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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title: Behavior of silver nanoparticles in wastewater treatment plants**	**Experiment number**: 01-01-859
Beamline: BM01B	**Date of experiment**: from: 24.11.2011 to: 29.11.2011	**Date of report**:
Shifts: 15	**Local contact(s)**: Sergey Nikitenko, Miguel Silveira	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Ralf Kaegi

Dr. Andreas Voegelin

Ms. Maline Elumelu

Report:

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 the transport of the AgNP in the sewer and during the wastewater treatment needs to be evaluated in detail (Kaegi et al. 2012). Applied studies performed on full- and pilot- scale wastewater treatment plants (WWTP) (Kim et al., 2010, Kaegi et al. 2011) as well as fundamental investigation on the lab scale (Levard et al. 2011) suggest that Ag-NP are readily transformed into Ag_2S for example 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) but could influence the kinetics of the transformation reactions. In our previous report (01-01-813) we have shown the feasibility to determine the speciation of Ag present in low concentrations (ppm) and in complex environmental matrices, such as sewage sludge. However, rather fast transformation kinetics could lead to artifacts regarding the speciation of Ag caused by the sample preparation. Therefore, we used different sample preparation techniques to assess the effect of the sample treatment on the speciation of the Ag-NP. In parallel we also performed electron microscopic analysis (scanning- and transmission electron microscopy in combination with energy dispersive x-ray analysis) to further contain our findings.

Experiments were performed with Ag-NP (10 and 100nm diameter) stabilized with citrate either under non-aerated (sealed reaction vessels with no headspace) or aerated (constant bubbling of air in open reaction vessels) conditions. The reaction time was 20 minutes.

The concentration of Ag in the spiked sewage sludge was only about 500 µg/l (ppb), and therefore Ag had to be concentrated in the samples to be analyzed at the synchrotron using XANES. Based on our previous studies we knew that the AgNP were dominantly attached to the sewage flocs and therefore, the AgNP were concentrated by centrifugation (10min at 3000 x g). The sediment was immediately frozen in liquid nitrogen. Samples were freeze dried and pressed into 13mm pellets in a glove box under anoxic conditions. Alternatively, selected samples were kept frozen and analyzed in the frozen state. An additional experiment was conducted by spiking AgNP into frozen sludge followed by a short period of mixing in a ball mill using liquid nitrogen cooled buckets, followed by freeze drying.

Samples for electron microscopy were prepared by either filtering 1mL of sludge using 0.05µm membrane filters or by putting a drop of sludge (100 µl) on a lacy TEM grid. To be comparable with the synchrotron preparation techniques, additional frozen samples were embedded in epon resin in the frozen state and 200nm thick TEM sections were cut with a diamond knife.

The Ag K-edge XANES spectra of these samples were recorded at 80K using a He-cooled cryostat in fluorescence mode at the DUBBLE beamline. For the interpretation of experimental spectra by linear combination fitting, a series of reference materials were analyzed (in transmission mode), amongst others metallic Ag, Ag-NP, AgCl, Ag_2S, Ag_2O, thiol-bound Ag+, Ag-lactate, and aqueous Ag+. LCF fits revealed Ag_2S and metallic Ag as the most relevant spectra. In Figure 1 these two spectra together with selected experimental spectra are given. Results from the LCF are reported in Table 1.

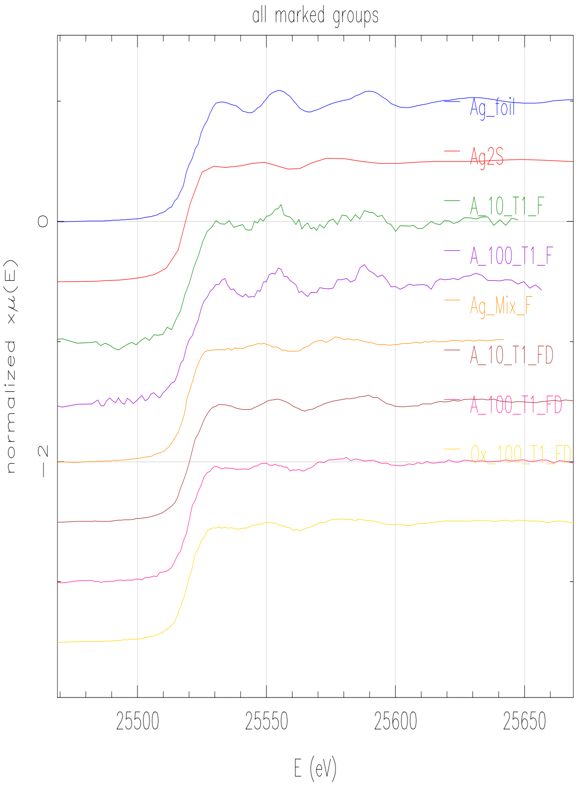

Figure 1: Experimental XANES spectra of references (Ag_2S, and Ag(0) and the experimental spectra

***Table 1**. Linear combination fit results for experimental XANES spectra using Ag_2S and Ag(O) as reference spectra. No constraints were put on the fits.*

samples	metallic Ag	Ag2S	sum
A_10_T1_F	81	20	101
A_100_T1_F	92	8	100
Ag_Mix_F	9	89	98
A_10_T1_FD	59	40	99
A_100_T1_FD	36	63	99
Ox_100_T1_FD	31	69	100

Legend of the samples:
A_10_T1_F: Ag 10nm, 20min, non-aerated, frozen
A_100_T1_F: Ag 100nm, 20min, non-aerated, frozen
Ag_Mix_F: Ag 15nm, spiked in frozen sludge, freeze dried
A_10_T1_FD: Ag 10nm, 20min, non-aerated, freeze dried
A_100_T1_FD: Ag 100nm, 20min, non-aerated, freeze dried
Ox_100_T1_FD: Ag 100nm, 20min, aerated, freeze dried

A visual comparison immediately reveals the similarity of the samples that were kept frozen and the samples that were freeze dried and pressed into pellets. The AgNP from the frozen samples remained dominantly metallic but the freeze dried samples always consisted of a significant proportion of Ag_2S. Even Ag-NP that where we spiked into the frozen sludge turned out be sulfidic after freeze-drying / pelleting the sample. Also the AgNP which were reacted with the activated sludge under aerated conditions (constant aeration of the sludge during the experiment) transformed into sulfidic species. These surprising results suggested that the freeze drying process severely affected the speciation of the AgNP.

To constrain these findings we carried out TEM analysis of a sample reacted under aerated conditions and embedded in epon resin in the frozen state without freeze drying. High resolution TEM images (Figure 2) and EDX analysis of individual Ag-NP (Figure 3) confirm that the particles remained metallic and did not sulfidice, in contrast to the XANES spectra that suggested near complete sulfidation after freeze drying.

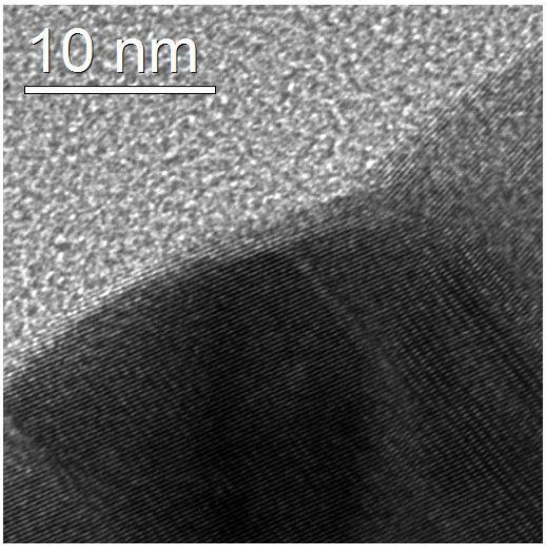

***Figure 2**: High resolution TEM images of a Ag-NP (100nm citrate, aerated)*

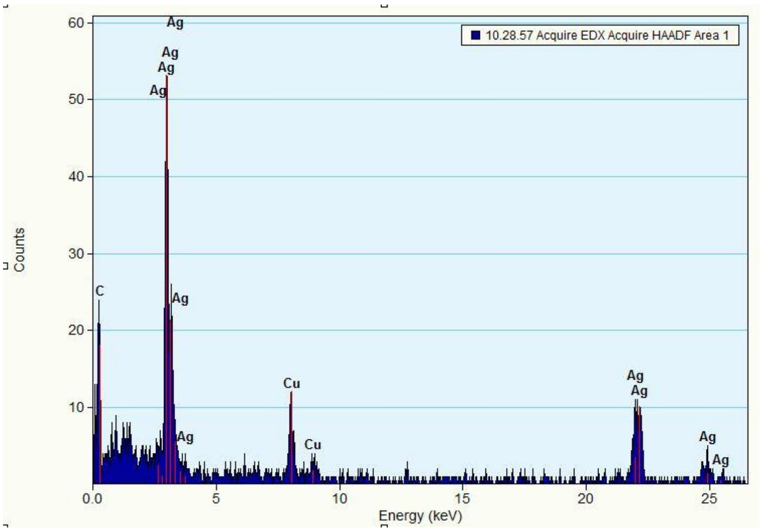

***Figure 3**: EDX spectrum of the Ag-NP shown on the right.*

These results suggest that the preparation technique, e.g. the freeze-drying process, can lead to severe artifacts in the speciation of Ag-NP as revealed by Ag K-edge XANES in combination with analytical electron microscopy. Freeze drying is a frequently used sample preparation technique for speciation analysis and therefore we will further investigate the effects of the freeze drying process on the speciation of Ag-NP is more detail. The results will possibly have a significant impact on the interpretation of published literature.

Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., and Siegrist, H. (2011) Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. Environmental Science & Technology, 110405125242089.

Kaegi, R., Voegelin, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M., and Siegrist, H. (2011) Fate and Behaviour of Silver Nanoparticles in Urban Wastewater systems, Waster Science and Technology, under review.

Kim, B., Park, C. S., Murayama, M., and Hochella Jr, M. F. (2010) Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. Environmental science & technology.

Levard, C., Reinsch, B. C., Michel, F. M., Oumahi, C., Lowry, G. V., and Brown, G. E. (2011) Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on Dissolution Rate. Environmental Science & Technology, **45**(12), 5260-5266.