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Nanoparticle tracking analysis of gold nanomaterials stabilized by various capping agents†

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Gold colloidal nanomaterials have been synthesized using different methodologies and characterized by a novel nanoparticle tracking analysis (NTA) technique as compared to conventional Transmission Electron Microscopy (TEM) characterisation. Results prove that NTA is a highly useful, simple, efficient and rapid characterisation tool for Au nanoparticles and nanorods, providing a highly reliable and fast alternative to traditional characterisation techniques. This approach provides a very simple way of studying nanoparticles and some of their properties. Studies on the stabilizing/capping effect of a variety of biomass-derived compounds also show different possibilities for the use of polysaccharide to stabilise colloidal Au solutions.

Introduction

Gold nanoentities are considered to be the most versatile nanomaterials today because of their excellent electronic and optical properties.[1] Their ability to form surface covalent bonds is currently being exploited for catalysis (self-assembled monolayers) along with several potential applications in biodiagnostics.[2,3] By attaching oligonucleotide complementary sequences to Au NPs, complementary target DNA (that of a target pathogen) can hybridize and change the color of the nanoparticles. Due to the instability of the nanoparticles in solution, they have thermodynamically favoured tendencies to form aggregates and form larger particles to stabilise their high surface energies.[4] Capping or stabilizing agents are added to the solution to play a role similar to nanoparticlecoating and thus avoid their aggregation in colloidal solutions.[4] These stabilizing agents include ionic liquids which have been shown to have the ability to control particle sizes,[5] organic and inorganic ligands (to control morphology and particle shape)[6,7] and a range of porous solids such as functionalized mesoporous carbonaceous supports for coupling applications.[4,8]

A range of stabilizing agents derived from renewable resources (*e.g.* sugars) have been previously reported[9–11] and proved to have excellent stabilizing properties for certain types of nanoparticles (*e.g.* silver). Our group has also recently reported the use of biomass-derived stabilizing/capping agents for the preparation of Ag colloidal nanoparticles.[12]

In terms of nanomaterial analysis and characterisation, several conventional techniques are generally employed to understand the surface properties, molecular structure, sizes and shapes of nanoentities. These methods include Transmission and Scanning Electron Microscopies (TEM and SEM), X-Ray Diffraction (XRD), Dynamic Light Scattering (DLS), *etc.* Importantly, most of them need an elaborate and time-consuming methodology for sample preparation and characterisation. Because of this, a simple, efficient and rapid alternative analysis able to provide a series of quick and reliable results in a very short period of time could be highly beneficial. Nanoparticle Tracking Analysis (NTA) developed by the company Nanosight has been shown to image a variety of diverse nanoparticles through efficient light scattering.[12] Equipped with a CMOS (Complementary Metal Oxide Silicon) camera, the software is able to detect colloidal nanoentities moving under Brownian motion and produce a size distribution. Basically, the instrument captures an image of a solution exposed to a laser of a specific wavelength and then records the flow of these particles in solution and subsequently averages the distances travelled by the particles. The software is then able to relate diffusion with size based on the Stokes–Einstein relation using the diffusion coefficient derived from the average distances, solution temperature, and viscosity.[13]

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Taking NTA as an interesting alternative to conventional characterisation techniques and in our continuing efforts to provide stabilizing agent alternatives, we report herein the use of similar renewable-derived agents for the stabilisation of colloidal solutions of gold nanomaterials. We demonstrate here that sugar/polysaccharides such as agar and glucose can be effective stabilizing/capping agents for gold colloidal solutions.

Materials and methods

Experimental

All chemicals used in this study were all reagent grade (with >99% purity) and were used upon received. The concentrated lignocellulosic syrup used in the study was obtained from the French Biorefinery Company CIMV (http://cimv.fr). The feedstock was basically a concentrated sugar solution containing 15% of monomeric sugars such as xylose and arabinose, 35% bonded sugars ranging from disaccharides to oligomers, 20% mineral salts, and other possible plant impurities.

Synthesis of Au colloidal solutions

To prepare the colloidal solutions, a 10 mL solution of 0.01 M $AuCl_3 \cdot 3H_2O$ was prepared. In parallel, 0.5 g of stabilizing agents (agar, glucose, syrup) were suspended in 20 mL of water. The gold solution and the stabilizing agents were mixed at 1 : 2 volume ratio (optimum ratio for the investigated conditions) and then analysed using the Nanoparticle Tracking Analysis by NanoSight. Citrate, a known gold reducing and stabilizing agent was also used to evaluate in its effect on the stabilizing properties of the other agents.

Synthesis of Au nanorods

In a typical synthesis, 140 mg (37 mM in the final growth solution) of CTAB and 31 mg (9.9 mM in the final growth solution) of sodium oleate were dissolved in 5 mL of warm water (~50 °C). The solution was allowed to cool down to 30 °C and 5 mL $HAuCl_4$ (1 mM) solution was subsequently added. The mixture turned colourless after 5 min of ultrasonication. Then, 250 μL $AgNO_3$ solution (4 mM) was added, while the solution was gently shaken. The pH was adjusted using 50 μL HCl (37 wt % in water, 12 M) to a final acidic pH. Then 15 μL of freshly prepared ascorbic acid solution (100 mM) was added to the mixture, ultrasonicated for 5 min until a homogeneous solution was achieved. One microliter of ice-cold $NaBH_4$ (10 mM) was used as a reducing agent. The solution was allowed to react unstirred for 6 h and the resulting colloidal products were collected by centrifugation and washed three times with doubly distilled water. Three types of gold nanorods were also synthesized based on the aforementioned general synthetic conditions with some variations:

Sample 1. *Au NRs with length/diameter: 87 nm/32 nm (as measured by DLS)*, 0.48 mM $HAuCl_4$, 37.2 mM CTAB, 9.9 mM sodium oleate, 96.9 μM $AgNO_3$, 58.1 mM HCl, 0.29 mM ascorbic acid, 0.97 μM $NaBH_4$.

Sample 2. *Au NRs with length/diameter: 79 nm/25 nm (as measured by DLS)*, 0.48 mM $HAuCl_4$, 37.2 mM CTAB, 9.9 mM sodium oleate, 96.9 μM $AgNO_3$, 58.1 mM HCl, 0.48 mM ascorbic acid, 0.97 μM $NaBH_4$.

Sample 3. *Au NRs with length/diameter: 76 nm/13 nm (as measured by DLS)*, 0.48 mM $HAuCl_4$, 37.2 mM CTAB, 9.9 mM sodium oleate, 96.9 μM AgNO3, 86.8 mM HCl, 0.15 mM ascorbic acid, 0.97 μM $NaBH_4$.

Characterization

The characterization of the nanoparticle solutions with their capping agents were performed using a NTA LM10HSBF instrument equipped with a 60 mW 450 nm laser, a high-resolution CMOS camera, and a thermal detector. The instrument also comes with a software that has the ability to capture several frames of the solution and then correlate the particle size with concentration of the particles. The software can differentiate the nanoparticles from mere dust and correct for common interferences in solutions. After sensitivity optimization of the instrument at the room temperature used, a highly diluted sample (10^6 dilution) is introduced into the measuring cell using a typical syringe. During the analysis, the software captures a video of the solution and subsequently analyses the particles in the solution using the Stokes–Einstein relation. The software will then provide a 2D plot of concentration *versus* particle size as well as a 3D graph of the same *y* and *x* axes including a relative intensity for the *z*-axis.

TEM characterisation

Transmission Electron Micrographs (TEM) were recorded on a JEOL JEM-2010HR instrument operated at 300 kV. Samples were suspended in ethanol and deposited on a copper grid prior to analysis.

Results and discussion

Au-colloidal solutions

The capping ability of a compound is highly dependent on its interaction with nanoparticles. Such interactions could be based on weak intermolecular interactions, coordinate covalent bonds or covalent bonds. Investigations on the stabilizing potential of a range of biomass-derived compounds on gold colloidal solutions were conducted in a swift manner with the NTA as compared to TEM (Fig. 1 and 2 *vs.* Fig. 3, see also Table 1). A gold blank run composed of an aqueous solution of gold chloride precursor exhibited a very low concentration of colloidal Au NPs with sizes over 200 nm (Table 1, Fig. 1). Interestingly, agar was found to have significant stabilizing/capping effects for colloidal Au nanoparticles, with a narrow and small particle size distribution centered at ~35 nm (Fig. 1, average particle size 50 nm). Comparably, glucose formed larger Au colloidal nanoparticles (~73 nm) with a broad distribution (Fig. 1, average 84 nm), while the use of the biorefinery-derived hemicellulosic syrup containing oligomers and a minor concentration of C5 sugars provided an unexpected polydispersed Au colloidal solution (Fig. 1, 184 nm average NP size). The smallest Au nanoparticles were however observed in the

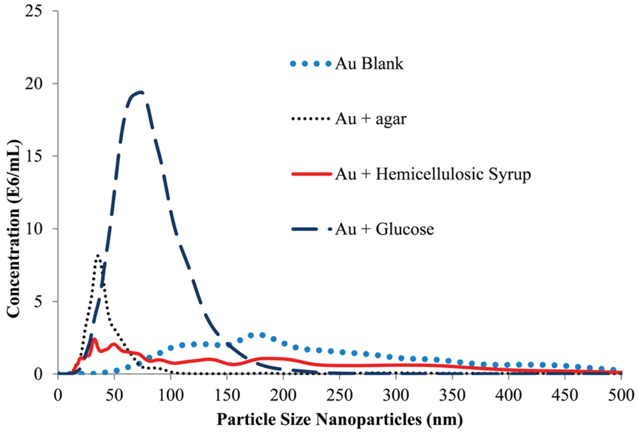

Fig. 1 NTA measurements for particle size distribution of different Au colloidal solutions in the presence of a variety of stabilizing agents.

experiment from the colloidal gold stabilized by the biorefinery syrup (~20 nm, see small peak in Fig. 1).

Citrates, previously reported as weak reducing[14,15] and stabilizing agent[16] (also found to play an important role in the stabilization of Ag colloidal solutions),[12] was subsequently added to evaluate a potential synergistic effect with the other capping agents (*e.g.* agar and glucose). Interestingly, while no significant effects were observed when it was added to Au–agar or to the Au–syrup solutions (results not shown), a remarkable synergistic effect was observed when the solution of sodium citrate was added to the Au + glucose mixture (Table 1, Fig. 2).

The AuNP sizes were observed to decrease following the addition of citrate (from 84 nm average particle size to 76 nm). The evolution of NP sizes was also followed with time as shown in Fig. 2 for the Au–glucose–citrate system. In all cases, the addition of mild capping agents was not sufficient to provide a long term stabilisation of colloidal gold nanoparticles with high surface energies. NP aggregation was found to follow a time-dependent pattern (Fig. 2); broader and larger polydispersed nanoparticles were observed through time. The phenomenon could be visualised with the unaided eye as a gradual change of colour of the colloidal gold solution from bright yellow to dark brown to almost black (*via* dark yellow, light green to light brown).

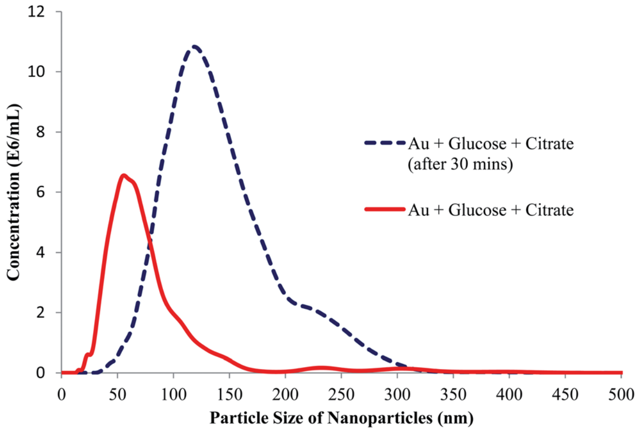

Fig. 2 Time evolution of particle size distribution of Au–glucose–citrate systems as measured by NTA.

The distribution curves from NTA analysis clearly suggest the formation of polydispersed nanoparticles in the solution. In order to validate the accuracy of the data which were obtained very quickly (only taking a few minutes from sample preparation to analysis), identical experiments were conducted and the solutions were submitted for Transmission Emission Microscopy (TEM). Representative TEM images of the investigated systems depicted in Fig. 3 show a very good agreement with the results obtained by NTA. Au NPs in stabilized colloidal solutions were found to be highly crystalline (C, F, I of Fig. 3, electron diffraction patterns) and sizes were observed to be in good agreement with those of NTA (Table 1). Due to the relatively high surface energy of gold, aggregation is unavoidable with time, which the formation of larger nanoparticles when visualised under TEM (Fig. 3D–G). Considering such rapid aggregation of gold nanoparticles, sizes measured between TEM and NTA are not quite far-off from each other, making the NTA an excellent technique for quick screening of nanoparticles in colloidal solutions.

Interestingly, the lines and regular patterns on the Au NPs in ED were remarkably changed after the addition of the capping agents, possibly indicating a change in the internal structure of the nanoparticle.[17,18]

The ability of the agar to avoid Au NP aggregation may be attributed to its potential to effectively coat the surface of the nanoparticle and significantly reduce its surface energy.[4,12] As reported by Shan *et al.*,[19] a chitosan polysaccharide does not only have the ability to interact with the surface but can also serve as a reducing agent. We believe that the observed NP stabilization in the case of agar is mainly due to a combination of a reduction in surface energy of the colloidal Au NPs and a mild reduction of Au^{3+} to Au species.[4,12] These results are also in good agreement with the observed synergistic effect of the addition of citrate to the Au–glucose system, with glucose playing the role of a capping agent and citrate as both a stabilising and mild reducing agent.[14–16]

Results obtained from the biorefinery-derived syrup were, however, remarkably different from previously reported Ag-stabilised nanoparticles using similar capping agents.[12] The syrup was found to be more efficient in stabilising Ag nanoparticles (as compared to sugars and related capping agents) with respect to the almost negligible capping/stabilising effect observed with Au nanoentities. A similar effect was observed for most capping agents utilized in our previous work with Ag colloidal NPs, which led to smaller and not so polydispersed Ag NP sizes.[12] In the case of the hemicellulosic syrup, low quantities of polydispersed colloidal particles could be observed in the systems (Fig. 4). We believe that the significant differences in terms of surface energies between Au and Ag colloidal nanoentities account for the different behaviour in the two colloidal systems based on measured experimental data for surface energies available for both metals (1.25 and 1.5 J m^{-2} for Ag and Au, respectively).[20]

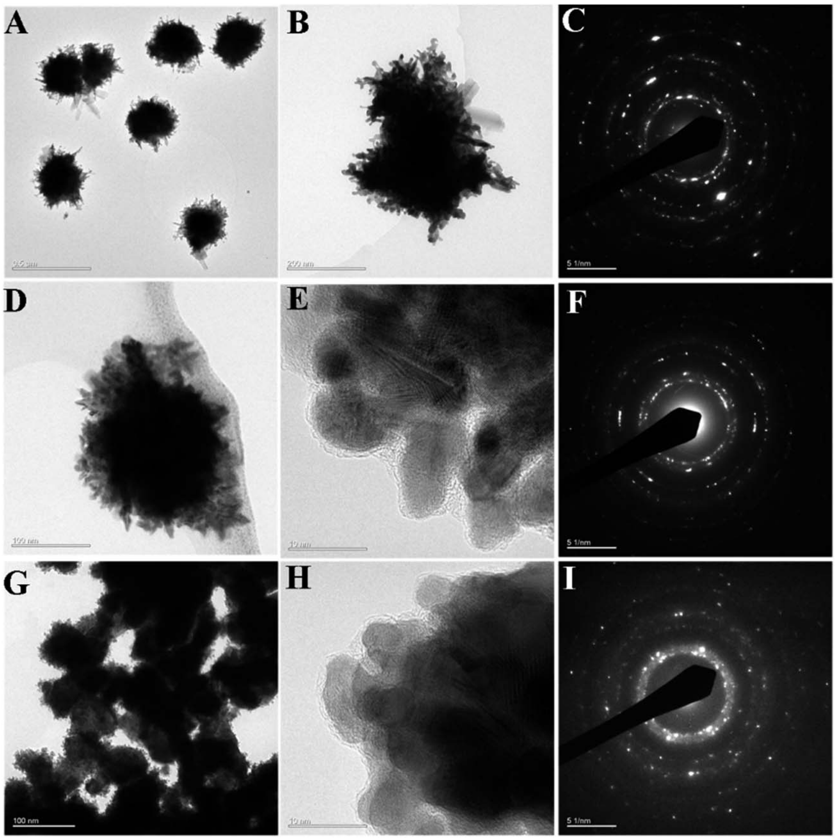

Fig. 3 TEM images and diffraction patterns of Au colloidal systems under different stabilising agents: (A–C) gold nanoparticles; (e–h) gold + glucose nanoparticles; (i–l) gold + glucose + citrate nanoparticles (pictures C, F, and I are diffraction patterns obtained using energy dispersive X-ray spectroscopy).

Table 1 Comparative NP sizes measured by both TEM and NTA for stabilised Au colloidal solutions

	Particle size (nm) – NTA		
	Mode (maximum)	Mean (average)	Av. article size (nm) – TEM
Au solution	—	233	Over 200 nm
Au + glucose	73	84	85–90
Au + glucose + citrate	56	76	70
Au + glucose + citrate (30 min)	119	144	140
Au + agar	35	50	50
Au + syrup	—	184	150–200

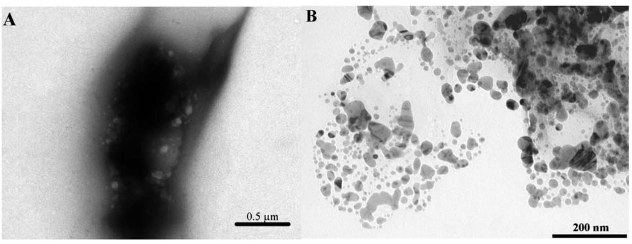

Fig. 4 TEM images of (A) colloidal Au in a syrup solution; (B) Ag colloidal nanoparticles stabilised by the biorefinery derived syrup.[12]

In fact, experiments with a significantly larger surface energy metal (Fe, 2.5 J m^{-2})[20] were unable to provide any stabilisation/capping in Fe-containing solutions.

Upon investigation of the capping abilities of various polyol compounds on Au colloidal solutions, we were prompted to study the reliability of the NTA by comparing its results with TEM.

Au nanorods (Au-NRs)

The three main types of Au NRs synthesized in this study were subjected to comparative experiments both under NTA fast analysis and TEM. Results of the analysis of selected samples of Au NRs are summarised in Table 2, Fig. 5, and Fig. 6 (NTA *vs.* TEM).

Being quite sceptical about the possibility of NTA to provide reliable data due to different dimensions (width and length) of Au NRs. Results obtained after comparison of NTA and TEM demonstrate the good agreement (even better as compared to colloidal Au NP systems) between both analytical techniques and the relatively good accuracy of employing NTA for nanoparticles in colloidal solutions. Au NRs exhibited a perfect crystalline fcc cubic structure (as depicted in the ED pattern of a representative sample, Fig. 6D).

Table 2 Comparative NP sizes measured by both TEM and NTA for stabilised Au colloidal solutions

	Particle size (nm) – NTA			
	Mode (maximum)	Mean (average)	Average particle size (nm) – TEM	DLS
Au NRs sample 1	52	82	80 × 20	76 × 13
Au NRs sample 2	59	69	85 × 30	79 × 25
Au NRs sample 3	65	85	90 × 40	87 × 32

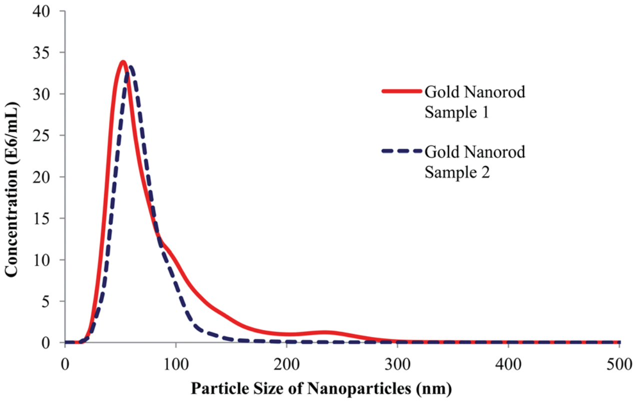

Fig. 5 NTA quantification of different Au NR samples using a NanoSight instrument.

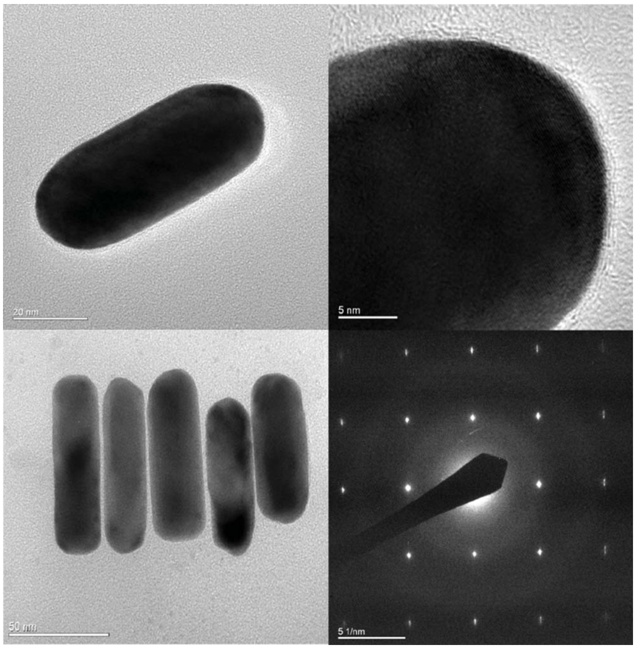

Fig. 6 TEM micrographs of Au NRs (samples 2 and 3); lower right image shows the EDX pattern of the Au NRs. The Electron Diffraction (ED) pattern shows a well-structured fcc cubic Au phase.

Conclusion

The study, along with the previous work of our lab, has shown that the nanoparticle tracking analysis is a simple, efficient and reliable method to approximate the size distribution of a range of various nanoentities in colloidal solutions. The use of polysaccharides (*e.g.* agar) and the combined sugars/citrate solutions as capping and reducing agents proved to provide promising media for the generation of colloidal gold nanoparticles. Contrary to previous results with Ag colloidal solutions,[12] a concentrated syrup derived from waste hemicellulosic biomass gave a poor particle distribution through NTA, making it a not so good capping agent because it generated polydispersity in terms of particle sizes. More importantly, NTA provides a reliable methodology for an efficient and swift characterisation of Au NRs in solution as compared to TEM. Further studies related to the utilisation of other nanoentities and types of nanomaterials for NTA analysis will be reported in due course.

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