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Fate and Behaviour of Silver Nanoparticles in Urban Wastewater Systems
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Abstract:	The increased use of metallic silver nanoparticles (Ag-NP) in many consumer products will eventually lead to the emission of such materials to the wastewater stream. As the toxicity of Ag strongly depends on its speciation, the physical-chemical transformation during their transport in the sewer and during the wastewater treatment needs to be evaluated in detail. Applied studies performed on full- and pilot- scale as well as fundamental investigation on the lab scale suggest that Ag-NP are readily transformed into Ag2S during anaerobic stages of the wastewater treatment. A surface coating, e.g. an organic coating, does not protect Ag-NP from corrosion (transformation into Ag2S or AgCl). First indication on the behavior of Ag in sewer system also indicates a rather fast conversion into Ag2S. The sewer channel and the WWTP act as a kind of a multi-barrier system and it seems rather unlikely that Ag-NP reach the environment in their pristine form via this pathway. If a surface coating can retard the corroding process significantly and also reduce the entrapment of AgNP into sewage sludge remains an open question. Under such conditions, however, a higher transmission of Ag-NP through the sewer system and the WWTP is conceivable.

FATE AND BEHAVIOUR OF SILVER NANOPARTICLES IN URBAN WASTEWATER SYSTEMS

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Abstract
The increased use of metallic silver nanoparticles (Ag-NP) in many consumer products will eventually lead to the emission of such materials to the wastewater stream. As the toxicity of Ag strongly depends on its speciation, the physical-chemical transformation during their transport in the sewer and during the wastewater treatment needs to be evaluated in detail. Applied studies performed on full- and pilot- scale as well as fundamental investigation on the lab scale suggest that Ag-NP are readily transformed into Ag_2S during anaerobic stages of the wastewater treatment. A surface coating, e.g. an organic coating, does not protect Ag-NP from corrosion (transformation into Ag_2S or AgCl). First indication on the behavior of Ag in sewer system also indicates a rather fast conversion into Ag_2S. The sewer channel and the WWTP act as a kind of a multi-barrier system and it seems rather unlikely that Ag-NP reach the environment in their pristine form via this pathway. If a surface coating can retard the corroding process significantly and also reduce the entrapment of AgNP into sewage sludge remains an open question. Under such conditions, however, a higher transmission of Ag-NP through the sewer system and the WWTP is conceivable.

Keywords
Silver nanoparticles, wastewater, sewer, speciation, fate

INTRODUCTION

Manipulation and shaping materials at the nanoscale allows for control of their physic-chemical properties which offers tremendous amounts of new applications in various fields. Several recent reviews and books well document this (e.g. Savage et al. (2009)). However, with all the potential benefits of these novel materials there also come unknown risks related to the potential release of such materials from already available consumer products to the aquatic environment.

Silver has been used for water disinfection and as biocide in wound dressings by the Greeks and the Romans (Silver, Phung, et al., 2006). More recently, the use of metallic Silver nanoparticles as biocide has received great attention due to its application in a variety of consumer products (http://www.nanotechproject.org/inventories/consumer/analysis_draft/). In the following we use the abbreviation 'Ag-NP' specifically for engineered, metallic silver nanoparticles and specify when referring to other forms of nanoscale silver. Recent reports, however, demonstrate that the release of Ag-NP into the aquatic environment cannot be avoided (Benn and Westerhoff, 2008; Kaegi, Sinnet, et al., 2010).

The toxicity of silver, generally attributed to the presence of silver ions (Ag+), and its compounds have been thoroughly reviewed (Ratte, 1999). Several recent studies report an additional particle-related bactericidal effect (Martinez-Castanon, Nino-Martinez, et al., 2008; Morones, Elechiguerra, et al., 2005; Pal, Tak, et al., 2007; Panáček, Kvítek, et al., 2006; Sondi and Salopek-Sondi, 2004). Choi and Hu (2008) suggested that the Ag-NP inhibited nitrification in activated sludge more strongly than the equivalent amounts of Ag in the form of Ag^+ indicating potential adverse effects on the performance of a wastewater treatment plant

(WWTP). Similar results were obtained in growth experiments with nitrifying bacteria (Choi, Deng, et al., 2008).

The fate of Ag-NP from the point of discharge into the wastewater to their release into surface waters critically depends on their physical-chemical transformation and retention during wastewater treatment as well as during their transport in the sewer system. Furthermore, the speciation of the Ag strongly influences its toxicity (Ratte 1999), calling for a thorough evaluation of the mobility and chemical transformation of Ag in the urban water cycle to address environmental risks from the use of Ag-NP.

Recently a few studies have been published addressing the behavior of Ag-NP in WWTPs. However, to date there are no experimental data on the behavior of Ag-NP in the sewer system. Thus, we will first discuss the current literature on Ag-NP transformation and retention in WWTPs and then present results on the behavior of Ag in a sewer system.

SILVER NANOPARTICLE BEHAVIOR IN THE WWTP

Mass balances established for Ag indicated a removal efficiency of $> 95\%$ in WWTPs (Shafer, Overdier, et al., 1998). Lower removal efficiencies were attributed to differences in the speciation of Ag in the influent or lower analytical accuracies at low Ag concentration levels (Lytle, 1984). However, these earlier studies addressed the fate of total Ag – mostly high Ag emissions from the photographic industry – and the results might not be directly transferrable to engineered nanoparticulate forms of Ag. Limbach, Breitner et al. (2008) concluded that up to 5 wt% of CeO_2 nanoparticles may pass through WWTPs as individual NPs. Based on these results, WWTPs have been identified as major hubs controlling the release of Ag-NP into surface waters (Gottschalk, Sonderer, et al., 2009; Gottschalk, Ort, et al., 2011). Kim, Park, et al. (2010) discovered exclusively Ag sulfide (Ag_2S) in the sludge of a full-scale WWTP, and speculated that Ag-NP may be transformed into Ag_2S during the wastewater treatment processes. Using a combination of bulk elemental analysis (inductively coupled plasma - mass spectrometry), transmission electron microscopy (TEM) and synchrotron based X-ray absorption spectroscopy (XAS) to monitor Ag-NP retention and transformation in a pilot WWTP, Kaegi, Voegelin et al. (2011) demonstrated i) that on a mass bases $> 95\%$ of the Ag is partitioned into the sewage sludge and dominantly present as Ag2S, ii) that the sulfidation of Ag-NP occurs during the anaerobic stages of the wastewater treatment process and iii) that the nanoscale Ag-particles (mostly Ag_2S) leaving the WWTP were attached to the wastewater biomass rather than being present as individual NPs. These findings have severe consequences regarding the risk associated with the release of Ag-NP; firstly as the toxicity of Ag_2S is orders of magnitude lower than Ag+ (Ratte, 1999) and secondly because the transport behavior of Ag-NP attached or incorporated into wastewater flocs is fundamentally different to the transport behavior of individual nanoscale particles. (Levard, Reinsch, et al., 2011) studied the sulfidation of PVP-coated Ag-NP in detail and revealed that - at least under laboratory conditions - the coating did not protect the Ag-NP against sulfidation. Also the study of (Ma, Levard, et al., 2011) suggested that the dissolution Ag-NP is controlled by their size but does not depend on the type of surface coating. (Kittler, Greulich, et al., 2010) on the other hand suggested that the rate and degree of the dissolution of Ag-NP depend upon their surface coating, their concentration, and the temperature. (Levard, Michel, et al., 2011) conclude that although the presence of organic coatings (PVP or PAA) does not prevent surface corrosion of Ag-NP, it may slow down the kinetics of the corrosion process by the formation of a passivating surface layer. These studies indicate that an organic surface coating does not protect the particles from the environment, but might decrease reaction rates between the particle and the surroundings. Based on experimental results of the sorption of NP to wastewater biomass, (Kiser, Ryu, et al., 2010) suggested that coated NPs are less effectively removed by biomass than uncoated NPs indicating that the surface coating significantly affects the fate of NP during the wastewater treatment process.

Ag-NP are readily removed from the wastewater stream and transformed into much less toxic Ag_2S in a WWTP. However, kinetic constraints slowing down the transformation / sulfidation during wastewater treatment combined with a reduced attachment efficiency might eventually result in a higher fraction of specifically stabilized Ag-NP in the wastewater effluent.

EXPERIMENTS IN A SEWER SYSTEM

Most of the available work on the fate and transport of Ag-NP are focused on the WWTP. However, before reaching the WWTP, the Ag-NP have to be transported in the sewer system, where high sulfide levels are frequently encountered. Own measurements revealed sulfide concentrations (AVS, acid volatile sulfide) in the order of 0.15 mM in the local sewer system. Sulfide is a well-known problem in sewer systems since decades due to corrosion and malodors (Ganigue, Gutierrez, et al., 2011). In a full scale study performed at an industrial laundry to evaluate the effects of silver spiked as particulate AgCl, we collected and characterized in detail the starting materials as well as the transformation products of the AgCl particles along the sewer channel. The layout of the study is schematically given in Figure 1.
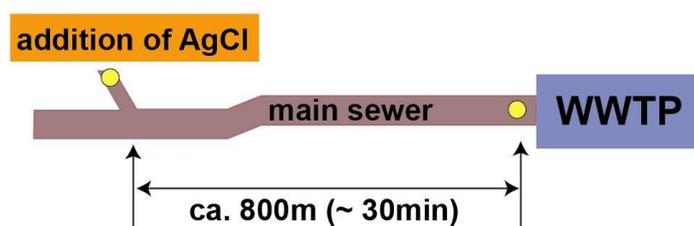

Figure 1: Schematic layout of the sewer study. The sampling points are indicated by yellow dots.

Materials and Methods

Electron Microscopy

Aliquots of 1-2mL taken from a larger sample volume were centrifuged directly onto TEM grids (Formvar C coated, 200 mesh, SPI). The applied centrifugation conditions (2h, ~ 3'000g) was sufficient to quantitatively remove nanoscale Ag-colloids (Ag(0), AgCl, Ag_2S) larger than 10 -15nm depending on the density of the Ag-species. The starting suspension was also centrifuged onto TEM grids using the same conditions. The TEM grids were either investigated on a TEM (Tecnai, F30ST, FEI) or an scanning electron microscope (SEM). The TEM was operated at an acceleration voltage of 300 kV. The microscope was operated in the scanning mode and the Ag-NP were localized using a HAADF (High-Angle Annular Dark Field) detector and analyzed with an EDX (Energy Dispersive X-Ray) system (EDAX). X-ray absorption Spectroscopy. Alternatively, TEM grids were investigated in a scanning electron microscope (S-4800, Hitachi, Japan), equipped with a secondary electron detector, a transmission electron detector and an EDX system for elemental analyses (INCA, Oxford Instruments, Oxford, UK). The SEM was operated at 25kV.

X-ray absorption spectroscopy

Solids contained in 200 - 400 mL of the laundry effluent and of the WWTP influent were separated from the liquid by centrifugation (10 min, 3000 x g), frozen and freeze dried. The dried samples from the laundry effluent and the WWTP influent were prepared in powder sample holders for XAS analysis. Reference materials included a metallic Ag foil as well as Ag_2S, AgCl, Ag-lactate, thiol-complexed Ag^+ (adsorbed to Ambersep™ thiol-resin). All reference materials were pressed into 13-mm pellets for XAS analysis. XAS analyses at the Ag K-edge were performed on the Dutch Belgian Beamline (DUBBLE) at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The energy of Si(111) double crystal monochromator was calibrated using an Ag metal foil by setting the first maximum of

the first derivative of the absorption edge to 25'518 eV. All spectra were measured at room temperature, sample spectra in fluorescence mode using a 5-element Ge solid-state detector, and reference spectra in transmission mode using Ar-filled ion chambers. For the extraction of the X-ray absorption near-edge structure (XANES) and the extended X-ray absorption fine structure (EXAFS) spectra and their analysis by linear combination fitting (LCF), we used the software code Athena. Linear combination fitting (LCF) analysis of XANES spectra was performed from -20 to +100 eV around E_0 and LCF analysis of the EXFAS spectra over the k-range of 3 to 8 Å^{-1} using the reference spectra of metallic Ag, AgCl, Ag$_2$S and thiol-Ag while constraining individual fractions to range between 0 and 100%.

Characterization of the starting Materials

The starting suspension was characterized using SEM (Hitachi S-4800). For image formation a transmission electron detector mounted underneath the sample was used. The starting suspension clearly consists of AgCl particles, which show a bi-modal size distribution. The smaller, - more abundant, - mode consists of nanoscale AgCl particles (<100nm) and the larger particles are a few 100 nm in diameter. It is worth noting that the AgCl particles are very sensitive to the electron beam irradiation leading to the growth of metallic particles at the expense of the AgCl particles within a few seconds. This artifact has to be kept in mind when assessing the transformation of the Ag in the sewer system based on electron microscopy analysis.

Results

Samples were collected at the point where the nanoscale AgCl containing suspension was discharged into the main sewer system. The use of the HAADF detector greatly facilitated the localization of individual nanoscale Ag particles present in the effluent (Figure 3) which appeared as bright spots due to the large density contrast between the nanoscale Ag particles and the dominantly organic matrix. Also in this sample particles in two distinct size ranges can be observed. The larger ones are larger than 100nm in diameter and the smaller ones are less than about 50 nm in diameter. The larger particles readily started to transform into metallic nanoscale Ag particles (bright dots at the rim of the larger particles in Figure 3). This electron beam induced transformation is even more severe than observed in the starting suspension, probably due to the higher energy of the electron in the TEM (300 keV) compared to the SEM (25 keV). However, besides the AgCl particles, also nanoscale Ag particles associated with significant amounts of S were observed in the TEM. These particles are in a comparable size range to the above described AgCl particles but are considerably more stable under the electron beam.
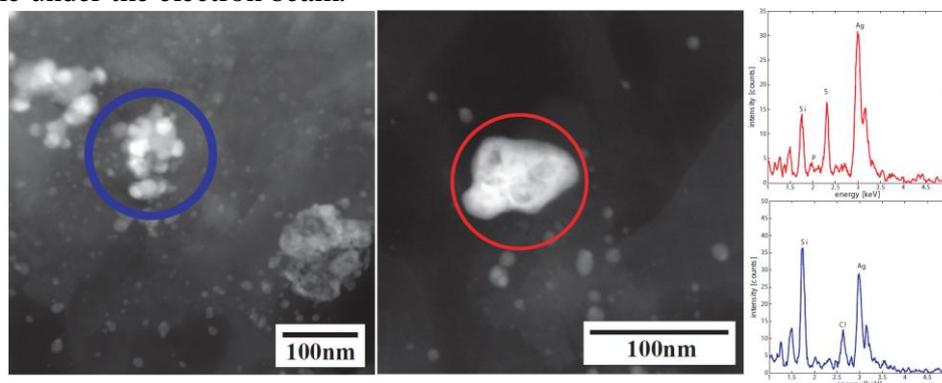

Figure 2: Nanoscale AgCl particles detected in the samples of the discharges to the main sewer channel. Left: AgCl particles already partly transformed to Ag(0).Right: nanoscale Ag particles associated with considerable amounts of S.

In the samples collected at the influent to the WWTP also bright, nanoscale particles were detected under the TEM and the elemental analyses of the respective particles confirmed the

presence of Ag, but also revealed significant amounts of S (Figure 3). This can be taken as a first indication that the nanoscale AgCl particles already transformed to Ag_2S during the transport in the sewer system.
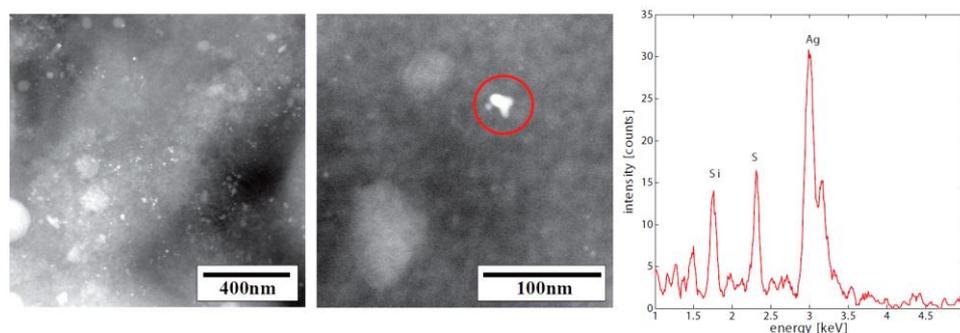

Figure 3: Nanoscale 'Ag₂S' particles detected in the samples of the influent to the WWTP. Left: The bright dots represent Ag bearing particles. Middle: High magnification of a bright particle seen on the left. Right: EDX spectrum of the bright particle shown in the middle.

In order to identify the different Ag-bearing phases present in the samples and to quantify their proportions, XAS analyses were carried out. The spectra (XANES and EXAFS, Figure 4) of Ag_2S and the thiol-Ag are very similar which does not allow us to distinguish between the two Ag species. We thus, treat them together referring to them as S-bound Ag (S-Ag). However, the spectra of Ag(0) and AgCl are very characteristic and fundamentally different from the spectra of Ag_2S and thiol-Ag. Comparing the spectra of the two samples, - point of discharge and influent to the WWTP, - reveals the strong similarity between these two spectra and the spectra of Ag_2S and thiol-Ag, suggesting that Ag in these samples was dominantly coordinated with S. This finding was confirmed by linear combination fitting (LCF, Table 1). Interestingly, LCF showed that already at the point of discharge, Ag was dominantly S-coordinated, and only one third was present as AgCl. This remaining AgCl fraction completely transformed to Ag_2S during Ag transport in the main sewer, which takes about 30 min. It is worth noting that the LCF results indicate a small but significant fraction of about 10% of metallic Ag in the influent of the WWTP, which was not present at the point of discharge. Beam damage, as observed in the electron microscope, could explain the detection of Ag(0) by XAS. However, considering that Ag(0) was neither detected in the AgCl standard, nor in the samples at the point of discharge makes this hypothesis rather unlikely, but cannot completely be ruled out due to the different matrices of the samples which might facilitate or prevent in situ reduction of Ag^+. An alternative explanation is that the Ag^+ was reduced by organic matter or bacteria present in the raw wastewater. In that case, it would be almost impossible to distinguish between engineered Ag-NP that might survive the transport in the sewer system and potentially enter the wastewater treatment, and metallic particles formed by an in-situ reduction of Ag^+. In fact, it is well known that microorganisms can synthesize metallic Ag nanoparticles, spherical in shape and with a rather narrow size distribution (Suresh, Pelletier, et al., 2010) and refs. therein)
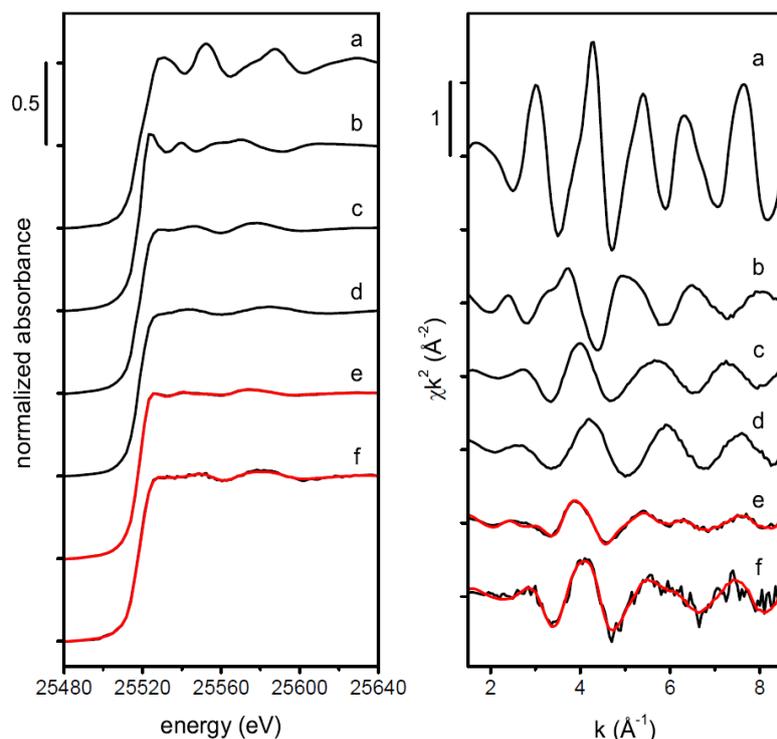
Figure 4. *XANES and EXAFS spectra of reference materials and samples (black lines) and LCF spectra (red lines). (a) Ag(0), (b) AgCl, (c) Ag$_2$S, (d) thiol-Ag, (e) laundry effluent, (f) WWTP influent*

Table 1. *Results from LCF analysis XANES and EXAFS sample spectra. Reference spectra used for LCF and LCF spectra are shown in Figure 4 together with sample spectra.*

	Ag metal (%)	AgCl (%)	Ag$_2$S (%)	thiol-Ag (%)	[a]sum (%)	[b]NSSR
XANES						
Laundry effluent	2%	35%	32%	31%	100%	0.000019
WWTP influent	10%	6%	63%	20%	100%	0.000050
EXAFS						
Laundry effluent	5%	34%	41%	20%	109%	0.033536
WWTP influent	11%	0%	77%	12%	113%	0.101333

[a]*Individual fractions constrained to 0-100%, but sum not constrained. Reported fractions for individual species were recalculated to sum to 100%.*
[b]*NSSR=normalized sum of squared residuals=$\sum(data_i-fit_i)^2/\sum data_i^2$.*

CONCLUSION

Ag-NP are retained to a very high degree (>95%) in WWTPs. This high removal efficiency is similar to the results from earlier studies which were based on mass balance calculations for total silver (Lytle, 1984). Furthermore, Ag-NP are mostly transformed into sparingly soluble Ag$_2$S during wastewater treatment. It has been shown that the Ag-NP are attached to the wastewater flocs also in the effluent of the WWTP. Studies on the corrosion, including transformations into Ag$_2$S and AgCl, of Ag-NP with different coatings revealed that the transformation processes cannot be prevented by an organic coating such as PVP or gum Arabic. However, it has also been reported that surface coatings negatively affect biosorption (Kiser, Ryu, et al., 2010), which might increase the fraction of Ag-NP in the effluent.

Much less is currently known about the behavior of Ag-NP in the sewer system. Based on the data gathered in a full scale experiment using nanoscale AgCl particles it is possible that also AgNP are already transformed during their transport in the sewer system. In addition to the surface functionalization which might affect the transport behavior in the sewer system also the presence of a biofilm in the sewer channel has to be taken into account. Ag-NP might be trapped in the biofilm and released later due to biofilm erosion during storm water events.

However, given the anaerobic conditions (Lens, De Poorter, et al., 1995) at the bottom of the sewer biofilm leading to the production and release of H_2S into the water column, it seems likely that AgNP deposited in the biofilm would eventually be transformed into Ag_2S.

Combining all these information it seems very unlikely that Ag-NP will enter the surface waters freely dispersed in metallic form. Ag-NP will rather be incorporated into the wastewater biomass, transformed into nanoscale Ag_2S particles and end up to a larger extent in the sewage sludge. The transformation of into Ag_2S leads to a strong reduction of the toxicity of Ag mainly caused by the extreme low solubility of Ag_2S. However, although it has been shown that the corrosion processes are independent of the surface coating of the Ag-NP, the kinetics of the transformation has not been addressed yet.

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