

A Highly Selective Fluorescent Sensor for Pb²⁺ Based on a Modified β -Cyclodextrin

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Abstract A modified cyclodextrin is synthesized as an anthracene derivative of 6-deoxy- 6-aminoethylamino- β -cyclodextrin and characterized using IR, NMR, and mass spectral techniques. The compound acts as a sensor of Pb²⁺ ions in a pool of several metal ions, by enhancement of fluorescence. The absorption spectrum does not show significant changes on the addition of metal ions. The stoichiometry and the binding constant of the complex are determined using fluorescence data. The enhancement of fluorescence is attributed to the aminoethyl and imino nitrogens of the spacer of anthracene and β -cyclodextrin. The compound is quite stable under the testing conditions and the sensing is attributed to the charge transfer mechanism.

Keywords Chemosensor · β -Cyclodextrin · Anthracene · Pb²⁺ sensing · Fluorescence spectroscopy

Introduction

Pb²⁺ ion causes health problems, affecting multiple targets in vivo [1]. Lead poisoning leads to muscle paralysis, anaemia, memory loss, and mental retardation. The Pb²⁺ concen-

tration leads in the Pb²⁺-contaminated sources and the role of Pb²⁺ in multicellular organisms in vivo can be clarified by the development of fluorescent chemosensors [2]. There are several reports on Pb²⁺ sensing [2–4]. However, the sensing using host molecule derivatives are very few in number [5–7]. Many heavy metals quench the fluorescence of receptor molecules through enhanced spin-orbit coupling, energy transfer, or electron transfer. The reported fluorescent Pb²⁺ sensors have used polypeptide scaffolds³ and DNAzymes [7], or considerably lengthy synthetic procedures [7–9]. Current practical methods of detection of lead use intense instrumental methods like inductively coupled plasmon resonance [10], atomic absorption spectrometry [11], and anodic stripping voltammetry [12]. However, these methods measure total lead content only. Hence, there exists a need for detecting and quantifying Pb²⁺ using a simple, inexpensive method.

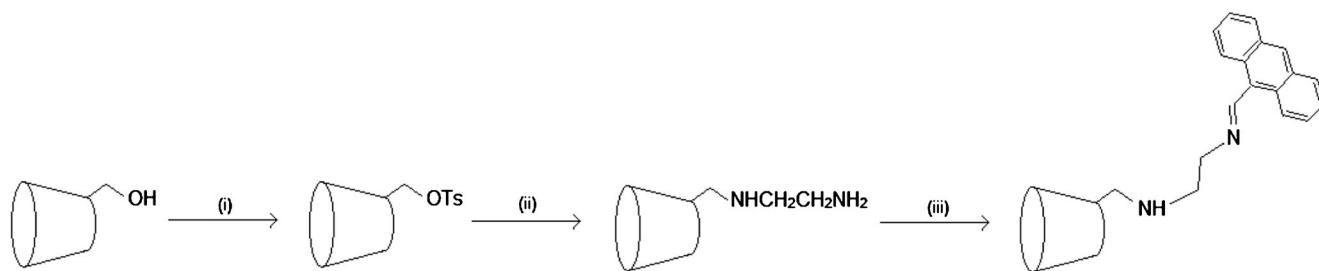
Cyclodextrins (CDs) are a class of cyclic oligosaccharides, which have mainly six to eight α -D-glucopyranose units [13–15]. These bucket-shaped compounds can encapsulate various organic guests within their hydrophobic cavities [16–18]. They can solubilize small molecules in aqueous solution [19]. Less work has been done on metal ion complexes of cyclodextrins or modified cyclodextrins acting as metal sensors because, on the one hand, their hydrophobic cavity is not suitable for metal ion encapsulation [20–22]; on the other hand, unmodified native cyclodextrins require a strong basic pH, where deprotonation of their hydroxyl groups occurs [23]. Hence, ion binding is usually achieved using a chelating group tethered with CD [24, 25]. Herein, we report an anthracene derivative of β -cyclodextrin (β -CD), synthesized using a simple method, and acting as a highly selective fluorescent chemosensor of Pb²⁺ among a pool of 15 metal ions.

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Scheme 1 Synthesis of compound **1**

Materials and Methods

β -Cyclodextrin, ethylene diamine, anthracene-9-carboxaldehyde were purchased from Sigma, India. Dimethyl sulfoxide and all the metal chlorides were products of Merck (India). The synthesis of 6-deoxy-6-[(anthracen-9-ylmethylidene)amino]-N-ethylmethanamino- β -cyclodextrin (Compound **1**) was carried out by refluxing 6-deoxy-6-

aminoethylamino- β -cyclodextrin (0.5 g) with an equimolar amount of anthracene-9-carboxaldehyde in ethanol at 70 °C for 2 h. The formation of the compound was observed by the single spot in thin layer chromatography using dimethyl sulfoxide as the solvent. The solvent was evaporated under vacuum and the compound was retrieved (melting point, 104 °C). The 6-deoxy-6-monoaminoethylamino- β -cyclodextrin was prepared by treating 6-deoxy-6-monosyl- β -cyclodextrin

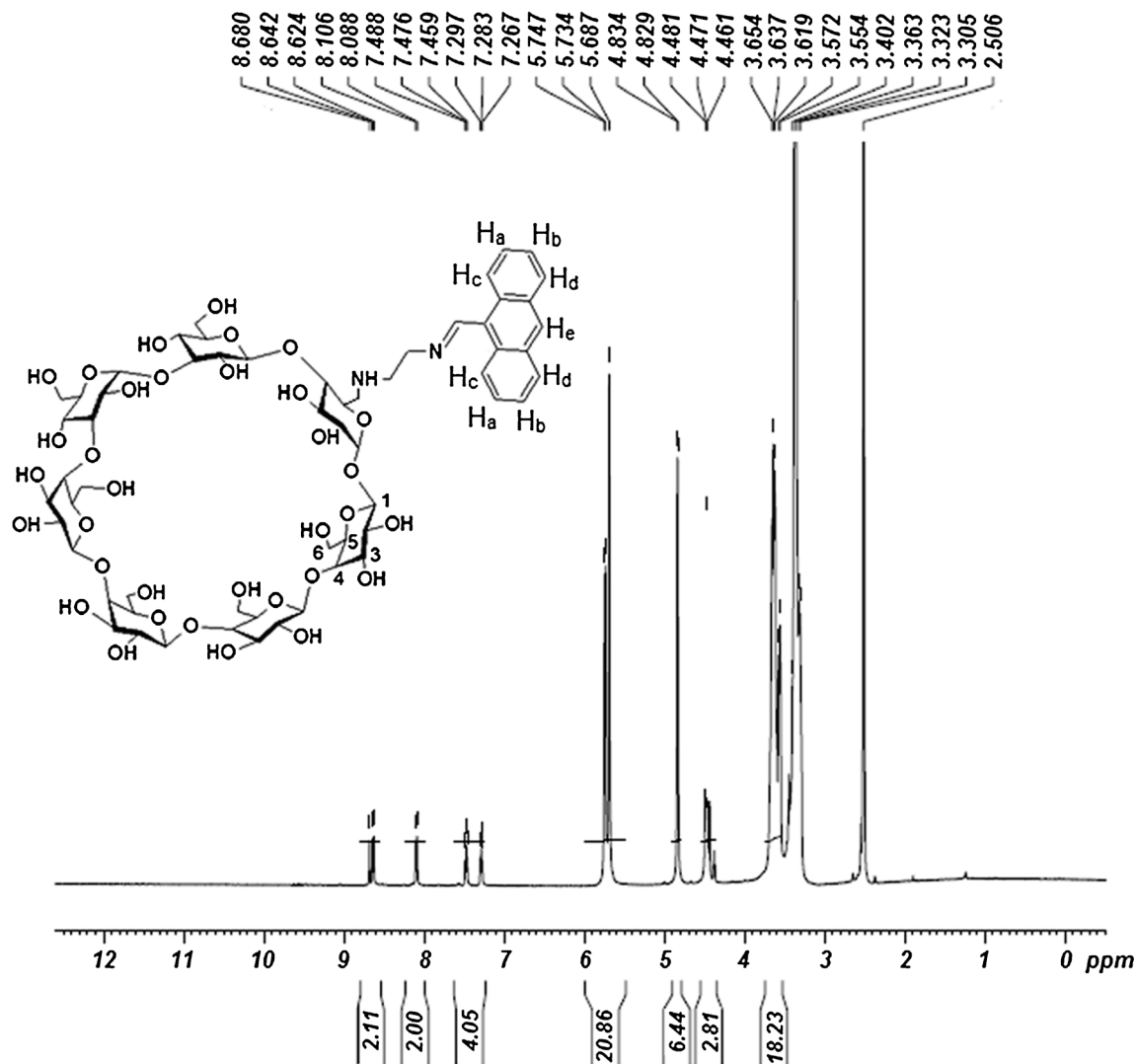


Fig. 1 ^1H NMR spectrum of compound **1**

with ethylamine using the reported procedure [26]. The test solutions for metal ion sensing were made in HEPES buffer.

The melting points of the compounds were recorded using a Labtronics (India) melting point apparatus with capillary tubes. NMR spectra were recorded on a Bruker AMX spectrometer operating at 500 MHz, using tetramethylsilane (TMS) as the reference compound. FT-IR Spectra were recorded using Bruker- α FT-IR spectrometer using KBr pellets. UV–Visible Absorption spectra was measured using a Jasco V 630 spectrophotometer. Fluorescence spectra were recorded using a Jasco FP 8300 spectrophotometer. The path length of the cells was 1 cm. The slit width was 4 nm for both the excitation and emission beams.

Results and Discussion

Compound **1** was synthesized by the treatment of anthracene-9-carboxaldehyde with mono- 6-aminoethylamino-6-deoxy- β -cyclodextrin (Scheme 1). The H^1 and C^{13} NMR

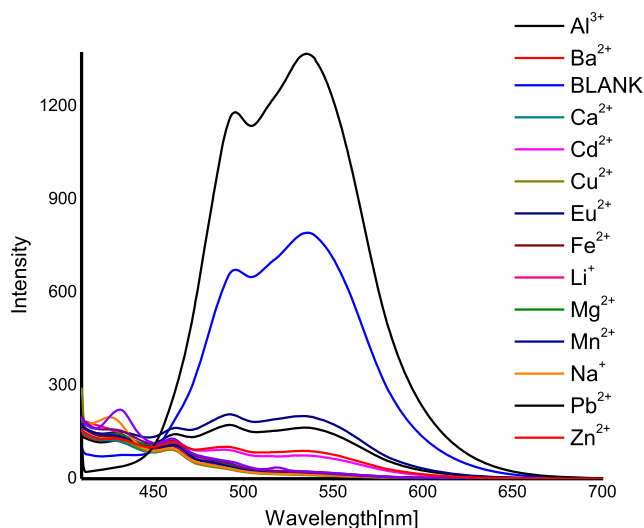


Fig. 3 Fluorescence spectra of compound **1** with various metal ions

spectra of **1** are shown in Figs. 1 and 2 respectively. The assignments of NMR peaks are as follows: H^1 NMR

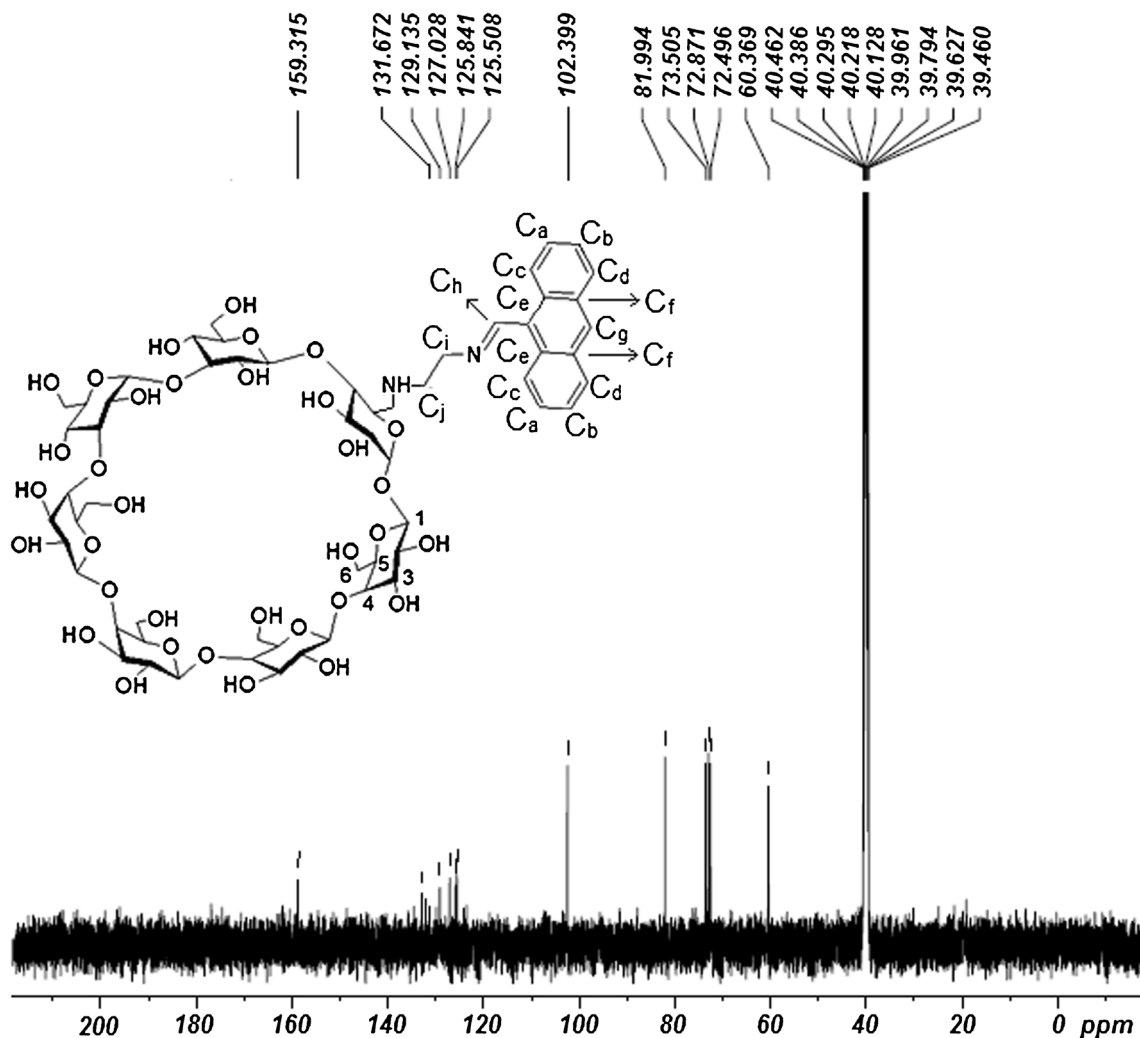


Fig. 2 ^{13}C NMR spectrum of compound **1**

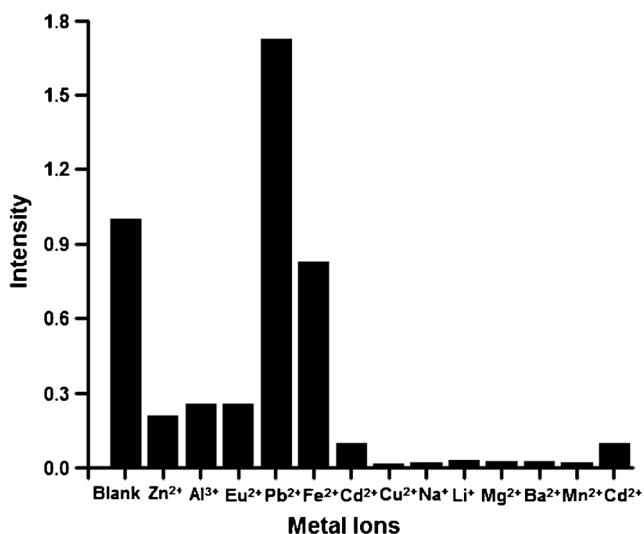
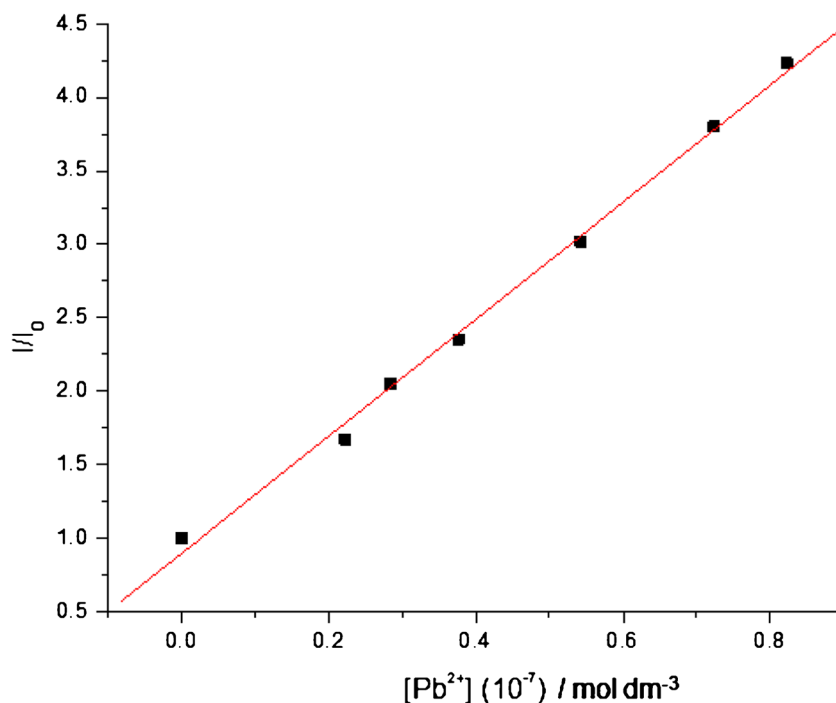


Fig. 4 Intensities of fluorescence showing the sensing of Pb²⁺ ions by compound **1**

(DMSO-D₆): δ =8.642 ppm (4H) – H_c and H_d of the anthracene ring; 8.106 ppm (2H) – H_c of anthracene; 7.283 ppm (4H) – H_a and H_b of the anthracene; 5.747 ppm (7H) – H-1 of β -CD; 4.834 to 4.829 ppm (6H) – methylene protons of the –CH₂NHCH₂CH₂N= moiety; 4.471 ppm (1H) – NH proton; 3.654 to 3.619 ppm (14H) – H-3 and H-5 of β -CD; 3.572 to 3.554 ppm (7H) – H-2 of β -CD; 3.402 to 3.305 ppm (7H) – H-4 of β -CD. C¹³ NMR (DMSO-D₆): δ =159.315 ppm – C_h of the anthracene ring; 131.67 ppm – C_e and C_f of anthracene; 129.135 ppm – C_c and C_d; 127.028 ppm – C_g; 125.841 and 125.508 – C_a and C_b; 102.399 – C-1 of β -CD; 81.994 ppm –

Fig. 5 Relative fluorescence intensity of compound **1** (Emission wavelength=548 nm) as a function of the Pb²⁺ concentration (0–9 × 10^{–8} mol dm^{–3}). Correlation coefficient=0.998, Standard deviation=0.079



C-4 of β -CD; 73.506 to 72.496 ppm – C-2/C-3/C-5 of β -CD/C_j of anthracene; 60.369 ppm – C-6 of β -CD. The significant IR spectral data are as follows: 3448 cm^{–1} band merged with 3380 cm^{–1} band – O-H stretchings of β -CD merged with amino N-H stretching; 2995 and 2910 cm^{–1} – aliphatic C-H stretching; 1675 cm^{–1} – C-N stretching.

The mass spectrum of **1** and the fragments corresponding to the prominent peaks are shown in S11 in the Supporting Information. The molecular ion peak at m/z 1379.81 shows the presence of the formed compound **1**.

The compound was yellow in color and its absorption band was centered at 412 nm. The fluorescence spectrum showed the existence of dual fluorescence, with the two bands centered at 486 nm and 537 nm respectively. The ability of compound **1** to sense metal ions was studied by mixing it with the metal ions Na⁺, K⁺, Li⁺, Ba²⁺, Ca²⁺, Mg²⁺, Mn²⁺, Pb²⁺, Cu²⁺, Cd²⁺, Eu²⁺, Fe²⁺, Ni²⁺, Zn²⁺, and Al³⁺. The absorption spectra are given in the Supporting Information (SI2). The absorbances of the spectra of **1** in the presence of various cations showed insignificant changes, compared to the spectrum of **1** without the metal ions added. However, the addition of Pb²⁺ ions showed a clear hyperchromic shift of absorption of **1**. This absorbance was slightly larger than that of the spectra of all the other the metal ion added compound. In order to evaluate the selectivity of Compound **1** towards any of the above-mentioned metal ions, we recorded the fluorescence spectra of **1** with the various metal ions. The changes of the fluorescence spectrum of **1** on metal ion addition were more pronounced than that of the absorption spectrum. The fluorescence was quenched by all the metal ions, whereas only the

Pb^{2+} ions enhanced it (Fig. 3). The intensity of the emission band was 1.75 fold higher than that of Compound **1** (Fig. 4). This leads to the inference that Pb^{2+} is the only metal ion readily binding to **1**, which enhances the fluorescence significantly. This enhancement of fluorescence permits a highly selective detection of Pb^{2+} . The enhanced fluorescence band (541 nm) is bathochromically shifted by 4 nm, from that of the chemosensor when the binding occurs with Pb^{2+} , whereas all the other metal ion bindings caused slight blue shifts of the band. The fluorescence enhancement might be due to the photoinduced charge transfer (PCR) and the conformational restriction offered by the metal ion binding. A possible photoinduced electron transfer (PET) due to the lone pair of electrons on the NH – and the enhancement of fluorescence due to the hindrance of PET on metal ion binding seems unlikely to occur in this compound. The electron delocalized imino group in conjugation with the anthracene moiety is manifested in the fluorescence spectrum. Diminishing the pH down to 4 does not show any enhancement of the fluorescence of **1**. A titration of **1** ($1 \times 10^{-5} \text{ mol dm}^{-3}$) by the addition of 0 to 110 equivalents of Pb^{2+} in water showed a saturation point when 110 equivalents of Pb^{2+} is added. Figure 5 shows the relative intensity versus the concentration of Pb^{2+} in the low concentration range up to $8.23 \times 10^{-8} \text{ mol dm}^{-3}$. Hence, this is the detection limit.

In order to study the influence of other metal ions on the Pb^{2+} ion binding by **1**, we studied the binding of Pb^{2+} ($1 \times 10^{-5} \text{ mol dm}^{-3}$) combined with the other metal ions ($1 \times 10^{-5} \text{ mol dm}^{-3}$). The fluorescence enhancement caused when Pb^{2+} was added in the presence of each of the other metal ions was similar to the enhancement due to the addition of Pb^{2+} alone (Fig. 6). This result is an indication that the other metal ions do not interfere with the Pb^{2+} ion binding to **1**.

Stoichiometry and Binding Constant of 1– Pb^{2+} Complex

Job's plot was made using the data obtained from the concentration dependent fluorescence intensity changes of the 1–

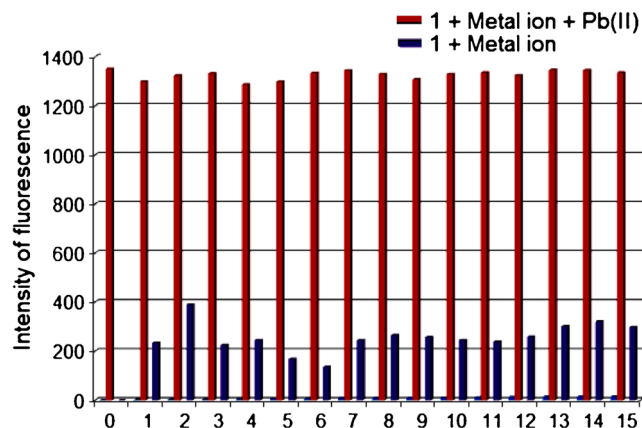


Fig. 6 Competitive binding of Pb^{2+} in the presence of other metal ions by compound **1**

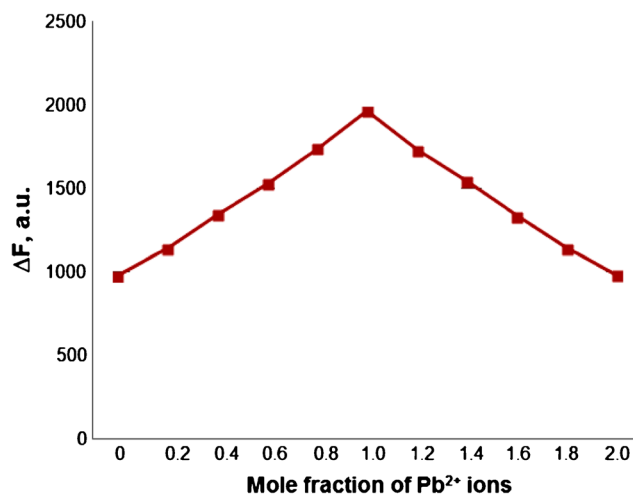
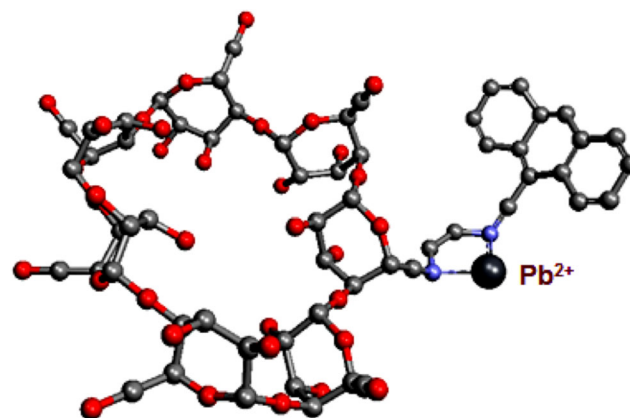


Fig. 7 Job plot of the compound **1**– Pb^{2+} binding

Pb^{2+} complex. The plot of emission intensity at 537 nm vs. Molar fraction of **1**, with a constant total concentration is shown in Fig. 7. The maximum emission intensity was found when the molar fraction was 1.0. This corresponds to a 1:1 ratio between **1** and Pb^{2+} . The $\log K$ value obtained was 9.2 ± 0.2 . The stoichiometry of the **1**– Pb^{2+} complex was 1:1, as indicated by the Job's plot. Based on the above results, the binding of Pb^{2+} ions to the receptor aminoethylmethanamino moiety of Compound **1** is depicted in Scheme 2.

Conclusions

We have developed a new derivatized β -cyclodextrin-based fluorescent sensor for Pb^{2+} using fluorescence enhancement, selectively of the lead ion amidst fluorescence quenching by all the other cations. The stoichiometry of the modified cyclodextrin : Pb^{2+} ion complex is 1:1 with the $\log K$ of 9.2 ± 0.2 . The side chain modification of aminoethylamino- β -cyclodextrin makes way for the application in biological systems, with the possible inclusion of deliverable small molecule in the cavity of β -cyclodextrin.



Scheme 2 Representation of the **1**– Pb^{2+} binding

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