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Development of a method for the analysis of nanoparticles in homogenized biota tissue from the German Environmental Specimen Bank

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Aim of this project was to adapt reported approaches to detect and characterize engineered nanoparticles (ENPs) in biota samples from the German Environmental Specimen Bank (ESB). These samples were already homogenized and stored in the ESB according to internal standard procedures for the German Environment Agency. For the analysis and characterization of nanoparticles in mussel samples of blue mussel (*Mytilus edulis*) and zebra mussel (*Dreissena polymorpha*) 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 the diffusion coefficients of particles and separates particles according to their hydrodynamic diameter without using a stationary phases. Possible changes in size distributions or losses of particles are largely avoided in contrast to conventional chromatographic techniques. Flow-FFF coupled to ICP-MS provides information on the elemental composition depending on the size of sample compounds. Both techniques 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 ENPs. Therefore, special emphasis was placed on the development of a mild method for the extraction of ENPs 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. Silver nanoparticles (AgNPs), CeO_2-NPs and TiO_2-NPs were chosen as model particles.

Complete tissue digestion of the mussel sample was achieved with both extraction procedures. For evaluation of the method, nanoparticles were measured in original samples as well as in spiked samples. First results showed minimal shifts compared to dispersions of the pristine particles and indicate that the original particle size distribution is not affected by the hydrolysate matrix according to spICP-QQQ-MS measurement series. For non-spiked samples we found Cer containing particle events. For samples with overlapping background signals additionally Flow-FFF-ICP-MS was applied.

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