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A simple and fluorescence turn-on recognition of Pb^{2+} ions involving pyrene-thiadiazole chemosensor probe

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In this present work, an exceptionally specific and selective chemosensor (Z)-5-((pyren-1-ylmethylene)amino)-1,3,4-thiadiazole-2-thiol (PCAT), has been designed and synthesized via simplest synthetic route. The synthesized probe is successfully portrayed by utilizing ^{1}H, ^{13}C NMR, ESI-MS techniques. The PCAT probe indicates colour changes which can be seen through naked-eye from colourless to pink and fluorescence turn-on response specifically towards Pb^{2+} ions with the possible contending metal ions. The binding stoichiometric proportion has been found as 1:1 from the Job's plot investigation. The detection limit for PCAT-Pb^{2+} ions is found to be 7.15×10^{-8} M. Besides, the real sample analysis is also done with the collected ecological water samples and has resulted in an excellent recovery percentage.

Keywords: Environmental water samples, fluorescence turn-on, Naked-eye, Selective, Sensitive

Fluorescence chemosensor has risen goliath attentions in the most latest years in the light of their high sensitivity, rapid detection and operational simplicity[1–3]. Specifically, perceiving heavy metal ions is contemplating about their non-biodegradability and incredibly destructive impact on the human flourishing, aquatic plants and animals[4,5]. Among the few heavy metal ions, Lead is a component with enunciated industrial utility and is broadly implemented in storage batteries, alloys, lead wires, paints, high quality glasses for welding of electronic contraptions and in foundries[6,7]. Nonetheless, it is far exceptionally second most adequate poisonous heavy metal that is appropriately acknowledged to cause a various sort of serious health impact including neurotoxin effects, reproductive dysfunction, gastrointestinal, anemia, memory loss, irritability and mental retardation even at very low concentrations, specifically most dangerous to kids[8]. The health impact has become passed off whilst people are consuming the compounds incorporates lead through inhalation, swallowing and rarely via skin[9]. Besides, an immense part of the industry produced and discharged Pb^{2+} into water and soil consistently that's moved into plants and accumulated in cereals and humans[10]. Likewise, cereals are the fundamental crop for half of the entire population. This method of food has pretty expanded human's consumption of heavy metals and threat of illness[11]. A collection of indications has been ascribed to lead poisoning inclusive of abdominal pain and vomiting. Based on the aforementioned toxicity of Pb^{2+} ions, the permissible limit for lead is 0.05 mg L^{-1} and 0.1 mg L^{-1} in drinking water has been set by the US environmental Protection Agency (EPA) and Bureau of Indian Standards respectively[12,13]. In this manner, to cope with all the expressed harmfulness and destructive impact of Pb^{2+}, it is far extremely urgent and of great importance to design simple cum cost-effective receptor to sense Pb^{2+} ion.

To date, some of traditional analytical techniques have been employed to detect Pb^{2+}ion such as atomic absorption/emission spectrometry[14], accelerator mass spectroscopy[15], inductively coupled plasma mass spectrometry[16], laser ablation microprobe mass analysis[17], electrothermal atomic absorption spectrometry[18], inductively coupled plasma-optical emission spectrometry[19,] square wave anodic stripping voltammetry[20]and several others[21,22]. These techniques are moderately unpredictable, often required expensive instrumentation and well trained personnel, making them fallacious for onsite detection. Therefore, the scientific research network has turned their attentions towards the fluorescence spectroscopic sensing technique which is effective over the other techniques and it has gigantic excitement in recent years for the detection of heavy metal ions due to its operational simplicity, environmental friendly, cost-effective, rapid pace detection, high selectivity and sensitivity[23,24].

In this present investigation, we have chosen the organic heterocyclic moieties built with nitrogen and sulphur to design a cost-effective with displaying incredible pharmacological activities receptor. Generally, five-membered ring heterocyclic frameworks contain two nitrogen atoms and one sulfur atom[25]. This has been pioneered and portrayed by Fischer in 1882[26]; however, the actual nature of these frameworks is clarified with explanation by Freund and Kuh in 1890[27]. Especially, these systems have showed a wide range of interesting biological activities. Moreover, 1,3,4-thiadiazole has shown well intense biological activities inclusive of antituberculosis, antifungal, diuretic, anticonvulsant, antihypertensive, antidepressant, antimicrobial, anti-inflammatory and anticancer and so forth[28-32]. In spite of their essentiality in biology and pharmacology, the mechanism of their action is still ineffectively known to the scientific society[33,34].

On the alternative hand, pyrene-1-carbaldehye has also been chosen for the structuring of receptor. Among various fluorophores, pyrene is an effective fluorogenic unit, chemical stability, low cost, strong absorption cum emission spectra, high quantum yield, long fluorescence lifetime and extended π electron delocalization[35-39]. It is increasingly beneficial because of its relatively efficient excimer formation and changes in emission properties. Plenty of pyrene based probes exhibits diverse promising applications in biolabelling, chemosensing, protein detection and so on. Nonetheless, there is a little number of reports comprising pyrene-based derivative as a sensor for heavy metal ions[40-42]. Therefore, it is essential to develop a pyrene-based naked-eye and fluorescence sensor for the detection of heavy metal ions. Besides, Schiff base is a form of organic probe which consists of an imine bond which is a unique covalent bond and can be efficiently synthesized[43,44]. Although many Schiff bases have robust emission and coordination capability with numerous metallic ions has broadly beenexamined[45,46]. Also, pinene molecules embedded on nanogels showed also be looked into for sensing properties[47-48]. Hence, in this present work, a novel chemosensor probe incorporated with the aforementioned moieties to design for highly selective and sensitive fluorescent sensor which is applied for real sample analysis in environmental water samples.

Experimental Section

Materials

Pyrene-1-carbaldehye, 5-amino-1,3,4-thiadiazole-2-thiol, all solvents, metal salts, analytical and spectroscopic grade reagents have been acquired and used from the suppliers from Sigma-Aldrich, Merck as received.

Instrumentation

^{1}H NMR and ^{13}C NMR have been recorded on a BRUKER 300 MHz instrument, the terms chemical shifts are referred in ppm, internal standard and solvent Tetramethylsilane and DMSO-d6 respectively have been utilized. Electronic absorption spectroscopic measurements have been performed by using JASCO V-550 UV-Visible spectrophotometer with a quartz cuvette having cell length 1cm in solution. High resolution electrospray ionization mass spectrometry analysis has been performed on the positive and negative ion modes on a Waters Q-Tof premier-HAB 213. Fluorescence spectra have been recorded through Agilent Cary Eclipse fluorescence spectrofluorimeter and DFT figuring and calculations have been also carried out by the utilization of Gaussian 09 package.

Synthesis of PCAT

A methanolic solution of Pyrene-1-carbaldehye (50 mg, 1 mmol) has been refluxed with 5-amino-1, 3, 4-thiadiazole-2-thiol (28 mg, 1 mmol) for about 2 hours. The development of the reaction is monitored through thin layer chromatography leading to getan yellowish orange color solid product namely (Z)-5-((pyren-1-ylmethylene)amino)-1,3,4-thiadiazole-2-thiol (PCAT). The acquired solid product has been recrystalized by the hot methanolic solution. (Yield 98%).

Preparation of stock solutions

Before proceeding to the analysis part, the stock solution of the PCAT has been prepared in DMSO at the concentration level of 1×10^{-4} M. The solution of metal ions from their chloride salts except silver nitrate, lead nitrate have been prepared at the concentration of 1×10^{-4}M using double distilled water then diluted accordingly, to respective techniques. The PCAT stock solution (30 μL) has been diluted with 2 mL DMSO to make a final concentration level of 15 μM which has been utilized for the entire spectroscopic studies carried out at room temperature.

Calculation of limit of detection and binding constant

The detection limit has been calculated from the standard formula 3σ/s; in which σ is the standard deviation of the blank titration and s is the slope which is derived by calibration curve. The binding constant has been determined from the fluorescence spectra results using Benesi-Hilderbrand equation,

(BH plot) $1/\Delta I = 1/\Delta I_{max} + (1/K[C] (1/\Delta I_{max})$, where $\Delta I = I-I_{min}$ and $I_{max}-I_{min}$, I_{min}, I and I_{max} are the emission intensities of the probe in the absence of Pb^{2+} ions, K is the binding constant and C is the concentration of Pb^{2+} ions. The binding constant k can be calculated from the plot of $(I_{max}-I_{min})/(I-I_{min})$ against $[C]^{-1}$ of Pb^{2+} ions.

Photophysical properties of PCAT

The PCAT photophysical response has been tested in the presence of diverse metal ions using absorbance and fluorescence spectroscopic techniques. Further, absorbance and fluorescence spectroscopy had been recorded to determine the selectivity, sensitivity and competitive sensing experiments. The PCAT fluorescence spectra have been recorded at λexc = 376nm and a slit width of 5nm.

Density functional theory calculations

Density functional theory (DFT) calculations have been performed by the usage of Gaussian 09 programme with 6-311G basis set. The B3LYP basis set has been used for the PCAT free probe and the LANL2DZ is the basis set employed for probe with lead ions. The geometries of all have been optimized using the respective basis sets.

Results and Discussion

Synthesis and characterization of Probe PCAT

The PCAT probe has been successfully synthesized by the condensation reaction of Pyrene-1-carbaldehyewith 5-amino-1, 3, 4-thiadiazole-2-thiol in methanol (Scheme 1). The spectroscopic technique results such as ^{1}H and ^{13}C-NMR and ESI-MS have confirmed the structure of PCAT probe effectively.

^{1}H NMR and ^{13}C NMR Spectral studies

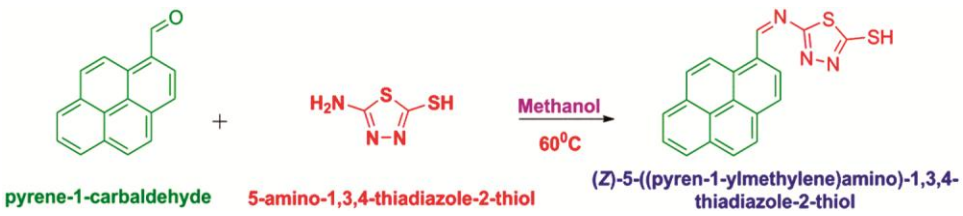
^{1}H NMR (300 MHz, DMSO): δ 8.25 (s, 1H), 6.80 (s, 1H), 6.02 (d, *J* = 8.0 Hz, 1H), 5.94 – 5.83 (m, 3H), 5.74 – 5.62 (m, 1H), 4.61 (s, 1H). ^{13}C NMR (75 MHz, DMSO: δ 194.61 (s), 135.77 (s), 131.84 (s), 131.60 (s), 131.32 (s), 131.01 (s), 130.63 (s), 128.19 (s), 128.19 (s), 128.05 (s), 127.79 (s), 125.69 (s), 124.53 (s), 123.98 (s), 123.32 (s). LC-MS (calculated 345.44, Found 344.03).

UV-Vis spectral selectivity and sensitivity studies of PCAT

At first, the specific sensing capability of PCAT with various metal ions in DMSO has been checked by utilizing the electronic spectra. PCAT probe displays two absorption bands at 296 nm and 445 nm which are ascribed to π-π* and n-π* transitions. These peaks did not show any noteworthy changes within the sight of diverse metal ions (Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Cr^{3+}, Al^{3+}, Mn^{2+}, Fe^{2+},Fe^{3+},Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, $Hg^{2+}$and Ag^{+}), however, the introduction of Pb^{2+}ions to the PCAT brought on significant spectral changes with red shift and a new peak has been found at 497nm that's shown in Fig. 1 a and b. This is probably attributed to the interaction between PCAT and Pb^{2+} ions. The colour changes of every addition can also be seen in the presence and absence of Pb^{2+} ions via naked-eye[49,50]. In order to verify the PCAT sensing potential with respect to Pb^{2+} ions, the titration experiments have been performed by utilizing UV-Vis absorption at room temperature. The incremental introduction of Pb^{2+} ions is done to the solution of PCAT (0 to120μM), Many spectral changes has been observed. The absorbance intensity is significantly quenched at the band 497nm. Based on these obtained spectroscopic changes the experiment indicates that PCAT certainly can have the interaction with Pb^{2+} ions. This significant observation surely distinguishes Pb^{2+} ions from the other metal ions[51] (Fig. 2).

Fluorescence spectral selectivity and sensitivity studies of PCAT

In fluorescence sensing systems, the specificity is the significant for assessing the exhibition of the fluorescence behaviour. Here, the PCAT fluorescence behaviour and selectivity of the probe PCAT in the presence of diverse metal ions have been examined in the solution state of DMSO. PCAT probe alone exhibits a peak in the fluorescence spectrum around at 422nm. There is no appreciable changes in the



Scheme 1 — Synthetic route for PCAT.

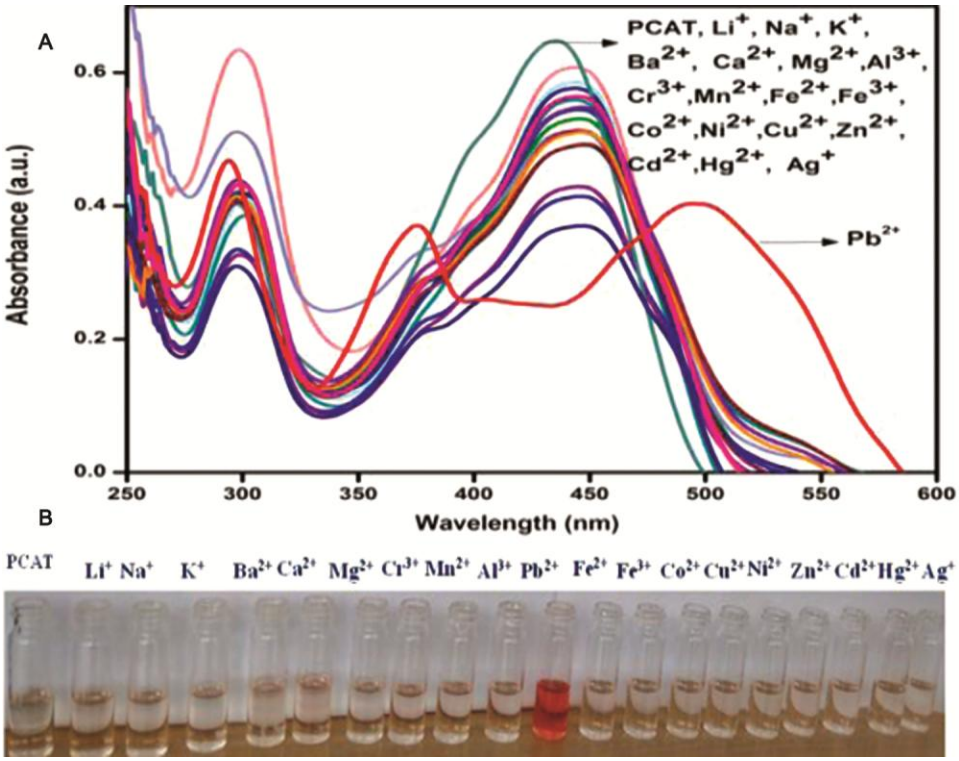


Fig. 1A) — UV-Vis spectra of PCAT (15 μM) in the presence of contending metal ions in DMSO (Li^+, Na^+, K^+, Ba^{2+},Ca^{2+}, Mg^{2+}, Al^{3+}, Cr^{3+},Mn^{2+}, Fe^{2+},Fe^{3+},Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, $Pb^{2+}$and Ag^+). B) The colour changes of PCAT (15μM) in the presence of contending metal ions under daylight in DMSO.

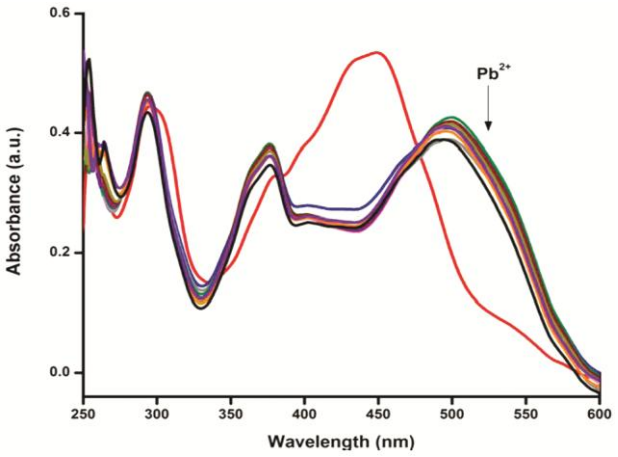


Fig. 2 — UV-Vis spectral changes of PCAT (15μM) upon the titration of Pb^{2+} ions 0 to120μM in DMSO.

presence of various metal cations (Li^+, Na^+, K^+, Ba^{2+},Ca^{2+}, Mg^{2+}, Al^{3+}, Cr^{3+},Mn^{2+}, Fe^{2+},Fe^{3+},Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Ag^+) except, the addition of Pb^{2+} ions culminated in an intense fluorescence band upon excitation at 376nm. As mentioned earlier, the fluorescence intensity is not much affected in the presence of other metal ions except Pb^{2+} ions. This fluorescence changes happen only because of the particular Pb^{2+} ions and at the same time as the alternative metal ions cause no changes in the spectroscopic outcome. This observed fluorescence results suggest that the PCAT has fantastic selectivity with respect to Pb^{2+} ions[52,53] (Fig. 3A-B).

The fluorescence spectroscopic titration experiments have also been carried out to understand the response of PCAT with respect to Pb^{2+} ions at room temperature under the identical conditions. A consistent incremental addition of Pb^{2+} ions to the solution state of PCAT, resulted in gradual enhancement in fluorescence intensity with predominant red shift, has been observed. These changes of fluorescence spectra only in the presence of Pb^{2+} ions happen only because of the chelation with the probe PCAT. This observed fluorescence enhancement is explained by the chelation enhancement of fluorescence (CHEF) process of the imine nitrogen presence in the PCAT[54,55] (Fig. 4).

Competitive binding experiments of Pb^{2+} ions

The competitive experiments have been performed by utilizing the electronic and fluorescence spectroscopic response of the PCAT sensing system towards Pb^{2+} ions in the presence of other contending metal ions of 10 equivalents to check the interference. The probe

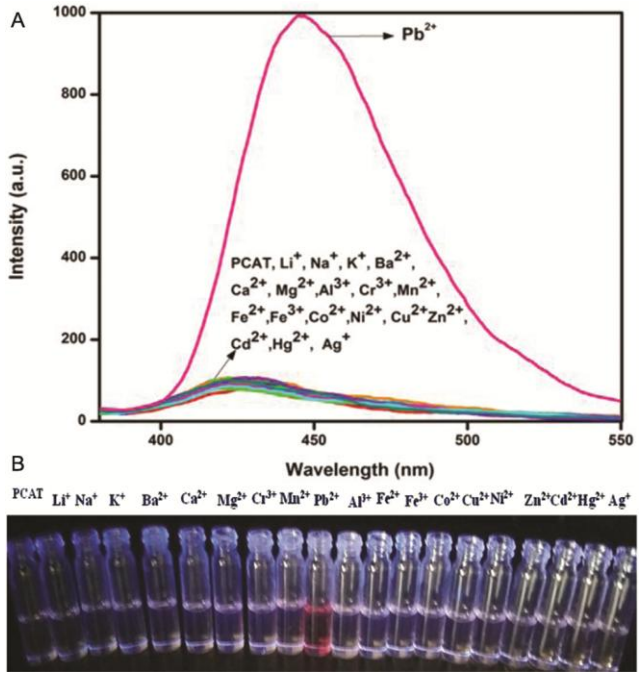


Fig. 3A) — Fluorescence spectra of PCAT (15 μM) in the presence of contending metal ions such as Li^+, Na^+, K^+, Ba^{2+},Ca^{2+}, Mg^{2+}, Al^{3+}, Cr^{3+},Mn^{2+}, Fe^{2+},Fe^{3+},Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, $Pb^{2+}$and Ag^+in DMSO. B) The color changes of PCAT (15μM) in the presence of contending metal ionsin DMSO under UV-light.

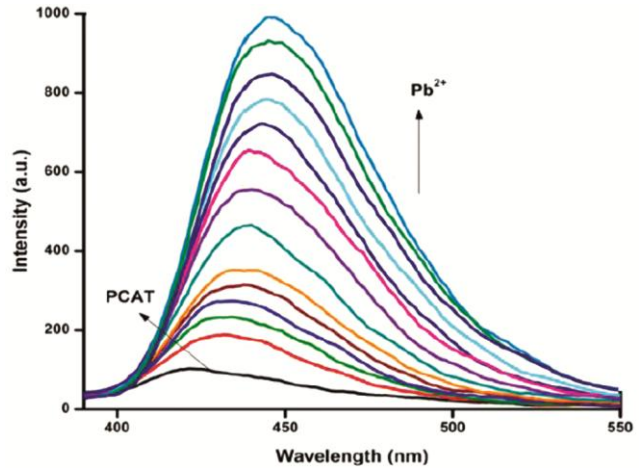


Fig. 4 — Fluorescence spectral changes of PCAT (15μM) upon the titration of Pb^{2+} ions 0 to 120μM in DMSO.

PCAT and contending metal ions presence indicate that, no metal ions interfere with Pb^{2+} ions. This result also exhibits that their coexisting metal cations have resulted in spectroscopic responses in significant obstruction on Pb^{2+} ions sensing by PCAT framework. Consequently, PCAT can provide, for sure, a specific and sensitive system for Pb^{2+} ions in a competing domain[56](Fig. 5).

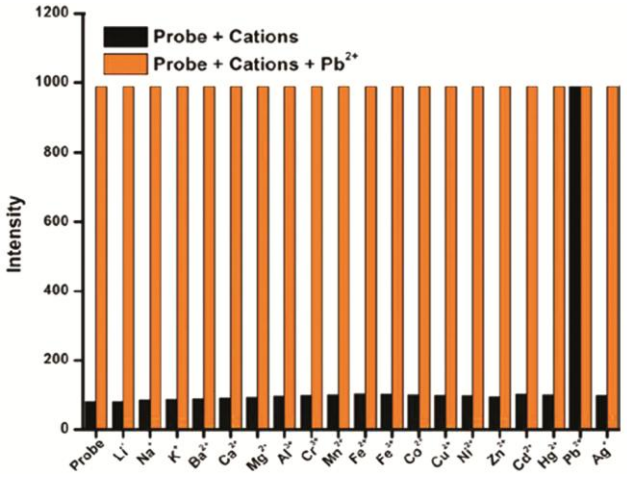


Fig. 5 — Bar diagram for the competitive experiments with PCAT in the presence of contending cations using fluorescence spectra. The black bars represent the competing cations with an added PCAT-Pb^{2+} ions in DMSO solution and the pink bars represent the absorbance intensity of PCAT-Pb^{2+} ions.

Scheme. 2 — Schematic representation of proposed plausible mechanism of PCAT with Pb^{2+} ion.

Plausible mechanistic pathway of PCAT towards Pb^{2+} ions

To explore the mechanistic pathway for the sensing system after successfully establishing the PCAT as an efficient chemosensor, is relatively specific sensing with respect to Pb^{2+} ions. The acquired spectroscopic results indicate that the Pb^{2+}ions sensing may be CHEF process. It may be attributed to the chelation of the imine nitrogen of the PCAT framework for CHEF process. Such a CHEF process is enhanced by the complexation of PCAT with Pb^{2+} ions. In this way, fluorescence intensity may be fantastically enhanced by the coordination of $Pb^{2+}$and also chelation of the donor atoms present in the PCAT. Hence, the CHEF process is the plausible mechanistic pathway for PCAT with Pb^{2+} ions interaction[57] (Scheme 2).

Stoichiometric analysis of PCAT towards Pb^{2+} ions

To confirm the stoichiometric proportion between PCAT and Pb^{2+} ions, Job's plot investigation has been completed by utilizing fluorescence spectroscopic method. The plot of the fluorescence intensity variation against mole fraction of the Pb^{2+} ions which is clearly indicated by the maxima of a mole fraction at 0.5, the

stoichiometric proportion has been determined as 1:1 which is further supported from DFT figuring with calculations[58] (Fig. 6).

Calculation of limit of detection and binding constant (BH plot)

The binding constant of PCAT for Pb^{2+} ions has been determined by utilizing Benesi-Hilderbrand equation k value to be 2.83×10^{-3} Mutilizing the titration profile of the fluorescence technique[59]as shown in Fig. 7A-B, Moreover, the detection limit has been determined by using the formula $DL=3\sigma/k$ where σ is the relative standard deviation from the blank titration, k is the slope of the calibration curve and it is to be 7.15×10^{-8} M for Pb^{2+}ions.

DFT calculations

DFT has played a significant role in understanding the sensing mechanism theoretically. In order to affirm and rationalize the obtained spectroscopic outcomes, DFT studies have been performed. The geometry of the PCAT and PCAT-Pb^{2+} ions is optimized using DFT-B3LYP 6-31G level for free probe LANL2DZ for metal binding probe utilizing Gaussian 09 package. In the free probe PCAT HOMO is localized over the entire moiety, though LUMO is slightly electron cloud which is moved to the thiophene moiety. After interacting with Pb^{2+} ions, the electron cloud in HOMO is present in the whole framework while in the LUMO electron cloud is highly over in the metal chelation binding site which confirms the proposed mechanism as CHEF process for PCAT-Pb^{2+} ions. The HOMO-LUMO energy gap also has been decreased when compared with the free probe as appeared in Fig. 8,

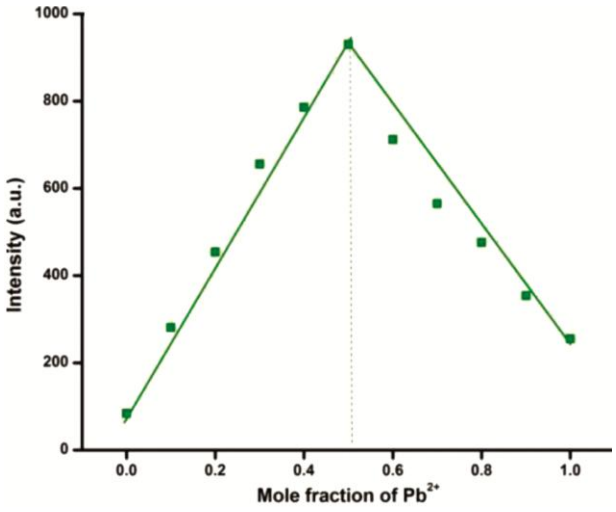


Fig. 6 — Job's plot analysis between PCAT and Pb^{2+} ions using fluorescence intensity.

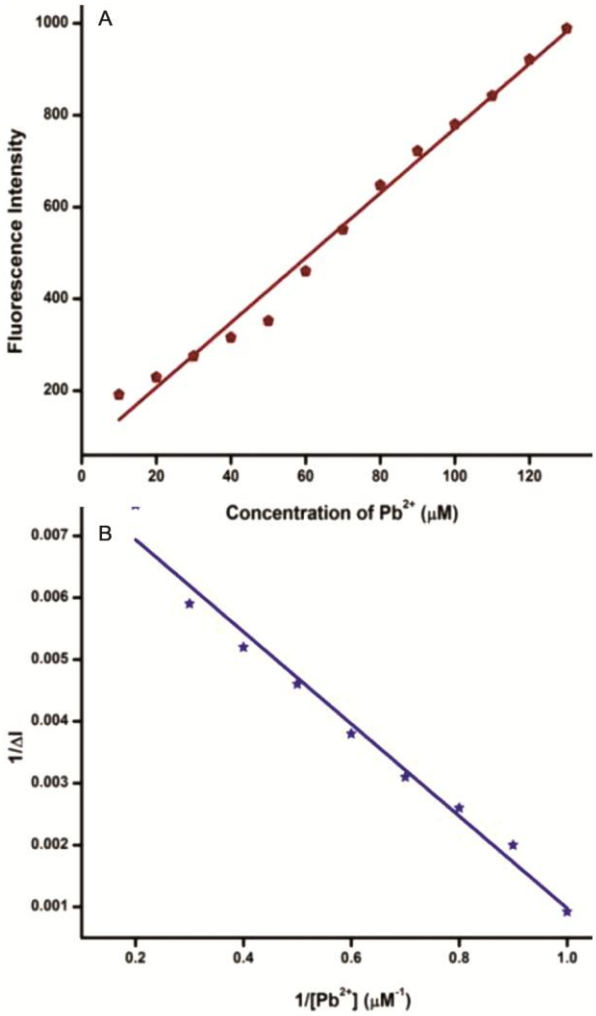


Fig. 7 (A) — linear fit obtained from the fluorescence titration of PCAT with Pb^{2+} ions, B) B-H plot from the fluorescence titration profile between PCAT and Pb^{2+} ions.

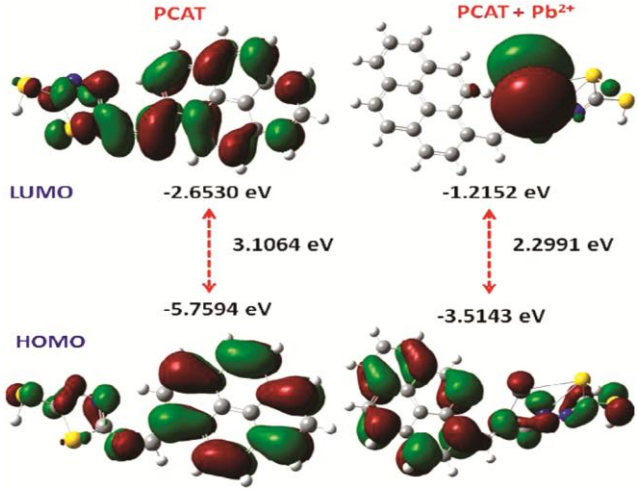


Fig. 8 — HOMO and LUMO of PCAT, PCAT-Pb^{2+}ions calculated with DFT at B3LYP/6-31G and LANL2DZ (d) level using Gaussian 09 package.

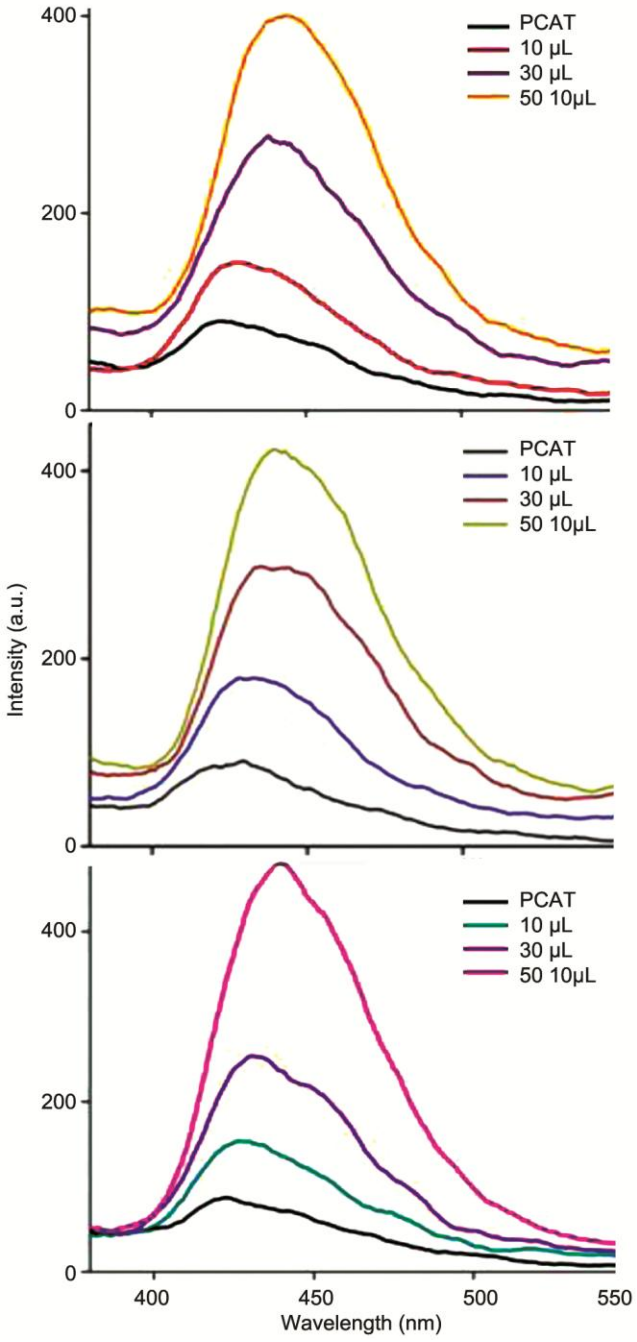


Fig. 9 — Fluorescence spectra of PCAT in (A) Thamirabarani, (B) RO and (C) tapwater samples for the determination of Pb^{2+} ions.

which absolutely shows that the complexes are exceptionally stable than the free probe[60].

Practical performance

In order to investigate the practical utility of PCAT, real sample analyses have led to determine Pb^{2+} ions. Three distinct water samples have been accrued from Thamirabarani river water in Tirunelveli, drinking water in the author department and tap water from Madurai Corporation have been utilized for the assessment. The collected water sample has been spiked with the standard Pb^{2+} ions and determined by a known standard addition method with the PCAT in fluorescence spectra[61] (Fig. 9). An extraordinary recovery of the spiked samples has been obtained. These results recommend that the PCAT proficiently assesses the measure of Pb^{2+} ions present in the ecological water samples and the observed results are shown in Table 1.

Table1 — Determination of Pb^{2+} ions in water samples.

Water sample	Pb^{2+} estimated (µL)	Pb^{2+} found (µL)	Recovery (%)
Tap water collected from Madurai corporation	0	0	--
	10	12	120
	30	34	113
	50	54	108
RO processed drinking water from Author department	0	0	--
	10	11	110
	30	33	110
	50	55	110
Thamirabarani River water collected from Tirunelveli	0	0	--
	10	13	130
	30	35	116
	50	55	110

Conclusion

In this work, a new turn-on fluorescence chemosensor with pyrene moiety has been developed. The absorption spectra show no remarkable changes upon the addition of 10 equivalents of different metal ions, except Pb^{2+} resulted with red shift and a new band formation. The fluorescence spectra show an obvious fluorescence enhancement response to Pb^{2+} with turn-on efficiency. The Job's plot analysis shows that PCAT chemosensor forms 1:1 binding stoichiometry to Pb^{2+} ions and the detection limit is 7.15×10^{-8} M indicating high sensitivity of PCAT-Pb^{2+} ions. Further, the spiked environmental water samples are also tested using fluorescence and received excellent recovery percentage. In addition, PCAT can be used for naked-eye detection of Pb^{2+} ions, which can greatly broaden its applications.

Conflict of interest

There are no conflicts to declare

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