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Nano-silica in selected food items

R.J.B. Peters

Business Unit: Residue & Contaminant analysis
Group: Veterinary Drugs

RIKILT - Institute of Food Safety
Wageningen University & Research centre
Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands
P.O. Box 230, 6700 AE Wageningen, The Netherlands
Tel +31 317 480 256
Fax +31 317 417 717
Internet www.rikilt.wur.nl

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Distribution list:

- National Institute for Public Health and the Environment (RIVM); A. Oomen
- Food and Consumer Product Safety Authority (VWA); D. van Aken
- Food and Consumer Product Safety Authority (VWA); J. Castenmiller

Summary

In this study the presence of silica NPs in food is studied. Although the commonly used definition of nano-materials refers to a size range in the order of 1 - 100 nm, nano-particles in this study were considered to be particles with a size up to 300 nm. This is because many nano-materials that are used consist of particles smaller than 100 nm which are dispersed in a product in an aggregated or agglomerated state with diameters up to 300 nm. Currently there are no special regulations for the application of nanotechnology in foods in the EU and US. Although recommendations for special regulations in the EU have been made, laws have yet to be changed.

Silica NPs have been reported to improve properties of several polymer matrices and are used in combination with anti-microbial agents in food packaging. In addition, silica (silicon dioxide - SiO_2) is the main component of food additive E551 that is used as anti-caking agent to thicken pastes and ointments, to maintain flow properties in powder products. Nano-sized silica particles were determined in a number of food items that contain E551 according to the declaration on the package, and in a few samples not containing E551. The results of dynamic light scattering shows that all samples, including those not containing E551, contain particles in a size range comparable to that of E551. Using hydrodynamic chromatography in combination with inductively coupled plasma mass spectrometry nano-silica was found in food items containing the food additive E551. In an additional experiment coffee creamer containing E551 was added to hot fresh black coffee, comparable to the normal use of coffee creamer. The results showed that nano-silica particles in the size range of 30-120 nm were present in the coffee in concentrations up to 22 mg/L.

1 Introduction

In its progress report and position paper on "Nanotechnology in Food Applications" the German Association of the Food Industry stated that, "there is currently no food ready for marketing or with market significance for the final consumer which is produced with the use of nanotechnology or from nano-materials"[1]. While the application of nano-particles (NPs) in food is still in the research and development stage[2,3,] silica NPs are relatively well-studied and have been reported to be used in food. In this study the presence of silica NPs in food is studied. Although the commonly used definition of nano-materials refers to a size range in the order of 1 - 100 nm, nano-particles in this study were considered to be particles with a size up to 300 nm. This is because many nano-materials that are used consist of particles smaller than 100 nm which are dispersed in a product in an aggregated or agglomerated state with diameters up to 300 nm. A recent ISO document on this topic describes such nano-objects as "weakly bound particles or aggregates"[4].

Silica NPs have been reported to improve mechanical properties[5], barrier properties[6] and water resistance[7] of several polymer matrices and are used in cooperation with anti-microbial agents in food packaging[8]. In addition to packaging materials, silica (silicon dioxide - SiO_2) is also the main component of food additive E551 that is used as anti-caking agent to thicken pastes and ointments, to maintain flow properties in powder products and as a carrier for fragrances or flavors presenting in non-food products (especially toothpaste), pharmaceuticals and food[2,3,9,10,11]. Besides, it has been reported that inorganic nano-materials such as food additive E551 with a coating are intended as moisture or oxygen barriers thereby improving the shelf life and flavor of confectionery products[2].

Till 2003, the maximum levels on the usage of E551 was 50 g/kg of food product according to the European Union (EU)[10]. However, currently there is no special regulations for the application of nanotechnology in foods in the EU and US. Although recommendations for special regulations in the EU have been made, laws have yet to be changed. Nonetheless, the European Commission (EC) intends to use existing food laws with respect to food products produced with nanotechnology, at the same time acknowledging that the technology will likely require modifications of the law.

2 Materials and methods

2.1 Materials and samples

2.1.1 Silica nano-particles

Colloidal silica, stabilised at pH 8.6, was obtained from the Institute for Reference Materials and Measurements (IRMM), European Commission Joint Research Centre, Geel, Belgium. Aerosil 200 F and Aerosil 380 F (E551) in the form of white powders were a kind gift of Petra Krystek, MiPlaza Materials Analysis, Philips, The Netherlands.

2.1.2 *Chemicals*

Sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$) was obtained from Fluka Anaytical. LC water was obtained from Biosolve, Valkenswaard, The Netherlands and Milli-Q water was prepared using a Milli-Q Gradient A10 system from Millipore, Molsheim, France.

2.1.3 *Food samples*

At the start of the study a series of powdered food products were purchased by RIKILT from local Dutch supermarket. According to the declaration on the packaging some of these products do contain E551 while others don't as indicated in table 1.

Table 1. Products purchased from a Dutch supermarket and used in this study

Code	Brand	Description	E551 on declaration
F-1	Honig	Mix voor Lasagnesaus	yes
F-2	Nissin	Cup noodles (soup powder)	yes
F-3	Albert Heijn	Gehakt kruiden mix	yes
F-4	Koopmans	Pannenkoek mix	yes
F-5	Albert Heijn	Asperge soep	yes
F-6	Albert Heijn	Pittige peper	yes
F-7	Bijenkorf	Sweets sticky rub	yes
F-8	Bijenkorf	Steak house rub	yes
F-9	Bijenkorf	Roast veg rub	yes
F-10	Bijenkorf	Sea food rub	yes
F-11	Albert Heijn	Burrito mix	yes
F-12	Friesland Food	Completa (coffee creamer)	yes
F-21	Dr. Oetker	Kook Pudding	no
F-22	Van Gilse	Poeder suiker	no
F-23	Maggi	Puree Naturel	no
F-24	Albert Heijn	Salt	no
F-25	Albert Heijn	Sugar	no

2.2 Methods and equipments

2.2.1 Sample preparation

Typically, food samples were suspended in LC or Milli-Q water using an ultrasonic liquid processor XL 2000 (Misonix, NewYork, US) for 15 min. The suspensions were further diluted in LC or MilliQ water with pH 5. Suspensions of IRMM silica and Aerosil 200F standards and spiked food samples were processed in the same way. Finally the diluted suspensions were filtrated though 0.45 µm filters prior to the measurements. When dilutions are kept for prolonged periods they are re-suspended using a Branson-3510 sonic bath for 15 min prior to the following measurements.

2.2.2 Dynamic light scattering

Dynamic light scattering (DLS) measurements to determine particle size and size distribution are performed using a Nanosight LM20 nano tracking analysis (NTA) instrument. Particle suspensions are injected with a syringe into the optical cell of the instrument and particle size is determined from the Brownian movement of the particles. Typical measurement time is 30s per sample and particle sizes are determined as the average of 6 measurements.

2.2.3 Hydrodynamic chromatography - Inductively coupled plasma mass spectrometry

Hydrodynamic chromatography (HDC) online coupled to inductive coupled plasma mass spectrometry (ICPMS) was used to determine nature and concentration of nano-particles in suspensions. The HPLC is an Agilent 1100 equipped with a PL-PSDA cartridge type 1 (800*7.5 mm) HDC column packed with non-coated, non-porous silica spheres. The eluens is an aqueous solution of sodium n-dodecyl sulphate (SDS, 10 mM) with a flow of 1.0 ml/min. Sample injection volumes typically are 50 µL. The ICPMS is a Thermo X series 2), equipped with platinum cones and a Babington nebulizer and an RF power of 1400 W.

3 Results

3.1 Screening of nano-particles using NTA

Suspensions of all food items and standards were measured using NTA as a screening method for the presence of nano-partices. Nano-particles were found in all samples, in those known to containing E551 (e.g. according to the declaration on the package) as well as in those not containing E551. The results are summarized in figure 1 were the particle size is shown along the vertical axes. Since the NTA analysis does not give any information regarding the chemical composition of the particles, the observed particles may be silica, but also large proteins, fibers or other organic particles like small oil droplets. While nano-particles found in a number of food items known to contain E551 have particle sizes in the range of Aerosil 200F, the particle sizes of particles found in food products not containing

E551 are generally smaller. However, the differences are statistically not significant and it is doubtful whether NTA analysis can be useful as a screening method for nano-particles in food.

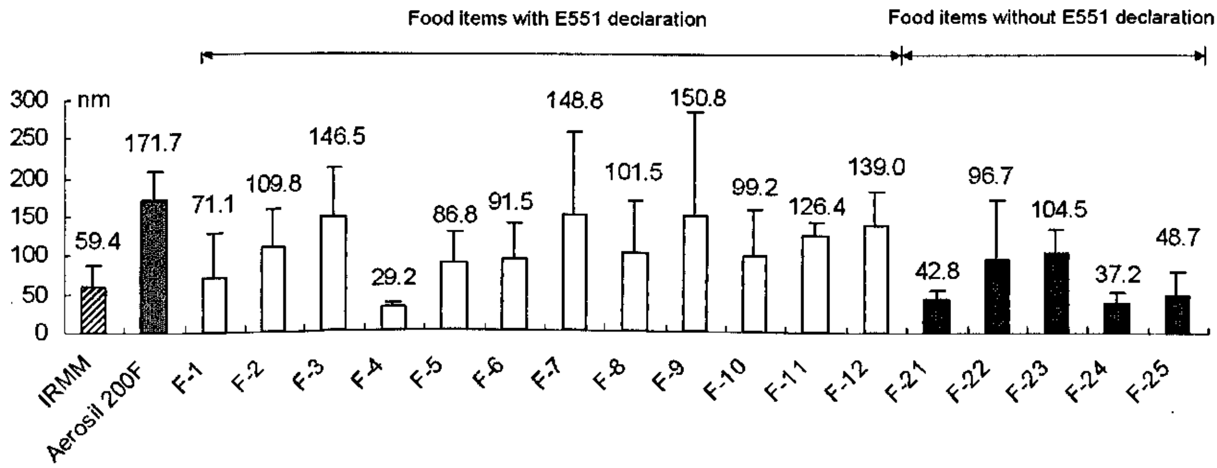

Figure 1. Median particle sizes in nm by NTA analysis (N>6) of standards (gray) and food items containing E551 as declared on the package (white) and food items probably not containing E551 (black).

3.2 HDC-ICPMS analysis of nano-particles in food

A method for the determination of silica nano-particles in food using HDC-ICPMS was developed. Following elution from the HDC silica particles are atomized in the ICP plasma and measured as silicium. Since the atomic mass of silicium coincides with that of N_2, the sensitivity of the method is limited and much less than for other elements like silver and gold. As a consequence the limit of detection for silicium is around 1 mg/L, also depending on the matrix. Another complication is the food matrix itself. Experiments showed that the recovery of silica nano-particles from samples fortified with nano-particles (IRMM or Aerosil 200F) depended on the composition of the food matrix. While the recovery was good from food sample F21 (pudding powder) and F6 (pepper) the recovery was only minimal from food sample F4 (pancake mix) as shown in figure 2. In addition the chromatographic peak of the IRMM standard was shifted to the left indicated a larger particle size. While the exact causes for these observations are unknown the results suggests that in some matrices like the pancake mix, adsorption to matrix constituents like fibers, or agglomeration processes may play a role.

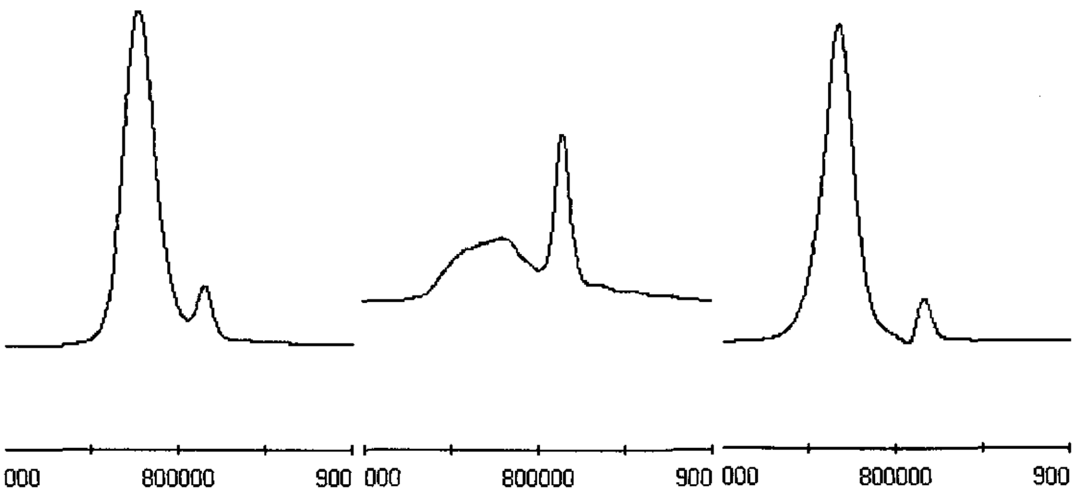

Figure 2. Samples F21 (pudding powder), F4 (pancake mix) and F6 (pepper) with addition of the IRMM standard show different chromatographic results.

The chromatograms in figure 2 all three show another peak, smaller than that of the IRMM standard and to the right of it. This peak was found not found in the blank LC-water and Milli-Q water, but was present in most of the samples. It was also found in tap water and in mineral water. The retention time of the peak suggests that these silica particles have sizes in the range of 5-10 nm. If water contains silica this is mostly in the form of ortho-sililic acid. Depending on the pH of the water part of this ortho-sililic acid will through a process of polymerization, nucleation and particle build-up be transferred into colloidal silica with particles sizes typically in the range of 5 nm.

Following further improvement of the analytical method all food samples in table 1 have been analyzed and the amount of silica in nano-form calculated. The results for the products containing E551 are presented in table 2 expressed in mg SiO_2/g product. The nano-silica content ranges from <0.1 to 1.0 mg/g product with particle sizes ranging from 50 to 200 nm. For all food items without E551 no peak was found in the chromatogram and the content of nano-silica was therefore <0.1 mg/g. For some food items the amount of nano-silica is compared to the total silicium content as determined by Petra Krystek from MiPlaza. If we assume that the total silicium content originates completely from silica (SiO_2) than we can estimate that for those products the percentages of silica in the nano-form ranges from 5% in the Burrito mix to 19% in the Coffee creamer. It should be kept in mind that a sonicator is used to prepare the suspensions and that the amount of the silica in nano-form is overestimated. On the other hand interference of the sample matrix in some cases (at least the pancake sample) can lead to adsorption and/or agglomeration processes and thus, an underestimation of the amount of silica in nano-form.

Table 2. Nano-silica contents in food items that contain E551 according to the declaration on the package

Code	Product description	nano-silica in mg/g	total Si* in mg/g	percentage silica in nano-form
F-1	Mix voor Lasagnesaus	0.26	2.5	5 %
F-2	Cup noodles (soup powder)	< 0.1		
F-3	Gehakt kruiden mix	0.17	1.2	7 %
F-4	Pannenkoek mix	< 0.1	1.3	< 4 %
F-5	Asperge soep	0.21	0.3	33 %
F-6	Pittige peper	0.13	0.5	12 %
F-7	Sweets sticky rub	0.43	2.8	7 %
F-8	Steak house rub	0.19	2.0	4 %
F-9	Roast veg rub	0.57	2.3	12 %
F-10	Sea food rub	0.53	2.2	11 %
F-11	Burrito mix	0.29	3.3	5 %
F-12	Completa (coffee creamer)	1.0	2.4	19 %
F-12	Completa coffee creamer, 2.0 g added to 200 ml hot freshly prepared coffee	2.2	2.4	43 %

**; These concentrations were determined and reported during the project by Petra Krystek, MiPlaza, Philips. Total silicium was re-calculated to total silica by multiplying with 60/28.*

For the determination of the exposure of the consumer to silica nano-particles in food, it is most relevant to determine the nano-silica content in the processed food, e.g. in the form it is consumed. This is done for coffee creamer following its addition to a freshly prepared cup of coffee. The contents of one sachet coffee creamer (2.0 g) was added to 200 mL of hot black coffee. A subsample was collected, filtered and analyzed directly with HDC-ICPMS. Different from the powdered food items, this sample was not sonicated. As shown in the last row of table 3 this resulted in a nano-silica concentration of 22 mg/L in the coffee, which translates to 2.2 mg/g for the coffee creamer. It is interesting to see that the amount of silica in the nano-form in this sample is more than two times higher than in the F-12 sample processed as the other food samples. Temperature may play a role here. Compared to the total silicium content of the coffee creamer this means that 43% of the silica in the coffee creamer (which is supposed to be mainly E551) is in the nano-form at the moment of consumption. Based on the HDC-ICPMS chromatogram the particle size of the nano-silica in the real coffee sample ranges from 30 to 120 nm. Figure 3 shows the chromatogram of a blank coffee sample and a coffee sample after addition of the coffee creamer.

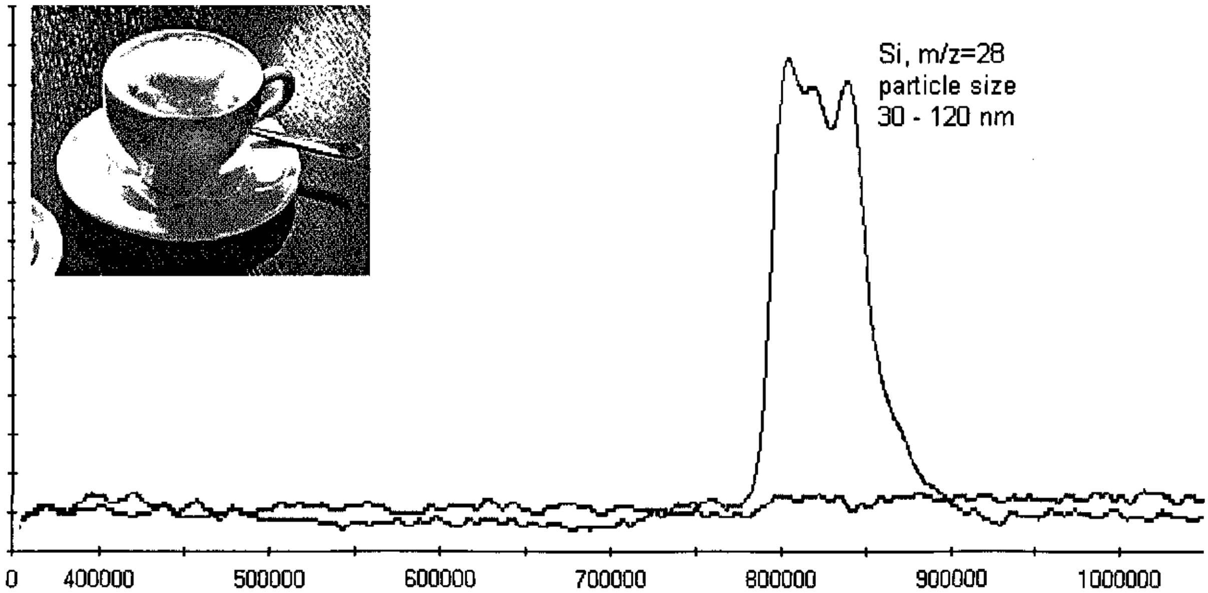

Figure 3. HDC-ICPMS chromatogram of a blank coffee sample (blue line) and a coffee sample after addition of the coffee creamer (green line).

4 Conclusions

Nano-silica particles were determined in a number food items that contain E551 according to the declaration on the package, and in a few samples not containing E551. The results of dynamic light scattering shows that all samples, including those not containing E551, contain particles in a size range comparable to that of E551. A method for the analysis of nano-silica in food items with HDC-ICPMS was developed. Using this method we found no nano-silica in food items without the food additive E551. On the other hand, 10 of the 12 food items that do contain E551 showed a peak in the chromatogram indicating the presence of nano-silica in concentrations of <0.1 mg/g to 1.0 mg/g and size ranges of 50 to 200 nm.

For coffee creamer an additional experiment was performed where coffee creamer was added to hot fresh black coffee, comparable to the normal use of coffee creamer. This resulted in a nano-silica concentration of 22 mg/L in the coffee with particles in the size range of 30-120 nm. Compared to the direct analysis of coffee creamer, the nano-particle concentration is higher and the nano-particle size smaller. This may be an affect of the higher temperature of the coffee and should be further studied.

5 References

[1] Bund für Lebensmittelrecht und Lebensmittelkunde. Progress report and position paper on "Nanotechnology in Food Applications", April 2008, http://www.bll.de/download/themen/nanotechnologie/progress_report_nanotechnolology.pdf

[2] Chaudhry Q et al. Applications and implications of nanotechnologies for the food sector. Food Additives and Contaminants, 2008. **25**(3): p. 241-258.

[3] Chen H, Weiss J and Shahidi F. Nanotechnology in nutraceuticals and functional foods Food Technol. 2007, 60 (3): 30-36.

[4] ISO/TS 27687, Nanotechnologies - Terminology and Definitions for Nano-objects - Nanoparticle, Nanofibre and Nanoplate, International Organization for Standardization, 2008.

[5] Wu CL et al. *Tensile performance improvement of low nanoparticles filled-polypropylene composites.* Composites Science and Technology, 2002. **62**(10-11): p. 1327-1340.

[6] Vladimirov V et al. *Dynamic mechanical and morphological studies of isotactic polypropylene/fumed silica nanocomposites with enhanced gas barrier properties.* Composites Science and Technology, 2006. **66**(15): p. 2935-2944.

[7] Tang SW et al. *Effect of nano-SiO2 on the performance of starch/polyvinyl alcohol blend films.* Carbohydrate Polymers, 2008. **72**(3): p. 521-526.

[8] Johnston JH et al. *Nano-structured composite calcium silicate and some novel applications.* 2008.

[9] OECD. *Synthetic amorphous silica and silicates*, in SIDS Initial Assessment Report for SIAM 19. 2004, OECD SIDS: Berlin, Germany.

[10] *Directive of the European Parliament and of the Council amending Directive 95/2/EC on food additives other than colours and sweeteners*, EU, Editor. 2002.

[11] Gibbons M, Angus F. *Acceptability of UK permitted food additives for special and religious diets.* Food Standards Agency, 2005.