separations

MDPI

Supplementary Materials

Challenges in Determining the Size Distribution of Nanoparticles in Consumer Products by Asymmetric Flow Field-Flow Fractionation Coupled to Inductively Coupled Plasma-Mass Spectrometry: The Example of Al_2O_3, TiO_2, and SiO_2 Nanoparticles in Toothpaste

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Table S1. Chemical composition of the tested toothpaste as labelled in the acquired products. The function of each ingredient is described [16].

Ingredient	Function
Aqua	-
Hydrated Silica	Abrasive agent (SiO_2)
Alumina	Abrasive agent (Al_2O_3)
CI 77891	Pigment (TiO_2)
Sorbitol	Humectant/sweetener
Hydroxyethylcellulose	Thickening/binding agent
PEG-40 Hydrogenated Castor Oil	Emulsifier/binding agent, surfactant, and fragrance
Stearic Acid	Emulsifier/binding agent, surfactant
Cocamidopropyl Betaine	Secondary surfactant
Citric acid	Therapeutical agent: synergist to enhance the effectiveness of antioxidants
3-(N-hexadecyl-N-2-hydroxiethylammonio)propyl-bis(2-hydroxiethyl)ammoniumdifluorid	Therapeutic agent/anti-carries
Olaflur	Therapeutic agent/anti-carries
Limonene	Fragrance
Aroma	Flavour
Sodium Saccharin	Artificial sweetener
Saccharin	Artificial sweetener

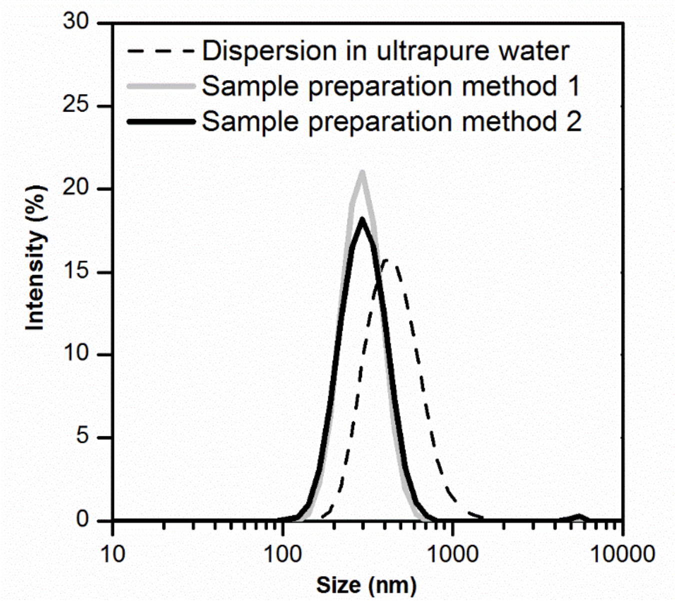


Figure S1. Intensity-based PSD obtained by DLS measurements of toothpaste dispersions (1 mg toothpaste / mL) in ultrapure water (dashed line), prepared by a two-step dilution in 0.1% w/w SDS (sample preparation method 1, gray line) and chemical oxidation with 30% H_2O_2 followed by dilution in 0.1% w/w SDS (sample preparation method 1, black line).

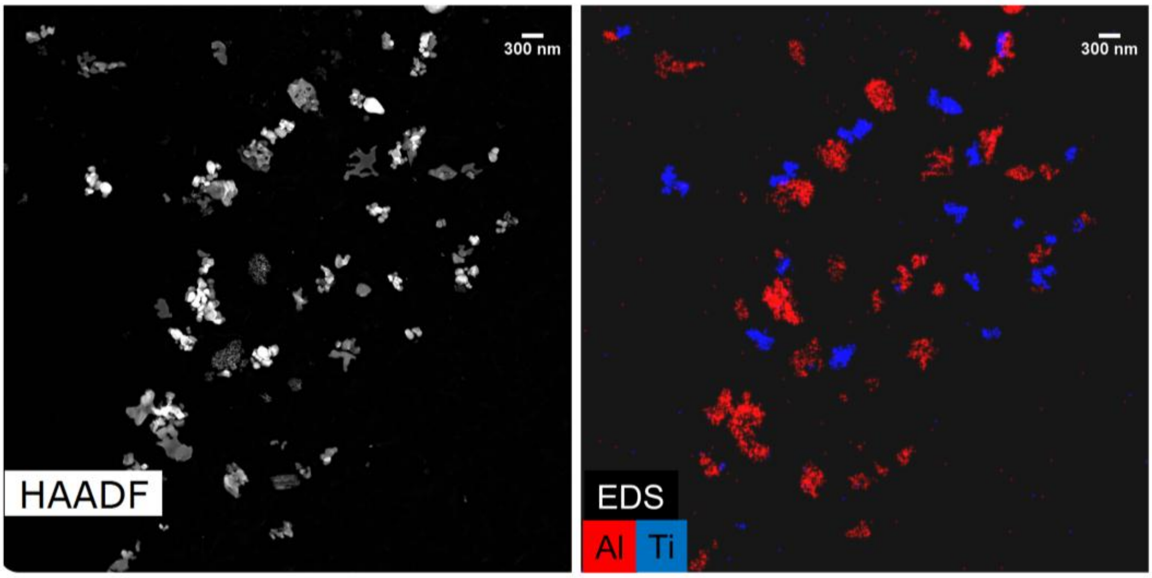


Figure S2. STEM-HAADF/EDX analysis of the toothpaste sample. The left panel shows an STEM-HAADF image of the particles present in toothpaste. The right panel shows the corresponding STEM-EDX mapping of the same area for Al and Ti. The Al- and Ti-containing particles are displayed in red and blue, respectively, and should correspond to the constituent Al_2O_3 and TiO_2 particles.

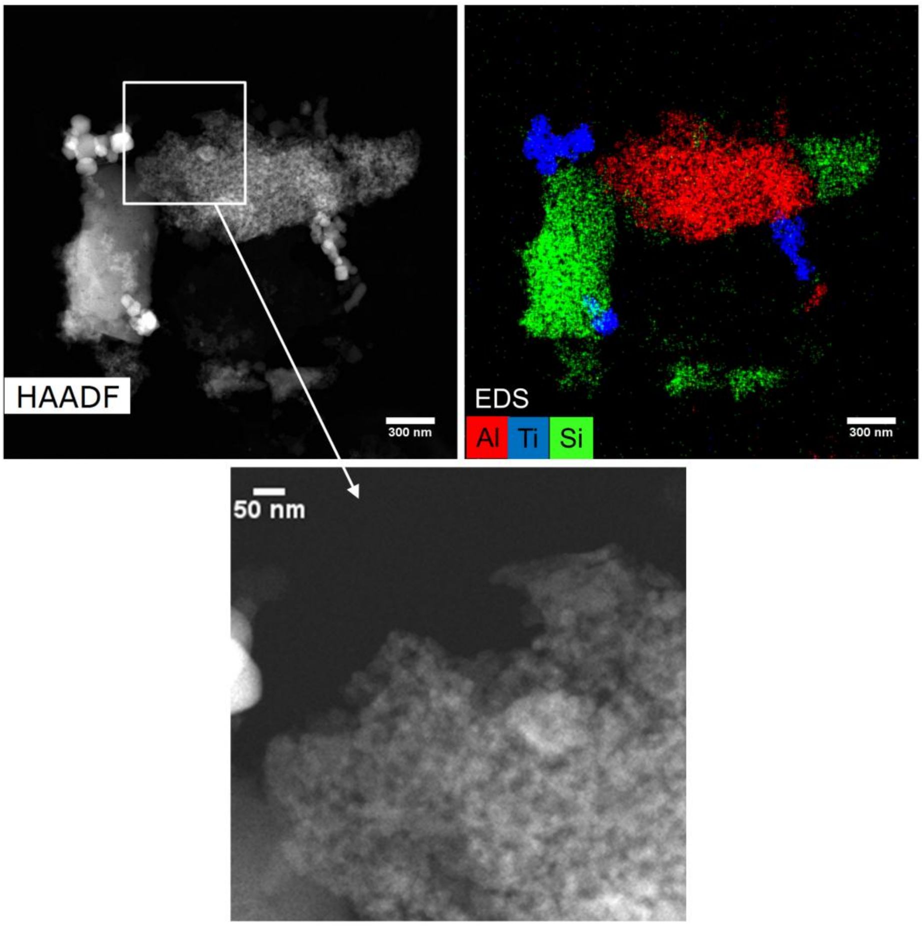


Figure S3. STEM-HAADF/EDX analysis of the toothpaste sample. The Al-, Ti- and Si-containing particles are displayed in red, blue and green, respectively, and should correspond to the constituent Al_2O_3, TiO_2 and SiO_2 particles. At the bottom a magnified HAADF image of the Al_2O_3 particles is shown, where their morphology can be seen in more detail.

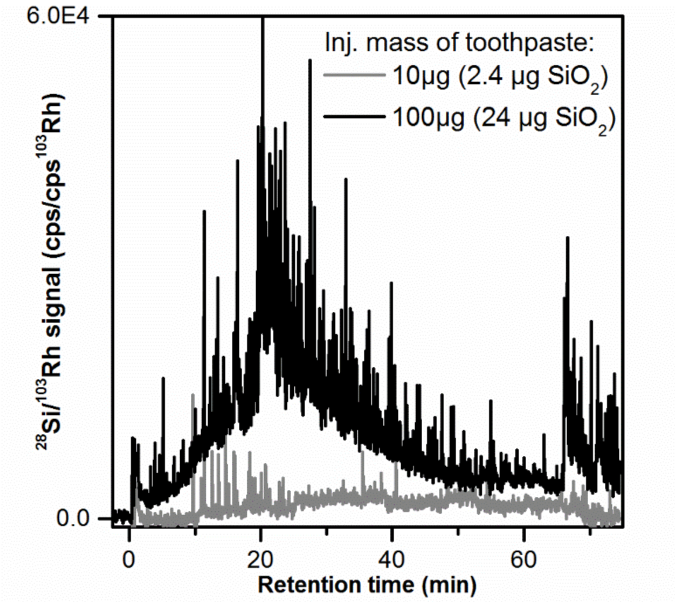
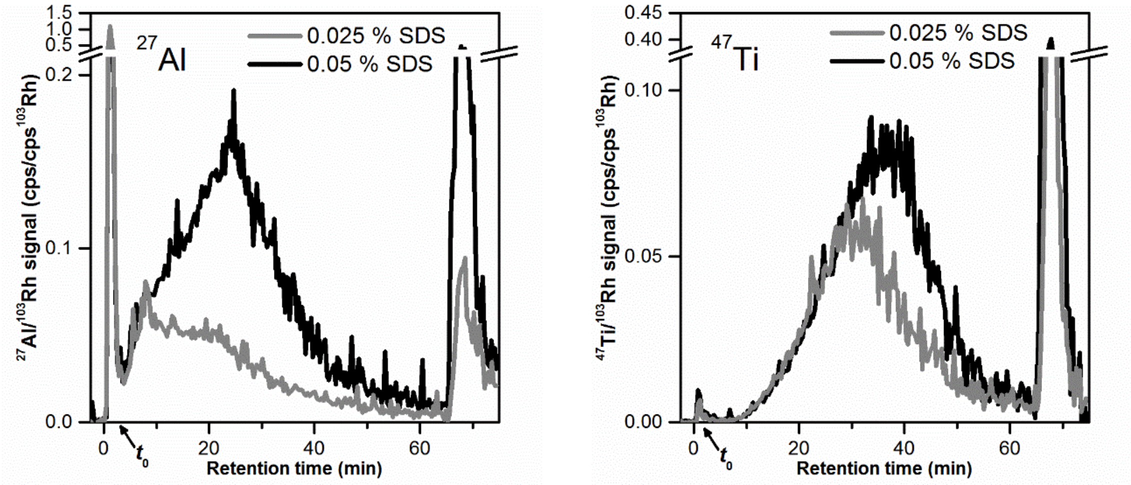
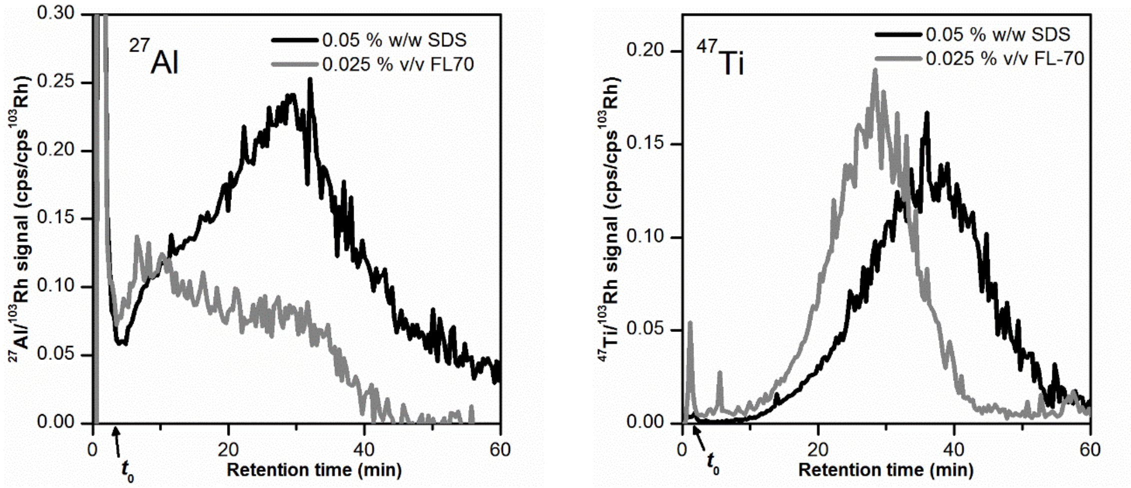


Figure S4. AF4-ICP-MS ^{28}Si fractograms obtained for 10 µg and 100 µg injected toothpaste mass values (corresponding to 2.4 and 24 µg of injected SiO_2, respectively). The AF4-ICP-MS measurements were performed on the same membrane.



(**a**) (**b**)

Figure S5. Influence of SDS concentration on the AF4 fractionation of the toothpaste particles (10 μg injected mass). The AF4-ICP-MS fractograms were obtained using 0.025% (gray line) or 0.05% w/w SDS (black line) or: (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal. The AF4-ICP-MS measurements were performed on two different membranes.



(**a**) (**b**)

Figure S6. Influence of carrier liquid composition on the AF4 separation of particles in toothpaste (10 μg injected mass). AF4-ICP-MS fractograms obtained using 0.05% w/w SDS (black line) or 0.025% v/v FL70 (gray line) as carrier liquid: (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal. The AF4-ICP-MS measurements were performed on two different PES membranes.

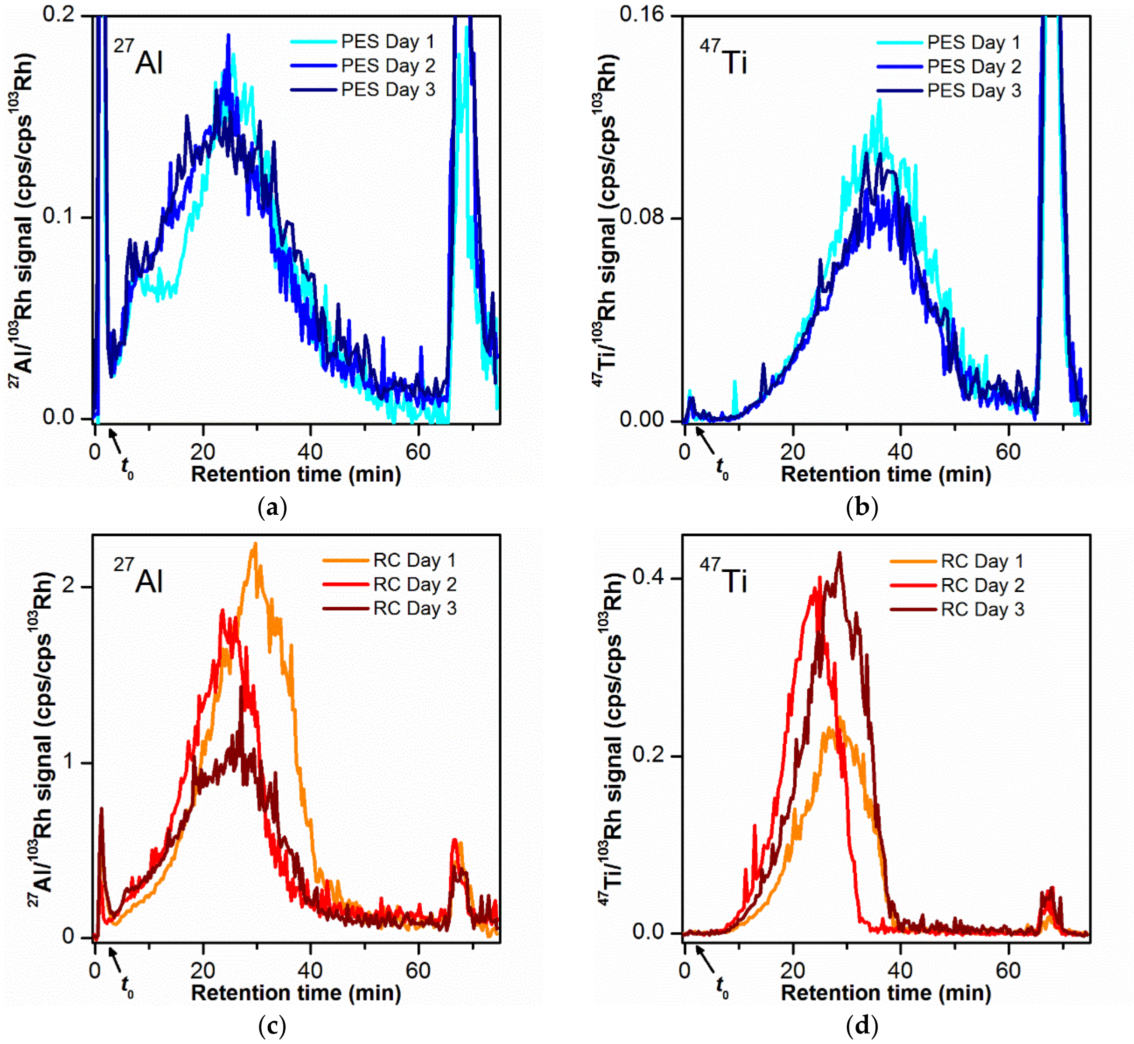
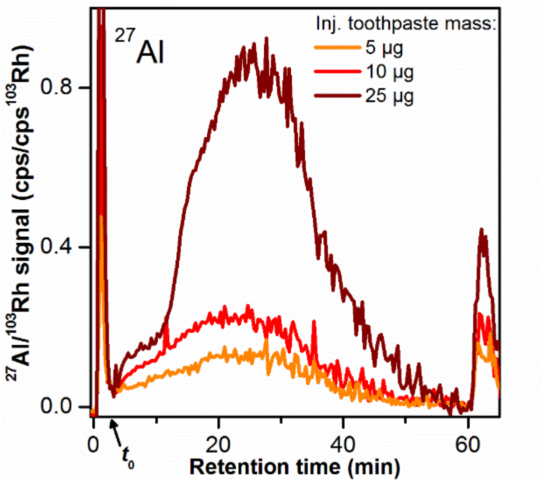


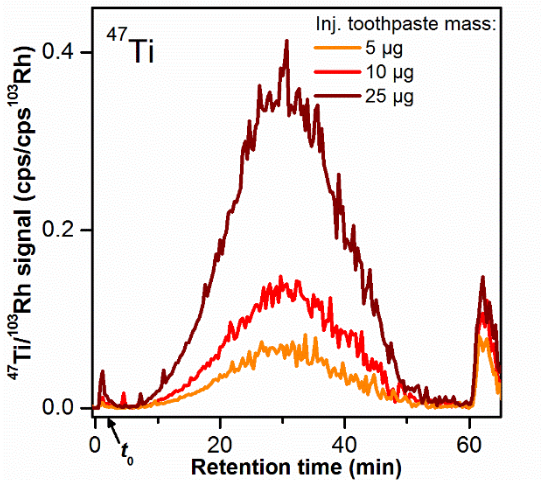
Figure S7. Influence of membrane composition on the AF4 fractionation of the toothpaste particles (10 µg injected mass). The AF4-ICP-MS fractograms were obtained using either a PES membrane (cyan, blue and navy lines, (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal) or RC membrane (orange, red and wine color lines, (**c**) ^{27}Al signal and (**d**) ^{47}Ti signal). For each of the AF4-ICP-MS measurements a new PES or RC membrane was used and the measurements were all performed on separate days.

Table S2. Retention times (t_r) and AF4 recoveries determined for Al and Ti for PES and RC membranes. For each of the AF4-ICP-MS measurements a new PES or RC membrane was used and the measurements were all performed on separate days (N = 3).

Element	**Membrane type**	t_r **main peak** (min)	***Recovery*** **void peak** (%)	***Recovery*** **main peak** (%)	***Recovery*** **release peak** (%)	***Recovery*** **total** (%)
Al	PES	24.3 ± 1.6	2.0 ± 0.0	10.7 ± 1.2	3.0 ± 1.0	15.7 ± 0.6
	RC	26.0 ± 2.6	0.7 ± 0.6	42.0 ± 22.6	2.3 ± 0.6	45.3 ± 23.9
Ti	PES	35.6 ± 0.2	2.0 ± 0.0	62.3 ± 6.4	29.3 ± 8.1	88.3 ± 1.2
	RC	26.3 ± 2.5	2.0 ± 0.0	84.3 ± 7.1	1.7 ± 0.6	86.3 ± 7.1

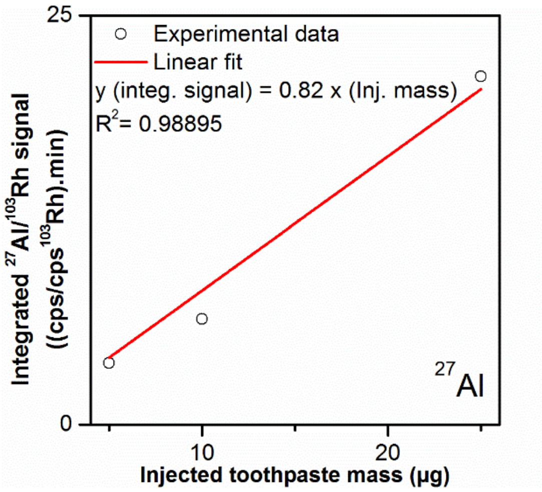


(**a**)

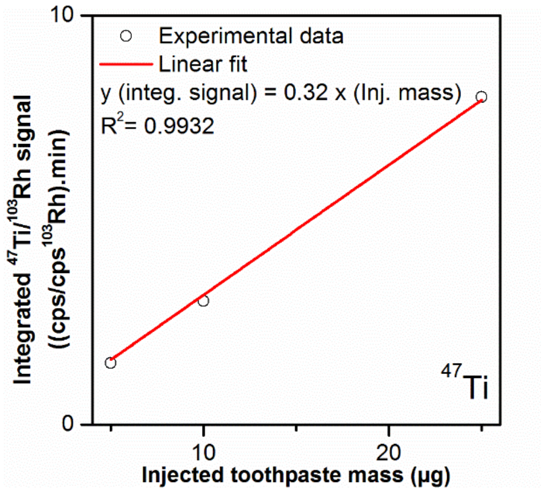


(**b**)

Figure S8. AF4-ICP-MS fractograms obtained for different injected masses of toothpaste (5, 10 and 25 µg injected mass): (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal. The AF4-ICP-MS measurements were performed using the same PES membrane and on the same day. In these experiments, the cross-flow rate was 0.4 mL/min.

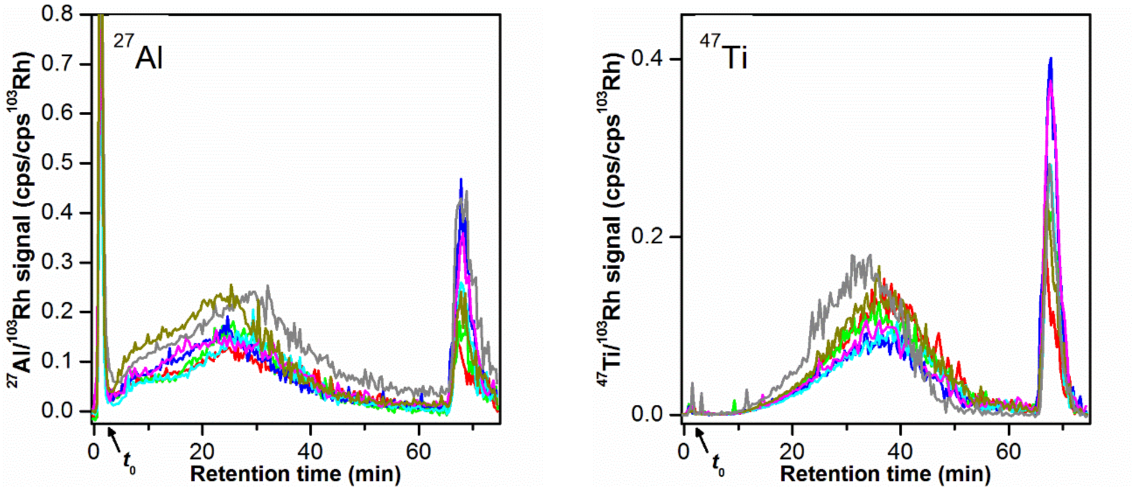


(**a**)



(**b**)

Figure S9. Linear dependence of AF4-ICP-MS signals (main peak area) on toothpaste injected mass (5, 10 and 25 µg injected mass): (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal. The experimental data points are displayed (open circles) and were fitted with a linear model (red trace).The AF4-ICP-MS measurements were performed using the same PES membrane and on the same day. In these experiments, the cross-flow rate was 0.4 mL/min.



(**a**) (**b**)

Figure S10. AF4-ICP-MS fractograms obtained using the final method (N = 7, where N is the number of independent measurements) for separation and detection of Al_2O_3 and TiO_2 particles in toothpaste (10 µg injected mass): (**a**) ^{27}Al signal and (**b**) ^{47}Ti signal. For each of the AF4-ICP-MS measurements a new PES membrane was used and the measurements were all performed on separate days.

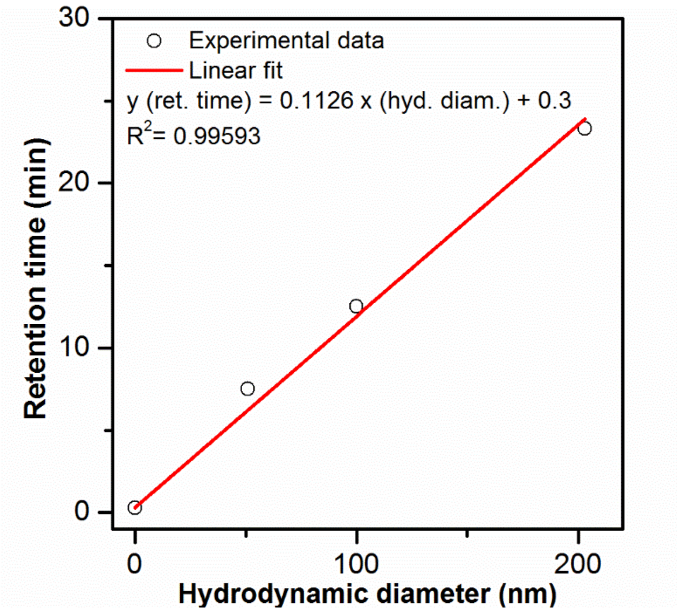


Figure S11. AF4 size calibration by polystyrene size standards (51 nm, 100 nm, and 203 nm). The calibration curve was based on a linear fitting of the retention times of the AF4-LS peak maxima obtained for the different size standards and the void time ("0 nm"). The size standards were analyzed separately using the same AF4 separation method that was used for the toothpaste.

Table S3. Results of spICP-MS analysis of the collected AF4 fractions and of the corresponding bulk digestate of the toothpaste sample.

		Dilution factor	Total number of detected particles	Minimum particle diameter (nm)	Median particle diameter (nm)	Maximum particle diameter (nm)	Particle mass concentration (ng/mL)
Al_2O_3	Sample prep. blank	$2 \cdot 10^6$	16	65	72	154	$0.01 \cdot 10^6$
	Bulk digestate	$40 \cdot 10^6$	**1696**	**55**	**113**	**653**	**$15.3 \cdot 10^6$**
		$20 \cdot 10^6$	**3039**	**58**	**121**	**877**	**$16.4 \cdot 10^6$**
	Carrier liquid	10	26	67	83	304	0.04
	Void	10	**1181**	**53**	**64**	**340**	**0.29**
	F1	10	27	67	79	159	0.01
	F2	10	**417**	**66**	**99**	**177**	**0.20**
	F3	10	96	61	116	300	0.13
TiO_2	Sample prep. blank	$2 \cdot 10^6$	0	-	-	-	0
	Bulk digestate	$40 \cdot 10^6$	**1056**	**57.8**	**136**	**731**	**$10.6 \cdot 10^6$**
		$20 \cdot 10^6$	**2041**	**56**	**143**	**886**	**$11.6 \cdot 10^6$**
	Carrier liquid	10	0	-	-	-	-
	Void	10	17	59	108	364	0.05
	F1	10	8	55.8	60	196	0
	F2	10	**698**	**56**	**152**	**951**	**1.51**
	F3	10	**341**	**59**	**224**	**546**	**1.79**

Values in grey – no statistic relevance because number of detected particles was not sufficient (< 100) for determination of a PSD.

Table S4. Results of HDC-ICP-MS analysis of the toothpaste sample prepared following method 1 and 2. Standard deviations (N = 3) are given right to the mode values.

Sample preparation	Element	Distribution mode (nm)	Recovery (%)
Method 1	Al	179 ± 8	17.7 ± 2.1
	Ti	248 ± 15	127 ± 61
Method 2	Al	148 ± 25	13.8 ± 0.2
	Ti	238 ± 16	54.0 ± 1.5

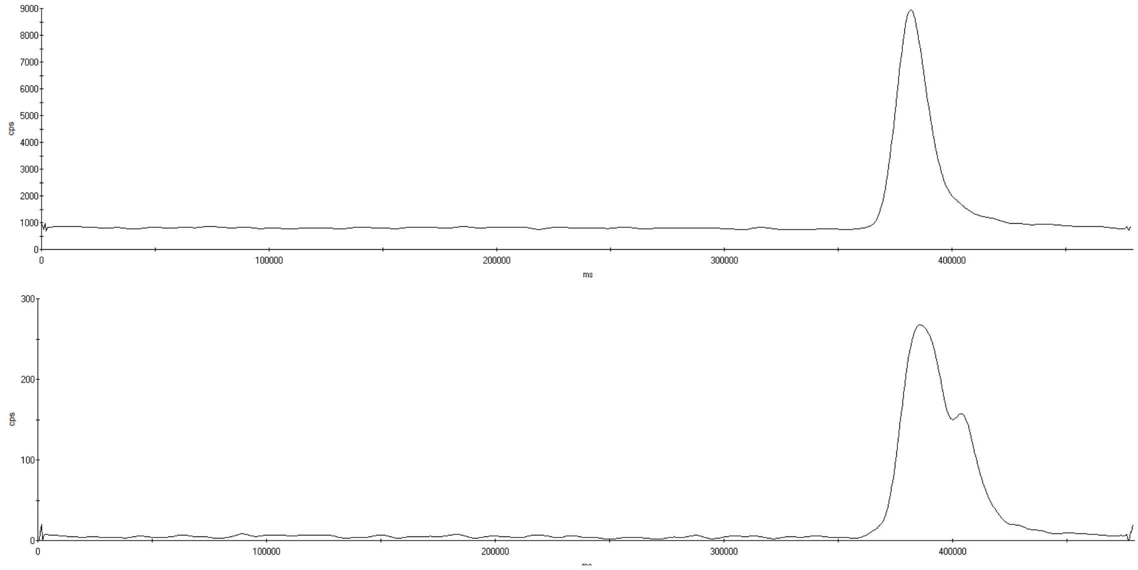


Figure S12. HDC-ICP-MS chromatograms for a toothpaste sample prepared by method 2 for Ti (upper graph) and Al (lower graph).

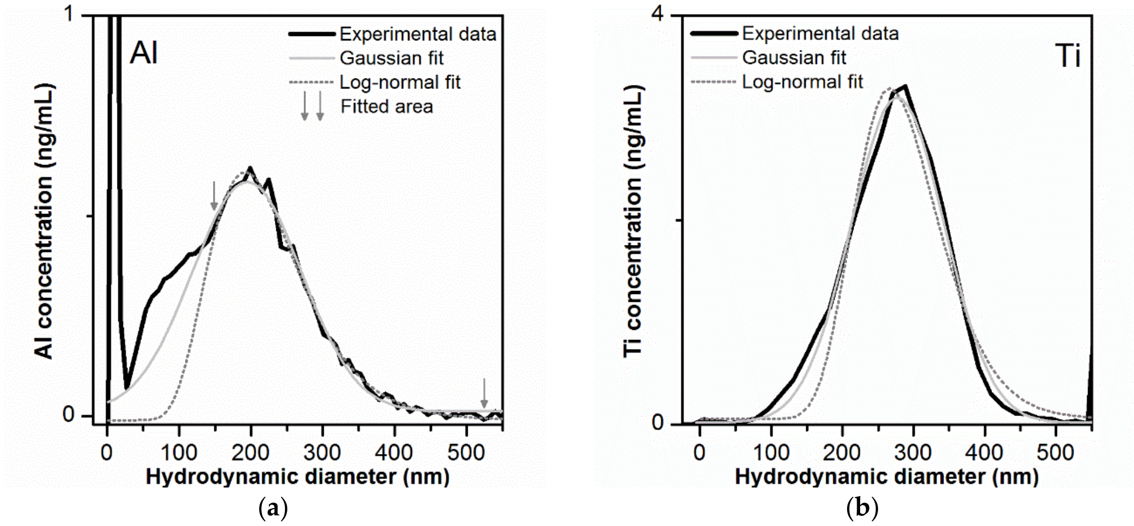


Figure S13. Fitting of the obtained Al and Ti AF4-ICP-MS mass-based particle size distributions (black lines in (**a**) and (**b**), respectively) with Gaussian and log-normal functions (solid gray and dotted lines, respectively). The Al mass-based particle size distribution was only fitted in the range 140–525 nm in order to exclude the shoulder/possible artifact observed at 40–120 nm. For the Gaussian fits, the adjacent R-square values of 99.3% for Al and 99.3% for Ti were obtained. For the log-normal fits, the adjacent R-square values were 99.4% for Al and 94.6% for Ti, respectively.