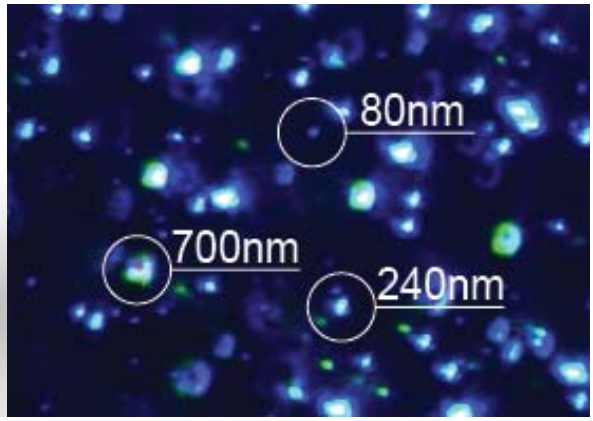
MANTA INSTRUMENTS
Better Nanoparticle Characterization
info@mantainc.com ■ www.mantainc.com

The Better Way to Characterize Nanoparticles – MANTA's *ViewSizer 3000*



MANTA's Most Advanced Nanoparticle Tracking Analysis technology performs individual particle analysis simultaneously on wide ranging nanoparticle sizes co-existing in liquid samples. This technology has been well validated and successfully commercialized in MANTA's first product the *ViewSizer 3000* which addresses the heretofore unmet needs of: (i) visualizing nanoparticles, (ii) measuring nanoparticle number concentration and size distribution, and (iii) measuring nanoparticle kinetic processes, even in highly polydisperse samples. This paper is focused on validation results from various experiments using the *ViewSizer 3000;* some of which include comparisons with the legacy nanoparticle characterization technologies.

Experiment 1: Nearly monodisperse nanoparticle size standards

The objective of experiment 1 was to evaluate the performance of the *ViewSizer 3000* for a suite of nearly monodisperse nanoparticle size standards covering a broad range of mean particle size. The size standards were polystyrene beads obtained from Thermo Scientific. Figure 1 shows example results obtained with *ViewSizer 3000* for three size standards with mean diameters ranging from about 80 nm to 700 nm. Each size standard was measured separately. The measured particle size distributions provide very accurate information on the dominant particle sizes. In this example the measured mode diameters for the three samples are within the size bins of 83-85, 256-262, and 712-730 nm which are in very good agreement with the nominal mean diameters of 81 nm (standard deviation SD = 9.5 nm), 269 nm (SD = 4.2 nm), and 707 (SD = 8.3 nm), respectively. The measured size distributions show a very good shape exhibiting nearly perfect symmetry, which is consistent with expectations for such nearly monodisperse particle size standards. It is important to emphasize that the presented distributions represent the real data from measurements with no smoothing, curve fitting, or any other operations or alterations applied to the original data. We note, however, that some degree of broadening of size distribution occurs with the *ViewSizer 3000* due to tracking individual nanoparticles in Brownian motion over finite period of time. This broadening effect is intrinsic for conventional NTA as well.

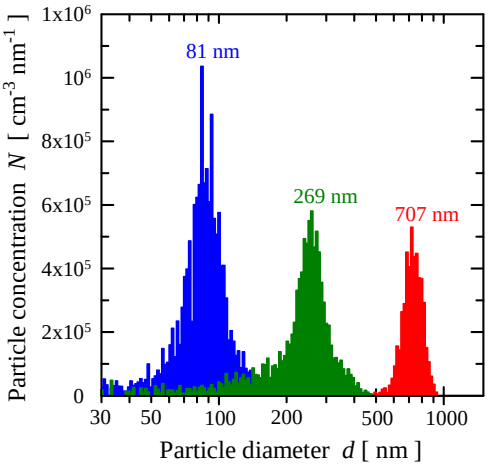


Figure 1. The particle number size distributions measured with MANTA's ViewSizer 3000 for three samples of polystyrene nanosphere size standards suspended in deionized prefiltered (0.2 μm) water. Each sample was measured separately. The size distributions are presented in the form of histograms as a semi-log plots of density functions that were obtained by counting particles within size bins and dividing by the width of size bins. The number concentration of particles per unit volume of sample is reported (rather than just particle counts) because the sample volume analyzed with ViewSizer 3000 is known. The original data were acquired in 1-nm size bins but for the purpose of the presentation the data were rebinned into logarithmically equal bins. The concentration of particles integrated between 0 and 350 nm in the 81-nm sample was 3.4 x 10^7 cm^{-3}. The concentration of particles integrated between 0 and 600 nm in the 269-nm sample was 6.0 x 10^7 cm^{-3}. The concentration of particles integrated between 500 and 1,000 nm in the 707-nm sample measured with ViewSizer 3000 was 8.7 x 10^6 cm^{-3}. For the purpose of this illustration the distribution for the 707-nm sample was rescaled by multiplying the measured distribution by a factor of 10.

Experiment 2: A mix of two nanoparticle size standards with similar size

The objective of experiment 2 was to evaluate the performance of MANTA's *ViewSizer 3000* for a mix of two nanoparticle size standards which are relatively close in size. The nominal mean diameters of these polystyrene standards were 100 and 152 nm. Figure 2 shows results for this sample which demonstrate the capability of MANTA's *ViewSizer 3000* to resolve two distinct particle populations whose dominant size differs by about 50 nm.

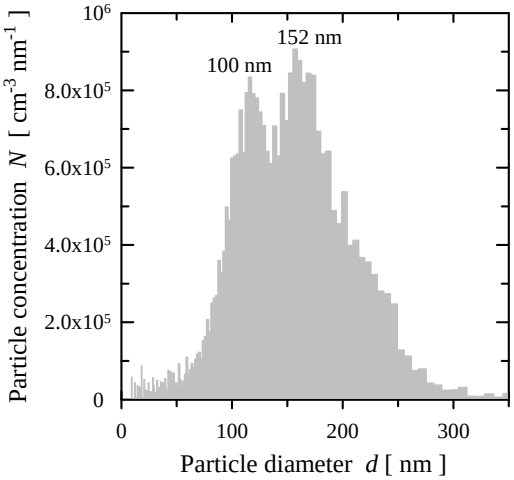


Figure 2. The results were obtained for a sample consisting of the mix of two polystyrene nanosphere size standards, 100 nm and 152 nm. The concentration of particles integrated between 0 and 1,000 nm for this sample was about 1.0 x 10^8 cm^{-3}.

Experiment 3: A mix of three nanoparticle size standards covering a broad size range

The objective of experiment 3 was to evaluate the performance of the *ViewSizer 3000* for a polydisperse sample representing a mix of a few nanoparticle size standards covering a broad size range. Similar to experiment 1, each component of the mix is nearly monodisperse. Figure 3 shows results obtained with a mix of three polystyrene size standards having mean diameters of 81, 240, and 707 nm. This example demonstrates the capability of MANTA's *ViewSizer 3000* to measure a broad size distribution of a highly polydisperse assemblage of nanoparticles consisting of distinct particle populations.

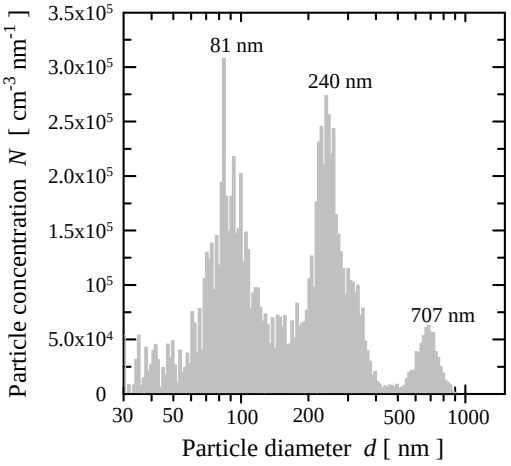


Figure 3. As in Figure 1 but the results were obtained for a polydisperse sample consisting of the mix of three polystyrene nanosphere size standards, 81 nm, 240 nm, and 707 nm. The concentration of particles integrated between 0 and 1,000 nm for this sample was about 5.0 x 10^7 cm^{-3}.

Experiment 4: A mix of nine nanoparticle size standards covering a broad size range

The objective of experiment 4 was to evaluate the performance of MANTA's *ViewSizer 3000* for a highly polydisperse sample representing a mix of many nanoparticle size standards. Similar to experiment 2 each component of the mix is nearly monodisperse, but overall the mix covers a broad range of sizes. Figure 4 shows results obtained with MANTA's *ViewSizer 3000* for a mix of nine polystyrene size standards covering the size range from about 150 nm to 800 nm. The nominal mean particle diameters for the nine standards used in the mix are: 151, 199, 240, 300, 404, 499, 600, 707, and 799 nm. The different size standards were mixed in proportions such that the particle number concentration, N, decreased with particle diameter, d, approximately as $N \sim d^{-3}$. Such distribution is similar to that exhibited by nanoparticles in environmental samples, for example in natural aquatic environments. Because there is no other ideal technique that can provide accurate size distribution for such highly challenging polydisperse samples covering such large portion of submicron range, we applied our own "reference" method to estimate the concentration of each size standard in the mix. This reference method combines the Mie scattering calculations for homogeneous spheres with optical measurements of the beam attenuation coefficient of nanoparticle suspension using a laboratory spectrophotometer equipped with appropriate geometry of measurement (i.e., illumination of sample with a collimated beam and detection of directly transmitted light with a very narrow angle of

acceptance). With this method we determined the approximate particle concentrations for samples of each size standard before they were mixed. Knowing the particle concentration of each size standard, the polydisperse sample was prepared by mixing the standards at preselected desired proportions. Figure 5 shows a remarkably good agreement between the distribution measured with *ViewSizer 3000* and the approximate concentrations of size standards in the mix as determined from the reference method. This example demonstrates the capability of *ViewSizer 3000* to make reliable measurements of highly challenging polydisperse samples with a broad size distribution consisting of many distinct particle subpopulations within the submicron range.

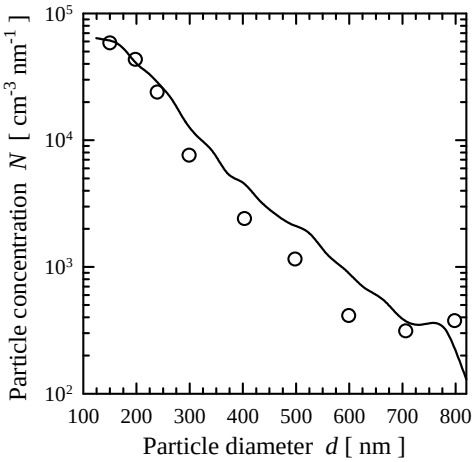


Figure 4. Particle number size distribution obtained with MANTA's ViewSizer 3000 (solid line) for a highly polydisperse sample containing a mix of nine differently-sized polystyrene size standards ranging in diameter from about 150 nm to 800 nm. As in Figure 1, the distribution is shown in terms of the density function but instead of histograms the curve obtained by connecting the mid-points of size bins is displayed. For comparison, approximate particle concentrations of size standards in the mix as determined from the spectrophotometric/Mie method are also shown (open circles). The circles are positioned at the nominal mean diameters of size standards.

Experiment 5: Lactalbumin nanoparticles

The objective of experiment 5 was to evaluate the applicability of MANTA's *ViewSizer 3000* for determining the number concentration and size distribution of polydisperse assemblage of nanoparticles whose chemical makeup has relevance to nanomedicine and pharmaceutical research and development. Specifically, protein nanoparticles of α-lactalbumin were selected for this test. The transmission electron microscopy (TEM) image of α-lactalbumin nanoparticles in the examined sample is shown in Figure 5. The presence of differently-sized nanoparticles is evident from this image.

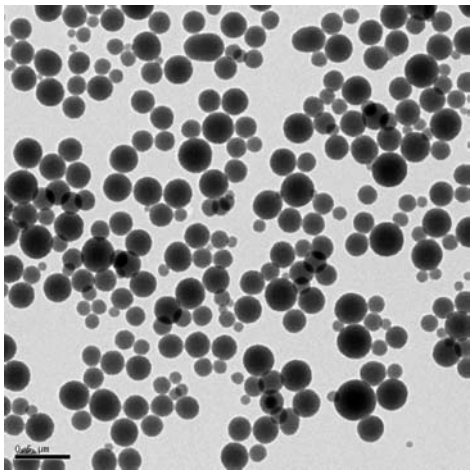


Figure 5. TEM image of α-lactalbumin nanoparticles in the examined sample (courtesy of Nia Bell of UCSD). The images were acquired with a FEI Tecnai G2 Sphera at 200 KV. The scale bar in the lower left corner of the image corresponds to 500 nm.

Experiment 5 also compared results from MANTA's *ViewSizer 3000* with those obtained with TEM, conventional NTA, and DLS methods. These results are presented in Figure 6. The best agreement in terms of both the total (integrated) number concentration of nanoparticles as well as the size distribution is observed between the MANTA and TEM results. Good agreement between the MANTA and TEM distributions was supported by Kolmogorov-Smirnov test. The comparisons involving the conventional NTA and DLS data failed this test. Note also that the TEM distribution shows increased "noise" in the data above 300 nm owing to reduced counting statistics. Thus, the MANTA-derived distribution is considered to provide the best measurement of the actual distribution.

The conventional NTA method considerably overestimated the presence of large nanoparticles. One consequence of this is that the total concentration and the mean size of α-lactalbumin nanoparticles were greatly overestimated with NTA. The DLS measurements were not reproducible and provided unreliable distributions, especially highly erroneous degree of polydispersity of the sample. The dominant size determined with DLS is shifted to larger size compared with the other methods. In addition the DLS measurements do not provide the absolute number concentration of nanoparticles. Therefore, for the purpose of this illustration, the measured DLS distributions were rescaled to enable comparison with other measurements.

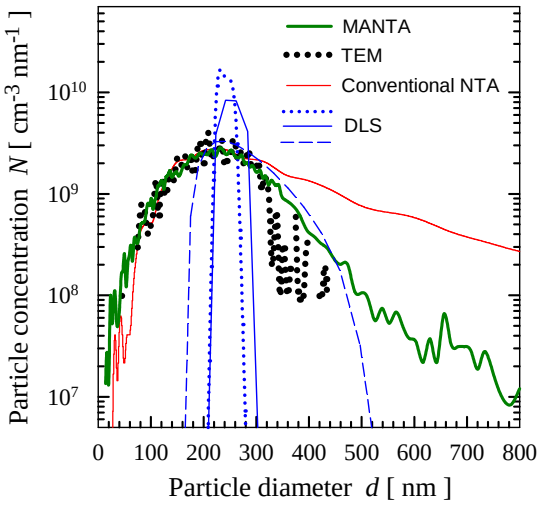


Figure 6. Comparison of particle number size distribution obtained with MANTA's ViewSizer 3000 with results from TEM, conventional NTA, and DLS methods for the sample of α-lactalbumin nanoparticles. As in Figure 1, the distributions are shown in terms of the density function but the mid-points of size bins were connected to produce the curves. An approximate estimate of total number concentration of nanoparticles in master sample of 5 x 10^{11} cm^{-3} was obtained from the combination of chemical assay and TEM analysis. The master sample was diluted 400,000-fold for MANTA, 10,000-fold for conventional NTA, and 100 to 1,000-fold for DLS measurements. For this comparative illustration, the original MANTA and conventional NTA measurements were rescaled back to master sample by multiplying the measured data by dilution factors. The TEM and DLS distributions (courtesy of UCSD's Nia Bell) were scaled to yield the total particle concentration of 5 x 10^{11} cm^{-3}. Three DLS replicates for the same sample are presented. Each replicate measurement is based on averaging 10 acquisitions, each of 10 seconds duration. The conventional NTA measurements were made with NanoSight LM10 using the standard algorithm with curve fitting (NTA version 2.3). The DLS measurements were carried out using a DynaPro NanoStar (Wyatt Technology) at a scattering angle of 90^o. The DLS size distributions were generated using a standard requlrization fit of the autocorrelation function in the instrument software (Dynamics Version 7.1.7.16).

Experiment 6: Kinetic stability of polymeric micelles

The objective of experiment 6 was to evaluate the applicability of MANTA's *ViewSizer 3000* for monitoring the stability of self-assembled polymeric micelles in aqueous solutions over time (from minutes to about 1.5 hours). One promising application of polymeric micelles is as a platform to encapsulate therapeutic drugs which can be tuned for drug delivery. One of the design criteria for success in such applications of micelles is their stability. The particle size is an important physical property for characterizing stability of micelle system. The sample of experiment 6 was prepared by dissolving a polymer $(PEG_4)_{76}(phenyl)_{25}$ in a binary solvent mixture of dimethyl sulfoxide (DMSO) and water (courtesy of UCSD's Nia Bell). A series of size distribution measurements of polymeric micelles within the sample were made with the *ViewSizer 3000* at different post sample preparation times for a period of about 1 hour. The sample was also analyzed with TEM. Figure 7 depicts the size distribution of micelles along with the corresponding TEM images for the elapsed times of 3, 18, 43 min, and 105 min. Initially, the size distribution was characterized by a dominant size of about 200 nm and very small contribution of micelles larger than 500 nm. With time, the distribution broadened owing to increasing numbers of larger micelles. Initially at 3 min and 18 min the mean diameter was about 250 nm. Then the mean diameter increased to 474 nm at 43 min and 592 nm at 105 min.

Interestingly, with the exception of higher particle concentration at 3 min, the total number concentration of micelles did not change significantly over time during the remaining portion of experiment. This demonstrates that micelles did not show significant levels of aggregation or disassembly and the increase in average particle size was caused by increasing size of individual micelles. The increase in size can be attributed to the presence of unreacted polymer in bulk solution. This test demonstrates that the combination of accurate determinations of the particle number concentration and the particle number size distribution provided by the *ViewSizer 3000* is a powerful tool for monitoring and understanding stability of nanoparticle systems.

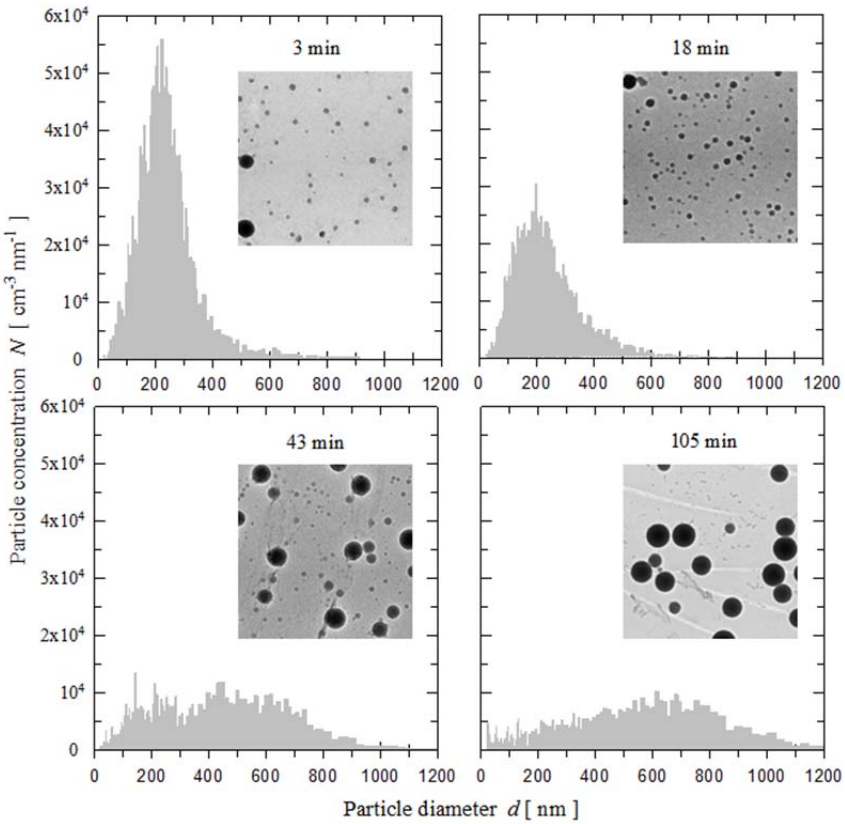


Figure 7. Particle number size distributions of micelle sample obtained with MANTA's ViewSizer 3000 for different elapsed times as indicated. As in Figure 1, the density function of particle size distribution is shown in terms of histogram. For comparison, TEM images are also shown (courtesy of Nia Bell of UCSD), which support the ViewSizer 3000 data that show a clear trend of increasing average particle size with elapsed time.

Summary

The ***ViewSizer 3000*** **can visualize a wide range of nanoparticle sizes simultaneously**. This unique capability provides various benefits including **accurate measurement of nanoparticle number concentration, size distribution, and kinetic processes** with one easy-to-perform test on particles in liquids.