Interfacial synthesis of luminescent 7 kDa silver clusters†‡

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We report the synthesis of luminescent Ag clusters through the *interfacial etching* of mercaptosuccinic acid (MSA) protected silver nanoparticles, Ag@MSA, with guanine at the water-toluene interface. The clusters exhibiting well-defined absorption emit in the near-infrared (NIR) region. Crude clusters were separated using polyacrylamide gel electrophoresis (PAGE). The cluster solid, prepared by freeze drying, is highly hygroscopic. Biomolecular markers were used to identify the approximate mass of the cluster which was found to be 7 kDa, as mass spectrometry did not reveal specific signatures. The clusters were investigated using UV-Vis spectroscopy, transmission electron microscopy (TEM), energy dispersive analysis of X-rays (EDAX), X-ray photoelectron spectroscopy (XPS), infrared spectroscopy (IR), and fluorescence spectroscopy. Elemental analysis and IR studies reveal the protection of the cluster by two types of ligands, namely MSA and guanine. Fluorescence of the cluster is highly temperature dependent, with an increase in intensity with decrease in temperature. Influence of different ratios of reactants, etching capacity of different nucleobases and effect of temperature on the synthesis as well as possible single-phase etching were investigated. Sensitivity of the cluster to certain metal ions has been monitored using fluorescence spectroscopy.

Introduction

Clusters are a class of materials considered as intermediates between atoms and bulk materials. In the case of ultra-small clusters, which have sizes smaller than 1 nm, the band structures of the extended solids get modified to discrete energy levels. When particle size reaches this regime, optical properties change most significantly. Quantum clusters like Au_{11}[1] and Au_{55}[2] were discovered decades ago. For the synthesis of metal clusters, different ligands such as thiols, amines, surfactants and phosphines have been used.[2,3] Au_8 quantum dots have been prepared using poly(amido)amine (PAMAM) dendrimers as a surface stabilizing agent[4] as well as with glutathione protection.[5]

Transition metal clusters, especially of Au, have been investigated intensely, mainly due to their remarkable catalytic activities arising from their large surface to volume ratio and modifications in the electronic structure.[6] For example, while bulk Au metal is chemically inert, mass-selected deposited Au clusters, Au_N ($N = 2$–20), on MgO (001) plays a catalytic role in the oxidation of CO.[7] Similarly, Ag clusters are of importance. However, only a few luminescent Ag clusters are known. The bioconjugated α-chymotrypsin Ag nanocrystals are of interest since they show intense luminescence in the visible region.[8] DNA template and oligonucleotide bound Ag clusters (Ag_1 to Ag_4) belong to this category and emit in the 400–600 nm range.[9]

Nanomaterials have been synthesized by a variety of methods. *Interfacial synthesis* has been considered as a viable option in the recent past. Such methods have been used for phase transfer, self organization, chemical reactions and phase transformation of nanoparticles.[10] Interfacial phenomena are complex involving several factors such as interfacial potential, surface tension, concentration gradients, *etc.* Liquid–liquid interfaces were employed in the preparation of nanocrystals.[11] Aggregates of CdS nanocrystals were obtained at the water–toluene interface.[12] It was shown that the interface between two immiscible liquids could be used for the preparation of films of metals such as gold and silver, chalcogenides and oxides.[13,14] Generally metal nanocrystals were prepared by taking the metal precursor in the organic phase and a reducing agent in the aqueous phase.[15] Nanocrystal superlattices can be formed at the air–aqueous interface.[16]

In the present work, we have explored the possibility of making luminescent Ag subnanoclusters using an interfacial etching reaction at the water–toluene interface. The etching capacity of some nucleobases was used here for this. The as-prepared nanoclusters are highly soluble in water and emit in the near-IR region. They are stable in solution phase at room temperature for long periods of time without aggregation. Effective core-size reduction of Ag@MSA (MSA = mercaptosuccinic acid) nanoparticles (NPs) has been attained using guanine sulfate as the ligand (note: A@B implies a core of A protected with a shell of B). From our study, it was found that an immiscible liquid–liquid interface is needed for the preparation of these kinds of small Ag nanoclusters starting from Ag@MSA

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‡ Electronic supplementary information (ESI) available: Absorption spectrum of guanine sulfate and TEM images of precipitate after etching reaction, expanded view of FT-IR, ESI-MS of MSA, guanine sulfate and the cluster, LDI MS of the cluster and the ligands, fluorescence spectra after metal ion addition and photographs of the cluster solution at different temperatures, UV-Vis spectra of the etching reaction of Ag@MSA with adenine, thymine and cytosine, absorption spectra of different ratios of reactants, absorption spectra of single phase etching and TEM images of the aggregated particles. See DOI: 10.1039/b900025a

precursor. As these small clusters show intense absorption and emission, we can use them as fluorophores. It may be noted that while interfacial etching is efficient for silver, gold clusters such as Au_{25} and Au_8 are made in gram quantities through homogeneous etching.[5]

Experimental

Materials

Silver nitrate ($AgNO_3$, 99.8%), MSA (97%), methanol, ethanol and toluene (all GR grade) were purchased from SRL Chemical Co. Ltd., India. Sodium borohydride ($NaBH_4$, 98%) was purchased from Sigma Aldrich. Guanine sulfate (G), adenine (A), thymine (T) and cytosine (C) were received from SPECTROCHEM Pvt. Ltd., India. Electrophoresis grade acrylamide, *N*,*N*′-methylenebisacrylamide, ammonium persulfate (APS), glycine, tris(hydroxymethylaminomethane), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and tris(hydroxymethylhydrochloride) were received from Sigma Aldrich. All chemicals were used without purification.

Synthesis of Ag@MSA nanoparticles (NPs)

The preparation method employed here for Ag@MSA NPs is similar to that reported previously.[17] About 0.5 mM of $AgNO_3$ was mixed with 1 mM of MSA in methanol (100 ml) to give a transparent solution. Freshly prepared 0.2 M aqueous $NaBH_4$ solution (25 ml) was added to it by drop by drop injection, under vigorous stirring. The colour of the solution changes from yellow to dark brown during stirring. After one hour of stirring, the solution was decanted and the dark brown precipitate was washed twice with a 20% water–methanol mixture. Then the precipitate was washed with ethanol several times to remove impurities. Excess solvent was removed using vacuum drying. The resulting brown Ag@MSA NPs showed characteristic surface plasmon resonance (SPR) around 400 nm.

PAGE separation[18] of a collection of clusters was carried out using the BIO RAD Mini-PROTEAN Tetra Cell gel electrophoresis unit. At a time we used single or double gels with 1 mm thickness. The procedure for the separation of the clusters using PAGE is described below. The separating and stacking gels were prepared with total contents of acrylamide monomers of 30 and 3 wt% (acrylamide/bis-acrylamide = 93:7), respectively. They were buffered at pH 8.7 and 6.8, respectively using Tris-HCl. The eluting buffer was made of 192 mM glycine and 25 mM tris(hydroxymethyl aminomethane). The as-prepared clusters were dissolved in 5% v/v aqueous glycerol solution. The sample solution was loaded onto the staking gel and continuously eluted for 5 hours at a constant voltage of 150 V. The elution was performed at room temperature (~300 K). Gels with lanes and without lanes were used and the same result was obtained after separation. The separating gel containing cluster fractions was cut out, crushed into pieces, dissolved in water and kept overnight under cold conditions. Suspended gel lumps were removed by centrifugation at 20 000 rpm. The solution was subsequently freeze dried to get a reddish brown powder. The material was highly hygroscopic but stable in the solid state and in solution at room temperature. As a result of the hygroscopic nature, accurate elemental analysis could not be performed.

Sensitivity of the cluster to metal ions

A water–methanol solution of the cluster was allowed to interact with metal ions such as Hg^{2+}, Mg^{2+}, and Ni^{2+}. Initially 3 ml of the cluster solution (in 1:1 water–methanol) was used. Final concentrations of the metal ions in the cluster solutions were made to 1 ppb, 10 ppb, 100 ppb, and 1000 ppb. Influence of ions on the fluorescence of the cluster was monitored as a measure of sensitivity. Fluorescence spectra were taken after cooling the sample using an ice bath. Chemicals used for this were mercurous chloride, magnesium nitrate, and nickel acetate tetrahydrate. They were obtained from commercial sources (CDH (P) Ltd., India for the first two and SD-Fine Chemicals Ltd., India for the last).

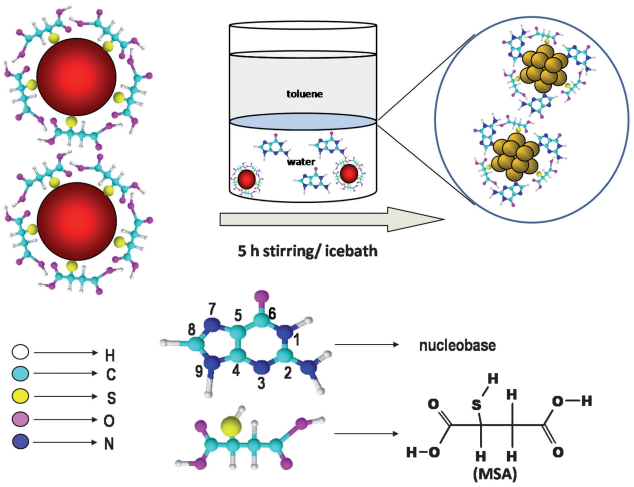
Instrumentation

Absorption spectra were recorded using a Perkin Elmer Lambda 25 spectrophotometer over the range 200–1100 nm. The FT-IR spectra were measured with a Perkin Elmer Spectrum One instrument. Samples were prepared by pressing KBr powder with the solid cluster powder into pellets. High resolution transmission electron microscopy (HRTEM) of the samples was carried out using a JEOL 3010 instrument with a UHR polar piece. TEM specimens were prepared by drop casting one or two drops of aqueous solution onto carbon coated copper grids which were allowed to dry at room temperature overnight. All measurements were done at 200 kV to minimize the damage of the sample. EDAX images of the cluster were taken with an SEM FEI QUANTA 200. X-Ray photoelectron spectroscopy (XPS) was done using an ESCA Probe TPD of Omicron Nanotechnology. Sample solution was spotted on a Mo sample plate and dried in vacuum. Monochromatic Al K_α was used as the X-ray source ($h\nu$ = 1486.6 eV). Spectra in the required binding range were collected and an average was taken. Beam induced damage of the sample was reduced by adjusting the X-ray flux. Binding energy was calibrated with respect to C 1s at 284.7 eV. Fluorescence spectrum was measured using aNano Log HORIBA JOBIN YVON spectrofluorimeter. A xenon lamp was used as the excitation source. The band pass for excitation and emission was set as 5 nm. Samples were cooled in a water bath to attain different temperatures. The mass spectrometric studies were conducted using a 3200 Q-TRAP LC/MS/MS (Applied Biosystems). 50% Water–methanol solutions of samples were used for electrospray ionization. Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) was done using an Applied Biosystems Voyage DE PRO Biospectrometry Workstation. Sinapinnic acid was used as the matrix. Mass spectra were also collected without matrix.

Results and discussion

Cluster synthesis

The parent Ag@MSA nanoparticles have a large core size distribution, in the range of 5–10 nm, and have been characterized adequately by a variety of techniques.[19] Such large core size distribution is typical of silver nanoparticles. These nanoparticles were subjected to interfacial reaction as shown in Scheme 1. In a typical process, Ag@MSA NPs (10 mg) and the nucleobase (40 mg) were taken in the aqueous phase (20 ml) and etching reaction was performed in the presence of toluene as the organic



Scheme 1 Schematic representation of the interfacial etching reaction of Ag@MSA with nucleobases (different weight ratios). Ag@MSA along with the nucleobase was taken in the aqueous phase and etching was performed in the presence of toluene as the organic phase in ice-cold conditions.

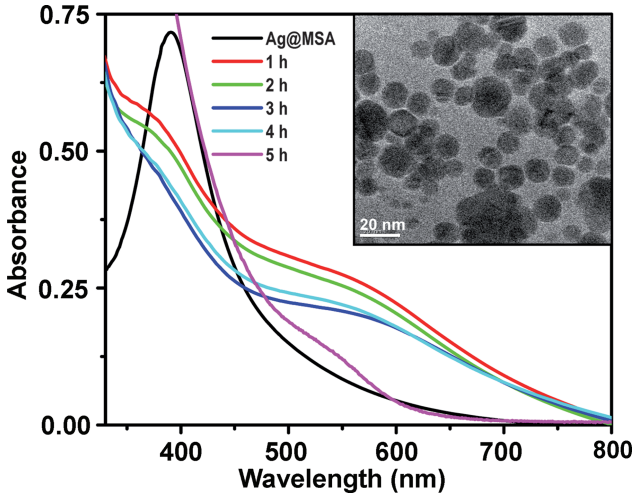


Fig. 1 Time dependent UV-Vis spectra of the interfacial etching reaction of Ag@MSA with guanine sulfate at 1:4 weight ratio at 0 °C. Time zero corresponds to the absorption of parent Ag@MSA. A TEM image of the parent Ag@MSA nanoparticles is shown in the inset.

phase (20 ml). Etching was done in an ice bath and the reaction was monitored by optical absorption spectroscopy. A faint pink layer was formed at the interface. The color of the aqueous phase changed to pale yellow. However, no characteristic change was observed for the organic phase. The resulting aqueous layer was separated and unreacted species were removed through centrifugal precipitation. The supernatant was dried to obtain a crude powder of the cluster. It was washed several times using methanol. A pale yellow powder was obtained after solvent evaporation. This mixture of clusters was separated by polyacrylamide gel electrophoresis (PAGE) according to their size and charge.

Time dependent study of the interfacial etching reaction

Time dependent UV-Vis absorption spectra during the etching reaction of Ag@MSA with guanine sulfate (in 1:4 weight ratio) were measured in an ice bath. The as-prepared Ag@MSA NPs showed a characteristic SPR around 400 nm, shown by the black trace in Fig. 1, typical of metallic particles. In the course of the reaction, the aqueous layer was centrifuged and the supernatant was analyzed periodically to check the formation of new NPs or clusters in it. As time progressed, the feature of Ag@MSA started disappearing and a new absorption was seen around 550 nm. This is likely to be due to the removal of surface atoms of Ag@MSA by the etching of ligand molecules, forming smaller clusters. The spectrum got broadened as well. After 5 h of the etching reaction, SPR of Ag@MSA was completely absent in the supernatant and only the new absorption around 550 nm was visible. It is shown by the magenta trace in Fig. 1. No change was observed in the absorption spectra after this, indicating the completion of etching reaction in 5 h. The reaction was stopped, the aqueous layer was centrifuged and the supernatant was used for further analysis. NPs with a diameter less than 3 nm are known to show a broadened SPR.[8] For smaller particles, the plasmon will be absent and some of the Au clusters show visible and near infrared (NIR) absorption. After etching, a precipitate was found at the bottom of the reaction vessel. This was dispersed in water and centrifuged. When the supernatant was analyzed by UV-Vis spectroscopy, it showed features of guanine sulfate. The dispersion was spotted on the TEM grid and images were taken. Images at different magnifications are given in ESI‡ (S1 B–D). They show lumps of particles with irregular shape. Thus a fraction of the nanoparticles do not get reacted.

The 550 nm absorption of the supernatant is attributed to the presence of small Ag clusters in the solution. The cluster solution was found to be stable at room temperature for a long time. DNA templated clusters of silver, such as Ag_2 and Ag_3, show absorptions at 424 and 520 nm.[8] Ag_4 to Ag_7 clusters bound by cytosine oligonucleotides have a strong peak at 440 nm.[20] But these absorptions are not broad when compared to those of the as-synthesized clusters seen here. No clusters were formed without the organic phase (see below). The supernatant after the etching reaction showed the absorption features of the ligand (guanine sulfate), which occur at 246 nm and 275 nm (ESI‡ Fig. S1A). It is likely to be due to the presence of excess ligand in the solution or due to ligand molecules attached to cluster core. This aspect will be discussed later in the paper. Mercaptosuccinic acid is not having any characteristic absorption in the visible region shown in Fig. 1.

PAGE separation

The PAGE separation yielded only one clear band which is likely due to one cluster (Fig. 2). A photograph of a typical PAGE band under normal illumination is shown in the inset of Fig. 2. A clear orange colored band was observed. The corresponding absorption spectrum is shown as trace b, having a broad maximum around 550 nm. The same absorption was seen for the supernatant also in the initial etching reaction (trace c). This confirmed the formation of a cluster in the aqueous phase. Electrophoretic separation was done for a number of cluster samples to confirm reproducibility. Only one band was clear and reproducible in each case.

The approximate molecular weight of the cluster was determined using a molecular weight marker molecule (see below).

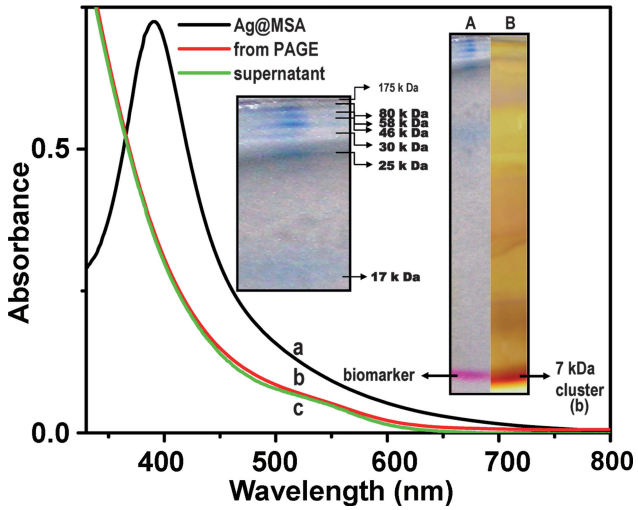


Fig. 2 Absorption spectra of a) Ag@MSA, b) cluster after PAGE separation and c) supernatant after 5 h of interfacial etching for comparison. Inset: Photographs of PAGE gel under normal illumination: (A) of the molecular marker and (B) of the cluster. High mass region of the biomarker is shown separately on the left to show the bands clearly. The spectrum b is from the band marked (b).

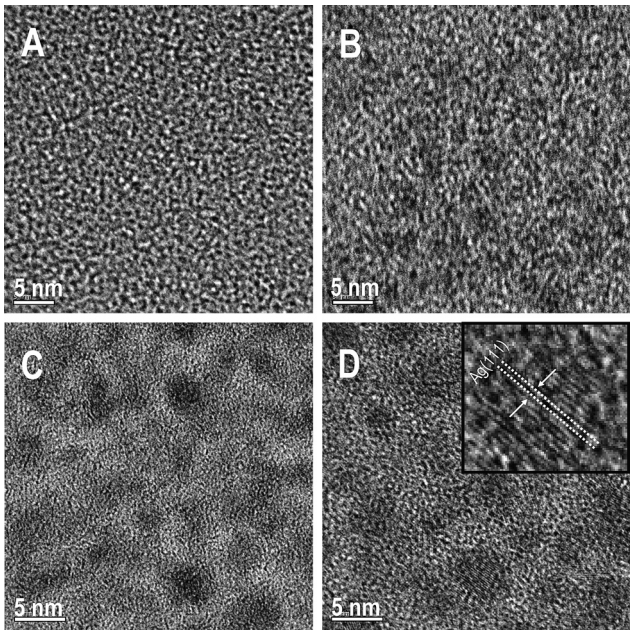


Fig. 3 TEM images of PAGE separated cluster obtained from interfacial etching reaction of Ag@MSA with guanine sulfate, as a function of electron beam irradiation at 200 kV. A, B, C and D correspond to increasing time of irradiation. Inset of D shows the Ag (111) lattice plane.

The marker molecule used is a prestained biomarker which is a mixture of 8 proteins. It resolves into 8 bands on the gel upon electrophoresis, with molecular weight ranging from 7 kDa to 175 kDa in Tris-glycine buffer. An expanded view of the biomarker upon PAGE separation is given in the inset of Fig. 2. Both the biomarker and the cluster were subjected to PAGE under similar conditions as described above. The relative movements of the separated bands of the cluster and the last band (pink coloured in the inset of Fig. 2) of the biomarker are comparable under the above conditions of electrophoresis. The band of the biomarker has a molecular weight of 7 kDa which can be assigned as the approximate molecular weight of the cluster. Several studies were done on this separated cluster.

Characterization

High magnification HRTEM images of the PAGE-separated Ag nanoclusters are given in Fig. 3. Small clusters are sensitive to electron beam irradiation and are known to produce larger NPs upon irradiation for a prolonged time.[21] Initially no NPs were present when sample was exposed to the electron beam (Fig. 3A). Note that initial Ag@MSA NPs had 5–10 nm diameter. The isolated clusters of sub-nanometer dimensions are not expected to be seen clearly. The grid was observed under a constant scale bar of 5 nm and the same area was focused upon throughout the analysis. After a period of 10 minutes, large NPs started to appear on the grid. It is believed to be due to the coalescence of small Ag clusters leading to the formation of large NPs. The same area was examined for more time to check any subsequent changes in the sample. Formation of still larger NPs was observed. A series of time dependent images are shown in Fig. 3B–D. The Ag(111) lattice plane in image D clearly shows the coalescence of clusters forming large nanoparticles.

A typical EDAX spectrum of a purified cluster sample is given in Fig. 4. The prominent Ag peak confirms the presence of Ag as the major constituent of the cluster. Elemental mapping of the sample revealed the presence of other elements such as S, N, C and O in the cluster. The atomic composition determined overestimates O and C due to the substrate (ITO coated glass) and atmospheric contamination of the highly hygroscopic material, respectively. However, the Ag:S atomic ratio determined is reliable, which was 1.58. The atomic ratio of C:N is 5.2, which could be an overestimate due to atmospheric carbon contamination.

XPS spectra show the various constituent elements of the cluster (Fig. 5). Binding energies of Ag $3d_{5/2}$ (368.1 eV) and Ag $3d_{3/2}$ (374.1 eV) are matching with the Ag(0) values. It is clear that the cluster is quite different from an Ag(I) complex. The same binding energy is seen in the parent silver NPs also. Binding energies of C 1s, N 1s, O 1s, and S 2p were also examined. Two components of nitrogen are shown in Fig. 5D, which are due to C–N and C=N present in the etching ligand used. The binding energies observed are 399.4 eV and 401.1, eV different from the typical N1s value of 400.0 eV. This reflects the nature of the nitrogen in guanine. The presence of C=O and C–O of MSA contributes to the larger width of O 1s. There can also be a contribution from the adsorbed water. S 2p is resolved into the two components, $2p_{3/2}$ and $2p_{1/2}$, as shown in Fig. 5F. The binding energy corresponds to the thiolate value as expected for MSA, which exists in the thiolate form. Thiolate does not show any X-ray induced oxidation to give sulfonate or sulfate, unlike in the case of nanoparticles.[22] The S 2p is not due to sulfate as it is expected only at 170 eV. Various types of carbons are reflected in the C 1s spectrum. As carbonaceous impurities are expected on the sample and conventional cleaning by argon ion etching or heating is not possible for such delicate cluster samples, the intensities of the C 1s features are not reliable. From the XPS data, it is clear that the ligands, MSA and guanine are present on

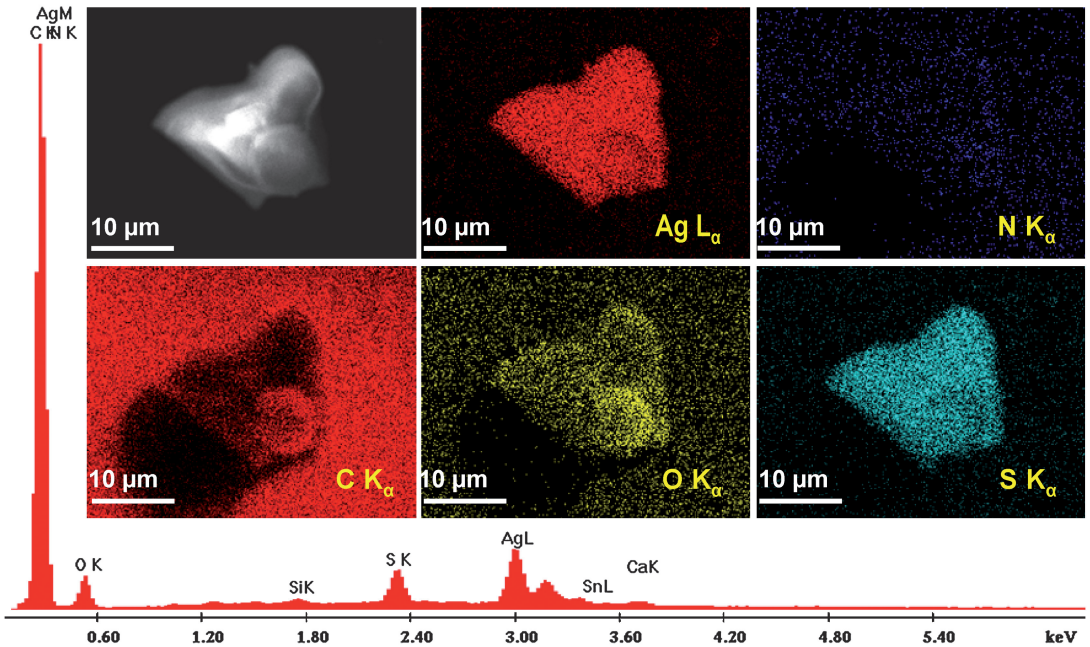


Fig. 4 EDAX spectrum of a cluster aggregate. Inset: SEM image and EDAX mapping of the cluster aggregate, using characteristic X-rays. The Si, Sn and Ca peaks in the EDAX spectrum are due to the conducting glass substrate. Part of the oxygen is also due to the substrate.

the surface and Ag in their integral form (except that MSA is in the thiolate form). Thus, both EDAX and XPS confirm the presence of MSA and guanine on the surface of the cluster. Some of the guanine molecules, during etching, may attach to the Ag core by replacing MSA molecules. But complete exchange of

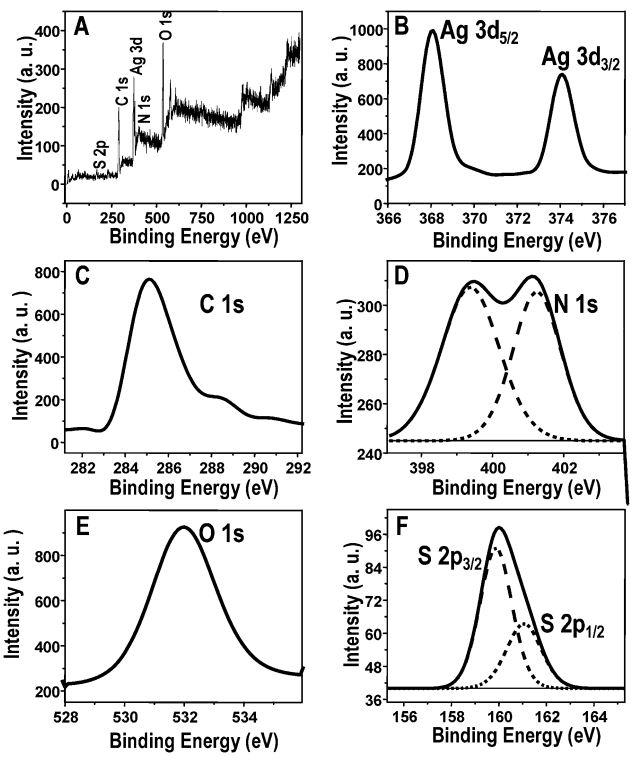


Fig. 5 A survey XPS spectrum and expanded regions of specific core levels of the PAGE separated Ag cluster.

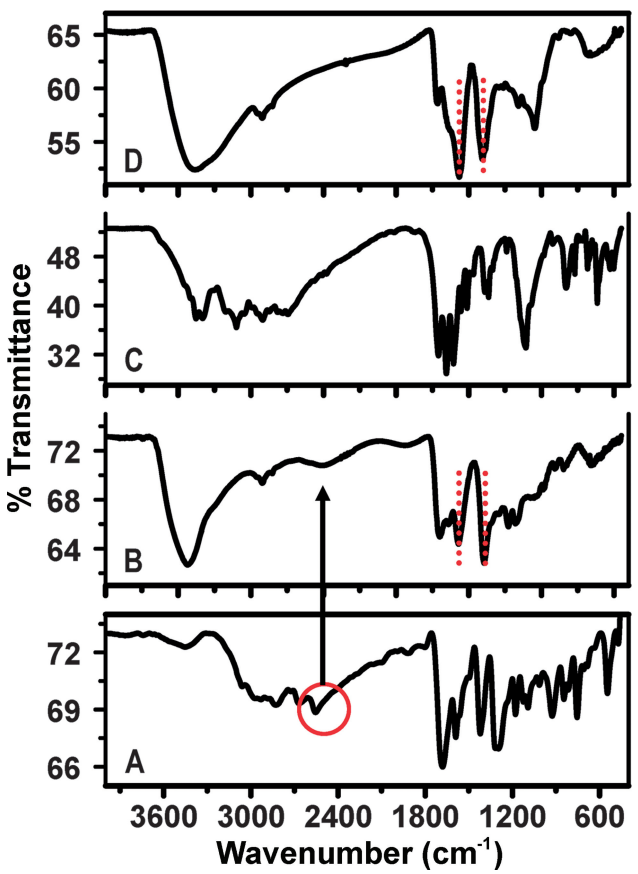


Fig. 6 FT-IR spectra: A) pure MSA, B) as-prepared Ag@MSA NPs, C) guanine sulfate, and D) silver clusters after PAGE. The –SH stretching frequency (marked by circle) of MSA is absent when it is on surface of the nanoparticles.

MSA molecules might not be achieved by etching, particularly due to the affinity of thiolates to silver. The EDAX data suggest nearly a 1:1 ratio for MSA:guanine on the cluster, as the observed atomic ratio of S:N was 2.39:10.74 in EDAX. Note that there are five N per guanine and one S per MSA.

Fig. 6 shows the FT-IR spectra of MSA, Ag@MSA, guanine sulfate and the cluster. FTIR spectroscopy was used to compare the characteristics of the cluster with the ligands used. Both Ag@MSA and the cluster have absorption in the OH vibrational (stretching) region. The broad peak around 3500 cm^{-1} is due to the existence of bound water in both Ag@MSA and the cluster. The peak at 2552 cm^{-1} (due to S–H stretching, marked by a red circle in Fig. 6A) present in MSA was absent in Ag@MSA indicating that MSA is attached to Ag through the S atom. The stretching frequency of –COOH of MSA appears as a peak around 1680 cm^{-1}, but it exists as the carboxylate ion ($–COO^-$) in Ag@MSA (presence of the bands at 1387cm^{-1} and 1571 cm^{-1}, due to symmetric and asymmetric stretching of COO^- in Ag@MSA).[17] This characteristic pattern is seen in the cluster too which is marked (red dotted lines in Fig. 6B and D).

It was found that N–H stretching frequencies of guanine sulfate are absent in the cluster. Expanded views of the FT-IR of the cluster in the regions, 600–900 cm^{-1}, 1000–1250 cm^{-1}, 660–1700 cm^{-1} and 3000–4000 cm^{-1} along with those of guanine sulfate are given in ESI‡ Fig. S2. The absence may be due to the fact that guanine is attached to the Ag core through N (N-9, see Scheme 1). Some similarity between the features of guanine and the cluster is observed in the bending region. Cluster also shows some similarity to the features of MSA and guanine. It confirms the presence of both MSA and guanine on the surface of the cluster.

ESI-MS of guanine sulfate shows a peak at *m/z* 152 (ESI‡ Fig. S3) due to protonated species. The corresponding spectrum of MSA shows a peak at *m/z* 149 in the negative mode. The cluster shows both the features of MSA and guanine in negative and positive modes, respectively (ESI‡ Fig. S3). LDI MS of MSA, guanine sulfate and cluster are given in ESI‡ Fig. S4. Both the analyses confirm the presence of MSA and guanine on the cluster. MALDI MS did not reveal integral features of the cluster unlike in the case of $Au_{25}SG_{18}$ or other such clusters[23] (SG = glutathione thiolate). The absence of integral cluster feature in mass spectra is not a surprise as these ions are expected to have poor stability. The cluster features are sensitive to the instrument configuration. For example, ESI MS gives good features for $Au_{25}SG_{18}$ for the cluster in an in-line geometry instrument[24] and does not give a molecular ion feature in orthogonal ESI and MALDI-ToF MS.[23]

Parent Ag@MSA NPs are not fluorescent since they are metallic. But the PAGE separated cluster emits near infrared (NIR) fluorescence as shown in the inset of Fig. 7. It reveals the molecular-like electronic transitions in the cluster. Both the ligands do not fluorescence in this window. The electronic transition can be attributed to be those between the higher excited states in the sp band to the lower-lying d band (interband transition).[7] Dickson and co-workers synthesized DNA bound Ag clusters which shows a prominent fluorescence at 638 nm with an excitation maximum of 560 nm.[9] Oligonucleotide stabilized red emitting clusters (ex. 550 nm, em. 650 nm) and NIR emitting clusters (ex. 650 nm, em. 705 nm) are also known.[9] Most of these clusters show several electronic transitions in contrast to the cluster reported here.

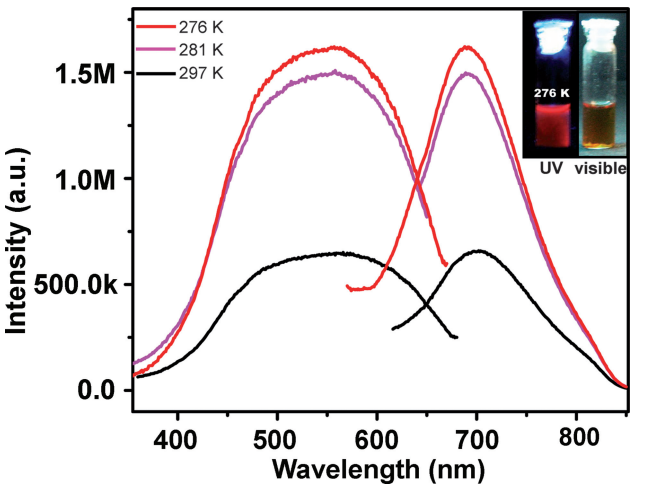


Fig. 7 Photoluminescence spectra of the cluster sample dissolved in 1:1 water-methanol mixture at different temperatures. Inset: photograph of the cluster under UV light at 276 K.

It was found that the intensity of the fluorescence depends highly on the temperature of the sample. Hence a temperature dependent study of fluorescence of the cluster was done to monitor this and the spectra are shown in Fig. 7. The cluster emits at 700 nm when excited at 550 nm and at room temperature (293 K). A blue shift of 10 nm in the emission maximum (690 nm) was observed when the temperature was decreased to 281 K. A further shift to 688 nm was observed when the temperature was changed to 276 K. Corresponding photographs of the cluster solutions under UV light are given in ESI‡ Fig. S5A. The quantum yield of the cluster was determined to be 0.015 at room temperature and 0.04 at 283 K.

Influence of metal ions such as Hg^{2+}, Ni^{2+} and Mg^{2+} on the fluorescence of the cluster was investigated. It was observed that addition of Hg^{2+} does not change the absorption features of the Ag cluster. The color of the cluster solution remained the same as before the addition of the ions. It was found that the intensity of fluorescence was quenched by the addition of Hg^{2+}. Fluorescence intensity was found to be decreased considerably with increase in concentration of metal ions in the cluster solution. The concentrations of the ions were varied from 1 ppb to 1000 ppb. The cluster was sensitive to the ions even at the ppb level. Fluorescence intensity decreased to more than half of the initial value upon the addition of 1000 ppb Hg^{2+}. Sensitivity to other metal ions was also studied. Fluorescence quenching was not prominent upon the addition of Mg^{2+} and Ni^{2+}ions, when compared to Hg^{2+}. Fluorescence spectra at different concentrations of metal ions are given in ESI‡ Fig. S5B, C and D.

It can be concluded that the cluster is highly sensitive to Hg^{2+} in comparison to others, although all ions show some sensitivity.

Additional experiments

Several additional experiments were performed with other nucleobases such as adenine, thymine, and cytosine as the etching ligands. We also tried the etching reaction at room

temperature, single phase etching and with different ratios of reactants.

Etching with different nucleobases. Here we followed the same procedure for etching as mentioned before in the 1:4 weight ratio. Adenine, thymine and cytosine were used as etching ligands in different trials. Time dependent optical spectra during the etching reaction using adenine, thymine and cytosine are shown in ESI‡ Fig. S6A, B and C, respectively. No characteristic change was observed for both the phases in all etching reactions. A red shift in the characteristic peak position of Ag@MSA NPs was found during etching when adenine was used as etching ligand. A weak absorption band around 550 nm was formed by the addition of thymine as the ligand. The intensity of absorption was slightly decreased which continued up to 5 h of the reaction in the case of cytosine. No other spectral changes were observed and hence the reaction was stopped in all etching reactions. A slight decrease in the intensity of the absorption was observed during the etching process.

But the nucleobases were unable to produce any reasonable change in the spectral feature of Ag@MSA NPs. The spectrum was not broadened during etching reactions. Combining all these observations, we note that the etching capacities of nucleobases adenine, thymine and cytosine were poor to remove the surface Ag atoms of Ag@MSA. In Ag@MSA, mercaptosuccinic acid is bound to Ag through the S atom. The strong binding may not be displaced by the amine group of these nucleobases.

Effect of different ratios. We tried the interfacial etching reaction of Ag@MSA with different ratios of the nucleobase, guanine sulfate. Various ratios such as 1:1, 1:0.1 and 1:0.2 were used to check whether the weight ratio of reactants influences the synthesis of the clusters. All the reactions were done in an ice bath for several hours. Corresponding absorption spectra are given in ESI‡ Fig. S7A, B and C, respectively. The intensity of the spectra decreased slightly upon the addition of the ligand in all cases. A new hump was formed around 480 nm within 1 h of the reaction in addition to the feature of Ag@MSA in the case of ratios 1:0.1 and 1:0.2. A new absorption was observed around 500 nm within 1 h of the etching reaction in the case of 1:1 ratio. But this feature is different from that obtained at 1:4 ratio. It may be due to formation of other clusters in the aqueous layer. No further spectral changes were observed in the supernatant in these reactions, which indicated completion of the reaction. The reaction was stopped and the supernatant was collected and dried to obtain the new materials. But we failed to get sufficient quantity of new clusters from this for further analysis. These points imply the fact that the ratios of reactants do play a role in the formation of subnanoclusters. Core etching of Ag@MSA was not achieved when the weight of the nucleobase was lesser than that of Ag@MSA.

Single phase etching. Single phase etching reaction was also performed to check whether the presence of an organic phase is needed for the synthesis of Ag subnanoclusters. For this, Ag@MSA with the nucleobase in the 1:4 weight ratio was taken in the aqueous phase and etching was performed in cold conditions. Time dependent spectra of the etching reaction are shown in ESI‡ Fig. S8A. A new absorption was observed in the supernatant around 580 nm. But this absorption was not stable after 1 day. It may be due to the instability of the products or they may be aggregated soon after formation. Hence the supernatant was dried to obtain the residue. This residue was dispersed in water and was analyzed using TEM.

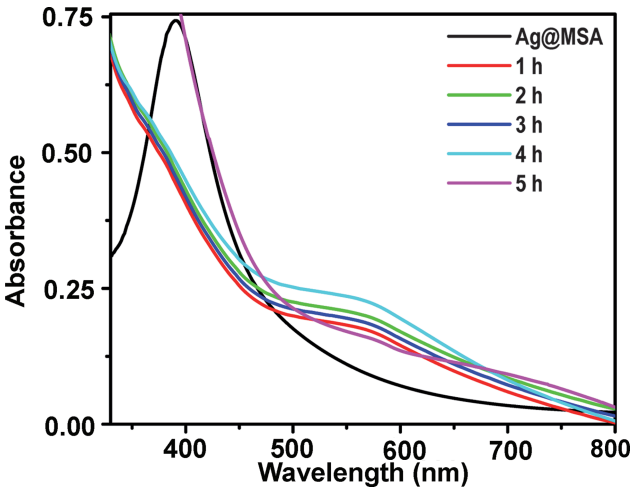


Fig. 8 Time dependent spectra of the interfacial etching reaction of Ag@MSA NPs with guanine sulfate (1:4) at room temperature.

TEM images showed the presence of aggregated particles. Images taken under different magnifications clearly show the existence of larger NPs (ESI‡ Fig. S8B, C and D). This aggregate warrants a separate study and is outside the scope of this paper.

Effect of temperature on the reaction. Interfacial etching reactions of Ag@MSA with guanine sulfate in the weight ratio 1:4 was performed at room temperature. UV-Vis spectra during this reaction were studied time dependently and are shown in Fig. 7. A faint pink colored layer was formed at the interface and the color of the aqueous phase changed to pale yellow during etching. It was found that the etching reaction was fast under these conditions. The feature of Ag@MSA disappeared within 10 minutes of etching and broadening of the spectrum around 580 nm was observed. The reaction was continued for 5 hours but it appears that the new cluster features are not stable. The cluster formed may be decomposing at room temperature. New clusters may be stabilized by varying the temperature. A more detailed study is necessary to isolate and confirm the formation of such clusters.

Conclusion

We synthesized Ag subnanoclusters by the interfacial etching of Ag@MSA using guanine sulfate. The clusters were separated using PAGE and a 7kDa cluster was isolated, which showed strong emission in the NIR region. Spectroscopic studies revealed the molecular nature of the cluster. The exact molecular formula of the cluster could not be determined from the mass spectra. Other nucleobases were not found to be effective in etching under similar reaction conditions. It was found that etching in a single phase did not yield stable clusters and an aqueous–organic interface was needed for the process. Different weight ratios of reactants could be used for the synthesis of

different clusters. Studies imply that etching at different temperatures may give other clusters. Fluorescence of the cluster is highly influenced by temperature. Quenching of fluorescence was achieved by the addition of heavy metal ions. The unique properties of these luminescent Ag clusters could be used in biolabeling, drug delivery, *etc.*

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