

Facile Synthesis of Water-Soluble Fluorescent Silver Nanoclusters and Hg^{II} Sensing

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Received January 18, 2010. Revised Manuscript Received June 3, 2010

A single step facile synthesis of highly emissive, water-soluble, fluorescent Ag nanoclusters has been reported using a small molecule, dihydrolipoic acid. These clusters were characterized using ultraviolet/visible (UV/vis) spectroscopy, photoluminescence spectroscopy, Fourier transform infrared spectroscopy (FT-IR), high-resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), and X-ray diffraction (XRD) studies. Mass spectrometric analysis shows the presence of a few atoms in nanoclusters containing only Ag₄ and Ag₅. The reported fluorescent Ag nanoclusters show excellent optical properties, including narrow emission profile, larger Stokes shift (more than 200 nm), and good photostability. Interestingly, these nanoclusters also exhibit semiconducting property. Moreover, as-prepared fluorescent Ag nanoclusters have been utilized as an indicator for selective and ultrasensitive detection of highly toxic Hg^{II} ions in water, even at subnanomolar concentrations.

Introduction

Recently, noble-metal nanoclusters have drawn considerable research interest, because of their unique function in bridging the "missing link" between atomic and nanoparticle behavior.¹ Noble-metal nanoclusters exhibit discrete molecule-like electronic transition and interesting optical properties, including fluorescence, because of their tiny size and the quantum confinement effect.² These nanoclusters find possible applications in single molecular spectroscopy, biological labeling, optical sensing, catalysis, and other fields.³ Although gold nanoclusters have been widely investigated^{2c,d,3f,4} over the past several years, only several methods have been developed for preparing fluorescent silver nanoclusters. Dickson and co-workers have synthesized water-soluble fluorescent Ag nanoclusters using poly-(amidoamine) (PAMAM) dendrimer and DNA as templates.^{2b,5} Different sequences of DNA templates have been used in the formation of Ag nanoclusters and their role in cluster formation has also been investigated.⁶ Kumacheva et al. and Frey et al. have separately reported the photogeneration of fluorescent Ag nanoclusters using poly (*N*-isopropylacrylamide-acrylic acid-2-hydroxyethyl acrylate) and multiarm star polyglycerol-block-poly(acrylic acid) copolymer-based microgel particles as templates.⁷ Dong and co-workers reported the synthesis of fluorescent Ag nanoclusters by common polyelectrolyte poly(methacrylic acid) as a template.⁸ Silver nanoclusters have been formed within silver exchanged zeolites using heat treatment⁹ and

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Article

within glass using a femtosecond laser irradiation¹⁰ and these are not well suited for bioapplication. Various other irradiation techniques have been used to prepare Ag clusters. Examples include the use of γ -ray irradiation for the preparation of Ag clusters in aqueous solution of polymers such as polyvinylacetone¹¹ and polyacrylate.¹² Similarly, electron irradiation, microwave irradiation, and polychromic irradiation have been used to make Ag clusters.¹³ The preparation of Ag clusters at room temperature, using the aqueous solution of an amphiphilic copolymer polyvinylacetone, has also been reported.¹⁴ Although these above-mentioned methods are efficient for the preparation of fluorescent Ag nanoclusters, all these cases involve the costly synthesis of macromolecular or dendrimeric templates and the sizes of these templates are also so large that their application in labeling biomolecules for the most dynamical experiments is limited.^{4g} Therefore, the development of a facile and convenient procedure is needed for making fluorescent Ag nanoclusters using a small molecule as a template.

In this study, we report a new method for simplistic and direct synthesis of highly fluorescent, water-soluble, Ag nanoclusters using the dihydrolipoic acid (which is a small molecule with two thiol groups) as a stabilizing agent. This is a very convenient approach to make stable, highly emissive, fluorescent Ag nanoclusters with control over the cluster size. Moreover, larger Stokes shift and good photostability are two important features of our synthetic fluorescent Ag nanoclusters, and, interestingly, these nanoclusters also exhibit semiconducting properties.

The highly toxic mercuric ion (Hg^{II}), which is the most stable form of inorganic mercury, causes serious health and environmental problems. Hence, quick and very sensitive detection of the Hg^{II} ion in water is essential. Different analytical methods for the detection of the

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highly toxic Hg^{II} ion have been reported. These include electrochemical methods,¹⁵ optical methods that involve Hg^{II}-sensitive fluorophores or chromophores,¹⁶ the use of proteins,¹⁷ functional polymer materials,¹⁸ metal nanoparticles,^{3d,19} and semiconductor quantum dots.²⁰ Our reported synthetic fluorescent Ag nanoclusters have been utilized as an indicator for the selective detection of the Hg^{II} ion in water, because Hg^{II} can solely quench the fluorescence intensity of Ag nanoclusters selectively and ultrasensitively, with a limit of detection of 10⁻¹⁰ M.

Experimental Section

Materials. $(\pm)\alpha$ -lipoic acid was purchased from Aldrich. Silver nitrate (AgNO₃) and sodium borohydride (NaBH₄) were purchased from Merck. The water used in all experiments was Millipore Milli-Q grade.

Synthesis of Ag Clusters. In a typical experiment, 5.26 mg of lipoic acid (LA) powder and 2 mL of Milli-Q water were placed into a vial to prepare fluorescent Ag nanoclusters. To this insoluble mixture, 0.24 mg of pure sodium borohydride was added, so that the molar ratio of LA:NaBH4 was 4:1 and this was stirred well until a clear solution was observed. In this step, lipoic acid is reduced in water to form soluble dihydrolipoic acid (DHLA). Now, to this freshly prepared aqueous DHLA solution, 100 μ L of 25 [mM] aqueous AgNO₃ solution was added and the mixture was well-stirred for 1 min. To this mixture, a slight excess of dilute aqueous sodium borohydride solution was added slowly. The stirring was continued for almost 2 h. At first, the mixture gradually became colorless to reddish pink color, then a deep reddish color was developed. The color of this soultion, over time (within 50 min), changed to a yellowish orange color (see inset of Figure 1a).

Instrumentation. UV/vis Spectroscopy. UV/vis absorption spectra were taken using a dilute aqueous solution of fluorescent Ag nanoclusters ($\sim 10^{-4}$ M concentration), and these spectra were recorded using a Varian Cary 50 Bio UV–vis spectrophotometer.

Photoluminescence (PL) Spectroscopy. Photoluminescence (PL) study was conducted using dilute aqueous solution of Ag clusters at a concentration of $\sim 10^{-5}$ M. PL spectra were recorded using a Horiba Jobin Yvon Fluoromax 3 instrument and a 1-cm-path-length quartz cell. The slit width for the excitation and emission was set at 5 nm. The Quantum yield was measured by a relative comparison method using the following equation:

$$\frac{Q_{\rm t}}{Q_{\rm s}} = \frac{(I_{\rm t}/A_{\rm t})\eta_{\rm t}^2}{(I_{\rm s}/A_{\rm s})\eta_{\rm s}^2}$$

where Q is the quantum yield, I the integral area under the PL spectrum, η the refractive index of the solvent, and A the absorption at the selected excitation wavelength. The subscripts "t" and "s" represent the test sample and the standard sample, respectively.

The PL lifetime was measured using a setup that involves a Horiba Jobin Yvon Fluoromax-P time-resolved fluorometer with multichannel scaling (MCS). The lifetime was measured using a xenon flash lamp.

FT-IR Spectroscopy. Fourier transform infrared (FT-IR) spectroscopy of dried DHLA and Ag nanocluster-DHLA

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Figure 1. (a) Time-dependent UV/vis spectra with the progress of the formation of Ag nanoclusters. (Inset: photographs of Ag nanoclusters in water under the exposure of daylight.) (b) Normalized photoluminescence (PL) and photoluminescence excitation (PLE) spectra of Ag nanoclusters. (Inset: photographs of fluorescent Ag nanoclusters in water under the exposure of UV light (excitation at 365 nm) and under exposure of fluorescence light using a blue filter (below).) (c) Time-dependent evolution of photoluminescence spectra, relative to the progress of the formation of Ag nanoclusters. (d) Plot of photoluminescence intensity of Ag nanoclusters versus time, relative to the progress of their formation.

nanoconjugate was performed using a Nicolet 380 FT-IR spectrophotometer (Thermo Scientific), using a KBr pellet technique.

High-Resolution Transmission Electron Microscopy (HR-TEM). High-resolution transmission electron microscopy (HR-TEM) studies of aqueous fluorescent Ag nanoclusters solution were carried out at a concentration of 2×10^{-5} M. HR-TEM images were taken with a JEOL electron microscope operated at an accelerating voltage of 200 kV. The as-prepared Ag nanoclusters were dried on carbon-coated copper grids (300 mesh size) by slow evaporation and then allowed to dry in a vacuum at 25 °C for two days. The average size of nanoclusters was statistically determined from the HR-TEM image by measuring the diameter of 100 clusters using ImageJ software.

X-ray Diffraction (XRD) study. The experiment was carried out using the dried sample of fluorescent Ag nanoclusters. Experiment was performed using an X-ray diffractometer (Bruker D8 Advance), with a conventional Cu K α X-ray radiation ($\lambda = 1.5418$ Å) source and a Bragg diffraction setup (Seifert 3000P).

Dynamic Light Scattering (DLS) Study. DLS study was performed in MELLERS GRIOT HeNe-LASER TER-BO-CORR CORILATOR, Model No. BI200SM-Goniometer VER-2.0 Instrument using dilute aqueous solution of Ag clusters at a concentration 2×10^{-5} M.

High-Resolution Mass Spectrometry (HR-MS). Mass spectrum was recorded on a Q-Tof Micro YA263 high-resolution spectrometer by positive-mode electrospray ionization using the water-methanol mixture of Ag clusters solution.

Current-Voltage (I-V) Measurement. A thin film of fluorescent Ag nanoclusters was prepared on glass substrate using spin coating method for electrical conductivity measurements. The film was dried well in vacuum for 2 days. The currentvoltage (I-V) measurements were carried out using Ag electrodes in coplanar configuration using a Keithley model 6517A Electrometer at room temperature. The thickness of the film was determined using a surface profilometer (STYLUS, Model No. DEKTAK 6M), and it was found to be more or less uniform in thickness (with an average thickness of $2.45 \,\mu$ m). Uniformity in thickness and absence of interstices were further checked by atomic force microscopy of this thin film (see Figure S1a in the Supporting Information). Electrical conductivity was measured putting the point I = 36.6 nA, V = 0.9 V from I-V curve (Figure S1b in the Supporting Information) using the following equation:

Conductivity
$$= \frac{1}{\rho} = \frac{Il}{VA}$$

where ρ is the electrical registivity, *I* the current, *l* the length, *V* the voltage, and *A* the cross-sectional area.

Results and Discussion

The time-dependent UV-Vis absorption spectroscopic and PL spectroscopic measurements have been used to monitor characteristics feature that have been observed during the formation of Ag nanoclusters.

Ultraviolet/Visible (UV-Vis) Spectroscopic Study. The time-dependent change in UV-Vis absorption spectra during the progress of the formation of fluorescent Ag nanoclusters are illustrated in Figure 1a. A broad absorption band was started to appear at ~460 nm after 3 min from the addition of sodium borohydride to the aqueous mixture of silver nitrate and dihydrolipoic acid (see Figure 1a and the subsection "Synthesis of Ag Clusters" in the Experimental Section). This broad absorption band gradually became sharper with the progress of the reaction, and the intensity of this band was also increased with time up to 25 min (Figure 1a). The absorption intensity was then steadily diminished up to 42 min, this broad peak was started to split: one at 435 nm with higher absorption intensity and another at 335 nm. Another shoulder peak at \sim 500 nm was also observed after 1 h. The area under the absorption band steadily decreased with time after 25 min. This indicates the gradual decrease of nanoparticle size.²¹ Moreover, it has been noticed that the absorption peak width gradually became narrower with time, and this suggests that the size distribution of newly formed Ag nanoparticles is narrow.²² Previously, Dickson and his co-workers reported that fluorescent silver clusters Ag1-Ag4 show absorptions at 440 and 357 nm.⁵ Silver clusters Ag₄-Ag₉ in microgel template showed a shoulder at 330-360 nm, with an intense peak at 490–520 nm in the absorption spectra.^{7a} Another report of microgel-templated fluorescent Ag clusters exhibited a peak at 400 nm with a shoulder at 345 nm.²³ Therefore, in our study, the observed absorption spectra (Figure 1a) may be responsible for the formation of ultrasmall Ag nanoparticles (Ag^0) .

Photoluminescence Study. Photoluminescence (PL) and photoluminescence excitation (PLE) spectra of dilute aqueous Ag nanoclusters colloids at room temperature are shown in Figure 1b. The PL spectrum shows an emission maximum at 652 nm upon excitation at 425 nm. The main absorption peak in the UV/vis spectrum appeared at 435 nm, with two shoulders, at 325 and 500 nm. Excitation at either 325 or 500 nm also gives rise to an emission maximum at 652 nm (see Figure S2 in the Supporting Information). It is evident that no change in emission maximum was observed by altering the excitation wavelength. This indicates that the obtained emission is a real luminescence from the relaxed states and it is not due to scattering effects. It is thought that the emissive nature of Ag nanoclusters can arise because of the fact that electrons can travel from the submerged and quasi-continuum 5d

band to the lowest unoccupied conduction band of Ag nanoclusters, and this is an interband transition.^{1b,24}

A time-dependent PL study was performed to probe the kinetics of the formation of Ag nanoclusters. The timedependent changes in fluorescence intensity during the progress of the formation of fluorescent Ag nanoclusters are given in Figure 1c. It is evident from the figure that fluorescence clusters started to form 42 min after the beginning of the reaction (i.e., 42 min after the addition of sodium borohydride to the aqueous mixture of silver nitrate and dihydrolipoic acid; see the subsection "Synthesis of Ag Clusters" in the Experimental Section). It is important to note that this is the time when the absorption spectra started to split (see Figure 1a). The fluorescence intensity steadily increases with time and ultimately reaches the saturation point after 97 min from the commencement of the reaction. Both fluorescence and absorption spectra did not change significantly after that time. Therefore, it can be stated that the formation of Ag nanoclusterts was completed at 97 min. PL intensities at different time intervals were plotted with respect to time, and a straight line was found (see Figure 1d). This indicates that the cluster formation kinetics follows an apparent zero-order rate law. Concentration-dependent fluorescence study was also carried out, and it shows that PL intensity increases linearly with the increase in cluster concentration (see Figure S3 in the Supporting Information). The quantum yield (Q) of these fluorescent Ag nanoclusters was found to be 2% in water using Acridine yellow (Q = 0.47 in ethanol) as a reference. The PL lifetime of these Ag nanoclusters was measured in this study and was found to be 36.96 μ s (see Figure S4 in the Supporting Information). The observed PL decay was fitted well with a single exponential decay.

These fluorescent Ag nanoclusters show interesting optical properties. First, the narrow emission profile (full width at half-maximum (fwhm) of 96 nm) of these fluorescent Ag nanoclusters indicates that the size distribution of these clusters is narrow.^{3d} The emission bandwidth is much narrower than the previously reported results obtained by different research groups.^{3e,7a,7b,8} However, it is comparable to a bandwidth obtained by Ras and co-workers.²⁵ Their study demonstrated the well-controlled synthesis of nanoclusters containing a small number of Ag atoms, such as Ag₂, Ag₃, and Ag₅.²⁵ Second, these Ag nanoclusters show strong red luminescence. The aqueous Ag nanoclusters exhibit a pronounced red photoluminescence upon the irradiation by UV light at 365 nm. The same red photoluminescence is also observed under fluorescence light using a blue filter (see inset in Figure 1b). This inherent bright PL property can allow the imaging of a single Ag nanocluster. It suggests that they may be used for the cell imaging purpose. Third, these Ag nanoclusters have a very large Stokes shift (> 200 nm). Furthermore, these emissive nanoclusters have shown good photostability. Note that

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Figure 2. FT-IR spectra obtained from (a) dihydrolipoic acid (DHLA) and (b) a Ag nanocluster–DHLA nanoconjugate.

the PL property of these Ag nanoclusters in water could still be sustained for 4 h in the presence of continuous UV irradiation. The advantage of large Stokes shift and good photostability of these fluorescent Ag nanoclusters opens up an opportunity to use these materials in optics and chemical sensing.

FT-IR Study. It is evident from FT-IR spectra (Figure 2) that the characteristic band at 2548 cm⁻¹ for ν (S–H) of dihydrolipoic acid (DHLA) disappeared, because of the formation of a Ag nanocluster-DHLA nanoconjugate. This clearly indicates that thiol groups of DHLA remain in the form of the thiolate in the nanoconjugate.^{4c} The presence of a band at 1564.3 cm^{-1} in both DHLA and Ag nanocluster-DHLA nanoconjugate suggests that the carboxylate ion remains in the free state (in unbound form). Therefore, we believed that freshly prepared reduced lipoic acid (DHLA) contains two free thiol groups per molecule that are very sensitive to the surfaces of Ag nanoclusters and these two thiol groups stabilize the fluorescent Ag nanoclusters very well. The hydrophilic nature of the carboxylate moiety present in the DHLA molecule at the surface makes the Ag nanocluster-DHLA nanoconjugate soluble in an aqueous medium (see Figure 3). Our reported fluorescent Ag nanoclusters are very stable and last more than 3 months in water. These clusters are not precipitated out from water, and the optical properties (absorption and emission) do not change significantly over this period of 3 months (see Figure S5 in the Supporting Information). Moreover, there is no substantial increase in cluster size after keeping them in water for 3 months, and this is evident from the HR-TEM study (see Figure S6 in the Supporting Information). The high stability is ascribed to the bidentate chelate effect afforded by the dithiol groups of dihydrolipoic acid (see Figure 3).

HR-TEM, XRD, and DLS Studies. The size distribution of the fluorescent Ag nanoclusters was characterized by two techniques, such as high resolution transmission electron microscopy (HR-TEM) and dynamic light scattering (DLS). HR-TEM was used to visualize the shape as well as measure the diameter of the inorganic Ag clusters.



Fluorescent Ag nanocluster

Figure 3. Scheme illustrating the formation of a Ag_5 nanocluster–DHLA nanoconjugate.



Figure 4. HR-TEM image of fluorescent Ag nanoclusters, showing the lattice fringes in the inset.

HR-TEM image of the fluorescent Ag nanoclusters are given in Figure 4. The as-prepared Ag nanoclusters had an average diameter of 2.44 nm. There is no formation of larger Ag nanoparticles or aggregation, as was observed by HR-TEM. This demonstrates that the synthetic procedure allows for control over the nanocluster size (i.e., the cluster size distribution; see Figure S7a in the Supporting Information). The majority of these Ag clusters are within the size range of 1-3 nm. The HR-TEM image (see inset of Figure 4) also indicates the presence of a lattice plane, which has an interfringe distance of 2.34 Å, corresponding to the (111) plane of fcc Ag. Selected-area electron diffraction study (SAED) shows the presence of the (111) plane for silver (See Figure S7b in the Supporting Information). This result from the SAED and HR-TEM analyses is consistent with that of X-ray diffraction (XRD) study. The XRD pattern for Ag nanoclusters showed the diffraction peaks at $2\theta = 38.2^{\circ}, 44.2^{\circ}, 64.5^{\circ},$ and 77.4°, all of which are very consistent with those for Ag (see Figure 5a). These diffraction peaks respectively correspond to the (111), (200), (220), and (311) Miller indices of fcc Ag. Generally, the intensity ratio between



Figure 5. (a) XRD pattern of dried Ag nanoclusters material and (b) DLS data of fluorescent Ag nanoclusters.



Figure 6. High-resolution mass spectroscopy (HR-MS) spectrum of fluorescent Ag nanoclusters, showing the exclusive presence of Ag_4 and Ag_5 clusters (here, L = DHLA (dihydrolipoic acid)).

the peaks corresponding to the (111) plane versus the (200) plane is 0.40 versus 0.24.²⁶ It can be mentioned in this study that the intensity ratio was 2.41 versus 0.61, which is higher than the conventional value. This observation reveals that Ag nanoclusters are primarily dominated by (111) facets. The size distribution obtained from DLS study (Figure 5b) suggests that the population of Ag nanoclusters is almost monodisperse. The narrow size distribution of clusters agrees well with the narrow emission bandwidth. The observed mean diameter of the clusters is 2.5 nm, which matches the average diameter obtained from TEM well (2.44 nm).

High-Resolution Mass Spectral (HR-MS) Study. The formation of nanoclusters was also confirmed by HR-MS analysis. The spectrum (Figure 6) shows the presence of nanoclusters containing only Ag_4 and Ag_5 . These two nanoclusters exhibit their peaks both free and in associated states with DHLA with an almost 1:1 molecular ratio. In this regard, it can be mentioned that Tsukuda and coworkers have reported that the stoichiometric ratio of [Au]:[ligand] is 1:1 in thiolated Au clusters. They obtained this ratio by analyzing the electrospray ionization (ESI)mass data.^{4c} In our study, generally no clusters other than Ag_4 and Ag_5 have been observed. Dickson and co-workers reported the presence of Ag_1 - Ag_5 clusters, using matrixassisted laser desorption/ionization (MALDI) mass spectrometry, which results in the observation of a broad emission band.^{3e} Ras et al. showed the presence of clusters containing a small number of Ag atoms, such as Ag₁, Ag₂, and Ag₅, because it was evident from their matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum analysis.²⁵ Mass analysis also confirms the narrow size distribution of our Ag nanoclusters, and this result is in good agreement with the observed narrow emission bandwidth in the PL study (see Figure 1b).

Semiconducting Study. Another interesting feature of these Ag nanoclusters is that they exhibit semiconducting properties. Theoretically, it has been reported that there is a trend to decrease the conductivity with reduction of the core size of metallic nanoparticles.²⁷ Recently, Whetten and co-workers reported, in a computational study, that a thiolated Au nanowire can be made semiconducting by considering the Au/thiolate interface and the electronic configuration of the ligand-protected Au nanoclusters.²⁸ The observed electrical conductivity of our fluorescent Ag nanoclusters was 2.48×10^{-5} S cm⁻¹ at room temperature, and this clearly indicates the transition from metal to semiconductor at a very tiny size dimension of Ag clusters. To the best of our knowledge, this is the first experimental evidence that demonstrates that a conducting Ag metal (with an electrical conductivity of $63 \times 10^4 \text{ S cm}^{-1}$ in the bulk state) has been transformed to semiconducting Ag

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Figure 7. (a) Fluorescence response of Ag nanoclusters (volume = 4 mL, concentration = 10^{-5} M) upon the successive addition of 10 μ L aqueous Hg^{II} ions with increasing concentration. (b) HR-TEM image of the aggregated Ag nanoclusters in the presence of Hg^{II} ions.

nanoclusters by size reduction. This type of material may be served as a promising candidate for the development of light-emitting diodes, solar cells, field-effect transistors, thermoelectric materials, and applications in other fields.²⁹

Metal Ion Sensing Study. The concentration of Ag nanoclusters in water that was used for the metal-ion sensing experiment was 1×10^{-5} M and the metal-ion sensing started with the metal-ion concentration from 10^{-10} M and it has been increased gradually to 10^{-5} M. We found that the fluorescence emission was rapidly quenched in the presence of Hg^{II} (see Figure 7 a). More and more quenching occurs with an increase in Hg^{II} and, surprisingly, fluorescence intensity has been decreased to zero (i.e., 100% quenching) up to additions of 10^{-5} M Hg^{II} and its quenching value was 87.6% up to additions of 10^{-7} M Hg^{II}. To check the selectivity of this sensor, we also carried out studies with other metal ions, such as K^I, Cs^I, Sr^{II}, Ba^{II}, Mg^{II}, Mn^{II}, Fe^{II}, Co^{II}, Pb^{II}, Cu^{II}, Zn^{II}, Sn^{II}, and Pd^{II} under exactly similar conditions that were used for the detection of Hg^{II}. The quenching data is presented meticulously in Figure S8 in the Supporting Information. For other metal ions, for additions of up to 10^{-7} M ions, a quenching effect like that of the Hg^{II} ion was not observed. Even the addition of more-concentrated solutions of other metal ions cannot reduce its fluorescence intensity to zero. The relative PL quenching of fluorescent Ag nanoclusters toward various common metal ions is presented in Figure 8. This result suggests that our fluorescent Ag nanoclusters are selective for Hg^{II} detection.

There is an excellent linear relationship between fluorescence quenching and the logarithm of the concentration of Hg^{II} ions within the range of $10^{-8}-10^{-5}$ M (see Figure S9 in the Supporting Information). This suggests that our fluorescence quenching effect of Ag nanoclusters by Hg^{II} ions abide by the Stern–Volmer equation. The limit of detection (LOD), down to 10^{-10} M, was reached. This LOD value is much lower than the maximum permissible limit of mercury in the drinking water (2 ppb, 10 nM) declared by the United States Environmental Protection





Figure 8. Fluorescence quenching effect $[(I_0 - I)/I_0]$ of Ag nanoclusters at 652 nm, up to an addition of 10^{-7} M different aqueous metal ions.

Agency (EPA) and by the European Union (1 ppb, 5 nM).³⁰ To the best of our knowledge, this is the supreme obtainable sensitivity for the detection of Hg^{II} ions in water, compared to other previously reported values.^{15–20}

The fluorescence quenching of Ag clusters may occur through the Hg^{II} mediated interparticle aggregation mechanism.¹⁹ The aggregation is conducted via the free carboxylic acid (present in reduced lipoic acid on the surface of Ag nanoclusters-ligand conjugate), which can easily interact with Hg^{II} ions. In the presence of Hg^{II} ions, aggregation occurred through an ion-templated chelation method.^{19a,b} The HR-TEM analysis shows evidence for the aggregate formation of Ag nanoclusters upon the addition of Hg^{II} (Figure 7b) and, in this case, particles < 7 nm in size was never observed. This explains the nonfluorescent behavior of the resulted solution of the aggregated species. The fluorescence intensity of Ag clusters also quenches to a small amount in the presence of other metal ions (except Hg^{II}). The maximum fluorescence quenching effect for Hg^{II}, among all the metal ions, is due to the fact that simple carboxylic acids have a much-stronger affinity toward Hg^{II}

⁽³⁰⁾ Dynamics of Mercury Pollution on Regional and Global Scales: Atmospheric Processes and Human Exposures Around the World; Pirrone, N., Mahaffey, K. R., Eds.; Springer: New York, 2005.

Article

ions in water (log $\beta_4 = 17.6$).³¹ The fluorescence quenching effect may be interpreted another way, in terms of the donation of electron density from the Ag nanoclusters to the adsorbed cations.³² In the presence of Hg^{II} ions, the electron donation leads to partial Ag oxidation and amalgam formation.³² Consequently, the fluorescent Ag nanoclusters goes to a nonfluorescent form and this results in a decrease in fluorescence signal of the sensor system. However, it can be mentioned that, unlike fluorescence, the presence of Hg^{II} ions has a less significant effect on the absorption spectra (see Figure S10 in the Supporting Information), because fluorescence is more sensitive to changes in the particle size than the absorption.

Conclusions

In summary, we have demonstrated an easy synthesis for water-soluble, semiconducting, fluorescent Ag nanoclusters using a small molecule (reduced lipoic acid) as a template and it can readily, selectively, and very sensitively detect mercury ions (Hg^{II}), even at subnanomolar concentrations. Our Ag nanoclusters have well-defined excitation and emission spectra, a narrow emission profile, a larger Stokes shift, and good photostability. Moreover, this

procedure does not require high temperature or toxic precursors. Thus, these newly prepared bright fluorescence Ag nanoclusters hold future promise of using this material as a good candidate for biolabeling, biosensing, and applications for other purposes.

Acknowledgment. B.A. thanks the CSIR, New Delhi, India, for financial assistance.

Supporting Information Available: AFM image of the surface of a Ag nanocluster-DHLA nanocomposite film (Figure S1a); current-voltage (I-V) plot of fluorescent Ag nanoclusters (Figure S1b); PL spectra of the Ag nanocluster at different excitation wavelengths (Figure S2); concentration-dependent photoluminsence of Ag clusters (Figure S3); measurement of the fluorescence lifetime of the Ag nanoclusters (Figure S4); a comparison of the fluorescence of Ag nanoclusters just after their synthesis and after 3 months of storage in water (Figure S5a, absorption spectra; Figure S5b, emission spectra); HR-TEM image of fluorescent Ag nanoclusters after 3 months of storage (Figure S6), cluster size distribution (Figure S7a) and SAED image from TEM (Figure S7b); fluorescence response of Ag nanoclusters upon the addition of different metal ions (Figure S8); plot of fluorescence quenching effect of Ag nanoclusters versus log[Hg^{II}] (Figure S9); absorption response of Ag nanoclusters upon the addition of HgII ions (Figure S10); and expanded view of FT-IR spectra (Figure S11) and HR-TEM image (Figure S12) of fluorescent Ag nanoclusters. This information is available free of charge via the Internet at http://pubs.acs.org.

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