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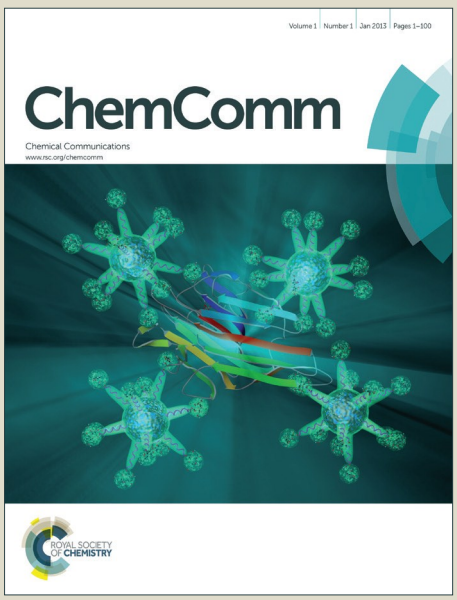

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Nitrogen Doped Thiol Functionalized Carbon Dots for Ultrasensitive Hg (II) Detection

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Abhishek Gupta, Abhishek Chaudhary, Pooja Mehta, Charu Dwivedi, Syamantak Khan, Navneet Chandra Verma, Chayan Kanti Nandi*

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Nitrogen doped PEGylated carbon dots (C-dots) have been synthesized for the detection of mercury ions (Hg^{2+}). The detection limit was found to be 6.8 nM, however upon functionalization with dithiothreitol (DTT), it reached to as low as 18 pM. The C-dots/Hg^{2+}system were also able to detect the biothiols efficiently.

Among different chemical forms such as metallic, ionic, organic and inorganic salts, the most stable solvated inorganic mercuric ion (Hg^{2+}) is one of the major health and environmental pollutant, which is routinely released from coal burning power plants, gold mining and many other industries.[1] The microbial bio-methylation in aquatic sediment of water soluble Hg^{2+} accumulates in human body, causing damage to the nervous system, immune system, liver, kidney and also have several other acute and chronic toxicity.[2-4] Because of such toxic and carcinogenic effect, United State Environmental Protection Agency (EPA) has set the limit of Hg^{2+} intake in drinking water less than 10 nM.[5] As a result, the detection of Hg^{2+} with ultrasensitivity and selectivity in water and food is a worldwide general concern to check and prevent Hg^{2+} poisoning. Over the past decades, several approaches such as inductively coupled plasma mass spectrometry (ICPMS),[6] cold vapour atomic absorption and fluorescence,[7,8] anodic stripping voltammetry,[9] auger spectroscopy[10] and X-ray absorption spectroscopy[11]have been extensively used for the detection of Hg^{2+}. Although, among all of the above, ICPMS provided the detection limit up to 0.1 nM,[6] but, all the techniques have their limited application for the accurate measurement of Hg^{2+} due to the costly instrumental facility, complexity in sample preparation, time consuming procedures and moreover requirement of trained personal.

To overcome these limitations, major focus of research is directed towards development of simple and cost effective colorimetric and fluorimetric sensors. Recently, thymine enrich ssDNA coordinated gold nanoparticles (GNP) have been developed as the fluoregenic and colorimetric sensors. A disposable strip biosensor for the visual detection of Hg^{2+} in aqueous solution has been constructed on the basis of Hg^{2+} triggered toehold binding and exonuclease III assisted signal amplification with a detection limit of 1 pM.[12] Gold nano rod based fluorescence resonance energy transfer assay showed a detection limit of 2.4 pM.[13] Lysozyme type VI stabilized gold nanoclusters assay showed the detection limit of 3 pM.[14] Reports are also available with a detection limit from 50 pM to 100 nM using DNA or mercaptooctanoic acid modified GNP (Table S1). However, the above techniques need expensive reagents, require harsh acidic or basic media and are tedious multistep time consuming process.[15] Further, most DNA-GNPs assays rely on accurate control of the detection conditions, such as temperature, which limits their practical application.

Here, we have shown a simple, low cost and less toxic nitrogen doped thiol functionalized PEGylated (PEG) carbon dots for the ultrasensitive detection of Hg^{2+} ion from distilled and real water. PEG is a hydrophilic linear polymer and is widely used as surface passivation agent for C-dots. It facilitates effective radiative recombination by stabilizing the various emission trap sites on the C-dots and hence increases the fluorescence intensity.[16,17] Therefore, PEGylated C-dots are synthesized using a simple synthetic protocol which requires only a generous microwave heating of a mixture of chitosan gel and PEG for 3 minutes and at a later stage a rather simple centrifugation process,[18] followed by functionalization with DTT (Supporting Information and Figure S1). With the surface functionalized DTT/C-dots, the detection limit was found to be 18 pM in distilled water, which is the so far best label free ultrasensitive report on Hg^{2+} sensing using any C-dots (Table S1). Our assay is extremely selective for Hg^{2+} even in the presence of high concentrations of other alkali or heavy metal ions. We have extended our work for biothiols (GSH and Cys) detection, as they are responsible for many oxidation, reduction and metabolic reactions in human body.[19]

The as synthesized C-dots (before DTT modification) showed the usual multicolor fluorescence with the maximum fluorescence intensity at around 412 nm, when excited at 280 nm (Figure S2a). The UV-Vis spectrum shows peak at 257 and 303 nm, which are attributed to the π-π* transition of the C-C structure, in agreement with previously reported carbon dots (Figure S2b).[20] The zeta potential of the C-dots was found to be -12.4 mV. The FTIR spectrum (Figure S2c) showed peaks at 1650 and 1574 cm^{-1} indicating the existence of -COOH and N-H bending respectively, while the broad peak with a maximum at 3331 cm^{-1} corresponds to the -OH and N-H stretching vibrations.[16] The peak at 2904 cm^{-1} corresponds to the -CH stretching vibration, while the peak at 1071 cm^{-1} corresponds to C-O-C bonds. The average size of the C-dots were found to be 8nm, which was confirmed by the height profile of the atomic force microscopy (AFM) image and the statistical analysis of more than 60-70 C-dots observed in the transmission electron microscope (TEM) (Figure S2d-f). The surface composition of the as-prepared C-dots was also characterized by X-ray photoelectron spectroscopy (XPS) (Figure S3a-d). The three peaks at 283.2, 401.0 and 529.8 eV in XPS spectrum (Figure S3a) can be attributed to C1s, N1s, and O1s, respectively.[21] This suggests that the synthesized C-dots majorly contains C and O and very small amount of N (% atom ratio of C: O: N is

67.04%: 30.50%: 2.46%). The three peaks of the C1s spectrum (Figure S3b) at 283.5, 284.8 and 286.5 eV are assigned to C−C, C−N and C=O respectively.[22,23] The two peaks at 530.7 and 531.3 eV in the O1s spectrum (Figure S3c) are assigned to C=O and C−OH/C−O−C groups, respectively, while the N1s spectrum (Figure S3d) shows two peaks at 397.8 and 398.2 eV which are assigned to the C−N−C and N−H groups, respectively.[24,25]Appearance of a new peak at 162.2 eV in the XPS spectrum, which is responsible for the bound thiol confirms the successful surface modification of C-dots by DTT (Figure S4a-b). A new band at 655 cm^{-1} in FTIR spectrum corresponding to C-S bonding also confirms the DTT functionalization on C-dots (Figure S4c). A weak band at 2426 cm^{-1} confirms the presence of the free SH group of DTT.[26] The zeta potential also reduced from -12.4 to -7.2mV upon DTT modification (Figure S4d). The DTT/C-dots showed the multicolor fluorescence like the synthesized C-dots, however the quantum yield (Φ) of C-dots decreased from 13.4% to 9.5 % after functionalization with DTT. Although, there is no significant change in the UV-Vis spectrum was observed (Figure S5a-b). The C-dots are readily water soluble, stable at high salt concentration and also showed maximum fluorescence intensity at physiological pH (Figure S6a-b). These results suggest that C-dots have great potential for sensing applications under physiological conditions. Extensive fluorescence quenching of DTT/C-dots is observed upon addition of Hg^{2+} indicating the interaction of DTT/C-dots with the added Hg^{2+} (Figure 1a & inset). This was further confirmed by the decrease in zeta potential of the system from -7.2 to -3.1 mV. The above complex formation is attributed to electron transfer reaction that occurs through chelation of Hg^{2+} with NH_2 and thiol group present on the surface.[27-28]

No change was observed in the UV-Vis absorption spectrum. However, a decrease in excited state lifetime after the Hg^{2+} complex formation confirms that the quenching process is dynamic in nature and takes place via an excited state electron transfer reaction (Figure S7a-b). To determine the detection limit, different concentrations of Hg^{2+} in the range from 0 to 2 × 10^4 nM were investigated. Figure 1b shows a gradual decrease in fluorescence intensity with increasing concentration of Hg^{2+}. The detection limit is estimated to be 18 pM at a signal-to-noise ratio of 3 and a fitting parameter of 0.998 (inset of Figure 1b). The kinetic experiment showed that Hg^{2+} could be detected within 5 minutes, just after the addition of Hg^{2+} in DTT/C-dots (Figure 1c). No interference of the halide ions such as chloride, bromide and iodide were observed on the fluorescence intensity of DTT/C-dots and hence Hg^{2+} detection (Figure S8a-e). Indeed, that the thiol group, which has the very strong affinity for Hg^{2+} bonding in the order of 10^{15} to 10^{20} (affinity for -NH_2 and carbonyl is 10 orders of magnitude less),[29] played the major role in the detection of Hg^{2+}. It is further confirmed by performing the same experiment with the as synthesized C-dots (without DTT modification). The sensitivity of the sensor is drastically reduced to 6.8 nM (Figure S9a-b), which is at least 300 times less than that of DTT/C-dots assay. Interestingly, in both the cases the sensitivity is much higher than the maximum level (10 nM) for Hg^{2+} in drinking water permitted by the EPA.

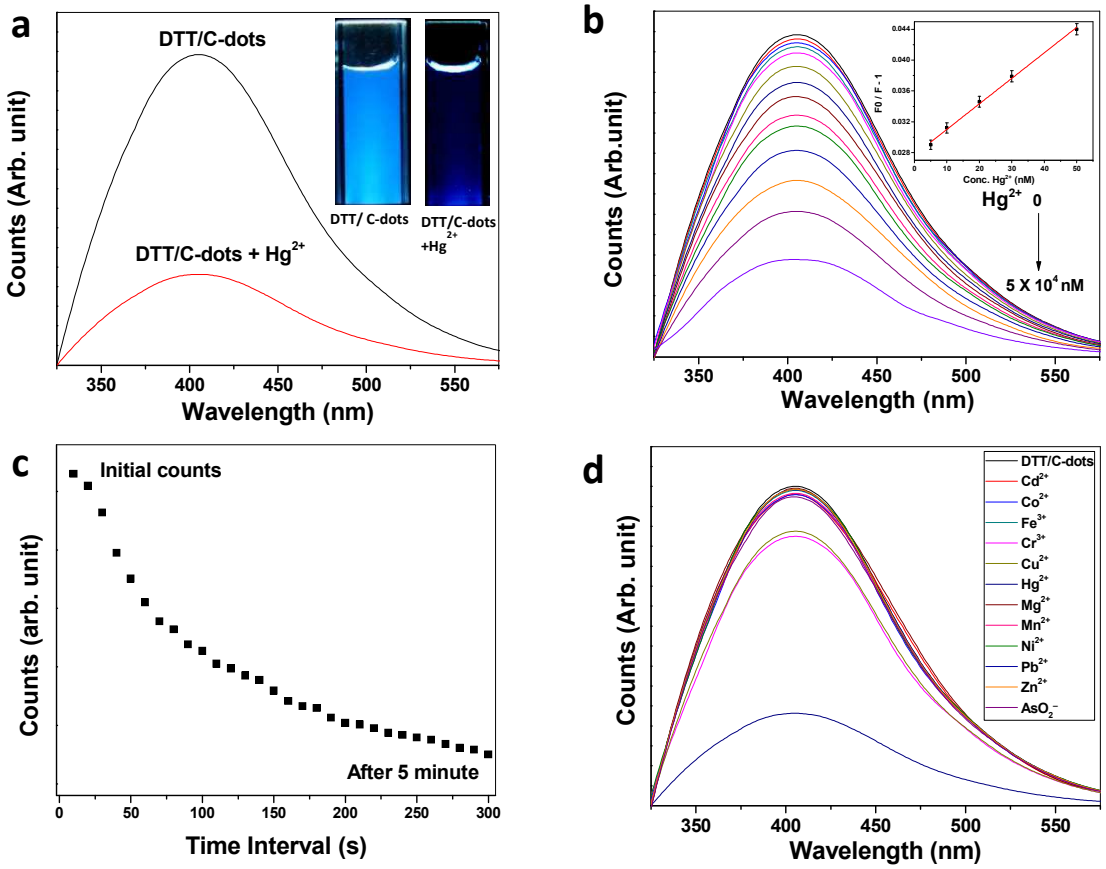

Figure 1. (a) Extensive fluorescence quenching of DTT/C-dots was observed upon addition of 50 μM of Hg^{2+}(inset shows the UV lamp images of DTT/C-dots without and with addition of Hg^{2+} (b) titrimetric quenching pattern of fluorescence intensity of DTT/C-dots with increasing concentration of Hg^{2+}(inset is the Stern-Volmer fitting to calculate the detection limit) (c) kinetic behaviour of the quenching pattern of DTT/C-dots and (d) fluorescence intensity of DTT/C-dots in the presence of various other metal ions (50 μM). individually to the DTT/C-dots. Each data point is presented as standard deviation from three replicate assays.

To determine the selectivity, which is one of the most important criteria for a good metal ion sensor, the influence of various relevant metal ions was checked. While Cr^{3+} and Cu^{2+} ions showed only little quenching, no other metal ions showed any obvious fluorescence changes (Figure 1d and Figure S10). These results clearly demonstrate that the DTT/C-dots based fluorescence sensor is highly selective toward Hg^{2+} over the other metal ions. On the other hand, thiol containing GSH and Cys modified C-dots do not show such high sensitive and selective Hg^{2+} detection. This may be due to the non-availability of the free thiol for Hg^{2+} bonding (Figure S11). Further, we also checked whether the C-dots assay could detect efficiently Hg^{2+} in the presence of other metal ions by monitoring the changes in the fluorescence spectra of DTT/C-dots in the presence of all metal ions including and excluding Hg^{2+}. Results showed that there was no change in the fluorescence intensity of the C-dots in the presence of all the metal ions (excluding Hg^{2+}), however, extensive quenching was observed when Hg^{2+} added to the system (Figure S12).

The excellent selectivity combined with high sensitivity and fast response of DTT/C-dots to Hg^{2+}suggested that our method might be directly applied for detecting Hg^{2+} in real samples, where several contaminants are present (Table S2 & Table S3). Therefore, we further examined the practicality of the assay by testing Hg^{2+}in real water.[30] For this purpose, river water was collected from the Uhl River in Mandi district, Himachal Pradesh province, India. The water sample, before performing any sensing experiment, was filtered through a 0.22 µm membrane and then centrifuged at 15000 RPM for 30 minutes. The supernatant water was spiked with standard solutions containing different concentrations of Hg^{2+}.[31-32] It is observed that the fluorescence intensity decreases with increased concentration of Hg^{2+} (Figure 2a**)** and the detection limit was determined to be 45 pM (inset of Figure 2a). Despite of the interference from numerous minerals and organics existing in river water, this sensing system can still detect Hg^{2+} very efficiently. In a similar way, the detection limit of Hg^{2+} spiked in tap water was also determined as 50 pM (Figure S13 and inset).

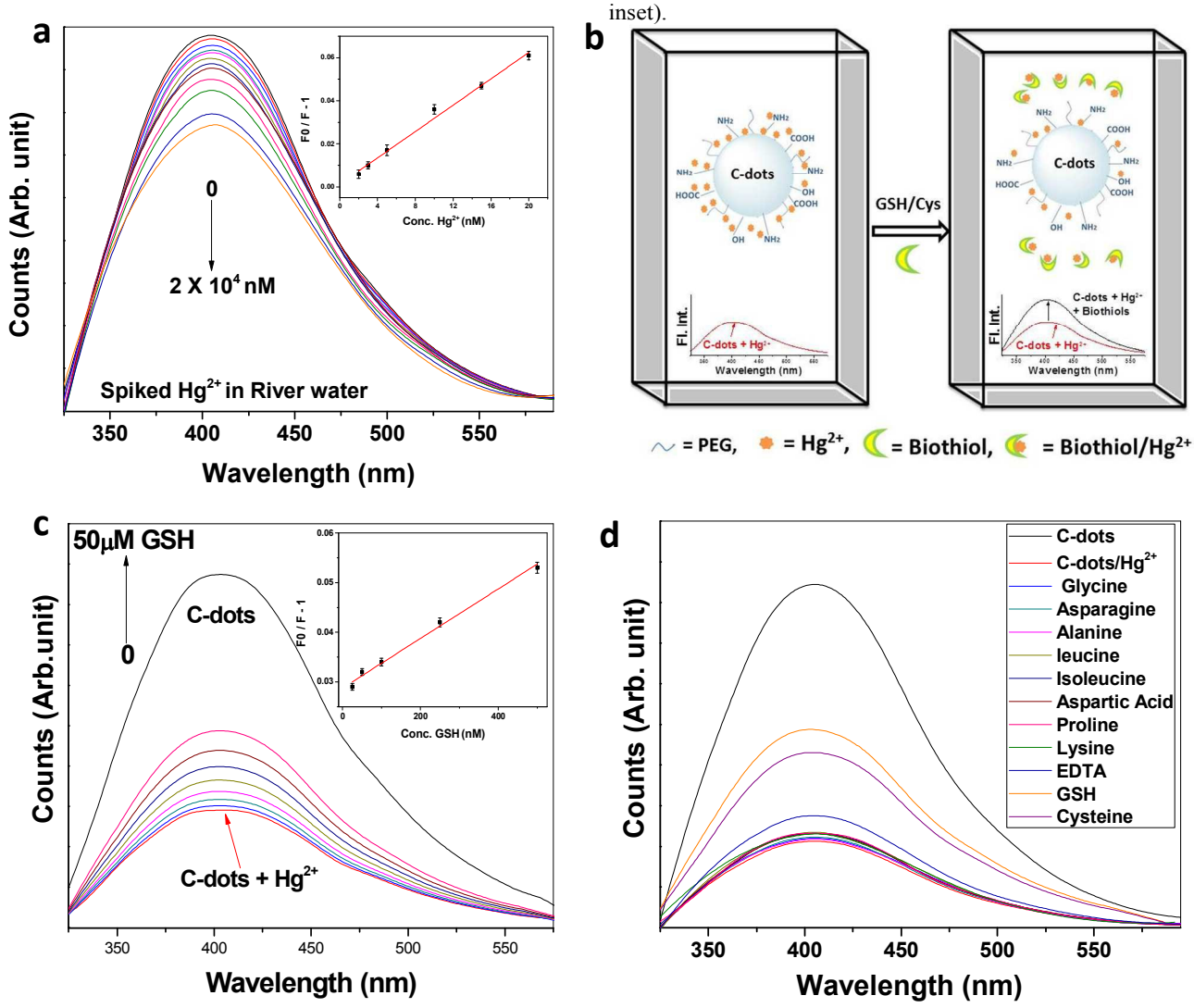

Figure 2. (a) Fluorescence quenching pattern of DTT/C-dots in presence of Hg^{2+} spiked in river water (inset is the Stern-Volmer fitting to calculate the detection limit) (b) Schematic diagram shows the recovery of quenched fluorescence in presence of biothiols (GSH or Cys) (c) Experimental fluorescence recovery of quenched C-dots /Hg^{2+}assay after the addition of GSH (inset is the Stern-Volmer fitting to calculate detection limit) and (d) Selectivity of the assay for the biothiols (GSH and Cys) in presence of several amino acids (50 µM). Each data point is presented as standard deviation from three replicate assays.

Finally, we used the unmodified C-dots/Hg^{2+} assay for biothiols sensing based on a competition mechanism by using reverse quenching process. Glutathione (GSH) and Cysteine (Cys) were chosen for the study. Here, 0-50 μM concentration of biothiols has been used. In the presence of biothiol, the fluorescence quenching of C-dots (due to C-dots/ Hg^{2+} complex formation) could be reversed as Hg^{2+} is released from C-dots surface and it complexes with GSH and Cys due to strong binding preference (Figure 2b).[33-34] The detection limits are 57 nM and 72 nM for the GSH and Cys respectively, with a linear range from 25-500 nM (Figure 2c and Figure S14). The recover tendency of GSH is more than Cys, which was similar to previous reports.[35] The reasons for the better restoration of fluorescence intensity by GSH than Cys could be due to the multi-dentate binding site and the chelating structure of GSH, which might lead to the more coordination with Hg^{2+}. Control experiment (without addition of Hg^{2+}) revealed that the fluorescence intensity of pure C-dots had a negligible change in the presence of biothiols (Figure S15). For confirming the specificity for thiol compound detection, the fluorescence response of C-dots/Hg^{2+} with various amino acids and EDTA, which do not contain any thiol group, was performed (Figure 2d). In this case, no recovery of fluorescence intensity was observed, suggesting that C-dots/Hg^{2+} assay is accurate and reliable for biothiols determination in practical samples analysis (Figure S16).

Conclusions

In summary, nitrogen doped thiol functionalized PEGylated C-dots were synthesized for the ultrasensitive picomolar level fluoregenic detection of Hg^{2+}. Additionally, this sensing assay exhibits high selectivity toward Hg^{2+}over several other metal ions. The unmodified C-dots/Hg^{2+} assay could detect the biothiols very efficiently. We expect that this strategy may offer a new approach for developing low cost and sensitive sensors in biological and environmental applications.

Notes and references

[a]*Chayan K. Nandi
School of Basic Sciences,
Indian Institute of Technology, Mandi,
Himachal Pradesh-175001, India
Email: chayan@iitmandi.ac.in

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