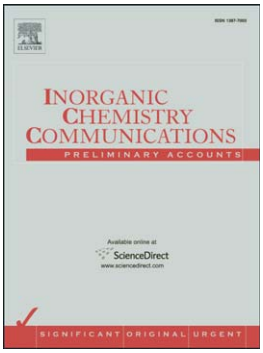

Selective detection of mercury ions using benzothiazole based colorimetric chemosensor

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Title: 1 SELECTIVE DETECTION OF MERCURY IONS USING BENZOTHIAZOLE BASED COLORIMETRIC CHEMOSENSOR

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Abstract: Three new receptors L1, L2 and L3 were designed and synthesized in a single pot synthesis. The synthesized receptors were characterized by ^{1}H NMR, FT-IR and Mass spectrometry. The cation binding affinity was studied using colorimetric and UV-Vis spectral studies for the three receptors. Receptor L1 showed high selectivity with a significant color change from yellow to colorless (turn-off response) in the presence of Hg^{2+} ions over the other cations such as, Ni^{2+}, Cr^{3+}, Pb^{2+}, Cu^{2+}, Cd^{2+} , Co^{2+}, $Zn^{2+}$and Fe^{2+} and the receptor L2, L3 did not show any color change or change in the absorption in UV-Vis spectral studies with Hg^{2+} ions and other tested metal ions, this may be due to lack of –OH group in receptor L2 and L3. The B-H Plot shown 1:1 complex between L1and Hg^{2+} ions.

Key Words: Chemosensors, Naked-eye detection and B-H Plot

Introduction: The development of new colorimetric receptors for selective recognition of heavy metal ions [1-3] has received considerable attention in past few decades. This may be because of the fact that certain metals when exceed permissible limit, become extremely toxic and hazardous to mankind. Among various heavy metal ions mercury, lead and cadmium ions are well known for their high toxicity towards environment and human health. Among them mercury is one of the most poisonous metallic contaminants and can exist in ionic (Hg^{2+}), elemental (Hg^{0}) and organic form methyl mercury (CH_3Hg^{+}) this powerful neurotoxin enters the food chain and finally accumulates in the human body[4].the so called " Minamata Disease" is a tough consequence of methyl mercury. Mercury is the most lethal for living organisms because it can easily enter through the skin and respiratory cell membranes, leading to DNA damage, myocardial infarction, some kind of autism, damage of the brain, kidneys and damage to the sensory parts of the nervous system. Knowing the seriousness of problem, significant research efforts have been dedicated to improve heavy metal ion detection [5]. Current industrial approaches count on costly, sophisticated instruments, complicated procedures or low sensitivity and selectivity approaches like atomic

absorption/emission spectroscopy or inductively coupled plasma mass spectroscopy. For these reasons, chromogenic chemosensors are especially attractive because they permit naked eye detection of color change without any use of spectroscopic instruments [6]. In addition these colorimetric sensors ideally have the benefits of low cost, easy synthesis and storage, and more tolerance towards different experimental conditions. Therefore, many researchers have focused on "colorimetric," and /or fluorometric chemosensors in this research area, for the detection of heavy metals.

Recent time's Ye won Choi group designed imidazole moiety as a metal ion receptor [7] thiazole moiety was often chosen as a metal ion receptor [8-11], and 2 hydroxy salicylaldehyde is well-known chromophore and has been frequently used for the colorimetric chemosensors [11-14]. Therefore, we planned to incorporate the Benzothiazole moiety into 5-nitro-2-hydroxy salicylaldehyde moiety to develop a novel chemosensor (L1), 2-(Benzothiazol-2-yliminomethyl)-4-nitro-phenol.

Herein we report a simple and reliable colorimetric chemosensor (L1) for the detection of Hg^{2+} ions in aqueous media. This work was motivated by the fact that most organic molecules reported as Hg^{2+}chemosensors perform the sensing process in a medium different than water, which limits their function in environmental and life sciences. This new sensor is based on a Schiff base derivative, this have been described as important tools for a long time, but their usage as a signaling subunit in chemosensors is limited due to their low solubility in aqueous media. Mercury is a soft metal and therefore favors sulfur-rich binding sites. It shows an intense color change after coordination with metals and offers a wide variety of design possibilities. Sensing characteristics of (L1) were investigated using UV-Vis absorption spectroscopy in aqueous solutions in the presence of biologically and environmentally relevant metals, i.e., Ni^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, $Zn^{2+}$and Fe^{2+} [15-23]. The results demonstrated that L1 can be employed for sensing Hg^{2+} in water with a limit of 0.15 μM even under the presence of other interfering ions. This is one of the lowest detection limit reported for mercury in aqueous media.

Experimental:

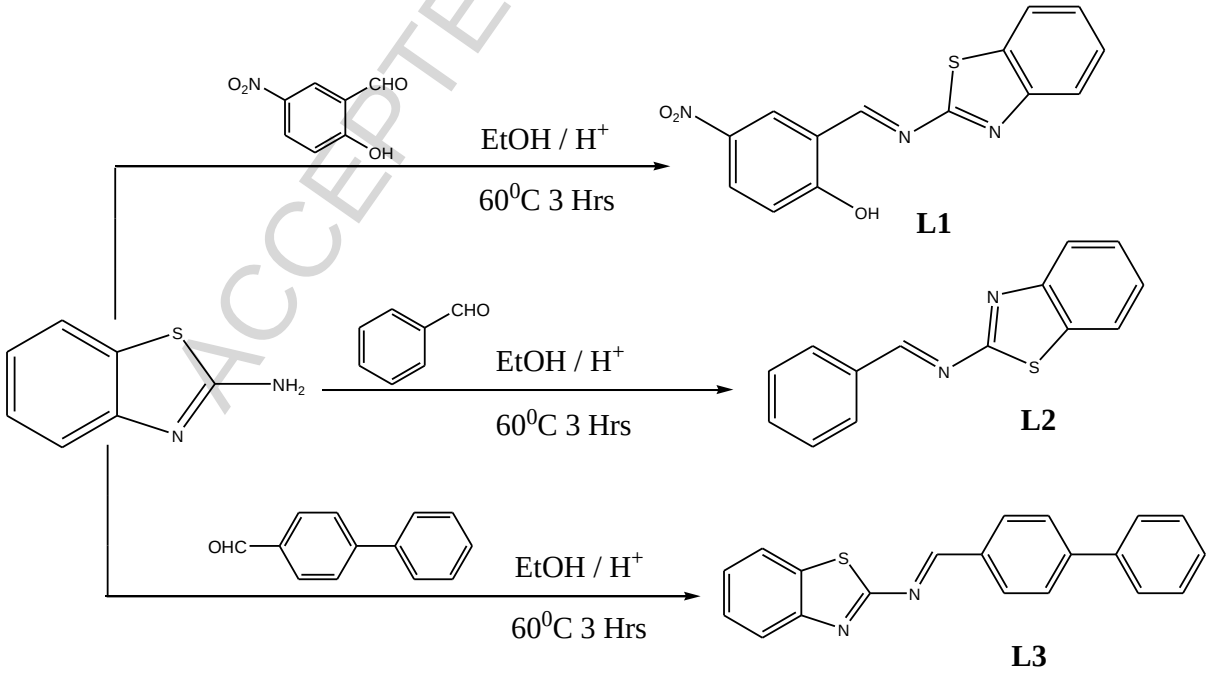
Materials and methods:

All chemicals were procured from Sigma-Aldrich, Alfa Aesar or from Spectrochem and used without further purification. All the solvents were procured from Spectrochem, SD Fine, India of HPLC grade and used without further distillation. The ^{1}H NMR spectra were recorded on a Bruker, (400MHz) instrument using TMS as internal reference and DMSO-d_6 as solvent. Resonance multiplicities are labelled as s

(singlet), d (doublet), t (triplet) and m (multiplet). Melting points were measured on a Stuart- SMP3 melting-point apparatus in open capillaries. Infrared spectra were recorded on a Perkin-Elmer FT-IR spectrometer; signal designations: s (strong), m (medium) and w (weak). UV-vis spectroscopy was carried out with Analytikjena Specord S600 Spectrometer in standard 3.5 mL quartz cells (2 optical windows) with 10 mm path length.

Synthesis of receptors L1-L3:

Receptor **L1-L3** was designed and synthesized as shown in Scheme 1, all receptors were synthesized in a single step, through a condensation reaction of 2-Amino benzothiazole (0.83 mM, 0.125 g) with 5-nitrosalicyaldehyde (0.83 mM, 0.138 g), Benzaldehyde (0.83 mM, 0.088 g) and Biphenyl-4-carboxaldehyde (0.83 mM, 0.152 g) in ethanol under reflux condition at 60°C for 3 hrs. The reaction was catalysed by a drop of acetic acid. After cooling, the solid was filtered and washed with ethanol to obtain the solid designed compounds. The obtained products were characterized by ^{1}H NMR, FT-IR spectroscopy, and Mass spectrometry (See the supporting information Fig. 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b and 4c).



Scheme-1: Synthesis of receptor L1, L2 and L3

Characterization data:

2-(Benzothiazol-2-yliminomethyl)-4-nitro-phenol, (L1):

Melting range: 247-250˚C; IR (KBr, frequency in cm^{-1}): (C=C) 1463 cm^{-1}, 1526 cm^{-1}, (C=N) 1608 cm^{-1}, (C-H aromatic str) 3075 cm^{-1}, (OH) 3377 cm^{-1}. ^{1}H-NMR (500 Mzdmso-d_6, δ_{ppm}):

7.01-7.25 (m,1H),7.38(m,4H), 7.2 (m, 1H), 7.1(m,1 H), 8.0(s HC=N, 1 H), 9.0 (s,-OH 1H).^{13}C NMR ($CDCl_3$, 125 MHz) 51.71, 58.42, 102.05, 110.22, 118.61, 140.79, 154.21, 167.02. MS (ESI, m/z) calculated 299.30, observed (M+1) 300. Elemental analysis calculated C-56.18, H- 3.03, N-14.04 Observed C- 56.32, H-3.08 and N- 13.85.

Benzothiazol-2-yl-benzylidene-amine (L2):

Melting range: 219-224˚C; IR (KBr, frequency in cm^{-1}): (C=C) 1543 cm^{-1}, (C=N) 1615 cm^{-1}, (C-H aromatic str), 3067 cm^{-1}. ^{1}H NMR (300Mzdmso-d_6, δ_{ppm}): 7.62 (m, 1H), 7.5 (m 4H), 7.0 (m, 1H),7.2 (m,1H) 6.89 (m, 1H), 8.95 (s,1H HC=N). ^{13}C NMR ($CDCl_3$, 100 MHz) 76.61, 119.44, 122.00, 126.03, 128.77, 137.43. MS (ESI, m/z) calculated 238.31, observed (M+1) 239.00. Elemental analysis calculated C-70.56, H- 4.23, N-11.76 Observed C- 70.42, H-4.28 and N- 11.87.

Benzothiazol-2-yl-biphenyl-4-ylmethylene-amine (L3):

Melting range: 220-226˚C; IR (KBr, frequency in cm^{-1}): (C=C) 1543 cm^{-1}, (C=N) 1615 cm^{-1}, (C-H aromatic str) 3062cm^{-1}. ^{1}H NMR (400Mz, dmso-d_6, δ_{ppm}): 8.29 (m, 2H), 7.82 (m, 2H) 7.67 (m, 3H), 7.59 (m, 3H), 6.79 (s,1H), 6.6(s, 2H) 5.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) 39.70, 117.50, 125.53, 139.79, 166.57, 189.04. MS (ESI, m/z), calculated 314.40, observed (M+1) 314.00, Elemental analysis calculated C-76.40, H- 4.49, N-8.91 Observed C- 76.32, H-4.41, N- 8.78.

Results and discussion: An individual colorimetric studies were conducted for the synthesized receptor L1-L3 in DMSO solvent, (2.5 x10^{-5} M) for the detection of different cations such as Ni^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+}, Zn^{2+} and Fe^{2+} in an aqueous solution (1.0 x 10^{-3} M). The receptor L1 showed a significant color change from yellow color to color less in the presence of Hg^{2+} ions which can be easily identified by the naked eye and the L1 did not show any color change in the presences of 3.4 equiv. of other tested cations. It indicates that the receptor L1 has selective and sensitive towards Hg^{2+} ions over the other tested metal ions. In the similar way the receptor L2 and L3 were investigated for colorimetric detection of cations and they did not show any color change in the presence 3.4 equiv. of Hg^{2+} and other tested cations.

The absorption spectra of receptor **L1** in DMSO show major band at 445 nm. The recoganization ability of receptor **L1** towards different metal ions investigated by UV-Vis measurement as shown in Fig.2a, receptor **L1** did not show any absorption spectra in the presence of 3.4 equiv. of an aqueous solution of different cations such as Ni^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Cr^{3+}, Zn^{2+} and Fe^{2+}. In the presence 3.4 equiv. of Hg^{2+} ions the receptor **L1** show

a new absorption band at 345 nm and decreased absorption intensity at 445 nm in the UV-Vis absorption spectra (Fig. 2a) and the solution of receptor **L1-Hg^{2+}** showed a significant color change from yellow to colorless, which could be easily detected by naked-eye as shown Fig. 1 This signifies the interaction between receptor **L1** and Hg^{2+} ions and the receptor **L1** sense Hg^{2+} in aqueous medium because of its thiophillic nature and having -OH group.

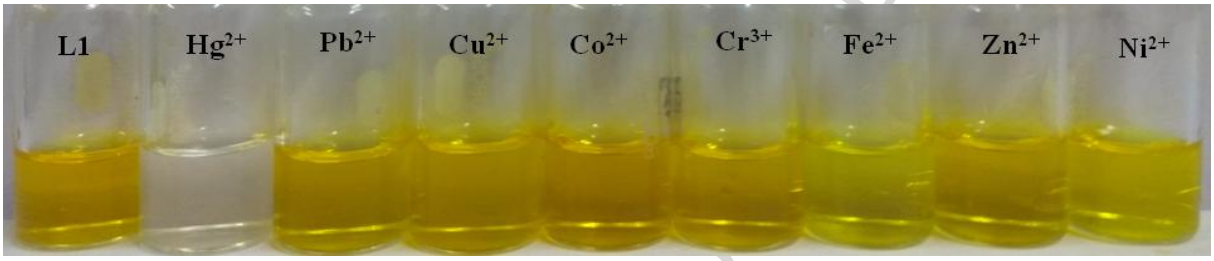


Fig. 1. The colorimetric changes observed by naked-eye of receptor L1 upon addition of 3.4 equiv. of various cations in an aqueous solution

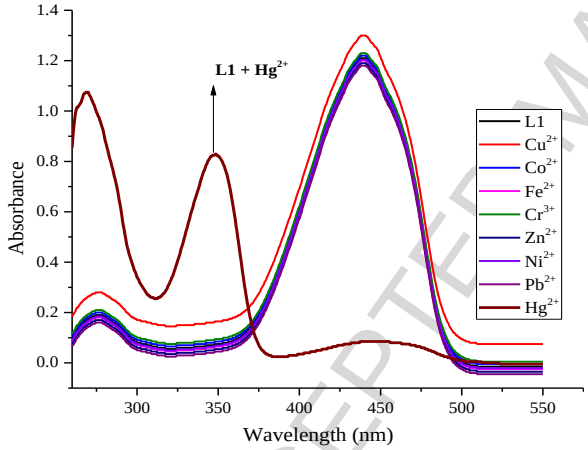


Fig. 2a. UV-Vis spectral change of receptor L1 (2.5×10^{-5} M in DMSO solvent) in the presence of 3.4 equiv. various metal ions. Insert showing the absorbance of receptor L1 and L1+Hg^{2+} ions

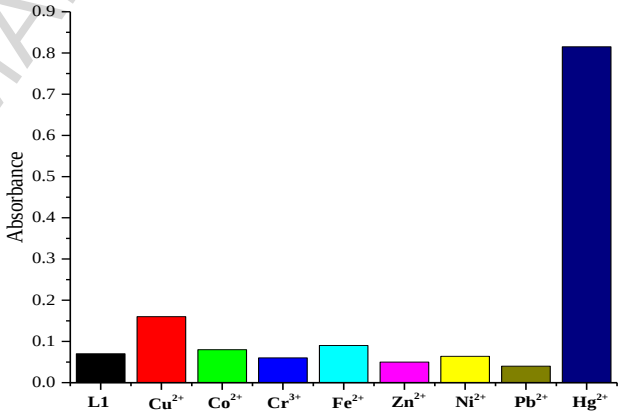


Fig. 2b. A bar chart representation of absorption change of L1 in the presence of 3.4 equiv. of various metal ions at wavelength 345 nm

To explore the sensing properties of receptor **L1** and Hg^{2+}, UV-Vis spectral titration experiment was performed between receptor L1 and Hg^{2+} ions as shown in Fig.3a. The incremental addition from 0 to 3.4 equiv. of Hg^{2+} ions (1.0×10^{-3} M in an aqueous solution) to a solution of receptor **L1** in (2.5×10^{-5} M in DMSO solution), the absorption intensity band at 445 nm corresponds to -OH group in the receptor **L1** gradually decreases and a new band at 345 nm gradually increases. This observation is due to the formation of the complexation of Hg^{2+} ions with receptor **L1**, the electron rich hydroxyl group functioned as a donor group and Hg^{2+} accepts electrons [16-18]. The binding modes of receptor **L1** and Hg^{2+} ions were supported by solid FT-IR studies. The free receptor **L1** displayed -OH str. frequency at 3377 cm^{-1} and the -OH str. frequency of complex (L1 - Hg^{2+}) at 3247 cm^{-1} (See supporting

information Fig.12), this decrimental -OH str. frequency results suggests that Hg^{2+} ions might bind to the phenolic O, $S_{thiazole}$ and N_{imine}.

The binding stoichiometry between receptor L1 and Hg^{2+} ions was determined by the Benesi-Hildebrand (B-H) method using UV-Vis spectrometric titration data wavelength at 345 nm. The linearity of the graph confirms the formation of stable 1:1 complexation of receptor L1 with Hg^{2+} ions as shown in Fig. 3b. The association constant (K) found to be 6.8×10^3 M^{-1} using following equation

$$\frac{1}{(A - A_o)} = \frac{1}{\{K (A_{max} - A_o) . [M_x^+]^n\}} + \frac{1}{[A_{max} - A_o]}$$

Where, A_0, A, A_{max} are the absorption considered in the absence of Hg^{2+}, at an intermediate, and at a concentration of saturation, K is binding constant, $[M_x^+]$ is concentration of Hg^{2+} ions and n is the stoichiometric ratio.

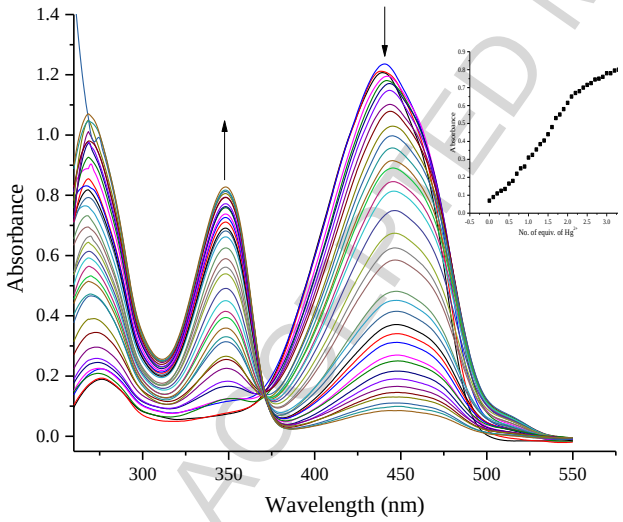


Fig. 3a: UV–vis spectra of L1 **(**2.5×10^{-5} M, DMSO) with increasing concentration of Hg^{2+} ions (0 – 3.4 equiv.) in an aqueous medium. Inset graph shows binding isotherm at selective wavelength (345 nm)

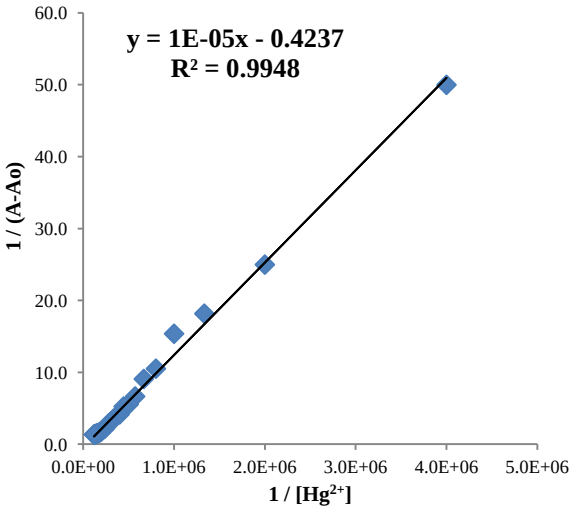


Fig. 3b: Benesi–Hildebrand plot of receptor L1assuming 1:1 binding stoichiometry with Hg^{2+} ions, wavelength at 345 nm

The Detection limit (DL) and Quantization limit (QL) for Hg^{2+} ion was determined using the a calibration plot between Hg^{2+} metal ion concentration (range from 2.5×10^{-7} M to 6.0×10^{-6} M) and corresponding absorbance of receptor L1-Hg^{2+}complex measured wavelength at 345 nm.

The DL and QL were determined based on the standard deviation of the response and the slope using the following equation mentioned by ICH quality guideline Q2R1 [24]

$$DL\ or\ QL = \frac{C \times \sigma}{m}$$

Where, σ = standard deviation of blank measurement**,** m = slope of the calibration curve, C = constant (for DL 3.0 and QL 10.0).

The calibration plot was drawn between the concentration of metal ion and absorbance using LINEST MS-excel as shown in the Fig. 4.

The calculated DL was found to be 0.15 µM and QL was found to be 0.51 µM. (See the supporting information Fig. 10).

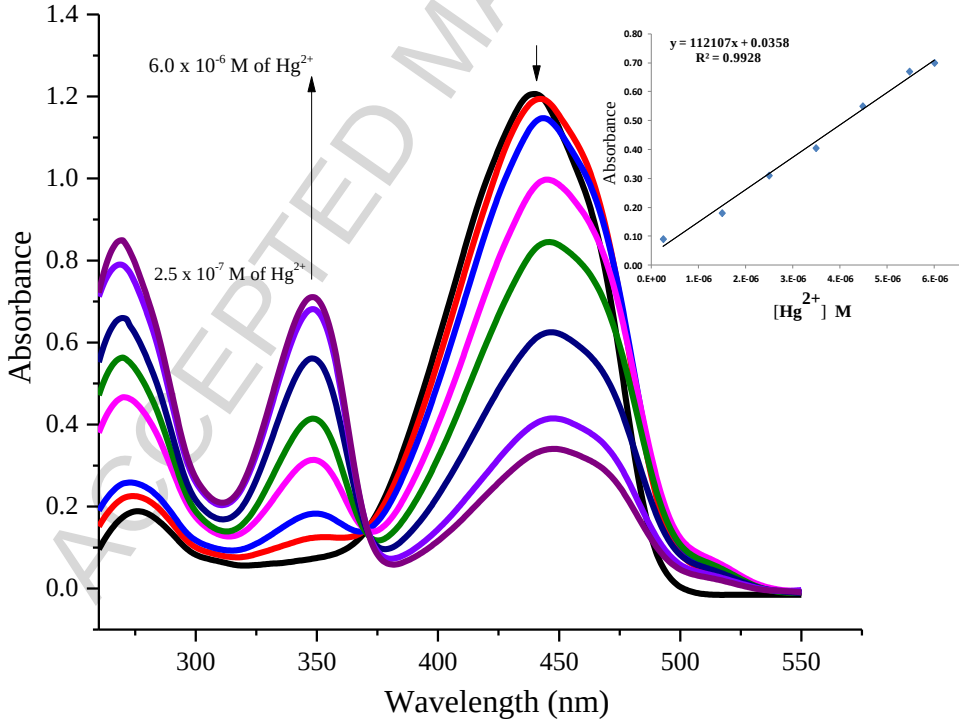


Fig. 4: Linearity of UV-Vis spectra of complex (L1-Hg^{2+}). Inset graph shows calibration curve plot of absorbance of complex (L1-Hg^{2+}) vs concentration of Hg^{2+} ions in M

Based on the UV-Vis titration studies experiment, solid FT-IR and ^{1}H NMR titrations were carried out to further identify the binding mechanism. The figure (ESI-16) shows the spectra of receptor L1 (5×10^{-4} mol /L) up on addition of 0-2 equiv. of Hg^{2+}. When treated with 1.0 equvi of Hg^{2+}, the peak of phenol OH upfield shifted from 9.0 to8.8,

while it completely disappeared after adding 2.0 equiv. of Hg^{2+} ion at the same time the aromatic proton signals were also affected which underwent a concomitant upfield shift. These observations clearly supported the –OH group involved in the binding mechanism. The binding mechanism of the Hg^{2+} ions to the receptor L1 can be proposed as showed in Scheme 2. The solid state FT-IR spectrum were recorded for the free receptor L1 and L1 - Hg^{2+} complex individuality, the observation reveals that the -OH Str. frequency of receptor L1 decreases in the presence 3.4 equiv. of Hg^{2+} ions, from 3377 cm^{-1} to 3247 cm^{-1} as shown in Fig. 5. From the UV-Vis titration and FT-IR studies results reveals that -OH group present in receptor L1 responsible for binding of Hg^{2+} ions. (25)

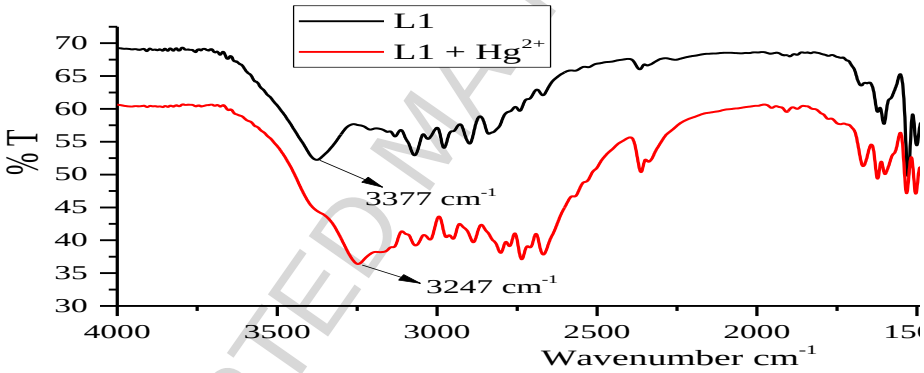
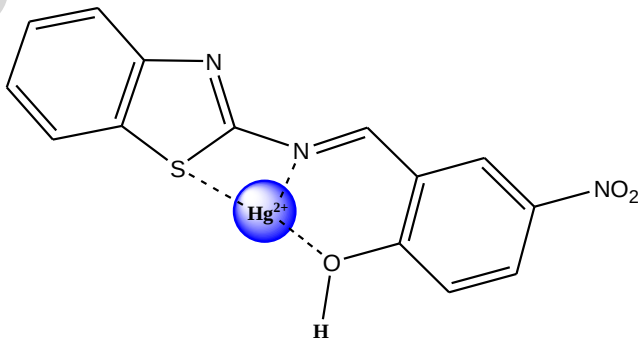


Fig. 5: Solid state FT-IR Spectra of free receptor L1 and L1 - Hg^{2+} complex



Scheme 2: Proposed binding mechanism of Hg^{2+} ions with receptor L1

Conclusion:

To summarize, herein we report, the design and synthesis of receptor L1 for the colorimetric detection of Hg^{2+} ions over other tested cations. The receptors L1 with phenolic-OH functional group as a binding site showed a significant color change from yellow color to

colorless, selectively in the presence of Hg^{2+} ions which can be easily identified by the naked-eye, where as other tested cations failed to show any color change with receptor L1. The color change was due to the formation of complex between Hg^{2+} ions and receptor L1, which was confirmed by UV-Visible spectral titrations and solid state FT-IR studies. The receptor L1 showed a reasonable detection limit of 0.15 μM and a quantization limit of 0.51 μM for Hg^{2+} ions.

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- Detection of Hg^{2+} ion gained significance because of its key role in biological sciences as well as in environmental sciences.
- On adding Hg^{2+} ions to the receptors L1 displayed instantaneous color change from yellow to colorless (Turn-Off Mechanism).
- The receptor L1 showed a reasonable detection limit of 0.15 μM and a quantization limit of 0.51 μM for Hg^{2+} ions.
- 4. The receptor L1 shown good linear range from 2.5 x 10^{-7} M to 7.9 x10^{-6} M of Hg^{2+} ions.

Graphical abstract