Supplementary Information (SI) for Environmental Science: Nano.

Supplementary Material

Evaluation of alternatives bases to TMAH tissue extraction of ENMs from tissues prior to spICP-MS analysis

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QAH Extraction Concentration Rangefinding Experiments

The optimal concentration of both TPAH and TBAH to extract 0.5 mg of ground beef tissue was determined using a 24-hour rangefinding experiment. Tissue aliquots were placed in 15 ml centrifuges tubes and 10 ml of base was added using the solvent to tissue ratio of 20:1. The concentrations of QAHs tested were 5, 10, 15, 20, and 25% for TPAH and 5, 10, 20, 30, and 40% for TBAH. Centrifuge tubes containing QAH and tissue were bath sonicated for 1 hour then placed on an orbital shaker table for 23 hours. Digestion completion was assessed visually where complete tissue digestion was determined by visually inspecting centrifuge tubes to see if any solid tissues remained. Images of rangefinding experiments at 0 and 24 hours are shown in Figure S1. Based on these experiments, TPAH digestions were conducted at 20% while TBAH digestions were conducted at 12%. TBAH concentration was selected as complete digestion of Tissues occurred at 15% TBAH, but not 10%, and 12% was selected to minimize QAH use. Subsequent tissue digestions using 12% TBAH resulted in completely digested tissues.

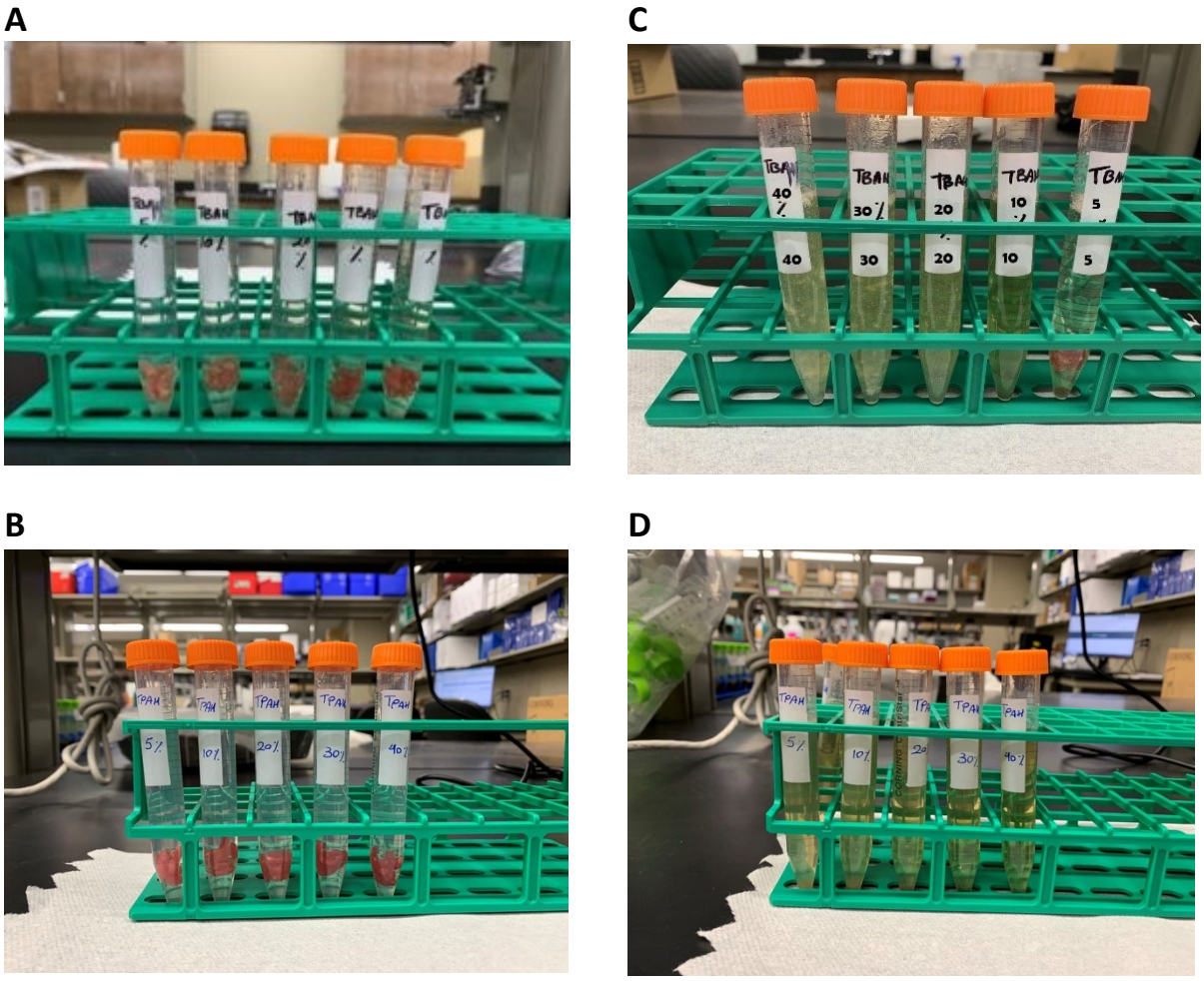

Figure S1. Images of QAH digestion concentration range-finding experiments for TPAH and TBAH. Images (A) and (B) show centrifuge tubes with tissue at 0 hours while panels (C) and (D) show digestion vessels after 24 hours.

Spiked Reference Material (SRM) Concentration

Aliquots of the in house SRM were microwave digested following EPA 3051A prior to analysis using ICP-MS to measure total Au and Ag tissue concentrations. Four replicate samples from different areas within the SRM were individually digested. The microwave digestion procedure and ICP-MS calibration are described in detail in the main manuscript. Tissues were nominally spiked to a concentration of 10 mg/kg wet tissue weight for both Au and Ag ENMs

Table S1. Total Au and Ag concentrations in spiked reference tissues after microwave digestion and ICP-MS analysis. Averages concentrations from 4 samples with ± one standard deviation.

Element	Spiked reference tissue concentration (mg/kg)	Standard Deviation
Au	8.17	0.25
Ag	8.35	0.65

Total Au and Ag concentrations in all QAH extractions.

The average total Au or Ag concentration for each QAH extraction is shown below in Table S3. Solution concentrations are calculated by converting the measured SRM concentrations listed in Table S2 for the volume of QAH (35 ml) and exact SRM mass added to each digestion test tube. The total concentrations of Au and Ag shown here are used to calculate total ENM mass recovery relative to spICP-MS measured ENM mass distributions shown in Figures 2,3, and 4.

Table S2. Average total Au and Ag solution concentrations for each QAH tissue extraction. Each concentration is the average of three replicate measurements± one standard deviation.

Element	QAH solution	Extraction Concentration (µg/L)	Std Dev
Au	TMAH	409.44	0.39
Au	TPAH	409.04	0.41
Au	TBAH	408.97	0.50
Ag	TMAH	418.11	0.23
Ag	TPAH	418.55	0.80
Ag	TBAH	418.59	0.60

ENM mass and particle number recovery calculations

Tabulated mass and particle number recovery values for Au and Ag ENMs extracted from spiked reference tissues using TMAH, TPAH, or TBAH. Solution mass was calculated relative to the appropriate value presented above in Table S3. Particle number recovery was calculated relative to the spICP-MS measured value at 1 day, therefore all Au and Ag particle number recovery reported below for day 1 is 100% for all QAHs tested.

Supplemental Table S3. Au particle number and mass recovery in TMAH, TPAH, and TBAH in 60 days. Recovery values reported here are from three replicate digestions with ± one standard deviation.

Time (day)	Au-TMAH		Au-TPAH		Au-TBAH	
	Particle No. Recovery	Mass Recovery	Particle No. Recovery	Mass Recovery	Particle No. Recovery	Mass Recovery
1	100.0 % (± 6.9)	99.3 % (± 6.2)	100.0 % (± 5.6)	107.6 % (± 4.9)	100.0 % (± 5.2)	90.3 % (± 5.4)
2	91.0 % (± 24.7)	99.9 % (± 22.1)	92.4 % (± 8.4)	111.9 % (± 8.4)	56.6 % (± 7.0)	82.4 % (± 12.5)
3	69.6 % (± 4.6)	77.2 % (± 2.5)	31.5 % (± 7.2)	74.6 % (± 14.8)	35.7 % (± 3.6)	63.0 % (± 8.2)
4	64.6 % (± 4.9)	69.1 % (± 8.0)	18.5 % (± 6.3)	58.1 % (± 10.2)	34.3 % (± 0.7)	66.9 % (± 8.8)
5	58.4 % (± 3.5)	60.5 % (± 2.0)	10.0 % (± 0.3)	45.4 % (± 6.1)	27.3 % (± 5.9)	58.4 % (± 15.0)
7	49.4 % (± 1.5)	33.0 % (± 5.6)	8.1 % (± 1.0)	30.6 % (± 11.1)	27.8 % (± 6.0)	62.1 % (± 12.3)
14	33.8 % (± 4.3)	7.9 % (± 7.6)	4.3 % (± 0.2)	16.4 % (± 1.4)	22.4 % (± 5.4)	45.9 % (± 8.8)
21	20.0 % (± 2.7)	5.1 % (± 2.2)	5.7 % (± 1.9)	20.5 % (± 7.4)	18.5 % (± 1.1)	43.8 % (± 7.4)
30	16.5% (± 4.3)	4.2 % (± 0.3)	4.9 % (± 0.8)	13.5 % (± 10.6)	16.0 % (± 3.8)	39.9 % (± 15.4)
60	10.2% (± 0.32)	2.8 % (± 0.4)	4.1 % (± 1.0)	6.5 % (± 2.9)	9.9 % (± 0.3)	19.9 % (± 0.8)

Supplemental Table S4. Au particle number and mass recovery in TMAH, TPAH, and TBAH in 60 days. Recovery values reported here are from three replicate digestions with ± one standard deviation.

Time (day)	Ag-TMAH		Ag-TPAH		Ag-TBAH	
	Particle No. Recovery	Mass Recovery	Particle No. Recovery	Mass Recovery	Particle No. Recovery	Mass Recovery
1	100.0 % (± 3.9)	100.5 % (± 9.3)	100.0 % (± 5.8)	74.7 % (± 4.9)	100.0 % (± 1.6)	72.2 % (± 5.5)
2	65.8 % (± 3.3)	96.2 % (± 14.2)	104.0 % (± 1)	77.9 % (± 1.2)	97.2 % (± 4)	71.8 % (± 6.6)
3	38.6 % (± 4.5)	74.1 % (± 2.8)	95.4 % (± 3)	75.3 % (± 3.6)	90.9 % (± 1.9)	71.7 % (± 2.4)
4	46.4 % (± 6.5)	73.0 % (± 6.9)	97.1 % (± 5.3)	65.0 % (± 3.4)	83.3 % (± 3.5)	59.0 % (± 2.1)
5	41.0 % (± 18.9)	49.8 % (± 2.0)	92.5 % (± 1.7)	63.8 % (± 2.7)	81.7 % (± 3.5)	56.0 % (± 3.2)
7	19.5 % (± 2.7)	48.4 % (± 7.1)	95.4 % (± 2.9)	75.4 % (± 4.3)	88.1 % (± 2.4)	68.9 % (± 3.2)
14	18.7 % (± 1.9)	29.7 % (± 7.8)	57.2 % (± 4.6)	73.1 % (± 6.6)	88.4 % (± 2.4)	66.9 % (± 1.4)
21	14.1 % (± 0.3)	23.7 % (± 2.2)	34.0 % (± 1.3)	64.7 % (± 1.6)	85.9 % (± 1.5)	66.7 % (± 2.8)
30	16.0 % (± 1.0)	24.7 % (± 4.5)	21.1 % (± 2.4)	42.8 % (± 7)	77.2 % (± 2.5)	67.1 % (± 2.6)
60	13.5 % (± 1.2)	20.2 % (± 1.4)	13.0 % (± 2.7)	42.0 % (± 9.2)	58.6 % (± 3.5)	61.8 % (± 0.9)