Green Chemical Synthesis of *N*-Cholyl-L-Cysteine Encapsulated Gold Nanoclusters For Fluorometric Detection of Mercury Ions

Kasthuri Jayapalan (✉ drkasthurishree@gmail.com)

Quaid-E-Millath Government College for Women Department of Chemistry <https://orcid.org/0000-0003-0976-8887>

Sivasamy Arumugam

Central Leather Research Institute: Central Leather Research Institute CSIR

Rajendiran Nagappan

University of Madras Taramani Campus: University of Madras

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Abstract

Here we report a simple, single-step, cost-effective, environmentally friendly, and biocompatible approach using sodium salt of *N*-choly-L-cysteine (NaCysC) capped gold nanoclusters (AuNCs) with green emission properties at above the CMC in aqueous medium under UV-light irradiation. The primary and secondary CMC of NaCysC was found to be 4.6 and 10.7 mM respectively using pyrene as fluorescent probe. The synthesized AuNCs exhibit strong emission maxima at 520 nm upon excitation of 375 nm with a large Stokes shift of 145 nm. The surface functionality and morphology of NCs are studied by Fourier transform infrared spectroscopy, dynamic light scattering studies and transmission electron microscopy. The formation of AuNCs was completed within 5 h and exhibit high stability for more than 6 months. The NaCysC templated AuNCs selectively quenches the Hg^{2+} ions with higher sensitivity in aqueous solution over the other metal ions. The fluorescence analysis of Hg^{2+} showed a wide linear range from 15 to 120 μM and a detection limit was found to be 15 nM.

Introduction

In the ever-expanding field of nanomaterial research, noble metal nanoclusters (MNCs) in particular silver (Ag), gold (Au) and copper (Cu) have emerged as a new class of functional materials and attracted a great deal of interest over the last two decades [1, 2]. These metal NCs are less than 3 nm in size which is comparable to the Fermi wavelength of the conduction electrons and show discrete electronic structures as compare to single metal atoms and large nanoparticles (NPs) [3]. Also they exhibit special molecule-like unusual intrinsic magnetism due to the strong quantum confinement effect, biocompatibility and size-dependent fluorescent properties over a wide range from the ultraviolet to near-infrared (NIR) region [4, 5]. Owing to their unusual properties, these NCs are widely used as promising fluorescent probes in biological and chemical sensing, bioimaging, drug delivery, catalysis, small biological molecule detection [6–10] and antibacterial agents [11]. Among the NCs currently under exploration, AuNCs have been focused as most widely studied material by many research groups during the last two decades due to easy to synthesis, low toxicity, water solubility, ultra-small size as compared to semiconductor QDs and good photostability, large Stokes shift and brightness than traditionally used organic dyes. Therefore, AuNCs used as potential fluorescent probe for metal ions sensing, biological imaging and drug delivery systems [3, 12, 13]. However, owing to the ultrasmall size, AuNCs are tend to aggregate into larger NPs and lost their associated photo-physical properties when the capping molecules are not effective [12]. Therefore, capping molecules plays a significant role in the formation of AuNCs, which can prevent the super atoms from aggregation and keep the size dependent fluorescence property from ultraviolet (UV) to near infrared (NIR) by modulating the core size of AuNCs [14]. In the past two decades, several protocols were developed to synthesize AuNCs using different kinds of ligands or capping molecules such as polymers [15], surfactants [16], DNA [17], amino acids [18], organic thiolate molecules [19] and dendrimers [20] with different core sizes, chemical compositions, surface ligands and emission properties. Zheng et al. reported water-soluble, monodisperse, blue-emitting Au_8 nano dots using biocompatible poly(amidoamine) (PAMAM) dendrimers [21]. Qu et al. elaborated about various synthetic

strategies on fluorescent AuNCs and recent advances in biological applications [22]. In recent years, AuNCs were extensively used as probe for sensing of toxic heavy metal ions owing to their size dependent luminescence properties which is one of the most characteristic features of NCs, high sensitivity in presence of analytes, simplicity, and low cost [23]. Regarding the heavy metals, they are highly poisonous even at trace levels, endangering to human health through the air, beverages, and the food chain, in which water plays a key role [24, 25]. Among the various heavy metals, mercury ion (Hg^{2+}) is of great concern since they exhibit high toxicity to humans and aquatic species even at low concentrations and bioaccumulation of Hg^{2+} ions can cause serious health problems [26]. Solvated mercuric ion (Hg^{2+}) is one of the most stable inorganic forms, which can damage the brain, heart, kidney, stomach, and intestines, even at very low concentrations [27]. Owing to their advantage of strong affinity of metallophilic $\text{Au}^+ \text{-Hg}^{2+}$ interactions that quench the fluorescence of AuNCs at trace level would be used for selective and sensitive of Hg^{2+} ions in environmental samples [28]. Qiao et al. reported L-Amino acid oxidase capped AuNCs based fluorescence probe for highly selective and sensitive detection of Hg^{2+} in aqueous solution [29]. Xie et al. demonstrated simple label-free method for the detection of Hg^{2+} ions with high selectivity and sensitivity by using fluorescent AuNCs in aqueous media [30]. Oligonucleotide templated AuNCs was prepared by Qing et al. for the detection of Hg^{2+} ions [31]. Hsu et al. demonstrated bovine serum albumin (BSA)–AuNCs for high sensitive detection of Hg^{2+} ion by fluorometric method [32]. Dihydrolipoic acid assisted AuNCs was prepared by Shang et al. for the detection of Hg^{2+} ions in living cells [33]. So far numerous reports available on the AuNCs using different types of capping molecules however, very few reports available on the surfactant encapsulated NCs. Previously we have reported simple, cost effective, bio-compatible and green approach for the synthesis of Ag and Au NCs using bile acid based biosurfactant as capping and reducing agent for the purpose of biological and chemical applications [34, 35]. Here we report for the first time the synthesis of NaCysC capped AuNCs under UV-light irradiation and used as effective fluorescent probe for sensing of Hg^{2+} ions.

Experimental Section

Materials and Methods

Instruments

UV-visible spectroscopy measurements were carried out on Techcomp UV-2301 spectrophotometer operated at a resolution of 1 nm. High-resolution transmission electron microscopic (HR-TEM) images were recorded with a JEOL JEM 2100 equipped with a Gatan imaging filter. The HR-TEM analysis were conducted by placing a drop of the NCs solution on carbon-coated copper grid and followed by solvent evaporation under vacuum as per manual instructions. Dynamic light scattering (DLS) measurement was performed for NCs solutions using Nanotrac Ultra NPA 253 from Microtrac, U.S.A. Pyrene fluorescence emission spectra were measured by monitoring the spectra from 350 to 500 nm at a scan rate of 30 nm/sec upon exciting the pyrene molecules near 340 nm using a Fluoromax 4 (Horiba Jobin Yvon) spectrofluorimeter equipped with 150 W Xenon lamp. The slit widths of excitation and emission were 20

and 1.5 nm, respectively. Band pass filter (10BPF10-340) was used to avoid white light from the monochromators. For FT-IR analysis the pressed pellet was prepared by grinding lyophilized NaCysC capped AuNCs powder with KBr in a 1:100 ratio and analyzed in the spectral range of 4000-500 cm^{-1} .

Materials

L-cysteine and hydrogen tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) were purchased from LOBA chemi, India. Salts of the different ions such as Na^+ , K^+ , Ca^{2+} , Li^+ , Cd^{2+} , Hg^{2+} , Fe^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} were procured from SRL chemicals Pvt. Ltd., India. All heavy metal salt solutions (1×10^{-5} M) used for the experiments were prepared by mixing the required amount of salt in triple-distilled water and pH of the solution was adjusted by adding 1N HCl and 1N NaOH. The glasswares were washed with aqua-regia ($\text{HCl}:\text{HNO}_3 = 3:1$ (v/v)) and rinsed well with triple distilled water prior to use.

Synthesis of Sodium Salt of *N*-CholyL-L-Cysteine

Sodium salt of *N*-cholyL-L-cysteine (NaCysC) was prepared by adopting our previous protocol with slight modifications [36]. Briefly, a magnetically stirred solution of 1.0 mM of L-cysteine methyl ester hydrochloride was added into the 0.5 mM cholic acid in dry DMF (2 mL). Then DEPC (0.6 mM) and Et_3N (0.8 mL) was added into the reaction mixture and the resulting suspension was stirred at room temperature for 2 h as shown in **Scheme-1**. After the completion of reaction, the product was extracted with EtOAc and the combined extracts were washed with double distilled water and evaporated to dryness. The residue was then refluxed for 30 min in 10 mL of 5% methanolic NaOH. The solvent was evaporated under reduced pressure and the hydrolyzed product was dissolved in 10 mL of water and acidified with 5% H_2SO_4 with constant stirring. The precipitated solid was filtered, washed with water and dried to give the crude amino acid conjugate of 80% yield, which was then recrystallized from an appropriate solvent.

Sample Preparation

Before all measurements, stock solution of 3×10^{-5} M aqueous pyrene was prepared by gentle evaporation of pyrene solubilized methanol solution, followed by addition of an appropriate volume of double distilled water in a volumetric flask and sonicated for 30 min. The synthesized NaCysC was hygroscopic and lyophilized salts were used to prepare defined concentration of stock solutions. The stock solutions of 16.0 mM of NaCysC was prepared using double distilled water and the actual pH of the solutions were found to be 6.5. Since NaCysC was soluble only in neutral and basic pH, NaOH was used to adjust the pH of the solution. Subsequent dilutions were made from this stock solution to obtain the desired final concentration. Aliquots of 300 μL stock solutions of pyrene were added to 4 mL of NaCysC to obtain a final concentration of 2.25×10^{-6} M of pyrene to measure the CMC. The solutions were kept for few hours for the encapsulation of pyrene molecules.

Synthesis of NaCysC Stabilized AuNCs

The fluorescent NaCysC stabilized AuNCs was prepared by mixing aqueous solution of 0.1 mL of HAuCl_4 (10 mM) with 2 mL of NaCysC (above the CMC) in 5 mL disposable glass vial containing 2.4 mL of triple distilled water. The reaction mixture was stirred vigorously for 2 min, followed by the addition of a certain amount of 1 M NaOH to adjust the reaction pH to 9.0. Thereafter, the resulting solution was kept under UV-light irradiation at 365 nm for 5 h and the formation of NCs was monitored by UV-visible spectroscopy and fluorescence spectroscopy.

Fluorometric Detection of Heavy Metal ions

Under optimized concentration the synthesized AuNCs was assessed as fluorometric probe owing to their size dependent emission properties and tendency to quenching the emission in the presence of analytes. Hence, the following metal ions were used to evaluate the selectivity of the AuNCs: Na^+ , K^+ , Ca^{2+} , Li^+ , Cd^{2+} , Hg^{2+} , Fe^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} at a fixed concentration of 70 μM . The changes in the emission intensity of solution was monitored by fluorescence spectroscopy.

Result And Discussion

Determination of CMC of sodium salt of *N*-Cholyl-L-Cysteine

Critical micellar concentration (CMC) is a crucial parameter for surfactants which is used to examine the changes in the chemical and physical properties of surface activator solutions upon increase in the concentrations. Bile acids are a class of naturally occurring steroidal nucleus forming a fused aliphatic tetracyclic rings with chemically nonequivalent hydroxyl groups and easily modifiable carboxylic group [37]. Owing to the special structure, chirality and rigid steroidal skeleton bile acids are exhibit primary and secondary CMC through hydrophobic and intermolecular hydrogen bonding [38]. Therefore, CMC for NaCysC was determined by employing fluorescence spectrophotometer using pyrene as a probe by measuring the intensity ratio of the third and first (I_3/I_1) highest vibronic bands in the emission spectra which are sensitive to the polarity of microenvironment. A very low concentration of pyrene (2×10^{-6} M) was used in this study, hence only negligible effect on the micellization process was noticed. The changes in the I_3/I_1 ratio of pyrene at a fixed concentration with increased concentration of NaCysC showed a sigmoid variation (**Fig.1**). The point of intersection of the horizontal line and the line of inflation represents formation of both primary and secondary CMC of NaCysC and are found to be 4.6 and 10.7 mM respectively. The significant decrease in the CMC of sodium salt of NaCysC solubilized pyrene as compare to pure NaC could be correlated with the increase in the hydrophobicity that favours the formation of CMC at a lower concentration

Optical Properties of AuNCs

The formation fluorescent AuNCs was confirmed by demonstrating the reaction between 1×10^{-6} M of HAuCl_4 and NaCysC in below and above the CMC at basic pH= 9 under UV light irradiation at 365 nm as shown in **Fig.2**. In below the CMC, step like feature was appeared in the UV region in addition to weak

surface plasmon resonance (SPR) between 500 to 600 nm could be due to the formation mixture of both AuNCs and AuNPs (**curve a**). However, at above the CMC no SPR peak in the visible region demonstrated that the as prepared NaCysC capped Au NCs is indeed NCs rather than NPs (**curve b**). When the solution was kept under UV light at 365 nm it exhibit a green emitting property could be due to the formation of AuNCs. Hence, above the first CMC of NaCysC (5.8 mM) was chosen as the optimum concentration for the preparation of green emitting AuNCs. As opposed to larger MNPs, these small NCs possess a distinctive feature like strong fluorescence due to their lower density of electronic states. Therefore, the well dispersed aqueous suspension of NaCysC stabilized AuNCs exhibits a strong emission peak at 520 nm with a full width at half maximum (FWHM) of 95 nm, when excited at 370 nm (**inset of Fig.2**), which confirms the existence of green emission properties of AuNCs. Also, the formation of AuNCs was monitored at various time intervals by fluorescence spectroscopy as shown in **Fig. 3**. Initially, the solution did not show any significant emission in the reaction mixture. Upon UV light irradiation for 20 min, the reaction mixture exhibits green emission with low intensity indicates the formation of AuNCs. The fluorescent intensity increased gradually at 520 nm without any shift in the emission maxima when the solution was irradiated up to 5 h. Soon after no significant change in the fluorescent intensity was noted upon further extending the irradiation time. This observation clearly indicates that the reaction was completed within 5 h. Besides, the synthesized AuNCs were exhibit stability for more than 6 months and hence tested for their applications as a fluorescent probe for the detection of metal ions.

Morphological Studies of NaCysC Capped AuNCs

The self-assembling behaviour of NaCysC was confirmed by microscopic studies. **Fig.4 (a&b)** shows the low and high magnification FE-SEM images of NaCysC at above the CMC in aqueous medium where the molecules are existed as aggregated form could be due to the hydrophobic and hydrophilic interactions. High resolution transmission electron microscope (HR-TEM) was used to characterize the morphology of AuNCs and their size distribution was observed using dynamic light scattering (DLS) studies. The TEM micrographs of NaCysC stabilized AuNCs with different resolutions are represented in **Fig.4 (c&d)** at above the CMC wherein the spherical shaped ultra-small particles with good monodispersity having size <3 nm was observed. Further the hydrodynamic radii of NaCysC capped AuNCs was measured using DLS analysis and found to be yielding 3.9 ± 0.4 nm (**inset of Fig.4d**). The difference in the HR-TEM and DLS measurement could be due to the fact that DLS measurements records higher values, since the light scattered from both core particle as well as layer on the surface of the NCs. Whereas in HR-TEM measurement only the metallic particle core is measured. The interactions of functional groups on the surface of metal NCs was studied by FT-IR spectroscopy. The changes in the shape and peak position after the formation of AuNCs was compared with pure NaCysC as shown in **Fig. 5**. In pure NaCysC, the peaks appeared at 3381, 2561, 1636, 1518 and 1453 cm^{-1} corresponds to the stretching vibration of -OH and -NH group, SH, -C=O, O=C-NH, and COO- groups respectively. After the stabilization of AuNCs, the intensity of -SH peak is completely vanished indicating the binding of SH group on the surface of NCs. Also, the characteristic frequency of -NH, C=O, and COO- are shifted towards higher frequency which confirms the interaction functional groups on the NCs surfaces.

Metal ions Recognition Ability of NaCysC Capped AuNCs

In recent years AuNCs were extensively used as fluorescent probe for the sensing of heavy metal ions because the intensity of fluorescence significantly altered in the presence of analytes. Therefore, the synthesized green emitting AuNCs was tested as fluorescent probe for the detection of heavy metal ion such as Na^+ , K^+ , Ca^{2+} , Li^+ , Cd^{2+} , Hg^{2+} , Fe^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} at fixed concentration in aqueous medium and the changes in the emission peak intensity was monitored by fluorescence spectroscopy as shown in **Fig.6a**. After the addition of 70 μM of metal ions with AuNCs, the solutions were incubated for 10 min then the changes in the emission properties was monitored upon excitation at 370 nm. Among the various metal ions tested only for Hg^{2+} ion quenched the fluorescence intensity about more than 80 % in presence of 70 μM of Hg^{2+} ion, while the rest of the metal ions no substantial change in the fluorescence intensity was observed (**Fig.6b**). The greater the quenching effect of Hg^{2+} over the other environmentally relevant metal ions indicates the high specificity and selectivity of this probe towards Hg^{2+} ions detection.

Sensitivity Detection of Hg^{2+} ions using AuNCs

To evaluate the sensitivity of NaCysC capped AuNCs, various concentrations of Hg^{2+} ions 15, 30, 45, 60, 75, 90, 105, and 120 μM were added into the AuNCs solution. The intensity of fluorescence progressively quenched without any changes in the emission maxima at 520 nm and spectral shape upon increase in the concentration of Hg^{2+} ions as shown in **Fig. 7a**. This could be due to the coordinating ability of -SH group containing in NaCysC with Hg^{2+} ions and strong $5d_{10}-5d_{10}$ metallophilic interactions between Hg^{2+} and Au^+ on the surface of NCs leads to the either destabilization or aggregation of NCs thereby the intensity of fluorescence quenched significantly[26]. A linear correlation existed by plotting the value of $(F_0-F)/F_0$ and concentration of Hg^{2+} in the range from 15 to 120 μM with a correlation coefficient (R^2) of 0.9926 (**Fig. 7b**). Where F_0 and F represent the fluorescence intensities of NaCysC-AuNCs before and after the inhabitation of Hg^{2+} ions, respectively. From this plot, a linear correlation is achieved between the range 15- 120 μM with a correlation coefficient (R^2) of 0.9926. The limit of detection (LOD) was found to be 15 nM which is less than the maximum permitted level of Hg^{2+} in drinking water by the World Health Organization (WHO) and Chinese National Standard [39-41]. Also, the intensity of fluorescence completely quenched with incubation time of 9 min, therefore the synthesized NCs could be used as suitable probe for the detection of Hg^{2+} ion in aqueous environment. **Scheme-2** represented the sensing mechanism of Hg^{2+} ions based on the quenching of fluorescence intensity of NaCysC stabilized AuNCs at 520 nm (turn off). Upon addition of Hg^{2+} into the NCs solution, there are two possible interactions between NCs with Hg^{2+} ions. The first one is NaCysC containing -SH group strongly interact with Hg^{2+} ions, thereby the capping molecules are removed on the surface of the NCs and the second possibility is that the Hg^{2+} ion has higher affinity to interact with AuNCs through strong $5d_{10}-5d_{10}$ metallophilic interaction between Hg^{2+} and Au^+ ions, resulting in fast adsorption of Hg^{2+} ion on the surface of AuNCs. Hence, the NCs becomes destabilized or aggregated owing to the removal of capping molecules and

metallophilic interactions as shown in the **Scheme-2**. As a result, the intensity of fluorescence decreases upon increase in the concentration of Hg^{2+} ions.

Interference of Metal Ions

To evaluate the comparative test of individual metal ions with Hg^{2+} ions, the changes in the emission spectra of AuNCs was monitored in the presence of competitive metal ions at fixed concentration (100 μM). The bar diagram in **Fig. 8** shows the selectivity and sensitivity among other competing metal ions. The interference test clearly indicates that Hg^{2+} ions efficiently quenches the AuNCs even in presence of other metal ions through strong $5d_{10}-5d_{10}$ metallophilic interaction between Hg^{2+} and Au^+ on the gold surface.

Conclusions

In conclusion, we have reported a simple, cost effective efficient and green approach for the synthesis of NaCyaC stabilized AuNCs with green emission properties in aqueous medium at above the CMC under UV light irradiation at 370 nm. Where NaCyaC act as effective template for the reduction and stabilization of NCs. The optical and morphological properties of NaCyaC capped AuNCs were studied by UV-visible, fluorescence spectroscopy FE-SEM, HR-TEM, DLS and FT-IR analysis. The synthesized AuNCs exhibit stable dispersion at basic pH (9.0) and exhibit high stability at room temperature for more than six months. The prepared AuNCs having size less than 3 nm with distinct excitation and emission properties. Further the prepared AuNCs were used as fluorescent probe selective and sensitive detection of Hg^{2+} over other metal ions and the detection limit was found to be 15 nM. Hence, it could be effectively used as fluorescent probes for the sensing of metal ions in both environmental and biological samples.

Declarations

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References

1. Zheng J, Nicovich PR, Dickson RM (2007) Highly Fluorescent Noble-Metal Quantum Dots. *Annu Rev Phys Chem* 58:409–431. doi:10.1146/annurev.physchem.58.032806.104546
2. Jin R, Zeng C, Zhou M, Chen Y (2016) Atomically Precise Colloidal Metal Nanoclusters and Nanoparticles: Fundamentals and Opportunities. *Chem Rev* 116:10346–10413. doi:10.1021/acs.chemrev.5b00703
3. Zhang L, Wang E (2014) Metal nanoclusters: New fluorescent probes for sensors and bioimaging. *Nano Today* 9:132–157. doi:https://doi.org/10.1016/j.nantod.2014.02.010
4. Zheng J, Zhang C, Dickson RM (2004) Highly Fluorescent, Water-Soluble, Size-Tunable Gold Quantum Dots. *Phys Rev Lett* 93:077402. doi:10.1103/PhysRevLett.93.077402
5. Huma Z-e, Gupta A, Javed I, Das R, Hussain SZ, Mumtaz S (2018) Cationic Silver Nanoclusters as Potent Antimicrobials against Multidrug-Resistant Bacteria. *ACS Omega* 3:16721–16727. doi:10.1021/acsomega.8b02438
6. Shiang Y-C, Huang C-C, Chen W-Y, Chen P-C, Chang H-T (2012) Fluorescent gold and silver nanoclusters for the analysis of biopolymers and cell imaging. *J Mater Chem* 22:12972–12982. doi:10.1039/C2JM30563A
7. Shang L, Xu J, Nienhaus GU (2019) Recent advances in synthesizing metal nanocluster-based nanocomposites for application in sensing, imaging and catalysis. *Nano Today* 28:100767. doi:https://doi.org/10.1016/j.nantod.2019.100767
8. Lin Y-C, Wu T, Lin Y-W (2018) Fluorescence sensing of mercury(II) and melamine in aqueous solutions through microwave-assisted synthesis of egg-white-protected gold nanoclusters. *Anal Methods* 10:1624–1632. doi:10.1039/C8AY00308D
9. Kumar V, Vaid K, Sarawagi N, Dhiman J (2021) Influence of Fe(III) on the Fluorescence of Lysozyme: a Facile and Direct Method for Sensitive and Selective Sensing of Fe(III). *J Fluoresc*. doi:10.1007/s10895-021-02813-1
10. Li N, He Y, Ge Y, Song G (2017) “Turn-Off-On” Fluorescence Switching of Ascorbic Acid-Reductive Silver Nanoclusters: a Sensor for Ascorbic Acid and Arginine in Biological Fluids. *J Fluoresc* 27:293–302. doi:10.1007/s10895-016-1957-2
11. Qian H, Zhu M, Wu Z, Jin R (2012) Quantum Sized Gold Nanoclusters with Atomic Precision. *Acc Chem Res* 45:1470–1479. doi:10.1021/ar200331z
12. Choi S, Dickson RM, Yu J (2012) Developing luminescent silver nanodots for biological applications. *Chem Soc Rev* 41:1867–1891. doi:10.1039/C1CS15226B
13. Liu S, Lu F, Zhu J-J (2011) Highly fluorescent Ag nanoclusters: microwave-assisted green synthesis and Cr³⁺ sensing. *Chem Commun* 47:2661–2663. doi:10.1039/C0CC04276E

14. Kawasaki H, Hamaguchi K, Osaka I, Arakawa R (2011) pH Dependent Synthesis of Pepsin-Mediated Gold Nanoclusters with Blue Green and Red Fluorescent Emission. *Adv Func Mater* 21:3508–3515. doi:<https://doi.org/10.1002/adfm.201100886>
15. Aldeek F, Muhammed MAH, Palui G, Zhan N, Mattoussi H (2013) Growth of Highly Fluorescent Polyethylene Glycol- and Zwitterion-Functionalized Gold Nanoclusters. *ACS Nano* 7:2509–2521. doi:10.1021/nn305856t
16. Shen J, Bi Y, Liu B, Xiao Q, Yu Y, Qi W (2019) Co-assembly of gold nanocluster with imidazolium surfactant into ordered luminescent fibers based on aggregation induced emission strategy. *J Mol Liq* 291:111275. doi:<https://doi.org/10.1016/j.molliq.2019.111275>
17. Liu G, Shao Y, Ma K, Cui Q, Wu F, Xu S (2012) Synthesis of DNA-templated fluorescent gold nanoclusters. *Gold Bulletin* 45:69–74. doi:10.1007/s13404-012-0049-6
18. Alkilany AM, Alsolari S, Alkawareek MY, Abulatefeh SR (2019) Facile Hydrophobication of Glutathione-Protected Gold Nanoclusters and Encapsulation into Poly(lactide-co-glycolide) Nanocarriers. *Sci Rep* 9:11098. doi:10.1038/s41598-019-47543-4
19. Jung J, Kang S, Han Y-K (2012) Ligand effects on the stability of thiol-stabilized gold nanoclusters: Au₂₅(SR)₁₈–, Au₃₈(SR)₂₄, and Au₁₀₂(SR)₄₄. *Nanoscale* 4:4206–4210. doi:10.1039/C2NR30501A
20. Tran ML, Zvyagin AV, Plakhotnik T (2006) Synthesis and spectroscopic observation of dendrimer-encapsulated gold nanoclusters. *Chem Commun*. doi:10.1039/B602079H.2400-1. doi:10.1039/B602079H
21. Zheng J, Petty JT, Dickson RM (2003) High Quantum Yield Blue Emission from Water-Soluble Au₈ Nanodots. *J Am Chem Soc* 125:7780–7781. doi:10.1021/ja035473v
22. Qu X, Li Y, Li L, Wang Y, Liang J, Liang J (2015) Fluorescent Gold Nanoclusters: Synthesis and Recent Biological Application. *Journal of Nanomaterials* 2015:784097. doi:10.1155/2015/784097
23. Zhang J, Cheng F, Li J, Zhu J-J, Lu Y (2016) Fluorescent nanoprobe for sensing and imaging of metal ions: Recent advances and future perspectives. *Nano Today* 11:309–329. doi:<https://doi.org/10.1016/j.nantod.2016.05.010>
24. Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QMR (2015) Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *Int J Mol Sci* 16. doi:10.3390/ijms161226183
25. Senthil Kumar P, Saravanan A. 11 - Sustainable wastewater treatments in textile sector. In: Muthu SS, editor. *Sustainable Fibres and Textiles* (2017) doi:<https://doi.org/10.1016/B978-0-08-102041-8.00011-1>. Woodhead Publishing 323-46
26. Sahu D, Mohapatra P, Swain SK(2020) Highly orange fluorescence emission by water soluble gold nanoclusters for “turn off” sensing of Hg²⁺ ion. *Journal of Photochemistry and Photobiology A: Chemistry*. 2020;386:112098. doi:<https://doi.org/10.1016/j.jphotochem.2019.112098>
27. Yang Y, Lu L, Tian X, Li Y, Yang C, Nie Y (2019) Ratiometric fluorescence detection of mercuric ions by sole intrinsic dual-emitting gold nanoclusters. *Sens Actuators B* 278:82–87. doi:<https://doi.org/10.1016/j.snb.2018.09.072>

28. Zhang Y, Jiang J, Li M, Gao P, Shi L, Zhang G (2017) Bright far-red/near-infrared gold nanoclusters for highly selective and ultra-sensitive detection of Hg²⁺. *Sens Actuators B* 238:683–692. doi:<https://doi.org/10.1016/j.snb.2016.07.118>
29. Qiao Y, Zhang Y, Zhang C, Shi L, Zhang G, Shuang S (2016) Water-soluble gold nanoclusters-based fluorescence probe for highly selective and sensitive detection of Hg²⁺. *Sens Actuators B* 224:458–464. doi:<https://doi.org/10.1016/j.snb.2015.10.080>
30. Xie J, Zheng Y, Ying JY (2010) Highly selective and ultrasensitive detection of Hg²⁺ based on fluorescence quenching of Au nanoclusters by Hg²⁺–Au⁺ interactions. *Chem Commun* 46:961–963. doi:10.1039/B920748A
31. Qing T, He X, He D, Qing Z, Wang K, Lei Y (2016) Oligonucleotide-templated rapid formation of fluorescent gold nanoclusters and its application for Hg²⁺ ions sensing. *Talanta* 161:170–176. doi:<https://doi.org/10.1016/j.talanta.2016.08.045>
32. Hsu N-Y, Lin Y-W (2016) Microwave-assisted synthesis of bovine serum albumin–gold nanoclusters and their fluorescence-quenched sensing of Hg²⁺ ions. *New J Chem* 40:1155–1161. doi:10.1039/C5NJ02263K
33. Shang L, Yang L, Stockmar F, Popescu R, Trouillet V, Bruns M (2012) Microwave-assisted rapid synthesis of luminescent gold nanoclusters for sensing Hg²⁺ in living cells using fluorescence imaging. *Nanoscale* 4:4155–4160. doi:10.1039/C2NR30219E
34. Chandirasekar S, Chandrasekaran C, Muthukumarasamyvel T, Sudhandiran G, Rajendiran N (2016) Biosurfactant templated quantum sized fluorescent gold nanoclusters for in vivo bioimaging in zebrafish embryos. *Colloids Surf B* 143:472–480. doi:<https://doi.org/10.1016/j.colsurfb.2016.03.067>
35. Chandirasekar S, Chandrasekaran C, Muthukumarasamyvel T, Sudhandiran G, Rajendiran N (2015) Sodium Cholate-Templated Blue Light-Emitting Ag Subnanoclusters: In Vivo Toxicity and Imaging in Zebrafish Embryos. *ACS Appl Mater Interfaces* 7:1422–1430. doi:10.1021/am507291t
36. Annadhasan M, SankarBabu VR, Naresh R, Umamaheswari K, Rajendiran N (2012) A sunlight-induced rapid synthesis of silver nanoparticles using sodium salt of N-cholyl amino acids and its antimicrobial applications. *Colloids Surf B* 96:14–21. doi:<https://doi.org/10.1016/j.colsurfb.2012.03.009>
37. Annadhasan M, Rajendiran N (2015) Highly selective and sensitive colorimetric detection of Hg(ii) ions using green synthesized silver nanoparticles. *RSC Advances* 5:94513–94518. doi:10.1039/C5RA18106B
38. Chandirasekar S, Dharanivasan G, Kasthuri J, Kathiravan K, Rajendiran N (2011) Facile Synthesis of Bile Salt Encapsulated Gold Nanoparticles and Its Use in Colorimetric Detection of DNA. *J Phys Chem C* 115:15266–15273. doi:10.1021/jp2044465
39. Pomal NC, Bhatt KD, Modi KM, Desai AL, Patel NP, Kongor A (2021) Functionalized Silver Nanoparticles as Colorimetric and Fluorimetric Sensor for Environmentally Toxic Mercury Ions: An Overview. *J Fluoresc* 31:635–649. doi:10.1007/s10895-021-02699-z

40. Hasan A, Nanakali NMQ, Salihi A, Rasti B, Sharifi M, Attar F (2020) Nanozyme-based sensing platforms for detection of toxic mercury ions: An alternative approach to conventional methods. *Talanta* 215:120939. doi:<https://doi.org/10.1016/j.talanta.2020.120939>
41. Bigdeli A, Ghasemi F, Abbasi-Moayed S, Shahrajabian M, Fahimi-Kashani N, Jafarinejad S (2019) Ratiometric fluorescent nanoprobe for visual detection: Design principles and recent advances - A review. *Analytica Chimica Acta*. 2019;1079:30-58. doi:<https://doi.org/10.1016/j.aca.2019.06.035>

Scheme

Schemes 1 and 2 are available in the Supplemental Files section.

Figures

Figure 1

Changes of I_3/I_1 ratio of pyrene monomer fluorescence as a function of various concentration of NaCysC in aqueous medium.

Figure 2

(a) UV-visible spectra of NaCysC capped AuNCs synthesized below (curve a) and above the CMC (curve b). The inset shows the emission spectra of AuNCs when excited at 370 nm.

Figure 3

Emission spectra of NaCysC stabilized AuNCs at different time intervals (0-5h) under UV-light exposure (365 nm). The inset shows the corresponding photograph of AuNCs solution under day and UV light (365 nm) respectively.

Figure 4

FE-SEM image of NaCysC at above the CMC with different magnification (a&b) and HR-TEM images of NaCysC stabilized AuNCs with different magnifications (c&d) and its corresponding particle size distribution of AuNCs (inset of d).

Figure 5

FT-IR spectra of pure NaCysC (a) and NaCysC stabilized AuNCs (b).

Figure 6

Fluorescence spectra of NaCysC capped AuNCs in the presence of different heavy metals ions. (a) The bars represent the fluorescence intensity ratio (F/F_0) of AuNCs incubated with different metal ions (b).

Figure 7

Fluorescence quenching of NaCysC capped AuNCs in presence of different concentrations of Hg^{2+} (a). Changes in the relative fluorescence ($F_0 - F/F_0$) as a function of Hg^{2+} concentrations at wavelength 520 nm (b).

Figure 8

The bars represent the fluorometric response of AuNCs incubated with various metal ions without (red bar) and with Hg²⁺ ions (blue bar).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)
- [Scheme2.png](#)