Supporting information

Intermethod Comparison of the Particle Size Distributions of Colloidal Silica Nanoparticles

Jani Tuoriniemi, Ann-Cathrin J. H. Johnsson, Jenny Perez Holmberg, Stefan Gustafsson,
Julián A. Gallego-Urrea, Eva Olsson, Jan B. C. Pettersson, and Martin Hassellöv

Table S1 Physical and chemical properties of the colloidal silica dispersions.

The information was provided by Eka Chemicals.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g cm$^{-3}$)</td>
<td>1.3024</td>
<td>1.3010</td>
</tr>
<tr>
<td>SiO$_2$ (wt %)</td>
<td>41.64</td>
<td>41.49</td>
</tr>
<tr>
<td>pH</td>
<td>9.18</td>
<td>9.28</td>
</tr>
<tr>
<td>Specific surface (m$^2$ g$^{-1}$)</td>
<td>122</td>
<td>135</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>6.6</td>
<td>8.8</td>
</tr>
</tbody>
</table>
**Figure S1** SEM image of a cluster of particles. Scale bar 30 nm. The indicated distances are in nanometers.

**Figure S2** TEM images of the particles seen in Figure S1. Scale bar 20 nm. Note that the image is rotated 30° clockwise compared to Figure S1.
Confirmation of magnification calibration of SEM and TEM

The Figures S1 and S2 are SEM and TEM images of an identical cluster of particles. The dashed lines denote measured distances between identical points. The solid lines are measured particle diameters. The distances appeared on average 3.4 % longer in SEM. The particles were on average 2.4 % larger, although the size difference varied considerably from particle to particle.

Calculation of NTA sensitivity as a function of particle size

Theory on the dependence of sensitivity of scattering power

The laser beam illuminating the sample has a Gaussian intensity profile and it has been pointed out that strongly scattering particles should be visible further from the beam center than weak scatterers.\cite{1} This entails a larger volume of detection, and consequently an increased sensitivity for strongly scattering particles. For particles in the Rayleigh regime this could lead to a bias towards larger sizes. To estimate in which degree the effects of inhomogeneous illumination could introduce bias in the size distribution the light scattering ability of silica particles as a function of their size was calculated using Mie theory in the Scatlab 1.2 software. The results were used to calculate the detection volume as a function of particle size using a model discussed in a recent article.\cite{2} For particles that are illuminated by a Gaussian beam described by:

$$I_0 = \frac{A}{\sqrt{\pi c}} e^{-\frac{r^2}{c}}$$  \hspace{1cm} (S1)

where $r$ is the distance from beam center and $I_0$ is its intensity. $A$ and $c$ are parameters controlled by the laser beam intensity and shape, respectively, with $c$ being $2*\sigma^2$. The $\sigma$ is the standard deviation of the presumably Gaussian beam.
The scattering power, $K$ of the particles can be defined as the fraction of incident light scattered by them:

$$I_{\text{scattered}} = K I_0$$  \hspace{1cm} (S2)

At the largest distance from the beam center where a particle is visible it scatters light at the threshold intensity for detection:

$$I_{\text{thresh}} = K I_0$$  \hspace{1cm} (S3)

By inserting equation S1 for $I_0$ into equation S3 the following equation is obtained:

$$I_{\text{thresh}} = \frac{KA}{\sqrt{\pi c}} e^{-\frac{r^2}{c}}$$  \hspace{1cm} (S4)

Solving for $r^2$ gives:

$$r^2 = c \ln \left( \frac{KA}{I_{\text{thresh}}\sqrt{\pi c}} \right)$$  \hspace{1cm} (S5)

The volume where particles are illuminated strongly enough for detection can be calculated knowing that the laser beam is cylindrical with radius $r$ and the particles are imaged in a segment of length $L$. An expression for the detection volume $V_{\text{det}}$ is then obtained using simple geometry:

$$V_{\text{det}} = L \pi r^2$$  \hspace{1cm} (S6)

Inserting equation S5 for $r^2$ gives:

$$V_{\text{det}} = L \pi c \ln \left( \frac{KA}{I_{\text{thresh}}\sqrt{\pi c}} \right)$$  \hspace{1cm} (S7)

The sensitivity of the NTA instrument is likely to be proportional to the detection volume. As $V_{\text{det}}$ increases with scattering power, the detection volume becomes first limited by boundaries of the imaged area. With further increase in the ability to scatter light, the depth of focus of the
microscope sets the limit of the detection volume in the direction of the optical axis. Therefore equation S7 can be used straightforwardly only close to the visualization limit.

**Calculations of NTA sensitivity.** The light scattering ability of the silica particles as function of size were calculated using Scatlab software with the option ”direct intensity graph”. The following parameters were entered to the Mie box (The rest being zero). Environment refractive index: \( n_1 = 1.3329 \), Real part of particle refractive index: \( n_2 = 1.50 \) Wavelength of incident light: \( \lambda = 0.650 \, \mu m \). To account for the range of angles for which the microscope collect light the calculations were repeated for scattering angles ranging from 70° to 110° with 5° spacing and averaged. Only the component polarized in the plane of the optical flat was taken into consideration. The detection volumes were calculated using equation S7. The scattering powers obtained in the calculations above were used as the parameter \( K \). The \( I_{\text{thresh}} \) was set to \( 5 \times 10^{-7} \) in order to produce visualization limits close to what seems to be the smallest silica particles detected. The calculations were repeated for two different intensity profiles \( c=1 \), and \( c=10 \). The value of the parameter \( A \) in equation S7 was 1. \( L \) was set to 2. Since the actual beam shape of the NTA instrument is not known, only qualitative agreement with experimental results can be achieved.

**References**
