



Synthesis and characterization of 2-[(hydroxy(4-nitrophenyl) methyl] cyclohexanone for its potential application as off-on fluorescent sensor for selective cadmium detection

Maria Sadia*, Muhammad Naveed Umar, Robina Naz, Jahangir Khan and Rizwan Khan
Department of Chemistry, University of Malakand, Chakdara, Lower Dir, Khyber Pakhtunkhwa, Pakistan
mariasadia@gmail.com

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Abstract

2-[hydroxy(4-nitrophenyl)methyl]cyclohexanone (*R*) has been synthesized, purified (column chromatography) and characterized by ¹H NMR. The fluorescence properties of chemosensor (*R*) were studied in acetonitrile for the sensing of alkali, alkaline and transition metal ions (Mg²⁺, Cd²⁺, Ni²⁺, Zn²⁺, Ba²⁺, Co²⁺, Cu²⁺, Pb²⁺, Na¹⁺). Interaction of Cd²⁺ with chemosensor *R* displayed a significant fluorescence enhancement as compared to other examined cations. Maximum emission was observed at pH 10. According to job's plot analysis binding ratio of the complex *R*-Cd²⁺ was found to be 1:1. No significant interference was observed in the presence of competitive metal ions. The fluorescence of chemosensor *R* exhibits a good linear fluorescent response towards Cd²⁺ in the range 1-140 μM. The chemosensor *R* showed good binding constant to Cd²⁺ calculated as 1×10⁶ M⁻¹ using Benesi-Hilderbrand equation. The synthesized fluorescent chemosensor *R* was used successfully to determine Cd²⁺ in aqueous solution with a detection limit in μ range.

Keywords: 2-[hydroxy(4-nitrophenyl)methyl] cyclohexanone, Fluorescent sensor, Cd²⁺, Chelation Enhanced Fluorescence.

Introduction

Water pollution due to heavy metals is a serious environmental concern and it requires great attention. Natural sources such as volcanoes and anthropogenic activities, the release of toxic compounds and industrial wastewater results in an introduction of heavy metals to the environment¹. Among the heavy metal cadmium is very toxic because it can cause damage to a number of biological systems, generally at lower dose than most toxic metals². Cadmium is bioaccumulative (having a biological half-life between 15 to 20 years in humans), has no biological function, and can persist in sediment and soils for decades^{3,4}.

In the general population diet and cigarette are the two major sources of exposure to cadmium. According to the classification by World Health Organization's International Agency for Research on Cancer and the United States National Toxicology Program, cadmium is designated as a human carcinogen that causes pulmonary cancer, renal cancer, cancer of the liver, urinary bladder, hematopoietic system and stomach⁵. The uptake of cadmium via ingestion and inhalation or cause kidney disorders from minor tubular dysfunctions to severe damage involving tubuli as well as glomerular⁶. Other adverse effects includes damage to the nerve system and bones, calcium metabolism disorders⁷, respiratory disorders like shortness of breath, lung edema, damage to the mucous membranes and gastrointestinal effects including vomiting and diarrhea⁸.

Considering its adverse biological effect an intense effort has been devoted to developing methods for the sensing and

monitoring of cadmium in biological samples, environmental sample and industrial waste effluents. Analytical techniques employed for detection of cadmium in various samples include flame atomic absorption spectrometry⁹, inductively coupled plasma mass spectrometry¹⁰ and atomic absorption spectroscopy¹¹. Recently the method of fluorescence sensing for the detection of heavy metal ions has attracted great attention due to its simplicity, high sensitivity, high selectivity, ease of operation, high quantum yield, lower limit of detection and its application in medicine, environmental chemistry and biology¹². A fluorescent sensor has two parts an ionophore and a fluorophore^{13,14}. Such sensors should have high selectivity and sensitivity must provide on-line and real-time analysis and should be capable of working in aqueous medium^{15,16}. Only few fluorescent chemosensors have been reported that can distinguish cadmium from zinc since both have similar chemical properties¹⁷. Our present work describes the synthesis and application of 2-(hydroxy(4-nitrophenyl) methyl) cyclohexanone as a fluorescent sensor for the selective detection of cadmium.

The 2-(hydroxy(4-nitrophenyl)methyl) cyclohexanone exhibits very weak fluorescence due to PET phenomenon. It exhibit a selective chelation enhanced fluorescence effect in the presence of cadmium. In this regard, we successfully explored the use of 2-(hydroxy (4-nitrophenyl) methyl)cyclohexanone chemosensor **R** as a turn on fluorescent chemosensor for selective detection of cadmium and may find application in detecting cadmium in real water samples from different sources.

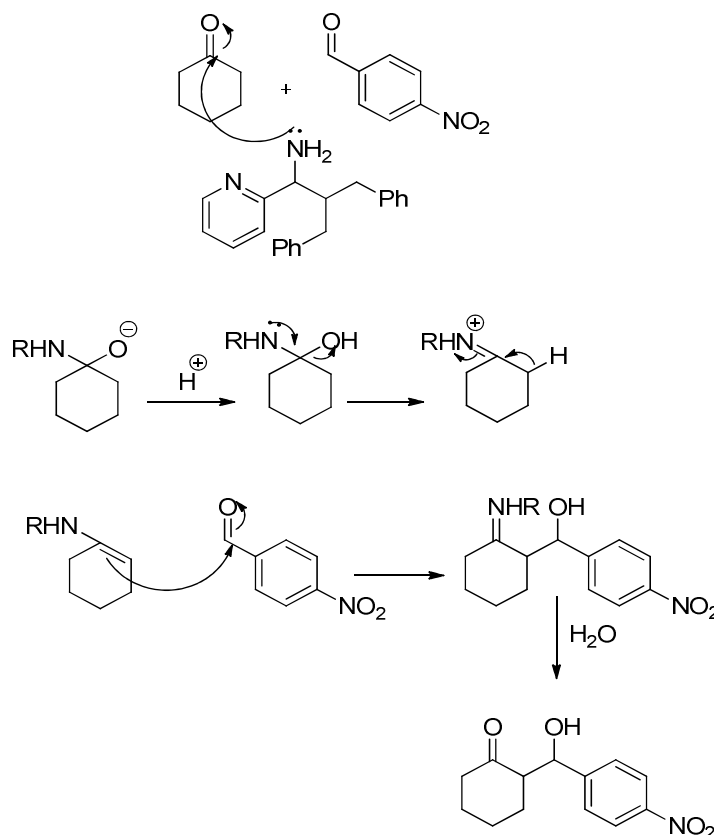
Materials and methods

Apparatus: UV/visible 1800 spectrophotometer was used for obtaining UV-Vis absorption spectra. Fluorescence spectra were generated on Shimadzu RF 5301 PC Fluorescence spectrometer. Using samples in solution, fluorescence free quartz cuvette of 1cm path length (in a volume of 3 mL) was used for spectra collection. Slit width both for excitation and emission were 5nm. The pH of the tests solutions were measured by a BANTE instrument pH meter. A ¹HNMR spectra of chemosensor R was obtained on Bruker 400 MHz.

Materials: All chemicals were purchased from commercial sources and were used without further purification. Distilled water and analytical reagent grade acetonitrile were used throughout the analysis. 1×10⁻²M stock solution of various mineral salts (Cd²⁺, Ba²⁺, Ni²⁺, Zn²⁺, Pb²⁺, Na⁺, K⁺, Co²⁺, Cu²⁺, Mg²⁺) were prepared in distilled water and 4×10⁻³M solution of the chemosensor R was prepared in acetonitrile. Different concentration of various metals ions and chemosensor R were prepared from stock solution by appropriate dilution for spectroscopic investigation. pH adjustments were made by using NH₃/NH₄Cl buffer. All of the measurements were conducted at room temperature. All types of glassware were washed first with acid, then detergent and distilled water and dried in the oven. Optically four sides' clear quartz cuvettes were used for fluorometric determination of Cd²⁺ and were washed with acetone before use.

Solution preparation: Cadmium nitrate hexahydrate (Cd(NO₃)₂·6H₂O) was used for stock solution preparation of cadmium. A stock solution of chemosensor R [2-(hydroxy(4nitrophenyl)methyl) cyclohexanone] was prepared by dissolving it in acetonitrile. To obtained desire and suitable concentrations for spectrofluorometric analysis stepwise dilution of stock solutions were carried out with distilled water. Using HCl (37%) and NaOH solutions (in distilled water) pH adjustment were made. Ammonia/Ammonium chloride buffer solution was prepared by using NH₄Cl (ammonium chloride) and NH₃ (ammonia) in distilled water.

Synthesis of chemosensor R: Chemosensor R was synthesized according to the method reported¹⁸. The 2,4-dinitrobenzenesulfonic acid (2,4-DNBSA) salt of (S)-PicAm (0.025 mmol, 0.0138 g) was added to brine (1.0 mL, 0.5 M). The cyclohexanone (3.30 equiv, 1.65 mmol, 0.17 mL) and 4-Nitrobenzaldehyde (1.00 equiv, 0.50 mmol, 0.0755 g) were then added. The mixture was stirred and heated at 45°C for 16 hr. The reaction mixture was quenched by simply adding EtOAc (10 mL) and water (10 mL). The resulting aqueous layer was extracted a total of three times with EtOAc (10 mL x 3). The organic extracts were isolated and dried over Na₂SO₄, evaporated (Rot Vap), and then exposed to high vacuum for 1 hr. Column chromatography technique was then employed for purification of the crude product.

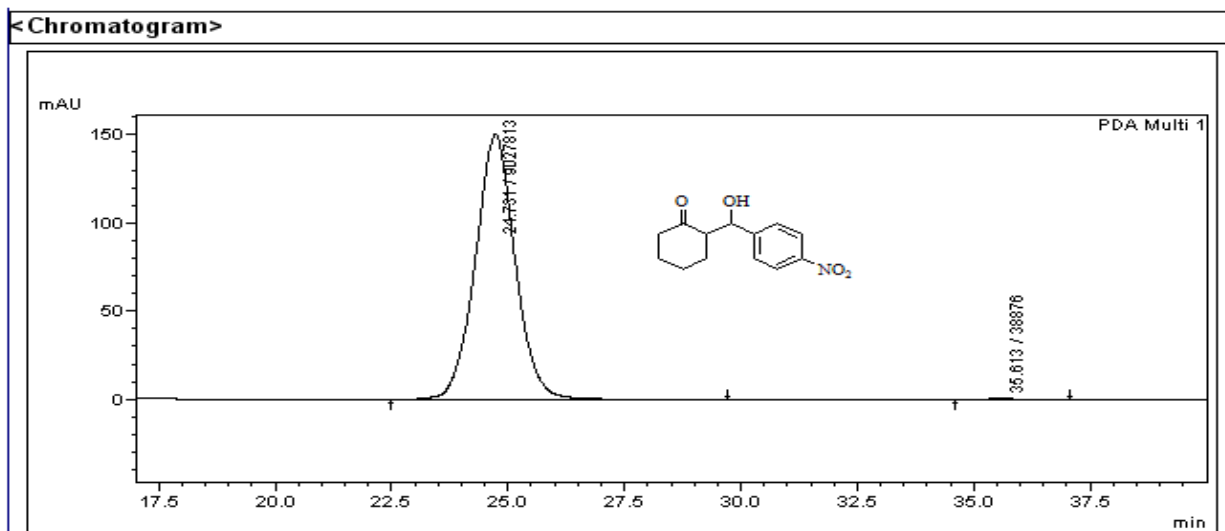


Scheme-1: The Synthetic route of chemosensor R.

Results and discussion

Characterization by ¹H NMR: ¹H NMR spectroscopy was conducted to confirm the synthesis of chemosensors R. Column chromatography technique was employed {Pet ether/EtOAc (9:1)} for the purification of crude product. For column chromatography separation silica gel 60 (0.040-0.063 mm) and petroleum ether (having boiling point in the range 60-80°C) were used. The resulting purified product was then assayed by HPLC to determine their *ee* (enantiometric excess). For this purpose a chiralpak OD-H column with eluent such as *n*-

heptane and *i*-propanol were used. Reaction time: 16 hr; flash column chromatography: (EtOAc/Pet ether = 10:90); yield: 92%; The *ee* was determined by chiral HPLC (Chiral OD-H, *i*-PrOH/heptane 5/95, flow rate = 1 mL/min, λ = 210 nm): *t*_{major} = 24.2 min, *t*_{minor} = 36.5 min, *ee* = 99%, *dr* = 22:1 (anti/syn), *R*_f = 0.29 EtOAc/pet ether (2:3). ¹H NMR (400 MHz, CDCl₃) (ppm): 1.26-1.41 (m, 1H), 1.54-1.66 (m, 3H), 1.82 (m, 1H), 2.10 (m, 1H), 2.36-2.49 (m, 1H), 2.53-2.61 (m, 2H), 4.11 (br s, 1H), 4.90 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 8.23 (d, *J* = 8.6 Hz, 2H).

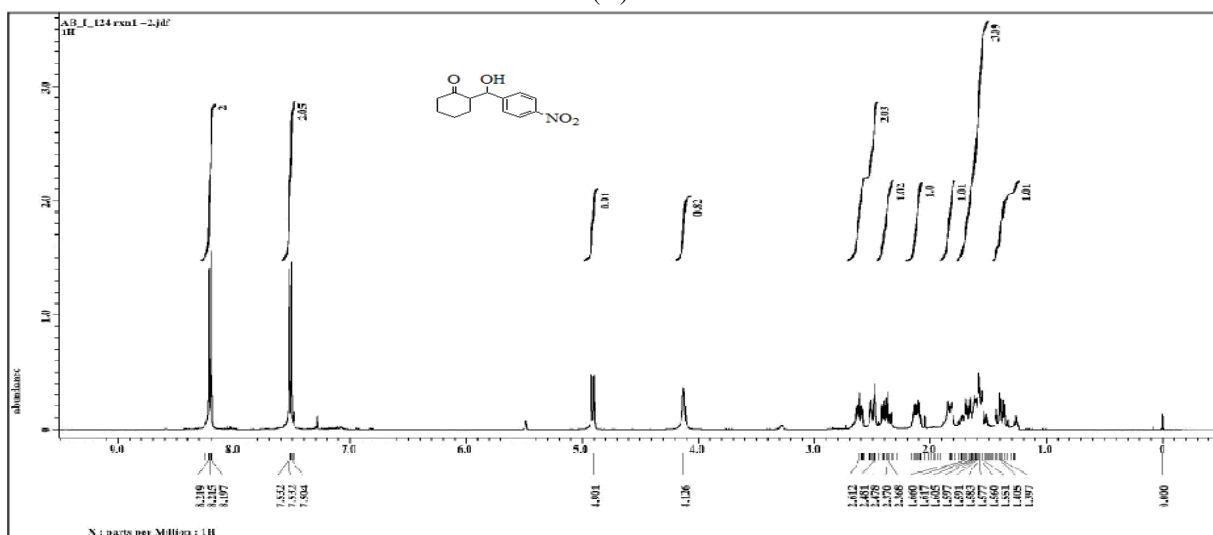


<Results>

PDA Ch1 210nm 4nm

Peak #	Ret. Time	Area	Height	Area %	Height %	Width at 10% Height	Mark
1	24.731	9027813	150581	99.571	99.624	1.782	
2	35.613	38876	568	0.429	0.376	1.825	
Total		9066689	151150	100.000	100.000		

(A)



(B)

Figure-1: HPLC (a) and ¹H NMR spectrum (b) of chemosensor R.

UV absorption measurement of chemosensor R and chemosensor R+Cd²⁺ complex: Chemosensor R [2-(hydroxyl (4-nitrophenyl) methyl) cyclohexanone] molecular weight is 249.262 g and its molecular structure is shown in the Figure-2(a). Chemosensor R solution was prepared in acetonitrile. The solution was of yellow color. Metal solution was prepared in distilled water. UV absorption spectrum of chemosensor R and

its complex with Cd²⁺ were scanned in the range 200-800 nm to find the λ_{\max} and are shown in Figure-2 (b) and (c) respectively. Absorption spectral analysis were performed at room temperature. The chemosensor R exhibit λ_{\max} at 297 nm in the absorption spectrum but upon interaction with Cd²⁺ ions a new peak was observed at 289 nm.

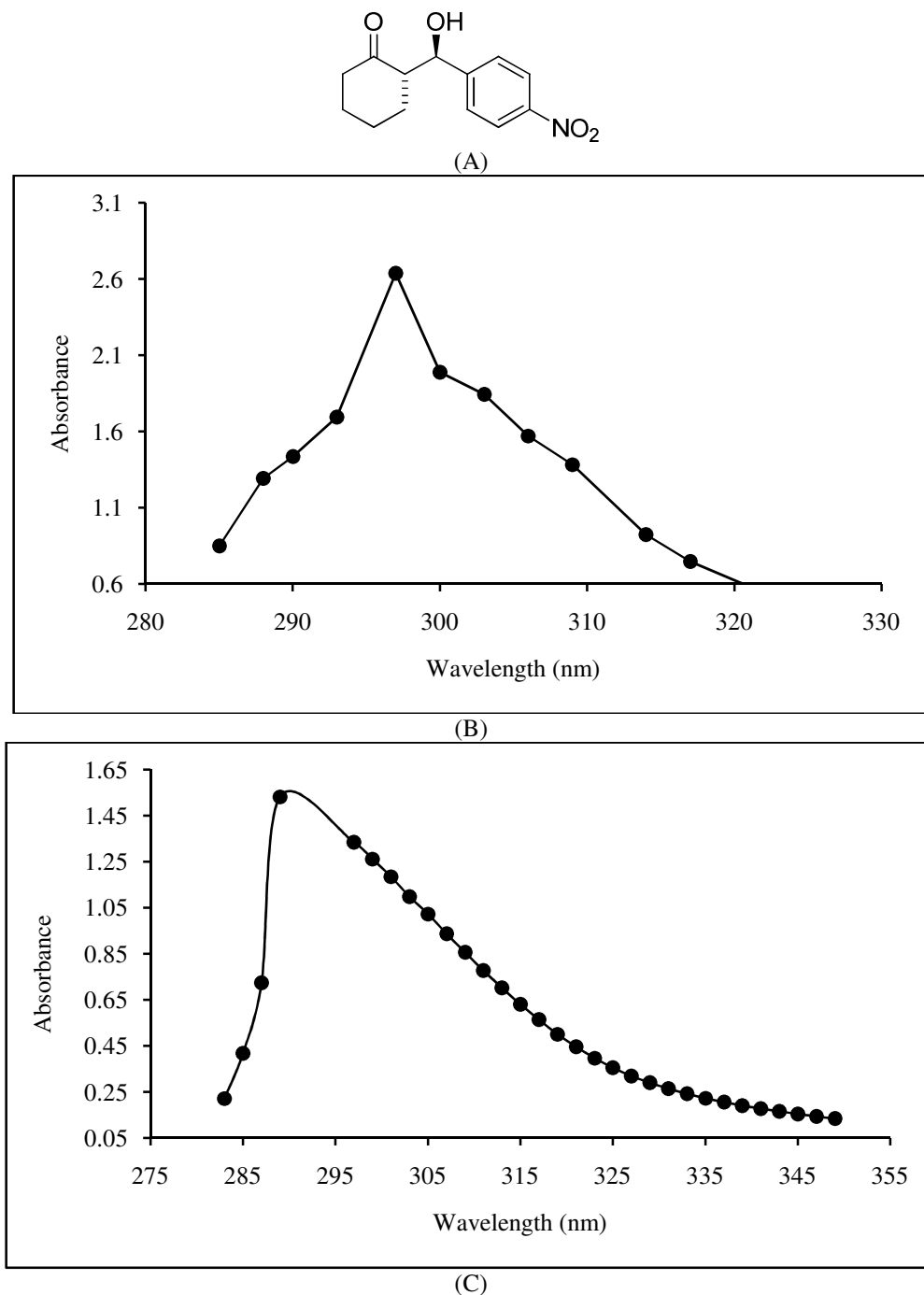


Figure-2: Molecular structure of chemosensor R (a) UV absorption spectrum of chemosensor R (b) and chemosensor R+ Cd²⁺ complex (c).

Preliminary study: To investigate the utility of chemosensor R as a metal ion-selective sensor the fluorogenic behaviour of chemosensor R in acetonitrile was examined using fluorescence spectroscopy against metal ions of environmental relevance such as alkali (Na^+ , K^+), alkaline (Ba^{2+} , Mg^{2+}) and transition (Pb^{2+} , Ni^{2+} , Zn^{2+} , Cu^{2+} , Cd^{2+} , Co^{2+}) metal ions as their salts in distilled water and the results are shown in the Figure-3. The reported compound (chemosensor R) displays very weak emission at 707 nm (in the range 691-718 nm) when excited at 352 nm and is almost non-fluorescent. The quenching of fluorescence observed may be probably due PET phenomenon from OH oxygen atom to phenyl ring. Upon interaction of the above mentioned metal ions with chemosensor R, a significant enhancement of fluorescence intensity was displayed by the chemosensor R at 707 nm. As compared to other tested metal ions enhancement caused by Cd^{2+} is more pronounced. Binding of Cd^{2+} with chemosensor R blocked PET phenomenon. Complexation leads to rigidity of the system and consequent enhancement in fluorescence of chemosensor R as shown in scheme-2. The difference in fluorescence emission intensity shows that chemosensor R has high affinity for Cd^{2+} as compared to other metal ions. The increase in the fluorescence intensity is due to metal chelation enhancement effect.

Cd^{2+} concentration effect on fluorescence emission intensity: For understanding the detection mechanism more accurately the fluorogenic response of chemosensor R towards Cd^{2+} was studied by an experiment of chemosensor R with the sequentially added different concentrations of Cd^{2+} in the range $1-140 \times 10^{-6}$ M. When excited at 352 nm, the free chemosensor R displays a weak emission band at 707 nm. Upon addition of the equivalent of Cd^{2+} ions, a gradual enhancement at 707 nm in fluorescence intensity was observed which may be attributed to the inhibition of PET phenomenon as a result of the formation of chemosensor R + Cd^{2+} complex. With gradual addition of Cd^{2+} fluorescence intensity of chemosensor R at 707 nm was enhanced steadily. The fluorescence response is linearly proportional to Cd^{2+} concentration in the range $1-140 \times 10^{-6}$ M as shown in Figure-4. Kaya I et al synthesized a fluorescent probe (HDI-Co-3-DHB-2-AP) for sensing of Cd^{2+} with a detection limit 8.86×10^{-4} M¹⁹. Yao. Y et al developed Quinoline-trizole linked gold nanoparticles as a fluorescent probe for the sensing of Cd^{2+} with a detection limit 1×10^{-5} M in aqueous solution²⁰. In our work limit of detection (LOD) of the free sensor for determining Cd^{2+} ion was calculated by using the equation $3 \times \sigma_{\text{blank}} / k$ where σ is the standard deviation of the blank measurement and k is the slope of the titration curve²¹ and was found to be 5.5×10^{-7} M which is lower than the literature mentioned.

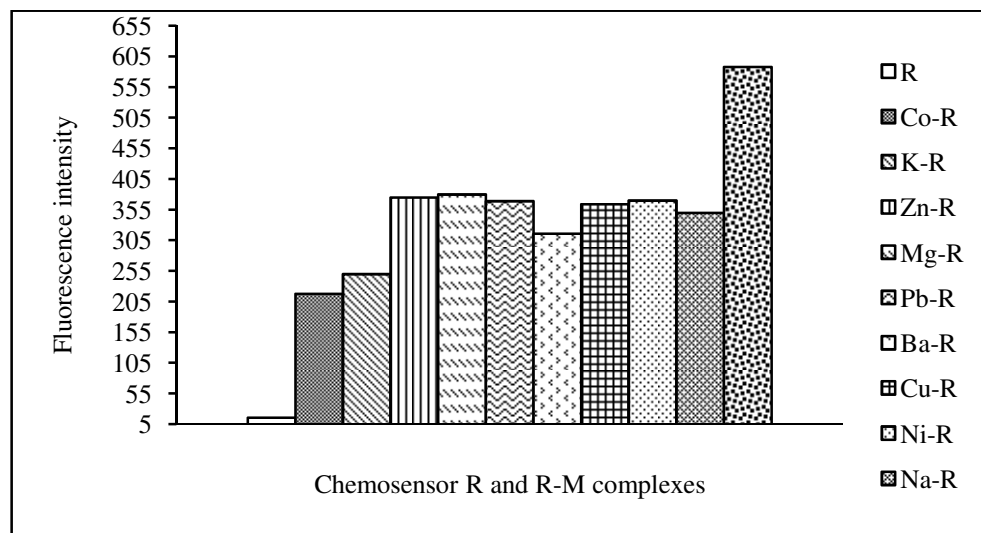
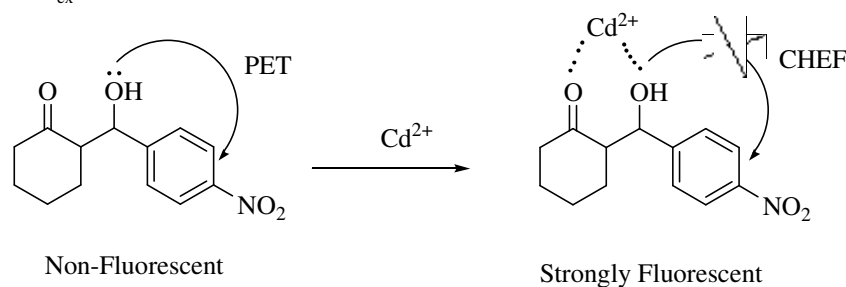


Figure-3: Fluorescence intensity of chemosensor R and relative changes in its intensity upon interaction with metal ions 200×10^{-6} M as their aqueous solutions. λ_{ex} is 352 nm.



Scheme-2: Proposed binding mechanism of chemosensor R with Cd^{2+} .

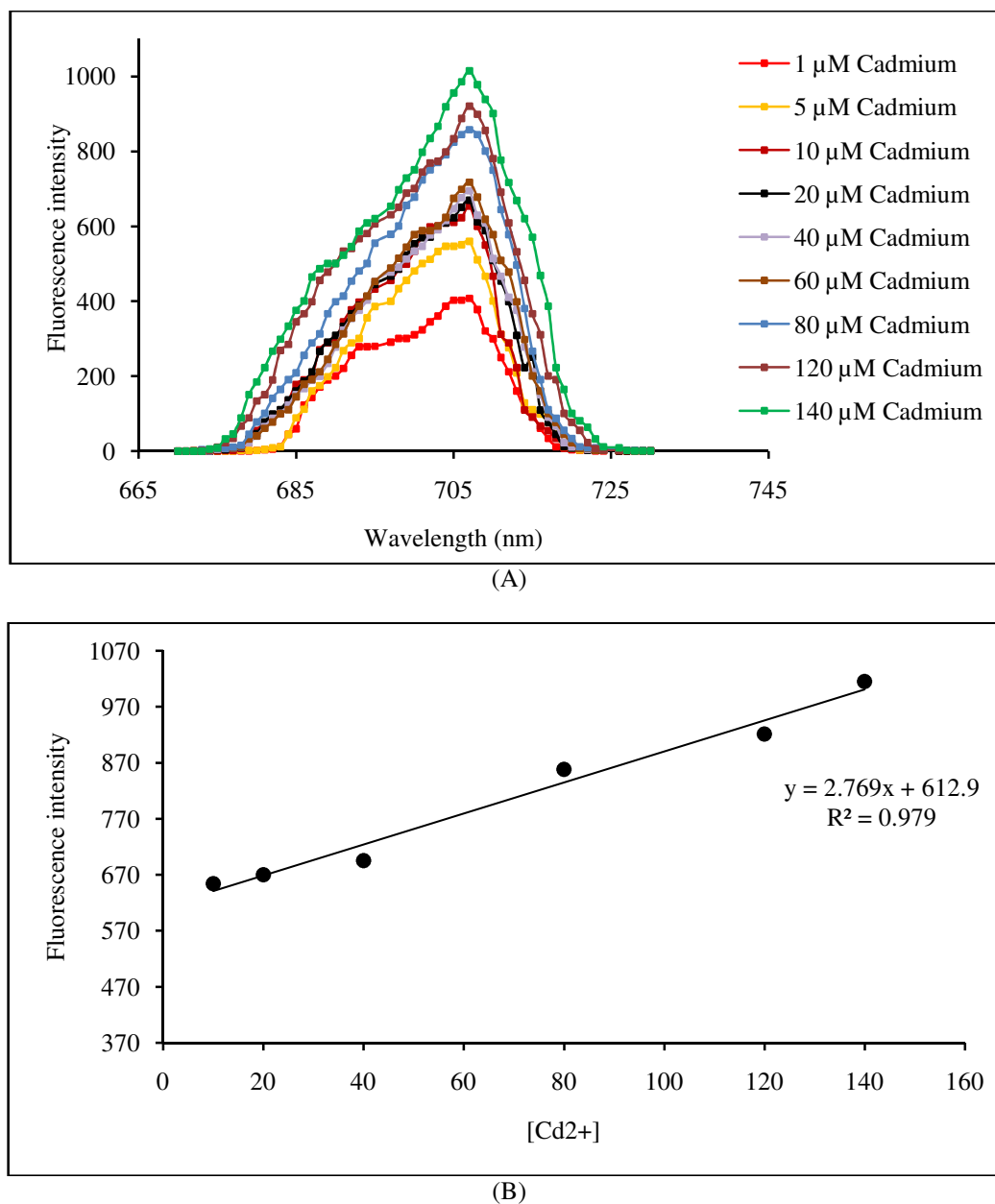


Figure-4: Enhancement of fluorescence intensity of chemosensor R at 707 nm with an excitation at 352nm (a) upon addition of increasing Cd^{2+} concentration ($1\text{-}140 \times 10^{-6}\text{M}$). Fluorescence intensity of chemosensor R (b) as a function of Cd^{2+} concentration.

Effect of pH on fluorescence response of chemosensor R: pH of the solution is an important factor which influences the fluorescence phenomenon. Oxygen and OH in the chemosensor R has a lone pair of electrons, so its fluorescence intensity might be affected by pH. To determine a suitable pH range over which chemosensor R can effectively detect Cd^{2+} an experiment was conducted by varying pH of R-Cd^{2+} complex solution in the range 2-12. The pH values of test solutions were adjusted by using 0.25M HCl and 0.01M NaOH and the alteration in fluorescence intensity were recorded at 707 nm. Figure-5 clearly demonstrate that the fluorescence intensity of chemosensor R- Cd^{2+} complex decreased drastically when pH is less than 4,

suggesting poor stability of the R-Cd^{2+} complex at low pH. Under acidic condition (pH 2 and 3) protonation of OH occurred that prevent the formation of the complex and results in quenching of fluorescence. However fluorescence emission intensity at 707 nm enhance significantly with increasing pH value which may be associated with deprotonation of OH in chemosensor R. The result also indicates that the chemosensor R-Cd^{2+} complex are formed at high pH values as a result of deprotonation process. The pH- controlled fluorescence measurements revealed that chemosensor R can respond to Cd^{2+} in the optimal pH range from 5 to 10.

Effect of concentration of chemosensor R: To investigate the influence of chemosensor R concentration, a series of fluorescence analysis were conducted varying the concentration of chemosensor R in the range $0.4\text{--}4\times 10^{-3}$ M and keeping the Cd^{2+} concentration constant (2×10^{-6} M). pH was adjusted at 10 and measurements were made at room temperature. It was observed that fluorescence enhances gradually by increasing concentration of chemosensor R. Replicate analysis measurement were conducted to evaluate the reproducibility of chemosensor R using 2×10^{-3} M chemosensor R and 2×10^{-6} M Cd^{2+} solution at pH 10. Replicate analysis measurement shows that RSD was 0.23% for eight repeated emission measurements.

Job's Plot and Benesi-Hilderbrand plot: To determine a suitable binding ratio between chemosensor R and Cd^{2+} Job's plot analysis were conducted. Keeping total concentration constant at 400×10^{-6} M, molar ratio of chemosensor R was varied from 0.1-0.8. The pH of test solution was adjusted at 10 and fluorescence intensity of each complex solution was plotted against the molar fraction of chemosensor R and the results were recorded. It is revealed from Figure-7(a) that the fluorescence intensity at 707 nm reached the maximum when the molar ratio was 0.5 that demonstrate a 1:1 binding ratio between Cd^{2+} and chemosensor R.

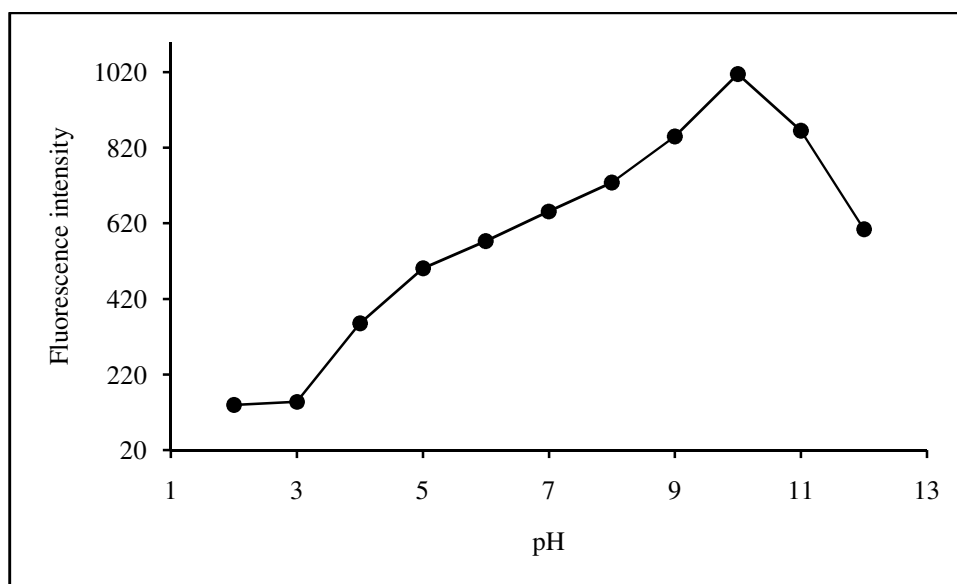


Figure-5: Fluorescence intensity of chemosensor R as a function of pH in the presence of Cd^{2+} (20×10^{-6} M), $\lambda_{\text{ex}}=352\text{nm}$, $\lambda_{\text{em}}=707\text{nm}$.

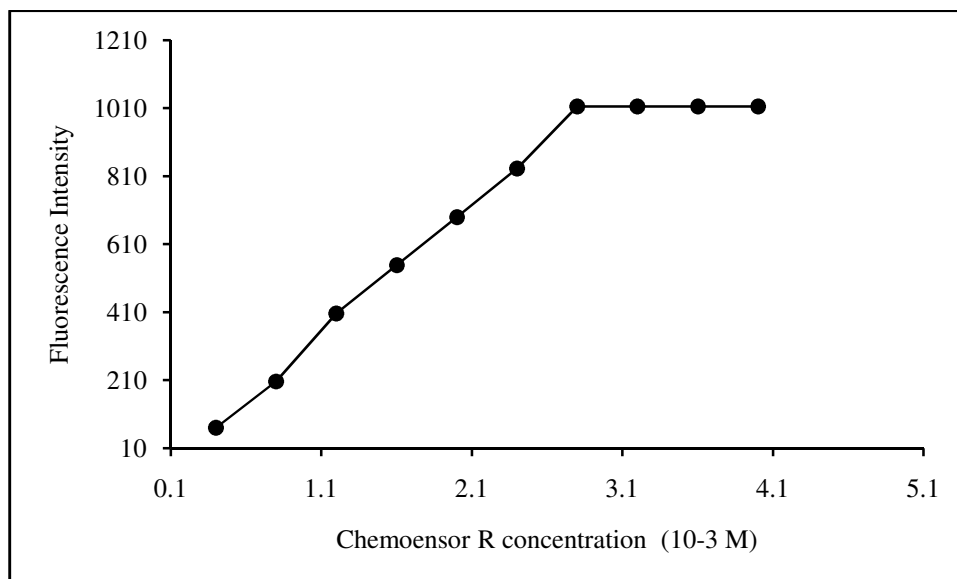
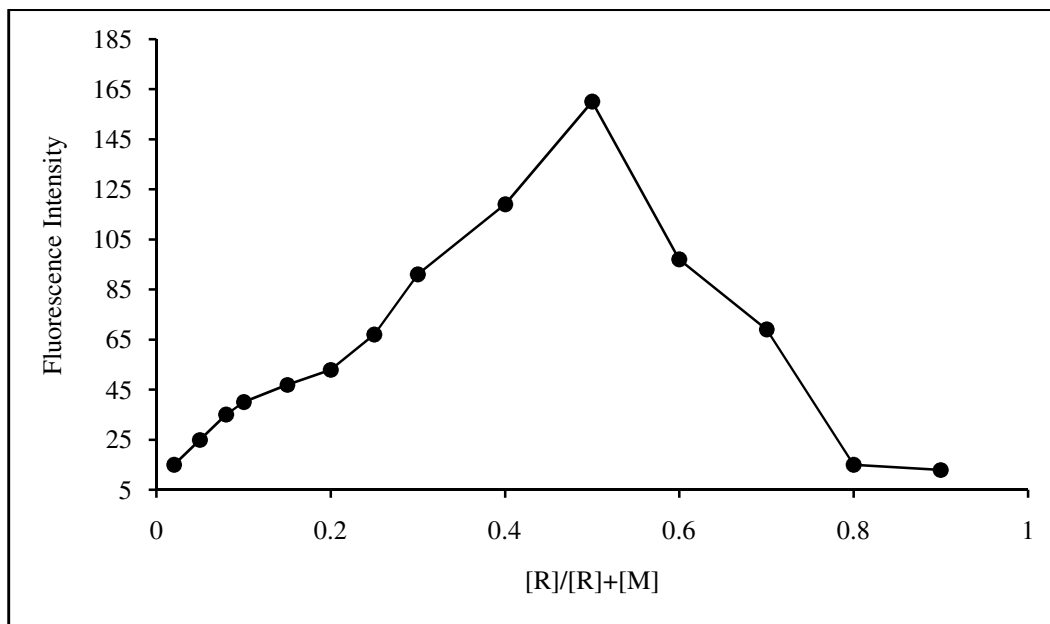


Figure-6: Fluorescence intensity at pH 10 as a function of chemosensor R concentration, Cd^{2+} concentration was kept constant (2×10^{-6} M).

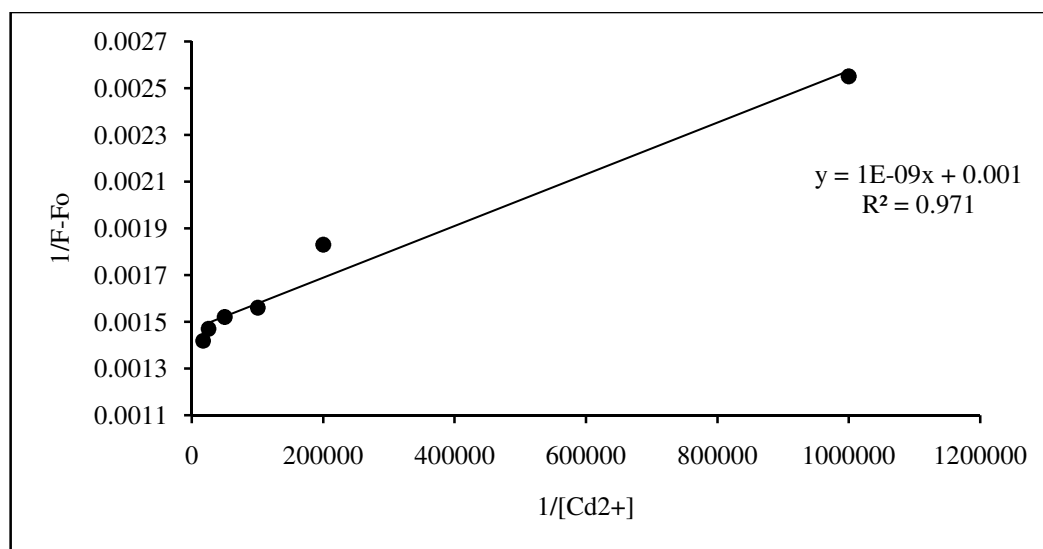
On the basis of 1:1 stoichiometry as suggested by Job's plot, K_a value of chemosensor **R** with Cd^{2+} was determined from experiment of Cd^{2+} concentration effect on emission intensity by the Benesi-Hildebr and plot. Using Benesi-Hildebr and equation²² the K_a value of chemosensor **R**- Cd^{2+} complex was calculated

$$\frac{1}{F-F_0} = \frac{1}{K_a \times (F_{max}-F_0) \times [Cd^{2+}]} + \frac{1}{F_{max}-F_0} \quad (1)$$

Where: F_0 represent fluorescence intensity of **R** in the absence of Cd^{2+} , F_{max} is the maximum fluorescence intensity of **R** in the presence of Cd^{2+} in solution, F is the fluorescence intensity of **R** at any given Cd^{2+} concentration at 707 and K_a is the association constant. By plotting $1/(F - F_0)$ versus $1/[Cd^{2+}]$ the association constant value (K_a) was figure out graphically. According to equation (1) Fluorescence intensity data was fitted linearly. K_a was found to be $1 \times 10^6 M^{-1}$, obtained from the slope and intercept of the line.



(A)



(B)

Figure-7: (a) Job's plot for complexation of chemosensor **R** with Cd^{2+} showing maxima at 1:1 obtained from fluorescence data at a total concentration of 400×10^{-6} M of **R** and Cd^{2+} and λ_{ex} is 352 nm (b) Benesi-Hildebrand plot of chemosensor **R** 4×10^{-3} M with Cd^{2+} $1-140 \times 10^{-6}$ M.

Response Time: Long response time is undesired in numerous fluorescent sensors. To evaluate the effect of reaction time on the fluorogenic response of chemosensor **R** towards Cd^{2+} , the fluorescence intensity of chemosensor **R**- Cd^{2+} complex was recorded at different intervals of time. Upon the addition of Cd^{2+} the fluorescence of chemosensor **R** at 707nm enhanced remarkably in one twenty sec but the response measured after it showed a saturation of emission signal as displayed in Figure-8. These measurements demonstrate that the chemosensor **R** can be used for real-time detection of Cd^{2+} .

Interference and Selectivity Study: Selectivity is an important feature for an effective chemosensor which mean to possess high affinity for the analyte of interest over competitive metal ions. Fluorescent sensor for detecting Cd^{2+} are also reported but most of them show interferences from transition metal ion especially interference from Zn^{2+} . Cd^{2+} and Zn^{2+} can cause similar spectral changes upon interaction with fluorescent sensor, as they belong to the same group and have similar properties^{23,24}. Soyoung S. et. al developed fluorescent sensor based on Rhodamine-hydroxamate platform showing a good detection limit 25nM but it shows interferences from metal ions such as Cu^{2+} , Hg^{2+} , and Zn^{2+} even at equimolar ratio²⁵. Chunliang L, et. al developed a chemosensor containing DPA (Di-2-picoylamine) as a binding site and 4,5-diamino-1,8-naphthalimide as a signaling unit but it also shows small interferences from Hg^{2+} and Ag^{1+} at equimolar ratio¹⁰. Hong-Yuan L et al developed Porphyrin-appended terpyridine as a selective fluorescent sensor for the detection of Cadmium, but the intensity was quenched in the presence of Cu^{2+} and Zn^{2+} even at the concentration lower than the equimolar. (The concentration of Cd^{2+} was 3.2×10^{-5} M and Cu^{2+} and Zn^{2+}

concentration was 1×10^{-5} M respectively)²⁶. Mathieu S. et. al used Rhod-5N as fluorescent sensor for Cd^{2+} showing good affinity, but Pb^{2+} comes out to be an interfering ion²⁷. In order to investigate the selectivity and practical applicability of chemosensor **R** for Cd^{2+} , the competitive experiments were conducted by keeping the ratio of the Cd^{2+} to interfering metal ions 1:10 and the alteration in fluorescence intensity of the chemosensor **R**- Cd^{2+} was observed at 707 nm in each case. For most of the competitive metal ions the fluorescence of chemosensor **R**- Cd^{2+} at 707 nm was not changed and no significant interference was observed. These analysis ascertain that most of the interfering metal ions have no effect on complexation of Cd^{2+} with chemosensor **R**. Thus, chemosensor **R** can be used as a selective fluorescent sensor for the detection of Cd^{2+} over a complex background of competitive metal ions found in biological system and in the environment.

Application for detection of Cd^{2+} in real water samples: Water resources may be contaminated with cadmium from industrial waste. The average level of Cd^{2+} in ocean water is between <5 and 110×10^{-9} M. EPA requires water suppliers to limit the Cd^{2+} concentration in water to $<5 \times 10^{-6}$ M. To check the effectiveness of chemosensor **R** we performed an experiment to detect Cd^{2+} in nearby river water and in tap water samples. Tap water was collected from our lab and river water from river Swat respectively. Using spike method, different concentration of Cd^{2+} in the range from 20×10^{-6} M to 80×10^{-6} M were added to these water samples. For each data set, the fluorescence intensity was recorded at 707 nm. As it is depicted in Figure-10, that fluorescence intensities were linearly proportional to the amount of Cd^{2+} added. From these experiments it is obvious that chemosensor **R** is able to detect Cd^{2+} even in complex matrices.

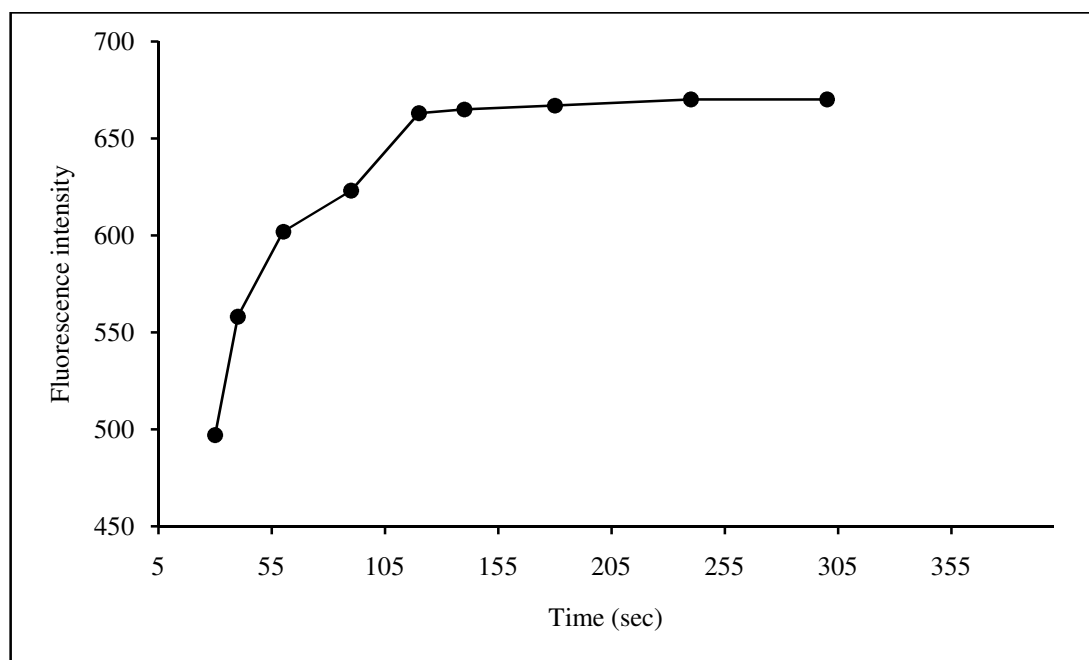


Figure-8: Fluorescence intensity of chemosensor **R**+ Cd^{2+} complex 20×10^{-6} M, at room temperature as a function of time.

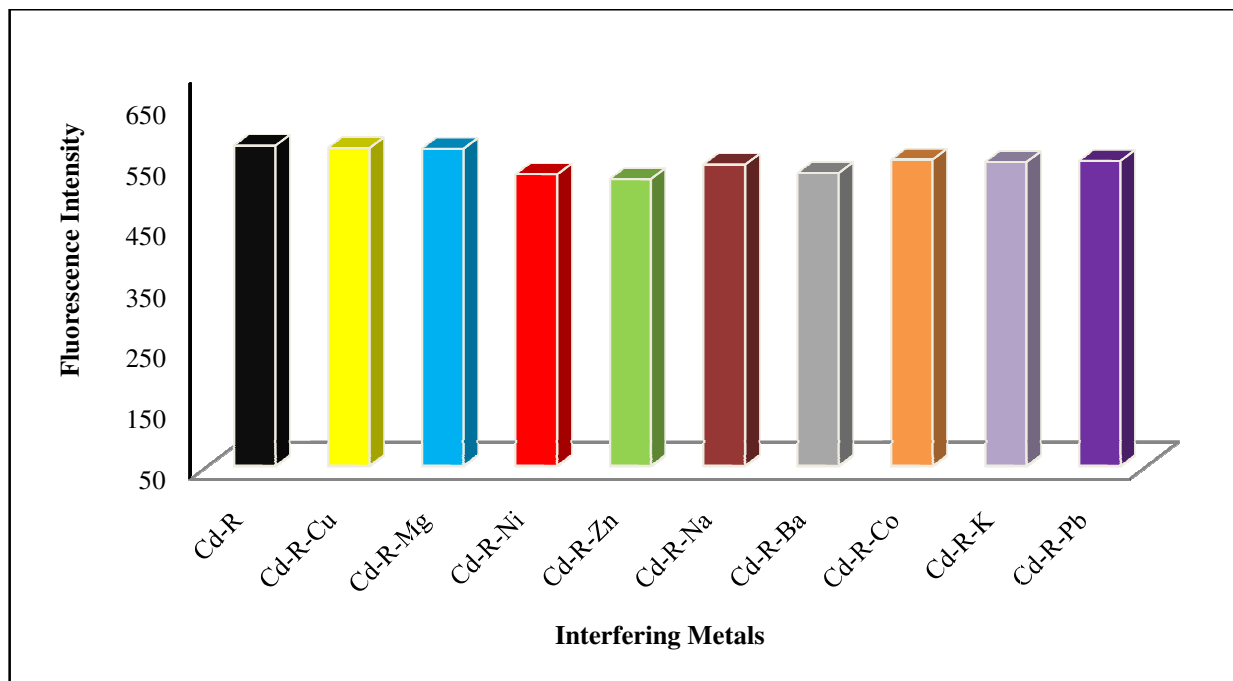


Figure-9: Fluorescence intensity chemosensor **R**-Cd²⁺ complex at 707 nm in the presence of interfering metal ions 200×10⁻⁶M, Cd²⁺ is 20×10⁻⁶M and pH is 10.

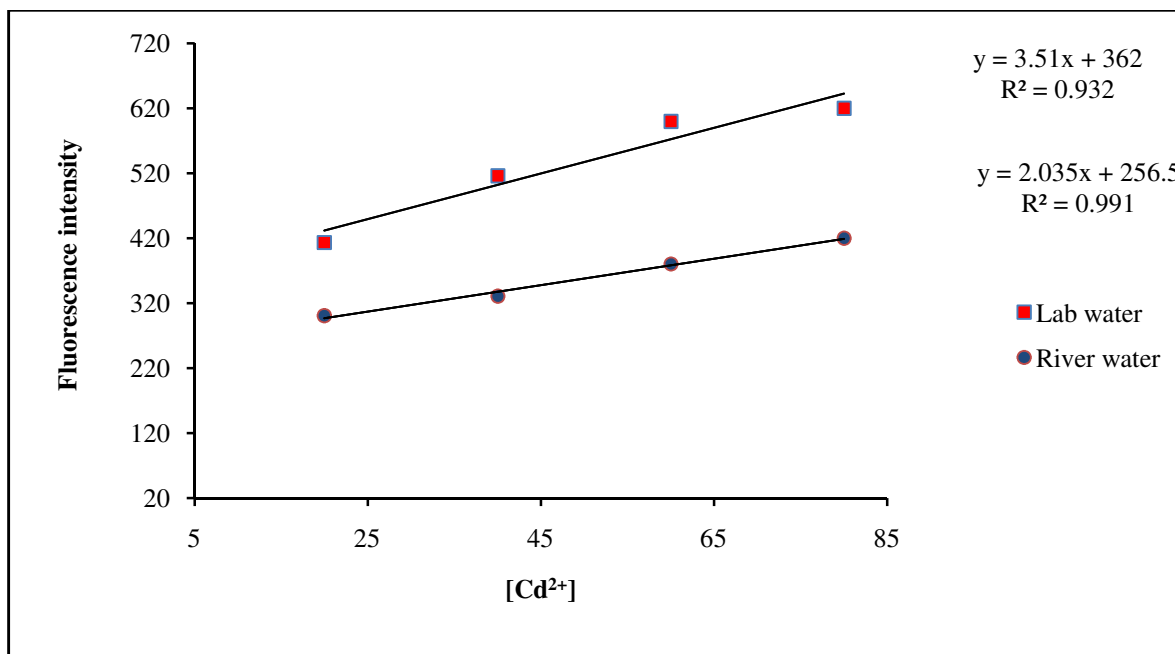


Figure-11: Changes in fluorescence of chemosensor **R** with various amount of Cd²⁺(20-80×10⁻⁶M) added to river water and tap water at 707 nm and pH 10, λ_{ex} is 352 nm.

Conclusion

In conclusion we have successfully synthesized chemosensor **R**[2 (hydroxy (4-nitrophenyl) methyl cyclohexanone)] as a turn-on fluorescence method to detect Cd²⁺ over (Mg²⁺, Cd²⁺, Ni²⁺, Zn²⁺, Ba²⁺, Co²⁺, Cu²⁺, Pb²⁺, Na¹⁺). The chemosensor **R** is almost non-fluorescent, but upon interaction with Cd²⁺ an

enhancement was observed. The enhancement is due to inhibition of PET phenomenon. The detection limit was found to be 5.5×10⁻⁷M. The co-existing metal ions do not cause significant interference. The method proposed in this paper is sensitive, simple and selective and was effectively applied for the detection of cadmium in real water samples.

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