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), cyamine (Zheng et al., 2012) and boron dipyrromethene difluoride (BODIPY) (Li et al., 2012). Among these, xanthene derivatives are of great interest due to their excellent photophysical properties such as long absorption and emission wavelengths, large absorption coefficient and high fluorescence quantum yield (Beaumont et al., 1993). Rhodamine is a well known dye of xanthene derivative having spirolactam ring which is color less and non-fluorescent, whereas its ring opened amide form gives strong color and fluorescent emission. Based on this, many rhodamine derivatives have been used as a fluorescent probe for the detection of various metal ions (Kim et al., 2008). The sensing behavior of most of the rhodamine based fluorescent probes was studied in the solution (Zhang et al., 2012; Lee et al., 2015; Lee et al., 2010; Weerasinghe et al., 2009; Ju et al., 2011). In order to use these probes in the fields, it should be materialized. In recent years, the preparation of rhodamine functionalized fluorescent particles for the removal of metal ions received much interest (Jung et al., 2011).

Molecular imprinting polymerization (MIP) is also known as template polymerization and widely used method for synthesizing host polymer which has been recognizing target species. Due to their chemical and mechanical stability, simple preparation, low cost and high selectivity, MIP has been used in wide range of fields such as chromatographic separation, solid-phase extraction, catalysts and sensors (Yang et al., 2009; Andersson, 2000; Li and Husson, 2006). The imprinting mechanism is explained as the trapping of template molecule into the polymer matrix during polymerization as a result the molecular information of the template is retained in the polymeric material in the cross linked powders after complete extraction of the template from the matrix membranes. Two types of interactions between the template...
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\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^{+}\), Cd\(^{2+}\), Hg\(^{2+}\), Pb\(^{2+}\), Al\(^{3+}\) 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(vinylpyrrolidone) (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, M\(_{w}\) = 1,300,000) were used as the initiator and stabilizer, respectively.

2.2. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded in CDCl\(_3\) unless otherwise stated, with tetramethysilane (TMS) as internal reference at ambient temperature, mainly on a BRUKER AVANCE III 300 Magnet: Ascend TM series, 14.1 Tesla, \(^{1}H\) 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 an Agilent 8453 spectrophotometer. 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.SPI 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 \(^{1}H\) NMR and mass spectroscopy (Fig. S1 and S2).
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 ofethylene 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 was 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 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.

![Absorbance spectra of 10 μM of RB-AC in ethanol/DMF (9:1) in the presence of 10 μM of different metal ions.](image1)

![Fluorescence spectra of 10 μM of RB-AC in ethanol/DMF (9:1) in the presence of 10 μM of different metal ions.](image2)

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 color less 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 \(3\delta/k\) (Lohani et al., 2010). Where, \(\delta\) 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 didn’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 spirolactam amide carbonyl, imine (C=N) and pyridine (C=N) stretching vibrations, respectively. Which confirms the Al\(^{3+}\) was opened the spirolactam 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 enhancement 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 hydrazine binding site as well as the affinity of the pyridine group toward Al\(^{3+}\).

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Appendix: Supporting information
**Fig. S1** ¹H NMR spectrum of RB-AC.

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

**Fig. S3** UV-Vis spectra of 10 μM of RB-AC in ethanol/DMF (9:1) in the presence of different concentrations of Al³⁺. Inset: Absorbance at 560 nm as a function of the concentration of Al³⁺.

**Fig. S4** Fluorescence spectra of 10 μM of RB-AC in ethanol/DMF (9:1) in the presence of different concentrations of Al³⁺. Inset: Fluorescence at 580 nm as a function of the concentration of Al³⁺.
Fig. S5  Benesi-Hildebrand plot and equation for binding of Al\textsuperscript{3+}.

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

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

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

Fig. S9  Job’s plot for RB-AC with Al\textsuperscript{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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