

## Development of Fluorescent Turn-On Sensor for Selective Detection of $\text{Cu}^{2+}$ in Aqueous Solution

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$\text{Cu}^{2+}$  is one of the most essential metal ions in biological systems, since it plays an important role in numerous biological processes and can induce various disorders, including some neurodegenerative diseases such as Alzheimer's, Prion, and Parkinson's diseases.<sup>1</sup> Thus, quantifying the amount of  $\text{Cu}^{2+}$  in a systems crucial for studying the pathological roles of  $\text{Cu}^{2+}$  in living systems. The development of fluorescent sensors for  $\text{Cu}^{2+}$  has received much attention in last decade because of their simplicity and high sensitivity.<sup>2-6</sup> In addition, fluorescent sensors can offer the potential application in real time detection in living systems with high spatial resolution that is difficult or impossible to obtain using conventional techniques such as atomic absorption spectroscopy and inductively coupled plasma mass spectroscopy. In particular, the development of a fluorescent turn-on sensor is critical to enhance detection sensitivities. Although there have been reports on fluorescent  $\text{Cu}^{2+}$  sensors, fluorescent turn-on sensors are still relatively rare because the paramagnetic nature of  $\text{Cu}^{2+}$  ( $3d^9$ ) tends to quench the fluorescence. Recently, several groups have proposed copper promoting reactions including hydrolysis,<sup>7</sup> oxidative cyclizations of thiosemicarbazone to develop fluorescent turn-on sensor.<sup>8</sup>

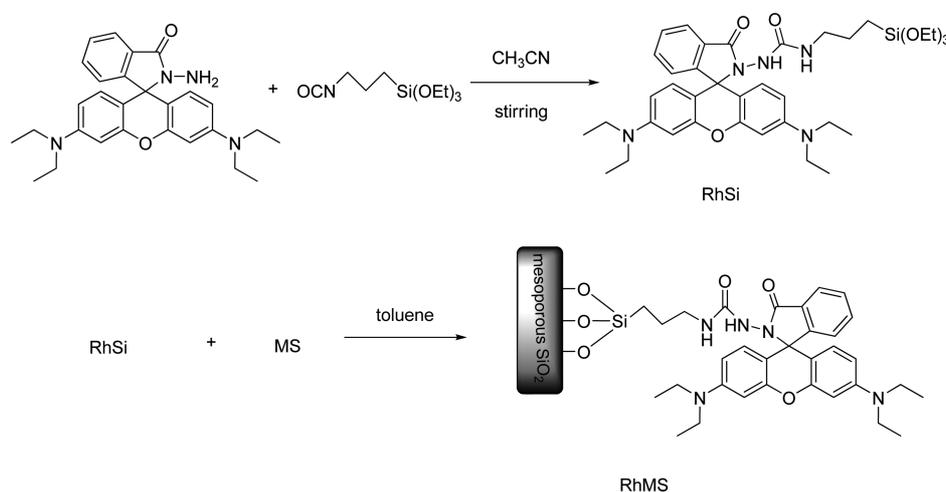
Rhodamine derivatives are one of the most widely used fluorescent dyes in the design of fluorescent sensors due to their great photo-stability, high quantum yield, emission at long wavelengths, and applicability in biological systems.<sup>9</sup>

Recently, Li *et al.* reported a rhodamine sensor containing a thiourea group immobilized on mesoporous silica for the detection of  $\text{Cu}^{2+}$ . However the sensor showed a lack of selectivity for  $\text{Cu}^{2+}$  versus  $\text{Hg}^{2+}$ , because a rhodamine B derivative containing a thiourea group involves the ring opening of spirolactam followed by cyclization in the presence of  $\text{Hg}^{2+}$ .<sup>10</sup>

Herein, we report on the development of rhodamine-immobilized mesoporous silica (RhMS) and the characterization of its metal recognition properties. To discriminate the  $\text{Cu}^{2+}$  from  $\text{Hg}^{2+}$ , we changed the thiourea group to a urea group to diminish the rhodamine derivative reactivity toward  $\text{Hg}^{2+}$ . We found that the fluorescence turn-on chemodosimeter RhMS exhibited a rapid response and good selectivity toward  $\text{Cu}^{2+}$  over other metal ions. Moreover, the RhMS exhibited simultaneous colorimetric and fluorescence enhancements upon the addition of  $\text{Cu}^{2+}$ .

The RhMS was synthesized as shown in Scheme 1. Figure 1 shows a TEM image of the synthesized mesoporous silica (MS) (a) and RhMS (b). As shown in Figure 1, the synthesized RhSi and RhMS were well-ordered. The immobilization process did not give rise to significant changes in the ordered structure. To confirm the successful grafting of Rh-Si onto the mesoporous silica, FT-IR spectroscopy, TGA analysis, and nitrogen adsorption-desorption measurements were carried out.

Figure 2 illustrates the IR spectra of the mesoporous silica, RhMS, and Rh-Si in solid form. The absorption bands of the



**Scheme 1.** Synthesis of rhodamine-immobilized mesoporous silica (RhMS).

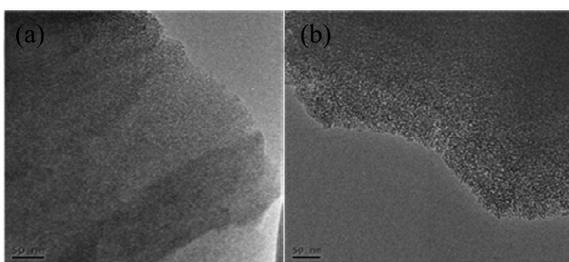


Figure 1. TEM images of the synthesized MS (a) and RhMS (b).

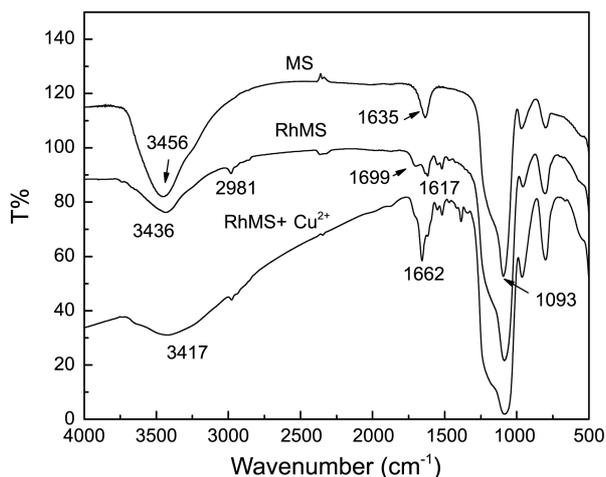


Figure 2. IR spectra of mesoporous silica, RhMS1 and Rh1.

mesoporous silica appear at 3456, 1635, and 1093  $\text{cm}^{-1}$  whereas the bands of RhMS appear at 3436, 2981, 1699, 1617, 1549, 1519, 1469, and 1083  $\text{cm}^{-1}$ . The appearance of new absorption peaks at 2981, 1699, 1549, 1519, and 1469  $\text{cm}^{-1}$  originated from the addition of the rhodamine, suggesting a successful grafting of Rh-Si onto the surface of the mesoporous silica. In addition, TGA analysis confirmed the formation of RhMS, where the amount of attached Rh-Si was estimated to be about 10%. To investigate the porosity changes of the mesoporous silica induced by the introduction of Rh-Si, the surface area, pore volumes, and pore diameters of both the mesoporous silica and the RhMS were measured with nitrogen adsorption–desorption isotherms. The Brunauer–Emmett–Teller (BET)<sup>11</sup> surface area and pore volume of the mesoporous silica were determined to be 728.2  $\text{m}^2/\text{g}$  and 0.720  $\text{cm}^3/\text{g}$ , respectively, whereas those of the fluorescent RhMS were determined to be 413.65  $\text{m}^2/\text{g}$  and 0.376  $\text{cm}^3/\text{g}$ , respectively. Furthermore, the Barrett–Joyner–Halenda (BJH) pore diameter decreased from 3.77 to 3.30 nm, indicating the attachment of rhodamine to the mesoporous silica.

The binding properties of RhMS toward various metal ions were investigated by UV/Vis absorption and fluorescence spectroscopy. In the absence of metal ions, the RhMS was colorless, but its color changed to pink upon addition of  $\text{Cu}^{2+}$  ions. The addition of other metal ions did not lead to any other color changes. Figure 3 shows the absorption spectra (a) and fluorescence spectra (b) of RhMS in both the absence and the presence of various metal ions ( $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,

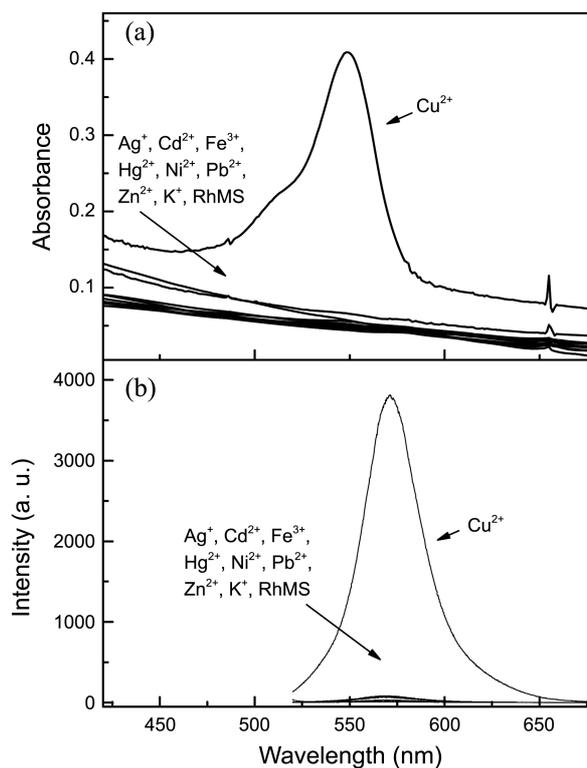
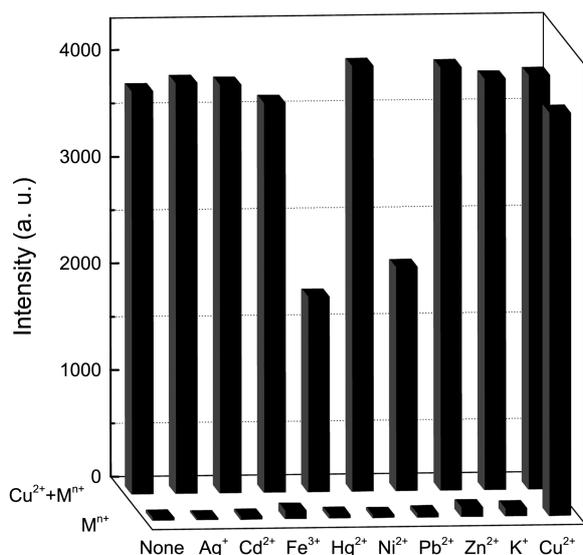


Figure 3. Absorption spectra (a) and Fluorescence spectra (b) of RhMS (20  $\mu\text{g}/\text{mL}$ ) in the absence and presence of various metal ions (75  $\mu\text{M}$ ) in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}=1:1$  pH 7, (excitation at 510 nm).

$\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{K}^+$ ) in an aqueous solution. In the absence of metal ions, RhMS was non-fluorescent and colorless because the rhodamine molecule was in a spirolactam form. In the presence of  $\text{Cu}^{2+}$  ion (75  $\mu\text{M}$ ), however, the suspension of RhMS showed remarkable enhancement of the UV absorption at 548 nm (Figure 3(a)) and the fluorescence centered at 571 nm (Figure 3(b)). From these spectroscopic changes, we noticed that the RhMS reveals a high selectivity for  $\text{Cu}^{2+}$  ions over other metal cations, such as  $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{K}^+$ . Upon the addition of  $\text{Cu}^{2+}$ , the chelating of  $\text{Cu}^{2+}$  with rhodamine led to the opening of the spirolactam ring. As a result, RhMS converted into an open ring state triggering a dramatic increase in the fluorescence intensity at 580 nm, as well as a strong absorption at 548 nm.

The fluorescence response of RhMS (2.8  $\mu\text{M}$ ) toward  $\text{Cu}^{2+}$  was explored by performing titration experiments at various concentrations of  $\text{Cu}^{2+}$  (0, 1, 2, 3, 4, 5, 7, 7.5, 8, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 75, and 100  $\mu\text{M}$ ). The fluorescence intensity increased continuously with increasing  $\text{Cu}^{2+}$  concentration. Figure 4 represents the selectivity and competition properties of the fluorescent sensor RhMS for the metal ions of interest. There was almost no interference from the  $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{K}^+$  ions, and  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  led to a slight decrease in the fluorescence intensity, compared to the  $\text{Cu}^{2+}$  alone. Yet, the presence of  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  strongly enhanced the fluorescence of RhMS- $\text{Cu}^{2+}$ .

To elucidate the sensing mechanism of RhMS toward  $\text{Cu}^{2+}$



**Figure 4.** Fluorescence response of RhMS (20 µg/mL) to various metal ions (75 µM), first row: only metal ion, second row: Cu<sup>2+</sup> and metal ions.

ions, the resultant pink mixture of RhMS and Cu<sup>2+</sup> solution was examined by FT-IR and fluorescence spectroscopy. After treatment with centrifugation at 3,000 rpm for 10 min, the precipitate was collected and re-dispersed in an acetonitrile/water mixture (1:1, v/v, pH 7). We found that the resultant solution was colorless and exhibited no fluorescence. We examined the precipitated nanostructure using FT-IR and found that the urea carbonyl (1699 cm<sup>-1</sup>) band shifted to a lower frequency (1662 cm<sup>-1</sup>), suggesting that an urea group had remained on the MS surface and had coordinated with the Cu<sup>2+</sup>. On the other hand, the supernatant solution was pink in color and showed strong fluorescence, indicating that an open-ring rhodamine existed. In addition, the fluorescence intensity was not affected by the addition of EDTA, suggesting that Cu<sup>2+</sup> was not involved in the complexation with free rhodamine. Based on the observed experimental results, we propose the sensing mechanism as shown in Figure 5. Upon addition of Cu<sup>2+</sup>, ring-opening, redox, and hydrolysis reactions occurred, which gave rise to the formation of free rhodamine from the RhMS.

In conclusion, we have developed a novel chemodosimeter for RhMS by covalent coupling of rhodamine with mesoporous SiO<sub>2</sub> and characterized as a fluorescent sensor for Cu<sup>2+</sup>. The RhMS showed a high sensitivity and selectivity toward the Cu<sup>2+</sup> ions over other metal ions in an aqueous

solution. Upon addition of the Cu<sup>2+</sup>, the RhMS underwent a hydrolysis reaction and ring opening process, which increased the fluorescence intensity by a factor of 1,000 and changed the color from colorless to pink.

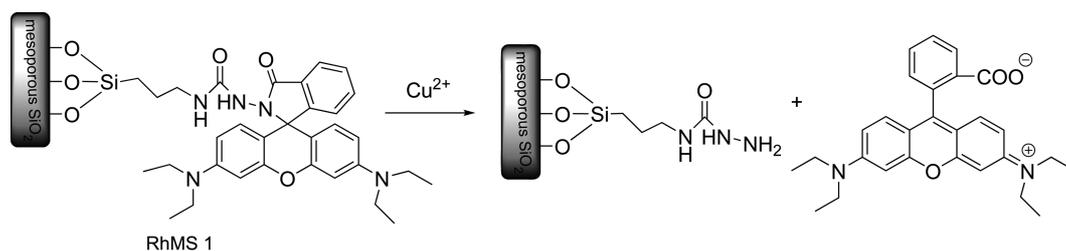
## Experimental

**Chemicals.** Tetraethyl orthosilicate 96% (TEOS) (TCI), 3-(triethoxysilyl)propyl isocyanate (TCI), Cetyltrimethylammonium Bromide (CTAB) 98% (TCI), and Rhodamine B (Aldrich) were used as purchased without further purification. All other reagents were purchased from Samchun and were used without further purification.

**Characterization.** The NMR experiments were performed with a JEOL AL-300 (300 MHz for <sup>1</sup>H-NMR and 75 MHz for <sup>13</sup>C-NMR) spectrometer. The chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. FT-IR experiments were conducted on a JASCO FT/IR-4200 spectrometer. TGA data were collected with TGA Q5000 IR. High resolution mass spectra were recorded on a JEOL JMS-700 spectrometer. UV-Vis absorption spectra and fluorescence spectra were recorded on a Shimadzu UV-3100 and a Hitachi FL-4500, respectively.

**Synthesis of Rh-Si.** Rhodamine B hydrazide<sup>12</sup> (0.228 g, 0.5 mmol) was dissolved in CH<sub>3</sub>CN (10 mL), and 3-(triethoxysilyl)-propylisocyanate (0.15 g, 0.62 mmol) in CH<sub>3</sub>CN (10 mL) was added dropwise in 1 h, and the reaction was stirred under N<sub>2</sub> for 24 h. Then, the solvent was evaporated *in vacuo*. CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added, and the organic layer was washed (2 × 20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent was removed. The crude product was further purified by column chromatography on silica gel (ethyl acetate/hexane, 1:1) to give 210 mg of yellowish solid Rh-Si with 64% yield. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.97 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.23 (m, 1H, ArH), 6.40 (m, 4H, xanthene-H), 6.29 (m, 2H, xanthene-H), 5.63 (s, 1H, NH), 4.63 (s, 1H, NH<sub>2</sub>), 3.34 (q, 6H, OCH<sub>2</sub>CH<sub>3</sub>), 3.33 (q, 8H, NCH<sub>2</sub>, NCH<sub>2</sub>), 2.83 (q, NHCH<sub>2</sub>), 1.20 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.17 (m, 21H, NCH<sub>2</sub>CH<sub>3</sub>, OCH<sub>2</sub>CH<sub>3</sub>) 0.43 (t, 2H, SiCH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 7.1, 12.3, 18.1, 22.7, 42.3, 44.2, 58.0, 66.3, 98.0, 104.2, 107.8, 123.3, 124.4, 127.5, 128.5, 129.3, 133.6, 148.8, 150.1, 153.9, 157.3, 166.9. FABMS: *m/z* (M<sup>+</sup>) calcd. For C<sub>38</sub>H<sub>53</sub>N<sub>5</sub>O<sub>6</sub>Si 703.9, found 704.5.

**Synthesis of RhMS.** The synthesis of mesoporous silica (MS) was previously described by Jung *et al.*<sup>13</sup> To remove the surfactant template (CTAB), 0.5 g of as synthesized MS



**Figure 5.** Proposed mechanism of RhMS for Cu<sup>2+</sup> ion.

was refluxed for 24 h in a methanolic solution (1:10 of concentrated HCl:methanol). The resulting material was filtered and extensively washed with deionized water and methanol. The surfactant-free MS material was dried at 100 °C in air. Rh-Si (0.1 g) and MS (0.1 g) was refluxed for 24 h in 30 mL of anhydrous toluene to yield RhMS material.<sup>13,14</sup>

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