

FACILE SYNTHESIS OF L-CYSTEINE FUNCTIONALIZED GRAPHENE QUANTUM DOTS AS FLUORESCENT SENSING PROBES FOR Zn^{2+} IONS DETECTION

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ABSTRACT—In recent years, carbon based nanomaterials have generated new research interest in detection of micrograms quantities of metal ions and in bio-fluorescence imaging and sensing. Graphene quantum dots have gained immense importance due to their facile synthesis, low toxicity and aqueous solubility. Graphene quantum dots are getting the tremendous attentions because of their unique key properties like tunable photoluminescence, high photostability, biocompatibility and small size. In present work, graphene quantum dots are prepared by a simple and green pyrolysis method employing ethylene glycol as carbon source, L-Cysteine as functional molecule. These functionalized graphene quantum dots are used as fluorescence sensing probe for detection of Zn^{2+} in μM concentration. This fluorescence sensor is evidence of Zn^{2+} due to the prominent fluorescence quenching. The interference of diverse ions is studied. Optimum pH is found to be at 7. This is a simple and very sensitive method for detection of Zn^{2+} ions in environmental samples.

Keywords: Graphene quantum dots, L-Cysteine, functionalized, fluorescence sensing probe, ethylene glycol and Zn^{2+} ions.

INTRODUCTION :

Zinc is found in earth crust. It is widely used in industries as well as in domestic operations. It is released in the environment from natural and anthropogenic source. It is used in electroplating ore processing. It is important component of brass, bronze, rubber and paints. mostly it is used for galvanizing of iron. Zinc is an essential element for health. excessive intake is not advisable as it causes stomach cramps, skin irritation, nausea, vomiting and anemia. Free zinc ions in water are highly toxic for plants and fishes. According to WHO the adult requirement of zinc is 15 -22 mg/day. Hence it is very essential to detect and remove zinc from aqueous streams.

Zinc is the transition metal which is second most abundant metal in human body after Fe^{2+} and Fe^{3+} [1,2,3] and are plays in numerous aspects in biological redox systems, enzymatic functions and cellular signal[1]. Zn^{2+} plays an important role in sense of taste and smell, normal growth of the human body, protection from accelerated aging[1]. Although zinc is a relatively essential and nontoxic element[2], but imbalance in the intracellular Zn^{2+} level is associated with various disease[1]. Deficiency of zinc in human body causes disease such as Alzheimer's disease, β -thalassemia, perkinson's disease, ischemic stroke and epilepsy[3]. Excess of Zn^{2+} concentration causes pulmonary manifestations, fever, chills, and gastroenteritis[2].

Many methods have been developed for the detection of Zn^{2+} , including chromatography (HPLC), capillary electrophoresis, electrochemistry and colorimetry[1]. Although for Zn^{2+} detection by these methods. Good results are obtained out few restrictions are there. These are costly, time consuming and required sophisticated and expensive instruments. Therefore, there is still acute demand to develop a simple procedure of Zn^{2+} in water sample for highly sensitive and selective detection [5]. Fluorescence methods for determining Zn^{2+} have attained much attention because of their simplicity, convenience and high sensitivity [1].

Graphene quantum dots (GQDs) are the most crucial carbon based fluorescence material, they have received tremendous attention in nanoscience and nanotechnology[3]. due to their unique luminescent properties, large surface area, low toxicity, excellent solubility, photostability against photobleaching and blinking, cheap cost, biocompatibility properties, highly tunable photoluminescence properties. GQDs have recently fired up the enhancing concern in detection of metal ion and metabolites by fluorescence method using as prepared and surface modified GQDs [5]. Although several reward can be scope by using GQDs as the sensing element to detect metal ions[5], the relative poor quantum yield of GQDs has limited the detection sensitivity compare with those standard method[5]. The methods is needs further improved. Cystein is the better amino acid with one carboxyl group, amino group and sulphur group. Such functionalization with rich functional group makes it very easy to make non-covalent interaction other molecules. Which advised great potential of cystein as a good functional molecule to improve the analytical performance of GQDs because extra S-group compared to other amino acid[1].

We have synthesized the cystien capped GQDs. A simple and green method by pyrolysis treatment of cystein and glycol was developed to synthesize cys-GQDs. With high quantum yield by pyrolysis method. It is used for sensitive and selective detection of picomolar level of Zn^{2+} in wastewater sample. The the $-NH_2$, $-COOH$, $-SH$ group improve the fluorescence quantum yield 40.3%. While S-atom serve as the active sites for Zn^{2+} coordination to enhance the selectivity and sensitivity of

Zn^{2+} . The cys-GQDs are applied for Zn^{2+} detection in real sewage and dye waste water. Result obtained in this study clearly indicate the superiority of as prepared cys-GQDs to detect picomolar level of Zn^{2+} in different matrices of water and wastewater.

MATERIAL AND METHOD:

Ethylene Glycol, acetone, acetic acid, sodium acetate, disodium hydrogen phosphate, HCl, NaOH, Standard solution of metal ions (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Hg^{2+} , Co^{3+} , Cd^{2+}) were procured from merck limited Mumbai. Cysteine, alanine, glycine were purchased from HiMedia Laboratories Pvt. Ltd. All chemicals were of analytical grade and as used received. All aqueous solutions were prepared with Millipore water.

INSTRUMENTATION AND MEASUREMENT:

All fluorescence spectra were surveyed on an F97 fluorescence spectrophotometer (agilent). UV-vis measurement were carried out on a TU-1810 spectrophotometer (Beijing) and the spectra were collected from 200nm to 500 nm. the time-resolved fluorescence decay and the fluorescence quantum yield were performed on an edinburgh FL980 spectrofluorometer (Edinburgh,uk) with a 360 nm LED as the excitation source.

SYNTHESIS OF L-CYSTEIN-GQDs:

L-Cysteine functionalized graphene quantum dots were prepared by a simple and green method using ethylene glycol as a carbon source in one step. 0.3g L-Cysteine was taken with 10ml ethylene glycol in a 20ml round-bottom flask up to 160°C on a hot plate with stirring. The color of the liquid changed from colorless to orange and finally reddish brown. Then heating was stopped. It was cooled to room temperature and centrifuged by a 10000 rpm/minute centrifuge to remove insoluble matter. The supernatant was diluted with same volume of millipore water.

THE SENSING PROCEDURE:

200 μl 0.1M phosphate buffer solution (pH=7) 37.5 μl 0.1M cys-GQDs stock solution were spiked into fluorescence cuvette and diluted with water to get a final volume of 3.00ml, after reaction for 4min, different volume of Zn^{2+} stock solution were added into the cuvette and reaction 4 min. The fluorescence intensity was measured at $\lambda_{\text{em}}/\lambda_{\text{ex}}=445/360$.

RESULT AND DISCUSSIONS:

OPTICAL PROPERTIES OF cys-GQDs:

UV-VISIBLE SPECTRA

The optical properties of cys-GQDs were investigated by UV-vis spectroscopy. Cys-GQDs show strong absorption band at 360nm, which is ascribed to the

$n-\pi^*$ transition of cys-GQDs. The cys-GQDs aqueous solution showed reddish brown under visible light and emitted strong blue fluorescence under 365nm UV lamp irradiation. As the excitation wavelength changed from 300nm to 400 nm with wavelength interval of 10nm the intensity increased when the excitation wavelength increased from 300nm to 360 nm and then decreased at excitation wavelength of 370 nm to 400 nm. The maximum excitation and emission wavelength of the cys-GQDs were revealed at 360nm and 450nm, respectively.

The effect of pH on fluorescence intensity of the cys-GQDs was studied a wide pH range from 4 to 10. When pH was 4 or 5, fluorescence intensity was low at such strong acidic conditions, whereas, it increased drastically with the increase of pH value. When pH was above 5, fluorescent intensity was slightly changed. The phenomenon might be ascribed to the ionization of carboxyl group. It is that electronic structure and charge density might affected the optical characteristics of GQDs. At pH 7 highest intensity of fluorescence obtain.

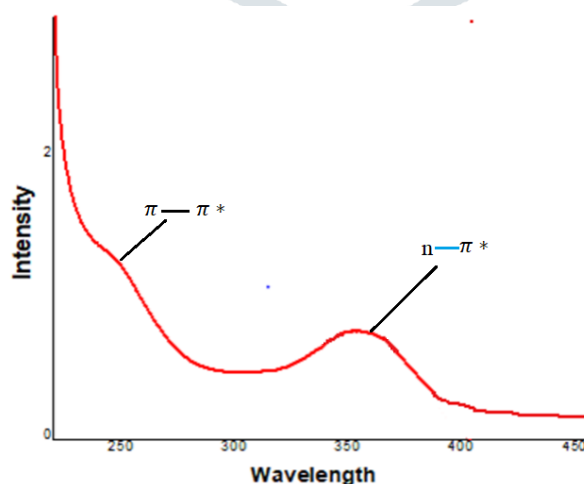


Fig. 1. UV-Vis spectra of GQDs/Cys

FTIR SPECTRA OF cys-GQD :

There are three FTIR curve in which 'a' represent spectra of aqueous solution of Zn^{2+} ion, curve 'b' represent FTIR spectra of pure cys-GQDs and curve 'c' indicate spectra of metal ion/GQDs complex. FTIR spectra of cys-GQDs shows peaks approximate at 3114.85cm^{-1} and 3448.51 which is assigned to N-H and O-H stretching frequency respectively. Absorption band 1401.88 and 1124.83 related to C=C and C-N vibration frequency respectively. 815 cm^{-1} and 615 cm^{-1} attributed stretching of C-H and C-S bond respectively. 1634.99 cm^{-1} indicate C=O stretching frequency of carboxylic acid. In curve 'c' 1744.48 cm^{-1} absorption band is result of interaction between metal ion and GQDs that why band shift to higher wavelength from 1634 cm^{-1} to 1744.48 cm^{-1} and below 100 cm^{-1} were disappear due to the vibration modes of metal-oxygen-metal stretching. Such as the M-O-H vibration represent at 523 cm^{-1} and the O-M-O vibration band around 426.50 cm^{-1} and further demonstration the successful formation of the desired M-GQDs.

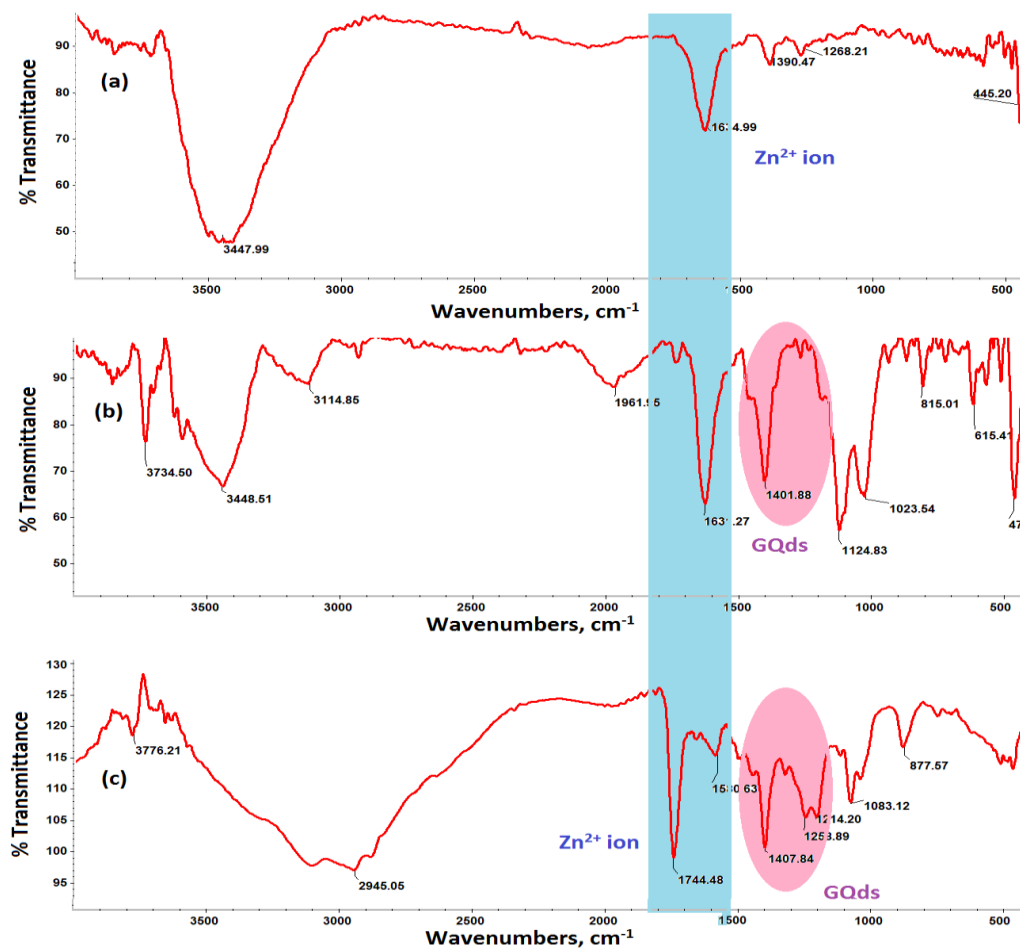


Fig2: FTIR spectra of (a) Zn^{2+} ion (b) cys-GQDs (c) cys-GQDs in presence of Zn^{2+} ion

SELECTIVE FLUORESCENCE RESPONSE OF cys-GQDs

The fluorescent characteristics in commonly used phosphate buffer solution and the presence of diverse metal ions, including K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Hg^{2+} , Co^{3+} , Cd^{2+} were studied. When the mixed solution was placed under observation of fluorescence spectrometer, remarkable fluorescent quenching observed in fluorescence intensity of mixture of cys-GQDs and Zn^{2+} , however no significantly fluorescence quenching effect was studies in the presence of other metal ions.

THE MECHANISM OF BINDING OF ZINC ION WITH GQDs:

Up to $1\text{ }\mu\text{M}$ by addition of Zn^{2+} ion solution fluorescence intensity of cys-GQDs gradually decreased. 90% quenching efficiency was observed. This is evidence of binding in between Zn^{2+} ion and GQDs. Which is due to consequently quenching of fluorescence of GQDs. Co-ordination of Zn^{2+} ion and functional group of GQDs such as amino acid, carboxylic acid, thio group and hydroxyl group is responsible for this quenching. To confirm the quenching of GQDs, quenching of Zn^{2+} ion GQDs system were investigated first. The reason for the fluorescence quenching is dynamic quenching or static quenching effect or both simultaneously. The fluorophore in the excited state is non-radiatively deactivated during dynamic quenching. Non-radiative complex is formed with the quencher molecule (Zn^{2+} ion) from fluorophore, which could be expressed by Stern-Volmer Equation as follows:

$$F_0/F = 1 + k_{sv}[Q] = K_q\tau_0$$

Where $[Q]$ is represent quencher concentration i.e. Zn^{2+} ion in this study, K_{sv} is quencher constant, F_0 and F are steady state fluorescence intensities in the absence and presence of quencher, respectively. τ_0 is fluorescence lifetime, K_q is the molecular quenching rate constant.

OPTIMIZATION OF cys-GQDs AS A FLUORESCENCE SENSOR FOR DETECTION OF ZINC ION:

For detection of Zn^{2+} ion in waste water sample by cystein functionalized GQDs were optimized under best experimental conditions. In present method the quenching properties of fluorescence intensity of GQDs is used for optimization. The parameters of reaction times, pH, and concentration of analyte (Zn^{2+} ion) were investigated.

For detection of metal ions by cys-GQDs pH is a important factors, which affects the quenching of fluorescence intensity. Therefore here shows effects of pH on the quenching intensity of GQDs in the presence of Zn^{2+} ion was studies by changing of pH of the solutions from 4.0 to 10.0 pH. It is observed the pH range 4.0 to 7.0 intensity of fluorescence was regularly decreased. In the range of 8.0 to 10.0 i.e. alkaline medium, there increasing of the intensity of GQDs. So, upto 3.0 to 7.0 it is pH quenching of intensity of fluorescence was occurred and slightly decreased beyond pH 7.0. The reason for quenching of cys-GQDs in neutral pH range might favor the binding the Zn^{2+} ion with cys-GQDs surface. Therefore, the 7.0 pH was chosen as optimization for further experiment.

For maximum quenching of intensity of fluorescence in the presence of the Zn^{2+} ion. The optimum concentration of cys-GQDs required. For this purpose different concentration of Zn^{2+} ion from $0.2 \mu\text{M}$ to $7.0 \mu\text{M}$ were taken in sample vial containing a $37.5 \mu\text{M}$ of 0.1 M cys- GQDs at pH 7.0. By using fluorescence spectrophotometer the intensity was observed when concentration of pH 7.0 of spectra of the solution mixture obtained was examined. The result of the decreasing in intensity were observed when the concentration of the Zn^{2+} ion was increased than $7 \mu\text{M}$. Thus $7 \mu\text{M}$ GQDs was the minimum to obtain the quenching of GQDs in the presence of Zn^{2+} ion.

Reaction time implies the maximum quenching of intensity of GQDs. When Zn^{2+} ion solution was added the time is required, during the reaction time Zn^{2+} ion interact with surface of GQDs. The intensity of GQDs at pH 7.0 were investigated in different time (1 min to 8 min). In reaction time 1 to 4 min the result showed quenching of intensity regularly, after 4 min quenching intensity of spectra of GQDs was remain constant. In fluorescence spectrophotometer the change in intensity of spectra of GQDs was examined. This for further investigation 4 min reaction time was selected.

For selective detection of Zn^{2+} ion in water sample, the effect of various metal ions were assessed for this purpose. At 7.0 pH and reaction time 4 min detection of Zn^{2+} ion was carried out in water spiked with different concentration of metal ions. The tolerance limit obtained for interfering metal was much higher than their actual levels in water sample. Thus it is observed that ions which may present in water sample was not interfere significantly in the detection of zinc ion.

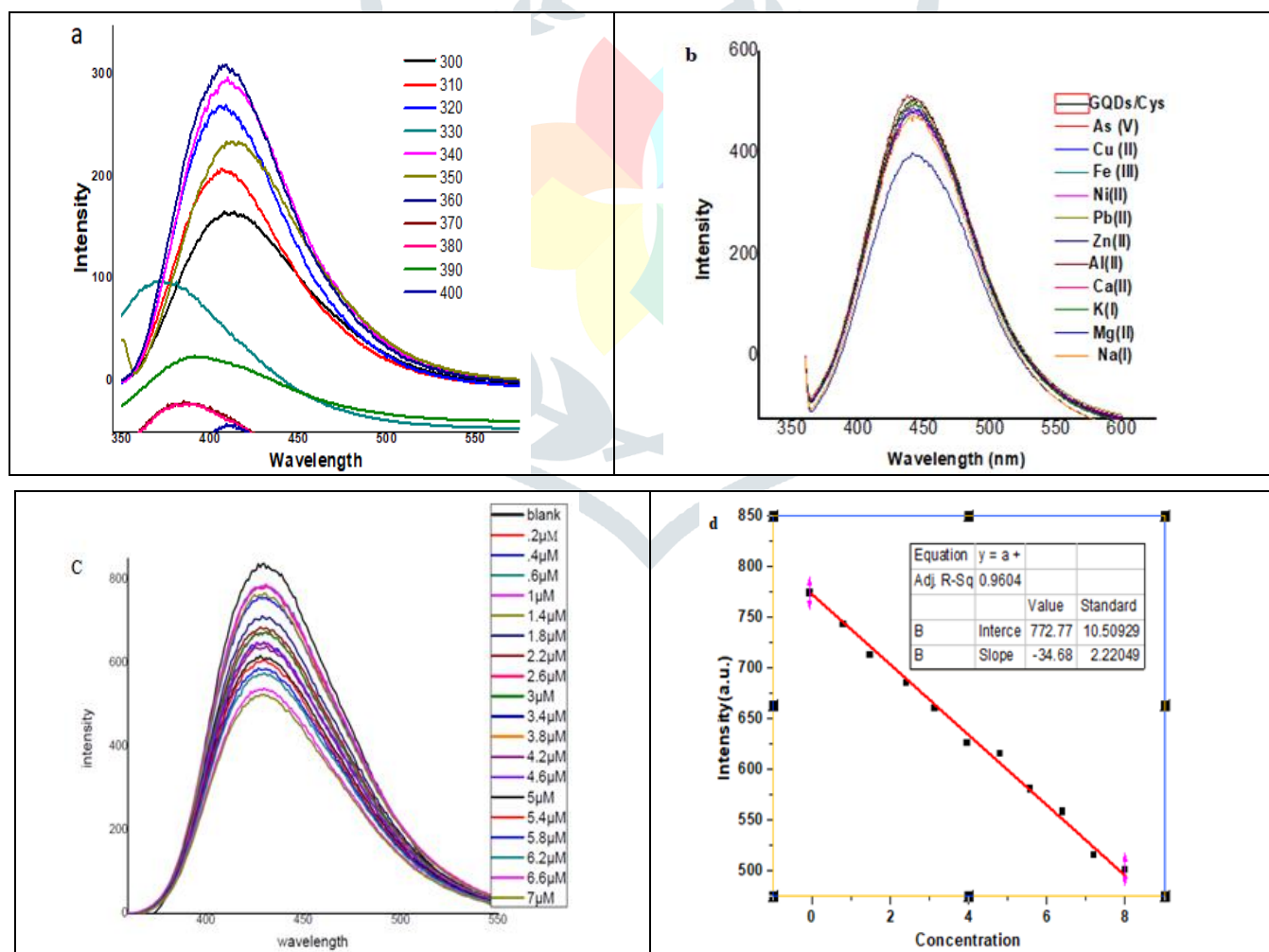


Fig: 3 (a) Fluorescence emission spectra of cys-GQDs with excitation of different wavelength (b) fluorescence emission spectra of cys-GQDs with various metal ion. (c) Fluorescence spectra of GQDs upon addition of different concentration of Zn^{2+} ion. (d) Linear range the plot intensity against concentration.

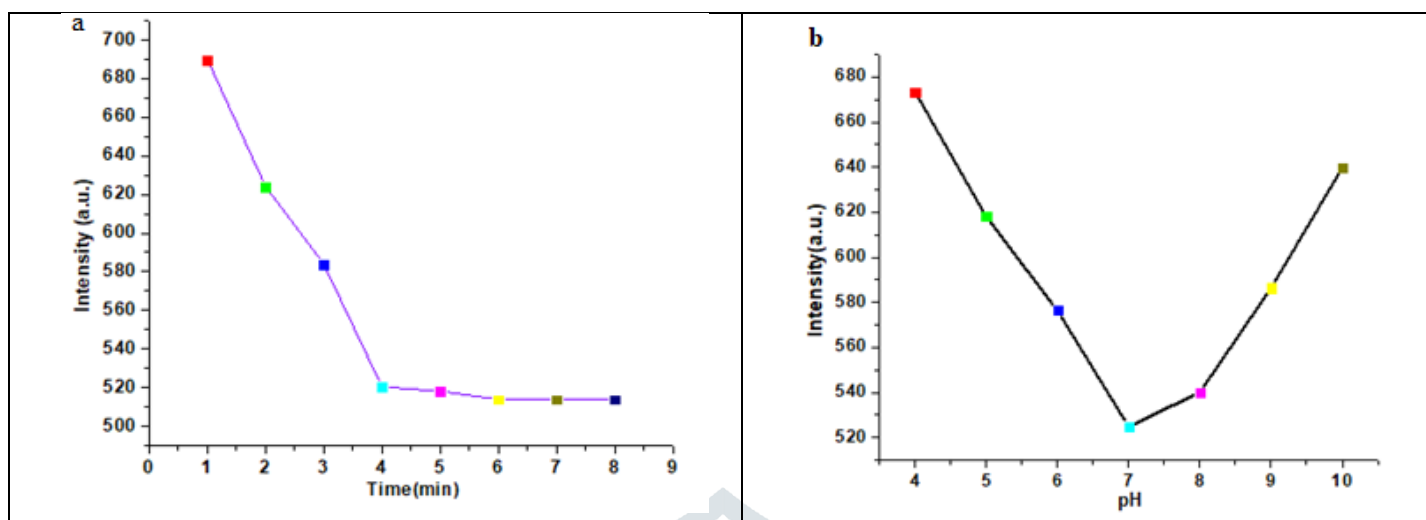


Fig:4 (a) Time dependent fluorescence response of cys-GQDs upon addition of Zn^{2+} ion. (b) Effect of pH on fluorescence intensity of cys-GQDs with Zn^{2+} ion.

CONCLUSION:

We have synthesized a simple and low cost photoluminescence sensor of cysteine capped GQDs by one step pyrolysis method. Cys-GQDs based fluorescence sensor was used for selective detection of Zn^{2+} ion by using quenching properties of fluorescence intensity of sample in the range of $0.2 \mu M$ to $70 \mu M$. The fluorescent based sensor cys-GQDs can be stored for a long time for detection applications. coefficient of 0.96 and detection limit is 1pM, for the cysteine capped GQDs.

ACKNOWLEDGEMENT :

The authors are thankful to Dr A. M. Rawani, Director NIT Raipur for laboratory and library facilities. One of the author Pradeep Kumar Dewangan gratefully acknowledges the JRF awarded by CSIR.

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