DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF NANOPARTICLES IN THE FRESHWATER CLAM *CORBICULA FLUMINEA*

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1. Introduction

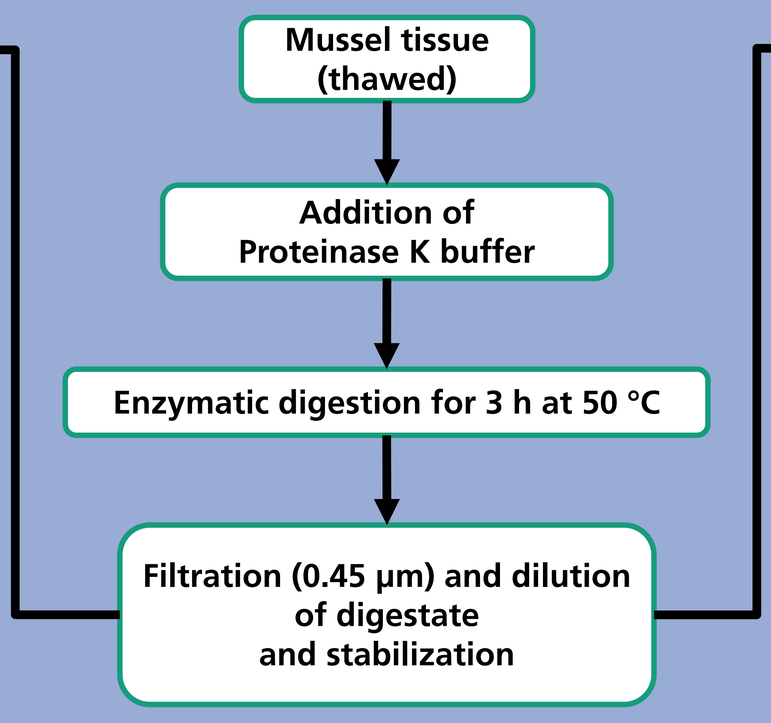
Bioaccumulation studies with manufactured nanomaterials (MNMs) were carried out with the freshwater clam *Corbicula fluminea*. Silver nanoparticles (AgNPs) and TiO_2-NPs were chosen as model particles. The aim of this project was to detect and characterize MNMs in the tissue of animals collected at the end of the exposure period. For the analysis and characterization of nanoparticles in the tissue samples, two promising analytical methods were applied: (i) single particle inductively coupled plasma mass spectrometry (spICP-QQQ-MS) as well as (ii) Flow-Field-Flow-Fractionation (Flow-FFF) coupled to ICP-QQQ-MS. Furthermore, a tissue extraction procedure had to be developed to transfer the particles into stable suspension, which is a prerequisite of both analytical techniques. Special emphasis has to be placed on the development of a mild method for the isolation of MNMs from the environmental matrices. A promising approach is the application of an enzymatic digestion method using Proteinase K.

2. Methods – digestion and analysis

spICP-QQQ-MS

- spICP-QQQ-MS measurements performed with an Agilent 8900
- Dwell time 0.1 ms
- 60 seconds transient signal measurement
- Monitored masses - ^{107}Ag, $^{63}(TiNH)$
- Different types of gas modes checked

ICP-QQQ-MS (© Fraunhofer IME)



Flow-FFF -ICP-QQQ-MS

- Flow-FFF Wyatt Eclipse Dualtec coupled to Agilent 8900 ICP-QQQ-MS
- Size dependent AgNP (NM 300K) fractionation
- Membrane regenerated cellulose (MWCO 10 kDa)
- Channel flow 1 mL/min; cross flow 0.7 mL/min
- Carrier 0.125% w/w FL-70 in ultra pure water

AF4-ICP-QQQ-MS
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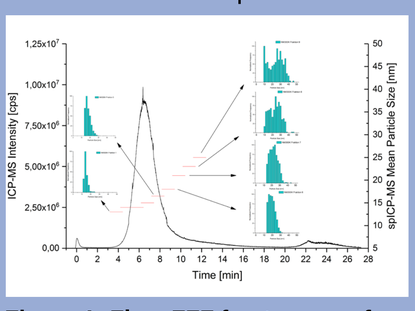

Figure 1: Flow-FFF fractogram of NM 300K and fractionations taken from the peak measured with spICP-MS.

3. Results

- Size distributions of NM 300K AgNPs spiked and injected to mussel tissue prior to and after enzymatic digestion were recovered (Figure 2).

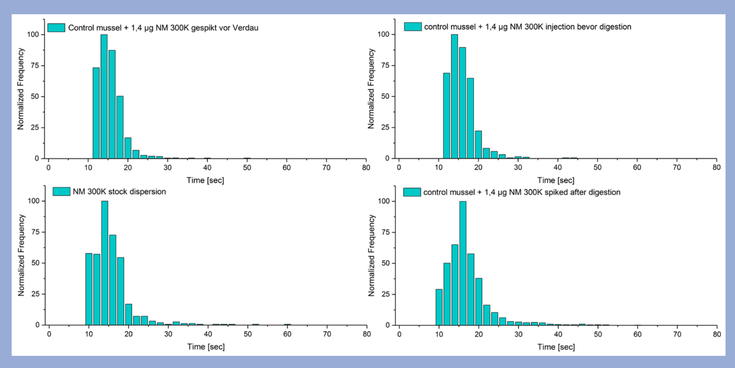

Figure 2: Effect of the enzymatic digestion on NM 300K size distribution in control mussels.

- Size distributions of NM 105 TiO_2-NPs in digested mussel tissue after 12 h and 120 h exposure shifted to smaller sizes (Figure 3A).
- Size distributions of NM 300K were slightly shifted to smaller sizes (Figure 3B).
- First results with Flow-FFF pre-fractionation of AgNPs for reduction of ionic background are promising (Figure 1).
- Proteinase K digestion is in principle feasible for the analysis of AgNPs and TiO_2-NPs in mussel tissue.

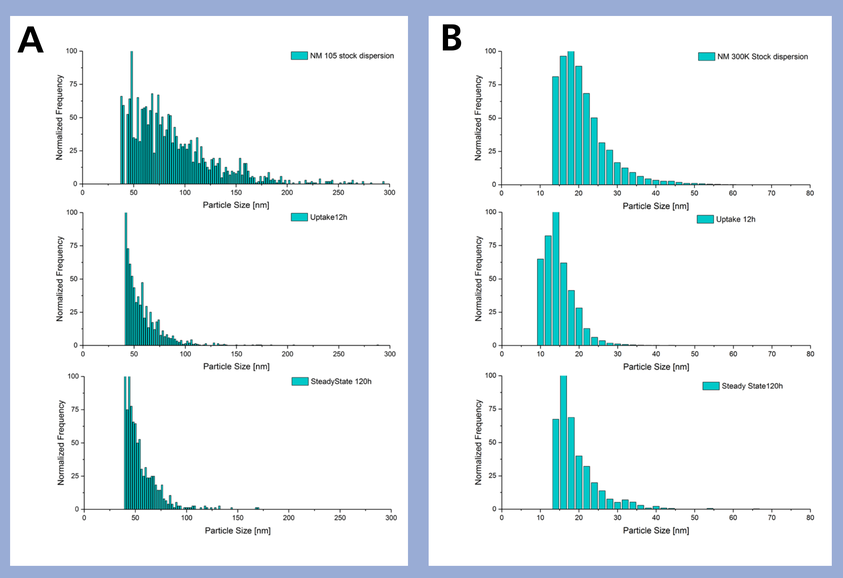

Figure 3: Size distribution of NM 105 (A) and NM 300K (B) stock dispersion and digested mussel tissue after 12 h and 120 h exposure.

4. Conclusion and Outlook

- ➢ The MNMs can be extracted from mussel tissue to determine uptake of MNMs and size distributions in the tissue using digestion by Proteinase K followed by spICP-MS analysis.
- ➢ Additional exposure studies with more replicates to address the individual variability of mussels in order to ensure a higher statistical certainty in the results are part of ongoing work.
- ➢ Flow-FFF-separation for pre-fractionation and removal of ionic silver for analysis of NM 300K by spICP-MS is part of ongoing work.

References

Gray, E. P., et al. (2013). "Extraction and analysis of silver and gold nanoparticles from biological tissues using single particle inductively coupled plasma mass spectrometry." Environmental science & technology 47(24): 14315-14323.

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Funding: German Environment Agency (UFOPLAN FKZ 37166 66 410 0).

Development of a method for the analysis of nanoparticles in the freshwater clam *Corbicula fluminea*.

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Bioaccumulation studies with manufactured nanomaterials (MNMs) were carried out with the freshwater clam *Corbicula fluminea*. Silver nanoparticles (AgNPs) and TiO2-NPs were chosen as model particles. The aim of this project was to detect and characterize MNMs in the tissue of animals collected at the end of the exposure period. For the analysis and characterization of nanoparticles in the tissue samples, two promising analytical methods were applied: (i) single particle inductively coupled plasma mass spectrometry (spICP-QQQ-MS) as well as (ii) Flow-Field-Flow-Fractionation (Flow-FFF) coupled to ICP-QQQ-MS. The spICP-QQQ-MS technique uses short acquisition times in the 0.1-10 ms range for the detection of individual particle events during transient analysis and enables the measurement of number-based particle size distributions. Flow-FFF is a separation technique based on the diffusion coefficients of particles and separates particles according to their hydrodynamic diameter without using a stationary phases. Both techniques can deliver complementary information about size and concentration of particles present in the samples.

Furthermore to the analytical method a tissue extraction procedure had to be developed to transfer the particles into stable suspension which is a prerequisite of both analytical techniques. Traditional digestion using strong acids likely leads to the dissolution of most MNMs. Therefore, special emphasis was placed on the development of a mild method for the isolation of MNMs from the environmental matrices. Two procedures were applied (i): enzymatic digestion with Proteinase K and (ii): additional alkaline hydrolysis with TMAH. The samples were examined with the former mentioned analytical method. Complete tissue digestion of the mussel samples were achieved with both extraction procedures. For evaluation of the methods, nanoparticles were measured in original samples as well as in spiked samples. The results obtained for the selected model particles are compared.