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Synthesis of Rhodamine based turn-on Fluorescent Sensors for the Detection of Chromium Ions

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ABSTRACT

Selective and sensitive rhodamine-based fluorescent Cr^{3+} sensor, A. ESI mass spectrometry, NMR, and elemental analysis were used to analyze Sensor A, which has been synthesized in high yield. Changes in fluorescence and absorption were used to assess its binding with different metal ions (Ca^{2+}, Na^{+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, Ni^{2+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Cr^{3+}, Cd^{2+}, Zn^{2+}, Sn^{2+}, and Hg^{2+}). Compared to other metal ions, A exhibits good sensitivity and high selectivity for Cr^{3+}. The selectivity regarding A is confirmed by its high binding constant value with Cr^{3+}. According to 1H NMR peak broadening tests, Cr^{3+} binds to the sensor's hydroxyl groups and the imine group's nitrogen. Cr^{3+} binding to sensor A's carbonyl oxygen is confirmed by the bright pink color that appears following Cr^{3+} addition. The fluorescence enhancement as dosed the new absorption beyond 400 nm is further confirmed by the quantum yield values for the Cr^{3+}-bound form and the ring-opened form of sensor A. According to the findings, the formation regarding the ring-opened form of sensor A upon Cr^{3+} binding is the mechanism responsible for the fluorescence enhancement and new absorption. It was found that A's sensitivity limit to Cr^{3+} was 568 nM. With excess EDTA, the pink color goes away and the binding of Cr^{3+} is reversible. As a result, this sensor could be employed as a reversible fluorescent sensor for Cr^{3+} that is visible to the human eye.

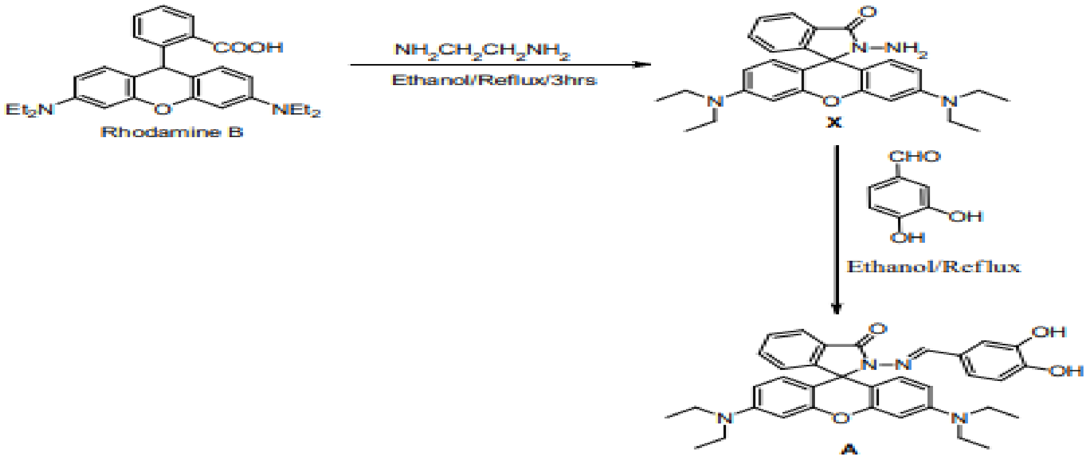
Keywords: Chromium Ion Detection, Rhodamine, Turn-On Fluorescent Sensor.

1. INTRODUCTION

Biological, environmental, biochemical, and analytical sciences have all shown a great deal of interest in the development of chemosensors for selective detection regarding metal ions.1 Although trivalent chromium (Cr^{3+}) is a pollutant in the environment, its detection is significant since it is necessary for many biological processes [1-6]. In mammalian body, Cr^{3+} is a necessary micronutrient that is crucial for the metabolism of proteins, carbohydrates, lipids, and nucleic acids [7]. It is crucial for preserving the human glucose tolerance factor, which is necessary for the stabilization of proteins as well as nucleic acids and the activation of certain enzymes [8]. Adults need between 50 and 200 µg of chromium per day, which is the estimated safe and adequate daily dietary intake (ESADDI) [7,9]. Because low levels of Cr^{3+} in the body impair immunological function and raise circulation levels of insulin, triglycerides, glucose, and total cholesterol, they raise the risk of diabetes and cardiovascular diseases [10]. Irreversible damage to cellular structures and functional ions could result from high quantities of Cr^{3+}, which is toxic [9,10].

Chromium has many industrial applications and it is used in the leather tanning, refractory industries, ceramic and glass industries, and in the manufacture of catalysts, fungicides, photography, wood treatment, pigments and paints, in photography and wood treatment, and in the making steel and other alloys.[6] It is this ubiquity of use that makes it a significant pollutant [11].

Chromium is essential because of the variety of roles it plays in biological systems, environment, and the industries. Since Cr^{3+} is paramagnetic, it quenches fluorescence and is regarded as one of the best fluorescent quenchers. Consequently, the development of fluorescence sensors for Cr^{3+} was inadequate [12]. In recent years, rhodamine compounds were used more often for Cr^{3+} sensing. Lately, Dhara et al. combined rhodamine B hydrazide with a benzo[d] [1,3] dioxole-4-carbaldehyde in a single molecule to create a turn-on fluorescent sensor for Cr^{3+}.9 [13]. This sensor exhibits low sensitivity for Fe^{3+} and Cu^{2+} and very high sensitivity for Al^{3+} along with Cr^{3+}. Another rhodamine-based FRET sensor for Cr^{3+} has been created by Lohar et al., however it also exhibits cross-sensitivity to Fe^{3+}. 10 [14-17]. A sensor created by Weerasinghe et al. exhibits cross sensitivity to Zn^{2+}, Hg^{2+}, and Pb^{2+}, yet is extremely sensitive to Cr^{3+} [18].



2. EXPERIMENTAL

2.1 Chemicals

Purchased from Sigma Aldrich, all of the compounds were of reagent quality and were utilized without additional purification. The buffer solution ions were created using nanopure water.

2.2 Synthesis of A

Ethanol (15 ml) was used to dissolve Compound X (0.46g, 1.00 mmol) and 2,3-dihydroxybenzaldehyde (0.14g, 1.00 mmol). A pink precipitate developed during the six hours that the reaction mixture was refluxed as well as refluxed. Filtration was used for separating the precipitate, and three 10 mL ethanol washes were performed. Following drying, a light pink solid in 90% yield has been obtained. Mp = 227-229 °C. ^{1}H NMR (400 MHz, CD_3CN): δ 11.07 (1H, OH.s), 8.85 (1H, s), 7.93 (1H, d, J = 6.4 Hz), 7.60 (1H, t, J = 7.3 Hz), 7.56 (1H, t, J = 7.7 Hz), 7.09 (1H, d, J = 7.1Hz), 6.82 (1H, d, J = 7.8 Hz), 6.70 (1H, t, 7.8Hz), 6.65 (1H, d, J = 7.8 Hz), 6.51 (2H, d, J = 8.8 Hz), 6.44 (2H, s), 6.40 (1H, OH. s), 6.35 (2H, d, 9.0Hz), 3.34 (8H, q, J = 7.0 Hz), 1.11 (12H, t, J = 7.0 Hz). ^{13}C NMR (100 MHz, CDCl3): δ 164.4, 153.5, 151.8, 151.1, 149.2, 145.3, 144.8, 133.7, 129.7, 128.7, 128.2, 124.2, 123.5, 122.3, 119.2, 118.4, 116.2, 108.3, 105.1, 97.9, 66.3, 44.4, 12.6. Analysis Calcd. for: $C_{35}H_{36}N_4O_4$: C, 72.90; H, 6.29; N, 9.72; O, 11.10. Found: C, 73.13; H, 6.73; N, 10.20. MS: m/z = 577.2.

2.3 Methods

A Shimadzu UV 2101 PC absorption spectrometer was used to evaluate optical absorption at room temperature. An Edinburgh F-2500 spectrofluorimeter was used for measuring fluorescence. Nitrates [$Zn(NO_3)_2$, $Cr(NO_3)_3$, $Hg(NO_3)_2$, $Pb(NO_3)_2$, $NaNO_3$] or chlorides [$CuCl_2$, $MnCl_2$, $FeCl_3$, $SnCl_2$, $CoCl_2$, $AlCl_3$, $CdCl_2$, $FeCl_2$, $CaCl_2$, $BaCl_2$, AgCl, KCl] in acetonitrile have been used for preparing stock solutions of metal ions (4 10-4 M). A produced a colorless solution in the 98% CH_3CN, 2% 0.01 M Tris-HCl buffer system (pH = 7.00) used for all spectroscopic investigations. Optical spectroscopy methods were used to assess the metal ion binding characteristics and selectivity of A (20 µM in 98% CH_3CN, 2% 0.01 M Tris-HCl buffer solution at pH 7) with the use of the metal ions listed above. A's selectivity was assessed independently for each of the designated metal ions. A's selectivity for Cr^{3+} was greater. For every emission study, 520 nm was the excitation wavelength. The widths of the excitation and emission slits were maintained at 2 nm. A JEOL Eclipse (400 MHz) equipment was used to acquire all of the 1H NMR, ^{1}H-^{1}H COSY NMR, and ^{13}C NMR spectra. A Thermo Scientific LTQ spectrometer was used to record the ESI mass spectra. A recognized method for determining relative quantum yields was used to assess the quantum yields of sensor A attached to Cr^{3+} and sensor A in its spirolactaum ring-opened form.15 The standard was rhodamine B in acetonitrile. At the excitation wavelength, the absorbance was limited to ≤0.1. The quantum yield was after that measured using the next relation:

$$\phi_{F,sample} = \phi_{F,std} \times \frac{\int I_{sample}(\bar{v})d\bar{v}}{\int I_{std}(\bar{v})d\bar{v}} \times \frac{A_{std}}{A_{sample}} \times \frac{\eta_{sample}^{2}}{\eta_{std}^{2}}$$

In which, ΦF is the fluorescence quantum yield, $\int Isample(\bar{v})d\bar{v}$ represent the area under the curve of fluorescence versus wavenumber, A represent the absorbance at the excitation wavelength and η the refractive index of the solvent. These measurements might be expected to have some experimental error (close to 5%) due to the sensitivity of the fluorescence spectrophotometer and other environmental conditions.

3. RESULTS AND DISCUSSIONS

3.1 synthesis of A

Through refluxing X with 3,4-dihydroxybenzaldehyde in ethanol, compound A was synthesized in high yield, as illustrated in Scheme 1. Compound 5 A's structure was verified with the use of ESI mass spectrometry, elemental analysis, ^{13}C NMR, and ^{1}H NMR [9].

3.2 Preparation of Metal Ion Solutions for UV Absorption and Emission Studies

With the use of the proper metal nitrates [$Zn(NO_3)_2$, $Cr(NO_3)_3$, $Hg(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, and $NaNO_3$] or chlorides [$CuCl_2$, $MnCl_2$, $FeCl_3$, $SnCl_2$, $CoCl_2$, $AlCl_3$, $CdCl_2$, $FeCl_2$, $CaCl_2$, $BaCl_2$, KCl, and AgCl] in acetonitrile, stock solutions of metal ions (4×10^{-4} M) were prepared. In order to create a colorless buffer, Compound A's stock solution (1.73 mM) was made in 98% CH_3CN and 2% 0.01M Tris-HCl. A 20M solution in 98% CH_3CN, 2% 0.01M Tris-HCl buffer, and the proper concentrations of metal ions have been used for spectroscopic investigations.

3.3 Steady-State Optical Properties

The 98% CH_3CN, 2% 0.01 M Tris-HCl buffer solution (pH = 7.00) was used for the spectroscopic investigations. In that solvent system, Compound A (which is bright pink in the solid state) created a colorless solution and proved to be extremely stable for almost a week. There was no peak above 400 nm in the absorption spectrum regarding A in the solvent system described above, suggesting that ring-closed spirolactone predominates. Furthermore, upon excitation at 520 nm, a very weak fluorescence signal was detected at around 580 nm, confirming the existence of ring-closed spirolactone. The characteristic carbon resonance at δ 66.3 ppm provides more evidence for the existence of the ring-closed form.

3.4 Selectivity to Metal Ions - Optical Absorption and Fluorescence Measurements

Figure 1 exhibits the absorption properties regarding A with different metal ions. In the non-presence of metal ions A has no absorption beyond 400 nm. Adding Cr^{3+} led to the appearance of a relatively intense peak centered at 558 nm with a shoulder around 525 nm. The solution also turned bright pink upon the addition of 1 equivalent of with Cr^{3+}. It also showed a very weak response with the addition of 1 equivalent of Sn^{2+}, but, did not show any change upon the addition of other metal ions. As shown in Figure 2, sensor A shows a 54-fold absorbance enhancement upon adding 1 one equivalent of Cr^{3+} and a 3-fold absorbance enhancement with 1 one equivalent of Sn^{2+}. Compared to the absorbance enhancement due to Cr^{3+}, the absorbance enhancement due to other metal ions (1 equivalent) is negligible. This confirms the higher selectivity of A towards Cr^{3+} over other metal ions, a major characteristic of a good sensor [19].

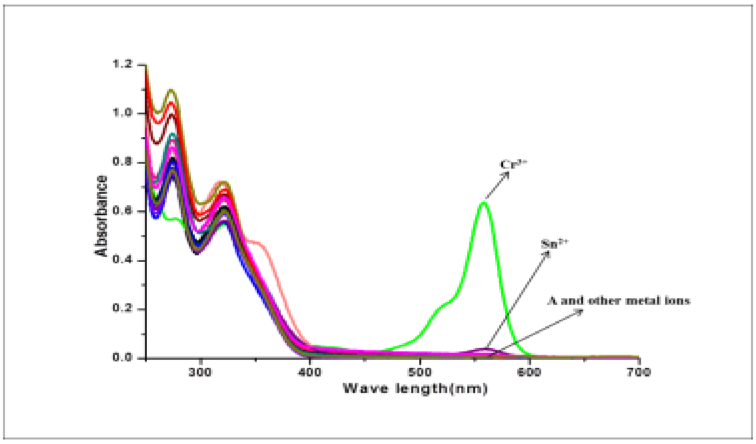


Fig.1. Absorbance changes of A (20 µM) with Na$^+$, K$^+$, Mg^{2+}, Ba^{2+}, Ca^{2+}, Al^{3+}, Ag$^+$, Cr^{3+}, Mn^{2+} , Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} (20 µM) in 98% CH_3CN, 2% 0.01 M Tris-HCl buffer

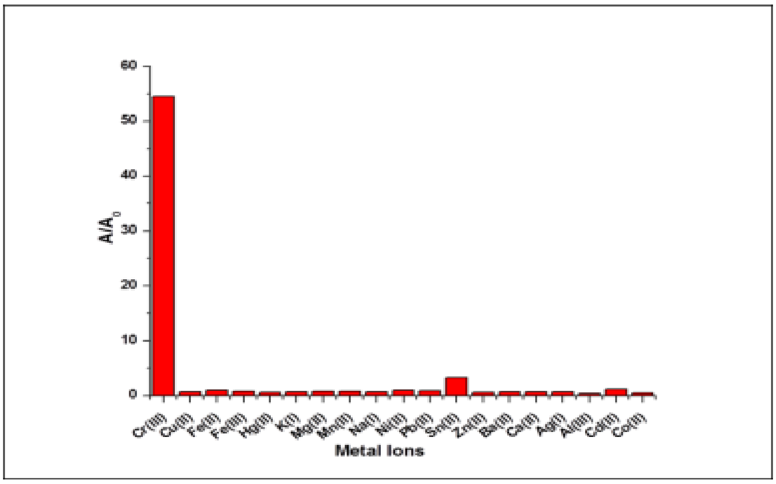


Fig.2. Absorption enhancement of A (20 µM) at 558 nm with metal ions (20µM)

To learn more about A's selectivity for various metal ions, fluorescence measurements have been conducted. Prior to the addition of metal ions, compound A exhibited extremely little fluorescent fluorescence, as seen in Fig 3, indicating that the ring closed form regarding A is predominant. All of metal ions (K$^+$, Na$^+$, Ba^{2+}, Mg^{2+}, Al^{3+}, Ca^{2+}, Cr^{3+}, Ag$^+$, Fe^{3+}, Mn^{2+}, Co^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Sn^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}) did not cause any change in A's fluorescence, with the exception of Sn^{2+} and Cr^{3+}. When 1 equivalent of Cr^{3+} was added, A displayed a very high fluorescence enhancement along with a dramatic color change from colorless to bright pink. When 1 equivalent of Sn^{2+} was added, A showed a very slight enhancement. Fig 4 illustrates that the fluorescence enhancement of A is 23 times greater with Cr^{3+} and 2 times higher with Sn^{2+}. This demonstrates once more how selective A is for Cr^{3+} in comparison to the other metal ions [20]. The formation regarding ring-opened spirolactam form of sensor A. is confirmed by the observed enhancements in both absorption as well as fluorescence with Cr^{3+}, and the significant color change from colorless to bright pink. Those observations also demonstrate that Cr^{3+} complexes with A.

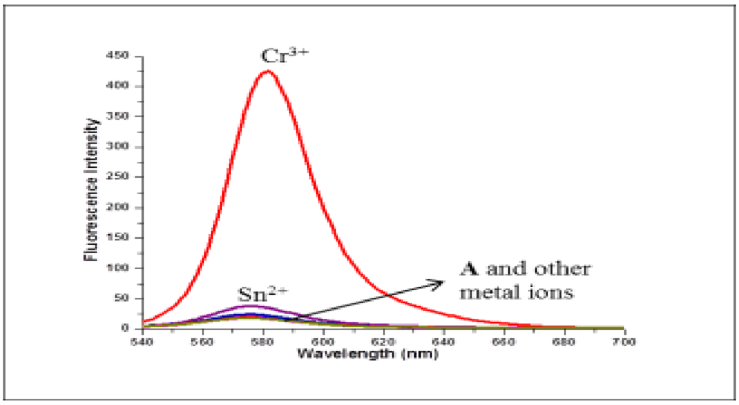


Fig.3. Fluorescence spectra of A (20 µM) with different metal ions (20 µM) in 98% CH_3CN, 2% 0.01 M Tris-HCl buffer (λex = 520 nm)

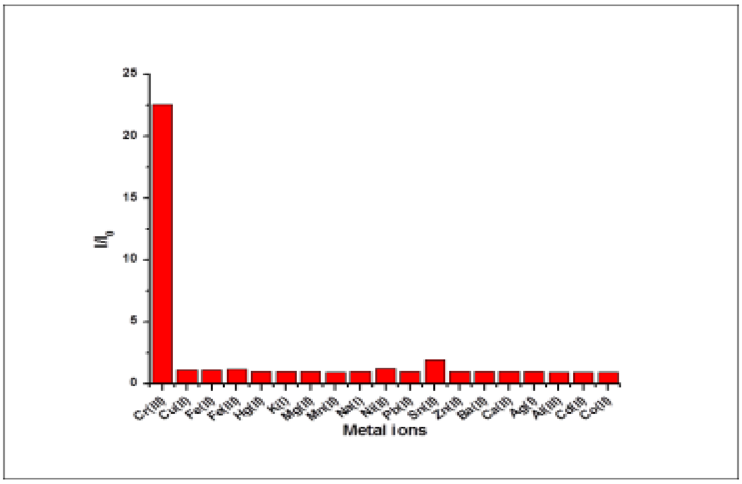


Fig.4. Fluorescence enhancement of A (20µM) at 580 nm with different metal ions (20 µM) in 98% CH_3CN, 2% 0.01 M Tris-HCl buffer (λex = 520 nm)

3.5 Binding Constants and Sensitivity to Cu^{2+}

From a Benesi-Hildebrand analysis [21]. the binding constant of sensor A with Cr^{3+} has been found to be $7.57 \times 10^4 \pm (1260)\ M^{-1}$ (i.e. a 1.6% error), Figure 6. Sensitivity measurements were performed with the addition of very small increments of Cr^{3+} to sensor A. The sensitivity was assigned as the concentration at which there is a threefold enhancement of the fluorescence. From the results of the analysis (Figure 5 and its inset), the sensitivity of A toward Cr^{3+} was determined to be 568 nM.

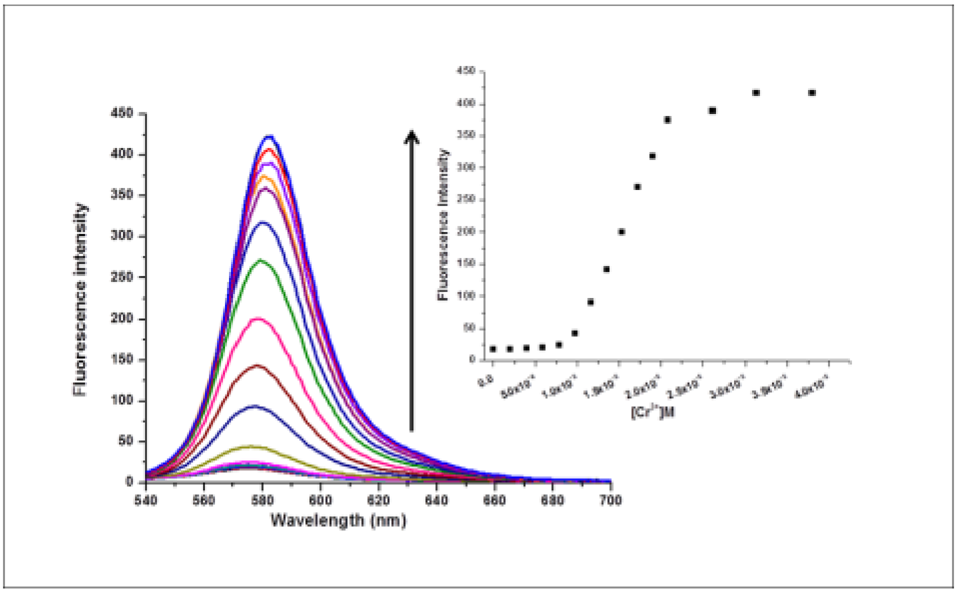


Fig.5. Fluorescence spectra of A (20 µM) with Cr^{3+} ions (0-31 µM) in 98% CH_3CN, 2% 0.01M Tris-HCl buffer. Inset: Fluorescence enhancement at 580 nm as a function of Cr^{3+} concentration

3.6 Mechanism of Cr^{3+} binding

3.6.1 Quantum yield values

The observation of turn-on fluorescence of the sensor with the existence of Cr^{3+} is quite interesting as the paramagnetism of the Cr^{3+} ion enhances the rate of intersystem crossing, which should normally quench the fluorescence. To explore the mechanism behind this unique sensitivity, further fluorescence measurements were carried out for both the ring-opened form of A and the Cr^{3+} –bound form, using Rhodamine B in acetonitrile as the standard. The results show that the ring-opened form related to sensor A has a quantum yield of 0.093 while the quantum yield of Cr^{3+} –A is 0.065. These quantum yield values further confirm the formation of ring opened form related to sensor A upon Cr^{3+} binding.

3.6.2 NMR Measurements

^{1}HNMR measurements were carried out in CD_3CN and in DMSO-d_6 to understand the binding of Cr^{3+} to sensor A. The ^{1}HNMR spectrum of sensor A in CD_3CN shows only one broad peak for one of the OH groups. For confirming the binding of Cr^{3+} to both OH groups of sensor A, another NMR titration was performed in DMSO-d_6 which further confirmed the formation of spirolactum ring-opened form regarding sensor A.

As shown in Fig.6, ^{1}HNMR peaks for two OH groups at 9.2 ppm and at 9.4 ppm were broadened and disappeared with the addition of 1 equivalent of Cr^{3+}. This confirms the binding of Cr^{3+} to both OH groups of sensor A [22-25] . The peak height of the peak for imine hydrogen at 8.65 ppm also decreased significantly and the peak was broadened with the addition of 1 equivalent of Cr^{3+}. This confirms the binding of Cr^{3+} with imine hydrogen of sensor A [21]. As the color of sensor A changes from colorless to bright pink upon Cr3+ addition, it also binds with oxygen of the spirolactam ring

The pink color of A-Cr^{3+} complex disappeared upon adding excess EDTA, confirming the reversibility of Cr^{3+} binding. Reversibility is another key advantage of this sensor.

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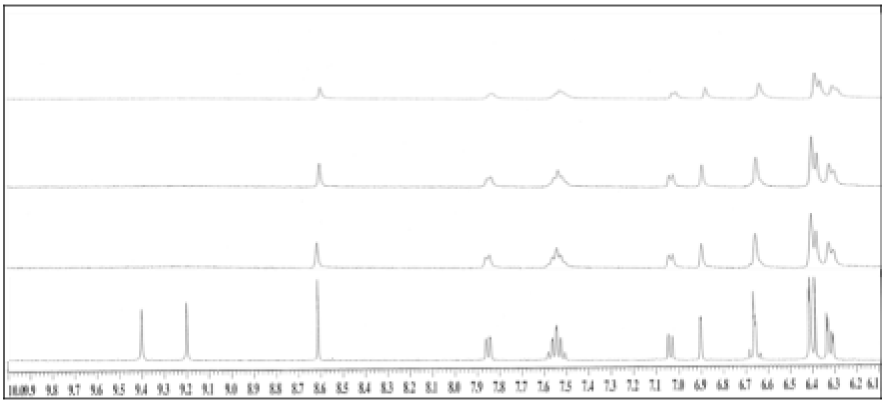

Fig.6. ^{1}HNMR titration of A with Cr^{3+} (0, 1, 3, and 6 equivalents from bottom to top) in DMSO-d_6

3.6.3 Binding ratio

With the use of job's plot, the binding ratio between Cr^{3+} and sensor 5 is found to be 1:1.

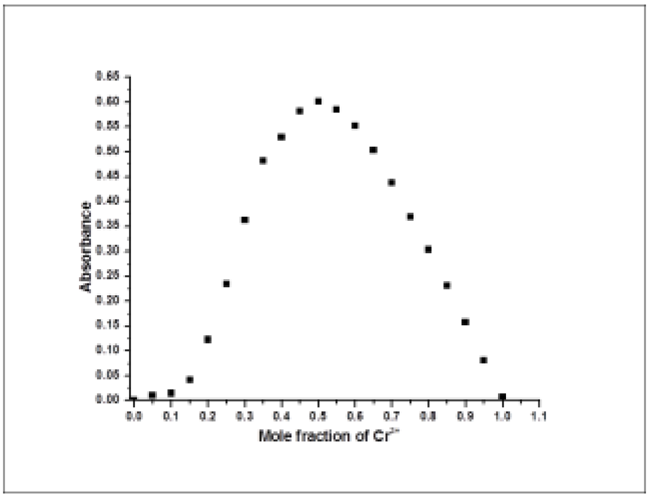

Fig.7: Job's plot of A (10 µM) with Cr^{3+} (10 µM) in CH_3CN

3.7 Possible Cr^{3+} binding model

The formation regarding the spirolactam ring opened form of sensor A is indicated by the observed improved fluorescence, new absorption beyond 400 nm, clear color change from colorless to bright pink, high quantum yield value, and ^{1}HNMR measurements with the existence of Cr^{3+}. The Job's plot indicates 1:1 complex formation of Cr^{3+} with sensor A. Based on all these observations, the possible mechanism of Cr^{3+} sensing is shown in Fig.8. Sensor A has three binding sites for Cr^{3+}. Cr^{3+} forms a 4- coordinated complex with 2 molecules of sensor A. The other coordination sites are most likely occupied by solvent molecules. This model agrees well with the observed low fluorescence, the lack of absorptions beyond 400nm, the low quantum yield and the colorless nature of free A, as well as with the observed turn-on fluorescence, new absorption beyond 400nm, high quantum yield and the clear color change from colorless to pink for the sensor A bound to Cr^{3+}.

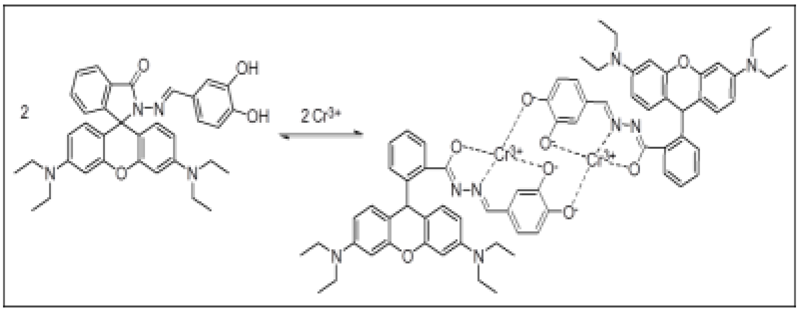

Fig.8: Mechanism of Cr^{3+} binding

Based on observed absorption and fluorescence, quantum yield values, NMR titration measurements, and the color of the solution of sensor A in the presence and absence of Cr^{3+}, and the observed job's plot, the possible Cr^{3+} binding model for sensor A is shown in Figure8. Sensor A has three binding sites for Cr^{3+}. Cr^{3+} forms a 4-coordinated complex with 2 molecules of sensor A. The other coordination sites are most likely occupied by solvent molecules.

4. CONCLUSION

Researcher has created a new turn-on fluorescence sensor for Cr^{3+} and synthesized and analyzed it. The sensor has a nanomolar sensitivity of 568 nM to Cr^{3+} and is very selective for it. The formation of spirolactaum ring opening form of sensor A upon binding to Cr^{3+} is responsible for the rise in fluorescence, new absorption, and obvious color change. It is a good turn-on fluorescent sensor for Cr^{3+} because, despite being a paramagnetic species which typically quenches fluorescence, Cr^{3+} can form the ring opening spirolactaum form of A, which enhances fluorescence. When binding with Cr^{3+}, the color changes dramatically, making it possible to detect Cr^{3+} with the naked eye. Sensor A could be reused because of the reversibility of Cr^{3+} binding.

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