Multi-Method Assessment of PVP-Coated Silver Nanoparticles and Artificial Sweat Mixtures

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Derek M Peloquin[1,2], Eric Baumann[3], Todd P Luxton[2]

[1]Oak Ridge Institute for Science and Education, Oak Ridge, TN; [2]US Environmental Protection Agency, Office of Research and Development, Cincinnati, OH; [3]Pegasus Technical Services, Cincinnati, OH

Derek M Peloquin | peloquin.derek@epa.gov | (513) 487-2670

Introduction

Research regarding exposure and toxicity of nanomaterials (NMs) to humans has been widely studied over the past two decades and whether engineered NMs produce adverse outcomes/responses when exposed to various microorganisms, tissues, and cells. However, the **majority of research conducted on the topic has failed to consider** how the immediate local environment and usage scenario impact the physical and chemical properties of NMs and how those alterations impact exposure. The current research presented utilizes silver nanoparticles (AgNPs) as **a case study for how the immediate local environment alters the physical and chemical properties of NMs**. The primary route for exposure to many of the **consumer products containing AgNPs** (e.g., hand sanitizer, body cream, foot deodorizer) would occur dermally, thus nanosilver exposure in these cases must **take into account interactions such as that between human sweat and AgNPs**. This interaction represents an important local environment that could alter NM speciation and the specific chemical/material that individuals would be exposed to as a basic assessment of **how perspiration alters the physical and chemical properties of AgNPs** in a simple mixture with artificial sweat.

Ionic silver and four different sizes of AgNPs were mixed with four different formulations of artificial sweat and water and were analyzed for changes in size using **dynamic light scattering (DLS), UV-Vis spectroscopy, and single particle inductively coupled plasma-mass spectrometry (spICP-MS)**. Additionally, each mixture was centrifuged using 3 kDa filters to assess speciation changes using **X-ray absorption spectroscopy**.

Silver Nanoparticles and Artificial Sweats

SILVER NANOPARTICLES	20 nm	50 nm	75 nm	100 nm
Shorthand Name	AgNPs-20	AgNPs-50	AgNPs-75	AgNPs-100
Coating	40 kDa PVP	40 kDa PVP	40 kDa PVP	40 kDa PVP
Supplier	NanoComposix	NanoComposix	NIST (via NanoComposix)	NanoComposix

ARTIFICIAL SWEATS	0.9% Sodium Chloride	Standard 15-2013 from the American Association of Textile Chemists and Colorists	Standard 105-E04:2013 from the International Organization for Standardization (acidic)	Standard 105-E04:2013 from the International Organization for Standardization (basic)
Shorthand Name	AS(NaCl)	AS(AATCC)	AS(ISOa)	AS(ISOb)
Sodium Chloride	0.900 g	1.000 g	0.500 g	0.500 g
L-Histidine Monohydrochloride Monohydrate	-	0.025 g	0.050 g	0.050 g
Sodium Dihydrogen Phosphate Dihydrate	-	-	0.220 g	0.250 g
Sodium Hydrogen Phosphate, Anhydrous	-	0.100 g	-	-
DL-Lactic Acid	-	0.100 g	-	-
Water	100 mL	100 mL	100 mL	100 mL
Required pH	-	4.3 ± 0.2	5.5 ± 0.2 (NaOH adjusted)	8.0 ± 0.2 (NaOH adjusted)

20 ppm **ionic or nanoparticulate silver was combined with each artificial sweat** in 1:1 ratio in foil-covered polypropylene centrifuge tubes and placed on a shaker table for 15 minutes.

- Withdrew solution to dilute the mixtures **1:10 for pH, Zeta potential, DLS, and UV-Vis** measurements
- Sequentially diluted the mixture **1:10000 for spICP-MS** analysis
- Filtered and rinsed each mixture using **3 kDa centrifuge filters to separate ionic and nanoparticulate fractions for XAS** analysis

Samples were then stored in a laboratory cabinet and **repeated the full analysis to obtain Day 0, 1, 4, and 7** samples.

Results

pH and Zeta Potential

In general, there was very little change over the course of the analysis, though several specific situations should be pointed out:

AS(ISOa) has a **slight raising in the pH** over 7 days that **is not mimicked** by the silver mixtures;

AS(ISOb) has a **slight lowering in the pH** over 7 days that **is mimicked** by the silver mixtures;

AS(AATCC) has a **slight raising in the pH** over 7 days that **is mimicked by all silver mixtures except** the one containing the 75 nm AgNPs. Zeta potential values were **all negative** but are drastically different based on the ionic composition, with the H_2O mixtures being the **most negative** and the AS(AATCC) mixtures being the **least negative**, and the Day 0 measurement usually the **most negative**.

	Day 0	Day 1	Day 4	Day 7
H_2O	6.79 / N/A	6.11 / N/A	6.54 / N/A	6.18 / N/A
+AgIon	6.01 / -43.7	5.93 / -38.5	5.29 / -41.3	5.60 / -33.1
+AgNPs-20	6.02 / -37.6	6.36 / -49.7	6.05 / -45.9	6.01 / -37.3
+AgNPs-50	5.35 / -28.9	6.24 / -36.4	5.71 / -37.4	5.79 / -26.6
+AgNPs-75	5.58 / -16.7	6.04 / -26.7	5.43 / -25.2	5.43 / -12.8
+AgNPs-100	5.75 / -39.5	6.15 / -38.3	5.49 / -40.4	5.63 / -28.8
AS(NaCl)	5.48 / N/A	5.34 / N/A	5.35 / N/A	5.62 / N/A
+AgIon	5.49 / -49.6	5.16 / -9.3	5.21 / -6.9	5.31 / -4.3
+AgNPs-20	5.65 / -26.7	5.80 / -24.4	5.53 / -30.6	5.72 / -23.8
+AgNPs-50	5.58 / -18.8	5.76 / -15.5	5.42 / -12.4	5.45 / -4.3
+AgNPs-75	5.53 / -13.9	5.68 / -8.6	5.35 / -9.5	5.40 / -9.3
+AgNPs-100	5.60 / -28.1	5.59 / -15.2	5.30 / -11.5	5.41 / -7.5
AS(ISOa)	5.53 / N/A	5.61 / N/A	5.77 / N/A	5.92 / N/A
+AgIon	5.64 / -34.2	5.75 / -20.6	5.66 / -8.2	5.70 / -5.6
+AgNPs-20	5.68 / -16.3	5.80 / -13.2	5.70 / -14.6	5.68 / -12.9
+AgNPs-50	5.66 / -15.9	5.70 / -14.2	5.65 / -13.9	5.59 / -15.5
+AgNPs-75	5.62 / -14.4	5.74 / -15.9	5.66 / -16.0	5.61 / -14.1
+AgNPs-100	5.63 / -22.6	5.75 / -19.2	5.66 / -14.8	5.68 / -12.1
AS(ISOb)	8.11 / N/A	8.16 / N/A	8.10 / N/A	7.97 / N/A
+AgIon	8.17 / -33.1	8.00 / -27.4	7.83 / -17.9	7.89 / -13.1
+AgNPs-20	8.19 / -22.9	8.10 / -22.8	7.98 / -21.3	7.85 / -20.8
+AgNPs-50	8.14 / -23.5	8.09 / -23.8	8.02 / -20.3	7.88 / -14.4
+AgNPs-75	8.15 / -14.0	7.95 / -13.1	7.99 / -13.9	7.84 / -13.7
+AgNPs-100	8.14 / -23.7	7.96 / -19.5	7.92 / -17.2	7.78 / -16.2
AS(AATCC)	4.40 / N/A	4.49 / N/A	4.80 / N/A	5.10 / N/A
+AgIon	4.78 / -25.6	4.72 / -6.3	4.80 / -3.2	5.10 / -3.4
+AgNPs-20	4.76 / -13.1	4.64 / -8.7	4.63 / -4.0	5.03 / -5.9
+AgNPs-50	4.69 / -8.9	4.63 / -7.3	4.78 / -3.4	4.98 / -2.9
+AgNPs-75	4.67 / -5.4	4.63 / -4.3	4.67 / -7.8	4.63 / -4.8
+AgNPs-100	4.69 / -6.2	4.60 / -6.2	4.84 / -4.6	4.93 / -2.8

- All values in this table are presented as:
  pH measurement / Zeta potential measurement (mV)
- Zeta potential values are the average of 5 consecutive measurements but the standard deviation has been omitted for clarity

Results

Dynamic Light Scattering

H_2O and AS(ISOb) samples showed a **fairly invariant response** for all NPs over 7 days, and **close to the certified value** for AgNPs-50 and AgNPs-75. AS(NaCl), AS(ISOa), and AS(AATCC) had **larger and variant values** for AgNPs-50 but **usually smaller and consistent values** for AgNPs-75.

	H_2O+AgNPs-50				AS(ISOb)+AgNPs-50				AS(AATCC)+AgNPs-50				AgNPs-50
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Certified value
d_{eq} (nm)	60	62	63	62	66	66	63	63	**98**	**145**	**236**	**438**	**62**
	H_2O+AgNPs-75				**AS(ISOb)+AgNPs-75**				**AS(AATCC)+AgNPs-75**				**AgNPs-75**
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Certified value
d_{eq} (nm)	**103**	**100**	**101**	**104**	**101**	**102**	**99**	**96**	**106**	**102**	**85**	**92**	**102**

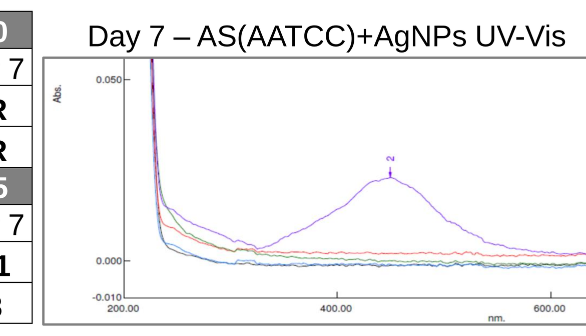
- DLS results are the average of 5 consecutive measurements but the standard deviation has been omitted for clarity

UV-Vis Spectroscopy

H_2O and AS(ISOb) samples showed a **fairly invariant response** for all NPs over 7 days. AS(NaCl) and AS(ISOa) had **no response** for all NPs. AS(AATCC) had an **invariant response** for AgNPs-75 but **no response** for all other NPs.

	H_2O+AgNPs-50				AS(ISOb)+AgNPs-50				AS(AATCC)+AgNPs-50			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
λ_{max} (nm)	431	429	430	428	428	432	432	427	**NR**	**NR**	**NR**	**NR**
d_{eq} (nm)	60	58	59	57	57	61	61	56	**NR**	**NR**	**NR**	**NR**
	H_2O+AgNPs-75				**AS(ISOb)+AgNPs-75**				**AS(AATCC)+AgNPs-75**			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
λ_{max} (nm)	**454**	**454**	**454**	**450**	**455**	**454**	**456**	**451**	**451**	**450**	**446**	**451**
d_{eq} (nm)	**80**	**80**	**80**	**77**	**81**	**80**	**82**	**79**	**78**	**77**	**74**	**78**

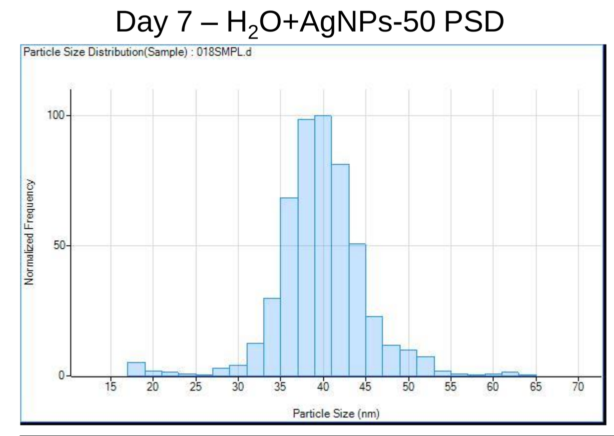
Day 7 – AS(AATCC)+AgNPs UV-Vis



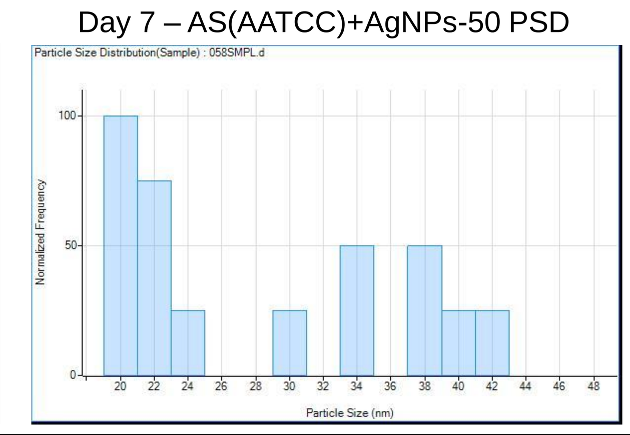
Single Particle Inductively Coupled Plasma-Mass Spectrometry

H_2O and AS(ISOb) samples showed a **fairly invariant response** for AgNPs-50 and AgNPs-75 over 7 days. AS(NaCl), AS(ISOa), and AS(AATCC) samples showed a **decrease in signal/stability** for AgNPs-50 but reported a **fairly invariant response** for AgNPs-75.

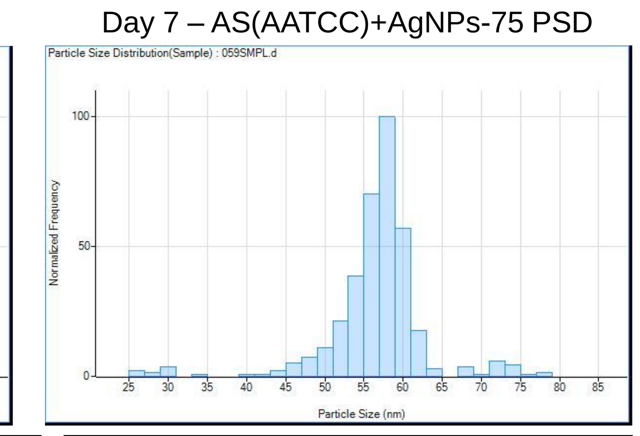
Day 7 – H_2O+AgNPs-50 PSD



Day 7 – AS(AATCC)+AgNPs-50 PSD



Day 7 – AS(AATCC)+AgNPs-75 PSD



	H_2O+AgNPs-50				AS(ISOb)+AgNPs-50				AS(AATCC)+AgNPs-50			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
d_{eq} (nm)	42	41	39	39	44	38	38	40	**43**	**34**	**31**	**28**
	H_2O+AgNPs-75				**AS(ISOb)+AgNPs-75**				**AS(AATCC)+AgNPs-75**			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
d_{eq} (nm)	**54**	**44**	**34**	**40**	53	53	52	57	58	52	48	56

AgNPs-50 QC Check					
ICV	CCV1	CCV2	CCV3	CCV4	End CCV
40	**40**	**40**	**40**	**40**	**40**
AgNPs-75 QC Check					
ICV	CCV1	CCV2	CCV3	CCV4	End CCV
44	**43**	**43**	**43**	**39**	**39**

X-ray Absorption Spectroscopy

Many samples were too low in concentration to give a good linear combination fit, but all reasonably-fit samples showed the **greatest contribution from silver** and sometimes a **small amount of silver chloride**.

Sample		Ag	AgCl	Ag_2O	Ag_3PO_4	Ag_2S	Total
H_2O+ AgNPs-50	Day 1	**95%**	NF	NF	**6%**	NF	101%
	Day 7	73%	12%	13%	NF	NF	98%*
AS(ISOb)+ AgNPs-50	Day 1	**89%**	**11%**	NF	NF	NF	100%
	Day 7	**95%**	NF	NF	**6%**	NF	101%
AS(AATCC)+ AgNPs-50	Day 1	36%	65%	NF	NF	NF	101%*
	Day 7	NF	20%	80%	NF	NF	100%*
H_2O+ AgNPs-75	Day 1	**98%**	NF	NF	**3%**	NF	101%
	Day 7	**92%**	**8%**	NF	NF	NF	100%
AS(ISOb)+ AgNPs-75	Day 1	66%	28%	NF	NF	NF	94%*
	Day 7	**90%**	**10%**	NF	NF	NF	100%
AS(AATCC)+ AgNPs-75	Day 1	**82%**	**11%**	NF	**7%**	NF	100%
	Day 7	**78%**	**16%**	NF	**4%**	NF	98%

*Fits were bad from low sample concentration

Conclusions

The size provided by each techniques followed the trend of $\mathbf{d_{eq\,(DLS)} > d_{eq\,(UV\text{-}Vis)} > d_{eq\,(spICP\text{-}MS)}}$ though reliable results for each technique varied depending on **coating thickness/integrity**, **chemical speciation** of the nanoparticle at the **surface or as a whole**, and the length it took for an analysis and the **possible exposure of the sample to light and air**.

The **75 nm AgNPs** seem to have a **different chemical and/or photostability** compared to the other particles, which perhaps come from somewhere in the lyophilization or reconstitution processes. Additionally, basic versus acidic pH presents stability even when other factors are unvaried.

No single technique is capable of giving a complete and reliable analysis of a silver nanoparticle's size and speciation, though the planned use of **scanning electron microscopy with energy-dispersive x-ray analysis could intersect** some of the data presented herein and validate its possible use for supplying the greatest amount of information.

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