Supplementary Information

The Potential of a Multi-Method Platform Centred on ICP-MS to Provide New Insights Into the Size-resolved Quantification of TiO₂ Particles in Food

David Ojeda, Dorota Bartczak, Malvinder Singh, Paul Hancock and Heidi Goenaga-Infante

LGC National Measurement Laboratory, Queens Road, Teddington, Middlesex, TW11 0LY, United Kingdom.

E-mail: Heidi.Goenaga-Infante@LGCGroup.com

Total Ti determination in food samples containing E171 by ICP-OES

Approximately 0.5 g of each sample was accurately weighed into the microwave vessels of the Mars 6 microwave system (CEM, UK). High purity grade chemicals were used throughout. Sample digestion was performed using 7 mL nitric acid (HNO_3) and 0.02 mL of hydrofluoric acid (HF), purchased from Romil, Inc. (Cambridge, UK). After the addition of the chemicals, vessels were allowed to stand for 20 min, closed and digested using a program with a temperature ramped to 180 ºC over 20 minutes, then hold at 180 ºC for 10 minutes and finally cooled to room temperature.

The digest solutions were transferred into 50 mL polyethylene tubes, made up to 50 g with ultrapure water. This resulted in a final acid concentration in the samples of 14% nitric acid (HNO_3).

A 7000DV ICP-OES instrument manufactured by Perkin Elmer was used for total Ti analysis with the settings listed in Table S2. The instrument was equipped with a glass Seaspray nebuliser and a cyclonic spray chamber.

Table S1 List of food samples for this study purchased from local supermarket (Tesco Ltd., Teddington, UK and Tesco Ltd., Feltham, UK) and their Ti total contents determined by ICP-OES.

Product	Ti concentration (mg kg⁻¹)*
White Iced Ring Doughnuts Icing Only	1419
Fondant Icing	538
Polo Mint Original	< LOQ

*LOQ of 20 mg Ti kg⁻¹.
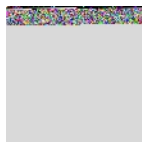
Table S2 ICP-OES operating conditions for single particle analysis.

Parameter	Value
RF power	1300 W
Plasma gas	15 L min^{-1}
Auxiliary gas	0.2 L min^{-1}
Nebuliser gas	0.7 L min^{-1}
Pump uptake rate	0.8 – 1.0 mL min^{-1}

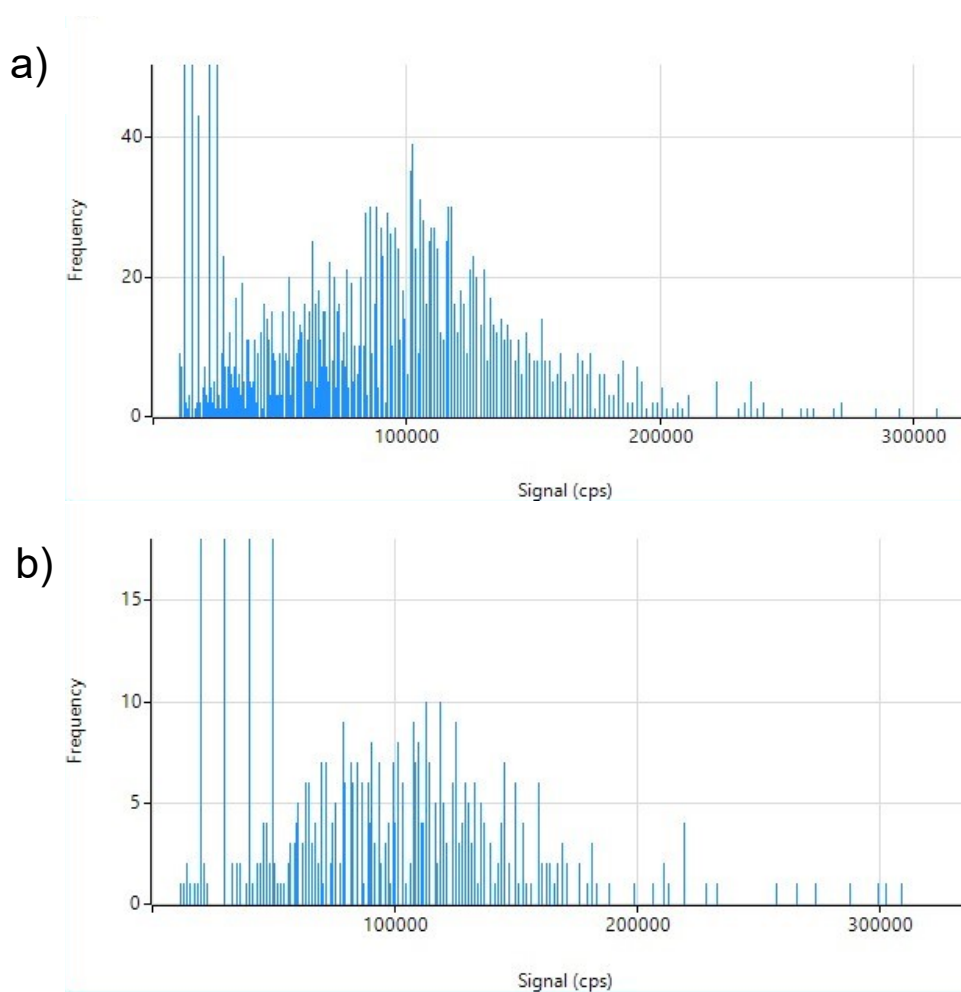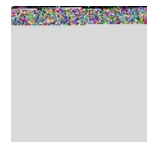**Signal distributions for different samples by spICP-MS**

Figure S1 Representative spICP-MS signal distribution of TiO$_2$ particles for the small fraction close to the background for: a) the NM-100 material and b) Iced ring food sample.

AF4 method optimisation for the fractionation of TiO_2 particles

AF4 separation method was optimised to allow simultaneous detection and baseline separation of the two size fractions seen with spICP-MS for NM-100 material, which included selection of the AF4 carrier and fine tuning of the AF4 separation programme. AF4 carrier 0.01% SDS at pH 8.0 was selected based on literature.[1-3] Other carriers like ultrapure water and 7.5 mM SDS in Tris-HCl (10 mM, pH 7.4) were also tested, but since no improvement in method's performance was seen (data not shown), 0.01% SDS pH 8.0 was selected as optimal for the purpose of the work described here.

A separation program with a reduction of the crossflow after the focus step was firstly selected based on the literature, where it has been successfully applied for different types of nanoparticles,[4-7] including TiO_2 in food samples.[2] Optimisation of the AF4 separation program included varying the focusing time, the crossflow in the elution step and the crossflow applied during the focusing step. Regarding the focusing time (Figure S2), 5 and 7 min were studied for the NM-100 material. Despite no peak was separated during the elution step in both cases, a reduction of the void peak was obtained when the focusing time was elongated, as an indication that the analyte focusing process improved. Therefore, 7 min condition was selected for further measurements. A reduction of the crossflow during the elution step from the starting conditions (from 0.5 to 0.1 mL min^{-1}) was tested. As can be seen in Figure S3, a peak from 12 to 35 min was detected for the lower crossflow condition, also reducing the peak area obtained at the end of the fractogram, during the washing step in which no crossflow is applied. Optimisation of the crossflow during the focusing step was also studied. By increasing the crossflow from 0.5 to 1.5 mL min^{-1}, the void peak was reduced (Figure S4). The modification of the crossflow in the focus step did not affect peak separated in terms of signals during the elution (same peak area), but its retention time (from 23 to 28 min, considering the peak maximum). Given these two factors, a better resolution of the main peak from the void peak was achieved, and 1.5 mL min^{-1} was selected for the separation program (specified in Table 2).
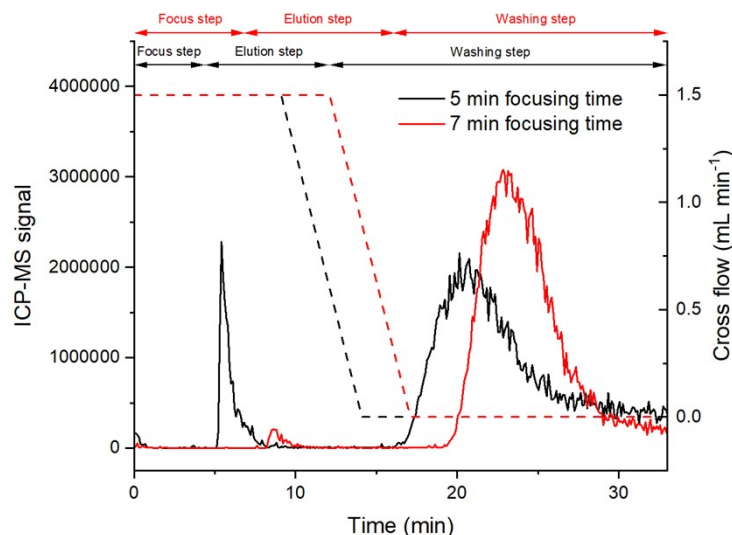

Figure S2 Focusing time optimisation for the AF4 separation method coupled to ICP-MS as detector system. Results for NM-100 material. Black line corresponds to 5 min, whilst red line to 7 min focusing time. Crossflow program is show in both cases with the dash lines (following the same colour code).
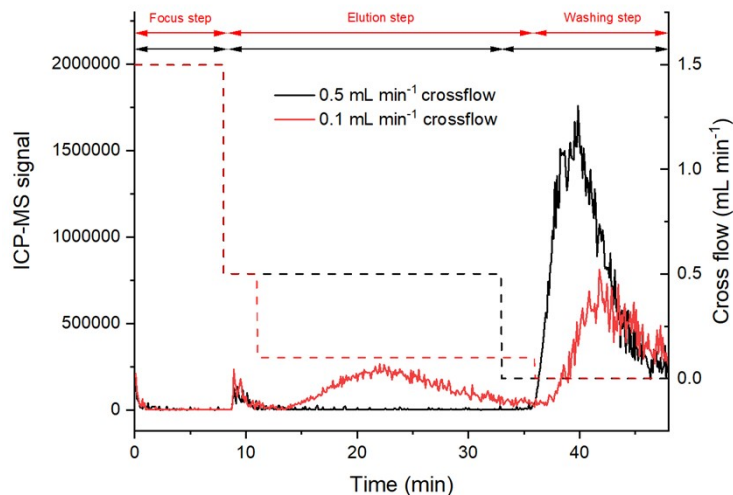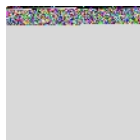
Figure S3 Crossflow optimisation during elution step for the AF4 separation method coupled to ICP-MS as detector system. Results for NM-100 material. Black line corresponds to 0.5 mL min^{-1}, whilst red line corresponds to 0.1 mL min^{-1}. Crossflow program is show in both cases with the dash lines (following the same colour code).
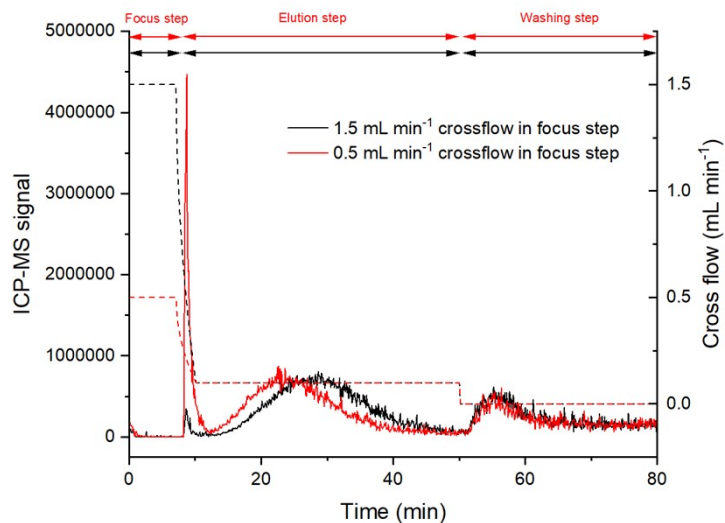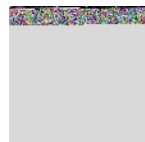

Figure S4 Crossflow optimisation during focusing step for the AF4 separation method coupled to ICP-MS as detector system. Results for NM-100 material. Black line corresponds to 1.5 mL min^{-1}, whilst red line to 0.5 mL min^{-1}. Crossflow program is show in both cases with the dash lines (following the same colour code).

References

1 I. de la Calle, M. Menta, M. Klein, B. Maxit and F. Seby, Towards routine analysis of TiO2 (nano-)particle size in consumer products: Evaluation of potential techniques, *Spectrochim. Acta Part B*, 2018, **147**, 28-42.

2 D. Ojeda, M. V. Taboada-López, E. Bolea, J. Pérez-Arantegui, P. Bermejo-Barrera, A. Moreda-Pineiro and F. Laborda, Size characterization and quantification of titanium dioxide nano- and microparticles-based products by Asymmetrical Flow Field-Flow Fractionation coupled to Dynamic Light Scattering and Inductively Coupled Plasma Mass Spectrometry, *Anal. Chim. Acta*, 2020, **1122**, 20-30.

3 B. Li, S. L. Chua, D. Yu, S. H. Chan and A. Li, Separation and size characterization of highly polydisperse titanium dioxide nanoparticles (E171) in powdered beverages by using Asymmetric Flow Field-Flow Fractionation hyphenated with MALS and ICP-MS, *J. Chrom. A.*, 2021, **1643**, DOI: 10.1016/j.chroma.2021.462059.

4 L. Sánchez-García, E. Bolea, F. Laborda, C. Cubel, P. Ferrer, D. Gianolio, I. da Silva and J.R. Castillo, Size determination and quantification of engineered cerium oxide nanoparticles by flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry, *J. Chrom. A.*, 2016, **1438**, 205-215.

5 I. Abad-Álvaro, E. Bolea, F. Laborda and J.R. Castillo, An ICP-MS-based platform for release studies on silver-based nanomaterials, *J. Anal. At. Spectrom.*, 2017, **32**, 1101-1108.

6 I. Abad-Álvaro, C. Trujillo, E. Bolea, F. Laborda, M. Fondevila, M.A. Latorre and J.R. Castillo, Silver nanoparticles-clays nanocomposites as feed additives: characterization of silver species released during in vitro digestions. Effects on silver retention in pigs, *Microchem. Journal*, 2019, **149**, DOI: 10.1016/j.microc.2019.104040.

7 M.S. Jiménez, M. Bakir, K. Ben-Jeddou, E. Bolea, J. Pérez-Arantegui and F. Laborda, Comparative study of extraction methods of silver species from faeces of animals fed with silver-based nanomaterials, *Microchim. Acta*, 2023, **190**, DOI: 10.1007/s00604-023-05777-0.