Magnetically Reusable and Well-dispersed Nanoparticles for Oxygen Detection in Water

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Abstract
In this study, we aimed to synthesize magnetically well-dispersed nanosensors for detecting dissolved oxygen (DO) in water, and explore their biological applications. Firstly, we synthesized two kinds of magnetic nanoparticle with average sizes of approximately 82 nm by one-step emulsion polymerization: polystyrene magnetic nanoparticles (Fe3O4@Os1-PS) and polymethylmethacrylate magnetic nanoparticles (Fe3O4@Os1-PMMA). Both types of nanoparticle present good dispersibility and fluorescence stability. The nanoparticles could be used as oxygen sensors that exhibited a high DO-sensitivity response in the range 0-39.30 mg/L, with a strong linear relationship. The nanoparticles have good magnetic properties, and so they could be recycled by magnet for further use. Recovered Fe3O4@Os1-PS still presented high stability after continued use in oxygen sensing for one month. Furthermore, Fe3O4@Os1-PS was employed for detecting the bacterial oxygen consumption of Escherichia coli (E-coli) to monitor the metabolism of bacteria. The results show that Fe3O4@Os1-PS provide high biocompatibility and non-toxicity. Polystyrene magnetic nanoparticles therefore present significant potential for application in biological oxygen sensing.

Keywords Magnetic · Nanosensor · Dissolved oxygen (DO) · Escherichia coli

Introduction
Dissolved oxygen (DO) plays a critical role in regulating multiple processes involved in complex biological systems [1–5]. Meanwhile, hypoxia usually leads to illness. Therefore, DO sensing is important for the diagnosis of certain diseases [6–8], and is usually realized by employing three methods: the Winkler titration method [9], the Clark electrochemistry method [10], and the optical method [11]. Among these, the optical method, which uses fluorescence sensors, is applied widely because of its non-invasive action on bacteria and tissues, high sensitivity, and real-time results [12]. Various oxygen probes that employ platinum/palladium porphyrins as such as polyfluorence (PFO) [13], oligofluorene [14], and other emitters [15–17] are available to detect dissolved oxygen. Most of these are hydrophobic [18, 19], and difficult to apply in biological fields. Although some hydrophobic probes can be modified to increase solubility, their quantum yield is rather low [20, 21]. Porphyrin compounds are usually used in probes for DO sensors because of their high quantum yields and long excitation times [22–24].

To enable biological applications, scientists have explored various approaches, including the generation of nanoparticles with the assistance of polymers [25, 26]. Polymer materials for the connecting probe mainly include polymer nanoparticles, micelle polymers, and polymer films. Micelle polymers composed of multi-armed polymers may exhibit various morphologies and assemblies. However, micelle polymers sensors present various disadvantages, such as a long period of toxicity in vivo, and the difficulty of synthesizing micelle [27, 28]. Polymer films are unstable sensors when used to detect dissolved oxygen, although they are highly sensitive [29, 30]. Polymer nanoparticles with specific surface areas, mechanical properties, and higher dissolved-oxygen permeability, have gained considerable
research attention, where the properties of polymer spheres largely depend on the polymer material employed [31, 32]. Although polyspheres with dissolved oxygen-sensitive probes have been reported in the literature, most of these are prone to agglomeration or precipitation, and have particular difficulty in forming a stable dispersion and solution system in water [33, 34]. Magental nanoparticles (NPs) are increasingly important in many biomedical applications, such as drug delivery, hyperthermia treatment, and magnetic resonance imaging (MRI) contrast enhancement [35, 36]. Otherwise, nanoparticles with magnetic properties can be recycled using magnetic separation.

In this paper, we synthesized two new kinds of nanoparticle using the incorporation of magnetic spheres and oxygen sensors, which employed PMMA and PS as the matrix. These two new nanoparticles named Fe3O4@Os1-PS and Fe3O4@Os1-PMMA were used for detecting DO. Both nanoparticle types exhibited good dispersity by optimizing and regulating amount of surfactants, while their non-toxicity indicates their potential for application in DO detection in biosystems such as bacteria or cells.

**Experimental**

**Materials and Reagents**

Sodium dodecyl benzene sulfonate (SDBS) and ferric acetylacetonate Fe(acac)3 were purchased from Energy Chemical (Shanghai, China). Styrene was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Prior to use, the polymerization inhibitor in the styrene was removed using 10 % NaOH solution. Polyvinylpyrrolidone (PVP, K30), methyl methacrylate, oleic acid, oleylamine, and benzyl ether were purchased from Aladdin Industrial Corporation (Beijing, China). 1,2-hexadecanediol was purchased from Chemical Industry Co., Ltd (Tokyo, Japan). Ammonium persulfate was purchased from Lingfeng Chemical Reagent Co., Ltd (Shanghai, China), which served as an initiator of the redox polymerization of styrene and methyl methacrylate. 5-(4-methacryloxyethoxycarbonyl phenyl)-10,15,20-triphenyl-porphyrin (Os1) was synthesized according to reported procedures [15].

**Instruments**

Fe3O4@Os1-PS and Fe3O4@Os1-PMMA were separated by an ultra centrifuge (MAX-XP). Apparent morphologies were characterized using a scanning electron microscope (SEM, MIRA3 TESCAN) and a transmission electron microscope (TEM, Tecnai G2 F20 S-TWIN (200 kV) and HT7700). A PerkinElmer fluorescence spectrophotofluorometer was used for the fluorescence measurements. Dynamic light scattering (DLS) (Nano ZS, Malvern, UK) was used to measure the particle diameters. A vibrating sample magnetometer (VSM, JDAW-2000C&D) was employed to measure the hysteresis loop for Fe3O4 and Fe3O4@Os1-PS. Plate Reader (BioTek Cytation 3, Winooski, VT, USA) was used to measure bacteria respiration for Escherichia coli (E-coli). Dissolved oxygen and nitrogen gases were mixed at various concentrations using gas flow meters. All measurements were performed at room temperature (25 °C ± 5 °C).

**Preparation of Fe3O4 Nanoparticles**

Superparamagnetic Fe3O4 nanoparticles were synthesized according to the following approach [35]: Fe(acac)3 (2 mmol, 0.7063 g), 1,2-hexadecanediol (10 mmol, 2.5845 g), oleic acid (6 mmol, 1.6948 g), oleylamine (6 mmol, 1.6049 g), and benzyl ether (20 mL) were mixed together, and further heated to 200 °C for 3 h under a nitrogen atmosphere. The product was then precipitated with ethanol and dispersed into 1.66 mL of hexane after centrifugation. The product was then placed in a refrigerator at −4 °C for further use.

**Preparation of Fe3O4@Os1-PS and Fe3O4@Os1-PMMA**

Fe3O4@Os1-PS was synthesized via the emulsiifer polymerization of styrene and Os1 initiated by ammonium persulfate (APS): Firstly, 12 mg of SDBS and 40 mg of PVP solution were mixed and dissolved in 10 mL of water. Then, 400 μL of the above Fe3O4 dispersion solution, 0.2766 g styrene, and 2 mg Os1 dissolved in 200 μL of ethanol were added to the mixture. After ultrasonication, the suspension was degassed with nitrogen. To initiate polymerization, 0.0066 g of APS was added and stirred at a speed of 400 rpm for 5 h at 60 °C with the protection of nitrogen gas. Finally, the resulting obtained magnetic nanoparticles were separated by centrifugation at 30000 rpm for 30 minutes. After washing twice with distilled water, 1 mg of magnetic nanoparticles was redispersed in 4 mL of distilled water for the detection of dissolved oxygen.

The synthesis method for the Fe3O4@Os1-PMMA nanoparticles is listed in supporting information, which is similar to the above methods with only the replacement of an equal amount of styrene with methyl methacrylate.

**Sensor Performance of Dissolved Oxygen**

To investigate the sensing property of dissolved oxygen, the emission spectra excited at a wavelength of 405 nm under different dissolved oxygen in aqueous solutions were recorded at room temperature. Different ratios of DO were regulated by introducing mixtures of N2 and O2 into the
solution using a cuvette and a pair of mass-flow controllers. The results for DO were analyzed quantitatively based on fluorescence intensities at 660 nm.

**Detection of E. coli Respirations and the Toxicity of Fe₃O₄@Os1-PS for E. coli**

*E. coli* were revived and cultivated in a liquid Lysogeny Broth (LB) medium at 37 °C with shaking at 200 rpm for one night. The bacteria density of *E. coli* was estimated by measuring the optical density at 600 nm, which is linearly proportional to *E. coli* density when OD600 is in the range of 0.1 to 1.0. A value of 1 indicated a bacteria density of 5.0 × 10⁸ colony-forming unit per milliliter (CFU/mL), based on the calibrations of the UV–vis spectrophotometer. The *E. coli* respiration and toxicity of Fe₃O₄@Os1-PS nanoparticles were analyzed using the plate reader. 100 μL of OD 0.025 were analyzed using the plate reader. 100 μL of OD 0.025 were revived and cultivated in a liquid Lysogeny Broth (LB) medium at 37 °C with shaking at 200 rpm for 5 hours under the reaction with protection of nitrogen. In order to know the sizes and morphologies of two types of nanoparticles, transmission electron microscope (TEM) and scanning electron microscope (SEM) were detected as they are the important means to demonstrate. TEM and SEM images for Fe₃O₄@Os1-PS and Fe₃O₄@Os1-PMMA are shown in Fig. 2. As shown in the TEM images of Fe₃O₄@Os1-PS (Fig. 2a) and Fe₃O₄@Os1-PMMA (Fig. 2b), it is evident that the Fe₃O₄ nanoparticles were wrapped in nanoparticles polymerized with styrene or methyl methacrylate as monomers, respectively. From Fig. 2c, d, both of these nanoparticles had diameter sizes of approximately 80 nm which can also be proved by the characterization of Dynamic light scattering (DLS). DLS (Fig. S3a, b) showed excellent dispersibility with low PDI, with 0.198 for Fe₃O₄@Os1-PS and 0.202 for Fe₃O₄@Os1-PMMA.

To study the oxygen-sensing properties of Fe₃O₄@Os1-PS and Fe₃O₄@Os1-PMMA in aqueous solutions, the emission spectra under different dissolved oxygen were recorded at room temperature. Dissolved oxygen was controlled by introducing different ratios of N₂ and O₂ into the solution within a cuvette, using a pair of mass-flow controllers (Fig. S4). We typically allowed 180 s between the changes of the N₂ and O₂ gas mixtures to ensure a new equilibrium. The solution was excited at a wavelength of 405 nm, and maximum emission was monitored at 660 nm. After using 405-nm light to continuously excite Fe₃O₄@Os1-PS and Fe₃O₄@Os1-PMMA for 30 minutes, the intensities of their maximum emissions at 660 remained almost unchanged (Fig. S5), suggesting that these two new kinds of oxygen sensor are particularly stable. It was observed that emission intensities of Fe₃O₄@Os1-PS (Fig. 3a) and Fe₃O₄@Os1-PMMA (Fig. 3b) at 660 nm (ascribed to Os1) decreased with increasing O₂ partial pressure from 0 % atm to 100 % atm. The emission ratios (Iₒ/I) and oxygen concentrations showed a linear relationship, following the Stern-Volmer Eq. (1).

\[
\frac{I₀}{I} = 1 + K_{SV}[PO₂]
\]  

where Iₒ and I are the steady-state luminescence signals at 660 nm, measured under nitrogen and under different O₂ partial pressures, respectively. KSV is the Stern-Volmer quenching constant, and PO₂ denotes the O₂ partial pressures. As
shown in Fig. 3c, the linear response to oxygen indicates a uniform distribution of the oxygen probes in the PS and PMMA matrix. The sensitivity of the oxygen sensor with nanoparticles is found to be 4.86-fold for Fe$_3$O$_4$@Os$_1$-PS and 2.68-fold for Fe$_3$O$_4$@Os$_1$-PMMA. Fe$_3$O$_4$@Os$_1$-PS presented higher sensitivity for sensing. To explore whether the sensors of Fe$_3$O$_4$@Os$_1$-PS can be reusable for detecting dissolved oxygen (DO) when they were conserved for some time and redispersed in water, we conducted the extra experiments for the detection of oxygen sensitivity. Interrestingly,
even after a month, Fe₃O₄@Os1-PS almost retained the same oxygen-detection sensitivity (Fig. S6), indicating that the sensor remained highly stable when redispersed in solution after a long time. Based on the advantages of fluorescence stability and reusable for the detection of dissolved oxygen (DO). Therefore, Fe₃O₄@Os1-PS was attempted to chosen as the exbacteriaent sensor for oxygen in the following experiment.

The magnetism characterization for Fe₃O₄@Os1-PS was performed using a vibrating sample magnetometer (VSM) at room temperature. VSM measurements showed that Fe₃O₄@Os1-PS could be used as a magnetic material because it presents a saturation magnetization (Sm) of 4.59 emu/g (Fig. 4a) which was decreased compared to the pure Fe₃O₄ nanoparticles due to the fact that small amounts of Fe₃O₄ was wrapped into the nanoparticles of Fe₃O₄@Os1-PS. Although the saturation magnetization (Sm) value of Fe₃O₄@Os1-PS has decreased increasingly, the nanoparticles can be quickly separated by less than 5 s by an external magnetic field. The magnetic nanoparticles may also be recycled using magnets for further use (Fig. 4b).

For biostudies, it is important to determine the toxicity assay for a new material. In this research, E. coli was cultured in LB broth at 37 °C, with shaking at 200 rpm for 12 h. We incubated the same E. coli density (OD 0.025) in the multi-well plate with different concentrations of Fe₃O₄@Os1-PS nanoparticles. Blank 1 was the control test without the sensing nanoparticles, and blank 2 was the control test without E.coli. OD600 is usually used as a standard indicator of the concentration of microorganisms in liquid culture medium. Absorbance at a wavelength of 600 nm (OD₆₀₀) was monitored using a plate reader. As shown in Fig. 5a, the time-dependent OD₆₀₀ of E. coli was not influenced by Fe₃O₄@Os1-PS concentration which showed that Fe₃O₄@Os1-PS could not affect the growth of E. coli. These results suggest the non-toxicity and biocompatibility of the nanosensors.

Bacteria respiration is a key process for bacteria metabolism. Bacteria at a higher concentration can consume dissolved oxygen more rapidly. Bacteria were gradually diluted with LB broth to OD₆₀₀ of 0.1, 0.05, 0.025 and 0.0125. Then 100 μL of the above-mentioned bacteria densities were mixed with 0.025mg/ml of Fe₃O₄@Os1-PS and placed in 96 well plates to test oxygen respiration, and the same intensity changes at a wavelength of 660 nm under excitation at 405 nm at different OD₆₀₀ of 0.1, 0.05, 0.025, and 0.0125 of E. coli, and without nanosensors (named “blank”)

Fig. 4 Magnetism characterization for Fe₃O₄@Os1-PS: a) VSM detection and b) a recycled Fe₃O₄@Os1-PS microsphere

Fig. 5 a) Bacteria toxicity test at different concentrations of Fe₃O₄@Os1-PS at 37 °C. Blank 1 indicates only 0.025 of E. coli in the media, without Fe₃O₄@Os1-PS nanoparticles. Blank 2 indicates the consequences without the presence of E.coli. b) Time-dependent emission intensity changes at a wavelength of 660 nm under excitation at 405 nm at different OD₆₀₀ of 0.1, 0.05, 0.025, and 0.0125 of E. coli, and without nanosensors (named “blank”)
volume of LB broth without bacteria was set as the blank control. To prevent the exchange of oxygen in the media with air, 100 μL of mineral oil was used to seal the well. For an excitation of 405 nm, emission intensity changes at a wavelength of 660 nm were recorded for the oxygen probe during bacteria growth. It was observed that the intensity of the oxygen probes’ emission increased much faster at higher bacteria densities, indicating that the oxygen was consumed much faster at these higher densities (Fig. 5b). After the dissolved oxygen in the medium was completely consumed, the fluorescence intensity did not change further. A slight decrease in the fluorescence intensities at an early stage of the experiments was observed because of the temperature effect [34]. Together, these data showed that the nanosensors could detect the respiration of bacteria, and thus the metabolism of bacteria.

**Conclusion**

In this paper, we first synthesized two new types of magnetic nanoparticle, named Fe₃O₄@Os₁-PS and Fe₃O₄@Os₁-P MMA, which employed PS and PMMA as a matrix, respectively. Both nanoparticles exhibited good dispersity and fluorescence stability. Fe₃O₄@Os₁-PS provided high sensitivity in terms of dissolved oxygen detection, and retained almost the same oxygen sensitivity even after a month. The sensors presented good biocompatibility and were successfully applied to the real-time monitoring of bacteria oxygen respiration for *E. coli*. It is expected that these new types of magnetic nanoparticles could be applied as oxygen sensors in related research into bacteria growth and metabolism.

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**Data Availability** The data and material are available within the manuscript.

**Declarations**

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

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**Consent for Publication** Not applicable.

**Conflict of Interest** The authors have no competing interests to declare that are relevant to the content of this article.

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