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ORIGINAL RESEARCH PAPER

Highly sensitive fluorescence biosensors for sparfloxacin detection at nanogram level based on electron transfer mechanism of cadmium telluride quantum dots

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Abstract A sensitive fluorescence biosensor for determining sparfloxacin (SPF) based on the electron transfer mechanism and the fluorescence quenching effect of SPF to cadmium telluride quantum dots (CdTe QDs) was developed. The mechanism of the interaction between SPF and CdTe QDs was investigated by UV/Vis absorption and fluorescence spectroscopy. The biosensor could be used for the determination of SPF with a high sensitivity. Under optimum conditions, the linear range was from 0.28 to 40 μg SPF ml^{-1} with a correlation coefficient of 0.9983, and the detection limit (3δ/k) was 83.7 ng SPF ml^{-1}. Furthermore, this method has been applied to the determination of SPF in the synthetic environmental water samples and the spiked human serum samples with good results.

Keywords Biosensor · Fluorescence "on–off" · Quantum dots · Sparfloxacin · Spectrum detection

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Introduction

Luminescent semiconductor materials, such as quantum dots (QDs), due to their unique optical and electronic properties, have grown considerably in both fundamental research and technical applications in the past decade (Ali et al. 2007; Zhang et al. 2014). Compared with other materials, QDs have distinct properties such as greater brightness, better stability, broad excitation spectrum, high quantum yield and size-tunable emission spectrum (Chidawanyika et al. 2010). Therefore, QDs have been increasingly exploited as fluorescent probes in the fields of analytical chemistry and biomedical science.

Sparfloxacin (SPF; molecular structure given in Supplementary Fig. 1) is a synthetic fluoroquinolone antibacterial agent with a broad spectrum of activity against gram-positive and negative bacteria, mycoplasmas and chlamydiae (Goa et al. 1997). It is widely used in the treatment of urinary tract infections local and systemic diseases of human beings, or as an illegal feed additive for animals (Barton 2000). Moreover, antibiotics, including SPF, have been found in the aquatic environment such as polluted water resources. This compound plays a critical role in the maintenance or extension of antibiotic-resistance bacteria, resulting in hazards to human health. Therefore, screening SPF in biological and environmental studies is of importance.

Many different methods have been reported to detect SPF, such as flow-injection chemiluminescence

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method (Sun et al. 2006), high performance liquid chromatography (Ramos-Payán et al. 2013) and capillary electrophoresis with fluorescence (Lombardo-Agüí et al. 2010). However, these methods are technically complex and expensive for routine analysis, which block the practical application of SPF. Thus, it is urgent to develop a sensitive, precise and convenient detection method for SPF.

Herein, a sensitive biosensor for detecting SPF based on the electron transfer mechanism and the fluorescence quenching effect of SPF to glutathione–cadmium telluride quantum dots (GSH–CdTe QDs) is proposed. It has been applied to detect SPF in synthetic environmental water samples and the spiked human serum samples with satisfactory results.

Materials and methods

Synthesis of glutathione (GSH)-capped CdTe QDs and real samples

Aqueous CdTe QDs were synthesized according to the previously reported method with a modification (Liu and Yu 2009). Firstly, under Ar atmosphere, Te powder (0.0383 g) was reacted with excessive $NaBH_4$ in water to produce sodium hydrogen telluride (NaHTe). Then, $CdCl_2{\cdot}2.5H_2O$ (0.1028 g) and GSH (0.1847 g) were dissolved in 150 ml water, and the pH of the mixture was adjusted to 11.2 by addition of 1 M NaOH l^{-1}. In addition, 0.5 M H_2SO_4 was introduced dropwise to NaHTe solution to produce H_2Te gas, which passed through the oxygen-free Cd^{2+} precursor with a slow Ar flow for 30 min. After being refluxed at 369 K for 90 min under open-air conditions, the GSH–CdTe QDs were obtained.

A 100 μg SPF ml^{-1} stock standard solution was prepared by dissolving SPF in ultrapure water. Tap water was analyzed without any treatment. River water samples were freshly collected and filtered with polyamide membrane filters of 0.45 μm to eliminate suspended solid matter and stored in dark at 4 °C. Prior to analysis, the water samples were spiked with the standard solutions (100 μg SPF ml^{-1}) and diluted to different concentrations, respectively. For human serum samples, no extraction process was needed except a deproteinization pretreatment step utilizing trichloroacetic acid. To prepare the spiked samples, certain amounts of SPF were spiked into 2 ml of protein-free serum and then diluted to different concentrations with ultrapure water.

Analytical methods

One milliliter GSH–CdTe QDs, 1 ml 50 mM Tris/HCl buffer, and SPF were mixed and made to 10 ml with deionized water. After incubation for 10 min (as shown in the supporting information), the spectra of solution were examined. The resulting solutions were investigated by fluorescence, UV/Vis absorption spectroscopy.

Results and discussion

Characterization of the synthesized GSH–CdTe QDs

The morphology of the GSH–CdTe QDs was investigated by AFM. As shown in Fig. 1a, b, the 2D and 3D images of the GSH-CdTe QDs indicate diameter of about 3–4 nm. GSH-CdTe QDs possessed good dispersed crystalline structures. The TEM image is shown in Fig. 1c also showed that the particles were monodispersed with diameters of ~3–4 nm.

UV/Vis absorption and fluorescence emission spectra of the QDs are shown in Fig. 2. The fluorescence quantum yield of GSH–CdTe QDs at room temperature was 48.5 % as determined using Rhodamine 6G in aqueous solutions as reference.

Fluorescence quenching of sparfloxacin to GSH–CdTe QDs

The fluorescence spectra of GSH–CdTe QDs are shown in Fig. 3. They exhibited strong fluorescence at 542 nm (excitation 350 nm). When SPF was added to the system, the fluorescence of GSH–CdTe QDs was quenched sharply, based on which, the possibility of developing a sensitive method for SPF was evaluated.

From the Fig. 3, we can see that the fluorescence quenching degree was not always in line relationship with the concentration of the SPF except for in a certain range. The *inset* of Fig. 3 shows that, within the range of 0–40 μg SPF ml^{-1}, the fluorescence quenching of the GSH–CdTe QDs by SPF fitted the following equation: $\Delta F = F_0 - F = 54 + 102.76\ c$ (c: μg SPF ml^{-1}), where F_0 and F are the fluorescence intensity of

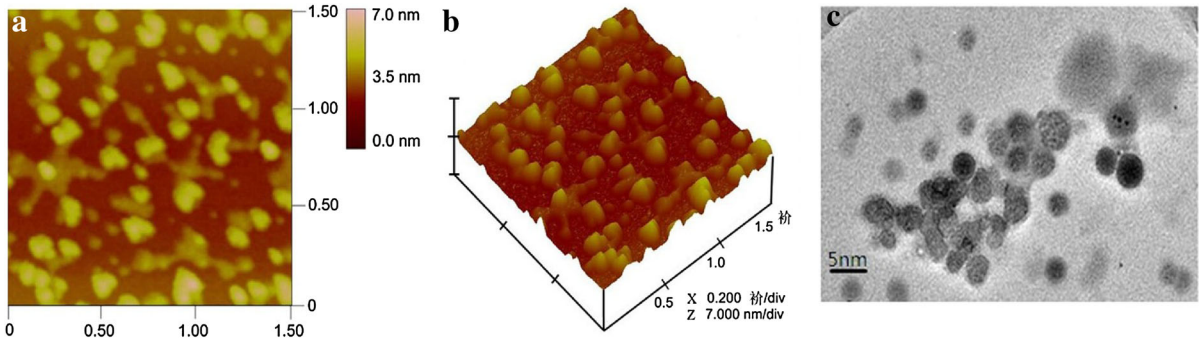

Fig. 1 AFM images (**a**, **b**), TEM image (**c**) of aqueous of as-prepared GSH–CdTe QDs

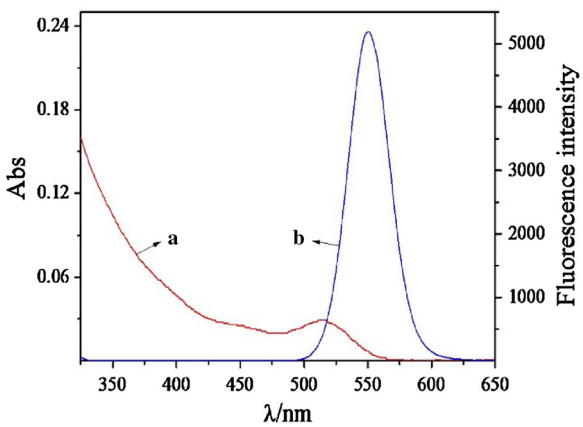

Fig. 2 UV/Vis absorption spectra (*a*) and fluorescence spectra (*b*) of GSH–CdTe QDs (excitation 350 nm)

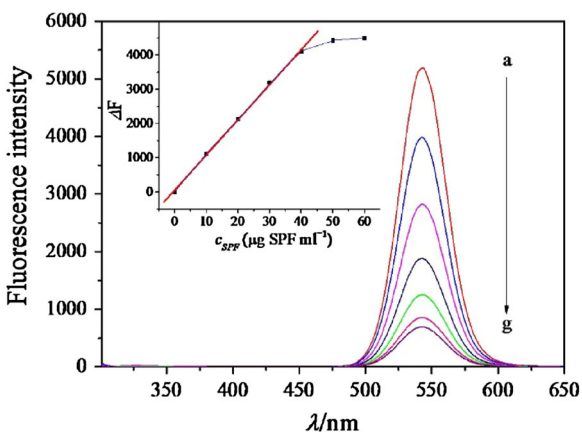

Fig. 3 The fluorescence spectra of GSH–CdTe QDs–sparfloxacin (SPF) system. The concentrations of SPF added for spectrum *a*–*g* were 0, 10, 20, 30, 40, 50 and 60 μg SPF ml^{-1}, respectively; *inset* the intensity variation (ΔF) of the fluorescence against the concentration (0–60 μg SPF ml^{-1}) of sparfloxacin

GSH–CdTe QDs in the absence and presence of SPF with certain concentrations, respectively.

Under optimal conditions, the linear ranges of GSH–CdTe QDs fluorescence intensity versus the SPF concentration were 0.8 μg SPF ml^{-1} (10δ/k) to 40 μg SPF ml^{-1} with correlation coefficient of 0.9983. The limit of detection (3δ/k) was 83.7 ng SPF ml^{-1}, where δ was the standard deviation of eleven replicate measurements of the ratio of fluorescence intensity of blank samples and k was the slope of the calibration.

Interaction mechanism

Fluorescence quenching mechanisms are usually divided into two categories: static and dynamic quenching, which can be distinguished by their differing dependence on temperature. For dynamic quenching, the quenching rate constants should increase with increasing temperature, while the reverse effect is observed in the case of static quenching. The fluorescence quenching mechanism can be analyzed quantitatively at different temperatures (280, 290 and 300 K) (as shown in Fig. 4) with the Stern–Volmer equation:

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

In this Eq. (1), F_0 and F was the fluorescence intensity of the QDs in the absence and presence of quencher, 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.

From Fig. 4, it can be seen that the Stern–Volmer quenching constant of the system increased with the

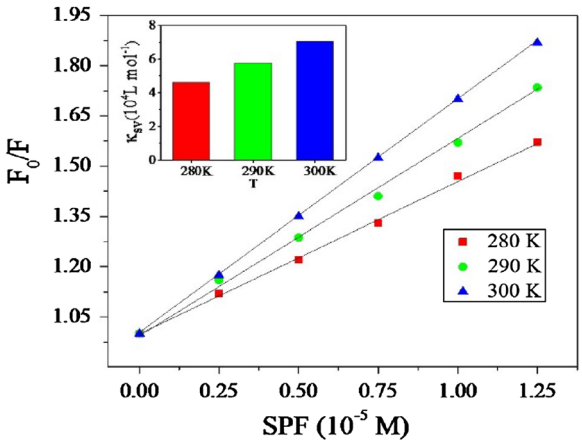

Fig. 4 Stern–Volmer plots for the GSH–CdTe QDs–sparfloxacin (SPF) system at three different temperatures (c_{QDs}: 5.0×10^{-5} M, pH 7.2); *inset* the relationship between the Stern–Volmer quenching constant (κ_{SV}) and the temperature (T)

rise of temperature indicating that quenching was dynamic.

Transfer of electron from GSH–CdTe QDs to SPF was also been explored to determine the mechanism of the reaction. Certain excitations to the GSH–CdTe QDs could cause the promotion of electron from its valence band to the conduction band and result in the formation of a positively-charged hole in its valence band and a free electron in the conduction band. In the absence of SPF, the recombination of the electron and hole would result in the fluorescence. Introducing SPF, which serves as efficient electron acceptor for the conduction band electron from the GSH–CdTe QDs (Kathiravan and Renganathan 2008), to the solution of QDs, would prevent the electron–hole recombination at the interfaces of GSH–CdTe QDs (Scheme 1) and cause fluorescence quenching (Jagadeeswari et al. 2011).

Scheme 1 Interaction mode of GSH–CdTe QDs with sparfloxacin (SPF) and the fluorescence quenching mechanism

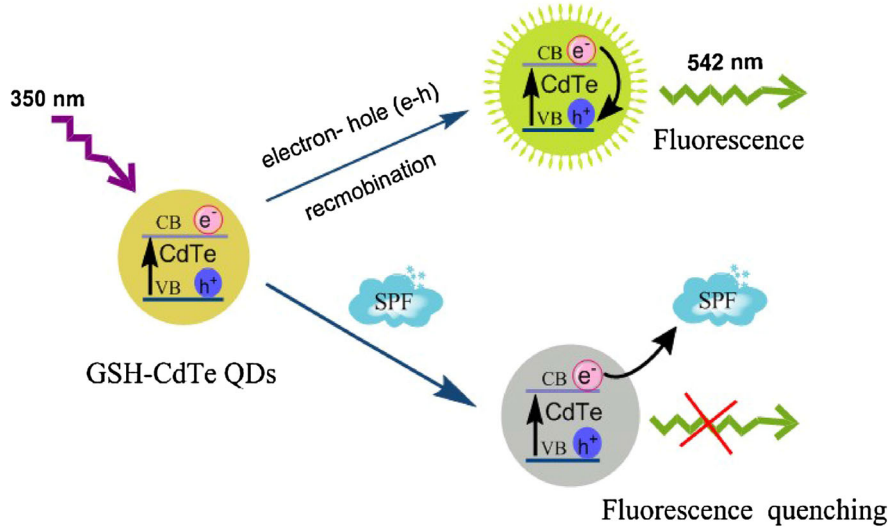

Table 1 Results for determination of SPF in environmental water samples and spiked human serum samples

Sample	Added (μg SPF ml^{-1})	Found[a] (μg SPF ml^{-1})	Recovery (%)
Water	0	Not detected	–
	5	4.83 ± 0.14	96.6
Tap water	5	4.89 ± 0.11	97.8
	10	10.15 ± 0.13	101.5
River water	5	4.76 ± 0.30	95.2
	10	10.27 ± 0.09	102.7
Human serum sample	0	Not detected	–
	5	4.87 ± 0.18	97.4
	10	9.81 ± 0.29	98.1

[a] Mean of six determination ± standard deviation

Analytical applications

In order to evaluate the applicability of the proposed method to real samples, the practical feasibility of the fluorescent probe was applied to determine SPF in environmental water samples, and spiked human serum. The results are shown in Table 1. Recoveries of the environmental water samples were from 95.2 to 102.7 % and the recoveries of spiked human serum was large than 97.4 %, which demonstrating a good precision on this method. All of these obtained results indicated the reliability and practicality of the method. The results determined by the fluorescence method were in agreement with those of pharmacopoeia method.

Conclusion

A sensitive SPF fluorescence biosensor based on the fluorescence quenching effect of SPF to glutathione–capped CdTe QDs was developed. The linear range of this detection method was from 0.28 (10δ/k) to 40 μg SPF ml^{-1} with a correlation coefficient of 0.9983. The detection limit (3δ/k) was 83.7 ng SPF ml^{-1}. The mechanism of the reaction was dynamic quenching. This method has been applied to the determination of SPF in the synthetic environmental water samples and the spiked human serum samples with satisfactory results.

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Supporting information Supplementary Fig. 1—Molecular structure of sparfloxacin.

Supplementary Fig. 2—**a** Effect of pH on the fluorescence intensity of the solution system in the presence (F) and the absence (F0) of sparfloxacin (SPF, c SPF: 5 μg SPF ml^{-1}, cQDs: 5×10^{-5} M) and **b** effect of incubation time (c SPF: 5 μg SPF ml^{-1}, cQDs: 5×10^{-5} M).

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