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CdTe QDs based fluorescent sensor for the determination of gallic acid in tea

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ABSTRACT

A new fluorescent light switch method, which based on *N*-acetyl-L-cysteine capped CdTe QDs (NALC-CdTe QDs), was developed for the detection of gallic acid (GA). The QDs possess a fluorescence emission wavelength at 520 nm and with symmetric fluorescence. When $KMnO_4$ is added, the high fluorescence of QDs could be effectively quenched for the electron transfer process between $KMnO_4$ and QDs. But with the addition of GA, the fluorescence of $KMnO_4$-QDs system could recover for the reason that redox reaction of GA and $KMnO_4$. Therefore, a fluorescent light switch method could be used for GA with a detection range of 0.6–12.6 $\mu g \cdot mL^{-1}$ and a detection limit of 0.56 $ng \cdot mL^{-1}$. Furthermore, the feasibility of the proposed fluorescence biosensor in tea was also studied and satisfactory results were obtained.

1. Introduction

Gallic acid (GA: 3,4,5-trihydroxybenzoic acid ($C_6H_2(OH)_3COOH$)) is an organic acid, which exist in gallnuts, grapes, sumac, witch hazel, tea leaves, hops and oak bark [1,2]. As a strong antioxidant, GA has large numbers of biological properties include antimutagenic, anticarcinogenic, antioxidant activities and other potential health effects [3,4]. Therefore, it is necessary to establish selectivity and sensitivity methods for the determination of GA in the food substances, and it is also important for understanding their health benefits.

Up to know, a wide variety of methods have been developed to detect GA, for example, High Performance Liquid Chromatography (HPLC) [5], flow injection analysis [6,7], resonance light scattering [8], thin layer chromatography [9] and electrochemical methods [10–16]. Although these techniques are capable of absolute identification and have low detection limits for GA, they are rather costly, sophisticated, time-consuming, and inappropriate for on-site field analysis.

As a novel fluorescent nanomaterial, Quantum dots (QDs) have many special optical properties, including high quantum yield, high molar extinction coefficient, wide range of absorption spectrum, narrow and symmetrical fluorescence spectrum, large stokes shift and high photochemical stability [17]. Based on the above special optical properties, much research has been devoted to the study of the synthesis and applications of quantum dots (QDs) [18–25], especially in fluorescent labeling, biological imaging and sensing system [26–28]. Among them, light switch QDs-based sensing systems have appeared as a novel type of sensors and have provided a broader platform for QDs in recent years [29–32]. In this type of sensing system, the fluorescence of the QDs is initially quenched (turned off), and recovered (turned on) in the next sequence step with analytes. It has the advantages of versatility and high selectivity in the light switch process. In this study, a new fluorescent sensor for the determination of GA based on the reversible "off-on" fluorescence change of *N*-acetyl-L-cysteine (NALC) capped CdTe QDs was build. $KMnO_4$ was utilized as it could efficiently quenched the fluorescence intensity of QDs (as shown in Fig. 1).

2. Experimental

2.1. Materials

All chemicals are analytically pure or above. Te power, *N*-acetyl-L-cysteine and gallic acid were produced by Sinopharm Chemical Reagent Co., Shanghai, China. $CdCl_2 \cdot 2.5H_2O$ was purchased from ShangHai Chemicals Reagent Co., Shanghai, China. $NaBH_4$ comes from Tianjin Huanwei Fine Chemical Co., Tianjin, China. Tris-HCl buffer solutions with different pH were prepared by mixing 0.2 $mol \cdot L^{-1}$ Tris and 0.1 $mol \cdot L^{-1}$ HCl in different proportions. The water used in all experiments had a resistivity greater than 18 $M\Omega \cdot cm^{-1}$.

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2.2. Instruments

Hitachi F-2500 spectrofluorophotometer (Hitachi Company, Tokyo, Japan), UV-2450 spectrophotometer (Tianmei Corporation, Shanghai, China). Hitachi-600 transmission electron microscopy (TEM, Hitachi Company, Japan), PHS-3C pH meter (Leici, Shanghai, China).

2.3. Synthesis of QDs

According to previous literature [33], CdTe QDs (capped by *N*-acetyl-L-cysteine) were synthesized by hydrothermal method with modification. In a 50 mL three-neck flask, N_2 was used as a protective gas, add in sequence with Te power (0.0255 g), which was magnetically stirred to dissolve in 10 mL of distilled water, and an excessive amount of $NaBH_4$ with slow speed. After a period of time, the black Te power disappeared and the reactant became a clear and transparent solution.

150 mL distilled water was added to a 250 mL three-neck flask containing 0.0488 g $CdCl_2 \cdot 2.5H_2O$. Then 0.0574 g NALC was added with the protection of N_2 and magnetic stirring, at the same time adjusted the pH to 11.25 with NaOH solution (1 $mol \cdot L^{-1}$). Under the appropriate stirring speed, add excessive H_2SO_4 (0.5 $mol \cdot L^{-1}$) to NaHTe solution drop by drop, and generated H_2Te gas was loaded into the $CdCl_2$ solution through N_2. Orange liquid was obtained after the reflux reaction at 369 K for 1 h. The final concentration of NALC-capped CdTe QDs, as measured by the Cd^{2+} concentration, was 1.425×10^{-3} $mol \cdot L^{-1}$.

2.4. Detection process

In a 5 mL colorimetric tube, 0.5 mL of prepared quantum dots, 0.5 mL of Tris-HCl buffer solution with a pH of 7.4 and $KMnO_4$ solution with different concentrations were added, diluted with water and shaken for 10 min, and the fluorescence of the system was determined.

Then research the effect of GA on the fluorescent restoration of the QDs-$KMnO_4$ system. 0.5 mL of CdTe QDs solution, 0.5 mL Tris-HCl buffer solution (pH 7.4), and a certain amount of $KMnO_4$,different amount of GA were sequentially added to a 5.0 mL colorimetric tube, thoroughly shaken and equilibrated for 10 min until fully mixed. The fluorescence spectra were recorded in the 480–660 nm emission wavelength range, and the fluorescence intensity of the maximum emission peak was used for quantitative analytical.

3. Results and discussion

3.1. Feasibility of the GA assay system

According to Fig. 1, the fluorescence spectrum of CdTe QDs fluorescence was narrow, and with nearly monodisperse and homogeneous, which could also be displayed by the TEM image and the absorption spectra of the QDs, as shown in Fig. S1. With the addition of $KMnO_4$, the photoluminescence intensity of CdTe QDs decreased obviously. For the fluorescence quenching of QDs by metal ions, it is a complicated process, and there are two main explanations [34–37]: (1) electron transfer from the photoexcited QDs to the cations bound at its surface; and (2) the formation of new nonradiative surface channels for electron annihilation within the QDs. In this work, the fluorescence quenching of QDs in the presence of $KMnO_4$, might be due to the nonradiative electron-hole recombination annihilation through an effective electron transfer process.

3.2. Fluorescence quenching effect of $KMnO_4$ on CdTe QDs

Fig. 2 shows the fluorescence emission spectra of quantum dots in the presence or absence of $KMnO_4$. As the concentration of

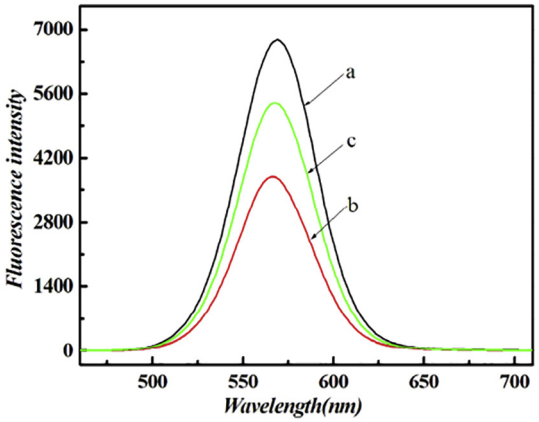

Fig. 1. The fluorescence spectra of NALC-capped CdTe QDs (*curve a*), NALC-capped CdTe QDs-$KMnO_4$ system (*curve b*) and NALC-capped CdTe QDs-$KMnO_4$ system in the presence of GA (*curve c*).

$KMnO_4$ increasing, the fluorescence intensity of CdTe QDs gradually decreased, which deduced that the QDs fluorescence quenching was mainly concentration dependent. But according to the inset of Fig. 2, with the $KMnO_4$ concentration increasing, the fluorescence intensity of QDs decreasing, when $KMnO_4$ concentration was 1.6×10^{-4} $mol \cdot L^{-1}$, the degree of quenching achieve the maximum value. Therefore, 1.6×10^{-4} $mol \cdot L^{-1}$ of $KMnO_4$ for further investigation and the GA was added to induce the fluorescence restoration of the CdTe QDs-$KMnO_4$ system.

3.3. Fluorescence recovery of CdTe QDs-$KMnO_4$ system induced by GA

GA with its strong reducing property, which could react with $KMnO_4$, as a result, the fluorescence of QDs was switch on after the addition of GA into the QDs-$KMnO_4$ system. Fig. 3A shows the fluorescence spectra of QDs-$KMnO_4$ complex upon the addition of various concentrations of GA. With the GA concentration increase, the fluorescence intensity of QDs-$KMnO_4$ complex gradually increased and could remained almost the same strength with a further increase of GA concentration. The Fig. 3B reveals a good linear correlation between $\Delta F = F_1 - F$ (F is the fluorescence intensity of QDs-$KMnO_4$ system, and F_1 is the fluorescence intensity of QDs-$KMnO_4$ system with the addition of various concentration of

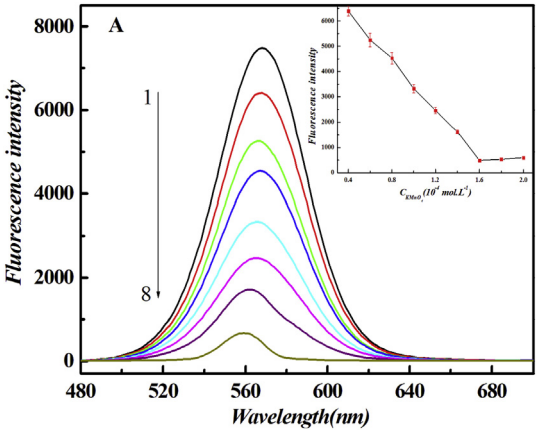

Fig. 2. (A) Fluorescence spectra of NALC-capped CdTe QDs in the presence of $KMnO_4$ in 0.5 mL Tris-HCl buffer solution at pH = 7.4; the inset shows the linear curve of the quenched fluorescence intensity of QDs against the concentration of $KMnO_4$. (NALC-capped CdTe QDs: 1.425×10^{-4} $mol \cdot L^{-1}$; 0.5 mL Tris-HCl buffer solution (7.4)).

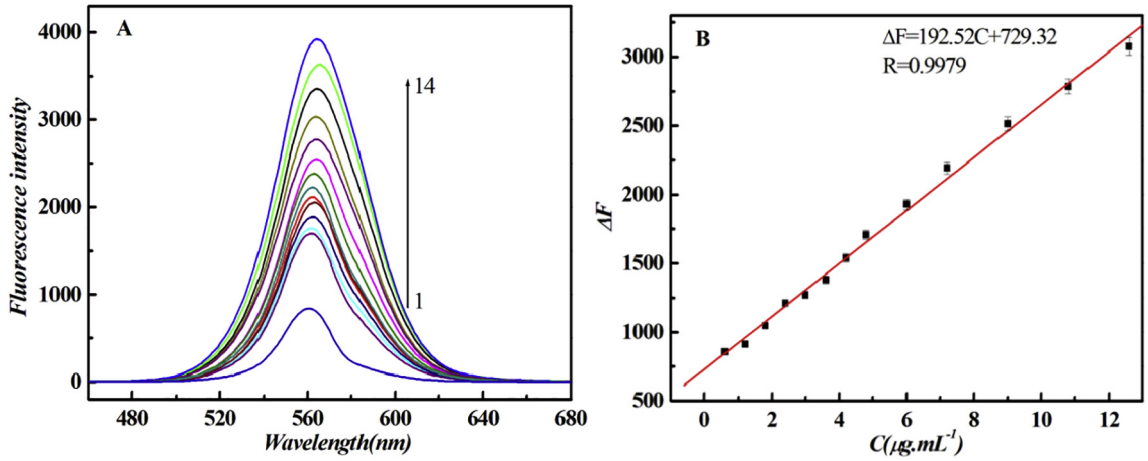

Fig. 3. (A) Fluorescence spectra of NALC-capped CdTe QDs with the addition of 1.6×10^{-4} mol·L^{-1} of $KMnO_4$ recover due to introducing GA in the concentration range of 0.6–12.6 μg·mL^{-1} (0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8, 6.0, 7.2, 9.0, 10.8, 12.6 μg·mL^{-1} respectively); (B) The linear calibration plot of the fluorescence intensity against the concentration of GA. (Concentration of NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}).

GA) and GA concentration ranging from 0.6 to 12.6 μg·mL^{-1}. The regression equation is $\Delta F = 729.32 + 192.52C$, with the regression coefficient for 0.9979, and the detection limit (LOD) for 0.56 ng·mL^{-1}. As shown in Table 1, compared with the other methods before, the method in this work has lower detection limit and wider detection range.

3.4. Optimization of the method

3.4.1. Influence of buffer solution

Usually, the acidity environment is an important factor for the reaction system and the fluorescence intensity of QDs itself. By measuring the influence of buffer solution with pH value between 7.0 and 8.0 in the QDs-$KMnO_4$ system, it is concluded that $KMnO_4$ can quench the fluorescence of QDs to the maximum extent in buffer solution with pH value of 7.4, as shown in Fig. S2A. Therefore, the optical pH of QDs-$KMnO_4$ system was 7.4. And from Fig. S2B, the maximum fluorescence recovery of the QDs-KMnO4 system after the addition of GA was obtained at pH 7.4, which fitted well with the optimum pH (7.4) of the QDs-$KMnO_4$ system. Hence, the buffer solution chose Tris-HCl (7.4) in this experiment.

3.4.2. Effect of reaction time

Reaction time is an indispensable factor in the reaction system. At room temperature, the effects of different reaction times on the system were studied. By recording the experimental phenomena, it was found that the whole system reacted completely after about 5 min, and the fluorescence intensity of the system remained almost unchanged in the following 90 min, which indicating that the QDs-$KMnO_4$ system exhibited good stability. Thus, the fluorescence intensity of QDs-$KMnO_4$ system was recorded after the addition of $KMnO_4$ for 5 min.

The effect of the reaction time on the fluorescence recovery of QDs-$KMnO_4$ system after the addition of GA was also discussed. And the results indicated that the fluorescence recovery of QDs by GA was completed in 10 min and lasted for more than 120 min.

Table 1
Comparison of the reported methods for GA.

Method	Detection limit (μg/mL)	Detection range (μg/mL)	Reference
HPLC	Not mention	0.052–0.26	[38]
Flow injection analysis	0.58	0.70–100	[39]
Chromatography	2.50	2.50–120	[40]
Polarography	0.28	0.85–204	[41]
Electrochemical methods	0.07	0.10–10	[42]
Fluorescence method	5.6×10^{-4}	0.60–12.6	This work

3.4.3. Effect of $KMnO_4$ concentration

For the ternary system, the addition of intermediate substances directly affects the sensitivity of the whole detection system. Theoretically, in order to provide an optimal "off" state of the biosensor, a higher concentration of quencher should be applied to completely turn off the fluorescence of CdTe QDs. However, a significant amount of quencher in the system will hamper the response sensitivity of subsequent GA detection. If the amount of $KMnO_4$ in the solution were excessive, some GA would react with free $KMnO_4$, therefore, the reminder GA reacting with $KMnO_4$, which induced the fluorescence quenching of CdTe QDs would decrease. According to the preliminary experimental results (as shown in Fig. S3), when the concentration of $KMnO_4$ was 1.6×10^{-4} mol·L^{-1}, the recovered fluorescence intensity induced by GA reached the maximum value. In addition, the inset of Fig. 2 shown that when the $KMnO_4$ was at a lower concentration, the fluorescence intensity decrease greatly, but with the increase of the concentration of $KMnO_4$, the extent of the decrease in fluorescence intensity diminished. When $KMnO_4$ concentration was 1.6×10^{-4} mol·L^{-1}, the fluorescence intensity variation did not enhanced. Thus, 1.6×10^{-4} mol·L^{-1} $KMnO_4$ was employed to be the quencher of the CdTe QDs in the biosensor preparation.

3.5. Possible reaction mechanism of the fluorescent light switch method

The fluorescence of QDs is caused by the combination of electron and hole pairs, that fluorescence quenching can be observed when the electron-hole pair recombination is blocked. This phenomenon may be due to the fact that atoms on the surface of quantum dots are cross-linked with molecules or atoms in the medium. The changes of the surface or environment of QDs would affect the efficiency of electron-hole recombination and consequently alter the optical properties of these particles. About the fluorescence quenching mechanism usually includes the dynamic quenching and static quenching [43]. Generally, in the dynamic quenching, fluorescence is subsequently quenched when the electron acceptor collides with the excited fluorophore, and charge transfer often occurred. Therefore, no changes in the absorption spectra are expected. But on the contrary, in static quenching, because of the ground state complex formation during the state

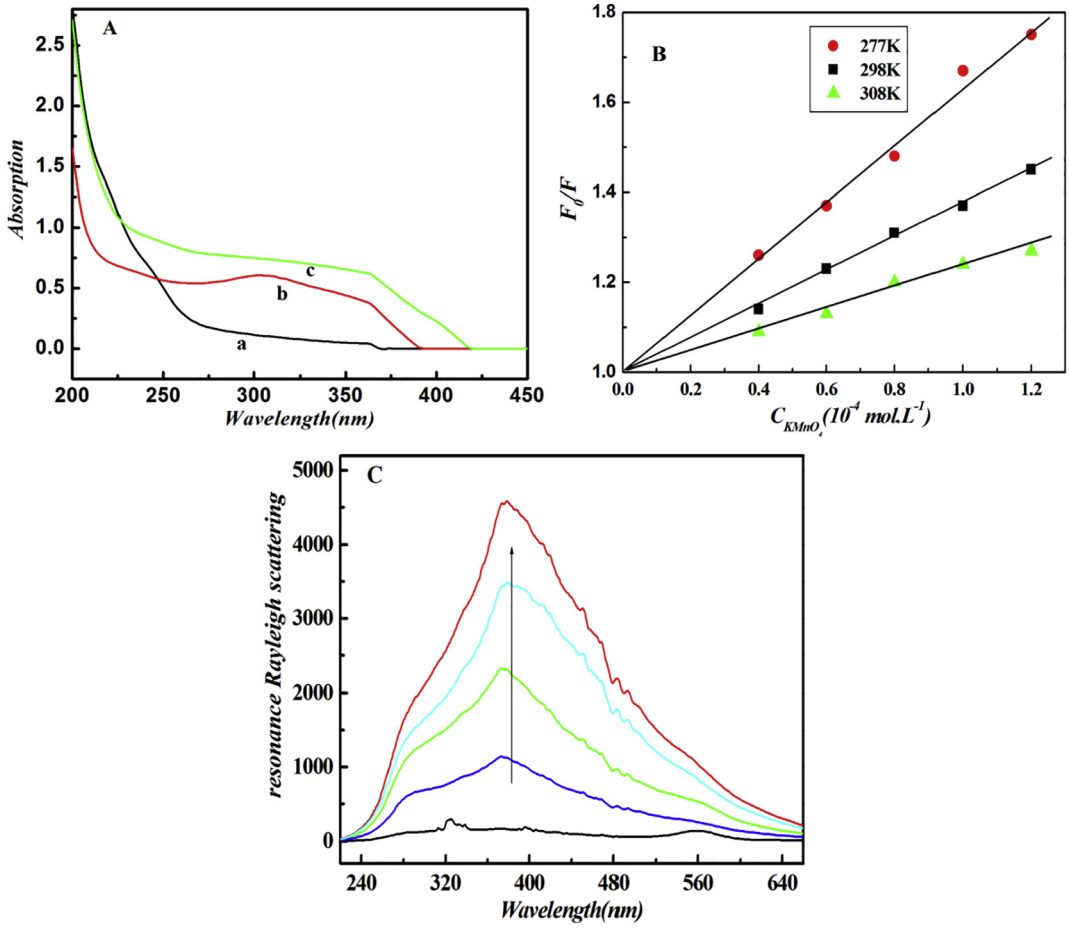

Fig. 4. (A) UV−vis absorption spectra of (a) NALC-CdTe QDs, (b) $KMnO_4$ (with distilled water as the reference), (c) mixture solution system (NALC-CdTe QDs and $KMnO_4$); (B)Stern-Volmer curves for the QDs-$KMnO_4$ solution system at three different temperatures (277 K, 298 K, 308 K); (C) RRS spectra of NALC-capped CdTe QDs in the presence of $KMnO_4$ in pH 7.4 Tris-HCl buffer solution.

quenching, the absorption spectra of the fluorophore will be perturbed. In order to precisely reveal the fluorescence quenching mechanism, the UV−vis absorption spectra of $KMnO_4$, QDs and $KMnO_4$-QDs system were recorded. As shown in Fig. 4A, the UV−vis absorption spectrum of QDs in the presence of $KMnO_4$ was higher, a bit flatter and with an observed red shift when compared with the single UV−vis spectrum of QDs, which indicated that the changes in size of surface properties of the QDs. Namely, the quenching type is static quenching. On the other hand, the well-known Stern-Volmer equation ($F_0 / F = 1 + Ksv[Q]$) is also a way to distinguish static quenching and dynamic quenching. In the equation, F_0 and F represent the fluorescence intensity of QDs in the absence and presence of quencher ($KMnO_4$), respectively. [Q] is the concentration of quencher. Ksv is the Stern-Volmer quenching constant. According to Fig. 4B, the linear plots for the QDs-$KMnO_4$ solution system at three different temperatures (277 K, 298 K, 308 K) were investigated. It can be seen that the quenching constants decreased with rise of temperature, which indicates that the probable quenching mechanism is static quenching.

The RRS scattering intensity of both $KMnO_4$ solution and QDs solution is very weak, but when the two are mixed together, the RRS scattering of the whole reaction system is significantly enhanced, and its maximum scattering peak is at 370 nm. The influence of solution ionic strength on the RRS strength of the whole reaction system was observed by adding NaCl solution (ion enhancer) to the reaction system. The results indicated that with the ionic strength enhanced, the RRS intensity of the system increased. Therefore, the electrostatic attraction played an important role in the reaction of $KMnO_4$ and NALC-CdTe QDs, which further prove the quenching type is static quenching.

On account of electron transfer of $KMnO_4$ to the surface of QDs, the QDs are then switched to the fluorescence "off" status. But the fluorescence of QDs could switch "on" again attribute to the redox and coordination reaction of $KMnO_4$ with GA. As shown in Fig. S4A,

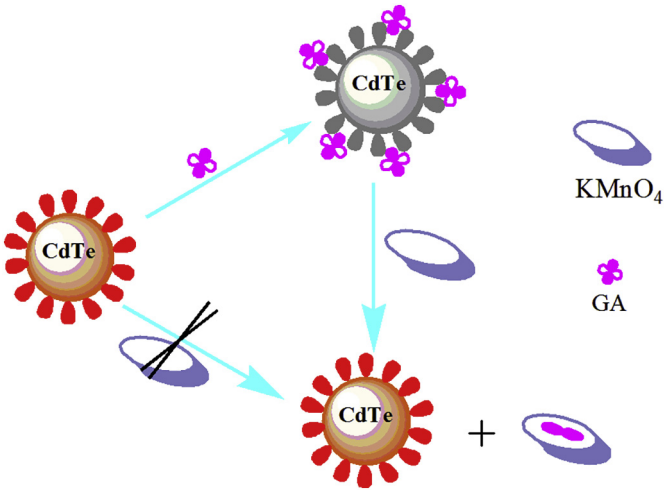

Fig. 5. The mechanism of the proposed "off-on" fluorescence biosensor.

Table 2
Determination of GA in green tea and black tea.

Sample	Found[a] (mg/g)		Added (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (%)
Green tea	8.71	8.65	2.00	2.87, 2.89, 2.84, 2.79, 2.82	97.34	1.40
	8.56		4.00	4.76, 4.90, 4.97, 4.94, 4.72	99.19	2.28
	8.68		6.00	6.98, 6.73, 6.85, 6.80, 6.78	95.72	1.40
Back tea	5.25	5.05	2.00	2.48, 2.42, 2.53, 2.45, 2.49	93.87	1.68
	4.92		4.00	4.59, 4.53, 4.43, 4.47, 4.35	93.86	2.01
	4.98		6.00	6.42, 6.61, 6.38, 6.57, 6.60	102.18	1.65

[a] The content of GA in every gram tea.

the fluorescence emission spectrum of QDs was not influenced by the addition of GA, which indicated that GA had no obvious influence on the fluorescence intensity of CdTe QDs, and further illustrated that the recovered fluorescence of the "turn-off on" system was caused by the interaction of $KMnO_4$ and GA. In addition, the absorption spectra of QDs (a), $KMnO_4$-QDs (b) and QDs-$KMnO_4$-GA system (c) (shown in Fig. S4B) were recorded. With the result that the addition of GA caused the decrease of $KMnO_4$-QDs absorption intensity, which also verify that the fluorescence recovery of the "turn-off on" system was caused by the interaction of $KMnO_4$ with GA through redox reaction. The worked principle of the biosensor was shown in Fig. 5.

3.6. Selectivity of the light switch fluorescence biosensor

Selectivity is crucial in measuring a test method. The effects of some common substances, such as amino acid, anion and cation, on fluorescence were investigated, and the results are shown in Fig. S5. Assuming the fluorescence intensity of NALC-CdTe QDs was 100% and $KMnO_4$-CdTe QDs is zero, GA exhibited a significant fluorescence enhancing effect on the proposed light switch biosensor. Cysteine and tryptophan could also recover the fluorescence of $KMnO_4$-CdTe QDs system, but compared with GA, the recovered efficiency of cysteine and tryptophan are strikingly lower. For the other kinds of amino acids, anion and cation, the recovered efficiencies are very low. Therefore, this proposed method had high selectivity of GA and can be used for the determination of GA.

3.7. Application of the proposed light switch biosensor

The developed fluorescent light switch method was used to determination of the content of GA in black and green tea to evaluate the feasibility. Weighed 1.0 g green and black tea accurately, then soaked them with 20 mL of boiling water three times, and finally filtered and transferred the samples to a 100 mL flask. The extract was diluted to 100 mL as the stock sample solution. For the determination with the proposed fluorescent light switch method, 0.5 mL of the sample solution was taken. The standard addition method was used for the determination of GA in the black and green tea real samples. As the results shown in Table 2, which indicated that the amount of GA in the green tea was higher than that of black tea. In addition, according to adding different amount of GA standards in the real samples, the recovery test had been performed. Based on the results shown in Table 2, the new light switch fluorescence biosensor was sensitive and accurate for the GA detection.

4. Conclusion

Summarily, a new fluorescent light switch method was fabricated in Tris-HCl buffer solution for the determination of GA. NALC-CdTe QDs with obviously fluorescence were synthesized successfully. After addition of $KMnO_4$, the fluorescence of QDs was quenched, but surprisingly when added GA, the quenched fluorescence could be recovered in the system. Therefore, a novel and simple fluorescent light switch was developed to detect GA in tea. The new method presented a high performance, high sensitivity and selectivity in a wide range of concentration.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2019.117356.

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