



# An effective approach for size characterization and mass quantification of silica nanoparticles in coffee creamer by AF4-ICP-MS

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## Abstract

Silicon dioxide (SiO<sub>2</sub>) has been used as a food additive (E551) for decades. However, some safety concerns have been raised recently due to the detection of silica nanoparticles (SiO<sub>2</sub> NPs) in a variety of foodstuffs and their unknown long-term health risk to humans. In order for risk assessment to be conducted, it is essential to establish a reliable, valid, and pragmatic method for analysis of SiO<sub>2</sub> NPs in foods for estimation of exposure. This paper presents an effective approach for both size characterization and mass quantification of SiO<sub>2</sub> NPs in commercial high-fat coffee creamer using asymmetric flow field-flow fractionation (AF4) coupled to inductively coupled plasma mass spectrometry (ICP-MS). SiO<sub>2</sub> NPs from coffee creamer were well extracted after cleanup with hexane in a two-phase (hexane vs. water) aqueous environment. Size determination of SiO<sub>2</sub> NPs was performed by on-line AF4-ICP-MS based on calibration with monodispersed standards. The dominant primary size of SiO<sub>2</sub> NPs in the studied sample was 36.5 nm. The mass percentages of SiO<sub>2</sub> NPs (vs. total SiO<sub>2</sub>) were 18.6% for the dominant primary nano-silica particles by prechannel calibration and 35.7% for total SiO<sub>2</sub> NPs (≤ 100 nm) by postchannel calibration, with recoveries of 89–96% for the former and 75% for the latter. The established approach was demonstrated to be efficient and practical for routine analysis of polydispersed SiO<sub>2</sub> NPs with wide nano-size distribution in coffee creamer. This method may be extended to monitor the presence of SiO<sub>2</sub> NPs in other similar complex food matrices.

**Keywords** Silica nanoparticles (SiO<sub>2</sub> NPs) · Coffee creamer · Size characterization · Mass quantification · AF4 · ICP-MS

## Introduction

In recent years, the application of nanomaterials (NMs) has increased intensely in many areas, such as in cosmetics and consumer products and in the food industry, due to technical benefits offered by the enhanced material properties [1]. In particular, food additives containing food-grade NMs have been widely applied in many foodstuffs and consumer

products [2]. Among the common food additives, synthetic amorphous silica (SAS) in the form of amorphous version of silicon dioxide (SiO<sub>2</sub>), reported to consist of primary particles and small aggregates with at least one dimension below the 100-nm size limit (i.e., silica nanoparticles (SiO<sub>2</sub> NPs)) [3] which is the size threshold of NMs [4], has been approved as a food additive (E551) in many countries since the 2000s [5]. It has been widely applied in foods particularly dry powdered products such as instant soups, milk powder, coffee creamer, etc. [6], as anticaking agent, antifoaming agent, thickener, and carrier of flavors. Due to the widespread usage, the European Food Safety Authority (EFSA) reviewed and upgraded the regulations of E551 regularly with the aim to better protect the consumers. For instance, composition and purity criteria were described in Regulation (EC) No. 1333/2008 [7], and detection and labeling of engineered NMs as ingredients was added in Regulation (EU) No. 1169/2011 [8]. Characterization of particle size distribution as well as the percentages (in number and by mass) of SiO<sub>2</sub> NPs was proposed to be included for re-evaluation and modifications of the EU specifications for E551 in the latest assessment by

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EFSA ANS Panel in 2018 [9]. Considering the possible existence of SiO<sub>2</sub> NPs under the nano-threshold of 100 nm in E551 and foods and the current knowledge gap in this field about their risk on human health over a long-term period, there is a need to evaluate ingestion exposure from food containing NMs including E551 for comprehensive risk assessment. In this regard, it is essential to establish an effective analytical method for detection, characterization, and quantification of SiO<sub>2</sub> NPs in foods particularly those with complex matrices.

Some reports have been published about the testing of SiO<sub>2</sub> NPs in foods [10–16], especially in coffee creamer matrix utilizing E551 as anticaking agent [3, 17, 18]. These studies explored different approaches for detection and characterization of SiO<sub>2</sub> NPs. However, there is still a lack of well-documented methodologies for fast, reliable, and practical size determination and mass quantification of SiO<sub>2</sub> NPs in foods, due to inherent deficiencies of some commonly used techniques. Dynamic light scattering (DLS) is a common size characterization technique which can detect monodispersed NPs at high concentration. Barahona et al. [6] reported that this technique offered low size resolution and was less suitable when samples containing polydispersed NPs with different sizes were analyzed. This was due to its inability to distinguish the presence of smaller particles from bigger ones, which could possibly lead to inaccurate size information or even false-negative detection of NPs in samples with complex matrix. Multi-angle light scattering (MALS) can independently measure the size of NPs without interference from co-eluted particle impurities or matrix components. However, the sensitivity of MALS detection drops significantly with decreasing size of NPs. The size detection limit by MALS was 20 nm as reported by von der Kammer and coworkers [19] or even 30 nm as observed by Wagner et al. [13], and they can only be achieved at sufficiently high concentration of NPs, which narrowed down the measurable size range of NPs. The single-particle inductively coupled plasma mass spectrometry (spICP-MS) technique has the same drawback of poor size detection limit of SiO<sub>2</sub> NPs, exemplified with the size detection limit of 200 nm using standard quadrupole ICP-MS as reported by Mattarozzi et al. [1] and Montañó et al. [20], respectively. Small angle X-ray scattering (SAXS) is a powerful method for measurement of size, size distribution, shape, and surface structure of individual nanoparticles [21]. It can be used to analyze nanoparticle dispersions as well as powders or solids with more detailed information about size distributions of primary nanoparticles. The difficulty in SAXS is that this method measures only the electron density difference. The upper dimensions of the detected nanoparticles are significantly smaller than those from other techniques, with the example of upper size limit of 50 nm when measuring SiO<sub>2</sub> NPs using SAXS as reported by Pabisch et al. [22]. Other than the abovementioned tools, electron microscopy (EM)-based techniques such as transmission electron microscopy (TEM) and

scanning electron microscopy (SEM) can well determine the size and aggregation status of NPs. They are costly, have low throughput, and require highly specialized technical support, which prohibits their routine use in NP size characterization, as concluded by Calzolari and coworkers [2]. Nevertheless, both techniques can be used to verify the presence of certain NPs detected by other sizing techniques.

Among all the available techniques, asymmetric flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry (AF4-ICP-MS) has emerged as a highly promising and universal technique for analysis of NPs, due to AF4's favorable nanoparticle size fractionation capability in complex samples combined with the outstanding sensitivity and selectivity in detection by ICP-MS. More importantly, size characterization and quantification of SiO<sub>2</sub> NPs can be conducted with AF4-ICP-MS by calibration with multiple certified size and concentration of SiO<sub>2</sub> NP standards. Since the potential interactions between NPs and permeation membrane together with losses of NMs caused by other factors may lead to inaccurate results, prechannel calibration with SiO<sub>2</sub> NP standards is proposed to overcome this drawback, enabling effective determination of size and mass content of SiO<sub>2</sub> NPs by AF4-ICP-MS, which is an improvement over previous reports in the analysis of pure monodispersed SiO<sub>2</sub> NP suspensions with single size [23, 24].

The present study aims to develop a practical AF4-ICP-MS method for both size characterization and mass quantification of SiO<sub>2</sub> NPs in commercial high-fat coffee creamer. Based on AF4 separation of a set of SiO<sub>2</sub> NP standards in the range of 20–180 nm with certified size and concentration, the hydrodynamic size characterization of SiO<sub>2</sub> NPs in the extract of coffee creamer was achieved. The combination of standard addition method with prechannel mass calibration using SiO<sub>2</sub> NP standards and postchannel mass calibration using ionic silicon standards resulted in more accurate quantitative profiling of SiO<sub>2</sub> NPs. Different recovery rates of SiO<sub>2</sub> NPs in measurement by AF4-ICP-MS were further evaluated in the nano-size range under study (20–100 nm). Finally, TEM together with energy-dispersive X-ray analysis (EDX) was also utilized for SiO<sub>2</sub> NP size estimation and to verify the results found with AF4-ICP-MS. To the best of our knowledge, there has not been any report on the analysis of polydispersed SiO<sub>2</sub> NPs in a complex food matrix like high-fat coffee creamer by using this proposed approach.

## Materials and methods

### Chemicals and materials

HPLC grade 2-propanol (IPA) was purchased from Merck (Darmstadt, Germany). Ammonium bicarbonate at AG grade was supplied by Sinopharm Chemical Reagent Co. Ltd.

(Shanghai, China). *n*-Hexane 95% was purchased from Tedia (Fairfield, USA). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (30%, ultrapure reagent) and nitric acid (HNO<sub>3</sub>) (70%, ultrapure reagent) were obtained from J.T. Baker, Avantor Performance Materials (Center Valley, USA). Silicon standard solution (1000 µg/mL) from Sigma-Aldrich (St. Louis, USA) and rhodium (Rh) standard solution (1000 µg/mL) from Reagecon Diagnostics Ltd. (Shannon, Ireland) were used for ICP-MS testing. Milli-Q ultrapure grade water was used in all the experiments.

SiO<sub>2</sub> NP standard (sicastar, plain) with nominal diameter of 30 nm was purchased from Micromod Partikeltechnologie GmbH (Rostock, Germany). SiO<sub>2</sub> NP standards (NanoXact) with 20, 50, 60, 80, 100, 120, and 180 nm nominal diameters were obtained from nanoComposix (San Diego, USA). All of these standards were supplied as suspensions in Milli-Q water.

A commercial coffee creamer with high content of fat (around 34%) and anticaking agent E551 was purchased from the market and used for this study.

## Instrumentation

For SiO<sub>2</sub> NP analysis by AF4-ICP-MS, an AF2000 system from Postnova Analytics (Landsberg am Lech, Germany) was utilized. The AF4 separation was achieved using a regenerated cellulose membrane with a molecular weight cutoff of 10 kDa and a spacer thickness of 350 µm. For on-line signal monitoring, channel outlet was sequentially connected to a UV/Vis detector and a MALS detector prior to ICP-MS, with settings of 254 nm and 90°, respectively. To achieve suitable fractionation of SiO<sub>2</sub> NPs, AF4 running conditions were optimized to ensure baseline separation of the smallest SiO<sub>2</sub> NPs (20 nm) used in this study from the void peak. Briefly, aqueous solution consisting of 0.25 mM ammonium bicarbonate and 2.5% IPA was used as the AF4 carrier. A constant cross flow of 1 mL min<sup>-1</sup> was applied at first together with the focus flow of 1.3 mL min<sup>-1</sup>, while the flow rate to detector was kept constant at 0.5 mL min<sup>-1</sup>. After a period of 6 min for sample injection and focusing and a transition time of 1 min, the cross flow started to linearly decrease to 0 mL min<sup>-1</sup> over 30 min, while the tip flow decreased as well until a flow rate of 0.5 mL min<sup>-1</sup> was reached and kept constant for 10 min, yielding the total AF4 run time of 47 min.

The AF4 instrument was coupled to Agilent 7900 ICP-MS equipped with a Micromist nebulizer and a Scott-type spray chamber. The ICP-MS system was operated in hydrogen mode using 100 sweeps, and isotope of *m/z* 28 for silicon was monitored with integration time of 0.5 s. The ICP-MS parameters were optimized and set as follows: RF power, 1550 W; plasma gas (Ar), 15.0 L min<sup>-1</sup>; carrier gas (Ar), 1.09 L min<sup>-1</sup>; and auxiliary gas (Ar), 0.9 L min<sup>-1</sup>.

DLS measurements were performed using a Zetasizer Nano ZS (Malvern, UK) for particle size analysis and evaluation of size distribution (based on hydrodynamic diameters).

## Sample preparation for extraction of SiO<sub>2</sub> NPs

The coffee creamer powder was thoroughly shaken for homogenization and about 0.05 g of sample was accurately weighed. Different experimental factors were then explored for optimized extraction of SiO<sub>2</sub> NPs. In general, there were 8 protocols investigated which fell into three categories (A–C) as below. Table 1 summarizes the comparison of these 8 sample preparation protocols.

(A) No defatting: 1 ml of water was added to the weighed sample, followed by vortex for 20 s with/without subsequent sonication. (B) Defatting under nonaqueous condition: 7 ml of hexane was added to the weighed powder. After manual shaking for 1 min followed by settling time of 1.5 h with/without subsequent centrifugation (5 min, 3000 rpm), the hexane containing fats was exhaustively removed; 1 ml of water was added to the solid residue then vortexed for 20 s with/without subsequent sonication. (C) Defatting under aqueous condition: 1 ml of water was firstly added to the weighed powder, followed by vortex for 20 s and 7 mL hexane was then added. The sample was defatted with hexane in existence of water by manually shaking for 1 min and settled for 1.5 h without subsequent centrifugation. The upper phase of hexane containing fats was carefully removed with/without subsequent sonication of the remaining aqueous suspension.

In addition, when the SiO<sub>2</sub> NP standard (30 nm, 1 mL, and 50 µg/mL) was spiked into coffee creamer powders at the same amount of about 0.05 g as above for sample preparation method evaluation, there was no other change except that water used in the above protocols was replaced by this SiO<sub>2</sub> NP standard dispersion. As discussed in the later sections, the optimal sample preparation protocol #8 listed in Table 1 was selected for extraction of SiO<sub>2</sub> NPs from coffee creamer powder in the following experiments.

## Silica nanoparticle size characterization by AF4-ICP-MS

To characterize the size of SiO<sub>2</sub> NPs in coffee creamer, 0.025 g of the sample was accurately weighed, followed by spiking the SiO<sub>2</sub> NP standard (20 nm, 1 mL, 50 µg/mL), homogenization, defatting, and extraction. The same experimental conditions and procedures were further applied to the other monodispersed suspensions of SiO<sub>2</sub> NP standards with nominal diameters of 30, 50, 60, 80, 100, 120, and 180 nm, respectively. The obtained coffee creamer extracts spiked with different SiO<sub>2</sub> NP standards were individually injected for AF4-ICP-MS analysis under the same conditions. By plotting the known hydrodynamic particle sizes against retention times

**Table 1** Optimization of experimental conditions for extraction of SiO<sub>2</sub> NPs from raw coffee creamer powder

Category	S/ N	Extraction environment <sup>a</sup>		Centrifugation (yes/no)	Sonication <sup>d</sup> (yes/no)
		Defatting under nonaqueous condition (yes/no) <sup>b</sup>	Defatting under aqueous condition (yes/no) <sup>c</sup>		
A	#1	No hexane, only water (or SiO <sub>2</sub> NP standard aqueous suspension) —no defatting		No	No
	#2	No hexane, only water (or SiO <sub>2</sub> NP standard aqueous suspension) —no defatting		No	Yes
B	#3	Yes	No	No	No
	#4	Yes	No	No	Yes
	#5	Yes	No	Yes	No
	#6	Yes	No	Yes	Yes
C	#7	No	Yes	No	No
	#8	No	Yes	No	Yes

<sup>a</sup> Extraction solvents include 7 mL of hexane under nonaqueous condition or 1 ml of ultrapure grade water (or SiO<sub>2</sub> NP standard aqueous suspension) under aqueous condition for all these evaluated experimental protocols, respectively

<sup>b</sup> Add hexane at first for defatting with/without subsequent centrifugation followed by its removal after defatting. Then, add water (or SiO<sub>2</sub> NP standard aqueous dispersion) to the defatted pellet for suspension

<sup>c</sup> Add water (or SiO<sub>2</sub> NP standard aqueous dispersion) firstly for thorough suspension. Then, hexane was added to the aqueous suspension for defatting followed by its removal after defatting

<sup>d</sup> The gentle sonication in a water bath was applied for 2 min to assist dispersion and disaggregation of SiO<sub>2</sub> NPs

at peak maxima of these SiO<sub>2</sub> NP standards, regression equation for modeling the relationship between retention time and hydrodynamic size was able to be established, and the size of SiO<sub>2</sub> NPs in the coffee creamer extract was characterized by polynomial interpolation processing.

### Quantification of SiO<sub>2</sub> NPs by prechannel and postchannel calibration

Quantification of SiO<sub>2</sub> NPs in coffee creamer was conducted by prechannel calibration using SiO<sub>2</sub> NP standard, as well as by postchannel calibration with ionic silicon standard. For prechannel calibration, the standard addition method was used by separately spiking SiO<sub>2</sub> NP standard (30 nm) stock suspension into 8 centrifuge tubes which individually contained the same amount of about 0.05 g of the coffee creamer sample to obtain a series of standard suspensions with its final concentrations of 0 (unspiked coffee creamer extract), 5, 10, 15, 20, 30, 40, and 50 µg/mL, and plotting these spiked concentrations against the peak areas obtained by their prechannel injection and on-line AF4-ICP-MS analysis. For postchannel calibration, the AF4 fraction of total SiO<sub>2</sub> NPs (≤ 100 nm) in unspiked coffee creamer extract was collected over their elution session. Meanwhile, background fraction of the blank control sample without any coffee creamer was prepared and analyzed in the same way as above to remove possible background interferences from sample preparation and

postchannel calibration. Then, under the same tuning conditions as those for hyphenation with AF4, ICP-MS data acquisition of these fractions was performed. The off-line quantification was conducted by routine external calibration with a series of ionic silicon standard solutions which was corrected with internal standard of Rh at *m/z* 103. The concentration of silicon in coffee creamer was calculated based on injection volume (20 µL) and dilution factor involving the volumes of the prepared sample suspension and collected fraction. The quantification data of ionic silicon (Si) was converted to SiO<sub>2</sub> if not stated otherwise.

### Microwave-assisted acid digestion and measurement of total silicon by ICP-MS

The coffee creamer sample was subjected to microwave-assisted acid digestion using a microwave reaction system (MARS 6, CEM Corporation, Matthews, USA). Coffee creamer powder (0.4 g) was accurately weighed into the microwave digestion vessel and 2 mL water was added with subsequent vortex and sonication; 2 mL H<sub>2</sub>O<sub>2</sub> and 10 mL concentrated HNO<sub>3</sub> were further added into the suspension followed by standing for 20 min. Digestion was performed by ramping from room temperature to 200 °C within 30 min and hold for 10 min. After cooled down to room temperature, the digest was diluted to a total volume of 50 mL with water and homogenized prior to injection for ICP-MS analysis.



Total silicon content in digested samples was further determined under the same conditions for postchannel calibration and off-line quantification as described in the above section.

### Determination of recovery rates of SiO<sub>2</sub> NPs in coffee creamer by AF4-ICP-MS

For recovery experiments of prechannel calibration, all potential sources accounting for total losses of SiO<sub>2</sub> NPs in the sample should be considered, including the effects of extraction of SiO<sub>2</sub> NPs and interference of coffee creamer matrix as well as the effects of interactions between SiO<sub>2</sub> NPs and the permeation membrane in AF4 channel. Hence, recovery evaluation was conducted by injection of coffee creamer extracts which were unspiked and spiked with SiO<sub>2</sub> NP standard for AF4-ICP-MS analysis with normal cross flow applied, followed by analysis of the same SiO<sub>2</sub> NP standard in pure water without cross flow applied. Consequently, the <sup>28</sup>Si ICP-MS peak areas were integrated and the overall recovery rate was defined and calculated as  $R (\%) = [(A_2 - A_1) / B_0] \times 100$ , where  $A_2$ ,  $A_1$ , and  $B_0$  represented the peak areas obtained in the analysis of spiked and unspiked coffee creamer extracts with normal cross flow applied as well as those of the same spiked monomodal SiO<sub>2</sub> NP standard in pure water without applying cross flow in AF4 separation, respectively. To further understand the recovery of prechannel calibration, there were the other two types of recovery rates, i.e.,  $R_A$  and  $R_B$ , which were covered by the overall recovery rate of  $R$ .  $R_A$  reflected the possible losses of SiO<sub>2</sub> NPs due to its extraction from raw coffee creamer powder and its potential interactions with coffee creamer matrix.  $R_A$  was defined and calculated as  $R_A (\%) = [(A_2 - A_1) / A] \times 100$ , where  $A_2$ ,  $A_1$ , and  $A$  represented the peak areas obtained in the analysis of spiked and unspiked coffee creamer extracts as well as those of the same spiked SiO<sub>2</sub> NP standard in pure water, respectively, with normal cross flow applied in AF4 separation.  $R_B$  reflected the possible losses of SiO<sub>2</sub> NPs due to its potential interactions with the permeation membrane in AF4 channel.  $R_B$  was defined and calculated as  $R_B (\%) = (B / B_0) \times 100$ , where  $B$  and  $B_0$  were peak areas of the same monomodal SiO<sub>2</sub> NP standard in pure water with or without cross flow applied in AF4 separation.

For the recovery evaluation of postchannel calibration, similar workflow and definition of recovery rate as described above were adopted, except that SiO<sub>2</sub> NP fractions were collected and submitted to off-line quantification calibrated by ionic silicon and that normal cross flow was applied throughout the AF4 separation process.

### TEM with energy-dispersive X-ray analysis

For TEM measurement of SiO<sub>2</sub> NP size, the defatted coffee creamer was further suspended in a mixed solvent of ethanol–

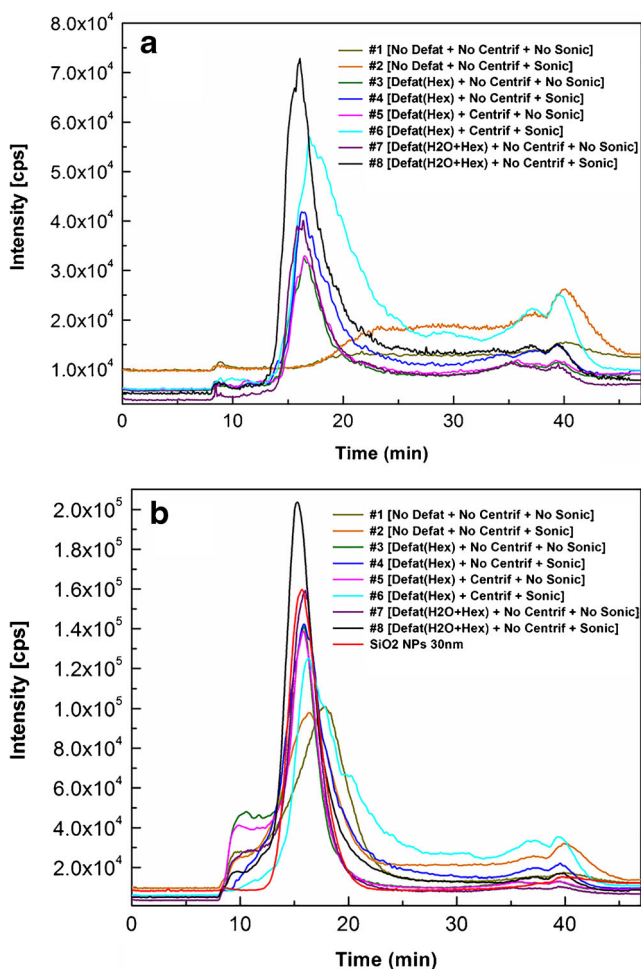
water (1:2) at a concentration of 10 mg mL<sup>-1</sup>. This adequately diluted particle suspension (2 mL) was dropped through a TEM copper grid which was then dried at 60 °C for 3 h prior to the following analysis. Images were obtained by using a JEOL 2100 TEM system (JEOL Ltd., Tokyo, Japan). The primary particle size of SiO<sub>2</sub> NPs on the recorded images was analyzed with ImageJ1.50i software (National Institute of Health, Bethesda, MD, USA). Results of the particles were processed with Microsoft Excel to obtain size histograms where the relative number frequency of SiO<sub>2</sub> NP size intervals was plotted. The presence of silicon in the particles characterized by TEM was confirmed by EDX.

## Results and discussion

### Optimization of the sample preparation protocol

To effectively characterize and quantify SiO<sub>2</sub> NPs, coffee creamer powder must be properly dispersed in liquid medium. Similar to previous reports [10, 15, 18], for foods with massive organic substances especially those with high content of fat, a defatting step with organic solvent like hexane was necessary and applied in this study. On the other hand, although filtration had been used for extraction of SiO<sub>2</sub> NPs by other researchers [3, 6, 15, 18], it was not adopted in this study due to significant losses of SiO<sub>2</sub> NPs after filtering of coffee creamer dispersion (data not shown) and the mass uncertainties introduced which may cause great inaccuracy in quantification.

In general, intensive cleanup and other sample treatment procedures may cause modification of size distribution of SiO<sub>2</sub> NPs owing to the complexity of food matrices. Hence, to explore the best SiO<sub>2</sub> NP extraction conditions, the effects of various protocols on AF4 separation performance were compared. As shown in Table 1, the application of three main types of experimental parameters was investigated because of their potential necessity and significant effects on SiO<sub>2</sub> NP extraction, including extraction environments for defatting by hexane, centrifugation, and sonication, and 8 proposed protocols were explored accordingly. As shown in Fig. 1a, for the unspiked coffee creamer extracts, comparison of fractograms obtained by these protocols demonstrated that protocol #8 was the best method, i.e., defatting by two-phase (hexane vs. water) aqueous extraction, omission of centrifugation but combined with sonication of the obtained aqueous suspension, since it provided SiO<sub>2</sub> NP extract with the highest signal intensity together with narrow and sharp AF4 peak with baseline separation without obvious peak broadening. In addition, the effects of sample preparation on size distribution of SiO<sub>2</sub> NPs in coffee creamer matrix were also studied based on separately spiking coffee creamer powder with SiO<sub>2</sub> NP standard (30 nm, 50 µg/mL) and subsequent extraction using the 8



**Fig. 1** Optimization of the sample preparation methods for AF4-ICP-MS analysis of coffee creamer. Overlaid SiO<sub>2</sub> NP fractograms of **a** unspiked coffee creamer extracts and **b** extracts of coffee creamer spiked with SiO<sub>2</sub> NP standard (30 nm, 50 µg/mL) which were obtained with protocol #1–#8. Fractogram of the SiO<sub>2</sub> NP standard in pure water (30 nm, 50 µg/mL) was also overlaid in **b** for comparison. Fractograms corresponding to the 8 protocols (as defined in Table 1) as well as that of SiO<sub>2</sub> NP standard in pure water were annotated in different colors

protocols. As shown in Fig. 1b, extract of the spiked coffee creamer with protocol #8 again represented the best AF4 fractogram. More importantly, it was worth noting that the fractogram of extract produced by protocol #8 was very similar in terms of silicon distribution shape to that of the pure SiO<sub>2</sub> NP standard (30 nm) in water but presented with higher signal intensity. The small difference in retention times of their AF4 peak maxima (0.3 min) was also displayed. These similarities suggested that there was no significant change in SiO<sub>2</sub> NP size distribution resulting from protocol #8.

All these phenomena originated from features of surface chemistry in SiO<sub>2</sub> NPs. When coffee creamer powder was firstly suspended in aqueous solution, water can stabilize the silica particles via a solvation layer and electrostatic repulsion formed on the silica surface through strong hydrogen bonding of water molecules to their surface silanol groups, which

protected the primary SiO<sub>2</sub> NPs against negative influences from the following treatments and minimized the risk of aggregation for SiO<sub>2</sub> NPs. In contrast, if coffee creamer powder was firstly suspended in hexane, silanols on adjacent silica particles were induced to interact directly by hydrogen bonding, which caused destabilization and aggregation for SiO<sub>2</sub> NPs [25]. Additionally, although centrifugation at low speed can benefit the collection of SiO<sub>2</sub> NPs suspending in hexane and avoid their potential loss, it may also cause irreversible aggregation of SiO<sub>2</sub> NPs in aqueous suspension, whereas sonication can break up agglomerates and assist the dispersion and stabilization of NPs. As a result, protocol #8 showed clear advantages and was utilized for the following investigations.

### Size characterization of SiO<sub>2</sub> NPs in coffee creamer

To conduct size characterization of NPs by AF4, two different approaches were generally used. The first one relied on AF4 theory in calculating the hydrodynamic size with parameters, such as retention time, diffusion coefficient, viscosity and flow rate of carrier, channel thickness, etc. [13, 18]. The second approach was based on size calibration with a series of NP standards, so that the relationship between retention time and size can be established under fixed AF4 conditions [23, 24, 26]. Size characterization based on AF4 theory was applicable only under ideal running conditions as it did not consider certain surface properties of particles that can induce interactions imposed on SiO<sub>2</sub> NPs and large shifts in elution time. The actual nonideal behavior of SiO<sub>2</sub> NPs in AF4 channel in the presence of complex food matrix may lead to inaccurate determination of SiO<sub>2</sub> NP diffusion and the consequent unreliable hydrodynamic size. In this regard, the use of SiO<sub>2</sub> NP standards showed more advantages because NPs in both standards and samples shared the same composition and underwent the same conditions for AF4 separation. The interactions among the sample SiO<sub>2</sub> NPs, food matrix, and AF4 membrane that affected the elution performance were theoretically compensated or at least the interfering effects were minimized.

To verify the hydrodynamic sizes of the commercial SiO<sub>2</sub> NP standard suspensions declared by the manufacturer and evaluate their size distribution, all the SiO<sub>2</sub> NP size standards were firstly analyzed by DLS. As shown in Table 2, the representative homogeneous hydrodynamic sizes in the form of Z-average of each SiO<sub>2</sub> NP standard were obtained. These results well cohered with the hydrodynamic diameters reported in the manufacturer's certificates, with relative deviations in the range of 0–4% except for the 20- and 80-nm-sized particles that showed values of 6.8% and 5.6%, respectively. Meanwhile, it was also found that their particle size distributions were unimodal by DLS analysis, with the measured polydispersity indexes (PDI) ranging from 0.013 to 0.203. All the results suggested that these commercial SiO<sub>2</sub> NP

**Table 2** Evaluation of the size distribution of commercial silica nanoparticle standard suspensions used for size calibration

Normal size (nm)	Sizes of SiO <sub>2</sub> NP standard <sup>a</sup>		DLS analysis value <sup>b</sup> (nm)		Relative deviation <sup>c</sup> (%)
	TEM diameter (nm)	Hydrodynamic diameter (nm)	Z-average (nm)	PDI	
20	21.4 ± 2.7	22.0	23.5 ± 0.4	0.139	6.8
30	— <sup>d</sup>	30.0	30.0 ± 0.5	0.203	0.0
50	47.0 ± 3.0	54.0	54.5 ± 0.3	0.106	0.9
60	60.3 ± 2.9	72.5	69.6 ± 0.5	0.059	4.0
80	76.0 ± 6.0	91.0	96.1 ± 0.2	0.026	5.6
100	104.0 ± 9.0	111.0	110.7 ± 0.9	0.029	0.3
120	118.5 ± 5.7	137.6	136.6 ± 1.1	0.013	0.7
180	185.8 ± 5.7	201.4	195.9 ± 2.4	0.032	2.7

<sup>a</sup> Size information as reported in the manufacturer's certificates

<sup>b</sup> Z-average and polydispersity index (PDI) obtained by DLS measurement ( $n = 3$ )

<sup>c</sup> Relative deviation obtained by comparison of the Z-average measured by DLS with the hydrodynamic size declared in the manufacturer's certificate

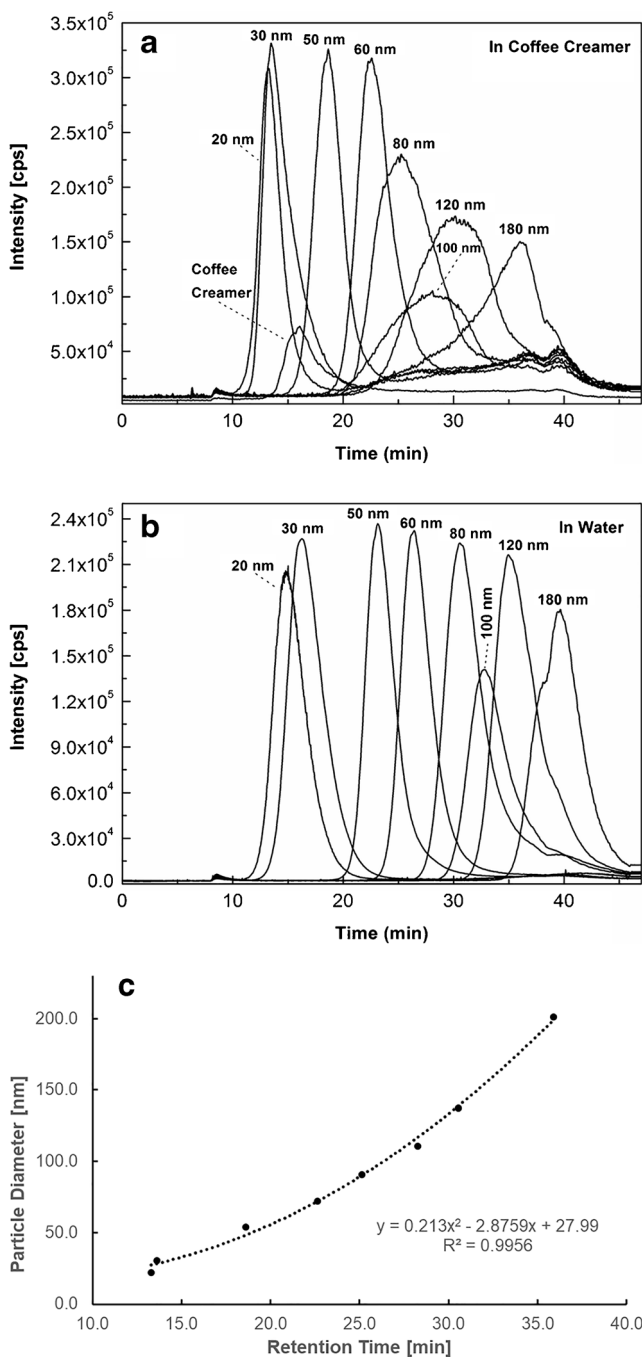
<sup>d</sup> Not available

standards presented highly monodispersed hydrodynamic sizes. Hence, the hydrodynamic diameters reported in the manufacturer's certificates were utilized as targeted values for size calibration in the following AF4-ICP-MS analysis.

Figure 2a shows the overlaid AF4-ICP-MS fractograms of eight SiO<sub>2</sub> NP standards with nominal sizes ranging from 20 to 180 nm which were separately spiked into coffee creamer sample and extracted for analysis, with the normalization of potential interferences from the food matrix. All peak maxima were separated with distinct retention time, including the partially resolved peaks of 20 and 30 nm SiO<sub>2</sub> NPs, hence allowing attribution of SiO<sub>2</sub> NP size in the unknown coffee creamer sample. A close examination of Fig. 2a revealed that size fractions corresponding to 100, 120, and 180 nm SiO<sub>2</sub> NPs were presented as fronting peaks, whereas the other size standards below 100 nm showed relatively narrow and symmetrical peaks. The fronting of these three peaks did not come from inherent higher polydispersity of the commercial SiO<sub>2</sub> NP standards according to the manufacturer's certificates. It was attributed to the interference from the coffee creamer matrix whose bigger particles with lower diffusion coefficient caused them to move to the slower stream lines of the AF4 channel and pushed some of these relatively smaller SiO<sub>2</sub> NPs of 100, 120, and 180 nm into faster stream lines and eluted earlier. The size calibration curve was then constructed by plotting hydrodynamic diameters of the SiO<sub>2</sub> NP standards from the manufacturer's certificates against their respective retention times of peak maxima. The modeling equation was determined and hereby used, i.e.,  $y = 0.213x^2 - 2.8759x + 27.99$  ( $y$  is the particle diameter in nm,  $x$  is Rt in min,  $R^2 = 0.9956$ ). This excellent polynomial fit between retention times and hydrodynamic diameters was predicted by theory and because of the fact that AF4 had parabolic flow velocity

profiles inside its channel [27]. It was in agreement with observations in other studies [23, 26] utilizing AF4 as the fractionation technique. Consequently, under the same AF4 conditions, the unspiked coffee creamer extract was also analyzed for size characterization of SiO<sub>2</sub> NPs, with the obtained fractogram shown in Fig. 2a. A hydrodynamic diameter of  $36.5 \pm 0.9$  nm was determined as the dominant primary particle size of the investigated SiO<sub>2</sub> NPs in crude coffee creamer extract, based on retention time of peak maximum measured by AF4 (Table 3). The nano-size distribution range of primary particles among the polydispersed SiO<sub>2</sub> NPs was estimated to be ranging from  $22.9 \pm 1.3$  to  $69.6 \pm 1.7$  nm, based on modeling calculation with the starting and ending retention times of these SiO<sub>2</sub> NPs. The measured prevailing size and nano-size range agreed with other studies on E551 [6, 14] and well overlapped with the detected size ranges of SiO<sub>2</sub> NPs in food matrices by other researchers [3, 10, 17]. Considering the potential effects of variation of the AF4 membrane on separation of SiO<sub>2</sub> NPs, the reproducibility of size characterization was also evaluated after the change of membrane following the same procedure as above. Based on recalibration with SiO<sub>2</sub> NPs size standards, it was found that the obtained hydrodynamic diameters of dominant primary SiO<sub>2</sub> NPs among different batches compared well with the above size of  $36.5 \pm 0.9$  nm, with the absolute deviations ranging within 1–3 nm (or relative deviations in the range of 2–6%). These insignificant variations indicated that the established size calibration method represented consistent and reliable performance.

In contrast, when the same SiO<sub>2</sub> NP standards as above were suspended in pure water and analyzed under same conditions of AF4-ICP-MS, as shown in Fig. 2b, their fractograms were generally displayed as well-defined and symmetrical peaks, but the peak maxima were all eluted



**Fig. 2** Size characterization with SiO<sub>2</sub> NP standards (size-ladder) by AF4-ICP-MS. **a** Overlaid fractograms of the investigated unspiked coffee creamer and SiO<sub>2</sub> NP standards (at 50 µg/mL for each) spiked in this coffee creamer. **b** Overlaid fractograms of SiO<sub>2</sub> NP standard dispersions in pure water at 50 µg/mL for each. **c** Regression curve for modeling the relation between retention times of the peak maxima of SiO<sub>2</sub> NP size standards spiked in coffee creamer plotted against particle diameters in nanometers

somewhat later than those of their counterparts spiked in coffee creamer in Fig. 2a. The difference in retention times could be attributed to the interferences from large coffee creamer matrix particles together with the separation features of AF4.

In brief, in Fig. 2a, the analytes were SiO<sub>2</sub> NPs in unspiked coffee creamer and coffee creamer spiked with SiO<sub>2</sub> NP standards, respectively. Compared with SiO<sub>2</sub> NPs, large particles in coffee creamer matrix had larger particle sizes and lower diffusion coefficient. As explained earlier, these big matrix particles were carried to the slower stream lines of the AF4 channel and pushed relatively smaller SiO<sub>2</sub> NPs out to the faster stream lines, making the SiO<sub>2</sub> NPs eluted earlier. The strength of this interference effect increased with the increment of the size of SiO<sub>2</sub> NPs since diffusion coefficient decreased and diffusion speed of SiO<sub>2</sub> NPs turned slower with the increment of size of SiO<sub>2</sub> NPs, which led to higher probability for the matrix components to interfere and interact with the larger SiO<sub>2</sub> NPs. All these interferences could make the SiO<sub>2</sub> NP standards in Fig. 2a eluted earlier than their counterparts in Fig. 2b where the analytes were pure SiO<sub>2</sub> NP standards in pure water without any matrix interferences. Hence, after the size calibration curve and modeling equation for these SiO<sub>2</sub> NP standards in pure water in Fig. 2b were created in the same way as above, a hydrodynamic diameter of  $28.0 \pm 0.2$  nm was determined as the dominant particle size of SiO<sub>2</sub> NPs in the same unspiked coffee creamer sample. The difference in elution behavior of these two scenarios indicated that, when SiO<sub>2</sub> NP standard dispersions in pure water were utilized for size analysis of SiO<sub>2</sub> NPs in coffee creamer, a smaller hydrodynamic size would be obtained and bias could be introduced in measuring particle size relative to the starting sample.

### Mass quantification of SiO<sub>2</sub> NPs in coffee creamer

As reported previously, prechannel [24, 26, 28] and postchannel [13, 18, 23] mass calibration had been frequently used for quantification of NPs mainly to account for possible sample material losses in AF4 separation for the former or the unavailability of size and concentration-certified nanoparticle standards for the latter. Each of the two strategies may solely work well in practice. However, when complex food samples were encountered, like high-fat coffee creamer containing SiO<sub>2</sub> NPs with wide nano-size distribution in the current study, the combination of these complementary approaches would enable comprehensive quantitative profiling of the total SiO<sub>2</sub> NP mass content.

Coffee creamer usually contains a complex matrix of ingredients at high levels. As an enhanced prechannel calibration with SiO<sub>2</sub> NP standards, the standard addition method can correct the matrix/aggregation effect by spiking different known amounts of analyte SiO<sub>2</sub> NP standard (with size matched) into samples for mass quantification. In this study, the diameter of the prevalent SiO<sub>2</sub> NPs in coffee creamer was measured to be around 36.5 nm as a hydrodynamic size rather than a physical size. Since hydrodynamic size was slightly larger than nominal size, a SiO<sub>2</sub> NP standard with nominal



**Table 3** Size characterization and quantification of SiO<sub>2</sub> NPs in coffee creamer by prechannel and postchannel calibration ( $n = 3$ )

SiO <sub>2</sub> NPs		Total Si in powder (mg/kg)	Total SiO <sub>2</sub> in powder (mg/kg) <sup>h</sup>	Mass percentage of dominant SiO <sub>2</sub> NPs (%) <sup>i</sup>	Mass percentage of total SiO <sub>2</sub> NPs (%) <sup>j</sup>					
Retention time (min) <sup>a</sup>	Hydrodynamic size	Concentration								
		Prechannel by SAM		Postchannel by ICP-MS						
		$C_m$ (powder) (mg/kg) <sup>d</sup>	$C_n$ (suspension) (particles/mL) ( $\times 10^{11}$ ) <sup>f</sup>	$C_m$ (powder) (mg/kg) <sup>g</sup>	MS $C_m$ (powder) (mg/kg) <sup>g</sup>					
Dominant diameter (nm) <sup>b</sup>	Size range (nm) <sup>c</sup>	$C_m$ (suspension) (mg/L) <sup>e</sup>								
16.0 ± 0.2	36.5 ± 0.9	(22.9 ± 1.3)-(69.6 ± 1.7)	291.3 ± 4.7	14.6 ± 0.2	2.9 ± 0.1	561.3 ± 34.9	733.2 ± 29.9	1571.2 ± 64.0	18.6 ± 0.7	35.7 ± 2.2

<sup>a</sup> Retention time at AF4 peak maximum of dominant primary SiO<sub>2</sub> NPs

<sup>b</sup> Dominant primary particles size of the detected polydispersed SO<sub>2</sub> NPs by measurement at peak maximum in AF4-ICP-MS fractogram

<sup>c</sup> Estimated size range of primary particles based on modeling equation at the starting and ending retention times of the main AF4 peak of polydispersed SiO<sub>2</sub> NPs

<sup>d</sup> The mass content of dominant primary particles in raw coffee creamer powder quantified by on-line AF4-ICP-MS with standard addition method (SAM)-based prechannel calibration using SiO<sub>2</sub> NP standard (30 nm)

<sup>e</sup> Mass concentration of dominant primary SiO<sub>2</sub> NPs in the prepared suspension which was determined by AF4-ICP-MS and prechannel calibration with SiO<sub>2</sub> NP standard

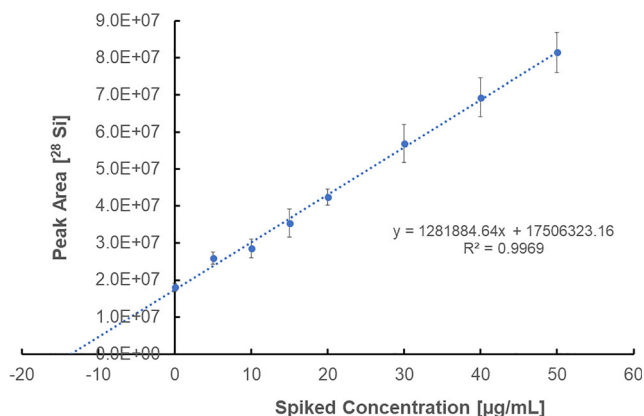
<sup>f</sup> The particle number concentration estimated with the mass concentration and hydrodynamic diameter of dominant primary SiO<sub>2</sub> NPs measured by AF4-ICP-MS and prechannel calibration with SiO<sub>2</sub> NP standards

<sup>g</sup> Total SiO<sub>2</sub> NPs containing primary particles and small aggregates ( $\leq 100$  nm) were collected as AF4 fraction over their whole elution session and quantified by subsequent off-line ICP-MS analysis with postchannel calibration using ionic silicon standards

<sup>h</sup> Concentration of total SiO<sub>2</sub> (molecular weight 60 g/mol) was obtained by multiplying that of total silicon (Si, molecular weight 28 g/mol) with 60/28

<sup>i</sup> Mass percentage of dominant primary nano-silica particles ( $\sim 30$  nm) vs. total SiO<sub>2</sub> in raw coffee creamer powder by prechannel calibration

<sup>j</sup> Mass percentage of total SiO<sub>2</sub> NPs ( $\leq 100$  nm) vs. total SiO<sub>2</sub> in raw coffee creamer powder by postchannel calibration



**Fig. 3** Calibration curve recorded with ICP-MS for isotope  $^{28}\text{Si}$  by standard addition method for quantification of  $\text{SiO}_2$  NPs in the coffee creamer extract

size of 30 nm was used for standard addition method, considering that NPs with similar sizes were supposed to have similar behavior in the same sample matrix.

Figure 3 shows the calibration curve with the standard addition method. The linear regression curve was extrapolated to the  $X$ -axis. The intersection value of the extrapolated curve and the  $X$ -axis (i.e.,  $x$ -intercept) was determined as the endogenous  $\text{SiO}_2$  NP concentration in the prepared coffee creamer suspension. The  $\text{SiO}_2$  NP mass content in raw coffee creamer powder was then calculated based on injection volume and dilution factor during sample preparation. Using this approach, for the dominant primary  $\text{SiO}_2$  NPs detected with nano-size range over  $22.9 \pm 1.3$  to  $69.6 \pm 1.7$  nm and centered around  $36.5 \pm 0.9$  nm, their mass content was quantified as  $291.3 \pm 4.7$  mg/kg (Table 3).

In the meantime, quantification of  $\text{SiO}_2$  NPs by postchannel calibration with ionic silicon standards was also conducted. According to the conclusions from other researches [23, 24, 29], the atomization of silica with particle diameters smaller than 500 nm was complete and they can be well ionized within the plasma. Aureli et al. [23] reported an average absolute deviation of 2.4% based on comparison of total silicon content from digested and undigested  $\text{SiO}_2$  NPs (20 and 50 nm). Barahona et al. [24] reported detected silicon signal ratios between digested and undigested  $\text{SiO}_2$  NPs ranging from 88 to 102% for all the studied  $\text{SiO}_2$  NP standards with sizes across 20 to 150 nm. Statistically, these reported differences of total silicon contents determined between acid-digested and undigested  $\text{SiO}_2$  NPs were insignificant, which suggested that ionic calibrants can be reliably used in postchannel calibration to quantify  $\text{SiO}_2$  NPs without acid digestion in the size range considered in this work. Consequently, the total nano-silica fraction of primary particles and small aggregates ( $\leq 100$  nm) in the coffee creamer was collected. Using external calibration with ionic silicon standards, the silicon content of the collected fraction was quantified by off-line ICP-MS. The  $\text{SiO}_2$  NP concentration

was thus determined as 561.3 mg/kg (Table 3) which represented total  $\text{SiO}_2$  NPs under the size threshold of NMs of 100 nm in the studied coffee creamer in accordance with the definition by the EU [8]. To evaluate the background noise from sample preparation and calibration due to use of glassware or other silicon sources, a fraction of the blank control sample without any coffee creamer was simultaneously collected and quantified in the same way as above. It was found that the ratios of total silicon content in the blank control sample vs. those in the coffee creamer sample ranged from 3 to 5% which indicated that the background signal was negligible. After subtraction of the background value, the  $\text{SiO}_2$  NP concentrations were obtained as above accordingly.

Separately, the total silicon content of raw coffee creamer powder was measured to be 733.2 mg/kg with microwave digestion, and the total  $\text{SiO}_2$  concentration was further determined as 1571.2 mg/kg (Table 3). Assuming that total silicon content only originates from  $\text{SiO}_2$ , the mass percentage of  $\text{SiO}_2$  NPs (relative to the total amount of  $\text{SiO}_2$  with all sizes beyond the limit of the nano-threshold of 100 nm) was found to be 18.6% for the dominant primary nano-silica particles ( $\sim 30$  nm) by prechannel calibration and 35.7% for total  $\text{SiO}_2$  NPs ( $\leq 100$  nm) by postchannel calibration. These measured mass percentages of  $\text{SiO}_2$  NPs vs. total  $\text{SiO}_2$  were in accordance with or close to previous reports ranging from 19% in coffee creamer [17] to 30% in coffee with creamer containing E551 [3].

The complementary performance of these two calibration strategies can be summarized. The standard addition method-based prechannel calibration enabled fast and simple quantification of dominant primary  $\text{SiO}_2$  NPs with certain size in a complex food matrix within a short time provided the size-matched  $\text{SiO}_2$  NP standard was available. The unexpected quantification errors from the sample material losses in AF4 separation were minimized and the food matrix effects can be effectively overcome. Nevertheless, the contents of  $\text{SiO}_2$  NPs or small aggregates whose sizes greatly differed from the prevailing diameter in the sample would likely be underdetected or even missed by this method. Conversely, postchannel calibration ensured that the overall  $\text{SiO}_2$  NPs smaller than 100 nm in a sample can be quantified without using  $\text{SiO}_2$  NP standards. However, this workflow was time-consuming and quantitative inaccuracy may be caused by different running conditions of ionic calibrants and analyte nanoparticles. Comparison of these features indicated the necessity in combining the strategies for quantification of highly polydispersed  $\text{SiO}_2$  NPs in a complex food matrix.

### Particle number concentration of $\text{SiO}_2$ NPs in coffee creamer

Besides mass concentration, particle number concentration is another important regulatory specification according to EC

recommendation on the definition of nanomaterial [4]. In this study, particle number concentration of the dominant primary SiO<sub>2</sub> NPs was also calculated based on the results of mass concentration from AF4-ICP-MS analysis. In this regard, mass concentration in the prepared aqueous suspension was firstly determined by standard addition method-based prechannel calibration, following the same procedure as in previous sections. Then, this mass concentration was mathematically converted to particle number concentration, based on the following equation [30]:

$$C_{n \text{ (suspension)}} = \frac{6 \times C_{m \text{ (suspension)}}}{\rho \times \pi \times d^3}$$

where  $C_{n \text{ (suspension)}}$  is the particle number concentration,  $C_{m \text{ (suspension)}}$  is the mass concentration,  $\rho$  is the density of SiO<sub>2</sub> NPs, and  $d$  is the hydrodynamic diameter of dominant primary SiO<sub>2</sub> NPs measured by AF4-ICP-MS and prechannel calibration as nominal size over the AF4 size distribution, assuming the spherical SiO<sub>2</sub> NPs with a density of 2 g/mL. The mass concentration and particle number concentration of dominant primary SiO<sub>2</sub> NPs in the sample suspension obtained were 14.6 mg/L and  $2.9 \times 10^{11}$  particles/mL (shown in Table 3) which were well accorded with the corresponding reported values for characterization of SiO<sub>2</sub> NPs in E551 products in a previous study by other researchers [6]. Consequently, it demonstrated that, although AF4-ICP-MS cannot directly lead to number-based quantification of SiO<sub>2</sub> NPs and the high uncertainties related to the approximate conversion from mass concentration into particle number concentration may lower the accuracy of resultant data, the number concentration of SiO<sub>2</sub> NPs estimated in this work presented verifiable reference values.

### Recovery experiments for measurement by AF4-ICP-MS

Corresponding to the mass quantification of SiO<sub>2</sub> NPs by prechannel and postchannel calibration, their recovery rates were evaluated, respectively. Prechannel calibration with NPs compensated for the possible losses of sample materials due to interactions between NPs and the permeation membrane or other wetted surfaces in the AF4 channel. The interactions were generally the major cause for incomplete

recoveries. Moreover, particle extraction efficiency and the interference by food matrix had to be considered as well for quantification of the analyte particles in complex samples. Hence, the losses of materials were investigated by separately using nano-sized monomodal suspensions of 20, 30, 50, and 100 nm SiO<sub>2</sub> NPs. As shown in Table 4, the overall recoveries ( $R$ ) were favorable for all these four sizes of SiO<sub>2</sub> NPs, ranging from 96% for the smallest size (20 nm) to 89% for the greatest size (100 nm). Although the recovery rate for 100 nm particles was slightly lower which can be attributed to the higher possibility of their irreversible interactions with the membrane, all the recoveries in Table 4 generally suggested that there was no obvious loss of SiO<sub>2</sub> NPs coming from either extraction and interference by the coffee creamer matrix or the application of cross flow in AF4 separation, and that the established prechannel calibration worked sufficiently. On the contrary, recovery rate of postchannel calibration reflected the total loss of silicon content of the test sample over sample preparation and AF4-ICP-MS analysis, in the form of elemental (Si) analysis and regardless of variations of particle sizes. Therefore, by using the representative SiO<sub>2</sub> NP standard of 30 nm, the recovery rate of postchannel calibration was measured as  $75 \pm 1\%$  ( $n = 3$ ) which was in well accordance with the reported recovery of total silicon at 72% on a silica suspension [23]. It was thus clearly demonstrated that postchannel calibration yielded less accurate quantification result, whereas prechannel calibration performed more accurately, which was probably due to the general requirement that samples and all calibrants undergo the same injection/separation procedure [26]. Nevertheless, in consideration of the good applicability for quantification of polydispersed nanoparticles with wide size distribution, postchannel calibration still had its advantages for mass quantification of SiO<sub>2</sub> NPs in cases where there was a lack of suitable SiO<sub>2</sub> NP standards.

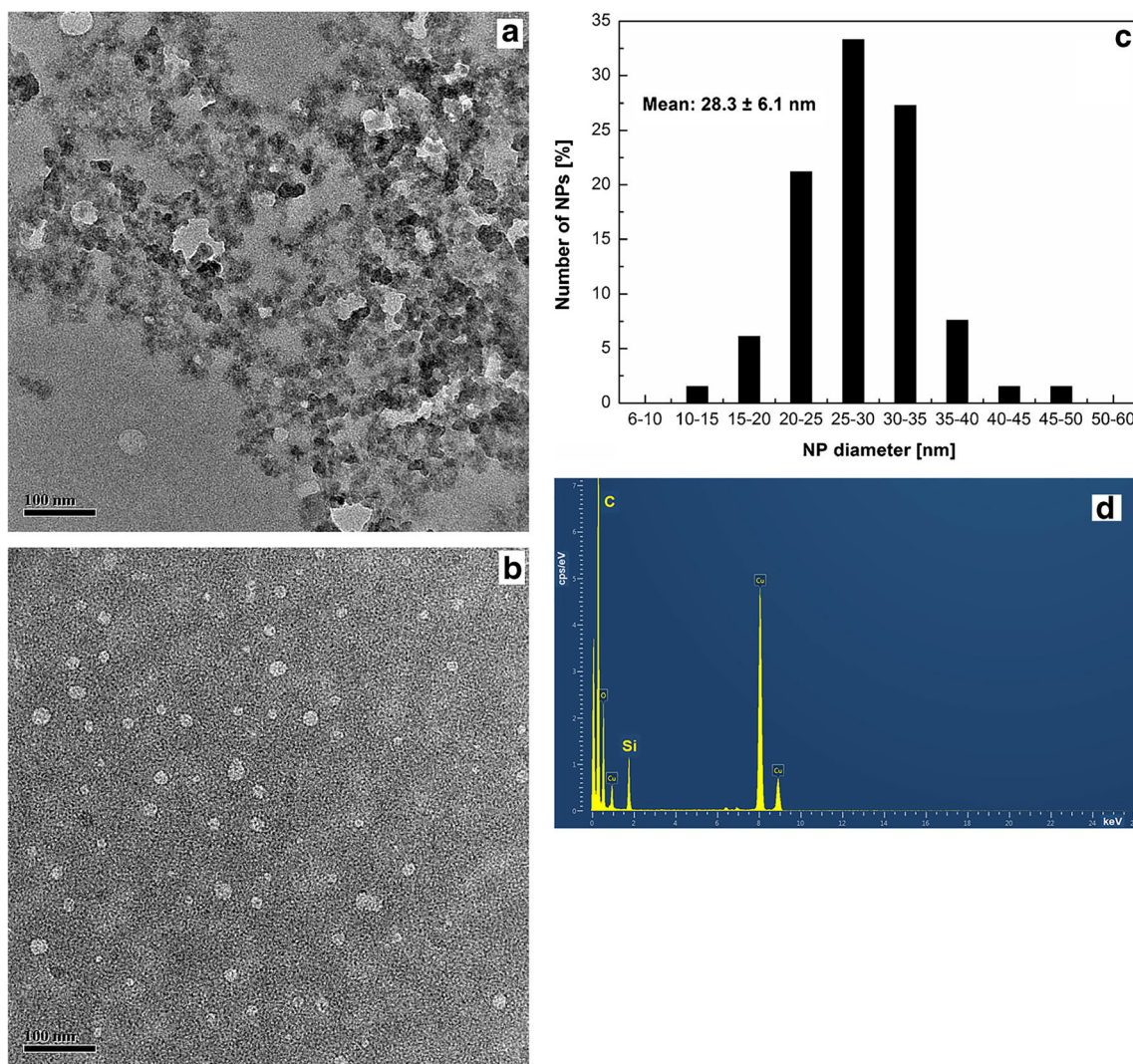
### TEM characterization

For verification of SiO<sub>2</sub> NPs size determined by AF4-ICP-MS, TEM characterization together with EDX was conducted. As shown in Fig. 4, the image by TEM revealed the presence of clear aggregation and/or agglomeration of NP clusters (Fig. 4a), but a lot of individual NPs can still be found and deemed as primary particles (Fig. 4b), and their corresponding size

**Table 4** Recoveries determined by AF4-ICP-MS for SiO<sub>2</sub> NPs in coffee creamer with nominal sizes in the range of 20–100 nm ( $n = 3$ ) based on prechannel calibration

Nominal size (nm)	Recovery rate ( $R$ )—overall (%)	Recovery rate ( $R_A$ )—extraction and matrix (%)	Recovery rate ( $R_B$ )—AF4 channel (%)
20	96 ± 8	99 ± 7	98 ± 2
30	94 ± 8	97 ± 6	97 ± 3
50	90 ± 8	92 ± 6	98 ± 3
100	89 ± 11	93 ± 8	96 ± 3





**Fig. 4** TEM characterization and EDX analysis for verification of SiO<sub>2</sub> NPs detected by AF4-ICP-MS, including **a** TEM image of SiO<sub>2</sub> small or large aggregates, **b** TEM image, and **c** its corresponding size distribution

distribution histogram was generated (Fig. 4c). According to this histogram, sizes of the primary SiO<sub>2</sub> NPs in the defatted coffee creamer extract were mainly distributed within the spanning of 10–50 nm, with a mean particle diameter of  $28.3 \pm 6.1$  nm and maximum particle number (%) at around 25–30 nm. These measured size and size range of SiO<sub>2</sub> NPs coincided well with the results from other reports on nano-silica size via TEM analysis [31–33]. Additionally, based on EDX analysis of the defatted coffee creamer extract, the presence of silicon in the particles sized by TEM was confirmed (Fig. 4d), further verifying the characterization of SiO<sub>2</sub> NPs in the studied samples.

Furthermore, it was worth noting that the size of 28.3 nm measured by TEM was very close to the hydrodynamic diameter of 28.0 nm obtained through size calibration with SiO<sub>2</sub> NP standards in pure water as described in an earlier section.

histogram of the primary SiO<sub>2</sub> NPs, as well as **d** EDX image of the defatted coffee creamer extract

Since the physical size determined by TEM was theoretically and practically smaller than the hydrodynamic diameter of NPs in AF4 separation, the equivalence of these two measured sizes around 28 nm indicated a shift of the characterized diameter of SiO<sub>2</sub> NPs toward a smaller value by size calibration using SiO<sub>2</sub> NP standards dispersed in pure water and its unsuitability for size determination of SiO<sub>2</sub> NPs in coffee creamer compared to the size calibration with SiO<sub>2</sub> NP standards spiked in coffee creamer.

## Conclusions

An effective and practical approach for both size characterization and mass quantification of SiO<sub>2</sub> NPs in a complex food matrix such as high-fat coffee creamer by AF4-ICP-MS has



been developed. Based on defatting with hexane followed by resuspension of the SiO<sub>2</sub> NPs in water and subsequent sonication, the established sample preparation method ensured the advantages in terms of simplicity, efficiency, and compatibility with AF4 separation. The on-line AF4-ICP-MS analysis was accomplished for accurate dimensional characterization of SiO<sub>2</sub> NPs in the coffee creamer extract with size calibrants. SiO<sub>2</sub> NP mass content was determined with good recovery rates by the combination of the standard addition method using on-line prechannel calibration with SiO<sub>2</sub> NP standard and off-line postchannel calibration with elemental standards due to their complementary benefits. In addition, TEM characterization further verified the size and size range measured by AF4-ICP-MS and confirmed the presence of primary nanosilica particles and small aggregates below 100 nm in the studied coffee creamer. In brief, this AF4-ICP-MS method provided reliable, economical, and pragmatic solution for monitoring the presence of SiO<sub>2</sub> NPs in coffee creamer. It is expected that the developed analytical methodology could also be applicable to other foodstuffs containing SiO<sub>2</sub> NPs with appropriate modifications.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Mattarozzi M, Suman M, Cascio C, Calestani D, Weigel S, Undas A, et al. Analytical approaches for the characterization and quantification of nanoparticles in food and beverages. *Anal Bioanal Chem.* 2017;409(1):63–80.
- Calzolai L, Gilliland D, Rossi F. Measuring nanoparticles size distribution in food and consumer products: a review. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2012;29(8):1183–93.
- Peters R, Kramer E, Oomen AG, Herrera Rivera ZE, Oegema G, Tromp PC, et al. Presence of nano-sized silica during in vitro digestion of foods containing silica as a food additive. *ACS Nano.* 2012;6(3):2441–51.
- EC recommendation on the definition of NM (2011/696/EU), Off J Eur Union, L275/38.
- Peters R, Brandhoff P, Weigel S, Marvin H, Bouwmeester H, Aschberger K, et al. Inventory of nanotechnology applications in the agricultural, feed and food sector. EFSA supporting publication. 2014:EN-621, 125 pp.
- Barahona F, Ojea-Jimenez I, Geiss O, Gilliland D, Barrero-Moreno J. Multimethod approach for the detection and characterisation of food-grade synthetic amorphous silica nanoparticles. *J Chromatogr A.* 2016;1432:92–100.
- Regulation (EC) No 1333/2008 of the European Parliament and of the Council on Food Additives. *Off J Eur Union.* 2008;L 354/16.
- Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the Provision of Food Information to Consumers. *Off J Eur Union.* 2011;L 304/18.
- EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). Scientific opinion on the re-evaluation of silicon dioxide (E 551) as a food additive. *EFSA J.* 2018;16(1): 5088.
- Contado C, Ravani L, Passarella M. Size characterization by sedimentation field flow fractionation of silica particles used as food additives. *Anal Chim Acta.* 2013;788:183–92.
- Grombe R, Charoud-Got J, Emteborg H, Linsinger TP, Seghers J, Wagner S, et al. Production of reference materials for the detection and size determination of silica nanoparticles in tomato soup. *Anal Bioanal Chem.* 2014;406(16):3895–907.
- Lim JH, Sisco P, Mudalige TK, Sánchez-Pomales G, Howard PC, Linder SW. Detection and characterization of SiO<sub>2</sub> and TiO<sub>2</sub> nanostructures in dietary supplements. *J Agric Food Chem.* 2015;63(12):3144–52.
- Wagner S, Legros S, Loeschner K, Liu J, Navratilova J, Grombe R, et al. First steps towards a generic sample preparation scheme for inorganic engineered nanoparticles in a complex matrix for detection, characterization, and quantification by asymmetric flow-field flow fractionation coupled to multi-angle light scattering and ICP-MS. *J Anal At Spectrom.* 2015;30:1286–96.
- Contado C, Mejia J, Lozano García O, Piret JP, Dumortier E, Toussaint O, et al. Physicochemical and toxicological evaluation of silica nanoparticles suitable for food and consumer products collected by following the EC recommendation. *Anal Bioanal Chem.* 2016;408(1):271–86.
- de la Calle I, Menta M, Klein M, Séby F. Study of the presence of micro- and nanoparticles in drinks and foods by multiple analytical techniques. *Food Chem.* 2018;266:133–45.
- Geiss O, Bianchi I, Senaldi C, Barrero J. Challenges in isolating silica particles from organic food matrices with microwave-assisted acidic digestion. *Anal Bioanal Chem.* 2019;411(22):5817–31.
- Dekkers S, Krystek P, Peters RJB, Lankveld DPK, Bokkers BGH, van Hoeven-Arentzen PH, et al. Presence and risks of nanosilica in food products. *Nanotoxicology.* 2011;5(3):393–405.
- Heroult J, Nischwitz V, Bartczak D, Goenaga-Infante H. 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. *Anal Bioanal Chem.* 2014;406(16): 3919–27.
- von der Kammer F, Legros S, Larsen EH, Loeschner K, Hofmann T. Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. *TrAC Trends Anal Chem.* 2011;30(3):425–36.
- Montaño MD, Majestic BJ, Jämting ÅK, Westerhoff P, Ranville JF. Methods for the detection and characterization of silica colloids by microsecond spICP-MS. *Anal Chem.* 2016;88(9):4733–41.
- Li T, Senesi AJ, Lee B. Small angle X-ray scattering for nanoparticle research. *Chem Rev.* 2016;116(18):11128–80.
- Pabisch S, Feichtenschlager B, Kickelbick G, Peterlik H. Effect of interparticle interactions on size determination of zirconia and silica based systems – a comparison of SAXS, DLS, BET, XRD and TEM. *Chem Phys Lett.* 2012;521:91–7.
- Aureli F, D'Amato M, Raggi A, Cubadda F. Quantitative characterization of silica nanoparticles by asymmetric flow field flow fractionation coupled with online multiangle light scattering and ICP-MS/MS detection. *J Anal At Spectrom.* 2015;30:1266–73.
- Barahona F, Geiss O, Urbán P, Ojea-Jimenez I, Gilliland D, Barrero-Moreno J. Simultaneous determination of size and

- quantification of silica nanoparticles by asymmetric flow field-flow fractionation coupled to ICPMS using silica nanoparticles standards. *Anal Chem.* 2015;87(5):3039–47.
25. Raghavan SR, Walls HJ, Khan SA. Rheology of silica dispersions in organic liquids: new evidence for solvation forces dictated by hydrogen bonding. *Langmuir.* 2000;16(21):7920–30.
  26. Geiss O, Cascio C, Gilliland D, Franchini F, Barrero-Moreno J. Size and mass determination of silver nanoparticles in an aqueous matrix using asymmetric flow field flow fractionation coupled to inductively coupled plasma mass spectrometer and ultraviolet-visible detectors. *J Chromatogr A.* 2013;1321:100–8.
  27. Makan AC, Spallek MJ, du Toit M, Klein T, Pasch H. Advanced analysis of polymer emulsions: particle size and particle size distribution by field-flow fractionation and dynamic light scattering. *J Chromatogr A.* 2016;1442:94–106.
  28. Sánchez-García L, Bolea E, Laborda F, Cubel C, Ferrer P, Gianolio D, et al. Size determination and quantification of engineered cerium oxide nanoparticles by flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry. *J Chromatogr A.* 2016;1438:205–15.
  29. Prestel H, Schott L, Niessner R, Panne U. Characterization of sewage plant hydrocolloids using asymmetrical flow field-flow fractionation and ICP-mass spectrometry. *Water Res.* 2005;39(15):3541–52.
  30. Petersen E, Montoro Bustos A, Toman B, Johnson M, Ellefson M, Caceres G, et al. Determining what really counts: modelling and measuring nanoparticle number concentrations. *Environ Sci Nano.* 2019;6:2876–96.
  31. Athinarayanan J, Periasamy VS, Alsaif MA, Al-Warthan AA, Alshatwi AA. Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. *Cell Biol Toxicol.* 2014;30(2):89–100.
  32. Athinarayanan J, Alshatwi AA, Periasamy VS, Al-Warthan AA. Identification of nanoscale ingredients in commercial food products and their induction of mitochondrially mediated cytotoxic effects on human mesenchymal stem cells. *J Food Sci.* 2015;80(2):N459–64.
  33. Yang Y, Faust JJ, Schoepf J, Hristovski K, Capco DG, Herckes P, et al. Survey of food-grade silica dioxide nanomaterial occurrence, characterization, human gut impacts and fate across its lifecycle. *Sci Total Environ.* 2016;565:902–12.

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