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High-throughput and rapid fluorescent visualization sensor of urinary citrate by CdTe quantum dots

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ABSTRACT

In this paper, we have presented a novel CdTe quantum dots (QDs) based fluorescent sensor for visual and turn-on sensing of citrate in human urine samples. The europium ion (Eu^{3+}) can lead to the fluorescence quenching of thioglycollic acid (TGA) modified CdTe QDs due to photoinduced electron transfer accompanied by the change of emission color from yellow to orange. Next, addition of citrate breaks the preformed assembly because citrate can replace the CdTe QDs, based on the fact that the Eu^{3+} ion displays higher affinity with citrate than the CdTe QDs. Thus the photoinduced electron transfer is switched off, and the fluorescence emission of CdTe QDs is rapidly (within 5 min) recovered, simultaneously, the orange emission color restores to yellow. Such proposed strategy may conveniently discriminate the patient of renal stone from normal person by naked eyes. In addition to visualization detection, the fluorescence responses can be used for well quantifying citrate in the range of 0.67–133 μM. So, the present, simple, low-cost and visualized citrate fluorescence sensor has great potential in the applications for earlier screening in clinical detection.

1. Introduction

Urolithiasis is an ancient and common disease caused by the breaking of the equilibrium of crystallization promoters (e.g. oxalate, urate, and cystine) and inhibitors (e.g. citrate, Tamm–Horsfall protein) [1], and characterized by high prevalence and incidence, high morbidity and high rates of recurrence. Citrate is an important inhibitor, which appears to direct inhibit the process of crystals growth [2,3]. According to previous reports, mean urinary citrate excretion levels of 83 normal subjects are 2.8 mmol/24 h, however, that of 130 subjects with stones are 1.2 mmol/24 h [4]. That is, the citrate excretion levels of patients with calcium oxalate renal stone are lower than that of normal subjects [5]. Thus, it is important to frequent monitoring of the urinary citrate levels for the evaluation and treatment of urolithiasic patients.

To date, various citrate assay systems have been designed based on different physicochemical principles, such as enzymatic technique [6], ion chromatography and high-performance liquid chromatography [7,8]. Of various chemosensory protocols, the color change observed by the naked eye is considered to be a conceivable way to indicate the presence of an analyte. Usually, the current visual fluorescence probes were designed by hybridizing dual-emission quantum dots (QDs), and the two fluorophores should be preconjugated or preassembled together by sophisticated procedures to overcome the errors caused by variations in sensor concentration [9–11]. So, a scientific question is whether visual sensing can be generally and reliably achieved by a single-emission QDs, because from “dual-emission” to “single-emission” not only simplifies sensing visual design but also will promote the corresponding applications.

However, the design and development of such single-emission color-based sensing system remains a challenge [12]. For fluorescent visualization sensor, two demands should meet simultaneously. First, an appropriate emission shift is necessary to obtain a measurable emission color change. Next, the emission maximum of the probe must be located within a suitable wavelength range to satisfy the visible color change with a given emission shift. In this regard, CdTe QDs are good candidates to design such fluorescent visualization sensor because of two reasons: (1) basically, the intrinsic size-dependent luminescence properties of QDs allow us to choose a proper emission wavelength through tuning the particle size, (2) and crucially, the large emission shift often results from the reversible aggregation/disaggregation process of QDs, which can be performed by tuning non-covalent interaction between QDs.

Herein, we present a simple but effective fluorescent visualization platform for urinary citrate sensing with TGA–CdTe QDs as

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the signal output. The fluorescence of TGA–CdTe QDs can be quenched by Eu^{3+} accompanied by the change of emission color from yellow to orange. Interestingly, the quenched fluorescence can be recovered rapidly (within 5 min) in the presence of citrate following an emission color regain (orange back to yellow). The proposed sensing system has been successfully used for the visual detection of citrate in human urine samples with naked eyes. Due to simplicity and effectivity, it exhibits great promise as a practical platform for urinary citrate sensing.

2. Experimental section

2.1. Reagents

Tellurium powder (99.999%), TGA, mercaptoethylamine (MEA) and europium nitrate hexahydrate were obtained from Jkchemical. Guanine, carbamide, and the amino acids were obtained form Aladdin. Cadmium chloride, sodium borohydride, sodium citrate, tris(hydroxymethyl)aminomethane, ascorbic acid, and other routine chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. Other reagents were of analytical reagent grade and were used without further purification. Water used throughout was doubly deionized water.

2.2. Instrumentation

UV–vis absorption spectra were recorded with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). Fluorescence spectra were measured with a Hitachi-F-4600 spectrofluorimeter. The transmission electron microscopy (TEM) was taken with Tecnai G^2 20 S-TWIN transmission electron microscope operating at 200 kV. Fluorescence lifetimes were measured with the time-correlated single-photon counting technique on the Combined Steady State and Lifetime Spectrometer (Edinburgh Analytical Instruments F900). All pH values were measured with a Model pHs-3C meter (Shanghai, China).

2.3. Synthesis of water-soluble CdTe QDs

The thiol-capped CdTe QDs were prepared using the reaction between Cd^{2+} and NaHTe solution at pH 11.0 with TGA as the stabilizing reagent (pH 5.6 for MEA), according to the previous report with slight modification [13]. The molar ratio of Cd^{2+}/Te^{2-}/TGA (MEA) was 1:0.5:2.4. Briefly, fresh NaHTe (0.3 mmol) prepared by reaction of 40 mg Te powder and 50 mg $NaBH_4$ in 1 mL water at 0 °C for 8 h, was injected into 50 mL oxygen-free $CdCl_2 \cdot 2.5H_2O$ aqueous solution. Then the resulting mixture was heated under reflux. The crude solution was washed with isopropanol and centrifuged to remove excess precursors. Finally, the prepared thiol-capped CdTe QDs were dispersed in water and diluted 10 times for use.

2.4. Treatment of the urine sample

Twenty-four-hour urine samples employed in this study were provided by the members in our group. The samples were collected with 6 M HCl as preservative (approximately 0.02 mL of HCl per 5 mL of sample) and diluted 100 times [2]. Then the samples were stored at −20 °C for further analysis.

2.5. Standard procedures

Into a 5 mL volumetric flask was transferred 150 μL of Tris–HCl (0.1 M) buffer solution, 100 μL of QDs, and an appropriate quantity of Eu^{3+} (20 μM) was added. Then, different amounts of citrate or 100 μL urine samples were added to the mixture. Finally, the mixture was diluted to 3 mL with water and thoroughly mixed. Then the fluorescence intensities were measured with the following settings of the spectrofluorimeter: excitation wavelength, λ_{ex}=380 nm; emission wavelength, λ_{em}=590 nm; excitation and emission band-passes, 10 nm.

3. Results and discussion

3.1. Characteristics of the TGA–CdTe QDs

The TEM image of the TGA–CdTe QDs is shown in Fig. 1, demonstrating that the as-prepared TGA–CdTe QDs are well-dispersed. Their size distribution ranges from 2 to 4 nm (inset of Fig. 1), and average diameter is 3 nm (100 random nanoparticles were accounted).

As described in Fig. S1 (Supporting information), the as-prepared QDs possess an absorption maximum of the first electronic transition at 534 nm. According to the following equation,

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - 194.84$$

where D (nm) is the size of QDs, and λ (nm) is the wavelength of the first excitonic absorption peak of the corresponding sample [14], the size of the CdTe QDs is calculated to be 3 nm, which is in good agreement with the result of TEM.

As can be seen from Fig. S1 in Supporting information, the yellow fluorescence of the QDs gradually changes to orange with the wavelength increases from 590 to 600 nm. In this study, the band-edge emission at 590 nm is employed because it is located within a suitable wavelength range to satisfy the visible color change with a given emission shift.

3.2. The interaction of TGA–CdTe QDs and Eu^{3+}

The fluorescence intensity of TGA–CdTe QDs decreases gradually with the increasing of Eu^{3+} because of high affinity of Eu^{3+} with QDs, which is nearly completely quenched within two minutes with the addition of 0.3 μM Eu^{3+} (Fig. 2). And it remains stable for more than 40 min (line a in Fig. S2 of Supporting information).

Inset B in Fig. 2 reveals that fluorescence intensity exhibites a good linear between F/F_0 and the concentration of Eu^{3+}, indicating the quenching ability of Eu^{3+} to the QDs. Importantly, there is an optical shift of maxima wavelength from 590 to 600 nm, which can induce the measurable color change from yellow to orange (inset A of Fig. 2). It provides a possibility to develop a method for citrate visual detection.

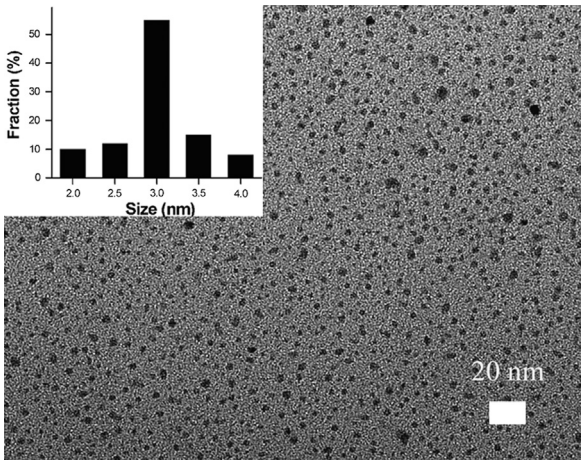

Fig. 1. The TEM image of the TGA–CdTe QDs. Inset: the particle size distribution.