STUDY FOR MINIMIZING MEASUREMENT UNCERTAINTIES OF NANOPARTICLE SIZE USING DYNAMIC LIGHT SCATTERING

B. -J. Park/Presenter, D. Khishigmaa and N. W. Song[*]

Center for Nano-Bio Convergence, Korea Research Institute of Standards and Science, 209 Gajeong-Ro, Yuseong-Gu, Deajeon, 305-340, Rep. of Korea, nwsong@kriss.re.kr

Abstract: This study is aimed to determine proper measurement parameters and procedures for accurate DLS measurement in determining the size of nanoparticles in a solution. We evaluated the effect of dust limit, upper dust limit and accumulation number on the relative repeatability uncertainty of DLS size measurement. Our proposed protocol reduced the relative repeatability uncertainty of size measurement down to 5% for suspension of gold nanoparticle (AuNP).

Keywords: gold, nanoparticle, size, dynamic light scattering, repeatability uncertainty

1. INTRODUCTION

Measuring the size of nanoparticles is currently of great interest in various scientific and industrial applications from material, chemical and colloidal sciences to health, food, electronics and computers. In these applications, recent experience has been revealed the need for a well-defined and accurate method of characterizing nanoparticles in suspension. Among the available experimental methods, there are very few techniques available for the measurement of nanoparticles in solution.[1]

Dynamic light scattering (DLS) is a very effective technique for characterizing the size of colloidal dispersions. DLS utilizes the illumination of a suspension of particles undergoing Brownian motion by a laser beam (ISO 1332).[2] The time-dependent fluctuations in the intensity of scattered light that occur are analyzed using an autocorrelator which determines the autocorrelation function of the signal. The autocorrelated intensity signal can provide information about the size of suspension of particles in the range from a few nanometers to several micrometers. [1]

Primary problem in DLS measurements is the existence of dusts (> 1 μm) especially in highly polar liquids such as water. Dust is a general name for any large scatterer that contributes to the light signal. The presence of undesired dust may seriously bias the result of nanoparticle size measurement. For example, a particle, which is ten times larger than the other one in size, scatters a million times more photons. Therefore it is generally expected that a few dusts will surpass nanoparticles in the scattered light intensity. The scattered light from dusts results in completely erroneous correlation function and therefore incorrect size analysis of nanoparticles in solutions.[2]

Although filtration or centrifugation can be used to minimize dusts in liquid samples, they are only available for limited types of liquids and frequently alter the characteristics of dispersed particles in liquids. For this reason, most of DLS instruments allow '*Dust Limit*' and '*Upper Dust Limit*' to reduce the effect of dusts in scattered light signals. The dust limit is the number of times to get the average strength for setting the dust limit. No dust limit will be set if this value is "0". The upper dust limit sets the maximal intensity of the scattered light, which will be accumulated as valid data, relative to the average scattered intensity obtained prior to the measurement. For example, if the upper dust limit is 10%, any scattered signal higher than 110% of the average intensity would be dismissed as invalid data.[3]

There is no doubt that the dust limit and upper dust limit might play important roles in determining accuracy and reliability in size measurements of nanoparicles in suspensions using DLS technique. With too low number for dust limit, the average scattered light intensity will not be able to represent the strength of real light signal from the nanoparticle suspension. On the other hand, too high value of dust limit allows inclusion of the contribution from dusts during the measurement. Similarly, the improper upper dust limit might result in under- or over-estimation of DLS size of nanoparticles.

In this study, AuNP suspensions were employed to investigate a practical protocol to determine the proper parameters including dust limit, upper dust limit and accumulation times of integration cycles for the measurement. Relative value of repeatability uncertainty to the average value of measurement was used to evaluate the effect of change in each parameter on the accuracy of DLS measurement. This paper suggests proper measurement parameters and procedures minimizing the dust effect to ensure the accurate DLS measurement to determine the size of nanoparticles in solutions.

2. EXPERIMENT

Sample preparation: The sample for the DLS measurement was citrate-stabilized AuNPs with diameter of 15 nm (TEM size) and concentration of 35 μg/ml. The

AuNP is widely used in chemical, biological, engineering, and medical applications and therefore added to one of manufactured nanomatierials extensively studied at the OECD WPMN. Prior to sample loading, potential dusts at disposable cuvette (or cell) were blown off inside and outside of the cells using nitrogen gas. The sample cell containing the AuNP suspension was degassed using negative pressure oil free pump to remove micro bubbles. The sample cells were then sealed with a parafilm to avoid dust contaminations.

Measurement method: The DLS apparatus was a commercial particle analyzer (ELS-Z, Photal Otsuka Electronics, Japan) with an input laser wavelength of 633 nm, scattering angle of 165° and 50 μm pinhole. Before the DLS size analysis, the instrument was pre-warmed for 30 min and all measurements in this study were performed at the ambient temperature (~ 25°C). The scattered intensity from the sample was automatically adjusted to be within 10 to 50 kcps (kilocounts per second) by controlling the input intensity of the laser. During the measurement, the scattered light intensity was collected as a data train of sampling time between two photon pulses. The autocorrelation function is then calculated for a specified number (channel number) of multiples of sampling times. The process is repeated for a number of times (accumulation times) and the autocorrelation function is summed to perform cumulant particle size analysis using the instrument software. In order to estimate the uncertainties of size measurements, the dust limit, upper dust limit, and accumulation numbers were varied in the range of 2 ~ 10, 0 ~ 100%, and 5 ~ 100, respectively. For comparison, the relative repeatability uncertainty (U_r) was obtained from the repeatability uncertainty (U_c) and average value of measurement according to Eqns. (1) and (2). [4]

$$U_c(\bar{x}) = k \cdot \frac{\sigma_s}{\sqrt{N}} \qquad (1)$$

$$U_r(\bar{x}) = \frac{U_c(\bar{x})}{\bar{x}} \qquad (2)$$

Where $\bar{x}$ is the average DLS size, σ_s is the standard deviation of DLS sizes from N- repeated measurements and k is the coverage factor for a given number of repeated measurements.

3. RESULTS AND DISCUSSION

Dust limit: As explained above, the dust limit is the first and critical parameter in DLS measurement to determine the average scattered intensity of particle suspension. Improper dust limit can result in the erroneous DLS size with high uncertainties.

In this study, we tested the dust limits from 2 to 10 in DLS measurement on AuNP suspension. The scattered light intensities were recorded five times to obtain the average scattered light intensities and repeatability uncertainties of measurements as a function of dust limits (N) at the confidential interval (CI) of 95% (Fig 1a). The average scattered light intensities were similar to each other irrespective of the dust limits, while relative repeatability
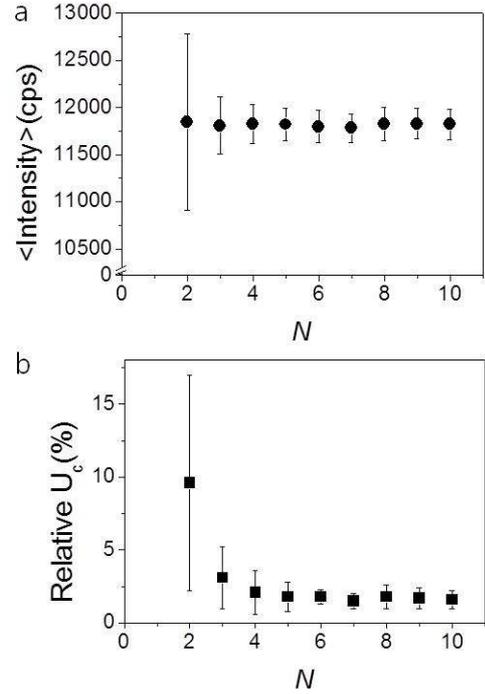

Figure 1. The average scattered light and relative uncertainty of measurements as a function of the dust limit. (a) the average scattered light intensity and uncertainty of measurements at the IC of 95%, (b) the relative uncertainty and standard deviation from three independent measurements at the CI of 95%.

uncertainties to average intensities decreased with the increase of dust limits. The relative uncertainty reduced below 5% when dust limit was higher than 4. According to the Eqn (1), it is reasonable to observe the decreased relative uncertainty with higher values of dust limit because dust limit is equivalent to the number of times to get the average intensity of scattered light.[3]

Therefore, we strictly selected dust limit as 7 for further studies since dust limit plays a very important role in determining the size of nanoparticles using DLS technique.

Upper dust limit: It is well known that the dusts in a sample solution lead to inaccurate size measurements by sudden upshifts of the autocorrelation functions in DLS experiments.[5] A complete removal of dust from nanoparticle suspension generally requires extreme efforts or sometimes impossible.[2, 5] To avoid the dust effects in the DLS measurement, upper dust limit can be used to discriminate the signals from dusts by shutting off the calculation of the correlation function when the scattered intensity exceeds a prescribed level. The critical point is setting the discrimination level since too high upper dust limit allows the inclusion of dust signals while too low value can remove the fluctuating signals from the particles of interest.

In this study, we therefore measured the DLS size of AuNP in suspensions with varying the upper dust limit from 5% to 100%. The accumulation number was 15 and 5 replicate measurements were performed. Here, the lower dust limit of 100% was used to incorporate all signals from the particles. Fig 2a shows the relative repeatability
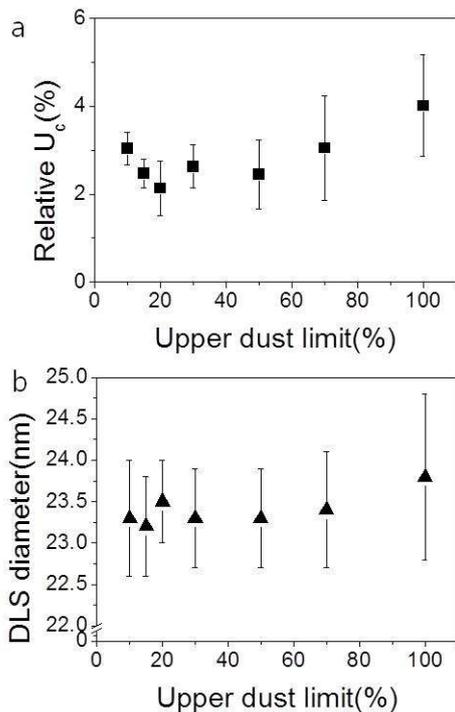
Figure 2. The relative uncertainty of measurements and DLS sizes as a function of the upper dust limit. (a) the average and standard deviation of relative repeatability uncertainties at CI of 95% from three independent measurements, (b) the average nanoparticle sizes and their repeatability uncertainties at CI 95%.

uncertainties of DLS sizes measured as a function of the upper dust limit at the CI of 95%. The relative uncertainties were obtained from three independent measurements. This result indicated that the relative repeatability uncertainty could be reduced below 5% with 70% of or less dust limits and further reduced below 2.1% (lowest) at the 20% of the dust limit. As described above, it was shown that too low or high values of upper dust limit could result in the erroneous DLS size. Fig 2b indicates that the result of DLS size measurement was almost similar ($p < 0.05$) irrespective of the upper dust limit. Therefore, we selected the upper dust limit as 20% for further studies.

Accumulation number: In order to obtain the DLS size of particles, the autocorrelation functions are fit using the cummulant method as defined by ISO 1332:1996(E).[2] The cummulant method is a useful technique for the analysis of scattered signals from polydisperse samples, which gives a polynomial expression for accumulated autocorrelation functions. The accumulation times or number of accumulations are equivalent to the integration cycles of autocorrelation functions and therefore very critical to obtain the accurate DLS size of nanoparticles.[3] Although there are some guidelines recommending 100 times for accumulation or 6 accumulations out of 12 measurements, the basis of determining the number of accumulation is still unclear.

In this study, we therefore measured the DLS size of AuNPs in suspensions to evaluate the effect of accumulation number on the measurement uncertainties. Accumulation number was varied from 5 to 100 at the dust limit of 7,
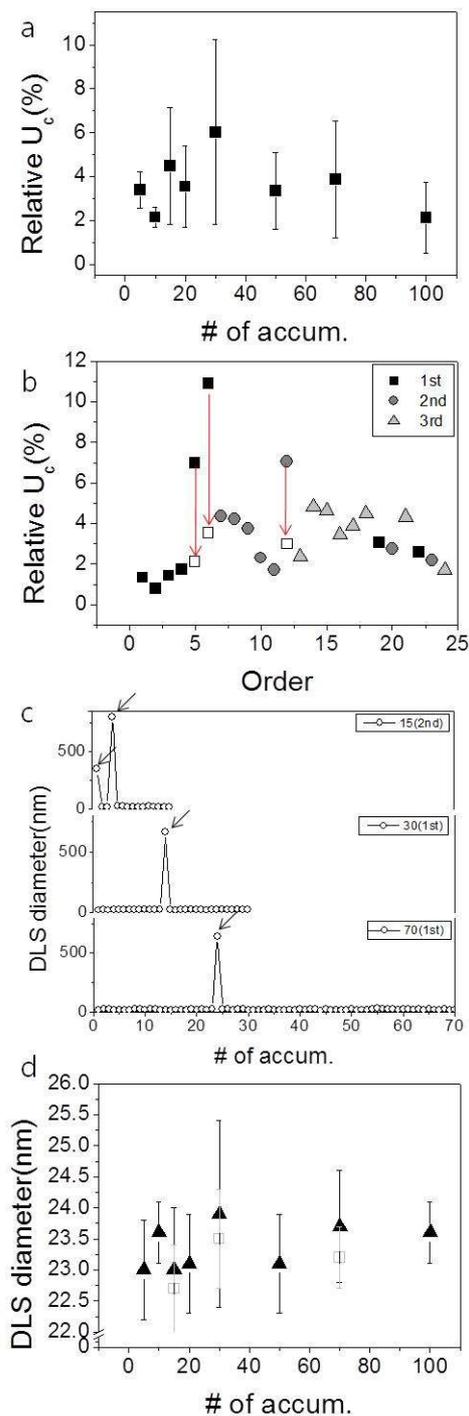

Figure 3. The repeatability uncertainty of measurements and DLS sizes as a function of accumulation number. (a) the average and standard deviations of relative repeatability uncertainties from three independent measurements at the CI of 95%, (b) the average and standard deviations of relative repeatability uncertainties as a function of measurement sequence (c) the real time DLS sizes at the number of accumulations of 15 (2nd), 30 (1st) and 50 (1st); dusts are indicated by arrows, (d) the average and standard deviation of DLS size as a function of accumulation number.

upper dust limit of 20%, lower dust limit of 100% and repetition of 5. Fig 3a and 3b shows the average and standard deviations of repeatability uncertainties in DLS size measurement as a function of accumulation number and

measurement sequence at the CI of 95%. The relative repeatability uncertainties were obtained from three independent replicate measurements. From the results, we were not able to find a certain trend or relationship between the relative uncertainty and the accumulation times, while relatively high relative uncertainties were found at the accumulation numbers of 15 (4.5%), 30 (5.2%) and 70 (4.0%), of which only 1st, 2nd and 1st measurements interestingly showed the highest relative uncertainties in Fig 3b.

The real time DLS size measurements further indicated that the top three relative uncertainties were caused by dusts (indicated as arrows in Fig 3c), and it was clear that the dust contaminated autocorrelations were not compensated even at accumulation number of 70. To confirm the effect of dusts at the relative uncertainties, we excluded the dust contaminated DLS measurements (similar to dust rejection filter) instead of compensating the autocorrelation functions since high number of accumulations might include additional dust contaminations. Once corrected with new DLS measurements, the top three relative uncertainties decreased from 4.5% to 3.1%, 6.0% to 3.6% and 3.9% to 2.3%, respectively, at the 15, 30 and 70 accumulation numbers (indicated by blank squares in Fig 3b) without significant changes of DLS sizes (Fig 3c). This further indicated that lower number of accumulations might be useful at the above method in terms of minimized measurement time with relatively low uncertainties.

Based on our results, we suggested following practical protocol for effective and reliable DLS measurement of suspension of nanoparticles. (1) dust limit of 7, (2) upper dust limit of 20%, (3) lower dust limit of 100%, (4) accumulation numbers of 5, (5) repetition of 12 DLS measurements, 6 highest DLS sizes were removed as a dust rejection filter, yielding a total measurement duration of analysis typically of 60 s. This practical protocol was validated by measuring the DLS sizes of well dispersed 15 nm AuNPs in solution (BBI international, England). The averaged DLS size of AuNPs was 18.2 nm ± 0.3 nm (uncertainty of measurement at the CI of 95%), which was close to the nominal 15 nm of AuNP. On the contrary, use of incomplete protocol (excluding step 5) showed 34.8 nm ± 21.4 nm that is significantly different and inaccurate results compared to the use of full protocol. Now it is very clear that our proposed protocol is relatively fast and effective on size measurement of nanoparticle suspension.

4. CONCLUSIONS

This paper suggested following proper measurement parameters and procedures excluding the effect of dusts and thus enhancing the reliability and efficiency of DLS measurement in determining the size of nanoparticles in a solution. The relative uncertainty of measurements reduced down to 5% at the CI of 95% by followings:

First, the dust limit of 7 was recommended while ≥ 4 might work well.

Second, the upper dust limit of $\leq 70\%$ was recommended while the minimum value of relative repeatability uncertainty was obtained at 20%.

Third, correction of the dust contaminated measurements with new DLS measurements was recommended for size evaluation of nanoparticle suspensions, which can lead relative uncertainty of measurements down to 5%.

Fourth, we highly recommend accumulation number of 5 with repetition of 12 DLS measurements to reject dust by removing 6 highest DLS sizes from measurements.[6]

5. REFERENCES

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