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Fluorescence Turn on Sensor for Sulfate Ion in Aqueous Medium Using Tripodal-Cu^{2+} Ensemble

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Abstract We report the selective recognition of sulfate anion in aqueous medium at biological pH 7.2 over the other interfering anions based on naphthoic acid bearing tripodal ligand by applying fluorescence *turn off-on* mechanism. The carboxylic acid groups in the ligand enhance the solubility in water and enable it to form complex with copper salt. Thus formed **L**-Cu^{2+} ensemble quench the fluorescence of the parent ligand and in turn recognize sulfate anion via revival of fluorescence intensity. The 1:2 stoichiometry was confirmed by ESI mass spectral data and Job's plot. The average binding constant was found to be 6.2×10^8 M^{-2}.

Introduction

The alarming increase in environmental pollutions and associated health hazards caused by chemical productions, pesticides, drug wastes and industrial wastes are of grave concern. Amongst all known sources of pollutions, sulfur containing compounds have drawn special attention as they are known to be present in upper atmosphere as a contaminant, in nuclear waste that can interfere treatment process, low solubility in borosilicate glass during vitrification and also responsible for permanent hardness of water [1, 2]. The most common soluble sulfur containing compound in aqueous environment is sulfate anion. Thus, the design of synthetic receptors capable of selective binding and sensing of sulfate anion in aqueous medium has emerged as a major endeavor in recent times. The recent trend to design synthetic receptor for anion sensing are based on H-bonding capable functionality viz. amide, urea, thiourea, imidazole, or pyrrole subunits [3–5]. However, it may be mentioned here that a vast majority of literature reports describe anion sensing in organic media. Thus it is of great challenge to design synthetic anion receptor in aqueous environment to have real life application. Hence a major motivation in supramolecular chemistry is to selectively sense the anions in a competitive aqueous medium. In this direction, Wu and Yang et al. developed receptors for sulfate anion and demonstrated that the binding ability decreases with addition of water [6–8]. Squaramide based ligand have been derived that senses sulfate anion in 10 % water-alcohol mixture and modification of those moiety resulted in enhanced sensing of sulfate anion in pure water [9, 10]. A fluorescent probe can be considered as a valuable sensing platform for anions in aqueous medium. Anions are strong hydrogen acceptor and in aqueous solution remains hydrated hence a big competition occur for H-bond with hydrogen of water and H-bonding site in the ligand that may be a major impediment of anion sensing in aqueous medium. In the recent years fluorescence *turn off-on* technique have been introduced for better sensing of anions on the basis of anion binding site and signaling unit, where in situ formed soluble coordination complex can be an efficient ensemble for subsequent anion sensing in aqueous medium. A fluorescence *turn off-on* technique may overcome this problem as evidenced in sensing of cyanide ion [11–13], sulfide ion [14–17], fluoride ion [18], iodide ion [19–21], and acetate ion [22] in different mixture of solvent. In continuation of our work on sensing [23–31] herein we report fluorescence *turn off-on* sensor for sulfate anion in aqueous medium using fluorogenic naphthoic acid containing tripodal host.

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In most of the cases of anion sensing study anions are taken as a tetra-alkylammonium salt of the corresponding anion and there is concern about the solubility of the receptors in water. The competition of H-bonding with water molecule and receptor reduces the sensing ability of the receptor for the anion in aqueous medium. In this regard, sensing property of the tripodal ligand for sulfate anion as a sodium salt using fluorescence *turn off-on* techniqueis a feasible key to overcome this issue. From that point of view our results epitomizes the meaningful application in practical purposes.

Experimental

Materials and Methods

Commercially available 1-Hydroxy-naphthalene-2-carboxylic acid and Triethanolamine were obtained from Sigma, USA and used as supplied. The solvents were distilled freshly following a standard procedure. Other chemicals were of reagent grade and used without further purification. The FT-IR (Fourier transform infrared) spectra were recorded on a Perkin Elmer-Spectrum One FT-IR spectrometer with KBr (potassium bromide) disks in the range 4,000–500 cm^{-1}. The absorption spectra were recorded on a Perkin Elmer Lambda- 25 UV-Visible Spectrometer at 298 K. While the fluorescence measurements were carried on a Horiba Fluoromax-4 spectrofluorimeter using 10 mm path length quartz cuvettes with a slit width of 5 nm at 298 K by exciting at 320 nm wavelength. The NMR (Nuclear Magnetic Resonance) spectra were recorded on a Varian FT-400 MHz (Megahertz) instrument. The chemical shifts were recorded in parts per million (ppm) on the scale using tetramethylsilane (TMS) as a reference. The following abbreviations are used to describe spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplate. All luminescence measurements were made using optically dilute solutions and were corrected for spectral imperfections of the instrument by reference to a standard lamp. The mass spectra were obtained using Waters Q-ToF Premier mass spectrometer.

Synthesis and Characterization

Synthesis of 1-Hydroxy-Naphthalene-2-Carboxylic Acid Methylester (1) 1-Hydroxy-naphthalene-2-carboxylic acid (0.9 g, 5 mmol) was thoroughly mixed in methanol and 1 mL conc. H$_2$SO$_4$ (sulfuric acid) was added slowly by portions with continuous stirring (Scheme 1). Finally whole reaction mixture was refluxed at 80 °C for 8 h. The solvent was evaporated under reduced pressure and brown solid mass was extracted with ethylacetate 3×20 mL. The organic layer was washed several times with water and dried over
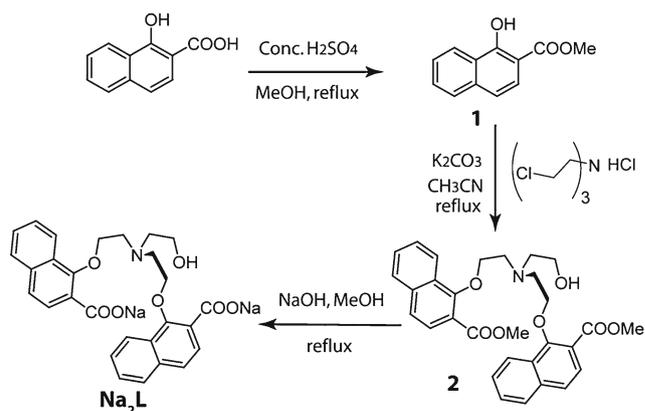
Scheme 1 Synthetic scheme for Na$_2$L

anhydrous Na$_2$SO$_4$ and solvents were removed under reduced pressure that leaves pure the brown solid product.

Yield 90 %, mp 77–79 °C. ^{1}H−NMR (400 MHz, CDCl$_3$) δ (ppm): 3.95 (s, 3H, Me), 8.40–7.19 (m, 6H, Ar) 11.97 (s, 1H, OH). ^{13}C-NMR (100 MHz, CDCl$_3$) δ(ppm): 51.15, 106.09, 118.36, 123.84, 124.85, 125.22, 127.55, 129.27, 137.35, 161.42, 173.77. FT-IR spectra: 1,635 cm^{-1} for ester −C=O (Fig. S1, Fig. S2, Fig. S6). Data for the compound are in agreement with published previously [32].

Synthesis of Tripodal Ester (2) A mixture containing 1-Hydroxy-naphthalene-2-carboxylic acid methylester (1.5 g, 7.4 mmol) and finely powdered solid K$_2$CO$_3$ (8 g) in 30 mL acetonitrile were refluxed at 80 °C for 1 h (Scheme 1). To the hot solution dissolved Tris(2-chloro-ethyl)amine hydrochloride salt (3.57 g, 14.7 mmol) added in portions over 15 min. The whole reaction mixture was refluxed for another 12 h for completion of the reaction. Then solid mass was removed by filtration and filtrate was evaporated in vacuum to give brown semi-solid product.

Yield 70 %, ^{1}H−NMR (400 MHz, CDCl$_3$) δ (ppm): 7.30–8.40 (m, 6H, Ar), 4.80 (t, 2H, OCH$_2$), 3.80 (t, 2H, OHCH$_2$), 4.20 (s, 3H, OMe), 3.30 (t, 2H, NCH$_2$). ^{13}C-NMR (100 MHz, CDCl$_3$) δ(ppm): 42.15, 52.83, 56.91, 63.64, 105.64, 118.65, 123.92, 124.30, 125.32, 126.70, 129.45, 137.24, 160.94, 171.47. FT-IR spectra: 1,712 cm^{-1} for ester −C=O. HRMS (positive mode ESI): found, m/z 518.2208 (cal 518.2173) for [LMe$_2$+H$^+$] (Fig. S3, Fig. S4, Fig. S7, Fig. S13).

Hydrolysis of Ester and Synthesis of Disodium Salt (Na$_2$L)

To hydrolyze the ester group the tripodal ester (2) was dissolved in 10 mL methanol and methanolic solution of sodium hydroxide (5 N, 40 mL) was added to it and resulting mixture was refluxed for 12 h at 80 °C. Then the solution was neutralized partially with dil. HCl to pH ~7.0 that yields colorless solid powder Na$_2$L from slow evaporation of the solvent.

Yield 70 %, mp 210–212°C ^{1}H–NMR (400 MHz, D_2O) δ (ppm): 7.30–8.20 (m, 6 H, Ar), 4.80 (t, 2 H, OCH_2), 3.76 (t, 2 H, $HOCH_2$), 3.36 (t, 2 H, NCH_2). FT-IR spectra: 1,592 cm^{-1} for carboxylate −C=O. HRMS (positive mode ESI): found, m/z. 490.1747 (cal 490.1866) for $[LH_2+H^+]$ and 512.1824 (cal 512.1685) for $[LH+Na^++H^+]$ (Fig. S5, Fig. S8, Fig. S14).

General Methodology Adopted for Spectroscopic Studies

Solutions of the sodium or potassium salts of the respective anions were used for the studies. For checking the relative, but qualitative, binding affinity of individual anions toward the **L**-Cu^{2+} (complex of **L** and Cu^{2+} salt), the effective anion concentration aqueous Tris–HCl buffer was maintained at 15 mmol, while that for the receptor **Na$_2$L** was maintained at 10.0×10^{-6} M. UV-visible study could not give satisfactory result of effect of anion towards **L**-Cu^{2+} ensemble. So we mainly focused on fluorescence spectroscopic study. A solution of the sodium or potassium salts of the respective ions in a spectroscopic-grade aqueous Tris–HCl buffer (15 mmol, pH 7.2) was used for the studies, while maintaining an effective concentration of the respective anions at 5×10^{-5} M. A solution of the receptor **Na$_2$L** with an effective concentration of 1.0×10^{-5} M was prepared in aqueous medium and was used for checking the relative sensing affinity with different anions. Relative Fluorescence Quantum Yield (Φ, It is defined as the ratio of the number of photons emitted to the number of photons absorbed.) was measured following the reported literature (fluorescein in 0.1 N NaOH as a reference) [33].
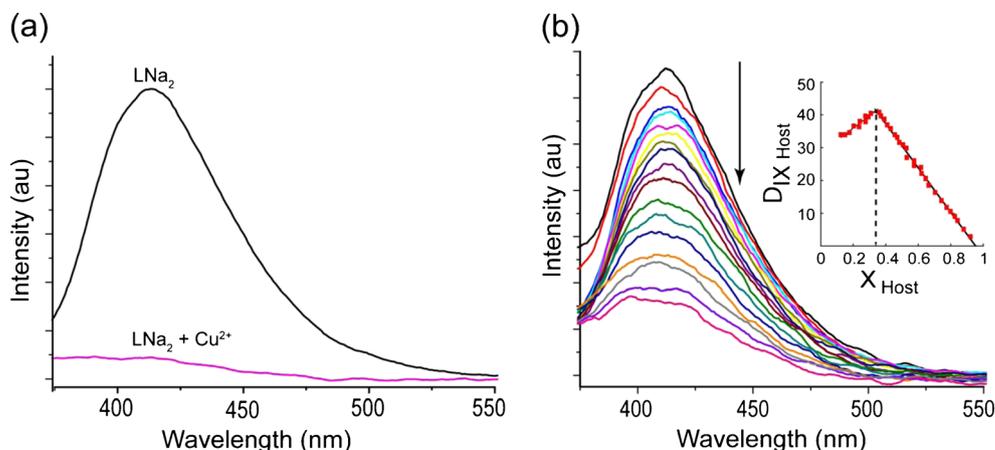
Determination of Stoichiometry Number of the Complex

The basic equation (1) for determination of the ligand-metal complexation is:

$$D + nM = C \qquad (1)$$

where D is the ligand; M is the metal ion, and C is the metal-complex.

Calculation of the Binding Constants

Receptor Na$_2$L with an effective concentration of 1.0×10^{-5} M in an aqueous Tris–HCl buffer (15 mmol, pH 7.2) was used for the emission titration studies with a Cu^{2+}/SO_4^{2-} solution. A stock solution of Cu^{2+} (as chloride salt), having a concentration of 2.0×10^{-4} M in an aqueous Tris–HCl buffer (pH 7.2) solution was used. The binding constant K of the metal complex was determined by equation (2), assuming the concentration of free metal is about equal to its total concentration $([M] \cong [M]_t)$,

$$(F-F_0)/(F_m-F) = [C]/[D] = K[M]^n \qquad (2)$$

Where F_o, F, and F_m are the corrected fluorescence emission intensity of the complex at initial, interval t, and the final state at which the complex was fully formed upon addition of metal ion, respectively. The binding constant K was determined from the plot of the linear regression of log $[(F-F_0)/(F_m-F)]$ vs. log [M] in equation (3), derived from equation (2), to obtain the intercept as log K and the slope as n. [34]

$$\log(F-F_0)/(F_m-F) = [C]/[D] = \log K + n \log [M] \qquad (3)$$

Evaluation of the Detection Limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of **L**-Cu^{2+} was measured for ten times and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensity at 412 nm was plotted as a concentration of SO_4^{2-}. So the detection limit was calculated with the following equation (4):

$$\text{Detection limit} = 3\sigma/k \qquad (4)$$

Where σ is the standard deviation of blank measurement, k is the slope between the ratio of Fluorescence versus $[SO_4^{2-}]$.

Fig. 1 **a** Fluorescence spectra of Na$_2$L in presence of Cu^{2+} ion (10 equiv) in 15 mmol Tris–HCl buffer, pH=7.2. λex=320 nm (slit=5/5). **b** Fluorescence emission spectra of Na$_2$L (10 μM) with increasing amount Cu^{2+} ion (0–2 equiv) in 15 mmol Tris–HCl buffer, pH=7.2). INSET: Job's plot showing formation of L-Cu^{2+} ensemble in 1:2 ratio

Fig. 2 **a** Effect of various anions on the Fluorescence emission spectra of L-Cu²⁺ ensemble. **b** Fluorescence emission spectra of L-Cu²⁺ ensemble (10 μM) with increasing amount SO₄²⁻ ion (0–2 equiv) in 15 mM Tris-HCl buffer, pH=7.2. INSET. Change in fluorescence intensity of L-Cu²⁺ ensemble with respect to equivalent of added SO₄²⁻ ion
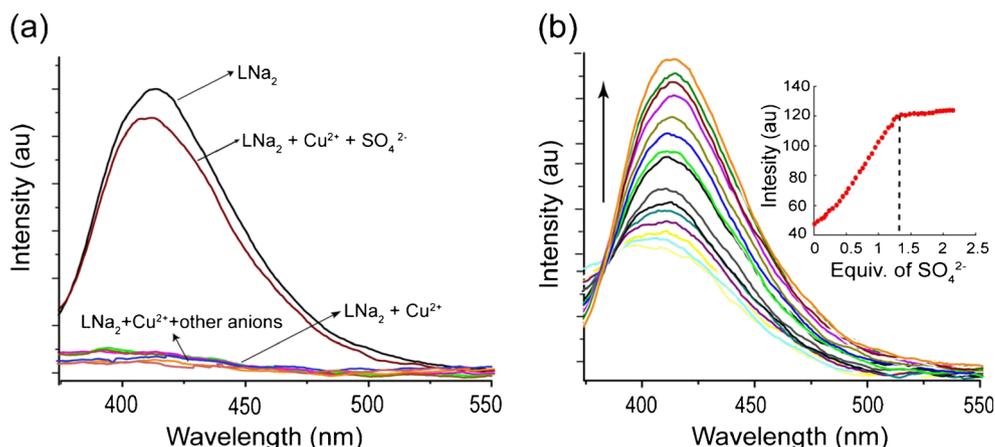
Results and Discussion

Owing to the concern of sensing property of the ligand in water development of sensor for anions in aqueous medium is a great challenge and we have chosen fluorescence *turn off-on* technique, hence we have focused to design a molecule which is sufficiently soluble in water and have some binding site for metal ion. Hence we have chosen naphthoic acid based acyclic nitrogen bridged tripodal ligand which satisfies our assumptions. On the other hand the flexibility of the tripodal receptor provides added advantage in changing its conformation to accommodate guest species in solution.

Absorption spectra of the ligand do not provide any significant outcome while interacting with metal ions and/or anions (Fig. S9). This suggests that ligand-metal interaction is unable to change any ground state interaction efficiently. Hence we mainly focused on fluorescence study to explore sensing property of the ligand. Fluorescence emission spectra of the receptor Na_2L (10 μM) in 100 % aqueous solution of Tris–HCl buffer, pH=7.2 was recorded at room temperature (RT). Upon excitation at 320 nm it exhibits a relatively strong fluorescence emission at 412 nm (Quantum yield, Φ=0.58). On addition of Cu²⁺ ions to Na_2L causes almost 95 % quenching of the emission intensity at 412 nm (Φ=0.03) (Fig. 1a). Titration of the ligand with copper salt resulted in gradual decrease in the fluorescence intensity of the ligand (Fig. 1b). Two equivalent of copper salt led to 16-fold quenching of fluorescence intensity and the Job's plot showed (Fig. 1b INSET) the complex formation in 1:2 binding mode between ligand and metal ion. The average binding constant determined on the basis of fluorescence titration data was found to be 6.2×10^8 M⁻² for a considering 1:2 binding model (Fig. S10). The chelation enhanced fluorescence quenching (CHEQ) behavior of Na_2L in presence of Cu²⁺ could be attributed to the effective binding of Cu²⁺ in the tripodal pseudo-cavity. Carboxylate group of the tripodal anionic ligand forms coordination complex with Cu²⁺ in a fashion as known for metal-carboxylate complexes [35–38]. Hence the

fluorescence intensity is essentially completely quenched by the addition of Cu²⁺ ion in aqueous medium. Coordination of the Cu²⁺ ion facilitates the metal to ligand charge transfer (MLCT) that is concomitant with the quenching of fluorescence intensity of the ligand.

Fluorescence emission of the disodium complex Na_2L was almost completely quenched by Cu²⁺ ion in Tris–HCl buffer at pH 7.2. The effect of different oxy anions and halides (viz. AcO⁻, NO₃⁻, SO₄²⁻, PO₄³⁻, ClO₄⁻, F⁻, Br⁻, and I⁻ of concentration 5.0×10^{-5} M) on L-Cu²⁺ ensemble were examined in Tris–HCl buffer at pH=7.2. As evident in Fig. 2a, selective recognition of sulphate anion was achieved by a significant enhancement of the fluorescence intensity of L-Cu²⁺ ensemble (Φ=0.52). Addition of other anions could not recover the diminished fluorescence intensity of L-Cu²⁺.

It was further conceived that reacting the L-Cu²⁺ complex with anions may replace Cu²⁺ ion from the ligand environment leading to recovery of the fluorescence of the free ligand in solution. It is known that binding affinity of the sulfate anion to Cu²⁺ is stronger than the anionic ligand which in turn
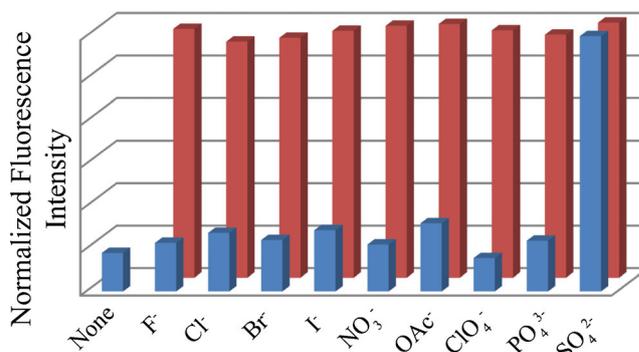

Fig. 3 Normalized fluorescence responses of L-Cu²⁺ (10 μM) to various anions in a 100 % aqueous Tris-HCl buffer solution (pH=7.2). The blue bars represent the emission intensities of L-Cu²⁺ in the presence of anions of interest (50 μM.). The red bars represent the change of the emission that occurs upon the subsequent addition of (30 μM) of SO₄²⁻ to the above solution. The intensities were recorded at 412 nm. Excitation wave length 320 nm, slit 5/5

Scheme 2 Probable displacement mechanism of turn off-on fluorescence sensing of sulfate anion as a sodium salt in aqueous medium
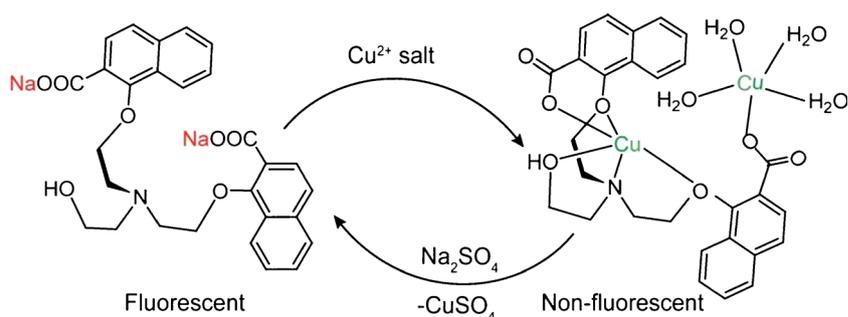
yields water soluble copper sulfate in aqueous medium. Only the addition of sulfate anion causes increase in the fluorescence intensity of **L**-Cu^{2+} complex. The fluorescence titration of **L**-Cu^{2+} complex by sulfate ion showed almost complete revival of fluorescence by 2 equivalent of sulfate anion (Fig. 2b, INSET). A linear relationship was observed from the plot of fluorescence intensity of **L**-Cu^{2+} with SO$_4^{2-}$ (at λ_{max} 412 nm) with respect to the concentration of sulfate and the detection limit was calculated to 514 nM, suggesting the **L**-Cu^{2+} ensemble is efficiently sensitive towards sulfate anion (Fig. S11). Sensing capability of the **L**-Cu^{2+} complex was tested in aqueous medium in presence of other competitive anions like AcO$^-$, NO$_3^-$, PO$_4^{3-}$, ClO$_4^-$, F$^-$, Br$^-$, and I$^-$ in much higher concentration (5.0×10^{-5} M) than the sulfate ion. However, no noticeable fluorescence enhancement was observed with these interfering anions. However, addition of SO$_4^{2-}$ anion to this mixture caused a dramatic increase in fluorescence intensity of **L**-Cu^{2+} complex (Fig. 3). Hence, the **L**-Cu^{2+} ensemble is highly selective towards the sensing of sulfate anion even in the presence of other competitive anions in aqueous condition. We have also carried out pH dependent study of the *turn off*-on sensing of sulfate anion in Tris–HCl buffer solution (Fig. S12), which indicates the rational use of the ensemble in a wide pH range.

These experiments showed good sensing property for sulfate anion over a pH range 6.5–8.5 where intensity of **L**-Cu^{2+} at 412 nm was fully recovered with addition of SO$_4^{2-}$ ion. The Cu^{2+} induced quenching of fluorescence of **Na$_2$L** and subsequent sulfate selective recovery of fluorescence is due to the formation of Cu^{2+} complex and subsequent de-complexation by SO$_4^{2-}$ ion in solution. This sequence of event is also confirmed by a detailed positive ion ESI mass spectral analysis. Mass spectra analysis (Fig. S15) of the **L**-Cu^{2+} suggested a 1:2 binding of metal with ligand having and a mass of m/z 685.0512 (calc. 685.0646) corresponding to [L+2Cu^{2+}+4H$_2$O] which was in agreement with Jobs plot obtained from fluorescence titration experiment. This also supports solution state coordination/chelation induced quenching of fluorescence. The sensing of sulfate anion was accomplished through the displacement of metal ion from ligand coordination environment with revival of fluorescence of free ligand, which also could be established from mass spectroscopy. The mass spectra of free ligand gave two fundamental peaks at m/z 490.1747 (calc. 490.1866) for [LH$_2$+H$^+$] and 512.1824 (calc. 512.1685) for [LH+Na$^+$+H$^+$]. Mass spectrometry data of the **L**-Cu^{2+} complex after addition of sulfate anion (Fig. S16) essentially matches with fundamental peak of the free ligand (Fig. S14). Based on the fluorescence and mass spectral data (685.0512 corresponding to [L+2Cu^{2+}+4H$_2$O]) plausible mechanism of sulfate ion sensing can be depicted as indicated in Scheme 2.

Conclusions

We have reported selective sensing of sulfate anion in 100 % aqueous medium by using fluorescence *turn off*-on mechanism. Our sensing component is a water soluble fluorophoric naphthoic acid based N-bridged tripodal ligand. Fluorescence of the ligand was quenched completely by adding copper(II) salt, which was retrieved selectively by sulfate anion in aqueous environment through de-metalation in a highly competitive environment with other interfering anions. The fluorescence *turn off*-on sensing property is well applicable in a physiological range of pH. We have also deduced the probable binding mode for copper from ESI mass and average binding constant from fluorescence titration data. Supporting characterization data also established copper mediated sensing of sulfate anion in solution.

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