Supporting Information

FRET based rhodamine-benzimidazole conjugate as Cu^{2+}selective colorimetric and ratiometric fluorescence probe with its function as cytoplasm marker

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1. General:

The 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 were uncorrected. ^{1}H-NMR and ^{13}C NMR spectra were recorded on Brucker 400 MHz instruments. For NMR spectra, $CDCl_3$ was used as solvent with 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 with a fluorescence cell of 10 mm path.

2. General method of Uv-vis and fluorescence titrations:

For UV-vis and fluorescence titrations, stock solution of the RBC was prepared (c = 1×10^{-5} ML^{-1}) in CH_3CN/aqueous HEPES buffer solution (8:2, v/v, pH 7.4). The solution of the guest metal ions like Cr^{3+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+}, Hg^{2+} using their chloride salts were prepared (c = 2×10^{-4} ML^{-1}) in CH_3CN. The original volume of the RBC solution was 2 ml. Solutions of the RBC of various concentrations and increasing concentrations of metal ions were prepared separately. Then the absorption and fluorescence sensing of metal ions were recorded.

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

Stock solution of same concentration of RBC in CH_3CN- HEPES buffer solution (10 mM HEPES, 8:2, v/v, pH 7.4) and Cu^{2+} were prepared in the order of 1.0×10^{-5} M in pure CH_3CN. 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).

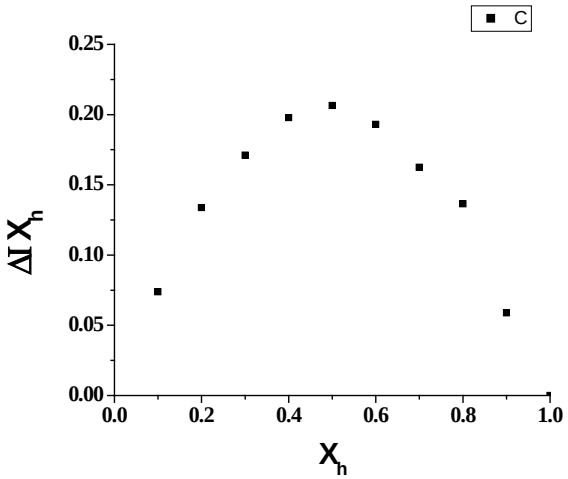


Fig-S$_1$: Jobs plot diagram of **RBC** for Cu^{2+}.

4. **Association constant determination using fluorescence titration:**

The binding constant value of Cu^{2+} with **RBC** has been determined from the emission intensity data following the modified Benesi–Hildebrand equation, $1/\Delta I = 1/\Delta I\ max + (1/K[C])(1/\Delta I\ max)$. Here $\Delta I = I - Imin$ and $\Delta I\ max = I_{max} - I_{min}$, where I_{min}, I and I_{max} are the emission intensities of RBC considered in the absence of Cu^{2+}, at an intermediate Cu^{2+} concentration and at a concentration of complete saturation where K is the binding constant and [C] is the

Cu^{2+} concentration respectively. From the plot of $1/(I-I_{min})$ against $[C]^{-1}$ for RBC, the value of K has been determined from the slope. The association constant (K_a) as determined by fluorescence titration method for RBC with Cu^{2+} is found to be 1.5×10^4 M^{-1} .

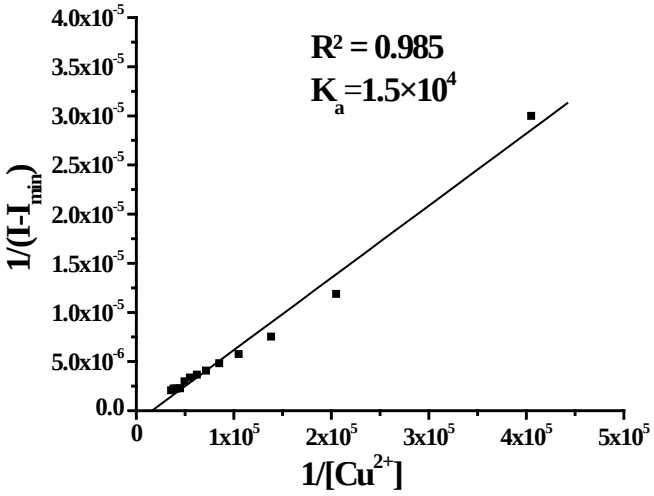


Fig. S_2: Benesi–Hildebrand plot from fluorescence titration data of **RBC** (c =1×10^{-5}M) with Cu^{2+} (c = 2×10^{-4}M).

5. Calculation of the detection limit:

The detection limit (DL) of **RBC** in emission spectra for Cu^{2+} was determined from the following equation[1]:

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.S_3, we get slope = 18105, and Sb1 value is 18798.

Thus using the formula we get the Detection Limit for Cu^{2+} = 3.1 µM in Fluorescence spectra.

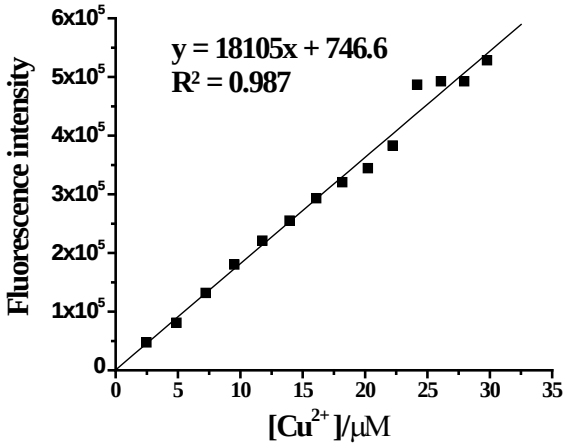


Fig. S_3 Changes of Fluorescence Intensity of **RBC** (c = 1×10^{-5}M) as a function of $[Cu^{2+}]$ (c = 2×10^{-4}M) at 580 nm.

Ref.1: Zhu, M.; Yuan, M.; Liu, X.; Xu, J.; Lv, J.; Huang, C.; Liu, H.; Li, Y.; Wang, S.; Zhu, D. *Org. Lett.* **2008,** *10*, 1481-1484.

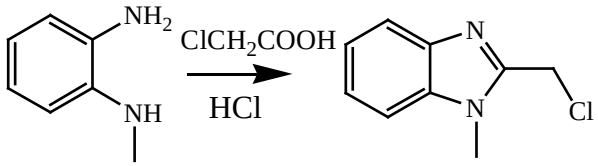
6. **Methods for the preparation of RBC:**

a) Synthesis of 2-amino-3',6'-bis(diethylamino)spiro[isoindoline-1,9'-xanthen]-3-one (1)

Rhodamine B (**1**) (1.0 g, 2.1 mmol), 80% hydrazine hydrate (2.7 g, 43 mmol) and EtOH (12 mL) were added to a flask, stirred and heated to reflux for 3 h, and then water (40 mL) added to the mixture. The mixture was extracted with ethyl acetate (40 mL) three times. The combined organic layer was dried over anhydrous magnesium sulfate and then filtered. The filtrate was concentrated to give product (**1**)

b) Synthesis of 2-chloromethyl-1-methyl-1*H*-benzimidazole (2)

2-Chloromethyl benzimidazole (2) was prepared by condensation of commercially available N-methyl-1,2-phenylenediamine with chloroacetic acid in the presence of hydrochloric acid. (Ref:*Synthetic Communications*, 2013,**43,** 1882–1895)



c) Synthesis of RBC

2-amino-3',6'-bis(diethylamino)spiro[isoindoline-1,9'-xanthen]-3-one **(1)** (500 mg, 1.1 mmol) and 2-chloromethyl-1-methyl-1*H*-benzimidazole **(2)** were refluxed in dry THF in presence of Et_3N for two days. The mixture was extracted with chloroform. The combined organic layer was dried over anhydrous magnesium sulfate and then filtered. The filtrate was concentrated and purified through column chromatography using 10% CH_3OH in $CHCl_3$ as 72% yield

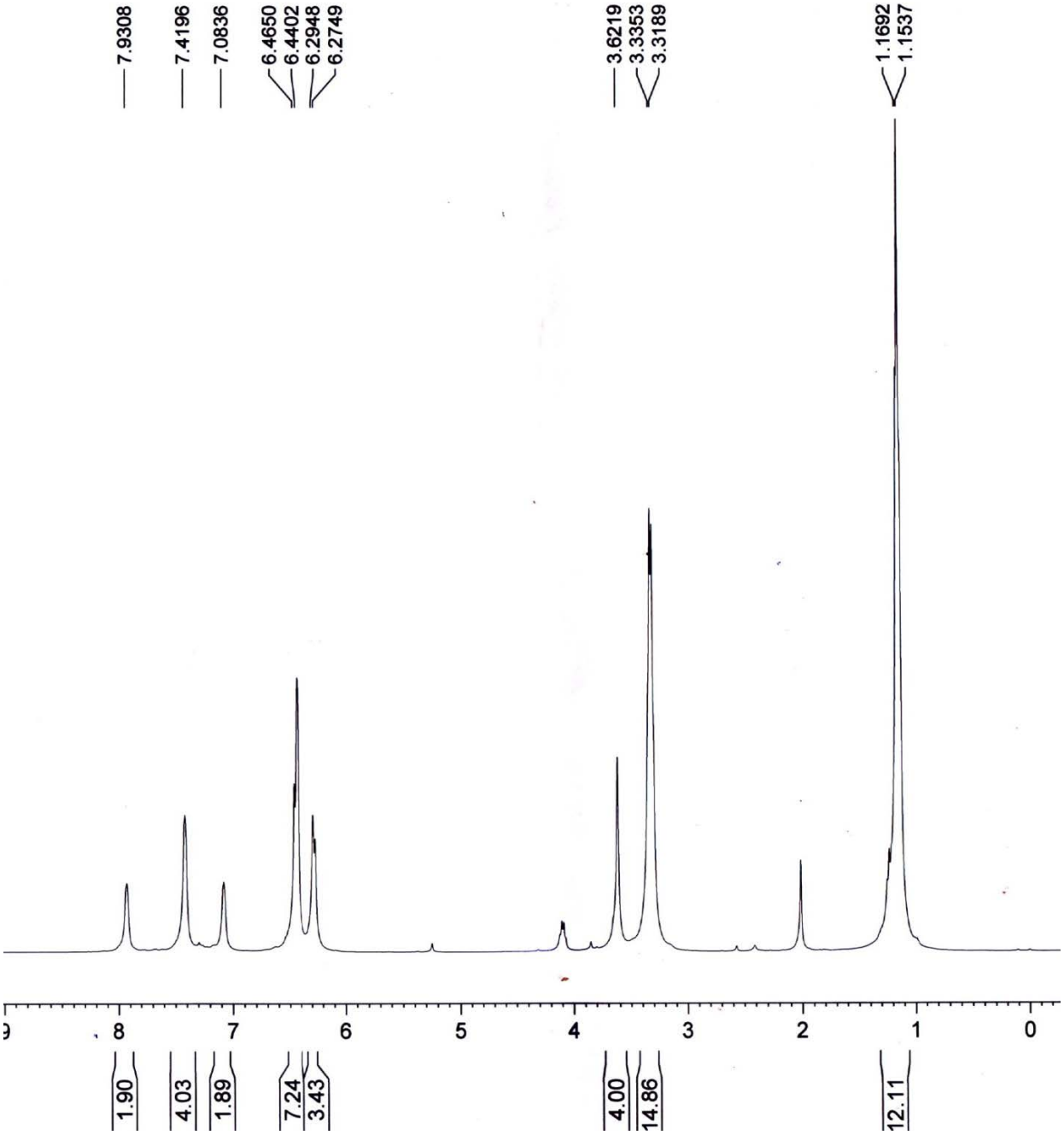
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 7.93 (s, 2H), 7.41 (s, 4H), 7.08 (s, 2H), 6.45(d, 7H, *J* = 8.00 Hz), 6.28 (d, 3H, *J*= 8.00 Hz), 3.62 (s, 4H), 3.32 (s, 14H), 1.16 (s, 12 H).

^{13}C NMR($CDCl_3$, 100 MHz):δ (ppm): 166.05, 153.84, 151.58, 148.84, 132.48, 130.03, 128.04, 123.50, 122.90, 108.01, 104.63, 98.00, 65.86, 44.35, 12.62.

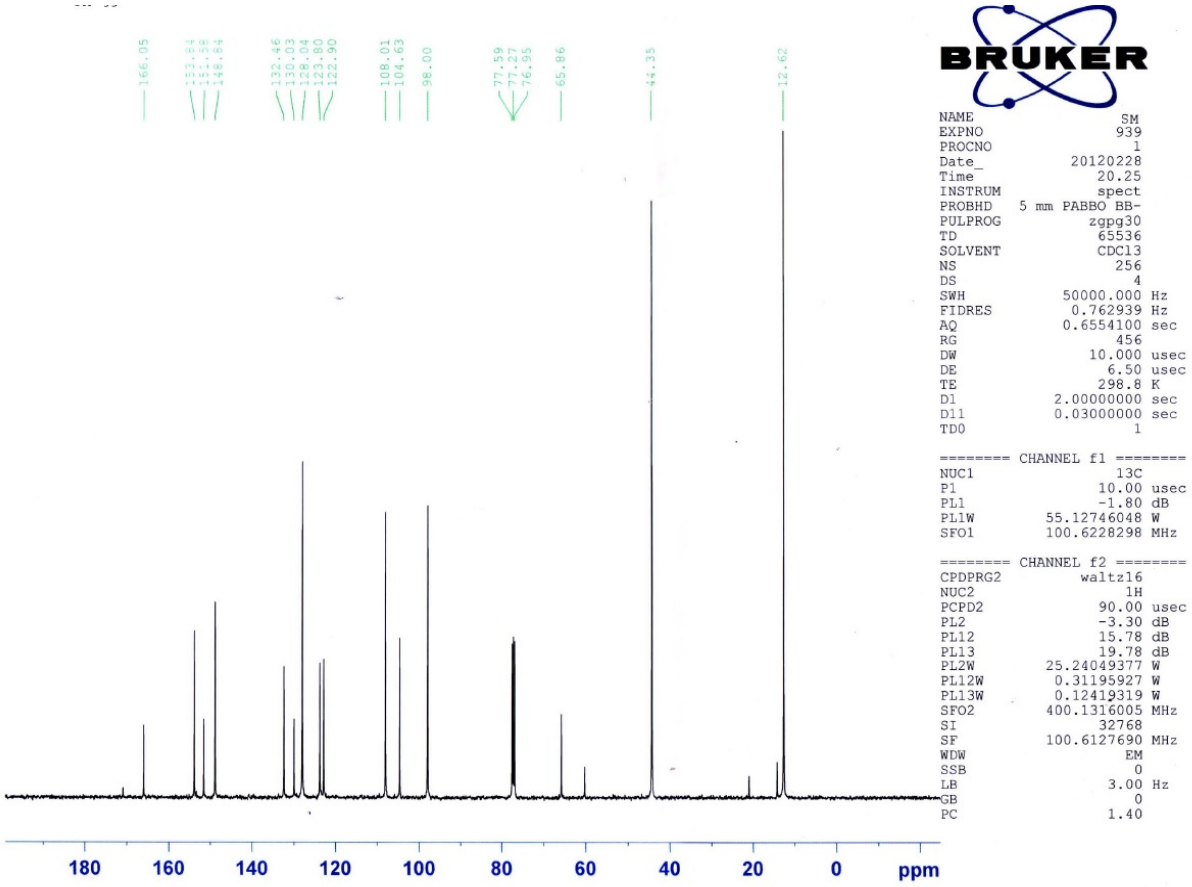
HRMS (M + H^+): calcd for 745.2978, found 745.2430.

Anal calcd for $C_{46}H_{48}N_8O_2$: 74.17% C, 6.49% H, 15.05% N, and 4.30% O; found: 74.26% C, 6.90% H, 15.18% N, and 4.68% O.

7. **a) ^{1}H NMR spectrum of RBC in $CDCl_3$ (400 MHz):**



b) ^{13}C NMR spectrum of RBC in $CDCl_3$ (100 MHz):



BRUKER

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SWH	50000.000 Hz
FIDRES	0.762939 Hz
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RG	456
DW	10.000 usec
DE	6.50 usec
TE	298.8 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
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P1	10.00 usec
PL1	-1.80 dB
PL1W	55.12746048 W
SFO1	100.6228298 MHz
======== CHANNEL f2 ========	
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NUC2	1H
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PL12	15.78 dB
PL13	19.78 dB
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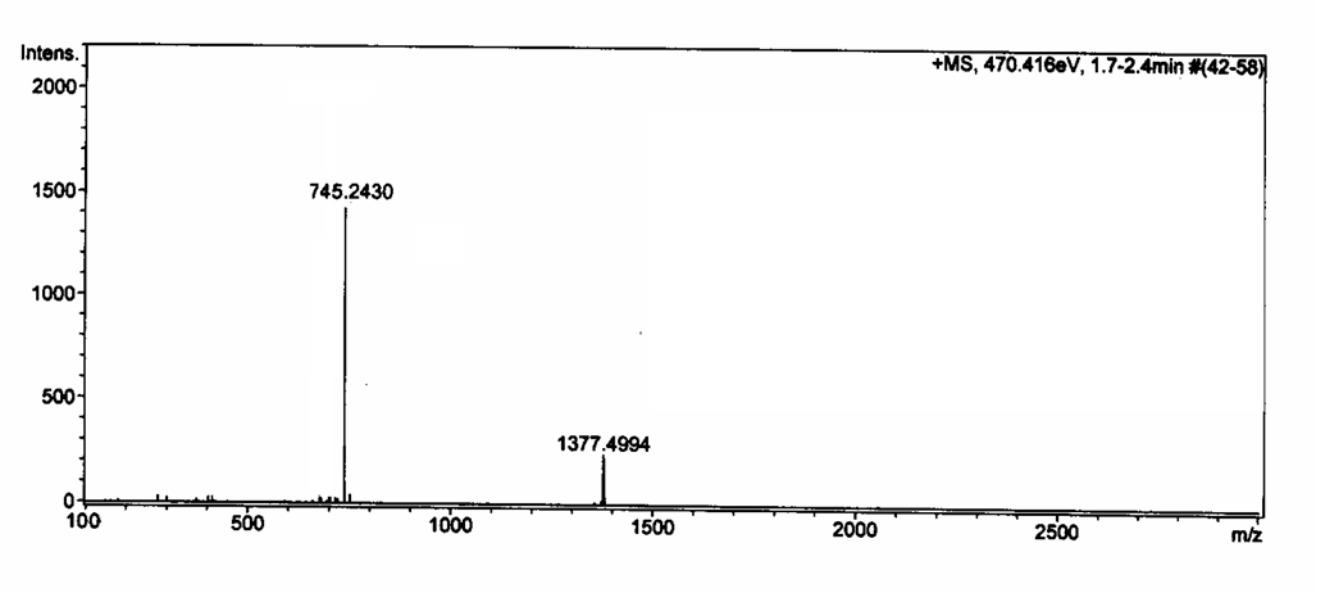
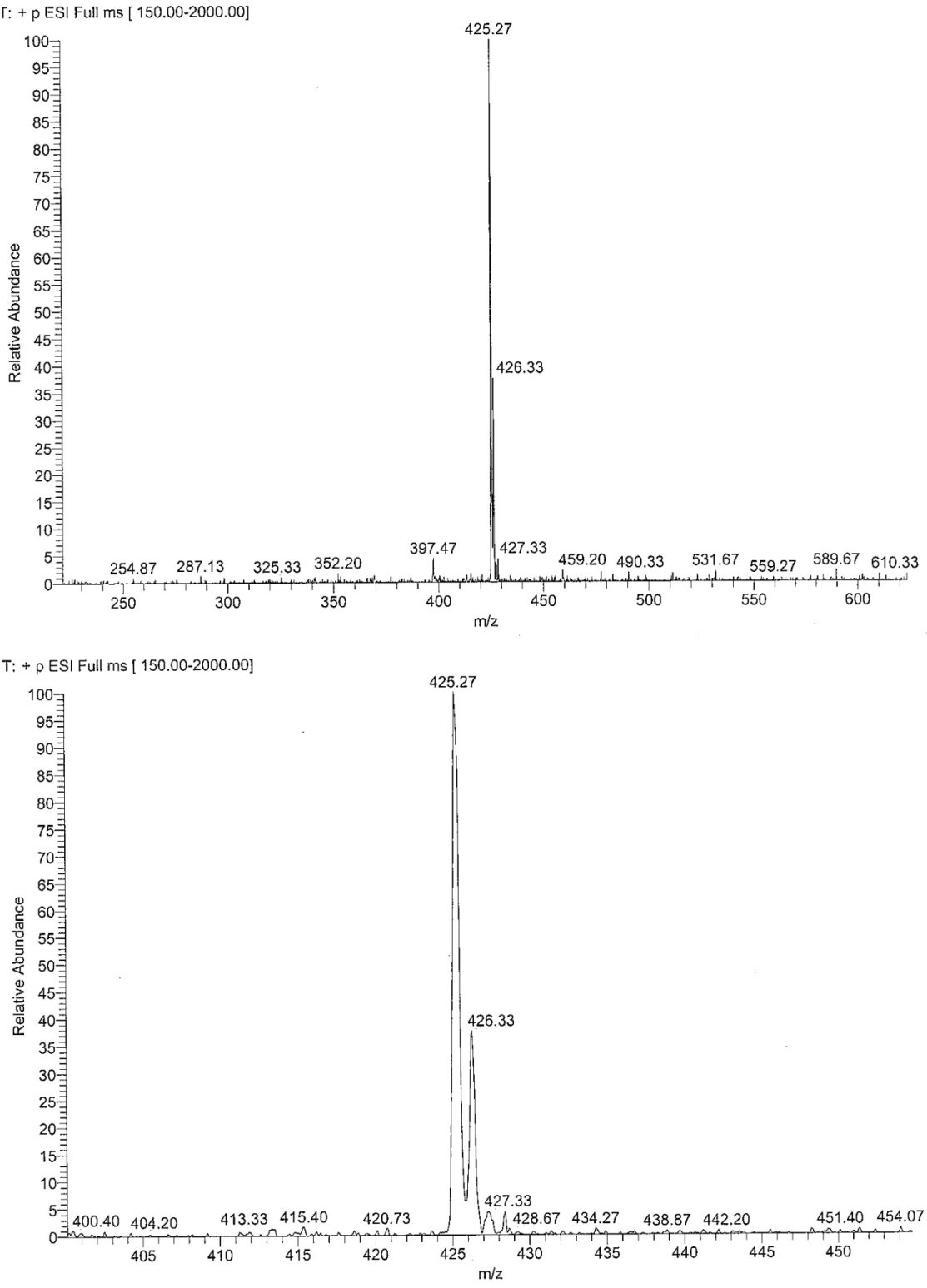
GB	0
PC	1.40

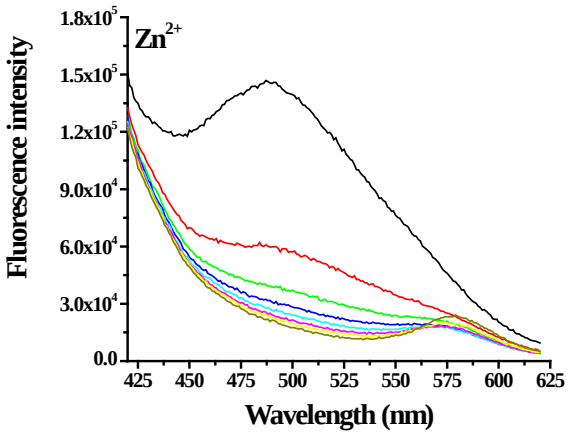
c) Mass spectra of RBC:

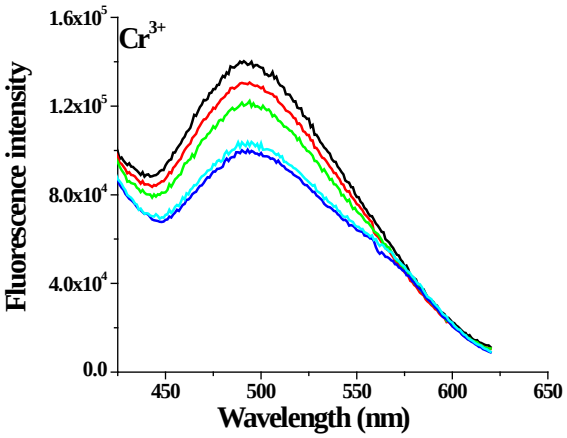


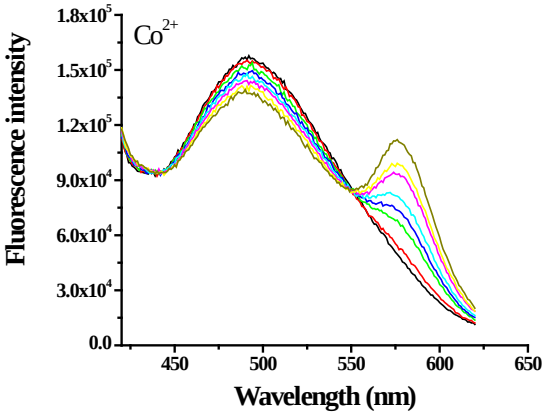
d) ESI mass spectra of Cu-complex with RBC: [$C_{48}H_{51}CuN_9O_2$ + 2H]$^{2+}$ = 425

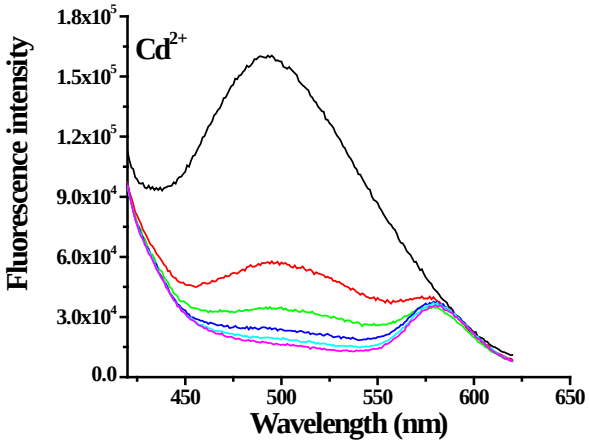


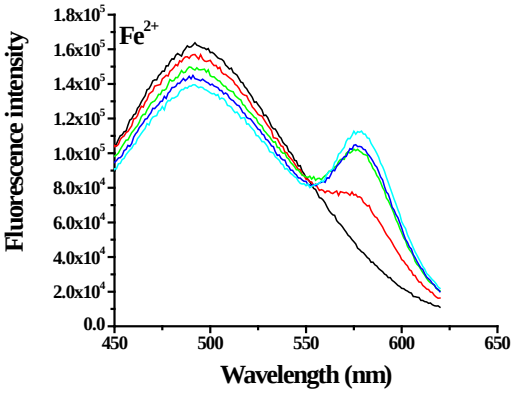
8. Fluorescence spectra of RBC with different guest metal ions in acetonitrile-HEPES buffer (pH 7.4)

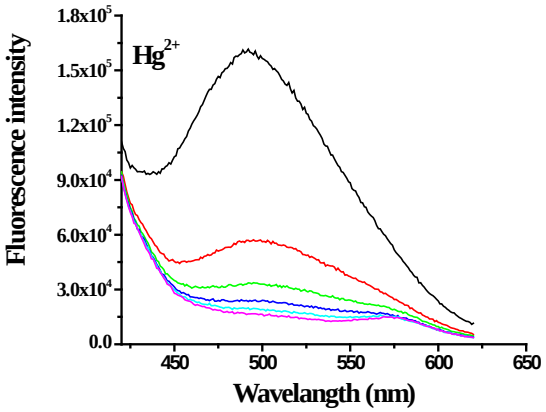


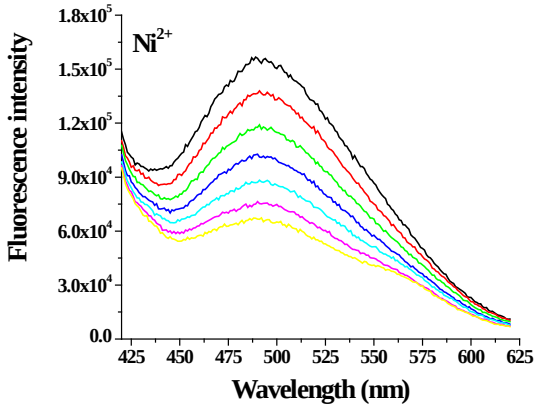


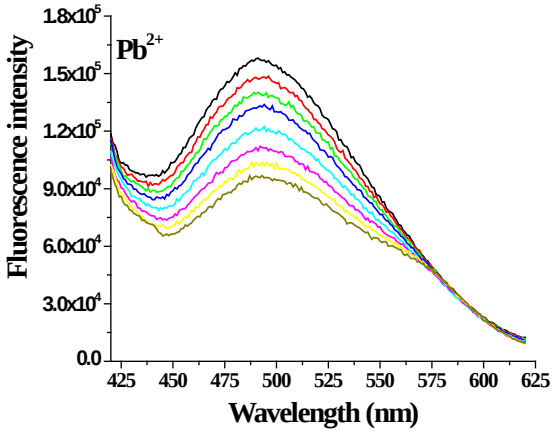


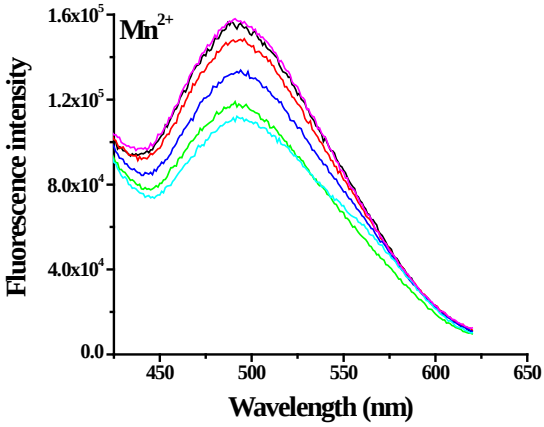












9. UV-vis spectra of RBC with different guest metal ions in acetonitrile-HEPES buffer (pH 7.4)