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COMMUNICATION

Highly selective and sensitive colorimetric detection of Hg (II) ions using green synthesized silver nanoparticles

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A facile, one-pot and green method has been developed for the synthesis of silver nanoparticles (AgNPs) using sodium salt of *N*-choly-L-cysteine (NaCysC) as capping and reducing agent under ambient sunlight irradiation. The synthesized nanoparticles was used for highly selective and sensitive colorimetric detection of Hg²⁺ in aqueous medium with a limit of detection of about 8 nM.

Contamination of the environment by heavy metal ions has been a severe problem faced by the world for the past several decades. Detection of heavy metal ions at concentration levels down to parts-per-billion is a problem of fundamental and practical interest due to the high toxicity of the metals and its role in environmental pollution.^{1,2} Among various heavy metals, mercury ion (Hg²⁺) is of great concern since its compounds are highly toxic. Solvated mercuric ion (Hg²⁺) is one of the most stable inorganic forms, which can damage the brain, heart, kidney, stomach, and intestines, even at very low concentrations.^{3,4} Hence, the development of new sensors capable of detecting traces of Hg²⁺ ions in wastewater and in biological systems is extremely appropriate for controlling mercury pollution. Various methods have been developed for the detection of Hg²⁺ using fluorophores,⁵ oligonucleotides,⁶ polymers,⁷ metal nanoparticles,⁸ and semiconductor quantum dots^{9,10} as sensing probes. Among them metal nanoparticles (MNPs) shows a promising way for the colorimetric detection of heavy metal ions even at trace levels.¹¹ In particular, noble metal nanoparticles silver and gold (Ag and Au NPs) have attracted much research interests owing to their size and shape dependent optical properties, high molar extinction coefficient, excellent stability, good biocompatibility and water solubility.¹²⁻¹⁶ However, the development of facile, low-cost, highly sensitive and eco-friendly sensors for the detection of Hg²⁺ ions in the environmental systems as well as in real-time still remains challenging.

To overcome these problems, attention has been paid on eco-friendly methods and greener energy sources like solar light for the preparation of these MNPs. Driven by the need of green chemistry

principles,¹⁷⁻¹⁹ significant growth has been achieved in interdisciplinary nanoscience to minimize/reduce the health risk of human and the environment. Only few research groups have laid effort to synthesize noble metal nanoparticles under solar light irradiation.²⁰⁻²³ Since, it is widely considered to be the largest source of carbon neutral renewable energy, non-toxic, non-polluting, economical and traceless in chemical processes. Cheng and co-workers reported the synthesis of AgNPs using sunlight irradiation and showed that toxicity of the AgNPs were significantly reduced.²⁰ AgNPs have been synthesized using garlic extract under sunlight irradiation by Rastogi *et al.*²¹ Recently we reported the synthesis of AgNPs and AuNPs under sunlight irradiation through a green chemical method in the view of chemical and biological application.²² Very recently Yin *et al.*, reported that ionic Ag and Au were converted to metallic Ag and Au nanoparticles using dissolved organic matter under sunlight irradiation.²³ These NPs were sensitive to their surrounding environment due to their unique localized surface plasmon resonances (LSPRs) and it can be used as a sensor for the colorimetric detection.²⁴⁻²⁹

There are many reports available for the colorimetric detection of Hg²⁺ ions using AgNPs. Wang *et al.*, reported the colorimetric detection of Hg²⁺ ions using unmodified silver nanoparticles in aqueous medium.³⁰ Green synthesized AgNPs has been used for the highly selective detection of Hg²⁺ without modification.³¹ Poly(amidoamine) dendrimers stabilized AgNPs has been used as sensor for the detection of Hg²⁺ in aqueous solution.³² The enhanced sensitivity of AgNPs using SERS technique for the detection of Hg²⁺ has been reported by Ren *et al.*³³ A label free colorimetric detection of Hg²⁺ has been carried out using gelatin stabilized AgNPs in waste water and blood samples.³⁴ 4-mercaptopyridine (4-MPY) functionalized AgNPs has been used as a SERS probe for the detection of Hg²⁺ ions.³⁵

Hence, in this communication, for the first time we report a size and shape controlled synthesis of AgNPs using sodium salt of *N*-choly-L-cysteine (NaCysC) as a reducing and stabilizing agent under sunlight irradiation in aqueous medium. The *N*-choly-L-cysteine was prepared by adopting our previously reported method^{22a} and the obtained product was characterized by FT-IR and NMR spectroscopy (Fig. S1 ESI†). The prepared NPs were used as a probe for the colorimetric detection of Hg²⁺ ions in environmental samples. In order to optimize the reaction conditions, 0.2 mL of AgNO₃ (1.0

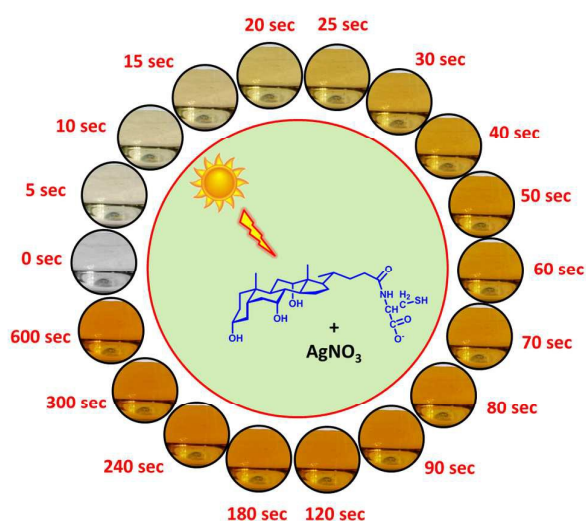
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mM) solution was added to different concentrations of NaCysC (2.0 mM) ranging from 6.6×10^{-5} M to 1.0×10^{-3} M and the final volume was adjusted to 3 mL using triple distilled water. Then the mixture was shaken well and kept under sunlight. The formation of NPs could be visually observed from the colour change of the reaction mixture.

Initially, the colourless reaction mixture change to lemon yellow within a minute and then the colour deepened into bright yellow at the end of the reaction. The whole reaction was completed within 5 minutes (Scheme-1). The Fig. 1 shows UV-visible spectra of AgNPs formation as a function of various concentration of NaCysC. The SPR peak intensity gradually increases at 420 nm with increase in the concentration of NaCysC up to 3.3×10^{-4} M, which could be due to the formation of NPs. Above which, no significant change in the SPR peak intensity was observed indicating that the reaction was completed. After the synthesis, the unreacted metal salts and capping agent was removed by centrifugation.



Scheme. 1. Schematic presentation of AgNPs formation using NaCysC at different time intervals under sunlight irradiation.

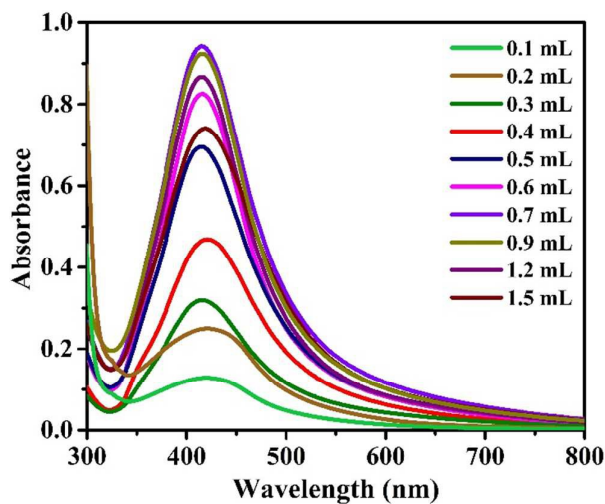


Fig. 1. UV-visible spectra of NaCysC stabilized AgNPs with different concentrations of NaCysC (6.6×10^{-5} to 1.0×10^{-3} M).

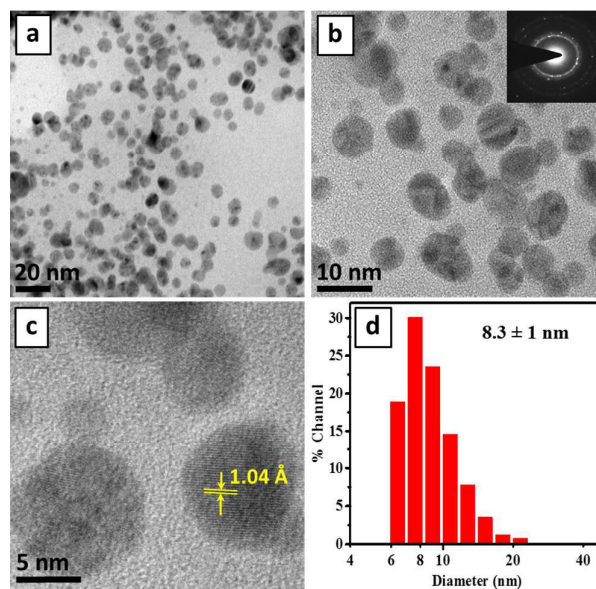


Fig. 2. TEM images of NaCysC stabilized AgNPs at different magnifications (a-d).

The purified NPs were dispersed in water and then stored at ambient condition for further uses. These NPs were highly stable for more than 6 months without any sign of aggregation (Fig. S2 ESI†). The size and shape of the prepared NPs were further characterized by TEM analysis as shown in Fig. 2. The TEM images clearly show that the NPs were spherical in shape with multi-dispersion.

The average size of the particles was found to be 7.8 nm. Also, from the DLS studies the hydrodynamic radii of the NaCysC stabilized AgNPs was measured as 8.3 ± 1 nm. The inset of Fig. 2b shows the corresponding SAED pattern of NaCysC stabilized AgNPs. The Scherrer ring pattern obtained for these AgNPs clearly shows that the acquired particles were nanocrystalline nature. Besides, the crystalline pattern of the prepared AgNPs was confirmed by powder XRD analysis. The XRD pattern of the AgNPs shows well defined peaks at $2\theta = 38.2, 44.3, 64.5, 77.4$ and 81.5 assigned to $\{111\}, \{200\}, \{220\}, \{311\}$ and $\{222\}$ lattice planes respectively for the fcc structure of AgNPs (Fig. S3 ESI†). The elemental composition of NPs was confirmed by EDAX and XPS measurements (Fig. S4 and S5 ESI†).

At optimized condition the prepared NPs was tested for their application as a colorimetric sensor because of their size/shape dependent optical properties and tendency to agglomerate in the presence of analyte. The interaction of AgNPs with various metal ions (100 nM) was monitored by UV-visible spectral change and also the colour change was visualized with naked eyes (Fig.3). Out of large number of metal ions tested ($\text{Ba}^{2+}, \text{Cd}^{2+}, \text{Co}^{2+}, \text{Cr}^{3+}, \text{Cu}^{2+}, \text{K}^{+}, \text{Li}^{+}, \text{Mg}^{2+}, \text{Mn}^{2+}, \text{Na}^{+}, \text{Ni}^{2+}, \text{Pb}^{2+}, \text{Zn}^{2+}$ and Hg^{2+}), only Hg^{2+} ions exhibited significant spectral change and the colour of the solution changed from yellow to colourless which are easily observed with naked eyes. This indicates that the prepared AgNPs can be used as colorimetric sensor to detect Hg^{2+} ions in aqueous medium without any modification. The selective interaction of NPs with Hg^{2+} ions could be due to the presence of thiol groups on the surface of the NPs. The responses of AgNPs to individual metal ions with and without addition of Hg^{2+} were evaluated based on the changes in the absorbance intensity at 420 nm. The bar diagram in Fig. 4 shows the

selectivity and sensitivity among other competing metal ions. It was noted that the coexistence of other competing metal ions shows significant spectral changes upon addition of Hg^{2+} ions. These results indicate that the sensing of Hg^{2+} ions is also possible in the presence of other metals. The inset of Fig. 4 shows the colloidal colour change of AgNPs after addition of different cations.

The quantitative analysis of Hg^{2+} was carried out by varying the concentration of Hg^{2+} to AgNPs solution. The changes in the absorbance intensity and shift in the SPR peak position was monitored by UV-visible spectroscopy as shown in Fig. 5. The SPR peak intensity decreased gradually and shifted towards lower wavelength was observed on increasing the concentration of Hg^{2+} from 5 nM to 200 nM, accordingly the colour of the solutions changed from light yellow to colourless. The photograph shows the visual color changes of AgNPs as a function of Hg^{2+} ions, which could be easily visualized by the naked eyes from 40 nM of Hg^{2+} . Similar observations have been reported in the earlier studies.^{36,37} This could be due to the strong affinity of thiol group in NaCysC with Hg^{2+} which led to the destabilization of AgNPs structure. The changes in the intensity of SPR band show an excellent linear response towards Hg^{2+} over the range of 5-50 nM as shown in inset of Fig. 5, above which there was a slight deviation from the linear response (Fig. S6 ESI†). This may be due to the interference of aggregated NPs present in the sensing systems. The linear regression coefficient (R^2) was found to be 0.9913 with the detection limit of 8 nM of Hg^{2+} ions (inset of Fig. 5).

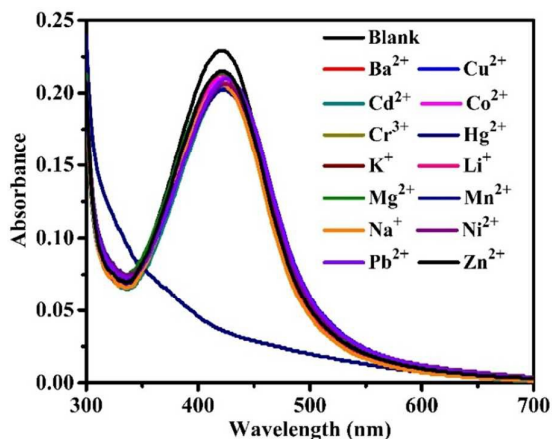


Fig. 3. UV-visible absorbance spectra of NaCysC stabilized AgNPs interaction with different metal ions.

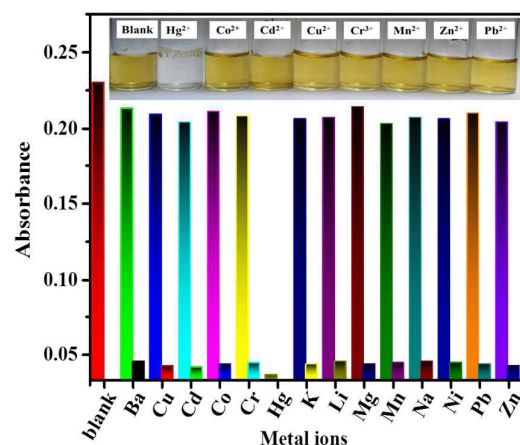


Fig. 4. The bars represent the colorimetric response of AgNPs incubated with various metal ions without and with Hg^{2+} ions.

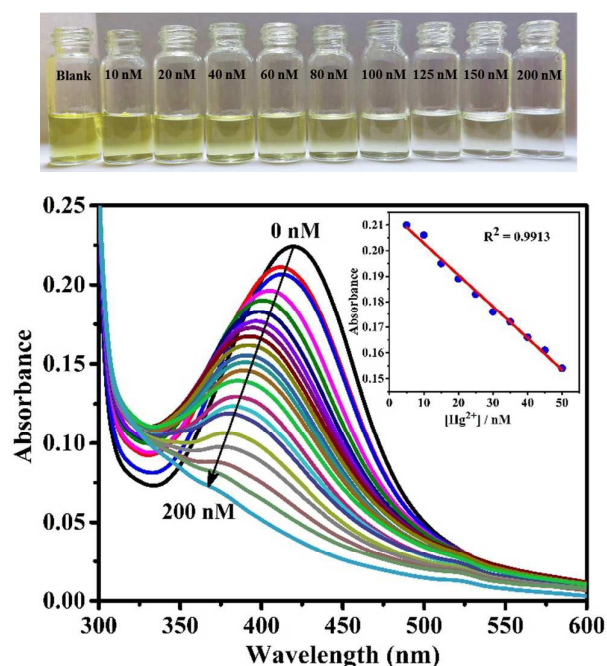


Fig. 5. UV-visible absorbance spectra of AgNPs interaction with various concentrations of Hg^{2+} ions. The inset shows the linear plot for absorbance difference versus Hg^{2+} concentration in nM level. The photograph shows the corresponding visual color changes of AgNPs as a function of Hg^{2+} ions.

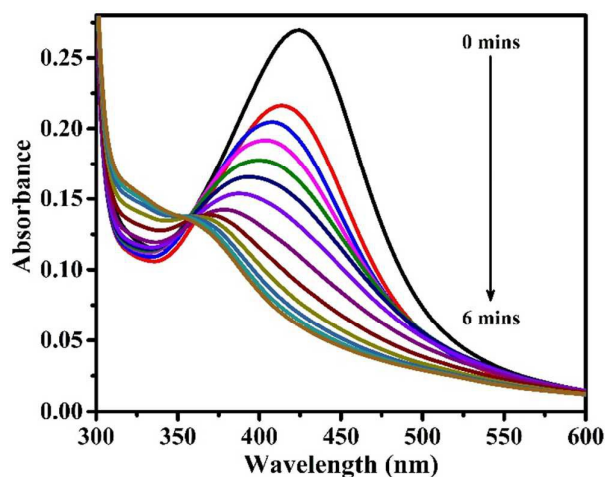


Fig. 6. Time dependant UV-visible spectral studies of AgNPs interaction with fixed concentration of Hg^{2+} ions (200 nM).

A time dependent spectral study was carried out in order to evaluate the response time of the detection probe under the optimum conditions. After the addition of Hg^{2+} (200 nM) into AgNPs solution an immediate decrease in the SPR peak intensity was observed and it reaches relatively to constant values in 3 mins (Fig.6), which was in good agreement with the colour change of NPs visualized by naked eyes. These results showing that the aggregation/partial oxidation of AgNPs was completed within 5 min, after that no significant change was observed. The fast response time and simplicity of this method makes it as an efficient probe for the detection of Hg^{2+} in real time analysis. The aggregation of NPs in presence of Hg^{2+} was confirmed by TEM analysis (Fig. 7). After the addition of 100 nM of Hg^{2+} to the AgNPs solution, aggregations occurred as evidenced from the TEM images (Fig.7). The TEM images clearly shows the aggregation of NPs and presence of larger NPs. This could be due to the effective interaction of Hg^{2+} to the thiol group of NaCysC results in the aggregation of NPs. Besides, the existence of both Ag and Hg^{2+} ions were confirmed by EDX analysis (Fig. 8).

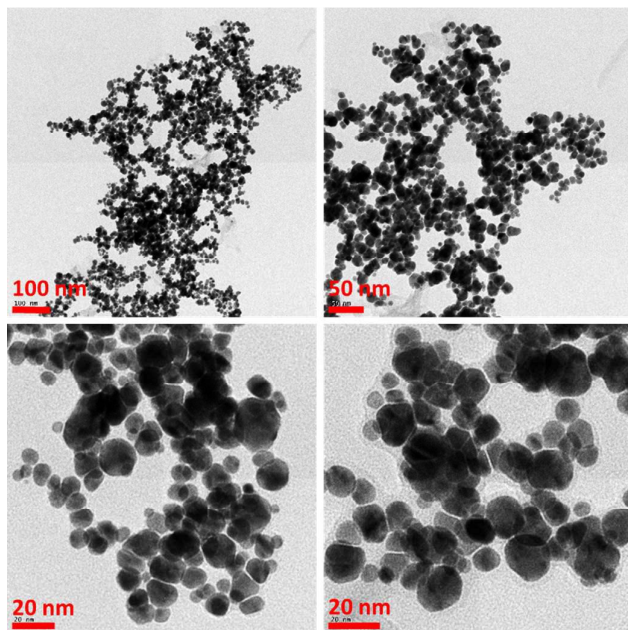


Fig. 7. TEM images of NaCysC stabilized AgNPs after the addition of 100 nM of Hg^{2+} ions with different magnifications.

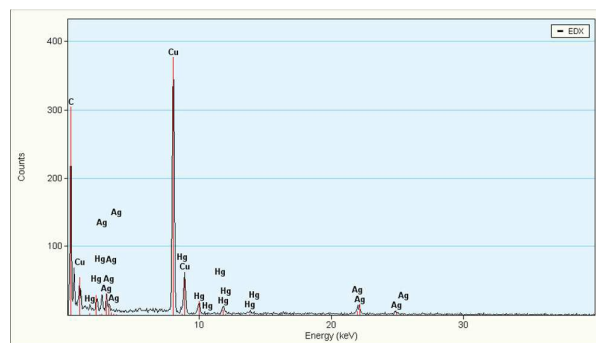
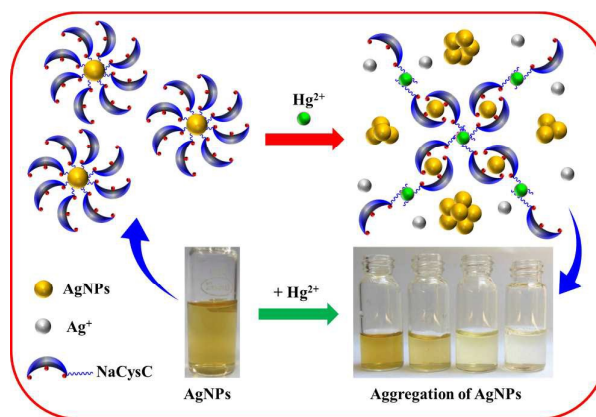


Fig. 8. EDX spectrum of AgNPs in the presence of 200 nM Hg^{2+} ions.

Scheme 2 illustrates the proposed sensing mechanism of Hg^{2+} with NaCysC stabilized AgNPs. While addition of Hg^{2+} into the NPs solution, initially the Hg^{2+} abstracts the thiol groups from the surface of the NPs because of the strong affinity of $-\text{SH}$ to Hg^{2+} . As a result, the protective layer of NaCysC was removed around the surface of the NPs that led to the destabilization of NPs. Then the unprotected NPs were fused together to form larger aggregated particles as evidenced from the TEM images (Fig. 7). At the same time, a partial oxidation proceeds on the unprotected NPs surface that led to the oxidation of Ag (0) to Ag^+ . This could be due to the standard reduction potential of Hg^{2+} (+0.92 V) is higher than the Ag (+0.7996 V). Then the Ag^+ ions react with Hg^{2+} led to the formation of Ag-Hg nanoalloy.³⁸⁻⁴² Accordingly, the color of the solution changed from yellow to colourless thereby the intensity of SPR band decreases with blue shift.



Scheme-2. Schematic representation of Hg^{2+} interaction with NaCysC stabilized AgNPs.

To evaluate the potential application of the developed colorimetric probe for the detection of Hg^{2+} ions in real samples such as tap water and drinking water, samples were collected in our campus and filtered through a 0.2 mm membrane prior to analysis. The samples were spiked with known concentration of Hg^{2+} . A plot of the absorption difference versus Hg^{2+} concentrations was shown in Fig. S7 (ESI[†]). The linear relationship was obtained over the range of 5-50 nM for both in drinking and tap water samples with the detection limit of 10 nM and 8 nM respectively (Fig. S7b ESI[†]). Real water showed more sensitivity towards Hg^{2+} ions

compared to deionized/distilled water. This could be due to the existence of some dissolved organic/inorganic matters; it interferes in the detection of Hg^{2+} in real water analysis. Compared to the previously reported methods, our method offers the advantages of simple, green, and economically feasible which led to the detection as low as below 10 nM.³⁶⁻⁴² These results demonstrate that this AgNPs probe is applicable for naked eye detection of Hg^{2+} ions in the water samples collected from different environment.

In conclusion, we have developed a simple, fast and an eco-friendly method for the synthesis of AgNPs under sunlight irradiation using NaCysC in aqueous medium. Since, NaCysC acts as a capping as well as reducing agent, no other external toxic reducing agent is required. The synthesized NPs were stable for more than six months. The AgNPs showed remarkably high selectivity to Hg^{2+} over other metal ions, and detected Hg^{2+} ions at concentrations as low as 8 nM. This simple, selective, cost-effective and rapid response to Hg^{2+} by the probe holds great potential for the detection of traces of Hg^{2+} in real environmental samples through the quenching of the colour and the plasmon band of silver nano colloid.

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