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Uncertainty Measurements in Chemically Synthesized Stable Uniform Sized Gold Nanoparticles for TEM/HRTEM Calibration

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The accuracy of an electron microscope depends on the magnification and resolution of the microscope. Hence in order to achieve high accuracy measurement results from the electron microscopic examination, a standard specimen is required to calibrate the transmission electron microscope (TEM). In the present work, gold nanoparticles of uniform size with narrow particle size distribution were synthesized with an aim to use them as reference material (RM) for TEM/high resolution TEM calibration and then release them in market as Bhartiya Nideshak Dravya (BND). As synthesized gold nanoparticles at 20 ± 2 °C temperature and 45± 5% relative humidityand the standard sample were characterized by using HRTEM, model: FEI-Tecnai F30 G2 STWIN at suitable magnification and areas. Both images exhibit spherical shaped nanoparticles with size varying in the range from (15.35-20.15 nm) and (5.10 -7.70 nm) respectively. Stability in particles size of the chemically synthesized gold nanoparticles was studied for a period of one year time span using UV-Visible spectrometer. TEM/HRTEM measurements were carried out in automated mode under similar experimental and environmental conditions at different magnifications to get accurate particle size. Overall uncertainty is estimated in small and big size particles as per standard GUM document guidelines.

Keywords: Gold Nanoparticles, Uncertainty Measurement, TEM/HRTEM, Bhartiya Nideshak Dravya (BND).

1 Introduction

The transmission electron microscope (TEM) is one of the very important instruments for the precise measurements of linear sizes of nano-objects and nanomaterials with small uncertainty as compared to other techniques. TEM’s point resolution and the resolution in the mode of the scanning transmission electron microscope (STEM-mode) can achieve the values in the range of 70–80 pm. Aberration correctors and image reconstruction methods have further improved the point resolution of TEM to about 0.05 nm. With the increasing demand of HRTEM images; an accurate magnification/ resolution calibration of TEM is becoming a more and more critical parameter. It is important to mention here that magnification and resolution are interrelated features and are always related to the efficiency of the microscope. In order to achieve a high level of accuracy and efficiency in the measurements for scientific research & development, innovations, calibration of certain parameters of the electron microscope has drawn considerable attention. Since the characterization of materials under the electron beam inside the microscope is dealing at sub nano scale, it is very important to have the equipment always with extremely high precision.

Uncertainty calculations in measurements for any quantitative parameter is must to get acceptance of high precision data globally as per ISO/IEC 17025 standard norms. All the existing measurements are taken into account for the estimation of overall uncertainty in gold nanoparticles size measurements by HRTEM technique. This reflects dispersion in the measured 98 % confidence level. The exhaustive work is going on reducing measurement uncertainty to have parameter value more near to its true value. The easiest way to reduce measurement uncertainty is to reduce the number of factors like instrumental, statistical, environmental and human which directly or indirectly influence the value of measuring parameters. In this paper we are focused on resolution calibration of HRTEM by chemically derived gold nanoparticles.

Gold nanoparticles (AuNPs) are the most studied nanomaterials because of its exceptional chemical stability, ease in processability, high catalytic activity, surface plasmon resonance, and metallic nature, which provide them the unique size-dependent electronic and optoelectronic properties[1]. As a result, AuNPs are used in a wide variety of technologies including nanoelectronics, catalysis, imaging,

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biosensors, photodynamic therapy, biodiagnostics, and biomedicine[2-4]. With expanding potential of AuNPs, it is important to stabilize the size and control shape of the nanoparticle. Several efforts have been made and henceforth reported for the synthesis of stabilized AuNPs, among them, the chemically synthesized colloidal AuNPs received widespread attention due to ease in synthesis[5-12]. The chemically synthesized AuNPs generally takes few minutes to hour for the synthesis and are consists of two-phase systems in which one of the phase has at least one dimension less-than 100 nm[5-6].

The particle size measurements for the synthesized nanoparticles are made by both indirect and direct measurement techniques. The indirect ways of determining the particles size is by absorption study, provides the mean particle size distribution of the nanoparticle, which is important for initial study and get the broad idea on the formation of nanoparticle, but this technique suffers with disadvantage of difficulty in making this number more precise[5,13]. Also the determination of internal structure of nano-sized particle is still out of the reach of any indirect methods. This sets the need for more suitable, accurate and direct reliable measurement techniques such as TEM (Transmission Electron Microscopy) for précised particle size measurement. The TEM measurement is a widely recognised method for measuring the particle size and size distribution of the nanoparticles.

Now a days, TEM is considered as highly accurate, widely used measurement tool for the characterization of variety of materials especially nanomaterials for their crystallographic structure, epitaxial growth, lattice imaging, particle size, shape analysis, phase identification and defects studies such as voids, dislocations, twinning, stacking and other structural faults at micro and nano levels[14-15]. By use of the TEM the size, shape, distribution of size, structure, and genetic relationships among the various morphological species can be determined. To maintain its accuracy in the data collected, the instrument needs to be calibrated at a proper interval of time. This requirement led to the development to highly précised, stable, mono dispersed nano-sized particles with minimum uncertainty in the data for the calibration of the instrument[13-17]. Thus, a calibration sample developed for transmission electron microscopy (TEM) have to perform the three pivotal task for instrument calibrations *i.e.* the image resolution calibration for measurements of images, the camera constant calibration for indexing diffraction patterns, and the image/diffraction pattern rotation calibration for relating crystal directions to features in the image[14]. The existing calibration standards such as polystyrene spheres which damages with radiation or the grating which changes with fabrication process, incites the researchers to prepare calibration sample which is more stable with changing temperature or radiation[16]. Nanostructured gold have been studied to sustain under adverse conditions, and thus picked for preparing calibration samples.

The citrate reduced colloidal AuNPs has gain significant attention in this regards, for its non-toxic composition and stability[1,6,17]. The citrate reduced nanoparticle shape, size and the nature of size-distribution have been shown to vary within limits. This made the AuNPs suitable for testing and calibration of instruments and became a mainstream demand of the scientists, engineers and industrialists involved in a variety of metrological measurements. In this article, we report the studies on the indigenously developed stable and easily synthesized AuNPs for the calibration of TEM/HRTEM. The chemically synthesized AuNPs were investigated for size, morphology and stability. The synthesis parameters for the stable AuNPs have been optimised in the present study.

2 Experimental Details

Gold (III) chloride hydrate procured from M/s Sigma Aldrich and Tri-sodium citrate from M/s LobaChemie were used in the synthesis of gold nanoparticles (AuNP) in order to produce stable nanoparticles. The synthesis was done in the month of April 2017.

In the chemical synthesis process, AuNPs were synthesized in the presence of tri-sodium citrate. For this synthesis, 2.2 mM of Tri-sodium citrate was dissolved in 100 ml of deionised water (DI-water) (Millipore, 18.2 MΩ·cm at 25°C) into a two-necked round bottom flask and reflux at 100 ºC followed with continuous stirring. Further, 1 ml of 25 mM Gold (III) chloride hydrate was added to this solution. The colour changes from transparent to violet and finally to wine red was observed. There was no further change of colour on prolonged boiling. The wine-red colour indicates the formation of colloidal AuNPs[6]. These AuNPs were further stored and investigated for variation in particle size using high resolution transmission electron microscope (HRTEM; JEOL, JEM-2100F and FEI, Technai G2 F30 STWIN) and

UV-Visible spectroscopy (Agilent Cary5000). The schematic for formation of citrate reduced AuNPs using wet chemical method is shown in Fig. 1.

3 Results and Discussion

Material Characterizations:

The microstructural investigations of chemically synthesized AuNPs deposited on carbon coated Cu grids have been carried out to reveal the size, shape, stability and separation between particles in the microstructure. The measurement was made at the time interval of 3 months (in July' 2017) from synthesis date. Figure 2a shows TEM (JEOL at CSIR NPL) micrograph of the AuNPs sample, recorded at suitable area and magnification. TEM micrographs of the AuNPs show uniformly distributed nano-sized spherical shaped particles. The spherical shape nanoparticles are found to have size in the range 10 to 20 nm. HRTEM image of the individual grain, shown in Fig. 2b, reveals the lattice planes having interplanar spacing(d-spacing) of about 0.23nm or 0.24 nm and 0.20 nm, which corresponds to (111) and (200) planes of cubic structure of gold. The results are found to be in good agreement with the standard data (JCPDS file no: # 040784).

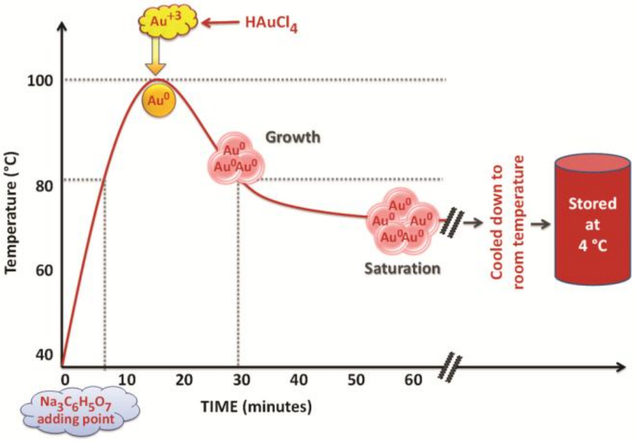

Fig. 1 — Schematic showing the formation of citrate reduced AuNPs using a wet chemical method

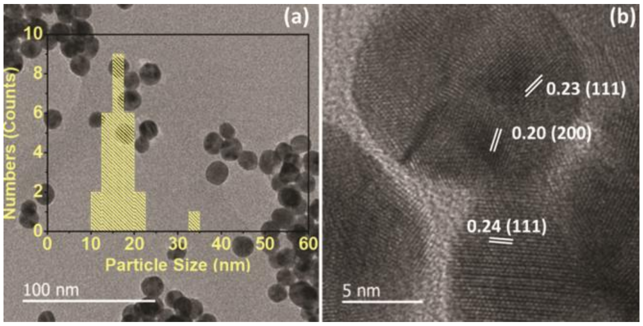

Fig. 2 — (a) TEM micrograph of chemically synthesized AuNPs and (b) HRTEM image of an individual AuNPs

To study the inter-instrument calibration, the same sample of AuNPs was again investigated for its shape and size using another TEM (TECNAI) at CSIR NPL. The measurement was made at the time interval of 6 months (in November' 2017) from the synthesis date. Figure 3a shows the size and distributions of AuNPs which are found to be similar to previous measurement results conducted in August 2017 using JEOL make TEM. Figure 3b represents the HRTEM image of AuNPs, depicting the formation of polycrystalline nature of AuNPs with interplanar spacing of 0.23 nm, 0.20 nm and 0.24 nm. On comparison of both the measurement data, it is observed that the shape, size and distribution of AuNPs are stable over a period of 6 months.

The aforementioned samples were further studied for the stability of their shape, size and distribution using TEM (TECNAI) at the time interval of another 9 months (in March'2018), from the synthesis date. Figure 4a shows nearly the similar particle size, shape and size distribution *i.e.* 10-20 nm, as observed in previous measurements. Figure 4b also shows the similar interplanar spacing in HRTEM image, illustrating the stability of AuNPs. Additionally, Figure 4c shows the selected area electron diffraction pattern (SAEDP) of AuNPs sample revealing ring pattern depicting polycrystalline nature. Detailed analysis of these diffraction rings resulted the presence of (111), (200), (220), (311), (222), (400), and (422) planes of Au having cubing structure, confirming the formation of Au structures. The similar planes have also been reflected in the previous measurements, suggesting the stability of the AuNPs. This indicates that the chemically synthesized AuNPs may be used as standard material for the calibration of TEM/HRTEM.

The same samples as investigated were further characterised for shape, size and distribution using

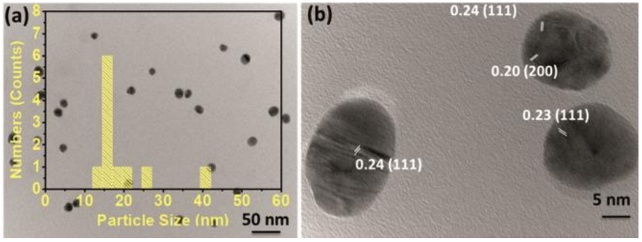

Fig. 3 — (a & b): TEM and HRTEM image of chemically synthesized individual AuNPs

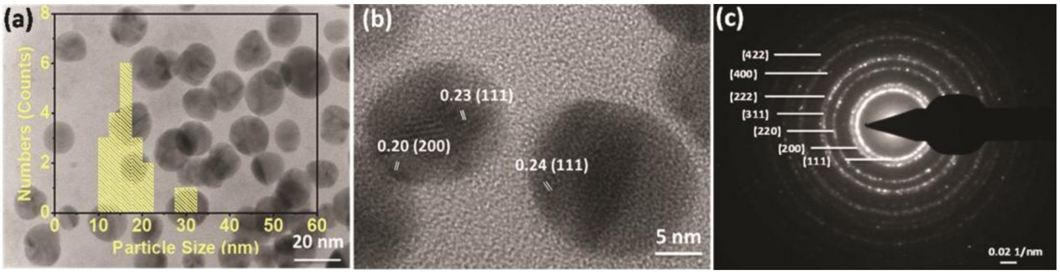

Fig. 4 — (a, b & c) TEM, HRTEM and diffraction pattern of chemically synthesized individual AuNPs, respectively

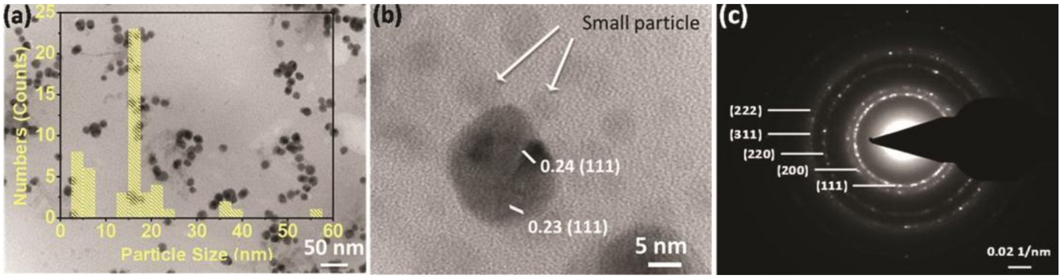

Fig. 5 — (a & b) TEM and HRTEM image, showing the presence of tiny particles in the background and (c) diffraction pattern of chemically synthesized AuNPs

HRTEM at the gap of another 3 months from last measurement (*i.e.* in June' 2018). Particle size distribution of the sample, shown in Fig. 5a, reveals the presence of tiny particles having the size around 5 nm along-with 10-20 nm range nanoparticles. The presence of tiny particles can be an outcome of Ostwald's ripening[18-19], where the smaller particles start shrinking the results into an increased number of free molecules and deposit over larger particles leading to the increase in particle size distribution. Further, from the HRTEM micrograph, Figure 5b, along with the particles 10-20 nm and d-spacing the existence of tiny particles can clearly be seen. SAEDP as shown in Fig. 5c indicates the presence of diffraction rings corresponding to (111), (200), (220), (311), (222), (400), and (422) planes of Au, confirming the presence AuNPs only.

From the EDS spectra (Fig. 6) of the gold nanoparticles deposited on the Silicon wafer, it is observed that a high amount of gold is present. Reflection of Silicon in the EDS spectra is due to the fact that the AuNP,s are deposited on the silicon substrate. Presence of other elements such as carbon , oxygen and sodium in the spectra is due to Tri-sodium citrate. No other element as impurities was observed in the EDS spectra.

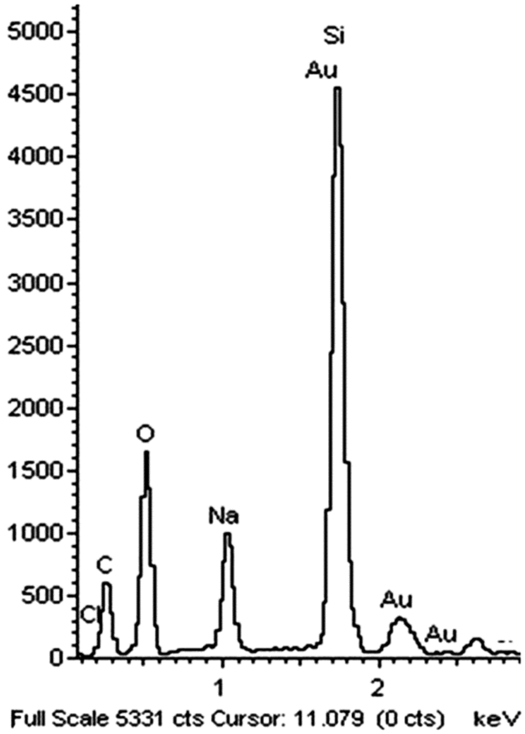

Fig. 6 — EDS spectra of the as synthesized gold nanoparticles

Further, the UV-Vis absorbance spectrum of AuNPs was carried out, on Cary 5000 (Agilent Technologies, USA) using quartz cuvette (3.5 ml, 10 mm path-length), to analyse the particle size of the sample. The absorbance spectrum of water was used for baseline correction. Figure 7 shows the UV-Vis absorbance data of AuNPs, recorded at the interval of three months, and exhibits the strong absorbance band in the visible region (450-600 nm)

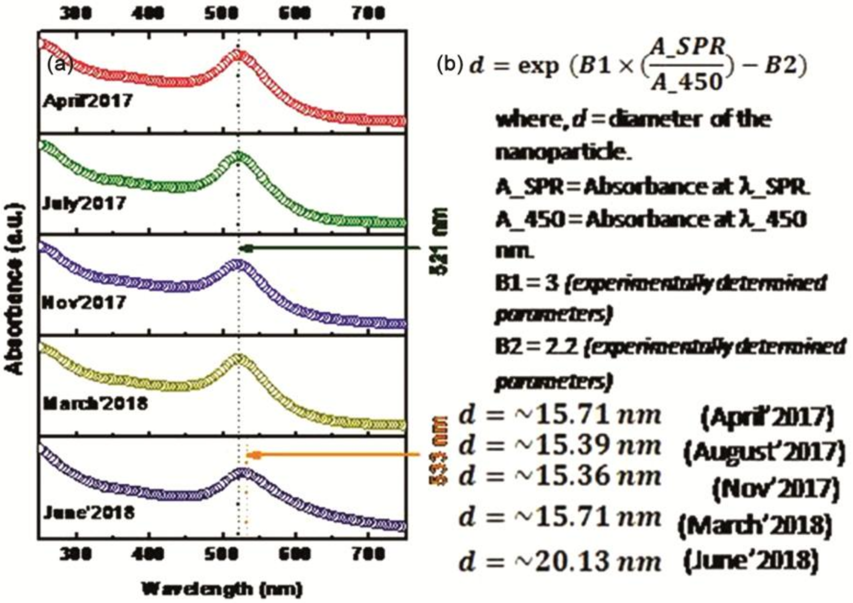

Fig. 7 — (a) UV-Vis absorbance data for AuNPs and (b) the associated particle size calculation.

with surface plasmon resonance (SPR) peak at 520 nm revealing the formation of AuNPs. The SPR of AuNPs recorded at different times has shown the same SPR peak position *i.e.* 521 nm, except for the July' 2018 where the SPR peak position was found a bit shifted to 533 nm, which is a clear indication of increase in average particle size. The data is further used for particle size calculation[7].The particle size was calculated from UV-VIS absorption data found to be ~15-16 nm for over the period of 1 year (which is the average of what calculated from TEM image data), after this period, an increase in average particle size was observed and found to be ~20 nm. All aforementioned results have suggested the formation of stable AuNPs of particle size in the range of 10-20 nm which retained its structure for the period of 1 year.

Uncertainty and Uncertainty Budget Calculations:

After optimizing the synthesis parameters and the stability of the AuNP's, we calculated the overall uncertainty and also prepared uncertainty budget for the synthesized material size measurements and compared it with the uncertainty calculation of standard Au nanoparticle samples (Gold on Carbon EMS Cat_80041), results are shown below:

Evaluation of measurements overall expanded uncertainty in standards and chemically derived Gold Nanoparticles images recorded by HRTEM for small and large size Au nanoparticles. The uncertainty in nanoparticles size measurements by HRTEM were made on the basis of GUM document guidelines[20]. Before beginning the calculations, first cause and effect fish bone diagram (Fig. 8) for quantifying the uncertainty components is prepared by considering the various parameters affecting the uncertainty in nanoparticle size measurement. Since all the measurements were done under similar environmental conditions by the same operator, the effects induced by environmental condition changes and human factor on AuNPs size measurements were ignored in the uncertainty estimation calculations. The TEM images were repeated ten times to check the repeatability at the same location for evaluation of Type A uncertainty value by variance and standard deviation.

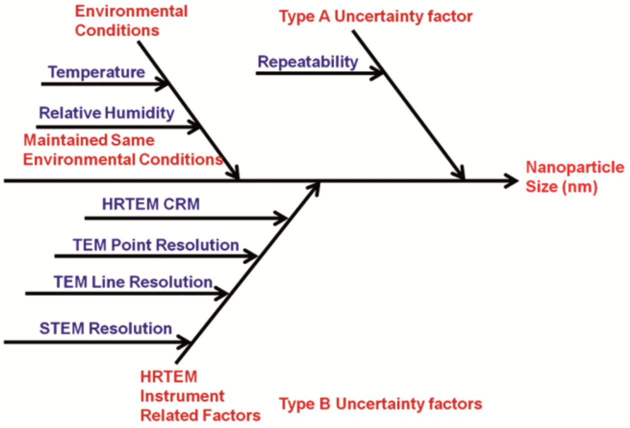

Fig. 8 — Cause and Effect Fish Bone diagram for quantifying uncertainty components in size measurements by HRTEM

Initially, particles shape and size of standard AuNPs samples were measured by using its TEM image (Fig. 9) and then studied for the uncertainty calculations. From the image, it has clearly been observed that there exist two different size distributions for the particles (for distinction the small and big sized AuNPs are shown by yellow and red colours respectively). We have calculated uncertainty in measurements for both size particles separately.

A. Estimation of the Overall Expanded Uncertainty of Standard Gold Nanoparticles

A.1 Type A Uncertainty Estimation

The uncertainty evaluated through the Type A method in the following steps in the measurement of particle size by TEM is shown in Table. 1.

The standard uncertainty associated with particle size is computed using relation:

$$u(x) = \sigma / (n)^{1/2} \quad \text{... (1)}$$

where *n* is the number of observations and σ is the standard deviation of the repeated measurements xi . The σ is computed as:

$$\sigma = [\Sigma(xi - \overline{x})^2/(n-1)]^{1/2} \quad \text{... (2)}$$

n

Where $\overline{x} = \Sigma$ xi/n is the arithmetic mean of the repeated measurements.

i = 1

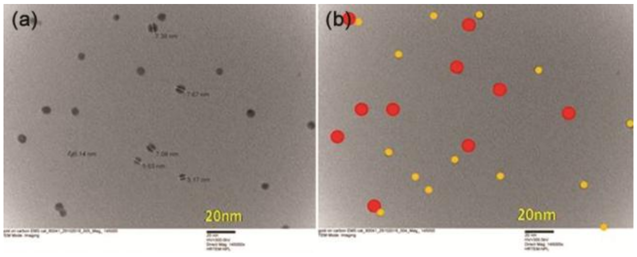

Fig. 9 — (a) TEM images of standard Gold Nanoparticles and (b) marked in to size categories

A.2 Type B Uncertainty Estimation

Type B uncertainty was evaluated from the contribution of three main sources (i) measuring instrument, (ii) operating procedure and (iii) characteristics of the sample under calibration. Uncertainty values for these components were generally taken from the calibration certificate provided by the manufacturer and the following procedure was followed.

In the particle size uncertainty measurements by TEM, the standard reference material and three types of different resolutions involved in measuring the particle size were considered.

A.2.1 Uncertainty in layer thickness of Reference standard for HRTEM: The calibrated values of layer thickness of MAG*I*CAL Reference standard provided by the supplier for the calibration of + 2 % *i.e.* + 0.02 nm. By assuming triangular distribution, the standard uncertainty in the particle size measurement, u1(δr1) = 0.02//√6 = 0.008165 nm with degree of freedom (ν1) = ∞.

A.2.2 Uncertainty in TEM Point Resolution: TEM point resolution value is 0.205 nm *i.e.* 0.205 x 10^{-9} m for HRTEM as provided by the supplier for thickness in the range of 50 to 150 nm. By using normal distribution, standard uncertainty in the value of measurement of particle size by this resolution: u2(δr2) = 0.205/2 = 0.1025 nm with degree of freedom (ν2) = ∞.

A.2.3 Uncertainty in TEM Line Resolution: TEM Line resolution value is 0.144 nm *i.e.* 0.144 x 10^{-9} m for thickness in the range of 50 to 150 nm for HRTEM as provided by the supplier. By assuming normal distribution, standard uncertainty in the value of measurement of particle size by this resolution: u3(δr3) = 0.144/2 = 0.072 nm with degree of freedom (ν3) = ∞.

A.2.4 Uncertainty in STEM resolution: STEM resolution value of is 0.17 nm *i.e.* 0.17 x 10^{-9} m

Table 1 — Type A uncertainty for small size Gold Nanoparticles

x_i	$\overline{x}$	$(x_i - \overline{x})$	$(x_i - \overline{x})^2$	$\sigma = [\Sigma(x_i - \overline{x})^2/(n-1)]^{1/2}$	Type A Uncertainty $u(x) = \sigma/(n)^{1/2}$
5.63	5.247	0.0	0.0000	0.2023	0.06398
5.14		0.0	0.0000		
5.17		-0.03	0.0009		
5.23		0.0	0.0000		
5.63		0.0	0.0000		
5.23		0.03	0.0009		
5.14		-0.04	0.0016		
5.13		0.0	0.0000		
5.14		0.04	0.0016		
5.13		0.0	0.0000		

for HRTEM as provided by the supplier. By assuming normal distribution, standard uncertainty in the value of measurement of particle size by this resolution: $u4(\delta 43) = 0.17/2 = 0.085$ nm with degree of freedom $(\nu 3) = \infty$.

Type A uncertainty and uncertainty budget has been prepared and shown in the following Table 1 and Table 2, respectively, for smaller size gold nanoparticles of standard gold sample.

Similarly, the overall expanded uncertainty of large size Standard Gold nanoparticles has also been derived. Type A uncertainty parameter calculations and uncertainty budget are shown in Table 3 and Table 4, respectively.

A.3 Overall Expanded Uncertainty

Type A and Type B uncertainty components are combined by using the following equation to assign single value of uncertainty in particle size measurement by TEM technique i.e. the combined measurement uncertainty:

Combined uncertainty (uc) = $[uA(x)2 + u1(\delta r1)\ 2 + u2\ (\delta r2)2 + u3(\delta r3)2 + u3(\delta r3)2]1/2 = 0.001871$ nm

Overall Combined expanded uncertainty associated in 5.247 nm particle size is given as:

Uc (particle size) = Particle size x 0.001871 = 0.009815 nm

Then the final result of measured value along with its overall expanded uncertainty is 0.0196 nm at 95% confidence level.

Similarly, the uncertainty calculations were made for bigger size standard Gold nanoparticles

B. Estimation of the Overall Expanded Uncertainty of chemically derived Gold Nanoparticles for BND development.

Like standard Gold nanoparticles samples, the prepared sample with synthesized nanoparticles were also found to exhibit (figure 3a) two different nanosized nanoparticles of about 14 nm and 17 nm range. Type A uncertainty calculated by repeating images 10 times for both sizes are presented in Table 5 and Table 6.

Table 2 — Uncertainty Budget for Small Size Standard Gold Nanoparticles

Quantity	Estimated value xi	Limits Δxi	Probability Distribution/ Type A & Type B	Sensitivity Coefficient	Degree of Freedom	Uncertainty Contribution	[ui2(y)]
Particle size (X nm)	5.247 nm	0.49	Normal, Type A, $\sqrt{10}$	1	9	0.06398	6.0863E-7
δu1	10.55 nm	1.9	Triangular, Type B, $\sqrt{6}$	1	∞	0.008165	5.9897E-7
δu2	100 nm	0.35	Normal Type B, 2	1	∞	0.1025	1.0506E-6
δu3	100 nm	0.05	Normal Type B, 2	1	∞	0.072	5.184E-7
δu3	100 nm	0.1	Normal, Type B, 2	1	∞	0.085	7.225E-7

Table 3 — Type A uncertainty for large size Standard Gold Nanoparticles

x_i	$\overline{x}$	$(x_i - \overline{x})$	$(x_i - \overline{x})^2$	$\sigma = [\Sigma(x_i - \overline{x})^2/(n-1)]^{1/2}$	Type A Uncertainty $u(x) = \sigma/(n)^{1/2}$
7.08	7.347	- 0.267	0.071289	0.2585	0.08175
7.67		0.323	0.104329		
7.38		0.033	0.001089		
7.08		- 0.267	0.071289		
7.08		- 0.267	0.071289		
7.67		0.323	0.104329		
7.38		0.033	0.001089		
7.38		0.033	0.001089		
7.67		0.323	0.104329		
7.08		- 0.267	0.071289		

Table 4 — Uncertainty Budget for Large Size Standard Gold Nanoparticles

Quantity	Estimated Value xi	Limits Δxi	Probability Distribution/ Type A & Type B	Sensitivity Coefficient	Degree Of Freedom	Uncertainty Contribution	[ui2(y)]
Particle size (X nm)	7.347 nm	0.59	Normal, Type A, $\sqrt{10}$	1	9	0.08175	8.2725E-7
δu1	10.55 nm	1.9	Triangular, Type B, $\sqrt{6}$	1	∞	0.008165	5.9897E-7
δu2	100 nm	0.35	Normal Type B, 2	1	∞	0.1025	1.0506E-6
δu3	100 nm	0.05	Normal Type B, 2	1	∞	0.072	5.184E-7
δu3	100 nm	0.1	Normal, Type B, 2	1	∞	0.085	7.225E-7

The corresponding uncertainty budget Table 7 and Table 8 were prepared.

C. Comparing Overall Expanded Uncertainty of Standard and Chemically Derived Gold Nanoparticles:

Estimated values of Overall Expanded Uncertainty of Standard and chemically derived Gold Nanoparticles size by HRTEM available in CSIR-NPL, New Delhi are expressed as:

For Standard Gold Nanoparticles

(i) 5.247 ± 0.0196 nm (for small size particles)

(ii) 7.347 ± 0.02023 nm (for large size particles)

Table 5 — Type A uncertainty of smaller size chemically derived Gold Nanoparticles

x_i	$\overline{x}$	$(x_i - \overline{x})$	$(x_i - \overline{x})^2$	$\sigma = [\Sigma(x_i - \overline{x})^2/(n-1)]^{1/2}$	Type A Uncertainty $u(x) = \sigma/(n)^{1/2}$
13.8	14.23	- 0.43	0.1849	0.33015	0.1044
13.8		- 0.4	0.1849		
14.2		0.03	0.0009		
14.5		- 0.27	0.0729		
14.7		- 0.47	0.2209		
13.8		- 0.43	0.1849		
14.2		0.03	0.0009		
14.4		0.17	0.0289		
14.4		0.17	0.0289		
14.5		- 0.27	0.0729		

Table 6 — Type A uncertainty of Large size chemically derived Gold Nanoparticles

x_i	$\overline{x}$	$(x_i - \overline{x})$	$(x_i - \overline{x})^2$	$\sigma = [\Sigma(x_i - \overline{x})^2/(n-1)]^{1/2}$	Type A Uncertainty $u(x) = \sigma/(n)^{1/2}$
19.3	19.56	- 0.26	0.0676	0.2366	0.07483
19.3		- 0.26	0.0676		
19.8		0.24	0.0576		
19.3		- 0.26	0.0676		
19.8		0.24	0.0576		
19.8		0.24	0.0576		
19.6		0.04	0.0016		
19.6		0.04	0.0016		
19.3		- 0.26	0.0676		
19.8		0.24	0.0576		

Table 7 — Uncertainty Budget for Smaller Size Gold Nanoparticles

Quantity	Estimated Value xi	Limits Δxi	Probability Distribution/ Type A & Type B	Sensitivity Coefficient	Degree Of Freedom	Uncertainty Contribution	[ui2(y)]
Particle size (X nm)	14.23 nm	0.9	Normal, Type A, √10	1	9	0.104403	5.8674E-7
δu1	10.55 nm	1.9	Triangular, Type B, √6	1	∞	0.008165	5.9897E-7
δu2	100 nm	0.35	Normal Type B, 2	1	∞	0.1025	1.0506E-6
δu3	100 nm	0.05	Normal Type B, 2	1	∞	0.072	5.184E-7
δu3	100 nm	0.1	Normal, Type B, 2	1	∞	0.085	7.225E-7

Table 8 — Uncertainty Budget for Large Size Gold Nanoparticles

Quantity	Estimated Value xi	Limits Δxi	Probability Distribution/ Type A & Type B	Sensitivity Coefficient	Degree Of Freedom	Uncertainty Contribution	[ui2(y)]
Particle size (X nm)	19.56 nm	0.5	Normal, Type A, √10	1	9	0.07483	8.1967E-7
δu1	10.55 nm	1.9	Triangular, Type B, √6	1	∞	0.008165	5.9897E-7
δu2	100 nm	0.35	Normal Type B, 2	1	∞	0.1025	1.0506E-6
δu3	100 nm	0.05	Normal Type B, 2	1	∞	0.072	5.184E-7
δu3	100 nm	0.1	Normal, Type B, 2	1	∞	0.085	7.225E-7

For Chemically Derived Gold Nanoparticles

(iii) 14.23 ± 0.0531nm (for small size particles)

(iv) 19.56 ± 0.0674 nm (for large size particles)

Results suggest that the as synthesized AuNPs are in correlation with available standard Au nano particle sample, and thus can be used as a standard sample for the calibration of resolution and camera length of TEM and HRTEM.

4. Conclusion

Preliminary studies on the synthesis and characterization of mono-dispersed, nanostructured, stable AuNPs prepared by chemical synthesis has been presented in the present study. The optimized AuNP samples were compared with the standard Gold Nanoparticles in order to obtain the traceability of the size measurement. In the present investigations, synthesis parameters have been optimised to obtain highly stable gold nanoparticles using chemical route. Estimation of the Overall Expanded Uncertainty of chemically derived Gold Nanoparticles has also been done in order to develop a standard reference material (BND). Results of the study suggest the comparable variations in both standard and chemically synthesized AuNPs. Therefore, the synthesized AuNPs are manifested to be used for calibration of the resolution and camera length of the TEM/HRTEM. As per best of our knowledge, at present there is no indigenously made standard sample available in our country. Also, a possible improvement in the studied synthesis process may further be used to narrow down the size distribution of the nanoparticle.

References

1 Piella J, Bastús N G & Puntes V, *Chem Mater*, 28 (2016) 1066.

2 Hvolbaek B, Janssens T V W, Clausen B S, Falsig H, Christensen C H & Norskov J K, *Nano Today*, 2 (2007) 14.

3 Homberger M & Simon U, *Philos Trans R Soc*, 368 (2010) 1405.

4 Sperling R A, Rivera P G, Zhang F, Zanella M & Parak W J, *Chem Soc Rev*, 37 (2008) 1896.

5 Turkevich & Hillier, *Anal Chem*, 21 (1949) 475.

6 Turkevich J, Stevenson P C & Hillier J, *Discuss Faraday Soc*, 11 (1951) 55.

7 Azubel M & Kornberg R D, *Nano Lett*, 16 (2016) 3348.

8 McFarland A D, Haynes C L, Mirkin C A, Van-Duyne R P & Godwin H A, *J Chem Edu*, 81 (2004) 544.

9 Ghosh D, Sarkar D, Girigoswami A & Chattopadhyay N, *J Nanosci Nanotechnol*, 11 (2011) 2.

10 Ghosh D & Chattopadhyay N, *Opt Photon J*, 3 (2013) 18.

11 Leng W, Pati P & Vikesland P J, *Environ Sci Nano*, 2 (2015) 440.

12 Tyagi H, Kushwaha A, Kumar A & Aslam M, *Nanoscale Res Lett*, 11 (2016) 362.

13 Haiss W, Thanh N T K, Aveyard J & Fernig D G, *Anal Chem*, 79 (2007) 4215.

14 Orji N G, et al., *Proc of SPIE*, 6518 (2007) 651810.

15 Singh S, Singh D & Singh M, *Indian J Pure Appl Phys*, 57 (2019) 157.

16 Filippov M N, Gavrilenko V P, Kovalchuk M V, Mityukhlyaev V B, Ozerin Y V, Rakov A V, Roddatis V V, Todua P A & Vasiliev A L, *Meas Sci Technol*, 22 (2011) 094014.

17 Senoner M, Wirth T, Unger W, Osterle W, Kaiander I, Sellin R L & Bimberg D, *Surf Interface Anal*, 36 (2004) 1423.

18 Frens G, *Nature*, 241 (1973) 20.

19 Liebig F, Thunemann A F & Koetz J, *Langmuir*, 32 (2016) 109.

20 International Organization for Standardization (ISO), Guide to the Expression of Uncertainty in Measurement, International Organization for Standardization (ISO), Geneva, Switzerland, 1993 (corrected and reprinted 1995).