Changes in silver nanoparticles exposed to human synthetic stomach fluid: Effects of particle size and surface chemistry

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Abstract

The significant rise in consumer products and applications utilizing the antibacterial properties of silver nanoparticles (AgNPs) has increased the possibility of human exposure. The mobility and bioavailability of AgNPs through the ingestion pathway will depend, in part, on properties such as particle size and the surface chemistry that will influence their physical and chemical reactivity during transit through the gastrointestinal tract. This study investigates the interactions between synthetic stomach fluid and AgNPs of different sizes and with different capping agents. Changes in morphology, size and chemical composition were determined during a 30 min exposure to synthetic human stomach fluid (SSF) using Absorbance Spectroscopy, High Resolution Transmission Electron and Scanning Electron Microscopy (TEM/SEM), Dynamic Light Scattering (DLS), and Nanoparticle Tracking Analysis (NTA). AgNPs exposed to SSF were found to aggregate significantly and also released ionic silver which physically associated with the particle aggregates as silver chloride. Generally, the smaller sized AgNPs (< 10 nm) showed higher rates of aggregation and physical transformation than larger particles (75 nm). Polyvinylpyrrolidone (pvp)-stabilized AgNPs prepared in house behaved differently in SSF than particles obtained from a commercial source despite having similar surface coating and size distribution characteristics.

1 Introduction

In recent years, there has been wide spread interest in AgNPs because of their antimicrobial properties. These unique properties have made AgNPs the most extensively used nanoparticles in consumer products finding applications in detergents, dietary supplements, fabrics, toothpaste, kitchen and medical appliances to name a few (PEN 2012; Ip et al., 2006). According

to the Project on Emerging Nanotechnologies, nanosilver accounts for at least one fifth of all the products claiming to incorporate some form of engineered nanomaterials (PoEN, 2012). Despite the burgeoning investment in nanotechnology and increasing commercialization of silver-based products, insufficient understanding remains about the health, environmental and safety impacts for these materials. Yet, given the forms, uses and potential misuse of these consumer products, the release of AgNPs into the environment and the potential for human exposure is high (Klaine et al., 2008; Mueller and Nowack, 2008). An increasing number of reports have appeared in recent years where the authors have investigated the toxicity of AgNPs for a range of organisms (AshaRani et al., 2009; Griffitt et al., 2009; Griffitt et al., 2008; Hussain et al., 2005). However, little progress has been made toward understanding the transformations and transit of AgNPs in the gastrointestinal tract (GI).

Studies have shown that ingestion of colloidal silver may cause moderate to severe cases of generalized argyria (deep blue/grey discoloration of the skin) (Chang et al., 2006; White et al., 2003). It is however; unclear as to whether colloidal silver simply functions as a delivery system for soluble forms of silver in the GI tract. Although previous studies have shown that citrate-stabilized AgNPs agglomerate in synthetic gastric juice, the form of silver that may cross the intestinal wall was not determined (Walczak et al., 2012). Further, intravenous administration of very high doses of colloidal silver to rats for four days resulted in organ failure and death (Schmaehl and Steinhoff, 1960). A recent study showed concentration-dependent mortality and developmental defects, including spinal deformation and cardiac arrhythmia to zebrafish models after exposure to silver nanoparticles (Asharani et al., 2008). These studies are a useful measure of the potential effects of human exposure to silver nanoparticles. However, in order

to adequately assess how particle characteristics affect the toxicity of these particles upon exposure to humans, it is important to consider the particle alterations that occur in the GI tract. The mobility, bioavailability and toxicity of AgNPs in any ecosystem is affected to a large extent by colloidal stability (El Badawy et al., 2010). Colloidal stability is in turn influenced by the particle capping agents and environmental surroundings such as; ionic strength, pH and electrolyte composition of the suspending media (El Badawy et al., 2010). In this regard, the highly acidic environment of the human stomach is likely to influence colloidal stability and hence the bioavailability of ingested nanoparticles. A central question in AgNPs bioavailability and toxicology studies is whether the AgNPs remain as monodispersed particles in the GI tract or whether they form aggregates and complexes, whose properties may be significantly different than those of the ingested particles.

Previously, we have shown that 40nm citrate-capped AgNPs aggregate and partially react to form silver chloride during exposure to synthetic stomach fluid (Rogers et al., 2012). The primary objective of the current study is to investigate the interaction between SSF and AgNPs of different sizes, capping agents and from different sources (i.e., synthesis protocols).

2 Methods and Materials

2.1 Materials

Polyvinyl pyrolidone (pvp)–stabilized (10 nm and 75 nm nominal diameter, Biopure, 1.0 mg/mL) and citrate (cit)-stabilized AgNPs (10 nm and 75 nm nominal diameter, Biopure, 1.0 mg/mL) were obtained from Nanocomposix (San Diego, CA). These AgNPs are sold as standard particles commissioned by the Organization for Economic Cooperation and Development

(OECD) and will be referred to as OECD standard particles. Characterization data for these particles is available on the manufacturer's website (www.nanocomposix.com). Branched polyethyleneimine (BPEI)-stabilized (1-20 nm, 70 µg/mL) and pvp-stabilized AgNPs (1-20 nm, 70 µg/mL) were synthesized and characterized as previously described (El Badawy et al., 2010; El Badawy et al., 2011). The in-house pvp-AgNPs will be referred to as PVP2-AgNPs. SSF was prepared as previously described (El Badawy et al., 2011) using deionized water and contained HCl (0.42 M) and glycine (0.40 M) pH 1.5.

2.2 TEM and SEM conditions

For TEM experiments, AgNPs were applied to amine functionalized silicon dioxide grids (Dune Scientific, Eugene OR) by leaning the chemically modified side of the grid against a sample drop applied to a hydrophobic surface (Parafilm) for 10 min. The grid was then rinsed by placement in deionized (DI) water for 15 s and allowed to dry before placing on the TEM sample holder. The time from sample grid preparation to image acquisition was 2 hr. Images were acquired at 300 kV using a FEI Tecnai F30 G2 TEM (Hillsboro, OR) with a 2k x 2k pixels) ORIOUS SC200D CCD camera, manufactured by Gatan Inc. (Pleasanton, CA). The imaged structures were analyzed by energy dispersive X-ray spectroscopy (EDS) for elemental composition using an EDAX detector.

SEM was performed using a Zeiss EVO MA SEM (Carl Zeiss SMT Ltd, Cambridge, UK) with an in-lens or SE2 arrangement at < 3keV working voltage and ~5 mm lens to detector distance. SiO_2 grids described above were mounted on the stage using a double sided carbon tape. To confirm the distribution of AgCl on the surface of particle agglomerates, X-ray mapping

examinations were carried out using QUANTAX Bruker energy dispersive X-ray spectrometer attached to the Zeiss microscope. The EDS mappings were carried out at a voltage of 8-10 kV under vacuum conditions.

2.3 Plasmon resonance and determination of hydrodynamic diameter (HDD)

UV-Vis spectra were collected on a Hewlett Packard single beam instrument (Model 8453, Agilent, Santa Clara, CA) equipped with a thermostated sample holder. Solution spectra were obtained by measuring the absorption of the prepared nanoparticle dispersions in a quartz cuvette with a 1 cm optical path. Concentrated AgNPs suspensions were diluted with DI water to an initial working stock of 20 μg/mL then diluted (1:10) in SSF. Spectra were recorded in a second to minute time frame. The hydrodynamic diameter (HDD) values for the AgNPs were measured using two complementary techniques. These included a Nanoparticle Tracking Analysis (NTA), NS500 series instrument (Nanosight, Amesbury, UK) and a Zetasizer nanoseries equipped with 633 nm laser source and a detection angle of 173° (HDD detection range 1 nm to 10 μm) (Malvern Instruments, Westborough, MA) .

3 Results

3.1 Surface plasmon resonance

Localized surface plasmon resonance (LSPR) of AgNPs (which contribute to their observed color) occurs when the nanoparticles are excited by electromagnetic radiation resulting in collective oscillations of their conduction electrons (Jensen et al., 2000). Changes in LSPR are highly dependent on the size, shape, and dielectric properties of the metal nanoparticles as well

as matrix conditions (Malinsky et al., 2001). Consequently, it was expected that particle agglomeration would influence the absorbance spectra of the particle suspensions.

Suspensions of AgNPs in DI water have been reported to show plasmon resonance peaks ranging from 380 nm to 460 nm depending on factors such as the nominal size and matrix properties, characteristic of monodispersed silver nanoparticles under 100 nm in size (Li et al., 2010). The absorbance spectra of AgNP suspensions used in our study showed absorbance peaks around 400 nm and were stable (in DI water) for at least 30 min. Upon exposure to SSF, however, the initial plasmon band rapidly decreased and a broad red-shifted peak was observed for all particle suspensions except for PVP2 (Fig. 1). Upon exposure to SSF, changes in absorbance peaks centered around 400 nm were normalized to the initial absorbance and plotted ($[Abs]_0/[Abs]_t$) versus time (Fig. 2). PVP2 particles did not exhibit significant shifts in absorbance even after 30 min exposure to SSF and only showed a slight decrease in absorbance of the initial peak. Addition of SSF to the AgNP suspensions immediately changed the observed color of each of the suspensions, except for PVP2, from yellow to gray or tan (Fig. 1S-A). Even though there was no obvious color change for the PVP2 suspension after 30 min exposure time (Fig. 1S-B), the color did eventually change after exposure to SSF for 72 hrs (Fig. 1S-C). The plasmon resonance results suggest that for OECD standard AgNPs, the smaller particles (10 nm) aggregated faster than the larger (75 nm) particles. These results also suggest that different stabilization chemistries affect the rate of AgNP transformations in the presence of SSF. These results further indicated that particles with nominally similar sizes and coatings (e.g., 10 nm OECD-pvp and PVP2) may display different time-dependent LSPR spectral changes when

exposed to SSF suggesting that different coating densities, specific polymer chemistries affected particle aggregation kinetics.

3.2 TEM and SEM

Representative TEM images of all the particles before exposure to SSF showed mostly individual spherical particles and a few clusters with distinct perimeters consistent with monodisperse suspension (example is shown in Fig. 3). Corresponding EDS elemental analysis confirmed the presence of Ag, Si and O which is consistent with AgNPs bound to the SiO_2 substrate grid. This is also reflected in the SEM/EDS mapping data for the 75 nm OECD-pvp particles which showed clusters of spherically defined particles on a SiO_2 substrate (Fig. 2S-A). The EDS data showed these particles to be devoid of immobilized Cl (Fig. 2S-B). False color maps indicated that the particles blocked the Si signal (Fig. 2S-C) and showed the presence of the Ag signal (Fig. 2S-D).

Upon exposure to SSF for 15 min, TEM and SEM images showed AgNPs to be morphologically different from the AgNPs not exposed to SSF. The 10 nm OECD-pvp particles that were exposed to SSF for 15 min formed aggregates fused together with few distinct boundaries defining individual particles (Fig. 4). The EDS spectrum for these AgNPs showed the additional presence of AgCl (Fig. 4). Comparison of SEM images for the 10 nm and 75 nm OECD-pvp particles indicated that after exposure to SSF for 15 min, the 75 nm particles, although aggregated, appeared to (for the most part) maintain independent particle perimeters (Fig. 5A) whereas the 10 nm particles appeared to coalesce into a single mass (Fig. 5B). By contrast, when the 75 nm OECD-pvp particles were exposed to SSF for 72 hrs, the larger particles (which

maintained their morphology after 15 min of exposure) now also appeared to coalesce into a single mass similar to the smaller particles (Fig. 3S-B). The EDS spectrum showed the presence of both Ag and Cl associated with the particle aggregate (Fig. 3S-A). False color EDS mapping also showed the presence of Ag and Cl associated with the particle aggregate (Fig. 3S-D, 3S-F). These observed changes in morphology for AgNP aggregates exposed to SSF and resulting in the formation of AgCl at the particle surface have been previously reported and characterized by X-ray diffraction spectroscopy (Rogers et al., 2012). Thus in the presence of SSF, dissolution and aggregation of AgNPs appear to occur simultaneously with the formation of AgCl precipitate on the particle surface. This is reflected in the X-ray mapping results of 75 nm OECD particles after extended exposure to SSF (72 hr) showing higher concentration of Cl on the regions with AgNPs clusters (Fig. 3S).

3.2 Dynamic Light Scattering and Nanotracking Analysis

Time-dependent changes in the HDD resulting from aggregation of AgNPs suspensions exposed to SSF were monitored by DLS and NTA techniques. Both techniques measure the HDD of AgNPs, which includes the Ag core, capping agents and layers of solvent molecules that are associated with the particle or particle aggregates based on their relative Brownian motion (MacCuspie et al., 2011). DLS measurements are based on Raleigh scattering, whereby the intensity of light scattered by a nanoparticle is proportional to its diameter raised to the sixth power. One inherent disadvantage with DLS lies within the basic principles of the technique. Since particle size is determined from fluctuations in scattered light intensity, larger diameter particles will dominate the intensity. This technique relies on instrument software to

accommodate skewing of the particle mean diameter. An advantage of this technique is its broad dynamic range (i.e., particle size range of 0.15 nm – 10 μm).

The NTA technique also depends on Brownian motion of AgNPs and measures particle size and particle number in real-time by tracking individual particle motion using a CCD camera operating at 30 frames per second. Based on a laser illuminated microscopic technique, the NTA software determines the location in the field of view of the center of the spot of light scattered by individual particles. Since each particle is visualized and analyzed, this method is not limited by scattering intensity as is the case with DLS. In addition, NTA provides approximate particle number concentrations, a feature which proved useful for the current study. One of the major limitations of NTA is that due to detector limitations, particles with diameters less than 20 nm yielded unreliable measurements.

Average HDD values for AgNPs of various sizes and with different coatings measured in DI water by DLS were similar to the average diameters measured by TEM (Table 1). In the cases of the 75 nm OECD-cit and 75 nm OECD-pvp particles the average HDD values by DLS were slightly larger than nominal TEM values reported by the manufacturer. The HDD value for the 10 nm OECD-cit particles was about twice the TEM value and the PVP2 particles were 84 nm compared to an average TEM value of 6.1 nm. For the NTA measurement of AgNP HDD in DI water, the values for the 75 nm OECD-cit and 75 nm OECD-pvp particles were similar to the nominal TEM values. For the smaller particles (10 nm OECD-cit, 10 nm OECD-pvp, PVP2 and BPEI), NTA determination of HDD prior to particle aggregation was not possible (Table 1).

Upon addition of SSF and as measured by DLS, the average HDD for the smaller (i.e., 10 nm) AgNPs showed an immediate increase (compared to the DI water control and prior to the first measurement), followed by a slower increase over a 30 min time frame (Fig. 6). Although particles of the smaller nominal sizes and with different coatings (i.e., citrate and pvp) showed an initial aggregation, the average PVP2 particle aggregate size did not increase over the 30 min exposure to SSF as compared to the more significant aggregations observed for the other particles. TEM images, however, showed that after 72 hr of exposure to SSF, the perimeters of the PVP2 particles had changed such that the perimeters of the particles in the aggregates were less distinct (Fig. 4S-B) and the presence of Cl was observed in the EDS spectrum. This change after extended exposure to SSF was also consistent with the observed color change (Fig. 1S-C). In most cases, similar results were observed with NTA (Fig. 7). Although the kinetics for aggregation as measured by increasing HDD values upon exposure to SFF were not identical for the two techniques, the trends were similar. For the NTA measurement of 10 nm OECD-pvp and 10 nm OECD-cit particles the particles continued to aggregate for 15 min then showed a decrease in the average HDD between 15 and 30 min (Fig. 7B). Because the vertical dimension for the NTA observation cell is small (< 1 mm), this decrease may have been due to the larger particles settling out of the scattering laser field after about 15 min yielding a smaller average HDD value. Although the herein reported values for DLS and NTA were not identical, they showed similar trends for particle aggregation in the presence of SSF. DLS and NTA rely on different mechanisms; consequently, different measurement values for identically sized particles and particle aggregates have been previously reported (MacCuspie et al., 2011, Rogers et al., 2012).

In addition to measuring HDD, the NTA technique also reported changes in the particle number over time. Upon exposure to SSF, each particle type except for the PVP2 particles showed a similar decrease in particle number (in a closed system) over a 30 min time frame (Fig. 8). The typical change in particle number ranged from about 9 x 10^8 particles/mL to 2 x 10^8 particles/mL. Although related to particle diameter, the decrease in particle number over time would not, for several reasons, be expected to directly correlate with increases in average particle aggregate diameter. First, it should be noted that determination of HDD by either DLS or NTA is subject to the fundamental assumption that the particles conform to spherical geometry. This assumption may not be completely accurate for particle aggregates that may form branched chain structures in addition to irregular spherical shapes. Next, even with an assumption of spherical structure, the increase in particle aggregate diameter is not a linear summation of diameters of particles added to the aggregate but rather is related to the three dimensional shape and volume of the growing aggregate. In addition, it cannot be assumed that the aggregates will grow simultaneously leading to larger polydispersity values.

4 Discussion

AgNPs of different sizes, from different vendors and with different capping agents were investigated with respect to their behavior in a simulated stomach environment. Although the model solution used to simulate human stomach fluid was less complex than expected to be present in the digestion process, the high acidity which is the dominant feature of the stomach fluid resulted in significant transformations in the AgNPs. Factors such as size and surface chemistry typically play an important role in the reactivity of AgNPs and to a large extent

determine their physical and chemical state in different media. In this regard, investigation of AgNPs of different sizes and with different coatings was considered in this study.

The LSPR spectra of the AgNP suspensions were similar to those previously reported , (El Badawy et al., 2010). When exposed to SSF, the AgNP suspensions visibly changed color and eventually became clear. In addition, the initial absorbance peaks were drastically reduced and replaced with a broad peak appearing at a longer wavelength. Similar observations have been reported to occur when AgNPs were exposed to low pH media (Prathna et al., 2011). These shifts towards longer wavelengths (red shift) indicate increased particle size presumably resulting from aggregation (Prathna et al., 2011) which is also reflected in the TEM, SEM, DLS and NTA measurements (Table 1). The spectral evidence of particle–particle interaction is consistent with the sintered appearance of SSF-exposed AgNPs in TEM and SEM micrographs (Fig. 4, Fig. 5, Fig.3-S). In addition to morphological changes in SSF-exposed AgNPs, the presence of particle bound chloride in the EDS spectrum suggests that AgCl is formed in the aggregate interface (Fig 4). The EDS data showed the presence of Cl associated with SSF-exposed AgNPs. In the case of the PVP2 particles, the LSPR spectra and HDD data did not show a significant change resulting from exposure to SSF over a 30 min time frame, however, the particle suspension became clear after 72 hr suggesting that the particles were likely aggregating and possibly transforming but that the kinetics were slower for these particles.

The relative rate and extent of aggregation (within the measurement time window) depended on the particle size, coating chemistry and the synthetic approach employed to prepare the nanoparticles. For example, while the 10 nm OECD-pvp AgNPs showed a relatively

rapid aggregation rate, the PVP2 particles with a similarly described coating and size distribution did not appear to further aggregate in the 30 min time frame after exposure to SSF. While the two types of pvp-stabilized particles showed a similar particle size distribution, it is likely that the synthetic approach employed imparted different surface properties to the particles. In comparing the commercially available particles, the 10 nm OECD-pvp and 10 nm OECD-cit coated AgNPs aggregated and were transformed faster than the larger 75 nm OECD-pvp and 75 nm OECD-cit coated particles. For example, as shown Fig. 5, the smaller particles (10 nm OECD-pvp) are morphologically different with no distinct boundaries while for the larger AgNPs (75 nm OECD-pvp) the spherical particles appeared to aggregate but the perimeter boundaries were still defined after 15 min of exposure to SSF. This might be explained by the surface area differences which may render the smaller particles more reactive than the larger particles. Surface area-based release kinetics have previously been used to rationalize the differences in apparent rates of oxidative silver dissolution between bulk silver and nanosilver (Liu and Hurt, 2010).

Our data suggest that several processes occur after exposure of AgNPs to the acidic environment of the human stomach. First, AgNPs rapidly aggregate possibly due to loss of surface coating in acidic media. Second, Ag^+ ions are released in an oxidation process involving the concerted effects of dissolved O_2 and H^+. Third, chloride ions present in solution react with the released Ag^+ ions and precipitate on the particle surface to form insoluble AgCl.

$$2Ag + \tfrac{1}{2}O_2 + 2H^+ \longrightarrow 2Ag^+ + H_2O$$
$$2Ag^+ + 2Cl^- \longrightarrow 2AgCl$$
$$\overline{\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad}$$
$$2Ag + \tfrac{1}{2}O_2 + 2Cl^- + 2H^+ \longrightarrow 2AgCl + H_2O$$

It should be noted that SSF used in these studies did not contain protein or added NaCl, the presence of which may have resulted in the formation of soluble silver complexes. Although changes in AgNP properties (e.g., size, shape, morphology and chemical composition) induced by synthetic GI tract environment are likely to alter bioavailability, significant uncertainties remain in this area.

For the smallest particles (10 nm) used in the current study, exposure to SSF for 30 min resulted in a dramatic aggregation and alteration in both the physical and chemical state of the AgNPs. Similar aggregation accompanied by morphological changes were observed when 75 nm OECD pvp-AgNPs and the PVP2 particles were exposed to SSF for extended time periods (72 hr). By integrating the SEM/EDS data with mapping results the distribution of AgCl on the surface of the samples were clearly visualized (see Figs. 3S, 4S) further confirming the formation of AgCl precipitate. The resident time of ingested AgNPs in the stomach may vary among individuals and will, along with the complex matrix, ultimately determine the degree of Ag^{+} ion release and its conversion to AgCl. In addition, surface features and the size of AgNPs will play a key role in any processes leading to the transformation of AgNPs in the gastrointestinal tract. Although only representative of the observed particle aggregates, Figs. 5, 3S and 4S suggest that the larger particles and more durable particle coatings may survive transit through the stomach. Walczak et al. (2012) similarly observed that in low pH simulated stomach fluid, 60 nm AgNPs agglomerated (with chlorine inter-particle bridges) but separated into individual particles when they transitioned into higher pH simulated intestinal fluid.

5 Conclusion

In the presence of SSF, AgNPs not only aggregate but also appear to release ionic silver resulting in the formation of AgCl on the particle surface. The present study demonstrates that ingested AgNPs may aggregate and become chemically modified in the stomach and that size and surface chemistry play an important role in the transformation of AgNPs in SSF. Moreover, this study demonstrates the differences in aggregation and transformation behavior of nanoparticles of different sizes, surface chemistries and synthetic methodologies highlighting the challenges for human exposure assessment. Several factors, which were not considered in the current study, such as ionic strength and complexing agents (e.g., carboxylates, sulfhydryl and amine groups) normally present in food may also influence the aggregation and dissolution of AgNPs. Further work is needed to address these issues in order to make informed conclusions on the transformations of ingested metal particles.

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6 References

Asharani PV, Lian Wu Y, Gong Z, Valiyaveettil S. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 2008; 19: 255102.

AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 2009; 3: 279-90.

Chang ALS, Khosravi V, Egbert B. A case of argyria after colloidal silver ingestion. Journal of Cutaneous Pathology 2006; 33: 809-811.

El Badawy AM, Luxton TP, Silva RG, Scheckel KG, Suidan MT, Tolaymat TM. Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions. Environmental Science & Technology 2010; 44: 1260-1266.

El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM. Surface Charge-Dependent Toxicity of Silver Nanoparticles. Environmental Science & Technology 2011; 45: 283-287.

Griffitt RJ, Hyndman K, Denslow ND, Barber DS. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. Toxicol Sci 2009; 107: 404-15.

Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 2008; 27: 1972-8.

Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 2005; 19: 975-83.

Ip M, Lui SL, Poon VK, Lung I, Burd A. Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol 2006; 55: 59-63.

Jensen TR, Malinsky MD, Haynes CL, Van Duyne RP. Nanosphere lithography: Tunable localized surface plasmon resonance spectra of silver nanoparticles. Journal of Physical Chemistry B 2000; 104: 10549-10556.

Klaine SJ, Alvarez PJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, et al. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 2008; 27: 1825-51.

Li X, Lenhart JJ, Walker HW. Dissolution-Accompanied Aggregation Kinetics of Silver Nanoparticles. Langmuir 2010; 26: 16690-16698.

Liu J, Hurt RH. Ion release kinetics and particle persistence in aqueous nano-silver colloids. Environ Sci Technol 2010; 44: 2169-75.

MacCuspie RI, Rogers K, Patra M, Suo Z, Allen AJ, Martin MN, et al. Challenges for physical characterization of silver nanoparticles under pristine and environmentally relevant conditions. J Environ Monit 2011; 13: 1212-26.

Malinsky MD, Kelly KL, Schatz GC, Van Duyne RP. Chain length dependence and sensing capabilities of the localized surface plasmon resonance of silver nanoparticles chemically modified with alkanethiol self-assembled monolayers. Journal of the American Chemical Society 2001; 123: 1471-1482.

Mueller NC, Nowack B. Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 2008; 42: 4447-53.

Nanocomposix Biopure. (Accessed 2012). (http://nanocomposix.com/nano_catalog/sphericalsilver/BioPure/citrate)

Prathna TC, Chandrasekaran N, Mukherjee A. Studies on aggregation behaviour of silver nanoparticles in aqueous matrices: Effect of surface functionalization and matrix composition. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2011; 390: 216-224.

Product on Emerging Nanotechnologies (PEN). (Accessed 2012). (http://www.nanotechproject.org/inventories/consumer/browse/products/)

Project on Emerging Nanotechnologies (PoEN). Nanotech-enabled Consumer Products Top the 1,000 Mark. (Accessed 2012). http://www.nanotechproject.org/news/archive/8277/)

Rogers KR, Bradham K, Tolaymat T, Thomas DJ, Hartmann T, Ma L, et al. Alterations in physical state of silver nanoparticles exposed to synthetic human stomach fluid. Science of the Total Environment 2012; 420: 334-339.

Schmaehl D, Steinhoff D. [Studies on cancer induction with colloidal silver and gold solutions in rats]. Z Krebsforsch 1960; 63: 586-91.

Walczak AP, Fokkink R, Peters R, Tromp P, Rivera ZEH, Rietjens I, Hendriksen PJM, Bouwmeester H. Behavior of silver nanoparticles and silver ions in an in vtro human gastrointestinal digestion model. Nanotoxicology, doi:10.3109/17435390.2012.72632.

White JML, Powell AM, Brady K, Russell-Jones R. Severe generalized argyria secondary to ingestion of colloidal silver protein. Clinical and Experimental Dermatology 2003; 28: 254-256.

Figure Legends

Fig. 1 Absorbance spectra of; (a) 10 nm, OECD cit-stabilized, (b) 10 nm OECD PVP stabilized (c) 75 nm OECD cit-stabilized, (d) 75 nm OECD PVP-stabilized (e) PVP2 (1-20 nm), (f) BPEI-stabilized (1-20 nm) AgNPs exposed to SSF for 30 min. The arrows indicate decreasing or increasing absorbance over time (peak absorbances around 400 nm and 800 nm). The spectra were collected after (10, 30, 60, 90 s, 2, 3, 4, 6, 8, 10, 15, 20, 25, 30 min).

Fig. 2 Ratio normalized to the initial absorbance (peak centered around 400 nm) vs time for AgNPs after exposure to SSF for a period of 30 min. (a) 10 nm, OECD cit-stabilized, (b) 10 nm OECD PVP-stabilized (c) 75 nm OECD PVP-stabilized, (d) 75 nm OECD cit-stabilized (e) BPEI (1-20 nm), and (f) PVP2 (1-20 nm).

Fig. 3 TEM micrographs and EDS spectrum of unexposed (control) 10 nm OECD-pvp particles. Particles were immobilized on amine-modified SiO_2 TEM grids.

Fig. 4 TEM micrographs and EDS spectrum of 10 nm OECD-pvp AgNPs exposed to SSF for 15 min. Particles were immobilized on amine-modified SiO_2 TEM grids.

Fig. 5 SEM images of AgNPs after 15 min exposure to SSF; (A) 75 nm OECD-pvp particles and (B) 10 nm OECD-pvp particles.

Fig. 6 Hydrodynamic diameter measurements by DLS for AgNPs exposed to SSF; (A) 75 nm OECD cit- & pvp-coated, (B) 10 nm OECD cit- & pvp-coated (C) 1-20 nm BPEI-coated, and (D) 1-20 nm PVP2 as measured by DLS.

Fig. 7 NTA measurement of hydrodynamic diameter measurements by NTA for AgNPs exposed to SSF. (A) 75 nm OECD cit- & pvp-coated, (B) 10 nm OECD cit- & pvp-coated (C) 1-20 nm BPEI-coated, and (D) 1-20 nm PVP2

Fig. 8 Change particle concentration (particles/mL) for different AgNPs exposed to SSF for a period of 30 min.