Supporting Information for

A Simple Cost Effective Carbazole-Thiobarbituric acid Conjugate as Ratiometric Fluorescent Probe for Detection of Mercury (II) ions in Aqueous Medium.

K. Kala, P. K. Vineetha and N. Manoj*

Department of Applied Chemistry, CUSAT, Kochi-682 022, Kerala, India, E-mail:

manoj.n@cusat.ac.in

1 Experimental Section

1.1 Reagents

Starting materials and reagents such as carbazole, 1-bromooctane, N-methylformanilide, and barbituric acid were purchased from Sigma-Aldrich and used as received. The solvents used in the synthesis procedures were obtained from Spectrochem pvt.Ltd. and distilled before use. Spectroscopic grade solvents from Spectrochempvt. Ltd. were used for photophysical studies.

1.2 Experimental General

The ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz on Bruker FT-NMR spectrometer with tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in parts per million (ppm) downfield to tetramethylsilane. High resolution mass spectra of new compounds were obtained using a WATERSSYNAPTG2S spectrometer.

1.3 Synthesis of CTBA

CTBA was also synthesized according to a reported procedure[1] by Knoevenagel condensation between 9-octyl-9*H*-carbazole-3-carbaldehyde and thiobarbituric acid in ethanol. Yield: 80%. mp 276 °C, FT- IR (cm^{-1}) 1533, 1642, 2922, 3436. ^{1}H NMR (DMSO d_6) δ (ppm): 0.80 (t, 3H), 1.12–1.35 (m, 10H), 1.75–1.81 (m, 2H), 4.45 (t, 2H), 7.31 (t, 1H), 7.56 (t, 1H), 7.70 (m, 2H), 8.20 (d, 1H,), 8.56 (s, 1H), 8.70 (m, 1H), 9.36 (s, 1H), 12.27 (s, 1H), 12.36 (s, 1H); ^{13}C NMR (DMSO d6) δ (ppm): 13.84, 21.94, 26.33, 28.44, 28.51, 28.62, 31.08, 42.61, 109.41, 110.34, 114.02, 120.42, 120.47,120.64 122.18, 122.35, 122.93,123.62, 126.82,130.05, 133.74, 140.78, 142.41, 143.56, 157.95, 160.14, 162.49, 178.10; HRMS (ESI MS) m/z: theoretical: 437.2137, found: 437.1669 ($[M + 4H]^{+}$ detected.

1.4 General Photophysical Studies

A stock solution of CTBA with concentration of 6.9×10^{-3} M was prepared in tetrahydrofuran (THF) and stored in a cold and dark place. This stock solution was used for all spectrofluorimetric titrations after appropriate dilution. Absorption spectra were recorded using Evolution 201 UV-visible spectrophotometer. Fluorescence emission spectrum of a sample was measured using a Perkin Elmer luminescence spectrophotometer (model LS 45). Metal ion stock solutions were prepared in the respective medium and used with appropriate dilution.

1.5 Determination of Association Constant for 1:2 Stoichiometry

$$CTBA + Hg^{2+} \overset{K_{11}}{\rightleftharpoons} CTBA\text{-}Hg^{2+}$$

$$CTBA\text{-}Hg^{2+} + Hg^{2+} \overset{K_{12}}{\rightleftharpoons} Hg^{2+}\text{-}CTBA\text{-}Hg^{2+}$$

The association constant[2] was calculated based on the absorbance or fluorescence titration curve of CTBA with metal ions. Association constant was determined by a nonlinear least squares fit of the data with the following equation as described elsewhere.

$$A = \frac{A0 + A\infty\, K12\, [G]2}{1 + K12\, [G]2}$$

Where A is absorbance/fluorescence signal, $A0$ and $A\infty$ are the initial and final absorbance/ fluorescence signal, [G] is total concentration of metal ion

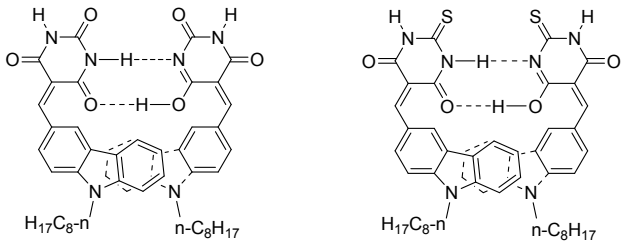
1.6 Job Plot by UV-vis Method

A series of solutions containing CTBA and $Hg(OAc)_2$ were prepared and kept sum of concentration [Hg^{2+}] ion and [CTBA] as a constant, whereby, the mole fraction (X) of Hg^{2+} was varied from 0.1 to 1.0. The Job's plot[1] is obtained by plotting the absorbance (Abs_{550} x $X_{Hg^{2+}}$) at 550 nm against the mole fraction of the Hg^{2+}. The value of mole fraction corresponding to the maximum on the Job's plot thus obtained was 0.67 is an indication of a 1:2 binding stoichiometry.[3]

1.7 Detection Limit Calculation's Experimental Procedure

The detection limit[3] was calculated based on the fluorescence titration. To calculate the S/N ratio, the emission intensity of CTBA in the absence of Hg(II) was measured 10 times and the standard deviation (**σ**) of blank measurements was determined. Three independent duplication measurements of emission intensity at 600 nm in the presence of Hg(II) and the average value of the intensities was plotted against concentration of Hg(II) for determining the slope (**m**). The detection limit is then calculated with the following equation.

Detection limit = 3σ/m



Scheme 1

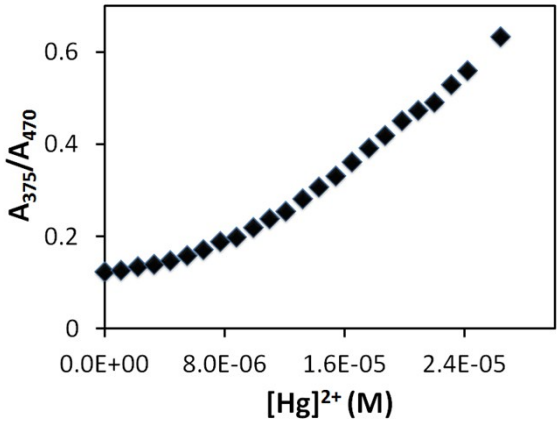


Figure S1 A plot of absorbance ratio (A_{375}/A_{470}) vs concentrations of Hg^{2+} in MeCN.

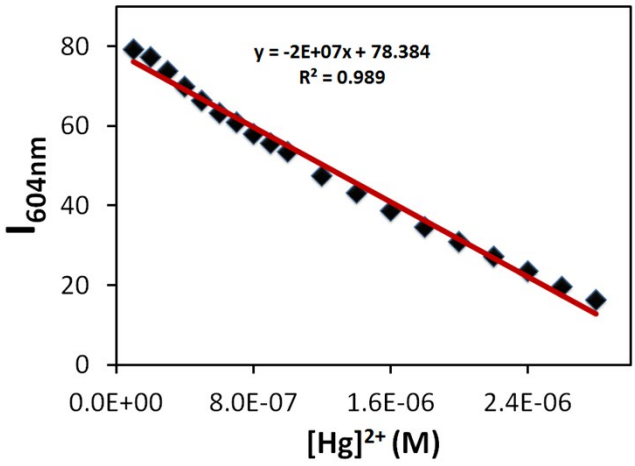


Figure S2 The fluorescence intensity changes at 604 nm as a function of Hg^{2+} concentration.

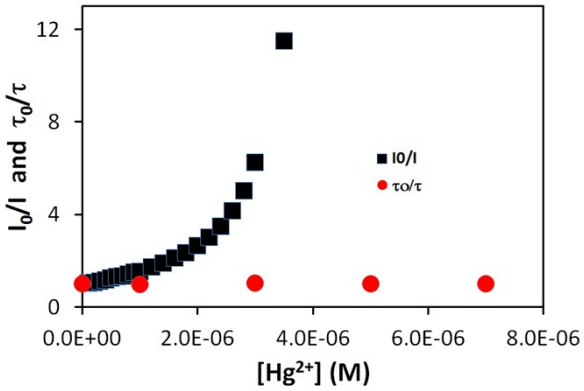


Figure S3 Stern–Volmer plot obtained for the quenching of fluorescence of CTBA by Hg^{2+} ions by Steady–State fluorescence measurements and time resolved emission measurements.

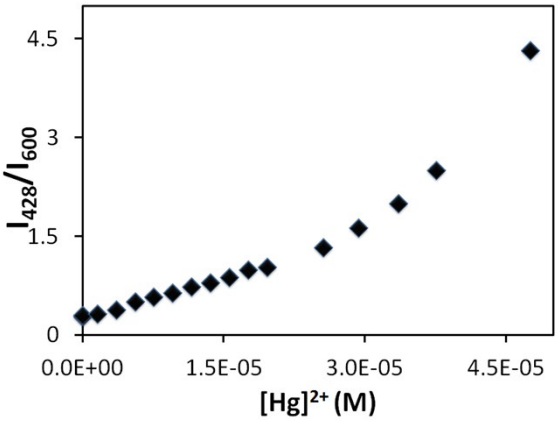


Figure S4 A plot of fluorescence intensity ratio (I_{428}/I_{600}) vs concentration of Hg^{2+} in 9:1 THF/H_2O, (λ_{ex} = 360 nm)

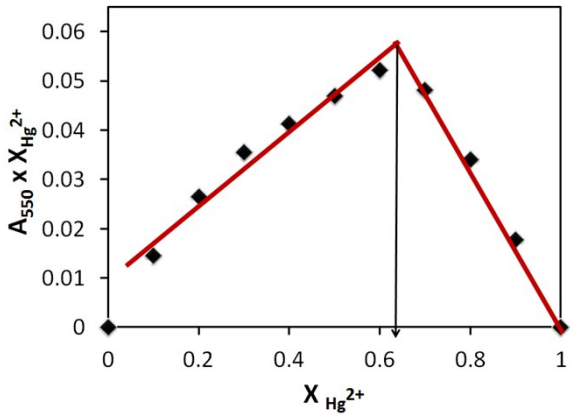


Figure S5 Job plot analysis of CTBA with Hg^{2+} showing a 1:2 binding geometry in 9:1 THF/H_2O

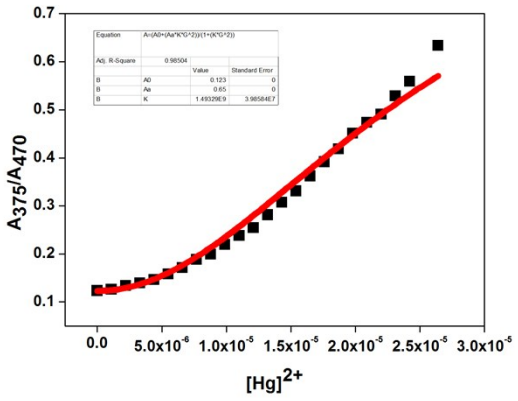


Figure S6 A plot of absorbance ratio (A_{375}/A_{470}) vs concentrations of Hg^{2+} in MeCN.

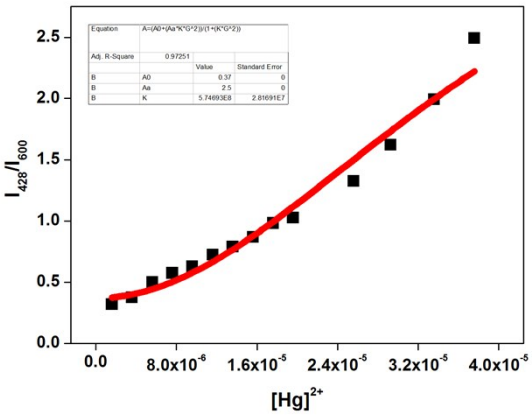


Figure S6 A plot of fluorescence intensity ratio (I_{428}/I_{600}) vs concentrations of Hg^{2+} in 9:1 THF/H_2O.

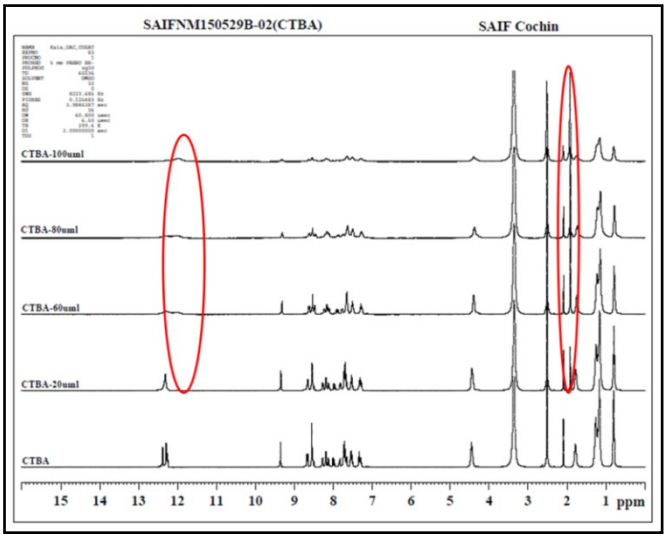


Figure S7 The ^{1}H-NMR spectra of the CTBA and CTBA in the presence of 0-2 equivalents of Hg^{2+} acetate (400 MHz, DMSO-d6)

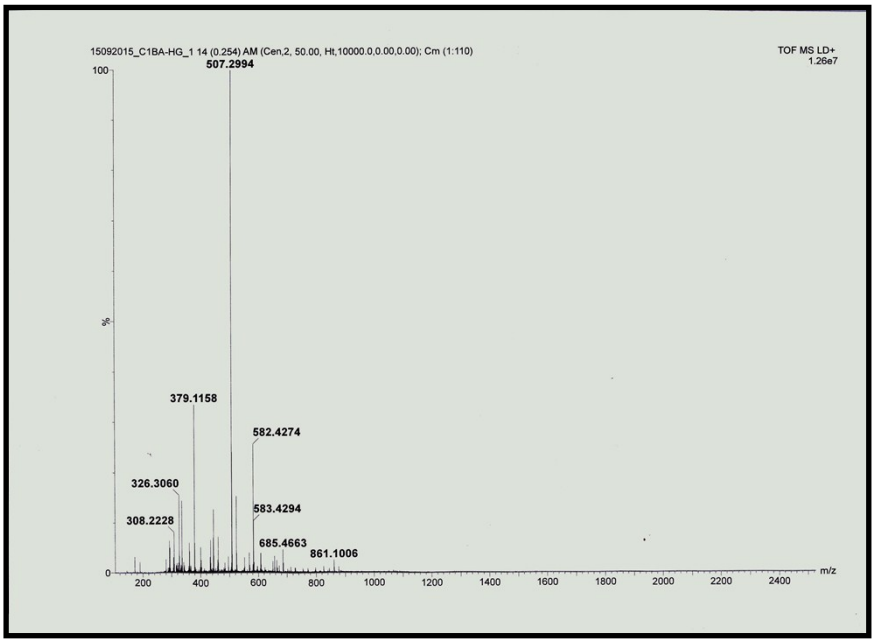


Figure S8 MALDI- TOF mass spectrum of CTBA –Hg complex

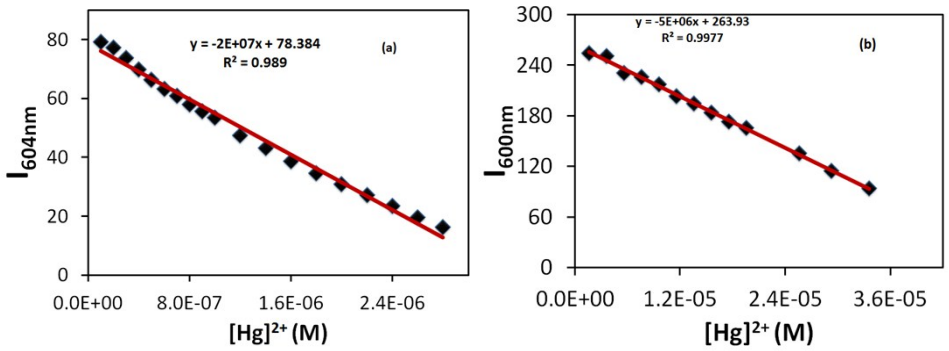


Figure S9 A plot showing variation of fluorescence intensity at 604 nm and 600 nm as a function of concentrations of Hg^{2+} in MeCN and 9:1 THF/H_2O respectively.

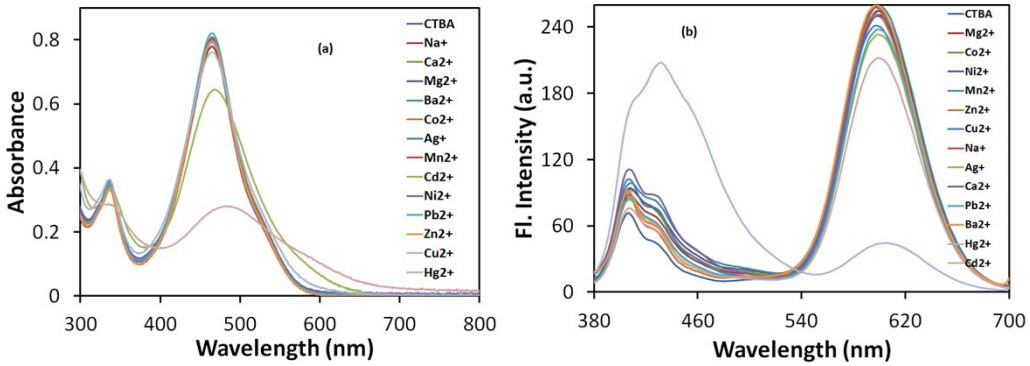


Figure S10 Absorption (a) and emission spectra (b) of CTBA recorded in the presence of various metal ions in 9:1 THF/H_2O. ((a) Metal ion concentration = 65 μM, [CTBA] = 30 μM. (b) Metal ion concentration = 65 μM, [CTBA] = 30 μM), λ_{ex}= 360 nm.)

Distribution of Probes in Micelles

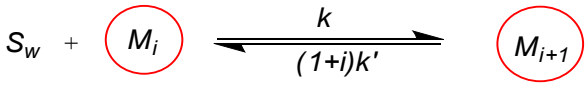
Given the discrete number of micelles present at a given total surfactant concentration, introduction of probes leads to their solubilization in the available number of micelles. Knowledge of this distribution of probes among the micelles is essential for the interpretation of various exited state bimolecular processes such as fluorescence quenching, excimer formation, energy and electron transfer.

Given the average number of solutes per micelle $\bar{n} = \frac{[CTBA]}{[M]}$ where [CTBA] is the total concentration of CTBA introduced, [M] is the concentration of micelle.

$$[M] = \frac{[SDS]}{Nagg}$$

Where [SDS] is the concentration of surfactant, N_{agg} is the aggregation number[4,5]

The determination of the probability P_i of finding i probes in a given micelle can find out by several distribution laws such as geometric, binomial and Poissonian distributions.[4,5] Poissonian distribution is the most widely used model for the distribution of probes in micelles. According to Poissonian distribution model for the distribution of probes in micelles the following dynamic equilibrium of probe S with micelle can be represented as



Where S_w is the probe molecule in water, M_i is the micelle assembly containing *i* number of probe molecules. The rate *k* is a second order rate constant which describes the rate of entry of probes in the micelles. The *k'* is the exit rate of one probe and is assumed to be independent of occupation no *i*. The rate at which the probes leave the micelle containing several of them is assumed to be linearly dependent on the number of probes, ie., *rate = (1+i) k'*. There is no limit to the maximum number of probes that may occupy a given micelle. Writing equilibrium expressions for all values of *i*, it is easy to obtain the fraction of micelles that are occupied by *i* probes as

$$\frac{[Mi]}{[M]} = \frac{\bar{n}\, e^{-\bar{n}}}{i!}$$

This equation shows that the distribution of probes in micelles to be governed by a Poisson distribution. At a low value of $\bar{n}$ = 0.1, most of the micelles are empty and only 10% of micelles contain one or more probes. When $\bar{n}$ = 1, ie., there is equal concentrations of probes and micelles, 37% of micelles are still empty but about 26% of them contain two or more probes. In this type of distribution aggregation of probes and bimolecular self-quenching of the excited states are possible. To avoid this $\bar{n}$ should be much less than 0.5. In our study, we used a surfactant concentration of 100 mM ie., concentration of micelle is 1.33×10^{-3} M and the probe concentration of $\sim 10^{-5}$ M which makes the $\bar{n}$ to have a value of 0.0132 which is << 0.1. Thus, the probe molecules are distributed in such a way that there is large excess of empty micellar assemblies ruling out multiple occupancies of probe molecules and thus aggregation effects

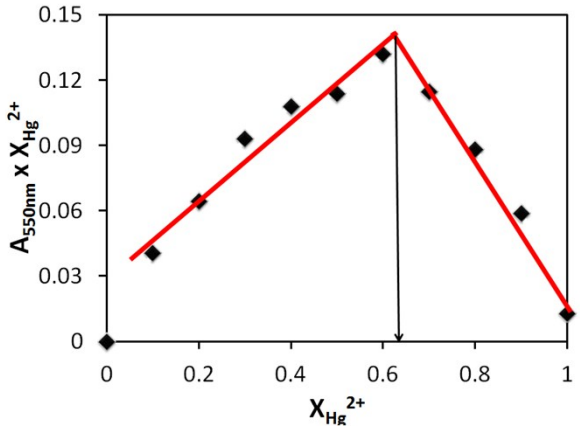


Figure S11 Job plot analysis of CTBA with Hg^{2+} showing a 1:2 binding stoichiometry in 100 mM SDS.

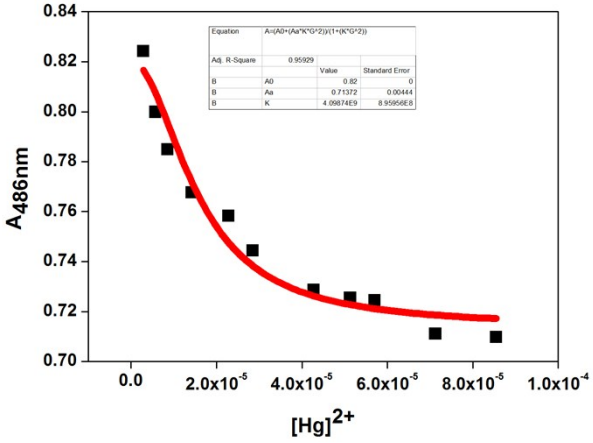


Figure S12 A plot of absorbance at 486 nm *vs*. concentrations of Hg^{2+} in 100 mM SDS.

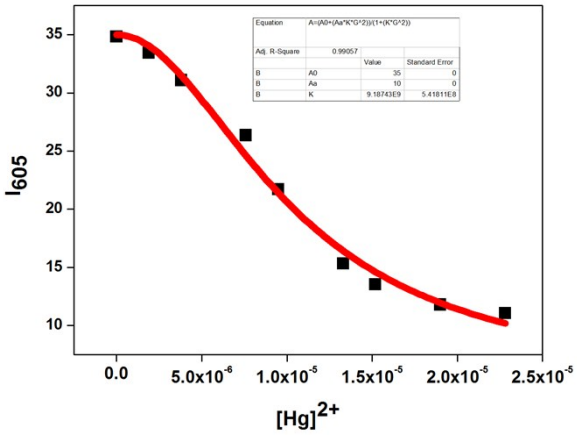


Figure S13 A plot of fluorescence intensity at 605 nm *vs*. concentrations of Hg^{2+} in 100 mM SDS.

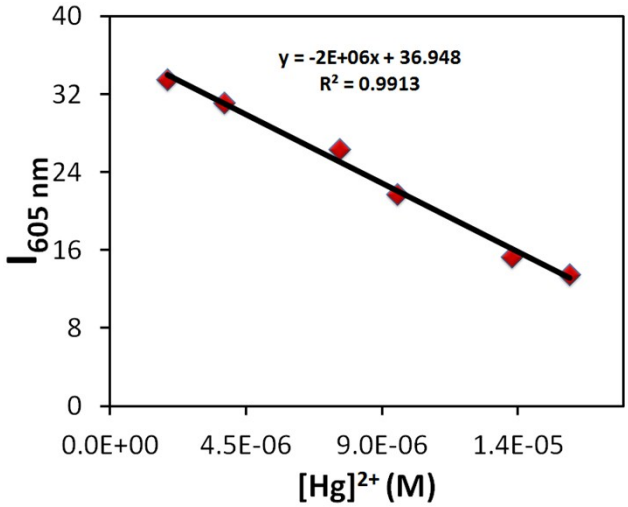


Figure S14 A plot showing variation of fluorescence intensity at 605 nm as a function of concentrations of Hg^{2+} in 100 mM SDS

Reference

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