# Sequential Studies of Silver Released from Silver Nanoparticles in Aqueous Media Simulating Sweat, Laundry Detergent Solutions and Surface Water

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**S** *Supporting Information*
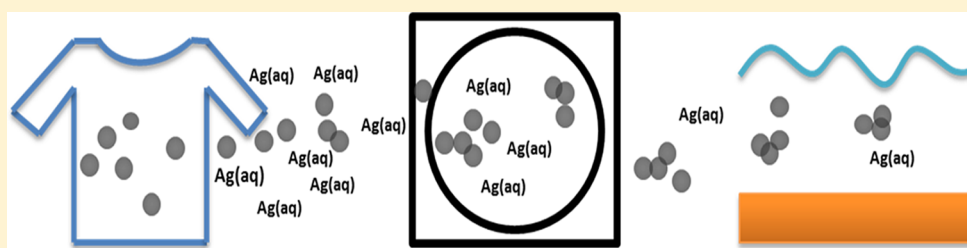
**ABSTRACT:** From an increased use of silver nanoparticles (Ag NPs) as an antibacterial in consumer products follows a need to assess the environmental interaction and fate of their possible dispersion and release of silver. This study aims to elucidate an exposure scenario of the Ag NPs potentially released from, for example, impregnated clothing by assessing the release of silver and changes in particle properties in sequential contact with synthetic sweat, laundry detergent solutions, and freshwater, simulating a possible transport path through different aquatic media. The release of ionic silver is addressed from a water chemical perspective, compared with important particle and surface characteristics. Released amounts of silver in the sequential exposures were significantly lower, approximately a factor of 2, than the sum of each separate exposure. Particle characteristics such as speciation (both of Ag ionic species and at the Ag NP surface) influenced the release of soluble silver species present on the surface, thereby increasing the total silver release in the separate exposures compared with sequential immersions. The particle stability had no drastic impact on the silver release as most of the Ag NPs were unstable in solution. The silver release was also influenced by a lower pH (increased release of silver), and cotransported zeolites (reduced silver in solution).

## ■ INTRODUCTION

The use of silver nanoparticles (Ag NPs) in consumer products, such as clothing, is expanding on the global market, primarily due to their antibacterial properties.[1] The estimated production rate of Ag NPs in Europe is in the range of 0.6−55 tonnes/year.[2] At the moment, the environmental concentrations of Ag NPs in general are in the ng/L range,[3,4] meaning no present acute risk for environmental hazard induced by Ag NPs. However, the large uncertainty in production volumes, which are increasing, together with the diversity of Ag NPs in terms of, for exmaple, size and coating, call for investigations of Ag NPs. Hence, there is a need for accurate assessment of their potential environmental dispersion and fate[3,4] as silver is potentially toxic, especially toward water organisms.[5] For example, a median effect concentration value (EC50) of 0.187 mg Ag/L for *Daphnia magna*,[6] and a median lethal concentration value (LC50) of 0.696 μg Ag/L for *Ceriodaphnia dubia*[7] have been established. Gottschalk et al. have estimated, based on modeling, that increased levels of silver released into the environment would result in elevated risks for, for example, surface water organisms.[8] The chemical speciation of released silver and nanoparticle characteristics, such as size and surface charge, largely influence silver toxicity.[9,10] Increased release of dissolved silver has been seen to increase toxicity.[9,10] Furthermore, Ag NPs may change speciation in different liquid media, which influences the extent and chemical form of released silver.[9−11] Parameters such as particle size, coatings/capping agents, solution pH and composition have been investigated in relation to dissolution of Ag NPs, all essentially underlining the complexity of silver release.[9−16] A dependency of solubility on particle size was found, with a higher solubility for smaller particles, related to the fact that small particles have a higher surface area.[15] Modeling attempts have shown that the

**Table 1. Ag NP Characteristics at Dry and Wet Conditions**

| | |
|---|---|
| isoelectric point (pH)[30] | ~3 |
| specific surface area[30] | 3.38 m$^2$/g |
| surface compound in air (Raman)[30] | Ag$_2$CO$_3$ |
| surface composition, mass% (XPS) | O: 8%, Ag: 72% (47% metallic Ag), C: 19%. |
| size and morphology | see TEM image, Figure 1 in Skoglund et al.[29] size range (after sonication): 2−150 nm. |
| d(50), volume based, artificial sweat | 52 ± 30 $\mu$m |
| d(50), volume based, detergent solution, no zeolites | 65 ± 20 $\mu$m |
| d(50), volume based, freshwater | 70 ± 3 $\mu$m |

extent of silver release does not follow expected values predicted from either loadings (mass Ag NP added per volume) or surface area,[16] due to varying particle properties with particle size. Solubility and release rates of silver from Ag NPs are also, as expected, highly dependent on solution composition.[9,11,14,17]

Reported findings of the environmental dispersion of silver and Ag NPs from consumer products show that significant amounts of silver are released from Ag NP impregnated clothing already after a few laundry cycles, both as particles and ionic species.[18−21] Released silver will inevitably change its properties during transport via different aquatic media. To the best of the knowledge of the authors, studies of sequential exposures of Ag NPs to different aquatic media of relevance for environmental exposure and fate scenarios are, so far, largely missing.

This study aims on simulating the environmental interaction of Ag NPs released from e.g. clothing, by monitoring changes in ionic release and changes in particle properties of uncoated Ag NPs in sequential contact with artificial sweat (human contact), model laundry detergent solutions (washing cycle), and synthetic freshwater, respectively. Artificial sweat (AS) was chosen to mimic transformation of Ag NPs, released from, for example, clothing, in human skin contact. Its composition is based on an established protocol.[22] To take into account the potential release of Ag NPs and silver species from clothing or the skin into the washing cycle, a laundry relevant medium with typical detergents was the second step.[23] Surface water was the final step of the sequence, a standard medium for dissolution/transformation, designed to have a general relevance for transformation of materials in aqueous media.[24]

Naturally, there are additional aquatic settings of relevance for the environmental fate of Ag NPs, including, for example, sewage sludge,[25] and media containing natural organic matter[14,26] to complete a more comprehensive investigation of the fate of Ag NPs. These media will be targeted in future studies, as this study is a first step toward introducing the approach of sequential immersions for investigations of environmental transformation of NPs.

## ■ MATERIAL AND METHODS

**Silver Nanoparticles.** A commercial noncoated Ag NP powder (EV NANO Technology Co., Ltd., China), produced by laser ablation, was investigated at a particle loading of 1.5 mg Ag NPs in 15 mL solution, that is, 0.1 g Ag/L. The specific surface area (3.38 m$^2$/g) of the Ag NPs was measured by the BET (Brunauer, Emmet, Teller) method, Table 1, which is described elsewhere.[27,28] The powder consisted of small particles sized less than 10 nm, and also larger particles and agglomerates of several hundred nm, as illustrated in the TEM image published by Skoglund et al.[29] Low angle laser light scattering (LALLS) (Supporting Information (SI) Table S1)

measurements at the onset of immersion in solution revealed wide particle size distributions with sizes up to several hundred $\mu$m with slight difference in observed d(50) values in artificial sweat, detergent solutions, and freshwater, respectively (Table 1). The surface composition (X-ray photoelectron spectroscopy, XPS, Table 1) showed metallic Ag signal in addition to oxidized Ag, indicating that the surface film (adventitious carbon and Ag$_2$CO$_3$) is in the order of 5 nm or less (see SI on details on XPS).

**Solutions and Exposure.** Artificial sweat (AS) was prepared according to the EN 1811 standard[22] and contained 5.0 g/L NaCl, 1.0 g/L urea and 1.0 g/L lactic acid. The pH was adjusted to 6.5 using NaOH. Approximately 3.6 mL 5 vol % NaOH was added to 1 L ultrapure water solution, resulting in 4.5 mM NaOH. The model laundry detergent solution consisted of 2 g/L Berol (Berol 266, Akzo Nobel), an alcohol ethoxylate with chain length C9−11 and 4−7 ethylene oxide units, 1.8 g/L dodecylbenzenesulfonate (LAS, Sigma-Aldrich), and 11 g/L zeolite (ball milled Zeolite A, size <10 $\mu$m, Sigma-Aldrich), adjusted to pH 10 using HNO$_3$ and NaOH. Approximately 0.4 mM NaOH and 30 $\mu$M HNO$_3$ was added. The freshwater solution contained 0.0065 g/L NaHCO$_3$, 0.00058 g/L KCl, 0.0294 g/L CaCl$_2$·H$_2$O, and 0.0123 g/L MgSO$_4$·7H$_2$O, based on the OECD transformation/dissolution protocol recommended for fresh water solutions of pH 6.0 (low salinity).[24] The solution was not pH-adjusted and had an initial pH of 7.2. Ultrapure water (Milli-Q, 18.2 MΩ cm) was used to prepare all solutions. All chemicals were of analytical or puriss p.a. grade.

The samples were exposed in a Stuart S180 incubator at bilinear shaking conditions (12°, 25 cycles/min) followed by particle separation using centrifugation (10 min at 3000 rpm (704 rcf)) after each step in the sequential exposures. Successful particle removal was ensured using ultracentrifugation performed with a Beckman Optima B-90K (Brea, CA) using a SW 55-Ti rotor. The centrifuging time was 90 min, the sample volume 6 mL, and the speed 50,000 rpm (corresponding to approximately 137 000 $g$), ensuring removal of NPs in solution.[31] No significant difference ($p < 0.05$) in silver concentration in solution was observed when using ultracentrifugation compared with the centrifugation procedure described above. After exposure, 10 mL of particle-free sample (supernatant) was collected for analysis. The remaining solution was removed using a pipet, followed by introduction of new solution. In this way was approximately only 0.1% of the solution transferred to the new medium. The vessel was then manually shaken for approximately 5−10 s in the new solution. Nonsequential exposures in each solution (detergent solution, detergent solution without zeolites, and freshwater) were conducted for comparison. Exposures were performed at 37 ± 0.5 °C for AS and for the detergent solutions with and without zeolite (close to 40 °C, which is a common temperature for
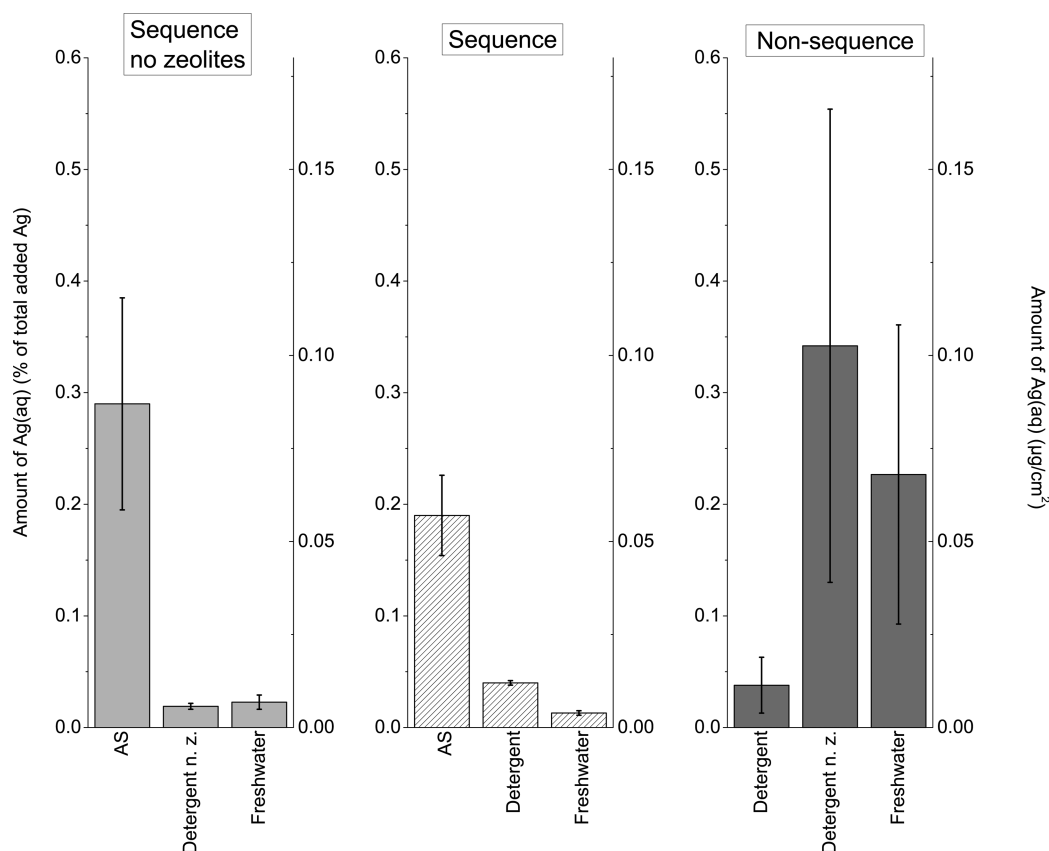
**Figure 1.** Amount of silver in solution (per amount of added Ag NPs in %, left *y*-axis, or surface area, right *y*-axis) after sequential and nonsequential exposures of Ag NPs in artificial sweat (AS), detergent solutions (with and without zeolites) and freshwater. Left: Successive exposure in AS (37 °C, 4 h exposure time), detergent solution without zeolites (37 °C, 4 h), and freshwater (25 °C, 18 h). Middle: Successive exposure in AS (4 h), detergent solution with zeolites (4 h), and freshwater (18 h). Right: Corresponding nonsequential exposures. Error bars show standard deviations of three separate exposures. All analyses were made using AAS of digested samples.

washing), and at 25 ± 0.5 °C for the freshwater. All exposures involved triplicate samples with a parallel blank sample (without any Ag NPs). The solution pH was measured before and after all exposures. All glassware and plastic vessels were acid cleaned with 10 vol % $HNO_3$ for at least 24 h and rinsed four times with Milli-Q water.

**Silver Release.** The total silver concentration in solution was determined using atomic absorption spectroscopy (AAS) in the graphite furnace mode (PerkinElmer AAnalyst 800). Calibration standards at 0, 7.5, 15, 30, and 45 µg Ag/L were prepared from a 1 g/L standard from PerkinElmer. The samples were first acidified to a pH < 2 with 65% $HNO_3$ (puriss p.a.), followed by digestion (2.5 mL sample, 1 mL 30 wt % $H_2O_2$ (puriss p.a.), 100 µL 30 wt % HCl (puriss p.a.), 6.4 mL Milli-Q water) via UV treatment (Metrohm 705 UV digester, 500 W, 95 °C for 1 h) to break up any possible strong silver complexes and ensure analysis of the total amount of silver in solution when measured by means of AAS. The detection limit was estimated to 5 µg/L in AS and freshwater, respectively, and to 10 µg/L in the detergent solution based on the t-value for a 99% confidence interval (3.49, n = 6) multiplied by the standard deviation of blank values.[32] Triplicate readings were analyzed for each sample and control samples of known silver concentration were analyzed in parallel, showing acceptable recoveries of added amounts (85−100%). All data is presented with the standard deviation of three independent samples and the blank value (matrix effect), if >0 µg/L, subtracted. All presented data significantly exceeded the blank values. The

blank values were on average 1.4 µg/L for AS, 3 µg/L for the laundry detergent solution, and 1.2 µg/L for freshwater.

The release rate of silver can be calculated with the following eq 1:

$$\text{release rate}\left(\frac{\mu g}{cm^2 h}\right) = \frac{c\left(\frac{\mu g}{L}\right) \times V(L)}{m(g) \times \text{BET}\left(\frac{cm^2}{g}\right) \times t(h)} \quad (1)$$

Here, BET is the specific surface area, *V* the volume, t the exposure time, *c* is the concentration, and *m* is the sample mass.

**Particle Size Distribution.** Photon cross-correlation spectroscopy (PCCS, NanoPhox, Sympatec, Germany) was employed to determine the particle size distribution in solution for the smaller (<100 nm) particles. Duplicate samples were measured three times, each at 25 °C. Data from the unique measurements was integrated to produce a single distribution with the PCCS software (Window 5.7, Sympatec). Standard Latex particles sized 20 ± 2 nm (Nanosphere, Thermo Scientific) and blank samples were tested prior to analysis to ensure the accuracy of the measurements (data not shown). Sonication with a Branson Sonifier 250 (30% duty cycle, output 5, for 5 min) followed by filtration (0.45 µm, cellulose acetate, Whatman) were performed prior to some of the PCCS measurements (SI Figure S1) to remove the largest particle agglomerates. This was necessary to ensure reproducibility and high enough scattered light intensity for detection of small particles (SI Table S2).[29,30] The proportion of the small

particles in the size distribution measurements may however be overestimated due to rotational diffusion that can occur for nonspherical particles.[33] This gives rise to a peak at small sizes (<10 nm).[33] The existence of Ag NPs sized less than 10 nm is however indisputable, as also proven in recent findings.[29] Detection limit of the PCCS technique, based on the scattered light intensity, was estimated to 30 ppb of Ag NPs. This was accomplished using known amounts of commercially available Ag NPs (RAS Chemicals GmbH, Germany) of 15 nm in size. The detection limit was calculated from the silver concentration (as Ag NPs) corresponding to the background intensity plus three standard deviations.

LALLS was performed using a Malvern Mastersizer 2000 equipment with a Hydro SM dispersion unit in order to study larger (>100 nm) particles and agglomerates in AS, laundry detergent solution (without zeolites), and freshwater solution. Duplicate samples were measured for each measurement.

**Surface Compounds.** Raman spectroscopy measurements were conducted using a Horiba HR800 instrument. A 514 nm laser was used together with an Olympus 50× objective. Wavenumbers in the range of 150−4000 cm$^{-1}$ were investigated. Measurements were performed on washed, air-dried, Ag NPs after exposure in the different solutions, as described above. Laser power was attenuated to avoid sample damage. Visual inspection (optical microscope) was made prior to and after each measurement to ensure no sample destruction.

**Equilibrium Calculations.** The Visual MINTEQ model, version 3.0, was used to estimate the solubility of silver in the different media. Due to the lack of some stability constants, it was not always possible to include all possible silver species in the calculations. For AS, only silver chloride species were considered. In the case of the detergent solution, no ionic species were introduced, and only the pH was set as an input value (pH 10). It has previously been noted that surfactants at similar concentrations as used in this study have not influenced the silver ion concentration in solution.[18] The freshwater solution was modeled for all different salts of the medium included. The Medusa software[34] was used to plot solubilities of different relevant compounds.

**Statistics.** To identify the significance of differences, a Student *t*-test of unpaired data (paired for sequencial immersions) with unequal variance was employed between two different data sets. Differences are counted as significant when $p < 0.05$, with higher significance for a smaller p-value.

## RESULTS AND DISCUSSION

**Sequential Exposures of Ag NPs in Aquatic Media of Different Chemistry Result in Lower Amounts of Released Silver in Solution Compared with Non-sequential Conditions.** The total amount (normalized to the amount of Ag NPs added, or surface area) of aqueous silver species after sequential exposures of Ag NPs in AS, the detergent solution, and freshwater, respectively, is presented in Figure 1. The normalization to surface area was based on the BET area, thus representing an estimation of the surface area, which might change due to dispersion and agglomeration throughout the immersions. Results from nonsequential exposures in the detergent solution, with and without zeolite, and in freshwater are included for comparison.

Both sequential experiments (with and without zeolites), starting with exposure in AS, resulted in significantly ($p < 0.05$) reduced amounts of silver in solution compared with the

nonsequential exposures, except in the case for the detergent solution containing zeolites. Even though differences in release patterns were observed, the total amount of released silver in solution was at low levels, <0.5% and 1% of the total amount of Ag NPs added for the sequential and nonsequential experiments, respectively. Significantly lower amounts of silver in solution were determined in both the detergent solutions and in freshwater when preceded by exposure in artificial sweat ($p < 0.05$). Liquid media in aqueous settings are hence necessary to take into account when assessing the fate of released silver from Ag NPs in, for example, consumer products. The difference between the three nonsequential (reference) exposures presented in Figure 1 were nonsignificant ($p < 0.05$), except when comparing detergent solutions with and without zeolites.

Observed differences between the sequential and non-sequential experiments cannot solely be explained by differences in pH before and after exposure (*c.f.* Figure S2 in SI). The pH after exposure was similar in the case of freshwater (pH 7 and 6.9), compared with the initial pH (7.2). It was also reduced after immersion into the sequential detergent solution (initial pH 10) without zeolites (pH 7.8) compared with the corresponding nonsequential exposure (pH 8.8) despite significantly higher levels of released silver for the non-sequential exposure. These findings are opposite of the expected effect of an increased pH. In the sequential exposure with detergent solution containing zeolites, the final pH of the freshwater was significantly higher (pH 7.9) compared with the nonsequential exposure and the initial solution pH (pH 7.2, $p < 0.05$). This is most probably attributed to cotransported laundry solution components. The increased pH will contribute to the lower release of silver in this medium, however the difference is larger than what is expected from a pH difference of 0.7 when comparing with the data of Liu et al.[14] The main difference is instead attributed to the initial release of easily dissolved surface silver compounds that only occurs in AS of the sequential immersion experiments. The initial release of easily soluble surface silver compounds will be discussed below (section "Weakly bound silver surface complexes desorb and dissolve in aqueous media.").

Cumulative released amounts of silver for the sequential and nonsequential exposures are presented in Figure 2. Significantly more silver in solution was determined in the nonsequential experiment in the laundry detergent solution without zeolites compared with zeolites ($p < 0.05$), and when compared with both sequential exposures ($p < 0.05$). Cumulative amounts of released silver in solution in relation to the total amount of added Ag NPs were 0.7% or lower, for all measurements (essentially the same particle loading, 0.1 g/L, in all experiments).

**Weakly Bound Silver Surface Complexes Desorb and Dissolve in Aqueous Media.** Raman results are presented in Figure 3 for washed Ag NPs before and after exposure into the different media. Since the acquirement of ex-situ spectra could possibly generate contamination peaks in the range between 1300 and 1600 cm$^{-1}$ and 2800−3000 cm$^{-1}$,[35] detailed assignments were only made in the low frequency region (<600 cm$^{-1}$) that includes information on compounds bound directly to silver. Full Raman spectra are presented in Figure S3 (SI) for all different media and combinations of sequential exposures.

As previously shown, peaks corresponding to silver carbonate (291, 704, 820, 1061, 1524 cm$^{-1}$), present at the surface of dry

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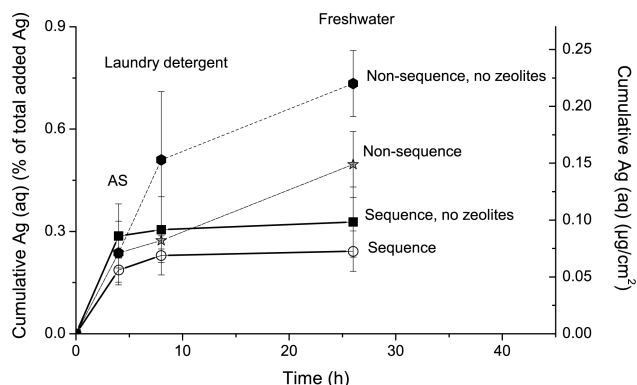dx.doi.org/10.1021/es500234y | Environ. Sci. Technol. 2014, 48, 7314−7322

**Figure 2.** Cumulative amount of aqueous silver measured in solution using AAS, released from Ag NPs exposed in artificial sweat (AS), detergent solutions (with and without zeolites), and freshwater, either sequentially or in each solution separately. Data is normalized to the amount of added Ag NPs (in %, left y-axis), or to their surface area (right y-axis). The average amount of aqueous silver of the two sequential exposures in AS was used to describe its nonsequential exposure. Error bars represent the standard deviation from three independent measurements.

Ag NPs, dissolved upon exposure to aqueous media.[30] This implies a very weakly bound surface compound.

None of the sequential and nonsequential exposures resulted in any significant changes in the low frequency region compared with parallel exposures in pure water with the peak at 242 cm$^{-1}$ assigned to Ag−O.[30] However, as spectra after exposure in AS and freshwater also were similar to AgCl (237 cm$^{-1}$),[36] precipitation of this compound cannot be ruled out. Exposures in the laundry detergent solution resulted in a slight shift of the Ag−O peak to 235 cm$^{-1}$, becoming broader at the low frequency side and with a possible shoulder at around 210 cm$^{-1}$. This shift cannot be attributed to the formation of bulk-like Ag$_2$O at the particle surface,[37] however it may originate from another form of silver oxide that readily can form at high pH values.

Low released amounts of silver in solution measured for the sequential exposures (Figures 1 and 2) also support the presence of a weakly bonded surface compound (silver carbonate), easily desorbed from the surface. Adsorbed silver species may desorb upon exposure into an aqueous medium and thus increase the silver content in solution.[38] As noted previously, this effect would only occur in the first step in the

sequential exposures, and thereby contributing to the lower amount of released silver, compared with the nonsequential experiment. Adsorbed silver ions at the Ag NP surface have been observed to hinder further oxidation.[13,14,39] Hence, if the Ag$^+$ at the surface are displaced or removed, for example by chloride ions, oxidation can be accelerated.[13,39]

Some similarities are evident when comparing generated data (Figure 2) for the sequential exposures with reported kinetics of silver release in a single medium, e.g. initial high release rates that with time level off to low quasi-equilibrium conditions.[9,10,15,16] However, the literature shows, that in many cases much longer exposure times than the here studied 26 h are needed to reach a quasi-equilibrium.[10] This makes it unlikely that it has been reached in these studies. Thus, longer exposures in each medium would, in the present study, be needed to verify whether quasi-equilibrium conditions have been reached or not.

**Solution Composition Influences the Silver Release and Presence of Silver Species in Solution.** The solubility of silver chloride complexes is essential to consider, when interpreting measured amounts of silver in chloride-containing media such as AS, due to the possible formation of soluble and insoluble chloride complexes in solution.[9,14,17] Exposures of Ag NPs were therefore also performed in NaCl (same concentration as in AS) and AS for periods up to 168 h exposure, Figure 4. The temperature was 30 °C (standard skin temperature[22]) for the data presented in Figure 4, instead of 37 °C (elevated skin temperature) for the measurements in Figure 1. However, only the relative comparison between NaCl and AS in Figure 4 is regarded, to identify trends in solubility (which also should be valid at 37 °C).

At 168 h, measured amounts of silver in solution were similar for AS and NaCl (no significant difference, $p > 0.05$). In NaCl, they were similar after 2 h and after 168 h of exposure. Thus, this indicates that the amount of chlorides determined the measured amounts of silver in solution. It can hence be concluded that NaCl governs the solubility (aqueous amount) of silver in the case of AS, most likely forming insoluble AgCl precipitates for exposures longer than approximately 10 h as the silver concentration in solution does not further increase beyond this point. NaCl can actually enhance the release of Ag NPs by the action of the chloride ion at the particle surface.[13,17,39] Lack of increase in soluble silver amount is supported by simplified equilibrium calculations using Visual MINTEQ, as the silver concentration, when stabilized, is
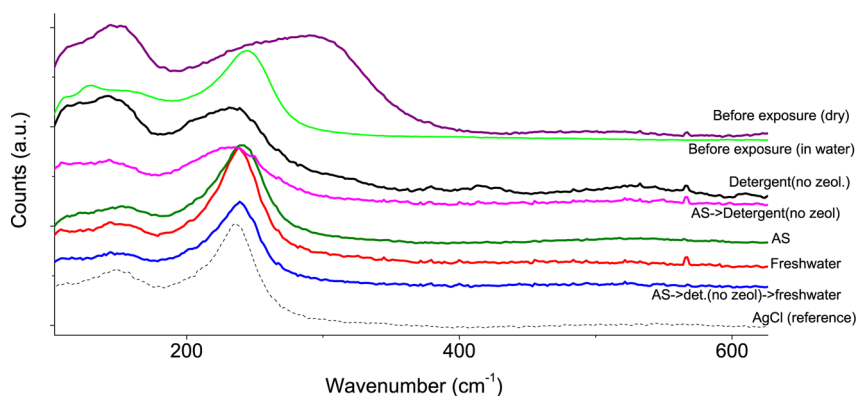


**Figure 3.** Raman spectra of Ag NPs after sequential and nonsequential exposures in artificial sweat (AS), detergent solution (with and without zeolites) and freshwater. Spectra were collected ex-situ on washed and dried particles unless noted. "Det." denotes detergent solution.
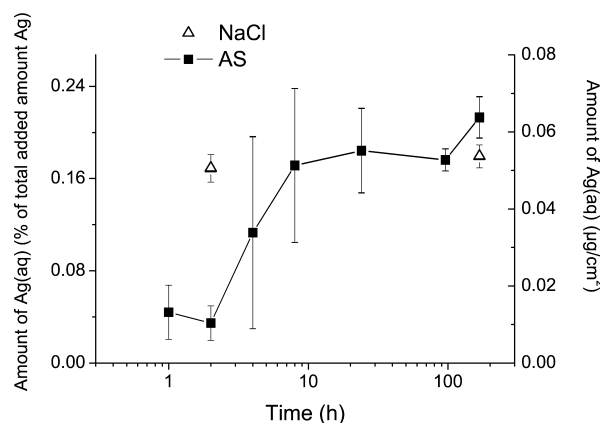
**Figure 4.** Amount of silver in solution (%, left; per surface area, right) after exposure of Ag NPs in artificial sweat (AS) and NaCl (5 g/L) up to 168 h, respectively. Measurements were performed after 2 and 168 h in NaCl, and after 1, 2, 4, 8, 24, 96, and 168 h in AS. Error bars represent the standard deviation of three individual samples. The lines are only guide for the eyes.

relatively close to the theoretical solubility limit (one-third), see Table 2. The estimated soluble amount corresponds to the case when added silver has been completely dissolved.

**Table 2. Comparison between Measured Silver Concentrations and Estimated Solubilities in Artificial Sweat (AS), Detergent Solution (Without Zeolites) And Freshwater**

|  | measured soluble silver species ($\mu$g/L) | estimated theoretical solubility ($\mu$g/L) | soluble silver compared to estimated solubility limit (%) |
|---|---|---|---|
| AS | 260 ± 98 | 780 | 33% |
| detergent, no zeolites (nonsequential) | 300 ± 98 | 21 400 | 1.4% |
| detergent, no zeolites (sequence) | 20 ± 2 | 21 400 | 0.1% |
| freshwater (nonsequential) | 240 ± 139 | 49 600 | 0.5% |
| freshwater (sequence, no zeolites) | 24 ± 5 | 49 600 | 0.05% |

Quadros et al. investigated the release of silver from fabric containing Ag NPs in AS. Their findings showed that the absence of either urea or lactic acid did not influence the release of silver, whereas their complete removal reduced the total amount of released silver. This suggests their combined effect on the release process.[19]

The estimated solubilities were significantly higher than observed concentrations of silver in solution for both freshwater and detergent (Table 2). This is most likely explained by the fact that only a small fraction of the Ag NPs dissolved. The possible transfer of small amounts of solution species to the next step of the sequence (approximately 0.1%, see Materials and Methods) did not affect the solubility of the new medium. Chlorides also play an important role for the release of silver from Ag NPs in the freshwater solution.[11−13,17] Estimated fractions of Ag$^+$ and AgCl(s) show that there is a possibility of formation of AgCl(s) in freshwater for observed concentrations of silver in solution (Figure S4, SI).

Zeolites in laundry detergents are known to form complexes with silver ions.[40] This explains the significantly lower presence of aqueous silver in the detergent solution with, than without, zeolites ($p < 0.05$), c.f. Figure 1. Previous findings show that the LAS surfactant of the model laundry detergent solution interacts with the silver surface and shifts the zeta potential to further negative values, hence stabilizing the particles in the solution.[29,30] Adsorbed surfactants are expected to desorb upon contact with the freshwater solution.[30]

**Rapid Agglomeration and Sedimentation of Ag NPs.** Particle stability measurements by means of PCCS revealed very rapid agglomeration and sedimentation of the Ag NPs in AS, as was evident from a low intensity of scattered light directly upon measurements (SI Table S2). This intensity was insufficient to extract any size distribution information, indicating a small fraction of particles present in solution. This trend with rapid sedimentation of larger agglomerates, leaving smaller particles in solution was confirmed with LALLS, Figure 5, top, also supported by decreased light intensity in LALLS (not shown).

Rapid particle agglomeration in AS is expected, since the electrostatically stabilized Ag NPs[30] will agglomerate to a high extent in a high ionic strength media such as AS.[41] Furthermore, agglomerated particles will have a smaller specific surface area compared with well-dispersed particles. This will limit the oxygen transport, thus slowing down dissolution kinetics.[16,42] Kent et al.[13] observed similar release rates of silver as herein (approximately 0.02 $\mu$g/h·cm$^2$), despite a higher pH (7) and lower temperature (25 °C), compared with this study (pH 6.5 and 37 °C). A lower pH and higher temperature in general increase the release of silver.[14,42] Agglomeration was however not a factor in Kent's experiments, using uncoated Ag NPs attached to a substrate.[13] This increases the surface area relative to this study and hence release rate. This is also seen to influence the similar release rates of silver ($\mu$mol·L$^{-1}$·h$^{-1}$) in the work of Zhang et al., despite a twice as high loading (mass silver/volume) in this study.[16] In addition, silver release findings of this study (~0.2% of added silver in detergent solution without zeolites) are comparable with observations of Geranio et al.,[18] who performed silver dissolution tests on uncoated Ag NPs at pH 10 in model laundry detergents with similar particle loadings (added silver amount/volume) as in this study. More well-dispersed carbonate coated Ag NPs rendered on the other hand approximately a ten times higher silver fraction in solution,[18] which emphasizes the importance of agglomeration on the release process.

As evident from a low intensity of scattered light of the Ag NPs in detergent solution and freshwater (SI Table S3), the Ag NPs were not redispersed in the sequential exposures (detection limit of approximately 30 ppb Ag NPs). Since all solutions were poorly dispersed into solution due to rapid agglomeration and sedimentation, observed differences in amounts of dissolved silver in the nonsequential and sequential exposures could not be explained by differences in surface area. Agglomeration of Ag NPs is a realistic scenario for particles released from clothes, since a significant fraction of the detected released Ag NPs tend to be larger than 450 nm when released from e.g. textiles.[18] However, this size of the agglomerates does not necessarily result in sedimentation since that process also depends on other parameters such as ionic strength. In real consumer products, the method of incorporation of Ag NPs, and material properties, will influence the release of the silver species. These factors are not taken into account in this study,
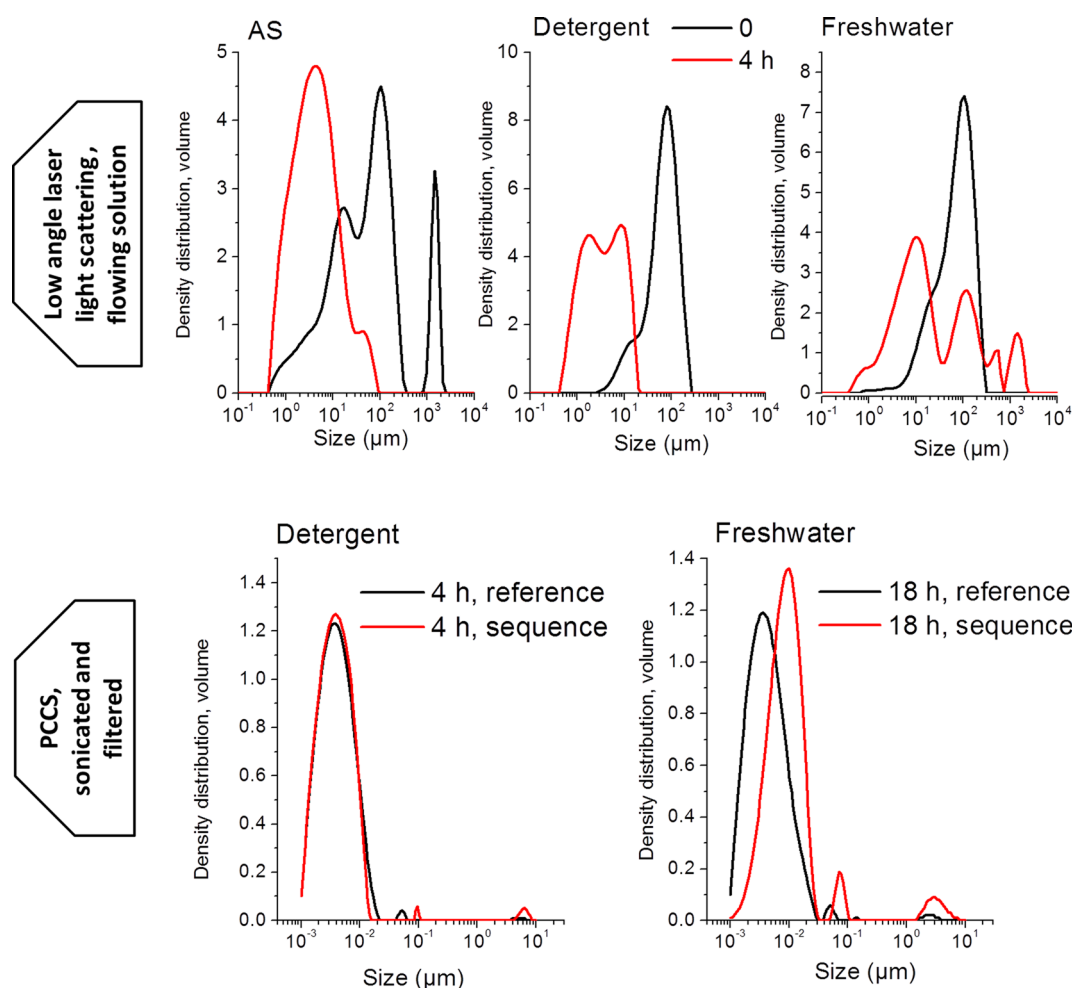
**Figure 5.** Top: Particle size distribution measurements of nonfiltered, nonsonicated, Ag NPs in artificial sweat (AS), detergent solution without zeolites, and freshwater, respectively, by means of LALLS after exposure for 0 and 4 h. Bottom: Particle size distribution measurements (based on volume distribution) of sonicated and filtered Ag NPs prior to nonsequential (reference) and sequential exposures in detergent solutions without zeolites (4 h) followed by freshwater (18 h), by means of photon cross correlation spectroscopy.

as it only considers a general exposure scenario where uncoated Ag NPs are interacting with aquatic media at a higher loading than what is expected in a real case.

Sonication (c.f. SI Figure S2 for experimental scheme) of particle suspensions generated dispersed Ag NPs in freshwater and laundry detergent without zeolites, evident from an increased scattered light intensity and also shown in previous investigations.[30] Particles sized between 1 and 10 nm, as well as agglomerates sized between 100 and 10 000 nm were present in solution (Figure 5, bottom). These sonicated Ag NPs were more prone to agglomerate in freshwater after sequential exposure in AS and detergent solution compared with the nonsequential exposures, Figure 5. This discrepancy in stability could be explained by an increased ionic strength of the freshwater (not measured) of the sequential exposures, originating from adsorbed and desorbed components from preceding solutions (AS, detergent solution). Generated findings show that the particle history and characteristics, as well as exposure conditions such as agitation, sonication, etc. are very important both for the release and particle stability, illustrated in, for example, Figures 1 and 5.

**The Assessment of Release and Transport of Silver Species and Ag NPs from Source to Recipient Should Involve Sequential Exposure Scenarios.** Sequential in vitro

studies have been performed on uncoated Ag NPs in different aquatic media (from artificial sweat to laundry detergent solutions to freshwater) aiming to mimic a potential exposure scenario for Ag NPs and silver released from impregnated clothing that are transported via different media in aqueous settings. Sequential exposures in these media resulted in different amounts of soluble silver compared with analogous nonsequential experiments, Figures 1 and 2. High release rates of silver were observed during the first few hours of the sequential exposures, followed by subsequent reduced rates. Release rates from parallel nonsequential measurements did not decrease in the same manner when added up. In this work, we emphasize the importance of dissolution of weakly bound soluble silver complexes present at the surface of the particles at dry conditions.[38,39] This effect will only be of importance during the first step in the sequential exposures, and result in lower cumulative amounts of released silver compared with the sum of nonsequential exposures.

Other factors influencing a lowering of silver release include reduction in surface area, and thereby a slower oxygen transport and hindering of the silver oxidation with adsorbed species and/or ions, or strongly adsorbed ions or polymers that reduce dissolution of Ag NPs (at least during relatively short time periods), possibly by blocking sites for oxidation.[10,11,42,43] In

general, it is conceivable that, when Ag NPs are transported to a different medium, an established quasi-equilibrium is disrupted due to redispersion of the NPs, or desorption of blocking molecules. However, this is not conclusively seen in this work, indicated by Raman (Figure 3), where no strongly adsorbed species were observed in the spectra, and PCCS results (SI Table S3), that showed that the particles remained sedimented.

For an even more realistic exposure scenario, the concentration of Ag NPs should be significantly lower than investigated in this study, and dilution effects that take place upon environmental entry should be considered. Recent estimates show Ag NP concentrations between 0.06 and 1.5 $\mu$g/L in influent water to wastewater treatment plants (WWTP).[44] Such low concentrations would, according to Visual MINTEQ predictions, at such conditions not result in any precipitation of AgCl either in AS, detergent solution, or freshwater solution. Precipitation of silver sulfide and interactions with organic ligands such as cysteine are expected to play more important roles for silver speciation under given conditions in a WWTP.[25,45]

Generated findings show that changes in particle characteristics in different transients influence the release behavior, essential aspects to consider for risk assessment and design of relevant case studies, not only for Ag NPs, but for NPs in general. To accurately quantify and monitor changes in released silver from Ag NPs in e.g. consumer products (both as ionic species in solution and as precipitated complexes) upon sequential environmental exposure, it is important to identify potential environmental hazards. This is valuable information for mitigating risks of Ag NP dispersion, given the case that its environmental release and accumulation would increase to potentially harmful levels. Further sequential studies are needed on, for example, changes in silver speciation in solution and particle surface characteristics for different types of Ag NPs in relevant aquatic media at relevant concentrations to mimic different exposure scenarios, and be able to scientifically assess the environmental fate of Ag NPs.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information
Additional LALLS data, Raman spectra, PCCS data, pH of solutions before and after exposure, and equilibrium calculations are availible. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes
The authors declare no competing financial interest.

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