Fabrication of L-Cysteine functionalized Graphene quantum dots as a fluorescent nanosensing probe for determination of mercury

Pradeep Kumar Dewangan¹, Fahmida Khan*¹, Vinayak Sahu¹, Komal kashyap¹, Ch Chandra¹, Khilawan Patel¹

¹Department of Chemistry, National Institute of Technology, Raipur, CG, 492010
*Corresponding author Email: fkhan.chy@nitrr.ac.in, pradeepdew2013@gmail.com

Abstract. A highly sensitive and selective nanosensing probe for mercury ion is reported. Herein highly luminescent cysteine(cys) functionalized Graphene quantum dots(GQDs) were fabricated through facile one-step pyrolysis method by using ethylene glycol and cysteine. The coating of cysteine not only increases quantum yield to 41% but also enhances selective detection of ppb level of mercury ion in the waste water. The fluorescence intensity of Cys-GQDs was sequentially quenched by different concentrations of mercury ion via forming non-luminescent complexes of Cys-GQDs-Hg(II). HR-TEM study clearly shows a monodisperse Cys-GQDs of sizes 2-5 nm with a spherical shape. Raman spectra of Cys-GQDs display the D and G bands at 1350 cm⁻¹ and 1580 cm⁻¹ respectively. Optimum pH is 8 for mercury detection. The minimum limit for accurate detection of mercury is 0.64 ppb

Keyword: Fluorescence probe, Cysteine, Mercury ion, Graphene quantum dots, pyrolysis

1. Introduction
Mercury (Hg) is a highly poisonous, nonradioactive transition heavy metal that is discharged into the environment from natural and anthropogenic sources [1-2]. It is indestructible and exists in inorganic, metallic and organic forms in nature [3]. It is discharged to the atmosphere from volcanic and geothermal activities, mining coal,oil, fossil fuel extractionand gas, mercury-dependent artisanal and dental clinics, small scale gold mining sector, thermometer factories[4-5]. The permissible detection of limit in potable water is 1ppb according to the world health organization [6]. Mercury has a high tendency to accumulate in the human body by ingestion of contaminated water and contaminated seafood. The mercury ion may cause serious problems in many human organs even at low concentration e.g. kidney brain, nervous system, endocrine system, and other organs [4-6]. The well-known case of the Minamata disease was due to the accumulation of the most common form of methylmercury, in Japan and Iraq[7]. Therefore, the discovery of highly sensitive and selective methods of detection of Hg(II) has challenges in recent years.

Up to now, for the detection of mercury many traditional methods have been reported. The commonly used analytical methods for detection of Hg(II) is inductively coupled plasma mass spectroscopy[8], chromatography-mass spectroscopy[9-10], graphite furnace atomic absorption spectrometry[11], surface-enhanced Raman scattering[12], atomic fluorescence spectroscopy[13-14],
localized surface Plasmon resonance spectroscopy\[15\], resonance light scattering spectrometry\[16\], and electrochemical methods\[17\]. However, in general, these methods have some limitations, e.g. most of them required expensive instrumentation or complicated sample preparation, the need time-consuming pretreatment, complicated detection processes sophisticated, shortcomings dramatically restricted their application in daily monitoring for their handling expert is required. Therefore it is still of great challenge to develop a high sensitivity, low cost, short analysis time, portable and simple sensing platform for rapid on-site Hg(II) analysis. Therefore tunable fluorescent, low bio-toxic GQDs can be required alternatives.

Graphene quantum dots(GQDs) are the recent most attracting small fluorescent nanoparticles of the carbon family. It shows unique optical magnetic and exciting electrical properties governed by the quantum confinement and edge effects\[18-21\]. They have received tremendous attention among researchers of various fields due to its various applications in optoelectronic, FET, Bioimaging devices and biolabeling. GQDs shows excellent characteristic including excellent luminescent properties, low toxicity, photostability against photobleaching and blinking, cheap in cost, high biocompatibility, chemical inertness, eco-friendly, and high solubility in the various solvent\[22-25\]. Therefore GQDs as a new style carbon-based nano-material extend the strong potential for the promising candidate to substitute traditional semiconductor quantum dots. Although GQDs have recently fired up the enhancing concern in the sensing of the metal ion. The poor quantum yield and nonspecificity of original GQDs have limited the detection sensitivity. Various heteroatom doped GQDs e.g. N, S, B, N-P, N-S \[26-30\]doped GQDs have been prepared and applied in the detection of heavy metal. However, these sensing probes have not good LOD and low quantum yield. The methods are a needs further treatment for better result. For this purpose cysteine is the better amino acid contain -COOH, -NH₂, and –SH group. Such a functional group makes a better sensing probe for selective detection of Hg(II). This advised great potential of cysteine as a good functional groupsto enrich the analytical carried out of GQDs because extra S-group compared to another amino acid.

We have synthesized the cysteine (Cys) capped GQDs. A green and simple method by pyrolysis method of cysteine and glycol was developed to synthesize Cys-GQDs with high quantum yield by pyrolysis method. Synthesized GQDs has been successfully applied as a highly selective and sensitivensensing probe for Hg(II) in the environmental sample. The -NH₂, -COOH, -SH group improves the fluorescence quantum yield. While S-atom enhances the selectivity of Hg(II) because S based ligand strongly binds with Hg(II) according to the hard-soft acid-base concept. So S-atom form a stable complex with Hg(II). Obtained GQDs selectively applied for detection of Hg(II) in the environmental sample with detection limit 0.64 ppb.

2. Experimental Sections
2.1. Materials and reagents
Cysteine, Histidine, glycine, Histidine, arginine, Lysine, L-tryptophan was purchased from HiMidia Laboratories Pvt. Ltd. Glutamate, L-glutathione, methionine were procured from Alfa Aesar. Ethylene glycol, acetic acid, acetone, disodium hydrogen phosphate, Hydrochloric acid, Sodium hydroxide, standard solution of metal ions (As(III), As(V), Mg(II), K(I), Na(I), Pb(II), Co(III), Zn(II), Cd(II), Hg(II), Ni(II), Cu(II), Fe(III)) were purchased from Merck limited Mumbai. By dilution of standard stock solutions of Hg(II), different concentrations were prepared. All aqueous solution was obtainedwith Millipore water.

2.2. Instrumentation
UV-Visible spectra were measured by carrying Eclipse spectrophotometer and the spectra were recorded from 200 nm to 800 nm. The optical property including fluorescence spectra measurement wasrecorded on an F97 fluorescence spectrophotometer. The fluorescence quantum yield and time-resolved fluorescence decay were calculated on an FL980 spectrophluorometer (Edinburgh, UK) with a 370 nm LED as the excitation source. Fourier transformed infrared spectroscopy (FTIR) study was measured with the Thermofisher DRS-FTIR instrument (10 mg catalyst, KBr wafer technique). The
morphology and size of the Cysteine capped GQDs were recorded by using JEOL 2012 high-resolution transmission electron microscopy (HR-TEM) at a stimulating voltage 200 kV. Raman spectra recorded by Research India RIAR-0403 Spectrometer RIRAMAN532.

2.3. Synthesis of L-cysteine capped GQDs:
L-Cysteine capped graphene quantum dots (cys-GQDs) were prepared using ethylene glycol as a precursor in one step by a pyrolysis method. In a 20ml round-bottom flask 0.3g L-Cysteine was taken with 10ml ethylene glycol up to 170°C on a hot plate with stirring. The color of the liquid changed from colorless to orange and finally reddish-brown. Then was cooled to room temperature and centrifuged by a 10000 rpm/minute centrifuge to remove insoluble matter. The supernatant was diluted with the same volume of Millipore water.

3. Result and discussion
3.1. UV-visible study of cys-GQDs
The excitation properties of cys-GQDs were measured by UV-visible spectroscopy. UV-visible absorption spectra of cys-GQDs show a sharp peak at 370nm (Figure 1). The sharp UV-vis absorption peak at 370 nm is indicated n-$\pi^*$ type transition of C=O, C≡N [31]. In UV-Chamber aqueous solution cys-GQDs irradiated at 365 nm exhibit bright blue fluorescence.

![Figure 1. UV-visible spectra of cys-GQDs](image)

3.2. HR-TEM Study of cys-GQDs
To study of surface morphology and size GQDs HR-TEM is one of the most important techniques. HR-TEM image of cys-GQDs indicates that the size of most of the particle of quantum dots is in range 3 to 6 nm. Figure 2 (a) exhibit particles of cys-GQDs are monodisperse and spherical. Figure 2 (b) indicates that particles of cys-GQDs aggregated after the addition of Hg(II) ion. Aggregation of particles proves that interaction between Hg(II) ion and cys-GQDs occur.
3.3. Raman study of cys-GQDs
D-band and G-band are characteristics property of the graphitic structure of GQDs. Raman spectra cys-GQDs show two peaks at 1350 cm$^{-1}$ and 1580 cm$^{-1}$ which represent D and G band respectively (Figure 3 (a)) [32]. The disordered (d) band at 1350 occurs due to sp$^3$ hybridized carbon atom defect, while the crystalline(G) band at 1580 represents the in-plane vibration of sp$^2$ hybridized carbon atom. G- band also represent the aromatic character of cys-GQDs. The intensities ratio of D-band and G-band (I_D/I_G) is found to be 0.85 for cys-GQDs, which means that cys-GQDs is highly graphitic material. TEM image and Raman spectra confirm that GQDs is successfully prepared by this method.

Figure 3. (a) Raman spectrum of cys-GQDs
Excitation spectra of GQDs
The emission fluorescence spectra were recorded to cys-GQDs by excitation range 310 nm to 400 nm (Figure 3 (b)). 310 nm to 370 emission intensity of GQDs increases and wavelength shows a red shift after 370 nm fluorescence intensity decreases. Figure 3 (b) clearly indicated that maximum emission intensity occur when cys-GQDs was excited at 370 nm. Therefore excitation and an emission wavelength of cys-GQDs is 370 nm and 443 nm respectively.

Figure 3. (b) Fluorescence emission spectra of cys-GQDs at different excitation wavelength ranging between 310 nm to 400 nm.

3.4. The mechanism of binding of Hg(II) ion with cys-GQDs:
After the characterizations, the sensitivity of cys-GQDs towards the detection of Hg(II) ion was examined by the change in photoluminescence intensity presence and absence of Hg(II) ion. Fluorescence intensity gradually decreases by addition to various concentrations of Hg(II) ion in the aqueous solution of cys-GQDs. Change in fluorescence intensity after adding Hg(II) ion is occurs due to both dynamic and static quenching[33]. After the Collision between Hg(II) ion and cys-GQDs results in energy transfer as well as quenching, this process is known as dynamic quenching. Collision force to excited fluorescence material back to ground state. While static quenching Hg(II) ion interact with GQDs and form non-radiative co-ordinate complex (Hg-cysGQDs). GQDs transfer electron density on to Hg(II) ion results from fluorescent material to non-fluorescent co-ordinate complex. The fictionalization of GQDs by cysteine increases the chance of complex formation due to the addition of –COOH, -NH₂, -OH and –SH functional group. Sulphur based ligand is highly specific for bond formation with Hg(II) ion due to the well known soft base-soft acid interaction. The quenching of fluorescence intensity of cys-GQDs after the addition of different concentrations Hg(II) ion ranging from 5 ppb to 60 ppb. The quenching of fluorescence intensity of cys-GQDs increases upon increasing Hg(II) ion concentration and 80% of the pure GQDs fluorescence intensity quenched at 60 ppb. A linear relationship between the intensity of cys-GQDs and Hg(II) ion well fitted in the stern-Volmer equation. The calibration curve in ranging 5ppb - 60ppb has an excellent linear relationship with a correlation co-efficient (R²) of 0.995. quenching in fluorescence intensity calculated by using a stern-Volmer equation. The stern-volmer equation is followed as-

\[ \frac{I_0}{I} = 1 + k_{sv}[Q] = K_q \tau_0 \]

Here, steady-state photoluminescence intensities are I₀ and I in the presence and absence of quencher, respectively. Quencher concentration is [Q] i.e. in this study mercury ion, Ksv represents quencher constant, fluorescence lifetime is \( \tau_0 \), the quenching rate constant is \( K_q \), value was calculated to be 6.50 and binding constant value \( K_b = 9.50 \times 10^8 \) also be calculated by plotting \( \log I - I/I \) and \( \log[Q] \) the value.
3.5. The selectivity of the cys-GQDs for Hg(II) ion

The selectivity and specificity is the most important factor for fluorescence sensing purpose. In this study, different interfering metal ions added to cys-GQDs for selectivity evaluation. The fluorescence intensity of cys-GQDs examined in the presence of As(III), As(V), Mg(II), K(I), Ca(II), Na(I), Pb(II), Co(III), Zn(II), Cd(II), Hg(II), Ni(II), Cu(II), Fe(III) ions (Figure 4). The concentration of each metal ions is 60 ppb. The interfering graph indicates that the fluorescence intensity of cys-GQDs is significantly quenched by only in case of presence Hg(II) ion, other metal ions have slight quenching. Here no metal ion interferes with the Hg(II) ion. Therefore cys-GQDs are successfully applied for sensing of Hg(II) ion. In the case of Hg(II) ion effectively quenching occur because of the special chemistry of cys-GQDs. Cys-GQDs have S-atom, which have a strong affinity to binding with Hg(II) ion. Hg(II) ion is soft acids and s- atom is soft base, so according to Pearson’s theory hard-soft acid-base S atom form co-ordination complex with Hg(II) ion is very rapidly. Therefore in the case of Hg(II) ion almost 80% quenching occurs.

![Figure 4](image)

Figure 4. The selectivity sensing response of cys-GQDs in the absence and presence of different interfering metal ions.

3.6. Optimization of cys-GQDs as a fluorescence sensor for detection of zinc ion:

For the detection of analyte suitable pH and reaction time is an important factor, pH and reaction time influence the interaction between Hg(II) ion and cys-GQDs, therefore an optimum pH and time are required. For this purpose, optimization is important for better results. Reaction time implies that interaction between bind site of cys-GQDs and Hg(II) ion required some time for the maximum quenching of fluorescence intensity. Fluorescence intensity of cys-GQDs analyzed at a different time (0 to 8 min). fig. implies that 0 to 3 min fluorescence intensity gradually decreases, then 3 min to 8 intensity is almost constant. Maximum quenching lies at 3 min that means all Hg(II) ions are bind with the active site of cys-GQDs. So optimum reaction time is 3 min, so this time is selected for the further investigation of Hg(II) ion.

In the order of detection of Hg(II) ion, the pH effect on the fluorescence intensity of cys-GQDs was investigated at different pH (2 to 12). The different pH of the buffer solution was prepared from dilute NaOH and H3PO4 solution. Figure 5 indicates the fluorescent intensity of cys-GQDs at a different pH. The fluorescence intensity gradually decreases from pH 2 to 8, after pH 8 fluorescence intensity increases. Maximum quenching occurs at a slightly alkaline medium. Fluorescence intensity of cys-
GQDs can be changed due to protonation and deprotonation of donor atom (-COOH, NH$_2$, -SH) present in the surface of GQDs. At acidic medium maximum donor atom protonated, therefore less Hg(II) ion interact GQDs while alkaline medium electron density increases. At alkaline medium more donor atom available on the surface GQDs, which is reacted with Hg(II), therefore maximum quenching takes place at pH 8. So pH 8 is chosen for further detection of Hg(II) ion. Fluorescence intensity cys-GQDs were investigated at different concentrations of Hg(II) ions by using a fluorescence spectrophotometer. Figure 5 indicated that fluorescence intensity regularly decreases with the increasing of concentration of Hg(II) ions. In this work, the concentration of Hg(II) ions is sensing from 10 ppb to 50 ppb. At 50 ppb approximately 80 % quenching occurs. Quenching of fluorescence intensity increases with the concentration of Hg(II) ion. Regularly quenching occur due to at high concentration of Hg(II) ions is interact with the maximum number GQDs and convert it in non-fluorescent material. Therefore fluorescence intensity of GQDs is decreasing.

Figure 5. (a) Emission fluorescence spectra of Cys-GQDs with different concentrations of Hg(II) ion (ranging from 10ppb to 50ppb). (b) Calibration curve of fluorescence quenching of cys-GQDs in the presence of a different concentration of Hg(II) ion (from 10 ppb to 50ppb). (c) the response of pH curve on the PL intensity in an aqueous solution of cys-GQDs (ranging from 2 to 12) (d) Time-dependent fluorescence emission spectra of cys-GQDs at pH 8.

4. Conclusion
In this study, a highly selective and sensitive cys-GQDs base nanosensor developed for rapid and robust detection of ppb level of Hg(II) ion in an environmental sample. The preparation of GQDs is a very easy and one step pyrolysis method. The size of the GQDs is 2-6 nm. Functionalization of GQDs surface by using cysteine amino acid enhances the quantum yield up to 41%. Thio functional group
enhances the selectivity and sensitivity of cys-GQDs towards Hg(II). It is found that the detection mechanism for this method is based on both dynamic and static quenching. The detection of the limit of Hg(II) ion is 0.64 ppb. Result optioned in this sensing method shows that cys-GQDs provides a selectively and sensitivity with reliable, cheap, excellent repeatability for detection Hg(II) ion.

Acknowledgment
Authors gratefully acknowledge the Director of National Institute of Technology Raipur, for providing laboratory facilities and financial support from Council of Scientific and Industrial Research (CSIR) in the form of SRF with Grant No. 09/1116(0001)2016-EMR-I, Pusa, New Delhi.

Reference
[1] Ali M, Hery S, and Putri S A 2018. In EDP Sciences 73-06002.
[2] Grandjean P, Satoh H, Murata K and Eio K 2010. Environmental health perspectives 118-1137.
[3] Vianna A D S, Matos E P D, Jesus I M D, Asmus C I R F and Câmara V D M 2019 Cadernos de saúde publica 35-00091618.
[4] Guzzi G and La Porta C A 2008 Toxicology 244-1.
[5] Riaz A, Khan S, Muhammad S and Shah M T 2019 Mine Water and the Environment 1-8.
[6] Ashraf M W and Mian A 2019 Bulletin of the Chemical Society of Ethiopia 33-573
[7] Xu H, Xia Y K, Li C J, Zhang J Y, Liu Y, Yi W, Qin Z Y, Chen L, Shi Z F, Quan K and Yang Z X 2019 Laboratory Investigation 99-588.
[8] Zangmo T and Siripinyanond A 2019 Analytica chimica acta 1085-29.
[9] Mishra S, Tripathi R M, Bhalke S, Shukla V K and Puranik V D 2005 Analytica Chimica Acta 551-192.
[10] Zhang D, Yang S, Cheng H, Wang Y and Liu J 2019 Talanta 199-620.
[11] Sun Z, Du J and Jing C 2016 J. of Envoir. Sciences 39-134.
[12] Leopold K, Foulkes M and Worsfold P J 2009 Analytical chemistry 81-3421.
[13] Sanchez-Rodas D, Corns W T. Chen B and Stockwell P B 2010 J. of Ana. Atomic Spectrometry 25-933.
[14] Huang H, Qu C, Liu X, Huang S, Xu Z, Zhu Y and Chu P K 2011 Chem. Communications 47-6897.
[15] Fan Y, Long Y F and Li Y F 2009 Ana. chimica acta 653-207.
[16] Hasanjani H R A and Zarei K 2019 Biosensors and Bioelectronics 128-1.
[17] Feng J, Guo Q, Liu H, Chen D, Tian Z, Xia F, Ma S, Yu L and Dong 2019 Carbon 155-491.
[18] Parlar M, Kayaaslan E, Ugus D, Bilen B, Erkturk H and Yazici T 2019 I. Soc. for Optics and Photonics 10929-109290.
[19] Mombrud, Romero M, Faccio R and Mombrud Á W 2019 Physica E: Low-dimensional Systems and Nanostructures 113-130.
[20] Pathan S, Jalal M, Prasad S and Bose S 2019J. of Materials Chemistry A 7-8510.
[21] Kalita H, Harikrishnan V and Aslam M 2014 Int J Nanotechnol 11-75.
[22] Wu Y, Wadia C, Ma W, Sadtler B and Alivisatos AP 2008 Nano Lett 8-2551.
[23] Dewangan P K, Khan F, Shrivas K and Sahu V 2019 J. of Radioanalytical and Nuclear Chemistry 320-757
[24] Alivisatos AP, Gu W and Larabell C 2005 Annu. Rev. Biomed Eng. 7-55.
[25] Yang Y, Xiao X, Xing X, Wang Z, Zou T, Wang Z, Zhao R and Wang Y 2019 Mat. R. Express 095615.
[26] Gu S, Hsieh C T, Tsai Y Y, Ashraf Gandomi Y, Yeom S, Kih K D, Fu C C and Juang R S 2019 ACS Applied Nano Materials 2-790.
[27] Du F, Sun L, Zen Q, Tan W, Cheng Z, Ruan G and Li J 2019 Sensors and Actuators B: Chemical 288-96.
[28] Chandra S, Laha D, Pramanik A, Ray Chowdhuri A, Karmakar P and Sahu S K 2016 Luminescence 31-81.
[29] Sadhanala H K and Nanda K K The J. of Phy. Chemistry C 119-13138.
[30] Han Y, Tang D, Yang Y, Li C, Kong W, Huang H, Liu Y and Kang Z Nanoscale7-5955.
[31] Fan M, Zhu C, Yang J and Sun D 2016Electrochimica Acta 216.
[32] Kamat P V 1993 Chemical Reviews 93-267.