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1 Test criteria for method development

1.1 Recovery calculation

Three different recoveries were defined: Si bulk mass recovery, sample recovery following AF separation, and total Si recovery.

1.1.1 Si bulk mass recovery (rec$_{Si,bulk}$)

The Si bulk mass recovery (rec$_{Si,bulk}$) is defined as

$$\text{rec}_{Si,bulk} = \frac{c_{Si,sample,ICP-OES}}{c_{Si,initial}} \times 100\%$$  \hspace{1cm} (Equation 1)

where ($c_{Si,sample,ICP-OES}$) is the Si mass concentration after the sample preparation procedure, as determined by ICP-OES analysis, and ($c_{Si,initial}$) is the initial Si mass concentration, calculated from the SiO$_2$ mass concentration in the stock solutions and converted into SiO$_2$ concentration of the respective sample (compare Table 1 of main the text).

1.1.2 AF$^4$ recovery (rec$_{AF^4}$)

The AF$^4$ recovery (rec$_{AF^4}$) is based on the MALS data acquired during AF$^4$ analysis. Assuming that no significant quantities of particles other than SiO$_2$-ENPs are present in the suspension, the MALS signal can be considered to be semi-quantitative. The AF$^4$ recovery after separation in the AF$^4$ system is defined as:

$$\text{rec}_{AF^4} = \frac{A_{FFF,sample}}{A_{Rec,sample}} \times 100\%$$  \hspace{1cm} (Equation 2)

where ($A_{FFF,sample}$) is the area under the peak for an AF$^4$ run with field force (i.e. cross flow) and ($A_{Rec,sample}$) is the area under the peak for an AF$^4$ run without field force.

There were usually three peaks in the fractogram following AF$^4$-separation, the void peak, the sample peak, and the release peak (Figure A-1). The void peak was due to unretained particles and the release peak to particles temporarily attached to the membrane. The sample peak represents the fractionated particles. All three peaks were used for the recovery calculations.
Figure A-1: AF₄-fractogram monitored with the MALS signal

Since AF₄-recovery is only semi-quantitative carry-over of particulate material introduces the risk that the SiO₂-ENP recovery might be overestimated due to the false detection of particles by MALS, which are not SiO₂-ENPs.

1.1.3 Total Si mass recovery (rec_{Si,total})

Total Si mass recovery (rec_{Si,total}) after size separation is determined by:

\[
\text{rec}_{\text{Si,total}} = \frac{n_{\text{Si,sample,ICP-MS}}}{n_{\text{Si,initial}}} \cdot 100 \ [\%] \quad (\text{Equation 3})
\]

where \(n_{\text{Si,sample,ICP-MS}}\) is the total Si mass content following size separation, as determined by Si mass quantification using ICP-MS coupled online to the AF₄-system, and \(n_{\text{Si,initial}}\) is the known initial Si mass content.

1.2 Particle size distribution calculation

1.2.1 Approach 1 based on MALS:

ASTRA Software (Version 5) provides eight different models (Zimm, Debye, Berry, Random Coil, Sphere, Coated Sphere, Rod, and Mie) for calculating the radii of particles from the MALS data. Additionally, it allows the user to select between various fitting orders such as Debye 3rd order. The
quality of size fitting for spherical particles (Nanosphere™ particle size standards) in the size range of the analysed SiO$_2$-ENPs decreases in the following order: Sphere > Debye 3$^{rd}$ >> Berry > Zimm $^1$. For heterogeneously shaped (essentially non-spherical) particles the quality of the size fitting decreases in the following order: Zimm > Debye >> Sphere & Berry. All models were tested and one particular model selected on the basis of the available information on particle shape, and on a comparison of the model results with size data (hydrodynamic radius) derived from the second approach. We decided to use the Debye 3$^{rd}$ order model to calculate the size distributions from the MALS data because of its robustness and fitting capabilities for both spherical and non-spherical particles $^1$. The Debye model also provides the radius of gyration, which is otherwise known as the root mean square radius ($r_{\text{rms}}$). The $r_{\text{rms}}$ (as defined in von der Kammer et al.$^1$) takes into account the mass distribution within the particle and is hence shape sensitive $^1$.

The size distribution function obtained by MALS is intensity weighted. However, since the MALS measurement is done after the particle size fractionation in the AF$^4$ small particles are still detectable.

1.2.2 Approach 2 based on AF$^4$ calibration:

The $r_h$ size distribution was determined after AF$^4$-calibration with polystyrene standards (PS standards with diameters of 50, 100, and 150 nm: Thermo Scientific). A linear size calibration method employed resembled the typical $\lambda = 6 \cdot R$ linear approximation (R retention factor) in FFF theory and follows the equation: $t_R = 0.0856 \cdot r_h + 0.0578$ with $R^2=0.999$.

2 Methodology for the separation of SiO$_2$ nanoparticles

2.1 Sample homogenization (step I)

Three different techniques were tested for homogenization of the tomato soup samples (either as stand-alone techniques or in combination):

(1) manual agitation: The sample was shaken by hand for 15 seconds. Fat and organic fiber material were dispersed and suspended in the aqueous solution.
(2) heating: The sample was heated at 50°C in a water bath for 30 minutes, during which time fatty constituents of the tomato soup were dispersed and dissolved.

(3) mechanical mixing: An IKA T10 basic Ultra Turrax stainless steel dispersing instrument was operated for 30 seconds at 20,000 - 25,000 rpm, broke down most of the organic fiber material and dispersed the fat.

Following each of the tests an aliquot of 1.5 g was removed from the stock suspension using a stainless steel sampling spoon.

2.2 Acid digestion of tomato soup matrix (step II.1)

Two different procedures were tested for acid digestion in order to optimize the energy input. The two types of acid digestion were performed with the samples SiO$_2$-ENP, TS+SiO$_2$-ENP, and TS+SiO$_2$-ENP$_{aged}$. Additional blank MQ-water and tomato soup samples were run for quality control. The initial SiO$_2$-ENP and organic matter concentrations were similar in all samples.

1) Heating and bath sonication. 2 mL of tomato soup, 0.5 mL of SiO$_2$-ENP, and 7.5 mL of MQ-water were first well mixed. The SiO$_2$ mass concentration in the mixture was 2 g$_{SiO_2}$ L$^{-1}$. Blanks were prepared with 2 mL of pure tomato soup and 8 mL of MQ-water. 2 mL of this solution were added to 8 mL of HNO$_3$ (65% Merck, Suprapure®) and 2 mL of H$_2$O$_2$ (30% Merck super pure), to make a total volume of 12 mL. The prepared suspension was then sonicated for 30 minutes at 90°C. Following temperature adjustment to 25°C the sample was diluted with MQ-water by a factor of 1:10. The sample was further diluted with MQ-water in two steps (1:50 and 1:100) to achieve 1:5,000 dilution for ICP-MS analysis.

2) Microwave-assisted digestion. 2 mL of the sample were first poured into the Teflon® digestion tubes. In order to oxidize the organic carbon matrix, 1 mL H$_2$O$_2$ (30%; Merck supra pure) and 5 mL HNO$_3$ (65%; Merck supra pure) were added prior to microwave assisted digestion (Microwave 3000, Anton Paar, USA), which results in an increase in pressure and temperature. Samples were heated stepwise for 27 minutes (0-5 min: 0-250 W; 5-7 min: 250 W; 7-12 min: 250-750 W; 12-27 min: 750 W; cooling: 27-42 min: 0 W), achieving a maximum temperature of 200°C. The pressure maximum in each digestion tube was set to 60 bar. After cooling down, the acidic samples were transferred from
the digestion tubes to volumetric flasks and filled to 100 mL using MQ-water, introducing a 1:50 sample dilution. The acidic samples were stored in 100 mL PE bottles.

To determine the Si bulk mass recovery the digested samples were tip sonicated for 90 seconds (0.05 kJ mL⁻¹, Bandelin Sonoplus, Germany) and 3 mL then diluted 1:10 with FFF carrier solution (0.025% (v/v) FL-70™). The diluted samples were then immediately analyzed for Si content by ICP-OES.

2.3 Colloidal extraction (step II.2)

During colloidal extraction the separation of particles from the matrix was attempted by the addition of extractants to obtain individual ENPs, by dilution, and using mechanical energy input to destroy aggregates of SiO₂-ENPs and matrix components (fat, fibers). The all type of colloidal extractions were performed with the samples SiO₂-ENP, TS+SiO₂-ENP, and TS+SiO₂-ENP aged. Each of the extraction agents was adjusted to a pH of 9 prior to the experiments. Indeed, SiO₂-ENP suspensions show improved colloidal stability in slightly alkaline conditions, the pH was adjusted to 9 when necessary, using NaOH. The ENP-surfaces are then negatively charged (PZC = 2; zeta potential (pH=9) < -30 mV) and aggregation of SiO₂–ENPs is less likely to occur. However, the ionic strength of the extractants was not matched.

For all colloidal extraction 1.5 g of samples (TS+SiO₂-ENP or TS+SiO₂-ENP aged) were mixed with 13.5 mL of extractants. 3 types of extracting agent were used:

MQ-water was used for baseline testing to determine the individual effects of dilution and mechanical energy input.

Ammonium carbonate (0.25, 2.5, and 25 mmol L⁻¹). Ammonium carbonate (AC) was selected as a buffer because of its compatibility with later ICP-MS analysis. High AC concentrations (c(AC)=25 mmol L⁻¹ and IS = 75 mmol L⁻¹) are likely to destabilize any other particles in the suspension because of the ionic strength. SiO₂-ENPs were, however, stable under these conditions as indicated by preliminary tests (Table A-2).

FL-70™ solution (0.025, and 0.05% (v/v)). The FL-70™ solution, as a mixture of a variety of surfactants (ionic and anionic), has the potential to stabilize particles with heterogeneously charged surfaces. Besides, FL70 has shown stabilizing properties during FFF separations.
In addition to the type of extraction agent used, the mechanical energy input and agitation time were also tested for their effects on Si mass recovery and method duration. In order to accelerate SiO$_2$-ENP extraction mechanical energy was provided either by agitation or by sonication. To determine the minimum extraction time required for maximum recovery the mixture was agitated for 0.5, 1, 3, 5, 8, 16, 24, and 72 hours at 200 rpm on a horizontal shaker. The mixture was then centrifuged for 30 minutes at 1,700 rpm (cut off equals 400 nm, Jouan CR422, Thermo Scientific, USA) and the Si content of the supernatant analyzed using ICP-OES. Particle characterization was performed using DLS and AF$^4$-MALS. Particulate matter content was further quantified by UV absorbance measurements at 280 nm (UV/vis Spectrometer, Lambda 35, Perkin Elmer).

2.4 Particle concentration enrichment (step III)

Particle concentration enrichment (III) and particle stabilization (IV) were carried out immediately after microwave digestion. Subsamples (each of 20 mL) of acid digested solutions were centrifuged at 4,500 rpm for 15 minutes (Jouan CR 422, USA) in order to enrich the ENP concentration. After centrifugation a 10 mL volume of the supernatant was analyzed by ICP-OES in order to determine the mass of Si which was not concentrated during centrifugation. From this measurement the Si mass in the remaining liquid (i.e. concentrate) was found to have increased by a factor of approximately 2.4. The ENP concentration in the colloidal extract (II.2) was not enriched.

2.5 Stabilisation of the particles (step IV)

The objective of the ENP stabilization was twofold: (a) to break up any aggregates that had formed during the separation, and (b) to prevent re-aggregation during further analysis. The colloidal extraction required no further particle stabilization as the pH and ionic strength in the extract did not promote particle aggregation or dissolution. However, acid digestion introduced marked changes in the hydrochemical conditions (pH, ionic strength). Indeed, the point of zero charge (PZC) of SiO$_2$-ENPs (between 2.2 and 3.4 $^2$) was crossed during acid digestion, aggregation of the particles was likely. Therefore, the particle suspensions needed to be stabilized by re-adjusting the pH and ionic strength in order to avoid particle aggregation or dissolution. Adjustment of the ionic strength was
achieved through the use of a dilution/extraction agent (e.g. salt or detergent solution). In practice the suspension was diluted by the factor of 10 in 0.025% FL-70™ solution. The pH adjustment to values between 7 and 8 was achieved by adding NaOH solution (0.1 or 0.01 mol L⁻¹). After 12 hours the suspensions of the stabilized particles were again characterized. Besides, sonication was used following pH adjustment to break up any possible aggregates/agglomerates. Size distributions after sonication for 0, 45, 90, and 120 seconds were compared with the original size distribution of the undigested reference sample.

3 Characterization of the pure SiO₂-ENP suspension

As a first step the pure particle suspension was characterized in terms particle size distribution and the recovery was calculated for the analytical method. The data were used as a benchmark and compared with the particle size distribution of the extracted SiO₂-ENPs from tomato soup by various sample preparation methods. Additional data on characterization of the pure particle suspension is also provided by Grombe et al.³

3.1 Recovery

Following water bath sonication and dilution to approximately 100 mg L⁻¹ the AF⁴ mass recovery (rec_{AF⁴}) from the pure SiO₂-ENP suspension was 90%. Total Si mass recovery (rec_{Si,total}) was in the same range (97%). The mass loss was attributed to an accumulation of material on the surface of the membrane, which was not released during sample fractionation. Regular system-cleaning runs were therefore performed (by injecting 10 µL of iso-propanol) in order to avoid long-term accumulation of SiO₂-ENPs within the FFF system. Recovery data indicated that the applied AF⁴ size separation worked sufficiently.

3.2 Particle size distributions

The hydrodynamic radius (r_{h,DLS}) obtained from the DLS measurements was 68 nm. The r₃-size distribution obtained from the AF⁴-calibration had its maximum at 63 nm, with a standard deviation (s.d.) of 2 nm from the mean value (Figure 2, main manuscript). The median hydrodynamic radius (r_{h,median}) derived from AF⁴-calibration of 70 ± 5 nm was larger than the mode and the mode/median
ratio was 0.90 ± 0.04, indicating a tailing of the size distribution. Since the MALS-derived light
scattering intensity is used to establish a size distribution from AF^4 fractograms, the distribution
function obtained is intensity weighted. This weighting becomes more pronounced as the detection
angle decreases (i.e. particle size increases), and the intensity-weighted size distributions of poly-
disperse samples are therefore biased towards larger radii. For particles with a constant, known
stoichiometry a true particle mass based size distribution (which is not affected by the particle size)
can be derived from the ^28Si ICP-MS signal, which was recorded online following size separation
using AF^4. As expected, the Si mass based size distribution (based on the r_h) derived from ICP-MS
quantification showed generally smaller particle sizes than the MALS-based size distribution. The
median r_h was determined to be 54 nm and the mode 43 nm, resulting in a mode/median ratio of 0.80.
The mass-based size distribution approached a lognormal distribution, as depicted in Figure 2 of the
main manuscript.

Similar SiO_2-ENPs were used to spike the tomato soup. The size distribution of SiO_2-ENPs extracted
from the tomato soup is expected to show similar properties. Therefore, the sample preparation was
adjusted until both size distributions (from the pure suspension and from particles extracted from the
tomato soup) were likewise. In order to identify possible bias of the size distribution due to sample
preparation both pure particle suspensions and tomato soup were treated by the respective sample
preparation procedure.

The r_mis based on MALS data increased linearly across most of the size distribution (Figure 2 a, main
manuscript). Exceptions were small particles with r_h < 30 nm and large particles with r_h > 100 nm. The
r_mis data in the lower range of the size distribution (< 30 nm) was likely influenced by incomplete void
peak separation, as indicated by increasing radii towards the void peak. The r_mis/r_h ratio, which can be
used as an indicator of particle shape, had values between 1.0 and 1.2 indicating an ideal particle size
separation, but also indicating that the fumed silica particles are not homogeneous spheres.¹
4 Acid digestion for extraction of SiO$_2$-ENPs from tomato soup

4.1 Recovery

The Si bulk recovery ($\text{rec}_{\text{Si,bulk}}$) was close to 100% for all samples using either sonication + heating or microwave-assisted digestion. However, organic matrix oxidation was more complete with microwave assistance than with sonication and heating, as was indicated by the loss of the yellowish colour in the sample suspension following microwave-assisted digestion. Microwave-assisted acid digestion was therefore used to further optimize sample preparation.

4.2 Particle morphology

Possible alteration of SiO$_2$-ENPs, caused acid digestion, were investigated by TEM analysis (CM 100 BioTWIN at 80kV). Image analysis did not indicate a change in particle morphology or size of the primary particle size due to acid digestion (Figure A-2).

![TEM analysis of a) SiO$_2$-ENPs and b) SiO$_2$-ENPs extracted from tomato soup via acid digestion after tip sonication (90 s), both size bars 200 nm](image)
4.3 Particle distribution after sonication (step IV)

Parameters of the size distribution of SiO$_2$-ENPs extracted from tomato soup after different times of tip sonication are provided in Table A-1. Indications that the composition of the dilution media had effect on the size distribution (0.025% FL-70$^\text{TM}$ or 0.25 mmol L$^{-1}$ AC) were observed. For practical considerations regarding AF$^4$ separation 0.025% FL-70$^\text{TM}$ was selected for use as dilution agent and stabilization agent for separated SiO$_2$-ENPs.

Table A-1: Peak evaluation after different sonication times for the pure SiO$_2$-ENP suspension after acid digestion based on the MALS signal; uncertainty is expressed as standard deviation from the mean value of triplicate analysis

<table>
<thead>
<tr>
<th>sonication time [s]</th>
<th>stabilization agent [-]</th>
<th>$r_h$, mode [nm]</th>
<th>$r_h$, median [nm]</th>
<th>peak shape</th>
<th>peak shape factor [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>FL-70$^\text{TM}$</td>
<td>137 ± 1</td>
<td>126 ± 1</td>
<td></td>
<td>1.09</td>
</tr>
<tr>
<td>45</td>
<td>FL-70$^\text{TM}$</td>
<td>111 ± 1</td>
<td>108 ± 1</td>
<td></td>
<td>1.03</td>
</tr>
<tr>
<td>90</td>
<td>FL-70$^\text{TM}$</td>
<td>97 ± 1</td>
<td>101 ± 1</td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>90</td>
<td>AC</td>
<td>107</td>
<td>126</td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>135</td>
<td>FL-70$^\text{TM}$</td>
<td>88 ± 1</td>
<td>93 ± 3</td>
<td></td>
<td>0.95</td>
</tr>
</tbody>
</table>

Colloidal extraction of SiO$_2$-ENPs from tomato soup

The objective of Colloidal extraction is separating the SiO$_2$-ENPs without any destruction or dissolution of the matrix. It is a far gentler and less invasive method that could potentially also be used for more vulnerable particles such as silver-ENPs or copper-ENPs.

5.1 Colloidal extraction: Si mass recovery

The maximum rec$_{Si,bulk}$ was obtained with extraction by 2.5 mmol L$^{-1}$ AC-solution, but this corresponds to only 12 ± 2% (Table A-2). The extraction efficiency followed the order of (AC) > (FL-70$^\text{TM}$) > (MQ-water) (Tab. A-2). The data suggest that none of the extraction agents were able to break
the bonds between silica particles and particulate tomato soup matrix within the 30 minute extraction period.

Table A-2: Si mass recovery ($\text{rec}_{\text{Si,bulk}}$ [%]) after a 30 minute extraction period for tomato soup spiked with SiO$_2$-ENPs (both aged and freshly spiked) and for pure SiO$_2$-ENP suspension, using different concentrations of ammonium carbonate and FL-70$^{\text{TM}}$. Errors are indicated by single standard deviations calculated from triplicates

<table>
<thead>
<tr>
<th>Type of extraction agent and concentration</th>
<th>SiO$_2$-ENP [%]</th>
<th>TS+SiO$_2$-ENP [%]</th>
<th>TS+ SiO$<em>2$-ENP$</em>{\text{aged}}$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ammonium carbonate (mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>90±9</td>
<td>n/a</td>
<td>2±0</td>
</tr>
<tr>
<td>2.5</td>
<td>94±4</td>
<td>90±3</td>
<td>12±2</td>
</tr>
<tr>
<td>25</td>
<td>93±5</td>
<td>93±2</td>
<td>7±4</td>
</tr>
<tr>
<td>FL-70 (%, v/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>85±4</td>
<td>n/a</td>
<td>1±0.2</td>
</tr>
<tr>
<td>0.25</td>
<td>90±3</td>
<td>n/a</td>
<td>5±1</td>
</tr>
<tr>
<td>2.5</td>
<td>84±4</td>
<td>n/a</td>
<td>5±3</td>
</tr>
<tr>
<td>MQ-water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>86±3</td>
<td>n/a</td>
<td>1±1</td>
</tr>
</tbody>
</table>

5.2 Colloidal extraction: particle size distribution

Following colloidal extraction, particles in the tomato soup extracts showed a six times higher UV-light absorbance at 280 nm (abs$_{280}$=0.59 mAU) than those in the pure particle suspension (abs$_{280}$=0.09 mAU), indicating poor separation of the target particles from the matrix and the presence of matrix residue within the extract. The total particulate matter content after colloidal extraction followed by centrifugation was too high for further size characterization with FFF-ICP-MS. Filtration (5 µm, nylon) was therefore used as an additional clean-up step, but this resulted in a significantly lower Si mass recovery. The Si mass recovery fell from 26% to 20% after filtration, which is equivalent to a Si mass loss of 21%. Incomplete separation during the sample preparation meant that particle size and concentration could not be correctly determined by FFF-MALS-ICP-MS.
6 Signal intensities during ICP-MS analysis of SiO$_2$-ENPs

The response of the mass detector depending on different particle size is depicted in Figure A-3. All particle sizes were measured at similar concentrations and there was no difference in signal intensity observed. The signal noise increased with increasing particle size and constant Si concentration which results in a decreased measurement precision of the ICP-MS.

Figure A-3: ICP-MS $^{28}$Si signal intensities from the dissolved standard and from 100, 500, and 1000 nm SiO$_2$-ENPs (Postnova analytics), all at similar mass concentrations

7 Stability of SiO$_2$-ENP suspension

Simovic & Prestidge$^4$ reported a critical coagulation concentration for hydrophobic silica (0.25% (m/m)) of between 10 and 100 mmol L$^{-1}$ ionic strength at pH values of 7 and 9, respectively. Preliminary tests with different ionic strength concentrations have confirmed the data shown here (Table A-3).

Table A-3: Hydrodynamic radii determined by DLS of SiO$_2$-ENPs in suspension (100 mg L$^{-1}$) at various ionic strengths (measured as triplicates)

<table>
<thead>
<tr>
<th>c(NaCl) mmol L$^{-1}$</th>
<th>1 h average (nm)</th>
<th>1 h std.dev. (nm)</th>
<th>3 h average (nm)</th>
<th>3 h std.dev. (nm)</th>
<th>5 h average (nm)</th>
<th>5 h std.dev. (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>67</td>
<td>0.5</td>
<td>65</td>
<td>0.5</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>64</td>
<td>1</td>
<td>65</td>
<td>0.5</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>66</td>
<td>1</td>
<td>76</td>
<td>1</td>
<td>85</td>
<td>1</td>
</tr>
<tr>
<td>150</td>
<td>110</td>
<td>4</td>
<td>254</td>
<td>8</td>
<td>181</td>
<td>7</td>
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</tbody>
</table>
8 References


