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ORIGINAL ARTICLE

A Simple Red Emitting "Turn-On" Optical Relay Detector for Al^{3+} and CN^{-}. Application in the Real Sample and RAW264.7 Cell Imaging

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Abstract

A quantitative and qualitative fluorogenic molecular probe (**R**) has been designed and synthesized using Rhodamine-B hydrazide and 3,5-dibromo salicylaldehyde. The probe **R** has been applied for detection of ionic species and probe **R** turned out to be a prominent distinguisher among divalent and trivalent cations Al^{3+}, Cr^{3+} and Fe^{3+} via the fluorometric response in acetonitrile medium. Colorimetric changes were observed for trivalent cations and Cu^{2+}. Among all trivalent cations, Al^{3+} is possessing relay recognition for CN^{-} even in the presence of other interfering anions with optimum precision. The association constant and the detection limit for **R**-Al^{3+} are $4.5 \times 10^{6}\ M^{-1}$ and 17.9 nM respectively. On the other hand, **R** + Al^{3+}+CN^{-} exhibits an association constant and detection limit of $5.77 \times 10^{5}\ M^{-1}$ and 4.6 nM respectively. The stoichiometry of Al^{3+} binding with **R** is found to be 1:1. Quantum efficiency of probe **R**, **R** + Al^{3+} and **R** + Al^{3+}+CN^{-} are found to be 0.2, 0.88 and 0.04 respectively. The receptor showed excellent real-time applicability for commercially available antiperspirant and apple seeds extract. The efficiency of the receptor further extended for fluorescent imaging of Al^{3+} and CN^{-} in RAW264.7 cells.

Keywords Antiperspirant · Red emission · Relay recognition · Rhodamine-B · Trivalent sensor

Introduction

Metal ions are supremely paramount in the field of medicinal, clinical and environmental research since metal ions are unavoidable components of environmental and biological processes [1–5]. Presence of trivalent metal ions specifically Al^{3+} are significantly minute in natural process, but absence or overdose of Al^{3+} ions can lead to fatal effects like generation of reactive oxygen species and its interference in cellular metabolism [6], neurodegenerative disorders including Wilson's disease [7], Alzheimer's disease [8], Parkinson's disease [9], Menkes syndrome [10], low oxygen delivery [11], low blood pressure, and decreased immunity [12]. Alongside cations, recognition of anions has taken the attention of many groups, since anions play pivotal roles in chemical, biological, industrial and environmental fields [13]. Among various anions, cyanide is of great concern due to its diverse chemical characteristics. Cyanide is a sufficiently strong nucleophile that can act as an ambidentate ligand with very high stabilization energy while being basic in nature. Cyanide is considered as one of the most life-threatening analytes due to its eminent binding capability with Fe^{3+} in hemoglobin and hence hamper the oxygen transportation process [14]. These strong side effects compel the NIOSH (National Institute of occupational safety and health) to suggest the permissible limit of exposure of CN^{-} ions of 5 mg/m^3 for about 10 mins period [15]. So, it is a universal problem to maintain cyanide concentration in environmental systems.

Currently, many techniques are available for quantitative analysis of metal ions such as atomic absorption spectroscopy, inductively coupled plasma-mass spectroscopy, inductively coupled plasma emission spectrometry, chromatography, and voltammetry. Most of these methods possess some major disadvantages such as troublesome sample preparation procedures, sophisticated instrumentation, and high maintenance cost. Whereas in contrast, colorimetric and fluorescent chemosensors have monumental advantages like easy usage, simple synthetic procedure, real-time bare-eye visualization, cost-effective, real-time applicability, time effective, mostly non-instrumental and portability [16–23]. The emission property has been used for the determination of

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human serum albumin (HAS) interaction with cyclophosphamide (Cyc) and estradiol (ES) [24], 2,4-dinitrophenol [25], colchicine (COL) and aspirin (ASA) [26], lomefloxacin using biophysical techniques [27] and three different sizes of silver nanoparticles on the binding of curcumin to lysozyme [28]. Alongside natural analytes, metal ions specifically Al^{3+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+} and Pb^{2+} have devastating effect on human health and are capable to interact with human serum albumin (HSA) in presence of Norfloxacin by giving fluorescence quenching optical response [29]. So, floresence is a useful technique for precise, rapid and qualitative determination of analytes.

In the recent decade, enormous outputs have been reported based on various luminophore for Al^{3+} recognition [30–41]. Among most of the luminophores, rhodamine-B derivatives attract mostly due to its intense visible wavelength emission, very high quantum yield, reversibility, intense naked eye observable colour change and most importantly water miscibility with less toxicity [42–55].

From the analytical perspective, determination of two analytes via relay recognition is of ample desire in lieu of single analyte detection. Sequential recognition of two analytes by an efficient receptor follows sensing mechanisms like chemodosimetric, reversible complexation. Along with different mechanisms, there are various combinations in detection sequence of analyses like M^{n+} followed by A^{n-}, A^{n-} followed by M^{n+}, A^{n-} followed by A^{n-}, M^{n+}/A^{n-} followed by neutral species like amino acids or glucose [56].

During the preparation of this manuscript, we came across one journal published by Daoyong Jiang et al., where they have reported ten Schiff bases based on rhodamine-B and various derivatives of salicylaldehyde. Among which 3,5-dibromosalicylaldehyde derivative turned out to be a promising detector of VO^{2+} via turn-on red emission and Cu^{2+} via an intense colour change from colourless to reddish pink in DMSO/Tris-HCl (7:3 *v*/v, pH = 7.4) among a library of alkaline earth and transition metals. And additionally, the adduct of VO^{2+} and Cu^{2+} can detect pyrophosphate anion (PPi). The receptor was applied for MCF-7 cell line fluorescence imaging of VO^{2+} [57]. Though they claimed that trivalent cations specifically, Al^{3+}, $Cr^{3+,}$ and Fe^{3+} did not show appreciable influence on the receptor in that specified condition, but the receptor is showing comprehensive recognition capacity for trivalent cations specifically, Al^{3+}, $Cr^{3+,}$ and Fe^{3+} via turn-on red emission in aqueous-acetonitrile medium and Al^{3+}-ensemble is efficient in CN^{-} recognition selectively among the library of competing toxic anions. Moreover, the receptor is efficient in biological pH for the marking real time application and acetonitrile is a greener solvent over DMSO since acetonitrile has low boiling point, better miscibility, and low toxicity in comparison to DMSO.

On the count of the facts, here we report a rhodamine-B based luminogenic receptor **R** for sequential detection of Al^{3+} and CN^{-}. There are no reports found on relay recognition of Al^{3+}/CN^{-} based on our knowledge. Receptor **R** has been characterized and confirmed by using various ubiquitous spectroscopic techniques such as FT-IR, ^{1}H & ^{13}C-NMR and HR-MS. The obtained results have been documented below.

Results and Discussion

The receptor 'R' was designed for naked-eye observable colour change and also for profound fluorescence response for environmentally abundant analytes (metal ions and anions). Firstly, the receptor **R** was employed for anion sensing application, as the receptor has a free [-OH] functional group which can be deprotonated by anions. But, the receptor did not show any observable response against anions. Followed by anion sensing, the receptor was applied for metal ion sensing and expectedly, the receptor **R** showed excellent noticeable naked eye responses which are documented in the following paragraph:

Optical Selectivity

To examine the optical response and sensitivity of receptor 'R' for metal ions, 2 equivalent of metal ions like, Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Al^{3+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, $Ag^{+,}$ and Mn^{2+} were added to 3 mL (1 μM) solution of 'R' in acetonitrile medium. Receptor 'R' does not possess any colour whereas, on the addition of Cu^{2+} and trivalent metal ions Al^{3+}, Cr^{3+}, and Fe^{3+}, an intense pink colour appeared and for other divalent metal ions, a slight change in colour is observed, which are incomparable to Cu^{2+} and trivalent metal ions (Fig. S6). In the case of fluorescence response, receptor 'R' is found to be exclusive and selective for trivalent cations over divalent metal ions. From the emission image (Fig. 1), it is evident that except trivalent cations, none of the divalent cations showed any sort of emission with receptor 'R', though the colour change has taken place. Among the trivalent cations, Al^{3+} is preferable over Cr^{3+} and Fe^{3+} since intensity in fluorescence for Al^{3+} is profound over others.

Spectroscopic Response

The electronic responses of receptor **'R'** with all cations were recorded. It is evident in the optical naked eye image, that Cu^{2+} addition showed the most intense pink colour from colourless which is supported by the electronic spectrum with the highest absorption intensity at λ_{max} 558 nm along with a hump at 530 nm. Among trivalent cations, Fe^{3+} showed more intense colour followed by Al^{3+} and Cr^{3+}, which are evident in the UV-Vis spectrum (Fig. S7a). On incremental titration against Al^{3+}, the receptor got saturated by one equivalent of Al^{3+} (Fig. S7b and S7c). Fluorescence spectrum is self-explanatory for the fact that receptor **'R'** can distinguish efficiently and selectively among trivalent metal ions and divalent metal ions by means of 'turn-on' emission response at around

Fig. 1 Naked eye image under UV-light of the turn on emission of **'R'** by trivalent cations Al^{3+}, $Cr^{3+,}$ and Fe^{3+}, where Al^{3+} shows intense emission in acetonitrile medium

583 nm (Fig. 2a). A red emission is because of the spirolactum ring-opening process of rhodamine-B by trivalent metal ions. Among trivalent cations, Al^{3+} shows its predominance over the other two trivalent metal ions. The predominance of Al^{3+} is due to its diamagnetic property, whereas Cr^{3+} and Fe^{3+} possess paramagnetic property. Incremental titration of receptor **'R'** against Al^{3+} shows its saturation limit with 1 equivalent of Al^{3+} (Fig. 2b and c).

Relay Recognition of Cyanide

As receptor **'R'** showed selectivity for trivalent metal ions over divalent metal ions via fluorescence response, the 'R + Trivalent metal ions' complexes were applied for sequential detection of anionic analytes. Among three trivalent metal ion complexes, only '$R + Al^{3+}$' showed precise selectivity for CN^- via 'turn-off' emission and dis-coloration of the solution (Fig. 3a and b). Firstly, 1 equivalent of Al^{3+} were added to all vials containing 3 mL of (15 μM) receptor '**R**'. After addition of 15 μM Al^{3+}, the solutions turned into pink colour and followed by 100 μL of (50 μM) anions like CN^-, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, and OH^- were added one by one into vials. Colour and emission of the complex '$R + Al^{3+}$' revert back to the original receptor '**R**'. From the electronic and emission spectrum, it is obvious that CN^- is interacting with '$R + Al^{3+}$' and regenerating the receptor 'R' (Fig. 3c and d). The absorption and emission spectra of the incremental titration showed that the $R + Al^{3+}$ complex gets saturated with three equivalences of CN^- (Fig. 4a and b). The absorbance spectra of $R + Al^{3+}$ showed a maximum at 558 nm followed by a hump at around 530 nm, which is attributed as the spirolactam ring opening took place on interaction with Al^{3+}. On incremental addition of CN^- into the $R + Al^{3+}$ adduct, the absorbance intensity kept on decrease which is due to the re-formation of the spirolactam ring. But excess concentration of CN^- in the medium leads to the deprotonation of the [-OH] group of salicylaldehyde moiety, resulting a new peak at around 558 nm with negligibly low intensity. The cycle of sequential recognition is effective up to two cycles, and thereafter receptor **'R'** is unable to show any sort of response (Fig. 4c).

Interference Studies

To examine the selectivity and specificity of receptor **'R'** towards Al^{3+}/CN^- analytes, an interference study is necessary. In the presence of other equivalent competitive analytes, the receptor must interact explicitly and efficiently with the desired analyte to ensure the sensing ability without any external interferences. To examine Al^{3+} selectivity, 2 equivalents of each cations like Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, Ag^+ and Mn^{2+} were added separately to 3 mL of receptor **'R'** (1.0×10^{-5} M) followed by 1 equivalent of Al^{3+} to each of the vials containing 'R + Metal ions'. Now to examine CN^- selectivity by '$R + Al^{3+}$', 2 equivalent of each anions like F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, and OH^- were added separately to 3 mL of complex '$R + Al^{3+}$' followed by 3 equivalent of CN^- to each

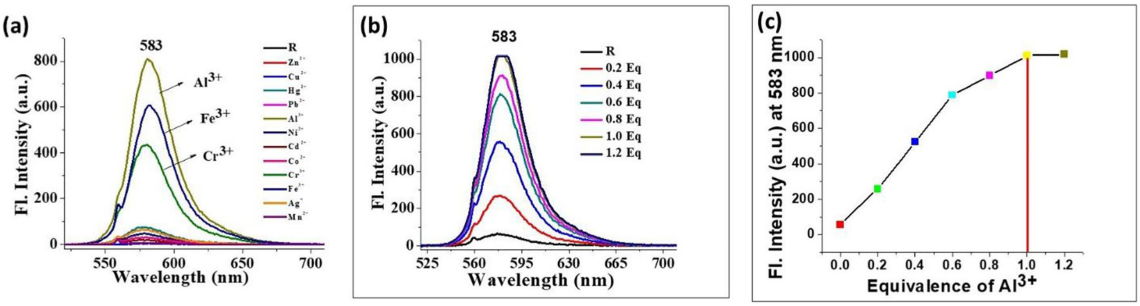


Fig. 2 **a** Emission spectrum of receptor **'R'** with metal ions like Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Al^{3+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, Ag^+ and Mn^{2+}. 30 μM of cations in water were added to 3 mL of receptor **'R'** (15 μM) in ACN. **b** Incremental addition of Al^{3+} from (0–1.2) eq., (**c**) Saturation limit and ratiometric detection of Al^{3+} by receptor **'R'** in 1:1 (Al^{3+}:R)

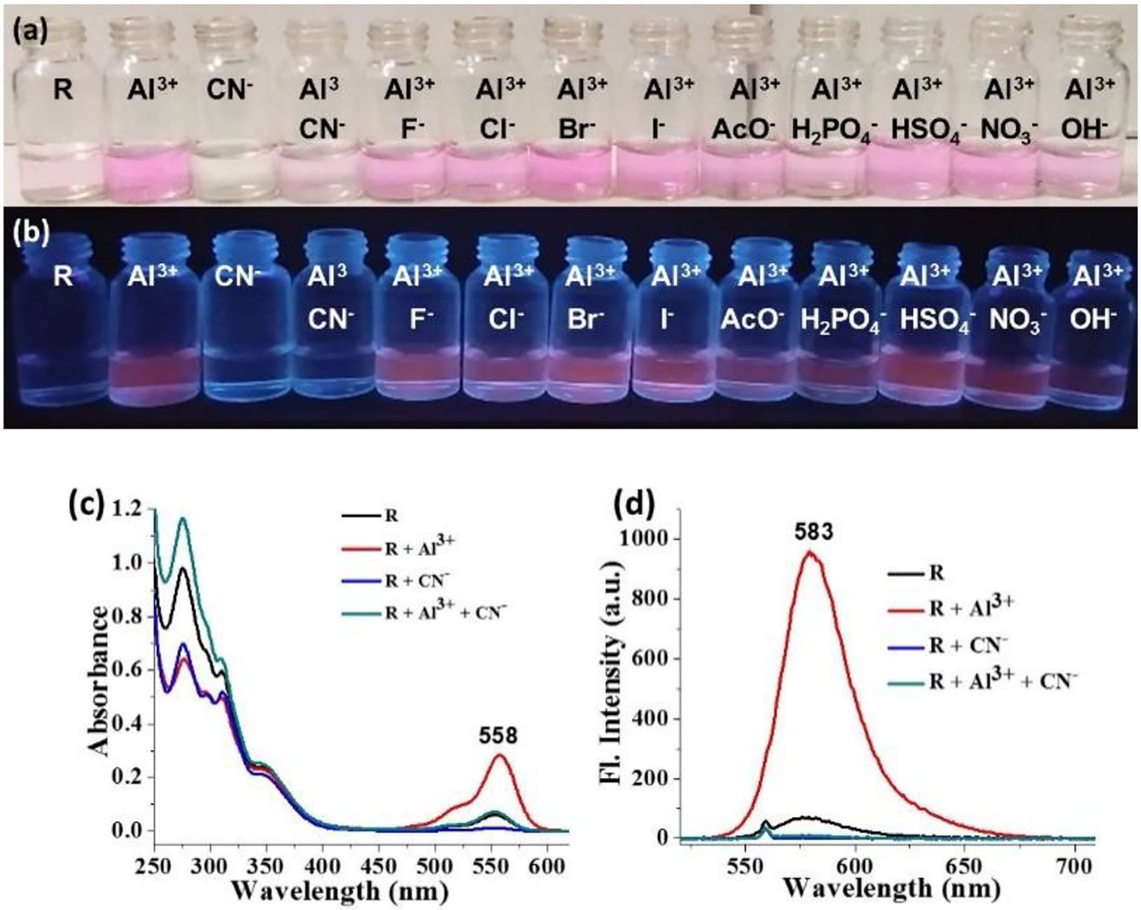


Fig. 3 **a** Naked eye observable colorimetric response and **b** fluorescence response under long-range UV-light for sequential "Off-On-Off" detection of Al^{3+}/CN^-, **c** Electronic spectrum and **d** Fluorescence response for Al^{3+}/CN^- relay recognition

of the vials containing 'R+ Al^{3+} + anions'. In fluorescence spectra, there is a steady increase in fluorescence intensity after addition of Al^{3+}, which implies hardly any interference by any metals, except Fe^{3+}, which is accountable from the naked eye selectivity experiment (Fig. 5a), whereas CN^- selectively quenches the emission of Al^{3+}-complex without any interference of other competing anions (Fig. 5b). The interference in selective sensing of Al^{3+}/CN^- pair is also investigated using absorption spectroscopy, which is supported by the obtained result (Fig. S8).

Kinetics of Sensing

The receptor **'R'** is capable of recognizing Al^{3+}/CN^-. So it is of utmost importance to find out the time required to show optimum intense response and quenching of fluorescence for sequential addition of Al^{3+}/CN^-. The kinetics of the sensing event has been studied and presented in Fig. 6. The maximum fluorescence intensity for '$R + Al^{3+}$' at 583 nm reached within 50 s after the addition of Al^{3+}. But for CN^- addition to the '$R + Al^{3+}$' complex, quenching of fluorescence took place within a few seconds and emission intensity is lower than that of receptor 'R'.

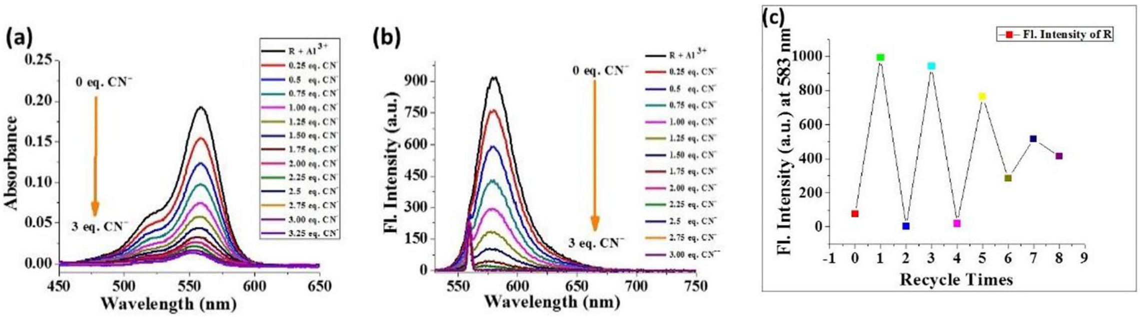


Fig. 4 **a** Electronic and **b** emission spectrum of incremental titration of CN^- to the ($R + Al^{3+}$) complex and **c** Emission response for reusability of receptor **'R'** for sequential recognition of Al^{3+}/CN^-

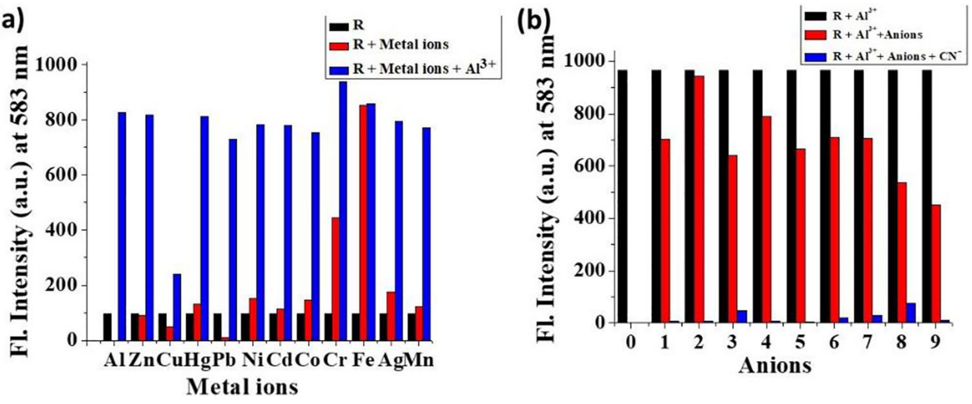


Fig. 5 **a** A comparative emission plot for only receptor 'R', 'R + Metal ions' and 'R + Metal ions + Al^{3+}, at 583 nm, and **b** A comparative emission plot for R + Al^{3+}, R + Al^{3+} + Anions and R + Al^{3+} + Anions + CN^- at 583 nm. In the interference plot of CN^- selectivity, 0 = R + Al^{3+}, 1 = R + Al^{3+}+F^-, 2 = R + Al^{3+}+Cl^-, 3 = R + Al^{3+}+Br^-, 4 = R + Al^{3+}+I^-, 5 = R + Al^{3+}+AcO^-, 6 = R + Al^{3+}+$H_2PO_4{}^-$, 7 = R + Al^{3+}+$HSO_4{}^-$, 8 = R + Al^{3+}+$NO_3{}^-$ and 9 = R + Al^{3+}+OH^-

Lifetime Measurement

The receptor **R** was examined for the lifetime decay experiment (λ_{em} = 583 nm) using a 550 nano-LED source, where the lifetime of **'R'** was found to be 9.7 × 10^{-10} s. On addition of Al^{3+}, the average fluorescence lifetime of 'R + Al^{3+}' system increased to 19.4 × 10^{-10} s. Followed by addition of CN^- to 'R + Al^{3+}, the average lifetime decreased to 1.34 × 10^{-10} s. The fluorescence decay curves were fitted to triple-exponential decay theoretical calculation and compared with experimental data. The resultant increment of the average lifetime before and after addition of Al^{3+} is approximately 10 × 10^{-10} s which implies that the excited state of 'R + Al^{3+-}' is more stable in comparison to the excited state of free receptor 'R'. As a result, there is a decrease of 10 × 10^{-10} s in the rate of decaying process when the spirolactum ring of the receptor gets opened by Al^{3+}; hence it is proved that there is a platform for the sharp enhancement of fluorescence in the case of 'R + Al^{3+}' interaction (Fig. 7a and b). Whereas upon addition of CN^-, a resultant decrease of an average lifetime took place of around approximately 17 × 10^{-10} s, which implies the stability of the excited state of the Al^{3+} complex gets decreased on the addition of CN^-. Hence it is an ideal condition for fluorescence quenching (Fig. 7b and c).

Quantification of Al^{3+}/CN^-

Qualitatively receptor **'R'** has shown a promising effect for sequential recognition of Al^{3+}/CN^-. Along with qualitative property, quantitative analysis is also an important aspect of a detector. During incremental titration of receptor **'R'** against

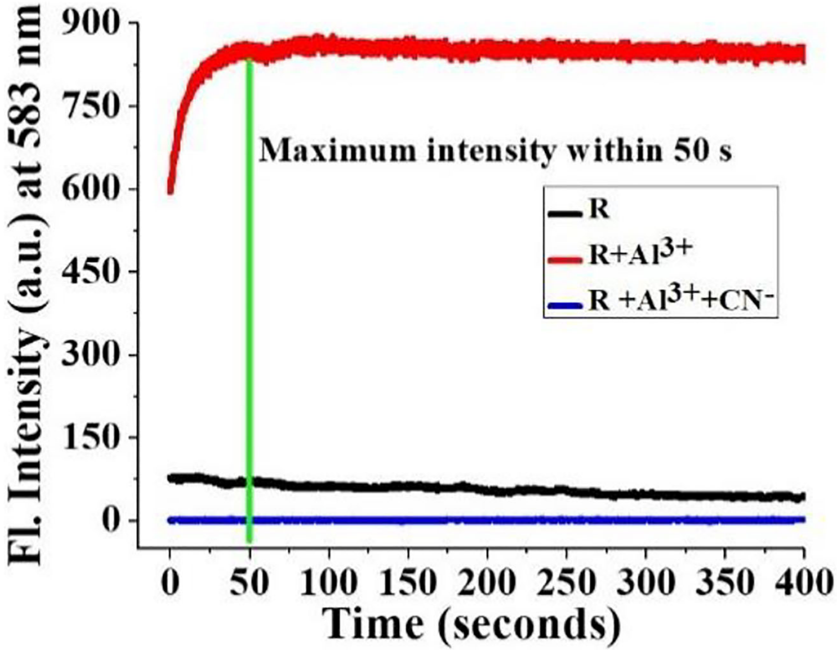


Fig. 6 Kinetics study for the time required to sense Al^{3+}/CN^- by receptor '**R**'. For Al^{3+}, **'R'** takes a maximum of 50 s to reach maximum fluorescence intensity whereas, 'R + Al^{3+}' takes no time to get quench by CN^-

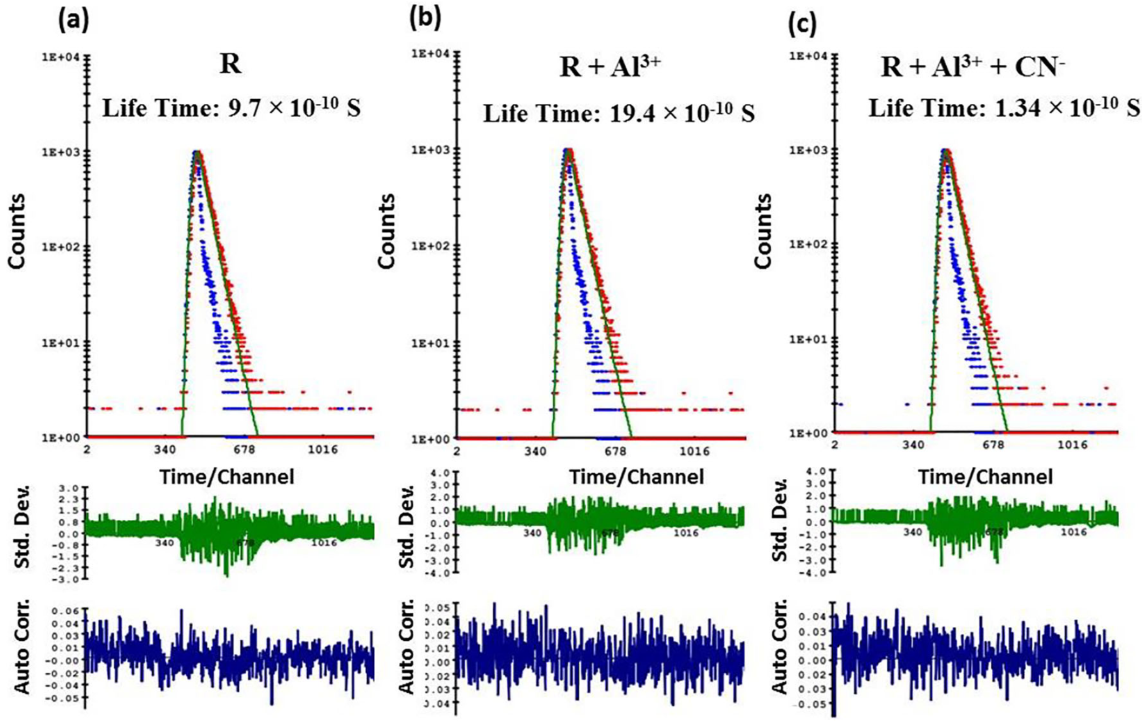
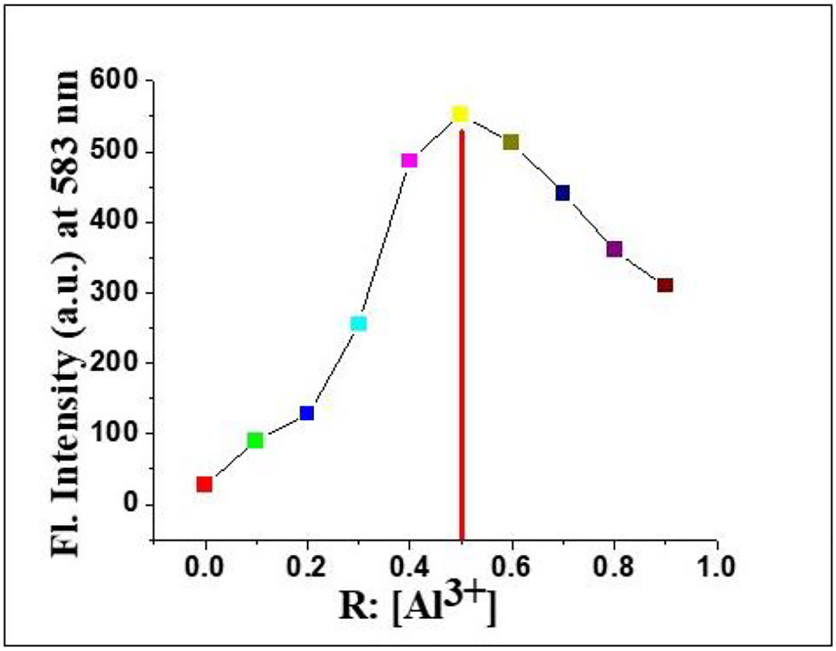


Fig. 7 Lifetime measurement studies of (**a**) 'R', **b** R + Al^{3+} and **c** R + Al^{3+} + CN^-. Standard deviation is the deviation between the experimentally obtained spectra with the theoretically calculated using a double exponential decay process. Autocorrelation is the parameter that defines the linearity between experimentally obtained spectra and theoretically calculated exponentials

Al^{3+}, it was observed that the receptor got saturated by the addition of one equivalent of Al^{3+}. To prove the stoichiometry, Job's plot experiment has been performed maintaining constant variance procedure. The concentration of receptor **'R'** and Al^{3+} kept at (1.0×10^{-5} M). From the Job's plot (Fig. 8), it is evident that maxima of the plot are obtained at the ratio of 1:1 between 'R': Al^{3+}. The binding constant (k_a) for Al^{3+} and CN^- are found as 4.5×10^6 M^{-1} and 5.77×10^5 M^{-1} respectively (Fig. S9 and S10). The limit of detection (LoD) for Al^{3+} and for CN^- are found as 17.9 nM and 4.6 nM respectively (Fig. S11 and S12). The Photochemical yield (ϕ) of **R**, R + Al^{3+} and R + Al^{3+}+CN^- of the sequential sensing process of Al^{3+} and CN^- are 0.2, 0.88 and 0.04 respectively (Fig. S13).

Fig. 8 Job's plot for R: [Al^{3+}]



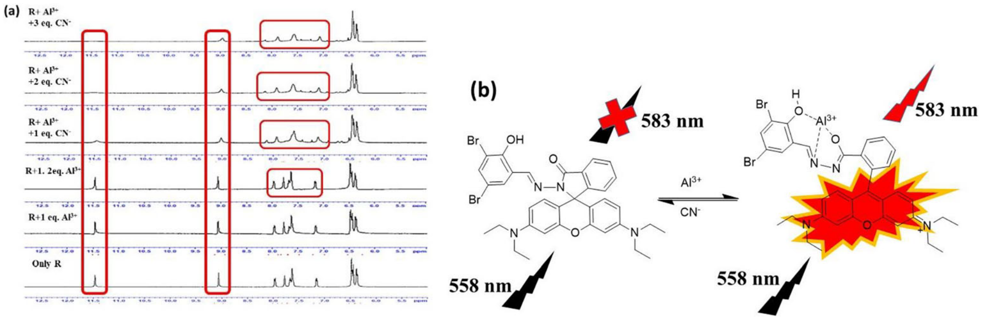


Fig. 9 **a** ^{1}H-NMR titration of **R** against Al^{3+}/CN^- analytes sequence. **b** The probable sensing mechanism for sequential recognition of Al^{3+}/CN^- in ACN medium

Sensing Mechanism

The detection mechanism is ascertained to be a ratiometric binding of Al^{3+} and upon addition of CN^- the complexation gets disturbed and Al^{3+} is coming out to yield the free receptor. To prove this, ^{1}H-NMR titration against Al^{3+}/CN^- pair has been performed in DMSO-d_6 solvent (Fig. 9a). On addition of 1.2 eq. of Al^{3+}, the chemical shift positions of protons of receptor '**R**' did not alter, but the splitting of the aromatic protons got broadened. On the contrary, in addition to 1 Eq. CN^-, proton peak at 11.5 ppm corresponding to [-OH] proton disappeared and a decrease in intensity for a peak at 9.1 ppm with 0.3 ppm downfield shift which is ascertained as [-N=CH] proton. On sequential addition of CN^- up to 3 Eq, there is hardly any change in the peak position of [-N=CH] functional group but proton peak of the aromatic group gets broaden. From the NMR titration, it is confirmed that upon addition of Al^{3+} to the receptor solution, one equivalent of added Al^{3+} forms a complex with the available binding sites such as amide carbonyl oxygen, imine nitrogen and free [-OH] from salicylaldehyde moiety. Doing so, it forced to open the spirolactum ring of rhodamine-B moiety, which renders in 'turn on' red emission near 583 nm. Whereas the addition of CN^- to the '$R+Al^{3+}$' solution, the [-OH] is deprotonated by CN^-, which facilitates regaining of the free receptor from the Al^{3+} complex with the quenching of the red emission (Fig. 9b).

Application

The effectiveness or utility of a detector will only be accepted if the receptor is equally efficient in a real-time environment. As receptor **'R'** shown efficiency towards relay recognition of Al^{3+}/CN^- in the presence of interfering analytes, then the same effect must be reflected in the real sample, MTT assay, and RAW264.7 cell fluorescence imaging. The results are as follows:

Real Sample

To prove the receptor's efficiency, the receptor is applied for real sample detection. Aluminum is an essential component of cosmetics and so for deodorants, where antiperspirant is used against sweat. The antiperspirant temporarily plugs sweat glands and that is how it stops sweat. But, this absorbing of antiperspirant has numerous life-threatening effects like breast cancer, kidney diseases, skin rashes and so on. So, for the real sample experiment, a commercially available anti-perspiration agent from "Sweat Guard- REXONA" was chosen as a real sample for Al^{3+} and apple seeds extract was chosen as a real sample for CN^-. The sweat guard was extracted using water-DCM through the solvent extraction process followed by centrifugation of the water extract part and was kept at room temperature for 1 day. The resultant supernatant water was used as a real sample for Al^{3+}. After addition 100 µL of real sample solution to the receptor solution, the colour of the solution turned into pink along with turn on emission. The electronic and emission spectra were recorded for the samples.

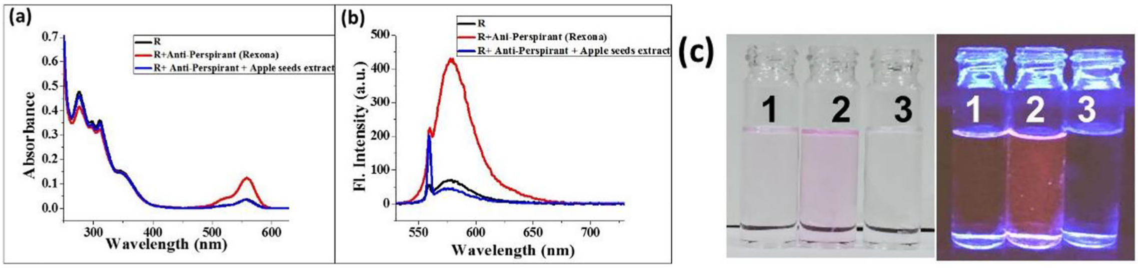


Fig. 10 **a** Electronic spectrum of receptor **'R'** with antiperspirant (real sample for Al^{3+}) and apple seeds extract (real sample for CN^-) in water were added to 3 mL of receptor **'R'** (1.0×10^{-5} M) in ACN. **b** Emission response of receptor **R** and respective real samples, **c** Naked eye colour and emission change for sequential recognition of Al^{+3} and CN^- by receptor **R** from the respective real samples. 1 = Only Receptor, 2 = Receptor + Antiperspirant (Al^{3+} source), 3 = Receptor + Antiperspirant (Al^{3+} source) + Apple seeds extract (CN^- source)

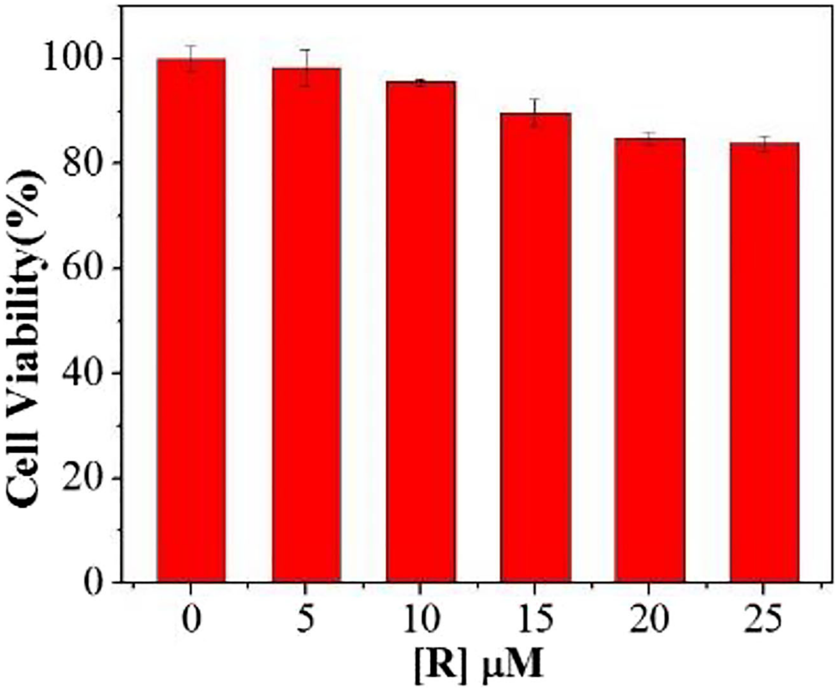


Fig. 11 MTT assay of RAW264.7 cells in the presence of **'R'** (0–25 μM) at 37 °C for 24 h

Followed by, 200 μL of apple seeds extract was added to that solution and again spectra were recorded. A change in colour alongside quenching of emission was observed after addition of apple seeds extract (Fig. 10).

MTT Assay and Cell Imaging

The receptor was employed for cytotoxicity experiment (MTT assay) against the RAW264.7 cell line. According to the in vitro MTT assay, the IC50 represents the concentration of the tested agent that is required for 50% inhibition of the cell viability. From Fig. 11, it is observable that toxicity level of receptor **'R'** at a concentration of 25 μM is around 10%, and the receptor is showing its utmost efficiency even in 10 μM where toxicity is below 7%. Hence receptor **'R'** can be applicable for fluorescence imaging of cells. Fluorescence imaging of cells is the proof for biocompatibility of the receptor and it can be useful for tracking Al^{3+} inside cells (Fig. 12). RAW264.7 cells were

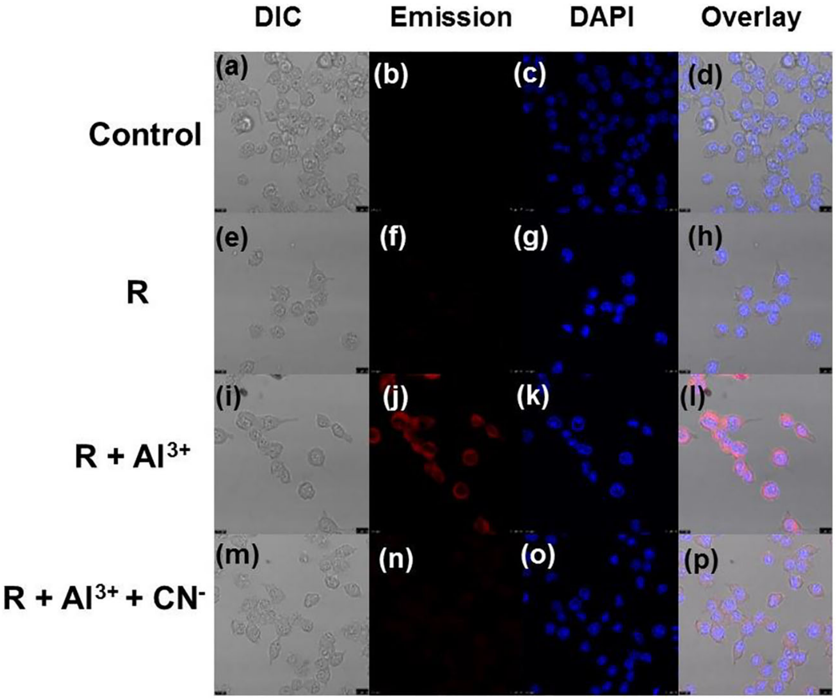


Fig. 12 Confocal fluorescence images of RAW264.7. **a–d** Control experiment of RAW264.7. **e–h** The cells were incubated with R (20 μM) for 30 min. **i–l** The cells were treated with R (20 μM) for 30 min and followed by incubation with Al^{3+} (10 μM) for 30 min. **m–p** Further incubation with CN^- (30 μM) for 30 min. Images were taken at λex = 558 nm. Scale bars = 10 μm

grown in DMEM media with 10% (*v*/v) FBS (fetal bovine serum) and penicillin/streptomycin (100 μg/mL) at 37 °C in a 5% CO_2 incubator. The cells were treated with 20 μM of **R** dissolved in DMSO and incubated for 30 min at 37 °C. After addition of Al^{3+} (40 μM) to the above cells, a strong red fluorescence in cells was observed. Further with the addition of CN^- (50 μM) in the culture medium the fluorescence in RAW264.7 cells disappeared. The culture medium was removed, and the treated cells were washed with 0.1 M PBS (2 mL × 3) before observation. The cells were excited with UV light below 558 nm and emission was collected at 583 nm ± 30 nm. The fluorescence images of '**R**', $R + Al^{3+}$ and $R + Al^{3+} + CN^-$ under emission field showed emission as expected. Inside cells also **'R'** is capable to act as Al^{3+}/CN^- detector via fluorescence 'off-on-off' response sequence.

Conclusions

In summary, we have developed a novel, ratiometric 'off-on-off' fluorescence marker for sequential detection of Al^{3+}/CN^- in acetonitrile medium. The marker is efficient to detect the analytes even in the interference of a library of competing analytes. The receptor **'R'** is observed to maintain a 1:1 stoichiometric with Al^{3+}. The receptor is applied for real sample analysis and found to be biocompatible as it shows more than 90% cell viability with 15 μM concentration. In fluorescence cell imaging of the RAW264.7 cell line, the receptor is found to be an efficient one. This receptor can be applicable for effective and selective detection of Al^{3+}/CN^- analytes pair.

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Compliance with Ethical Standards

Conflict of Intrest The authors do not have any conflict of interest.

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