ZetaView® Scanning NTA

Reliability of concentration measurements with the Particle Metrix ZetaView® Nanoparticle Tracking Analyzer

S. Kreutel[1], and G. Linz[2]

[1] Particle Metrix GmbH, Meerbusch, Germany
[2] Particle Metrix Inc., Mebane NC, USA

Abstract

Concentration levels of extracellular vesicles have a high clinical potential as a reference for disease (1). However, concentration measurements by nanoparticle tracking analyzers (NTA) are often inaccurate due to inadequate concentration calibration of the instruments. Here we report a concentration comparison on various pre-calibrated Particle Metrix ZetaView® instruments using a silica-based concentration reference source. The results of this study verify a very accurate concentration calibration method for the ZetaView system and thus accurate concentration measurements.

Introduction

The ZetaView NTA system by Particle Metrix is widely used in life science and medical research for measuring the size and concentration of biological nanoparticles, as well as fluorescence detection and zeta potential measurements. This includes the measurement of extracellular vesicles (EV), *aka* exosomes, as well as protein aggregates, viruses, prions, and other cell-derived nanoparticles (2, 3, 4). By tracking their Brownian motion, the diffusion coefficient of nanoparticles is directly measured, which leads directly to a calculation of size (5,6). Conversely, measuring particle concentration is not a direct measurement, as one must assume an “effective” volume of measurement, and is thus a challenge that often leads to inaccurate measurements. As such, NTA systems are best calibrated by benchmarking against a concentration standard. Polystyrene latex (PSL) beads are often used as calibration material, as PSL has a couple of advantages when compared to biological reference materials, including handling, stability and storage; however, the scattering efficiency of PSL is much greater than cell-derived biological particles such as EV (2). To evaluate this material difference on calibrations, the accuracy of concentration measurements for the ZetaView system was tested on five different and independently calibrated ZetaView instruments using a silica reference standard that has a scattering power much closer to EV (i.e., RI silica 1.445 vs RI Vesicles 1.48 vs RI PSL 1.605 (2)).

Methods

A NIST traceable and prediluted silica reference standard (size: nominal = 90 nm, mode = 85,1 nm; conc. 4.0 x 107 beads / ml, KANOMAX FMT, USA) was introduced into five different

ZetaView® PMX-110 Analysers (Particle Metrix, Germany). All instruments use a 405 nm excitation laser and were pre-calibrated for concentration with a 100 nm PSL reference standard (Applied Microspheres, Netherlands). All NTA measurements were carried out using exactly the same camera settings as well as the same tracking parameters, with values recommended for EV detection (sensitivity: 85, Shutter: 70, min Brightness: 20, min Size: 10, max. Size: 200). Videos were taken at 30 frames per second and analysed for size and concentration using the ZetaView software (Particle Metrix, Germany). The measured silica concentration values we report include all particles ≥ 60 nm, as defined in the certificate provided by KANOMAX FMT.

Results & Conclusions

Using nominal 90 nm silica beads with a refractive index close to extracellular vesicles, our measurements of concentration on different ZetaView instruments show essentially the same concentration within experimental error (see figure 2). This extreme similarity in measured values, while a welcome surprise, was not initially expected to be this good, given that the industry-accepted error in concentration measurements is supposed to be roughly 10%. Additionally, the independent measurements of the corresponding sizes show also a high conformity among each other (see figure 1). The average size was calculated to 85,6 nm ± 2,25 nm with a variance of only 5,9 %. Those results are in great agreement with the certified values of 85,1 nm for the mode size. Arriving at equivalent concentration and size values on 5 different ZetaView systems validates the approach of Particle Metrix to empirically benchmark concentration and size measurements against an established reference standard.

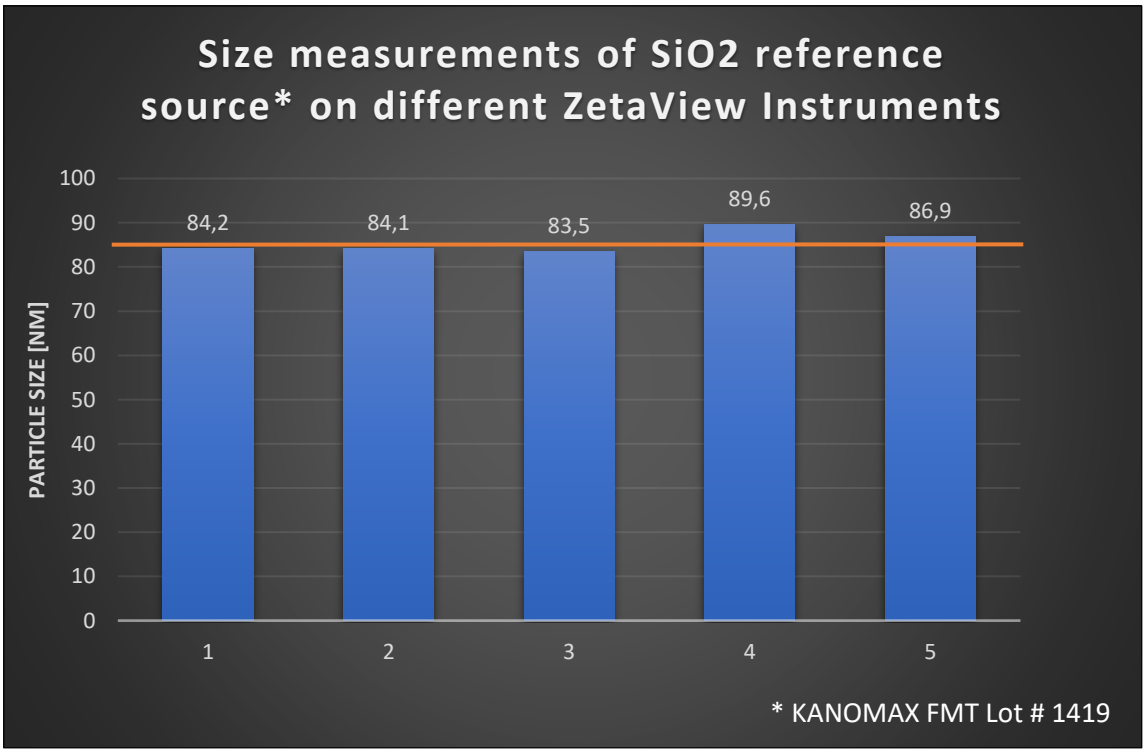

Figure 1: Size measurement of SiO2 reference source on 5 different ZetaView 110 instruments. mode size according certificate 85.1 nm (orange line)

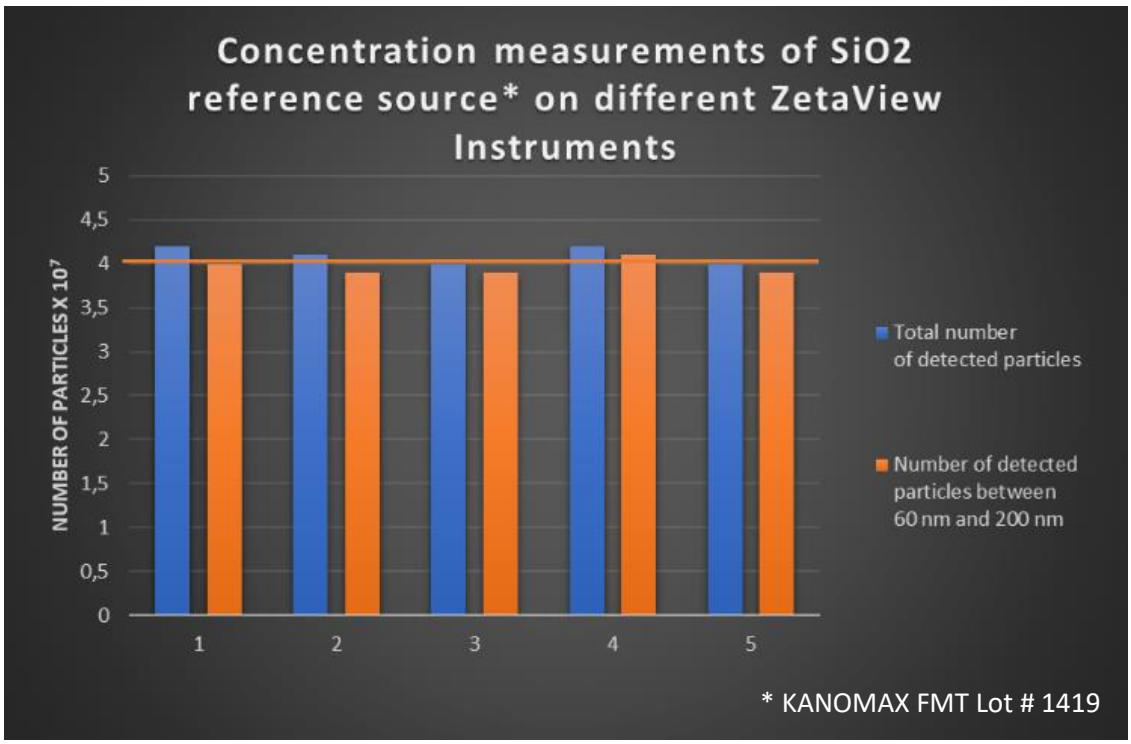

Figure 2: Concentration measurement of SiO2 reference source on 5 different ZetaView 110 instruments. Shown are the data for the total number of particles (blue) and the number of particle in the certified size range above 60 nm (orange). The Concentration according certificate 4,0 x 10^7 particles / ml is depicted as the orange line.

While these results show success with 90 nm silica on different PMX 110 Model systems, newer PMX 120 Model systems that have a CMOS camera do in fact benefit from using a separate concentration calibration against 90 nm silica beads. To clarify, a separate calibration factor can be determined using 90 nm silica as the benchmark reference standard for biologics, rather than using a PSL standard. The CMOS camera is much more sensitive than standard CCD cameras, and thus the difference in scattering power between PSL and silica is significant enough to warrant using silica for a separate calibration factor, thus further improving on ZetaView system concentration measurements of EV and other biologics.

References

1. **van der Pol et al. (2014)**
   Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing.
   J Thromb Haemost 12: 1182–92.

2. **van der Pol et al. (2012)**
   Single vs. swarm detection of microparticles and exosomes by flow cytometry.
   J Thromb Haemost 2012; 10: 919–30.

3. **Shu Liu *et al.* (2017)**
   Prions on the run: How extracellular vesicles serve as delivery vehicles for self templating protein aggregates
   Prion., Mar 4;11(2):98-112. doi: 10.1080/19336896.2017.1306162.

4. **Kordelas, L. *et al.* (2014)**
MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia, 28, 970- 973 doi:10.1038/leu.2014.41

5. **NOURI, M. *et al.* (2012)**
Effect of Partially Hydrolyzed Kappa-Casein on Physicochemical and Sensory Properties of Heated Milk. Journal of Dispersion Science and Technology, 33. Jg., Nr. 8, S. 1204-1209.

6. **ISO 22412:2008**
Particle Size Analysis - Dynamic Light scattering (DLS)

7. **ASTM2834-12**
Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA)

Acknowledgements

We thank Dr. Clemens Helmbrecht, Dr. Hanno Wachernig, Dr. Sascha Raschke, Ray Eby and David Palmlund for their support, scientific input and for critical reading of the manuscript.