Estimate the Population Density of Gold Nanoparticles in Suspensions from Experimental Data

Lei Yu*

Department of Chemistry and Biochemistry, Rowan University, Glassboro, New Jersey, 08028, USA

Gold Nanoparticles (AuNP) have been found many applications in chemistry and biochemistry, for example, as labels for bioanalysis or as payload carriers for biotherapeutics. Recently, investigations of the potential risks of the applications of AuNP and its products to human health and to the environment are also dynamic. It is very critical to know the mass or the number of nanoparticles in a sample in order to understand these biological and environmental effects. This requires analytical methods that focus on the average size of the particles, number of particles, and the total mass of particles in a liquid or solid sample.

Gold content in solutions can be measured by methods such as electrochemical methods, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), and Flame Atomic Absorption Spectrophotometry (FAAS). These methods measure the amount of total gold element in a solution after the chemical digestion of gold, usually by aqua regia or HBr/Br_2 solutions. In 2008, National Institute of Standards and Technology (NIST) released AuNP standards with known gold concentration and average particles diameter of 10, 30, and 60 nm [1]. The gold content was measured by using inductively-coupled plasma optical emission spectrometry (ICP-OES) after digestion. On the other hand, the average diameter of the AuNP was measured by Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Differential Mobility Analysis, Dynamic Light Scattering, and Small-Angle X-ray Scattering. In several recent reports the average diameter, total gold content, and number of AuNP have been characterized in natural water systems and uptake in cell lines. In these reports, a combination of TEM and ICP-MS, ICP-OES, or atomic absorption Spectrophotometry (AAS) was used for the AuNP characterization and determination.

Given the diameter of the AuNP and the total amount (in mole, mol/L, number of gold atoms, or gram) of gold, it seems not very hard to calculate the number of AuNP in a suspension:

$$\text{Number of AuNP} = \frac{\text{(Total number of gold atoms)}}{\text{(Average number of gold atoms in a single AuNP)}}$$

$$= \frac{\text{(Total mass of gold)}}{\text{(Total mass of gold/Average mass of single AuNP)}}$$

Absolute monodisperse AuNP suspension does not exist. The diameter of the AuNPs in a synthesized suspension varies and usually follows a discrete log-normal distribution. Therefore, the diameter of the AuNP that has been used for the calculations refers to the mean value of the AuNPs in a suspension. The mean value is usually obtained by measuring the diameters of tens or hundreds AuNPs in their TEM image. The mean diameter was calculated by the individual diameters of measured AuNPs. Considering that there are many different statistical ways to calculate the mean value of a set of data, an accurate statistical mean should be used to calculate the number of AuNPs in a suspension and a nominal value that can be used to describe the size of AuNPs.

In this statistical discussion, AuNP population refers to the number of all the particles. Population equals to N means there are

N nanoparticle in a suspension. Given a suspension of AuNP has population of N; each individual AuNP can be identified as P_i, *where i* = 1 ~ N. Assume all the AuNPs are geometrically spherical. Particle P_i has a radii of r_i.

The arithmetic average, or the numerical average, radius of the AuNPs, $\bar{r}$ should be:

$$\bar{r} = \frac{\sum_{i=1}^{N} r_i}{N} \tag{1}$$

In this case, all the AuNPs have the same weight contributed to the average. The weight is assigned to be unity.

When the weight of AuNP P_i equals to the radius (r_i) of the AuNP, the weighted average can be called radius average, which can be calculated as:

$$\bar{r}_r = \frac{\sum_{i=1}^{N} r_i \cdot r_i}{\sum_{i=1}^{N} r_i} = \frac{\sum_{i=1}^{N} r_i^2}{\sum_{i=1}^{N} r_i} \tag{2}$$

When the weight of AuNP P_i equals to the cross-section area (πr_i^2) of the AuNP, the weighted average can be called cross-section-area (CSA) average, which can be calculated as:

$$\bar{r}_r = \frac{\sum_{i=1}^{N} r_i \cdot r_i}{\sum_{i=1}^{N} r_i} = \frac{\sum_{i=1}^{N} r_i^2}{\sum_{i=1}^{N} r_i} \tag{3}$$

When the weight of AuNP P_i equals to the surface area ($4\pi r_i^2$) of the AuNP, the weighted average can be called surface-area (SA) average, which can be calculated as:

$$\bar{r}_{CSA} = \frac{\sum_{i=1}^{N} 4\pi r_i^2 \cdot r_i}{\sum_{i=1}^{N} 4\pi r_i^2} = \frac{\sum_{i=1}^{N} r_i^3}{\sum_{i=1}^{N} r_i^2} \tag{4}$$

When the weight of NP P_i equals to the volume ($4/3 \cdot \pi r_i^3$) of the NP, the weighted average can be called volume (V) average, which can be calculated as:

$$\bar{r}_V = \frac{\sum_{i=1}^{N} \frac{4}{3}\pi r_i^3 \cdot r_i}{\sum_{i=1}^{N} \frac{4}{3}\pi r_i^3} = \frac{\sum_{i=1}^{N} r_i^4}{\sum_{i=1}^{N} r_i^3} \tag{5}$$

For example, we have a set of 21 AuNPs. Each of these AuNP has different radius. We assuming r_1 = 4.00 nm, while $r_{i+1} - r_i$ = 0.10 nm. Then r_{21} is 6.00 nm. Following the above equations, the arithmetic and weighted average radius are calculated and summarized in table 1.

*Corresponding author: Lei Yu, Department of Chemistry and Biochemistry, Rowan University, Glassboro, New Jersey, 08028, USA, E-mail: Yu@rowan.edu

Average radius	Value (nm)
$\bar{r} = \dfrac{\sum_{i=1}^{N} r_i}{N}$	5.00
$\bar{r} = \dfrac{\sum_{i=1}^{N} r_i^2}{\sum_{i=1}^{N} r_i}$	5.07
$\overline{r_{CSA}} = \dfrac{\sum_{i=1}^{N} r_i^3}{\sum_{i=1}^{N} r_i^2}$	5.14
$\overline{r_{SA}} = \dfrac{\sum_{i=1}^{N} r_i^3}{\sum_{i=1}^{N} r_i^2}$	5.14
$\overline{r_V} = \dfrac{\sum_{i=1}^{N} r_i^3}{\sum_{i=1}^{N} r_i^3}$	5.21

Table 1: Arithmetic and Weighted average radius.

Weighted mean radii of AuNP should be obtained and used depending on the measurement methods. In these methods, the probabilities of each particle are picked and measured are identical or different. The probability may proportional to the radius, cross-section area, surface area, or volume, which are the weights of the mean. Currently, TEM is the prevailing method of AuNP radius measurement. When TEM is used, the probability of each AuNP is picked and measured is the same either by software or by operator. Therefore, the arithmetic mean values should be obtained by a number average of the measured radii of all AuNPs. However, depend on how these measured radii of AuNPs are used, statistical moment means should be considered when calculating the mean value. The moment mean radius of AuNPs can be calculated by equation:

$$\bar{r}[j,k] = {}^{(j-k)}\!\sqrt{\dfrac{\sum_{i=1}^{N} r_i^j}{\sum_{i=1}^{N} r_i^k}} \tag{6}$$

Therefore, the arithmetic mean, $\bar{r}$, corresponds to $\bar{r}$ [1,0]; while the weighted means $\overline{r_V}, \overline{r_{SA}}, \overline{r_{CSA}}$, and $\overline{r_r}$ correspond to $\bar{r}$ [4,3], $\bar{r}$ [3,2], and $\bar{r}$ [2,1], respectively. With the concept of moment mean, the number of AuNPs can be calculated more accurately. In experiments, the total amount (mass or volume) of gold can be measured by ICP-MS, ICP-OES, electrochemistry, or AAS. The mean radius of AuNPs can be measured by TEM. The number of the AuNP can be calculated by dividing the total volume by the mean volume. For a spherical particle, the volume equals $\frac{4}{3}\pi r^3$. Here, the radius r should not be the arithmetic mean of the radii measured by TEM; rather, it should be the number-volumetric mean of the measured radii. In the context of moment mean, the number-volumetric mean is $\bar{r}$ [3,0]:

$$\bar{r}[3,0] = {}^{3}\!\sqrt{\dfrac{\sum_{i=1}^{N} r_i^3}{\sum_{i=1}^{N} r_i^0}} = \sqrt[3]{\dfrac{\sum_{i=1}^{N} r_i^3}{N}} \tag{7}$$

Therefore, the mean volume of AuNPs should be calculated by the $\bar{r}$ [3,0] value of all the measured AuNP radii, instead of the arithmetic mean of the measured radii.

The size distribution of synthesized AuNPs in dispersions is usually a log-normal distribution that produces a "skewed" distribution curve.

A geometric standard deviation (σ_g) should be calculated and provided. Then the number-volume mean radius can be calculated by the Hatch-Choate equation [2].

$$\bar{r}[3,0] = \bar{r} e^{(3.5 \ln^2 \sigma_g)} \tag{8}$$

In summary, in this essay, different ways to calculate the mean radii of AuNPs from experimental data are discussed. The calculations are based on statistical concepts of arithmetic mean, weighted means, and moment means. Previously, the mean values and the distributions of particles' size have been discussed intensively in statistics and engineering. As the application of AuNP becomes more and more popular, the characterization of the mean size of AuNP and the number of AuNP in the areas of chemistry, biochemistry, physiology, environmental science, and food science will be more and more frequent. We believe these different ways of calculation should be noticed when experimental data are processed.

References

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2. Hatch T, Choate SP (1929) Statistical description of the size properties of non-uniform particulate substances. J Franklin Inst 207: 369-387.