Preparation of novel core-shell silica particles for pH sensing using ratiometric fluorescence approach

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Core-shell silica particles encapsulating two luminescent dyes with different emission wavelengths were prepared for ratiometric fluorescence sensing of pH. The composite core-shell structure particles contained a polyacrylonitrile (PAN) core surrounding a silica shell. Fluorescein isothiocyanate (FITC) encapsulated in the silica shell was taken as an indicator. Fluorescence emission from Ru(dpp)3(ClO4)2 immobilized in PAN core was used as a reference light. The ratiometric fluorescence signal from the core-shell silica particles were found with a linear range from pH 5.80 to 7.50 ($R^2 = 0.997$). In addition, the morphology of the core-shell silica particles, the effects of temperature and co-existing substances were investigated. The core-shell silica particles were successfully applied to determination of pH value in water and urine samples.

1. Introduction

Many important biological processes including calcium regulation, chemotaxis, neuronal function, cell growth and division, and tissue oxygenation etc. are sensitive to pH change. To date, several measurement methods and techniques of intracellular pH have been developed, one of the most common being optical fluorescence methods. There are many fluorescent indicators for pH. Among them, fluorescein is one of the most widely used indicators for pH measurement. There have also been reports of fluorescein modified and derivatized to be more useful in cellular applications; however, several problems still exist that prevent indicators from being ideal for such cellular determinations. Most fluorescence pH sensors are based on the measurement of the fluorescence intensity change using single emission light. Unfortunately, their measurements are generally compromised by the heterogeneity in the excited field, background fluorescence or fluctuations of individual signals. Ratiometric fluorescence approach is one of the most commonly used methods for intrinsic fluorescence referencing due to its reducing or cancelling out variations in fluorescence signal compared to the sensors based on a single intensity-base response to analyte. In addition, many fluorescence pH sensors suffer from oxygen quenching, indicator leaching or photobleaching, and less biocompatibility. Thus, it becomes important to establish an accurate ratiometric fluorescence pH sensor with good selectivity, reproducibility and anti-oxygen quenching.

In this paper, we established a ratiometric fluorescence pH sensor based on simultaneous excitation of a fluorescent indicator and a luminescent reference dye. As shown in Scheme 1, the key point of this approach is to encapsulate the pH-sensitive luminophore (fluorescein isothiocyanate, FITC) on the surface of particles, and the luminescent reference dye, Ru(dpp)3(ClO4)2, was immobilized in the cores of the PAN particles. The fluorescence of FITC on the surface of particles sensitively responds to the change of environmental pH, but the fluorescence intensity of Ru(dpp)3(ClO4)2 is constant in the pH sensing process. Ru(dpp)3(ClO4)2 embedded in oxygen-impermeable PAN

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Scheme 1 Synthetic procedure to coat PAN spheres with silica.
nanomaterials could minimize the effects of oxygen or other undesired interactions, and ensure the constant fluorescence intensity of Ru(dpp)$_3$(ClO$_4$)$_2$. Based on the consideration, a ratiometric method using dual dye was set up for pH sensing. The pH sensing approach presented good selectivity, easy usage, good reproducibility and physiological pH detection in the range of 5.80~7.50. Hence, the core-shell silica particles show potential for in vivo or real-time determination of H$^+$ in cells.

2. Experimental

2.1 Materials

Ru(dpp)$_3$(ClO$_4$)$_2$ (dpp = 4,7-diphenyl-1,10-phenanthroline) was synthesized and purified in the laboratory of the Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University. Acrylonitrile (AN) of analytical-grade was obtained from Chemical Reagent Factory of Tianjin Fu Chen (Tianjin, China). Fluorescein isothiocyanate (FITC), tetraethylorthosilicate (TEOS) and polyvinyl pyrrolidone-40 (average molar masses of 40 kg mol$^{-1}$, PVP-40) were ordered from Sigma-Aldrich (Milwaukee, WI, USA). 3-aminopropyltrimethoxysilane (APTMS) was obtained from TCI (Tokyo, Japan). Sodium dodecyl sulfate (SDS), potassium persulfate (PPS), ammonium hydroxide and ethanol (EtOH) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Phosphate buffer solutions (PBS) were prepared with potassium dihydrogen phosphate/disodium hydrogen phosphate (KH$_2$PO$_4$/Na$_2$HPO$_4$) and their pH values were measured using a commercial digital pH meter (CyberScan pH 510, EUTECH). Unless specified otherwise, their ionic strength (IS) was kept at 100 mM. Pure water was obtained from a Millipore Autopure WR600A system (Millipore Co., USA), and used throughout the experiments. All experiments were performed at 25 °C unless noted otherwise. All other reagents were of analytical reagent grade and were used without further purification.

2.2 Instrumentation and analysis

Transmission electron microscope (TEM, Tecnai F30, FEI Co. USA) was used to characterize the particle morphology. Fluorescence profiles were recorded from a Hitachi F-4600 fluorometer (Hitachi Co. Ltd., Japan).

2.3 Preparation of PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles

PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles were prepared according to Lior Boguslavsky report. To prepare PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles, 0.3 g SDS was dissolved in 28 mL water, then 1 mg mL$^{-1}$ (1.86 mL) Ru(dpp)$_3$(ClO$_4$)$_2$ AN solution was added, finally 30 mg of PPS was added. The polymerization of AN was accomplished in a 50 mL vial equipped with a magnetic stir bar. The vial was vigorously stirred under nitrogen gas at 65 °C for 10 h. The resulting particles were washed either by intensive dialysis against water, or by intensive centrifugation cycles with water. The sediments were re-dispersed in 20 mL ethanol.

2.4 Preparation of FITC-APTS

To prepare the silica core, FITC was first covalently linked to silane coupling agent APTMS. FITC-APTS was prepared according to the method developed by Christina Graf et al. There were two steps for preparation: in the first step, PVP-40 (amphiphilic, nonionic polymer) was used as a coupling agent, and adsorbed onto the PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles after being ultrasonified; in the second step, the stabilized particles obtained from the first step were transferred into the solution of ammonia in ethanol. Silica shells on the particle surfaces were grown by additions of TEOS. The detailed procedure includes: PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles were dissolved in ethanol and ultrasonified for 30 min before use. 0.32 g PVP-40 was dissolved in 32 mL ethanol, then, 1% PVP-ethanol solution and 4 mL of PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ colloidal solution was mixed and ultrasonified for 2 h at room temperature. The solution was centrifuged and the supernatant was removed. The sediment was dispersed in 32 mL ethanol, subsequently, 0.48 mL TEOS solution and 40 μL FITC-APTS were added, stirring 10 min. After this process, 0.96 mL ammonia solution (28 wt% in water) was immediately added under stirring. The reaction mixture was ultrasonified for 1 h and then stirred for another 2 h. The prepared sample was centrifuged to collect the particles, and the sediment was further washed with ethanol several times to remove the unreacted chemicals. Finally, the sediment was dispersed in 20 mL ethanol.

2.5 Preparation of PAN-Ru(dpp)$_3$(ClO$_4$)$_2$@SiO$_2$-FITC particles

PAN-Ru(dpp)$_3$(ClO$_4$)$_2$@SiO$_2$-FITC particles were prepared according to the method developed by Christina Graf et al. There were two steps for preparation: in the first step, PVP-40 (amphiphilic, nonionic polymer) was used as a coupling agent, and adsorbed onto the PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles after being ultrasonified; in the second step, the stabilized particles obtained from the first step were transferred into the solution of ammonia in ethanol. Silica shells on the particle surfaces were grown by additions of TEOS. The detailed procedure includes: PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles were dissolved in ethanol and ultrasonified for 30 min before use. 0.32 g PVP-40 was dissolved in 32 mL ethanol, then, 1% PVP-ethanol solution and 4 mL of PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ colloidal solution was mixed and ultrasonified for 2 h at room temperature. The solution was centrifuged and the supernatant was removed. The sediment was dispersed in 32 mL ethanol, subsequently, 0.48 mL TEOS solution and 40 μL FITC-APTS were added, stirring 10 min. After this process, 0.96 mL ammonia solution (28 wt% in water) was immediately added under stirring. The reaction mixture was ultrasonified for 1 h and then stirred for another 2 h. The prepared sample was centrifuged to collect the particles, and the sediment was further washed with ethanol several times to remove the unreacted chemicals. Finally, the sediment was dispersed in 20 mL ethanol.

3. Results and discussion

3.1 Characterization of particles

As shown in Fig. 1A and 1B, the diameter of prepared PAN-Ru(dpp)$_3$(ClO$_4$)$_2$ particles is about 150 nm. Typical HRTEM images of PAN-Ru(dpp)$_3$(ClO$_4$)$_2$@SiO$_2$-FITC spheres are shown in Fig. 1C and 1D. The thickness of the outer shell is about 10 nm, which could be controlled by the amount of TEOS added. PAN-Ru(dpp)$_3$(ClO$_4$)$_2$@SiO$_2$-FITC in ethanol solution was well-dispersed without aggregation.

3.2 Characterization of the pH sensor

3.2.1 Fluorescence characteristics. The maximum excitation wavelength of FITC (pH indicator) and Ru(dpp)$_3$(ClO$_4$)$_2$ (reference dye) is at 485 nm and 477 nm, respectively, indicating that both dyes can be excited at the same wavelength (480 nm). The maximum emission of the pH indicator was found to be at 523 nm, while 596 nm for the reference dye. Thus, the ratio of different emission intensities is available for the quantitative pH measurements. FITC exhibits pH-dependent fluorescent characteristics. No fluorescence could be observed from its acidic
from the lactonization, while its basic form displays strong green fluorescence, and the fluorescence intensity significantly changes within different pH values.

As shown in Fig. 2, with pH increase, the fluorescence intensity of FITC increased while the reference dye remained nearly constant. Experimental results revealed that PAN-Ru(dpp)₃(ClO₄)₂@SiO₂-FITC particles presented pH-sensitive responses in the pH range from 3.18 to 8.65. A linear dependence of fluorescence intensity ratio on the pH was found to be from 5.80 to 7.50, with a correlation coefficient of 0.997 (inset in Fig. 2).

3.2.2 Sensing stability. In the experiments, the sensing stability was evaluated using the fluorescence ratios obtained from 10 continuous measurements in pH 7.14 buffer solutions. Experimental results revealed that the pH sensor presented good stability with a relative standard deviation (RSD) smaller than 5%, which assured the reproducibility and accuracy of the data acquired.

3.2.3 Effect of temperature. Temperature is another important factor affecting emission intensities. To quantify this effect, fluorescence intensity ratios at pH 7.14 were measured under varying temperature at 25, 30, 35, 40, 45 and 50 °C. As shown in Fig. 3, no significant change of fluorescence intensity ratio was found in the temperature range from 25 to 50 °C (RSD = 3.56%). Therefore, temperature showed only a slight effect on the fluorescence intensity ratio of the pH sensing.

3.2.4 Effect of existing substances. The ability of the method established above to selectively detect pH is an important requirement for practical applications. Therefore, changes in the fluorescence characteristics of PAN-Ru(dpp)₃(ClO₄)₂@SiO₂-FITC caused by co-existing metal ions in pH 7.14 were investigated in the study. No obvious effects could be found in the presence of 1 μmol L⁻¹ of Na⁺, Ca²⁺, Mg²⁺, Zn²⁺, Ni²⁺, Al³⁺, Hg²⁺, Cu²⁺, Pb²⁺ and Cd²⁺. As shown in Table 1, the selected co-existing metal ions did not have any obvious influence on the performance of the pH sensor.

3.2.5 Sample analysis. The method proposed was applied to the determination of pH in water samples. Water samples were detected using both commercial electrochemical pH meter and the proposed method at room temperature. Before the detection, the water samples were filtered three times through qualitative filter paper before use. As shown in Table 2, the water pH could easily be determined using the proposed method. Compared with the electrochemical pH meter, the results obtained from the proposed method were accurate and the analytical performance of the pH sensor was satisfied. The results indicate that the proposed method had excellent performance for pH detection in water.

In addition, the proposed method was applied to the pH determination of human urine samples. Before pH detection, the urine samples were filtered through qualitative filter paper. The pH values were obtained by comparing the ratios between the fluorescence signals of fluorescein at 523 nm and 596 nm, respectively.
Ru(dpp)$_3$(ClO$_4$)$_2$ at 596 nm to the calibration curve. The obtained results of pH values, 6.95/0.04, indicate that there was only 0.1 pH deviation compared to those from a commercial pH meter.

### 4. Conclusions

In summary, a ratiometric fluorescence method was developed to monitor the pH change. The silica particles comprised Ru(dpp)$_3$(ClO$_4$)$_2$-doped PAN cores, and an outer FITC-doped silica shell. The core-shell silica particles displayed well-resolved dual fluorescence emission, with the FITC at 523 nm and the Ru(dpp)$_3$(ClO$_4$)$_2$ at 596 nm. The prepared core-shell particles presented pH sensible and the pH dynamic range was 3.18 to 8.65. The sensing approach presented excellent selectivity, stability and high reproducibility in pH optical sensing, indicating its great potential in physiological pH detection.

### Acknowledgements

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### References


### Table 1

<table>
<thead>
<tr>
<th>Metal (M$^{n+}$)</th>
<th>ΔF (%)</th>
<th>Metal (M$^{n+}$)</th>
<th>ΔF (%)</th>
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<tr>
<td>Na$^+$</td>
<td>0.36</td>
<td>Cu$^{2+}$</td>
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<td>Hg$^{2+}$</td>
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<tr>
<td>Al$^{3+}$</td>
<td>−1.76</td>
<td>Pb$^{2+}$</td>
<td>−4.68</td>
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</tbody>
</table>

$^a$ Metal ions were all at a concentration of 1 μmol L$^{-1}$. $^b$ The fluorescence data for several typical ions in pH 7.14 solution. $ΔF = (F_\text{after} − F_\text{before}) / F_\text{before} \times 100$, $F$ is fluorescence intensity ratio after adding co-existing ions and $F_\text{before}$ is the fluorescence intensity ratio without co-existing ions.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH Meter</th>
<th>pH Sensor</th>
<th>RSD$_{\text{pH}}$ (%)</th>
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<tr>
<td>Water sample-1</td>
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<tr>
<td>Water sample-2</td>
<td>6.55</td>
<td>6.51 ± 0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>Urine sample</td>
<td>6.92</td>
<td>6.95 ± 0.04</td>
<td>0.51</td>
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