Mesoporous silica nanoparticle-based protein delivery systems for biomedical applications

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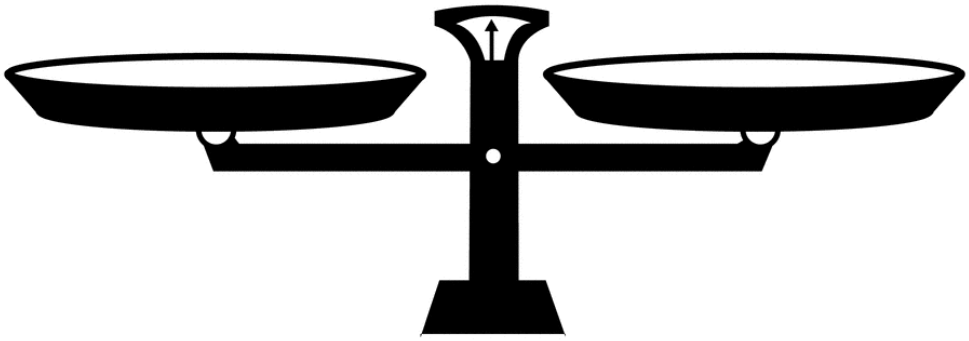
Chapter VI (Appendix)

Weight Estimation of a Single Mesoporous Silica Nanoparticle



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6.1 Introduction

Characterization of building blocks of cell's like proteins by their mass is essential for the discovery of disease biomarkers and the development of early disease diagnostic tools.[49, 50] In (bio)nanotechnology, individual nanoparticles are as unique as people's fingerprints,[51] therefore it becomes imperative to find methods for the full characterization of nanoparticles. In the field of nanomedicine,[52] knowing the mass of nanoparticles could result in more precise *in vivo* administration.[51] One of the most widely used mass-sensing methods is the quartz crystal microbalance,[53-56] with a total mass resolution of ~1 ng.[51, 57] However, rapid developments of nanotechnology in biology require a more sensitive technique, with a mass detection limit preferably at the level of nano-sized objects.[49, 58] In the last decade, mechanical resonator based nanomechanical mass sensors have been developed and used to weigh cells, biomolecules, bacteria and viruses.[49, 57, 59, 60] Burg[57] demonstrated that suspended microchannel resonators (SMR) can be used to weigh single particles or cells in water with sub-femtogram resolution; such as gold nanoparticles (100 ± 8 nm, 10 fg), *Escherichia coli* (110 ± 30 fg) and *Bacillus subtilis* (150 ± 40 fg). In principle, added mass from a sample of interest induces a downshift of the resonance frequency that is proportional to the ratio between the added mass and the resonator's mass.[49, 60] However, this technique isn't suitable for measuring the weight the particle size with small diameter (< 50 nm).[61] In recent years, with the development of nanomechanical devices, the ultimate mass detection limit rapidly shifted from pictograms (10^{-12} g)[57, 62] to yoctograms (10^{-24} g).[59] However, most of these techniques require complex high-vacuum conditions and are therefore, not suitable for analyzing biomolecules or nanoparticles in solution.[49, 57, 59, 63]

Here, a simple and non-destructive method to estimate the weight of a single particle in solution using Nanoparticle tracking analysis (NTA) is described. Nanosight, a laser-illuminated light scattering microscopy,[51] is capable of directly sizing and visualizing nanoscale particles in liquids with high-resolution, providing the size, total number of particles and the concentration of the measured samples.[51, 64] The NTA software can identify and track individual nanoparticles moving under Brownian motion and relates the movement to a particle size according to the formula derived from the Stokes-Einstein equation.[65] Taking the advantages of this technique and combined with a gravimetric measurement yields a simple and complementary method to determine the colloidal stability and estimate a single nanoparticle's weight of the sample of interest in solution such as the MSNs described in Chapter 2, 3, 4 and 5.

6.2 Materials and method

6.2.1 Materials

Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, M_n~5800 g/mol), tetraethyl orthosilicate (TEOS, ≥98%), hydrochloric acid (HCl), 1,3,5-trimethylbenzene (TMB) were purchased from Sigma-Aldrich and used as received. Fluorocarbon surfactant FC-4 was purchased from Yick-Vic Chemicals & Pharmaceuticals (HK) Ltd, China. Milli-Q water (18.2 MΩ/cm, Millipore Co., USA) was used throughout the experiments.

6.2.2 Preparation of large-pore MSNs

MSNs were synthesized as follows. 0.5 g of surfactant Pluronic P123 and 1.4 g of FC-4 were dissolved in 80 mL of HCl (0.02 M), followed by the introduction of 0.48 mL of TMB. After stirring for 6 h, 2.14 mL of TEOS was added dropwise. The resulting mixture was stirred at 30 °C for 24 h and transferred to an autoclave at 120 °C for 2 days. Finally, the solid product was isolated by centrifugation, and washed with ethanol and water. The organic template was completely removed by calcination at 550 °C for 5 h.

To determine the colloidal stability and concentration in particles (1×10^8/mL), MSNs (1 mg/mL) were sonicated (10 min) and dispersed in MilliQ. The sample was diluted with MilliQ to final concentrations ranging from 1 to 10 μg/mL. All the suspensions were sonicated for 10 min (Branson 1510 ultrasonic cleaner) before the measurements. The mean size, standard deviation (SD), and total concentration values were measured using a NanoSight LM20. Four measurements were taken from each sample and averaged. The weight of a single particle was determined using the following equations.

$$m\ (single\ MSN\ weight) = \frac{M}{N} \tag{6.1}$$

When the volume of the MSNs suspension is 1 mL,

$$m\ (\text{weight of a } single\ MSN) = \frac{particle\ concentration\ by\ weight}{particle\ concentration\ by\ counts} \tag{6.2}$$

M: Weight of MSNs (mg) as determined by micro balance (Sartorius),
N: Number of MSN particles as determined by NTA.

6.2.3 Particle analysis

The porous structure of the as-prepared MSNs was characterized using transmission

electron microscopy (TEM) operated at 70 kV (TEM, JEOL 1010, USA). The hydrodynamic diameter of the MSNs was measured with a Malvern Nano-ZS instrument. Nanoparticle tracking analysis (NTA) measurement was performed by using a NanoSight LM20 (NanoSight, Amesbury, United Kingdom). The software used for capturing and analyzing the data was the NTA 2.0 Build 127. Data analysis was performed using NTA 2.0 Build 127. All the samples were measured for 40 s at room temperature.

6.3 Results and discussion

The morphology and mesoporous structure of the MSNs was visualized by TEM (Figure 6.1a). Analysis of the TEM images revealed the MSNs had lengths of 90 ± 20 nm and widths of 43 ± 7 nm, giving them an elongated cuboidal-like geometry. Dynamic light scattering (DLS) measurements revealed MSNs with a unimodal distribution that possessed an average hydrodynamic diameter of 146 nm (Figure 6.1b). These sizes were slightly larger than those determined by DLS, since TEM provides the size distribution of dehydrated particles while DLS measurements yield an average hydrodynamic diameter of the particles in solution.[66] Nanoparticle tracking analysis (NTA) enables the determination of the hydrodynamic diameter distribution of the particles and in addition counts the number of individual nanoparticles.[51, 65] Therefore, the colloidal stability of the MSNs as a function of concentration was determined using NTA (Figure 6.1c and d). A dilution series of MSNs (1-10 μg/mL) was prepared and the concentration of MSN was determined to be 4.6×10^{8} - 2.9×10^{9} particles/mL were determined by NTA (Figure 6.1d, red dots curve). The mean size, standard deviation (SD), and molarity (n/v) as a function of MSN concentration (w/v) were also measured by NTA (Figure 6.1c, d, table 6.1). Surprisingly, an increase in MSN concentration, resulted in a decrease in the observed mean size of the MSN from 148 to 87 nm, which fits with both the transmission electron microscopy (TEM) and the dynamic light scattering (DLS) data results (Figure 6.1a, b). Since the mean size and SD values obtained by NTA correspond to the arithmetic values calculated with all the particles analyzed, the decrease in mean size may be due to a more accurate calculation at higher particle concentrations.[65]

Table 6.1 Mean size, size distribution and concentration in particles of MSNs from NTA measurements

Particle *conc.* (μg/mL)	Mean (nm)	SD (nm)[a]	Particle *conc.* (1×10^8/mL)[b]
1	146.8 ± 4.3	63.8 ± 1.9	4.6 ± 0.7
2	131.5 ± 5.2	58.5 ± 1.0	8.5 ± 0.2
3	138.3 ± 5.7	63.0 ± 6.8	9.4 ± 0.7
4	131.3 ± 6.7	68.5 ± 2.4	13.6 ± 1.2
5	122.5 ± 4.0	69.5 ± 1.0	15.4 ± 1.3
6	118.8 ± 2.1	68.0 ± 1.6	18.0 ± 2.1
7	106.5 ± 5.8	64.5 ± 4.2	19.5 ± 0.8
8	107.0 ± 1.2	70.3 ± 0.5	22.2 ± 1.8
9	106.5 ± 7.5	72.3 ± 8.4	26.0 ± 2.1
10	86.5 ± 6.1	64.8 ± 3.8	29.4 ± 3.4

[a]*SD* standard deviation calculated by the NTA software; [b]*Conc.* Concentration in particles 10^8/mL as measured by NTA. Numbers represent average values ± standard deviation (n = 4 measurements).

Figure 6.1d (red curve) shows, the linear (R^2 = 0.99) relationship between particle concentration by weight and by count rate (y = 2.61x + 2.315). The calculated weight of a single particle using equation 6.2 (red dots) increased steadily until they reached a plateau (Table 6.1). The weight of a single particle was calculated according to equation 6.3.

$$m\,(single\ MSNs' weight) = \frac{x}{2.61x + 2.315} \tag{6.3}$$

The slope reflects the weight of a single MSN. When a higher particle concentration is used for the NTA measurements, the value of a single MSN weight is closer to the real weight. When x goes to ∞, the weight of a single MSN is the reciprocal of the slope and thus the weight was calculated to be 3.8 fg. As no aggregation was observed over the used particle concentration range during all the measurements, the MSNs showed good colloidal stability.

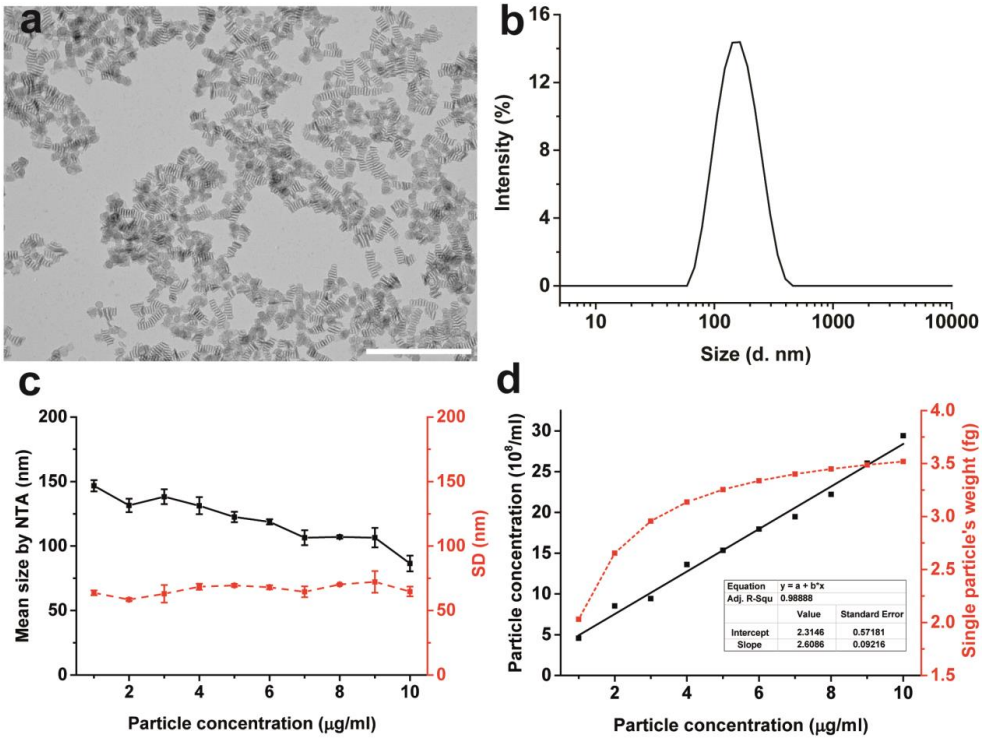

Figure 6.1 *(a) TEM image of MSNs, scale bar = 500 nm; (b) hydrodynamic diameter by DLS; (c) mean MSN size and standard deviation (SD) calculated by the NTA software; (d) NTA particle concentration (10^8 particles/mL) as a function of particle concentration (weight/volume, 1-10 µg/mL) from NTA measurements and estimation of singular particle weight. Concentration in particle number (10^8 particles/mL) as measured by NTA. Numbers represent average values based on 4 measurements.*

6.4 Conclusion

In conclusion, we developed a simple method to measure the colloidal stability of MSNs and estimate the weight of a single MSN at the same time. The detection limit for the nanoparticle size is determined by the sensitivity of the camera of NTA and the accuracy of the micro balance. This complementary and non-invasive method uses the advantage of NTA and provides a new and easy method for determining the weight of single nanoparticles and biomolecules in solution with a femtogram resolution.

6.5 References

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