

Application Note

Food, personal
care products



Analysis of TiO₂ Nanoparticles in Foods and Personal Care Products by Single Particle ICP-QQQ

Using the 8900 ICP-QQQ in MS/MS mass-shift mode to resolve ⁴⁸Ca⁺ isobaric interference on ⁴⁸Ti⁺



Authors

Janja Vidmar¹

Katrin Loeschner¹

Raquel Larios²

¹National Food Institute,
Technical University of Denmark
(DTU Food), Denmark

²Agilent Technologies, Spain

Introduction

Titanium dioxide (TiO₂) is classified as a food additive within the European Union (E171) and in the USA (INS171), where it is widely used as whitening and brightening agent. In the EU, E171 can be used in several food categories with no maximum upper limit (*quantum satis*) (1). E171 mainly consists of larger TiO₂ particles, but it also contains a fraction of nanoparticles (NPs), which are defined as particles with one or more dimensions less than 100 nm (2). TiO₂ is also used in food contact materials, cosmetics, and personal care products, such as toothpaste.

With increasing production and use of TiO₂ based additives, there is concern among regulatory authorities and consumers about the potential impact of human exposure to TiO₂ NPs. Several toxicological studies on TiO₂ NPs have been carried out (3–5), but no general agreement on the effects of TiO₂ NPs on biological systems has been reached. The European Food Safety Authority (EFSA) has released several scientific opinions (6, 7). EFSA concluded that the use of TiO₂

as a food additive does not raise a genotoxic concern and established a No Observed Adverse Effect Levels (NOAEL) of 2.250 mg/TiO₂ kg/bw per day. However, when the French food safety agency, ANSES, reviewed recent studies on TiO₂, they found a lack of conclusive results on the safety of the E171 additive. Following the appraisal, the French government announced that the sale of food products containing TiO₂ (E171) will be banned from January 1, 2020 (8). EFSA recently released a scientific opinion stating the need for further research on the particle size distribution of E171 in food (9).

European regulations state that all ingredients present in the form of engineered nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word 'nano' in brackets (10). In 2011, the European Commission published a recommendation for the definition of a nanomaterial suggesting that for regulatory purposes, the size distribution and number concentration of nanomaterials should be known (11). To keep pace with evolving regulations, effective analytical methods are needed for the characterization of TiO₂ NPs in a range of sample types.

ICP-MS is a powerful tool for the characterization of NPs, providing composition information that complements established techniques such as transmission and scanning electron microscopy (TEM/SEM), and dynamic light scattering (DLS). When used in single particle (sp) mode, spICP-MS provides simultaneous determination of the particle number and size distribution (assuming spherical particles). In addition, spICP-MS provides the concentrations of both the particles and the dissolved content of the element or elements of interest. The ease-of-use of modern ICP-MS instruments makes it the technique of choice for regulatory purposes, while its flexibility ensures that the same instrument can be used for multi-element analysis and speciation studies.

Titanium is a challenging element to measure by conventional single quadrupole ICP-QMS. The isobaric interference on the most abundant isotope, ⁴⁸Ti, by ⁴⁸Ca cannot be mass resolved by ICP-QMS. At its highest resolution setting, High Resolution (HR) ICP-MS can theoretically resolve ⁴⁸Ca from ⁴⁸Ti, as the required resolution (M/ΔM) is 10,458. However, ⁴⁸Ca is present at high concentrations in many food samples, such as milk, making separation of the adjacent peaks more difficult. Also, the combination of low sensitivity at high resolution, and peak tailing due to the poor abundance sensitivity of HR-ICP-MS means that this measurement is often not practical. Other elements such as sulfur, phosphorus, silicon, and carbon, which are also present in many foods at significant concentrations, form polyatomic interferences that

hinder the accurate measurement of ⁴⁸Ti. Typically, the less interfered isotopes ⁴⁷Ti or ⁴⁹Ti are measured by ICP-QMS. However, these less abundant isotopes provide lower sensitivity, which limits the detection of smaller-sized TiO₂ NPs by ICP-QMS, leading to biased size distribution results.

The Agilent 8900 Triple Quadrupole ICP-MS (ICP-QQQ) can operate in MS/MS mode with reactive cell gases to resolve the spectral interferences on ⁴⁸Ti, including the isobaric interference from ⁴⁸Ca. The first quadrupole, Q1, positioned before the collision/reaction cell (CRC), operates as a unit mass filter, allowing only the ions with target *m/z* to enter the cell and rejecting all ions of different masses. Analyte-ion selection by Q1 enables excellent control of the reaction chemistry in the cell. Also, the low background, high sensitivity, and very short dwell times (100 μs) of the 8900 enable the ICP-QQQ to detect small-sized particles.

In this study, TiO₂ NPs present in different food samples and toothpaste were characterized by spICP-MS using an Agilent 8900 ICP-QQQ. The results show the benefits of triple quadrupole (MS/MS mode) technology with O₂/H₂ cell gases to resolve polyatomic and isobaric interferences on ⁴⁸Ti.

Experimental

Standards and reference materials (RM)

NIST 8012 gold NPs RM (Gaithersburg, MD, USA) and ionic gold standards (PlasmaCAL standard, SCP Science, Baie D'Urfé, QC, Canada) were used. The gold NP RM, with a known average particle diameter of 27.6 nm as determined by TEM, and the ionic gold standard were analyzed to calculate the nebulization efficiency.

To determine the response factor for titanium, ionic titanium standard (PlasmaCAL standard, SCP Science) was used. Solutions of 5 to 200 ng Ti/mL in 100 times diluted milk (for milk samples) or 0.1% nitric acid (for TiO₂ NPs in water and food samples) were analyzed.

Anatase and rutile are two naturally occurring mineral forms of TiO₂. Two TiO₂ nanomaterials with different size distributions were used to spike milk samples and check the performance of the method. The nanomaterials included a TiO₂ representative test material, JRCNM 10200a (referred to as JRC NPs), from the Joint Research Centre's Nanomaterial Repository. The JRC NPs material contains primary particles of 115 nm and consists of anatase. NIST 1898 TiO₂ NPs SRM (referred to as NIST NPs) was also used. The NIST NPs SRM has a primary size < 50 nm and consists of 76% anatase and 24% rutile.

Samples and sample preparation

The concentrations of Ca, S, and P in cow's milk are in the range of 1070–1330 µg/mL, 320 µg/mL, and 630-1020 µg/mL, respectively (12). A matrix mimicking a diluted (100 times) milk matrix was prepared by diluting ionic Ca, S, and P standards (PlasmaCAL standards, SCP Science) in water. The final concentrations were 10 µg/mL for Ca, 3 µg/mL for S, and 10 µg/mL for P (individually and as a mix). In addition, 10 ng/mL of ionic Ti was spiked into the 100-fold diluted simulated milk matrix, and in 0.1% nitric acid (HNO₃).

Different food samples were bought from local stores in Denmark. The foods included skimmed milk with 0.1% fat content, salad dressing, and cake decorations (edible gold stars). A commercial toothpaste from a Dutch supermarket, which was extensively studied in the EU FP7 Project "NanoDefine" (13), was also analyzed. Details of the sample preparation for each sample type were as follows:

- Milk spiked with TiO₂ NPs: Stock suspensions of 1 mg TiO₂/mL were prepared. After appropriate dilution, TiO₂ was spiked into undiluted skimmed milk at 200 and 400 ng TiO₂/mL for JRC NPs, and 15 and 30 ng TiO₂/mL for NIST NPs.
- Toothpaste: The sample preparation protocol was adopted from Correia *et al.* (14). 100 mg of toothpaste was dispersed in 10 mL of ultrapure water (UPW) by vortexing. The solution was further diluted 10 times in 0.1% sodium dodecyl sulfate (SDS, Sigma-Aldrich, St. Louis, MO, USA), followed by vortexing.
- Salad dressing: 100 mg of salad dressing was dispersed in 5 mL of 0.1% SDS placed in a high intensity cup horn (Branson Digital Sonifier SFX 550). The solution was further diluted 10 times in 0.1% SDS, followed by vortexing.
- Cake decorations: One golden star was added to 5 mL of UPW and dissolved, with assistance from a high intensity cup horn. The solution was further diluted 10 times in UPW, followed by vortexing.
- Before analysis, all samples were diluted with UPW (milk 100x, toothpaste 2500x, salad dressing 500x, and cake decoration 250x).

Instrumentation

An Agilent 8900 ICP-QQQ (#100, Advanced Applications configuration) was used with an SPS 4 autosampler. The instrument was equipped with a MicroMist concentric nebulizer, Scott type double-pass quartz spray chamber, a quartz torch with a small internal diameter (1.0 mm) injector, and standard nickel cones. Samples were introduced directly into the ICP-QQQ via the standard peristaltic pump and tubing. The operating conditions of the Agilent 8900 ICP-QQQ are detailed in Table 1.

Table 1. ICP-MS operating conditions.

Parameter	Value
RF Power (W)	1550
Sampling Depth (mm)	8
Nebulizer Gas (L/min)	0.70
Sample Inlet Flow (mL/min)	~ 0.35
Spray Chamber Temperature (°C)	2
Dwell Time (ms)	0.1
Settling Time (ms)	0
Acquisition Mode	MS/MS (Q1: <i>m/z</i> 48, Q2: <i>m/z</i> 64)
Gas Flow Rates (mL/min)	O ₂ : 0.15 (10% of full scale) H ₂ : 7.0
Axial Acceleration (V)	1.0
Octopole Bias Voltage (V)	-6.0
Energy Discrimination (V)	-15.0
Acquisition Time (s)	60

Data was acquired for 60 s with a dwell time of 0.1 ms for each sample. The major titanium isotope, ⁴⁸Ti, was measured in MS/MS mass-shift mode, using a mixed cell gas containing oxygen and hydrogen. The method was used to resolve all the isobaric interferences—mainly arising from ⁴⁸Ca—and the matrix-based polyatomic interferences, such as those derived from C, S, and P. Q1 was set to *m/z* 48 (the mass of the precursor ⁴⁸Ti ion) and Q2 was set to *m/z* 64 (the mass of the target product ion ⁴⁸Ti¹⁶O). O₂ promoted the formation of the ⁴⁸Ti¹⁶O⁺ product ion, and H₂ helped with the formation of ⁴⁸Ca¹⁶O¹H⁺, avoiding interference on ⁴⁸Ti¹⁶O⁺ by ⁴⁸Ca¹⁶O⁺ (Figure 1). The instrument operating parameters were also used in a previous study (15). After each run, the sample introduction system was rinsed using UPW, diluted acids, and Triton X-100.

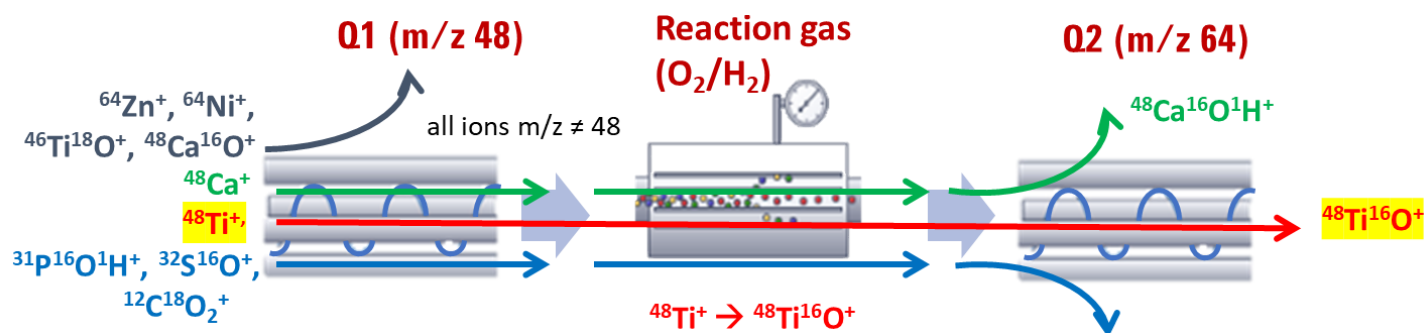


Figure 1. Determination of $^{48}\text{Ti}^+$ as $^{48}\text{Ti}^{16}\text{O}^+$ product ion using the 8900 ICP-QQQ operating in MS/MS mass-shift mode with O_2/H_2 as the reaction cell gases.

The Single Nanoparticle Application Module of the Agilent ICP-MS MassHunter software was used for method setup and data analysis. Analysis was performed in peak integration mode. The particle baseline was determined automatically by the software. The particle detection threshold was adjusted manually, and the same value was used for samples of the same type. The application of the same threshold ensured that a direct comparison of particle mass concentrations and median particle diameters between samples was possible. A particle density of 3.9 g/cm^3 and an analyte mass fraction (the value of molecular mass divided by analyte mass) of 1.67 was used for the calculations. The sample inlet flow was determined gravimetrically.

Results and discussion

Interference check in relevant matrices containing ionic Ti

To check the effectiveness of the MS/MS mass-shift mode to remove potential interferences on Ti, solutions containing single interfering elements and a mixture simulating 100 times diluted milk were analyzed (Table 2). The apparent Ti concentration was measured in all the solutions, as $^{48}\text{TiO}^+$ in MS/MS mode with O_2/H_2 , and as $^{48}\text{Ti}^+$ without any reaction gas (in Single Quad and MS/MS modes).

The results in no gas mode (SQ and MS/MS) show a large positive error in all matrices containing interfering elements, with the most pronounced errors in the presence of Ca. In contrast, O_2/H_2 in MS/MS mode effectively reduced all interferences, allowing the accurate measurement of Ti, even in the presence of Ca and in the simulated milk matrix. The highest Ti sensitivity and the lowest detection limit (DL) were also achieved in O_2/H_2 MS/MS mode. The results suggest that the 8900 ICP-QQQ operating in MS/MS mass-shift mode with O_2/H_2 would detect smaller sized particles compared to the two no gas modes.

Analysis of TiO_2 NPs spiked into milk samples

TiO_2 has been considered as a whitener for low-fat milk (16), although its addition to milk is currently not permitted in Europe. To check the effectiveness of the method to accurately measure TiO_2 NPs of different size distributions and concentrations, two different TiO_2 NPs (JRC and NIST) were spiked into the milk samples. JRC NPs, which contain larger sized particles with a broader size distribution than NIST NPs, resemble E171, which consists primarily of anatase. The fraction of NPs in E171 ranges between 17 and 36% and the mean diameters of the primary particles range between 115 to 145 nm (17).

Figure 2 shows the comparison of the measurement of the same TiO_2 NPs spiked at the same concentration into diluted milk or UPW and analyzed in MS/MS mode with O_2/H_2

Table 2. Interference check results for ^{48}Ti in various matrices, with and without cell gas.

Matrix	Ca 10 $\mu\text{g/mL}$	S 3 $\mu\text{g/mL}$	P 10 $\mu\text{g/mL}$	Mix Ca+S+P	10 ng/mL Ti in Mix Ca+S+P	10 ng/mL Ti in 0.1% HNO_3		
Cell Mode	Apparent Ti Measured Concentration, ng/mL						Sensitivity ¹ (cps/ ng/mL)	DL ² (ng/mL)
No gas MS/MS	35.6	0.395	0.264	36.3	41.7	9.58	207,044	4.1
No gas SQ	35.0	0.341	0.224	34.2	45.8	11.1	294,872	8.2
O_2/H_2 MS/MS	0.017	0.004	0.012	0.031	10.0	10.2	399,949	0.0075

1. Based on calibration curve of six ionic standards from 0.1 to 50 ng/mL ionic Ti in 0.1% HNO_3

2. DL calculated as three times the standard deviation of four blanks (mix Ca + S + P)

reaction gas. The particle size distributions were similar in UPW and in the milk matrix for both particle types. In water and the milk-matrix, the mean particle diameters were 226 ± 7 and 223 ± 2 nm (JRC NPs), and 78 ± 1 and 72 ± 2 nm (NIST NPs), respectively. The similarity of the results shows that interferences from Ca, S, or P in the milk matrix were resolved.

The particle size data agreed with existing data for the two particle types. According to the certificate of analysis for the NIST NPs, the stable nanoscale species is a monomodal aggregate of fused crystallites with a modal size of 70 nm. The JRC NPs are similar to NM-100 (18), which contain primary particles ranging from 20 up to 300 nm and aggregates ranging from 30 up to 700 nm. The mean diameter obtained by TEM was 190 nm (18).

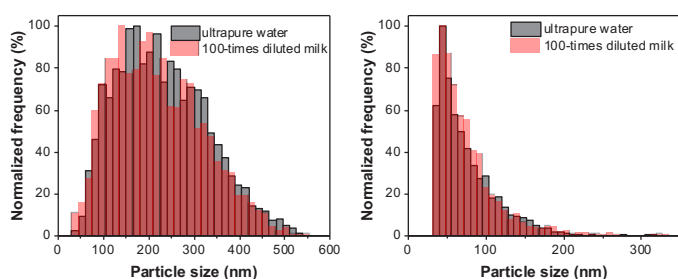


Figure 2. Particle size distributions for 4 ng/mL JRC NPs (left) and 0.15 ng/mL NIST NPs (right) in UPW and 100 times diluted milk measured in MS/MS mass-shift mode.

Similar particle size results were obtained by comparing the two spiked levels for both NP types (Table 3). The average mass recoveries, which were in the range of 71 to 85%, were considered satisfactory.

Table 3. spICP-MS results for measuring JRC and NIST NPs in 100 times diluted milk at two different concentration levels (n=3 for each spike level).

	JRC NPs 2 ng/mL	JRC NPs 4 ng/mL	NIST NPs 0.15 ng/mL	NIST NPs 0.30 ng/mL
Number of Detected Particles (particles/min)	917 ± 135	1624 ± 113	1648 ± 199	2988 ± 90
Particle Number Conc (E+07 particles/L)	4.40 ± 0.55	7.80 ± 0.54	7.60 ± 0.92	13.80 ± 0.42
Particle Mass Conc (ng/mL)	1.55 ± 0.26	2.91 ± 0.11	0.13 ± 0.01	0.25 ± 0.02
Mass Recovery (%)	76 ± 13	71 ± 6	85 ± 7	81 ± 4
Median Particle Size (nm)	204 ± 3	210 ± 2	60 ± 2	62 ± 1
Mean Particle Size (nm)	216 ± 0	223 ± 2	72 ± 2	74 ± 0

Effects of interferences on measurement of ^{47}Ti and ^{48}Ti in no gas mode

To show the interference removal benefits of MS/MS mass-shift mode compared to SQ or MS/MS in no gas mode, ^{47}Ti and ^{48}Ti were measured without cell gas in the same spiked diluted milk samples. NIST NPs were not detectable due to the high background on ^{47}Ti and ^{48}Ti . The larger JRC NPs were detectable (Figure 3), but the minimum detectable size increased from 40 nm (MS/MS mass-shift mode) to 213 nm (^{47}Ti) and 160 nm (^{48}Ti) in no gas mode. There was also an increase in the median diameters from 210 nm (MS/MS mass-shift mode) to 292 nm, and 210 nm (MS/MS mass-shift mode) to 260 nm, respectively.

The high (ionic) background on ^{48}Ti could be attributed to the isobaric interference of ^{48}Ca , as shown in Table 2. For ^{47}Ti , a high particle background was observed in both the spiked and non-spiked milk matrix. As no particles were detected in the non-spiked milk sample in MS/MS mass-shift mode, it could be concluded that these were not Ti-containing particles, but likely caused by polyatomic interferences.

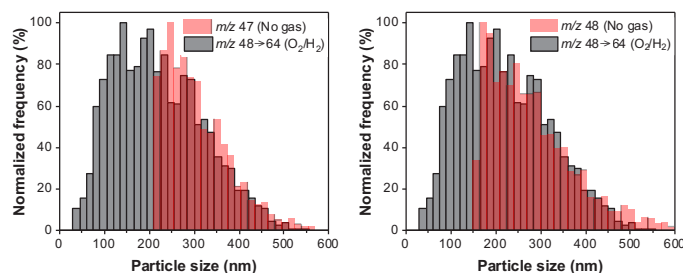


Figure 3. Particle size distributions for 4 ng/mL JRC NPs in 100 times diluted milk determined by analyzing ^{47}Ti in no gas mode (left) and ^{48}Ti in no gas mode (right). For comparison purposes, the size distribution obtained in MS/MS mode with O_2/H_2 gas is shown in grey in both graphs.

Analysis of TiO_2 NPs in toothpaste, salad dressing, and cake decorations

Other products that include TiO_2 in the list of ingredients were analyzed. Toothpaste, salad dressing, and a cake decoration (edible golden star) were selected as examples. The particle size distribution results, which are presented in Figure 4, show that each of the samples contains TiO_2 NPs with different size ranges. The cake decoration contained the smallest sized NPs (median size of 54 nm), while the salad dressing contained the largest particles (median size of 334 nm). The particle sizes determined in toothpaste (median size 155 nm) agreed with previously published results (14). The fraction of particles < 100 nm was 22, 3, and 70% for toothpaste, salad dressing, and the cake decoration, respectively. The average TiO_2 mass concentrations were in the mg/g sample range.

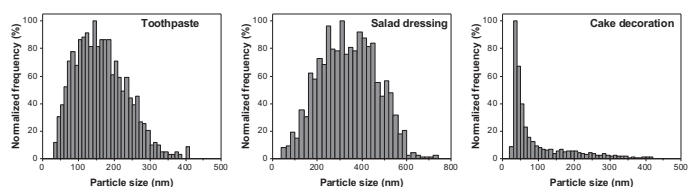


Figure 4. Particle size distributions for TiO₂ NPs in toothpaste (left), salad dressing (middle), and a cake decoration (right).

Conclusion

A single particle ICP-MS method was developed for the determination and characterization of TiO₂ NPs in foods and personal care products. An Agilent 8900 ICP-QQQ was used in the study, since its high sensitivity and short dwell times provide fast analysis times and excellent detection limits for the determination of small-sized particles. Also, the advanced interference-removal capabilities of ICP-QQQ using MS/MS allowed the analysis of the most abundant titanium isotope, ⁴⁸Ti, enabling the accurate analysis of small-sized TiO₂ NPs.

Operating the ICP-QQQ in MS/MS mode with O₂/H₂ cell gas, isobaric (⁴⁸Ca) and polyatomic interferences on ⁴⁸Ti were successfully resolved in matrices containing high concentrations of P, S, and Ca. By measuring ⁴⁸Ti rather than the less abundant Ti isotopes, a particle size detection limit of 30 nm was achieved for TiO₂ NPs.

The method was used for the characterization of TiO₂ NPs in foods that are known to contain the food additive E171, including toothpaste, salad dressing, and a cake decoration. spICP-MS can be used to indicate the elemental composition and size of particles in a sample, complementing the information on particle size and shape provided by electron microscopy.

Having access to reliable analytical methods for the characterization of NPs is important, as France will ban the sale of food products containing E171 from January 1, 2020. EFSA has also identified the need for further research on the particle size distribution of TiO₂ NPs in food.

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