

The influence of pH and media composition on suspension stability of Ag, ZnO, and TiO₂ nanoparticles and immobilization of *Daphnia magna* under guideline testing conditions

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(Technical University of Denmark) / Department of Environmental Engineering; N.B. Hartmann, A. Baun, Technical University of Denmark DTU / DTU Environment. In aquatic toxicity testing of engineered nanoparticles (ENPs) the process of agglomeration is very important as it may alter the bioavailability of the ENPs and hence their toxicity. In this study we evaluated test conditions that are more favorable in maintaining a stable and low agglomerate size profile of ENPs in aquatic media applicable in OECD guideline. In this study we focus on controlling stability (as point of zero charge) by employing changes in pH to media of different ionic strength (M7, and Very Soft EPA medium) and documenting the influence of these parameters on acute immobilization of *Daphnia magna*. Despite being sterically stabilized, test suspensions of Ag NPs were found to consist of large agglomerate sizes (close to μm range) for both VS EPA and M7 media. The toxicity of the AgNPs was found to be higher in VS EPA medium than in M7 medium caused by an increased dissolution in VS EPA medium. Maintaining a constant pH throughout the testing period of 48 hrs proved to be challenging. Especially for ZnO NPs a constant and stable pH is crucial since a large degree of dissolution was observed below pH 6.5. Pzc for ZnO ENPs was observed at pH ~8. Increased dissolution of ZnO NPs tested in VS EPA medium (at pH 7) rendered them more toxic compared to M7 medium. TiO₂ ENPs revealed a pzc at pH values between 7-8 and were present in relatively low-size agglomerates (~200 nm) in VS EPA medium (pH 7), whereas the agglomerate size in M7 medium reached μm range. While various studies have classified TiO₂ ENPs non-toxic, we saw that the low-size stable agglomerates caused immobilization to *D. magna* revealing EC50 of 13.7 mg L^{-1} . Aquatic toxicity studies used for regulatory purposes should ideally comprise a set of testing parameters under which the lowest size of ENPs can be maintained. This could be achieved by employing a medium of low ionic strength and measuring the point zero charge of the specific suspension, in order to test for toxicity related to nano-size properties.