NANOPARTz™

Technical Note 06-01

Comparison of Nanopartz™ Gold Nanoparticles to a Competitor's Citrate Stabilized Gold Nanoparticles

SIZE AND SHAPE MONODISPERSITY AND SURFACE REACTIVITY

OVERVIEW

Gold nanoparticles may be characterized by their size and shape monodispersity, along with their surface reactivity. These properties are important in the fields of molecular labeling, particularly in diagnostics such as lateral flow, resonance light scattering (RLS), and surface enhanced Raman (SERS). Good nanoparticles for these applications should be consistent in size and shape within the batch, and from batch to batch. They should also be highly reactive towards applying specific molecules or coatings onto the surface and yet not so reactive that they aggregate. This technical note investigates and compares these properties between the Nanopartz™ recently released, patent pending and proprietary gold nanoparticles and those purchased from an established competitor in the field.

COMPARISON

Nanopartz™ has recently released a complete line of gold nanoparticles from 30 to 90 nm in size. As opposed to the conventional citrate stabilized method of gold nanoparticle production, Nanopartz™ has developed a method utilizing a carboxylic acid functionalized hydrocarbon capping agent. This new proprietary and patent pending manufacturing method provides a means to produce gold nanoparticles of exquisite size and shape monodispersity.

SIZE MONODISPERSITY COMPARISON

Results have shown that Nanopartz™ can produce gold nanoparticles of the exact size requested by the customer, with batch to batch size variations of less than 1 nm. The competitor is specified to be better than 10% CV (center variation), but our measured %CV was larger than this for their 60 nm size (refer to Table 1).

Table 1. Comparison of actual size all sizes (nm). All sizes calculated through TEM image and Scion software calculations.

Size intended	**Nanopartz**™actual size
32	32.4
42	41.5
62	62.0
82	82.4
102	102.0

Size purchased	Competitor's actual size
20	17.1
60	52
80	74.9
100	92

Nanopartz™ batch to batch variations are less than 1 nm. Consequently, customers interested in any size, for example 45 nm, can purchase 45 nm nanoparticles that are ± 1 nm from batch to batch. When comparing these properties to those of the established competitor, we notice the competitor provides no specification of batch to batch variation. We have measured batch to batch size variations of ± 8 nm on over ten 60 nm gold nanoparticles purchases over the last few years. This means that purchasing 60 nm gold colloids from the competitor could result in nanoparticles as small as 52 nm or as large as 68 nm.

Next, the variation within the batch (in-batch variation) was compared. Using TEM, Nanoparts™ standard deviation within the batch varied from 1.1 nm for the 32 nm size, up to 2.6 nm for the 102 nm size nanoparticles. This compares to the competitors in batch variation which are specified to be better than 10% CV (center variation). Given

$$\text{Std dev} = \%CV * \text{average} / 100 \qquad (1)$$

Then the following in batch variation comparison is shown in Table 2 (and Appendix A):

Table 2. In batch variation for Nanoparts™ gold nanoparticles versus competitor.

Size (nm)	Nanopartz™ in batch std. deviation (± nm)	Competitors in batch std. deviation (± nm)
30	1.1	3
40	1.4	4
60	1.9	6
80	2.6	8

Consequently, the size monodispersity benefits of Nanopartz™ brand gold nanoparticles over the competitors include:

1. **Batch to batch variation is reduced from 10% CV to less than 1%, allowing the user to purchase exact sizes of the nanoparticles desired.**

2. **In batch variation is reduced from 10% CV to less than 4% CV, improving size monodispersity by over 300%.**

Size is very important in RLS (plasmon resonance light scattering), SERS (surface enhanced Raman spectroscopy), and LTF (lateral flow) applications. The peak light scattering wavelength used in RLS applications is highly dependent on the center nanoparticle size. Also, the intensity of the scattering is dependent on a very low standard deviation of size within the batch.

Size is also very important in SERS as the peak Raman cross-section is highly dependent on the excitation wavelength and the nanoparticle size.

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SHAPE COMPARISON

To define a specification for shape monodispersity is difficult. Other vendors claim statements such as “<5 % not round.” We believe the best way to compare shape is through TEM images. Figures 1 and 2 compare four sample batches of similar sizes between Nanopartz™ and the competitor.

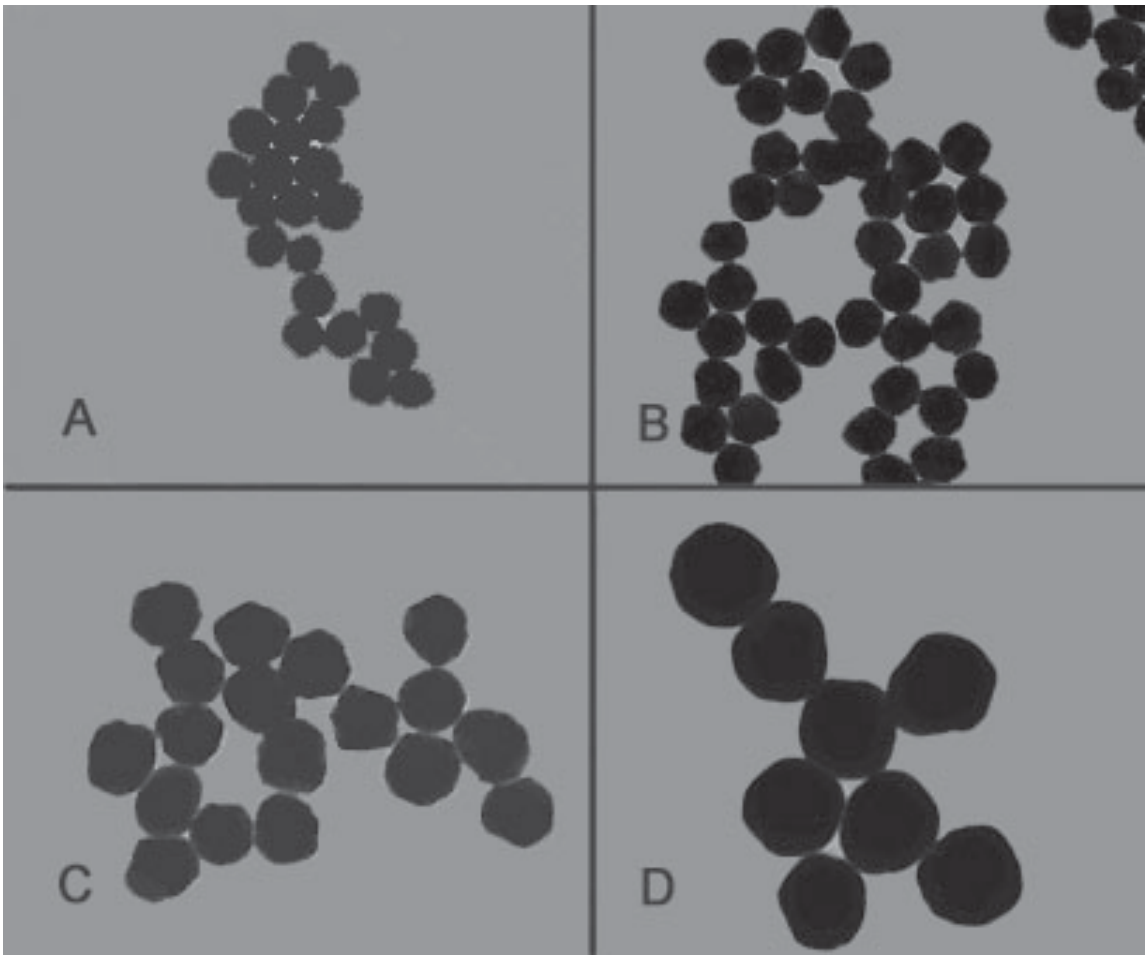

Figure 1 Nanopartz™ gold nanoparticles (a) 32 nm (b) 42 nm (c) 62 nm (d) 82 nm

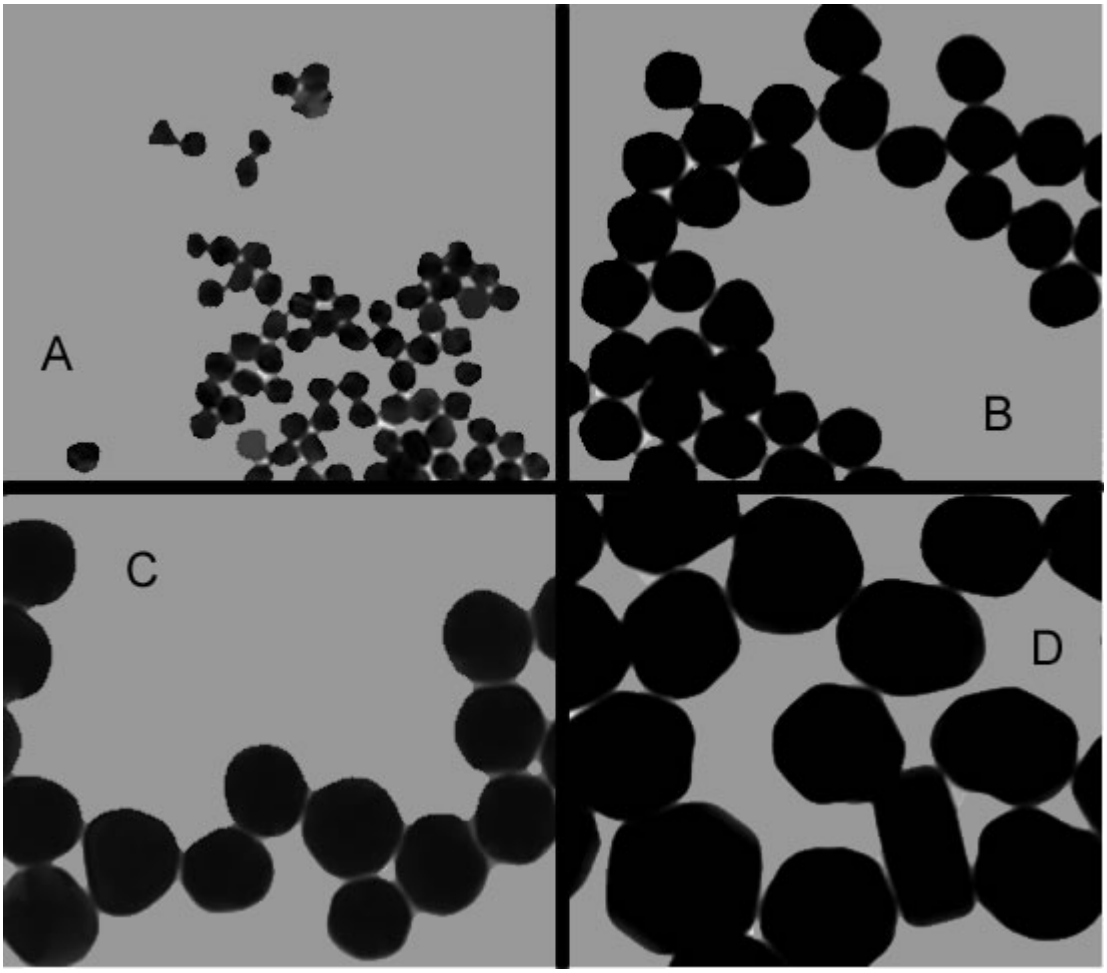

Figure 2 TEM pictures of competitors (A) 20 nm, measured 17 nm (B) 60 nm, measured 52 nm (C) 80 nm, measured 74 nm and (D) 100 nm, measured 92 nm.

Consequently, the shape monodispersity benefits of Nanopartz™ brand gold nanoparticles over the competitors include:

1. **A higher percentage of spheres.**

Shape is very important in RLS and SERS applications. The peak light scattering wavelength used in RLS applications is highly dependent on the shape, and the intensity of the scattering is dependent on a very uniform shapes within the batch.

Shape is also very important in SERS as the peak Raman cross-section is highly dependent on the excitation wavelength and the nanoparticle shape. Interestingly, we have found that particular non spherical shapes are better for SERS. But, to achieve this improvement, all of the shapes must be the same.

REACTIVITY COMPARISON

In most molecular labeling applications, the application demands a surface coating to be applied to the gold nanoparticle. The user demands a highly reactive surface that will yield complete coverage of the surface coating or selective immobilization of the desired molecules on the surface in controllable ways. At the same time, if the gold nanoparticle is too reactive to be controllable, then they will aggregate, thereby reducing their functionality. Therefore a balance is needed. This balance is achieved through capping agents and other additives to the stock solution. The competitor uses a citrate capping agent that possesses a -3 charge on each particle and undisclosed additives. Nanopartz™ gold nanoparticles use a proprietary capping agent that possesses a -1 charge and no other additives but distilled water. The benefits of the Nanopartz™ method are two fold.

1. With a -1 charge, the nanoparticles are much more reactive with added surface coatings.

2. Without added undisclosed additives, the user is not required to spin out the nanoparticle upon receipt and replace the buffer with distilled water.

EXPERIMENTAL RESULTS

Experiment 1: SERS utilizing a dye molecule as a surface coating.

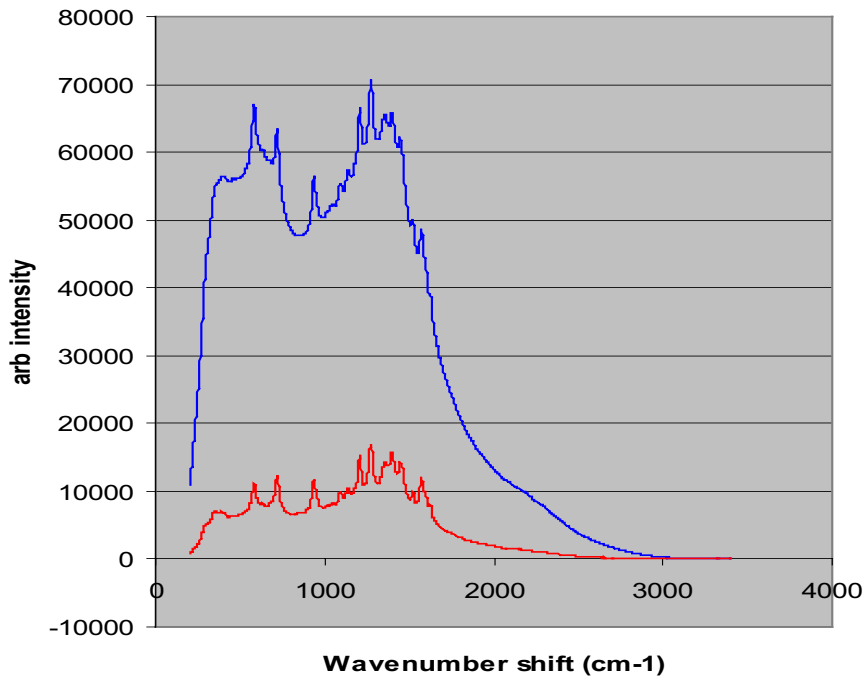

Figure 3 Comparison of SERS response from a dye molecule, indolenine cyanine dye, on competitors gold nanoparticles (blue) versus Nanopartz™ (red).

In this experiment, a monolayer of dye molecules was applied to both the competitors gold nanoparticles and a similar size Nanopartz™ gold nanoparticle solution. Briefly, a 100 mL of stock solution of indolenine cyanine dye was mixed with a solution of 60-nm Au nanoparticles which was 33% dilution of the stock nanoparticles. The final concentration of the dye was 0.7 mM. The adsorption of the dye on the nanoparticle results from an electrostatic interaction between the cationic dye and the Au nanoparticles capped with a monolayer of negatively-charged molecules. The SERS spectra of the mixed solution was taken after allowing the reaction for ~15 minutes. Figure 3 shows the resulting spectra for the nanoparticles of the two different sources. Note that the difference in the spectral background was reproducible even when the fluorescent quenching was observed to be similar for nanoparticles of the different sources.

The most obvious difference between the spectra is their baseline. The increase in the competitors baseline is due to fluorescence from the dye molecule. However, the purpose of the gold nanoparticle is to quench the fluorescence while providing an increase in Raman signal. The huge difference in fluorescence is due to the fact that Nanopartz™ brand nanoparticles react better with the dye molecules to provide better fluorescence quenching. Overall when comparing S/N of the particular peaks, the Nanopartz™ nanoparticles provide an increase of four times (most of it due to increased fluorescence induced noise). The reduction in fluorescence is over ten times.

Experiment 2. SERS utilizing a Thiol based Molecule: Mercaptobenzoic Acid (MBA)

In this experiment, a 30 mL of stock solution of MBA was mixed with a solution of 60-nm Au nanoparticles which was 33% dilution of the stock nanoparticles. The final concentration of MBA was 0.1 mM. A drop of the mixed solution was cast on an Au thin film slide, and was allowed to dry before the SERS spectrum was taken. Figure 4 shows the resulting spectra for the nanoparticles of the two different sources.

Note again the reduction in background fluorescence and the improvement in response. The S/N improvement of this experiment using Nanopartz™ brand gold nanoparticles was five times better. Again the background fluorescence may be attributed to unreacted MBA due to the lower reactivity of the competitor's nanoparticles.

Consequently, the reactivity benefits of Nanopartz™ brand gold nanoparticles over the competitors include:

1. **An improvement of 2 to 4 times in signal/noise.**
2. **A reduction of up to 10 times in background fluorescence.**

Reactivity is very important in RLS and SERS applications. Reactivity is important in all applications where the requirement for surface coating exist. (Refer to Application Notes).

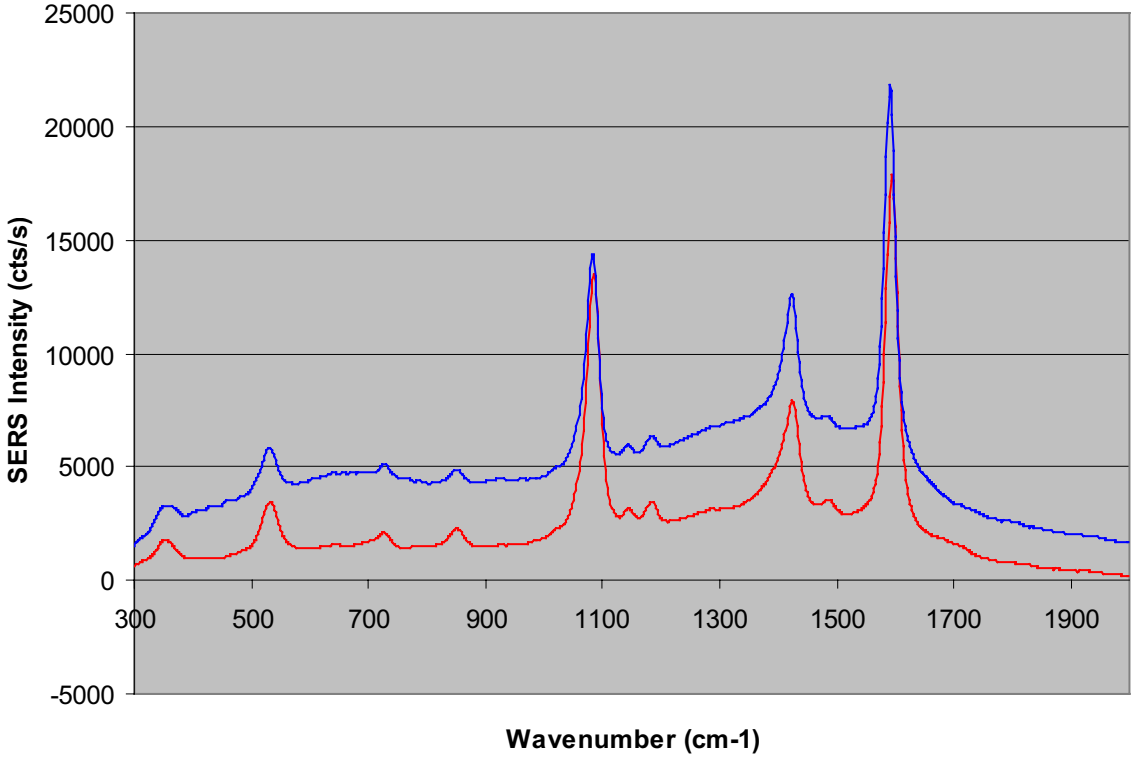

Figure 4 Comparison of SERS from a thiol based Mercaptobenzoic Acid (MBA) with competitors gold nanoparticles (blue) versus Nanopartz™ gold nanoparticles (red).

SUMMARY

Nanopartz™ brand gold nanoparticles, a patent pending new manufacturing method of making gold nanoparticles is shown to result in significantly improved size and shape monodispersity from batch to batch and within batch when compared to an existing competitors gold nanoparticles. Further, Nanopartz™ brand gold nanoparticles have shown to provide a much more reactive surface to conjugate surface coatings.

Nanopartz™ brand gold nanoparticles will result in better nanoparticles for RLS and SERS applications where these properties are important.