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Carbohydrate assisted fluorescence *turn-on* gluco–imino–anthracenyl conjugate as a Hg(II) sensor in milk and blood serum milieu†

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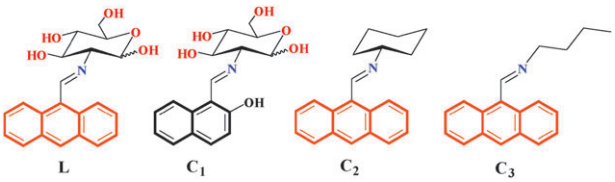
A new anthracenyl–imino–glucosyl conjugate (L) selectively senses Hg^{2+} by *turn-on* fluorescence with a 13 ± 1 fold enhancement by forming a 2 : 1 complex in pH 5 to 10 even in the presence of several biologically and ecologically relevant metal ions, with a 25 ± 2% fluorescence enhancement at the EPA limit of 2 ppb. L is equally sensitive towards Hg^{2+} in the presence of albumin proteins and in blood serum and milk.

A variety of environmental contaminants can cause serious ecological and health hazards. Among these, mercury is highly toxic and is widespread globally.[1] Mercury and some of its species could easily pass through biological membranes, such as skin, respiratory system, and gastrointestinal tissues, causing damage to liver, kidney, immune and nervous system and other organs.[2] Excessive exposure and accumulation of mercury[3] could result in DNA damage and impair mitosis.[4] All these provide sufficient impetus to design new chemical receptors for detection of mercury. Fluorescence is a powerful technique for detecting low concentrations of species.[5] Most of the literature Hg^{2+} receptors known are based upon the fluorescence quenching[6] and utilizes fluorescein,[7] rhodamine,[8] BODIPY,[9] or naphthalimide[10] as reporting groups. However, these generally suffer from poor water solubility, biocompatibility and also from the nonspecific interference arising from other ions such as Cu^{2+}, Ni^{2+}, *etc*. In this regard a molecular system based on carbohydrate would be of great advantage. To our knowledge there are two carbohydrate based reports available in the literature[11] for Hg^{2+} sensing by fluorescence quenching. One of these is based on small molecular one, wherein, the ions such as Cu^{2+}, Co^{2+} and Ni^{2+} exhibit competition against Hg^{2+}.[11a] The second one is a water insoluble polymer supported one, wherein, the studies were not extended to other ions.[11b] In this communication, for the first time, we demonstrate a water soluble glucose based imino–anthracenyl receptor system (**L**) that has been shown to recognize Hg^{2+} by *switch-on* fluorescence. The sensitivity of **L** in the recognition of Hg^{2+} in the presence of albumin proteins, such as BSA and HSA, human blood serum and milk, has also been demonstrated and hence its utility in the biological medium.



Scheme 1 Schematic representation of the structures of reporter **L** and three control (**C_1**, **C_2** and **C_3**) molecules. All the syntheses details are given in ESI 01.†

The receptor (**L**) has been synthesized by a one-step condensation reaction between 9-anthracenaldehyde and C2-deoxy-C2-amino glucose (glucosamine) in ~80% yield (Scheme 1).[12] The control molecules (**C_1**, **C_2** and **C_3**) have accordingly been synthesized. All these have been characterized by analytical and spectral techniques (ESI 01†). **L** differs from that of the **C_2** and **C_3** by having a carbohydrate in place of a non-coordinating cyclohexyl or *n*-butyl moiety. **L** differs from **C_1** by having an anthracenyl moiety in place of a naphthyl one. The presence of the carbohydrate moiety imparts **L** water solubility and biocompatibility. Therefore, the selective recognition of **L** towards Hg^{2+} in aqueous solution has been demonstrated primarily using fluorescence spectroscopy and was further supported by absorption, ^{1}H-NMR, and ESI-MS.

In order to explore the selectivity of **L** towards the metal ion, fluorescence titrations (ESI 02†) were carried out against thirteen ions of biological and ecological importance, *viz*., Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} in aqueous methanol by adding incremental concentrations of metal ions to a fixed amount of **L** (17 μM). Among these ions only Hg^{2+} showed a 13 ± 1 fold fluorescence enhancement (410 nm band), where all other ions exhibited no significant change in the emission intensity. The relative fluorescence intensity (I/I_0) *vs*. mole ratio plot demonstrates the selectivity of **L** towards Hg^{2+} as shown in Fig. 1a. The quantum yield in ethanol was found out to be 0.0015 and 0.0042 for **L** and its Hg^{2+} complex, respectively, using anthracene as standard. In the present case, besides observing a higher fluorescence enhancement, **L** was found to be insensitive towards other ions, including those of Ni^{2+}, Cu^{2+}, and Pb^{2+}.[7b–d,11] In order to prove that the Hg^{2+} binds **L**, absorption titration was carried out in aqueous methanol (ESI 03†).

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† Electronic supplementary information (ESI) available: Synthesis, characterization, absorption, mass spectra, NMR data of all compounds, titration and computational data. See DOI: 10.1039/c0cc04967k

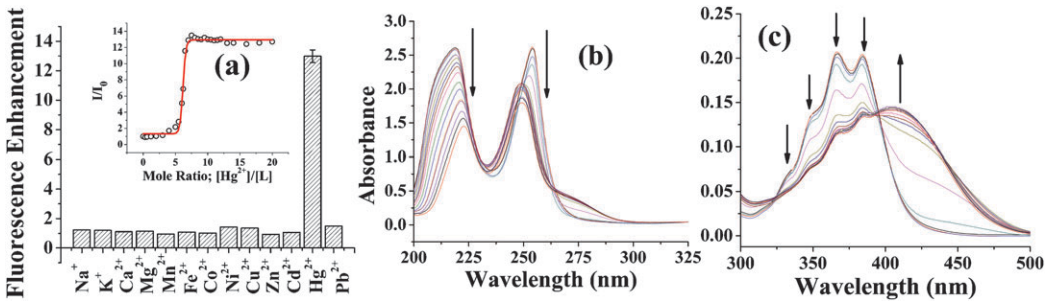

Fig. 1 (a) Fluorescence enhancement observed when **L** was titrated with metal ions. The inset corresponds to (I/I_0) *vs.* mole ratio of Hg^{2+} added to **L**. (b) and (c) are the absorption spectral traces observed in the titration of **L** with Hg^{2+}.

The presence of isosbestic points observed at 395, 325 and 264 nm (Fig. 1b and c) is indicative of the complex formation between Hg^{2+} and **L**. During the course of the titration, the absorbance of the characteristic bands for an anthracenyl-moiety observed at 383, 366, 348 and 331 nm decreases and the shoulder that arises from the imine moiety at 409 nm increases indicating the involvement of both these moieties in Hg^{2+} binding. This result has been checked against all the three control molecules studied, *e.g.*, $\mathbf{C_1}$, $\mathbf{C_2}$ and $\mathbf{C_3}$ (ESI 04†). The interaction between **L** and Hg^{2+} yields K_{ass} of 5032 ± 299 M^{-1}.

Distinguishable colour change was observed with the solutions of **L**, in visible as well as UV light, only in the presence of Hg^{2+} (Fig. 2, ESI 03†) and not with other ions. Hence **L** can also act as a naked eye sensor for Hg^{2+}.

Job's plot derived based on the absorption studies suggested the formation of a 2 : 1 complex between **L** and Hg^{2+}, which was further confirmed by ESI MS wherein a molecular ion peak at $m/z = 933$ exhibits the characteristic isotopic peak pattern for the presence of Hg (Fig. 3a). To find out the selectivity of **L** towards Hg^{2+} over other M^{n+}, competitive titrations were carried out in the presence of 30 equivalents of alkali and alkaline earth ions as well as 5 equivalents of transition ions. No significant change was observed in the fluorescence intensity of $\{\mathbf{L} + Hg^{2+}\}$ in the presence of these competitive ions as can be seen from Fig. 3b. In the ^{1}H NMR spectrum, the down-field shift observed in the protons of imine and C10- of anthracene, and the up-field shift of C1- and C8- protons are suggestive of the interaction of the Hg^{2+} with the imine as well as the anthracenyl moiety present in **L** (ESI 05†).

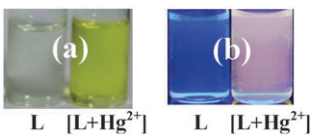

Fig. 2 Colour of the solutions of **L** and $\{\mathbf{L} + Hg^{2+}\}$ under (a) visible and (b) UV light.

Results of titration experiments carried out between $\mathbf{C_1}$ or $\mathbf{C_2}$ or $\mathbf{C_3}$ and Hg^{2+} in comparison with that obtained in the case of **L** and Hg^{2+} are suggestive of the binding of Hg^{2+} through imine as well as cation$\cdots\pi$ interactions (ESI 04†) and a minimum of the anthracenyl moiety is required. In spite of the presence of a number of –OH moieties which normally quenches fluorescence through PET, the observed fluorescence enhancement in the presence of Hg^{2+} by **L** is several fold higher than that observed by $\mathbf{C_2}$ or $\mathbf{C_3}$ which lacks –OH groups (Fig. 4a). Even the absorption spectral changes are much greater in the case of **L** as compared to $\mathbf{C_2}$ or $\mathbf{C_3}$ (Fig. 4b). Both the fluorescence and absorption studies clearly demonstrate that the carbohydrate moiety indeed facilitates the sensor property of **L** by imparting aqueous solubility and biocompatibility when compared to the control $\mathbf{C_2}$ or $\mathbf{C_3}$.

In order to see the sensitivity of **L** towards Hg^{2+} in the biocompatible medium, the titrations were repeated in aqueous ethanol and found that the receptor system detects Hg^{2+} in this medium with the same sensitivity as it exhibited in aqueous methanol. Sensitivity of **L** towards Hg^{2+} has been found to be constant in the biologically relevant pH 5 to 10 (ESI 06†). Since **L** recognizes Hg^{2+} equally well in ethanol, the sensitivity of **L** towards Hg^{2+} has been further carried out in the presence of albumin proteins, blood serum and milk by carrying out titrations, such as $\{Hg^{2+} + \mathbf{L}\}$ *vs.* X and $\{\mathbf{L} + X\}$ *vs.* Hg^{2+} (where X = protein, blood serum or milk) (ESI 06†). Both these types of titrations clearly suggested that there is no significant change observed in the sensitivity of **L** towards Hg^{2+} in the

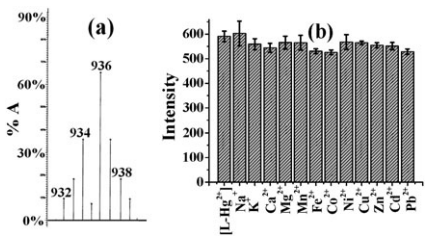

Fig. 3 (a) Molecular ion peak observed for the complex $[(\mathbf{L})_2Hg]^+$ formed in the titration of **L** *vs.* Hg^{2+} in ESI MS, (b) observed fluorescence intensity in the titration of $\{\mathbf{L} + x$ equiv. of $M^{n+}\}$ by Hg^{2+} $\{x = 30$ equiv. in the case of Na^+, K^+, Ca^{2+} and Mg^{2+} and $x = 5$ equiv. in the case of other ions$\}$.

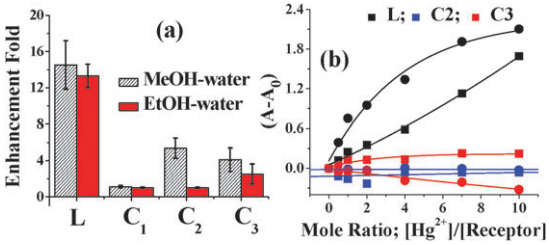

Fig. 4 (a) Observed fluorescence enhancement in the case of the titration of **L**, $\mathbf{C_1}$, $\mathbf{C_2}$, and $\mathbf{C_3}$ by Hg^{2+}; (b) difference absorbance of $\{\mathbf{L} + Hg^{2+}\}$ with that of $\{\mathbf{C_2} + Hg^{2+}\}$ and $\{\mathbf{C_3} + Hg^{2+}\}$ as obtained from the corresponding titrations. The symbols and colours: square (248 nm band) and circle (218 nm band); and black (**L**), blue ($\mathbf{C_2}$) and red ($\mathbf{C_3}$).

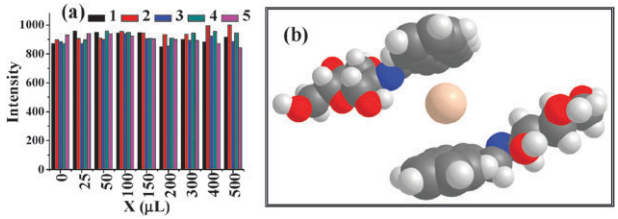

Fig. 5 (a) Fluorescence intensity observed in the titration of {**L** + Hg^{2+}} against incremental amount of X added (where X = BSA, HSA, blood serum or milk), where **1** = BSA, **2** = HSA, **3** = blood serum, **4** = milk protein, and **5** = milk. (b) DFT optimized [**L**–Hg^{2+}–**L**] complex.[13]

presence of any of these biological molecules or medium (Fig. 5). Similar titrations could not be carried out with the control molecules, since there is a precipitation in the medium owing to the absence of the carbohydrate moiety. This indicates the superiority of **L** over $\mathbf{C_2}$ or $\mathbf{C_3}$ as a sensor molecule for Hg^{2+}, wherein the carbohydrate moiety indeed assists the recognition. The detection limit for Hg^{2+} has been determined using fluorescence titration by keeping the **L** to Hg^{2+} ratio at 1 : 2 and was found to be 110 ± 16 nM (50 ± 10 ppb) (ESI 07†). This is certainly lower than 300 to 10 000 nM reported in the literature.[7] Since the EPA limit for Hg^{2+} has been 2 ppb in drinking water, the same has been checked by fluorescence spectra and found to be 25 ± 2% of enhancement in the intensity (ESI 08†) at this concentration. The enhancement observed in the present case is higher than that reported in the literature.[7d] Owing to its water solubility and biocompatibility, **L** would find a wide range of applications including those of environmental concern.

A new and simple glucose based receptor molecular system **L** linked to anthracene through an imine moiety has been synthesized and characterized. **L** shows selective sensing of the Hg^{2+} ion by exhibiting a 13 ± 1 fold fluorescence enhancement among the thirteen ions of biological and ecological importance studied in aqueous solutions of methanol and ethanol. None of these ions was found to compete with Hg^{2+}. The binding of Hg^{2+} to **L** has been shown by absorption, ESI MS and ^{1}H NMR titrations and found that the Hg^{2+} interacts both through the imine moiety (by binding) as well as the aromatic moiety (through cation$\cdots\pi$) and forms a 2 : 1 complex between **L** and Hg^{2+} and the molecular ion peak observed in the ESI MS supports the presence of mercury based on the isotope peak pattern. The interactions present between Hg^{2+} and **L** have been modeled by DFT computations (Fig. 5b, ESI 09†).[13] The reversibility of the Hg^{2+} binding to **L** has been demonstrated by titrating the solution with 50 mM solution of Na_2EDTA (ESI 10†). Hg^{2+} sensing by **L** has been found to be intact even in the presence of human blood serum and milk and also in the presence of albumin proteins. All these qualify the water soluble and biocompatible **L** to be a selective sensor for Hg^{2+} that can perhaps be used even in biological fluids, up to a minimum detection limit of 110 ± 16 nM (50 ± 10 ppb), in the pH range 5 to 10. At the 2 ppb limit of Hg^{2+} in drinking water, the fluorescence enhancement has been found to be 25 ± 2%. The possible application of **L** in sensing Hg^{2+} is further amplified because of the colour change that it exhibits under visible as well as UV light and hence the same may be extendable to even biological cells and tissues.

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