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Lysine and Dithiothreitol Promoted Ultrasensitive Optical and Colorimetric Detection of Mercury Using Anisotropic Gold Nanoparticles

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Abhishek Chaudhary[†], Charu Dwivedi^{†*}, Mohit Chawla, Abhishek Gupta, Chayan K. Nandi*

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Using Lysine (Lys) and dithiothreitol (DTT) as aggregation promoters, an ultrasensitive and highly selective colorimetric assay for detection of mercury (Hg^{2+}) ions has been developed with anisotropic gold nanoparticles (GNP). The detection limit of the assay was determined to be 27 pM and 58 pM for Hg^{2+} in the deionized water and tap water, respectively. The mechanism of the sensing is explained on the basis of possible mercury-gold amalgam formation and subsequent aggregation of the GNP on addition of Lys and DTT. The kinetics data indicate that the sensor can be effectively used for on-site and real-time Hg^{2+} detection.

Mercury has been widely recognized as the major environmental pollutant in all of its physical and chemical forms which causes toxic effects and leads to serious and permanent damage of the central nervous system.¹ The world health organization (WHO) and the U.S. environmental protection agency (EPA) has set the maximum allowable levels of Hg^{2+} in drinking water at 30 and 10 nM, respectively.² Therefore, it is imperative to develop methods for the highly sensitive and selective determination of mercury for the environment and human health. Towards this goal, new materials and processes are under consideration and development around the world. Many methods have been proposed to detect Hg^{2+} in environmental and biological samples based on fluorophores, proteins, oligo-nucleotides, polymers, etc. (Table S1, Supporting Information).³⁻⁵ However, these methods have a number of associated disadvantages limiting their practical application for example higher sample analysis time, complicated analysis procedure, requiring intricate and expensive instruments, poor selectivity and low sensitivity towards Hg^{2+} ions than the standard of the EPA.

With the fast development of nanotechnology, it is possible to tailor the morphology and surface chemistry of metallic nanoparticles by introducing suitable reagents, which

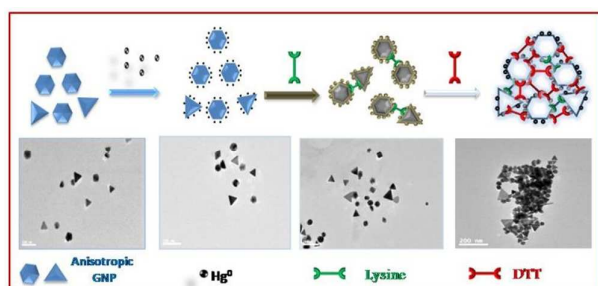
have opened new opportunities to detect metal ions selectively even in the nano molar range.⁶⁻⁹ In the past decades various colorimetric and fluorometric detection systems have been demonstrated for sensing of mercury ions in contaminated water (Table S1, Supporting Information).¹⁰⁻¹¹ Particularly colorimetric assays have attracted considerable attention for the toxic metal ion detection because of their simplicity, low cost and on-site application for environmental monitoring.¹²⁻¹⁴ Among several metal nanoparticles, GNP have received great attention in the development of visual sensing schemes owing to their surface plasmon resonance (SPR) which is extremely sensitive to their nature, size, shape, interparticle distances, and the nature of the surrounding media.¹⁵ Recently, Kim et al. have reported dithioerythritol-modified gold nanoparticles for the selective recognition of Hg^{2+} in aqueous media with detection limit (DL) of 100 nM.¹⁶ Li et al., have demonstrated quantum dots/DNA/GNP ensemble based nanometal surface energy transfer sensor for Hg^{2+} detection with DL of 2 nM.¹⁷ Chen and co-workers have proposed gold Nano rod-fluorescence resonance energy transfer based fluorometric sensor for Hg^{2+} with the DL of 2.4 pM.⁸ Kong et al. and Cai et al., in separate works, have developed sensor, for selective and ultrasensitive detection of Hg^{2+} ions in aqueous solution, based on the strong and specific binding of Hg^{2+} by two DNA thymine bases, using GNP, to achieve signal amplification with DL of 50 pM and ~3 nM, respectively.¹⁸⁻¹⁹ Although, these sensors have shown quite high selectivity and sensitivity, but these systems involve complex synthetic procedure. Moreover, the DNA oligomers used are quite expensive and need specific sample pre-treatment which limits their applications for many real samples. However, the main step involved in the sensing action of GNP based sensors is because of the fact that the SPR band of the GNPs is highly sensitive to the distance between each nanoparticle. The Hg^{2+} induced aggregation of alkanethiol or carbonyl capped or amino group functionalized GNP, results in the formation of a new SPR band at longer wavelengths and corresponding color change. The affinity constant for mercury bonding to thiolate anions is in the order of 10^{15} to 10^{20} . In

School of Basic Sciences, Indian Institute of Technology Mandi, Himachal Pradesh, 175001, India

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

contrast, the affinity constants for mercury bonding to oxygen- or nitrogen-containing ligands (e.g., carbonyl or amino groups) are about 10 orders of magnitude lower.¹

In this work, the high affinity of mercury towards GNP, amino and alkanthiol groups has been utilized to detect Hg^{2+} ions in as low as picomolar concentration. We have also exploited the variation in the surface energies of the different facets of the anisotropic GNP together with Lys and DTT (both acting as aggregation promoter) to develop a colorimetric sensor for Hg^{2+} ions. Schematic of the sensing action is described in Scheme 1. Firstly, Hg^{2+} in the water sample is reduced to Hg^0 by using strong reducing agent NaBH_4 .²⁰ Then subsequent introduction of this reduced mercury followed by Lys and DTT addition to the GNP solution induced huge red shift in the SPR band at 590 nm of GNP and color transition from blue to colorless within 15 minutes of total contact time. This phenomenon is supposedly occurring due to adsorption of Hg^0 onto the specific facets of GNP accompanied with binding of Lys with GNP, and finally DTT induced aggregation of the sensing system (GNP- Hg^0 -Lys).



Scheme 1: Schematic showing detection of Hg^{2+} ions by anisotropic GNP in presence of Lys and DTT.

The anisotropic GNP are synthesized by the protocol developed by Chen et al., in 2014.²¹ The synthesis method used is one pot, seedless growth process with high yield. The UV-Vis spectra of the synthesized GNP are shown in Figure 1a (trace a).

The synthesized GNP shows a sharp SPR band at 590 nm along with weak shoulder at 577 nm, assigned to in plane and out of plane dipole resonances, respectively. The average size of the synthesized GNP is determined from TEM analysis and found to be ~ 40 nm (Figure S1, Supporting Information). Upon addition of reduced mercury, a decrease in the absorption intensity of the GNP is observed, which could be due to the deposition of mercury on the surface of gold nanoparticles and resulting in the change in the SPR of the same (Figure 1a (trace b)). It is reported that the (111) facet of hexagonal and triangular plate GNP are of low energy and hence any incoming moiety will bind preferentially on the same facet.²²⁻²³ Because of this preferential binding, the local concentration of the incoming chemical moieties will be higher on these planes. Based on these facts, it is believed that the Hg^0 is getting adsorbed preferentially on the (111) facets of the anisotropic nanoparticles resulting change in local environment around GNP and hence decrease in the absorption spectra. Our speculation is further confirmed by HRTEM analysis of anisotropic and spherical GNP before and after Hg^0 adsorption (Figure 2a and 2c). Figure 2b and 2d shows the lattice resolved image of anisotropic and spherical GNP before and after Hg^0 addition, respectively. In the individual HRTEM image of GNP, a lattice spacing of 2.4 \AA is seen which corresponds to (111) facet of GNP (Figure 2b).²⁴ But after addition of Hg^0 , otherwise smooth edges of the GNP becomes highly corrugated indicating amalgamation and

the nanoparticle also exhibits the lattice spacing of 2.23 \AA , which is attributed (101) plane of Au_3Hg alloy (Figure 2d).²⁴

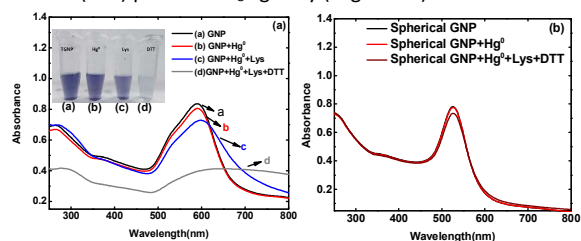


Figure 1: UV-Vis spectra of (a) anisotropic GNP and (b) spherical GNP, in the absence and the presence of Hg^0 , Lys, and DTT, respectively.

To further confirm the Hg^0 -Au amalgamation, XRD of the GNP before and after Hg^0 adsorption was recorded. A new weak peak at 2θ value of 40.7° is observed in the XRD pattern of the GNP corresponding to Au_3Hg alloy formation (JCPDS 04-0808) (Figure S2, Supporting Information). After adsorption of Hg^0 onto the GNP, Lys is introduced in the system and a red shift of 9 nm along with decrease in the absorption intensity by 15% is observed in the SPR band at 590 nm (Figure 1a (trace c)). This could be due to the surface complexation of the amino acids and possibly some agglomeration of the GNP consequent to surface modification.

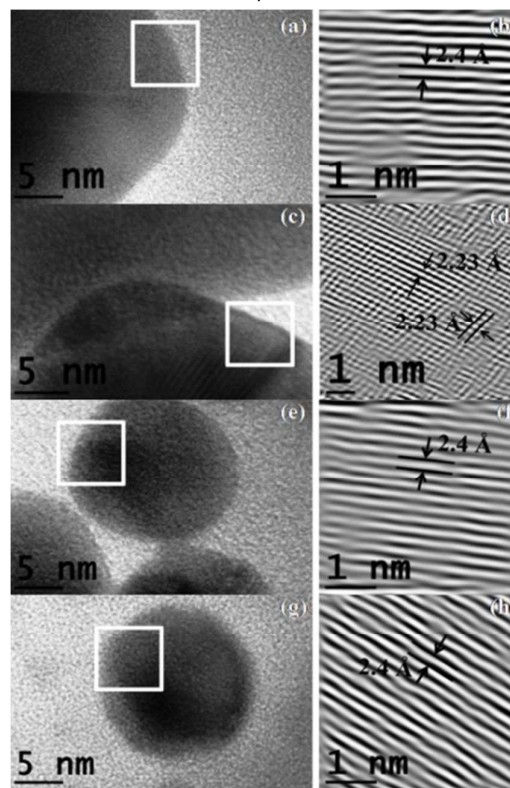


Figure 2: HRTEM image of (a-d) anisotropic GNP and (e-h) spherical GNP before and after addition of Hg^0 , respectively. Image (d) shows distortion in the lattice fringes of the anisotropic GNP after addition of Hg^0 .

In the last step, DTT is added as aggregation promoter which completes the color transition from blue to colorless. DTT is a strong chelating reagent and the presence of two thiol groups allows it to form specific and very stable complexes with mercury as well as GNP.²⁵ As it is evident from Figure 1a (trace d), on addition

of DTT, there is a huge red shift of 50 nm in the SPR band at 590 nm. To thoroughly investigate the role of anisotropy, Lys and DTT in the developed assay, similar experiments were performed with spherical GNP. It is clear from the Figure 1b that there is no significant shift in the absorption spectra of the spherical GNP even after addition of Hg^0 , Lys and DTT. Only slight decrease in the absorption intensity at 524 nm is observed indicating change in the dielectric constant of the surrounding media. Moreover, no change in the lattice spacing of the spherical GNP before and after addition of Hg^0 is observed in the HRTEM image, denying possibility of amalgamation at such low concentration of Hg^0 (Figure 2e-2h).

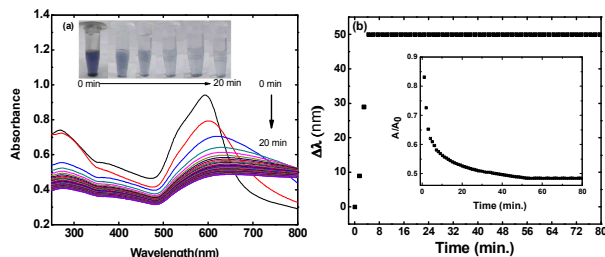


Figure 3: Effect of contact time on (a) UV-vis spectra and (b) SPR band at 590 nm of GNP/ Hg^0 /Lys/DTT system.

This could be due to the reason that the spherical GNP are not able to undergo preferential surface binding at certain sites due to its isotropic nature and hence the extent of the Hg^0 adsorption and coverage with Lys and DTT is also less as compared to the anisotropic particles.²²⁻²³ Therefore, the spherical GNP are not able to generate any colorimetric response on addition of Hg^0 , Lys and DTT. To check the effect of Lys on inducing particles aggregation, the experiments are performed at different concentration of Lys and with different amino acid as well. It is evident from Figure S 3a and S 3b, Supporting Information, only Lys is capable of inducing colorimetric response among the other 6 amino acids tested. It is notable here that Lys contains two primary amino groups which can interact with more than one Hg^0 adsorbed GNP and promote aggregation.²⁶ The effect of Lys concentration and contact time is investigated and 8.5 μM is chosen as the optimum concentration which does not induce any significant change in the SPR of GNP system, even after 2 h, in the absence of Hg^0 , DTT or both. (Figure S 4a and S 4b, Supporting Information). This observation is also supported by TEM analysis of the GNP- Hg^0 -Lys in which no significant aggregation is observed after 2 h of contact time (Figure S 5a and S 5b, Supporting Information).

Therefore, to achieve Hg^0 induced change in the SPR in a workable time frame, additional chemical agent, DTT, is added into the system. The possibility of only DTT induced aggregation, in the absence of Hg^0 , is overruled by checking effect of contact time on the SPR of GNP with the optimized concentration of DTT (16 μM) (Figure S 6a and S 6b, Supporting Information). On the other hand, when Hg^0 (30 nM) is added into the GNP solution along with Lys and DTT, the extinction spectrum shows a massive red shift along with color transition from blue to colorless) indicating particle aggregation (Figure 1 a, (trace d)). The aggregation of the GNP in presence of Hg^0 , Lys and DTT is further confirmed by DLS and TEM measurements (Figure S 7a and S 7b, Supporting Information). The response time of the assay is also standardized by monitoring the changes in SPR of the GNP- Hg^0 -Lys-DTT system with respect to time. From Figure 3a and 3b it is clear that, the absorption intensity of the mentioned system is reduced by 66 % at 590 nm along with a very prominent red shift of 50 nm, within 5 min of contact time. These results show that, the developed assay is quite sensitive and can detect Hg^{2+} in the sample within 15 min of total contact time (including equilibration of Hg^0 with GNP). To establish the selectivity of the developed sensor for Hg^{2+} ions over a variety of environmentally relevant metal ions, As^{3+} , Cr^{3+} , Cd^{2+} , Pb^{2+} , Ni^{2+} , Ba^{2+} , Zn^{2+} , Mg^{2+} and Ba^{2+} were analyzed under identical conditions. The results indicate that the developed assay is highly selective for Hg^{2+} even in the high concentration of other metal ions (1 μM), as no significant change in the SPR as well as color of the sensing system is observed (Figure 4a & inset).

The sensitivity of the developed sensor was realized by examining the response of GNP/Lys/DTT solution towards different concentrations of Hg^{2+} . A good linear response ($R=0.982$) of absorbance change against the Hg^{2+} ion concentration over the range from 0.01 to 1.0 nM is observed (Figure 4b and 4c). From the slope of the linear plot (inset Figure 4c), the detection limit was estimated to be 27 pM at a signal-to noise (S/N) ratio of 3, which was much lower than the EPA standard for the maximum allowable level (10 nM) in drinking water. To demonstrate the practical application potential of the developed assay in environmental analysis, we analyzed tap water samples, sampled from Mandi, India (see Table S2, Supporting Information for ion composition of the tap water) after spiking with Hg^{2+} . And, a linear correlation ($R^2=0.991$) between ratio A/A_0 and concentration of Hg^{2+} spiked into drinking water over the range 0.01 – 50 nM is obtained (Figure S 8, Supporting Information). The response of the sensor in double

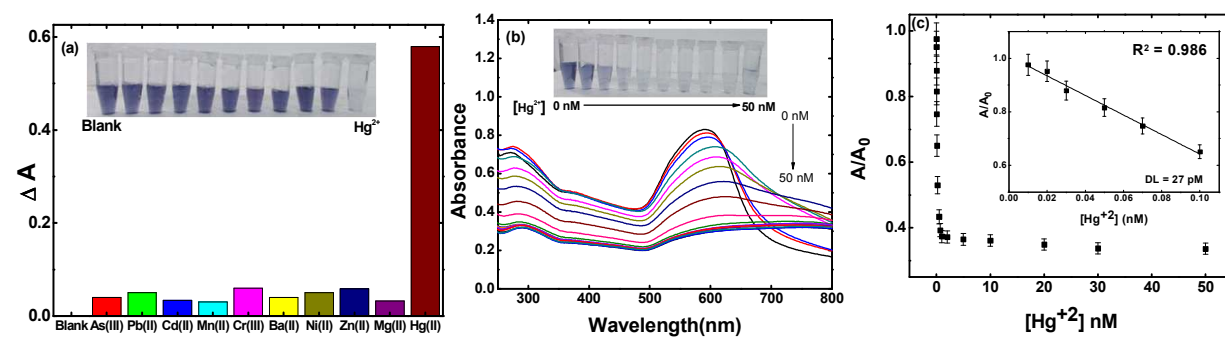


Figure 4: (a) Change in the absorption intensity ($\Delta A=A_0-A$) of the SPR band at 590 nm of GNP in the absence (A_0) and presence (A) of different metal ions and (inset) change in the color of GNP/Lys/DTT system in the presence of different metal ions, (b) UV-vis spectra of GNP/Lys/DTT system equilibrated for 15 min, with different concentrations of Hg^{2+} (0.01 nM-50 nM) (inset) change in the color of GNP/Lys/DTT system in presence of Hg^{2+} (0.01 nM-50 nM) (c) change in absorption intensity of the SPR band at 590 nm with change in concentration of Hg^{2+} , each data point is presented as standard deviation from three replicate assays.



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distilled water and tap water are very similar suggesting that the developed colorimetric assay can detect Hg^{2+} even in the interfering matrix of the tap water. The DL for Hg^{2+} in tap water experiments is determined to be 58 pM ($S/N=3$). Although, a slight decrease in the response of the assay in real sample is observed, still the DL is much below the EPA defined maximum allowable level in drinking water.

Conclusions

In brief, this study reports a new, simple and sensitive colorimetric assay for detection of mercury ions in water samples using anisotropic gold nanoparticles. This assay utilizes Lys and DTT as aggregation promoter of GNP. The most distinctive thing about this assay is the fact that it is based on the anisotropic GNP synthesized by seedless growth process which is used without any tedious surface modification steps. In addition to the excellent sensitivity ($DL=27$ pM), and selectivity, the ease of synthesis and application makes the sensor practical and cost-effective for monitoring low level contamination of Hg^{2+} in environmental samples.

Acknowledgments

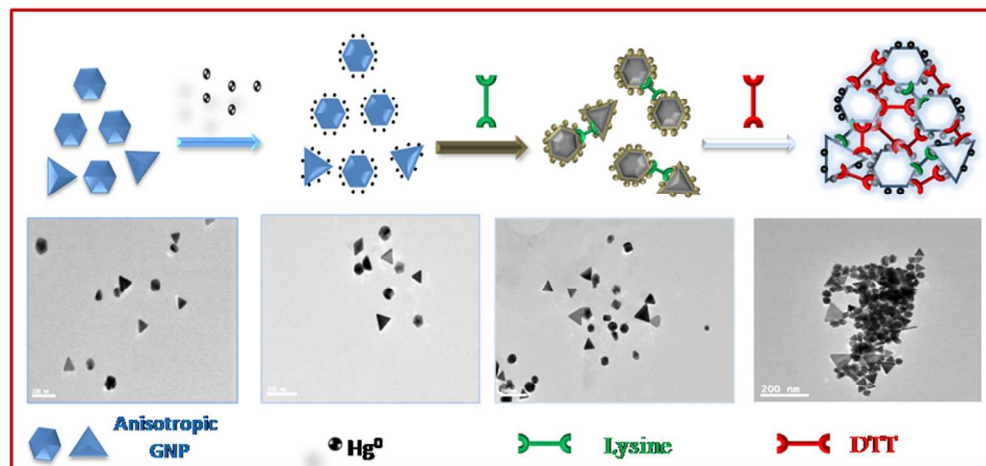
The authors acknowledge home institute, Indian Institute of Technology Mandi (IIT Mandi) and Department of Science & Technology India (Project SR/FT/CS-152/2011) for all the financial support. Advanced Material Research Centre (AMRC) Facilities of IIT Mandi is also being acknowledged.

Notes and references

Materials and methods; TEM image of anisotropic GNP before addition of Hg^0 ; X-ray diffraction patterns of GNP in the presence Hg^0 and absence of Hg^0 ; change in hydrodynamic radius of GNP on addition of Hg^0 , Lys and DTT; effect of amino acids on the UV-vis spectra and color of the system; optimization of the concentration of Lys and DTT and the contact time for the sensing assay; physicochemical properties of the tap water of Mandi, India; colorimetric assay performed on the tap water samples, spiked with different concentrations of Hg^{2+} .

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