A Novel Fluorescent Chemosensor Based on β-(2-Pyridyl)acrolein-Rhodamine B Derivative: Polymer Particle Interaction with an Enhanced Sensing Performance†

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Abstract
A novel, fluorescent chemosensor based on β-(2-pyridyl)acrolein-rhodamine B (RB-AC) derivative was synthesized and its sensing performance with poly(ethylene glycol) dimethacrylate (PEGDMA) polymer particle was investigated. The prepared β-(2-pyridyl)acrolein-rhodamine B/poly(ethylene glycol)dimethacrylate (PEGDMA/RB-AC) particle was used for sensing of Al3+. The PEGDMA/RB-AC particles showed immediate “off–on” fluorescent responses toward Al3+. The fluorescent response was attributed to the spirolactam ring opening of RB-AC. This sensor particle showed high selectivity towards Al3+ in the presence of other competing metal ions. The sensitivity of PEGDMA/RB-AC particle was demonstrated by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The binding stoichiometry and binding mode of the metal complex was established by Job’s plot and FT-IR spectroscopy.

Keywords: β-(2-pyridyl)acrolein-rhodamine B derivative, rhodamine/polymer particles, aluminium sensing, fluorescent sensor

1. Introduction
The development of selective and sensitive probe for the detection of biologically and environmentally important species has emerged as a significant goal in the field of chemical sensors (Zhou et al., 2014; Xu et al., 2010; Wang et al., 2014). So far, a number of fluorescent probes with different excitation and emission wave lengths have been used as signal receptors of chemosensors such as coumarin (Chen et al., 2014), pyrene (Wang et al., 2013), 1,8-naphthalimide (Dai and Xu, 2011), xanthenes (Chen et al., 2012), squaraine (Akkaya, 1997), cyanine (Zheng et al., 2012) and boron dipyrromethene difluoride (BODIPY) (Li et al., 2012). Among these, xanthenes derivatives are of great interest due to their excellent photophysical properties such as long absorption and emission wavelengths, large absorption co-efficient and high fluorescence quantum yield (Beaumont et al., 1993). Rhodamine is a well known dye of xanthenes derivative having spirolactam ring which is color less and non-fluorescent, whereas its

† Received 14 April 2015; Accepted 18 May 2015
J-STAGE Advance published online 30 June 2015
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2. Experimental section

2.1. Materials

The chemicals for the synthesis of β-(2-pyridyl)acrolein-rhodamine B derivative (RB-AC) were purchased from Aldrich and Kanto Chemicals Japan and were used without further purification. All reagents and solvents are of analytical grade and used without further purification. The metal ions such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺, Pb²⁺, Al³⁺ as perchlorate salts were purchased from commercial suppliers and used without further purification. Ethylene glycol dimethacrylate (EGDMA) was obtained from the Sigma Chemical Company, USA, and was used as received without purification. Azobisisobutyronitrile (AIBN) and poly(vinyl pyrrolidone) (PVP) were obtained from Acros Organics, New Jersey, USA. Ethylene glycol dimethacrylate (EGDMA) was used as the monomer. 2,2-Azobisisobutyronitrile (AIBN) and poly(vinyl pyrrolidone) (PVPK-85-95, Mw = 1,300,000) were used as the initiator and stabilizer, respectively.

2.2. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded in CDCl₃ unless otherwise stated, with tetramethylsilane (TMS) as internal reference at ambient temperature, mainly on a BRUKER AVANCE III 300 Magnet: Ascend TM series, 14.1 Tesla, ¹H resonance frequency 300 MHz, Top Spin 3.1 (software) spectrometer Germany spectrometers. HR-MS spectrum was recorded with Bruker autoflex III mass spectrometer. FT-IR spectra were recorded with an FTS-175C spectrometer. UV-Visible absorption spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Fluorescence emission spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. The slit width was 1.5 nm for both excitation and emission. Samples were contained in 10.0 nm path length quartz cuvettes (3.5 mL volume). Fluorescence imaging was performed by confocal fluorescence microscopy on LSM5Live, (Germany) with Zeiss LSM5 Live Release version 4.2.SP1 Image Browser software were used. The scanning electron microscope (SEM) images were obtained by JEOL, JSM-7000F.

2.3. Synthesis of RB-AC

Rhodamine B hydrazide and β-(2-pyridyl)acrolein were prepared following the literature method (Zhang et al., 2011; Krasnaya et al., 1997). The synthesis of RB-AC is shown in Scheme 1. In a 100 mL three necked round bottom flask, rhodamine B hydrazide (1.23 g, 2.6 mmol) was dissolved in hot ethanol (30 mL). Then, a solution of 0.5 g (3 mmol) of β-(2-pyridyl)acrolein in 20 mL of ethanol was added drop wise to the flask in 1 h. After the addition of β-(2-Pyridyl)acrolein, 1 ml of acetic acid was added to the reaction mixture. Under stirring, the reaction mixture was refluxed for 5 h. After 5 h, the reaction mixture was cool to room temperature and the formed precipitate was filtered and washed with cold ethanol. The crude product was purified by column chromatography using hexane-ethyl acetate (60:40) as eluent to get pure product (1.01 g, yield = 69.1%). The Product was confirmed by ¹H NMR and mass spectroscopy (Fig. S1 and S2).

![Scheme 1 Synthesis of RB-AC.](image-url)
2.4. Synthesis of PEGDMA/RB-AC particles

The dispersion medium (stabilizer) was prepared by dissolving 1.5 g of poly(vinyl pyrrolidone) (PVP) in deionized water (150 mL). The synthesis was performed in a three-necked flask equipped with a stirrer, water condenser and thermometer. A mixture containing 17 g of ethylene glycol dimethacrylate (EGDMA), initiator, 0.255 g of azobisisobutyronitrile (AIBN) and 0.0017 g of RB-AC was diluted with a mixed solvent (toluene (8 mL)/butanol (8 mL)). The reaction mixture transferred to the PVP dispersion medium and this reaction mixture was purged with N₂ gas for 10 min after that the reaction mixture was sealed under this nitrogen atmosphere and heated at 80 °C for overnight. The produced polymer was filtered using a Whatman filter number 1 and washed with acetone and methanol to get the RB-AC encapsulated PEGDMA (PEGDMA/RB-AC) as white powder.

3. Results and discussion

3.1. Preliminary studies

In order to evaluate the sensing behavior of PEGDMA/RB-AC, we have examined the absorbance, fluorescence and visible color change of the RB-AC in solution in the presence of various metal ions including alkali, alkaline-earth and transition-metal ions such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺, Pb²⁺ and Al³⁺, in ethanol/DMF (v/v = 9/1). The absorbance spectrum of RB-AC doesn’t show any peaks longer than 500 nm which was attributed to the ring closed spirolactam form of RB-AC (Fig. 1) (Chen et al., 2012). As shown in Fig. 1, the absorbance intensity of RB-AC was significantly increased at 560 nm upon the addition of Al³⁺ whereas no changes in absorbance intensity of RB-AC were observed upon the addition of other competitive metal ions. Fig. 2 shows the fluorescence changes of RB-AC in the presence of different metal ions. The RB-AC alone doesn’t show any fluorescence peak around 580 nm. The addition of Al³⁺ into RB-AC showed the considerable enhancement in fluorescence intensity at 580 nm. Further addition of other competitive metal ions doesn’t lead the fluorescence enhancement of the RB-AC.

Furthermore, the sensing behavior of RB-AC with different significant metal ions was checked by ‘naked eye’ detection methods. As shown in Fig. 3, the synthesized RB-AC displays a ‘naked-eye’ color change from colorless to pink (Fig. 3, top) after the addition of 10 μM of Al³⁺ in ethanol/DMF (9:1) mixture. While in the case of other cations such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ does not produce any significant color changes. Further, the addition of Al³⁺ exhibits intense yellow fluorescence under illumination of UV-light (Fig. 3, bottom). Thus, the sensing of Al³⁺ by RB-AC can be detected by the naked eye without the assistance of any instrument.

3.2. Spectroscopic recognition of RB-AC towards Al³⁺

UV-Vis and fluorescence spectral methods were used to study the recognition of RB-AC towards Al³⁺. UV-vis spectra of RB-AC in ethanol/DMF (9:1) mixture in the presence of different concentrations of Al³⁺ are shown in Fig. S3. In the absence of Al³⁺, the absorption spectrum of RB-AC doesn’t exhibit any characteristic absorption of rhodamine moiety whereas upon the addition of Al³⁺, a new absorption band centered at 560 nm was observed. Further addition of Al³⁺ into RB-AC leads to the increase in absorbance at 560 nm. This indicated a clear and gradually color change from colorless to pink due to the opening of the closed rhodamine spirolactam ring.
Due to its high sensitivity, fluorescence spectroscopy has been widely used in the study of molecular interactions between rhodamine and metal ions (Chen et al., 2009). Fig. S4 shows the fluorescence spectra of RB-AC in ethanol/DMF (9:1) mixture was recorded against the different concentrations of Al\(^{3+}\). As shown in Fig. S4, while increasing the concentration of Al\(^{3+}\), the fluorescence intensity at 580 nm was gradually increased. The binding constant of RG-HN with Al\(^{3+}\) was determined using Benesi-Hildebrand equation (Fig. S5). It was found to be 4.70 \times 10^4 which was within the range of reported Al\(^{3+}\) chemosensors (Kim et al., 2014).

The detection limit of the receptor RB-AC towards Al\(^{3+}\) was calculated based on 3\(\bar{\sigma}/k\) (Lohani et al., 2010). Where, \(\bar{\sigma}\) is the standard deviation of the blank solution and \(k\) is the slope of the calibration plot. A linear response was observed when the concentration of Al\(^{3+}\) is plotted against fluorescence intensity with a correlation coefficient of 0.982 (Fig. S6). The detection limit was calculated from slope and it was found to be 5.4 \times 10^{-8} M.

### 3.3. Sensing property of PEGDMA/RB-AC particles towards Al\(^{3+}\)

The results discussed in previous sections indicated that the synthesized RB-AC has good recognition towards Al\(^{3+}\) and it can be used for the preparation of sensing probe for Al\(^{3+}\). In order to improve the sensing performance of RB-AC, we have addressed the task of designing of RB-AC encapsulated PEGDMA particles. To demonstrate the encapsulation of RB-AC by PEGDMA, we have studied the color change of PEGDMA/RB-AC particles in ethanol/DMF (9:1) mixture with and without Al\(^{3+}\). In the absence of Al\(^{3+}\), PEGDMA/RB-AC particles in ethanol/DMF (9:1) mixture doesn’t show any color. After the addition of Al\(^{3+}\) to the PEGDMA/RB-AC particles in ethanol/DMF mixture (9:1), the color of the particles was changed colorless to pink and the particle showed yellow fluorescence under the illumination of UV-light as shown in the Fig. S7. Further, the sensing performance was examined by the addition of 1 mL of 10 \(\mu\)M of Al\(^{3+}\) in ethanol/DMF (9:1) mixture into the 1.0 g of synthesized PEGDMA/RB-AC particles. Besides that the mixture was stirred for 5 min up to attain the pink solid and filtered through the filter paper. As shown in Fig. 4, after the addition of Al\(^{3+}\), the color of the PEGDMA/RB-AC was turned from colorless to pink and it showed yellow emission under illumination of UV light, which is sensible via naked eye. The reversibility of PEGDMA/RB-AC was checked by the addition of 1 mL of 10 \(\mu\)M of EDTA extraction. Upon addition of EDTA in to Al\(^{3+}\) bounded PEGDMA/RB-AC, the color of the particle was turned from pink to color less and the yellow fluorescent emission was also turned off (Fig. 4). Further, the selectivity of PEGDMA/RB-AC particles towards Al\(^{3+}\) in the presence of 10 \(\mu\)M of other competitive metal ions was checked. As depicted in Fig. 5, Al\(^{3+}\) only changes the color of PEGDMA/RB-AC particles from color less to pink in visible light and yellow fluorescence under UV light whereas other competitive metal ions did not showed any changes. This indicates that the
PEGDMA/RB-AC particles show the fluorescence turn on by sensing of Al$^{3+}$ with good selectivity and reversibility.

### 3.4. Depicting the sensing property of PEGDMA/RB-AC by confocal laser scanning microscopy (CLSM)

Consequently, it was of great interest to investigate the sensory property by CLSM (Tao et al., 2011). The 1 mL of $1 \times 10^{-6}$ M Al$^{3+}$ (ethanol/DMF (9:1)) was dropped on the surface of a dry PEGDMA/RB-AC particles and allowed to stand for 1 min, resulting in the immediate adsorption of the Al$^{3+}$ by particles, after drying the particles in an oven at 150 °C, the CLSM was performed. As shown in Fig. 6A, PEGDMA/RB-AC particles did not show any fluorescence under the selective excitation whereas after the dropping of Al$^{3+}$ (1 mL) in to the PEGDMA/RB-AC particles, a significant increase in the fluorescence was observed from the selective excitation area (Fig. 6B).

### 3.5. Al$^{3+}$ binding on PEGDMA/RB-AC by SEM analysis

The CLSM results confirmed that the sensing property of PEGDMA/RB-AC towards Al$^{3+}$, to get further insight, SEM analysis was carried out. Fig. 7 showed the SEM images of prepared PEGDMA polymer encapsulated RB-AC in the absence and presence of Al$^{3+}$. The morphology of prepared PEGDMA/RB-AC particles is spherical in shape, and was arranged in an orderly three-dimensional shape and the particle size distribution is given in Fig. S8. There is only a little bonding between the particles (Fig. 7A). While in the presence of $1 \times 10^{-5}$ M of Al$^{3+}$, the PEGDMA/RB-AC particles were aggregated together (Fig. 7B). The difference in the surface morphology of these samples may be due to the formation of complex between RB-AC and Al$^{3+}$ followed by ring opening. Energy-dispersive analysis of X-rays (EDAX) was used to analyze the elemental constituents of prepared PEGDMA/RB-AC particles in the presence of Al$^{3+}$. Fig. 8 illustrates the EDAX spectra of PEGDMA/RB-AC particles after the sensing of Al$^{3+}$. The presence of aluminum in elemental analysis confirmed that the Al$^{3+}$ has been entrapped into PEGDMA/RB-AC by formation of complex with RB-AC.

### 3.6. Mechanism for the sensing of Al$^{3+}$ by PEGDMA/RB-AC

Since the sensing part of PEGDMA/RB-AC is RB-AC, to investigate the recognition of PEGDMA/RB-AC towards Al$^{3+}$, the stoichiometry and complex formation of RB-AC to Al$^{3+}$ were studied. The stoichiometry of RB-AC to Al$^{3+}$ was calculated by Job’s plot analysis and the complex formation was explained by FT-IR binding study. Total concentration of RB-AC and Al$^{3+}$ was kept constant at 10 μM according to the continuous variations changing the mole fraction of RB-AC from 0.1 to 0.9 (Fig. S9). The stoichiometry ratio of RB-AC with Al$^{3+}$ was analyzed by absorbance method. The maximum of curve...
was showed at 0.5 mole fraction, indicating that the formation of 1:1 complex between RB-AC and Al$^{3+}$. Fig. 9 shows the FT-IR spectra of RB-AC before and after binding with Al$^{3+}$. The FT-IR spectrum of RB-AC alone shows the peaks at 1678, 1610 and 1550 cm$^{-1}$ corresponding to spiro lactam amide carbonyl, imine (C=N) and pyridine (C=N) stretching vibrations, respectively. While the addition of 1 eq of Al$^{3+}$, the stretching frequency of these peaks was changed. Upon the addition of 1 eq Al$^{3+}$ the characteristic stretching frequency of spiro-
lactam rings amide carbonyl at 1698 cm$^{-1}$ was completely disappeared and the imine (C=N) and pyridine (C=N) stretching vibrations are shifted to 1643 and 1580 cm$^{-1}$, respectively. Which confirms the Al$^{3+}$ was opened the spiro lactam ring, formed the complex with oxygen and imine and pyridine nitrogen. It has been well established that Al$^{3+}$ forms complex with oxygen and nitrogen rich ligands (Dhara et al., 2014; Ghosh et al., 2014). Based on this, we have proposed the complex formation of Al$^{3+}$ with RB-AC (Scheme 2).

4. Conclusion

In conclusion, we have developed a novel fluorescent probe β-(2-pyridyl)acrolein-rhodamine B/poly(ethylene glycol) dimethacrylate (PEGDMA/RB-AC). The PEGDMA/RB-AC particles showed good selectivity and sensitivity towards Al$^{3+}$. Fluorescence enhance mechanism is presumably due to the chelation of Al$^{3+}$ with the oxygen atoms of the amide groups of RB-AC results in the formation of the open-ring form, which leads to the enhancement of fluorescence of PEGDMA/RB-AC particles. The selectivity for Al$^{3+}$ can be attributed to the rigid hydrazone binding site as well as the affinity of the pyridine group toward Al$^{3+}$.

Acknowledgements

This work was supported by research fund of Chungnam National University.
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Appendix: Supporting information
Fig. S1  $^1$H NMR spectrum of RB-AC.

Fig. S2  HR-MS spectrum of RB-AC.

Fig. S3  UV-Vis spectra of 10 $\mu$M of RB-AC in ethanol/DMF (9:1) in the presence of different concentrations of Al$^{3+}$. Inset: Absorbance at 560 nm as a function of the concentration of Al$^{3+}$.

Fig. S4  Fluorescence spectra of 10 $\mu$M of RB-AC in ethanol/DMF (9:1) in the presence of different concentrations of Al$^{3+}$. Inset: Fluorescence at 580 nm as a function of the concentration of Al$^{3+}$.
Fig. S5  Benesi-Hildebrand plot and equation for binding of $\text{Al}^{3+}$.

Fig. S6  Calibration plot for fluorescence intensity against concentration of $\text{Al}^{3+}$.

Fig. S7  Photographs of PEGDMA/RB-AC particles in ethanol/DMF mixture (9:1) (A) absence and (B) presence of $\text{Al}^{3+}$.

Fig. S8  Size histogram of PEGDMA/RB-AC particles.

Fig. S9  Job’s plot for RB-AC with $\text{Al}^{3+}$ ion.
**Author’s short biography**

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Kyo-Sun Ku received his B.Sc. (Advanced Organic Materials Engineering) in 2015 at Chungnam National University, Daejeon, South Korea, under the supervision of Prof. Dr. Young-A Son. He is currently a master candidate in the Department of Advanced Organic Materials and Textile System Engineering, Chungnam National University. His research interests lie in the synthesis of rhodamine, pyridine derivatives and their sensing applications towards cation and anion.

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Young-A Son is a professor in the Department of Advanced Organic Materials Engineering, Chungnam National University. He received his doctoral degree in color chemistry from the Department of Color Chemistry, University of Leeds, United Kingdom in 2001. His current research interests include luminescent organic materials, chemosensors, thermochromic dyes, color filter, functional dye materials and biosensors.