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ORIGINAL PAPER

Aqueous to Organic Phase Transfer of Au_{25} Clusters

M. A. Habeeb Muhammed · T. Pradeep

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Abstract Aqueous to organic phase transfer of water soluble sub-nanocluster, $Au_{25}SG_{18}$ (-SG, glutathione thiolate) is demonstrated using the phase transfer reagent, tetraoctylammonium bromide. The phase transfer occurred by the electrostatic attraction between the hydrophilic carboxylate anion of the glutathione ligand on the cluster surface in the aqueous phase and the hydrophobic tetraoctylammonium cation in the toluene phase. Detailed spectroscopic characterization of the phase transferred cluster using optical absorption, photoluminescence and X-ray photoelectron spectroscopy showed that the cluster retains its integrity during the phase transfer. The interaction of the cluster with the phase transfer reagent can be studied with infrared spectroscopy. The phase transferred cluster can be dried and redissolved in an organic medium, just as the original cluster. This is the first report of the phase transfer of a sub-nanocluster, keeping the cluster core intact. The effect of dilution and pH on phase transfer of this cluster is studied in detail. This method promises several possibilities to explore the properties, reactivity and applications of sub-nanoclusters both in the aqueous and organic phases.

Keywords Gold · Nanocluster · Phase transfer · pH

Introduction

In recent years, many new types of nanomaterials with interesting properties and applications have been discovered. Sub-nanoclusters or molecular clusters of

Dedicated to Prof. C.N.R. Rao on his 75th birthday, whose work on phase transfer of nanoparticles has inspired this work.

M. A. Habeeb Muhammed · T. Pradeep (✉)
DST Unit on Nanoscience, Department of Chemistry and Sophisticated Analytical Instruments Facility, Indian Institute of Technology, Madras, Chennai 600 036, India
e-mail: pradeep@iitm.ac.in

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metals, especially of silver [1–4] and gold [5–17] are the most recent additions. They are composed of a few to several tens of atoms, with size in the sub-nanometer range. In this size regime, optical, photophysical, electrochemical, magnetic and chiroptical properties differ dramatically in comparison to the metallic nanoparticles. They exhibit discrete energy levels with molecule-like transitions and thus provide a "missing link" between atomic and nanoparticle behavior. They are non-metallic, highly polarizable, and strongly fluorescent and can be used as a new class of biolabels. Nowadays, these molecular clusters can be synthesized in large quantities by several techniques including ligand exchange [10], chemical etching [11–15], template assisted synthesis [5–8], polyacrylamide gel electrophoresis [17], etc. and thus it is possible to explore their properties in detail. Among the various clusters, Au_{25} is a widely studied molecular cluster which consists of 25 gold atoms in the core protected with various ligands such as glutathione [9–15], phenylethane thiol [16], etc. While protection with organic thiols make Au_{25} organic soluble, a cover of glutathione makes them water soluble. Totally different methods are followed for the synthesis of organic and water soluble Au_{25} clusters. While organic soluble Au_{25} clusters are synthesized by reducing the precursor Au^{3+} ions in presence of various organic thiols [16], water soluble Au_{25} clusters are synthesized by the ligand etching of glutathione capped nanoparticles of $\sim$2 nm diameter in presence of excess glutathione [12–14] or by polyacrylamide gel electrophoresis separation of a mixture of clusters prepared by the reduction of Au^{3+} in presence of glutathione [17]. Here we present a method to transfer water soluble glutathione protected Au_{25} clusters from aqueous to toluene medium by the phase transfer reagent, tetraoctylammonium bromide (TOABr) keeping the cluster core intact. The phase transfer happens by the electrostatic attraction between the hydrophilic carboxylate anion of the glutathione ligand on the cluster surface in the aqueous phase and the hydrophobic tetraoctylammonium cation in the toluene phase.

There are several reports for the phase transfer of various nanostructures from aqueous to organic phase and vice versa. The famous Brust–Schiffirin method which was widely used for the synthesis of gold nanoparticles involve the phase transfer of Au^{3+} ions to the organic layer by TOABr and their subsequent reduction in presence of organic thiols [18]. Rao and co-workers developed an elegant method to synthesise thiol-derivatized nanoparticles of Au, Pt and Ag by the acid facilitated phase transfer of well-characterized particles in a hydrosol to a toluene layer containing the thiol [19]. Even though bigger nanoparticles [20] and other nanostructures such as nanorods [21] have been phase transferred, this is the first report of the phase transfer of a sub-nanocluster keeping the cluster core intact. As clusters in general are highly sensitive to chemical environment, phase transfer with no change in core structure is important, which promises several possibilities to explore their properties, reactivity and applications in the aqueous and organic phases.

Experimental

1. *Synthesis of Glutathione Capped Gold (Au@SG) Clusters*: Glutathione capped gold clusters were synthesized according to a reported method [17]. To a

100 mL, 5 mM $HAuCl_4 \cdot 3H_2O$ in methanol, 20 mM reduced glutathione (GSH) was added. The mixture was then cooled to 0 °C in an ice bath for 30 min. An aqueous solution of $NaBH_4$ (25 mL, 0.2 M), cooled at 0 °C, was injected rapidly into this mixture under vigorous stirring. The mixture was allowed to react for another hour. The resulting precipitate was collected and washed repeatedly with methanol through centrifugal precipitation and dried to obtain the Au@SG clusters as a dark brown powder. This product is a mixture of small nanoparticles and different clusters.

2. *Synthesis of $Au_{25}SG_{18}$*: $Au_{25}SG_{18}$ was synthesized from the as prepared Au@SG clusters by ligand etching [12–14]. The as prepared Au@SG cluster was dissolved in 25 mL water. GSH was added (20 mM) and stirred at 55 °C. The reaction was monitored by optical absorption spectroscopy. Heating was discontinued when the absorption features of $Au_{25}SG_{18}$ appeared in the UV–visible spectrum. The solution was centrifuged and methanol was added to the supernatant to precipitate the cluster. The precipitate was dried to obtain $Au_{25}SG_{18}$ clusters in the powder form.
3. *Phase Transfer of $Au_{25}SG_{18}$*: Phase transfer of Au_{25} was carried out by an immiscible biphasic method as follows.
    a. One mg of Au_{25} was dissolved in 20 mL of distilled water. One mL of 1 mM TOABr in toluene was added to make an immiscible layer above the water phase. The mixture was stirred for 2 min.
    b. Ten mg of Au_{25} was dissolved in 2 mL of distilled water. pH was adjusted to 11 by the addition of NaOH. One mL of 10 mM TOABr in toluene was added. The mixture was stirred for 2 min.
4. *Purification of Phase Transferred Clusters*: Ethanol was added to the toluene layer to precipitate the phase transferred clusters. The precipitate was washed two times with ethanol, centrifuged and dried.
5. *Instrumentation*: UV/Vis spectra were measured with a Perkin Elmer Lambda 25 instrument in the range of 200–1100 nm. Fluorescence measurements were carried out by a HORIBA JOBIN VYON NanoLog instrument. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an Omicron ESCAProbe spectrometer with monochromatized Al Kα X-rays ($h\nu$ = 1486.6 eV). The samples were spotted as drop cast films on a sample stub, and the surfaces were scraped in situ to remove any surface contamination that could arise from components of the atmosphere, such as water and CO_2. Constant analyzer energy of 20 eV was used for the measurements. Laser desorption ionization (LDI) mass spectrometric studies were conducted using a Voyager DE PRO Biospectrometry Workstation of Applied Biosystems MALDI-TOF MS. A pulsed nitrogen laser of 337 nm was used for the studies. Mass spectra were collected in positive ion mode and were averaged for 50 shots. The FT-IR spectra were measured with a Perkin Elmer Spectrum One instrument. KBr crystals were used as the matrix for preparing the samples.

Results and Discussion

Au_{25} clusters underwent immediate and complete phase transfer from the aqueous to the toluene layer by TOABr after 2 min of vigorous stirring. The phase transfer can be observed visibly by the color changes in the aqueous and the toluene phases. The colorless toluene layer turned dark brown and the aqueous layer which was originally dark brown turned colorless after stirring. Figure 1 is the schematic representation of phase transfer. Photographs of the bi-phase mixture before and after phase transfer are also shown.

The phase transferred cluster was analyzed thoroughly. Figure 2 compares optical absorption spectra of the Au_{25} both in the aqueous and the toluene layers. $Au_{25}SG_{18}$ has a well-structured optical absorption spectrum with an absorption maximum at 672 nm. The peak at 672 nm (1.55 eV) arises due to a LUMO ← HOMO transition which can be called as an intra-band (*sp* ← *sp*) transition [17]. The features in the lower wavelength region arise due to inter-band transition (*sp* ← *d*). All the absorption features of Au_{25} in the visible and the ultraviolet regions were seen after the phase transfer. The retention of the absorption profile after the phase transfer confirms the retention of the Au_{25} core. While the optical absorption maximum of Au_{25} in the aqueous layer is 672 nm, it was 678 nm in the toluene layer. This red shift may be due to the covering of tetraoctyl ammonium cation on the cluster surface in the toluene layer. However, all the absorption features, including the minor ones are reproduced in the phase transferred system. The cluster exhibits photoluminescence with a quantum yield of 10^{-3} which is very high when compared with metallic nanoparticles [17]. The origin of emission is not clearly understood as of now but can be correlated to the *sp* intra-band excitation. Au_{25} retains its photoluminescence after the phase transfer. Figure 3 depicts the photoluminescence profile of the cluster before and after phase transfer. There is a red shift in both the excitation and emission maxima. While excitation maximum was shifted from 535 to 575 nm, emission maximum was shifted from 700 to 720 nm after phase transfer. The cluster after phase transfer can be dried into redissolvable powder (Fig. 3).

Figure 4 compares the Au 4f core level photoemission spectra of Au_{25} before and after phase transfer. The 4f features were similar in both the cases, implying that the cluster core did not undergo any change after the phase transfer. The binding energy (BE) positions are similar in both the cases. Observed binding energy values are

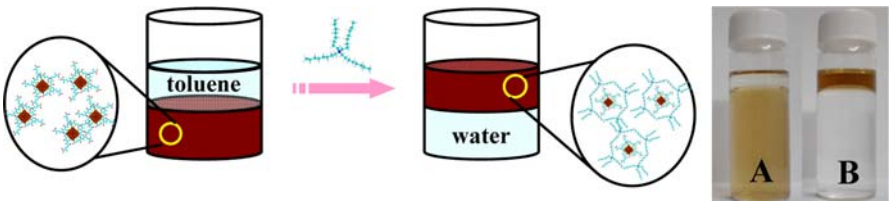

Fig. 1 Schematic representation of the phase transfer of Au_{25} clusters from aqueous to toluene layer by TOABr. Photographs of the bi-phasic mixture before (*A*) and after (*B*) phase transfer are also given

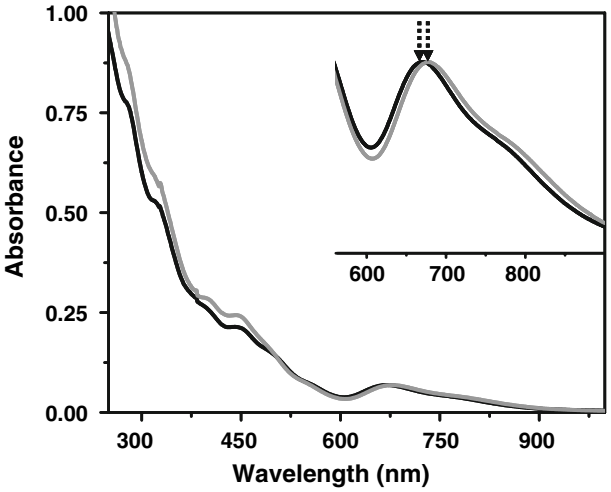

Fig. 2 Comparison of the optical absorption spectra of Au_{25} in aqueous (*black trace*) and toluene (*grey trace*) layers. Inset gives the enlarged spectra which show a red shift of 6 nm

close to those reported before [13] with Au $4f_{7/2}$ at 84.4 eV and Au $4f_{5/2}$ at 88.0 eV. These data suggest that the cluster core is similar before and after phase transfer. There are added Br 3d and enhanced N1s features due to the ammonium ion in the case of phase transferred cluster. The BE position of N1s of parent cluster appeared at 399 eV and that of phase transferred cluster appeared at 405 eV, with a shoulder at 402 eV. Br 3d feature, which is from TOABr, is absent in the parent cluster and present in the phase transferred cluster.

The positive ion mode laser desorption ionization mass spectral analysis of the phase transferred sample gave a peak due to tetraoctylammonium ion at *m/z* 467 in addition to gold and glutathione features at *m/z* 197 and 306, respectively (Fig. 5). Parent Au_{25} showed features due to gold; glutathione and sodium adduct of glutathione. Gold dimer (*m/z* 394) and trimer (*m/z* 591) were also seen both in the parent and phase transferred clusters. The intact cluster is not observed in laser desorption.

Figure 6 compares the FT-IR spectra of the Au_{25} clusters before and after phase transfer with that of TOABr and glutathione. While the parent Au_{25} showed features due to glutathione, phase transferred Au_{25} showed features due to both glutathione and TOABr. The peak at 2526 cm^{-1}, which correspond to the S–H stretching vibration of glutathione, disappeared in the parent $Au_{25}SG_{18}$ clusters confirming the binding of glutathione on Au_{25} core through the thiol group. The phase transferred cluster showed features between 3500 and 3000 cm^{-1} due to glutathione protection, same as in the case of parent $Au_{25}SG_{18}$ (Fig. 6A). On the other hand, free TOABr has no features above 3000 cm^{-1}. The peaks at 2850 and 2922 cm^{-1} are due to the methylene symmetric (d^+) and antisymmetric (d^-) stretching of the TOABr. These peaks are present in the phase transferred cluster at the same position. The peaks at 2873 and 2955 cm^{-1} are the peaks due to the symmetric and antisymmetric stretching modes of the methyl group. The peaks due to methyl stretching are absent

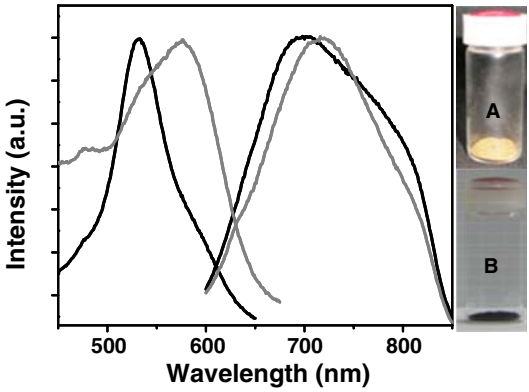

Fig. 3 Comparison of the photoluminescence spectra of Au_{25} in aqueous (*black trace*) and toluene (*grey trace*) layers. The photographs of the solid cluster before (*A*) and after (*B*) phase transfer are also given

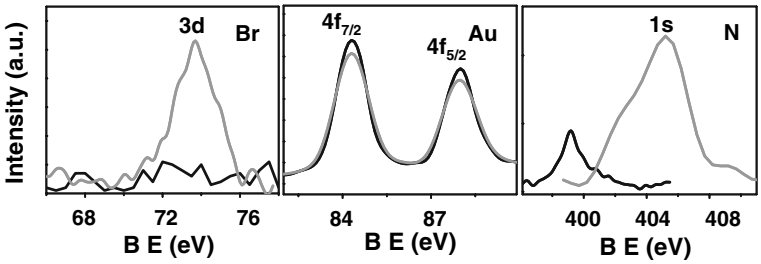

Fig. 4 Comparison of the Br 3d, Au 4f and N 1s core level photoemission spectra of Au_{25} before (*black trace*) and after (*grey trace*) phase transfer

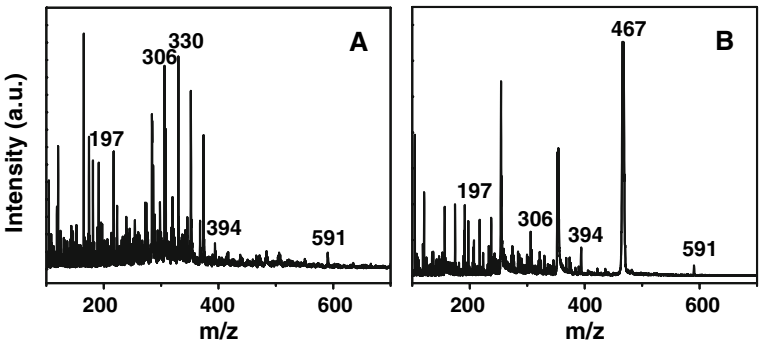

Fig. 5 Positive mode laser desorption ionization mass spectra of Au_{25} before (*A*) and after (*B*) phase transfer

in the parent Au_{25} and present in the phase transferred cluster. The peaks due to C=O stretching of the amide linkage of glutathione at 1650 cm^{-1} are present in the phase transferred cluster, same as in parent Au_{25}. There is a bunch of peaks between 1300 and 1000 cm^{-1} in the phase transferred cluster due to the progression bands arising from the CH_2 wagging modes of the TOA group. These peaks were absent in the parent cluster. All these features confirm the presence of both glutathione and

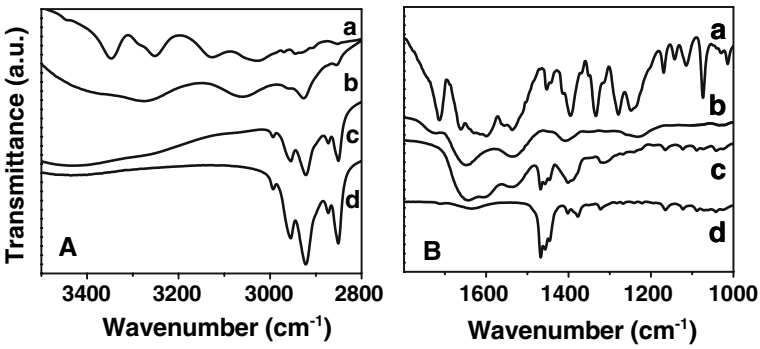

Fig. 6 Comparison of the FT-IR spectra of Au_{25} before (trace *b*) and after (trace *c*) phase transfer with that of TOABr (trace *d*) and glutathione (trace *a*). The traces are shifted vertically for clarity

TOA on the phase transferred cluster. The features due to glutathione in the parent Au_{25} and features due to glutathione and TOABr in the phase transferred clusters are less intense than in free glutathione or TOABr since they are bound on the cluster surface. The glutathione features are less intense than the TOABr features in the case of the phase transferred cluster owing to the fact that while glutathione is bound on the cluster surface strongly through covalent bonding through the thiol group of the cysteine moiety, TOABr is covering the cluster surface through electrostatic interaction.

It is found that the extent of phase transfer increases with dilution at constant amount of cluster and phase transfer reagent. To establish this, four batches of the biphasic mixture were made. All the batches consist of 1 mg of Au_{25} and 1 mL, 1 mM TOABr (0.547 mg). One mg of the cluster was dissolved in 2, 5, 15 and 20 mL of distilled water in batches A, B, C and D, respectively (left panel of Fig. 7). TOABr solution was added. The mixture was stirred for 2 min. It was found that extent of phase transfer increases from A to D. No phase transfer happened in batch A and complete phase transfer happened in batch D. The complete phase transfer was confirmed by the analysis of the aqueous layer which did not show any characteristic absorption of Au_{25} after phase transfer. The reason for the increase in the phase transfer with dilution at constant amount of TOABr can be explained as follows. Au_{25} cluster is protected with 18 glutathione molecules and hence they tend to form aggregates through hydrogen bonding between the COOH groups. As the volume increases, the clusters stay far apart from each other and hence the extent of hydrogen bonding between the clusters decreases. As a result, they get phase transferred into the toluene phase via ion-pair formation with the tetraoctylammonium cations. When large amount of cluster has to be phase transferred, large amount of water has to be used. One can overcome this problem by adjusting the pH of the solution. An experiment was carried out at various pH keeping the concentration of cluster and phase transfer reagent constant to check the effect of pH on phase transfer (right panel of Fig. 7). It was found that the extent of phase transfer increases with increase in pH of the solution with maximum phase transfer

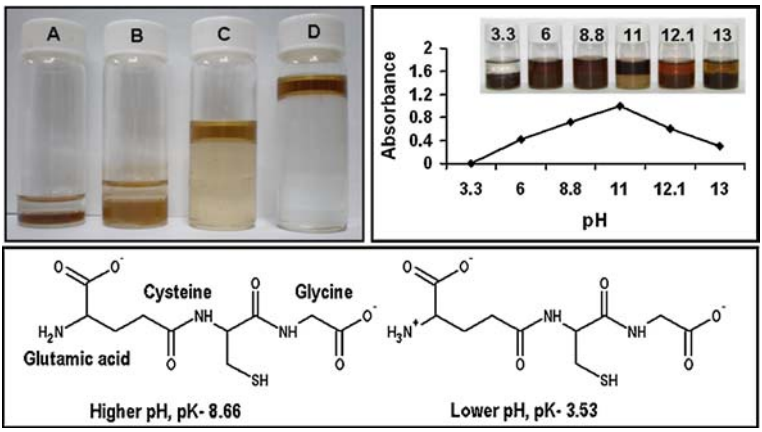

Fig. 7 *Left panel* shows the effect of volume during the phase transfer. Extent of phase transfer increases with dilution (*A* to *D*). *Right panel* shows the effect of pH on phase transfer. Extent of phase transfer increases with increase in the pH of the aqueous phase. *Lower panel* shows the two distinct forms of glutathione at higher and lower pH

occurring at pH 11 and decreasing afterwards. The reason for the increase in the extent of phase transfer with increase in pH can be explained as follows. At lower pH, the net charge of glutathione molecule will be −1 because of the existence of zwitter ion at the glutamate residue and COO^- at the glycine residue [22]. The p*K* value is 3.53. On addition of OH^- ions, zwitter ionic nature of glutathione is lost and there will be two carboxylate anions, one at glycine residue and another at glutamic acid residue and the net charge of the glutathione will be −2. The p*K* value is 8.66. The two ionization forms of the glutathione at lower and higher pH are given in the lower panel of Fig. 7. Thus at higher pH, more number of tetraoctylammonium cations can bind with each cluster than at lower pH, increasing the extent of phase transfer.

Conclusion

The sub-nanocluster, Au_{25} was transferred from the aqueous phase to the organic phase by the phase transfer reagent, TOABr by employing the electrostatic interaction between the hydrophilic carboxylate anion of the glutathione ligand on the cluster and the hydrophobic tetraoctylammonium cation. The cluster core remains intact after the phase transfer which was evident from the spectroscopic data. It was found that extent of phase transfer increases with dilution and pH. The phase transferred material could be dried and redissolved. This method allows the chemistry of $Au_{25}SG_{18}$ to be explored in both the aqueous and organic phases.

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