Supporting Information

'PET' vs. 'Push-Pull' induced ICT: A remarkable Coumarinyl-appended Pyrimidine based Colorimetric and Fluorimetric Sensor for detection of Hg^{2+} ion in aqueous media with test trips

Shyamaprosad Goswami*, Avijit Kumar Das and Sibaprasad Maity

Department of Chemistry, Bengal Engineering and Science University, Shibpur, Howrah 711103, West Bengal, India E-mail: spgoswamical@yahoo.com; Fax: +91-3326682916.

CONTENTS

1. General:

Unless otherwise mentioned, chemicals and solvents were purchased from Sigma-Aldrich chemicals Private Limited and were used without further purification. Melting points were determined on a hot-plate melting point apparatus in an open-mouth capillary and are uncorrected. ^{1}H-NMR spectra were recorded on Brucker 300 MHz instrument. For NMR spectra, $CDCl_3$ was used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. UV-vis titration experiments were performed on a JASCO UV-V530 spectrophotometer and fluorescence experiment was done using PerkinElmer LS 55 fluorescence spectrophotometer using a fluorescence cell of 10 mm path. The pH titration was carried out by using Agilent 8453 pH meter.

2. General method of UV-vis and fluorescence titration:

By UV-vis method:

For UV-vis titrations, stock solution of the sensor was prepared (c = 2 x 10^{-5} M) in CH_3CN-HEPES buffer (7/3, v/v, 25ºC) at pH 7.4. The solution of the guest cations like Cr^{3+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Hg^{2+} using their chloride salts were also prepared in the order of (c = 2x10^{-4}M). Solutions of various concentrations containing sensor and increasing concentrations of cations were prepared separately. The spectra of these solutions were recorded by means of UV-vis methods.

Association constant determination:

Binding constant was calculated according to the Benesi-Hildebrand equation[1]. *Ka* was calculated following the equation stated below.

$$1/(A\text{-}Ao) = 1/\{K(Amax–Ao)\,[M]_n\} + 1/[Amax\text{-}Ao]$$

Here Ao is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, Amax is absorbance in presence of added [M]max and K is the association constant (M^{-1}). The association constant (K) could be

determined from the slope of the straight line of the plot of 1/(A-Ao) against $1/[M]_n$. The association constant (K_a) as determined by UV-vis titration method for sensor with Hg^{2+} is found to be 4.0 x 10^4 M^{-1}.

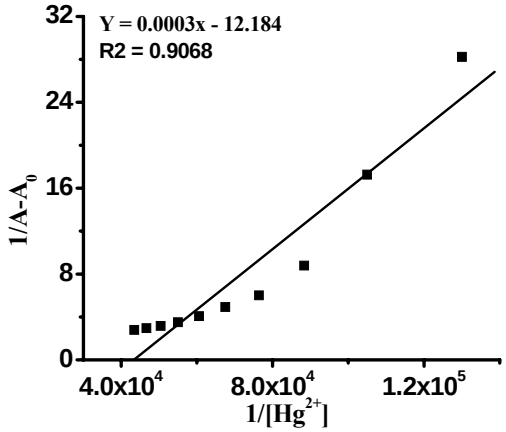


Figure S_1: Benesi–Hildebrand plot from UV-vis titration data of **PYC** (c = 2x10^{-5}M) with Hg^{2+}(c = 2x10^{-4}M).

General procedure for drawing Job plot by UV–vis method:

Stock solution of same concentration of **PYC** and Hg^{2+} were prepared in the order of ≈ 2.0 x 10^{-5} M in CH_3CN-HEPES buffer (7/3, v/v, 25ºC) at pH 7.4. The absorbance in each case with different *host–guest* ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the absorbance spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively).

By fluorescence method:

For fluorescence titrations, stock solution of the sensor (c = 2 x 10^{-5} M) was prepared for the titration of cations in CH_3CN-HEPES buffer (7/3, v/v, 25ºC) at pH 7.4. The solution of the guest cations using their chloride salts in the order of 200 μM were also prepared. Solutions of various concentrations containing sensor and increasing concentrations of cations were prepared separately. The spectra of these solutions were recorded by means of fluorescence methods.

3. Calculation of the detection limit:

The detection limit (DL) of **PYC** in absorption and emission spectra for Hg^{2+} was determined from the following equation:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph Fig.S3(a), we get slope = 0.0169, and Sb1 value is 0.0318.

Thus using the formula we get the Detection Limit for Hg^{2+} = 5.96 μM in UV-vis absorption spectra.

From the graph Fig.S3(b), we get slope = 5×10^{10}, and Sb1 value is 167178.98.

Thus using the formula we get the Detection Limit for Hg^{2+}= 10 μM in Fluorescence spectra.

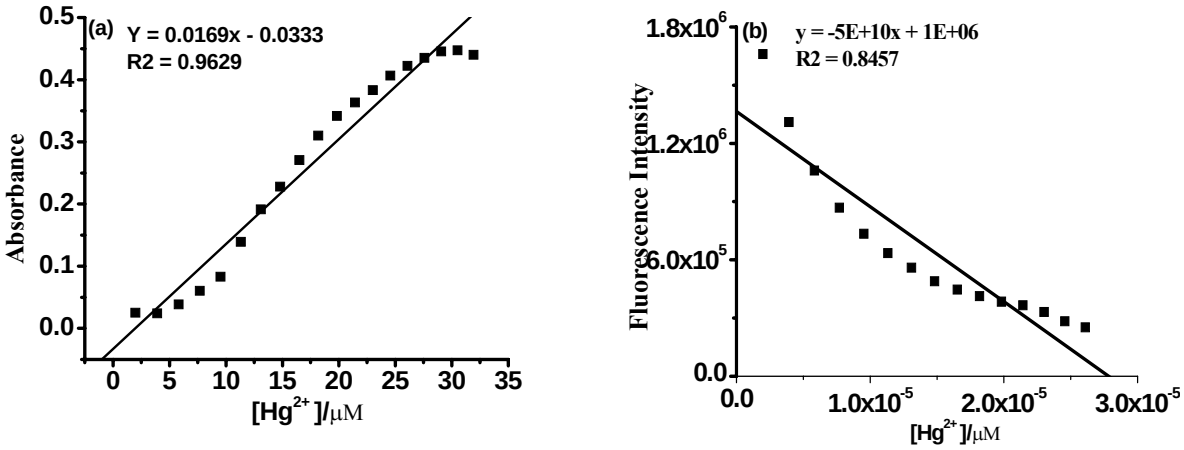


Figure S_2: (a) Changes of absorbance of **PYC** (c = $2x10^{-5}$M) as a function of $[Hg^{2+}]$(c = $2x10^{-4}$M) at 525 nm. **(b)** Changes of Fluorescence Intensity of **PYC**(c=$2x10^{-5}$M) as a function of $[Hg^{2+}]$ (c = $2x10^{-4}$M) at 509 nm.

4. Methods for the preparation of the receptor (PYC):

Synthesis of AC (3-acetyl-7-diethylamino-2H-chromen-2-one):

Synthesis of **AC**(3-acetyl-7-diethylamino-2H-chromen-2-one) was prepared by the reported literature. [1] 4-N,N-Diethylaminosalicyaldehyde (5.0 g, 0.025mol), ethyl acetoacetate (5.5 g, 0.15 mol), and 1 mL of piperidine were dissolved in 30 mL of absolute ethanol. After the mixture solution was refluxed for 5 h, the solvent was removed under reduced pressure. The yellow solid was precipitated and collected, and the product was recrystallized from absolute ethanol to afford compound **AC** (yield: 80%).

Synthesis of the receptor PYC:

AC(3-acetyl-7-diethylamino-2H-chromen-2-one)(2.5 g, 9.6 mmol)was added to a toluene (20mL) solution of $ZnCl_2$ (0.14g, 1mmol),triethyl orthoformate (5mL, 30mmol) and ammonium acetate (1.54g, 20mmol). The mixture was heated at 100°C under a nitrogen atmosphere for 48h. A saturated aqueous solution of $NaHCO_3$ (100mL) was added to the mixture to quench the reaction. The mixture was extracted with $CHCl_3$, and the organic extracts were dried over anhydrous Na_2SO_4, filtrated, and concentrated. The crude product was purified by silica gel column chromatography using 50% ethylacetate in pet-ether(v/v)to give a light yellow compound **PYC**(600mg, 26%).

Mp. 230-235°c.

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 9.01 (s, 1H), 8.91(s, 1H), 7.99 (d, 1H, J=6.0),7.46(d, 1H, J=9.0), 6.63(t, 2H, J=6), 6.51(s,1H), 3.43(t, 4H, J=6), 1.23(m, 6H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm):163.16, 161.56, 157.34, 155.09, 153.98, 151.94, 144.96, 130.91, 125.89, 111.24, 109.67, 105.18, 97.79, 96.85, 45.20, 27.14, 12.74.

MS (ESI-TOF): (m/z, %): M+ Calculated for $C_{17}H_{17}N_3O_2$ is 295.13; Found: 296.23 $(M+H)^+$;

Elemental analysis:

Calculated value: C, 69.14; H, 5.80; N, 14.23; O, 10.83.
Observed Value: C,69.16; H,5.79; N, 14.24; O,10.81.

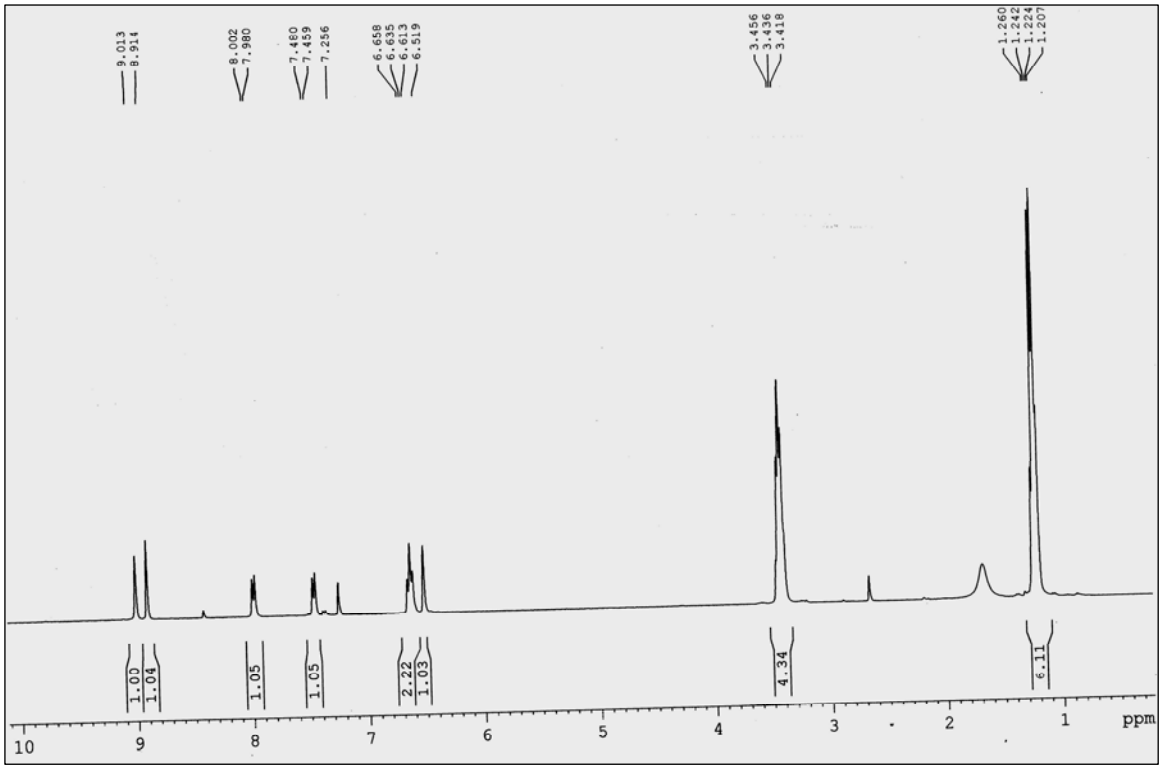
Synthesis of the Hg^{2+}–complex with PYC:

Hg^{2+} complex of **PYC** was synthesized by adding the sensor (100 mg, 0.33 mmol) into a methanol solution of $HgCl_2$ (90 mg, 0.33 mmol) and the whole mixture was refluxed for 1 hr. The solvent was removed under vacuum and the whole mass was washed with diethyl ether several times. Finally a brown colored solid was obtained (70 mg, 60%) which was characterized by mass spectroscopy (ESI–TOF) and elemental analysis.

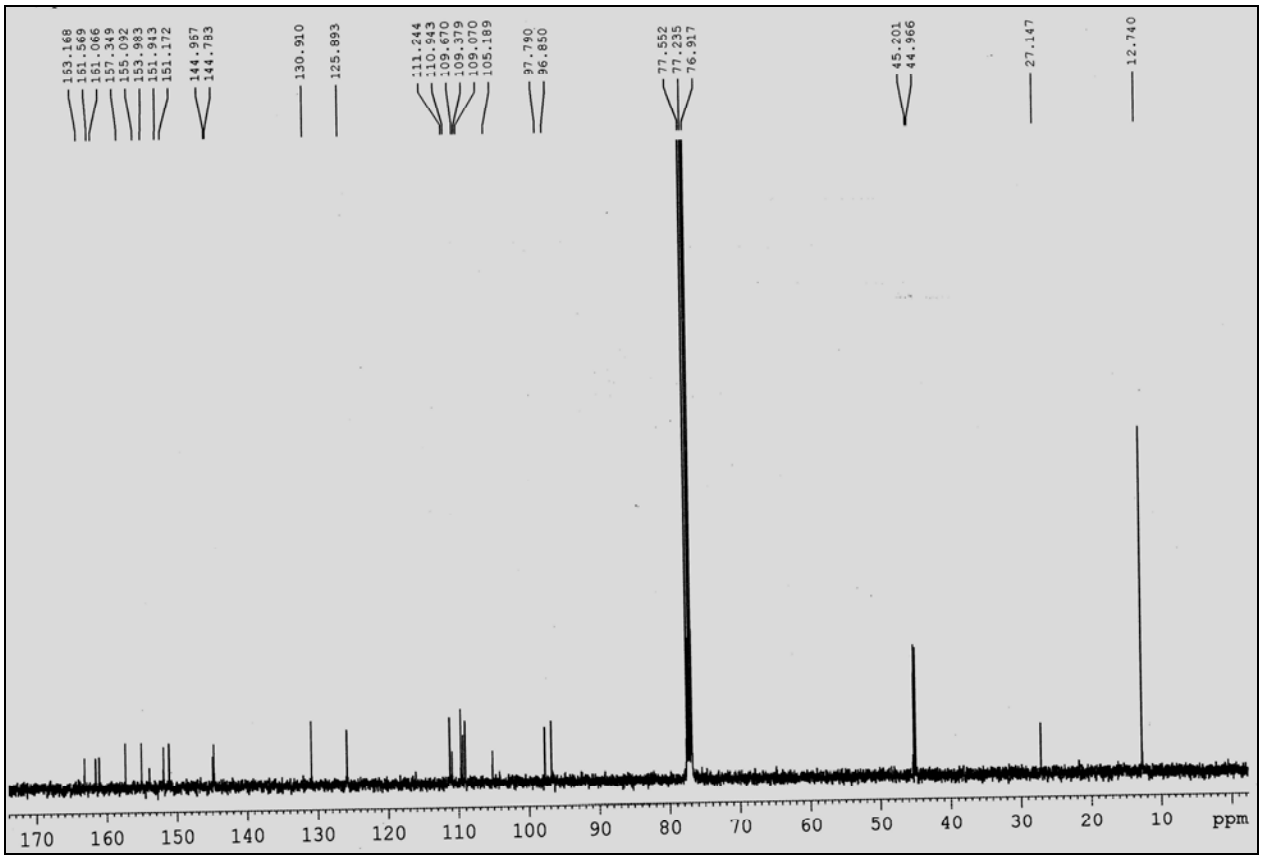
MS (ESI-TOF) :(m/z, %): M+ Calculated for $C_{17}H_{19}ClHgN_3O_3^+$ is 550.08; Found: 551.33.

Elemental analysis:
Calculated value: C, 37.16; H, 3.49; Cl, 6.45; Hg, 36.51; N, 7.65; O, 8.74.
Observed value: C, 37.19; H, 3.50; Cl, 6.43; Hg, 36.50; N, 7.67; O, 8.71.

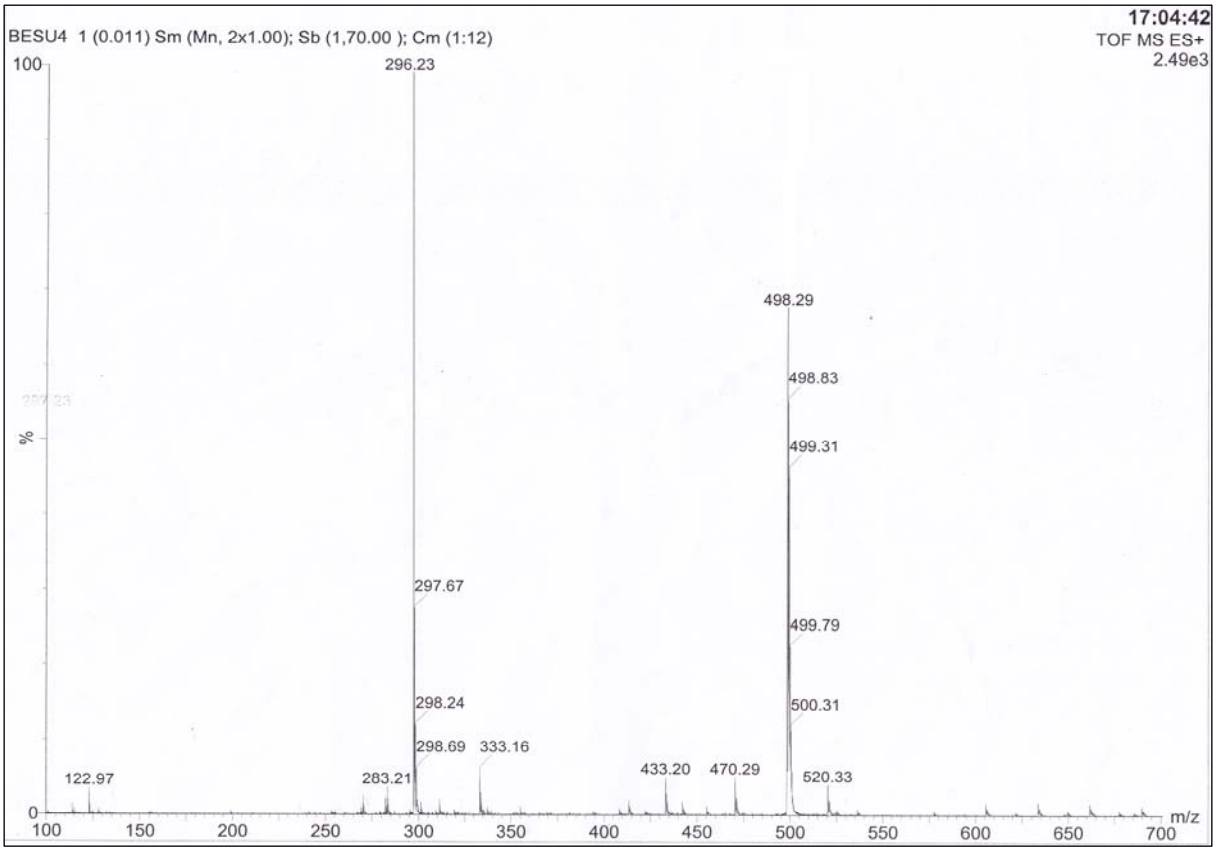
5. ^{1}H NMR spectrum (S_3) of PYC:



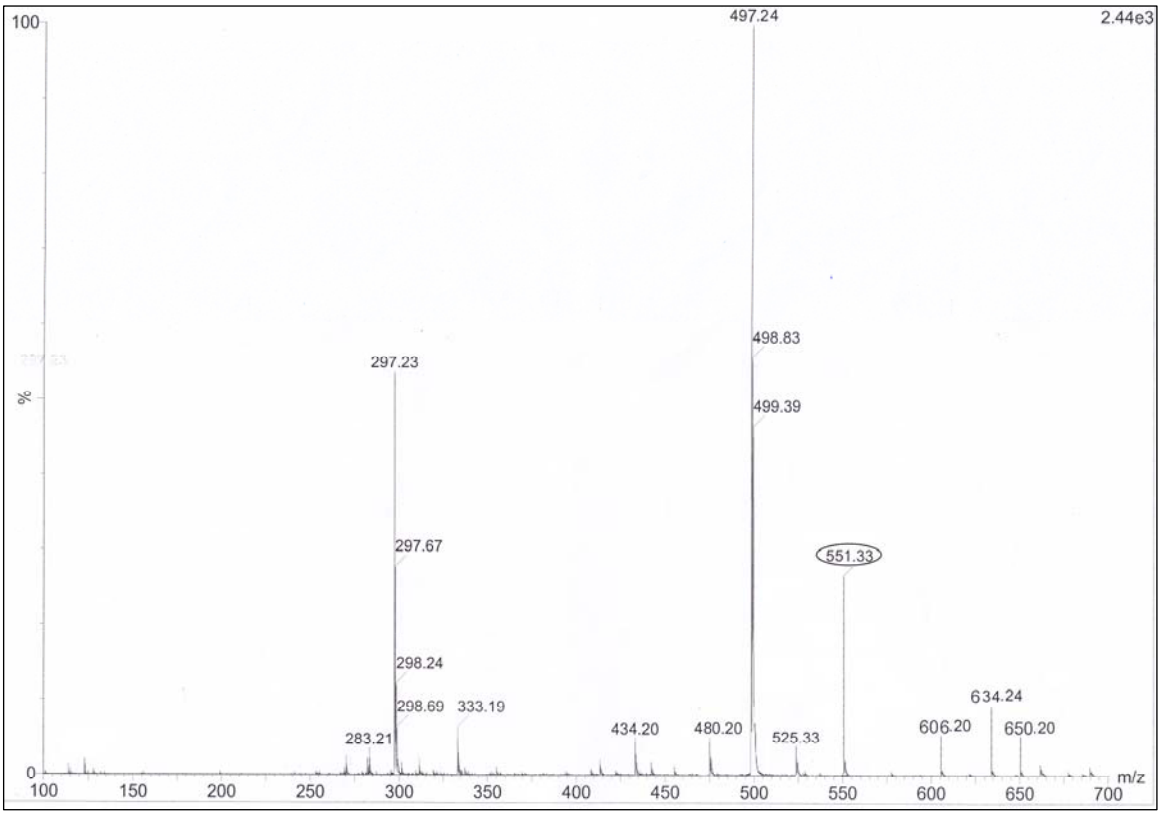
6. ^{13}C NMR spectrum (S_4) of Compound PYC:



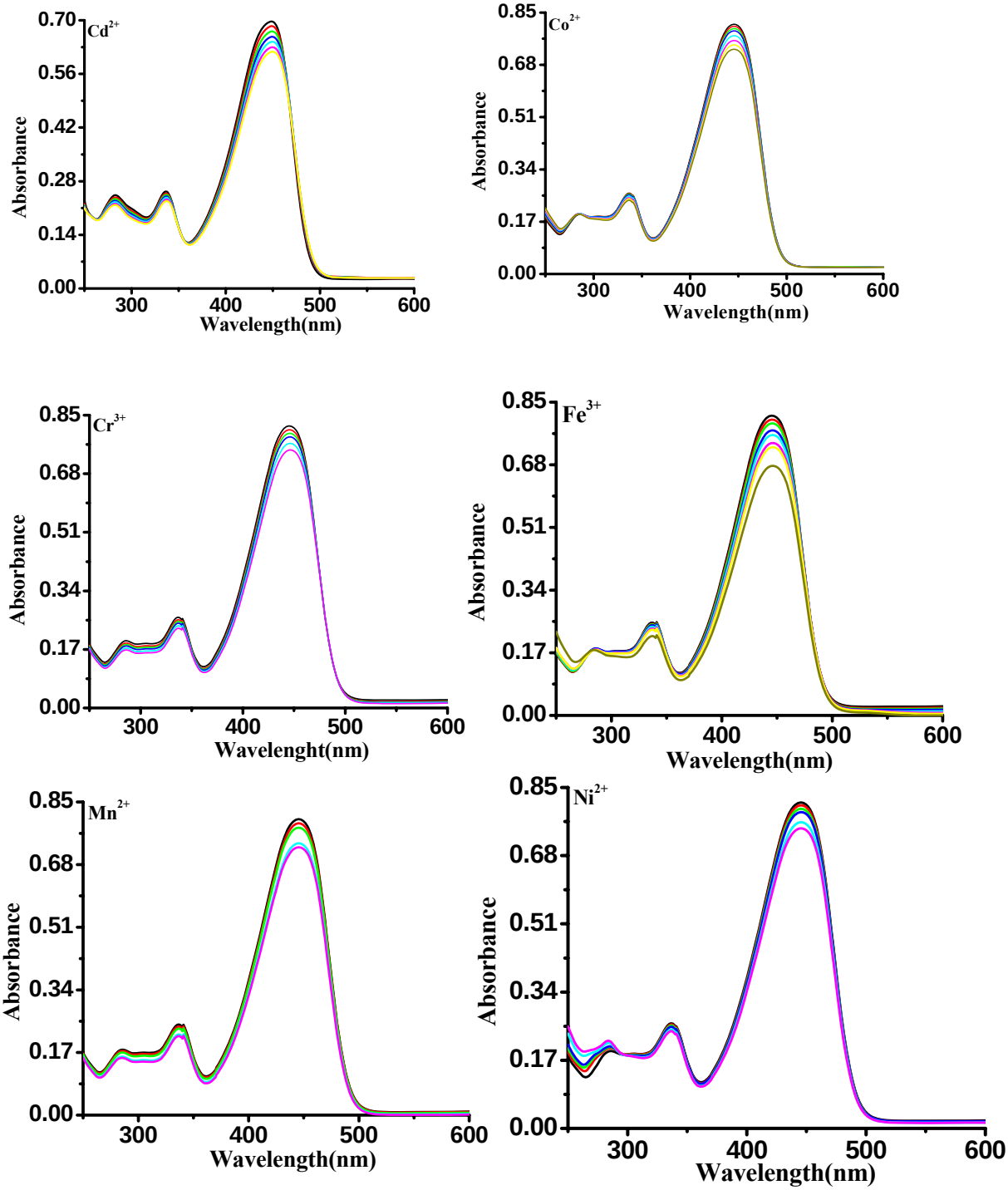
7. Mass spectrum (S_5) of Sensor PYC:

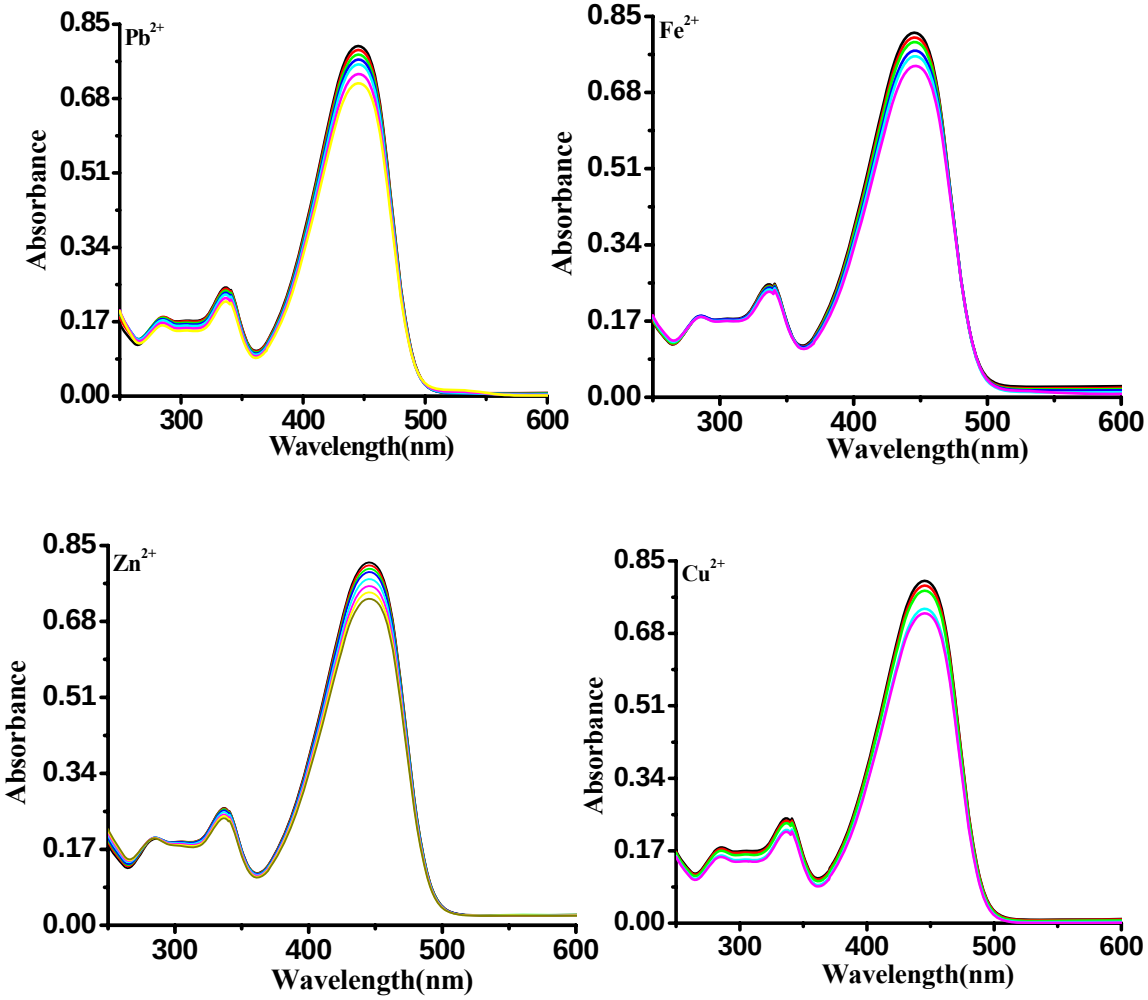


8. Mass spectrum (S_6) of Sensor PYC+Hg^{2+} complex:

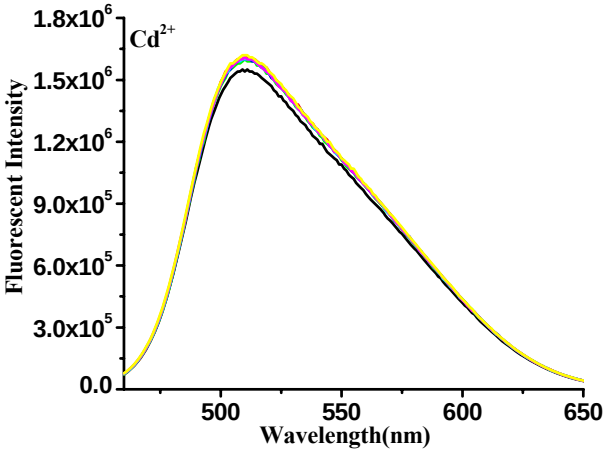


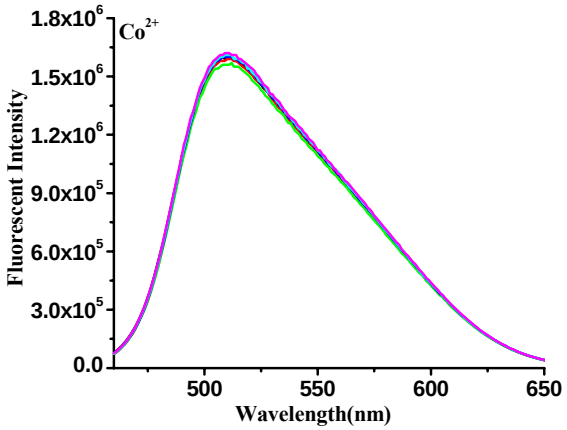
9. UV-vis absorption spectra of PYC (S_7)(c = $2x10^{-5}$M) with different cations (c = $2x10^{-4}$M) in CH_3CN-HEPES buffer (7/3, v/v, 25 º C) at pH-7.4:

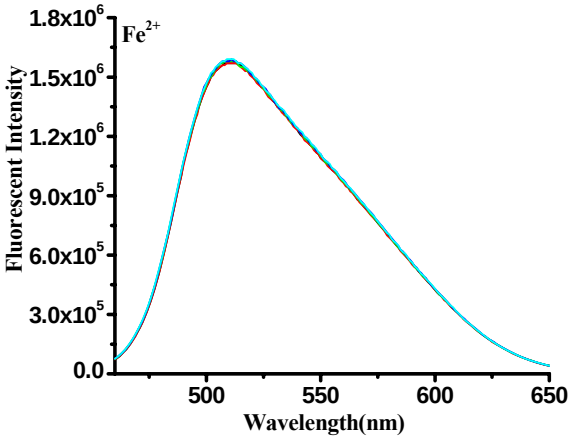


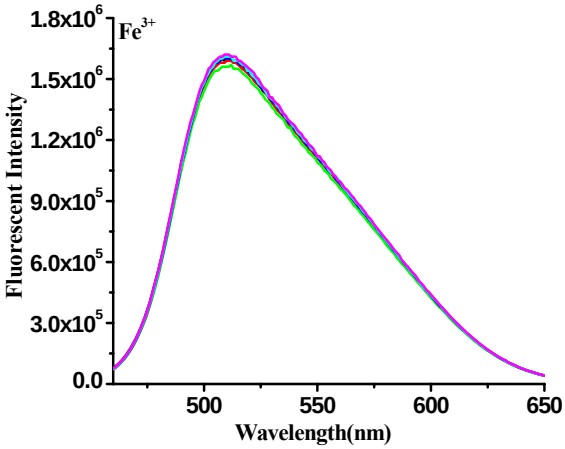


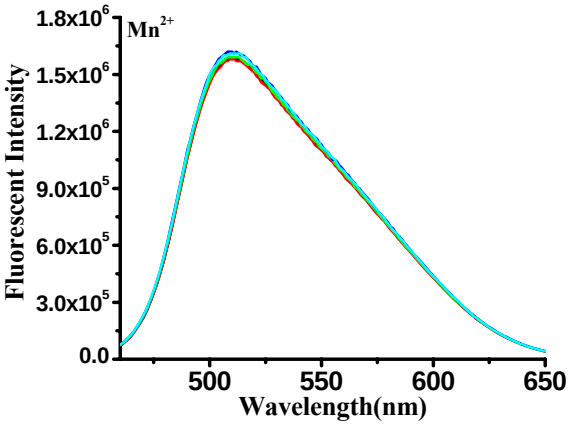
10. Fluorescent spectra of PYC (S_8)(c = $2x10^{-5}$M) with different cations (c = $2x10^{-4}$M) in CH_3CN-HEPES buffer (7/3, v/v, 25 ° C) at pH-7.4:

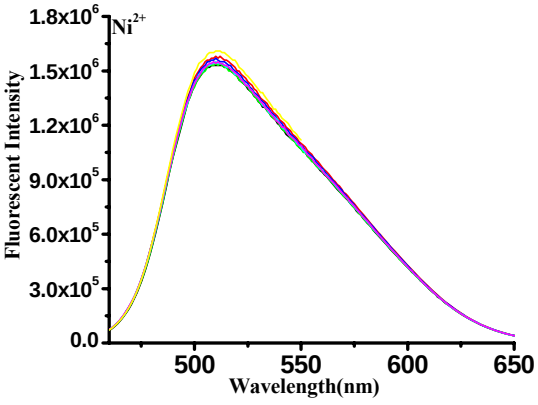


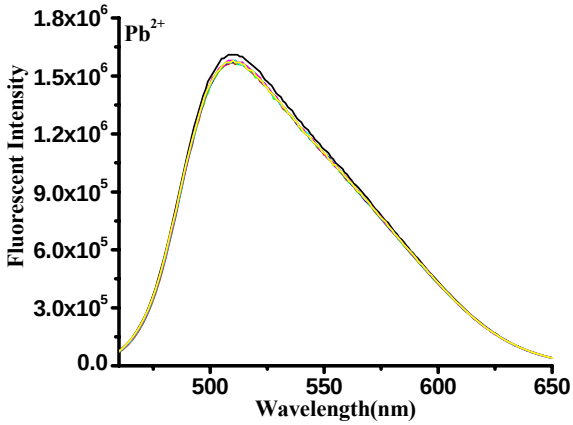


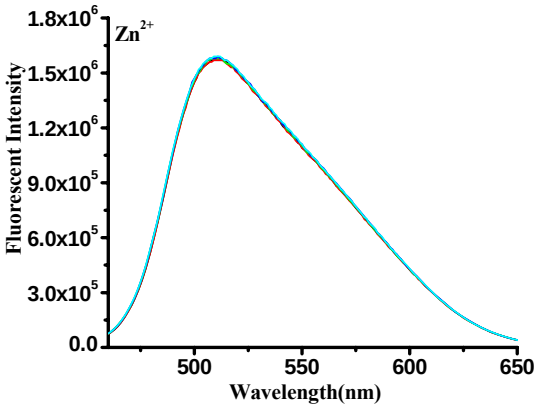


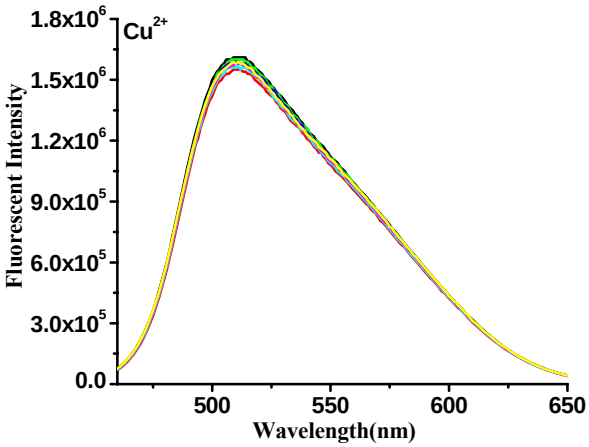


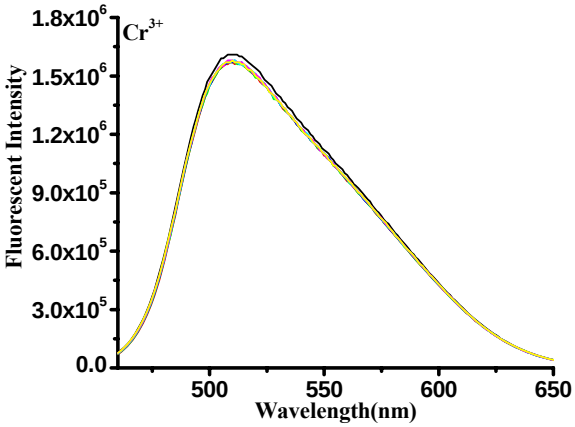












11. Counter ion-effect study of PYC with different salts of Hg^{2+} :

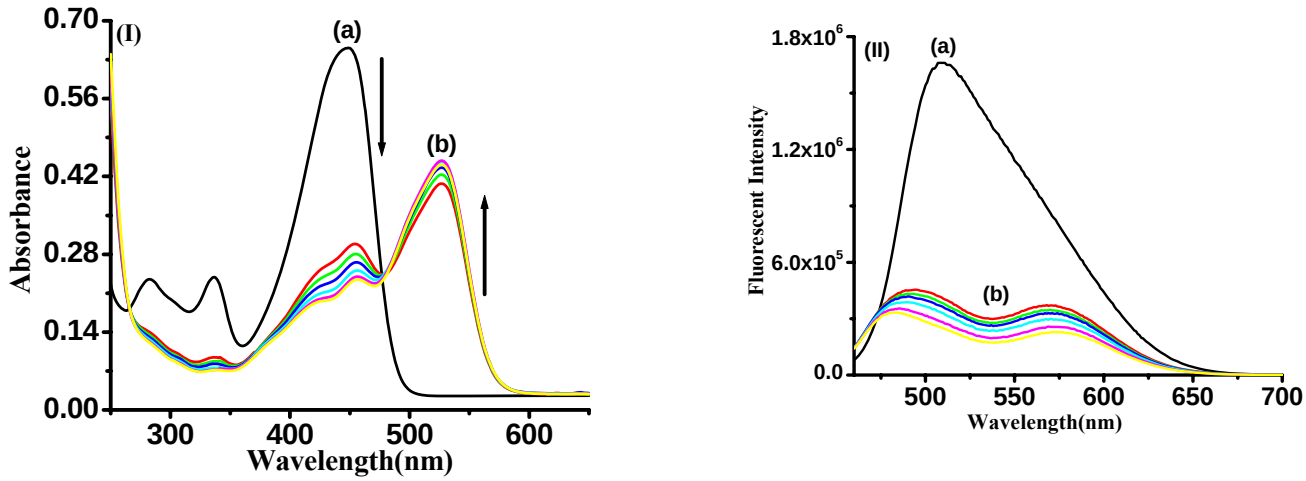


Fig. S9: (I) UV-vis absorption spectra of **PYC** (c = $2x10^{-5}$M) with different salts of Hg^{2+} (c = $2x10^{-4}$M) in CH_3CN-HEPES buffer (7/3, v/v, 25 º C) at pH-7.4.(a) **PYC** itself. (b) **PYC** + Hg^{2+}(Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, AcO^-).

(II) Fluorescent spectra of **PYC** (c = $2x10^{-5}$M) with different salts of Hg^{2+} (c = $2x10^{-4}$M) in CH_3CN-HEPES buffer (7/3, v/v, 25 º C) at pH-7.4.(a) **PYC** itself. (b) **PYC** + Hg^{2+}(Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, AcO^-).

From the above experiment it is observed that the addition of different Hg^{2+} salts to the receptor solution does not affect the sensing property of **PYC** towards Hg^{2+}. So the counter ion effect is not observed in the titration.

12. pH titration curve:

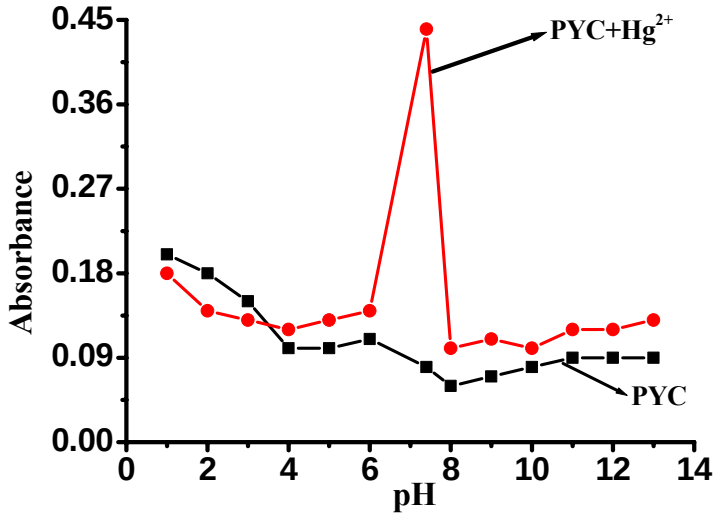


Fig. S_{10}: Absorbance of **PYC** (20 μM) at various pH values in the absence and presence of Hg^{2+}(5.0 equiv, c = 2.0 x10^{-4}M).

The sensing ability of **PYC** with Hg^{2+} in absorption spectra was also investigated at different pH values and itself left almost innocent to the overall pH range which was shown by black box. At lower pH values i.e. in acidic environment(made by different concentration of perchloric acid) due to protonation to the nitrogen of -NEt_2 group, the receptor becomes slightly disturb and gives the above changes which is shown by black box. Satisfactory Hg^{2+} sensing abilities were exhibited perfectly when the pH was increased from 6 to 8 and it reached the maximum value at pH-7.4 indicating that the **PYC** possessed high selectivity and sensitivity towards Hg^{2+} under the physiological pH window (red circles).

Ref:

1. J. Wu, R. Sheng, W. Liu, P. Wang , H. Zhang, J. Ma, *Tetrahedron*., 2012, 68, 5458-5463.