RESEARCH PAPER

The potential of asymmetric flow field-flow fractionation hyphenated to multiple detectors for the quantification and size estimation of silica nanoparticles in a food matrix

Julien Heroult · Volker Nischwitz · Dorota Bartczak · Heidi Goenaga-Infante

Abstract This work represents a first systematic approach to the size-based elemental quantification and size estimation of metal(loid) oxide nanoparticles such as silica (SiO$_2$) in a real food matrix using asymmetric flow field-flow fractionation coupled online with inductively coupled plasma mass spectrometry (ICP-MS) and multi-angle light scattering (MALS) and offline with transmission electron microscopy (TEM) with energy-dispersive X-ray analysis (EDAX). Coffee creamer was selected as the model sample since it is known to contain silica as well as metal oxides such as titania at the milligramme per kilogramme levels. Optimisation of sample preparation conditions such as matrix-to-solvent ratio, defatting with organic solvents and sonication time that may affect nanoparticle size and size distribution in suspensions was investigated. Special attention was paid to the selection of conditions that minimise particle transformation during sample preparation and analysis. The coffee creamer matrix components were found to stabilise food grade SiO$_2$ particles in comparison with water suspensions whilst no significant effect of defatting using hexane was found. The use of sample preparation procedures that mimic food cooking in real life was also investigated regarding their effect on particle size and particle size distribution of silica nanoparticles in the investigated food matrix; no significant effect of the water temperature ranging from ambient temperature to 60 °C was observed. Field-flow fractionation coupled to inductively coupled plasma-mass spectrometry (FFF-ICP-MS) analysis of extracts of both unspiked coffee creamer and coffee creamer spiked with food grade silicon dioxide, using different approaches for size estimation, enabled determination of SiO$_2$ size-based speciation. Element-specific detection by ICP-MS and post-FFF calibration with elemental calibration standards was used to determine the elemental composition of size fractions separated online by FFF. Quantitative data on mass balance is provided for the size-based speciation of the investigated inorganic nano-objects in the complex matrix. The combination of FFF with offline fractionation by filtration and with detection by ICP-MS and TEM/EDAX has been proven essential to provide reliable information of nanoparticle size in the complex food matrix.

Keywords Asymmetric flow field-flow fractionation · MALS · ICP-MS · TEM · Mass balance · Quality control · Silica nanoparticles · Coffee creamer

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Introduction

The presence of nanomaterials (with size range from approximately 1 to 100 nm) [1] in food is not new. The addition of nanomaterials to food has increased over the last decade since they may offer benefits to both industry and consumers. For instance, inorganic nanoparticles have been used as food additives for enhancing colour or flavour for preservation and to facilitate manufacturing processes. Examples of relevant nanomaterials in the food industry are Ag nanoparticles, which are used in food packaging to improve conservation due to antibacterial activity, TiO$_2$ (whitener) added to sweets, candies and chewing gums or silica (SiO$_2$) (anticaking) added

to salt, soups or coffee creamers [2–5]. Despite their wide use, nanomaterials may pose new risks as a result of their novel properties. In view of this potential hazard, regulatory and scientific assessment requires an understanding of exposure to both humans and the environment.

Despite the growing awareness of the use of nanomaterials in food preservation and processing and the concern about their possible toxic effects, there is no definitive proof for toxicity. This is due to the lack of standardised protocols and validated methods for nanotoxicity and human risk assessment [6, 7]. Moreover, until recently, metrology focused on the development of methods to characterise nanomaterials for their physical properties in their powder forms or in simple matrices. Therefore, there is an increasing need for traceable methods and reference materials for quality assurance of nanoparticle characterisation [8] in complex matrices including food [9, 10]. Such methods will help support upcoming regulation and enable quality control of existing products. In particular, the extraction of nanomaterials from the solid food sample without altering their properties is an important challenge [9, 10].

Given the complexity of nano-objects and of the matrix in most consumer products, the use of a single measurement technique has often resulted in their ambiguous detection and characterisation. The use of multi-method approaches has, therefore, been proven essential to provide improved information and reduced bias, especially for nano-objects in complex matrices. Such approaches, although time consuming and expensive, have the advantage of including a range of techniques that provide information on properties of nano-objects (e.g. size, size distribution, elemental composition and isotopic ratio, surface charge, shape, agglomeration and aggregation, etc.), which may be useful to their unambiguous detection and characterisation in their complex environment. Amongst the range of techniques available for nanomaterial characterisation, field-flow fractionation in combination with elemental and sizing detectors has emerged as a highly promising approach for size-based elemental speciation in complex environmental samples and consumer products [5, 10–12]. Whilst there are already several examples of using FFF for nanomaterials in environmental samples [10], the literature dealing with NPs in food is relatively scarce; a few examples include the use of FFF for the analysis of milk suspensions [13] and liposomes [14]. More recently, the use of FFF combined offline with atomic absorption spectrometry and sizing detectors has been proposed for the characterisation of silica particles used as food additives [15]. Quality control of the proposed methodology, e.g. by spiking experiments and/or mass balance calculations, was not undertaken in this study. To the authors' knowledge, no report on a systematic comparison and optimisation of extraction conditions on the reliable quantitation and size characterisation of inorganic nanoparticles in a real food matrix using FFF combined online with multiple detectors has been published so far. Moreover, most FFF applications to complex samples so far pay very little attention to quantitative element size-based fractionation and mass balance determination.

This work aims to overcome some of these remaining challenges by developing a first systematic optimisation of sample preparation and analysis conditions for the size-based elemental quantification and size estimation of metal(loid) oxide nanoparticles such as SiO_2 in a real food matrix using asymmetric flow field-flow fractionation coupled online with multiple detectors. Silica was selected as target nanomaterial due to the fact that amorphous silica has been used for many years in food applications, such as for clearing beers and wines and as an anticaking agent to maintain flow properties in powder products [3]. Commercially available coffee creamer was selected as a model sample for a complex food matrix. The effect of sample preparation conditions such as matrix-to-solvent ratio, defatting using hexane and sonication time on nanoparticle size and nanoparticle size distribution in suspensions was investigated. Spiking experiments with food grade SiO_2 nanomaterials of known primary size were performed to assist in the optimisation of sample preparation and size-based speciation analysis using FFF coupled to inductively coupled plasma mass spectrometry (ICP-MS). Special attention was paid to the selection of conditions that minimise particle transformation during sample preparation and analysis. The use of sample preparation procedures that mimic food cooking in real life was also investigated to obtain meaningful results regarding particle size and particle size distribution of SiO_2 nanomaterials in the coffee creamer matrix. Element-specific detection by ICP-MS and post-FFF calibration with Si calibration standards was used to determine the elemental composition of size fractions separated online by FFF. Quantitative speciation data was used to generate mass balance data for the Si additive in a real food matrix. The potential of FFF in combination with multi-angle light scattering (MALS), ICP-MS, and transmission electron microscopy (TEM) with energy-dispersive X-ray analysis (EDAX) was investigated for size estimation of the Si-containing fraction detected by field-flow fractionation coupled to inductively coupled plasma-mass spectrometry (FFF-ICP-MS).

Experimental

Chemicals and standards

Nitric acid (ultrapure), hydrogen peroxide (ultrapure), hydrofluoric acid (suprapure) and single element stock solution of Si (970 mg kg^{-1}) were purchased from Romil, Cambridge, UK. Hexane (HPLC grade) was obtained from Rathburn, Walkerburn, UK. Boric acid (suprapure) was supplied from VWR International, Darmstadt, Germany.

Deionised water (18 MΩ cm) was prepared by an ELGA PURELAB Flex system (ELGA, Veolia Water, Marlow, UK). The commercial surfactant mixture FL-70 (containing tetrasodium ethylenediaminetetraacetate, sodium oleate, sodium bicarbonate, sodium carbonate, triethanolamine oleate, water, polyethylene glycol and alcohols C12-14-secondary, ethoxylated) was purchased from Fisher Scientific UK Ltd, Loughborough, UK. NovaChem 100 surfactant (mix of ionic and nonionic surfactants) was purchased from Postnova, Landsberg, Germany.

Aqueous suspensions of gold nanoparticles with nominal particle sizes of 10 nm (RM 8011), 30 nm (RM 8012) and 60 nm (RM 8013), used only for the daily check of the FFF performance, were purchased from the National Institute of Standards and Technology (NIST). Food grade SiO_2 nanoparticles (Aerosil 200F, fumed hydrophilic silica) with 12 nm average primary particle size were supplied by Lawrence Industries, Tamworth, UK.

Sample and sample preparation protocol

A commercially available low-fat coffee creamer containing SiO_2 and TiO_2 (as shown on the label) was purchased from a local shop. Before taking a subsample, the container was thoroughly shaken. About 0.5 g of sample was accurately weighed in a 50-mL tube, and 20 mL of deionised water was added and the weight accurately recorded. The tube was thoroughly shaken for 1 min to ensure homogeneous suspension of the particles and then vortexed for 30 s. This suspension was filtered using a 5-μm syringe filter (cellulose acetate, Sartorius Stedim, Epsom, UK) prior to analysis by asymmetric flow field-flow fractionation. All extractions were performed at least in duplicate.

Defatting of the sample prior to extraction

Optionally, the sample was first defatted by adding 10 mL of hexane to 0.5 g of sample, shaking thoroughly for 1 min and sonication for 2 min, followed by settling time of at least 1 h and subsequent centrifugation at 3,000 rpm for 10 min. The hexane was carefully removed from the solid residue using a pipette. Then, 20 mL of deionised water was added, followed by thorough manual shaking and vortexing prior to sonication for 30 min. The suspension was finally filtered through a 5-μm syringe filter (cellulose acetate) prior to analysis.

Sample spiking experiments

Spiking with Aerosil SiO_2 particles was performed by suspending an accurately weighed amount of the particles (typically 1 to 3 mg) first in water and subsequently adding this suspension to the solid coffee creamer prior to extraction with water. Direct addition of an exactly known small amount of the solid Aerosil particles to the solid coffee creamer was not possible due to the electrostatic properties of the particles.

Sequential filtration for sample cleanup prior to size determination by FFF-MALS

The coffee creamer suspension (without defatting and without sonication) was first filtered through 5-μm syringe filter (cellulose acetate). The filtrate was then subjected to filtration through 0.45 μm (cellulose acetate), followed by 0.2 μm (cellulose acetate) and finally by 0.1 μm (PVDF). Each intermediate filtrate and the final filtrate was analysed by FFF-MALS-ICP-MS.

FFF combined with ICP-MS, MALS and TEM with EDAX

A metal-free AF2000 MT asymmetric flow field-flow fractionation (Postnova) instrument was coupled online to MALS and ICP-MS detection. FFF was operated with a regenerated cellulose (RC) membrane with 10 kDa molecular weight cutoff and a spacer of 350 μm. Deionised water (filtered through 0.1-μm membrane) was used as carrier. Manual injection was performed using a 20-μL loop and 4-min focusing time (tip flow 0.2 mL min^{-1}, focus flow 2.3 mL min^{-1}). Prior to injection, the samples were thoroughly mixed to ensure homogeneous distribution of suspended particles. A constant cross flow of 2 mL min^{-1} was applied, and the flow rate to the detector was kept at 0.5 mL min^{-1}. A transition time of 0.2 min was used. After 28.8 min, the cross flow was reduced to 0 mL min^{-1} within 2 min and kept at 0 mL min^{-1} for 5 min to remove any residual particles from the separation channel (40 min total run time). The fractograms are shown including the focus step to prove that there are no particles eluting during this step. The channel outlet was connected to multi-angle light scattering detection (PN3621, Postnova) and to ICP-MS.

ICP-MS detection was performed using an Agilent 7700 (Agilent Technologies, Tokyo, Japan) instrument (in hydrogen mode) equipped with a micromist nebuliser and Scott spray chamber. Before entering the ICP nebuliser, the FFF eluate was mixed with 3 % nitric acid solution containing 10 μg kg^{-1} of Ge as internal standard via a polyether ether ketone (PEEK) tee-piece. Isotopes m/z 28 and 29 were monitored for Si with integration time of 0.5 s as well as isotope m/z 72 for the internal standard.

The Si eluting from the FFF channel was quantified by a post-channel calibration approach as described before [11, 16]. Briefly, calibration was performed by replacing the post-column diluting nitric acid with calibration standards containing the same amount of nitric acid and internal standard but increasing concentrations of Si. ICP-MS measurement was performed running the FFF with the same cross flow and detector flow as during sample analysis but without injection. The resulting calibration was applied to convert the

FFF fractograms into mass flow chromatograms. The eluting amount of Si was determined by integration and the concentration in the sample calculated using the injection volume. For daily check of the FFF performance, a mixed standard of NIST gold nanoparticles (10, 30 and 60 nm nominal size) was prepared daily in deionised water (150-fold dilution) and analysed with the same FFF conditions as the sample extracts monitoring m/z 197 with ICP-MS in standard mode.

For TEM/EDAX analysis, the FFF nano-silica fraction was collected over 2 min (total collected volume of 1 mL). An aliquot of 0.5 mL was placed in a centrifugal filter tube (Amicon Ultra, 3 kDa) and centrifuged for 30 min at 14,000×g, until the sample was concentrated to 50 μL. A droplet of the concentrated coffee creamer suspension was deposited on a TEM grid (carbon film on 400-mesh copper, Agar Scientific) and air dried. TEM grids were imaged with FEI Technai-12 Transmission Electron Microscope operating at 80-kV bias voltage and equipped with TEAM™ EDS Appollo XLT X-ray microanalysis platform (Biomedical Imaging Unit, Southampton, UK).

Microwave digestion for total Si determination

Samples were digested using an Ethos microwave (Milestone, Sorisole, Italy). Coffee creamer (0.1 g) or 5 g of coffee creamer suspension was accurately weighed in Teflon digestion vessels in duplicate. Four milliliters of nitric acid, 4 mL of hydrogen peroxide and 50 μL of hydrofluoric acid were added. Deionised water was used to top up to an equal total volume (minimum requirement of the vessels is 9 mL). Digestion was performed by increasing the microwave power within 10 min to 1,000 W and keeping this power for 20 min with a maximum temperature limit of 180 °C. After cooling down to room temperature, the vessels were opened briefly to add 500 μL of saturated aqueous solution of boric acid, and then, the digestion programme was repeated to ensure complete reaction of excess HF with boric acid [17]. The digests were then transferred to 50-mL sample containers and topped up to 40 g with deionised water. In absence of a suitable matrix reference material, recovery measurements on samples spiked with inorganic silica standards prior to digestion were performed for quality control of the methodology used for total Si determination.

Determination of total Si by ICP-MS

Total Si in the digested sample, extracts and standards was determined by collision reaction cell ICP-MS (Agilent 7700) operated in hydrogen mode using the same conditions as for hyphenation with FFF. The isotopes ^{28}Si and ^{29}Si were monitored and ^{72}Ge was used as internal standard. Quantification was performed by external calibration with internal standardisation. Standard addition was applied on selected samples to check for matrix effects.

Results and discussion

Optimisation of asymmetric flow field-flow fractionation coupled online to ICP-MS

As a first step, a FFF methodology recently published by our group [11] was revisited for monitoring the size-based distribution of Si in a coffee creamer extract, prepared as described above, by using online ICP-MS detection. The primary size of food grade silica is in the order of 7−12 nm. Therefore, a FFF membrane of 10 kDa (regenerated cellulose) was selected for this work. The same type of membrane was recently shown [9] to provide not only relatively good recoveries for TiO$_2$ nanoparticles in complex sunscreen extracts but also higher recoveries for Au nanoparticles (>85 %) in comparison with other types of membrane surfaces [18].

Different FFF carriers were compared in terms of size distribution, background signal and recovery for Si as eluted from the FFF channel. They included water (commonly used in food preparations), a 0.1 % aqueous solution of a commercial surfactant mixture (FL-70), recently used for sedimentation FFF analysis of silica nanoparticles [19] and a second commercial mixture of nonionic and ionic detergents (NovaChem). The use of FL-70 carrier was found to lead to strong retention of SiO$_2$ particles and high blank signal intensity as detected by ICP-MS. Application of the NovaChem carrier resulted in significant particle aggregate formation, as observed for food grade silica added to coffee creamer by FFF-ICP-MS. Therefore, ultrapure water, which is highly compatible with ICP-MS detection and coffee creamer preparations in real life, was selected for further work. Using the same solvent for sample preparation and FFF separation reduces the probability of nanoparticle transformation due to particle aggregation, surface modification or dissolution during FFF analysis.
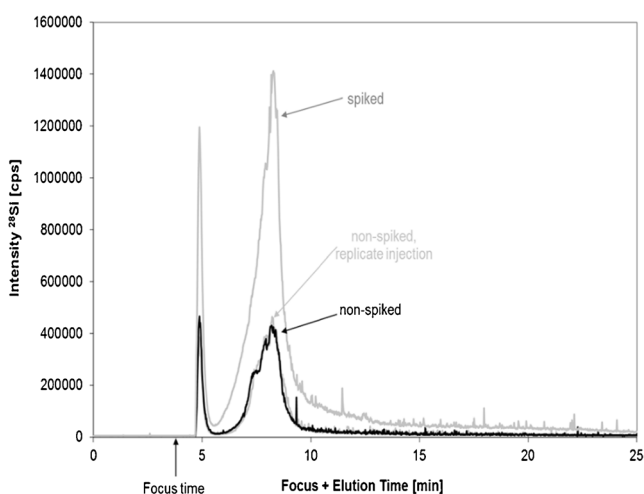
The effect of the cross flow in the selective separation and retention of silica particles was investigated. Figure 1 shows fractograms obtained for a coffee creamer extract using online detection of Si by ICP-MS. As clearly shown in this figure, the resolution of a retained silica peak from the elution peak seems to increase with the increasing cross flow. A cross flow of 2 mL min^{-1} provided baseline separation of the retained silica fraction from the elution peak (likely to correspond to large size particles eluting in steric mode). The use of cross flow rates higher than 2 mL min^{-1} did not result in further improvement of resolution between these Si-containing fractions but in a decreased recovery of Si from the FFF channel. Therefore, a cross flow rate of 2 mL min^{-1} was selected as optimal for further work.

Fig. 1 Optimisation of the cross flow for FFF-ICP-MS analysis of an extract of coffee creamer. The sample was defatted with hexane and then suspended in water using sonication. Regenerated cellulose with 10 kDa MWCO was used with deionised water as carrier and a detector flow of 0.5 mL min^{-1}
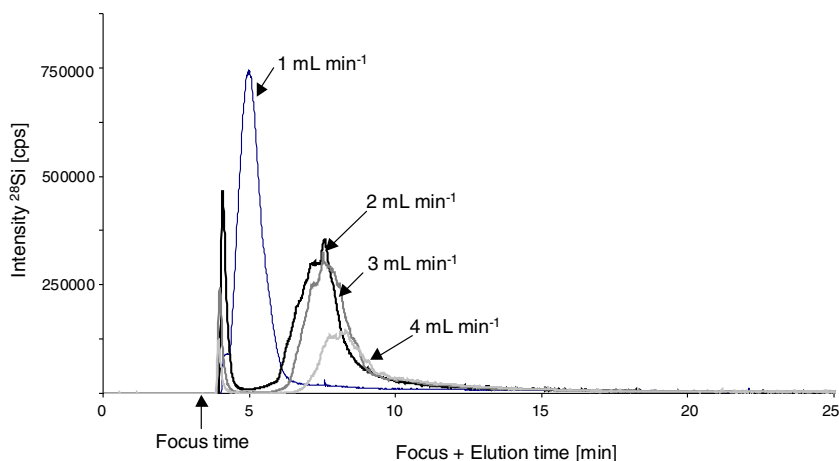
Comparative fractograms obtained for an unspiked coffee creamer extract and an extract spiked with food grade silica (primary size of 12 nm) using the optimal conditions described above are presented in Fig. 2. This figure shows an increased signal intensity for the Si peak detected at 8.3 min (focus and elution time) for the spiked extract. Moreover, no additional Si peak at larger elution times was observed for the spiked extract in comparison with the unspiked extract, suggesting that the matrix components seem to stabilise both the native and added silica particles with a size at the low end of the range 1–100 nm in the aqueous suspension of coffee creamer that passed through the 5-μm filter.

It is interesting to note that the elution time of the nano-silica fraction (8.3 min) detected by FFF-ICP-MS is shorter than that of the 10-nm gold calibrant (data not shown). This could be explained by the difference in elution behaviour between the Au and SiO$_2$ particles, which may be caused by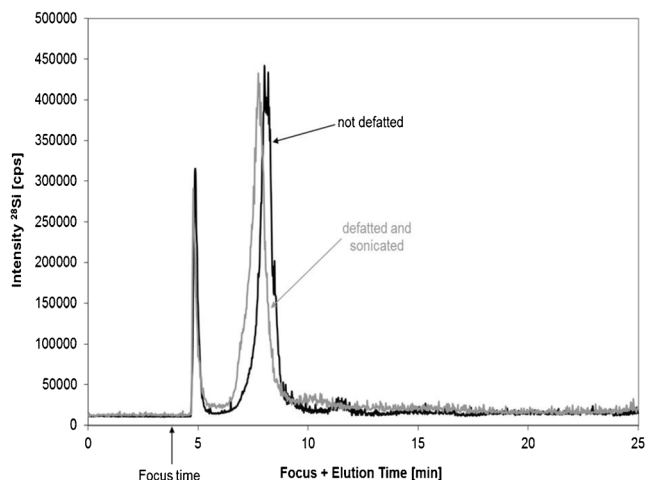 differences in particle–membrane interactions between these particles due to their different physicochemical properties (e.g. the surface of the citrate-coated gold particles in water is negatively charged as it is the FFF hydrophilic cellulose membrane under the conditions used and the surface of the hydrophilic SiO$_2$ particles is likely to be modified by organic components from the matrix). Such observed differences make the use of gold nanoparticles inappropriate for external size calibration in this work. Size-independent effects in nanoparticle retention behaviour during asymmetric flow field-flow fractionation have also been reported elsewhere [20].

Systematic optimisation of SiO$_2$ nanoparticle extraction from the coffee creamer matrix

Since the investigated coffee creamer matrix is known to contain high content of organic compounds including remaining fats, efforts were made to perform sample cleanup prior to FFF analysis of the aqueous suspension in order to prolong the lifetime of the FFF membrane. This was undertaken by studying the effect of sample defatting using an organic solvent such as hexane on the size distribution of nano-silica. Gentle sonication in a water bath was applied to assist particle dispersion and particle disaggregation. A suspension of the crude creamer in water was also prepared using mechanical shaking and analysed in parallel for the purpose of comparison. The fractograms obtained using the compared extraction conditions are presented in Fig. 3. As shown in this figure, the fractogram of the hexane-defatted coffee creamer is similar to that of the crude coffee creamer suspended in water; the small difference in elution time (0.2 min) is within the precision (5 %, $n=5$) obtained for the elution time of the nano-silica fraction . This suggests that no significant change in particle size distribution was observed by defatting the sample using hexane in comparison with sample preparation conditions that mimic coffee creamer preparation in real life. Alternatively, filtration of a creamer aqueous suspension (with and without previous hexane defatting) using 5-μm cellulose acetate

Fig. 2 FFF-ICP-MS ^{28}Si fractograms of an extract of coffee creamer spiked with Aerosil SiO$_2$ of 12 nm average primary particle size (prior to extraction) and of an unspiked extract. The samples were defatted with hexane prior to suspension in water and sonication. An optimal cross flow of 2 mL min^{-1} was used

Fig. 3 Fractograms obtained by FFF-ICP-MS for a coffee creamer extract prepared by simple suspension in water versus defatting and sonication

membranes was performed to minimise clogging of the PEEK connecting tubing between FFF and ICP-MS and to avoid increased system back pressure since both effects were observed after several sample injections onto the FFF system. This helped increase the robustness of the methodology over multiple batch analyses without affecting the Si size distribution observed for unfiltered extracts by using FFF-ICP-MS. Moreover, an average recovery of 102 ± 3 % (as Si) was obtained for food grade silica added to coffee creamer before filtration using the methodology developed in this work.

In real life, coffee creamer is consumed after mixing it with water at different temperatures, depending on each individual taste. Silicon fractograms were obtained by FFF-ICP-MS for coffee creamer suspensions in water at room temperature, 40 °C and 60 °C in order to investigate the effect of the water temperature on the size-based Si speciation. Results of this experiment (see Supplementary Electronic Material Fig. S1) suggest that there is no significant effect of the water temperature on the particle size distribution of silica nanoparticles in coffee creamer extracts; the peak widths at the peak base as well as the elution times at the maximum Si intensity are very similar for the compared fractograms.

It is interesting to note that $\geq$2-fold dilutions of the coffee creamer extract, prepared using the conditions described above (see Fig. 2), resulted in an increase of the elution time

of the retained Si fraction (see Supplementary Electronic Material Fig. S2). This is most likely due to change in the particle properties and/or aggregate formation with the matrix dilution, affecting the elution behaviour of the SiO_2 particles. This effect was observed for both native and food grade Si particles spiked to the creamer matrix. The applied optimal ratio of 0.5 g of sample to 20 mL of water, which also mimics real life preparations, was found to provide a stabilising environment for the silica particles leading to stable suspensions with low aggregation state.

Quantification of nano-silica (detected by FFF-ICP-MS) and mass balance calculations

The total Si content of Aerosil silica particles (food grade silica) was determined in a water suspension with and without microwave digestion. Recoveries from the expected gravimetric value were found to be 96 ± 4 % ($n=3$) and 91 ± 5 % ($n=3$), respectively. The small difference observed for total Si between undigested particles and dissolved silica (after microwave digestion) using ICP-MS detection suggests that using inorganic calibrants added post-FFF would seem appropriate for the purpose of Si quantification by FFF coupled to ICP-MS.

The concentrations (mg kg^{-1}) of Si in the solid sample (on a wet weight basis) in aqueous suspension (after 5 μm filtration) and in the nano-silica fraction detected by asymmetric flow field-flow fractionation coupled to ICP-MS are summarised in Table 1. As shown in this table, only approximately 20 % of the total Si in the sample passes through the 5-μm filter, suggesting that most Si in the solid sample is present in a larger size fraction. As mentioned earlier, this is not expected to affect recovery of the nano-silica fraction of primary size since an average recovery of 102 ± 3 % (as Si) was obtained for food grade silica (added to coffee creamer before filtration) using the methodology developed in this work.

From the total Si injected onto the FFF system, 73 ± 3 % was found to elute from the FFF channel, of which approximately 53 % corresponds to nano-silica and the remaining 20 % was found to elute in the void peak. This means that the nano-silica fraction detected by FFF-ICP-MS represents approximately 11 % of the total Si in the solid sample. From this result, we can conclude that the solid coffee creamer contains at least 11 % of Si within the size range of 1–100 nm.

Table 1 Concentration (mg kg^{-1}) of Si in the solid sample (on a wet weight basis), in the aqueous suspension after 5 μm filtration and the nano-silica fraction as detected by FFF-ICP-MS. Precisions are SD ($n=3$). Si fractionation in the coffee creamer extracts was determined by FFF-ICP-MS. Total Si in the solid sample and extracts was determined by ICP-MS using the conditions described above

Sample	Total Si (mg kg^{-1}) in the solid sample	Si (mg kg^{-1}), in aqueous suspension (after 5 μm filtration)	Nano-silica (mg kg^{-1}) detected by FFF-ICP-MS
Coffee creamer	493±2	99.6±3.0 (20 % of the total Si in the solid sample)	52.8±2.9 (11 % of the total Si in the solid sample)
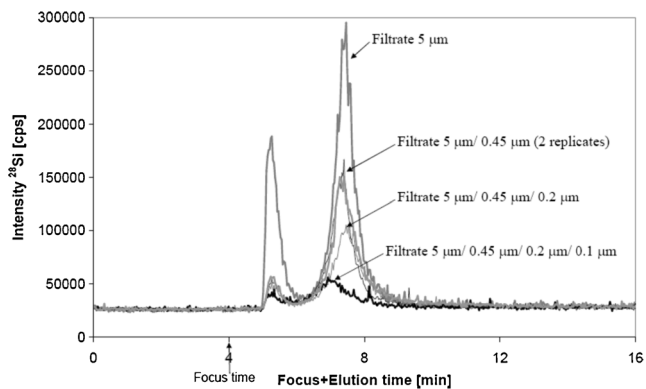
Fig. 4 Sequential filtration of a coffee creamer extract prior to analysis by FFF-ICP-MS/MALS. Ge was used as internal standard to correct for drift of the Si signal

The remaining 27±3 % of the injected Si that was not detected could be attributed to the presence of soluble Si which is transported by the cross flow, so it does not reach the ICP-MS or to the loss of Si particles by adsorption on the FFF membrane. Further studies involving the use of isotopically enriched silica nanoparticles should be pursued to investigate which of the above-described processes are responsible for the relatively low biased recovery achieved.

FFF combined with multiple detectors for size estimation of nano-silica in a coffee creamer matrix

For size estimation, the feasibility of different approaches was investigated. They include the use of the FFF theory equation and the coupling of FFF with standardless sizing detectors such as MALS and TEM (with EDAX for Si detection).

The FFF theory can be applied to calculate size from elution time provided that viscosity of carrier, channel thickness and flow rates are well known. Postnova analysis software was applied to nano-silica in a coffee creamer extract. The channel thickness was approximately determined by

Fig. 5 TEM (**A**) and EDAX (**B**) images of the nano-silica fraction from coffee creamer
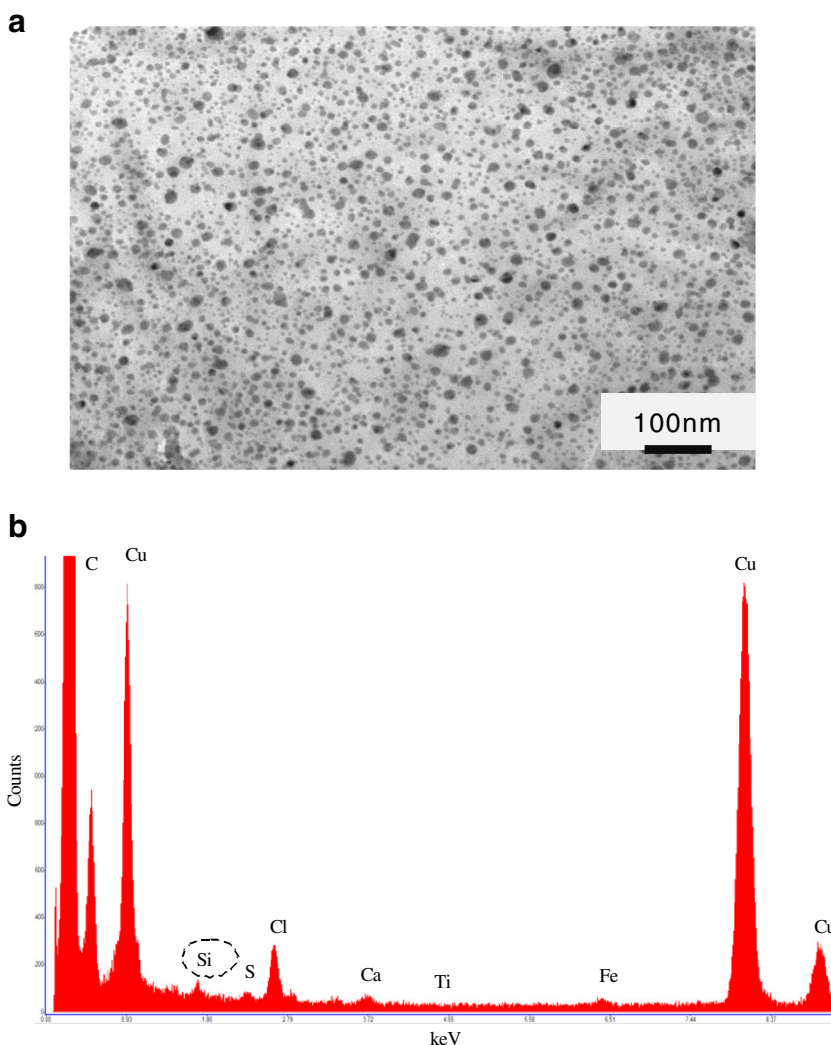
applying the analysis software to a surface-capped SiO_2 standard of known size and modifying the channel thickness until the calculated size agreed with the known size. This resulted in a thickness of 150 μm which is less than half of the nominal spacer thickness. Most likely, the silica particles do not pass in an ideal way through the channel but undergo some interaction with the membrane. Therefore, this determined channel thickness has the function of a correction factor to correlate theoretical elution time with experimentally observed elution time using silica particles with known size. Using this corrected channel thickness, an average size of 13±4 nm (n =2) was obtained for the nano-silica peak in crude coffee creamer extracts.

In addition to ICP-MS, online detection by MALS was also applied. The 21-angle MALS available for this work has been shown to be able to deliver reliable and accurate size results starting as low as 8-nm radii [21]. This is due to its unique broad angular range (in particular, due to the larger number of angles between 0° and 35°) in comparison with other commercial systems. Therefore, we investigated its feasibility, when combined with FFF, for size estimation of silica nanoparticles in of the aqueous coffee creamer suspension. However, analysis of the coffee creamer extracts with or without defatting and sonication caused saturation of most angles of the MALS detector, and therefore size determination was not possible. As discussed earlier in the text, dilution of the sample was not an option since it resulted in the formation of particle aggregates. Alternatively, sequential filtration of the coffee creamer extract prior to injection onto FFF was investigated to achieve extra selectivity in the separation of the nano-silica fraction from the matrix components prior to MALS detection. Verification of the presence of Si in the FFF fraction eluting at 8.3 min was undertaken by FFF-ICP-MS, and the resulting fractograms are presented in Fig. 4. As shown in this figure, all the fractograms show the Si fraction with maximum peak intensity at 8.3 min. However, the peak area of this Si fraction for the extract successively filtered through 5, 0.45, 0.2 and 0.1 μm represents only approximately 20 % of that obtained for the 5-μm filtrate. This suggests that losses of nano-silica occur during successive filtration of the extract, probably due to nanoparticle interaction with and/or coating by much larger components of the matrix. Although filtration-assisted matrix cleanup improved the selectivity of MALS detection, the formation of particle aggregates during the prolonged sample handling made nano-silica size characterisation by MALS an impossible task. Current work in our laboratory focuses on further improvement of sample preparation protocols and FFF separation conditions (e.g. by using sample hydrolysis or physiological buffers to prepare suspensions and/or as FFF carriers) in order to achieve improved selectivity in the separation/detection (e.g. using MALS) of silica nanoparticles, especially in complex matrices that contain other nanomaterials.

Finally, size characterisation of the FFF-ICP-MS detected and collected nano-silica fraction was attempted using TEM with EDAX under the conditions described above. The TEM image obtained for the collected fraction is presented in Fig. 5A, and the EDAX spectrum of the targeted particles is presented in Fig. 5B. The histogram of this TEM image (see Supplementary Electronic Material Fig. S3) suggests that the particle number distribution for the TEM image is predominantly observed for sizes <20 nm with the maximum particle number (%) at about 7 nm and a secondary maximum at about 12 nm. This agrees well with the size detection range reported for food grade or Aerosil silica particles [3] and overlaps with the interval defined by the average size provided by the FFF theory and its associated standard deviation. As shown in Fig. 5B, the EDAX spectrum shows the presence of Si in the particles sized by TEM.

Conclusions

The development of a systematic approach for the size-based elemental quantification and size estimation of silicon dioxide nanoparticles in a highly complex food matrix is reported here for the first time. The potential of FFF-ICP-MS has been demonstrated for the online determination of size-resolved elemental composition of nano-silica in "real-life" coffee creamer preparations. This represents one of the very few efforts to report quantitative data on mass balance for the size-based speciation of inorganic nanoparticles in food-type samples.

The combination of FFF with offline fractionation by filtration, and with detection by online ICP-MS and offline TEM/EDAX, has proven essential to provide reliable information of nanoparticle size in a complex food matrix.

Further studies should be pursued to improve sample preparation protocols and FFF separation conditions (e.g. by using sample hydrolysis or physiological buffers to prepare suspensions and/or as FFF carriers) in order to achieve improved selectivity in the separation/detection of silica nanoparticles, especially in complex matrices that contain other nanomaterials. However, special precautions have to be taken in order to prevent artifact formation due to the use of conditions that are irrelevant to preparations in real life.

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