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A Novel Nano-Sensor Based on Rhodamine-β-Isothiocyanate – Doped Silica Nanoparticle for pH Measurement

Feng Gao, Lun Wang*, **Lijuan Tang,** and **Changqing Zhu**

College of Chemistry and Materials Science, Anhui Key Laboratory of Functional Molecular Solids,
Anhui Normal University, Wuhu 241000, P.R. China

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Abstract. The preparation of fluorescent pH-sensitive nanoparticles and monitoring the pH in real samples is described. A novel fluorescent core-shell nanoparticle was developed by encapsulating the rhodamine-β-isothiocyanate (RITC) in a silica shell with a reverse microemulsion technique. The RITC encapsulation reduces RITC leaching from the silica matrix when immersed in water. The hydrophilic silica shells are made by hydrolyzing and polymerizing tetraethoxysilane (TEOS). The fluorescent core-shell nanoparticles are pH-sensitive, and the pH dynamic range of the sensing nanoparticles is between 5.0–10.0. The method offers the advantages of adequate sensitivity, accuracy and rapid detection of pH. The results are in good agreement with those using the standard glass electrode method. They show excellent stability and high reproducibility when used as pH sensors.

Key words: Water-in-oil microemulsion; fluorescent core-shell nanoparticles; pH sensor.

Nanotechnologies are now poised to revolution the electronic, chemical and biotechnology industries and biomedicals. There are many interesting areas in nanotechnology [1, 2]. One of the most important aspects in this field is the preparation and development of nanomaterials, such as nanoparticles [3–7]. Nanoparticles can be made with different materials as small as 1 nm [1–15]. Nanoparticles are useful in biochemical analysis, such as in sensors and biomarkers.

In the past twenty years, the development of studies on chemical sensors and biosensors was very rapid [16]. One of the most widely studied sensors, the pH sensor, was focused on because pH measurement is closely linked to modern industry, agriculture, medicine, bioengineering, environment, disease diagnostics and scientific studies. Several methods for pH sensors have been developed so far [17–20].

Among many techniques, water-in-oil microemulsion is one of the most widely used methods of preparing nanomaterials of small size [3, 21–24]. With this method, reversed micelles are formed, i.e. water nanodroplets are formed in an organic medium and used as nanoreactors for the formation of nanoparticles. The water/oil ratio can be carefully adjusted to control the particle size. Particles prepared by the reverse microemulsion method show great promise in size control and further miniaturization.

This study proposes a nano-pH sensor based on fluorescent silica nanoparticles developed by encapsulating the rhodamine-β-isothiocyanate (RITC) using the microemulsion technique at room temperature.

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* Author for correspondence. E-mail: summit8848cn@hotmail.com

The dye-doped silica nanoparticles can prevent fluorophores from photobleaching, which will affect the emission intensity. The result shows excellent stability and high reproducibility as a pH optical sensor.

Experimental

Materials

Rhodamine-β-isothiocyanate (RITC) and Triton X-100 (TX-100) were purchased from ICN Biomedical Inc., and n-hexanol, tetraethyl orthosilicate (TEOS) and cyclohexane were obtained from Shanghai No. 3 Chemical Co. All other chemicals were of analytical grade. Distilled, deionized water was used for the preparation of all aqueous solutions.

Instrumental

The size of the nanoparticles was measured using a Hitachi H-600 transmission electron microscope (TEM). Emission spectra were recorded on a Hitachi spectrofluorometer, model F-2500, and a pHS-3c pH meter was used for pH measurements (Shanghai, China).

Nanoparticle Synthesis

The w/o microemulsion was prepared by first mixing 1.77 mL of TX-100, 7.5 mL of cyclohexane, 1.8 mL of n-hexanol, and 340 µL of water. Aqueous luminophore solution was then added in such a way that the water-surfactant molar ratio was kept constant at 10. The final luminophore concentration in the mixture was 1.2 mM. In the presence of 100 µL of TEOS, a polymerization reaction was initiated by adding 60 µL of NH_4OH. The reaction was allowed to continue for 24 h. After the reaction was complete, the RITC-doped nanoparticles were isolated by acetone, followed by centrifuging and washing with ethanol and water several times to remove any surfactant molecules. Ultrasonication was performed while washing the nanoparticles in order to remove any physically absorbed luminophores from the particles' surface.

Results and Discussion

Nanoparticle Characterization

The microemulsion method yielded uniform luminophore-doped silica nanoparticles. These nanoparticles were characterized using microscopic and spectroscopic methods. The nanoparticles were uniform in size, 60 ± 4 nm in diameter (Fig. 1), as characterized by TEM.

In addition, spectrofluorometric measurements were used to characterize the nanoparticles. The excitation and emission spectra of pure RITC and the nanoparticles were measured in the aqueous solution. RITC exhibited an emission at 603 nm when excited at 542 nm in aqueous solution. However the emission maximum of the embedded nanoparticles shifted 6 nm to the longer wavelength. (Fig. 2), indicating that the spectral characterization of the fluorescence dye did not change to a great extent when it was doped inside the nanoparticles. This clearly shows that the luminophores are well protected inside the silica network under normal atmospheric conditions.

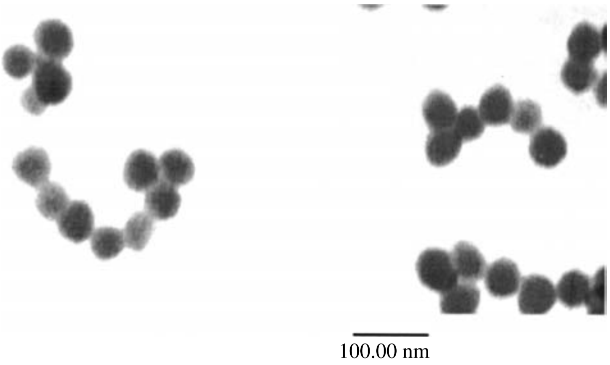

Fig. 1. TEM image of RITS-doped silica nanoparticles at 100 000 × magnification

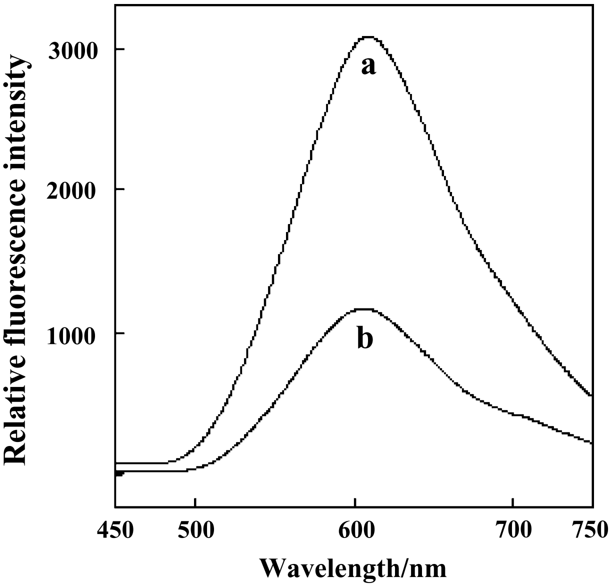

Fig. 2. Fluorescence emission spectra of fluorescent core-shell (*a*) and free dye (*b*) in aqueous phase

Application of the Nanoparticles in pH Sensors

The fluorescent nanoparticles were scattered into a symmetrical suspension solution in water by ultrasonication. 6 µL of nanoparticle suspension solution and 2 mL of PBS buffer was mixed, incubated for 5 min at room temperature, and then the fluorescence

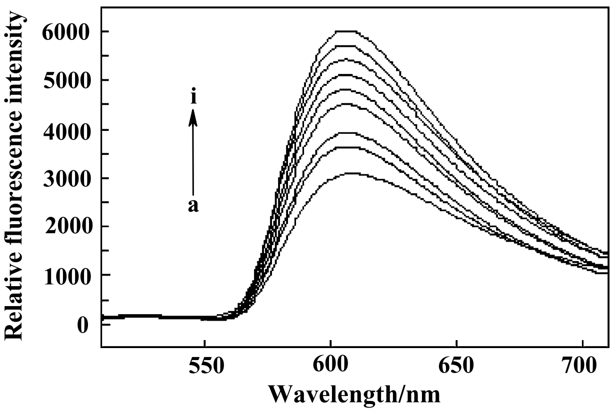

Fig. 3. Relative fluorescence intensity of nanoparticles-PBS buffer pH value ($a \rightarrow i$): 5.0; 6.0; 6.5; 7.5; 8.0; 8.5; 9.0; 9.5; 10.0

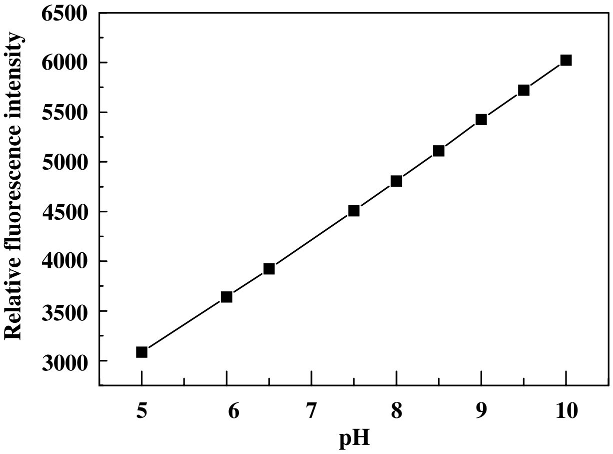

Fig. 4. Response curve between fluorescence intensity of nanoparticle and pH value

intensity of the mixed solution was measured at 609 nm ($\lambda_{EX} = 542$ nm). The result showed that the fluorescence intensity increased when the pH value increased in the range of 5.0–10.0 (Fig. 3). As show in Fig. 4 the fluorescence intensities varied linearly with the pH value of PBS buffer.

Response Time

Figure 5 shows the variation of the resistance of the pH sensor with respect to the response time for a sensor under different pH conditions. It was found that the fluorescence intensity ratio F_t/F_0, where F_t and F_0 are the fluorescence intensity of the sensor after and before soaking in a pH buffer, respectively, reached a steady state within 4 minutes. In order to ensure a complete equilibrium for the reaction of the pH sensor with the buffer solution, it is advisable to keep to a reaction time of 5 minutes for all pH measurement.

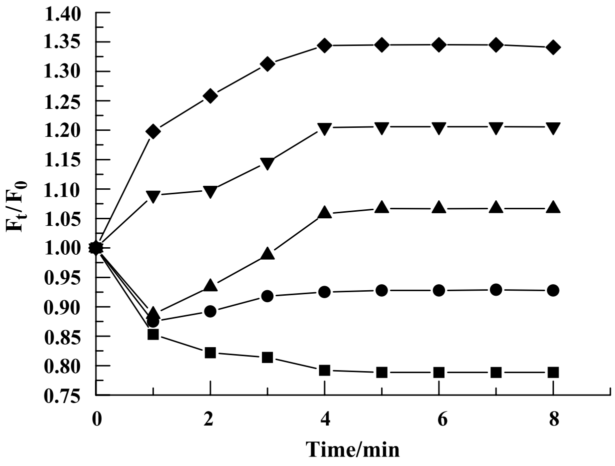

Fig. 5. Relationship of the response time of the pH sensor under different pH values of PBS buffer, —■— pH = 5.5; —●— pH = 6.5; —▲— pH = 7.5; —▼— pH = 8.5; —◆— pH = 9.5

Effect of Ionic Strength

The effect of solution ionic strength on the pH sensor performance was examined by exposing a pH sensor to 0.01–0.5 M sodium chloride solutions kept at a constant pH of 6.5 (see Fig. 6). The relative fluorescence intensity of the sensor changes by less than 5% ranging from 0.01–0.5 M sodium chloride, indicating that ionic strength has little effect on the pH sensor.

The effect of buffer solution type on the pH sensor response was also tested, using HAC–K_2HPO_4,

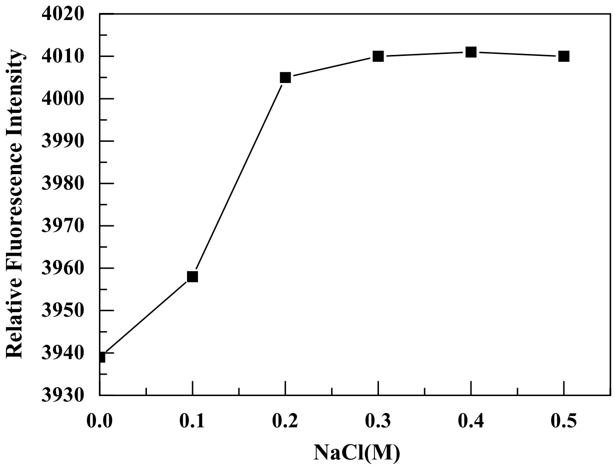

Fig. 6. Effect of ionic strength in a series of NaCl solutions of different concentrations

Briton-Robison, and $(CH_2)_6N_4$–HCl buffers. The change in fluorescence intensity of the pH sensor ranging from pH 5.0 to pH 6.0 for $0.05\,mol\,L^{-1}$ of HAC–K_2HPO_4, Briton-Robison, and $(CH_2)_6N_4$–HCl buffers was 588.6, 590.7, and 584.6, respectively. The result showed that the type of buffer solution has little influence on the pH sensor.

Repeatability and Reproducibility

The repeatability and reproducibility of the sensor were studied at pH 8.00. Sensor repeatability refers to the successive runs made using a single sensor to evaluate discrepancies in its response. Sensor reproducibility on the other hand refers to the sensor discrepancies in response between individual members of a batch of similarly constructed sensors. The repeatability of the sensor was studied by using the same sensor to repeatedly measure seven different buffer solutions of the same pH (pH 8.00). At the same time, the reproducibility of the sensor was studied by using seven similarly constructed sensors to measure the same buffer solution of pH 8.00. The relative standard deviation (R.S.D.) for repeatability and reproducibility of the sensor was evaluated to be 2.7 and 3.8%, respectively. Variation in the response of the pH sensor has been observed to occur from the main source, i.e. construction variation and operational variation [25].

Photostability

The sensor stability was evaluated by immersing the sensor in buffer solution of pH 7.00 for 5 h and measuring the fluorescence intensities at 509 nm. The sensor was found to be very stable with an R.S.D. value of 2.4%. A study of long-term stability and the effects of storage conditions was not conducted.

Reversibility Study

The reversibility of the constructed sensor was assessed by immersing the nanoparticles in buffer solutions of pH 5.5–9.5 followed by solutions of pH 9.5–5.5. As shown in Fig. 7, the response of the pH sensor demonstrated no hysteresis when the sensor was used to measure different pH values in the cycles from pH 5.5–9.5–5.5(acid–base–acid region). The results confirm the reversibility.

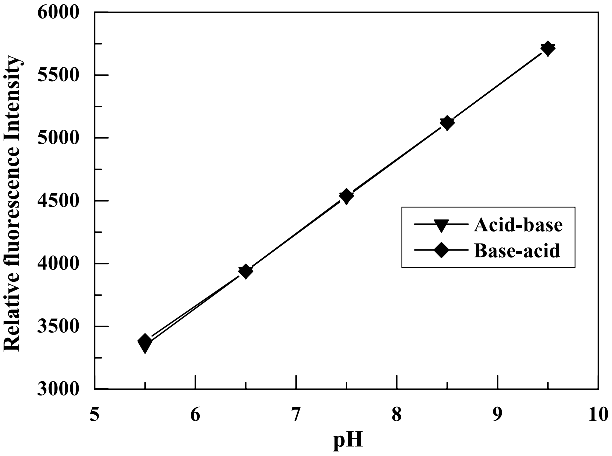

Fig. 7. Reversibility response of the sensor

Table 1. pH determination results for samples

Samples	This method	Glass electrode method	RSD (%)
Synthetic water sample ($1^{\#}$)	5.83	5.79	1.1
Synthetic water sample ($2^{\#}$)	7.82	7.80	2.3
Synthetic water sample ($3^{\#}$)	9.80	9.79	1.4
Tap-water	6.37	6.35	1.8
Distilled water	6.94	6.91	2.1
Mineral springs water	5.81	5.68	1.3

Calibration and Sample Analysis

Under the optimum experimental conditions, the sensor showed a linear response. A calibration curve can be described by the following equation:

$$F = 99.2759 + 590.7217\ pH$$

where F is the relative fluorescence intensity. The linear relative coefficient is $r = 0.9998$.

With the calibration curves, six samples were determined simultaneously under the same conditions. The determination results are listed and compared with those of the glass electrode method in Table 1. As Table 1 shows, all the results were satisfactory.

Conclusions

The nano-pH sensor based on the RITC core-shell nanoparticles offers the advantages of adequate sensitivity, accuracy and rapid detection of pH. The results are in good agreement with those using the standard glass electrode method. They show excellent stability and high reproducibility when used as pH sensors. In addition, the nanoparticles were easily synthesized, making this method suitable for practical applications.

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