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Electron transfer and Fluorescence "turn-off" based CdTe quantum dots for vancomycin detection at nanogram level in aqueous serum media

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Youqiu He[a*]

Abstract

A simple and sensitive fluorescence "turn-off" biosensor for detection of vancomycin at nanogram level was proposed based on the electron transfer mechanism and the fluorescence quenching of the CdTe quantum dots (QDs). The electron transfer process during the interaction between vancomycin and GSH-CdTe QDs was investigated not only by ultraviolet/visible (UV/vis) absorption and fluorescence (FL) spectroscopy but also by fluorescence lifetime. The degree of the electron transfer and as resulted fluorescence quenching was proportional to the increasing of vancomycin concentrations in of range 1.534 ng mL^{-1}–20 μg mL^{-1}, with a corresponding detection limit of 0.4605 ng mL^{-1}. This proposed a biosensor that could be applied to determine vancomycin in environmental water samples, pharmaceutical formulation and spiked human serum with all of the recoveries over 95.8%. The mechanism about the detection was dynamic quenching with an electron transfer (ET) process. The experimental conditions, key affecting factors and the influence of the coexisting substances have also been optimized and studied.

***Keywords*:** Vancomycin; Electron transfer; Fluorescence quenching; "ON-OFF" Quantum dots;

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Introduction

The interest in luminescent semiconductor quantum dots (QDs), due to their unique optical and electronic properties, has grown tremendously in both fundamental research and technical applications.[1] Compared with other luminescent materials, QDs have distinct properties such as greater brightness, better stability, higher quantum yield, broad excitation spectrum, narrow and size-tunable emission spectrum.[2] All of these properties provide them more advantages over traditional organic dyes and lanthanide probes in fluorescent applications. Subtle changes of the QDs result in dramatic changes in their optical properties, which would offer many opportunities for detecting various specific analytes. Therefore, QDs have been increasingly exploited as fluorescent probes and have been widely used in the field of analytical chemistry such as cell labeling, genomic and proteomic detection, DNA analysis, optical sensors etc.[3]

Vancomycin (as shown in Fig. 1) is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria.[4] Due to early observations of its nephrotoxicity and ototoxicity as well as its intravenous dosing requirements, vancomycin has traditionally been viewed as a drug of "last resort", applied only after treatment with other antibiotics had failed.[5] However, the emergence of vancomycin-resistant strains which requires higher concentrations in order to be effective has aroused concerns. Monitoring vancomycin in pharmaceutical products is of great importance to prevent side effects in patients under treatment and to achieve optimal therapeutic concentrations.[6] Moreover, antibiotics including vancomycin have been found in the aquatic environment such as waste and polluted water resources. This compound plays a critical role in the maintenance or extension of antibiotic resistance bacteria, finally resulting in hazards to human health.[7] Therefore, screening vancomycin both in biological and environmental studies is very important.

Many different methods have been used to detect vancomycin in different samples, such as spectrophotometry,[8] immunoassay,[9] voltammetry,[10] high performance liquid chromatography (HPLC) with ultraviolet detection,[11] capillary electrophoresis (CE),[12] mass spectroscopy detection[13] and electrochemical detection.[14] However, the above ways for the determination of vancomycin still have some imperfections. The electrochemical detection requires complicated

modifications of the electrodes and the HPLC detection needs long time and complex operation. Besides, high cost instrumentation, time consuming procedure and unsuitability for automatic analysis and the need for large amounts of expensive and toxic solvents are some of the reported drawbacks of these methods.[15] These also block the practical application of vancomycin. Thus, it is urgent to develop a sensitive, precise, simple and convenient detection method for vancomycin.

It is well known that fluorescence approach has attracted interests due to its high sensitivity and simplicity as useful analytical technique in recent years.[16] Nowadays, with the rapid development of nanotechnology, great attention has been focused on the using of nanoparticles and QDs as the fluorescence probes.[17] Herein, a novel, fast and simple vancomycin measuring method based on the fluorescence of QDs has been unprecedentedly constructed. The interaction between GSH-CdTe QDs and vancomycin has also been investigated. It was found that the fluorescence intensity of CdTe QDs was quenched at 542 nm in the presence of vancomycin, and the quenched intensity was proportional to the concentration of vancomycin in the large range with a low detection limit. The method has been applied to determination of vancomycin in environmental water samples and spiked human serum and satisfactory results were obtained. The mechanisms of the proposed reaction have also been discussed.

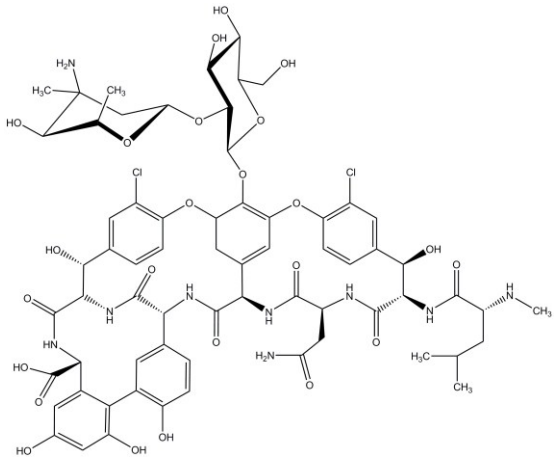


Fig. 1 Structure of the vancomycin.

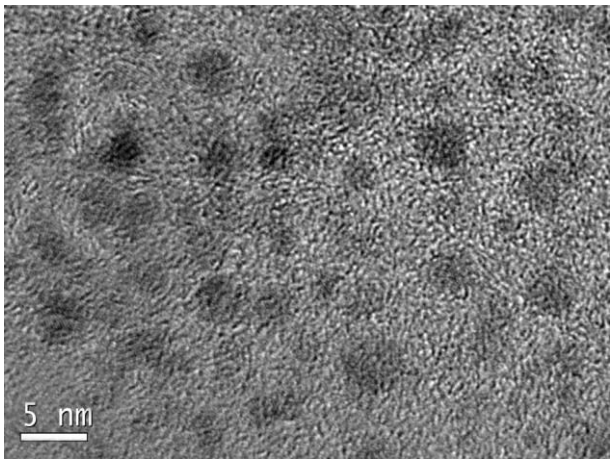
Results and discussion

GSH-CdTe QDs and the vancomycin samples were synthesized and prepared in the experimental partment. The simple vancomycin measuring method based on the fluorescence of QDs was

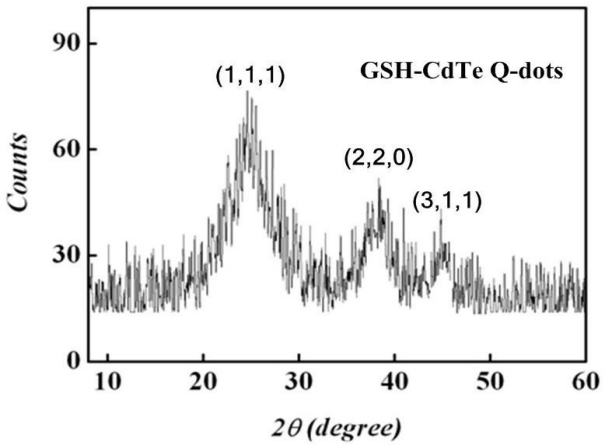
constructed based on the quenching effect of vancomycin to the GSH-CdTe QDs. The detail operation process of the system was performed according to the analytical applications section.

Morphology features characterization of GSH-CdTe QDs

The morphology features and diameter of the aqueous GSH-CdTe QDs were characterized by the TEM and XRD (Fig. 2). As shown in TEM image of Fig. 2 (A), the as-prepared GSH-CdTe QDs are uniform and mono-dispersed in shape. The diameters of the GSH-CdTe QDs have average narrow sizes of about 3–4 nm. X-ray diffraction (XRD) was used to characterize the crystal structure of the as-prepared CdTe Q-dots, and the XRD patterns are illustrated in Fig. 2 (B). The three diffraction peaks of CdTe Q-dots at 24.60◦, 38.56◦, and 47.01◦ can be indexed to the (1 1 1), (2 2 0), (3 1 1) planes of cubic CdTe lattice(JCPDS card: 01-075-2086). According to the method described in Ref.,[21] the quantum yield of GSH-capped CdTe Q-dots determined by using rhodamine 6G as a criterion (QY = 95 %) was about 47.5 %.



(A)



(B)

Fig. 2 (A) The TEM image of aqueous GSH-capped CdTe QDs. (B) XRD pattern of as-prepared GSH-CdTe QDs.

In addition, the UV/vis absorption spectra (a) and fluorescence spectra (b) of GSH-CdTe QDs were obtained as shown in Fig. 3. The UV/vis absorption spectrum revealed a strong excitonic absorption in the region of ultraviolet, and the characteristic absorption peak is located at 516 nm. Meanwhile, the fluorescence emission spectrum further confirmed that GSH-CdTe QDs were nearly monodisperse and homogeneous because of its favorable symmetry and narrow FWHM (about 42 nm), and the observed fluorescence band centered at 542 nm (excitation 350 nm). The as-prepared GSH-CdTe QDs exhibited narrow fluorescence bandwidth as the consequence of homogeneous size distribution and uniform crystallinity, which could be confirmed by TEM in Fig. 2(A).

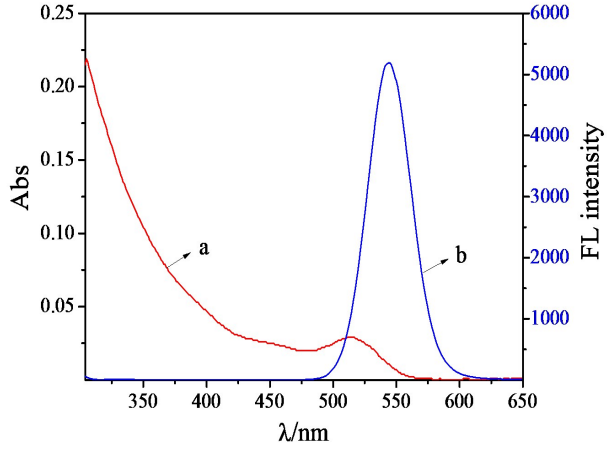


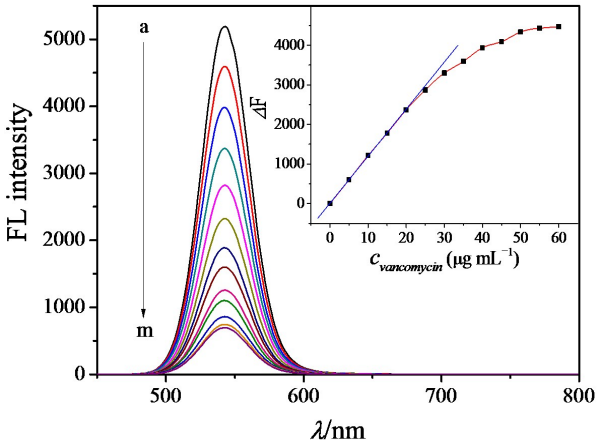
Fig. 3 The UV/vis absorption spectra (a) and fluorescence spectra (b) of GSH-CdTe QDs (excitation 350 nm).

As it could be seen from Fig. 3 that the emission maximum was close to its characteristic absorption peak, which indicated that the fluorescence emission of the QDs was due to the direct recombination of the electrons in conduction bands and the holes in valence bands. The particle size of the synthetic GSH-CdTe QDs was calculated about 3.12 nm based on the formula (1).[22] Obviously, the particle sizes obtained by calculation were well consistent with that got from TEM.

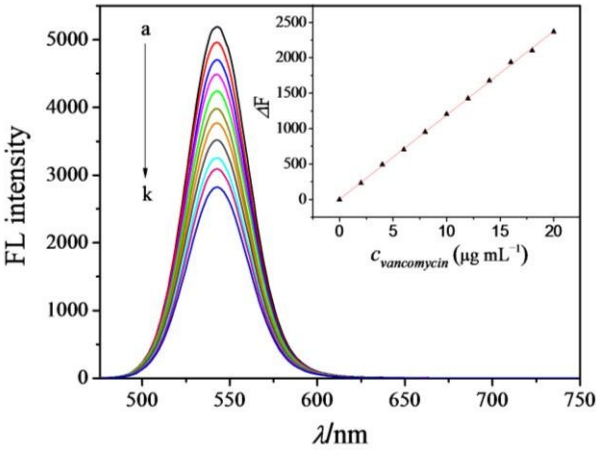
$$D = \left(9.8127\times10^{-7}\right)\lambda^3 - \left(1.7147\times10^{-3}\right)\lambda^2 + \left(1.0064\right)\lambda - 194.84 \qquad (1)$$

Fluorescence detection of vancomycin with GSH-CdTe QDs

As control experiment, the fluorescence spectra of the GSH-CdTe QDs were recorded in the absence and presence of vancomycin in Fig. 4 (A). It also can be seen from it that the as-prepared GSH-CdTe QDs exhibited strong fluorescence intensity and the observed fluorescence band centered at 542 nm (excitation 350 nm). After been mixed with vancomycin, the fluorescence intensity of the system decreased gradually. The quenching of the fluorescence signal intensity is directly related to the concentration of vancomycin added in a certain range. Based on this principle, the possibility of developing a sensitive method for vancomycin has been established.



(A)



(B)

Fig. 4 The fluorescence spectra of GSH-CdTe QDs-vancomycin system.

(A) The concentrations of vancomycin added for spectrum a – n were 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 μg mL^{-1}, respectively; Inset: the intensity variation (ΔF) of the

fluorescence against the concentration (0–60 μg mL^{-1}) of vancomycin.

(B) The concentrations of vancomycin added for spectrum a – n were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg mL^{-1}, respectively; Inset: the intensity variation (ΔF) of the fluorescence against the concentration (0–20 μg mL^{-1}) of vancomycin.

Optimization of the reactions

The influence of acidity on the fluorescence intensity of the aqueous GSH-CdTe QDs–vancomycin solution system was investigated and the results were shown in Fig. S1. In this experiment, Tris-HCl buffer solution (0.05 mol L^{-1}) was used to control the acidity of analytical system. The results showed that the maximum value of ΔF ($\Delta F=F_0-F$) was obtained at pH 7.4. In order to obtain a lower detection limit, so pH 7.4 was chosen to be the optimal reaction pH in this experiment.

The influence of the GSH-CdTe QDs concentrations on the fluorescence intensity of GSH-CdTe QDs-vancomycin system was investigated by means of keeping the vancomycin concentrations, Tris-HCl buffer concentrations and the pH constant while changing GSH-CdTe QDs concentrations. The results were shown in Fig. S2, which suggested that the GSH-CdTe QDs concentration strongly affected the fluorescence intensity of the solution system and the optimal concentration of GSH-CdTe QDs was 5.0×10^{-5} mol L^{-1}. The value of ΔF decreased when the addition amount of GSH-CdTe QDs went beyond this concentration. Accordingly, the GSH-CdTe QDs concentration of 5.0×10^{-5} mol L^{-1} was chosen as the optimal concentration.

The interaction between the GSH-CdTe QDs and vancomycin was monitored at different time scales at room temperature and the results were shown in Fig. S3. The results demonstrated that the fluorescence spectrum intensity of the mixture presented a rapid decrease after the vancomycin was added. The interaction process reached equilibrium after the reactions lasted for 10 min and the equilibrium remained stable for over 100 min, indicating that certain time was need to complete the interaction. Accordingly, it was not until 10 min later that we recorded the fluorescence spectra to ascertain that the reactions reached equilibrium. The time scale of 10 min was adopted in all of these experiments.

Calibration curves and sensitivity

As shown in the insert of Fig. 4 (A), within the concentration range of 0–60 μg mL^{-1}, the fluorescence quenching of the GSH-CdTe QDs by vancomycin was not always in line relationship with the concentration of the vancomycin, though the quenching intensity of the fluorescence signal was directly proportional to the concentration of vancomycin added in a low concentration range about 0–20 μg mL^{-1}. So, the relationship between the fluorescence quenching extent of the GSH-CdTe QDs and the concentration of the vancomycin was investigated in a small range of concentration as shown in Fig. 4 (B).

It clearly can be seen from the Fig. 4 (B) that, under the optimal conditions, the fluorescence spectra of aqueous GSH-CdTe QDs with different concentrations of vancomycin were recorded. The intensity quenching of the fluorescence signal was directly proportional to the concentration of vancomycin added. As shown in the insert of Fig. 4 (B), within the range of 0–20 μg mL^{-1}, the fluorescence quenching of the GSH-CdTe QDs by vancomycin fitted the following equation: $\Delta F = F_0\text{-}F = 4.8636 + 118.5772\ c$ (c: μg mL^{-1}), F_0 and F are the fluorescence intensity of GSH-CdTe QDs in the absence and in the presence of a given vancomycin concentration, respectively. Under the optimal conditions, the linear ranges of GSH-CdTe QDs fluorescence intensity versus the vancomycin concentration were 1.534 ng mL^{-1} ($10\delta/k$) – 20 μg mL^{-1} with a correlation coefficient of 0.9994, and the limit of detection ($3\delta/k$) was 0.4605 ng mL^{-1}, where δ was the standard deviation of eleven replicate measurements of the ratio of fluorescence intensity of the blank samples, and k was the slope of the calibration. It is noteworthy that beyond the concentration range, the fluorescence quenching of the system was no longer present proportional linear relationship.

In order to verify the accuracy of the method, we diluted the vancomycin to the exact concentration of 1.534 ng/ml and detected the fluorescence intensity of quantum dots system (as shown in Fig. S4). As it can be seen from Fig. S4 that the fluorescence intensity of the GSH-CdTe QDs changed from 5192.0 to 5187.32 after the addition of vancomycin with a concentration of 1.534 ng/ml, the intensity variation (ΔF) of the fluorescence was 4.68. While calculated value of the fluorescence intensity got by $\Delta F = F_0\text{-}F = 4.8636 + 118.5772\ c$ (c: μg mL^{-1}) was 5.0455. Compared with the calculated value got by the relational expression and the exact value got from the actual measurement, we found that the accuracy of the method was up to 92.76%, which

indicating that the good accuracy and practicability of the method. Besides, As it can be seen from the Fig.S5 that the fluorescence intensity of the GSH-CdTe QDs quenched gradually from 5192.0 to 5127.0 after the addition of vancomycin with concentration of 0, 5, 10, 50, 100, 200, 300, 400, 500 ng mL^{-1}, respectively. Uniform change of the quenching value reveals the sensitivity of the GSH-CdTe QDs to vanconmycin even at low concentration far less than 1 μg mL^{-1}. This contribute to the good accuracy and practicability of the method Therefore, the present method was reliable and practical in detection of vancomycin with detection limit at the level-ng ml^{-1}.

Compared with other different determined methods for vancomycin, as shown in Table 1, our proposed FL method displayed relatively low detection limit and wide application linear range, indicating that the FL method of using GSH-CdTe QDs as probe had its advantages compared with other methods, which might be attributed to the greatly quenched fluorescence signals of the GSH-CdTe QDs.

Table 1

Method	Matrix	Linear range (μg mL^{-1})	LOD[a] (μg mL^{-1})	Refs.
Spectrophotometry	Pharmaceutical formulation	2.0–18.0	0.020	[8]
Voltammetry	Pharmaceutical formulation	4.0–32.0	2.7	[10]
HPLC/UV	Human plasma	0.4–100.0	0.20	[11(a)]
HPLC	Rat plasma and human plasma	1.0–80.0	0.50	[11(b)]
HPLC/EC	Human plasma	0.5–50.0	0.25	[14]
LC/MS	Human serum	0.05–10.0	0.0010	[6]
LC/MS/MS	Water samples	0.016–2.0	0.010	[7]
	Rat plasma	0.01–20.0	0.0070	
HPLC/FL	Human plasma	0.005–1.0	0.0020	[11(c)]
CE/UV	Pharmaceutical formulation	0.25–5.0	0.10	[12(b)]
CL	Human serum and water samples	0.5–40.0	0.10	[20]
FL	Human serum, and water samples	0.001534–20.0	0.0004605	This work

[a] Limit of detection

Table 1 Analytical performance data of previously reported works for the determination of vancomycin.

Selectivity of the method

The selectivity and anti-interference ability of the FL method using GSH-CdTe QDs as probe for vancomycin detection were investigated by analyzing standard solution in existing interfering species. The influences of the foreign substances such as relevant metal ions, amino acid,

inorganic anions, and bio-molecules were investigated as shown in Table 2. If the coexisting substances caused a relative error of less than ±5% on the FL intensity change of the GSH-CdTe QDs, they were considered to have no interference with the detection of vancomycin. It was found that ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Ba^{2+} CO_3^{2-}, HCO_3^-, PO_4^{3-}, SO_4^{2-}, NO_3^-, Cl^-) and bio-molecules (Urea, common amino acids, Protamine and Albumin egg) posed no significant interference on the determination at relatively high concentration levels. Whereas, compared to other ions, Cu^{2+} has remarkable larger impact on the system, only below the concentration of 2.5×10^{-6} mol L^{-1}, can it has no obvious influence. Besides, among the analogues of vancomycin, both Tygacil and Benzylpenicillin have little interference on the determination at relatively high concentration levels. Whereas, Ofloxacin with high concentration will has some remarkable impacts on the detection of vancomycin in the system. It has no obvious influence only below the concentration of 0.5 μg mL^{-1}. Thus, when detecting vancomycin in the solutions containing a mixture of Ofloxacin, Ofloxacin should be removed or sheltered by certain Ofloxac chelant firstly. Additionally, Adenine hosphate and Human Serum Albumin could be allowed at lower concentration levels without significant interference. Apparently, the experimental results demonstrated that the proposed method possessed high selectivity and might be applied in the detection of vancomycin in the quality control of bulk drugs and their pharmaceutical preparations with satisfactory results.

Table 2

Coexistence material	Concentration (10^{-4} mol L^{-1})	Relative error (%)	Coexistence material	Concentration (10^{-3} mol L^{-1})	Relative error (%)
$Na^+(Cl^-)$	60	+1.07	Alanine	35	+1.35
$K^+(Cl^-)$	45	–2.67	Tryptophan	50	–4.50
$Mg^{2+}(Cl^-)$	10	+1.73	l-Glutamic acid	20	+1.10
$Ca^{2+}(Cl^-)$	9	–3.37	Glycine	15	–2.50
$Zn^{2+}(Cl^-)$	15	+2.28	Isoleucine	20	–1.15
$Cu^{2+}(SO_4^{2-})$	0.025	–3.85	Sarcosine	60	–2.44
$Ba^{2+}(Cl^-)$	5	–2.25	Creatine	50	–2.45
$CO_3^{2-}(Na^+)$	20	–2.30	Adenine hosphate	10	–3.12
$HCO_3^-(Na^+)$	10	+1.45	Human serum albumin	8 μg mL^{-1}	–3.55
$PO_4^{3-}(Na^+)$	16	+3.60	Albumin egg	35 μg mL^{-1}	–2.05
$SO_4^{2-}(Na^+)$	30	+2.90	Tygacil	5 μg mL^{-1}	–3.05
$NO_3^-(Na^+)$	100	–4.00	Ofloxacin	0.5 μg mL^{-1}	–4.30
$CO(NH_2)_2$	28	–3.35	Benzylpenicillin	5 μg mL^{-1}	–1.80

The concentration of vancomycin was 5 μg mL^{-1}.

Table 2 Effects of coexisting substances.

Mechanism investigation

It is known to all that fluorescence quenching mechanisms are usually be classified into two categories, either static quenching or dynamic quenching, which can be distinguished by their differing dependence on temperature.[23] The quenching rate constants increased with increasing temperature for dynamic quenching, whereas the reverse effect was observed in the case of static quenching.[24] To the best of our knowledge, the fluorescence quenching categories of vancomycin on GSH-CdTe QDs could be analyzed quantitatively at different temperatures with the Stern-Volmer equation.[25] :

$$\frac{F_0}{F} = 1 + K_q \tau_0 [\mathrm{Q}] = 1 + \kappa_{SV} [\mathrm{Q}] \quad (1)$$

In the expression of Eq. (1), F_0 and F was the fluorescence intensity of the QDs in the absence and presence of quencher (vancomycin), respectively, K_q was the quenching constant, τ_0 was the fluorescence lifetime in the absence of quencher, κ_{SV} was the Stern-Volmer quenching constant, and [Q] was the concentration of quencher.

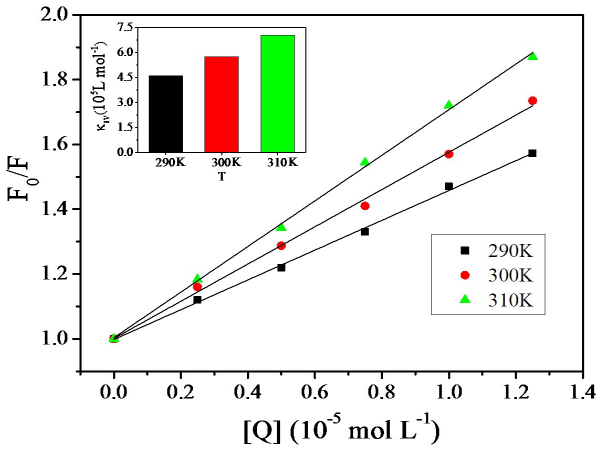


Fig. 5 Stern-Volmer plots for the GSH-CdTe QDs-vancomycin system at three different temperatures (c_{QDs}: 5.0×10^{-5} mol L^{-1}, pH =7.4); the inset: the relationship between the Stern-Volmer quenching constant (κ_{SV}) and the temperature (T).

As shown in Fig. 5, κ_{SV} of the GSH-CdTe QDs-vancomycin system was determined according to Eq. (1) at three different temperatures (290K, 300K and 310K) by linear regression of a plot of F_0/F against [Q], which was the concentration of vancomycin. The values of quenching constants (κ_{SV}) and correlation coefficient were listed in Table 3. From the Fig. 5, it clearly could be seen

that the Stern-Volmer quenching constant of the system increased with the rise of temperature, which indicated that the quenching type of GSH-CdTe QDs-vancomycin system was dynamic quenching.

Table 3

Temperature (K)	Sterm-Volmer linear equation	κ_{SV} (mol L^{-1})	R[a]	R.D[b]
290	$F_0/F = 0.998+4.5994\times10^4$ [Q]	4.5994×10^4	0.9977	0.0075
300	$F_0/F = 1.001+5.7463\times10^4$ [Q]	5.7463×10^4	0.9965	0.0114
310	$F_0/F = 0.998+7.0389\times10^4$ [Q]	7.0389×10^4	0.9984	0.0095

[a] R is the correlation coefficient.

[b] S.D. is the standard deviation for κ_{sv} values.

Table 3 Stern-Volmer quenching constants for the GSH-CdTe QDs-vancomycin system at different temperatures (c_{QDs}: 5.0×10^{-5} mol L^{-1}, pH=7.4).

It was known to all that measurement of the fluorescence life time was the most persuasive way to distinguish static and dynamic quenching. To further confirm the fluorescence quenching type of GSH-CdTe QDs-vancomycin system, measurement of the fluorescence lifetime of the GSH-CdTe QDs in absence and presence of vancomycin had also been performed (as shown in Fig. 6).

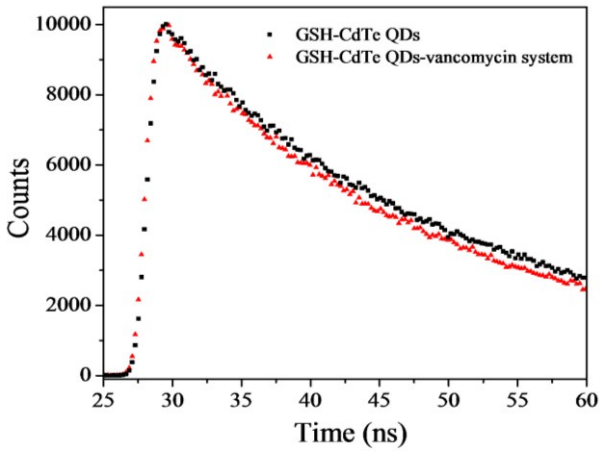
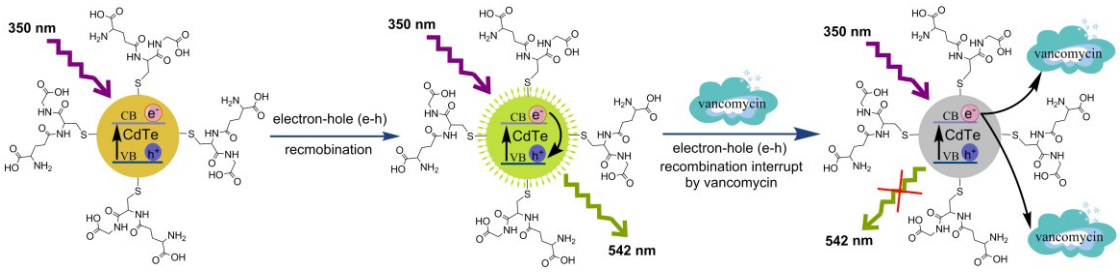


Fig. 6 Fluorescence decay of GSH-CdTe QDs (5.0×10^{-5} mol L^{-1}) in the absence (a) and presence (b) of vancomycin (10 μg mL^{-1}).

It was believed that if the static quenching takes place, the life time of the fluorescence molecule on excited state has no any change. Reversely, if dynamic quenching occurs, changes of life time must take place and it would become shorter.[26] It can be seen from the fluorescence emission decay curves in Fig. 6 that, after interacted with vancomycin, the life time of GSH-CdTe QDs (got by calculating method) on excited state did have changed obviously from 30.27 ns to

28.15 ns, which effectively demonstrated that the mechanism of the fluorescence quenching of GSH-CdTe QDs was indeed dynamic quenching rather than static quenching.

To explore the mechanism of quenching reaction, the electron transfer from GSH-CdTe QDs to vancomycin has also been explored. As known that upon excitation of GSH-CdTe QDs, it would lead to the promotion of electron from its valence band to conduction band, which results in the formation of a positively charged hole in its valence band and a free electron in its conduction band. The recombination of the electron and the hole of the GSH-CdTe QDs would take place and result in the fluorescence without the existence of vancomycin. Reversely, while introducing vancomycin, which serves as efficient electron acceptor for the conduction band electron from the GSH-CdTe QDs,[27] to the solution of QDs, it would prevent the electron-hole recombination at the interfaces of GSH-CdTe QDs (as shown in Scheme 1), which as a result caused the fluorescence quenching.[28]



Scheme 1 Mechanism of the fluorescence quenching process.

In terms of dynamic quenching, it was the collision between the electron acceptor collides and the excited fluorophore that result in the occasion of charge transfer which directly lead to the fluorescence quenching. Because the collision between the quencher and the fluorophore affects only the excited state of the fluorophore, so, no changes in the absorption spectrum could be expected. On the contrary, the formation of ground-state complex in static quenching will perturb the absorption spectra of the fluorophore.[29] Thus, distinguishing of static and dynamic quenching could be attempted by careful examination of the absorption spectrum.

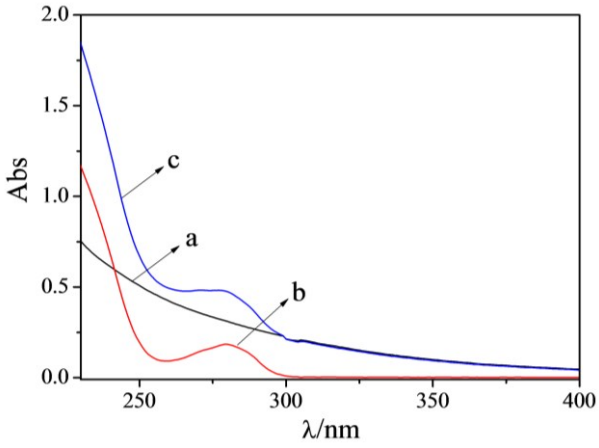


Fig. 7 UV/vis absorption spectra of (a) GSH-CdTe QDs (5.0×10^{-5} mol L^{-1}), (b) vancomycin (40 μg mL^{-1}) and (c) GSH-CdTe QDs-vancomycin system.

As shown in Fig. 7, the absorption spectra of GSH-CdTe QDs, vancomycin and QDs – vancomycin system were separately obtained. In the spectrum of pure QDs, there is strong absorption in the UV/vis at wavelengths < 400 nm, whereas the absorption is relatively weak in the visible. In the spectrum of pure vancomycin, there is a strong absorption peaks at 265 nm. As seen, no indication of a spectral shift is observed after vancomycin was added into GSH-CdTe QDs. In short, for the mixture of QDs and vancomycin, the absorption spectrum is simply a superposition of the QDs and vancomycin absorption spectra. As illustrated in Fig. 7, the absorption spectrum of the mixture is a linear combination of the spectra of each component, indicating that the mechanism to be dynamic quenching.

Analytical applications

To evaluate the applicability of the proposed FL method to real samples, the method was applied to determine the vancomycin in environmental water samples, and spiked human serum. The samples were prepared according to the general procedure described in the experimental section. The obtained results for environmental water samples and spiked human serum were shown in Table 4. The accuracy of the method was evaluated by performing recovery experiments for samples solution. The results presented in Table 4 showed that the recoveries of the environmental water samples were from 96.8% to 103.4% and the relative standard deviations were less than 4.33%, and the recoveries of spiked human serum were from 95.8% to 98.3% with

relative standard deviations of less than 4.35%, which demonstrating a good precision on this method. All of these obtained results indicated the reliability and practicality of the method. Meanwhile, the results determined by FL method are in agreement with those of pharmacopoeia method.

Table 4

Sample	Added (ng mL^{-1})	Found[a] (ng mL^{-1})	Recovery (%)
Water	0	Not detected	–
	5	4.92±0.12	98.4
Tap water	5	4.89±0.08	97.8
	10	10.25±0.13	102.5
Ground water	5	4.84±0.21	96.8
	10	9.75±0.36	97.5
River water	5	4.83±0.18	96.6
	10	10.34±0.09	103.4
Human serum sample	0	Not detected	–
	5	4.87±0.17	97.4
	10	9.83±0.36	98.3
	20	19.16±0.56	95.8

[a] Mean of five determination ± standard deviation.

Table 4 Results for determination of vancomycin in environmental water samples and spiked human serum samples.

Conclusions

Based on the fluorescence quenching effect of vancomycin on the GSH-CdTe QDs and the electron transfer mechanism, a Fluorescence method for determination of vancomycin with the detection limit of 0.4605 ng mL^{-1} was reported. The preparation of GSH-CdTe quantum dots was simple and inexpensive. Under the optimal conditions, the proposed method was rapid with a linear dynamic range of 1.534 ng mL^{-1}–20 μg mL^{-1} Moreover, it was successfully applied to the determination of vancomycin in environmental water samples, and spiked human serum with a relative standard deviations less than 4.33% and 4.35%, respectively, which characterized by simplicity, rapidity, and high sensitivity.

Experimental

Apparatus

The absorption spectra of QDs and vancomycin were measured on a UV-8500 spectrophotometer (Tianmei Corporation, Shanghai, China). All fluorescence spectra were recorded on a Hitachi F-2500 spectrofluorophotometer (Hitachi, Japan). JEOL JEM-2100 high

resolution transmission electron microscopy (TEM, Hitachi, Japan) was adopted to examine the appearance and size of nanoparticles. XD-3 X-ray diffraction (XRD, Purkinje General Instrument Co., Ltd., Beijing, China) were adopted to examine the appearance and size of nanoparticles. A FL-TCSPC Fluorolog-3 fluorescence spectrometer (Horiba Jobin Yvon Inc., Paris, France) was used to measure the fluorescence lifetime of the GSH-CdTe QDs in the absence/presence of vancomycin at room temperature. A PHS-3C pH meter (Leici, Shanghai, China) was used to measure the pH values of the aqueous solutions.

Chemicals

Cadmium chloride hemi (pentahydrate) ($CdCl_2 \cdot 2.5H_2O$) and Te powder were purchased from Aladdin Reagent Co. (Shanghai, China). Sodium borohydride ($NaBH_4$) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Glutathione (GSH) and all other chemicals and reagents used in this work were obtained from Aladdin Reagent Co. (Shanghai, China). Tris-HCl buffer solutions with different pH values were prepared according to suitable proportions. All reagents used were of at least analytical grade and used without further purification. Drug-free human serum used in this study was taken from healthy volunteers and stored in freezer until analysis. Ultrapure water (18.2 MΩ cm) was used throughout the experiment.

Synthesis of GSH capped CdTe QDs

GSH modified aqueous CdTe QDs were synthesized according to the previously reported method with a slight modification.[18] Firstly, Te powder (0.0383 g) and water (10 mL) were placed in a 50 mL three-necked flask. Excess amount of $NaBH_4$ was added under magnetic stirring. Subsequently, the colorless solution of NaHTe was obtained. Then $CdCl_2 \cdot 2.5H_2O$ (0.1028 g) and GSH (0.1847 g) were dissolved in 150 mL ultrapure water, and the pH was adjusted to 11.20 by addition of 1 mol L^{-1} NaOH solution dropwise. In addition, H_2SO_4 (0.5 mol L^{-1}) was introduced dropwise to NaHTe solution to produce H_2Te gas, which passed through the cadmium precursor with a slow Ar flow for 35 min. At this stage, CdTe precursors were formed. The resulting mixture was subjected to reflux for 90 min under open-air condition with a condenser. The GSH-CdTe QDs with a concentration of 2.0×10^{-3} mol L^{-1} were obtained. The concentration of GSH capped CdTe QDs was dependent on the HTe^- concentration.[19]

Pretreatment of real samples solution

A 100 μg mL^{-1} stock standard solution of vancomycin was prepared by dissolving 25 mg vancomycin in 500 mL ultrapure water and stored at 4 ℃ in refrigerator. Tap water was analyzed without any retreatment. Ground and river water samples were freshly collected and filtered with polyamide embrane filters of 0.45 μm to eliminate the suspended solid matter and stored in dark at 4 ℃ in the refrigerator.[20] They were used within 1 week. Prior to analysis, the water samples were spiked with the standard vancomycin solutions (100 μg mL^{-1}) and diluted to different concentrations, respectively. For human serum samples, an extraction process was not needed except a deproteinization pretreatment step utilizing trichloroacetic acid was performed. To prepare the spiked samples, certain amounts of vancomycin were spiked into 2.0 mL of protein-free serum and then diluted to different concentrations with ultrapure water.

Analytical procedure

In order to determine the interaction between GSH-CdTe QDs and vancomycin, a series of different concentrations of vancomycin samples and an appropriate amount of Tris-HCl solution were mixed into a 10 mL volumetric flask. Then certain amount of above GSH-CdTe QDs was added and the mixture was diluted to the calibration mark on the neck of the flask with ultrapure water and shaken thoroughly to be mixed completely. The typical reaction mechanism was shown in Scheme 1. After incubation for 10 min, the fluorescence intensity of the resulting solution was measured at the excitation wavelength of 350 nm. The resulting solutions were investigated by fluorescence, UV/vis absorption spectroscopy and fluorescence lifetime.

Acknowledgements

This work was supported by Chongqing Municipal Key Laboratory on Luminescence and Real-Time Analysis, Southwest University (CSTC, 2006CA8006) and the National Natural Science Foundation of China (No. 21175015).

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