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Efficient fluorescence resonance energy transfer between oppositely charged CdTe quantum dots and gold nanoparticles for turn-on fluorescence detection of glyphosate

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ABSTRACT

We designed a turn-on fluorescence assay for glyphosate based on the fluorescence resonance energy transfer (FRET) between negatively charged CdTe quantum dots capped with thioglycolic acid (TGA-CdTe-QDs) and positively charged gold nanoparticles stabilized with cysteamine (CS-AuNPs). Oppositely charged TGA-CdTe-QDs and CS-AuNPs can form FRET donor–acceptor assemblies due to electrostatic interactions, which effectively quench the fluorescence intensity of TGA-CdTe-QDs. The presence of glyphosate could induce the aggregation of CS-AuNPs through electrostatic interactions, resulting in the fluorescence recovery of the quenched QDs. This FRET-based method has been successfully utilized to detect glyphosate in apples with satisfactory results. The detection limit for glyphosate was 9.8 ng/kg (3σ), with the linear range of 0.02–2.0 μg/kg. The attractive sensitivity was obtained due to the efficient FRET and the superior fluorescence properties of QDs. The proposed method is a promising approach for rapid screening of glyphosate in real samples.

1. Introduction

Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum, non-selective, postemergence and systemic organophosphorus herbicide that is used extensively worldwide in various applications for weed and vegetation control [1]. Glyphosate acts by inhibiting the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the biosynthesis of aromatic acids precursors of many proteins [2]. Its high herbicidal activity and relatively low toxicity to mammals result in extensive agricultural, forestry, and aquatic applications. However, this indiscriminate application raises the potential for residue accumulation in both soil through adsorption, and water due to their high solubility and leaching, which generates some concerns regarding the possible health hazard and environmental contamination. Toxicological risks from human exposure to pesticides may occur through contaminated drinking water and agricultural products. Although glyphosate presents a lower acute toxicity than other herbicides, recent studies suggest that it is a potential endocrine disruptor [3]. The US Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of glyphosate in drinking water at 0.7 μg/mL (4.14 μM) [4]. The maximum residual level (MRL) of glyphosate in most crops is set at 0.1 μg/g by the European Union [5]. In China, the MRL of glyphosate in fruits such as apple was set at 0.5 mg/kg [6]. Therefore, monitoring of glyphosate in crops, fruits, vegetables, and drinking water has become increasingly important.

Among numerous analytical methods, high performance liquid chromatography (HPLC) [7–9], gas chromatography (GC) [10–12], and capillary electrophoresis (CE) [13,14] are widely used for the detection of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA) in various samples. However, the high polarity, high water solubility, low volatility, and the lack of chromophore or fluorophore in the molecular structure of these compounds made derivatization a standard procedure employed for their determination to improve volatility, enable chromatographic separation, and/or to increase detection sensitivity. Other methods have been proposed, such as ion exchange chromatography coupled to a pulsed amperometric detector (PAD) [15], ion chromatography (IC) [16], and enzyme-linked immunosorbent assay (ELISA) [17,18]. Although all these methods mentioned above can offer sensitive and accurate detection results, many of them are complicated, time-consuming, or require bulky instrumentation and have to be performed by highly trained technicians. Moreover, they are not cost-effective. Therefore, it is of considerable significance to develop sensitive, simple, and low-cost methods for the detection of glyphosate.

Optical methods, especially fluorimetric methods, offer many advantages such as high sensitivity, simple instruments, easy operation, and the ability to measure multiple fluorescence properties.

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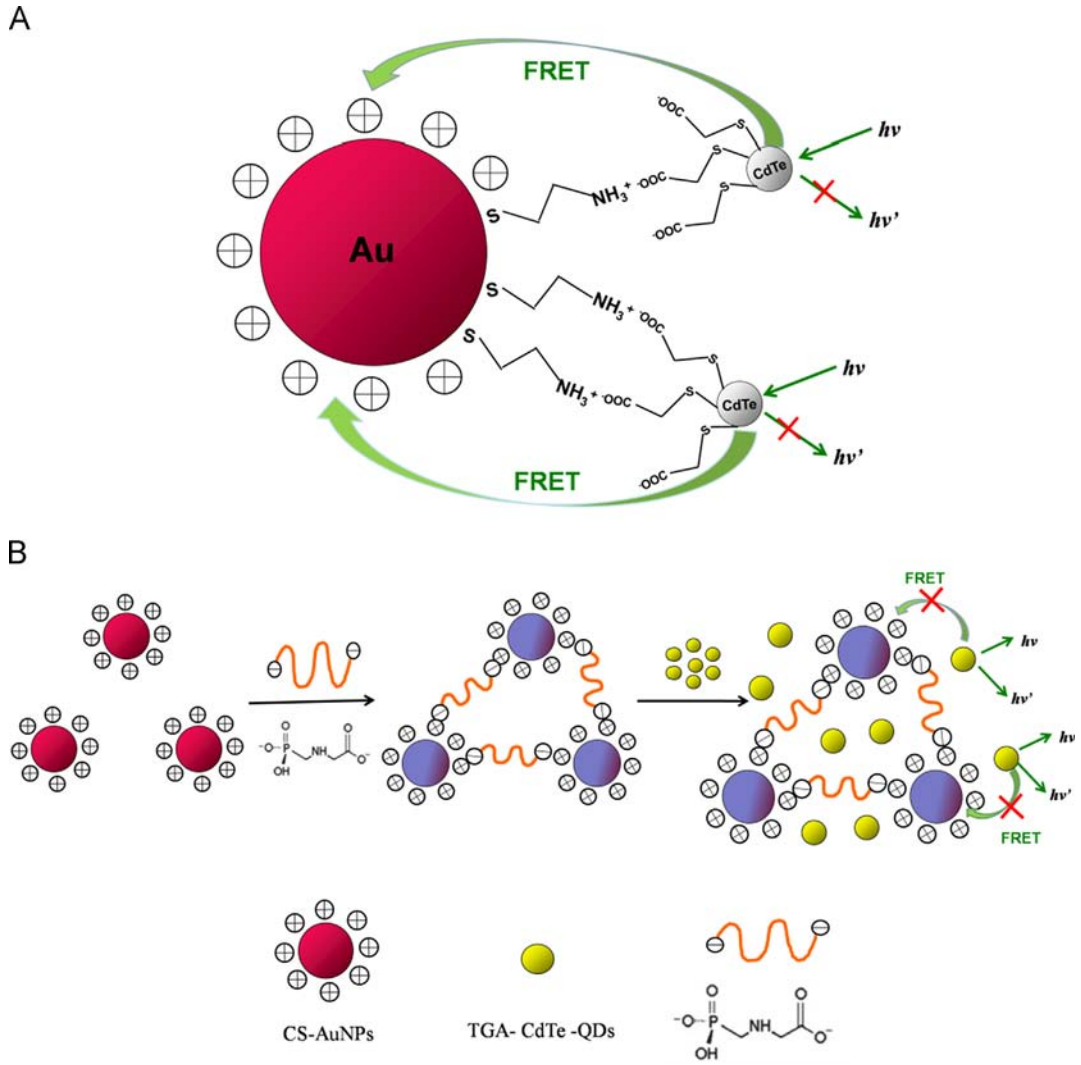
More particularly, fluorescence resonance energy transfer (FRET), has attracted more and more attention, which involves the nonradiative transfer of excitation energy from an excited donor fluorophore to a proximal ground-state acceptor. It is very sensitive to nanometer-scale changes in donor–acceptor separation distance and their relative dipole orientations. Generally, an effective FRET process could only occur when the emission spectrum of the donor and the absorption spectrum of the acceptor overlap appreciably and the mutual distance of the donor–acceptor pair is near enough (1–10 nm). Until now, because of the specificity and the intrinsic sensitivity of FRET to small changes in donor–acceptor distance, it has been utilized in various research areas, especially in analytical and bioanalytical chemistry [19]. Recently, AuNPs have been used as excellent acceptors to replace traditional organic quenchers in FRET-based analytical applications because of their high extinction coefficient and a broad absorption spectrum within the visible region which is superimposed on the emission wavelength of usual FRET donors such as organic dyes and QDs [20]. On the other hand, quantum dots (QDs) have been widely employed as donors in FRET-based systems for signaling probes due to several distinguished properties over common organic fluorescent dyes, which include high quantum yield, broad absorption spectra, narrow emission spectra, size-dependent emission wavelengths, and high photochemical stability [21]. The superiority of QDs-AuNPs donor–acceptor pairs designed as nanosensors for chemical and biological analyses with high sensitivity has attracted increasing attention. For example, specific interactions to form the QDs-AuNPs assembly were inhibited by the biological substances such as avidin [22], DNA [23], glucose [24], glycoprotein [25], and proteases [26], which attenuates the FRET process and thus cause the fluorescence recovery. In addition, the QDs-AuNPs assemblies formed by electrostatic interaction and H-bonding were used to selectively detect lead ion [27], fluoride anion [28] and concanavalin A [29] in the FRET inhibition assay.

Compared to some other complicated and costly biological method in constructing QD-AuNP assemblies [22–26], the FRET strategy based on electrostatic interaction is very simple and practical. The distinguished advantages of this kind of FRET technique intrigue us to develop a novel FRET system for the determination of glyphosate which contains negatively-charged functional groups of carboxyl (–COOH) and phosphonyl ($-PO_3H_2$). Scheme 1 outlines the principle of this method based on the attenuation of FRET efficiency between the thioglycolic acid-capped CdTe QDs (TGA-CdTe-QDs) and the cysteamine-stabilized AuNPs (CS-AuNPs) in the presence of glyphosate. When TGA-CdTe-QDs and CS-AuNPs were mixed together, oppositely charged TGA-CdTe-QDs and CS-AuNPs can form FRET donor–acceptor assemblies due to electrostatic interactions, which effectively quench the fluorescence intensity of TGA-CdTe-QDs (Scheme 1(A)). However, in the presence of glyphosate, negatively charged glyphosate was inclined to adsorb onto the surface of



Scheme 1. Scematic illustration of (A) FRET between TGA-CdTe-QDs and CS-AuNPs, and (B) glyphosate-induced attenuation of FRET and fluorescence recovery of quenched QDs .

positively charged CS-AuNPs by electrostatic interactions, rapidly inducing the aggregation of CS-AuNPs. Therefore, the FRET efficiency between TGA-CdTe-QDs and CS-AuNPs was attenuated, resulting in the fluorescence recovery of the quenched QDs (Scheme 1(B)). Since the degree of attenuation on the FRET efficiency was strongly dependent upon the amount of glyphosate, highly sensitive assay for glyphosate detection was developed.

2. Experimental

2.1. Reagents and materials

Te powder, sodium borohydride ($NaBH_4$) and thioglycolic acid (TGA) were obtained from Sinopharm Chemical Reagent (Shanghai, China). Cadmium chloride ($CdCl_2 \cdot 2H_2O$), $AuCl_3 \cdot HCl \cdot 4H_2O$, vitamin C, vitamin B_2, $FeCl_3$, NaH_2PO_4, $MgCl_2$, $ZnCl_2$, $CaCl_2$ and KCl were purchased from Beijing Chemical Reagent Company (Beijing, China). Cysteamine hydrochloride (2-amino-ethanethiol) was obtained from Sangon Biotech (Shanghai, China). Glyphosate (N-[phosphonmethyl] glycine), glufosinate and aminomethylphosphonic acid (AMPA) were purchased from Sigma-Aldrich (St. Louis, USA). If not specifically stated, all the chemicals were of analytical grade and triply distilled water was used in all experiments. Organic apples free from pesticides were purchased from the local supermarket.

2.2. Apparatus

The absorption spectra were recorded on a 2550 UV–vis spectrophotometer (Shimadzu, Tokyo, Japan). The fluorescence spectra were acquired on a RF-5301 fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) at the excitation wavelength of 400 nm, with both of the exciting and emission slits set at 5 nm. The fluorescence lifetime measurements were conducted using a FLS 920 spectrometer (Edinburgh Instruments, UK). Zeta potential and dynamic light scattering (DLS) were performed with a Malvern Nano-ZS apparatus for characterization of the surface charge and size distribution of nanoparticles in solution. Transmission electron microscopy (TEM) measurements were made on a JEM-2100F (JEOL Co., Japan) operated at an accelerating voltage of 200 kV. The samples for TEM characterization were prepared by placing a drop of colloidal solution on carbon-coated copper grid and dried at room temperature. A WVFY-201 microwave reactor of 800 W power (Zhize Equipment Factory, Shanghai, China) was used in the experiments. All pH measurements were carried out with a Model pHS-3C (Chenghua Equipment Factory, Shanghai, China). The ultrasonic treatment was carried out on a 125KQ-300DE ultrasonicator (Kunshan Ultrasonic Instrument Co., Shanghai, China).

2.3. Preparation of citrate-stabilized AuNPs and cysteamine-stabilized AuNPs (CS-AuNPs)

The solution of 13 nm citrate-stabilized AuNPs was prepared according to our previous work [30] and stored in a brown bottle at 4 °C. CS-AuNPs was prepared according to the reported literature [31] as following. All glassware was soaked overnight in freshly prepared concentrated HCl/HNO_3 (3:1, v/v) and thoroughly rinsed with doubly distilled water prior to use. Briefly, 500 μL of 0.213 M cysteamine hydrochloride and 50 mL of 1.40 mM $HAuCl_4 \cdot 4H_2O$ were mixed in a 100 mL round-bottom flask. The mixture was stirred for 20 min at room temperature in the dark. 12.5 μL of freshly prepared $NaBH_4$ solution (10 mM) was then quickly added into the above aqueous solution under vigorous stirring, and the mixture was further stirred for 30 min. The resulting wine-red solution was filtered by 0.45 μm filter and stored 4 °C before use. The molar extinction coefficient at ~528 nm for ~40 nm spherical CS-AuNPs is $7.99 \times 10^9\ M^{-1}\ cm^{-1}$ [32], thus the molar concentration of CS-AuNPs was calculated to be approximately $5 \times 10^{-10}\ mol\ L^{-1}$ according to the Lambert Beer's law.

2.4. Synthesis of water-soluble TGA-CdTe-QDs

TGA-capped CdTe QDs were synthesized according to the procedure described previously with some slight modification [33]. Briefly, 0.0256 g Te powder and 0.0386 g $NaBH_4$ was firstly added into 1 mL water in a three-neck flask with a condenser attached, and reacted at 50 °C for 45 min to get Te precursor (NaHTe). Cd precursor was prepared by mixing a solution of $CdCl_2$ (0.09134 g) with 66 μL TGA, and the solution was diluted to 100 mL, which was then adjusted to pH 11 by 1 M NaOH and deaerated with N_2 for 20 min. The Cd precursor was added into NaHTe solution while stirring vigorously at room temperature. The molar ratio of Cd^{2+}:Te^{2-}:TGA is 1:0.5:2.4. Under the protection of N_2 atmosphere, the mixed solution was stirred for 10 min and then heated with microwaves at 50% output power for 30 min. The concentration of as-prepared CdTe QDs is approximately $2 \times 10^{-5}\ mol\ L^{-1}$ according to the excitonic absorption peak value and the extinction coefficient per mole ($\varepsilon = 10{,}043\ (D)^{2.12}$) of CdTe nanoparticles [34].

2.5. General procedures for fluorescence detection of glyphosate

A typical FRET-based analysis of glyphosate was performed as follows. 0.2 mL CS-AuNPs ($5 \times 10^{-10}\ mol\ L^{-1}$) and 1.4 mL water were added into 4 mL centrifuge tubes with 0.2 mL different concentrations of glyphosate. The mixture was incubated at room temperature for 15 min, and then 0.2 mL CdTe QDs ($1 \times 10^{-5}\ mol\ L^{-1}$) was added into the system. Afterwards, the fluorescence emission spectra were recorded with the excitation of 400 nm. The calibration curve for glyphosate was established according to the fluorescence enhancement efficiency, which was monitored by $(F–F_0)/F_0$ where F_0 and F are the maximum emission intensity of the system in the absence and presence of glyphosate, respectively.

2.6. Procedures for glyphosate sensing in apple samples

Glyphosate in apple samples was measured to evaluate the potential of this assay for pesticide screening in real-world applications. The apple samples were pretreated according to the method of GB/T 5009.199-2003 [35]. 1 g of apple samples was weighed and finely chopped to 1 cm^3, then dissolved in 5 mL purified water and ultrasonicated for 2 min. After standing for 3–5 min, different concentrations of glyphosate standard solutions were added into the obtained matrix of apple samples. The supernatant was collected for analysis according to the method in Section 2.5. For recoveries experiment, known quantities of glyphosate were injected into the finely-chopped apples, then pretreated and analyzed according to the above procedures.

3. Results and discussion

3.1. Optical characteristics of TGA-CdTe-QDs and CS-AuNPs

Water-soluble TGA-CdTe-QDs were synthesized via a microwave-assisted heating method. The absorption and emission spectra of TGA-CdTe-QDs are shown in Fig. 1 (curves a and b). The average particle size is about 1.9 nm, derived from the wavelength of the first excitonic absorption peak (486 nm) in the absorption spectrum, based on an empirical fitting function from a previous

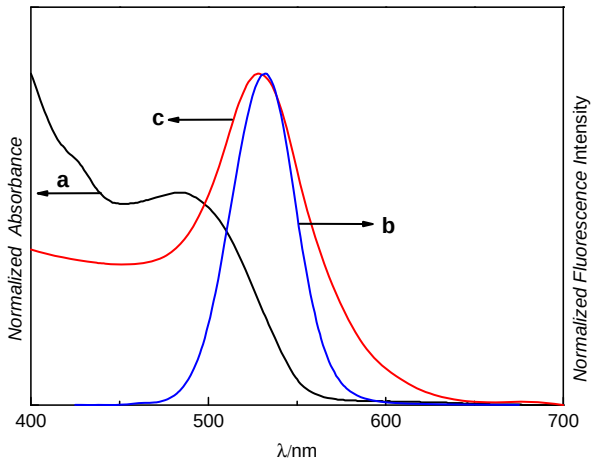


Fig. 1. Absorption (a) and fluorescence emission (b) spectra of TGA-CdTe-QDs; absorption spectrum of CS-AuNPs (c).

report [34]. The emission spectrum of TGA-CdTe-QDs displayed the maximum fluorescence centered at 532 nm. It can also be seen that the fluorescence spectrum band is relatively narrow and symmetric, which indicates that the obtained TGA-CdTe-QDs are nearly monodisperse and homogeneous.

CS-AuNPs display an intense plasmon absorption centered at 528 nm that renders the suspensions red (curve c in Fig. 1), which is just near the maximum fluorescence emission of TGA-CdTe-QDs. It is obvious that the absorption spectrum of CS-AuNPs overlaps well with the fluorescence emission spectrum of TGA-CdTe-QDs, demonstrating that it is possible to form FRET between them.

3.2. FRET-based nanoscale assembles of TGA-CdTe-QDs and CS-AuNPs

FRET is a process that involves non-radiative energy transfer from a photoexcited donor molecule, after absorption of a higher energy photon, to an acceptor molecule (brought into close proximity), which may relax to its ground state by emitting a lower energy photon [36]. The rate of energy transfer is highly dependent on many factors, such as the extent of spectral overlap, the relative orientation of the transition dipoles, and most importantly, the distance between the donor and acceptor molecules [19]. Recently, it has been demonstrated that the positively charged QDs could form FRET donor–acceptor assemblies with negatively charged AuNPs by electrostatic interactions, in which the fluorescence intensity of QDs was effectively quenched, because the electrostatic interaction could shorten the distance between the QDs donor and the AuNPs acceptor [27]. Herein, the zeta potentials of TGA-CdTe-QDs and CS-AuNPs were respectively −35.9 mV and 15.6 mV in pH 7.0 solution, due to the ionization of the –COOH group in the TGA ligand and the protonation of the $-NH_2$ group in the CS ligand. Therefore, an efficient FRET process could occur between TGA-CdTe-QDs as donors and CS-AuNPs as acceptors, because the distance between them is shortened by electrostatic interaction, and the spectral integral overlap between the QDs emission and AuNPs absorption is large (Fig. 1). The fluorescence of TGA-CdTe-QDs was noticeably quenched in the presence of CS-AuNPs (Fig. 2(A)). However, negatively charged citrate-stabilized AuNPs was used as the substitute of positively charged CS-AuNPs to carry out the control experiment, and almost no obvious luminescence change could be observed (Fig. 2(B)).

Fluorescence lifetime measurements can provide additional proof for an efficient FRET process because non-radiative energy transfer is expected to substantially alter the exciton lifetime of the donor. As shown in Fig. 3, the presence of positively charged

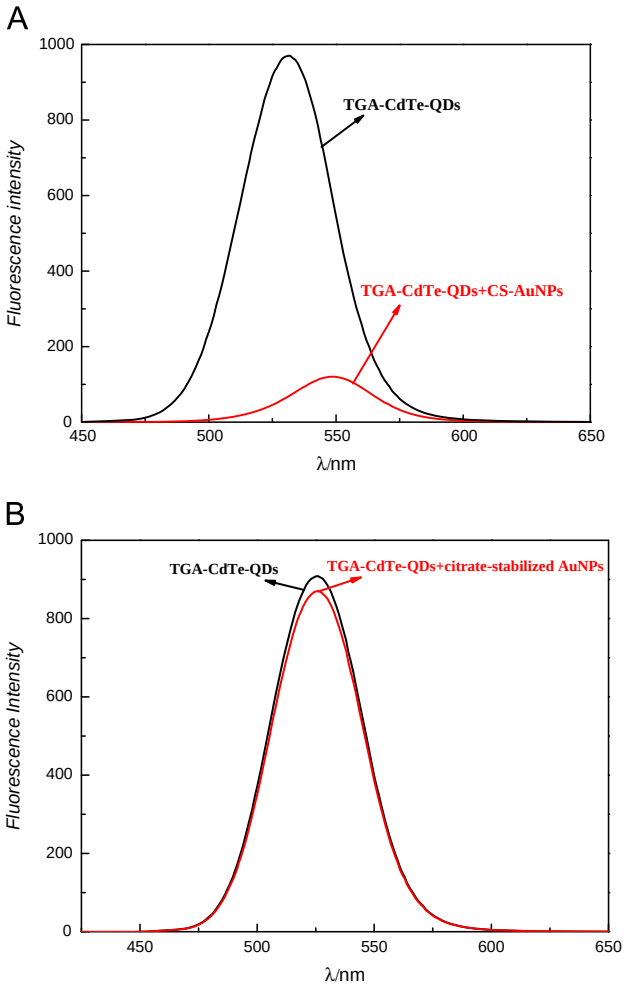


Fig. 2. Effects of AuNPs (5×10^{-11} mol L^{-1}) capped with different ligands on the fluorescence emission of TGA-CdTe-QDs (1×10^{-6} mol L^{-1}). (A) CS-AuNPs; (B) citrate-stabilized AuNPs.

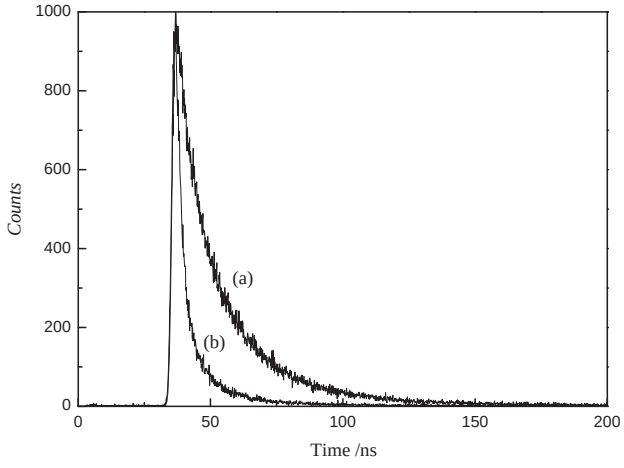


Fig. 3. Effects of CS-AuNPs on the fluorescence lifetime of TGA-CdTe-QDs. (a) TGA-CdTe-QDs (τ=36.8 ns); (b) TGA-CdTe-QDs in the presence of CS-AuNPs (τ=24.8 ns).

CS-AuNPs dramatically shortened the average lifetime of negatively charged TGA-CdTe-QDs from 36.8 ns to 24.8 ns. As expected, the average lifetime of negatively charged TGA-CdTe-QDs was hardly changed in the presence of negatively charged citrate-coated AuNPs. These results indicate that an efficient FRET process occurs between oppositely charged QDs and AuNPs, in which the

electrostatic interaction brings AuNPs acceptors into the proximity of QDs donors with the formation of QDs-AuNPs assemblies.

3.3. Effects of glyphosate on the optical characteristics of CS-AuNPs

The CS-AuNPs solution appeared red in color and exhibited an absorption peak at 528 nm (Fig. 1), which was ascribed to the surface plasmon resonance of the AuNPs. Due to the superficial $-NH_3^+$ groups, the CS-AuNPs were positively charged, resulting in high stability against aggregation because of the electrostatic repulsion force. As shown in Scheme 1, negatively charged glyphosate is inclined to adsorb onto the surface of positively charged CS-AuNPs by electrostatic interactions, resulting in the aggregation of CS-AuNPs. When glyphosate was added to the CS-AuNPs solution, it could be observed that the absorption spectrum of CS-AuNPs exhibited an obvious decrease at 528 nm accompanied with the red-to-purple (or blue) color change within several minutes, indicative of the CS-AuNPs aggregation (Fig. 4(A)). The glyphosate-stimulated aggregation of CS-AuNPs was further confirmed by the TEM observations and DLS measurement. The as-prepared CS-AuNPs are highly dispersed with an average size of about 40 nm (Fig. 4(B) and (D)). However, after addition of glyphosate, CS-AuNPs aggregate together (Fig. 4(C) and (E)). The results of TEM and DLS analysis are consistent with the changes of the absorption spectra. In fact, the aggregation and corresponding color change of AuNPs driven by glyphosate have been employed for the visual detection of glyphosate in environmental water samples [31]. However, the colorimetric techniques for the analysis of target objects generally display lower sensitivity.

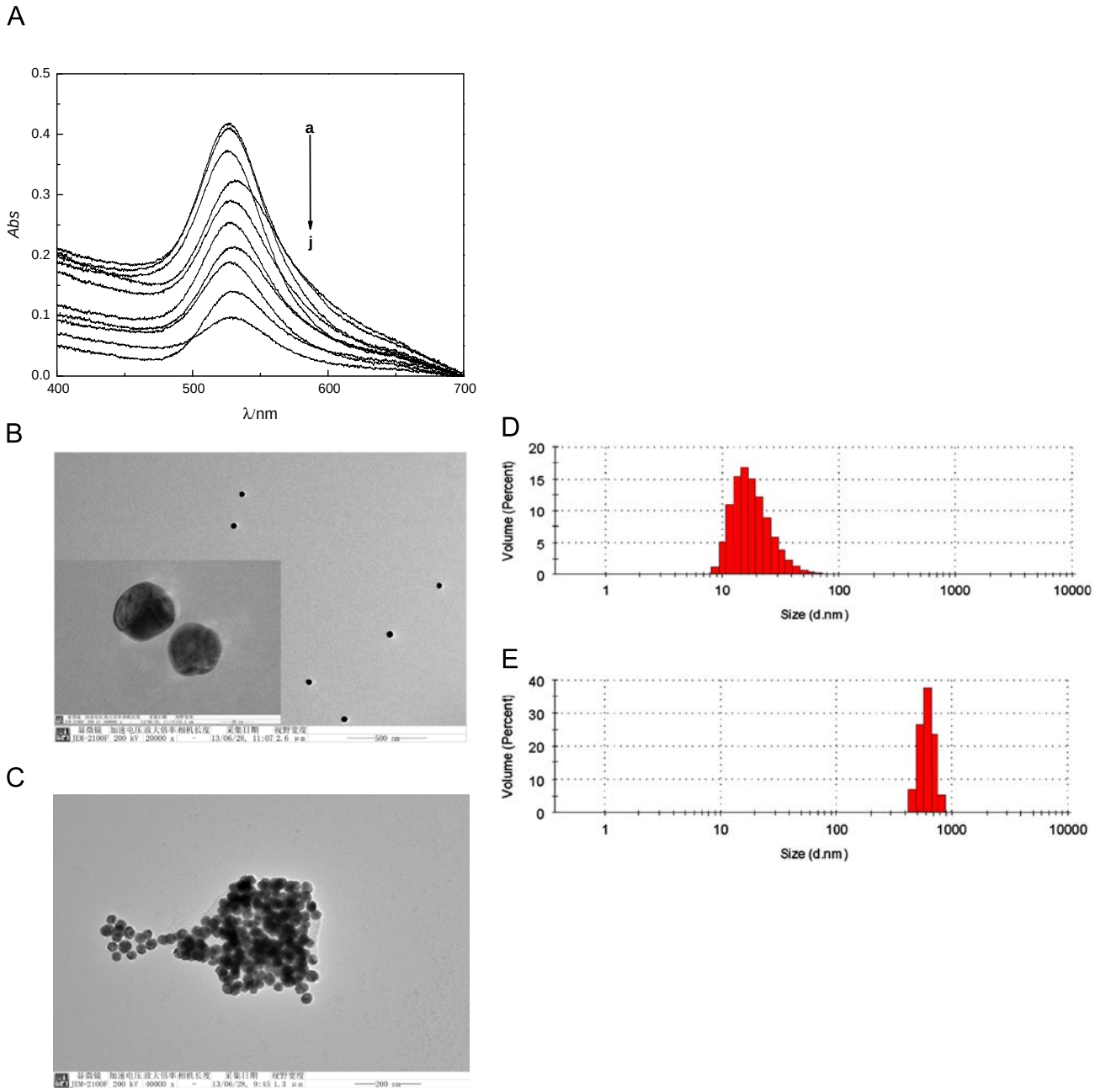


Fig. 4. (A) Absorption spectra of CS-AuNPs (5×10^{-11} mol L^{-1}) in the presence of glyphosate at various concentrations. The glyphosate in samples (a)–(j) is 0, 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 μg mL^{-1}, respectively; (B) TEM image of CS-AuNPs. Inset is an enlarged view of CS-AuNPs at the scale bar of 20 nm; (C) TEM image of CS-AuNPs after addition of 5 μg mL^{-1} glyphosate; (D) DLS size characterization of CS-AuNPs; (E) DLS size characterization of CS-AuNPs in the presence of 5 μg mL^{-1} glyphosate.

3.4. FRET-based QDs emission response to glyphosate

As discussed above, oppositely charged TGA-CdTe-QDs and CS-AuNPs can form FRET donor–acceptor assemblies due to electrostatic interactions, resulting in effective quenching of QDs emission, while glyphosate can induce the aggregation of CS-AuNPs accompanied with the absorption spectral changes. Therefore, it can be deduced that the presence of glyphosate could affect the FRET process between TGA-CdTe-QDs and CS-AuNPs, thus the FRET-based QDs emission would respond to glyphosate concentration. To illustrate this expected principle for fluorescent detection of glyphosate, the absorption spectra of this system were comprehensively investigated (Fig. 5). The absorption band of CS-AuNPs showed a very slight red-shift after mixing with TGA-CdTe-QDs (curves a and b), which might be due to the adsorption of QDs onto the surface of AuNPs via electrostatic interaction leading to the formation of nanoscale assembles of QDs and AuNPs. Glyphosate induced no changes of the absorption spectrum of TGA-CdTe-QDs (curves c and d), which indicated that there was no interaction between TGA-CdTe-QDs and glyphosate. Therefore, the glyphosate-induced spectral changes of CS-AuNPs were almost identical with or without the presence of TGA-CdTe-QDs (curves e and f), which indicated that CS-AuNPs came into aggregation driven by glyphosate.

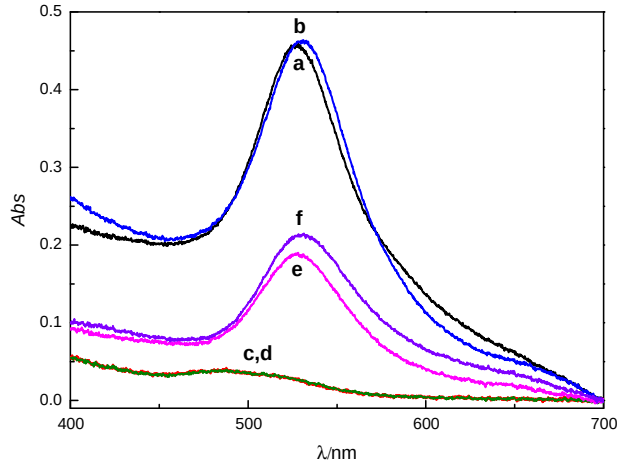


Fig. 5. Absorption spectra: (a) CS-AuNPs; (b) CS-AuNPs and TGA-CdTe-QDs; (c) TGA-CdTe-QDs; (d) TGA-CdTe-QDs and glyphosate; (e) CS-AuNPs and glyphosate; (f) mixture of CS-AuNPs, glyphosate and TGA-CdTe-QDs. TGA-CdTe-QDs, 1×10^{-6} mol L^{-1}; glyphosate, 5.0 μg mL^{-1}; CS-AuNPs, 5×10^{-11} mol L^{-1}.

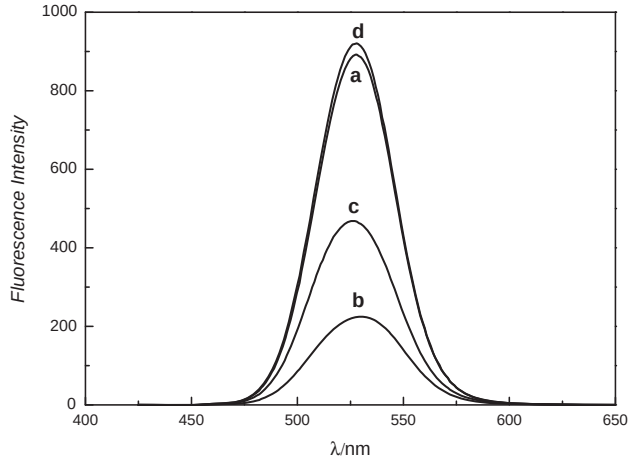


Fig. 6. Fluorescence emission spectra: (a) TGA-CdTe-QDs; (b) TGA-CdTe-QDs and CS-AuNPs; (c) mixture of CS-AuNPs, glyphosate and TGA-CdTe-QDs; (d) TGA-CdTe-QDs and glyphosate. TGA-CdTe-QDs, 1×10^{-6} mol L^{-1}; glyphosate, 0.2 μg mL^{-1}; CS-AuNPs, 5×10^{-11} mol L^{-1}.

As shown in Fig. 6, when TGA-CdTe-QDs was mixed with CS-AuNPs, the fluorescence emission was significantly quenched (curves a and b) due to the FRET between them. However, the FRET-decreased emission of TGA-CdTe-QDs was recovered obviously with the presence of glyphosate (curve c). Meanwhile, no discernible change in the shape of the emission spectra of TGA-CdTe-QDs could be observed, indicating that the recovered emission came from TGA-CdTe-QDs rather than any other newly formed emission centers. Since glyphosate had no obvious effect on the fluorescence emission of TGA-CdTe-QDs in the absence of AuNPs (curve d), it is confirmed that the fluorescence recovery should totally originate from the interaction between glyphosate and AuNPs, which can attenuate the FRET and "turn-on" the fluorescence emission of TGA-CdTe-QDs. Besides, the control experiments were carried out using glufosinate and aminomethylphosphonic acid (AMPA) which have a similar molecular structure to glyphosate as substitutes. As shown in Fig. 7, neither glufosinate nor AMPA could induce the absorption spectral changes of CS-AuNPs and recover the FRET-decreased emission of TGA-CdTe-QDs. Compared to glyphosate, the AMPA molecule just contains a negatively charged phosphonyl group ($-PO_3H_2$), and an amino

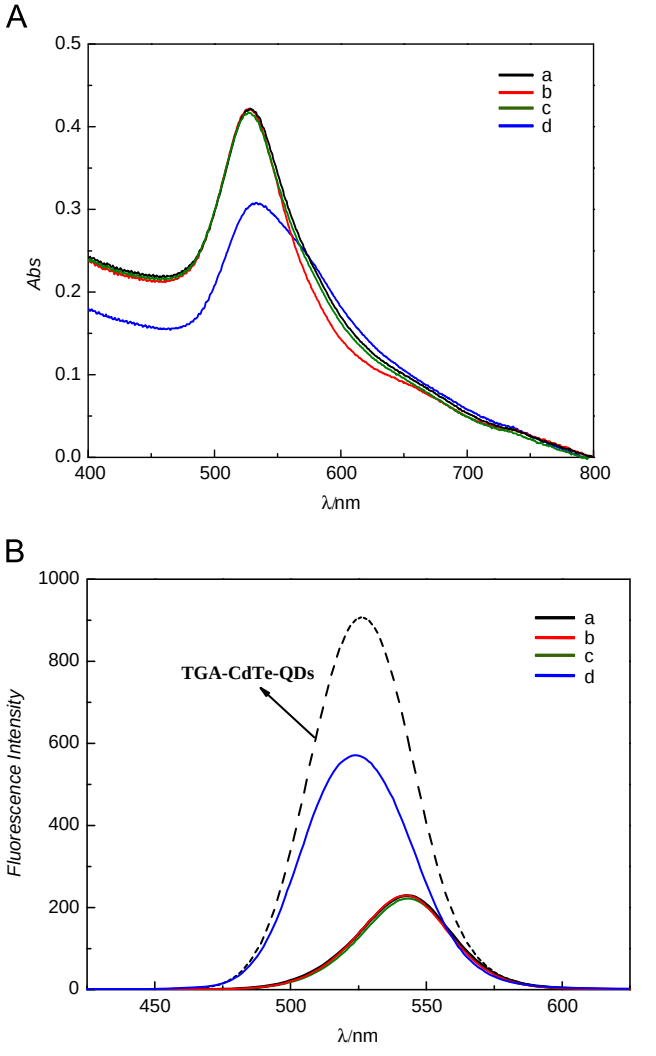


Fig. 7. (A) Absorption spectra: 5×10^{-11} mol L^{-1} CS-AuNPs solution (a), CS-AuNPs solution containing 2 μg mL^{-1} AMPA (b), 2 μg mL^{-1} glufosinate (c) and 2 μg mL^{-1} glyphosate (d). (B) Fluorescence emission spectra: solution of 1×10^{-6} mol L^{-1} TGA-CdTe-QDs and 5×10^{-11} mol L^{-1} CS-AuNPs (a), solution of TGA-CdTe-QDs and CS-AuNPs containing 0.5 μg mL^{-1} AMPA (b), 0.5 μg mL^{-1} glufosinate (c) and 0.5 μg mL^{-1} glyphosate (d). The experiments were performed at room temperature, the reaction time of 15 min and pH of 7.0.

groups (–NH_2) instead of carboxyl group (–COOH). Thus, AMPA molecules could not change the surface charge density of CS-AuNPs, or cross-link the neighbor CS-AuNPs [31]. As to glufosinate, because of the static resistance of methyl and the electrostatic repulsion force between $-NH_3^+$ groups, it could not bridge up the neighbor CS-AuNPs smoothly [31]. Therefore, only glyphosate could cause the aggregation of CS-AuNPs, accompanied with appreciable changes in absorption property, which decreased the FRET efficiency between CS-AuNPs and QDs. These results further validate the FRET-based mechanism for assay of glyphosate depicted in Scheme 1.

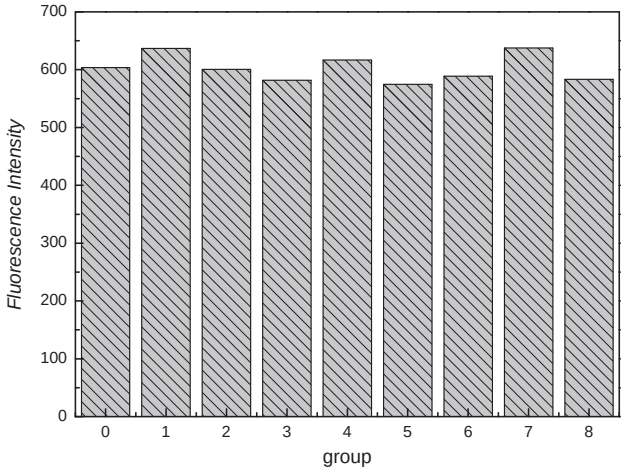


Fig. 8. The corresponding fluorescence intensity of AuNPs-CdTe QDs in the presence of 0.5 μg mL^{-1} glyphosate premixed with different substances. Substances: 0 control (AuNPs-CdTe QDs-glyphosate); 1 vitamin B_2 (0.28 μg mL^{-1}); 2 Ca^{2+} (0.05 mg mL^{-1}); 3 Fe^{3+} (7 μg mL^{-1}); 4 K^+ (0.9 mg mL^{-1}); 5 Mg^{2+} (0.04 mg mL^{-1}); 6 PO_4^{3-} (0.11 mg mL^{-1}); 7 vitamin C (0.04 mg mL^{-1}); 8 Zn^{2+} (5 μg mL^{-1}).

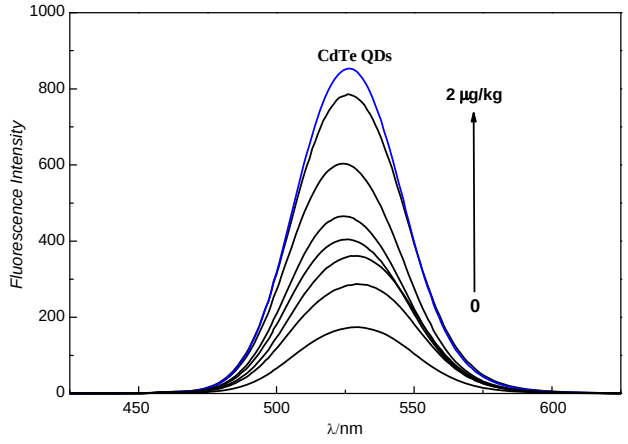


Fig. 9. Fluorescence emission spectra of CdTe QDs-AuNPs in the presence of increasing concentrations of glyphosate in apple matrix (0, 0.02, 0.1, 0.2, 0.4, 1.0, 2.0 μg/kg).

Thus, a novel fluorescent assay for glyphosate could be developed based on the FRET between QDs and AuNPs assembly. The pH values of the system greatly affect the QDs-AuNPs assemblies, since these donor–acceptor assemblies are formed by electrostatic interactions. TGA has one carboxyl group with pKa of 3.53 [37], and CS has one amino group with pKa of 10.75 [38]. So the favorable pH range would be from 5.0 to 8.0, in which TGA-CdTe-QDs and CS-AuNPs have stable optical properties, and they can form FRET donor–acceptor assemblies efficiently. On the other hand, the electrostatic interaction between CS-AuNPs and glyphosate is intensively pH-dependent. Experimental results demonstrate that glyphosate could induce absorption decrease of CS-AuNPs to a great extent at pH 7.0. Therefore, the optimal pH was chosen to be 7.0 for further experiments. The reaction time of CS-AuNPs-glyphosate was optimized by recording the absorption spectrum of CS-AuNPs every 2 min after mixing with glyphosate. The aggregation and spectral variation of CS-AuNPs could be completed within 15 min. Therefore, the reaction time of CS-AuNPs-glyphosate was chosen as 15 min.

3.5. FRET-based fluorescent sensing of glyphosate in real samples

Interference studies were done in order to explore the specific detection of glyphosate in apples using the proposed assay. These experiments included investigation of most commonly found substances in real samples, such as vitamin C, vitamin B_2, Fe^{3+}, Mg^{2+}, Zn^{2+}, K^+, Ca^{2+}, PO_4^{3-}. As shown in Fig. 8, no obvious interferences were noticed with the presence of these selected ions and compounds for determination of glyphosate (i.e., the relative error in all the cases was less than 6%). Therefore, the results showed no interferences from these substances in concentration levels usually found in apple samples.

To investigate the practical application of this FRET-based fluorescent method, the detection of glyphosate in apples was carried out. Different concentrations of glyphosate standard solutions were added into the matrix of apple samples obtained by the procedure in Section 2.6, and analyzed according to the FRET-based method mentioned in Section 2.5. The recovered fluorescence variation of QDs-AuNPs induced by glyphosate was shown in Fig. 9. The calibration curve ($(F–F_0)/F_0=1.2912c+0.9348$, $r^2=0.9903$) exhibited a linear correlation to glyphosate concentration in apple samples in the range from 0.02 to 2.0 μg/kg. The detection limit (3σ) was found to be 9.8 ng/kg, which is well below the safety limit, demonstrating that the FRET-based fluorescent method was sensitive enough to monitor glyphosate concentration. The relative standard deviation (RSD) was 7.3% for the determination of 1.0 μg/kg ($n=11$). In order to validate its reliability, the proposed method was applied to analyze glyphosate in the spiked apple samples. As listed in Table 1, the recoveries vary from 88.5% to 102.6% with the variation coefficient of 1.8–4.1%, indicating that the proposed fluorescence assay is highly reproducible and accurate for rapid screening of glyphosate in real samples in a simple manner. It is noteworthy that the present method provided a much lower detection limit than the recently reported absorbance-based method for glyphosate detection using AuNPs as colorimetric probes [31].

Table 1
Application of the proposed method for the determination of glyphosate in apple samples spiked with different amounts of glyphosate ($n=3$).

Sample	Amount added (μg/kg)	Amount found (μg/kg)	(Recovery ± RSD) (%) ($n=3$)
Apples	1.0	1.03	102.6+2.6
	1.5	1.36	90.8+4.1
	2.0	1.77	88.5+1.8

4. Conclusion

In this work, we have demonstrated a sensitive, selective fluorometric assay to detect glyphosate based on the efficient FRET between oppositely charged TGA-CdTe-QDs and CS-AuNPs. Oppositely charged TGA-CdTe-QDs and CS-AuNPs can form FRET donor–acceptor assemblies due to electrostatic interactions, which effectively quench the fluorescence intensity of TGA-CdTe-QDs. In the presence of glyphosate, negatively charged glyphosate can rapidly induce the aggregation of CS-AuNPs though electrostatic interactions, thus destroy the QDs-AuNPs assemblies and attenuate the FRET efficiency between them. This method is easy to operate with remarkably high sensitivity. Under the optimum conditions, the response is linearly proportional to the concentration of glyphosate in the range of 0.02–2.0 μg/kg, and the detection limit is found to be 9.8 ng/kg, which could satisfy the needs for on-site rapid monitoring of trace glyphosate. This simple and reliable assay would possess great potential applications in agricultural and environmental fields.

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