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NANOPARTICLE SIZE MEASUREMENT BY NANOPARTICLE TRACKING ANALYSIS (NTA) METHOD

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Abstract: Nanoparticle Tracking Analysis (NTA) is a newly developed method, and in the measure process, there are several factors and parameters which have significant effect on the accuracy of final mean particle size result. In this paper, we analysis the effect of sample concentration, camera parameters (shutter, gain and duration) and analysis parameter (threshold) on the obtained NTA results. Based on the work, appropriate methods about sample preparation and parameters settings in video capturing and analysis process are developed by measuring a polystyrene sample with nominal value of 80nm.

Keywords: Nanoparticle Tracking Analysis (NTA), Particle size, Measurement, Parameters.

1. INTRODUCTION

Nanoparticle Tracking Analysis (NTA) is a newly developed method in the field of particle sizing, which is available since 2006[1]. Generally, NTA method can measure particle size from 30nm to 1000nm[2]. Comparing with DLS method, NTA method is also based on Brownian motion of particles in liquid, but it is fitted with a unique laser-illuminated microscope and a CCD camera. By using a dedicated particle tracking image analysis programme, Brownian motion of particles can be analyzed in real-time, each particle being simultaneously but separately visualized and tracked. Because of this advantage of NTA method, it is being increasingly applied in a wide range of different applications in recent years and is now being used in over 150 laboratories worldwide[1].

In NTA method, there are some factors and parameters which can seriously affect the accuracy of final particle size result, for example, sample concentration, camera parameters and analysis parameters setting, therefore how to set and select those parameters seems much important for obtaining accurate and reliable results. In this paper, we develop methods on sample preparation and parameters setting in video capturing and analysis process, by measuring a polystyrene sample with nominal value of 80nm. Meanwhile, two kinds of techniques are utilized to verify the reliability of developed methods. The first verification technique is to use particles size reference materials (RMs), whose values are well determined by manufacturers. And the second verification technique is to use SEM method, which is a common and accurate technology in particle size measurement, and has been widely used in the determination of particle size Certified Reference Materials (CRMs) by National Metrology Institute (NMI) in many countries.

2. MATERIALS AND INSTRUMENTS

Polystyrene particle sample with nominal value 80nm was synthesized by emulsion polymerization, and SEM and TEM images of obtained samples are showing in Figure 1.

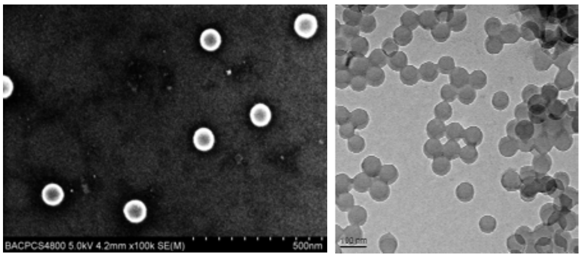

Figure 1 SEM and TEM images of sample

The zeta potentials of sample were measured by Zetasizer Nano ZS instrument, which can indicate whether the particles within a liquid will tend to flocculate (stick together) or not. In our zeta potential measurement, bulk solutions of nominal 80nm samples were measured. All the samples were measured in dip cell, and the general purpose mode is selected in data processing dialogue. Determination result of mean zeta potential for 80nm sample is -45.9mV.

Particle size measurements by NTA method were performed with a NanoSight LM20 (NanoSight, Amesbury, United Kingdom), equipped with a 640nm laser and with a sample chamber, which is approximately 250μL in volume. Before measurement, the sample was diluted using 10mmol/L NaCl solution, and then was injected in the sample chamber with sterile syringes until the liquid reached the tip of the nozzle. All measurements were performed at controlled room temperature of (21±0.5) °C.

In SEM measurement, the samples were characterized by Hitachi 3500 SEM operating 5.0kV, and Buler image analysis software 86-3000. Before measurement, the samples were diluted with amount of ultrapure water, and then one droplet (about 20μL) of the suspension was placed on a silicon surface, dried in room temperature. SEM magnifications were calibrated by using micrometer standard with nominal value of 400nm. The line width of micrometer standard is certified by National Institute of Metrology China with uncertainty of 0.2nm. The area and area-equivalent diameter of each particle are measured by image analysis system, and number mean particle size and particle size relative standard deviation can be calculated. In sample measurement, at least 1000 particles were analyzed for obtaining its mean particle size.

3. RESULTS OF NTA METHOD AND DISCUSSION

3.1 Sample concentration For a statistically significant number of particles to be present on the beam, sample concentrations should lie in an optimal concentration. In low concentration, because of fewer particles might being captured, the obtained mean particle size of each measurement may easily be effected and fluctuated. As showing in table 1, standard deviation of measurement results of sample 6 is much larger than the others. Accordingly the obtained results will not be reliable in low concentration sample. But too high concentration also brings some problems in video analysis. First of all, high sample concentration can suffer from a large amount of background noise and the diffraction patterns can clearly be seen around a number of particles, which can increase the risk of false centre appearing in video analysis. Secondly and importantly, in the captured video, small particle's tracks will more easy be affected and terminated by the neighbour particles -- due to the increased cross over rate of the particles, and the lifetimes of particle trajectories will get shorter, even get less than the setting value of min track length, thus those small particle tracks might be missed and will not be include in mean diameter calculation, and accordingly cause the increase of mean particle size (sample 1 and 2 as showing in table 2). So from the results and analysis, the optimal concentration of 80nm sample for NTA measurement is in the range of (3.5~7.0) $\times 10^8$ particles/mL.

Table 1 Obtained particle size with different sample concentration

Sample number	concentration /particles·mL^{-1}	Mean particle size /nm	Standard deviation of measurement results /%
1	14.11×10^8	86.9	1.44
2	11.82×10^8	85.1	1.51
3	7.00×10^8	83.1	1.17
4	5.51×10^8	83.2	0.95
5	3.45×10^8	82.6	1.63
6	2.83×10^8	84.6	1.86

3.2 Parameters selecting and setting In NTA method, camera parameters setting (shutter, gain and duration) and analysis parameters setting (mainly is threshold) can cause a significant effect on measurement results.

Camera gain can increase the numerical difference between the particle intensity and that of the noise. Generally, the gain should be set high to allow the shutter to be reduced to a minimum in order to reduce particle blurring[3]. In Nanosight software, the maximum value for gain is 680. With this value, there are no obviously increased background noise and diffraction patterns around particles, and this indicates that the selected gain value is optimal for nominal 80nm sample measurement.

Camera shutter determines the length of time the shutter is open for and therefore how much light is captured from the particles[3]. For increasing the accuracy, the images should be captured using the lowest shutter speed compatible with visualizing the particles[3]. But if it is too lower (in the range of 300~700 as showing in table 2), some clearly visible dim particles (associated with small particles or particles with a low refractive index) can not be captured and therefore missed in mean particle size calculating, so the obtained particle size might be larger than the true value. While if it is too higher and reach to 1400, an over exposed video was obtained, in which the centroid of intensity profile for some particle can not be founded accurately by Nanosight software, and accordingly the risk of obtaining incorrect results might increase. For 80nm sample, when the camera shutter is set in 800~1000, a good capturing video can be obtained.

Table 2 Obtained particle size with different camera shutter

number	a	b	c	d	e	f
Camera shutter	300	500	700	800	1000	1400
Mean particle size/nm	90	90	89	85	83	84

Camera gain: 680, Capture duration: 20s

Camera duration determines the length of video which is captured and longer videos allow more accurate particle distributions to be produced. Generally, when the camera duration increased, more tracking of particles will be captured, but long camera duration is not necessary and recommended. Because the same particle may appear in the capture screen many times and reanalyzed by Nanosight software. For get optimal camera duration in capturing 80nm particle image, batch capture is used with different camera duration. For each batch capture, 6 times individual capturing are carried out, and therefore repeatability are calculated according to the obtained results. From the results as showing in table 3, a conclusion can be drawn that as the increasing of camera duration, measurement repeatability get better, while when duration equal to and larger than 20s, repeatability get almost consistent, because of enough tracking of particles are captured and analyzed. So for the 80nm sample measurement, 20s is enough in video capture.

Threshold value determines the minimum intensity required for an area of light intensity to be determined as a particle[3]. That is to distinguish the particles and background. And in the software, when a particle has been correctly identified as a traceable object, a red cross will appear at the particle centre. So according to this characterizes, the detection threshold be finally selected and set according to all clearly recognizable particles have been captured with a red cross.

Table 3 Obtained particle size with different capture duration

number	1	2	3	5
Camera duration	10s	20s	30s	50s
Mean particle size/nm	82.5	83.3	83.8	83.7
repeatability	2.20%	1.79%	1.83%	1.70%

Camera shutter: 800, Capture gain: 680

3.3 Measurement results Based on the established methods for sample preparation and parameters setting, the number mean particle size of polystyrene particle sample with nominal value 80nm was measured by NTA method. And during the measurement process, for obtaining accurate results, much more particles are captured and measured in 10 times measurements by injecting new sample to the instrument's detection volume. The final mean value and standard reference deviation of total 10 times measurements are 83.2nm and 0.95%.

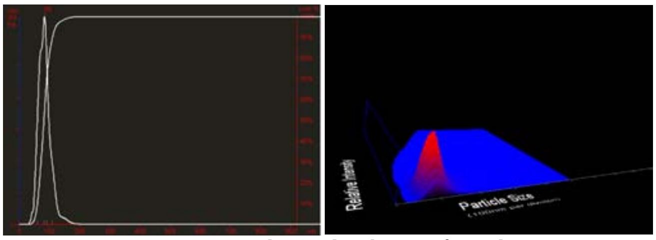

Figure 2 particle size distribution of sample

4. VERIFICATION OF NTA METHOD RESUTLS

4.1 Particle size RMs measurement Four kinds Thermo particle size Reference Materials (RMs) with nominal value of 60nm, 70nm, 80nm and 90nm are used and measured, whose values are well determined by manufacturer using TEM and DLS methods. In our verification measurements, the same sample preparation and parameter setting methods as described in 3.1 and 3.2 section of this paper are used. From the obtained results as showing in table 4, a conclusion can be drawn that, for 80nm and 90nm samples, NTA obtained results are well consistent with RMs' certified value, and which can indicate that our developed methods on sample preparation and the parameters settings in video capturing and analysis process are quite reliable and appropriate.

Table 4 Obtained particle size by measuring different RMs

Information of RMs		Obtained mean particle size of by NTA /nm	
Catalog	Mean particle size and uncertainty range of RMs /nm	NTA instrument	developed NTA instrument
3060A	60±2.7(TEM)/59~65(DLS)	67.1	61.2
3070A	73±2.6(TEM)/71~77(DLS)	76.6	74.3
3080A	81±2.7(TEM)/79~85(DLS)	81.1	81.5
3090A	92±3.7(TEM)/89~94(DLS)	91.0	91.2

While for 60nm and 70nm samples, the obtained results by NTA method are 67.1nm and 76.6nm, which are much larger than their certified values. The possible reason for causing this result is the low relative light scattering property of polystyrene sample which indicates that 60nm is close to the minimum measurement limit of Nanoparticle Tracking Analysis for polystyrene sample, not because of the sample preparation and parameter setting methods. For the purpose of verification this supposes, a more high sensitivity camera and a new sample chamber (equipped with a 403nm laser) are built and replaced the old one (a 640nm laser). In theory, with those developments the NTA instrument should have a significant improvement in sensitivity and an extended minimum measurement range. With this developed NTA instrument, same measurements were carried out. In each measurement, samples preparation and parameter settings are carried out according to the procedure prescribed in section 3.2 of this paper. The obtained results are showing in table 4. In the range of 60nm~90nm, the developed NTA instrument has well consistent results with the certified value of those RMs, and all the NTA results are in the tolerance range of certified values by TEM and DLS methods.

4.2 Comparison with SEM method Before measurement, SEM magnification (×100000) was calibrated using nominal 400nm micrometers. And the micrometers' line width was certified by National Institute of Metrology (NIM) China using frequency stabilized lasers and dividing scale of goniometer, the certified result can be traceable to national length primary standard. The certified value and uncertainty are 416.6nm and 0.2nm (*k*=2). The calibrated pixel size in 100000 magnification is 1.01nm/pixels. Subsequently, at least 1000 particles were analyzed, and their area and area-equivalent diameter of each particle are measured by image analysis system. Finally the obtained mean particle size and standard deviation are individually 84.2nm and 1.6%, and the curve of particle size distribution is shown in Figure 4.

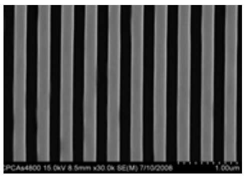

Figure 3 SEM images of micrometer

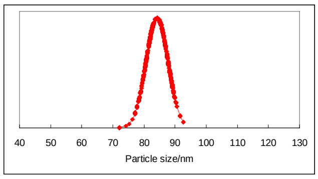

Figure 4 particle size distribution of 80nm sample measured by SEM

The obtained SEM result has a good traceability, which can traceable to national length primary standard of China. Additionally, uncertainty of SEM result was analyzed in this paper for the purpose of comparing SEM result with NTA result well, which is also a tolerance rang and can tell us both of results by SEM and NTA is well consistent or not. In SEM measurement process, there are five main uncertainty sources, including micrometer calibration uncertainty, distortion of SEM view field, SEM resolution, image analysis system, threshold setting and repeatability of measurements.

1) As mentioned before, the uncertainty of certified result is 0.2nm (k=2), so the relative uncertainty δ_1 caused by micrometer calibration is:

$$\delta_1 = \frac{0.2/2}{416.6} \times 100\% = 0.024\% \cdot$$

2) Magnification is not equally uniform in all SEM view field because of spatial in homogeneity of the scanner magnification, and different magnification along the horizontal and vertical direction. So in different locations of SEM view field, the measurement results could be different slightly in the measurement of micrometer and particles. In this research work, a single particle is measured individually at nine different locations of SEM view field. The obtained

nine measurement results in the unit of nm are 84.46, 84.80, 84.39, 84.83, 84.80, 84.81, 84.83, 84.80 and 84.60, and their standard deviation is 0.15nm. In particle size determination process, the micrometer and particles are measured separately, so the relative uncertainty δ_2 caused by distortion of SEM view field is:

$$\delta_2 = \sqrt{2} \times \frac{0.15}{84.2} \times 100\% = 0.26\%$$

3) SEM resolution is an ability of distinguish a minimal space between two spots, generally SEM resolution can be verified by measuring the minimal space of two particles in SEM pictures. In determination procedure, nominal 80nm particle size CRMs was a measurand, and a minimal space S between two particles in one horizontal was selected and measured. According to equation 1, in our measurement condition, the SEM resolution is not larger than 2.01nm.

$$R = \frac{S}{M} \qquad (1)$$

Where M is the SEM magnification. In magnification calibration and particle size measurement, ten different area of micrometer and more than 1000 particles were measured, so the uncertainty introduced by SEM resolution δ_3 is:

$$\delta_3 = \sqrt{\left(\frac{2.01/\sqrt{10}}{416.6}\right)^2 + \left(\frac{2.01/\sqrt{1000}}{84.2}\right)^2} \times 100\% = 0.17\%$$

4) In image analysis process, the threshold is selected and set to determine the edge of measurand and background according to half-amplitude method of threshold level setting (in ISO 13322-1: 2004). In this method, a small region of the background located a few pixels away from the boundary of a typical particle is selected, and the threshold level at which approximately half the pixels in the selected region are detected is recorded.

In SEM images of micrometer (as showing in Figure 5), the edge of the micrometer is sharp and clear, and the selected threshold to determine the edge of micrometer is 178. Between typical boundary of micrometer (threshold=255) and the considered boundary (threshold=178), there is only one pixels, so the bandwidth of 1 pixel (1.01nm) becomes the interval of the corresponding error. While in the SEM images of 80nm particles the edge of the particle is of little blurring, and the selected threshold to determine the boundary of particle can be in the range of 106~115. And when the threshold is out of this range, in the obtained binary image by image analysis software, either typical background is considered as particle boundary or typical particle boundary is considered as background. When the threshold are varied in 106~115, the obtained results also varied slightly, and the relative standard deviation is 0.21%. So the uncertainty introduced by image analysis system δ_4 is:

$$\delta_4 = \sqrt{\left(\frac{2.01}{416.6} \times 100\%\right)^2 + (0.21\%)^2} = 0.53\%$$

5) Because the actual magnification can vary between every measurement at the same nominal magnification in the same instrument, the repeatability of measurement procedure could make an effect on certified results. Through measure the same particle many times, the repeatability can be easily obtained, and in our determination the uncertainty of repeatability δ_A is 0.40%.

So, according to the above analysis, the combined uncertainty is,

$$u_c = \sqrt{\delta_1{}^2 + \delta_2{}^2 + \delta_3{}^2 + \delta_4{}^2 + \delta_A{}^2} = 0.74\%$$

And the Expanded Uncertainty is U_r=1.48% or U=1.3nm (k=2). So the SEM measurement results for nominal 80nm sample is (84.2±1.3) nm.

Comparison with obtained NTA and SEM results, NTA results of 83.2nm is slightly lower than SEM results of 84.2nm, but it is in the tolerance rang of SEM result. And this also indicates that our developed methods on sample preparation and the parameters settings in video capturing and analysis process are quite reliable and appropriate.

5. CONCLUSION

In NTA measurement method, sample concentration and parameters (shutter, gain, duration and threshold) settings in video capture and video analysis are important factors, which might have a significant effect on the accuracy and reliability of final results. For 80nm polystyrene sample, the optimal concentration for NTA measurement is in the range of (3.5~7.0)×10^8 particles/mL, and when the camera parameters of gain, shutter and duration are equal to 680, 800~1000, 20s, a good capturing video can be obtained. While in video analysis, the detection threshold should be finally selected and set according to all clearly recognizable particles have been captured with a red cross. In this way, an accurate and reliable NTA result could be obtained, which is well consistent with SEM results.

6. ACKNOWLEDGMENTS

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7. REFERENCES

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