Supplemental Information

Tracking dissolution of silver nanoparticles at environmentally relevant concentrations in laboratory, natural, and processed waters using single particle ICP-MS (spICP-MS)

Denise M. Mitrano, James F. Ranville, Anthony Bednar, Karen Kazor, Amanda S. Hering, Christopher P. Higgins

Methods

Instrumentation – Asymmetrical Flow Field Flow Fractionation (AF4)-ICP-MS

For AF4-ICP-MS analysis, an Agilent 7500 ICP-MS was used as an online detector. Standard operating and tuning procedures were used in maintaining and calibrating the instrument. Only one Ag isotope was monitored for detection (^{107}Ag), with an integration time of 2000 ms, alternating with a Bi internal standard (with a dwell time of 1000 ms), resulting in data being collected at approximately one reading every 3 s for the entire length of the fractogram. An asymmetrical FFF, AF 2000 AT, from Post Nova Analytics (Salt Lake City, UT) was used with a 10kDa regenerated cellulose membrane, changed approximately every 25 runs, and with a carrier fluid consisting of 0.025% FL-70 surfactant and 0.01% sodium azide as an antibacterial agent. A 100 µL injection loop was used to load samples onto the channel, and flushed continuously throughout analysis. The AF4 was directly plumbed into the ICP-MS. The channel flow conditions allowed direct connection of the AF4 effluent to the ICP-MS nebulizer without a flow splitter. The AF4 separation conditions were as follows: 10 min relaxation period (focusing step), followed by 40 - 60 min elution (0.7 – 1. 0 mL/min cross flow and 1.0 mL/min detector flow), and a 10 min flush (field-off, 1.0 mL/min) between each experimental run. The theoretical basis of FFF is found in a number of locations [1-4] in addition to details of its interfacing with ICP-MS. [5-7]

Data collection, conversion to particle size, and quality assurance – spICP-MS

For all spICP-MS analysis, raw intensity data were plotted as pulse intensity versus number of events to create a pulse distribution histogram. Very low intensity readings were considered to be instrument background, or, for slightly higher intensity values, dissolved metal. It is noted, however, that the term dissolved metal is used operationally in this context to refer to both dissolved Ag^+ species and any Ag ENPs that are smaller than can be distinguished as NPs by the spICP-MS method, which was approximately 25 nm diameter particles in this study considering all matrices analyzed. After background/dissolved metal was subtracted from the pulse intensity, ENPs were sized using spICP-MS theory. [5,8] Pulses that register at higher intensities are associated with larger diameter ENPs, which plot with approximate lognormal distribution around a mean as a function of NP size. Deviation from this shape may be an indication of particle coincidence in a given dwell time, or a polydisperse sample set, and thus would require further sample dilution and characterization to differentiate between these two occurrences. As suggested in our previous published work, we ensured that no more than 15% of the measurements be considered NP pulses. [5,9]

For all 100 nm dissolution samples < 24 h, the concentrations of Ag^+ and ENPs were independently quantified for each sample. An increasing concentration of Ag^+ can be recognized by an increase in intensity of the lower intensity counts. Once the dissolved Ag is distinguished from the Ag ENP pulses, the background intensity can be directly compared to the calibration curve to quantify the concentration of dissolved metal in the sample. In solutions containing both dissolved and Ag ENPs, the Ag ENP pulse registers as the summation of Ag^+ background and Ag ENP pulse intensities (i.e. the intensities are additive). Since the increase of dissolved metal not only presents itself by increasing the background concentration at low counts, but also by shifting the ENP pulses to higher intensities, Ag ENPs can be correctly sized only after subtracting the background intensity from the Ag ENP pulse intensity before sizing.

TOC and IC Results				
Analyte	**DL (mg/L)**	**Tap**	**Creek**	**WWTP**
DOC		0.92 ± 0.42	2.49 ± 0.015	166
F	0.05	0.47	0.44	1.15
Cl	0.1	9.08	6.35	971.7
Br	0.1	BDL	BDL	BDL
NO_3	0.1	0.95	0.43	0.7
PO_4	0.5	BDL	BDL	79.21
SO_4	1	52.1	24.56	5.69
ICP-OES Results				
Analyte	**DL (mg/L)**	**Tap**	**Creek**	**WWTP**
Ag	0.0002	BDL	BDL	0.0002
Al	0.0138	0.0232	0.0405	BDL
Ca	0.0041	27.6578	14.6538	40.0532
Fe	0.0004	0.0022	BDL	0.0390
K	0.0315	2.7639	1.3960	65.8117
Mg	0.0003	6.8475	3.2773	2.6462
Na	0.0211	23.3971	6.4513	43.4222
P	0.0197	BDL	BDL	12.1776
S	0.0061	29.9759	8.5558	2.9709
Si	0.2195	2.0525	3.5983	0.3664
Zn	0.0003	1.2883	0.0246	0.1146

pH Results	
Solution	**pH**
DI	6.7 ± 0.2
1 mg/L Cl-	7.0 ± 0.3
Equimolar Cl-	6.8 ± 0.2
1 mg/L S2-	8.9 ± 0.1
Equimolar S2-	6.9 ± 0.4
20 mg/L DOC	4.2 ± 0.3
2 mg/L DOC	4.8 ± 0.2
EPA Mod Hard	7.9 ± 0.5
Clear Creek	7.3 ± 0.1
Tap	7.9 ± 0.3
WWTP centrate	7.8 ± 0.2

Table S1. Water chemistry composition of various samples. Concentrations in mg/L.

	Avg. Particle Size (nm)	
Treatment	**T=12 hr**	**STDEV (nm)**
Ambient Laboratory Light	77.6	1.4
Dark	81.2	0.7
Window	78	3.2

Table S2: Particle size as determined by spICP-MS. 100 nm citrate coated Ag NPs suspended in DI water, aged 12 hours in various light treatments (triplicate samples). No significant size difference detected regardless of sample lighting conditions.

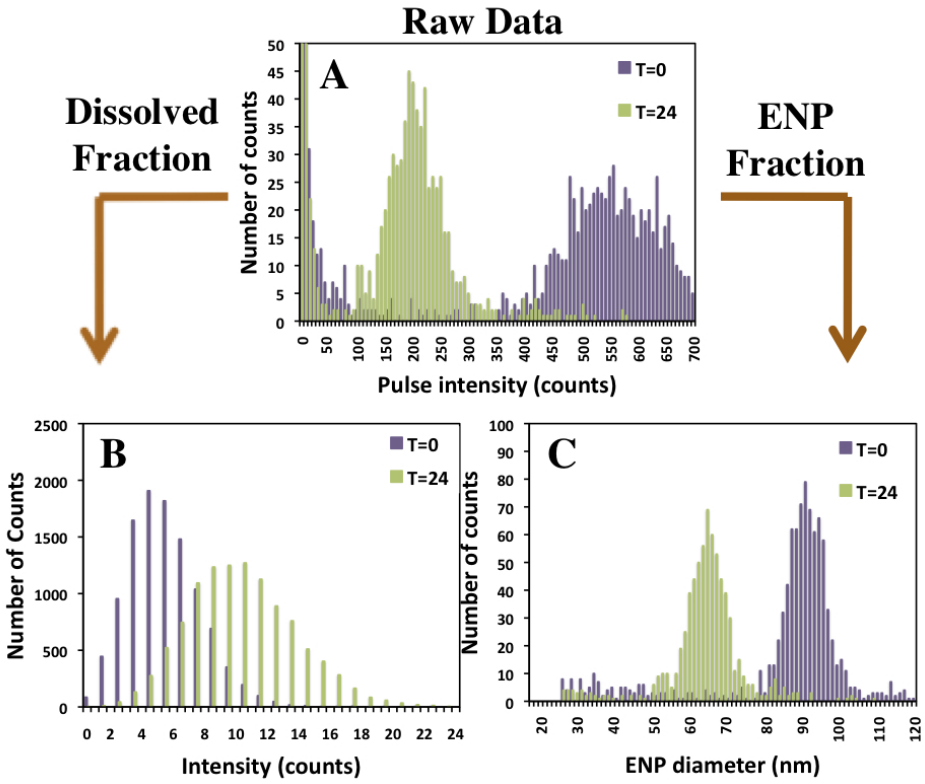

Figure S1: 100nm Ag ENP, PVP coating. Observed dissolution process over 24 hours via spICP-MS. A) Raw ICPMS output, binned according to pulse intensity. Low counts are considered background counts while higher counts are considered ENP pulse events. The first minimum of the histogram, in the case at approximately 75 counts, is considered the differentiating point. B) Background counts can be separated from the main data set, compared directly to the dissolved Ag calibration curve, and [Ag^+] can be determined. C) ENP pulse events can be separated from the main data set, and through spICP-MS theory, can be converted to particle size.

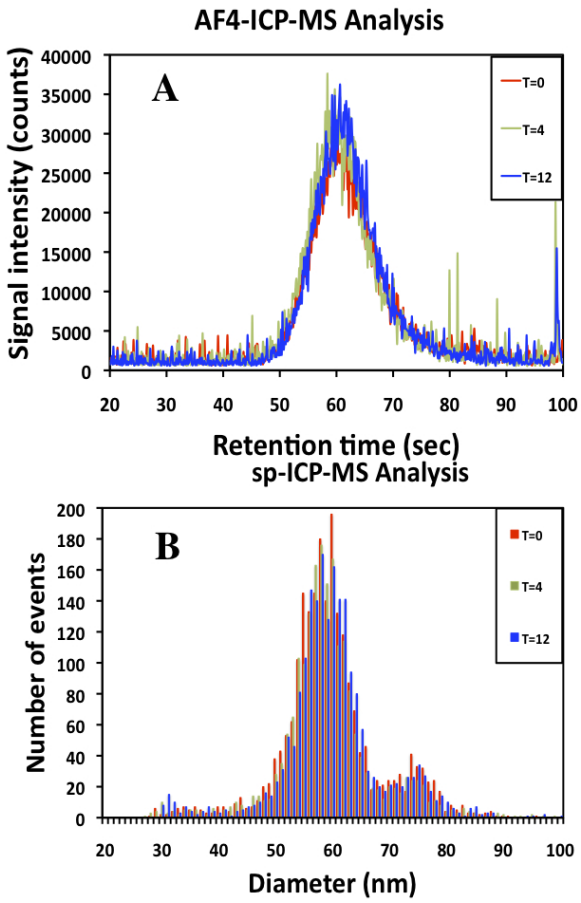

Figure S2: 60 nm tannic acid coated Ag ENPs, initial concentration 50 µg/L. Analyzed over time (0, 4 and 12 hours). A) Sample direct injection for AF4-ICP-MS analysis B) Sample dilution to 50 ng/L for spICP-MS analysis, with secondary coincidence peak observed due to high particle number concentration.

Explanation of Table 1: Example Calculations

Goal: Determine rate of Ag^+ lost from original (i.e. T=0) Ag NP

Step 1: Using the instantaneous mean diameter, calculate the mass of Ag lost from the original particle size at each time point.

Step 2: Normalize by surface area

Step 3: Plot the Ag mass lost per surface area by time.

Step 4: Log transform the slope, derive dissolution rate constant.

Calculating Ag Concentration and Mass Balance:

Goals:

1. Calculate total Ag (i.e Ag^+ and Ag NP) in samples as analyzed.
2. Determine Ag ENP number decrease over time.
3. Account for particle number losses to total Ag, i.e. adjust total Ag concentration by fixing particle number to initial particle number analyzed.

Step 1: Determine individual particle masses analyzed from pulse intensity using measured transport efficiency and dissolved Ag calibration curve.

Step 2: Determine total Ag ENP mass by summing up the calculated masses in Step 1 for all particles analyzed. Divide by transport efficiency to correct for the fact that not all particles are analyzed. This is the total measured ENP mass concentration.

Step 3: Determine the number of particles measured in each sample, correcting for transport efficiency. If this particle number does not match that which is measured for T=0, then ENPs are being lost from the dispersed phase and the percentage of ENPs remaining should be calculated. Factoring this loss into the calculations for ENP mass concentration from Step 2, this is the adjusted ENP mass concentration.

Step 4: Determine the amount of dissolved Ag^+ from each individual particle by subtracting the instantaneous particle mass (i.e. at T=X) from the initial particle mass (i.e. at T=0). Multiply the Ag^+ dissolved from each individual particle from the total number of particles in the sample and divide by the transport efficiency. This is the total calculated dissolved Ag^+.

Step 5: Multiply the mass lost per particle in each sample (Step 4) by the initial particle number (at T=0) to calculate the total amount of dissolved Ag that should be present. This calculation assumes that all ENPs in the system (whether they were counted in Step 4 or not) are dissolving at the same rate. This is the adjusted calculated dissolved Ag^+.

Step 6: Measure the dissolved Ag present in each sample by evaluating the "background" and using the dissolved Ag calibration curve. If the numbers from Step 4 or Step 5 do not match Step 6, this suggests loss of dissolved Ag in the system.

Step 7: Mass Balance Calculation. Sum Step 2 (total measured ENP mass concentration) and Step 6 (total measured dissolved Ag mass concentration) for each time point. If the sum of these does not equal the initial sum value (i.e., at T=0), then either NPs are being lost from suspension and/or dissolved Ag is being lost from the system.

Step 8: If mass balance is not achieved in Step 7, repeat Step 7, but divide by the fraction of particles recovered at that time point (calculated in Step 3). This modified mass balance calculation assumes that the ENPs lost from suspension (i.e., Step 3) are dissolving at the same rate as NPs present in suspension. If this modified sum does not equal the initial sum value (i.e., at T=0), then dissolved Ag is being lost from the system.

Treatment	100-DI-C	100-DI-T	100-DI-P	100-Tap-C	100-Tap-T	100-Tap-P	100-Crk-C	100- Crk -T	100- Crk -P	100-DOC-C	100- DOC -T	100- DOC-P	60-DI-C	60-DI-T	60-DI-P	60-Tap-C	60-Tap-T	60-Tap-P	60-Crk-C	60- Crk -T	60- Crk -P
100-DI-C		1.000	1.000	0.014	0.000	0.932	0.002	0.000	0.000	0.000	0.000	0.001	0.000	1.000	0.000	1.000	1.000	0.918	0.000	0.000	0.000
100-DI-T			0.917	0.193	0.001	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.854	0.000	1.000	0.982	0.227	0.000	0.000	0.000
100-DI-P				0.002	0.000	0.516	0.016	0.000	0.000	0.000	0.000	0.007	0.001	1.000	0.000	0.963	1.000	1.000	0.000	0.000	0.000
100-Tap-C					0.995	0.542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.138	0.004	0.000	0.000	0.000	0.000
100-Tap-T						0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
100-Tap-P							0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.414	0.000	1.000	0.716	0.047	0.000	0.000	0.000
100-Crk-C								0.023	0.790	0.051	0.001	1.000	1.000	0.025	0.000	0.000	0.007	0.273	0.000	0.350	0.563
100- Crk -T									0.931	1.000	1.000	0.049	0.259	0.000	0.981	0.000	0.000	0.000	0.665	0.999	0.990
100- Crk -P										0.987	0.344	0.923	1.000	0.000	0.095	0.000	0.000	0.001	0.023	1.000	1.000
100-DOC-C											0.999	0.103	0.434	0.000	0.911	0.000	0.000	0.000	0.477	1.000	0.999
100- DOC -T												0.003	0.025	0.000	1.000	0.000	0.000	0.000	0.992	0.784	0.569
100- DOC -P													1.000	0.011	0.000	0.000	0.003	0.152	0.000	0.539	0.758
60-DI-C														0.001	0.004	0.000	0.000	0.025	0.001	0.944	0.992
60-DI-T															0.000	0.924	1.000	1.000	0.000	0.000	0.000
60-DI-P																0.000	0.000	0.000	1.000	0.367	0.202
60-Tap-C																	0.995	0.314	0.000	0.000	0.000
60-Tap-T																		0.993	0.000	0.000	0.000
60-Tap-P																			0.000	0.000	0.000
60-Crk-C																				0.104	0.051
60- Crk -T																					1.000
60- Crk -P																					

Table S3. P-values obtained from conducting pair-wise comparisons of < 24 h log dissolution rates across unique pairs of testing conditions using Tukey's HSD test. P-values are adjusted such that values less than or equal to 0.05 (colored) are significant at a 95% family-wise confidence level across all comparisons. Red (blue) shaded values indicate that log dissolution rates were significantly larger (smaller) for the treatment listed to the left as compared to those treatments listed at the top of the table.

Water Chemistry	Capping Agent	Average Mass Balance Overall (unadjusted) (< 24 hr)	STDEV	Average Mass Balance Overall (Adjusted for "Lost" Particles) (< 24 hr)	STDEV
Creek	Citrate	62%	10%	86%	6%
Creek	PVP	64%	16%	92%	6%
Equimolar Cl-	Citrate	72%	11%	74%	9%
EPA Mod Hard	TA	75%	12%	87%	7%
Creek	TA	75%	13%	94%	4%
Equimolar Cl-	TA	76%	11%	79%	9%
Equimolar S2-	Citrate	78%	12%	83%	9%
1 ppm Cl-	Citrate	79%	11%	83%	9%
DI	Citrate	80%	22%	87%	20%
DI	TA	80%	7%	84%	6%
EPA Mod Hard	PVP	81%	9%	93%	5%
DI	PVP	82%	26%	82%	22%
1 ppm Cl-	TA	83%	13%	88%	10%
Equimolar Cl-	PVP	83%	12%	84%	10%
EPA Mod Hard	Citrate	85%	12%	96%	7%
Equimolar S2-	PVP	86%	13%	87%	10%
1 ppm Cl-	PVP	88%	7%	94%	3%
20 mg/L DOC	Citrate	91%	8%	95%	6%
2 mg/L DOC	Citrate	91%	7%	93%	4%
Equimolar S2-	TA	92%	14%	96%	12%
Tap	TA	92%	19%	95%	19%
2 mg/L DOC	TA	92%	4%	99%	1%
20 mg/L DOC	PVP	93%	5%	95%	2%
1ppm S2-	Citrate	93%	10%	99%	6%
20 mg/L DOC	TA	95%	3%	94%	3%
2 mg/L DOC	PVP	99%	4%	103%	2%
Tap	PVP	103%	12%	108%	13%
1ppm S2-	TA	107%	9%	103%	4%
1ppm S2-	PVP	110%	16%	111%	11%
Tap	Citrate	139%	15%	159%	15%

Table S4: Mass balance for 100 nm Ag ENP dissolution under 24 hours in all water chemistries including both unadjusted particle number (raw particle number as measured) and adjusted particle number based on particles detected in T=0 hr samples. Adjusted particle number refers to correcting the total particle number at a given time point to match the initial measured particle number at T=0 in each time set. Percent recoveries are organized from lowest to highest recovery.

Separate averages of the unadjusted and adjusted mass balances were made for an entire dissolution set (time series) of each particle size/capping agent/water chemistry. Subsequently, the standard deviation values apply to the standard deviation of the percent recovery for each analysis.

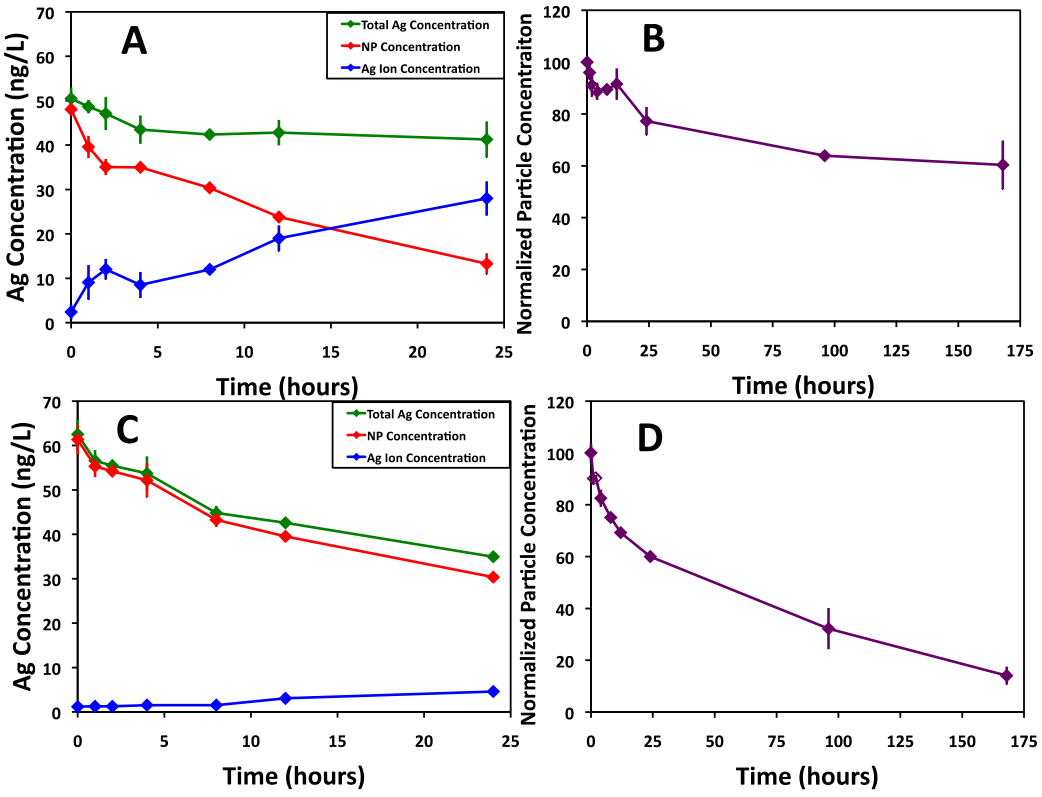

Figure S3: 100 nm TA Ag ENPs. Ionic and ENP concentrations in A) DI water and C) Creek water. Observed decrease in particle number, panels B and D, DI and Creek water respectively. Error bars represent standard deviation from triplicate experiments.

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