

Remarks on the Particle-Size Determination of NanoAmando by Dynamic Light Scattering

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Abstract. A polydisperse system containing minor but larger components, as in the case of single-nano diamond particles accompanying small amounts of spontaneous aggregates, often shows apparent but misleading concentration dependence of particle-size distribution when subjected to dynamic light scattering (DLS) analysis. An empirical countermeasure is suggested.



Background

Among a number of methods capable of sizing nanoparticles, quasi-elastic light scattering (QELS), or dynamic light scattering (DLS), is by far the most convenient to obtain the first-line information on the dispersion and aggregation of nanoparticles [1]. According to DLS analysis, our NanoAmando[®] consists of a dominant and narrow peak corresponding to single-nano diamond particles (4.7 ± 0.7 nm), and a small but wide centered at 50-60 nm. The weight-based ratio of the two components used to be 8 : 2 to 9 : 1 but recently decreased to as good as 98 : 2. Although the DLS method of determining the size of nanoparticles has been praised to be so accurate that it is used to determine the sizes of standard particles [1b], the above statements on the particle-size distribution of NanoAmando were obtained only after hard work. We believe that the presently commercialized DLS equipments do not warrant reliable results unless due attention is paid to every details including sample preparations and data analysis. This small report proposes a practical strategy to secure acceptable results, which is to find an optimum concentration range for every sample. It is strongly recommended to adopt the strategy when a minor constituent has larger size than the major one.

Let us begin with the general situation of DLS determination of particle-size distribution, which can be best described as the state of art. Anyone who begins using DLS for nanoparticles will soon encounter with a series of problems. The most serious of these are as follows.

- (1) At the moment no standard colloid sample is commercially available to calibrate the small nano-range, especially the most interesting single-nano range. This problem has not much to do with the instrumental defects, but arose simply by the lack of suitable substance qualified for standard nano-colloid regarding stability.
- (2) Results of DLS determination generally scatter widely and often irreproducible and neither

the reasons nor countermeasures are given in the manuals of DLS instruments.

- (3) Not only the poor reproducibility, but also the reliability is often questioned. Descriptions on the choice of appropriate concentration range, length of repetition, and the recommended handling of data, are not clearly given.

Such a frustrating situation certainly reflects the developing state of art in the DLS analysis of nanoparticles. Nonetheless, here we would like to give answers to all three critical questions mentioned above. Answers are not yet perfect and well-grounded, but rather practical and based on experience. For the first problem on the lack of standard sample, we will shortly make our dispersed particles of single-nano diamond available as the standard with its size determined by statistical treatments of TEM images of dispersed nanodiamond.

The second problem of poor reproducibility was resolved by increasing the number of determination. We recommend the following procedure. For each sample, 3-minute continuous measurement session is repeated 5 times after 20 seconds of interval between two consecutive sessions. Within each session, 88 measurements are repeated. Thus, in all 440 measurements are performed for each determination. Averaged set of the results from each session is treated as independent; hence we have five independent averages. We will come back later to the reproducibility of the set of five averages.

About the third problem, we have previously experienced significant variation in the DLS results taken in different concentrations of the same sample. Sometime ago we intentionally measured particle size of a sample in widely different concentrations and obtained very different results. However, the results on the middle concentration range gave reasonable results. Hence we thought there may be an optimum concentration range and decided to perform some systematic study on the concentration dependence of DLS results.

Before moving into the major body of the report, we mention about the pretreatments of samples. Dispersion of low-nano particles is sensitive to various processes of preparing its colloidal solution including centrifugal separation, dilution, concentration, filtration and even storage and transportation to induce weak aggregation. In general such aggregates can be readily dissolved by supersonic treatments. Hence we recommend to immersing all the samples in supersonic washing bath for certain period of time before submitting them to DLS determination (see Experimental).

Experimental

1. General

Freshly distilled water was used throughout this report as the solvent or, to be stricter in wording, dispersing medium. Dispersed single-nano diamond particles were manufactured at Nippon Kayaku Company, Ltd. in her pilot plant of nanodiamond [2], and coded BCE211NK001P26. Detailed specification of this sample is omitted here because the test production is still not in the

final stage, thus should be regarded as temporary. After receiving the sample, we subjected it to filtration through a nylon membrane-filter capsule POLYCAP 75 AS with 0.45 μm pore size from Whatman Co., to centrifugation in a Tabletop Plate-Spin Centrifuge Type 5200 with a maximum capacity of 1L and 3,000 rpm-speed, or in a Micro Refrigerated Centrifuge Type 3700 with a maximum capacity of 50 mL and 15,000 rpm-speed, both manufactured by Kubota Corporation, and to high-energy sonication with an Ultrasonic Processor UP-400S (400W, 24kHz) equipped with sonotrode H3 or H22 manufactured by Hielscher Ultrasonics Co., Tiltow, Germany, then to concentration on a rotary evaporator to obtain a diaphanous, black and viscous 9.06 wt% aqueous colloidal stock solution of single-nano diamond particles.

2. DLS Measurements in a Particle-Size Analyzer for Concentrated Solutions with Automatic Sampler

A portion of the stock solution prepared above was diluted to 4.53 wt% to use as mother liquor in the present study. Nine 2-ml sample solutions were prepared out of the mother liquor at concentration intervals of 0.5 wt% down to the lowest concentration of 0.57 wt% by using a Eppendorf pipette (precision 5 μl) and diluting with water. Each sample solution was placed in a 2.5 ml straight scent tube, type STS-2 from MaruEmu Co., Osaka, Japan, and closed tightly with a polyethylene cap, and immersed in a ultrasound cleaning bath, type MK-II, 24 and 31kHz, 110W, manufactured by Honda Electronics Co., or in a Ultrasonic Cleaner Type T490DH, 40 kHz, 360W, manufactured by Elmar, Singen, Germany, for 99 min for final re-dispersion. DLS measurements were carried out on a Particle Analyzer FPAR1000 equipped with an automatic sampler FP3000 manufactured by Ohtsuka Electronics Co.

3. DLS Measurements in a Particles-Size Analyzer for Diluted Solutions under Batch Operation

Dilute samples having 1.0 to 0.01 wt% concentrations were prepared in a similar way as mentioned above and subjected to batch DLS measurements on a Particle Analyzer FPAR1000.

Results

Particle-size distributions thus obtained for 16 samples in different concentrations are given in Table 1.

Table 1. Effects of Concentration Change on the Particle-Size Distribution

Options of DLS	No.	Conc. wt%	First group, nm (abund in wt % ^a)	Second group, nm (abund in wt % ^a)	Judgements
Autosampler	1	4.53	6.3 \pm 0.9 (11.6)	102.4 \pm 66.4 (88.4)	×

(fiber probe for conc. soln used)	2	3.96	7.3±1.1 (81.7)	125.3±57.6 (17.8)	×
	3	3.40	4.9±0.8 (79.5)	75.8±36.7 (20.5)	×
	4	2.83	5.4±0.9 (98.8)	71.6±30.9 (1.2)	⊙
	5	2.27	5.3±0.0 (95.9)	66.2±26.3 (4.1)	⊙
	6	1.70	4.6±0.9 (99.4)	56.2±21.7 (0.6)	⊙
	7	1.13	2.7±0.4 (99.8)	38.0±12.0 (0.2)	⊙
	8	0.85	6.1±1.2 (98.4)	43.9±16.5 (1.6)	○
	9	0.57	12.4±1.7 (99.9)	172.7±16.7 (0.1)	×
	Batch operation (fiber probe for dilute soln used)	10	1.0	4.5±0.7 (99.5)	57.3±21.5 (0.5)
11		0.75	5.4±1.0 (99.2)	50.8±20.2 (0.8)	⊙
12		0.50	8.5±1.9 (96.8)	45.1±18.2 (3.2)	○
13		0.25	6.1±1.1 (97.1)	36.8±14.1 (2.9)	○
14		0.20	-	31.3±13.8 (99.7)	×
15		0.10	-	29.8±14.3 (99.9)	×
16		0.075 ~0.01	immeasurable	immeasurable	×

^aRelative abundance of the peak group in weight%.

NanoAmando colloids generally contain a maximum of three particle-size groups:

The first group: average size smaller than 10 nm, distribution range narrower than 1 nm, and the weight-based population higher than 95%.

The second group: average size 30 to 80 nm, normal distribution over a few tens of nm, and population less than 10%. The origin of this peak group is still unconfirmed but the most likely explanation seems to be dynamic or spontaneous aggregates of the primary particles.

The third peaks group: average size a few hundred nm to a few μm . Supposed to represent uncracked agglomerates, population generally less than 0.1%. Very often remains unobserved.

The third group is insignificant and rarely appears, hence was omitted from Table 1. Each entry in Table 1 represents a set having the middle value in terms of the average of the first group among those of the five measurement sessions as mentioned in Background. More will be discussed below on the validity of such a selection.

A glance at Table 1 immediately reveals surprising tendency that both average particle-size and weight-based abundance are significantly affected by concentration change in the samples. As we have carefully removed weak aggregates immediately before the DLS measurements, it is likely that the diversity of particles-sizes is artifacts. Closer look at Table 1 suggests some correlation in the movements between the peaks; whenever the first group shifts towards larger size, the second group

shifts to the same direction while greatly increasing the population relative to the first group. This correlation seems to reflect the effect from the well-known increase in the scattering intensity with increase in the particle-size. The artifact appears itself more clearly when available scattering light intensity became insufficient for low concentrations below 0.2%, or too much for concentrations above 3%. For example, Table 2 shows highly diverse results of five separate measurement sessions on sample No. 2 having a too-high concentration of 3.96%.

Table 2. Averaged particle-size distributions obtained in five separate measurement sessions on sample No. 2

Order of measurement	First group, nm (pop in wt %)	Second group, nm (pop in wt%)	Third group, nm (pop in wt%)
1	7.3±1.1 (81.7)	125.3±57.6 (17.8)	2,806±410 (0.5)
2	10.7±1.6 (61.4)	119.7±58.2 (38.6)	—
3 ^a	5.1±0.7 (94.9)	25.2±2.2 (0.4)	2,811±413 (0.2)
4 ^b	7.6±1.3 (86.2)	116.3±52.7 (13.7)	2,784±403 (0.3)
5	6.4±1.2 (88.2)	88.1±55.8 (11.8)	—

^aAdditional peak at 145.2±57.9 nm (4.5 wt%).

^bAdditional peak at 1235±145 nm (<0.1 wt%).

Between the high and low concentration ranges, where apparent artifact results appeared as mentioned above, we found certain range wherein constant and stable results are obtained regarding both average size and population (blue ink in Table 1). In these concentrations, not only the middle set but also the other sets from a contiguous measurement session display excellent reproducibility. A typical example of Sample No. 6 (1.70%) is given in Tab. 3, which demonstrate almost perfect reproducibility. High reproducibility, or more correctly uniformity, among the separate measurement sessions seems to provide a good criterion for the correct concentration range. By the same token Table 2 represents a case of poor uniformity among the sessions, and it is quite difficult to choose representative values. Thus poor uniformity indicates a wrong concentration. The results for concentrations below 0.2% by the batch measurements using a fiber for dilute solution are too poor (Table 1), indicating there exists even ‘forbidden’ concentration range.

Table 3. Five consecutive intermediate particle-size distributions obtained for sample No. 6

Order of measurement	First peaks group, nm (pop in wt %)	Second peaks group, nm (pop in wt %)	Third peaks group, nm (pop in wt %)
1	4.6±0.9 (99.4)	56.2±21.7 (0.6)	—

2	5.1±0.8 (99.2)	56.6±23.1 (0.8)	—
3 ^a	4.4±0.7 (99.2)	53.3±23.3 (0.8)	—
4 ^b	6.3±1.4 (98.0)	57.3±23.0 (2.0)	—
5	4.4±0.7 (99.4)	54.8±20.9 (0.6)	—

At this point, two unexpected but remarkable conclusions are reached:

- (1) If we could identify a ‘correct’ concentration range for a sample of single-nano nanodiamond, quite uniform and reproducible results are obtained by DLS measurements. If the high uniformity in the large number of measurements can be taken as the assurance for correct measurements, then we can overcome the third problem of unreliability posted in Background.
- (2) The correct concentration range is surprisingly narrow. In the present case, 1.0~2.5 wt% when using fiber for concentrated colloid, and 0.5~1.0 wt% when using fiber for diluted colloid.

Although we used the word ‘correct concentration range’ above, we wish to confirm if the identified range is really right or not, namely the size of the first group obtained here agrees with the true value. In the absence of standard sample of NanoAmando, it is desirable to determine the particle-size distribution by different methods and compare the results. In 1997, Vul’ and his group determined the coherent scattering region of detonation nanodiamond produced in Russia by small angle X-ray scattering method and obtained a value of 4.5±0.5 nm [3]. We received their sample, dispersed by our beads-milling method, took a TEM picture of the dispersed product and visually determined the average size with the aid of a particle-size distribution analysis software Mac-View, version 3.5, from Mountech Co., Tokyo, by manually picking up 300 images of primary particles from the photograph. The result of this graphical analysis was 4.8±0.8 nm. These two distributions agreed with simple average of six data points in the correct concentration range for the first group given in Table 1 (blue ink), 4.6±0.8 nm with population of 99.3 wt%, within error range. Hence we may assume the following:

- (1) The size of primary particles or coherent scattering region of detonation nanodiamond is always constant, irrespective of the country of production. This means that detonation and diamond growth processes are uncontrollable as long as the same bursting explosive (Composition B) is used.
- (2) Our beads-milling method of destroying covalent aggregation among the primary particles works effectively and gives dispersed primary particles as the predominant product. This means that our beads-milling condition was adequate. Actually our present conditions have been chosen as the results of elaborate multi-variant optimization

technique [4].

- (3) The DLS Particle Analyzers used in the present work give correct particle-size distribution if the colloidal concentration was in the correct range. This means that our Analyzer has been well calibrated by the manufacturer.

Discussion

It is clear that the instability of DLS results for our NanoAmando is caused by the presence second distribution peaks in 50-60 nm range. As the Particle Analyzer we used adopts He-Ne laser of 632.8 nm as the scattering light source,

The enigmatic dependence of particle-size upon concentration arose, at least in the colloidal solution of our single-nano diamond particles, from the presence of the second distribution with larger particle-size around 57 nm, which produced much stronger scattering of incident probing laser than the first distribution at 4.6 nm, and obstructed us from evaluating the particle-size of the latter in a straightforward manner. Although the nature of this second peak at 57 nm is still to be confirmed, at the moment we think it likely that this peak group represents spontaneous formation of primary *van der Waals* aggregates. It is reasonable to expect aggregation-prone single-nano particles often coexist with small amounts of their light aggregates. Then we may conclude that DLS measurements of single-nano particles should be generally concentration-dependent, and the precautions suggested above should be carefully observed.

“We are still unable to prove these assumptions, hence recommend repeating the determination of particle-size distribution over a wide concentration range to find a ‘correct’ answer. In the correct concentration range, it is likely that the second group is correctly determined as well. Thus, the most reliable size distribution is obtained so far for the detonation nanodiamond is 56.7 nm with average population of 1.2%.”

For the moment, we will have to repeat DLS measurements over considerably wide range of concentrations for one sample. In order to obtain one particle-size distribution data, DLS analysis may have to be carried out a few thousand times which will require a few hours. It is advisable to use automatic sampler option and run the unmanned determination at night. It would be practically impossible to determine correct concentration range by batch operation. Hence we cannot handle dilute solutions and may have to concentrate them up to higher than 1% solutions before determining particle-size distributions. It is necessary to control re-aggregation during the concentrating operation.

We strongly suggest describing concentration of the sample whenever reporting the DLS particle-size distributions.

Summary

Recommended concentration ranges of single-nano diamond aqueous colloids for judicious determination of their particle-size determination

Option	Correct concentration range
Autosampler equipped with a fiber for concentrated solutions	1.0~2.5
Batch operation using a fiber for dilute solutions	0.5~1.0

Acknowledgments

We thank Mr. Sachio Inaba, Nippon Vilene Co. for assistance in graphic analysis of TEM images for particle-size distribution, and Mr. Makoto Takahashi, Nippon Kayaku Co. for many technical advices.

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光量次第で測定時間を延長すればよい、ということがマニュアルに書いてある。また多分散指数の使い方、解析方法の検討なども行う必要あり。サンプルを変えて（日本化薬はまずい）測定をやり直すこと。平成20年3月21日(金)