Fate and affects of engineered nanoparticles in aquatic environments

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1. Introduction

Nanoparticles are found in increasing numbers of products, making it all the more likely that they will also be released into the aquatic environment. This may occur in the course of production, use or disposal, either directly (e.g. as a result of accidents) or via wastewater. Although the level of synthetic nanoparticles in the environment remains low, compared with natural particles *(Gottschalk and Nowack 2011)*, their potential environmental risks need evaluation based on a fundamental understanding of the behavior, fate, bioavailability and interaction with organisms.

2. Release of nanoparticles in the environment?

With increasing use of nanomaterials in consumer products their dispersion in the environment is predictable. Thus, engineered nanoparticles (NP) might be released along the lifecycle of consumer products during their production, use, and disposal. Recent studies have demonstrated that for instance titanium dioxide (TiO_2) NP and silver(Ag) NP, which are used as whitening pigments and as bactericides in paints, leach from house facades releasing the particles in facade runoff and in urban stormwater runoff which is discharged into receiving waters *(Kaegi et al. 2008; Kaegi et al. 2010)*. Assessment of AgNP released from textiles was shown to occur upon washing. Current material flow analysis models indicate that most of the NP derived from consumer products will be released into sewer systems and reach wastewater treatment plants or eventually directly discharged into receiving waters *(Gottschalk and Nowack 2011)*. Although the behavior of NP in wastewater treatment plants remains to be examined for most types of particles, first studies with AgNP show that once introduced in a wastewater treatment plant particles are being transformed fast into silver sulfide *(Kaegi et al. 2011)* indicating the need to consider physical and chemical transformations in order to evaluate particle bioavailability in natural systems.

3. The fate of NPs in aquatic systems

To assess the effects of nanoparticles on aquatic organisms, their fate in aquatic systems under various natural conditions needs to be understood. The most important processes affecting the fate of nanoparticles in aquatic systems are agglomeration and /or aggregation, dissolution and chemical reactions including oxidation or reduction, ligand exchange and formation of new solid phases *(Handy et al. 2008; Klaine et al. 2008; Navarro et al. 2008 a)*.

The principles of colloid chemistry can assist to explain current observations on the fate of NP in aquatic systems (Hotze et al. 2010). Thus, the surface properties of NP including size and charge, are the most important factors determining their stability as colloidal suspensions or their aggregation into larger particles. The charge of nanoparticles originates either in reactions at their surfaces, in particular by acid-base reactions at the surface of oxide nanoparticles (e. g. TiO_2, CeO_2, Fe-oxides), or in the coating of nanoparticles (e.g. Ag-nanoparticles with carbonate or citrate coating). Oxide nanoparticles have thus a pH-dependent charge, which may be positive or negative. Metallic nanoparticles such as Ag(0), Au(0) may also be either negatively or positively charged, depending on the coating composition and on the pH dependence of this coating *(Figure 1)*.

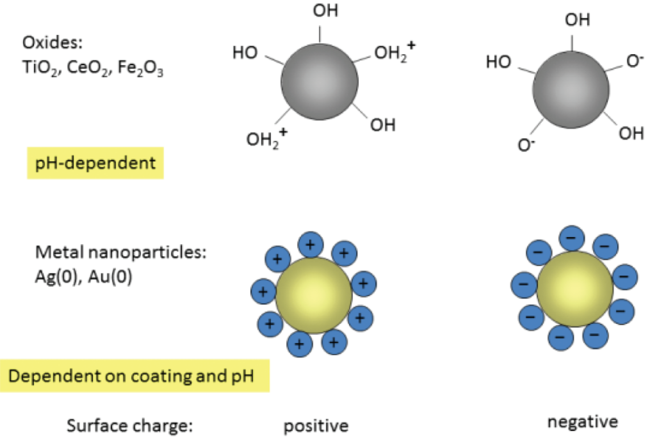

figure 1. Surface charge of nanoparticles resulting from pH-dependent surface reactions for oxides and from the coating for elemental metallic nanoparticles.

According to the classical DLVO theory of aggregation, the sum of attractive and repulsive forces determines interaction between particles. An electrostatic double layer (EDL) surrounding the particles as determined from the particle physico-chemical properties and the ionic strength of the aqueous medium (sum of all ions in the medium), controls the extent of particle interaction. Low ionic strength results in a large EDL and particle repulsion while high ionic strength conditions compress the EDL fostering particle attraction and aggregation *(Figure 2)*. However, a variety of physico-chemical properties of engineered nanoparticles including shape, size, structure, composition, and organic coatings do challenge the application of classical collision theories to predict aggregation of engineered NP *(Hotze et al. 2010)*.

Engineered NP are usually coated with inorganic or organic compounds, such as carbonate, citrate, cysteine, chitosan or surfactants such as sodium dodecyl sulfate to increase repulsion among particles and in that way to maintain particles as stable colloidal suspension. Yet, a number of laboratory studies indicate that nanoparticles will aggregate to some extent when introduced in aqueous systems (for a Review see *(Lin et al. 2010)*. The most important factors to be considered for aggregation of nanoparticles in natural waters are pH, ionic strength and behavior of the coating molecules. Conditions in which the surface charge of the nanoparticles is neutralized favor agglomeration, as well as high ionic strength. For example, natural waters with high calcium content are expected to induce agglomeration of nanoparticles, whereas waters with low ionic content may rather favor suspension stability. In case of silver nanoparticles, agglomeration has been observed in particular at low pH, when the negative surface charge of the coating becomes neutralized, and at high ionic strength *(Gao et al. 2009; Badawy et al. 2010; Elzey and Grassian 2010; Piccapietra 2010)*. These factors should also be considered in toxicological experiments with nanoparticles.

Reaction of NP with inorganic components of natural waters is expected to lead to the formation of new solid phases. Thus, AgNP may react with chloride and sulfide in solution to form the corresponding particular silver chloride or silver sulfide phases,

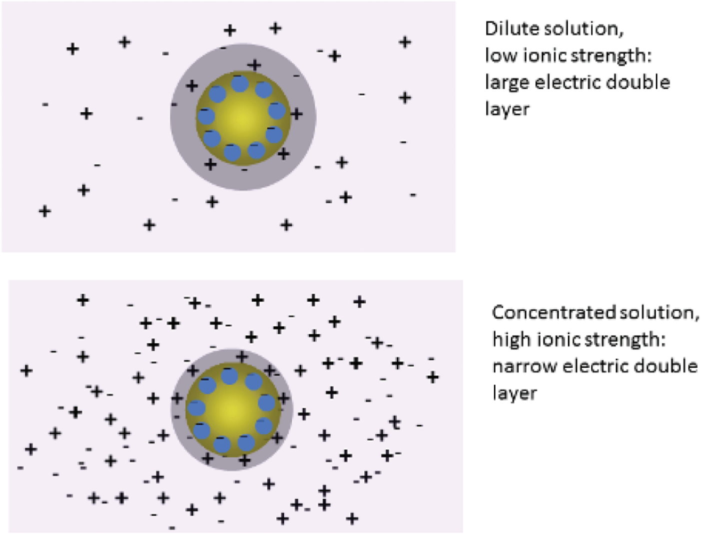

figure 2. Schematic view of the electric double layer surrounding a charged nanoparticle. At high ionic strength, the narrow electric double layer favors particle attraction and induces agglomeration.

which are only sparingly water soluble *(Scheckel et al. 2010)*.

Humic acids which are main components of natural organic matter in aquatic systems are expected to influence the stability of particle suspensions by altering the surfaces properties and by steric effects. Some studies have reported the stabilizing effects of humic acids on fullerenes and multi walled carbon nanotubes which was attributed to steric effects *(Chen and Elimelech 2008; Chappell et al. 2009; Schwyzer et al. 2011)*. Fulvic acids showed stabilizing and destabilizing effects on TiO_2 suspensions that were pH and ionic strength dependent and explainable by corresponding changes in particle and fulvic acid charge *(Domingos et al. 2009)*. Stabilization of bare gold nanoparticles as colloidal suspensions by albumin was attributed to steric effects though repulsion due to the protein coating cannot be excluded *(Brewer et al. 2005)*. This last result is indicative that similar effects are expected when NP interact with biomolecules once taken up by organisms.

Dissolution reactions of metal-based NP are of particular ecotoxicological relevance considering the toxicity of metal ions to aquatic organisms. Particle dissolution depends on their water solubility and might occur rapidly as in case of ZnO and CuO NP *(Franklin et al. 2007; Miller et al. 2010)* with solubility rate inversely proportional to the particle size *(Meulenkamp 1998; Bian et al. 2011)*. In case of elemental NP, such as Ag(0), chemical oxidation is required for dissolution *(Liu and Hurt 2010; Miller et al. 2010)*. Release of Ag^+ ions from AgNP is shown to be a very important factor for toxicity *(Navarro et al. 2008 b)*. While dissolution reactions in simple aqueous systems will be partly predictable by classical theories *(Bian et al. 2011)*, the dissolution dynamics in aquatic systems remains to be examined considering that rate and extent of dissolution is expected to depend on various solution conditions, such as pH, presence of oxygen and effects of ligands *(Liu and Hurt 2010)*.

Some nanomaterials may lead to the formation of reactive oxygen species (ROS), in particular TiO_2, which is photochemically reactive. These properties of TiO_2 are used for pollutant degradation *(Hoffmann et al.*

1995), but may also affect organisms *(Auffan et al. 2009 a)*. Other redox-active nanomaterials include cerium oxide CeO_2, in which the redox couple Ce(III)/Ce(IV) may result both in production or elimination of reactive oxygen species *(Auffan et al. 2009 b)*.

The example above provides evidence that NP are prone to dynamic changes upon release in aquatic systems. While providing a fundamental understanding of the fate of NP, studies on the behavior also provide information to guide ecotoxicological studies. Agglomeration of nanoparticles leads to larger particles, which may present an altered bioavailability compared to single particles. Dissolution of metal-based NPs might pose an additional risk to organisms exposed to both particulate metal and dissolved metal ions. Furthermore, particles might be predicted to change upon interaction of NP with biological molecules. Once in contact with cells, organelles, membranes and proteins, NP will contribute to the establishment of NP/biological interfaces that depend on colloidal forces as well as physicochemical interactions *(Lynch et al. 2007; Lundqvist et al. 2008)*. Reactions with biological molecules can on one hand be expected to impact on NP properties, such as reactivity and stability, and on the other hand, on biomolecule structure and function.

4. Uptake and effects to aquatic organisms

In response to international recommendations, an increasing number of studies is examining the ecotoxicity of engineered nanoparticles to aquatic. A very important point that emerges from the studies of NP is that as discussed above for their environmental fate, bioavailability and toxicity seem to be dependent on the chemistry and on particles characteristics such as size, surface area and reactivity, charge and agglomeration state. Questions that need to be addressed to evaluate ecotoxic risks associated with the release of engineered NP in the environment are: *a)* how are particles taken up by organisms; *b)* are particles toxic and by which mechanisms? and *c)* do particles effects on organisms translate into effects at the ecosystem level?

Knowledge on the uptake of NP is mainly available from mammalian toxicology while little information is available for aquatic organisms. NP may be absorbed by aquatic organisms through their diet or from the water. Potential routes of entry will include absorption from the gut following direct ingestion or entry across epithelial body surfaces such as gills, olfactory organs or skin. At the cellular level, endocytosis, is considered to constitute a major process for intracellular particle uptake *(Moore 2006; Zhao and Wang 2011)*. Endocytosis encompasses a variety of mechanisms by which cells absorb large hydrophilic biomolecules and particular matter by their engulfment into pockets of the cell membrane which pinch off inside the cell releasing a biomolecule/particle containing vesicle.

Some experimental evidence indeed is indicative that NP can be absorbed by aquatic organisms upon water and dietary exposure *(Roberts et al. 2007; Ramsden et al. 2009; Ward and Kach 2009)*. While studies on mammalian systems suggest that cellular internalization depends on particle size *(Zhao and Wang 2011)*, absorption and retention in the gut of clams was considerably more efficient upon exposure to large aggregates than to dispersed NP *(Ward and Kach 2009)*. Examination of cellular particle uptake into a rainbow trout gill cell line was shown to occur regardless of whether cells were exposed to NP agglomerates or to single particles *(Kühnel et al. 2009)*. Furthermore, carbon nanotubes were found to agglomerate when retained in the gut of water fleas *(Roberts et al. 2007)*. These examples illustrate changes experienced by the NP upon contact with organisms and indicate the need to understand the underlying mechanisms determining particle absorption, cellular uptake and biological transformation as important steps in understanding effects of NP to aquatic organisms.

The potential toxicity of various NP to aquatic organisms including plants, fungi, algae, invertebrates and fish has been reported in the last few years in an increasing number of publications (for Reviews see *(Perez et al. 2009; Kahru and Savolainen 2010)*. Most studies to date have focused on assessing the concentrations of NP affecting the survival, growth and a variety of physiological processes. These studies indicate that sensitivity to NP differs among species, with filter-feeding invertebrates being markedly more susceptible compared to larger organisms *(Griffitt et al. 2008)*. While being of direct relevance to the risk assessment of engineered NP, the applicability of these data is so far uncertain due to a generally poor characterization of the physico-chemical state and changes of experimental NP in biological media used for toxicity testing. In fact, low levels of reproducibility of toxicity data are generally observed in experiments with NP susceptible to high aggregation in biological media *(Hartmann et al. 2009)*.

Among the most challenging questions when examining particles toxicity is whether NP themselves induce toxicity, and which specific physico-chemical properties induce toxicity. In the case of metallic nanoparticles, it is possible that nanoparticles cause toxicity either directly by their chemical composition and surface reactivity or indirectly, by acting as a source of metal ions, reactive oxygen species, or merely by physical interactions *(Franklin et al. 2007; Navarro et al. 2008 a; Bastian et al. 2009; Kühnel et al. 2009; Auffan et al. 2009 a)*. Furthermore, all NP, even those considered being chemically and biologically inert might produce general negative impacts that are independent of the chemical composition of the NPs. Deposition of particles on sensitive body surfaces, adsorption onto the surface of cell membranes and cellular internalization might generally impair cellular processes.

In case of AgNP, the available information on the ecotoxicity of AgNP to date, indicates both particles and silver ions as causes of assessed effects *(Figure 3)*. Specific effects to bacteria were found to be caused by the intrinsic properties of the silver nanoparticles *(Morones et al. 2005; Fabrega et al. 2009)*. Similarly, the contribution of silver ions to lethal toxicity of silver nanoparticles to zebrafish, daphnids, and an algal species was found to be minor *(Griffitt et al. 2008)*. Dose-dependent developmental toxicity of AgNP to zebrafish embryos *(Asharani et al. 2008; Yeo and Yoon 2009)* was not found upon exposure to Ag^+. Toxicity to fathead minnow embryos was reported to be caused by particulate forms of Ag *(Laban et al. 2010)*. On the other hand, studies with freshwater and marine algae have demonstrated the toxicity to algal photosynthesis being caused by dissolved silver ions in freshwater and marine species *(Navarro et al. 2008 b; Miao et al. 2009)*. However, toxic effects of AgNPs were not fully explained by the extracellular silver ion concentration *(Navarro et al. 2008 b)*, suggesting significant interactive effects of nanoparticles and algae, which lead to increased silver dissolution.

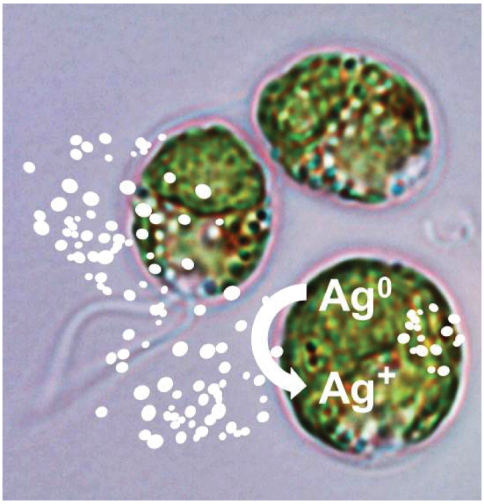

figure 3. The dissolution of metal-based NP is illustrated for elemental silver NP and algae. Formation of toxic Ag^+ ions upon oxidative dissolution of the NP might occur in the exposure water or within the organisms for particles internalized in cells.

To date, the general lack of mechanistic knowledge on interactions between NP and biochemical constituents prevent understanding the ecotoxicity of NP that still needs to be examined also considering concentrations of NP projected to be realistic in aquatic sytems. Furthermore, to be able to increase the environmental relevance of results obtained with single species, ecotoxicity studies with NP should also examine consequences to biological communities, where more than one single species interact to provide specific ecosystem services *(Bernhardt et al.*

2010). As long as this information is not available, the diffusion of engineered NP in the environment should be avoided.

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