

Quantitative characterization of silica nanoparticles by asymmetric flow FFF-MALS-ICP-QQQ using the Agilent 8800 ICP-QQQ

Application note

Materials, Environmental, Food

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Introduction

Synthetic amorphous silica (SAS) is one of the most commonly used nanomaterials. It is an authorized food additive and is also found in a wide variety of industrial and consumer products from polymers, paints, and textiles to toothpastes, detergents and cosmetics. Silica nanoparticles (NPs) also have great potential for a variety of other applications, including diagnostic and therapeutic applications in medicine [1].

Although silica NPs have been in use for a long time, their potential long-term effects on human health and the environment are not well understood. There is an increasing need for analytical methods that are capable of identifying, characterizing and quantifying NPs, and for the development of reference materials for quality assurance of measurements [2, 3]. Methods



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are required to support upcoming regulation [4], to enable quality and compliance control of nano-products, and in the assessment of nano-safety. A recent risk assessment of synthetic amorphous silica raised concerns on the long-term health effects due to its presence in food, highlighting that further investigation on this topic is warranted [5].

Field-flow fractionation (FFF) in combination with elemental and particle size detectors is a highly promising approach to separate and characterize NPs [6], but it has rarely been applied to nanosilica. In this study, quantitative characterization of silica NPs was accomplished by on-line coupling of asymmetric flow FFF (A4F) with multi angle light scattering (MALS) and triple quadrupole ICP-MS (ICP-QQQ) for silicon detection. Element-specific detection using the Agilent 8800 ICP-QQQ provides quantitative Si concentrations in each of the size fractions separated online by FFF. In this new method, the ICP-QQQ was used in MS/MS mode with oxygen (O₂) cell gas to successfully avoid the polyatomic interferences affecting *m/z* 28-30, enabling fractograms of all three silicon isotopes (²⁸Si, ²⁹Si, ³⁰Si) to be measured.

Experimental

Instrumentation

A metal-free Postnova Analytics AF2000 MT asymmetric flow FFF (A4F) system equipped with a flat separation channel (320 mm × 60 mm) of 280 mm length was coupled online to a Postnova 3621 MALS detector. A 350 μm spacer was used along with a 10 kDa molecular weight cut-off regenerated cellulose membrane (Postnova Analytics), as an accumulation wall.

An Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ), equipped with an octopole-based collision/reaction cell (CRC) was used for detection of the Si isotopes. The ICP-QQQ is a tandem mass spectrometer configuration, with two quadrupole mass analyzers (Q1 and Q2) separated by the CRC. This configuration means that the instrument can be operated in MS/MS mode, where both quadrupoles are acting as mass filters (1 amu resolution), to allow precise control of the reaction chemistry that takes place in the cell. Si measurements were performed using mass-shift mode with O₂ (99.999% purity grade) as the reaction gas. The sample

introduction system comprised inert components, including a demountable torch with platinum injector and a PFA concentric nebulizer with a double-pass PFA spray chamber cooled to 2 °C. The outlet of the MALS was connected to the inlet of the ICP nebulizer and the FFF eluate was mixed via an inert T-piece with an internal standard solution containing 2 μg/L Ge in 0.1% nitric acid, delivered by the ICP-QQQ's peristaltic pump. The ICP-QQQ operating and acquisition parameters are summarized in Table 1. All sample handling and analytical measurements were carried out in clean room conditions.

Table 1. Optimized ICP-QQQ parameters

Parameter	Setting
RF power (W)	1550
Carrier gas flow rate (L/min)	0.90
O ₂ reaction gas flow rate (mL/min)	1.15
Q1 bias (V)	-1
Octopole bias (V)	-5.3
Acquisition mode	Time Resolved Analysis
Integration time/mass (sec)	0.5
Sampling period (sec)	2
Total acquisition time (min)	50
Selected mass pairs (Q1 → Q2)	28 → 44, 29 → 45, 30 → 46, 72 → 72

Materials and reagents

Near-monodisperse, non-functionalized silica nanoparticle suspensions ('NanoXact') with nominal diameters of 20, 50, 80, 100, 120, 140, 160, and 180 nm were used (Nanocomposix, San Diego, CA). Certified Si and Ge standards at 1000 mg/L (High Purity Standard, Charleston, SC) were used for the preparation of the silicon calibration and internal standard solutions. Ultrapure HNO₃, HF (Carlo Erba Reagenti, Rodano, Italy) and H₂O₂ (Merck KGaA, Darmstadt, Germany) were used as reagents in closed-vessel microwave digestion according to Aureli et al. [7].

Determination of particle size and mass concentration of silica NPs by FFF-MALS-ICP-QQQ

The FFF method was developed to achieve controlled separation of particles over a size range of one order of magnitude (~20-200 nm as hydrodynamic diameter, *d_h*) with maximum recovery of analyte material. The silica nanoparticle suspensions with nominal diameters of 20, 50, 80, 100, 120, 160, 180 nm were characterized by FFF-MALS-ICP-QQQ in terms of size and mass fraction.

Particle size was calculated by converting the radius of gyration (r_g) obtained by MALS into d_h according to the relation $r_g/r_h = 0.775$ ($d_h = 2r_h$) based on the consistent spherical shape of the particles used. The results compared well with the d_h values reported by the manufacturer (Table 2). As a result, the d_h values of the certificate were used as the reference values for particle size calibration. Calibration was performed by injecting single near-monodisperse silica suspensions and plotting the known particle size against the resulting elution time. A curve-fitting equation was then determined to allow calibration of the particle sizes in the test samples. A typical calibration curve is shown in Figure 1.

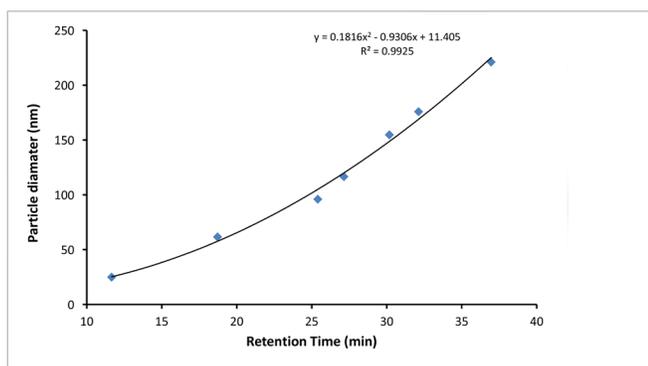


Figure 1. Calibration curve for silica nanoparticles

Particle mass concentrations of the near-monodisperse silica suspensions were determined by ICP-QQQ, and were found to be in the range 5.7–8.9 mg/mL. These values were used to calibrate the particle mass measured in the test samples.

The total silicon content of the 20 and 50 nm particle suspensions was also determined after microwave digestion. The average difference between the total silica concentration (after digestion) and the particle concentration of the undigested particles was only 2.4%, suggesting that ionic calibrants added post-FFF could be used to quantify silicon measurements by ICP-QQQ. In addition, pre-channel calibration of particle mass was used to account for possible losses occurring, e.g. in the FFF channel [8], by performing injections of the 20, 50, 100, 180 nm NP dispersions at a concentration of 1.2, 2.5, 6, 12 $\mu\text{g/mL SiO}_2$.

Table 2. Silica NP suspensions used for size calibration

Nominal size ^a (nm)	TEM diameter ^a (nm)	d_h reference value ^a (nm)	d_h found value ^b (nm)
20	23.2 ± 2.4	24.9	31.2 ± 8.3
50	47.7 ± 3.7	61.6	60.4 ± 10.6
80	82.6 ± 4.7	96.0	103.0 ± 14.7
100	101.7 ± 9.0	116.6	111.2 ± 14.2
120	119.9 ± 16.7	154.7	152.8 ± 16.0
160	156.0 ± 18.1	175.8	167.0 ± 10.8
180	186.8 ± 13.1	221.1	203.1 ± 19.6

^aHydrodynamic diameter per the manufacturer's certificate.

^bHydrodynamic diameter as assessed by converting r_g values obtained by MALS into d_h according to the relation $r_g/r_h = 0.775$ ($d_h = 2r_h$) based on the consistent spherical shape of the particles used.

Results and discussion

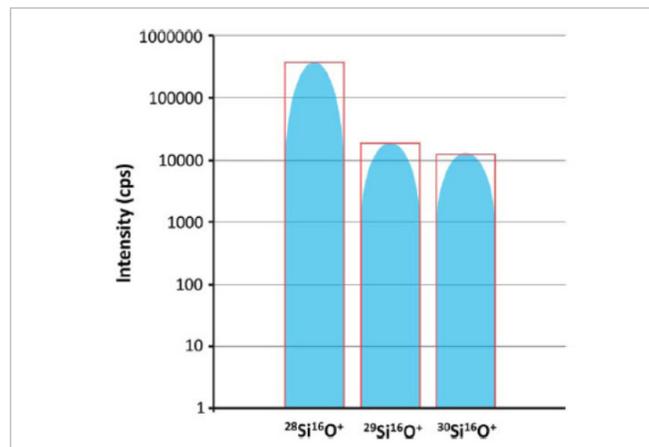
Removal of interferences on silicon

Sensitive and accurate silicon determination by single quadrupole ICP-MS is hindered due to polyatomic interferences affecting the three naturally occurring isotopes ^{28}Si ($^{14}\text{N}^{14}\text{N}^+$, $^{12}\text{C}^{16}\text{O}^+$), ^{29}Si ($^{14}\text{N}^{15}\text{N}^+$, $^{14}\text{N}^{14}\text{NH}^+$, $^{13}\text{C}^{16}\text{O}^+$, $^{12}\text{C}^{16}\text{OH}^+$), and ^{30}Si ($^{15}\text{N}^{15}\text{N}^+$, $^{14}\text{N}^{15}\text{NH}^+$, $^{14}\text{N}^{16}\text{O}^+$, $^{13}\text{C}^{17}\text{O}^+$, $^{12}\text{C}^{17}\text{OH}^+$). The natural abundances of the three isotopes are 92.2%, 4.7%, and 3.1%, respectively.

The Agilent 8800 ICP-QQQ with MS/MS capability can use mass-shift to selectively shift the target analyte ion away from intense spectral interferences at the analyte ion's original native mass. With oxygen (O_2) MS/MS mass-shift mode, the reaction product ion is shifted from its original analyte ion mass by +16 amu by an O-atom addition reaction, and can therefore be detected free from the original interference. In this case, Q1 was set to m/z 28, 29 and 30 to allow each of the Si isotopes into the cell in turn, and O_2 was added into the cell to convert the Si^+ ions into SiO^+ product ions, which were measured at the corresponding m/z of 44, 45 and 46 (Q2). As illustrated in Figure 2, the signal intensities obtained at m/z 44, 45 and 46 followed the natural isotopic pattern of Si, confirming that no mass bias was introduced in the reaction cell. Table 3 shows that satisfactory results in terms of LoD and BEC were obtained for the three silicon isotopes.

Table 3. Linearity, sensitivity, LoD and BEC of silicon determination by ICP-QQQ using O₂ mass-shift mode

Analytical conditions	Precursor and Product Ion	Q1 → Q2 m/z	R	Sensitivity cps/μg/L	b (blank) cps	LoD μg/L	BEC μg/L
O ₂ 1.15 mL/min	²⁸ Si → ²⁸ Si ¹⁶ O ⁺	28 → 44	0.9998	4876	13472	0.09	2.8
	²⁹ Si → ²⁹ Si ¹⁶ O ⁺	29 → 45	0.9998	260	782	0.31	3.0
	³⁰ Si → ³⁰ Si ¹⁶ O ⁺	30 → 46	0.9994	183	1112	0.56	6.1

**Figure 2.** Measured silicon isotopes (blue peaks) with the theoretical isotopic abundance template overlaid. Reproduced by permission of The Royal Society of Chemistry.

In the absence of a biological-matrix reference material with certified concentration values for silicon, an internal quality control material ISS-BL (bovine liver sample spiked with soluble silicon) was prepared to check the accuracy of the total silicon determinations [7]. The 8800 ICP-QQQ was used to analyze Si using the oxygen MS/MS mass-shift mode, and the results are shown in Table 4. The measured Si concentrations based on the SiO⁺ product ions (*m/z* 44, 45 and 46) derived from all three Si isotopes were in good agreement with the target values established using three independent techniques (ICP-DRC-MS, ICP-OES and HR ICP-MS) [7].

Table 4. Accuracy of total silicon determination by ICP-QQQ (n=5).

	Target value	²⁸ Si ¹⁶ O ⁺	²⁹ Si ¹⁶ O ⁺	³⁰ Si ¹⁶ O ⁺
Measured Si concentration (μg/g)	20.4±1.9	20.9±1.8	21.2±2.1	20.0±1.8

Optimization of the FFF method

The FFF focusing time and cross flow values were optimized to ensure a clear separation of the void peak from the peak originating from the smallest particle size used in this study (20 nm), while minimizing losses in the void peak and achieving satisfactory peak resolution.

Figure 3 shows the overlaid fractograms for the seven NanoXact silica particle size suspensions from 20 to 180 nm (see Table 2) measured at *m/z* 44 (²⁸SiO⁺) using ICP-QQQ. All peak maxima are separated, allowing the accurate determination of particle diameter in unknown samples in a size-range covering one order of magnitude. In particular, separation of size fractions in the nano-range (≤100 nm) is good.

The baseline signal of the fractogram is relatively high, indicating the presence of Si contamination in the FFF eluent, as other ions that could potentially contribute to the signal at *m/z* 44 should have been removed by the O₂ mass shift mode of the ICP-QQQ. To investigate this background signal, the pure eluent was analyzed for total silicon content. The FFF eluent consisted of a 0.1-μm filtered mixture containing 0.02% v/v of FL-70 - a commercially available alkaline surfactant mixture. The surfactant was found to contain 64.2 μg/L Si, which accounts for the high background signal. Reducing the Si contamination in the eluent would improve the analytical performance of the method.

Analysis of test samples

The FFF-MALS-ICP-QQQ method was applied to the characterization of two test samples, the reference material ERM-FD100 and the NanoXact silica suspension with 140 nm nominal diameter. Firstly, the recovery of the particle mass in two samples was investigated by both pre-channel calibration with silica NPs and post-channel calibration with elemental standards. Quantification using post channel-calibration with ionic silicon was less accurate than pre-channel

calibration using silica NPs. The latter approach ensures accurate quantification as the calibrants undergo the same injection/separation procedure as the samples.

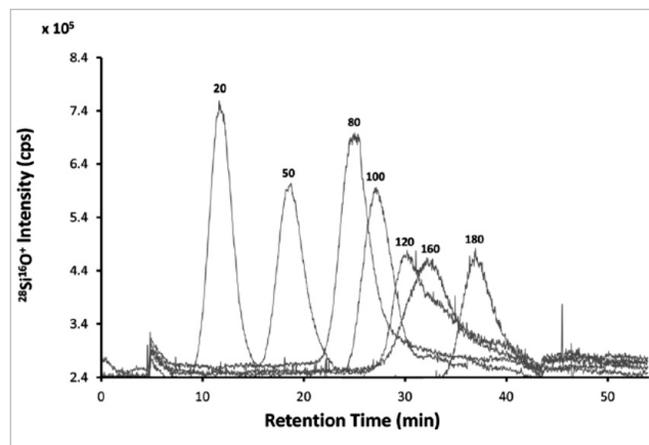


Figure 3. Overlaid FFF-ICP-QQQ fractograms of individual near-monodisperse NanoXact silica particle solutions. Numbers above the peaks represent the nominal size of each fraction in nm. Reproduced by permission of The Royal Society of Chemistry (RSC)

The hydrodynamic diameter of the silica NPs in the two test samples was calculated both from the radius of gyration (MALS) and by size calibration (FFF-ICP-QQQ). The results are compared with the reference values in Table 5.

In general, good agreement was found between the two experimentally determined values and with the reference value. It has to be noted that in the FFF-MALS analysis of ERM-FD100, the measured value was close to the size detection limit of the technique [9, 10] and a high sample concentration had to be used. Overall, the availability of two measurement approaches in the present method gives greater confidence in the estimation of reliable size values.

Table 5. Size characterization of two test samples by asymmetric FFF-MALS-ICP-QQQ.

Test sample	Reference diameter (nm)	d_h calculated from r_g as measured by FFF-MALS (nm)	d_h obtained by size calibration in FFF-ICP-QQQ (nm)
ERM-FD100	19.4 ± 1.3^a	22.9 ± 4.2	23.3 ± 3.8
NanoXact silica 140 nm	150.4^b	142.3 ± 10.6	167.4 ± 8.9

^a Certified value measured by electron microscopy.

^b Reference value reported on the manufacturer's certificate, measured by dynamic light-scattering.

Conclusions

On-line coupling of asymmetric flow FFF with multi-angle light scattering and ICP-QQQ detection was used successfully for the quantitative characterization of silica NPs. Accurate dimensional characterization of the particles separated by FFF was achieved by means of both ICP-QQQ detection with size calibrants, and standardless sizing by MALS. ICP-QQQ was used to provide element-specific quantitative measurement of the silicon present in the size fractions separated online by FFF. Calibration of particle mass was performed using both pre-channel calibration with silica NPs and post-channel calibration with elemental standards. The newly-developed FFF-MALS-ICP-QQQ method enabled dimensional and mass determination of silica particles over a size range of one order of magnitude with satisfactory recoveries of analyte material.

The Agilent 8800 ICP-QQQ with MS/MS mode is able to measure all three silicon isotopes as their SiO^+ product ions. This is an important feature of the method and opens application areas involving the use of isotopically enriched silica NPs, and absolute quantification of silica using isotope dilution (ID) analysis. The method is currently being used for the detection of nano-sized silica in foodstuffs.

More information

For a full account of this application see publication: [Quantitative characterization of silica NPs by asymmetric flow field flow fractionation coupled with online multi-angle light scattering and ICP-MS/MS detection](#), Federica Aureli, Marilena D'Amato, Andrea Raggi and Francesco Cubadda, *J. Anal. At. Spectrom.*, 2015, 30, 1266-1273. DOI: 10.1039/C4JA00478G

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References

1. D. Napierska, L.C.J. Thomassen, D. Lison, J.A. Martens, P.H. Hoet, *Particle and Fibre Toxicology* 2010, 7, 39.
2. T.P.J. Linsinger, G. Roebben, C. Solans, R. Ramsch, *Trends Anal. Chem.*, 2011, 30, 18–27.
3. T.P.J. Linsinger, Q. Chaudhry, V. Dehalu, P. Delahaut, A. Dudkiewicz, R. Grombe, F. von der Kammer, E.H. Larsen, S. Legros, K. Loeschner, R. Peters, R. Ramsch, G. Roebben, K. Tiede, S. Weigel, *Food Chem.*, 2013, 138, 1959–1966.
4. L. Calzolari, D. Gilliland, F. Rossi, *Food Addit. Contam.*, 2012, 29, 1183–1193.
5. P.C.E. van Kesteren, F. Cubadda, H. Bouwmeester, J.C.H. van Eijkeren, S. Dekkers, W.H. de Jong, A.G. Oomen, *Nanotoxicology*, 2014, Epub ahead of print July 18, doi:10.3109/17435390.2014.940408.
6. F. von der Kammer, S. Legros, E.H. Larsen, K. Loeschner, T. Hofmann, *Trends Anal. Chem.*, 2011, 30, 425-436.
7. F. Aureli, M. D'Amato, A. Raggi, M. Ciprotti, S. Nisi, A. Sorbo, F. Cubadda, 2016. In preparation.
8. O. Geiss, C. Cascio, D. Gilliland, F. Franchini, J. Barrero-Moreno, *J Chromatogr. A*, 2013, 1321, 100-108.
9. A. Zattoni, D.C. Rambaldi, P. Reschiglian, M. Melucci, S. Krol, A. M. Coto Garcia, A. Sanz-Medel, D. Roessner, C. Johann, *J. Chromatogr. A*, 2009, 1216, 9106-9112.
10. M. J. Spallek, A. Wallner, R. Jünger, Application Note 0028, Postnova Analytics GmbH.

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