Contribution to a sustainable usage of nanomaterials: release of nanomaterials from products and their dispersion into the environment

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Abstract

Nanomaterials (NMs) are defined as materials with a size less than 100 nm in one or more dimensions. These materials can, due to *e.g.* their small size, give rise to new and useful phenomena and material properties. More and more NMs are developed and used in an increasing number of consumer products. In this work two consumer products (mouth spray, skin cream) containing silver nanoparticles (Ag NPs) were tested for in scenarios for the user-phase to investigate NP size in solution as well as the release of silver from the NPs in the consumer products. These results were compared with pristine Ag NPs both with and without a capping agent. Also, more general trends of dissolution of Ag NPs were investigated in terms of its dependence on surface chemistry and capping agents.

The results of this report show that Ag NPs were released from the skin cream over time and that the NP concentration increasing over time. At the same time these NPs are dissolving in the artificial sweat. The Ag NPs in mouth spray agglomerated and dissolved continuously in artificial saliva. The faster dissolution of the Ag NPs in the consumer products compared with the pristine Ag NPs was mainly related to the smaller primary size of these NPs

In all, the results show that the studied consumer products release NPs during the user phase and that these retain their NP properties at least during the first 24 h in solution. Dissolution is on-going for the NPs during this user-phase and should be considered in risk assessments since the potentially toxic effects of ionic silver species.

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Background

Nanomaterials (NMs) are defined as materials with a size less than 100 nm in one or more dimensions. These materials can, due to *e.g.* their small size, give rise to new and useful phenomena and material properties. More and more NMs are developed and used in an increasing number of consumer products.[1] The usage of NM-containing products is therefore increasing each year and there are today thousands of NM-containing products on the market.[1, 2]
The potential for high potency of NMs,[3] as well as possibilities of increased environmental concentrations of these materials in the future,[4] underlines the need for environmental fate studies of NMs.[5] This is because the risk of NM dispersion is the product of hazard and exposure, with the environmental fate being closely connected to exposure. The project proposed in this application is linked to the exposure part of the risk assessment by considering release of NMs from consumer products. As mentioned, the Swedish Chemical Agency has in 2018 initiated a rule that producers of NM-containing products must register properties of added NMs in the products (*e.g.* size and charge) in order to increase the knowledge on which NMs that are present in consumer products on the market.[6] However, this database, or other NM-product databases, lacks information on release rates of NMs from these products, and of properties of released material (size, composition, morphology).[7] This lack of data is also evident from the scientific literature where 67 % of the studies on the release of NMs from consumer products do not include any information on the transformation products of the NMs after release.[8] The evident shortage of data on NM concentrations, size, and composition upon release from consumer products makes it impossible at the stage for any conclusive exposure risk assessments for NMs released from NM-containing products. This is an apparent road block for making the use of NMs sustainable and safe from an environmental perspective.
NMs will, upon environmental dispersion, arrive into different, compartments, including wastewater treatment plants[9] (followed by surface water[10]), soil,[11] waste incineration,[12] to name a few important examples. More specifically, the NM properties and treatment (*e.g.* crystallinity, surface oxide, coating) and history (*e.g.* exposure time, effects of light, changes in aquatic settings during transport) of the NMs and changes upon environmental interactions (*e.g.* water chemistry) are of large importance for the assessment of risks induced by NMs, both in terms of hazard and exposure. The properties of the NMs as they are released (*e.g.* from a consumer product) will influence environmental fate, and hence also the exposure of NMs to *e.g.* humans and animals. This has for instance been shown by the applicants for Ag NPs in artificial sweat and their further transformation from the laundry cycle to surface water, where the different exposure scenarios and history (treatment, exposure in different solutions, effect of light, etc.) largely affected their properties .[13] Other investigations have *e.g.* shown the influence of light on ageing of Ag NPs.[14] Knowledge of such interactions and effects of changes in realistic environmental exposure conditions is crucial to assess risks related to NMs released from consumer products, because NM properties are closely linked to *e.g.* toxic potential and mobility, and therefore largely affect any hazard and exposure assessment. This makes a strong case for the importance of the investigations in this project, as it considers release rates of NMs and their properties for consumer products which have not been investigated to date, as well as subsequent transformation in scenarios for environmental dispersion.
Eventually, experimental data coupled with fate modelling,[15] toxicity studies, material flow, and production volumes, has the opportunity to provide scenarios that can be included in risk assessments.[8, 16]

Experimental

Materials and exposure

Ag NP-containing skin cream and mouth spray are the two products that have been investigated. Manufacturer of the products is MaxLab/Sundoft and they are marketed under the name "*Silversalva*" (Ag ointment) and "*Silversept munsprej*"(Ag mouth spray). The Ag ointment and the Ag mouth spray contain silver: 30 ppm and 20 ppm, respectively.

The pristine Ag NPs consisted of PVP (40 kDa) capped Ag NPs obtain from Nanocomposix (San Diego, USA), and had a primary size of 50 nm according to the manufacturer. The bare Ag NPs were purchased from American Elements (Cleveland, USA) and the purity was 99.9%.

Each product was tested with two suitable solutions. Artificial saliva and ultra-pure water are used as solutions for mouth spray. Artificial sweat and ultra-pure water are used for the skin cream. Both products and solutions were exposed in three rounds for 20 min, 1 h and 24 h at 37 ºC. For each time condition with related product and solution, triplicate samples were taken and a blank sample. The temperature of 37 ºC was chosen considering the products exposed to body temperature.

The sample containers were acid-washed prior to use in a bath with 10% HNO_3 bath for at least 24 hours. After that, the sample containers were removed and rinsed with ultra-pure water 4 times to remove the acid.

Solutions were prepared for the products according to the recipe below.

Table 1. Composition of artificial sweat and artificial saliva solutions.

	Artificial sweat[17]	**Artificial saliva**[18]
pH	6.5	6.75
NaCl (g/L)	5.0	0.4
Lactic acid (g/L)	1.0	
KCl (g/L)		0.4
Urea (g/L)	1.0	1.0
$CaCl_2 \cdot H_2O$ (g/L)		0.795
$NaH_2PO_4 \cdot H_2O$ (g/L)		0.78
$Na_2S \cdot 9H_2O$ (g/L)		0.005

The pH was adjusted to 6.75 dropwise with 5% by volume NaOH.

Exposure to mimic user phase of consumer products

For each sample, two AAS samples were taken: one filtered and one unfiltered sample. Through the filtered sample, the concentration of silver ions can be obtained. The concentration of the filtered samples subtracted with the concentration of the filtered sample gives the concentration of Ag NPs. The filtered sample was prepared by passing 6 ml of the sample solution through an alumina filter (20 nm pore size, Anotop filter, Whatman). Then 15 µL of 65% HNO3 was added to preserve the samples. When preparing the unfiltered sample, 6 ml of the sample solution was taken and added with 15 µL of 65% HNO3 to preserve the AAS samples and to dissolve the Ag NPs.

Nanoparticle size

NTA Nanoparticle Tracking Analysis (NTA) was used to measure NP size in solution. The size range of nanoparticles that NTA can measure is between 10-1000 nanometers. NTA analyzes a sample via a video sequence and sends out a laser beam through the test chamber. The particles scatter light which can be read by a CCD camera. The camera records a video sequence of how the particles move under Brown motion. For each particle the size is calculated and then concentration and size distribution can be obtained.

Metal concentration in solution

Atomic absorption spectroscopy, AAS, is method used to determine the metal ion concentration insolution. A Perkin Elmer AAnalyst 700 was used in graphite furnace mode to determine silver concentrations. Silver calibration standards were 7.5 µg/L, 15 µg/L, 30 µg/L, 45 µg/L, prepared from a 1 g/L certified standard (Perkin Elmer).

Surface characterization

A Horiba Yvon Jobin HR800 Raman spectrometer with laser wavelength of 532 nm and a 50X objective was used for surface characterization of the mouth spray, PVP Ag NPs, and bare Ag NPs. Three different spots on were investigated for each particle type with laser beam focused softly to avoid beam damage. The samples were checked by means of optical microscopy before and after the measurement to assure no laser induced damage. For the mouth spray a drop of the spray was deposited on a glass slide and left to evaporate before Raman investigations. The measurements were focused on agglomerates of Ag NPs which could be identified through the optical microscope and also by the very large signal owing to the surface-enhanced Raman effect.35 The PVP Ag NPs were investigated in the stock solution as a drop on a glass slide. The Raman investigations of the bare Ag NPs were conducted in the form of dry powder.

Zeta potential

A Zetasizer Nano ZS instrument (Malvern Instruments, U.K.) was used to estimate the zeta potential of the NPs. The measurements were conducted at 25°C in triplicate readings. The Smoluchowski approximation was used for modelling the zeta potential. Measurements in AS and ASW only gave no results, ensuring that the measurements with Ag NPs are related to the zeta potential of the NP themselves. The PVP Ag NPs and mouth spray Ag NPs were diluted in 10 mM NaCl to a concentration of 0.1 g/L Ag NPs. The bare Ag NPs were sonicated.

Chemical equilibrium calculations

The chemical equilibrium calculations were performed using Medusa. The input was the constituents of the AS and ASW solution as detailed in Table 1, as well as different silver ions concentrations.

Electron microscopy

Scanning electron microscopy (SEM) was employed using a FEI-XL 30 series. In order for a sample to be analyzed with SEM, it needs to be conductive; hence the ointment samples were gold plated in order to obtained conductivity. The SEM instrument was also supplemented with energy dispersion spectroscopy (EDS), which by analysis shows the presence of different elements in the sample. For the part of the project, the purpose was to identify silver with SEM / EDS. The ointment was made gold-plated while the mouth spray was considered to have sufficient conductivity.

The Ag NPs in skin cream and mouth spray were imaged using a JEOL 200 kV 2100F field emission microscope operated in scanning beam mode (STEM). This was combined with energy dispersive

spectroscopy (EDS) microanalysis using a windowless silicon drift detector X-MaxN TLE from Oxford Instruments and the Aztech software, in order to confirm the identity of the imaged Ag NPs by EDS. The skin cream was prepared by heating the skin cream at 500 °C for 30 min in a muffle furnace in order to reduce the organic matter content. The ash was dispersed in butyl in a sonication bath for 20 min, and then deposited onto the copper grid followed by evaporation at ambient conditions. The mouth spray was prepared placing a drop of the stock solution on the grid, followed by blotting using a paper.

Results and discussion

Properties of Ag NPs before exposure

Figure 1 shows TEM images of the studied Ag NPs.

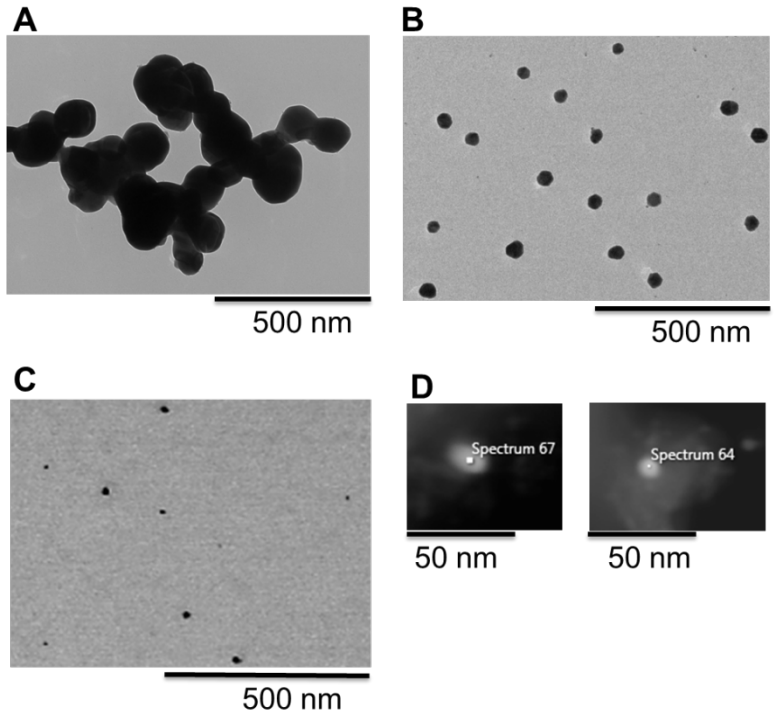


Figure 1. TEM images of bare Ag NPs (A), PVP Ag NPs (B), mouth spray Ag NPs (C), and skin cream Ag NPs (D). All images were collected in bright field mode except for the skin cream Ag NPs which were obtained using dark field mode. The noted spots in D were confirmed to contain silver using EDS.

The properties of the studied Ag NPs, before any exposure, are summarized in Table 2, including the primary sizes obtained by the TEM images.

Table 2. Summary of Ag NP properties in terms of primary size, zeta potential and surface compounds obtained by Raman.

Particle	Primary size (nm)	Zeta Potential (mV)	Surface compounds
Bare Ag NPs	50-150	0	Ag-O, Ag carbonate
PVP Ag NPs	50-70	-46	PVP
Mouth spray Ag NPs	2-20	-24	Organic compounds
Skin cream Ag NPs	15-25	N/A	N/A

Dissolution and particle agglomeration kinetics for Ag NPs in skin cream compared with bare and PVP-capped Ag NPs

Figure 2 shows the dissolution kinetics in AS for Ag NPs in skin cream, bare Ag NPs, and PVP Ag NPs. The total concentration of Ag NPs is 2 mg/L for all experiments, which is in the same range as tested in other works[19] and represents a dilution of the content in the consumer products of ca. 10 (skin cream) and 15 (mouth spray) The dissolution is here defined as what is left after passing through a 20 nm pore size filter ("ionic Ag"). Noted in the Figure 2 is also a line indicating the estimated solubility of silver in the solution at equilibrium, calculated using Medusa.

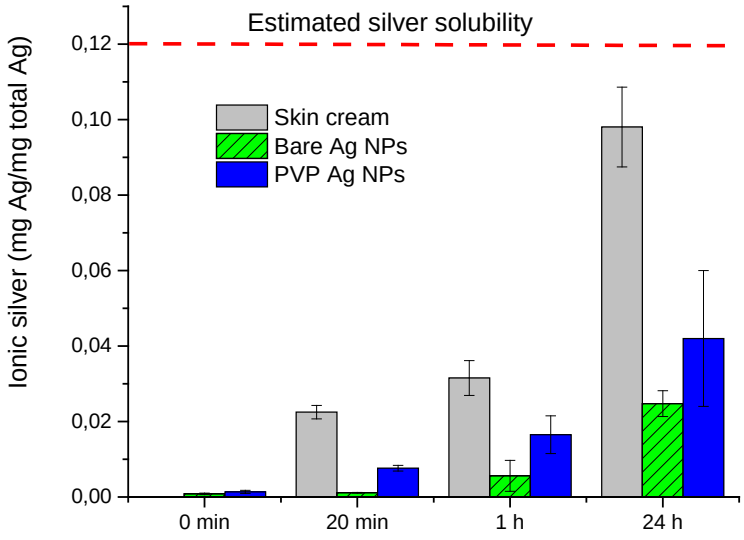


Figure 2. Dissolution kinetics of Ag NPs in skin cream, bare Ag NPs, and PVP Ag NPs in ASW. Silver solubility at equilibrium is given as dashed line, as estimated by Medusa. The results displayed are the average from three independent samples and the error bars correspond to the standard deviation between these samples. The 0 min time point corresponds to the ionic silver in the sonicated stock solution of the bare Ag NPs and the stock solution of PVP Ag NPs, just before exposure in ASW. The ionic Ag content in the skin cream could not be detected prior to any exposure and it's data point is there for missing in the category.

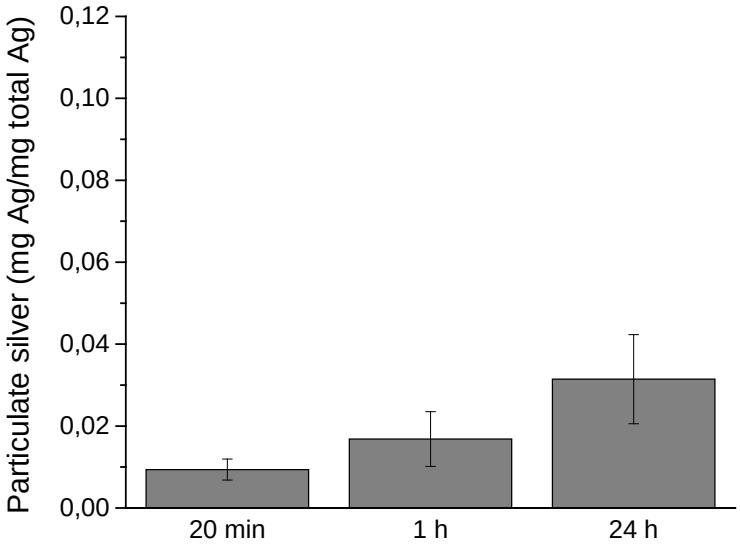


Figure 3. Kinetics of release of particulate silver particles (>20 nm size) in skin cream containing Ag NPs for exposure in ASW. The results displayed are the average from three independent samples and the error bars correspond to the standard deviation between these samples.

The dissolution kinetics in Figure 2 show that the dissolved Ag increased over time for all the studied Ag NPs and that it was this highest for the skin cream, reaching 10% of dissolved Ag after 24 h immersion time, followed by the PVP capped Ag NPs, and the bare Ag NPs.

Particulate silver (defined as the fraction retained by 20 nm pore size filter, see experimental section) released from the skin cream is presented in Figure 3, showing gradual increase at levels up to 3% particulate Ag after 24 h. The total release of particulate and ionic Ag adds up to ca. 1 mg Ag/kg product for the skin cream after 1 h in ASW, which is within the range as seen by Quadros *et al.* (0.14-18.5 mg Ag/kg) for a collection of Ag NP-containing products tested in ASW.[19] The work of Quadros *et al.* used a different mass ratio to liquid ratio (1:50) compared with this work (1:20), in addition to a 2 h immersion time compared to 1 h in this work.[19]

The particle size kinetics, investigated with NTA, for the NPs released from skin cream Ag NPs and PVP-capped are shown in Figure 4A and B, respectively. Note that the difference between the time points in Figure 4A are not statistically significantly in terms of particle concentrations different due to variations between replicates (Student's t-test, $p<0.05$). The bare Ag NPs did not give any reliable results in NTA due to extensive agglomeration and sedimentation, which shows that particles micron-sized and larger had been formed. These particles were also investigated with dynamic light scattering with the same outcome. The very fast agglomeration of bare Ag NPs in ASW has been seen earlier,[13, 20] and is related to the relatively high ionic strength in ASW (Table 1) which shield electrostatic forces, and the relatively high attractive van der Waals forces for metal NPs.[21]

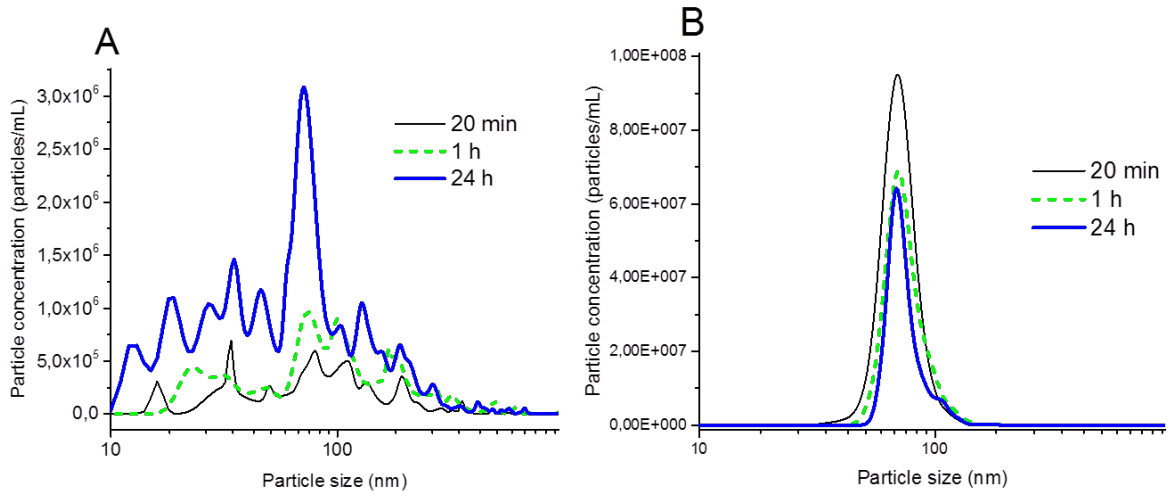


Figure 4. NTA results for particle size distribution in ASW. A: Ag NPs released from skin cream, B: PVP Ag NPs.

The PVP-capped Ag NPs showed only little agglomeration as the main peak was centered close at their primary size, ca. 67 nm, due the stabilizing effect of the PVP capping agent. The Ag NPs released from the skin cream were conversely of many different sizes, ranging from ca. 15 nm to 500 nm, showing agglomeration compared with primary sizes (Figure 1). These results demonstrate that the Ag NPs released from the skin cream did not have a capping agent as efficient as PVP with respect to agglomeration prevention. The stability was however greater compared with the bare Ag NPs, which implies that there was residue of skin cream formulation attached to the NPs, supplying a bit of agglomeration prevention. The skin cream formulation contains various organic compounds (see Materials and methods).

The surface area of the PVP Ag NPs will be larger than the bare Ag NPs due to to smaller particle sizes and hence in that sense favor dissolution of the PVP Ag NPs,[22] which will contribute to the relatively higher concentration of ionic Ag compared with the bare Ag NPs. The adsorbed PVP itself will also impact the chemical stability and in turn the dissolution.[23, 24] and has been suggested to enhanced dissolution compared with *e.g.* citrate-capped Ag NPs.[24, 25]

Considering that the skin cream only released ca. 3% of its particles into solution (Figure 4), the amount of ionic Ag is high compared with the bare Ag NPs and PVP capped Ag NPs who were fully exposed to the solution which should result in a higher surface area for these NPs compared with the skin cream Ag NPs. Mechanisms that can contribute to this difference is smaller primary size in skin cream which can make dissolution faster,[26] and the possibility that adsorbed organic matter can decrease chemical stability of the Ag NPs from the skin cream.[24]

Dissolution and particle agglomeration kinetics for Ag NPs in mouth spray compared with bare and PVP-capped Ag NPs

The dissolution kinetics in AS are displayed in Figure 5 for Ag NPs in mouth spray, bare Ag NPs, and PVP NPs. Included in the figure is also the estimated equilibrium concentration of soluble Ag (silver ions and soluble silver chloride complexes), see supporting information for details. These results are meant to have implications on a scenario for when spray products containing Ag NPs release ions and NPs into the saliva, and not for the aerosols, which has been investigated in other studies.[27]

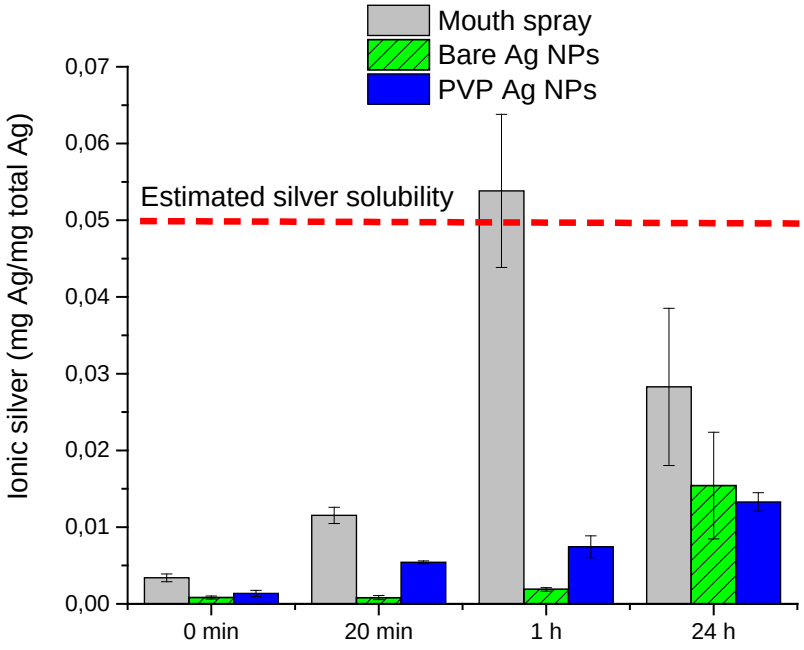


Figure 5. Dissolution kinetics in artificial saliva for Ag NPs in mouth spray, bare Ag NPs, and PVP Ag NPs. The 0 min time point corresponds to the ionic silver in the sonicated stock solution of the bare Ag NPs and the stock solution of PVP Ag NPs, just before exposure in ASW. The ionic Ag content in the skin cream could not be detected. The results displayed are the average from three independent samples and the error bars correspond to the standard deviation between these samples. Silver solubility at equilibrium is given as dashed line, as estimated by Medusa.

The dissolution kinetics in Figure 5 show a faster initial dissolution for the mouth spray compared with the bare Ag NPs and PVP Ag NPs. The estimated solubility of Ag in artificial saliva is close to the level of dissolved Ag for the mouth spray after 1 h exposure, before the dissolved Ag in mouth spray drops down significantly at 24 h ($p<0.05$, Student's t-test) These results indicate that there is precipitation of AgCl which results in lowering of the concentration of ionic Ag in solution over time. This behavior highlights the importance of considering the Cl^-/Ag^+ ratio when conducting dissolution experiments of Ag NPs.[28, 29] Chlorides can also enhance the dissolution, especially at under saturated conditions.[30]

The NTA results for particle size in artificial saliva are displayed in Figure 6, and the bare Ag NPs are again missing due to extensive agglomeration and sedimentation which prohibits quantification of particle sizes.

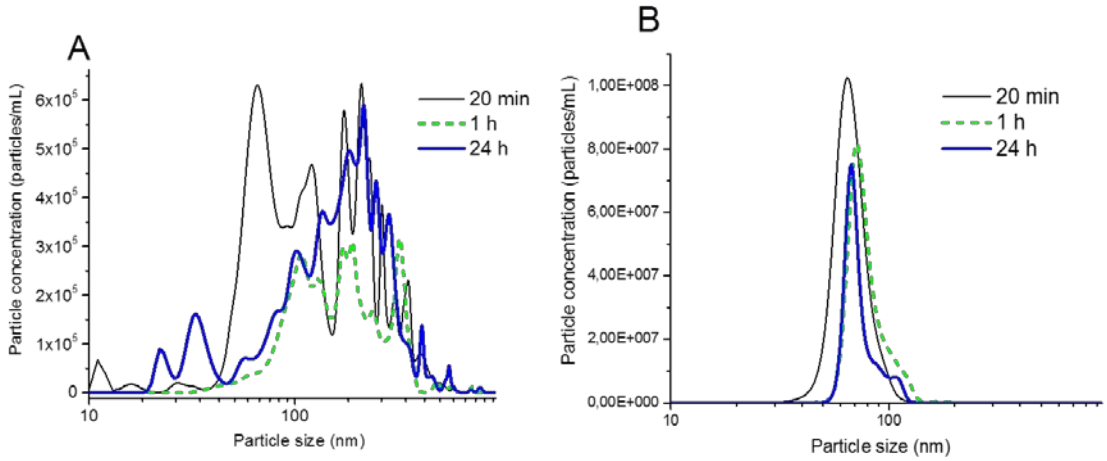


Figure 6. NTA results for particle size distribution in ASW. A: Ag NPs in mouth spay, B: PVP Ag NPs. The results displayed are the average from three independent samples.

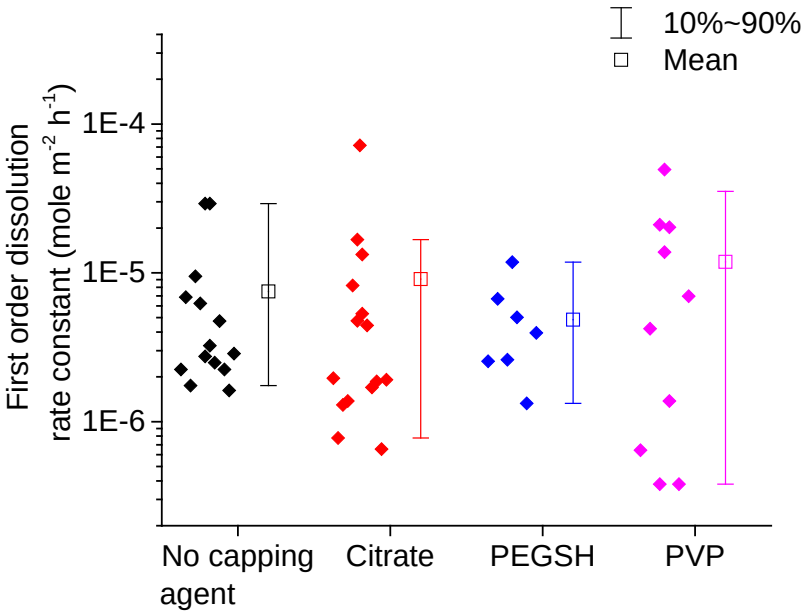
The results for the mouth spray in Figure 6A for different times points are not statistically different in terms of particle concentrations due to scatter between replicas. The particle sizes for the Ag NPs in mouth spray were largely poly dispersed with sizes ranging from around 10 nm to 800 nm. The PVP Ag NPs were conversely more narrowly distributed, and centered close to the primary size (65 nm), with also some agglomeration see from the tail of the size distribution up to ca. 110 nm. The higher colloidal stability for the Ag NPs in mouth spray compared with the bare Ag NPs can to some extent be explained by the presence of hydrocarbons on the surface of the NPs (Table 2), but the stabilization still not as efficient as the PVP as seen for the presence of relatively large colloids in Figure 6.

The population of very small primary particles (2 nm) present in the mouth spray (Figure S4) is from theory expected to have very rapid dissolution rates compared with the 60-70 nm PVP and 50-100 nm bare Ag NPs due the well-known size effect which in gives higher solubility and dissolution rates for particle size <20 nm.[24, 31] Agglomeration can however reduce this nano specific effect to some extent.[31, 32] In this work there is evidently agglomeration for the Ag NPs in mouth spray (Figure 6), but the NTA cannot detect particle as small as 2 nm, which opens up possibility that the rapid initial dissolution kinetics (Figure 5) is influenced by the small NPs sized around 2 nm in the mouth spray. The surface compounds before exposure (Figure 1) will also influence the outcomes of the dissolution results, but the exact nature of the coating is however unknown, which negates any discussion around this topic.

In this work, we have used a relatively simple formulation for the artificial saliva (Table 1). In order to be more relevant, there are work that use for example authentic saliva, which has been seen to have more of a stabilizing effect on the Ag NPs in terms of colloidal stability.[33] Future work of Ag NPs in for example mouth spray would benefit the use of such more authentic simulation of Ag NP transformation.

General trends for dissolution of Ag NPs of relevance for consumer products

A critical review article has been published on the dissolution of NPs in freshwater and similar media.[26] Part of this work was related to Ag NPs, e.g. in terms of dissolution rate constants for AG NPs with different capping agents (Figure 7).



Figur 7. Collected first-order dissolution rate constants for Ag NPs with and without different capping agents at freshwater-like conditions.

Reported dissolution rate constants in Figure 7 were generated under largely varying experimental conditions, for example in terms of primary particle size and presence of NOM. The non-functionalized Ag NPs show comparable dissolution rate constants compared with the functionalized Ag NPs. A study of Liu *et al.* investigated dissolution rates of PEG or BSA-coated and non-coated Ag NPs, conversely showed significantly lower dissolution rates for capped NPs compared with non-functionalized NPs.[23] To study effects of the different capping agents, the Ag NPs were attached to a substrate that excluded the effect of agglomeration.[30] Other investigations have shown that adsorbed molecules of Tween (a non-ionic surfactant), sodium dodecyl sulfate, and BSA on Ag NPs hindered the dissolution compared with bare NPs.[34, 35] No hindrance was however observed in the case of using citrate as capping agent.[34] The lack of connection between these studies (slower dissolution rate for functionalized Ag NPs) and the data presented in Figure 7 observation can have several explanations: *i)* some data of non-functionalized NPs was generated at different experimental conditions, including higher NOM concentrations, pH, and larger primary size, which may hinder dissolution,[36] *ii)* the capping agents may prevent from significant particle agglomeration and thereby enhance the extent of dissolution (see previous section), and *iii)* the capping agent is not stable in the exposed solution and detaches from the surface with time.[37, 38]
A capping agent, such as PVP, which enhances the colloidal stability can make nano-specific effects more evident as agglomeration can be avoided.[24, 39] On the same time can the capping agent reduce the chemical stability of the NPs by weakening metal oxide bonds as described above for NOM adsorption.[24]

Conclusions

This report does not claim to make a generalized model for choosing Ag NPs that can serve as models for Ag NPs released from consumer products. Instead, the results herein can be used as a starting point for further investigations with the aim to find, when possible, suitable model systems for Ag NPs in consumer products to be further employed in risk assessment.[40] In this part we discuss of the possibilities of using pristine Ag NPs as proxies for characteristics of Ag NPs released from consumer products, based on the results in this work. We will separate the discussion into separate a user-phase type of scenario (in the order of hours typically[19]) with the more long term results (24 h).

In the short term (<1 h) there were large variations in the release of ionic Ag when comparing the consumer products and the pristine Ag NP with the consumer products releasing significantly more ionic Ag (Figures 2 and 5). The ionic Ag in artificial saliva was >5 times faster for the consumer product (mouth spray) and >2 times faster in artificial sweat (skin cream). This is partly related to the smaller primary sizes in the consumer products (Figure 1) as well as the difference in agglomeration (Figure 4 and 6), as discussed previously. A wide range of primary Ag NP sizes is expected from previous observations in consumer products,[19, 41, 42] which makes it difficult to choose a simple pristine Ag NP as proxy in this case as the Ag NP primary sizes in consumer products varies considerably. The pristine Ag NPs may hence not always be a good proxy for consumer product in the short-term, for example when estimating any acute toxicity, which to some extent is dependent on ionic Ag.[43] Skin diffusion of NPs and toxicity is moreover highly size-dependent which may further hamper the use of Ag NPs for proxies as Ag NPs released from consumer products.[44]

The longer term behavior (24 h) was conversely more similar than the short term in terms of dissolution. Here, the difference was not significant when comparing PVP capped NPs and the Ag NPs in mouth spray ($p<0.05$, Student's t-test). For the skin cream there was still significantly more ionic Ag after 24 h compared with the pristine Ag NPs ($p<0.05$). The Ag NPs will be further transformed for example in wastewater treatment,[9, 45] by interaction with sulfide,[46] or coating degradation over time,[37] making the starting characteristics of lower importance in the long term compared with the short term. Ag NP-induced lung cell toxicity has moreover showed that the type of capping and agglomeration did not influence toxicity, whereas the primary particle size was a more important characteristic.[38]

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