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# High temperature nucleation and growth of glutathione protected $\sim \text{Ag}_{75}$ clusters<sup>†</sup>

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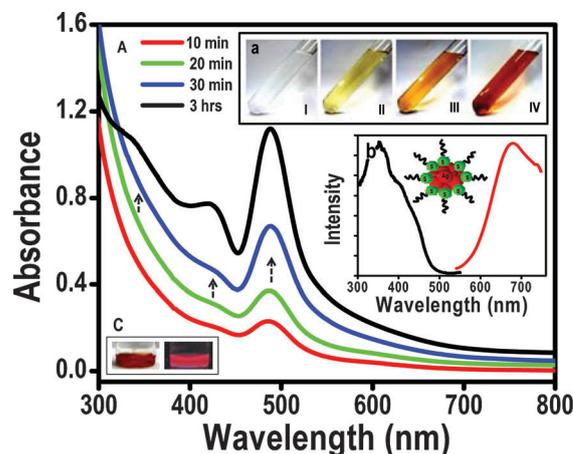
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We report the first high temperature solution state synthesis of glutathione (–SG) protected atomically precise silver clusters. Noble metal cluster synthesis from metal ions generally requires ice cold temperatures as they are extremely sensitive and high temperature routes are used only for core reduction methods, starting from nanoparticles. The clusters formed by the new route have distinct features in their absorption profile and they exhibit red luminescence. They are characterised by other spectroscopic and microscopic techniques and a tentative formula of  $\text{Ag}_{75}(\text{SG})_{40}$  has been assigned.

Noble metal quantum clusters<sup>1–8</sup> belong to a new category of materials with well defined optical absorption features and intense luminescence. While studies of atomically precise clusters with specific nuclearities such as  $\text{Au}_{25}$ ,  $\text{Au}_{38}$  and  $\text{Au}_{102}$  have progressed considerably,<sup>9–15</sup> their silver analogues are yet to be synthesized. In view of their reduced stability, novel routes are needed to make such relatively unstable clusters. As most of the clusters are metastable, nucleation at low temperature and rapid protection by covalent chemistry are the desired steps used in their synthesis.<sup>16</sup> As a result, reducing agents such as  $\text{NaBH}_4$  are used around 0 °C in a solution containing high concentration of the protecting agent.<sup>16–20</sup> Relatively high temperature etching of pre-formed metallic nanoparticles can also produce such clusters.<sup>11,21</sup> However, direct high temperature synthetic routes for clusters in solution, starting from ions, are yet to be reported. In this communication, we show that direct synthesis of atomically precise clusters is possible even at a high temperature of 70 °C. This study suggests that such clusters are likely to be stable even at elevated temperatures which expands the possibility of cluster research.

These clusters were synthesized by a new method which involves the mixing of 20 mL of 5.88 mM  $\text{AgNO}_3$  with 20 mL of 2.034 mM GSH and the mixture was stirred for 5 min at 70 °C followed by the addition of 20 mL of 0.0112 mM formic acid (all in water). Temperature was kept constant throughout the reaction. The color of the solution changed with time



**Fig. 1** UV-vis absorption spectra of the growth of clusters with time (A). Gradual increase in the intensity of the 350, 420 and 478 nm peaks is seen. The growth was complete in 3 h and no further increase in the intensity of the absorption bands occurred. Insets: (a) Photographs of the reaction mixture during synthesis at 0 min (I), 10 min (II), 20 min (III) and 3 h (IV); (b) luminescence excitation and emission spectra (black and red respectively) of as-synthesized clusters which emit at 670 nm upon excitation at 400 nm and (c) photographs of clusters under visible and UV light, respectively. Inset of inset (b) is a cartoon representation of the cluster.

indicating the nucleation and growth of clusters. After 3 h, no further color change was observed although stirring was continued for 24 h, which shows that the reaction was completed in 3 h. The final solution was reddish-brown. Photographs of time dependent reaction products are given in Fig. 1a and Fig. S1 (ESI<sup>†</sup>). The nucleation and growth of these clusters was monitored by UV-vis spectroscopy (Fig. 1A). Even at the initial stages of the reaction, emergence of a peak at 478 nm and shoulder peaks at 350, 420, 540 and 625 nm was observed. We ruled out the possibility of the 420 nm peak to be due to plasmonic nanoparticles as this kind of shoulder peak was observed for some glutathione protected silver clusters as well.<sup>5,22</sup> Besides, no nanoparticles were seen in TEM (see below). Our recent work on silver clusters also clarifies that the new peaks belong to a cluster with molecular behaviour.<sup>23</sup> The shoulder peak at 625 nm disappeared upon continuation of the reaction. A prominent feature at 478 nm and shoulder peaks at 540, 420 and 350 nm were observed after completion of growth. This solution was highly stable for

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several months even at higher temperatures. Even if we heat the clusters at 70 °C, a couple of months after synthesis, the solution was found to retain its optical properties. However, all the reported silver clusters are less stable and do not exist above 50 °C in solution.<sup>24,25</sup> The band gap of the cluster is estimated to be 1.73 eV (a plot of absorbance against energy in eV is given in Fig. S2, ESI†). The cluster exhibits emission at 670 nm. The excitation spectrum gives three band maxima at 360, 400 and 430 nm (Fig. 1b) and each of these excitations give the same emission. Multiple excitation maxima are seen in QCs such as Au<sub>25</sub>.<sup>14</sup> The cluster solution shows bright red luminescence (Fig. 1c) under UV light as expected from its emission wavelength. During the synthesis, emission intensity got increased and became stable after 3 h (Fig. S3, ESI†), in concordance with the UV-vis observation. Quantum yield was measured to be  $4 \times 10^{-3}$ , comparable to silver clusters synthesized by core etching.<sup>21</sup>

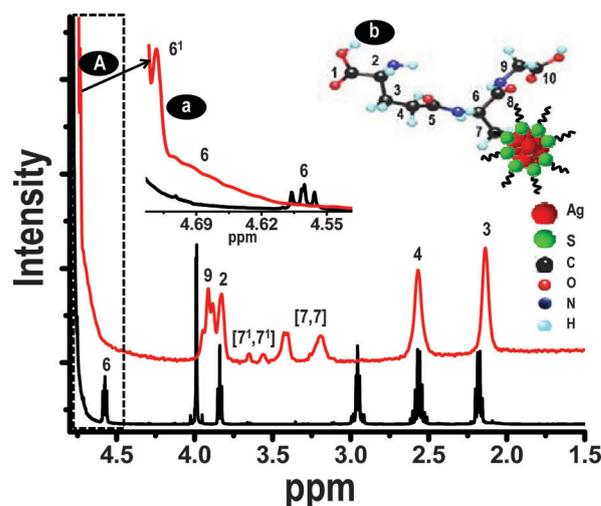
As the reaction proceeds, pH of the solution changes from 5.8 to 2.4. This may be due to the production of H<sup>+</sup> in the reaction medium while formic acid reduces silver ions to form silver clusters. In view of this, the following reaction for the decomposition of formic acid is suggested.



The  $E^\circ$  of this half cell (HCOOH/CO<sub>2</sub>, H<sup>+</sup>) at 298 K is -0.25 V [with respect to  $E^\circ(\text{H}^+, \frac{1}{2}\text{H}_2)$ ], which is quite negative, but higher reaction temperature may make the reduction facile ( $-T\Delta S$ ). It may also be noted that the electrochemical potential of a cluster ( $\text{Ag}_n \rightarrow \text{Ag}_n^+ + \text{e}^-$ ) is substantially lower than that of bulk silver ( $\text{Ag}_{\text{bulk}} \rightarrow \text{Ag}_{\text{bulk}}^+ + \text{e}^-$ ). This would enable the reduction of silver ions to silver clusters.

Control experiments were carried out by varying the luthathione concentration, keeping the silver concentration constant. In the absence of glutathione, nanoparticles (of 10–15 nm in diameter) were formed (Fig. S4, ESI†). With increase in concentration of GSH, clusters started to nucleate but after some concentration, thiolates were formed which precipitated in the test tube and formic acid was not able to reduce them. Extension of the reaction to gold clusters was also tried (Fig. S5, ESI†). After complete reaction, clusters showed UV-vis peaks at 350 and 680 nm. The solution exhibited strong red luminescence under UV exposure. We tried to get the material in the solid state but only a blackish powder, insoluble in water, could be obtained. It is known that it is not possible to get powders out of citrate capped gold nanoparticles<sup>26</sup> but it is very much feasible for monolayer protected gold or silver clusters.<sup>24,25</sup> This suggests that the clusters may be charged.

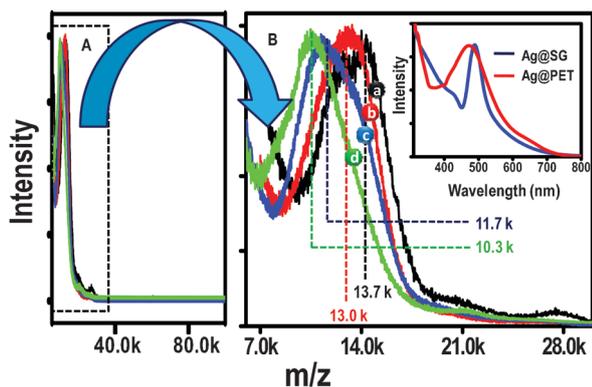
The <sup>1</sup>H NMR spectrum of the cluster was recorded. The sample was prepared in D<sub>2</sub>O and the resonances were compared with those of pure GSH (Fig. 2). All the peaks corresponding to the protons are broadened and weakened in the cluster. It should be noted that a strong peak at around 4.75 ppm arises from residual H<sub>2</sub>O and HDO in D<sub>2</sub>O. The resonances are labelled in the spectra. The strong singlet for H-9 (inset of Fig. 2) at 3.92 ppm, and a multiplet for H-3 and H-4 at 2.11 and 2.44 ppm, respectively, resemble closely the spectra of GSH. C-10, C-8, C-5, and C-1 do not bear hydrogen. As both H-6 (β position) and H-7 (α position) are very close to the silver core, it is likely that these protons are significantly shifted



**Fig. 2** (A) <sup>1</sup>H NMR spectra of Ag@SG clusters (red) and GSH (black) taken in D<sub>2</sub>O. For clarity, the 4.5 ppm region was expanded in (a). All the protons are marked, along with a schematic of the cluster. Downfield shift of H-6 and H-7 confirms the binding of GSH with Ag. The inset shows the structure of GSH attached with the cluster.

downfield. There are a set of broad peaks in the range of 3.0–3.8 ppm, assigned to H-7. These peaks can be compared with the data of Au<sub>25</sub>(SG)<sub>18</sub><sup>11,27,28</sup> and can be discussed in the light of the different types of ligands present on the cluster surface. QCs in general are composed of two types of ligands. There are six numbers of  $-\text{[SR}_C\text{-Au-SR}_B\text{-Au-SR}_C\text{-}]_n$  units decorating the Au<sub>13</sub> core giving the Au<sub>25</sub>(SG)<sub>18</sub> composition, where SR<sub>C</sub> and SR<sub>B</sub> are core-linked and bridged thiolates, respectively. <sup>1</sup>H NMR of Au<sub>25</sub>(SG)<sub>18</sub> shows two peaks in the 2:1 ratio with different chemical shifts for SR<sub>C</sub> and SR<sub>B</sub>, respectively.<sup>27,28</sup> Each peak is again split by chirality of the cluster, making four peaks (one pair with equal intensity). In our case, a similar situation arises. Here the number of core-linked thiolates is more, as expected in the case of a larger core.<sup>14</sup>

Mass spectrometric analysis of the cluster in aqueous medium was not successful so far using electrospray ionisation (ESI) and matrix assisted laser desorption ionisation (MALDI). Due to that reason, we choose an alternative way to find the molecular formula of the cluster. As-synthesised clusters were phase transferred to the organic (toluene) medium using phenylethanethiol (PET). MALDI MS of these clusters gave distinct features (Fig. 3A). From the previous studies, it is known that *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (popularly called DCTB) gives a good mass spectrum for clusters protected with PET.<sup>29,30</sup> Even after ligand exchange, all absorption bands are present in the PET protected cluster which proves that the cluster core is the same as in the case of GSH (Fig. 3B). Ligand exchange without core change is known in several cases.<sup>11,31</sup> The slight broadening of the absorption peak in the exchanged product is due to the overlap of two peaks (at 420 and 480 nm). In MALDI MS, the PET exchanged Ag cluster gives a molecular ion peak at 13.7 kDa at a threshold (nominal) laser intensity of 2850. Below this intensity, no cluster was detected. Peak position depends on the laser intensity. From lower (2850) to higher (3950) laser intensity, peaks were shifted to the low mass region (13.7 to 10.3 kDa). This can be understood from the gradual fragmentation of the cluster, as expected at higher



**Fig. 3** MALDI MS data of ligand exchanged Ag@SG clusters in the negative mode (A). It shows a molecular ion peak at 13.7 kDa (black color). The expanded view of the molecular peaks (B). Fragmentation occurs with increase in laser intensity. The traces a, b, c, and d are at laser intensities of 2850, 2910, 3174, and 3974, respectively (numbers refer to instrument settings and not laser power). Inset of B shows the UV-vis spectra of as-synthesized clusters and PET exchanged clusters. It shows that cluster is intact even after ligand exchange.

laser intensities (Fig. 3B). It is important to note that the MALDI MS feature is broader than that of a well-defined Au cluster such as Au<sub>25</sub>(SG)<sub>18</sub>. We believe that the weaker Ag–S bond strength in comparison to Au–S makes ligand desorption more probable in the silver analogues, making the spectrum broad.

As the core dimension is small, these clusters do not appear well in TEM.<sup>24</sup> Upon longer electron beam irradiation, they aggregate to form nanoparticles.<sup>21</sup> HRTEM images confirm the formation of the cluster with an average diameter of 0.85 nm (Fig. S6, ESI<sup>†</sup>). HRTEM images of PET exchanged silver clusters are given in Fig. S7 (ESI<sup>†</sup>). GSH protected clusters show increased aggregation (Fig. S6, ESI<sup>†</sup>).

The XPS survey spectrum of the as-synthesized silver cluster sample shows the expected elements (Fig. S8, ESI<sup>†</sup>). The expanded spectrum shows a state closer to Ag(0) with a binding energy (BE) of 368.2 eV (Fig. S8, ESI<sup>†</sup>). Note that between Ag(I) and Ag(0) states, there is not much difference in BE, unlike in the case of Au. S 2p exists at 162.5 eV, corresponding to thiolate. Peaks corresponding to O 1s, C 1s and N 1s appeared at the expected positions (Fig. S9, ESI<sup>†</sup>). The Ag to S ratio is 1.87 : 1.

Based on this information and the MALDI MS data, probable composition of our cluster is Ag<sub>75</sub>(SG)<sub>40</sub>. SEM/EDAX data given in Fig. S10 (ESI<sup>†</sup>) showing an Ag : S ratio of 1.83 : 1 also support this composition (close to the ratio in XPS). As the cluster could not be made into a free flowing powder, CHNS analysis was not performed.

The Ag@SG cluster shows optical chirality in its circular dichroism (CD) spectrum (Fig. S11, ESI<sup>†</sup>). It appears to arise from the cluster core as the ligand (GSH) does not show peaks in that region.

In conclusion, formic acid mediated high temperature synthesis of glutathione protected silver clusters is reported for the first time, starting from Ag<sup>+</sup>. A molecular mass of 13.7 kDa was confirmed from MALDI MS data of the ligand exchanged cluster. In conjunction with XPS and SEM-EDAX, a probable cluster composition of Ag<sub>75</sub>(SG)<sub>40</sub> was assigned.

The cluster exhibits chirality in the solution state. As all reagents are bio-compatible, this cluster may be useful in biological applications.

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