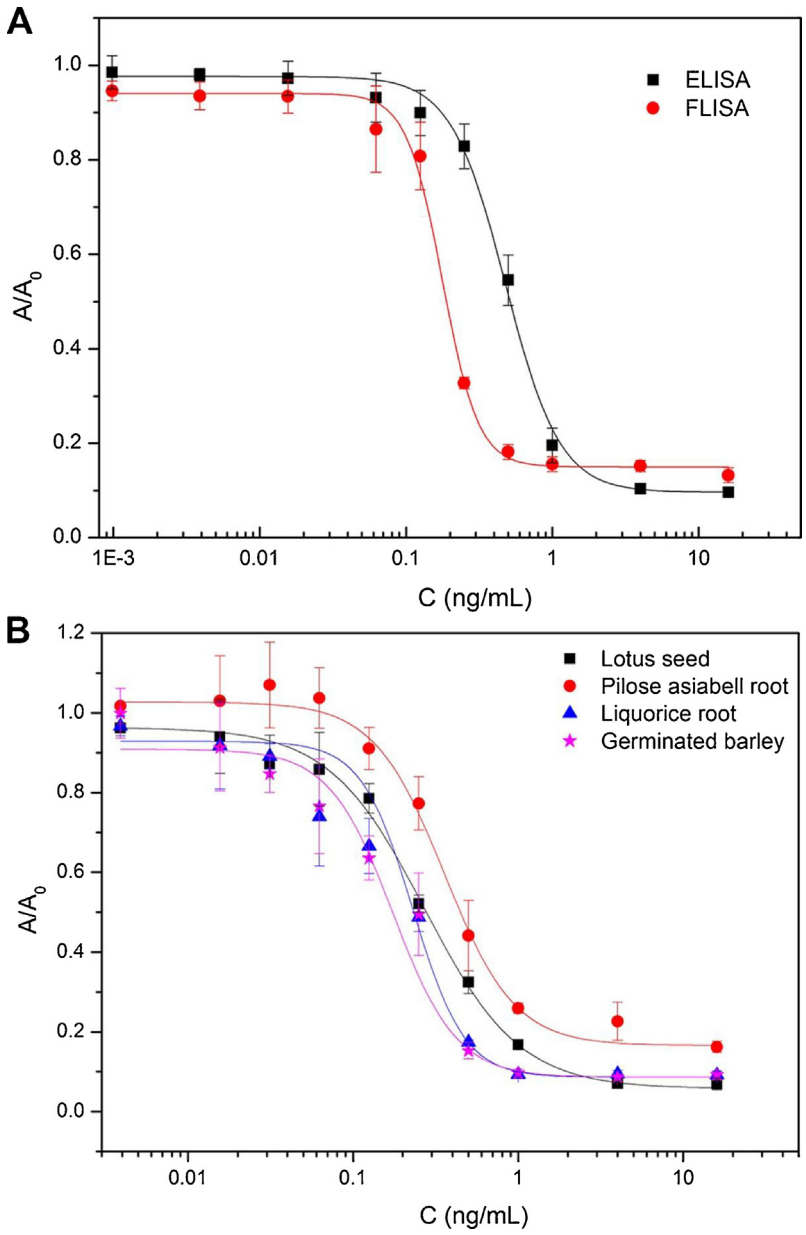


Fig. 3. (A) Calibration curve for quantifying AFB_1 by ELISA and FLISA. (B) Matrix-matched calibration curve for quantifying AFB_1 by FLISA. Error bars refer to standard deviation (n = 3).

Table 1
Analytical parameters and recovery tests for AFB_1 in four medicinal herb matrices.

Matrix				
	Lotus seed	Germinated barley	Liquorice root	Pilose asiabell root
LOD ($ng\,mL^{-1}$)	0.07	0.04	0.06	0.12
IC_{50} ($ng\,mL^{-1}$)	0.27	0.17	0.23	0.35
IC_{20}-IC_{80} ($ng\,mL^{-1}$)	0.10–0.82	0.09–0.32	0.14–0.38	0.18–0.70
Recovery (%) (added amount: 5 $\mu g\,kg^{-1}$, n = 6)	101.8	79.7	90.8	88.9
RSD (%)	7.7	5.4	18.9	13.2

replicates were used to evaluate test precision. It was found recovery for the four types of spiked samples ranged from 79.7 to 101.8% with a relative standard deviation (RSD) of 5.4–18.9% (Table 1). According to the Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits proposed by the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration Federal Grain Inspection Service [43], the acceptable range of recovery at 5.0 $\mu g\,kg^{-1}$ level is 50–150% with an RSD of no more than 25%. Therefore, the accuracy and precision of this proposed method were acceptable.

3.6. Measurement of AFB_1 in real samples using the developed QD-FLISA

To test the feasibility of the proposed approach using real samples, we analyzed nineteen samples randomly collected from medicinal centers in China. Three of the 19 samples were found to contain AFB_1 with an average content of <LOQ, 66.7, and 1.76 $\mu g\,kg^{-1}$, respectively (Table S2), where one of these samples contained 10 times the legal maximum residue levels (5.0 $\mu g\,kg^{-1}$) set by the European Union. Any potential AFB_1-containing samples mentioned above were also analyzed by the HPLC-FLD method (LOD: 0.2 $\mu g\,kg^{-1}$, LOQ: 1.0 $\mu g\,kg^{-1}$) established in our previous report [44]. No significant differences were noted between these two methods (Table S2). The antibody against AFB_1 showed 94.4% of cross-reaction with the similar analyte (aflatoxin G_1), which may influence the cross-sensitivity of the sensor. According to Commission Regulation (EU) No 519/2014 [45], the positive result screening by those rapid methods shall be verified by a full re-analysis by a confirmatory method. The positive results in the study were further confirmed by LC–MS/MS (see supporting information S4 and Fig. S7). We finally identified the carcinogen in those positive samples came from AFB_1. These results further support the efficacy of the QD-FLISA proposed in this work in accurately quantifying aflatoxin concentrations in complex medicinal herb matrices.

4. Conclusion

In this study, we developed a rapid screening immunoassay using PEG-modified QDs with signal enhancement by glycine for AFB_1 detection in medicinal herbs. To the best of the authors' knowledge, this is the first report on successful rapid detection of AFB_1 in medicinal herb matrices using a QD-based FLISA. In order to effectively water-solubilize hydrophobic oleylamine-capped QDs, a novel method was performed that combined MPA and mPEG-HS and significantly improved stability of the obtained water soluble QDs under harsh conditions. The primary advantages of this method were that the procedure was simple and easy to perform, and the shape and size of the obtained MPA-PEG-QDs remained unchanged. Meanwhile, it is worth noting that glycine solution was found to effectively enhance the PL of MPA-PEG-QDs, and therefore is a benefit as it increases the sensitivity in subsequent applications. Furthermore, we developed a rapid FLISA using these MPA-PEG-QDs as a label to detect AFB_1. The proposed assay method was sensitive, and compared to ELISA, the IC_{50} of FLISA was about three times lower. After validation, the FLISA based on QDs was successfully applied to determine AFB_1 in naturally contaminated medicinal herb samples. The novel QD label described in this study has the potential to serve as an important tool in clinical biology, trace toxicology tests, and drug discovery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2018.01.124.

References

[1] I. Rizzo, G. Vedoya, S. Maurutto, M. Haidukowski, E. Varsavsky, Assessment of toxigenic fungi on Argentinean medicinal herbs, Microbiol. Res. 159 (2004) 113–120.
[2] J.B. Calixto, Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents), Braz. J. Med. Biol. Res. 33 (2000) 179–189.
[3] W.-B. Shim, K. Kim, J.A. Ofori, Y.-C. Chung, D.-H. Chung, Occurrence of aflatoxins in herbal medicine distributed in South Korea, J. Food Protect 75 (2012) 1991–1999.
[4] V. Lippolis, O. Irurhe, A.C.R. Porricelli, M. Cortese, R. Schena, T. Imafidon, et al., Natural co-occurrence of aflatoxins and ochratoxin A in ginger (Zingiber officinale) from Nigeria, Food Control 73 (2017) 1061–1067.
[5] S. Ashiq, M. Hussain, B. Ahmad, Natural occurrence of mycotoxins in medicinal plants: a review, Fungal Genet. Biol. 66 (2014) 1–10.
[6] L. Santos, S. Marín, V. Sanchis, A.J. Ramos, Screening of mycotoxin multicontamination in medicinal and aromatic herbs sampled in Spain, J. Sci. Food Agric. 89 (2009) 1802–1807.
[7] IARC, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer, Lyon, 1993.
[8] X. Wang, R. Niessner, D.P. Tang, D. Knopp, Nanoparticle-based immunosensors and immunoassays for aflatoxins, Anal. Chim. Acta 912 (2016) 10–23.
[9] R. Eivazzadeh-Keihan, P. Pashazadeh, M. Hejazi, M. de la Guardia, A. Mokhtarzadeh, Recent advances in Nanomaterial-mediated Bio and immune sensors for detection of aflatoxin in food products, TrAC-Trend, Anal. Chem. 87 (2017) 112–128.
[10] A. Nabok, A.M. Al-Jawdah, A. Tsargorodska, Development of planar waveguide-based immunosensor for detection of low molecular weight molecules such as mycotoxins, Sens. Actuators B Chem. 247 (2017) 975–980.
[11] A.V. Orlov, A.G. Burenin, N.G. Massarskaya, A.V. Betin, M.P. Nikitin, P.I. Nikitin, Highly reproducible and sensitive detection of mycotoxins by label-free biosensors, Sens. Actuators B Chem. 246 (2017) 1080–1084.
[12] X.F. Chu, X.W. Dou, W.J. Kong, M.H. Yang, C. Zhao, M. Zhao, et al., Contamination level of aflatoxin B_1 in lotus seeds rapid screening by indirect competitive ELISA method, China J. Chin. Mater. Med. 40 (2015) 704–709.
[13] Y. Yang, Y.J. Xie, W.J. Kong, M.H. Yang, J. Wang, Determination of aflatoxin B_1 in lotus seeds by colloidal gold test strip based on lateral flow immune chromatography technique, Centr. South Pharm. 23 (2015) 779–780.
[14] F. Akhgari, H. Fattahi, Y.M. Oskoei, Recent advances in nanomaterial-based sensors for detection of trace nitroaromatic explosives, Sens. Actuators B Chem. 221 (2015) 867–878.
[15] H. Bhardwaj, C. Singh, M. k. Pandey, G. Sumana, Star shaped zinc sulphide quantum dots self-assembled monolayers: preparation and applications in food toxin detection, Sens. Actuators B Chem. 231 (2016) 624–633.
[16] W.C. Chan, S. Nie, Quantum dot bioconjugates for ultrasensitive nonisotopic detection, Science 281 (1998) 2016.
[17] T. Zhang, J. Ge, Y. Hu, Y. Yin, A general approach for transferring hydrophobic nanocrystals into water, Nano Lett. 7 (2007) 3203.
[18] R.E. Anderson, W.C. Chan, Systematic investigation of preparing biocompatible, single, and small ZnS-capped CdSe quantum dots with amphiphilic polymers, ACS Nano 2 (2008) 1341–1352.
[19] S. Wang, C. Li, P. Yang, M. Ando, N. Murase, Silica encapsulation of highly luminescent hydrophobic quantum dots by two-step microemulsion method, Colloids Surf. A Physicochem. Eng. Asp. 395 (2012) 24–31.
[20] J. Aldana, Y.A. Wang, X. Peng, Photochemical instability of CdSe nanocrystals coated by hydrophilic thiols, J. Am. Chem. Soc. 123 (2001) 8844–8850.
[21] V.V. Goftman, T. Aubert, D.V. Ginste, R. Van Deun, N.V. Beloglazova, Z. Hens, et al., Synthesis, modification, bioconjugation of silica coated fluorescent quantum dots and their application for mycotoxin detection, Biosens. Bioelectron. 79 (2016) 476–481.
[22] Y. Kobayashi, H. Matsudo, T. Nakagawa, Y. Kubota, K. Gonda, N. Ohuchi, In-vivo fluorescence imaging technique using colloid solution of multiple quantum dots/silica/poly (ethylene glycol) nanoparticles, J. Sol-Gel Sci. Technol. 66 (2013) 31–37.
[23] D. Wang, J. Qian, F. Cai, S. He, S. Han, Y. Mu, 'Green'-synthesized near-infrared PbS quantum dots with silica-PEG dual-layer coating: ultrastable and biocompatible optical probes for in vivo animal imaging, Nanotechnology 23 (2012) 245701.
[24] E.S. Speranskaya, N.V. Beloglazova, P. Lenain, S. De Saeger, Z.H. Wang, S.X. Zhang, et al., Polymer-coated fluorescent CdSe-based quantum dots for application in immunoassay, Biosens. Bioelectron. 53 (2014) 225–231.
[25] K. Susumu, H.T. Uyeda, I.L. Medintz, T. Pons, J.B. Delehanty, H. Mattoussi, Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands, J. Am. Chem. Soc. 129 (2007) 13987–13996.
[26] E.L. Bentzen, I.D. Tomlinson, J. Mason, P. Gresch, M.R. Warnement, D. Wright, et al., Surface modification to reduce nonspecific binding of quantum dots in live cell assays, Bioconjug. Chem. 16 (2005) 1488–1494.
[27] P.F. Zhang, H.X. Han, Compact PEGylated polymer-caged quantum dots with improved stability, Colloids. Surf. A Physicochem. Eng. Asp. 402 (2012) 72–79.
[28] N. Zhan, G. Palui, H. Grise, H. Tang, I. Alabugin, H. Mattoussi, Combining ligand design with photoligation to provide compact, colloidally stable, and easy to conjugate quantum dots, ACS Appl. Mater. Interfaces 5 (2013) 2861–2869.
[29] G.K. And, R.W. Murray, Ligand effects on optical properties of CdSe nanocrystals, J. Phys. Chem. B 109 (2005) 7012–7021.

[30] C. Bullen, P. Mulvaney, The effects of chemisorption on the luminescence of CdSe quantum dots, Langmuir 22 (2006) 3007–3013.
[31] M. Jones, S. Ding, M. Tucker, Y. Kim, S. Zhang, M. Himmel, et al., Stabilization of (CdSe) ZnS Quantum Dots in Water Using Amino Acid Capping Groups, 205th Meeting of the Electrochemical Society, 2006, pp. 340–349.
[32] X. Ai, Q. Xu, M. Jones, Q. Song, S.-Y. Ding, R.J. Ellingson, et al., Photophysics of (CdSe) ZnS colloidal quantum dots in an aqueous environment stabilized with amino acids and genetically-modified proteins, Photoch. Photobio. Sci. 6 (2007) 1027–1033.
[33] W.N. Nan, Y. Niu, H.Y. Qin, F. Cui, Y. Yang, R.C. Lai, et al., Crystal structure control of zinc-blende CdSe/CdS core/shell nanocrystals: synthesis and structure-dependent optical properties, J. Am. Chem. Soc. 134 (2012) 19685–19693.
[34] X.F. Chu, X.W. Dou, R.Z. Liang, M.H. Li, W.J. Kong, X.H. Yang, et al., A self-assembly aptasensor based on thick-shell quantum dots for sensing of ochratoxin A, Nanoscale 8 (2016) 4127–4133.
[35] M. Grabolle, M. Spieles, V. Lesnyak, N. Gaponik, A. Eychmüller, U. Resch-Genger, Determination of the fluorescence quantum yield of quantum dots: suitable procedures and achievable uncertainties, Anal. Chem. 81 (2009) 6285–6294.
[36] N.V. Beloglazova, E.S. Speranskaya, S. De Saeger, Z. Hens, S. Abe, I.Y. Goryacheva, Quantum dot based rapid tests for zearalenone detection, Anal. Bioanal. Chem. 403 (2012) 3013–3024.
[37] A. Sukhanova, J. Devy, L. Venteo, H. Kaplan, M. Artemyev, V. Oleinikov, et al., Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells, Anal. Biochem. 324 (2004) 60–67.
[38] W.Z. Lin, Y. Niu, R.Y. Meng, L. Huang, H.J. Cao, Z.X. Zhang, et al., Shell-thickness dependent optical properties of CdSe/CdS core/shell nanocrystals coated with thiol ligands, Nano Res. 9 (2016) 260–271.
[39] E.E. Lees, T.L. Nguyen, A.H. Clayton, B.W. Muir, P. Mulvaney, The preparation of colloidally stable, water-soluble, biocompatible, semiconductor nanocrystals with a small hydrodynamic diameter, ACS Nano 3 (2009) 1121–1128.
[40] S. Jeong, M. Achermann, J. Nanda, S. Ivanov, V.I. Klimov, J.A. Hollingsworth, Effect of the thiol-thiolate equilibrium on the photophysical properties of aqueous CdSe/ZnS nanocrystal quantum dots, J. Am. Chem. Soc. 127 (2005) 10126–10127.
[41] W.L. Zhou, W.J. Kong, X.W. Dou, M. Zhao, Z. Ouyang, M.H. Yang, An aptamer based lateral flow strip for on-site rapid detection of ochratoxin A in *Astragalus membranaceus*, J. Chromatogr. B 1022 (2016) 102–108.
[42] T. Dannhauser, M. O'neil, K. Johansson, D. Whitten, G. McLendon, Photophysics of quantized colloidal semiconductors. Dramatic luminescence enhancement by binding of simple amines, J. Phys. Chem. 90 (1986) 6074–6076.
[43] USDA/GIPSA, Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test Kits, U. S. Department of Agriculture, Federal Grain Inspection Service, 2016.
[44] L. Zhang, X.W. Dou, W.J. Kong, C.M. Liu, X. Han, M.H. Yang, Assessment of critical points and development of a practical strategy to extend the applicable scope of immunoaffinity column cleanup for aflatoxin detection in medicinal herbs, J. Chromatogr. A 1483 (2017) 56–63.
[45] Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis. Off. J. Eur. Uion, 2014, L 147/29.

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