

# Fluorogenic Chemo-Sensor-Based Sensing of Heavy Metal ion by Using Gold Coated Lower Rim Amino Functionalized Calix [4] arene

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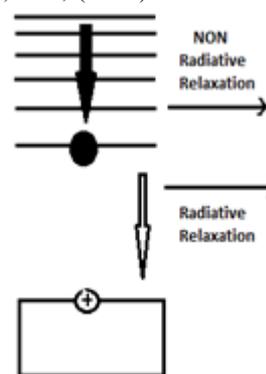
**Abstract:** In recent years, there has been increasing ecological and global public health concern associated with heavy metal ions even at lower concentration. These heavy metals are non-biodegradable and remain in the ecological system and food chain system at high levels of pollution. The chemical recognition of heavy-metal ions is the base for the mentioned techniques to enable a proper sensing through specific substances/species called receptors of the Fluorogenic chemo-sensor. So Fluorogenic chemo-sensor sensing technique having high reliability and sensitivity for analysis of target non biodegradable heavy metal ions detection by utilizing Gold Nanoparticles (AuNPs) surface coated with calix [4] arene amine derivative. Signaling event of chemo-sensor after binding with analyte of interest was characterized by <sup>1</sup>H NMR, Fourier-transform infrared spectroscopy (FTIR), Photo luminescence (PL) and Transmission electron microscope (TEM) analysis. Calix [4] arene amine derivative capped gold nanoparticle (C [4] A3@AuNPs) in Tetrahydrofuran (THF) dispersible at low pH with specific size and zeta potential of 50±2 MeV make these nanoparticle has potential for various application. Calix [4] arene amine derivative capped gold nanoparticle (C [4] A3@AuNPs) were found to be fluorometric sensor for Zn<sup>2+</sup> metal ion among these metal ions i.e. Li<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>, Which can detected at very low concentration level of 1 μM in facile way of fluorescence quenching via colorimetric sensor relies real time quantitative detection of Zn<sup>2+</sup> metal ion with detection limit of 1.5×10<sup>-6</sup> M.

**Keywords:** Calix [4] arene, AuNPs, Colorimetric sensor, Chemo-sensor, Photo luminescence

## 1. Introduction

As a result of strong interaction between electromagnetic radiation and Gold Nanoparticles enhancing absorption and scattering due to coherent oscillations of the free electron of the Gold Nanoparticle is known as SPR (Surface Plasmon resonance) (Kerbig *et. al.* 1985). As a result of SPR (Surface Plasmon Resonance) the amplitude of oscillation reaches maximum depending upon size, shape (Jin *et. al.*, 2003) and refractive index of solution, at certain frequency of light absorption (Link *et.al* 1999). Gold Nanoparticle (AuNP) display SPR band position at 520nm (Huang *et. al.*, 2010) with result of red shift of plasmonic band (Raether *et. al.*, 1988) due to aggregation of Gold Nanoparticles (Zhong *et.al* 2004). This SPR band shift is attributed to dipole-dipole interaction (Campbell *et. al.*, 2007) and coupling between plasmons of the neighboring particles in the aggregation (Link *et. al* 1999). When inter-particle distance is greater than the average particle diameter then SPR band appears to be red while on the other hand when interparticle distance is lesser than average particle diameter then SPR band appears to be blue band shift (Kreibig *et.al* 1985 ). This phenomenon has well adopted in various detection scheme with specific analyte of interest elements decorated on the nanoparticle surface for target of interest heavy metal ion (Darbha *et.al* 2008). The fluorescence-based AuNPs sensor detection strategy relies on the fluorescence change (Kattke *et al.*, (2011). When chemo-sensor sensing heavy metal ion (Pan *et. al.*, 2014) with emission of radiated light under surface modified fluorescence process or fluorescence resonance energy transfer (FRET) process. (Figure 1), The Surface Modified fluorescence (SMF) is the modification of the fluorescence by electromagnetic environment, where resonating energy transfer occurring between an excited

donor and acceptor fluorophore due to induced dipole-dipole interactions (Yu, J., *et al.*, (2014).



LIGHT EMISSION BY Q, D FLUORESCENCE

**Figure 1:** Displacement of Florescence quenching by resonance energy transferred for analyte sensing by the FRET process (Herrera *et.al.* 2001).

This fluorescence is affected by the nearby presence of a metal particle or metal surface (Silva *et. al.*, 1997). Surface Modified Surface (SMF) has been applied to measure the fluorescence change caused by excited fluorophore. If the distance between fluorophore and metal surface is less than 10nm, the latter absorb the energy and cause the change of fluorescence signal (Zhou *et.al.*, 2012). It is possible to design a colorimetric sensor for detection of heavy metal ion using self-reporting plasmonic shift upon binding with nanoparticle. Such type of interaction gives signaling interpretation about presence of heavy metal ion in human resources (Wonju *et.al.*, 2017). This chapter describes method to develop the Gold Nanoparticles (AuNPs) for the colorimetric detection of heavy metal ion based on

interaction (Cao *et al.*, 2008) between calix [4] arene and metal ion expressed on the surface of AuNPs based on binding capacities.

## 2. Materials

### 2.1 Equipment

**TEM** (Transmission Electron Microscope 200keV) FEI, Tecnai G2F30.

**UV-Vis spectrometer** 2450 Shimadzu,

**Weighing Balance** (Precisa Gravimetric AG, Swiss Made, 1mg-120g±0.1mg) Shimadzu

**<sup>1</sup>H-NMR** on Advance -11 (Bruker) instrument 400MHz.

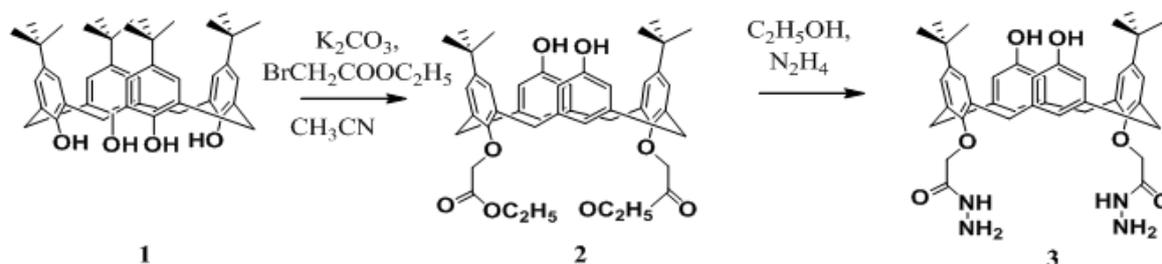
**FTIR** bruker Tensor 27 FTIR for liquid state compound.

**REMI** Centrifuge CPR-24 REMI Electronic limited

**HI Microplate reader** Gen 5 Software using biotek biocell plate (300-700nm).

### 2.2 Chemical requirement

ODA (Octadecyl amine), HAuCl<sub>4</sub> (gold (III) chloride trihydrate), Cu (NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, Ni (NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, Pb (NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, Fe (NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O, Hg (NO<sub>3</sub>)<sub>2</sub>.H<sub>2</sub>O, AgNO<sub>3</sub>, NaCl, Zn (NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O, Co (NO<sub>3</sub>)<sub>2</sub>.H<sub>2</sub>O, KNO<sub>3</sub>, Ca (NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O, Al (NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O, were obtained from sigma-Aldrich st Louis, MO. Aqua regia, distilled water, Toluene, NaBH<sub>4</sub> (Sodium borohydride), MeOH (Methanol), CHCl<sub>3</sub> (chloroform), N<sub>2</sub>H<sub>4</sub> (Hydrazine hydrate), DMSO (Dimethyl sulphoxide), Calix [4] arene, Ethanol, Ice flake, THF (tetrahydrofuran). ODA (Octadecylamine) All chemicals purchased from one chemical company of Mumbai **Loba Chemie Pvt. Ltd.**



**Figure 2:** Synthesis of functional group of Calix [4] arene derivative (2) and (3) (Vyas *et al.*, 2012).

### 3.2 Synthesis of Gold nanoparticle (AuNPs) :

- 1) Use dried glassware after thoroughly rinsed with aqua regia and milli-Q water.
- 2) 6.7mg (0.034mmol) auric chloride (HAuCl<sub>4</sub>) in 2gm (0.7mmol) Octadecylamine (ODA) with constant heating and stirring at 180°C for 10 mint.
- 3) Add NaBH<sub>4</sub> (0.178 g in 500 µl water) in Homogeneous solution.
- 4) The color of solution will change from black to brown then red color.
- 5) From prepared solution add THF in whole content and centrifuge it at 5000rpm for 20 mints at 25 °C.

### 3.3 Calix [4] arene amine derivative capped AuNPs (C [4] A3@AuNPs) Synthesis:

- 1) Use dried glassware after thoroughly rinsed with aqua regia and milli-Q water.

## 3. Method

### 3.1 Calix [4] arene amine derivative synthesis:

The receptor calix [4] arene amido amine derivative **3** was synthesized from calix [4] arene by two step synthesis.

#### (A) Synthesis of Diethyl ester p-tert-butyl-calix [4] arene derivative:

- 1) A solution of calix [4] arene (3.24g, 0.50 mmol), Compound (1) and K<sub>2</sub>CO<sub>3</sub> (0.690g, 0.50 mmol) in acetone (150 ml) in round bottom flask was stirred under reflux for 30 minutes.
- 2) With the completion of ½ hour condensations add subsequently Ethyl 2-bromoacetate (1.105 ml) to the solution and refluxed for 8 hours.
- 3) The reaction progress was monitored by observing the TLC (thin liquid chromatography) in 1: 2 ratio solvent CHCl<sub>3</sub>: C<sub>6</sub>H<sub>6</sub> (v/v, chloroform: hexane).
- 4) Upon completion of the reaction the solvent was filtered while hot.
- 5) The filtrate was distilled under vacuum to furnish solid residue. The residue was dissolved in Chloroform (CHCl<sub>3</sub>) and re-crystallized using methanol (MeOH).

#### (B) Synthesis of calix [4] arene amine derivative (2) :

In second step, ester derivative (2) of calix [4] arene (500mg) in ethanol (5ml) undergo amino ethylation in presence of hydrazine (N<sub>2</sub>H<sub>4</sub>) (5ml) by reflux reaction for 24 h at more than 80°C temperature.

The synthesized structure of compound 2 and 3 was confirmed from its spectroscopic and analytical data.

- 2) 6.7mg (0.034mmol) auric chloride (HAuCl<sub>4</sub>) in 2gm (0.7mmol) Octadecylamine (ODA) with constant heating and stirring at 180°C for 10 mint.
- 3) Add NaBH<sub>4</sub> (0.178 g in 500 µl water) in Homogeneous solution.
- 4) The color of solution will change from black to brown then red color.
- 5) From prepared solution add THF in whole content and centrifuge it at 5000rpm for 20 mints at 25 °C.
- 6) From above solution pipette out 998 µl amount and add 1 µl amine derivative of calix [4] arene.
- 7) Finally the product was centrifuged and dispersed to obtain the 1, 3-alternate calix [4] arene functionalized AuNP. Which was characterized by IR, UV-Visible, TEM and PL technique for further heavy metal ion analysis (Arneche *et al.*, 1997).

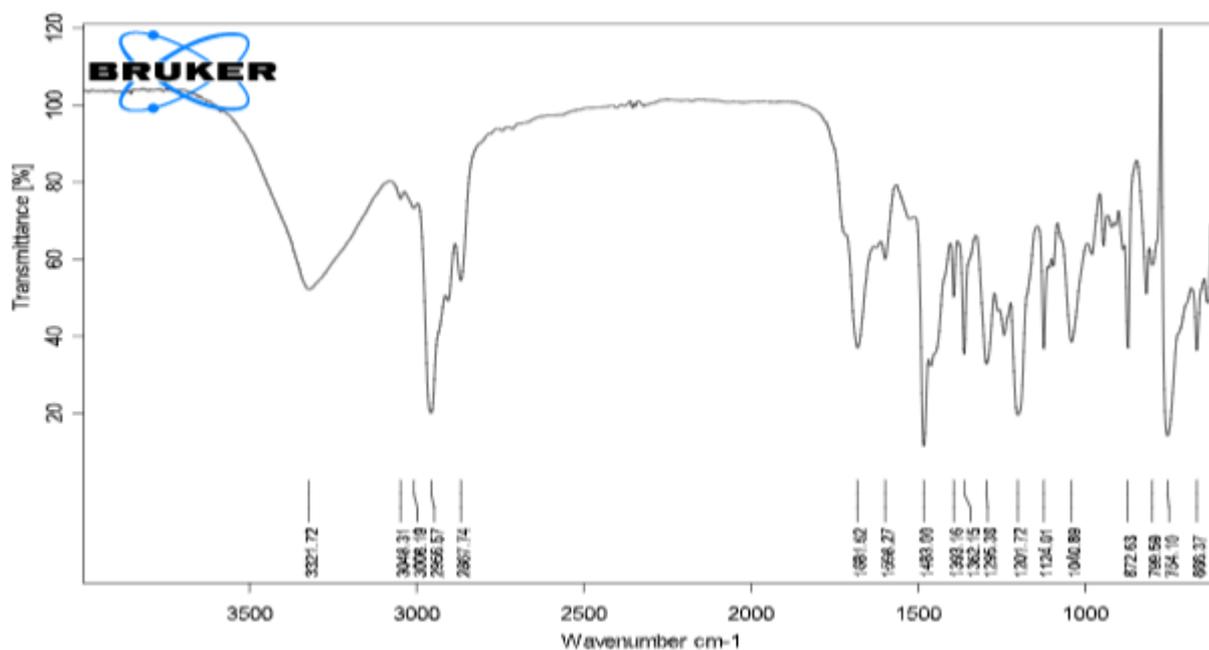
### 3.4 C [4] A3@AuNPs characterization and particle size analysis:

Reduction of by calix [4] arene occur via oxidation of amino group, transfer of electron from the amine of calix [4] arene to  $\text{Au}^{3+}$  ion. ODA stabilizes the surface of Au nanoparticle with the co-ordination of nitrogen from amine derivative. The resulting calix [4] arene amine derivative capped gold nanoparticle AuNP [C [4] A3@AuNPs] get stabilized electrostatically as well as characterised by FTIR spectra.



AuNP synthesis in THF with ODA show FTIR absorption peaks at 3440, 2968, 1645, 906  $\text{cm}^{-1}$  peak correspond to C-C, C-H, C-N, N-H. The absorption peak due to amide C=O was absorbed at 1683  $\text{cm}^{-1}$  and 3320  $\text{cm}^{-1}$  correspond to the amine, amide and phenolic moieties of compound shows the confirmation for AuNP capped calix [4] arene amine

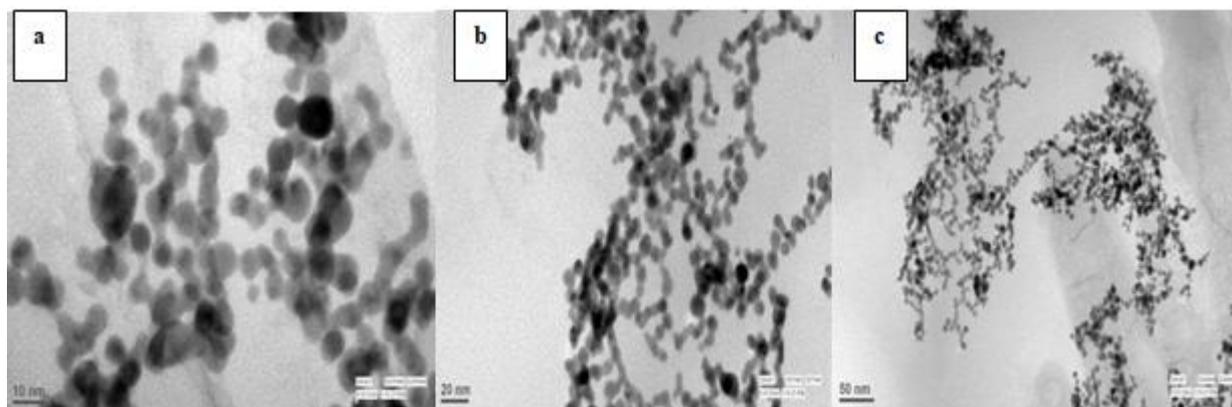
derivative (3) in THF solvent (figure: 3). Characteristic absorption peak due to the amide C=O ( $1645\text{cm}^{-1}$ ) indicate strong binding between the calix [4] arene derivative amide moieties over the surface of C [4] A3@AuNPs (McKervery *et. al.*, 1993). The synthesized gold nanoparticles (AuNPs) were characterized by infrared spectroscopy (IR) (Figure 3) and Transmission electron microscope (TEM) (Figure 4). From the comparison of the IR spectra of ODA modified gold nanoparticle (and 1, 3-alternate calix [4] arene was modified onto gold nanoparticle surface (Vyas *et. al.*, 2012), the characterized peak of ODA modified gold nanoparticle at 2927  $\text{cm}^{-1}$  and 2859  $\text{cm}^{-1}$  disappeared and the characteristic peak of carboxyl group (-COOH) at 1756  $\text{cm}^{-1}$  and benzene ring at 1634  $\text{cm}^{-1}$  and 2954  $\text{cm}^{-1}$  (-C-H-) and 1200  $\text{cm}^{-1}$  (Ar-O) (figure: 3) appeared which demonstrate that 1, 3-alternate calix [4] arene amine derivative has been successfully attached to the surface of gold nanoparticle (Morzherin *et. al.*, 1993).



**Figure 3:** FTIR spectra for amine derivative of calix [4] arene amine derivative capped gold nanoparticle (C [4] A3@AuNPs)

The TEM image showed in figure (4) revealed that the presence of 1, 3-alternate calix [4] arene have been

successfully attached to the surface of Gold Nanoparticle (AuNPs) (Kim *et. al.*, 2012)



**Figure 4:** TEM image of Calix [4] arene amine capped with gold nanoparticle (C [4] A3@AuNPs) at (A) 10 nm (B) 20 nm and (C) 50 nm scale

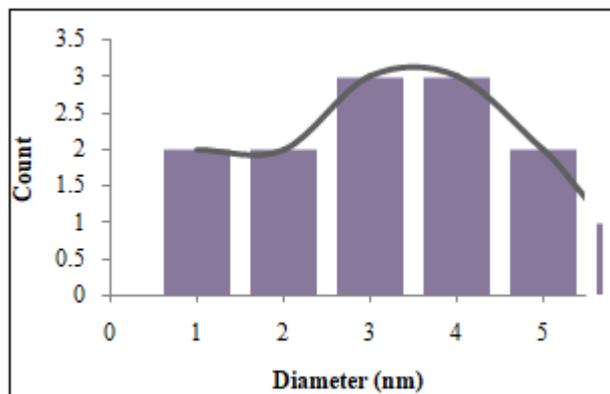


Figure 5: Histogram of (C [4] A3@AuNPs).

The key property of AuNPs is their Plasmon absorption in the near infrared region. Where Surface Plasmon Resonance (SPR) from the free electron at the metal surface resonating with the photon larger than the size of gold nanoparticle. Then surface electron get oscillate with respect to nuclei of gold nanoparticle. As a results of attraction between gold nanoparticle surface electron and nuclei specified with Localized Surface Plasmon Resonance (LSPR) (Peng *et al.*, 2007). Due to strong binding interaction between the calix [4] arene derivative amine moieties with AuNPs on its surface possess the functional group such as  $-NH_2$ . Which can interact effectively with the surface of AuNPs for form suitable ion recognition coordinating sites as chemo-sensor (Diming Zhang *et al.*, 2015). Functionalized chemo-sensor sustain maximum stability without any Coagulation and suspension. Synthesis of Gold nanoparticle (AuNP) in biphasic solvent water and THF 0.8: 1 ratio in presence of

water dissolved  $NaBH_4$  and hydrazine ( $N_2H_4$ ). Presence of surface carbonyl group of calix [4] arene amine derivative capped gold nanoparticle (C [4] A3@AuNPs) shows decrease frequency by  $38cm^{-1}$  toward blue shift. Because of spectral peak shift from  $1645cm^{-1}$  to  $1683cm^{-1}$  in figure: 3.FTIR characterization for tagging of compound (C [4] A3@AuNPs) leads toward particle size analysis via TEM analysis.

$$\text{Diameter} = 3.0 \times 10^{-7} \text{ m (figure: 5)}$$

$$\text{Radius} = 1.5 \times 10^{-7} \text{ m}$$

$$\text{Volume} = 4/3\pi r^3$$

$$V = 4/3 \times 22/7 \times (1.5 \times 10^{-7})^3$$

$$= 1.414 \times 10^{-20} \text{ cm}^3$$

$$\text{Mass} = \text{density} \times \text{volume}$$

$$\text{Density} = 19.3 \text{ g cm}^{-3}$$

$$\text{Mass of each particle} = 2.72902 \times 10^{-19} \text{ per particle}$$

$$\text{Amount of gold taken (Auric chloride)} = 6.7 \text{ mg}$$

$$\text{ODA (octadecylamine)} = 2 \text{ g}$$

$$\text{Solvent} = 30 \text{ ml}$$

$$6.7/303.5 = 0.034 \text{ millimole}$$

$$\text{Auric chloride (HAuCl}_4) = 0.034/30 \times 1000$$

$$= 0.7359 \text{ mM}$$

$$\text{Mass} \times \text{Avogadro no.} = 2.72902 \times 10^{-19} \times 6.023 \times 10^{23}$$

$$= 164368.9 \text{ Mol. wt.}$$

$$\text{AuNPs concentration} = 0.7359 * 196.7 / 164368.9$$

$$= 0.000880650354$$

$$= 0.8807 \text{ } \mu\text{M}$$

$$\text{Calix [4] arene derivative } 8.7 \text{ mg in } 30 \text{ ml} = 8.7 * 1000 / (30 * 792) = 366 \text{ } \mu\text{M (Wang et al., 2001)}$$

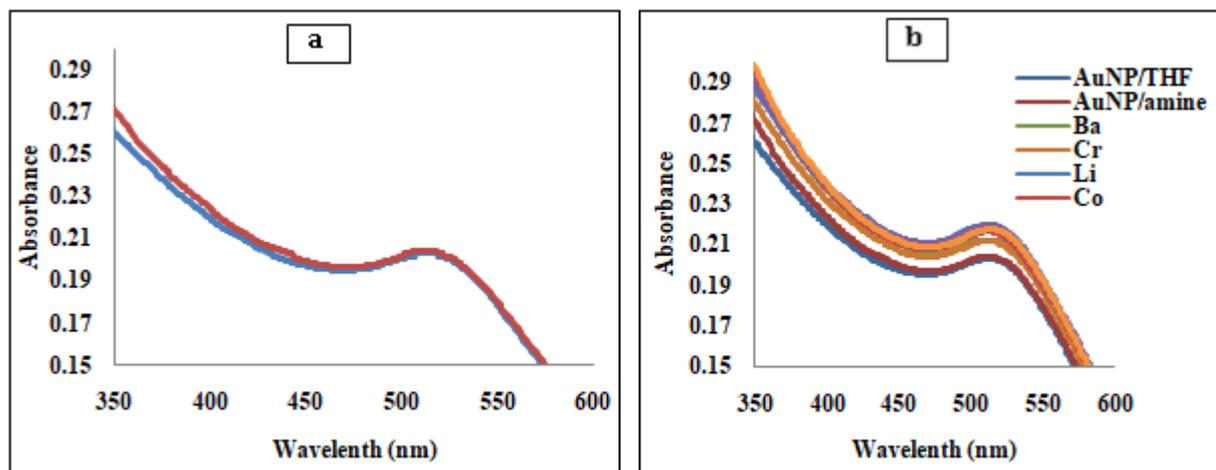
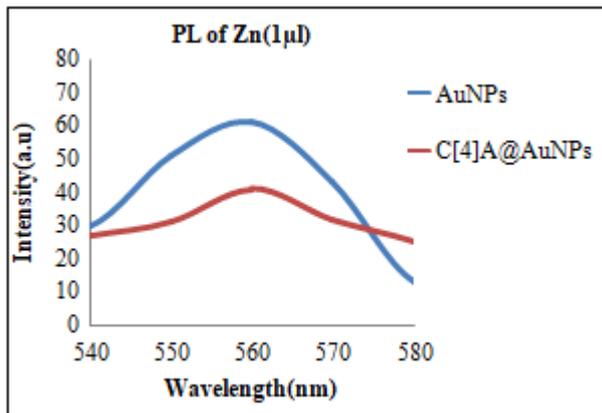
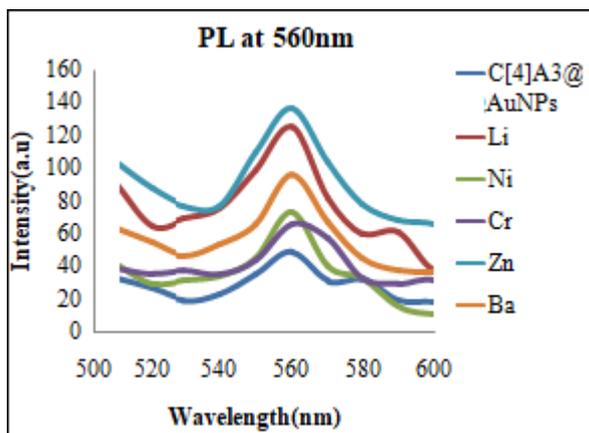


Figure 6: Absorption spectra in the range 350-600nm for detection of metal ions dissolved in water ( $1 \times 10^{-1} \text{ M}$ ) with the addition of  $1 \text{ } \mu\text{l}$  metal solution every time in C [4] A3@AuNPs.

Cation binding properties of compound **3** were investigated by UV-Vis (Figure 6) and fluorescence spectroscopy (Figure 7-8). The titration experiment were carried out in biphasic solvent water and THF (0.8: 1v/v) ratio by adding aliquots of different metal ion. The UV-Vis absorption spectrum of compound **3** ( $1 \times 10^{-1} \text{ M}$ ) exhibit typical  $Zn^{2+}$  absorption peak band at  $\lambda_{\text{max}} = 510 \text{ nm}$  (Figure 6-a & b)

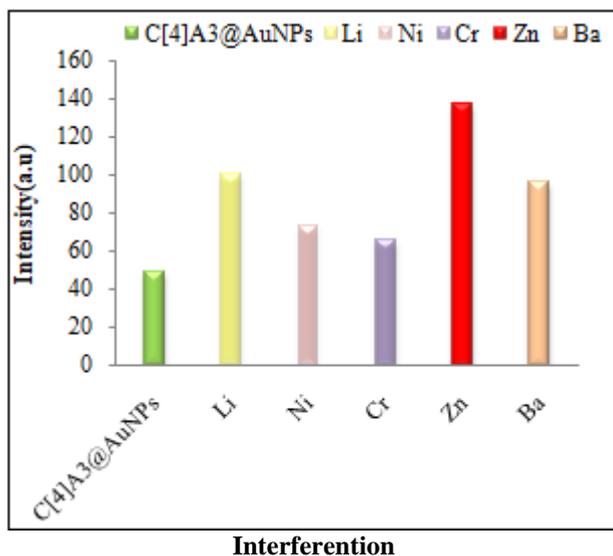


**Figure 7:** PL emission spectra of C [4] A3@AuNPs synthesis using ODA, NaBH<sub>4</sub> AuNPs (1mMol) with C [4] A3 (6.7mg in 100  $\mu$ l THF)



**Figure 8:** PL emission spectra of C [4] A3@AuNPs gold (Calix [4] arene amine derivative) study for comparative metal cations such as (Li<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>) show quenching and selectivity for Zn<sup>2+</sup> ion at 560nm with concentration range 1.0 $\times$ 10<sup>-5</sup>.

As a result of PL spectra it can justify under mentioned figure: 8 that there is three fold significant sensing enhancement in the fluorescence spectra in presence of Zn<sup>2+</sup> ion under the presence of other specified metal ions i.e Li<sup>+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>. which slightly interfere as well as leading to quenching of fluorescence spectra due to presence of Zn<sup>2+</sup> ion.



**Figure 9:** Fluorescence response of (C [4] A3@AuNPs) receptor (1 $\times$ 10<sup>-5</sup>M) to Zn<sup>2+</sup> (0 $\mu$ l to 10 $\mu$ l) concentration over selected metal (Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>, Li) cations having concentration (1 $\times$ 10<sup>-1</sup>  $\mu$ l to 1 $\times$ 10<sup>-5</sup>  $\mu$ l).

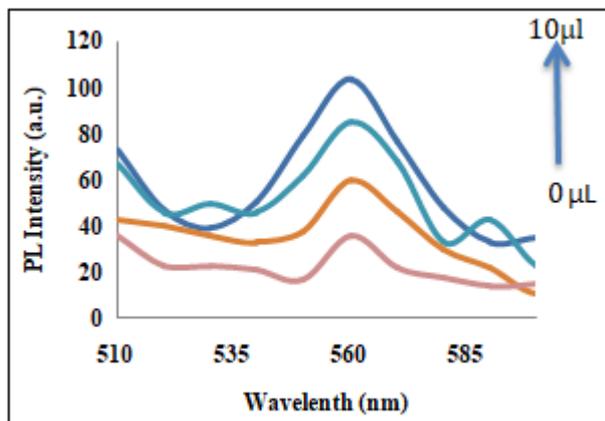
1, 3-alternate Calix [4] arene amine derivative gold nanoparticles (C [4] A3@AuNPs) were found to be fluorogenic colorimetric sensing with high sensitivity and selectivity give quenched sensing peak for Zn<sup>2+</sup> ion among various metal ion i.e Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>, Li which results that lowest detection limit at very low concentration level of 1  $\mu$ M in facile way of fluorescence quenching shown (Figure: 9) reveals real time quantitative detection of metal ion with detection limit of 1.5 $\times$ 10<sup>-6</sup> M.

### 3.5 Fluorogenic sensing behavior of C [4] A3@AuNPs against Zn<sup>2+</sup> cation

- 1) To determine the different metal ion concentration between 1 $\times$ 10<sup>-1</sup> to 1 $\times$ 10<sup>-5</sup> M 1 $\times$ 10<sup>-1</sup> were recorded using UV-Vis and PL spectrophotometer at different time interval.
- 2) All titration were carried out in THF solvent with successive addition of 1 $\mu$ l heavy metal ion prepared in Potassium bi-phosphate (KH<sub>2</sub>PO<sub>4</sub>) buffer solution of different metal ion (Li<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>) dissolved as their nitrate (NO<sub>3</sub>) salt.
- 3) The UV-Vis absorption spectra of compound (amine derivative) show typical shift absorption band from 363 to 451nm (Joseph *et. al.*, 2011) due to interaction of Zn<sup>2+</sup> ion with amino nitrogen leading to the ICT (intra-molecular charge transfer) phenomenon from imino group to CH<sub>2</sub>COO-anion group. Similar behavior was observed on addition of Zn<sup>2+</sup> ion (0.1-10.0 equiv). There is no colour change upon addition Li<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup> metal ion. But there is small increase in relative ratio I/I<sub>0</sub> or I<sub>415</sub>/I<sub>498</sub> of ligand (Babu *et. al.*, 2009) was 0.17 and it increase by 33.0 fold (figure: 9) upon addition of 10.0 equivalent of Zn<sup>2+</sup> ion. The binding properties of compound were investigated by UV-Vis, Fluorescence and 1H-NMR spectroscopy (Myung *et. al.* 2005).
- 4) The blue shift band at 415nm is attribute to the complexation induced deprotonation of calix [4] arene phenol in receptor. The deprotonation of phenol (OH) group has the fluorescence response of compound to the other metal ion shows no significance fluorescence change in presence of other metal ion (Li<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>3+</sup>).

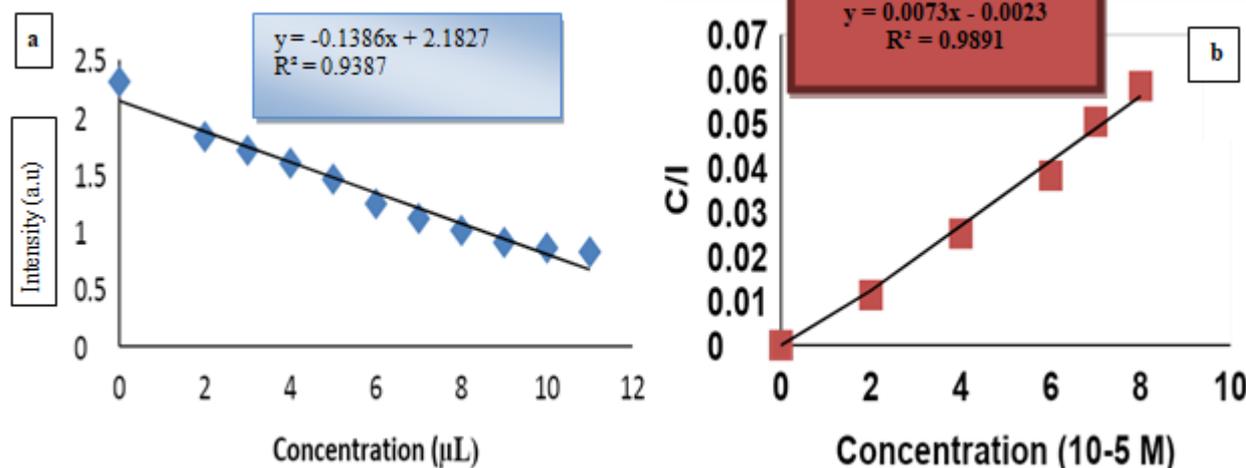
### 3.6 Colorimetric and fluorescence sensing of Heavy metal cation by (C [4] A3@AuNPs)

There is interaction between calix [4] arene amine derivative and heavy metal ions cluster complex.



**Figure 10:** Change in Fluorescence upon addition of increased  $Zn^{2+}$  concentration of (0  $\mu$ l-10  $\mu$ l) equivalent showing the binding isotherm over the chemosensor C [4] A@3AuNPS at 560nm.

The sensor response linearly increased with increasing  $Zn^{2+}$  concentration (figure: 10). Chemo-Sensor response in distilled water and suggesting that colorimetric assay can detect  $Zn^{2+}$  without being affected by the interfering other metal ion and organic environment. Accordingly, we



**Figure 11 (a) :** Linear regression correlation graph for study of  $Zn^{2+}$  metal ion with increasing concentration of  $Zn^{2+}$  0 $\mu$ l to 10  $\mu$ l for ( $1 \times 10^{-1}$  to  $1 \times 10^{-5}$  mM) solution of metal ion in potassium phosphate buffer in presence of other metal ion. **(b)** study of  $Zn^{2+}$  metal ion concentration (at lowest detection limit range with C [4] A3@AuNPs

**Note:** The fluorescence emission spectra of the C [4] A3@AuNPs were recorded at 560nm were studied over a wide range of pH (1.5-6.7).The maximum intensity was observed at 6.7pH for  $Zn^{2+}$  ion among other metal ion such as  $Li^+$ ,  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Ba^{2+}$ ,  $Cr^{3+}$ .The linear range of minimum and maximum detection of  $Zn^{2+}$  ion was determined by means of fluorescence titration of C [4] A3@AuNPs with increasing concentration of  $Zn^{2+}$  metal ion. It was noted that fluorescence intensity at 560nm gradually decreases with an increase in the concentration of Zn (II) ion. The maximum fluorescence quenching was observed at 1 $\mu$ M concentration of  $Zn^{2+}$  ion, used as a highly sensitive fluorescence ‘turn off’ sensor for  $Zn^{2+}$  ion without further modification. This highly sensitive and selective sensing may result from varying range from low concentration  $1 \times 10^{-4}$  to  $1 \times 10^{-5}$   $\mu$ M of  $Zn^{2+}$  concentration show linear regression correlation coefficient 0.93 were studied over wide range of pH (1.5-6.7) but the maximum intensity was observed at pH- 6.7.

calculate a limit of detection value (LOD) (1.0mM) and tested in the assay At low concentration  $1 \times 10^{-1}$  to  $1 \times 10^{-5}$  mM of  $Zn^{2+}$  concentration, the assay response demonstrated a high linearity ( $R^2=0.93$ ) (Figure: 11 (b)).To further demonstrate the applicability of our assay in practical application, with its LOD value which is below its EPA (10mM) value (Figure: 10). This low concentration and low standard deviation in the experiment indicate the high accuracy of our colorimetric assay. To determine the sensitivity of the colorimetric assay we tested several  $Zn^{2+}$  ion concentration in presence of other metal ion,  $Li^+$ ,  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Ba^{2+}$ ,  $Cr^{3+}$ , added with keeping their concentration range is same as 1 $\mu$ l  $Zn^{2+}$  each time (Figure 11b). So the fluorescence and colorimetric response for  $Zn^{2+}$  ion signaling its sensitivity and selectivity rather than other metal ions. The linear regression correlation coefficient of 0.93 (Figure11a) with the increasing concentration of  $Zn^{2+}$  from 0  $\mu$ l to 10  $\mu$ l for  $1 \times 10^{-1}$  mM solution of metal ion in potassium phosphate buffer give the results for its maximum selectivity and sensitivity at low concentration limit and without the interference of other metal ion.

#### 4. Acknowledgement

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