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Ultrasensitive determination of 1,4-dihydroxybenzene based on fluorescence resonance energy quenching of luminescent quantum dots modified on surface of silica nanoparticles

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ABSTRACT

An ultrasensitive solid-phase fluorescence resonance energy quenching (FREQ) method for determination of 1,4-dihydroxybenzene (DHB) using mercaptosuccinic acid (MSA)-capped CdTe quantum dots (QDs) immobilized on silica nanoparticles (NPs) as donors was developed. In the method, silica NPs were first modified with 3-aminopropyltriethoxysilane (APTS). Then, MSA-capped CdTe QDs were immobilized on the surface of the APTS-modified silica NPs. Finally, DHB in the solution was attached to the empty sites on the surface of silica NPs with QDs through electrostatic interaction. The fluorescence emission of the QDs was quenched by the proximal DHB molecules on the silica NPs. The quenching efficiency of the solid-phase FREQ method was 200-times higher than that of the solution-phase FREQ method. Using the ultrasensitive solid-phase FREQ method, DHB as low as 2.4×10^{-12} mol/L could be detected. The method was applied to quantify trace DHB in water samples.

1. Introduction

1,4-Dihydroxybenzene (DHB) widely used in many fields, such as tanning, dye, cosmetic, chemical, pesticide and pharmaceutical industries is a kind of conspicuous environmental pollutant. It is difficult to degrade DHB in the ecological environment [1]. A variety of methods such as chromatography [2,3], spectrophotometry [4] and electrochemical methods [5–8] have been used to determine DHB. The limit of detection (LOD) of these methods is in the range of 10^{-8}–10^{-5} mol/L. In order to detect ultratrace DHB, new sensitive methods should be developed. Fluorescence resonance energy quenching (FREQ) is a powerful technique used in analysis for the detection of nonemissive compounds [9,10]. In the FREQ methods, the donors are excited from the ground state to the excited state by an external excitation source. The donors go back from the excited state to the ground state to emit light. In the presence of nonemissive acceptors, the excited donors transfer energy to the proximal ground-state acceptors through long-range dipole–dipole interactions, quenching the light of the donors. This leads to a reduction in the emission of the donors. Thus, the nonemissive compounds are detected. In conventional FREQ methods for nonemissive small-molecule compounds, the most commonly used donors are fluorescent organic dye molecules [9,10]. The use of photostable probes with highly fluorescence intensity can enhance the signal of donors. Recently, luminescent semiconductor nanocrystals (quantum dots, QDs) as a new kind of luminophores have been developed. They offer advantages such as exceptional resistance to photobleaching and chemical degradation, high quantum yield, tunable size-dependent emission-wavelength, broad absorption spectra, large Stokes shift, as well as sharp and symmetrical emission spectra [11]. QDs as the donors can be used in FREQ for determination of different kinds of compounds [12,13]. The FREQ method with QDs as the donors has been used to detect phenolic compounds with a limit of detection (LOD) of ~10^{-7} mol/L [14].

If DHB can quench the emission of donors, this FREQ protocol should enable the determination of DHB. We found that DHB could quench the emission of CdTe QDs. In this work, a new FREQ method using QDs as donors to detect DHB was developed. In order to realize the FREQ, donors and acceptors have to spatially close to each other. For solution-phase FREQ, FREQ efficiency is low due to low collision probability between acceptor molecules and donor molecules. In order to enhance FREQ efficiency, a solid-phase FREQ method was adapted in this work. In this method, QDs as the donors and DHB as the acceptors were immobilized on the surface of nanoparticles (NPs). Since silica NPs are optically transparent and photophysically inert, they were used here to serve as the FREQ carriers. When the QDs on the silica NPs were excited, the fluorescence emission of the QDs was quenched by proximal DHB molecules. The quenched fluorescence signal was used to quantify

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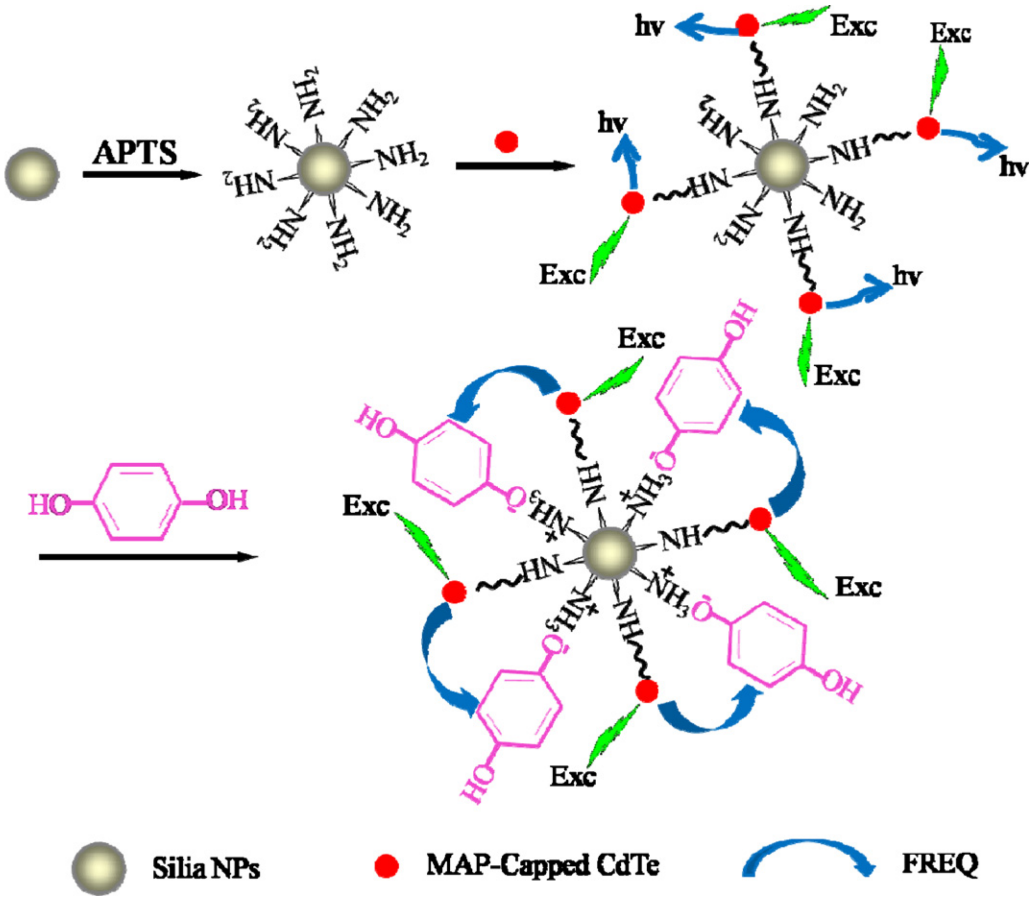

Fig. 1. Strategy of determination of DHB based on solid-phase FREQ method using QDs as donors on silica NPs.

DHB. The LOD of the solid-phase FREQ method for determination of DHB was down to 2.4×10^{-12} mol/L. The application of the proposed method for quantification of trace DHB was also performed to demonstrate the solid-phase FREQ protocol.

2. Materials and methods

2.1. Materials

DHB from Shanghai Chemicals Ltd. (Shanghai, China), tetraethylorthosilicate (TEOS) from Zibo Jiu Yu Chemical Co. Ltd. (Zibo, China), 3-aminopropyltriethoxysilane (APTS, 99%) from Qufu Huarong New Chemical Materials Co. Ltd. (Qufu, China), *N*-hydroxysuccinimide (NHS) from Huifeng Chemical Industry Ltd. (Weinan, China) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) from Shanghai Medpep Co. Ltd. (Shanghai, China) were used in this work. Mercaptosuccinic acid (MSA)-capped CdTe QDs were provided by Professor Jinhua Zhan (School of Chemistry and Chemical Engineering, Shandong University, China). Other chemicals (analytical grade) were obtained from standard reagent suppliers. 0.1 mol/L phosphate buffer (PB) (pH 7.0) consisted of 6.1×10^{-2} mol/L Na_2HPO_4 and 3.9×10^{-2} mol/L NaH_2PO_4. PB (pH 8.0) consisted of 9.47×10^{-2} mol/L Na_2HPO_4 and 0.53×10^{-2} mol/L NaH_2PO_4. All aqueous solutions were prepared with doubly distilled water.

2.2. Apparatus

Hitachi UV-4100 spectrometer was used to obtain UV–visible absorbance spectra. Perkin-Elmer luminescence spectrometer LS-55 was used to record fluorescence emission and excitation spectra. Bruker VERTEX-70 spectrometer was used to obtain Fourier transform infrared (FT-IR) spectra.

2.3. Preparation of silica-QD conjugates

First, silica NPs were synthesized by the hydrolysis of TEOS with aqueous ammonia according to the procedure reported in Ref. [15]. Then, the silica NPs were modified with APTS according to the Ref [16]. Briefly, 0.1 g of silica NPs, 2 mL of APTS and 48 mL of anhydrous toluene were mixed. The mixture was refluxed for 12 h under dry nitrogen. The resulting APTS-modified silica NPs were collected by centrifuging for 5 min at 4300 rpm. After washing with toluene thrice, the APTS-modified silica NPs were dried and stored. Before use, the silica NPs were dispersed in PB buffer (pH 7.0) by ultrasonication. In order to fabricate silica-QD conjugates, the MSA-capped QDs were activated by incubating 0.8 mL of 2.5×10^{-6} mol/L QDs with 8 μL of 10 mg/mL EDC and 8 μL of 10 mg/mL NHS for 0.5 h under shaking with a rate of 36 rpm. After 600 μL of 7.2 mg/mL APTS-modified silica NPs was added, the mixture was incubated for 4 h under shaking at 36 rpm. Then, the resulting silica-QD conjugates were separated by centrifuging for 5 min at 4300 rpm, followed by washing with PB (pH 7.0) thrice by centrifuging for 5 min at 4300 rpm. The silica-QD conjugates were dispersed in 300 μL of PB (pH 7.0).

2.4. Fluorescence measurements

After 300 μL of 14.4 mg/mL silica-QD solution prepared above was added to 6 mL of DHB standard solution or water samples that

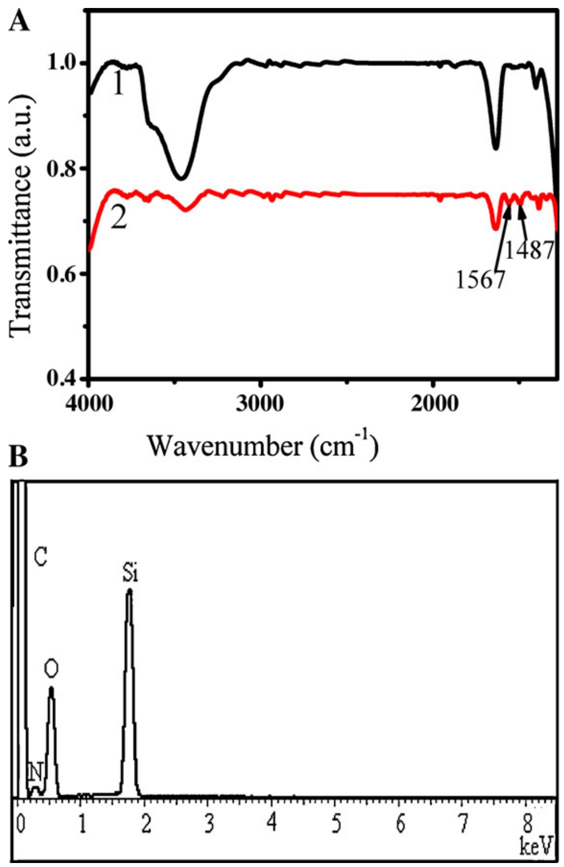

Fig. 2. (A) FT-IR spectra of silica NPs before (1) and after (2) modified with APTS and (B) EDX spectrum of the APTS-modified silica NPs.

had been passed through a 0.22 μm filter and adjusted the pH value to 8.0 with 0.15 mol/L NaOH, the solution was incubated for 10 h under shaking at 36 rpm. Then, the silica-QD conjugates with DHB were separated by centrifuging for 5 min at 4300 rpm, followed by washing with PB (pH 8.0) thrice by centrifuging for 5 min at 4300 rpm. After the silica-QD conjugates with DHB were dispersed in 1.6 mL of PB (pH 8.0) fluorescence spectrum of the solution was recorded immediately.

3. Results and discussion

3.1. Preparation and characterization of silica-QD conjugates

The strategy of the solid-phase FREQ method is illustrated in Fig. 1. First, silica NPs were modified with 3-aminopropyltriethoxysilane (APTS). Next, mercaptosuccinic acid (MSA)-capped CdTe QDs were immobilized on the surface of APTS-modified silica NPs. After that, DHB in the solution was attached to the empty sites on the surface of silica NPs with QDs through electrostatic interaction. When the QDs on the silica NPs were excited, the fluorescence emission of the QDs was quenched by proximal DHB molecules. The quenched fluorescence signal was used to quantify DHB. In order to perform the strategy, the silica NPs were synthesized using the method reported by Stöber et al. [15]. The diameter of the silica NPs (∼200 nm) was similar to that synthesized by Gao et al. [10] using the same method. In order to conjugate MSA-capped QDs with a diameter of ∼3 nm [17] onto the surface of silica NPs, the silica NPs were modified with APTS containing amino-groups. By comparing the FT-infrared spectra of silica NPs with and without APTS (Fig. 2A), the presence of bands around 1487 and 1567 cm^{-1} in the infrared spectra demonstrated the existence of the amino group on the surface of the NPs [18]. The conclusion was also proved by energy-dispersive X-ray (EDX) spectrum of the APTS-modified silica NPs (Fig. 2B). The amide reaction between carboxyl groups of MSA-capped QDs and amino groups modified on the silica NPs was used here to fabricate the silica-QD conjugates in the presence of EDC and NHS. Since MSA have two carboxyl groups, MSA-capped CdTe QDs could be easily bound to the APTS-modified silica NPs. The same absorption and emission spectra for QDs and the silica NPs with QDs (Fig. 3A) indicated that the MSA-capped QDs had been bound to the surface of the APTS-coated silica NPs.

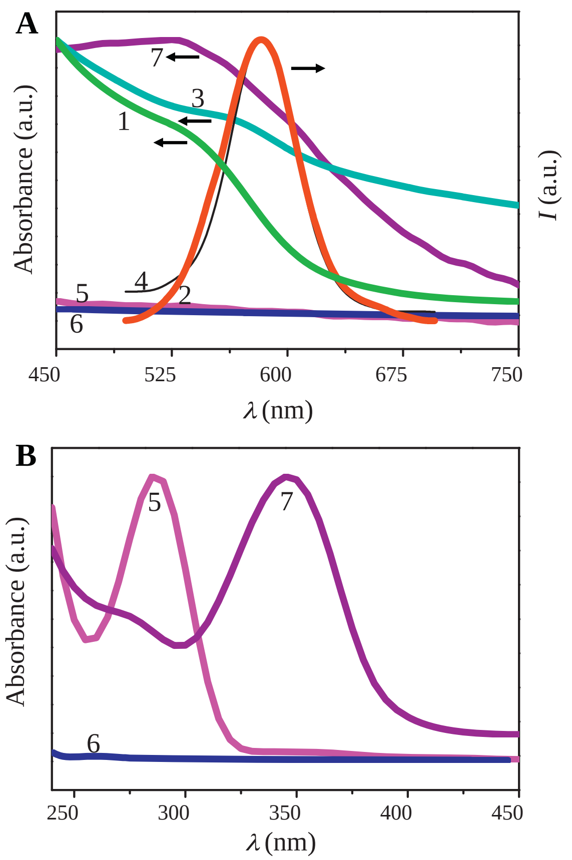

Fig. 3. Absorption (1, green line) and emission (2, red line) spectra of QDs, absorption (3, bluish-green line) and emission (4, black line) spectra of silica NPs with QDs as well as absorption spectra of DHB (5, pink line), APTS (6, blue line) and DHB + APTS (7, violet line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. FREQ of QDs on silica NPs in the presence of DHB

First, we examined the possibility of the interaction between DHB and APTS. When the colorless DHB solution was mixed with the colorless APTS solution, the solution changed to violet, indicating their interaction. Additionally, the great difference between absorption spectra of DHB solution before and after addition of APTS shown in Fig. 3B demonstrated the molecular interaction between DHB and APTS. This process was similar to the interaction between pentachlorophenol and APTS, and attributed to that the acidic hydroxyl group of the DHB molecules was deprotonated by the basic –$(CH_2)_3NH_2$ group of APTS [19]. The electrostatic interaction leaded to the formation of zwitterion complexes between DHB and APTS as indicated in Fig. 1. The fluorescence emission of the QDs on silica NPs had a spectral overlapping with the absorption spectrum of DHB-APTS complexes (Fig. 3A). Thus, the FREQ could occur between QDs as the energy donors and DHB as the energy acceptors on the surface of the silica NPs. The fluorescence quenching of

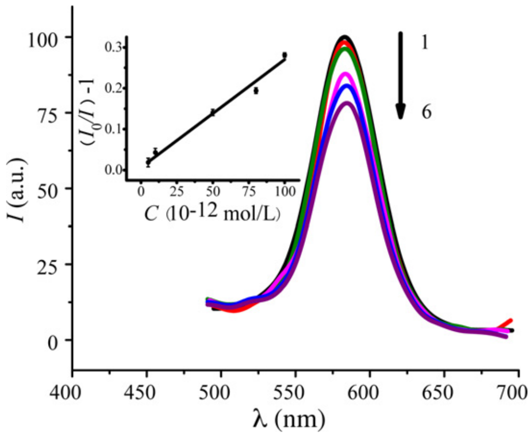

Fig. 4. Fluorescence spectra of QDs on silica NPs for different DHB concentrations (10^{-12} mol/L): (1) 0, (2) 5, (3) 10, (4) 50, (5) 80 and (6) 100. Insert, relationship between $(I_0/I-1)$ and DHB concentration.

QDs may occur via charge transfer. It was demonstrated that the quenching efficiency of DHB that was not attached on the surface of the QDs for emission of excited-state QDs via charge transfer was very low [14]. In the system, the FREQ was predominant. The FREQ followed the Stern–Volmer equation:

$$\frac{I_0}{I} = 1 + K_{SV}C_q \tag{1}$$

where I_0 and I are the fluorescence intensity of QDs before and after attaching DHB, respectively, and K_{SV} and C_q are the quenching constant and the concentration of the quencher, respectively. This equation was used to quantify DHB.

The FREQ efficiency depended on concentration of the silica-QD conjugate except for the concentration of DHB. When 0.68 mg/mL silica-QD conjugate that was fabricated using 0.68 mg/mL APTS-modified silica NP and 3.2×10^{-10} mol/mL MPA-QD was used, the maximum FREQ efficiency $(1-I/I_0)$ was obtained. The concentration was used here for quantification of DHB. The fluorescence spectra of the QDs on the silica NPs before and after attaching DHB at different concentrations are shown in Fig. 4. As expected, the fluorescence intensity of the QDs decreased with increasing DHB concentration. For the solid-phase FREQ method, the linear dynamic range was 5.00×10^{-12}–1.00×10^{-10} mol/L with a correlation coefficient of 0.989 (Fig. 4, insert). The relative standard deviation (RSD) was 5.4% for 5.00×10^{-11} mol/L ($n = 3$). The LOD was 2.4×10^{-12} mol/L which produced a fluorescence quenching three times the standard deviation of the blank signal. If DHB was not attached to silica NPs with QDs, DHB in the solution could also quench the fluorescence of the QDs on the silica NPs. In the solution-phase FREQ method, LOD was 200 times higher than that of the solid-phase FREQ method, suggesting that the solid-phase FREQ method had higher FREQ efficiency. This result was very reasonable. For the solution-phase FREQ method, only the DHB molecules in the solution, which collided with the QDs on the silica NPs, could quench QD fluorescence. For the solid-phase FREQ method, the DHB molecules were attached to the empty sites between QDs on the surface of the silica NPs. In this case, the demand for the donor–acceptor distance of <10 nm could be met for a majority of DHB molecules. Therefore, the solid-phase FREQ method for determination of DHB had higher sensitivity. Additionally, the LOD of the solid-phase FREQ method for determination of DHB is 7–3 order of magnitude lower than those of the methods reported in literature (Table 1).

Table 1
LODs of DHB in a variety of methods.

Methods	LOD (mol/L)	References
Chromatography	3.9×10^{-9}	[2]
Chromatography	5×10^{-7}	[3]
Spectrophotometry	1.4×10^{-8}	[4]
Electrochemical method	8.1×10^{-6}	[5]
Electrochemical method	5×10^{-7}	[6]
Electrochemical method	1.2×10^{-5}	[7]
Solution-phase FREQ method	2.4×10^{-10}	This work
Solid-phase FREQ method	2.4×10^{-12}	This work

3.3. Determination of DHB in water samples

In order to evaluate the selectivity of the FREQ method for different kinds of organic pollutants, we compared the $(I_0/I-1)$ values for pyrocatechol, resorcinol, β-naphthol, p-chloronitrobenzene, chlorophenol, 4-cresol and phenol with that for DHB. The concentrations of these compounds except for phenol, which had the same $(I_0/I-1)$ values as that for 5×10^{-11} mol/L DHB, were 2.5–7.5 order of magnitude higher than that of DHB (Table 2). Additionally, no FREQ was observed for 1×10^{-3} mol/L phenol. The results demonstrated that the solid-phase FREQ method had high selectivity for DHB and could be applied to determine DHB in water samples. Since no DHB in the collected lake water and river water samples was detected, DHB and organic pollutants (pyrocatechol, resorcinol and phenol) were spiked into the samples with different final concentrations. The average DHB concentrations determined using the proposed solid-phase FREQ method and the recoveries of

Table 2
Concentrations of different kinds of compounds for selective determination of 5×10^{-11} mol/L DHB.

Compounds	Pyrocatechol[a]	Resorcinol[a]	β-Naphthol[a]	p-Chloronitrobenzene[a]
Concentrations (mol/L)	1×10^{-8}	1×10^{-6}	1×10^{-6}	1×10^{-4}
Compounds	Chlorophenol[a]	4-cresol[a]	Phenol[b]	
Concentrations (mol/L)	1×10^{-5}	1×10^{-3}	1×10^{-3}	

[a] With the same $(I_0/I-1)$ values as that for 5×10^{-11} mol/L DHB.
[b] No FREQ.

Table 3
DHB values determined in different samples.

Samples	Sample concentration (10^{-11} mol/L)	Determined concentration (mean ± s, 10^{-11} mol/L)*	Added concentration (10^{-11} mol/L)	Recovery (%)
Lake water 1	1.1	1.06 ± 0.11	1.50	106
Lake water 2[a]	5.4	5.16 ± 0.26	3.50	103
River water 1	2.7	2.51 ± 0.08	1.50	102
River water 2[b]	4.6	4.57 ± 0.31	3.50	106

* Mean values for three measurements.
[a] The prepared samples containing 1×10^{-10} mol/L pyrocatechol, 1×10^{-9} mol/L resorcinol and 1×10^{-7} mol/L phenol.
[b] The prepared samples containing 5×10^{-10} mol/L pyrocatechol, 5×10^{-9} mol/L resorcinol and 5×10^{-7} mol/L phenol.

the method are listed in Table 3. The DHB concentrations detected in the spiked samples were in good agreement with spiked those of DHB. The recovery of the method was within 102–106%. These experimental results indicate that the method based on the solid-phase FREQ of QDs on silica NPs can be used to quantify DHB in real water samples.

4. Conclusions

The solid-phase FREQ method using QDs as the energy donors and silica NPs as the carriers of the donor–acceptor pair is very sensitive for quantification of DHB. The LOD is as low as 2.4×10^{-12} mol/L due to photostable QDs with high quantum yield as the energy donors and higher FREQ efficiency. Additionally, it is possible that the high-selective method may be used for the determination of DHB in the presence of pyrocatechol, resorcinol, β-naphthol, p-chloronitrobenzene, chlorophenol, 4-cresol and phenol.

Acknowledgments

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