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Development of a Europium (III) Ion Functionalized Silica Nanoprobe for Highly Sensitive Detection of Tetracycline

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Abstract: Tetracycline (TC) is a broad-spectrum antibiotic that has been widely used in numerous infection treatment due to its strong inhibitory effect on pathogenic microorganisms, low toxicity and low-cost. However, the abuse of TC may cause its residues in foods, such as meat and milk. Intake of these TC-contaminated foods could promote bacterial resistance to antibiotics. In this work, we report a simple and low-cost nanoprobe for TC detection with high selectivity and sensitivity. The nanoprobe is developed by chelating europium (III) ion onto the surface of silica nanoparticles (SiNPs-Eu^{3+}). The β-diketone configuration of TC can further coordinate with surface Eu^{3+} steadily, then absorb and transfer the excitation energy to Eu^{3+} via "antenna effect" upon UV light irradiation. The SiNPs-Eu^{3+} nanoprobe shows weakly luminescent in buffer solution. In the presence of TC, a strong emission at 615 nm is observed upon the excitation at 390 nm. This SiNPs-Eu^{3+} nanoprobe is featured with a wide linear range (100 nM - 5 μM), high sensitivity (limit of detection, 10 nM), quick response (75 min), allowing it to be potential used for TC detection in real-world samples.

Keywords: Tetracycline; europium (III) ion; luminescence; nanoprobe

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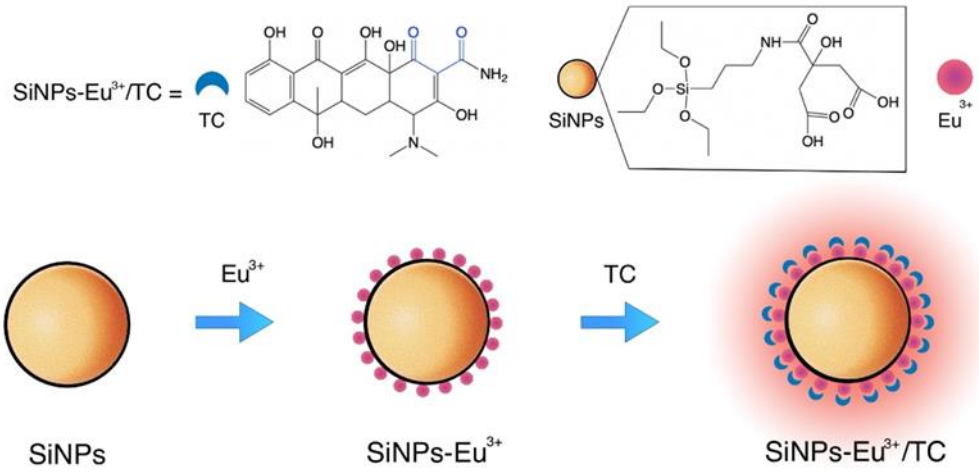
1. Introduction

Tetracycline (TC) is a huge family of antibiotics that mainly used for infection treatment, such as acne, brucellosis, cholera, malaria, plague and syphilis. This antibiotic has been widely utilized around the world due to its broad spectral activity pathogenic microorganisms, low toxicity and low-cost [1]. TC has been described as a generic medication by the World Health Organization (WHO). However, residues of TC in meats [2], milk [3] and honey [4] have been one of the key food safety issue because consuming large amounts of TC contaminated foods could cause a series of side effects, such as allergic reactions [5] and potentially promote the development and distribution of bacterial resistance to antibiotics [6]. The maximum residue limits (MRLs) of TC have thus been established in numerous countries to minimize the health impacts. Therefore, detection of TC in real-world samples is necessary for strict management of this antibiotic but remains challenging due to the lack of robust methods. In the past few years, a number of methods have been reported for TC detection, such as capillary electrophoresis [4], chemiluminescence [7], dipstick colorimetric method [8] and high-performance liquid chromatography [9,10], and photoluminescence [11-13]. Of these methods, photoluminescence analysis using responsive probe molecules and/or nanoparticles have attracted considering attention due to its high sensitivity, selectivity, and simplicity [14-16].

Specific interaction/binding/reaction between TC and the probe's recognition unit is key consideration for the development of responsive probes for TC detection. In considering of the specific coordination of TC's β-diketone configuration with europium (Eu^{3+}) ion, the responsive probes for TC detection can be readily developed by a Eu^{3+} functionalized nanostructure. As shown in Scheme 1, we report a Eu^{3+} functionalized silica nanoprobe (SiNPs-Eu^{3+}) for TC detection in this work. In this nanoprobe, the Eu^{3+} serves as both

emitter and the recognition unit for TC coordination. The coordination of TC allows the absorption of UV-vis light and then transfer the excitation energy to Eu^{3+} via "antenna effect" [6,17-19]. The silica nanoparticle (SiNPs) is served as solid support for Eu^{3+}, minimizing the luminescence quenching by water molecules interference. The SiNPs-Eu^{3+} nanoprobe is weakly luminescent in buffer solution. In the presence of TC, a strong emission at 615 nm is observed upon the excitation at 390 nm. This SiNPs-Eu^{3+} nanoprobe was featured with a wide linear range (100 nM - 5 μM), high sensitivity (limit of detection, 10 nM), quick response (75 min), allowing it to be potentially used for TC detection in real-world samples.

2. Materials and Methods



Scheme 1. Schematic illustration the design of luminescent SiNPs-Eu^{3+} nanoprobe for tetracycline (TC) detection.

2.1. Reagents and Instruments

Tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), ammonia solution (28 wt %), ethanol, europium chloride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), citrate acid, dimethylformamide (DMF), and tetracycline (TC) were purchased from Sigma-Aldrich. Unless stated, other chemicals and solvents were obtained for commercial sources and were used without further purification. Mili-Q water was used throughout. The morphology of SiNPs-Eu^{3+} nanoprobe was characterized using Hitachi HT7700 TEM operated at an acceleration voltage of 120 kV. The samples for TEM images were dispersed in water and then dropped on a copper grid. Luminescence spectra were reported using SHIMADZU RF-5301 PC spectrometer with excitation and emission slits of 5 nm.

2.2. Synthesis of SiNPs-Eu^{3+} Nanoprobe

SiNPs were synthesized by the modified Stöber method. Briefly, 420 μL ultrapure water and 1 mL ammonia solution (28 wt %) were added into 20 mL absolute ethanol on a stirrer for 0.5 h to prepare the base solution. Then, 1 mL TEOS and 53 μL APTES were slowly added into the base solution (TEOS/APTES with a molar ratio of 20:1). The mixture was stirred for 6 h at 50 °C and 1 h at room temperature. After repeating the process of centrifuge at 13,000 rpm for 5 min and wash with water for three times, the SiNPs (SiNPs-NH_2) was obtained.

To modify the surface of SiNPs-NH_2, 9 mg of citrate acid and 3 mg of EDC were added into 11 mL water/dimethylformamide (DMF) mixture (volume ratio = 1:1) containing 55 mg SiNPs (concentration = 5 mg/mL). After being stirred at room temperature overnight and washed with water, the nanoparticles in water (50 mg of SiNPs, 2.5 mg/mL) was added with 0.5 mM $Eu(NO_3)_3$ solution. The mixture was stirred overnight and then the excess (uncoordinated) Eu^{3+} ions were removed by centrifugation and washing with water for three times. The prepared SiNPs-Eu^{3+} nanoprobe was then dispersed in 50 mM Tris-HCl buffer for further use.

2.3. General procedure for luminescence spectrometric analyses

For TC detection in aqueous solution, the SiNPs-Eu^{3+} nanoprobe in 50 mM Tris-HCl buffer was added with TC at concentration of 0 to 5 μM. The mixture was stirred at the room temperature for 75 min before subjecting to spectrometric analysis. The calibration curve was generated through plotting the emission intensities at 615 nm against the concentrations of TC.

3. Results and Discussion

3.1. Preparation and Characterization of the SiNPs-Eu^{3+} Nanoprobe

Following the synthesis procedure of SiNPs by Stöber method, the surface modification by EDC chemistry, and the Eu^{3+} coordination with surface carboxylate of citrate acid, SiNPs-Eu^{3+} nanoprobe was prepared. The morphology of SiNPs-Eu^{3+} nanoprobe was characterized by TEM images. As shown in Figure 1A, the prepared nanoprobe was monodisperse, spherical and uniform in size. The size was determined to be 170 nm by TEM image analysis (Figure 1B).

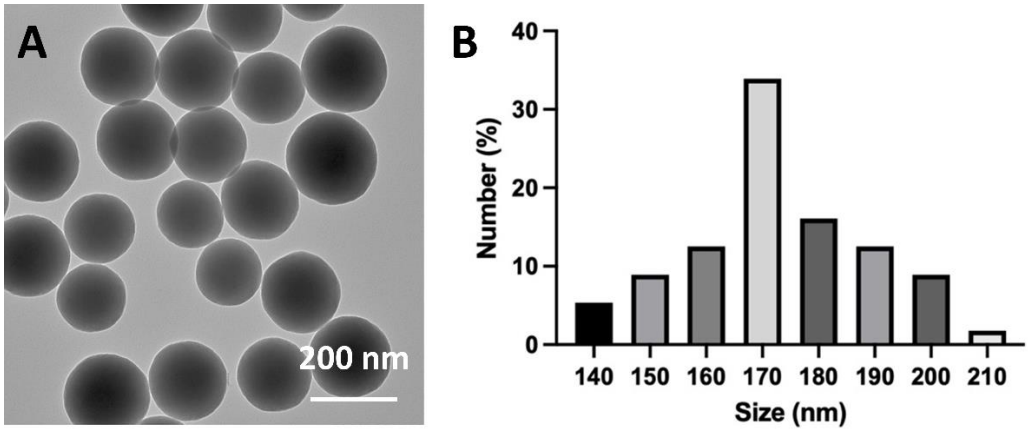


Figure 1. Characterization of SiNPs-Eu^{3+} nanoprobe. TEM image (A) and size distribution (B) of SiNPs-Eu^{3+} nanoprobe.

3.2. Optimization the Conditions for TC Detection in Aqueous Solution

Conditions for TC detection in aqueous solution, including pH and response time, were first optimized prior to the TC detection. In 50 mM Tris-HCl buffer of different pH (2 to 12), SiNPs-Eu^{3+} nanoprobe was added, followed by the addition of 5 μM TC. The emission intensity at 615 nm was recorded after 75 min incubation. As shown in Figure 2A, in the absence of TC, the SiNPs-Eu^{3+} nanoprobe exhibited weak luminescence in pH 2-12. In the presence of TC, the emission intensity was unchanged at pH < 3, which can be attributed to limited coordination of Eu^{3+} of nanoparticle with TC due to the protonation of citrate acid's carboxylate. Obvious enhancement in luminescence was noticed in pH 4-12, and highest luminescence intensity was obtained at pH 8.5, indicating the optimized pH for TC detection is 8.5. In Tris-HCl buffer of pH 8.5, the time-dependent luminescence response of SiNPs-Eu^{3+} nanoprobe to TC was then investigated. As shown in Figure 2B, in

the absence of TC, SiNPs-Eu^{3+} nanoprobe showed weak and stable luminescence in Tris-HCl buffer. Upon addition of TC, the emission intensity of SiNPs-Eu^{3+} nanoprobe was significantly increased, and the emission intensity reached plateau after 75 min incubation, indicating the optimized response time is about 75 min.

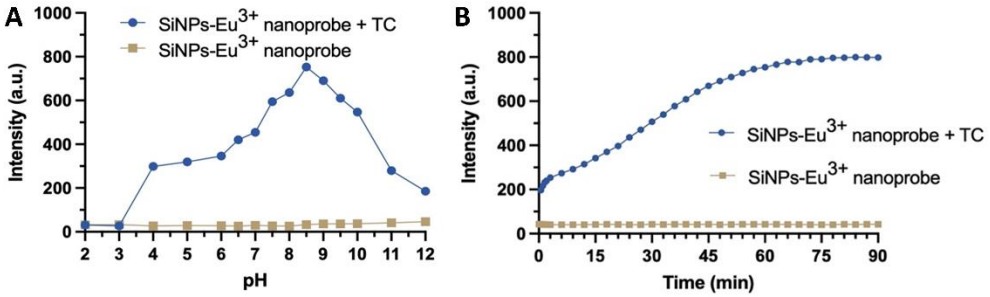


Figure 2. Emission intensities at 615 nm of SiNPs-Eu^{3+}/TC samples at different (A) pH condition and (B) time point. (A) Luminescence intensity of 1 mg/mL SiNPs-Eu^{3+} nanoprobe in the presence and absence of 5 μM TC in 50 mM Tris-HCl buffer of different pH. (B) Time-dependent luminescence response of 1 mg/mL SiNPs-Eu^{3+} nanoprobe to 0 and 5 μM TC in 50 mM Tris-HCl buffer of pH 8.5, respectively.

3.3. Spectrometric Analysis of TC in Aqueous Solution

Luminescence spectrometric response of SiNPs-Eu^{3+} nanoprobe to TC was then conducted in Tris-HCl buffer of pH 8. The SiNPs-Eu^{3+} nanoprobe in Tris-HCl buffer was added with different concentrations of TC and then the emission spectra were recorded after 75 min incubation. As shown in Figure 3A, upon addition of TC at increasing concentrations, the emission intensity was increased gradually. The emission intensity of SiNPs-Eu^{3+} nanoprobe at 615 nm exhibited a good linearity against the concentration of TC in the range of 0 to 5 μM (Figure 3B). The detection limits, calculated as the concentration corresponding to three standard deviation of the background signal, is 10 nM. The results indicate that the SiNPs-Eu^{3+} nanoprobe can be used for sensitive TC detection in aqueous solution.

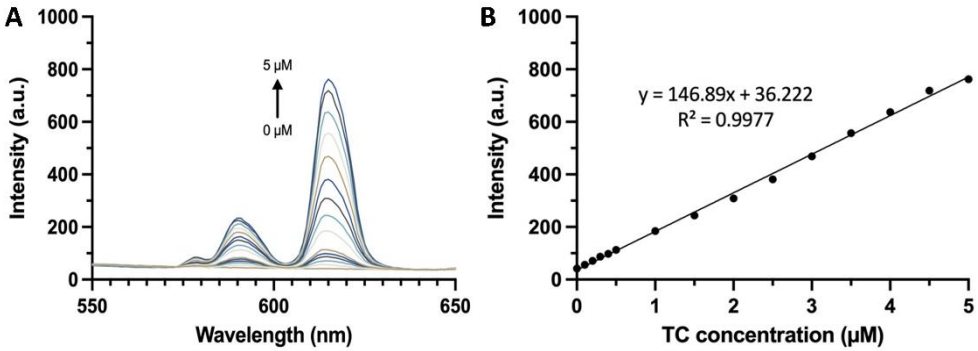


Figure 3. Luminescence response of SiNPs-Eu^{3+} nanoprobe to TC in 50 mM Tris-HCl buffer of pH 8.5. (A) Emission spectrum of 1 mg/mL SiNPs-Eu^{3+} nanoprobe in the presence of different concentration of (TC concentrations are 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 μM). (B) Emission intensities at 615 nm of SiNPs-Eu^{3+} nanoprobe against the concentrations of TC.

4. Conclusion

In conclusion, a Eu^{3+} functionalized silica nanoparticle (SiNPs-Eu^{3+}) was developed as the responsive luminescence probe for TC detection. The SiNPs-Eu^{3+} nanoprobe was developed based on the coordination of TC to Eu^{3+} ions on the surface of silica nanoparticle and the luminescence response mechanism of TC's antenna effect. The SiNPs-Eu^{3+} nanoprobe showed weak luminescence in the absence of TC, while significant enhancement

of luminescence at 615 nm was obtained. The optimized TC detection condition was Tris-HCl buffer of pH 8.5 and incubation time of 75 min. The SiNPs-Eu^{3+} nanoprobe was featured high sensitivity (detection limit of 10 nM), which allowed future applications of this nanoprobe for sensitive TC detection in real-world food samples.

Author Contributions: Z. Z., A. Q., J. Y., and M. W. performed the experiments and analyzed the data; Z. Z., and R. Z. wrote and revised the manuscript; Z. P. X., and R. Z. supervised the students. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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