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A quantum dot-spore nanocomposite pH sensor

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ABSTRACT

A new quantum dot (QD)-based pH sensor design is investigated. The sensor is synthesized based on the self-assembly of green QDs onto treated spores to form QD@spore nanocomposites. The nanocomposites are characterized using laser scanning confocal microscopy, transmission electron microscope, and fluorescence spectroscopy, among others. Fluorescence measurements showed that these nanocomposites are sensitive to pH in a broad pH range of 5.0–10.0. The developed pH sensors have been satisfactorily applied for pH estimation of real samples and are comparable with those of the commercial assay method, indicating the potential practical application of the pH sensors.

1. Introduction

The measurement and control of pH are essential in chemical, biomedical, and environmental sciences and industrial applications. The development of new techniques for pH detection is attracting increasing interest [1,2]. Potentiometric techniques employing a pH-sensitive membrane, such as glass membrane electrodes, are most commonly used for pH measurement. However, glass electrodes are subject to several issues, such as the influence of ionic strength, certain organic matter, and electromagnetic interference. An alternative involves developing new fluorescence pH sensors. Fluorescence sensors offer numerous advantages, such as small size, immunity to electromagnetic and radio frequency interference, and multiplexing capability.

In recent years, interest in the development of quantum dot (QD)-based pH sensors has increased because of the unique optical advantages of QDs in comparison with traditional organic dyes. The advantages of these sensors include the following: broad excitation spectra; tunable, narrow, and symmetric emission spectra; high resistance to photochemical degradation; and high photostability [3–5]. In this regard, a series of QDs has been tested as fluorescent platforms for pH sensing with different pH-responsive ranges, such as pH 7.1–8.5 for CdSe–ZnS core–shell QDs [6], pH 6.0–9.0 for thioglycolic acid (TGA)-capped CdTe QDs [7], pH 4.0–8.0 for mercaptoacetic acid-capped CdSe/ZnSe/ZnS QDs [8], and pH 6.0–8.0 for glutathione-coated CdSe/CdZnS QDs [9]. These studies indicated that QD-based pH sensors in aqueous solutions often lack the ability to sense responses over a wide pH range. In addition, colloidal QDs are not easily removed from solutions by conventional methods, such as filtration and centrifugation. Therefore, the use of colloidal QDs results in undesirable leaching, pollution, and inconvenient manipulation. To overcome these limitations, the development of hybrid materials based on the incorporation of QDs into solid matrices has drawn increasing interest. Previous studies demonstrated that the symbiotic effects of hybrid materials can yield novel properties and potential applications [10–14]. Among these techniques, polymer microparticles are commonly used as a QD carrier to form nanocomposites for the detection of biomolecules [15–21]. However, the use of QD-microparticle nanocomposites for pH sensing was rarely reported. In addition, polymer microspheres are traditionally prepared by physical and chemical methods [22,23]. These traditional methods suffer from the requirement of hazardous chemical usage, which is harmful to the environment and human body.

Bacterial spores possess a rigid core and display remarkable resistance to malnutrition, heat, radiation, chemicals, and desiccation. These properties contribute to their applicability as vehicles for cell-based biosensors. In general, spore-based biosensors were developed based on a genetically engineered technique [24,25]. However, the applications of engineered spore-based biosensors are still limited

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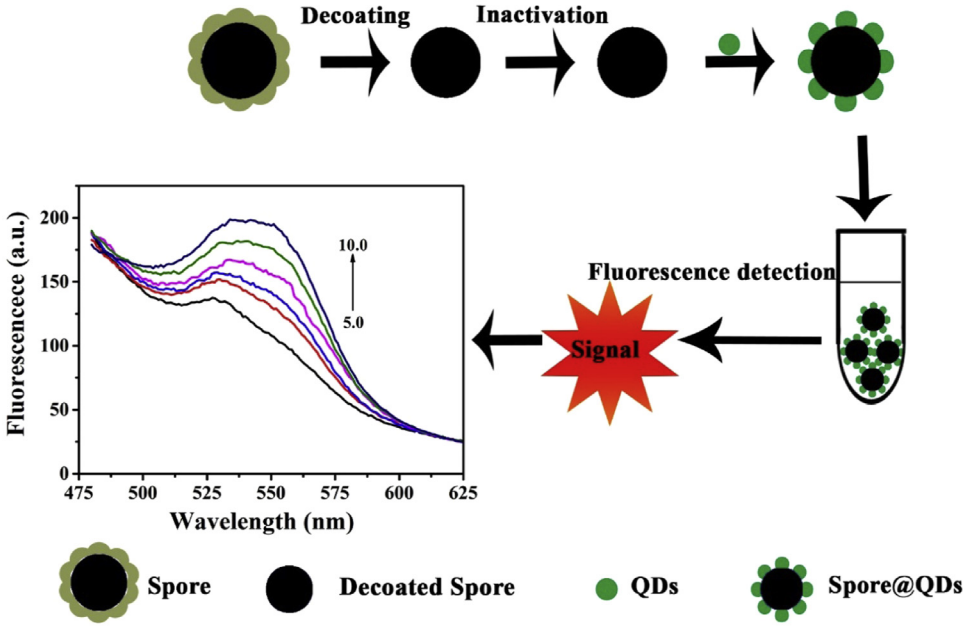

Scheme 1. Schematic of QD@Spore nanocomposite for pH detection.

because of the long detection time, low sensitivity, and need for professional training, spore generation, and optimization for batch production, among others [26]. Recently, we successfully demonstrated that wild-type bacterial spores can be used for the antioxidant capacity [27] and phenol assay [28] on the basis of the existence of laccase (i.e., CotA protein) on the spore surfaces of *Bacillus subtilis* and *Bacillus amyloliquefaciens*.

Previously developed spore-based biosensors require living spores for analyte detection. In this study, we introduced a novel spore-based sensing method using the "dead" (inactivated) spores of *B. subtilis* as nanocarriers for pH detection. The procedure of pH sensing is shown in Scheme 1. First, *B. subtilis* spores were peeled off the loosely attached, balloon-like outer layers, which belonged to the exosporium [29,30]. Second, the as-prepared spores were inactivated by using a high-pressure steam sterilizer for 30 min at 121 °C. The treated spores (TSs) could be considered as a bio-polymer microsphere for different applications. The proposed biological method for preparing spore-based microspheres presents several advantages, such as high-throughput, cost-effectivity, and ecological friendliness, over traditional physical and chemical methods. Third, TGA-modified CdTe/ZnS QDs were self-assembled onto TS surfaces to form QD@spore nanocomposites (i.e., QD microspheres) as detection devices. QD microspheres are expected to exhibit great potential for use as convenient, cheap, and effective pH-sensitive fluorescent probes for application in pH meters with a wide responsive range. Finally, the developed fluorescence microspheres were successfully used to determine the pH of water samples, yielding comparable results with those of commercial assay method.

2. Materials and methods

2.1. Apparatus

A RF-5301PC fluorescence spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 1 cm quartz cells was used for fluorescence measurements. A platform constant-temperature shaking incubator was purchased from Shanghai Jing Hong Laboratory Instrument Co. Ltd. (Shanghai, China). All solutions were prepared and diluted using ultrapure water (18.2 MΩ cm) produced by a Model Cascada IX laboratory ultrapure water system (Pall Co., Ltd., Washington, NY, USA). The pH of all buffer solutions was measured by a PB-10 pH meter (Sartorius, Gottingen, Germany). A transmission electron microscope (TEM; JEM-100CX II, JEOL Ltd., Tokyo, Japan) and a laser scanning confocal microscope, FV1000 IX81 (Olympus, Kyoto, Japan), were used to observe the morphology of the obtained particles. The leaching of QDs was determined by inductively coupled plasma mass spectrometry (ICP-AES; PerkinElmer, Waltham, MA, USA). An X-ray photo-electron spectroscopy (XPS) was performed on a VG Multilab 2000 (Thermo Fisher Scientific, Waltham, MA, USA) spectrometer using monochromatic Al Kα radiation as the X-ray source for excitation.

2.2. Materials

The *B. subtilis* CCAM 080032 strain was provided by Dr. Zhao Shumiao's group (Huazhong Agricultural University). Trypsin and Tween-20 were obtained from Sigma-Aldrich (St. Louis, MO, USA). 1,4-Dithiothreitol (DTT) was purchased from Hefei Bomei Biotechnology Co., Ltd. (Anhui, China). CdTe/ZnS (TGA) QDs were purchased from Beida Jubang Science & Technology Co., Ltd. (Beijing, China).

2.3. Preparation of spores

B. subtilis strains were cultured in lysogeny broth agar growth medium at 37 °C. The spores were harvested after a week of incubation at 37 °C. The spores were scraped from plates and successively washed via centrifugation with deionized water. The collected spores were resuspended in deionized water. The number of TSs (3.7×10^9 particles mL^{-1}) was determined by direct counting with a Burker chamber under an optical microscope.

2.4. Formation of QD microspheres

The as-prepared spores were diluted with deionized water, treated by ultrasound for 6 min, and centrifuged at 8000 rpm for 10 min, and then the supernatant was removed. The remaining spores were respectively incubated in trypsin buffer (1% trypsin in 1 mmol L^{-1} HCl solution) for 7 h at 37 °C and in decoating buffer