Supporting Information

Detection and Quantification of Silver Nanoparticles at Environmentally Relevant Concentrations Using Asymmetric Flow Field–Flow Fractionation Online with Single Particle Inductively Coupled Plasma Mass Spectrometry

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Additional Details on Experimental Section

Nanomaterials. Citrate coated silver nanoparticles (AgNPs) (40, 60, 80, and 100 nm – Citrate NanoXact^{TM} Silver) and Ag-SiO_2 core-shell nanoparticles (Ag core and SiO_2 shell, Silica Shelled 50 nm NanoXact^{TM} Silver) were purchased from nanoComposix, Inc. (San Diego, CA). The manufacturer stated that the AgNPs and Ag-SiO_2 nanoparticles (NPs) were suspended in 2 mM sodium citrate and isopropyl alcohol, respectively. Reference gold nanoparticles (AuNPs) with particle size of 60 nm were purchased from the National Institute Standards and Technology (Gaithersburg, MD). The AuNP suspensions were originally shipped in a 5 mL glass ampoules. For experiments, a AuNP suspension was transferred from the ampoule to a 15 mL polypropylene conical tube (Corning, Corning, NY). Extensive characterization of these nanomaterials were conducted by the manufacturers[1] and some of the results are presented in Table S1. Size distributions of AgNPs and Ag-SiO_2 NPs in the stock suspensions, which were provided by the manufacturer, are presented in Figure S1. All the stock suspensions were stored in tightly capped containers and kept in the dark at 4 ºC. To homogenize the stock suspensions before used, the AgNPs containers were shaken vigorously for *ca.* 30 s, while the AuNP suspension was sonicated for 15 min in an ultrasonic cleaner (Model 5510, Branson Ultrasonics, Danbury, CT). All the stock suspensions were used within less than 6 months after opening.

Converting Ag Signal to Particle Size. To convert Ag spike signal to particle size, calibration cures were generally prepared using spICPMS (5 ms dwell-time) and diluted suspensions of 40, 80, and 100 nm AgNPs (*ca.* 2 ng/L). These diluted suspensions were injected directly into the ICPMS using a syringe pump (WPI SP100i, World Precision Instruments, Sarasota, FL) and 20 mL disposal syringes (Thermo Fisher Scientific, Waltham, MA) at a flow rate of 1.5 mL/min, which was also the rate of the flow entering the ICPMS from the AF4 during AF4-spICPMS analyses. To minimize the effect of dissolution, the diluted AgNP suspensions were prepared less than 5 min before spICPMS measurements. The sampling time for one suspension was 4 min/run and triplicate runs were performed. Then, all the spikes having a signal higher than the cut-off value were averaged. Since extremely diluted AgNP suspensions were used, the maximum nanoparticle detection rate through spICPMS was about 5 – 6 particles/s. Within one dwell-time, the detected Ag mass was therefore expected to equal the mass of a single AgNP. The mass of AgNPs used for constructing calibration curves were calculated based on (i)

the assumption that AgNPs are spheres, (ii) their average TEM size, provided by the manufacture (Table S1), and (ii) a density value of 10,500 kg/m^3.

Determination of ICPMS Transport Efficiency. When a sample is analyzed by spICPMS, the number of particles detected is equal to the number of particles in the sample multiplied by the ICPMS transport efficiency (TE).[2] Therefore, TE is needed to estimate the number nanoparticle concentrations in unknown samples. In this study, the TE values were determined by using 60 nm AuNPs. Diluted AuNP suspensions with AuNP concentrations ranging from *ca.* 1.4×10^6 to 6.7×10^5 particles/L were injected into the ICPMS at a flow rate of 1.5 mL/min with a 20 mL syringe and a syringe pump. The spICPMS dwell-time was 5 ms and the sampling time was 4 min. Each suspension was analyzed three times. Within a spICPMS run, spikes having signal higher than the predetermined cut-off value were counted as detected AuNPs. Figure S3c shows a representative result a TE determination experiment. The number of detected AuNPs was found to be linearly proportional to the number of injected AuNPs. The TE was the slope of the linear regression curve and was found to be 0.495 in this specific case.

Constructing Contour Plots. To construct a contour plot, the AF4-spICPMS retention time and count of each spike were first converted into hydrodynamic and mass-based diameters using suitable calibration curves. Afterward, the two-column data (hydrodynamic and mass-based diameters) was transformed into a matrix. The matrix row labels were hydrodynamic diameters, starting at zero and ending at 303 nm, with an interval of 3 nm for each row label (total 101 rows). The matrix column labels were mass-based diameters, starting at the closest diameter value corresponding to the daily signal cut-off value for AF4-spICPMS (*e.g.*, 30, 35, or 40 nm) and ending at 135 nm. The interval of each column label was 5 nm (total maximum of 21 columns). Excel software then scanned the entire two-column data, and filled each matrix element with the total number of particles satisfying the hydrodynamic and mass-based diameter constrains of that matrix element (COUNTIFS function). Finally, a contour plot was created from the newly obtained matrix using Origin software.

Determination of t^0 and t_R. To determine the void times (t^0) and nanoparticle retention times (t_R), AF4–spICPMS spike fractograms were converted to particle number fractograms, which are similar to the constructed conventional chromatograms presented in Pergantis et al.[3] The y-axis of the particle number fractogram is the sum of the number of particles detected in 5 s windows and the x-axis is the average retention time of each sum. A representative particle

number fractogram made from Figure 1a spike fractogram data is presented in SI Figure S3a. Within the void region on the particle number fractogram (0 – 200 s), the occurrence time of the highest frequency of Ag spikes was interpreted as the AF4–spICPMS void time. The particle retention time are defined as the times at which the particles occur with the highest frequency within the sample eluting region (200 – 1800 s).

Evaluation of Hydrodynamic Diameter Accuracy. Ag-SiO_2 NPs and 40, 60, and 80 nm AgNPs were used to construct the hydrodynamic diameter – retention time calibration curve (Figure 1b). Figure 3c shows a contour plot constructed using this 4-point calibration curve and the original AF4–spICPMS data of a mixed suspension containing 60 nm AgNPs and Ag-SiO_2 NPs (Figure 3b). To evaluate the hydrodynamic diameter accuracy of a nanoparticle not included in the calibration, a 2-point calibration was constructed from only 40 and 80 nm AgNPs and used to calculate the hydrodynamic diameters of 60 nm AgNPs and Ag-SiO_2 NPs. Despite the use of different hydrodynamic diameter – retention time calibrations, the two contour plots in Figure 3c and SI Figure S5 were similar.

Difficulties When Analyzing Silver Nanoparticles

The spICPMS software used in this study only allows 30 min of continuous spICPMS measurements. As a result, it was not possible to use a higher cross flow rate and a lower channel flow rate to obtain better AF4 resolving power. However, this time constraint has its own advantages, such as relatively short time for AF4–spICPMS analyses and nanoparticles – AF4 membrane interaction. AF4–spICPMS analyses longer than 30 minutes might be possible with future releases of spICPMS software. With long spICPMS runs, signal drifting can possibly occur. Significant drift was not observed during a day of the experiments in this study. Any drift could be compensated by running a single standard of dissolved silver between AF4–spICPMS runs.

Sample aging or dissolution is a major concern when working with readily dissolved nanomaterials, such as AgNPs as discussed in the manuscript. Therefore, reducing the duration of AF4-spICPMS analyses and slowing down the dissolution of AgNPs are some options to obtain better, reproducible results. Reducing duration of AF4-spICPMS analysis, while keeping the same resolving power, requires changing channel and cross flow rates, channel thickness, membrane material, and many more parameters. The dissolution of AgNPs could be potentially reduced by storing the nanoparticle samples in tightly capped containers at 4 ºC or in glove boxes without

1 oxygen. Adding a reasonable amount of citrate in the samples is likely a good option as the
2 dissolution of AgNPs was reported to be slower in the presence of citrate.[4]

Table S1. Characterization results of nanoparticles (provided by the manufacturers)

Particle Name	**Diameter[a], nm**	**Hydrodynamic Diameter,[b] nm**	**Ag/Au Mass Concentration, mg/L**
40 nm AgNPs	39.0 ± 5.2	41.8	24
60 nm AgNPs	60.8 ± 6.6	62.5	20
80 nm AgNPs	79.0 ± 8.7	81.2	22
100 nm AgNPs	103.0 ± 11.0	106.5	22
Ag-SiO_2 NPs	51.0[c]	111.0	99
60 nm AuNPs	55.43[d]	55.3 ± 8.3	51.86 ± 0.64

[a]: Determined by using images from transmission electron microscopy (TEM), except for 60 nm AuNPs
[b]: Determined by dynamic light scattering
[c]: Silver core diameter
[d]: Average diameter value obtained from atomic force microscopy, scanning electron microscopy, and TEM.

Table S2. Composition of FL-70 detergent[5]

Chemical	**Percent**
Water	88.8
Triethanolamine oleate	3.8
Sodium carbonate	2.7
Alcohols, C12-14-secondary, ethoxylated	1.8
Tetrasodium ethylenediaminetetraacetate	1.4
Polyethylene glycol	0.9
Sodium oleate	0.5
Sodium bicarbonate	0.1

Table S3. Summarized results of three consecutive AF4-spICPMS runs of a mixture containing 60 nm AgNPs (291 ng/L) and Ag-SiO_2 NPs (219 ng Ag/L). Detected particles having diameters larger than 80 nm were considered Ag-SiO_2 NPs.

Run number	**60 nm AgNPs**			**Ag-SiO_2 NPs**		
	No. of detected particles	**Ag-based dia., nm**	**Hydrodynamic dia., nm**	**No. of detected particles**	**Ag-based dia., nm**	**Hydrodynamic dia., nm**
1	155	55.2	65.3	336	54.7	119.8
2	171	53.4	63.5	332	55.5	118.3
3	113	54.1	66.4	350	55.3	122.0

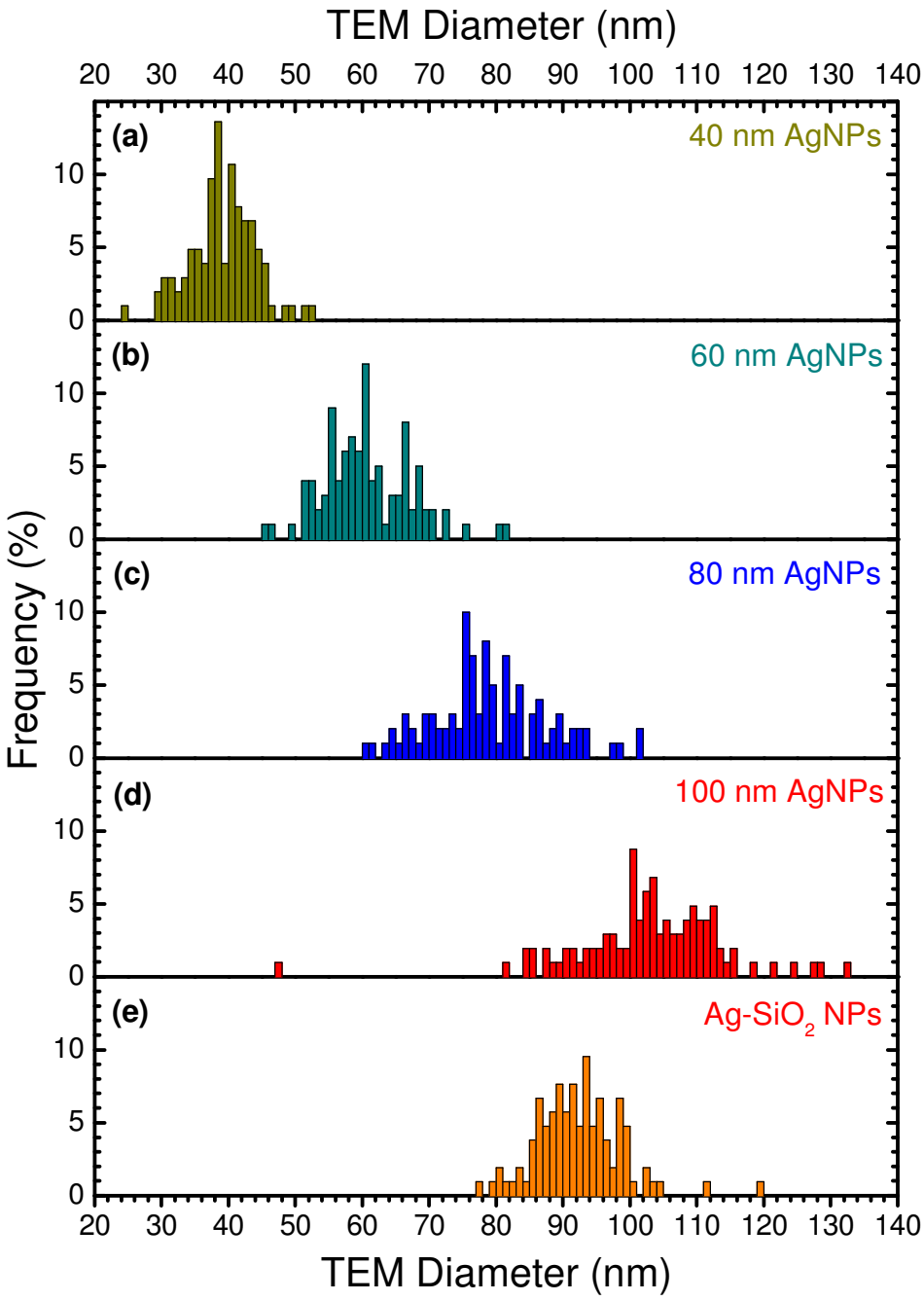

Figure S1. Nanoparticle size distributions (provided by the manufacturer – nanoComposix).

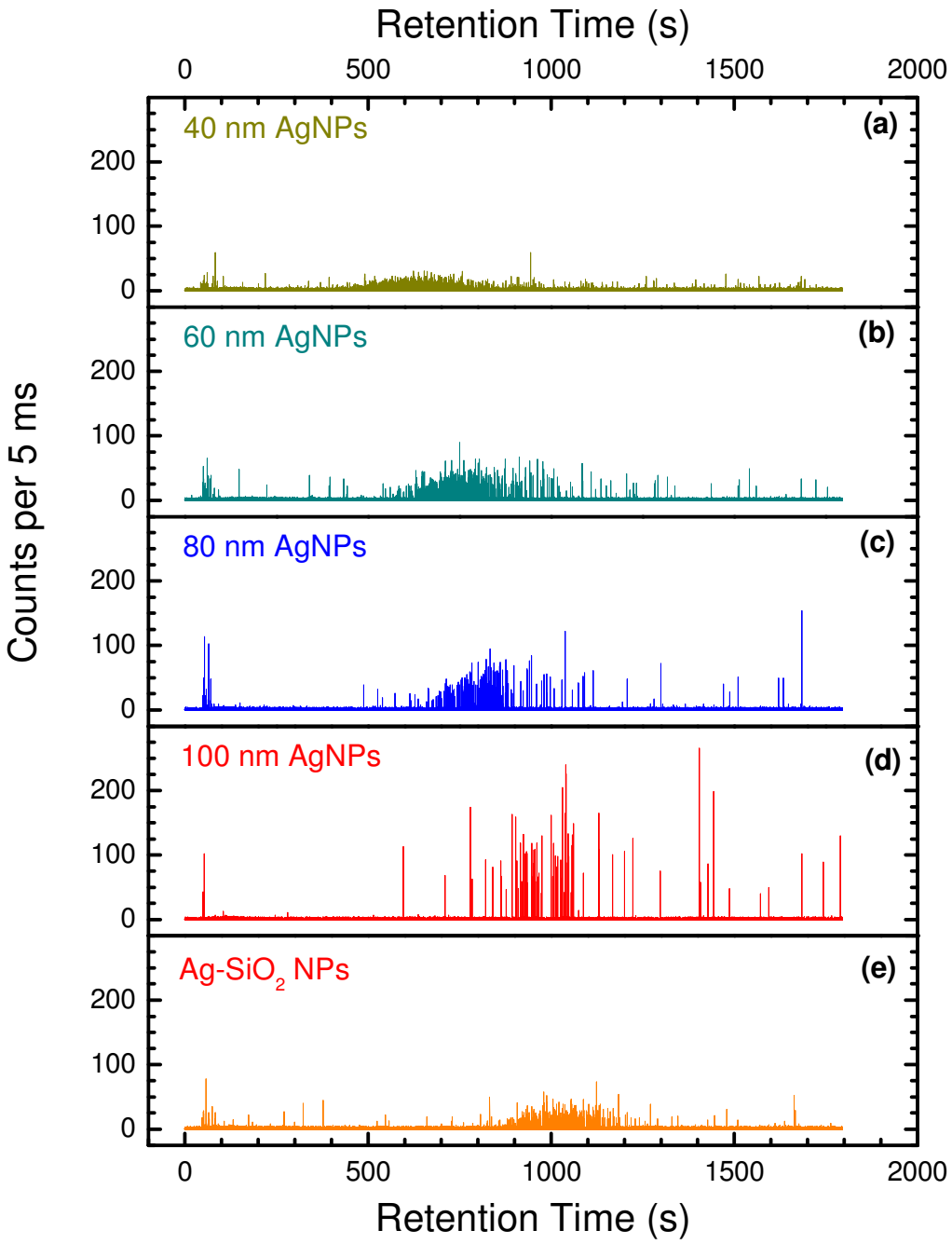

Figure S2. Representative AF4-spICPMS spike fractogram obtained from the analysis of (a) 40 nm AgNPs, (b) 60 nm AgNPs, (c) 80 nm AgNPs, (d) 100 nm AgNPs, and (e) Ag-SiO_2 NPs. The Ag-based mass concentrations of these NPs were *ca.* 500 ng/L.

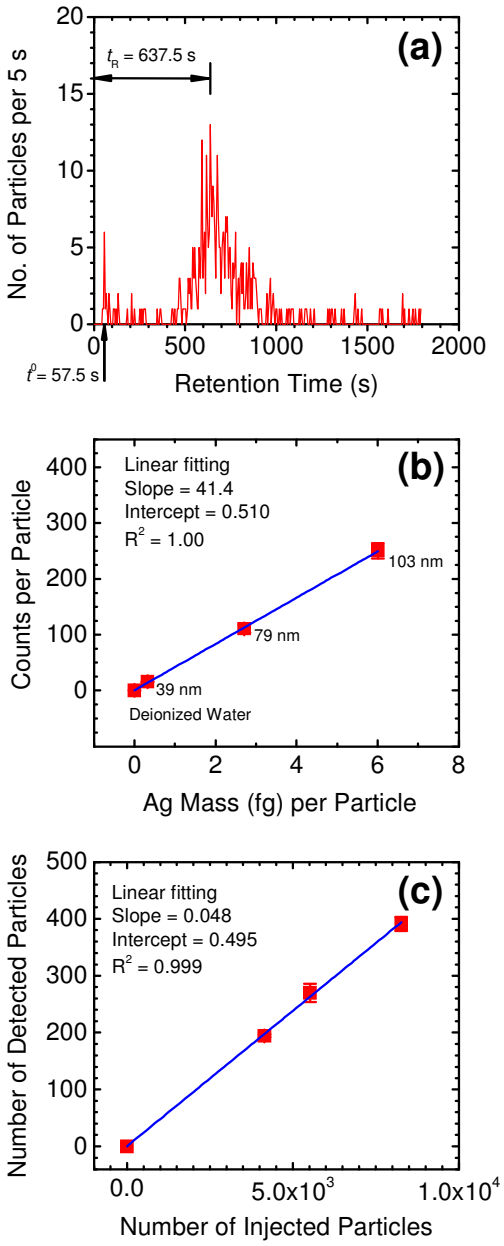

Figure S3. (a) A particle number fractogram constructed from data shown in Figure 1a. This graph is used for determining the void time (t^0) and nanoparticle retention time (t_R) from an AF4-spICPMS analysis. (b) Representative calibration curve converting spICPMS spike signal to AgNP mass. Error bars represent standard deviations of average signal obtained from three consecutive spICPMS runs (4 min/run). (c) Representative calibration curve used to obtain ICPMS transport efficiency. This experiment employed 60 nm AuNPs. Error bars represent standard deviation of number of detected AuNPs from three consecutive spICPMS runs (4 min/run).

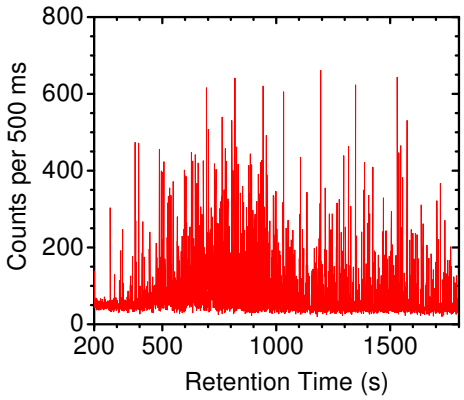

Figure S4. Conventional AF4-ICPMS fractogram constructed from original AF4–spICPMS data presented in Figure 2a. Data integration (dwell time) in this case is 500 ms, which is typical value for conventional ICPMS.

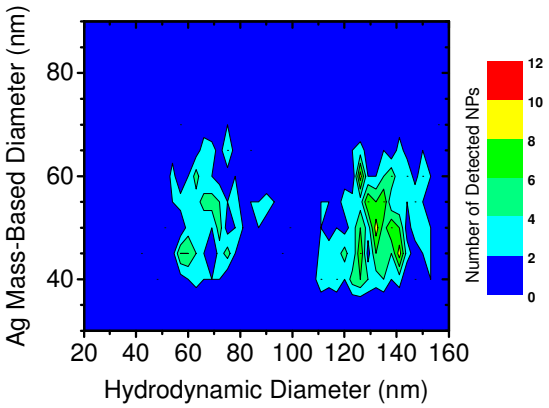

Figure S5. Contour plot obtained from data shown in Figure 3b. Only 40 nm and 80 nm AgNPs were used to construct hydrodynamic diameter – particle retention time calibration curve (slope = 5.54 and intercept = 308).

References

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(3) Pergantis, S. A.; Jones-Lepp, T. L.; Heithmar, E. M. *Anal Chem* **2012**, *84*, 6454-6462.
(4) Liu, J.; Sonshine, D. A.; Shervani, S.; Hurt, R. H. *ACS nano* **2010**, *4*, 6903-6913.
(5) pp Fisherbrand™ FL-70™ Concentrate https://www.fishersci.com/shop/products/fisherbrand-fl-70-concentrate-72/p-84423.