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Preparation and application of ratiometric polystyrene-based microspheres as oxygen sensors

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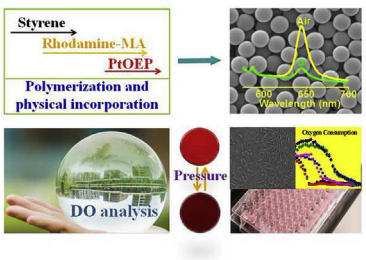
HIGHLIGHTS

- Uniform polymer microspheres with two different fluorophores as ratiometric oxygen sensors were prepared and characterized.
- The sensors can be used for accurately analyzing dissolved oxygen in some liquids.
- The sensors can respond well to air pressure.
- The microspheres were tested for monitoring cell respiration.

GRAPHICAL ABSTRACT



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ABSTRACT

Mono-dispersed polystyrene-based microspheres with diameters about 1 μm encapsulating rhodamine moieties as oxygen insensitive internal reference probes and platinum octaethylporphyrin units as oxygen sensitive probes were synthesized as new ratiometric oxygen sensors (Rhod-PtOEP-PS). The dual luminophors of rhodamines and platinum porphyrin moieties exhibited emissions maxima at 585 nm and 644 nm, respectively. The microspheres showed good oxygen sensing properties in different oxygen partial pressures (pO_2) and dissolved oxygen (DO) concentrations. It was found the oxygen probes and reference probes in microspheres showed higher photo-stability than their corresponding free fluorophores in solution. The microspheres also showed good sensitivity for air-pressure and cellular oxygen in cell culture medium. These microspheres were used to detect DOs in a few kinds of liquids including some daily drinks and it was found the measured errors were within positive/negative 11% as compared with the measured results using traditional oxygen electrodes.

1. Introduction

Oxygen is one of the most important substances in our human life. Monitoring oxygen concentration accurately is very important in many fields, such as food packaging [1–3], environmental monitoring [4–6], medical science [7,8], cell metabolism [9–11],

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and so on. Finding an accurate and also simple way to detect oxygen concentrations is a goal of many scientific researches. For now, Winkler titration method [12], Clark electrochemistry method [13], and luminance detection method [14,15] are commonly used to detect oxygen concentrations. Winkler titration method is a standard method to test oxygen concentration in some degrees [16]. But its steps are cumbersome and would consume lots of time, limiting its applications in many fields [17]. Clark electrochemistry method bases on reduction reaction of oxygen in anode [18]. It would have some errors because of magnetic field's interference or other substances' adherence to electrodes [19]. Also, due to their relatively large volumes, electrodes are difficult to be used in some narrow spaces or small volumes in a few microliters. Electrodes are either inapplicable for non-invasive intracellular applications. Photoluminescence detection method is an emerging method in recent years because of its sensitiveness, real-time, easy operation, no oxygen consumption, and miniaturization of application. Therefore, more and more people pay attentions to this method [20–22]. It has been well used in biological detection and aerospace. For achieving accurate measurements, ratiometric approach is attracting more and more attentions for oxygen sensing by using one oxygen insensitive probe as an internal reference for the oxygen sensitive probe [23]. Ratiometric approach alleviates the influence of sensors' concentrations, the light intensities of excitation sources, and even media to achieve higher accuracy of the measurement results. Some ratiometric oxygen sensors were reported by using platinum/palladium porphyrins as oxygen probes and conjugated polymers like polyfluorence (PFO) [24,25], oligofluorene [26], aggregation induced emitters [27], and other emitters [28–31].

In materials and polymer fields, polymer microspheres as a class of interesting polymeric materials caused significant scientific attentions. Polystyrene microspheres with variable sizes are commonly used in biological study due to its adsorbability and acid-base resistance property. A few kinds of oxygen sensitive polystyrene-derived microspheres synthesized by using different methods [32,33] were reported by the consideration of their specific surface area, good mechanical property, and higher oxygen permeability [34]. Herein, we report the synthesis of new oxygen sensitive polystyrene derived microspheres by using Pt(II) octaethylporphine (PtOEP) as an oxygen probe and a polymerizable methacrylate-substituted rhodamine derivative (Rhod-MA, Fig. 1) as an internal oxygen insensitive probe for realizing new ratiometric oxygen sensitive microspheres. The Rhod-MA was chemically immobilized in the microspheres, while the PtOEP was physically absorbed in the microspheres. These microspheres were used for air-pressure measurements, for *in-situ* monitoring cell oxygen respiration, and for analyzing the DO concentrations in a few kinds of liquids including drinks.

2. Experiment

2.1. Materials

2.1.1. Chemical reagents

Platinum octaethylporphine (PtOEP), styrene (St, 99%), and azoisobutyronitrile (AIBN, 99.8%) were purchased from Aladdin (Beijing, China). Styrene was purified by a basic alumina column to remove the inhibitor in it. Polyvinylpyrrolidone (PVP, Mw 40,000) was obtained from Fluka (Milwaukee, WI, USA). Rhod-MA was prepared according to the procedure as our previously reported [35]. pH buffers were prepared by the system of NaH_2PO_4, Na_2HPO_4, Na_2CO_3, and $NaHCO_3$. Silica-based thin layer chromatography (TLC) sheet as the substrates of pressure sensitive paints was purchased from Xinming materials Co., Ltd (Qingzhou, Shangdong, China).

2.1.2. Experimental instruments

Scanning electron microscope (TESCAN MIRA3, Kohoutovice, Česká Republika) was used for micropsheres' morphologies observation, Fluorescence Spectrofluorophotometer (PerkinElmer LS 55, Shelton, CT, USA) was used for fluorescence intensities measurements. Centrifuger (Hunan Xiangyi H1850, Changsha, Hunan, China) was used for centrifuging the particles. Dynamic light scattering (Malvern Nano ZS, Malvern, WR, United Kingdom) was used for measuring the particle sizes in solutions. Plasma Cleaner (Harrick, PDC-002, Ithaca, NY, USA) was used for surface treatment. Plate Reader (BioTek Cytation 3, Winooski, VT, USA) was used for measuring cell respiration. Dissolved Oxygen Analyzer (VD-O1 electrodes, Beverly, MA, USA) was used to measure dissolved oxygen concentrations. Incubator shaker (Crystal

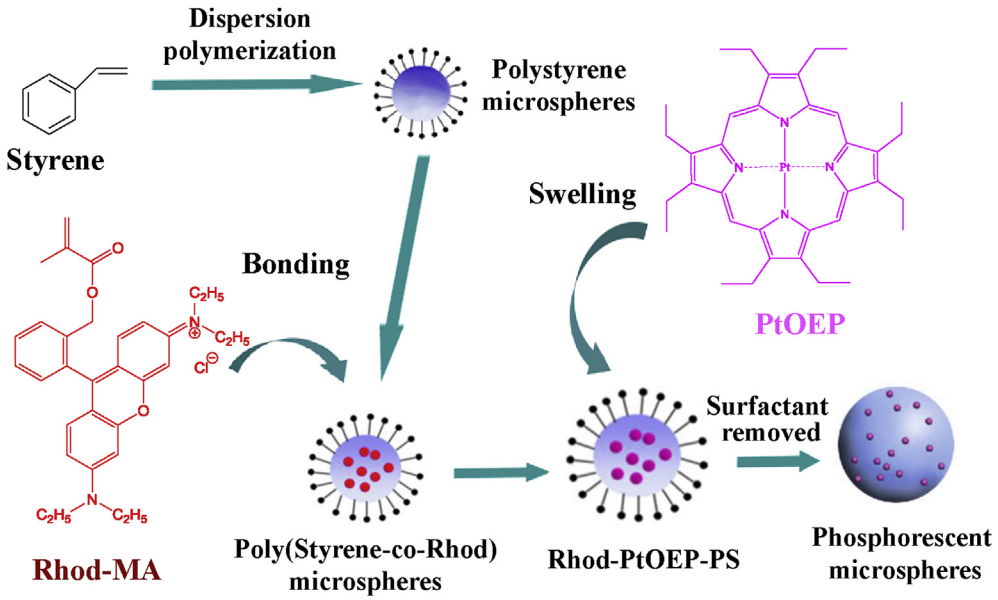

Fig. 1. The schematic drawing of the preparation of microspheres using a three-step procedure.

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