Supporting Information

Highly reactive (< 1 min) ratiometric "naked eye" detection of hypochlorite with real application in tap water

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CONTENTS

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}CNMR were recorded on a Brucker 400 MHz instrument respectively. 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 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. IR spectra were recorded on a JASCO FT/IR-460 plus spectrometer, using KBr discs.

2. General method of UV-*vis* and fluorescence titrations:

By UV-vis and fluorescence method:

For UV-vis and fluorescence titrations, stock solution of the sensor was prepared (c = 2 x 10^{-5} M) in CH_3CN: H_2O (4:6, v/v). The solution of the guest anions and oxidants were prepared (2 x 10^{-4} M) in CH_3CN: H_2O (4:6, v/v) at pH 7.4 by using 10 mM HEPES buffer. The original volume of the receptor solution is 2 ml. Solutions of the sensor of various concentrations and increasing concentrations of anions and oxidants were prepared separately. The spectra of these solutions were recorded by means of UV-vis and fluorescence methods.

3. pH effect on CDH moiety :

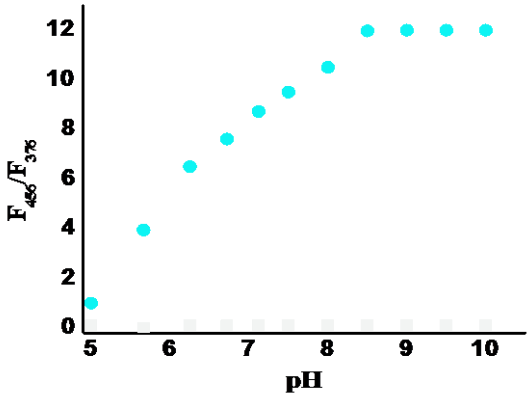


Figure S1: Emission ratio of (F_{456}/F_{376}) of probe CDH (5 μM) vs. pH values in absence (■) or presence (■) of OCl^- (40 equiv.) in CH_3CN:H_2O (4:6, V/V).

In the range of pH 5 to 10, CDH itself shows very weak fluorescence, but the fluorescence intensity increases significantly in the presence of hypochlorite. At pH 7.4, probe CDH exhibited a drastic change of the emission ratio (F_{456}/F_{376}) from 0.10 in the absence of OCl^- to 12.14 in the presence of OCl^- (40 equiv.), a very large (121-fold) enhancement. This indicates that the probe may be suitable for bio-applications at the physiological pH. The free probe is highly stable under the assay conditions.

4.Absorption and fluorescence spectra of CDH, CDH + OCl^- and Carbazoledialdehyde :

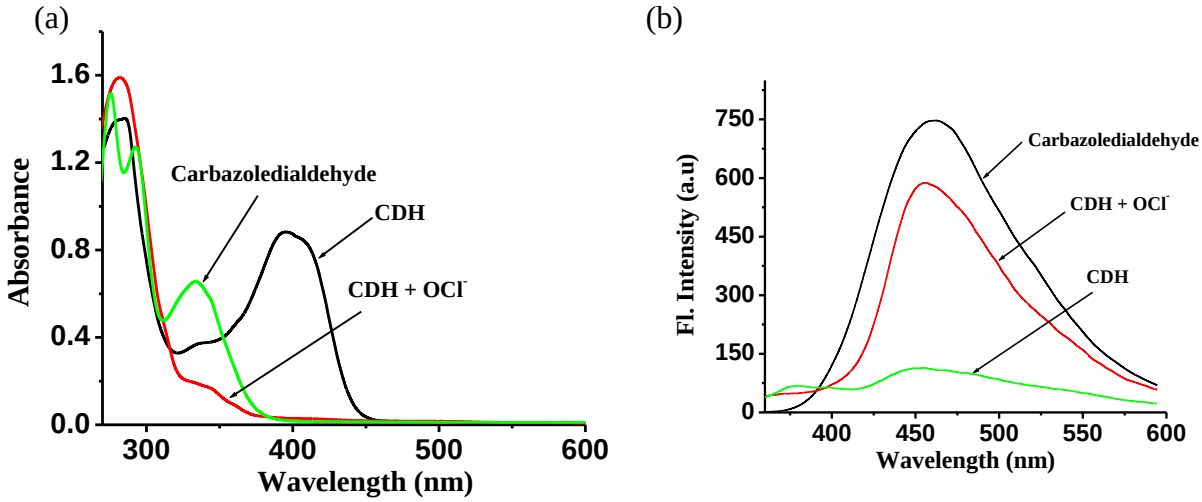


Figure S2: (a) Absorption spectra of CDH (C = 2 x 10^{-5} M), CDH + OCl^- (100 μM) and Carbazoledialdehyde (C = 2 x 10^{-5} M); (b) Fluorescence spectra of CDH, CDH + OCl^- and Carbazoledialdehyde with same concentration as used in absorption spectra.

5. Experimental Section:

Synthesis of Receptor (CDH): Compound (B) (1gm, 3.58 mmole) was refluxed with diaminemaleonitrile (773.5mg, 7.16mmole) in ethanol solvent for 8h. The solvent was removed under vacuum, and the residue was purified by filtration through a short column of silica using 10% MeOH in chloroform to get a pure yellow product, 1.399 mg (Yield: 85%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.489 (s, 2H), 8.125 (s, 2H), 7.932 (d, 2H, J=10 Hz), 7.494 (d, 2H, J=6.8 Hz), 4.332 (t, 2H, J=9.2 Hz), 2.050 (s, 4H), 1.877 (m, 2H), 1.341 (m, 2H), 0.961 (t, 3H, J=9.6 Hz)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 164.35, 130.47, 125.07, 121.12, 119.64, 119.04, 115.25, 114.70, 113.05, 111.15, 110.01, 60.64, 32.10, 20.54, 13.63.

MS (ESI MS): (m/z, %): 459.47 [(M^+, 100 %]

Elemental analysis: C= 67.94%, H= 4.59%, N= 27.47% (Calculated value : C= 67.96%, H= 4.61%, N= 27.43%).

FT-IR (KBr): 3449, 3290, 3166, 2891, 2208, 1595, 1478, 1361, 1227, 1124, 803, 725 cm^{-1}.

Melting Point: 265ºC

Synthesis of hypochlorite adducted CDH: CDH is mixed with two equivalents NaOCl in acetonitrile at room temperature to give a colorless solution. On removing the solvent, got a solid product which was used for ^{1}H-NMR and ^{13}CNMR, MASS and IR spectroscopy.

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.810 (s, 2H), 8.093(d, 2H, *J* = 6.8 Hz), 7.432 (m, 4H), 7.232 (t, 2H, *J* = 6 Hz), 4.282 (t, 2H, *J* = 10 Hz), 1.857 (m, 2H), 1.302 (m, 10H), 0.858 (t, 2H, *J* = 8.6 Hz).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 179.53, 135.35, 133.37, 121.12, 119.42, 113.05, 111.51, 60.60, 32.14, 20.53, 13.82.

MS (ESI MS): (m/z, %): 279.126 [($CDH+OCl^-$),[M^+], 100 %]

Elemental analysis: C= 77.38%, H= 6.13%, N= 5.09%, O= 11.40% (Calculated value : C= 77.40%, H= 6.13%, N=5.01%, O=11.46%.

FT-IR (KBr) : 2930, 2844, 2789, 2711, 1705, 1588, 1471, 1336, 1235, 1179, 1117, 890, 803, 732, 575 cm^{-1}.

Method for the preparation of TLC plate sticks:

It was easily prepared by immersing a TLC plate into the solution of CDH (2 X 10^{-4} M) in CH_3CN (1 mM) and then exposing it to air to evaporate the solvent. The detection of OCl^- was carried out by inserting the TLC plate to the different concentration of OCl^- (1 mM) and evaporating solvent to dryness. The color of the TLC plate changed from orange to colorless (Figure 8). Development of such dipsticks is useful as instant qualitative information is obtained without resorting to the instrumental analysis.

6. Determination of fluorescence quantum yield:

Here, the quantum yield φ was measured by using the following equation,

$\varphi_x = \varphi_s (F_x / F_s)(A_s / A_x)(n_x^2/ n_s^2)$

Where,

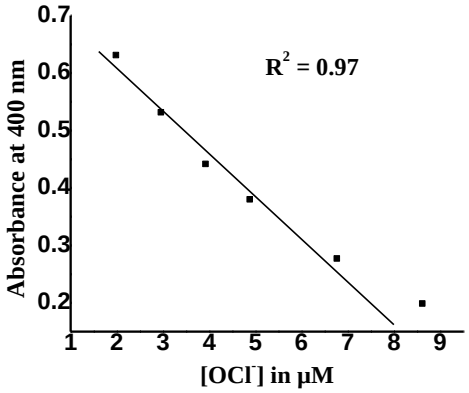
X & S indicate the unknown and standard solution respectively, φ = quantum yield,

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 **CDH** itself was 0.01 in presence of OCl^- it changed to 0.65, an enhancement around 65 fold is observed.

7. Calculation of the detection limit:



The detection limit DL of **CDH** for OCl^- was determined from the following equation[1]:

DL = K* Sb1/S

Where K = 2 or 3 (we take 2 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 = 0.0583, and Sb1 value is 0.031369.

Thus using the formula we get the Detection Limit = 1.07 µM i.e. CDH can detect OCl^- in this minimum concentration.

8. The bar diagram and absorbance ratio vs. conc of hypochlorite plot :

(a) (b)

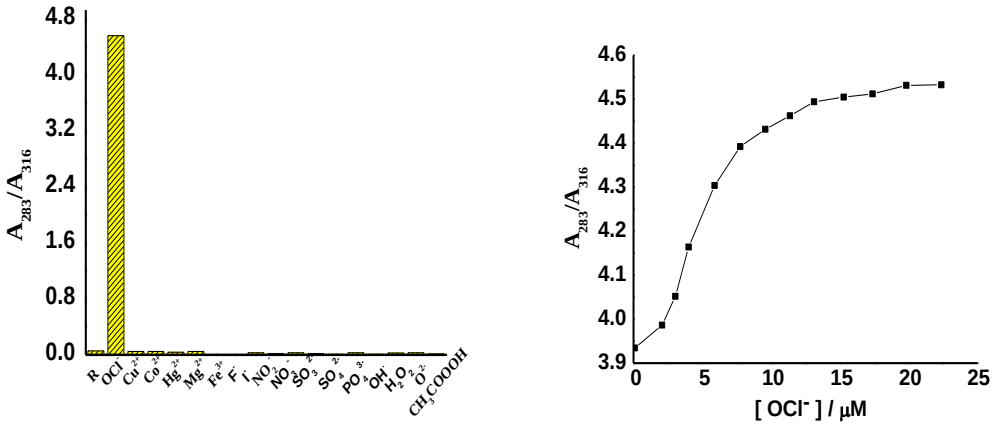


Figure S3: (a) The variation of absorbance ratio (A_{283}/A_{316}) for probe CDH in CH_3OH – H_2O solution (4: 6, v/v, 10 mM HEPES, pH 7.4) in presence of various ions and oxidizing species. (b) Absorbance ratio changes (A_{283}/A_{316}) of CDH upon gradual addition of OCl^-.

9. Time dependent absorbance curve:

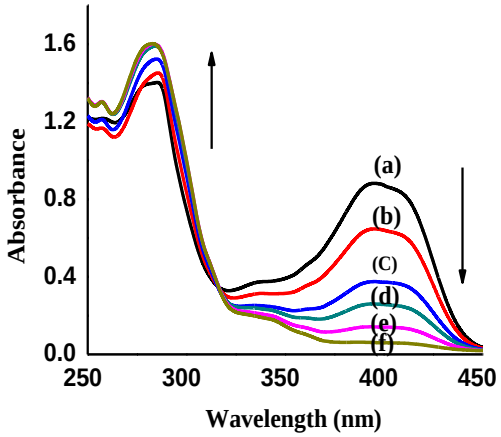


Figure S4: The time vs. absorbance spectra of (a) CDH (c = 2.0 x 10^{-5} M) in presence of OCl^- (c = 2.0 x 10^{-4} M) at pH 7.4 in CH_3CN:H_2O (4:6, V/V) at different times [(b) 20 (c) 30 (d) 40 (e) 50 (f) 60 sec.

10. Calculation of rate constant:

From the time vs. absorbance plot at fixed wavelength (283nm) using first order rate equation (Figure 3), we get rate constant K = slope X 2.303 = 0.0454 X 2.303 = 10.4 X 10^{-2} sec^{-1}.

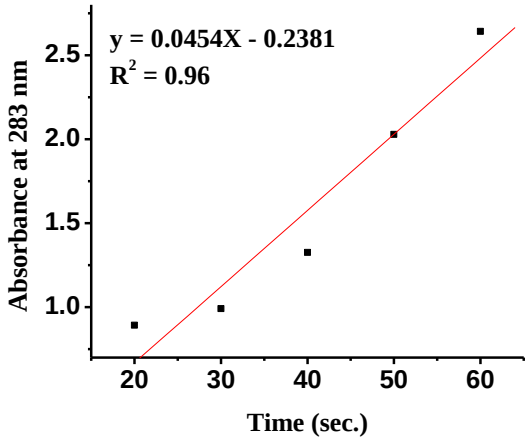


Figure S5: The time vs absorbance at a fixed wavelength (283nm) plot using first order rate equation

11. Time dependent fluorescence plot:

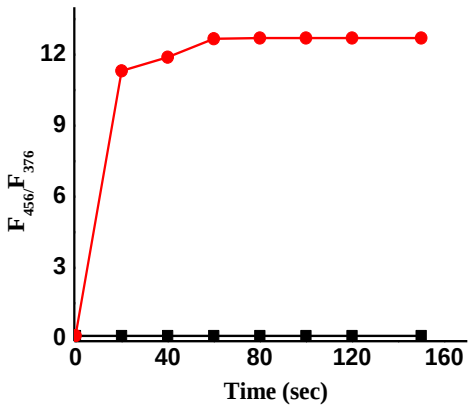
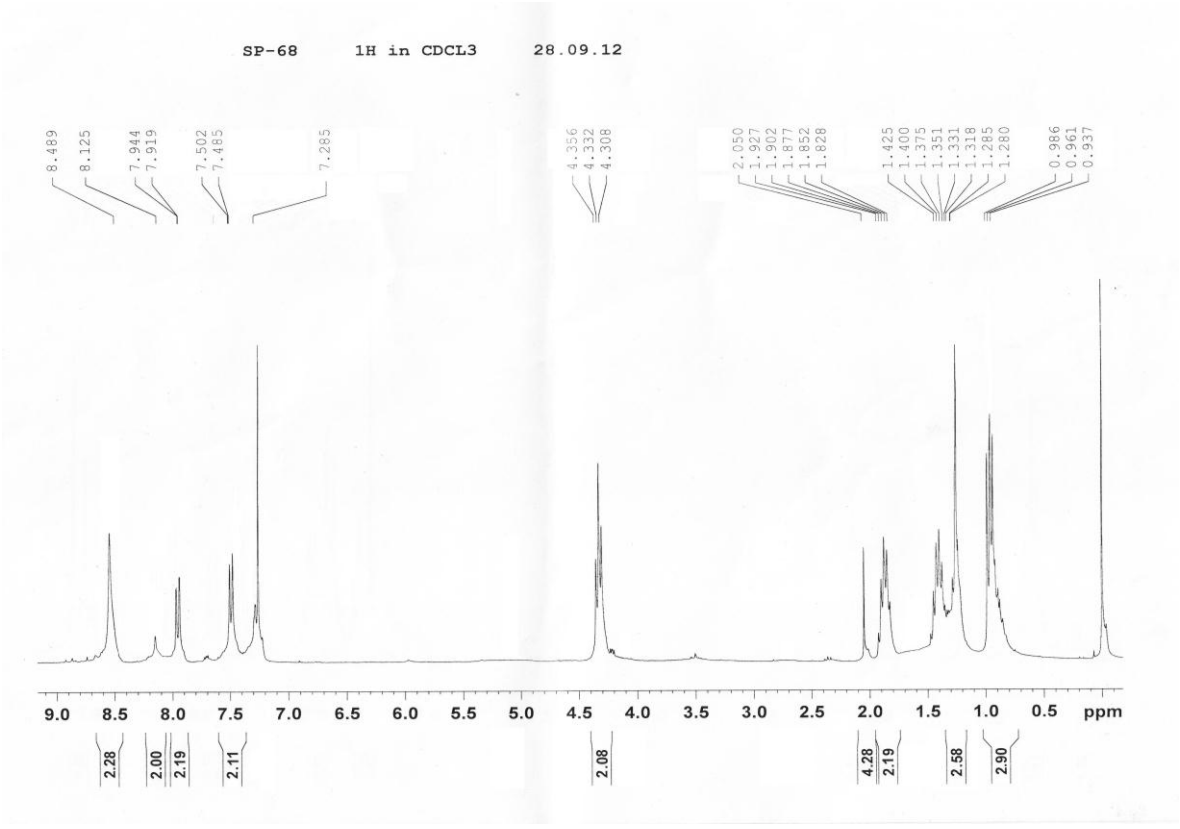


Figure S6: The time dependent fluorescence intensity changes of CDH in absence (■) or presence (●) of OCl^- (2 equiv.) at pH in CH_3CN:H_2O (4:6, V/V).

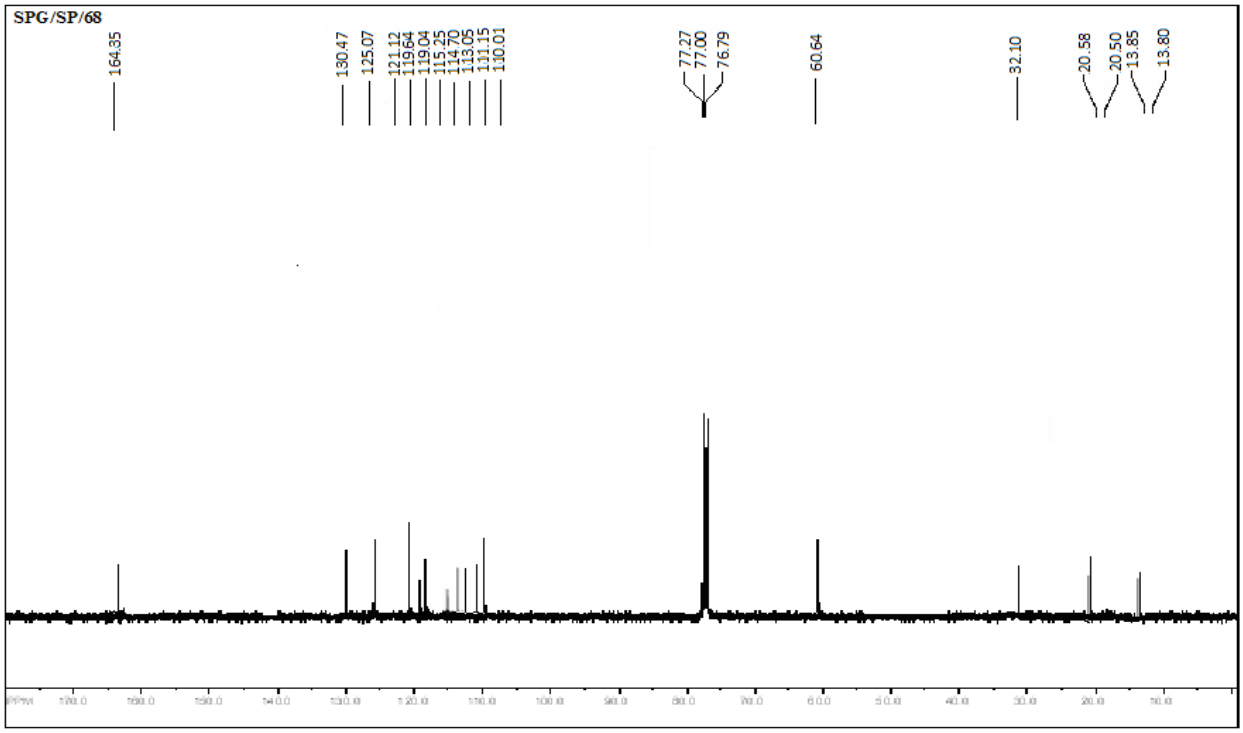
Upon addition of OCl^- a dramatic increase in the ratio was observed within 20 sec, and the ratio signal essentially reached maximum within 1 min and then becomes saturated. This finding suggests that probe CDH is a ‘‘fast-response’’ probe for OCl^- and may be suitable for real-time sensing of OCl^-.

12. ^{1}H NMR, ^{13}C NMR and ESI MS spectra of CDH and CDH + OCl^- :

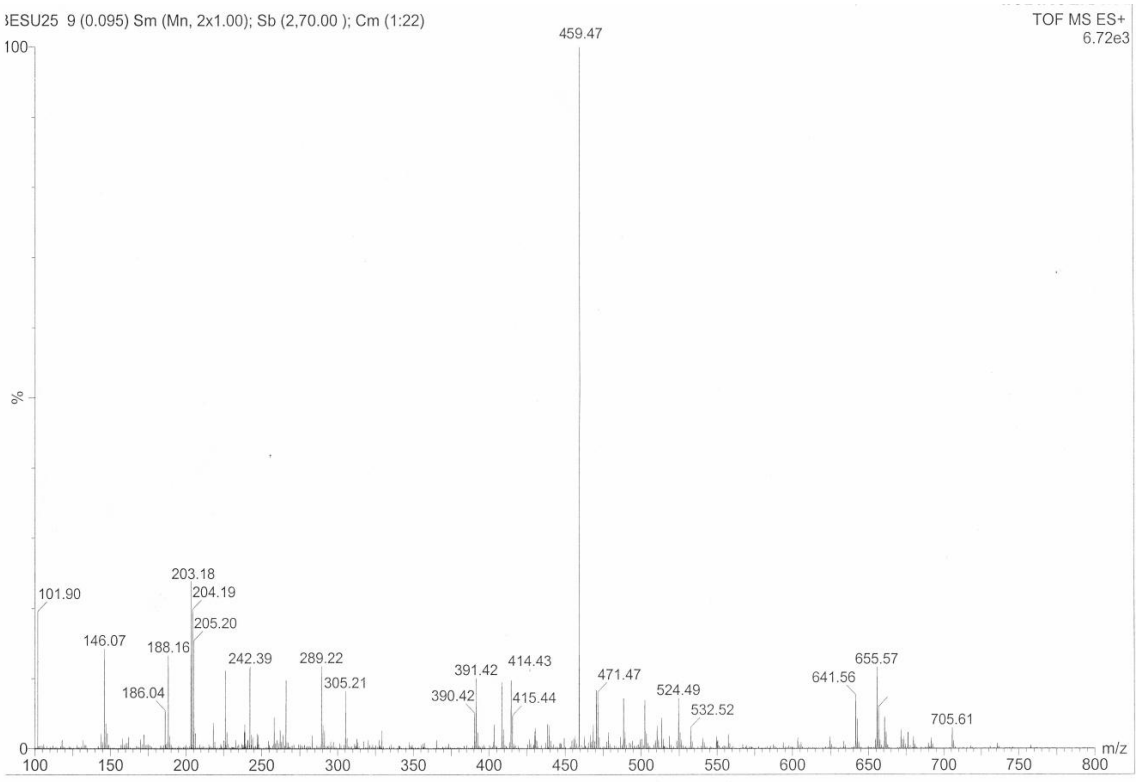
^{1}H NMR spectrum of Receptor i.e. CDH:



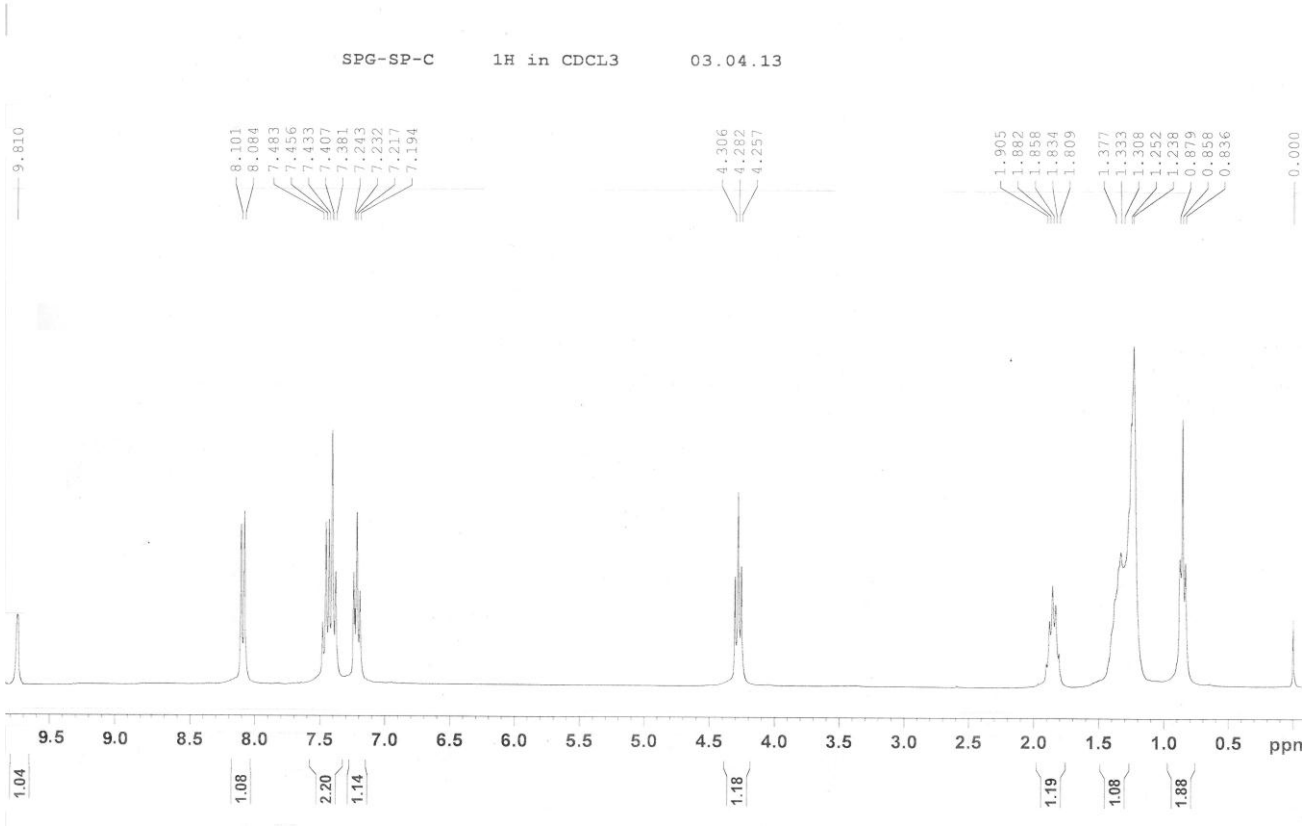
^{13}C NMR spectrum of Receptor:



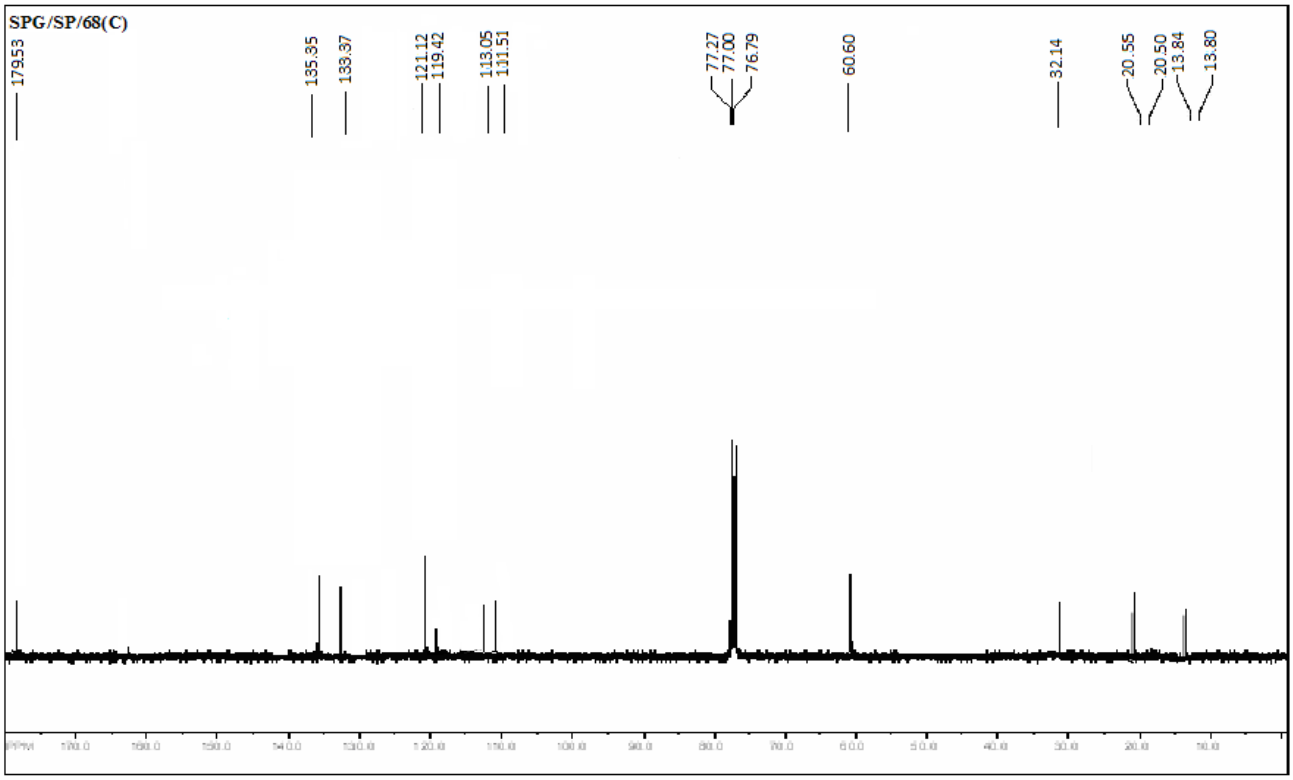
ESI MS Mass Spectra of Receptor:



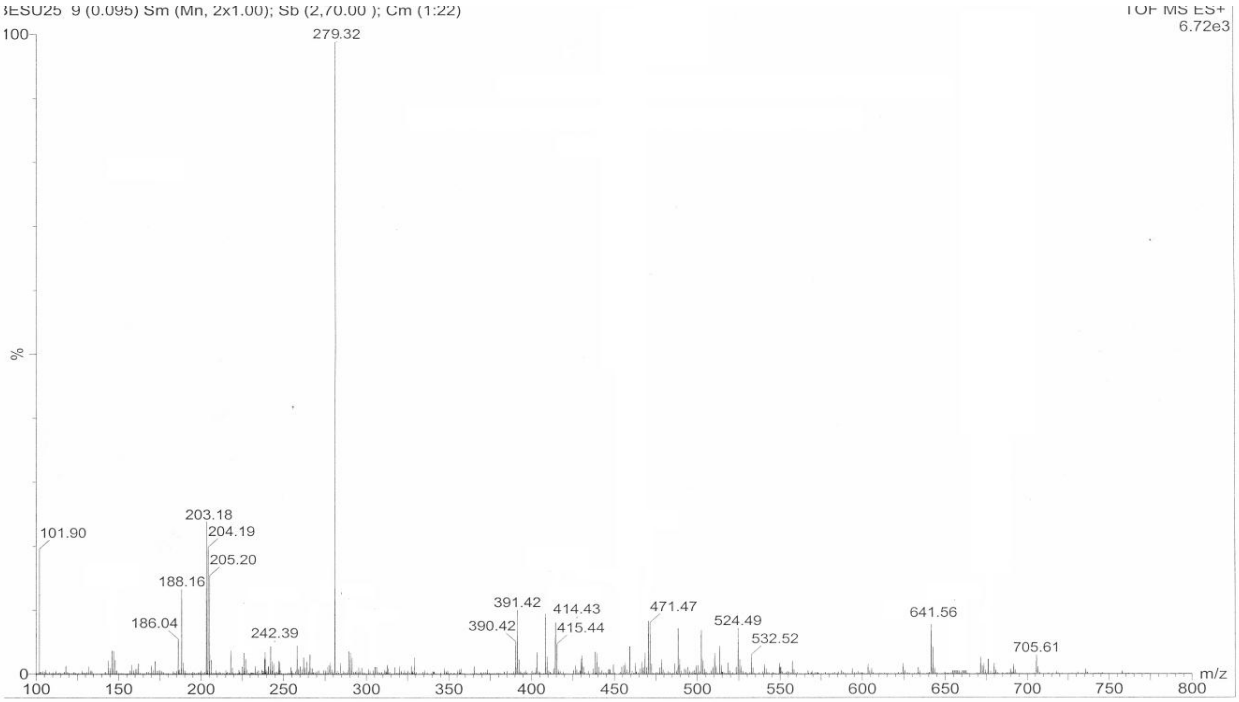
^{1}H NMR spectrum of CDH + OCl^{-}:



^{13}C NMR spectrum of CDH + OCl^{-}:

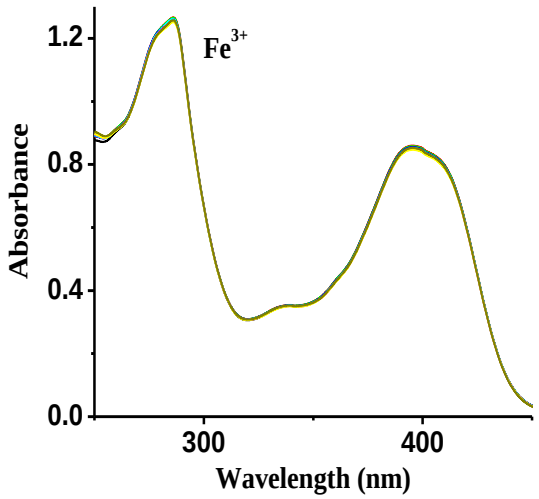


ESI MS Spectra of CDH + OCl^- :



13. UV-vis absorption spectra of CDH with different anions and oxidants as Fe^{3+} Co^{2+}, Hg^{2+}, Cu^{2+}, Mg^{2+}, F^-, I^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, SO_4^{2-}, Super oxide, H_2O_2, Hydroxyl radical, CH_3COOOH in CH_3CN : H_2O (4:6, v/v) (The solutions of anions and oxidants were prepared from $FeCl_3$, $Co(ClO_4)_2{\cdot}6H_2O$, $HgCl_2$, $Cu(ClO_4)_2{\cdot}6H_2O$, $MgSO_4.7H_2O$, HF, KI, $NaNO_2$, $NaNO_3$, Na_3PO_4, Na_2SO_3, Na_2SO_4 respectively in CH_3CN-H_2O)

(a)



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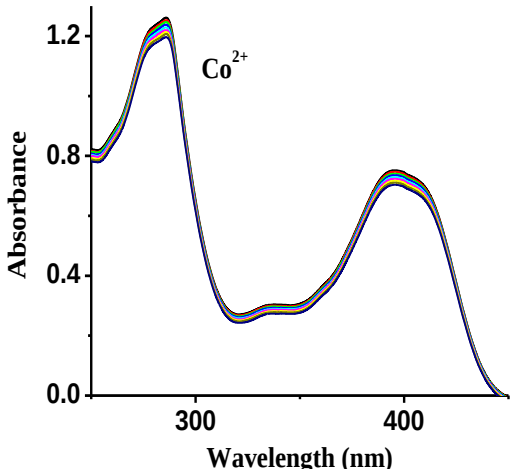

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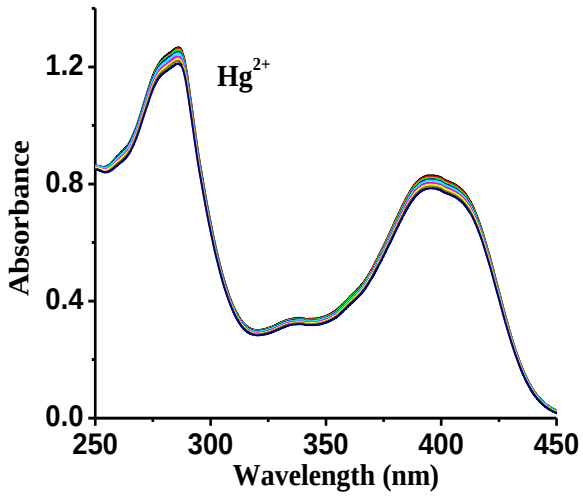

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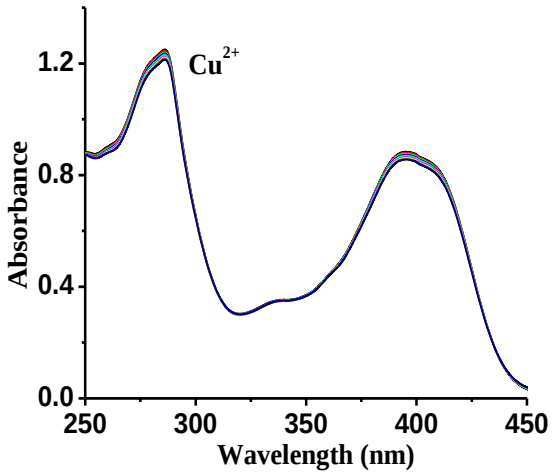

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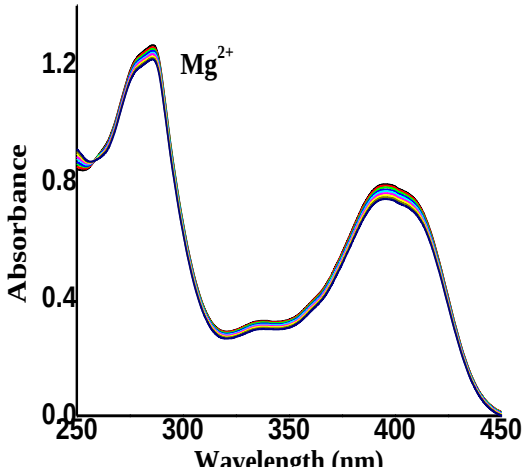

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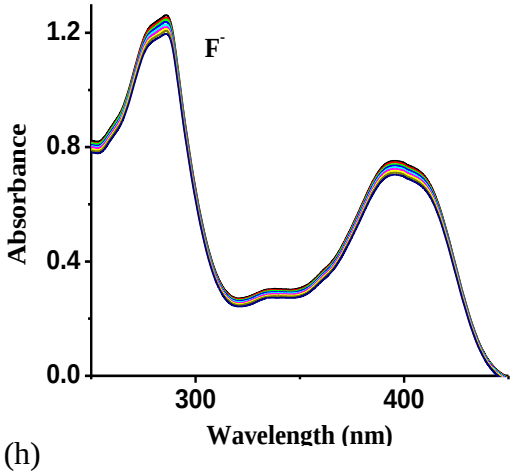

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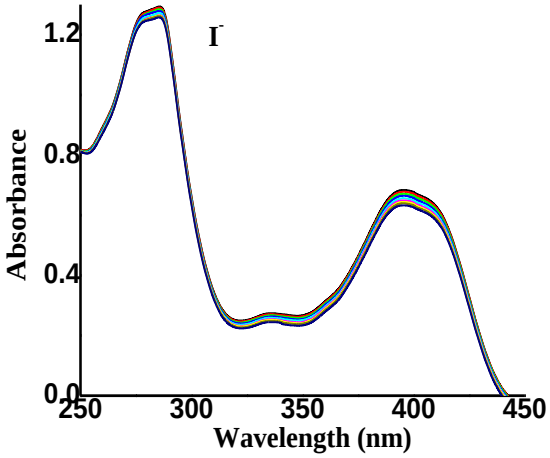

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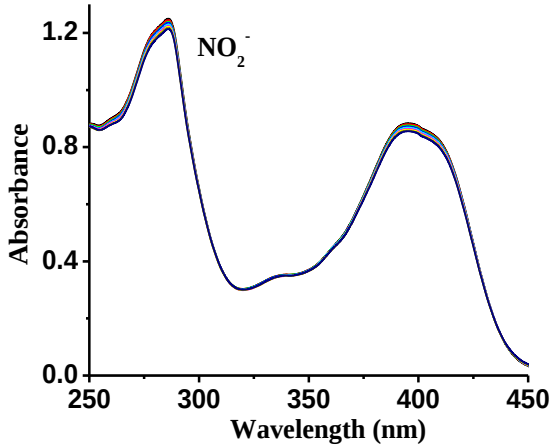

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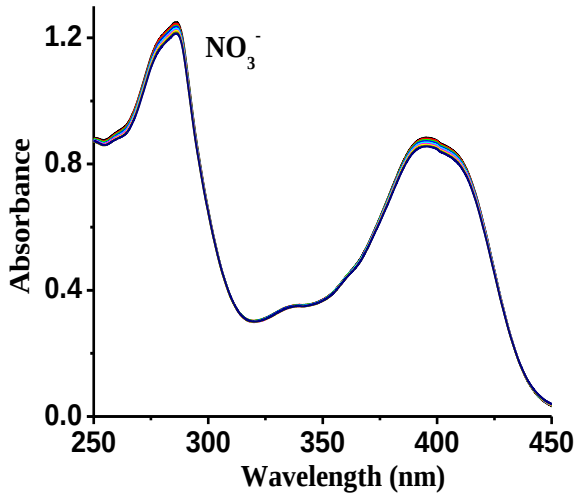

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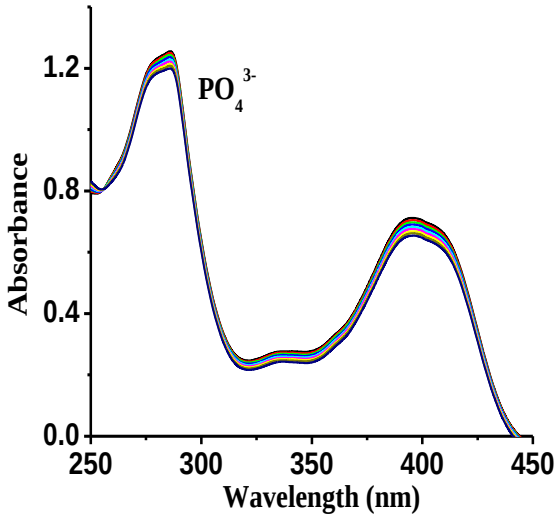

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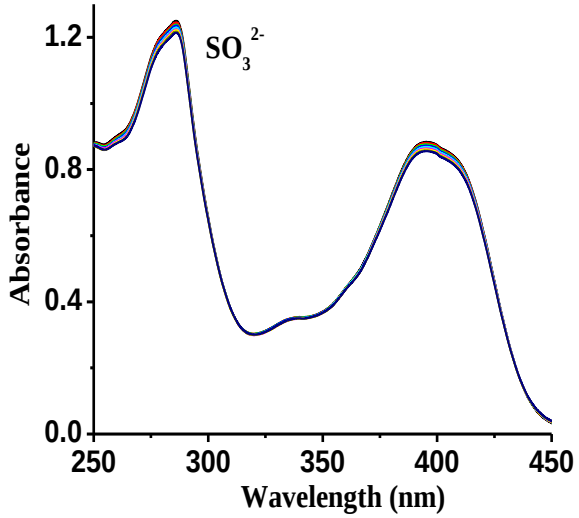

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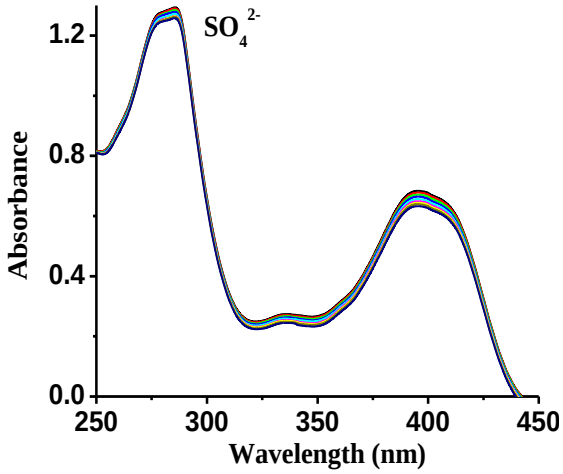

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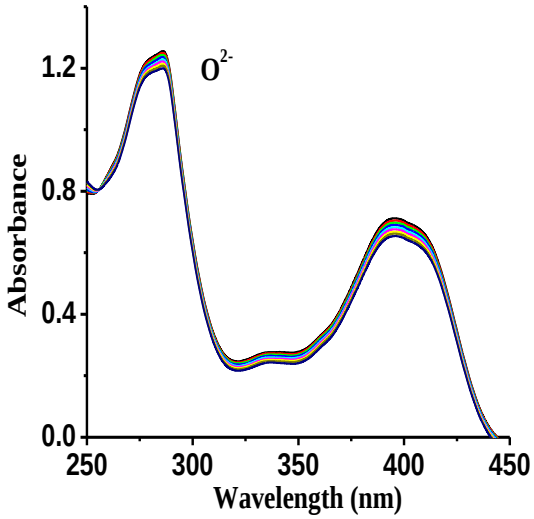

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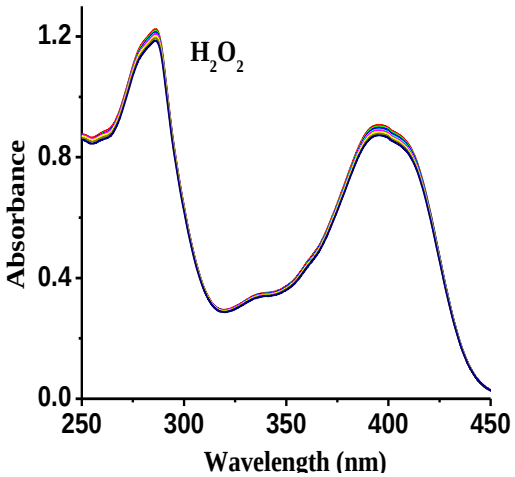
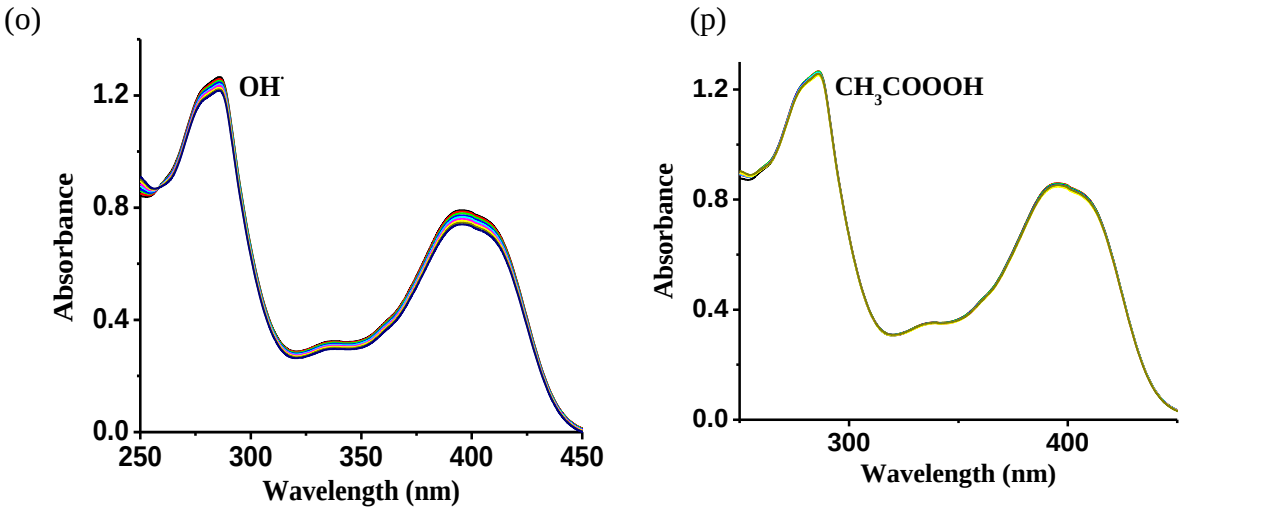
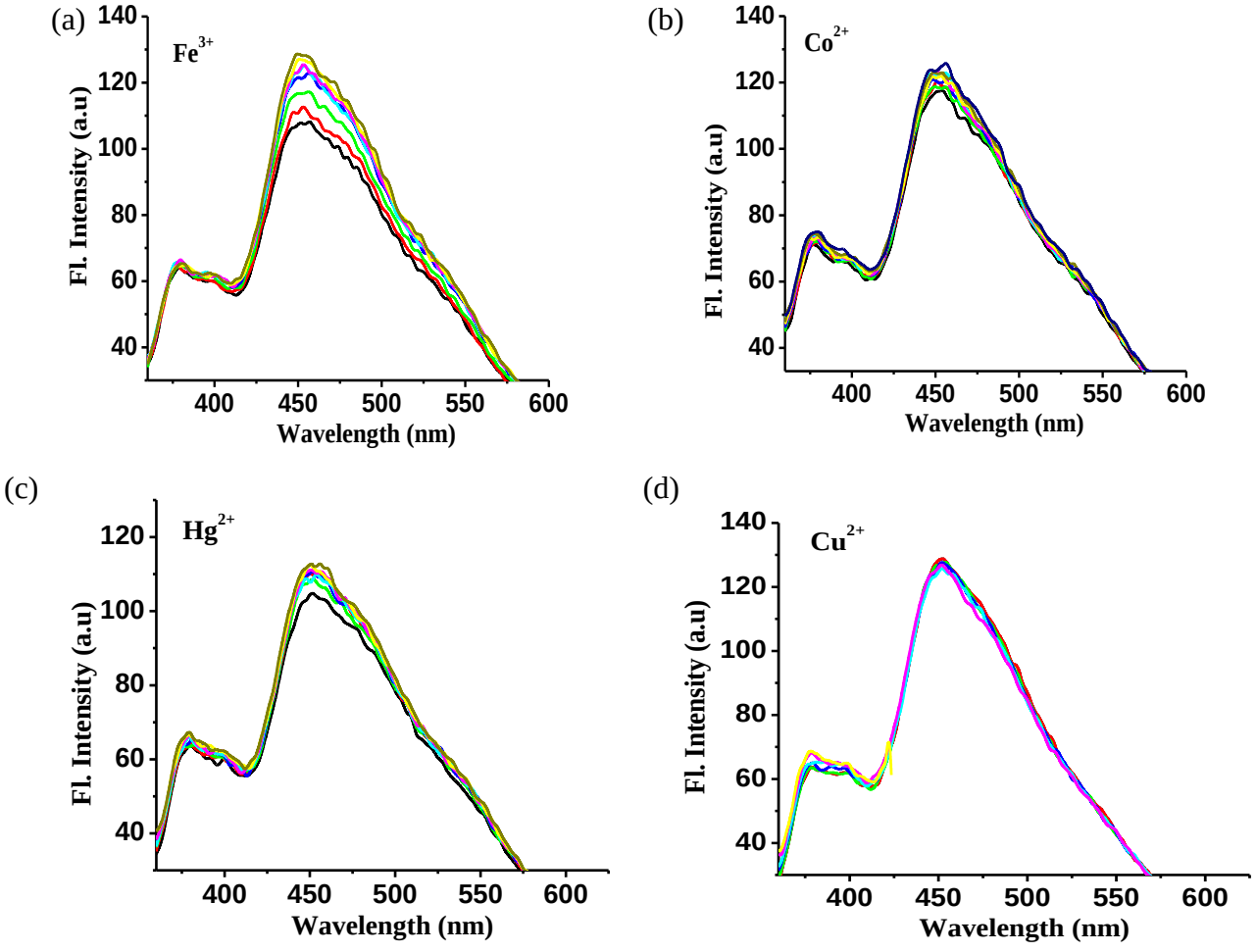




14. Fluorescence emission spectra of CDH with different anions and oxidants as Fe^{3+} Co^{2+}, Hg^{2+}, Cu^{2+}, Mg^{2+}, F^-, I^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, SO_4^{2-}, Super oxide, H_2O_2, Hydroxyl radical, CH_3COOOH in CH_3CN : H_2O (4:6, v/v) (The solutions of anions and oxidants were prepared from $FeCl_3$, $Co(ClO_4)_2 \cdot 6H_2O$, $HgCl_2$, $Cu(ClO_4)_2 \cdot 6H_2O$, $MgSO_4.7H_2O$, HF, KI, $NaNO_2$, $NaNO_3$, Na_3PO_4, Na_2SO_3, Na_2SO_4 respectively in CH_3CN-H_2O)



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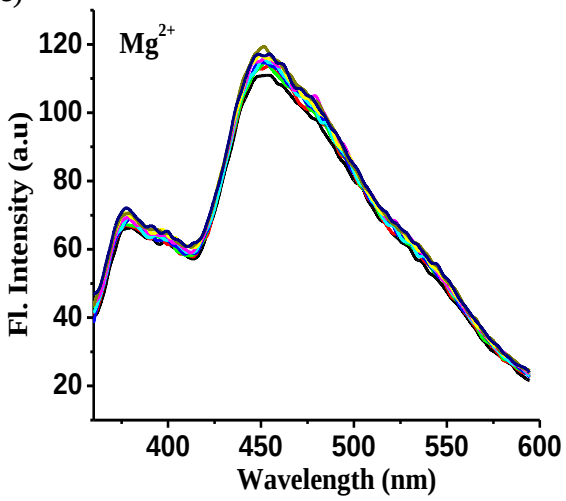

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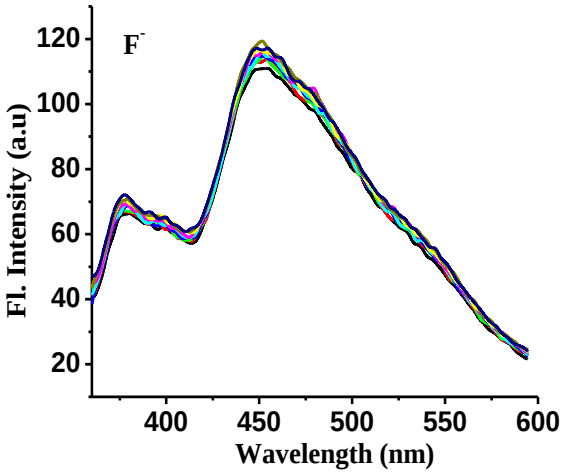

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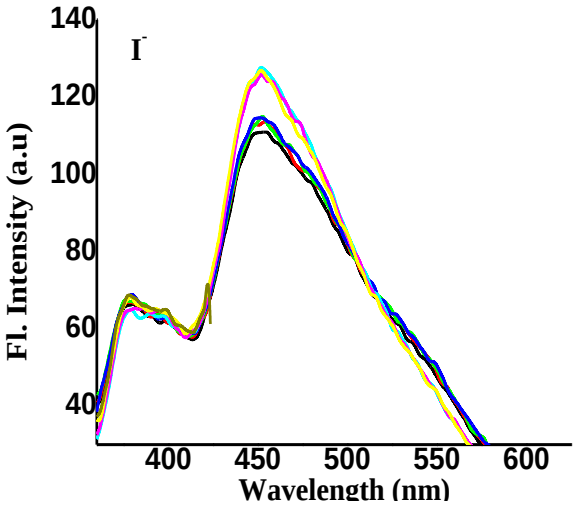

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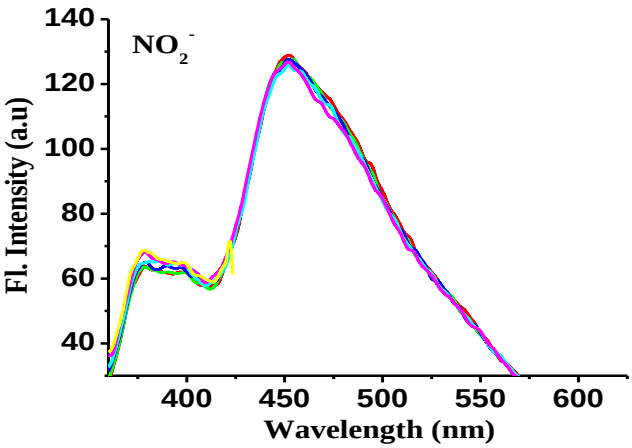

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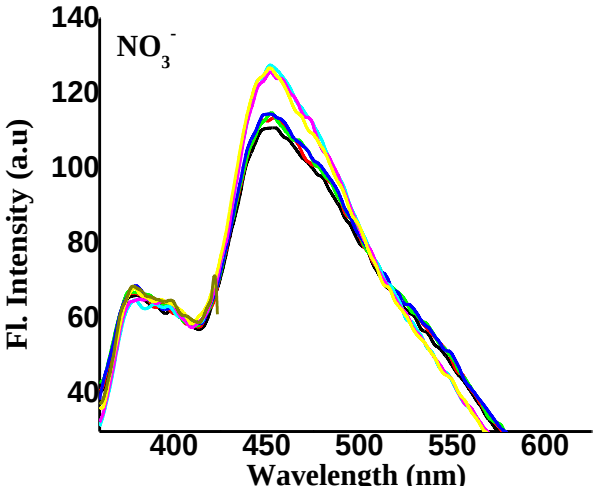

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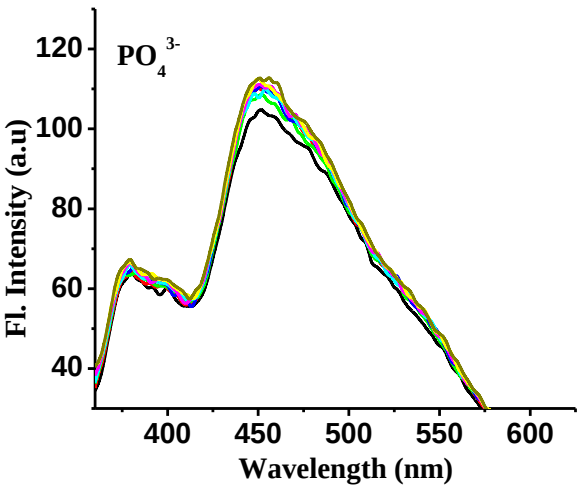

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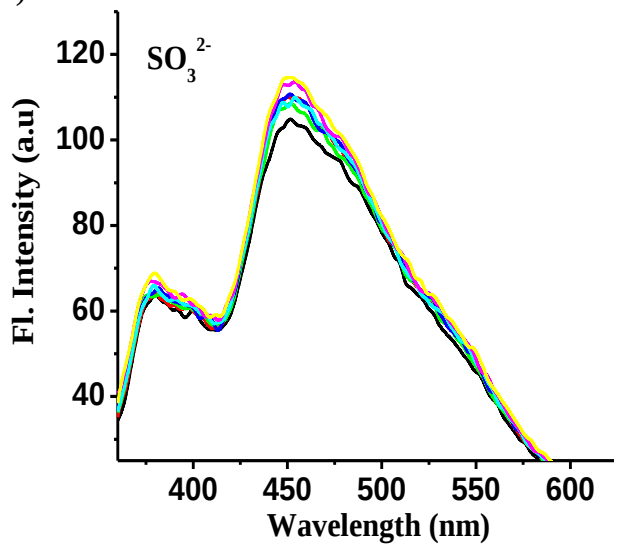

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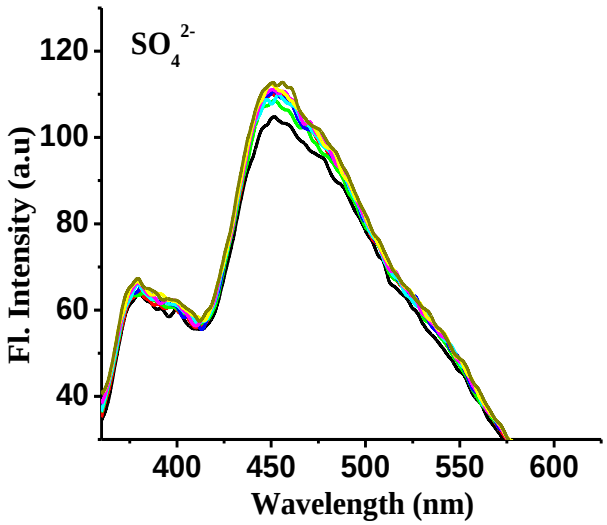

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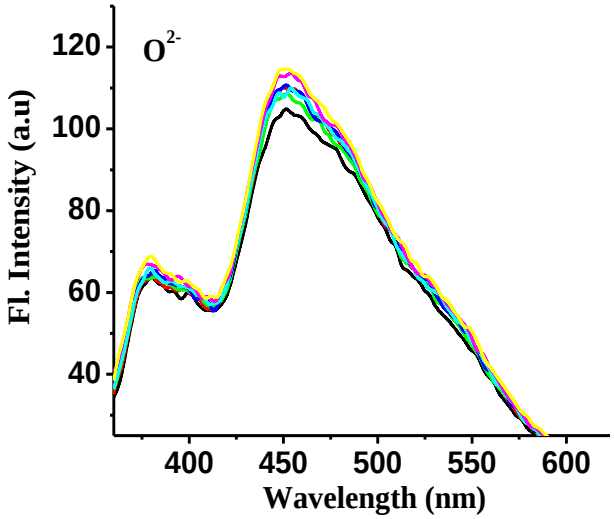

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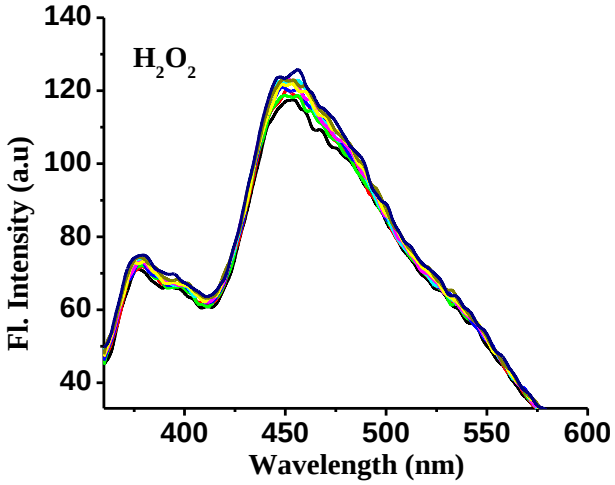
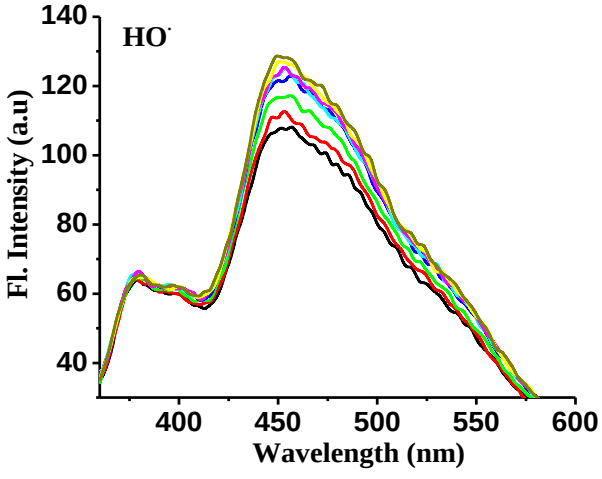


(o)



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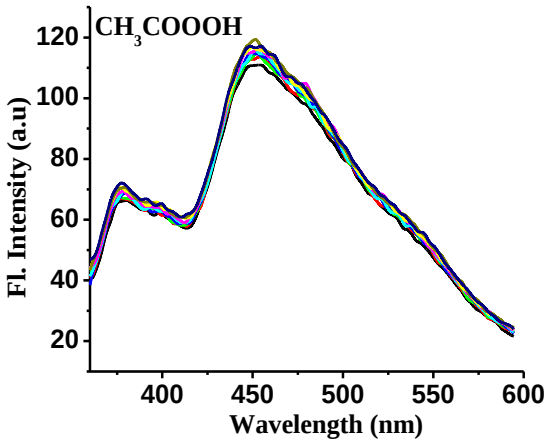

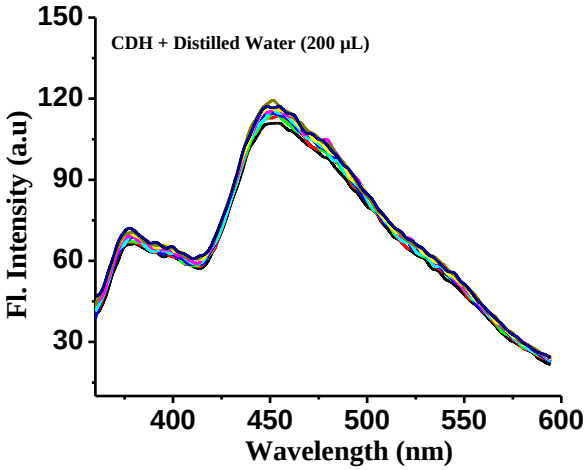

Fig S7: Fluorescence emission spectra of CDH (c = 2.0 x 10^{-5} M) in the presence of 200 µL distilled water at pH 7.4 in CH_3CN:H_2O (4:6, V/V).

15. 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**, 1481-1484