Supporting Information

A new pyrene based highly sensitive fluorescence probe for copper(II) and fluoride with living cell application

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1. General procedure for drawing Job's plot by fluorescence method:

Stock solution of same concentration of sensor and Cu were prepared in the order of ≈ 1.0 x 10^{-5} ML^{-1} CH_3CN-H_2O (7:3, v/v, at pH 7.5) media. The fluorescence in each case with different *host–guest* ratio but equal in volume was recorded. Job's plots were drawn by plotting $\Delta I.X_{host}$ *vs*. X_{host} (ΔI = change of fluorescence intensity of the spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively).

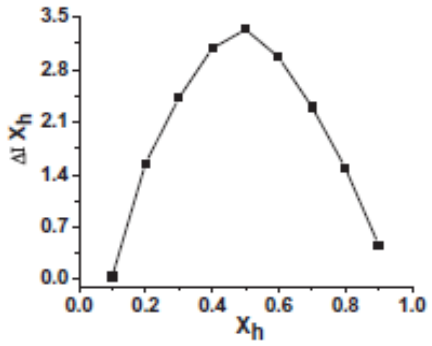


Figure (a)

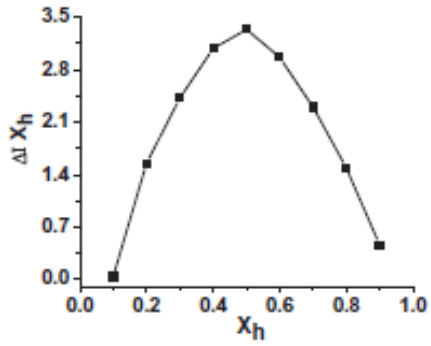


Figure (b)

Figure S1: Job's plot diagram of the (a): Cu^{2+}-complex for BMPA; (b): F^--complex for BMPA determined by fluorescence method in CH_3CN (where X_h is the mole fraction of the host and DI is the change of emission intensity).

2. UV–vis absorption spectra of receptor

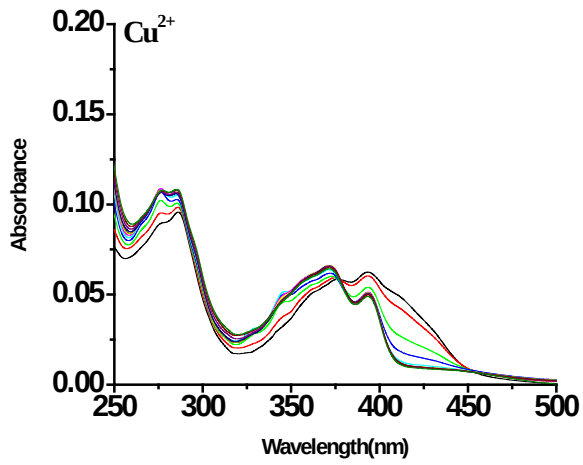


Figure (a)

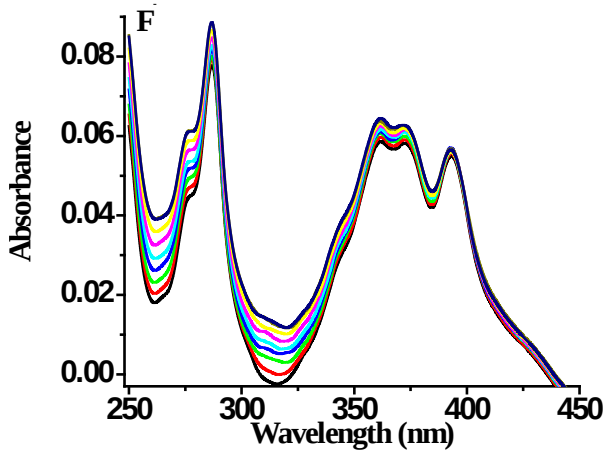


Figure (b)

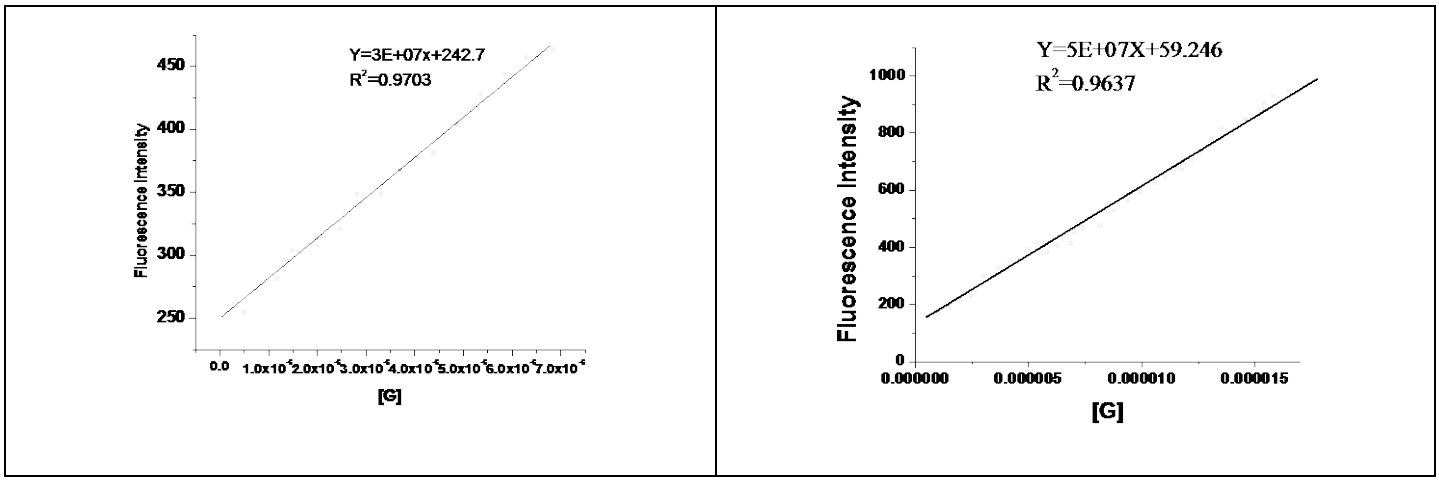
Figure S2: (a) UV–vis absorption spectra of receptor (**BMPA**) (c = 1X 10^{-5} ML^{-1}) upon gradual addition of Cu^{2+} (c = 2X 10^{-4} ML^{-1}) in CH_3CN. (b) UV–vis absorption spectra of receptor (**BMPA**) (c = 1X 10^{-5} ML^{-1}) upon gradual addition of F^- (c = 2X 10^{-4} ML^{-1}) in CH_3CN

3. Determination of Detection Limit:

The detection limit DL of **L** 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 we get slope = 3E+07, and Sb1 value is 12.12. Thus using the formula we get the Detection Limit = 1.21 µM i.e. L can detect Cu^{2+} in this minimum concentration.

From the graph we get slope = 5E+07, and Sb1 value is. 48.558. Thus using the formula we get the Detection Limit = 2.91 µM i.e. L can detect F^- in this minimum concentration



4. Determination of Association constant:

The spectra of these solutions were recorded by means of fluorescence methods. Binding constant was calculated according to the Benesi-Hildebrand equation. *Ka* was calculated following the equation stated below.

$1/(F-Fo) = 1/\{K(Fmax–Fo)\ [Cu^{2+}]n\} + 1/[Fmax-Fo]$

$1/(F-Fo) = 1/\{K(Fmax–Fo)\ [F^-]n\} + 1/[Fmax-Fo]$

Here Fo is the fluorescence of receptor in the absence of guest, F is the fluorescence recorded in the presence of added guest, Fmax is fluorescence in presence of added $[Cu^{2+}]$max and $[F^-]$max, K is the association constant (M-1). The association constant (Ka) could be determined from the slope of the straight line of the plot of 1/(F-Fo) against 1/$[Cu^{2+}]$n and 1/$[F^-]$n respectively. The association constant (*Ka*) as determined by

fluorescence titration method for sensor with Cu^{2+} and F^- is found to be 1.0×10^5 M^{-1} and $1.07x10^5$ M^{-1} respectively.

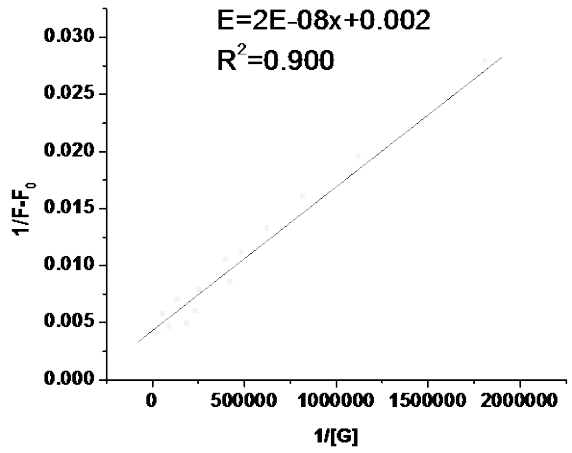


Figure (a)

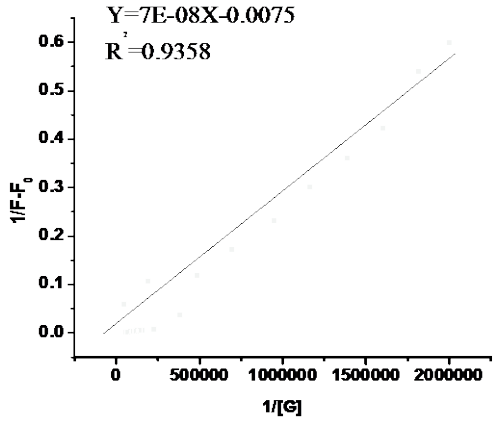


Figure (b)

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Figure S3: Benesi–Hildebrand plot from Fluorescence titration data of receptor **BMPA** ($1x10^{-5}$M) with (a) Cu^{2+} and (b) F^- respectively.

5. Determination of fluorescence quantum yield:

Here, the quantum yield φ was measured by using the following equation, φx = φs (Fx / Fs)(As / Ax)(nx2/ ns2) where, X & S indicate the unknown and standard solution respectively, φ = quantum yield, F = area under the emission curve, A = absorbance at the excitation wave length, n = index of refraction of the solvent. Here φ measurements were performed using anthracene in ethanol as standard [φ = 0.27] (error ~ 10%).

The quantum yield of **BMPA** itself is 0.88 which remarkably changed to 1.52 on the formation of a complex with Cu^{2+} metal ion and 4.02 due to binding with F^- respectively.

6. Fluorescence vs. pH plot

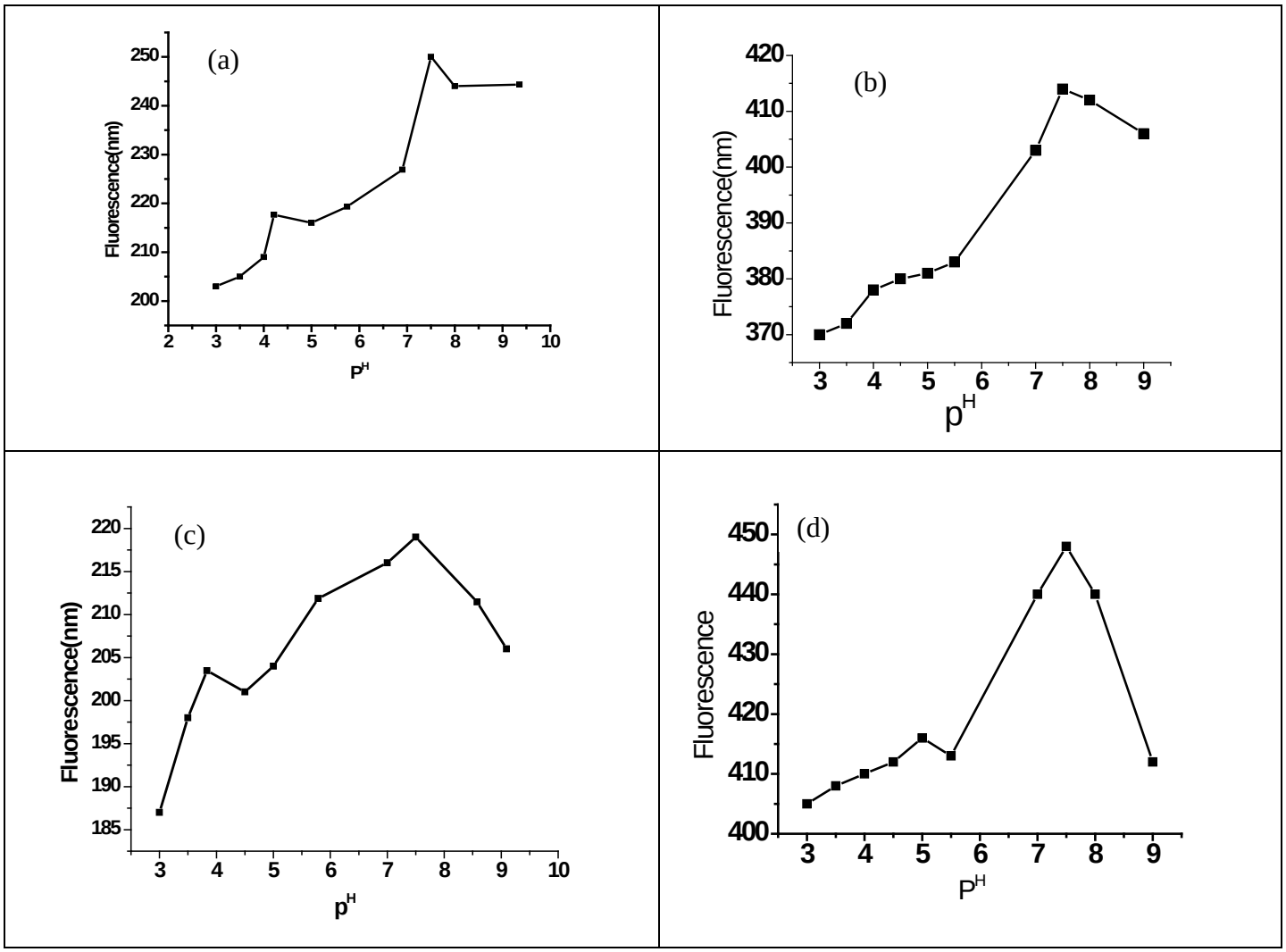
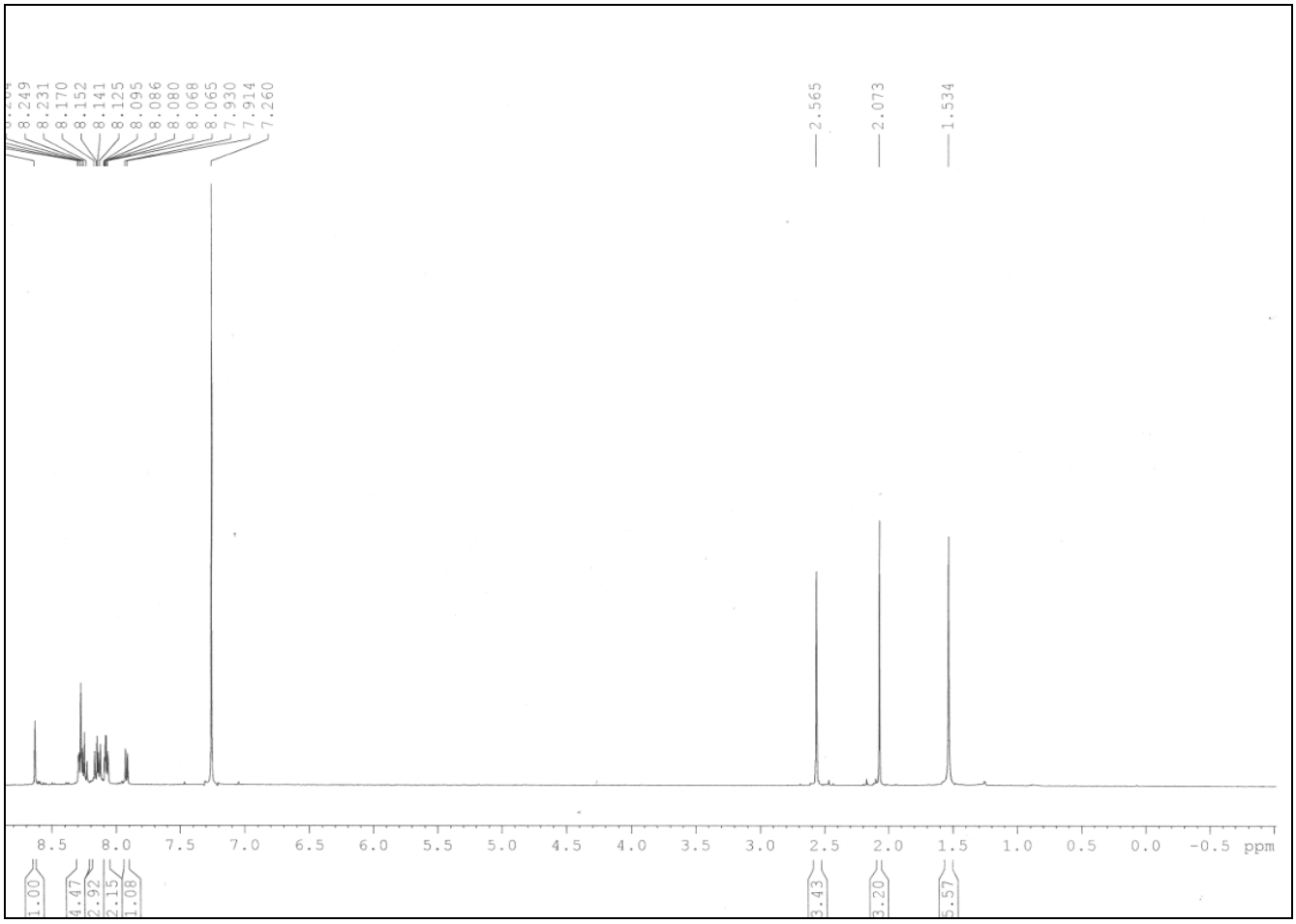


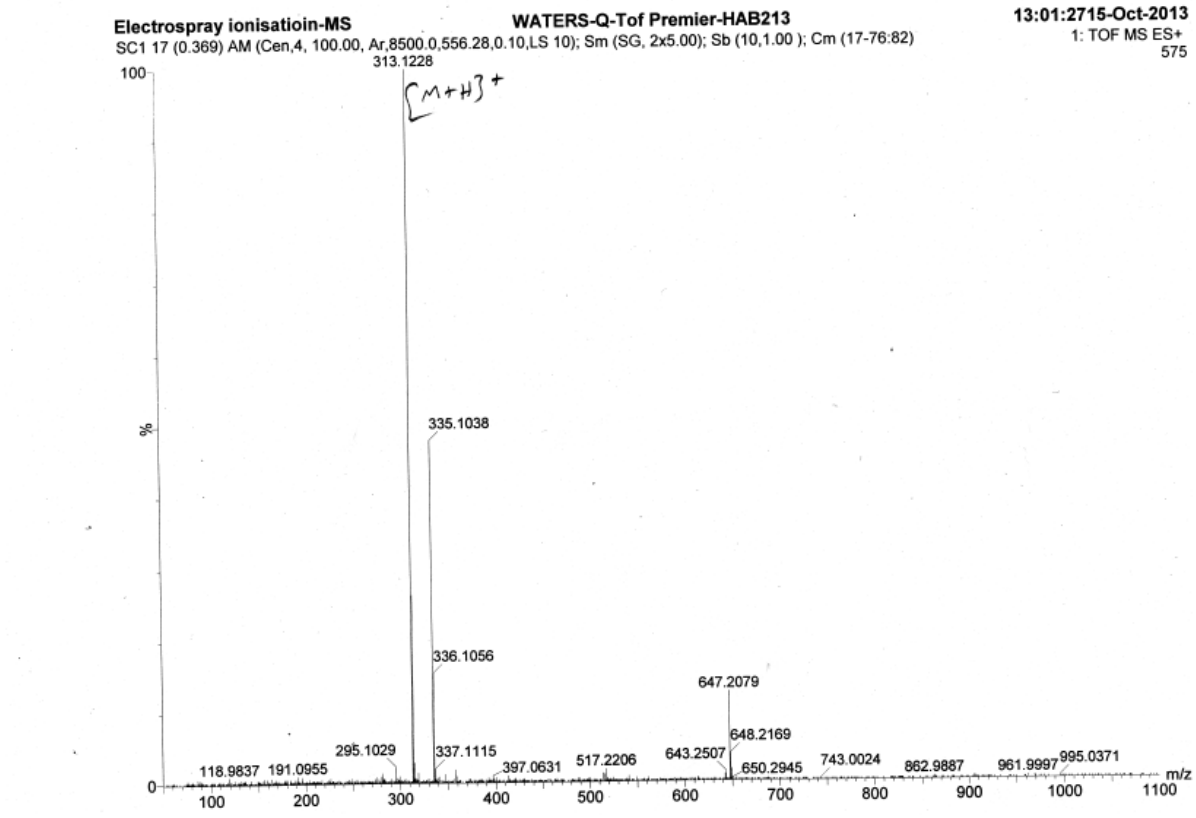
Figure S4: (a) Plots of fluorescence at 414 nm of **BMPA** as a function of pH. The solid line is the best fit pH titration curve with **p*Ka* 5.97**. (b) Absorbance intensity of **BMPA** (10 μM) at various pH values in CH_3CN-H_2O (7:3, v/v) medium in presence of Cu^{2+} (2.0 equiv., 2.0 x10^{-4} M) at 414 nm. (c) Plots of fluorescence at 448 nm of **BMPA** as a function of pH. The solid line is the best fit pH titration curve with **p*Ka* 6.24**. (d) Absorbance intensity of **BMPA** (10 μM) at various pH values in CH_3CN medium in presence of F^- (1.0 equiv., 2.0 x10^{-4} M) at 448nm.

7. ^{1}H NMR and HRMS spectra of compound (1):

^{1}H NMR of compound (1):

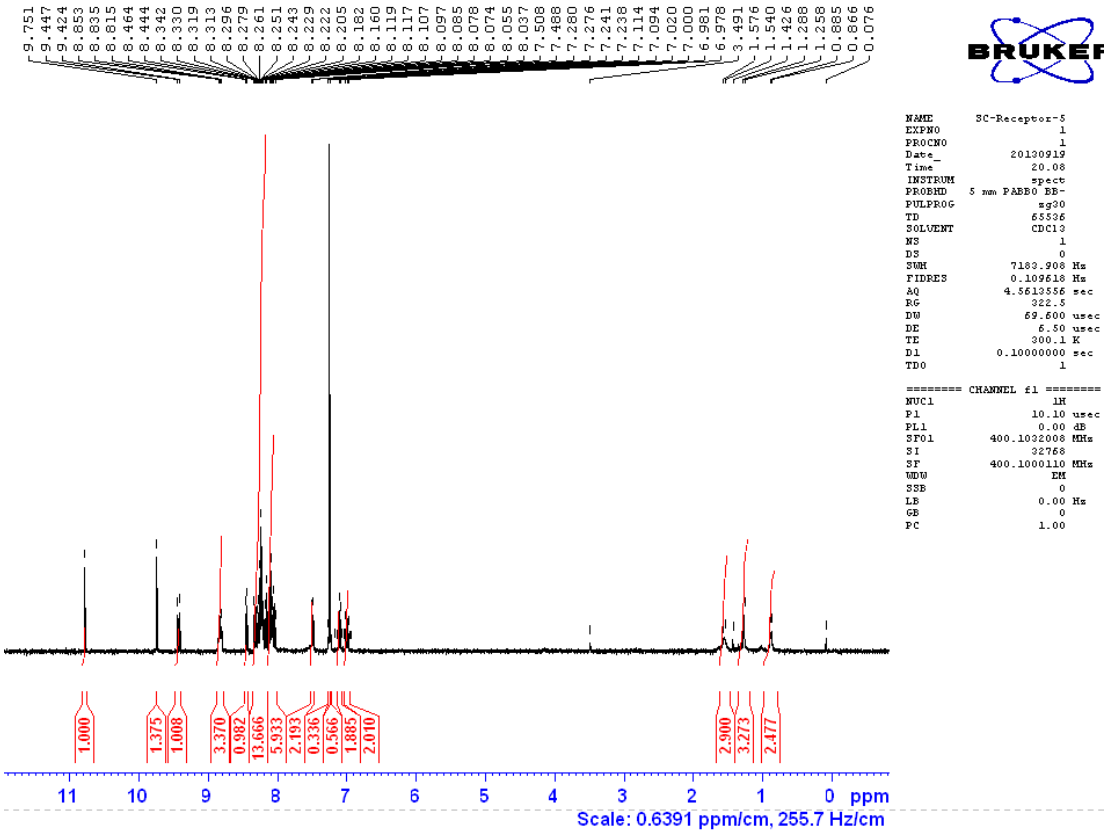


HRMS of compound (1):

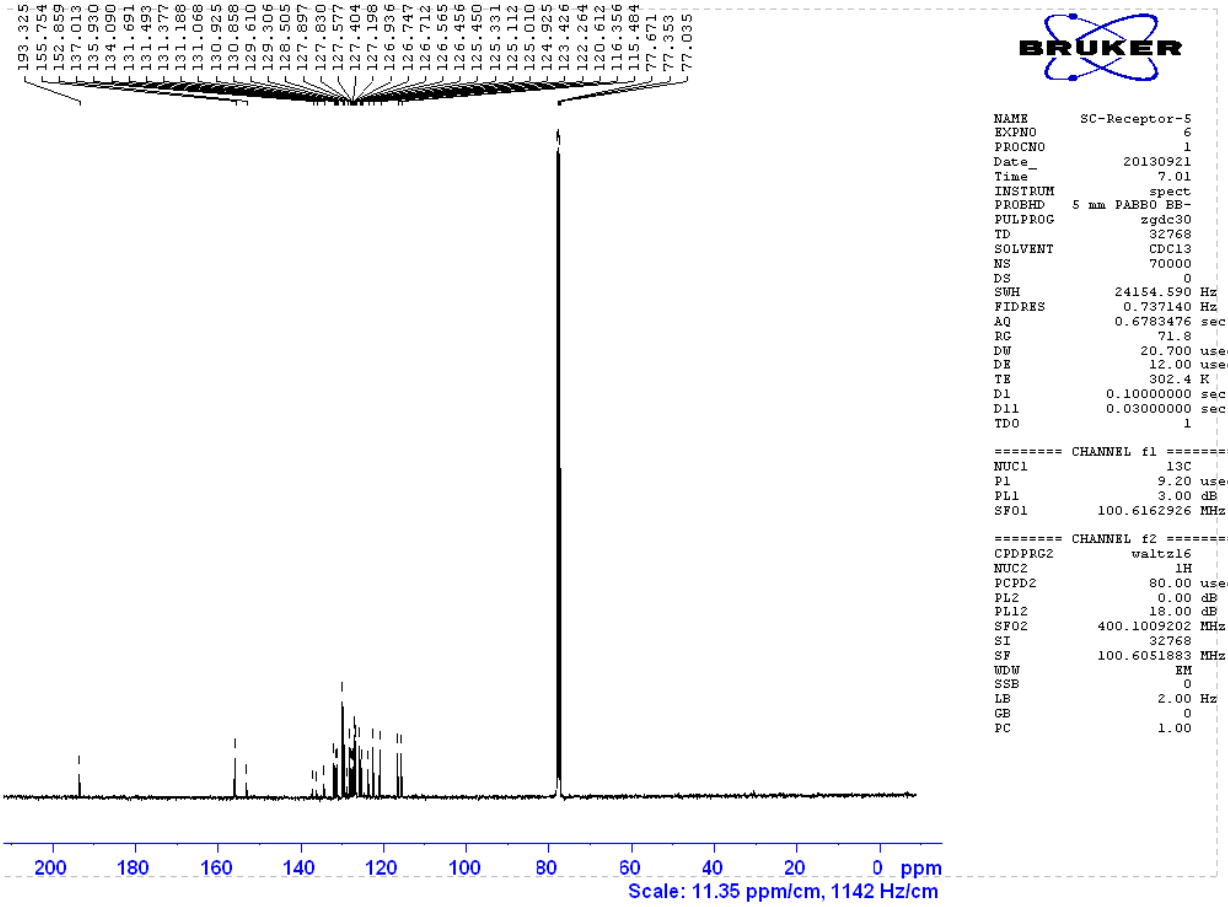


8. ^{1}H NMR, HRMS and FT-IR spectra of receptor and receptor + Cu(II):

^{1}H NMR of receptor:

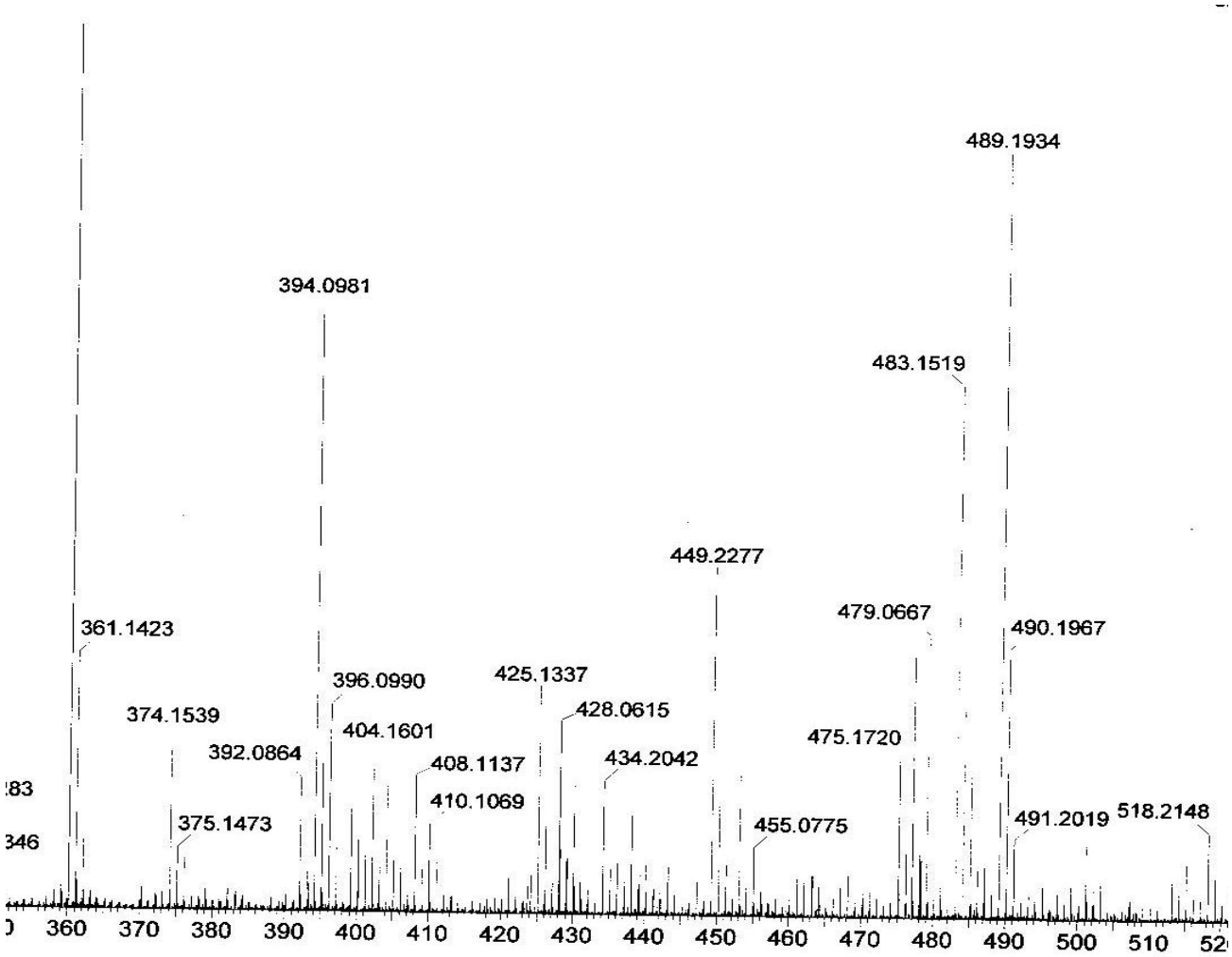


^{13}C NMR of receptor:

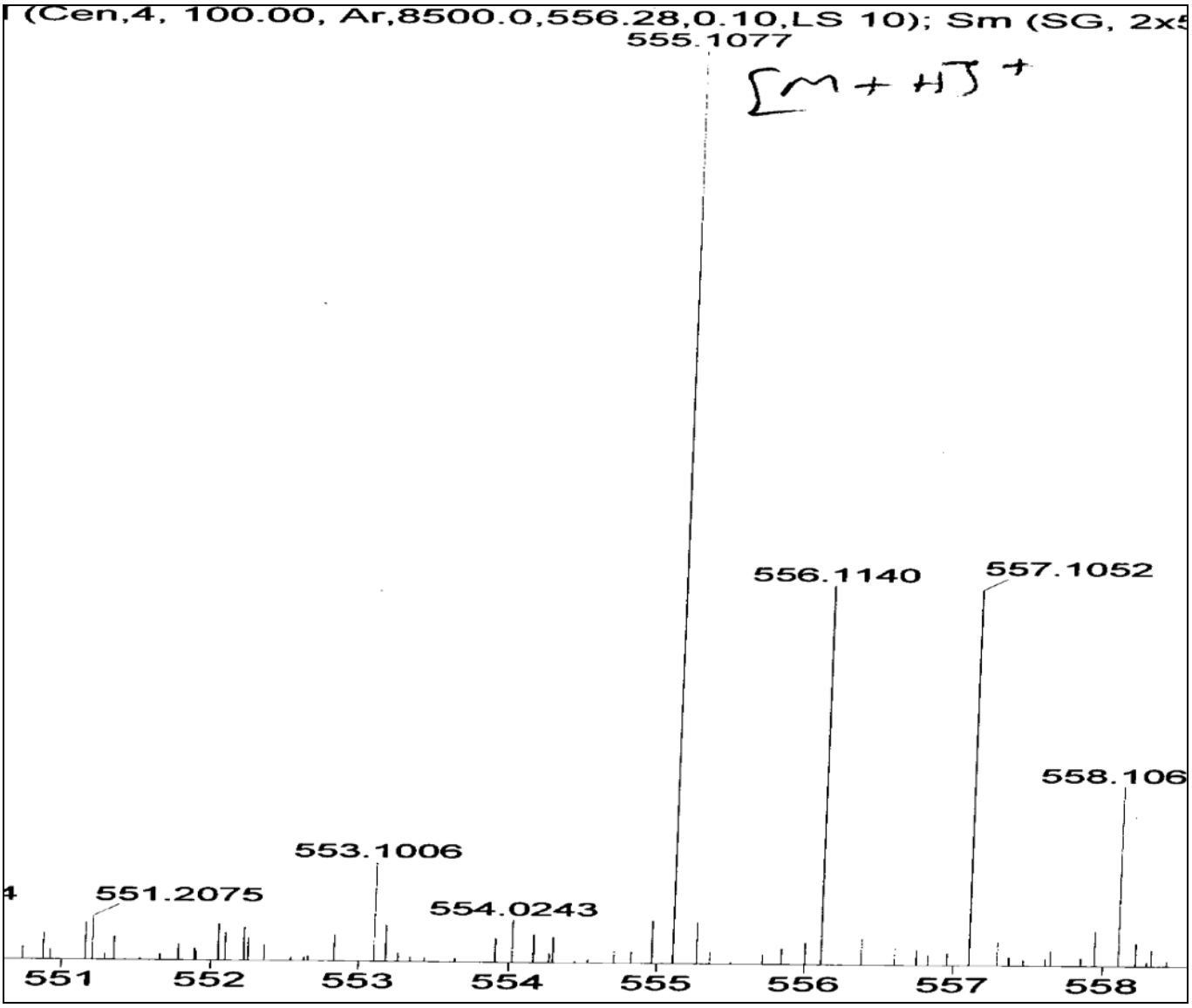


HRMS spectra of receptor:

(BMPA+Na^++1)

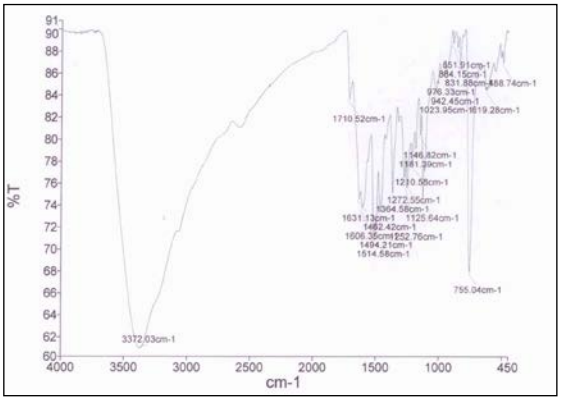


HRMS spectra of receptor + Cu:

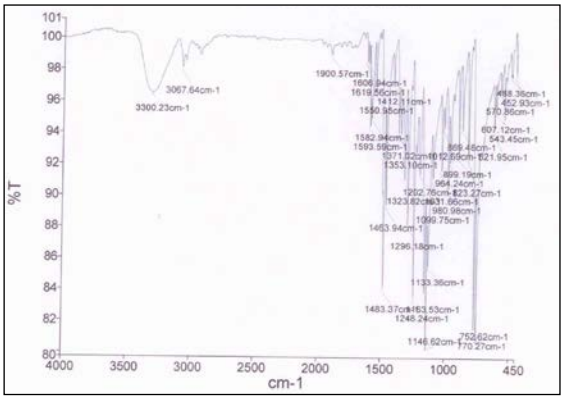


FT-IR data of (a) receptor and (b) receptor + Cu

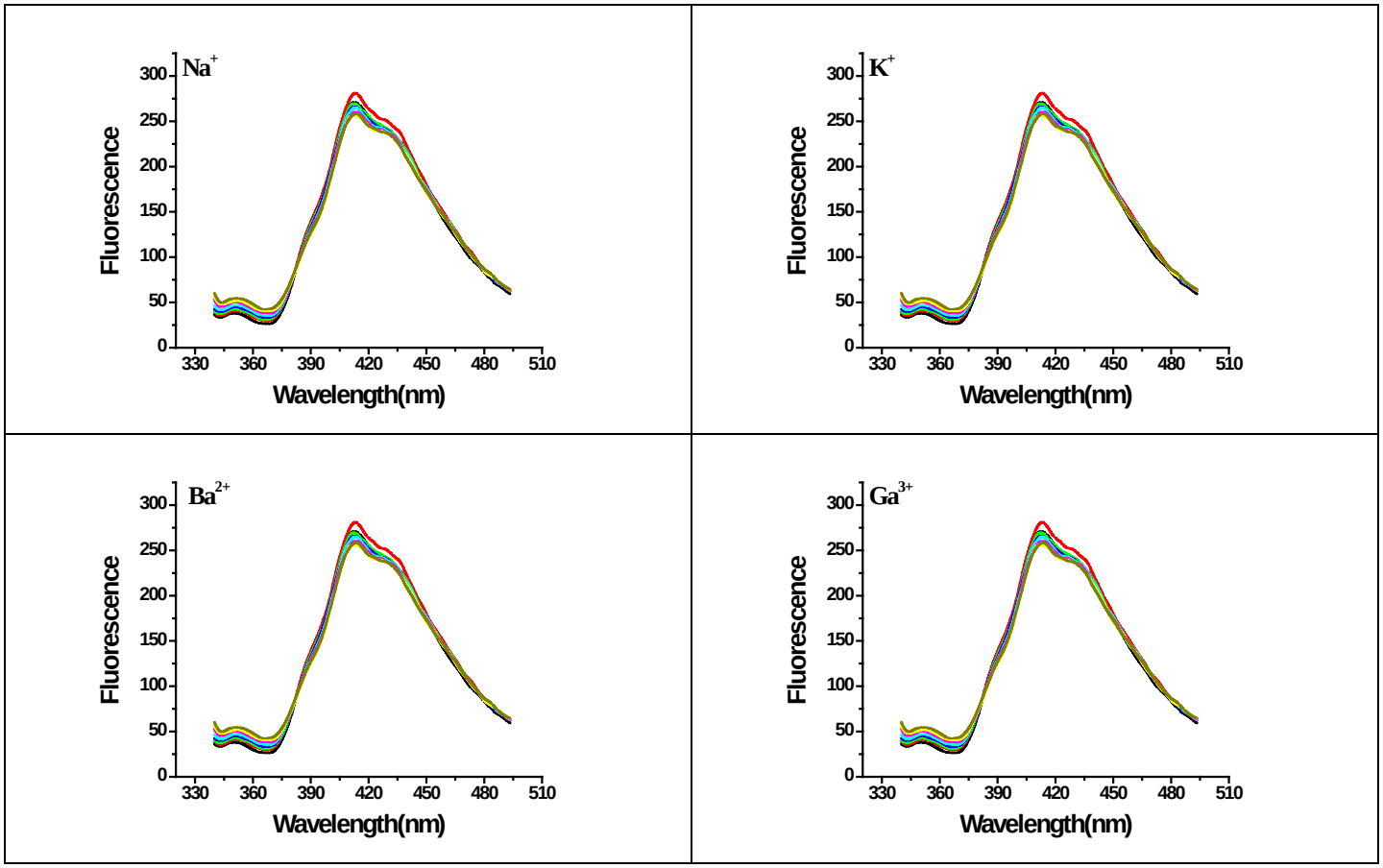
(a)

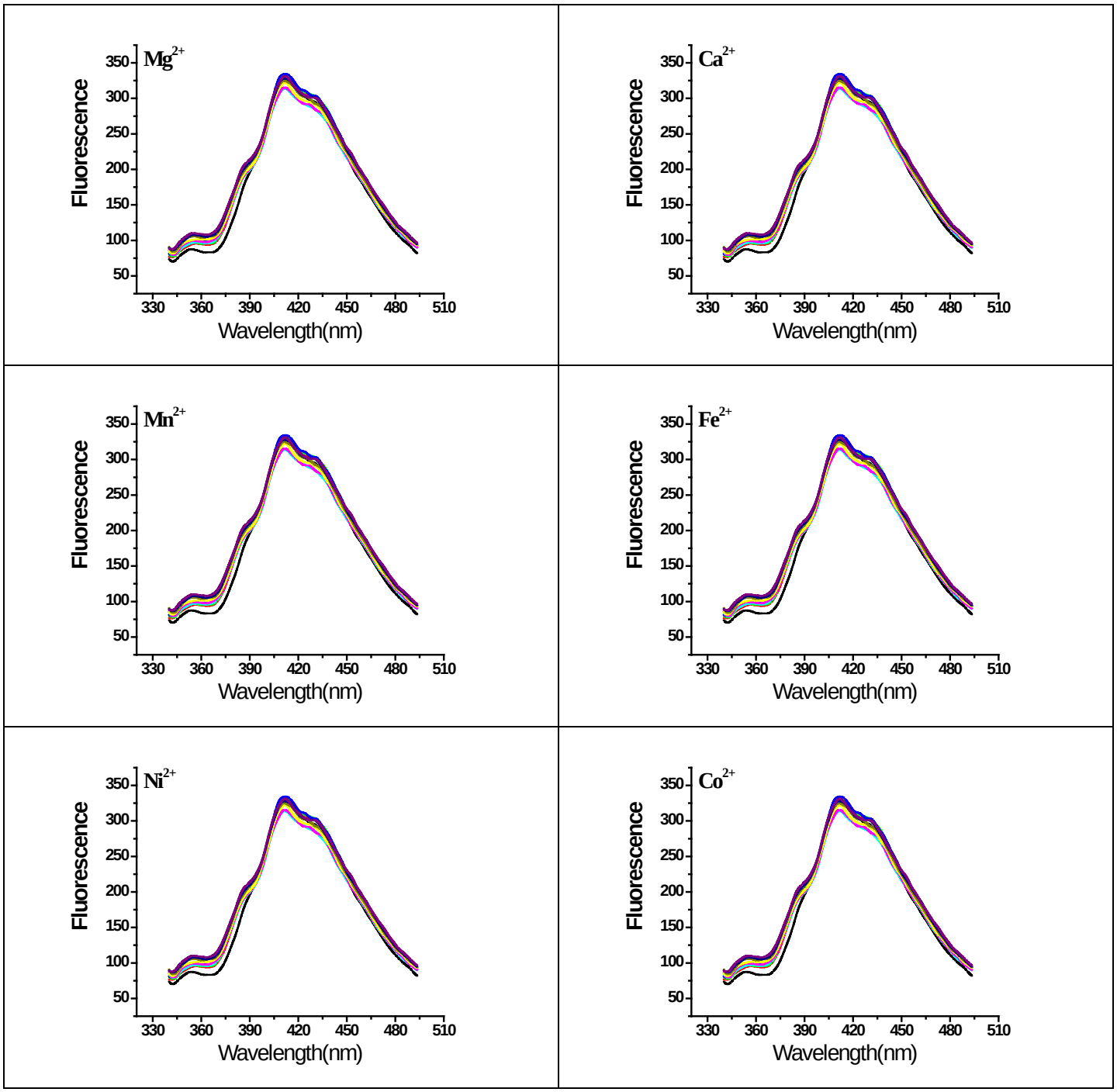


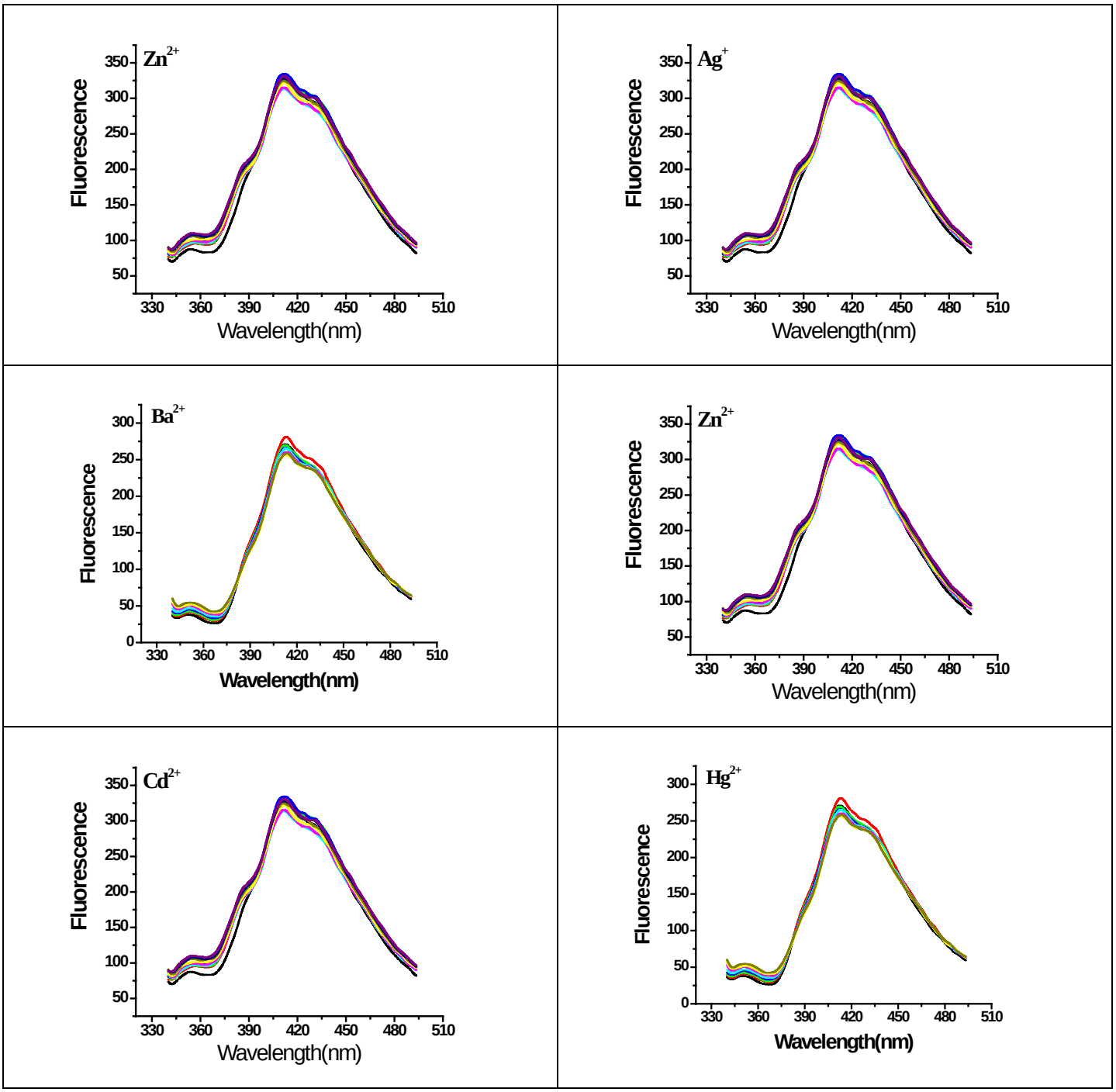
(b)

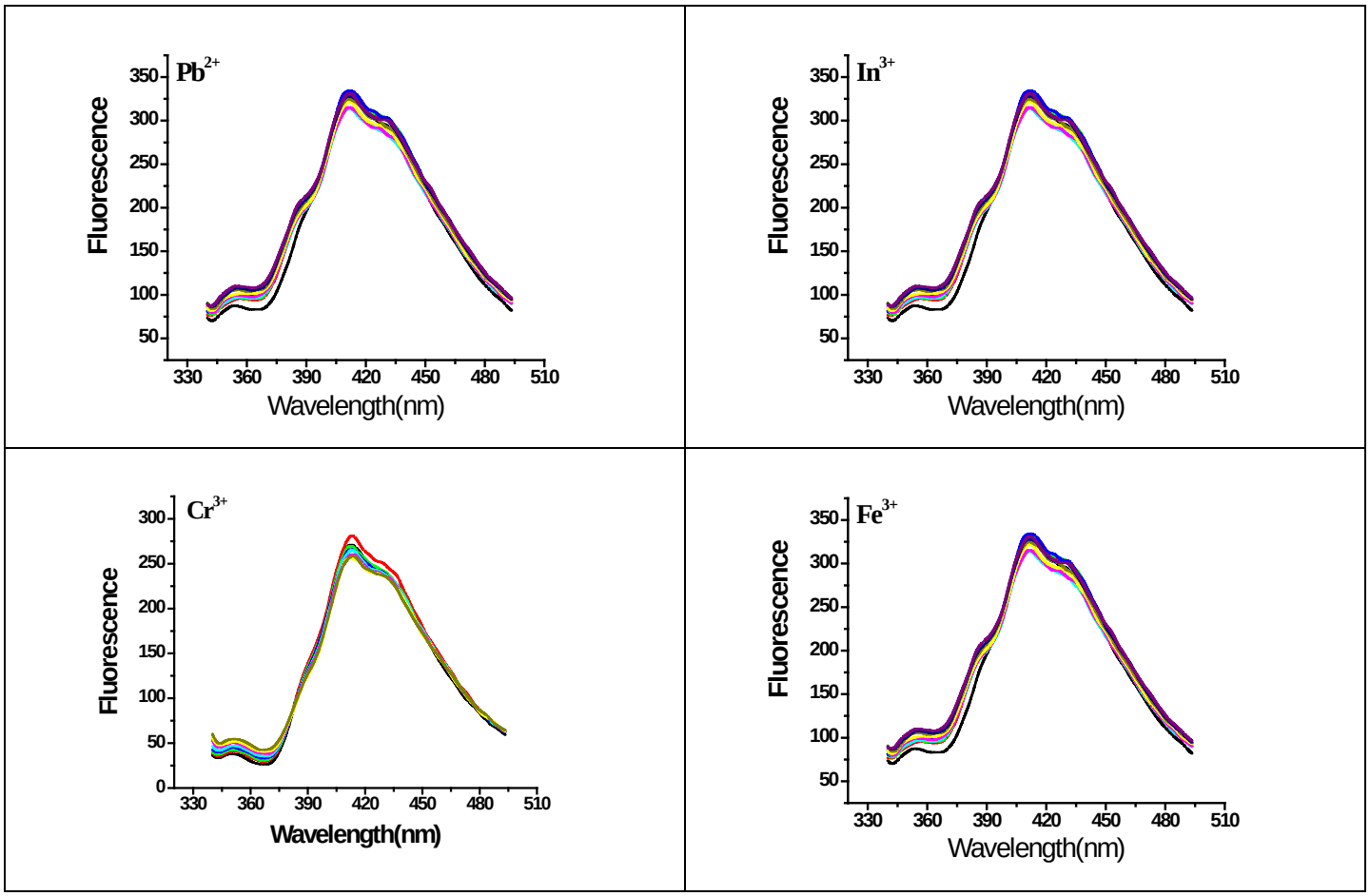


9. Fluorescence emission spectra of receptor (C = 1x 10-5 M, in CH_3CN : H_2O(7:3, v/v, at pH 7.5) with different cations as Na^+, K^+, Ba^{2+}, Ga^{3+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, Pb^{2+}, In^{3+}, Cr^{3+} and Fe^{3+} in CH_3CN : H_2O (C = 2x 10^{-4} M, 7:3, v/v, at pH 7.5). The solutions of metal ions were prepared from $NaClO_4$, $KClO_4$, $Ba(ClO_4)_2.4H_2O$, $GaCl_3$, $Mg(ClO_4)_2{\cdot}6H_2O$, $Ca(ClO_4)_2.4H_2O$, $Mn(ClO_4)_2{\cdot}6H_2O$, $Fe(SO_4)_2$, $Ni(ClO_4)_2{\cdot}6H_2O$, $Co(ClO_4)_2{\cdot}6H_2O$, $Zn(ClO_4)_2{\cdot}6H_2O$, $AgNO_3$, $Cd(ClO_4)_2{\cdot}H_2O$, $HgCl_2$, $Pb(ClO_4)_2$, $InCl_3$, $CrCl_3.6H_2O$ and $FeCl_3$ respectively.

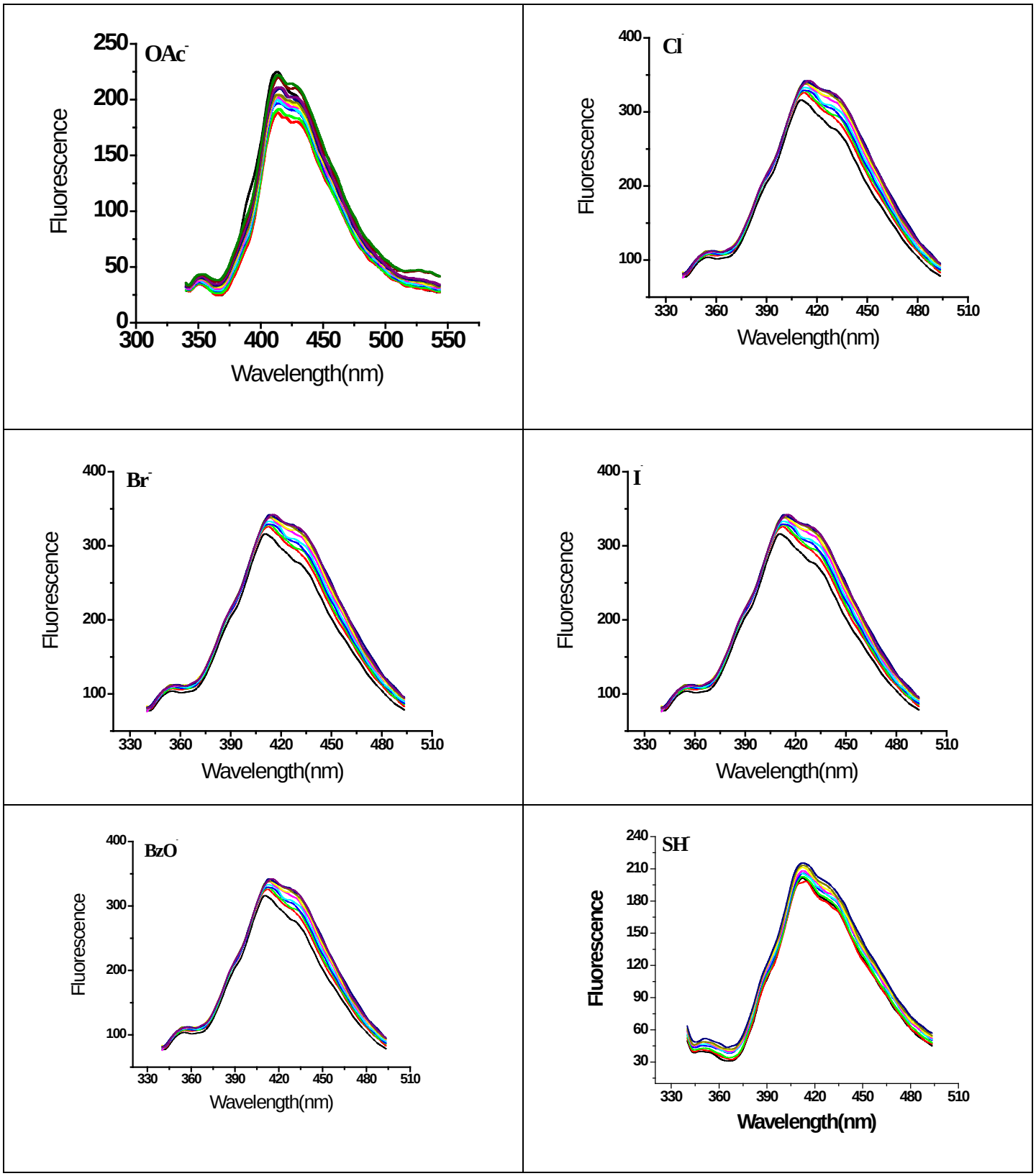


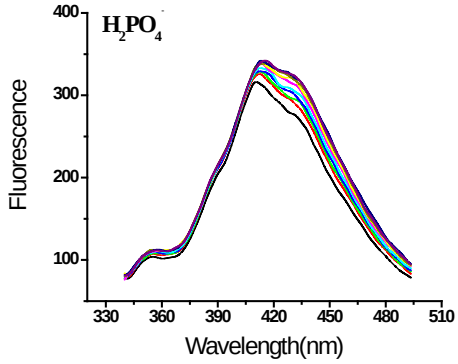

Mg2+
Ca2+
Mn2+
Fe2+
Ni2+
Co2+
Fluorescence
Wavelength(nm)
50
100
150
200
250
300
350
330
360
390
420
450
480
510

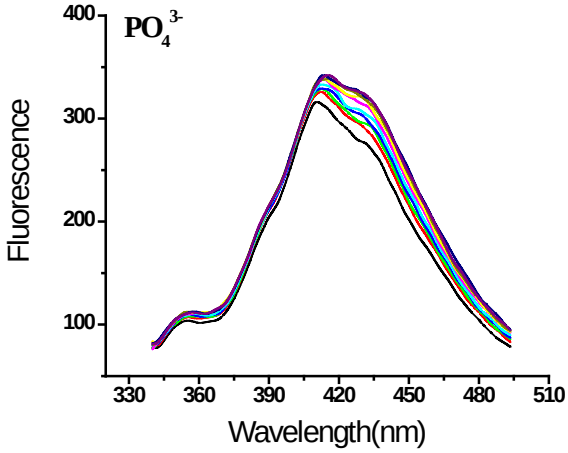

Zn2+
Fluorescence
Wavelength(nm)
Ag+
Fluorescence
Wavelength(nm)
Ba2+
Fluorescence
Wavelength(nm)
Zn2+
Fluorescence
Wavelength(nm)
Cd2+
Fluorescence
Wavelength(nm)
Hg2+
Fluorescence
Wavelength(nm)

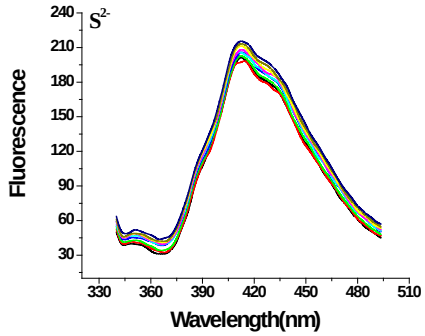


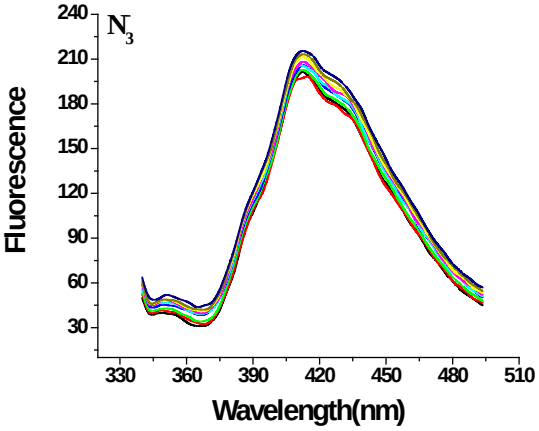
10. Fluorescence emission spectra of BMPA (C = 1x 10^{-5} M, in CH_3CN) at pH 7.5 with different anions as AcO^-, Cl^-, Br^-, I^-, BzO^-, SH^-, $H_2PO_4^-$, PO_4^{3-}, S^{2-}, N_3^-, $P_2O_7^{4-}$, SCN^- , ADP and ATP in MeOH : H_2O (C = 2x 10^{-4} M). The AcO^-, Cl^-, Br^-, I^-, and BzO^- as their tetra butyl salts; SH^-, $H_2PO_4^-$, PO_4^{3-}, S^{2-}, N_3^-, $P_2O_7^{4-}$ and SCN^- as their sodium salts; ADP and ATP as their potassium salts in CH_3CN.

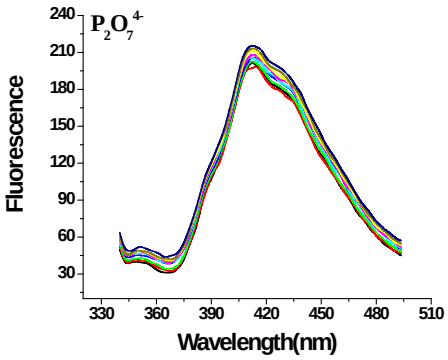

OAc-
Fluorescence
Wavelength(nm)
Cl-
Fluorescence
Wavelength(nm)
Br-
Fluorescence
Wavelength(nm)
I-
Fluorescence
Wavelength(nm)
BzO-
Fluorescence
Wavelength(nm)
SH-
Fluorescence
Wavelength(nm)

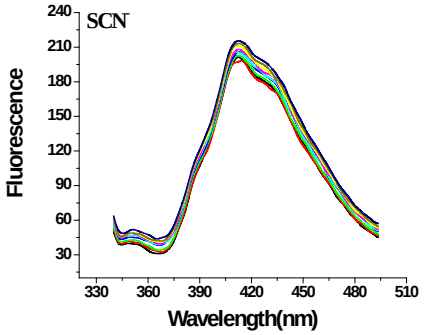

$H_2PO_4^-$
Fluorescence
Wavelength(nm)

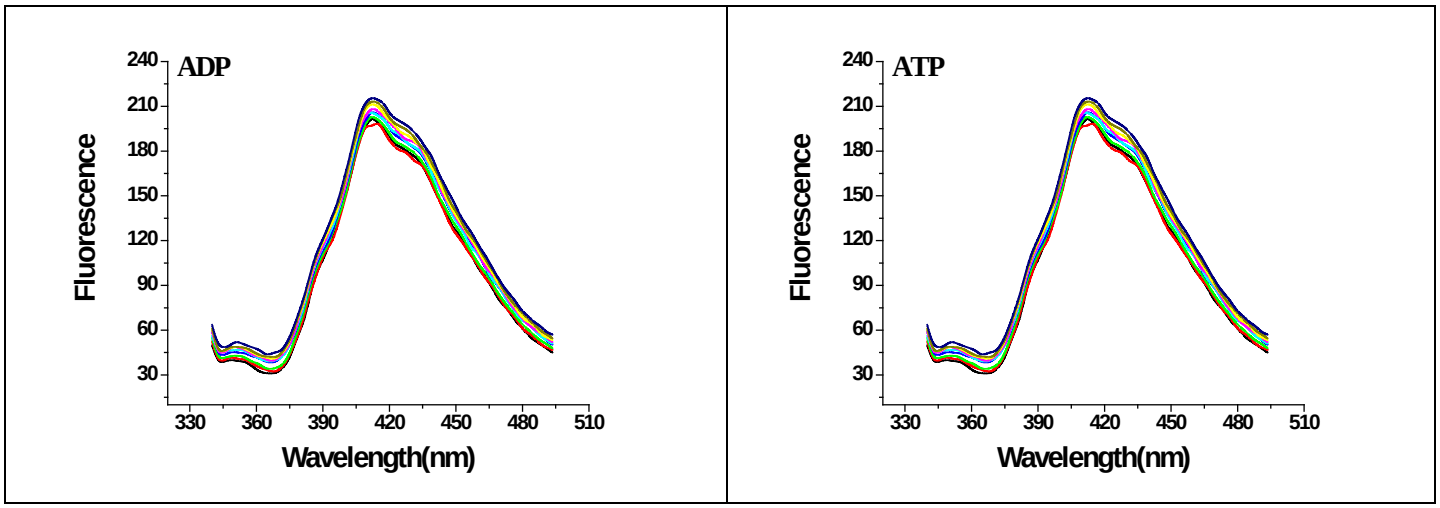

PO_4^{3-}
Fluorescence
Wavelength(nm)


S^{2-}
Fluorescence
Wavelength(nm)

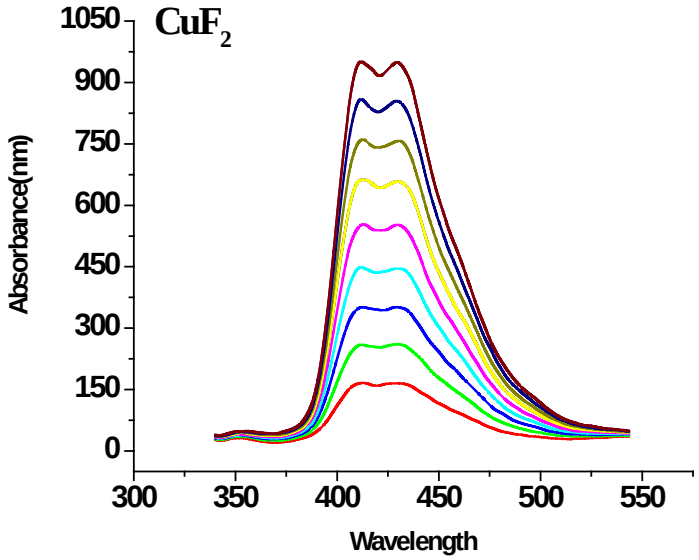

N_3^-
Fluorescence
Wavelength(nm)


$P_2O_7^{4-}$
Fluorescence
Wavelength(nm)


SCN^-
Fluorescence
Wavelength(nm)



11. Fluorescence emission spectra of BMPA (C = 1x 10^{-5} M, in CH_3CN) at pH 7.5 with Copper fluoride:



12. Fluorescence titration spectra of receptor with Cu^{2+} and F^- respectively and vice versa

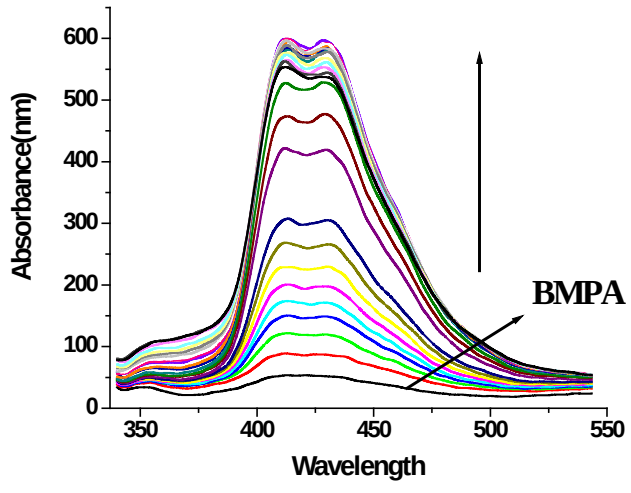


Figure (a)

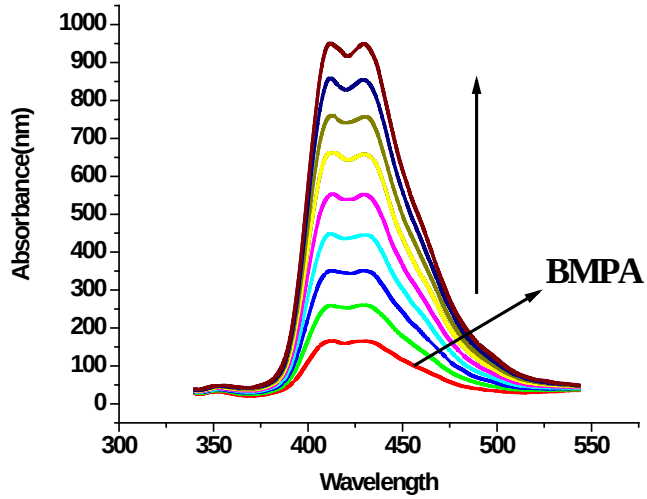


Figure (b)

Fig. 7 (a) Fluorescence spectra of (a) [BMPA + Cu^{2+}] upon addition of F^- and (b) [BMPA + F^-] upon addition of Cu^{2+} in CH_3CN.

13. Biological study

Preparation of *Tecoma stans* pollen cells

Pollen grains were obtained from freshly collected mature buds of *Tecoma stans*, a common ornamental plant with bell shaped bright yellow flower by crashing stamens on a sterile Petri plate and suspending them in normal saline. After crushing the stamina debrishes are removed by filtering through a thin layer of non absorbant cotton and the suspended pollens are collected by centrifugation at 3000 rpm for five minutes. The pollen pellet was then washed twice in normal saline and then incubated in a solution of copper perchlorate salt (1 mg/ml) for 45 minutes at ambient temperature. After incubation they are again washed in normal saline as mentioned above and then treated with the receptor **BMPA**(1 mg/ml) solution for 45 minutes. Then the cells were washed with normal saline and photographed under various objectives using UV filter in a Leica DM1000 Fluorescence microscope in presence and absence of the ligand. Cells without treatment were used as control.

Preparation of *Candida albicans* cells

Candida albicans cells (IMTECH No. 3018) from exponentially growing culture in Potato Dextrose Broth medium (pH 6.0, incubation temperature, 37 ^{0}C) were centrifuged at 3000 rpm for 8 minutes, washed twice with 0.1 M HEPES buffer at pH 7.4. Then, it was treated with copper perchlorate salt (1 mg/ml) for 45 minutes. After incubation the cells were washed again with HEPES buffer at pH 7.4. These salt treated cells were treated with the receptor (1mg/ml) for 45 minutes at ambient temperature. After incubation the cells were washed again with HEPES buffer at pH 7.4. The cells were then mounted on grease free glass slide and observed under a Leica DM1000 fluorescence microscope equipped with UV filter. Cells without treatment were used as control.

14. References:

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