A novel rhodamine-based colorimetric and fluorometric chemosensor for selective detection of Al$^{3+}$ ion in CH$_3$CN-H$_2$O solution

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Abstract: A novel 2-nitrobenzaldehyde appended rhodamine-based chemosensor (L) for selective detection of Al$^{3+}$ ions has been synthesized and characterized by various spectroscopic techniques. The chemosensor L showed high sensitivity towards Al$^{3+}$ ions without interference from other biologically significant cations examined in CH$_3$CN/H$_2$O (8 : 2, v/v) media by changing the color from colorless to deep pink which was very easily observed by naked eye. While other metal ions, alkali metal and alkaline earth metal cations did not induce such a change. The 1 : 1 binding stoichiometry of the metal complex was established by UV-Vis spectroscopy.

Keywords: Chemosensor, rhodamine B, UV-Vis absorption, association constant.

Introduction

Metal ions play vital roles in about one third of enzymes. Without the appropriate metal ion, a biochemical reaction catalyzed by a particular metalloenzyme would proceed very slowly, if at all. Therefore, a minute quantity of metal ions are essential for living organisms, but excessive accumulation of these metal ions in the body may cause fatal damage of several organisms. Therefore, development of molecular sensors for the selective detection of traces of these metal ions is of tremendous current research interest. In the last decade, the development of chromogenic and fluorogenic chemosensors for metal ions detection has attracted a great deal of attention in research because they allow a fast and selective detection of metal ions in solution by distinct color change and fluorescence enhancement, which can be clearly visible by simple naked-eye such that these sensors can be employed for selective detection of metal ions by simple naked-eye applications without the use of any expensive instruments. Thus, colorimetric sensor seems to be very promising candidate in the development of low costs, simple to use and in the field diagnostic tools for metal ions. Therefore, great efforts have been devoted in developing colorimetric chemosensor for sensing various metal ions. Aluminum is a non-essential element for living systems though extensively used in modern life. Excessive decomposition of Al$^{3+}$ in brain is believed to cause neuronal disorder leading to dementia, myopathy, Alzheimer’s disease and Parkinson’s disease, and dialysis encephalopathy. Besides these, the toxicity of aluminium is a hazard towards aquatic life and retards agricultural production in acidic soils. Therefore, developing fast and selective methods for Al$^{3+}$ detection is of vital significance in medical diagnostics, and has attracted
much attention. Among several methods\textsuperscript{8–11} available for the detection of Al\textsuperscript{3+}, spectrofluorimetric method\textsuperscript{12} has attracted a great deal of attention to the chemists, and a significant effort has been devoted to the development of Al\textsuperscript{3+}-selective fluorescent chemosensors because they allow a rapid detection of metal ions in solution with sufficient selectivity by simple absorption and emission enhancement or quenching without special instrumentation. During the last decade, many fluorescent probes for Al\textsuperscript{3+} have been reported for the purpose. Although fluorescent chemosensors such as Schiff bases\textsuperscript{13}, triazoles\textsuperscript{14}, triazole-pyridyl\textsuperscript{15}, calixarene\textsuperscript{16}, and secondary/tertiary amines\textsuperscript{17} and others have been reported for detecting Al\textsuperscript{3+}, unfortunately many of these turn-on sensors are faced with the following problems: (i) sensing mechanisms operate only in an organic solvent or at non-physiological pH, (ii) potential off-target responses and poor selectivity, (iii) limited bioactivity studies, (iv) behave as chemodosimeters i.e. irreversible binding to the receptor, and (v) interference caused by Fe\textsuperscript{3+} and Cu\textsuperscript{2+} with similar chemical properties. It must be noted that the poor coordination ability of Al\textsuperscript{3+} in compared to other transition metal ions makes the development of suitable and specific fluorescent chemosensors for Al\textsuperscript{3+} very daunting task\textsuperscript{18}. Consequently, the design and synthesis of a more versatile and new fluorescent chemosensors for Al\textsuperscript{3+} with a high selectivity and possessing sensitive signalling mechanism remains highly desirable.

We have taken care to design a 2-nitrobenzaldehyde-appended rhodamine based scaffold as a chemosensor to overcome these problems. Rhodamine B and its derivatives are extensively used as the potential fluorophoric and chromophoric chemosensor because of their excellent photophysical properties\textsuperscript{19}, where the signal transduction pathway mostly exploits its contrast structure-function correlation in lactonized-delactonized conformations\textsuperscript{20}. This unique structural feature makes rhodamine B a good candidate in constructing a “turn-on” fluorescent probe for Al\textsuperscript{3+}.\textsuperscript{21} Herein, we report a new turn-on colorimetric chemosensor based on 2-nitrobenzaldehyde appended rhodamine dye (L) (Scheme 1), which sensitively and selectively detects Al\textsuperscript{3+} in mixed solvent. It displays remarkably enhanced fluorescence intensity via CHEF process\textsuperscript{22} in a (HEPES) buffer [pH = 7.2] at 25°C and it shows prominent colour changes upon recognition of ions. The rhodamine B skeleton was used the potential fluorophore and chromophore. The 2-nitrobenzaldehyde was chosen as the recognizing group to get better selec-

\begin{center}
\textbf{Scheme 1.} Synthesis of chemosensor L.
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tivity and to facilitate the opening of the spirolactam ring of the Rhodamine moiety by electron attracting effect of ω-nitro group and generation of the delocalized xanthene moiety followed by the formation of complex by metal ion coordination.

Experimental

Materials:
All chemicals (rhodamine B, 2-nitrobenzaldehyde, ethanol, methanol, hydrazine hydrate etc.) were of reagent grade and purchased from Sigma-Aldrich Chemical Company, USA and used without further purification. All the solvents were of analytical grade.

Physical measurements:
Elemental analyses (carbon, hydrogen and nitrogen) were determined with a Perkin-Elmer CHN analyzer 2400. Melting points were determined using a Buchi 530 melting apparatus. The UV-Vis spectra of all samples were studied with UV-Vis spectrophotometer (Model: Evolution 201). Fluorescence spectra of the metal complex and the Schiff base ligand were measured with XenoFlash (Power Technology International) fluorimeter at room temperature (298 K). IR spectra (KBr pellet, 400–4000 cm⁻¹) were recorded on a Perkin-Elmer infrared spectrophotometer (Model: 883). ¹H NMR spectra were recorded on a Bruker spectrometer at 300 MHz in CDCl₃. Chemical shifts (δ values) were reported in ppm down field from internal Me₄Si.

Procedure for synthesis of chemosensor L:
Rhodamine B hydrazide was synthesized according to reported method²³. A mixture of rhodamine B hydrazide (0.50 g, 1.095 mmol) and 2-nitrobenzaldehyde (0.165 g, 1.095 mmol) was taken in 20 ml of dry methanol. The resulting solution was refluxed for 6 h using a fused CaCl₂ guard tube. The resulting maroon color solution was cooled to room temperature and then, the volume of the solution was reduced to 5 mL. The resulting precipitate was filtered off and washed with methanol/ether (1 : 1) by three times and dried under vacuum. Yield: 0.31 g (55%), m.p. 196 ºC; ¹H NMR (300 MHz, CDCl₃) (Fig. S1) δ (ppm): 1.14–1.19 (12H, t, N-CH₂-C₃H₈), 3.31–3.37 (8H, d, N-C₃H₂-CH₃, J 6 Hz), 6.26–6.28 (2H, d, xanthene-H, J 9 Hz), 6.50–6.56 (4H, m, xanthene-H), 7.12–7.14 ((1H, d, Ar-H, J 6 Hz), 7.35–7.40 (1H, t, Ar-

Fig. S1. Proton NMR spectra of the sensor L.
H, J 9 Hz), 7.46–7.55 (3H, m, Ar-H), 7.84–7.86 (1H, m, Ar-H), 8.03–8.05 (1H, d, Ar-H, J 6 Hz), 8.17–
8.20 (1H, d, Ar-H, J 9 Hz), 8.83 (1H, s, Ar-CH=N-);
ESI-MS : m/z Calcd. for C_{35}H_{35}N_{5}O_{4} [M+H]^+ (m/z) : 590.27, Found 590.54 (100 %) (Fig. S2). Anal. Calcd.
for C_{35}H_{35}N_{5}O_{4} : C, 71.29; H, 5.98; N, 11.88; Found : C, 72.53; H, 5.79; N, 11.85.

Association constant :
The binding constant of the [L-Al^{3+}] was evaluated from the spectrophotometric titration data using the well
known Benesi-Hildebrand (B-H) plot (eq. (1)).

\[
1/(A - A_o) = 1/[K(A_{\text{max}} - A_o)C] + 1/(A_{\text{max}} - A_o) \quad (3)
\]

where, \(A_o\) is the absorbance of L at absorbance maxima (\(\lambda_{\text{max}} = 558\ nm\) in the presence of Al^{3+} ion, \(A\) is the observed absorbance at that particular wavelength in the presence of a certain concentration (say, \(C\)) of the Al^{3+} ion. \(A_{\text{max}}\) is the maximum absorbance that was obtained at \(\lambda_{\text{max}} = 558\ nm\) during titration with varying concentration of metal ions, \(K\) is the association constant (M^{-1}) which was determined from the slope of the plot of 1/
\((A - A_o)\) versus 1/[Al^{3+}] and \([C]\) is the concentration of the Al^{3+} ions successively added during titration studies.

The goodness of the linear fit of the B-H plot of 1/(\(A - A_o\)) versus 1/[Al^{3+}] for 1 : 1 complex formation con-

Procedure for metal ion sensing :
The stock solutions of chemosensor L and metal salts of Na^{+}, K^{+}, Ba^{2+}, Mn^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+},
Co^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and Al^{3+} were prepared in CH_{3}CN-water (8 : 2, v/v) using HEPES buffer,
pH 7.2 at 25 °C. In titration experiments, each time a 2.5
ml solution of L (40 \(\mu\)M) was filled in a quartz cuvette (path length, 1 cm) and metal ions (100 \(\mu\)M) were added
into the quartz cuvette by using a micro-pippet. The re-
sulting solution was shaken well and incubated for 30
min at room temperature before recording the UV-Vis
spectra. For fluorescence spectral studies, same solutions
taken into the quartz cuvette were placed inside the fluo-
rometer in which excitation was done at 350 nm.

Results and discussion

Synthesis :
Chemosensor L was synthesized (Scheme 1) from rhodamine B by a two-step reactions sequence and char-
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characterized through were characterized by CHN, IR and mass spectrometry technique. In the first step of synthesis, rhodamine B was reacted with hydrazine hydrate to yield rhodamine hydrazide (RBH) following literature procedure. In the next step, chemosensor L was prepared as light yellow crystalline solid by refluxing RBH in the presence of 2-nitrobenzaldehyde using dry methanol as the solvent.

Absorbance studies:

We have studied the absorbance responses of the chemosensor L towards Al\(^{3+}\) ion and other cations in aqueous CH\(_3\)CN solution. Initially the chemosensor behavior of L was investigated by monitoring the UV-Vis absorption spectral behavior upon addition of various metal ions such as Na\(^+\), Mn\(^{2+}\), Mg\(^{2+}\), Al\(^{3+}\), Pb\(^{2+}\), Hg\(^{2+}\), K\(^+\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cu\(^{2+}\) and Cd\(^{2+}\) ions to the 40 \(\mu\)M solution in CH\(_3\)CN-H\(_2\)O (8 : 2, v/v), pH = 7.2] at 25 \(^\circ\)C.

As shown in the Fig. 1, in the absence of metal cations, chemosensor L exhibited no absorption peak at 558 nm. This observation is indicating that the rhodamine core is in the ring closed isomeric form. Our preliminary quantitative experiment revealed that the addition of 100 \(\mu\)M of metals ions such as Na\(^+\), Mn\(^{2+}\), Mg\(^{2+}\), Pb\(^{2+}\), Hg\(^{2+}\), K\(^+\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), and Cd\(^{2+}\) ions to the 40 \(\mu\)M solution in CH\(_3\)CN-H\(_2\)O (8 : 2, v/v), resulted no detectable changes the UV absorption spectrum of L. Although a weak absorption band of L was observed at \(\lambda_{\text{max}} = 553\) nm in the presence of Cu\(^{2+}\) ions, this perturbation was poorly detected by the “naked-eye” inspection (Fig. 3a). However, the most remarkable spectroscopic and visual response of L was observed in the UV-Vis spectrum of L upon addition of Al\(^{3+}\) ions. The appearance of a strong absorption band of L at \(\lambda_{\text{max}} = 558\) nm in the absorption spectrum suggests that the absorption changes may be observable to the naked-eye. And the result is that the colorless sensor solution immediately turned to pink solution when Al\(^{3+}\) ion was introduced into it and the pink color produced was sufficiently dark enough to be detected through the naked-eye (Fig. 3a). These results suggest that molecule L could be exploited as a colorimetric “naked-eye” sensor for Al\(^{3+}\) ion discrimination among various typical transition-metal ions. The relative intensity of the absorption bands of the chemosensor L upon addition of aforementioned metal ion is also shown by the column plot as shown in the Fig. 2.

![Fig. 1](image-url) UV-Vis spectral nature of L (40 \(\mu\)M) upon addition of different metal ions (Na\(^+\), Mn\(^{2+}\), Mg\(^{2+}\), Al\(^{3+}\), Pb\(^{2+}\), Hg\(^{2+}\), K\(^+\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cu\(^{2+}\), and Cd\(^{2+}\)) in a HEPES buffer [50 \(\mu\)M, CH\(_3\)CN-H\(_2\)O (8 : 2, v/v), pH = 7.2] at 25 \(^\circ\)C.

![Fig. 2](image-url) Column plot for the UV-Vis spectral intensity of L (40 \(\mu\)M) upon addition of different metal ions (Na\(^+\), Mn\(^{2+}\), Mg\(^{2+}\), Al\(^{3+}\), Pb\(^{2+}\), Hg\(^{2+}\), K\(^+\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cu\(^{2+}\), and Cd\(^{2+}\)) in a HEPES buffer [50 \(\mu\)M, CH\(_3\)CN-H\(_2\)O (8 : 2, v/v), pH = 7.2] at 25 \(^\circ\)C.

Upon exposure by a UV lamp of long wavelength (360 nm), the solution of L in the presence of Al\(^{3+}\) showed a dramatic color change from pink to bright orange which could easily be detected by the “naked-eye” (Fig. 3b). Similar UV exposure turned the solution of L itself and the solution of L in presence of Cu\(^{2+}\) ions to colourless. Appearance of a new absorption spectral band at \(\lambda_{\text{max}} = \)}
558 nm and an enhancement in intensity by UV exposure on binding to these metal ions also suggests the opening of the spirolactam ring and generation of the delocalized xanthene moiety followed by the formation of Al\(^{3+}\)-complex by metal ion coordination.

The chemical reversibility behaviour of chemosensor \(L\) was also studied to examine the reusability of the chemosensor. Now to demonstrate this fact the absorption titration experiment was conducted under UV-Vis spectrometer. Up on gradual addition of Al\(^{3+}\) ions (0–100 \(\mu\)M) to chemosensor \(L\) (40 \(\mu\)M) in \(\text{CH}_3\text{CN}-\text{H}_2\text{O}\) (8 : 2, v/v) generation of an absorption band in the spectral position at \(\lambda_{\text{max}} = 558\) nm was observed along with the gradual increase in the absorption intensity (Fig. 4) and the formation of the pink color which may be due to the opening of spirolactum ring of the rhodamine moiety to the delocalized xanthene moiety followed by the formation of complex by metal ion coordination (Scheme 2). The absorption titration of the resulting pink colour solution of the \(L\)-Al\(^{3+}\) complex with \(\text{Na}_2\text{EDTA}\) was also carried out. From this experiment it was observed that the intensity of the absorption band at \(\lambda_{\text{max}} = 558\) nm was gradually decreased (Fig. 5), and the pink colour of \(L\)-Al\(^{3+}\) complex was disappeared with increasing concentration of \(\text{Na}_2\text{EDTA}\). This disappearance of the pink colour indicates the decomplexation of \(L\)-Al\(^{3+}\) complex as \(\text{Na}_2\text{EDTA}\) strips away Al\(^{3+}\) from the binding zone with concomitant closing of the delocalized xanthene moiety to regenerate the spirolactum ring. The above results indicated that the compound \(L\) could be repeatedly used for selective and sensitive naked-eye detection of Al\(^{3+}\) ions.

To check the practical applicability of chemosensor \(L\) as a selective sensor for detection of Al\(^{3+}\) ion, we have carried out competitive experiments in the presence of Al\(^{3+}\) ions mixed with other different metal ions (Na\(^{+}\), Mn\(^{2+}\), Mg\(^{2+}\), Pb\(^{2+}\), Hg\(^{2+}\), K\(^{+}\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Cu\(^{2+}\), and Cd\(^{2+}\)). The absorbance response of the \(L\)-Al\(^{3+}\) system remains the same by comparison with or without the other metal ions (Fig. 6).
These findings confirmed the selectivity and effective interaction of sensor L with Al$^{3+}$.

To explore the binding mechanism, the Job’s plot of the UV-Vis titrations of Al$^{3+}$ ion with a total concentration of 20 µM was revealed as shown in Fig. 7. A maximum absorption was observed when the molar fraction reached 0.5, which is indicative of a 1 : 1 stoichiometric complexation between L and Al$^{3+}$ ion for the newly formed species. The association constant of the newly formed complex at 298 K was then determined as 4.9264 × 10$^4$ M$^{-1}$ with a good linear relationship with $R^2 = 0.99514$ by a 1 : 1 binding mode (Fig. 8). The possible binding mode of L and Al$^{3+}$ ion is shown in Scheme 2. In the [L-Al$^{3+}$] complex, the metal ion possibly coordinates with the nitrogen of the C=N Schiff group as well as the amide oxygen atom. It was further supported by the fact that the characteristic carbonyl amide stretching frequency appeared at 1720 cm$^{-1}$ in the IR spectrum of L was shifted to 1687 cm$^{-1}$ in the IR spectrum of L-Al$^{3+}$ complex.
**Fluorescence studies:**

The fluorescence behaviour of the sensor L and its complex were also studied. Upon excitation at 350 nm, the fluorescence spectrum of L (40 μM) in CH$_3$CN-H$_2$O (8 : 2, v/v) buffered with HEPES, pH = 7.2, and in the presence of various metal ions such as Na$^+$, Mn$^{2+}$, Mg$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, K$^+$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, and Cd$^{2+}$ exhibited no observable emission band above 500 nm, indicating the existence of only the lactam form in this media.

As shown in Fig. 9, the presence of Al$^{3+}$ ion resulted in a remarkable enhancement of fluorescence emission intensity at $\lambda_{\text{max}} = 582$ nm. Whereas addition of Cu$^{2+}$ ion into the sensor solution, a much weaker spectral response was observed relative to Al$^{3+}$ at the same concentration. The observed fluorescence occurred due to the metal binding event induced an electronic rearrangement within the dye that opened the spirolactam ring and yielded a highly conjugated rhodamine dye. The solution showed an intense orange fluorescence, with an approximately 50-fold enhancement in fluorescence intensity. Up on gradual addition of Al$^{3+}$ ions (0–100 μM) to chemosensor L (40 μM) in CH$_3$CN-H$_2$O (8 : 2, v/v) generation of an emission band in the spectral position at $\lambda_{\text{max}} = 582$ nm was observed along with the gradual increase in the emission intensity. The Fig. 10 shows the dependence of the emission intensity at 582 nm on increase in concentration of Al$^{3+}$ ion. To examine the reversibility behaviour, the fluorescence titration of the sensor L (40 μM) using Na$_2$EDTA was conducted in CH$_3$CN-H$_2$O (8 : 2, v/v, pH = 7.2) solution. The experimental result (Fig. 11) showed that in the presence of an excess amount of Na$_2$EDTA the bright fluorescence immediately turned off and the pink color of the L-Al$^{3+}$ complex solution disappeared. These findings imply reversible binding of sensor L with Al$^{3+}$ ion and that the sensor L could be used as an ‘off–on’ fluorescent chemosensor for Al$^{3+}$.
Fluorescence quantum yields ($\Phi_f$) of the sensor $L$ and its complex were also calculated using anthracene as the reference with a known $\Phi_f$ (0.27 in ethanol solvent)$^{25}$, according to the following equation$^{26}$:

$$\Phi_S/\Phi_R = [A_S/A_R][\text{Abs}_S/\text{Abs}_R][\eta_S^2/\eta_R^2]$$

where $\Phi_S$ and $\Phi_R$ are the fluorescence quantum yield of the sample and reference, respectively; $A_S$ and $A_R$ are the area under the fluorescence spectra of the sample and the reference, respectively; $\text{Abs}_S$ and $\text{Abs}_R$ are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation; $\eta_S$ and $\eta_R$ are the refractive index of the sample and the reference, respectively.$^{27}$

Since the sensor $L$ has a too low emission intensity to calculate the quantum yield, we have only calculated quantum yields for the [L-A$^{3+}$] complex and the fluorescence quantum yield ($\Phi_S$) of [L-A$^{3+}$] complex was found to be 0.31. From this quantum yield data it is clear that the quantum yields of the complex was increased with enhancement of the fluorescence intensity.

**Effects of pH :**

As pH dependence of fluorescence is undesirable in biological applications, the effect of pH on the fluorescence response of this new sensor $L$ was investigated. Experimental result showed that no obvious characteristic absorption or fluorescence could be observed for sensor $L$ between pH = 5–12 (Fig. S3). However, upon addition of A$^{3+}$ ion, sensor $L$ responds stably to A$^{3+}$ ion within this pH range and we did not observe any interference by protons. Thus, we can conclude that the sensor $L$ has optimal sensing response at physiological pH suggesting that this sensor could be promising for biological applications.

**Conclusion**

In summary, we have developed a novel colorimetric and fluorometric chemosensor $L$ based on rhodamine B derivative which exhibits highly selective and sensitive recognition toward A$^{3+}$ ion. Interaction of A$^{3+}$ with sensor $L$ enhanced the UV-Vis absorption at $\lambda_{\text{max}} = 558$ nm in mixed solvent and induced a turn-on response which was clearly visible to the naked-eye. That is the recognition of A$^{3+}$ ion gave rise to color change from colorless to pink in aqueous CH$_3$CN solution without resort to any spectroscopic instrumentation. We believe that this sensor with sensitive and selective naked-eye responses can be used for many practical applications in chemical, environmental and biological systems.

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