Goldschmidt 2025 Abstract
https://doi.org/10.7185/gold2025.29422

Evaluation of a Method for *In Situ* Monitoring of the Nanoparticles Homoaggregation in Surface Water

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Upon release into the environment, anthropogenic nanoparticles (NPs) undergo aging processes such as chemical transformation, aggregation, and disaggregation which significantly influence NP behavior, affecting their mobility, bioavailability, and potential ecotoxicity. Consequently, understanding their environmental fate, particularly their aggregation behavior, is essential for assessing their impact on aquatic ecosystems.

Previous studies investigating NP aggregation have been conducted under controlled laboratory conditions using model nanoparticles. However, it remains uncertain whether these results can be extrapolated to natural environments, where dynamic factors— including variable water chemistry and interactions with natural organic matter—can alter NP surface coatings and influence electrosteric interactions between aggregating particles. Currently, no standardized method exists for studying NP aggregation under real environmental conditions.

To address this limitation, we tested the validity of an in situ dialysis bag method previously developed in our group, which allows nanoparticles to be exposed directly to natural river water while preventing their loss due to advection. The method was evaluated by monitoring the homoaggregation of low-concentration citrate-coated silver nanoparticles inside and outside the dialysis bag, using dynamic light scattering and single-particle inductively coupled plasma mass spectrometry. The results demonstrated that aggregation rates and final aggregate sizes of silver nanoparticles with concentration of 10 mg/L were identical inside and outside the dialysis bag when exposed to the tap water for over 3 hours, confirming the method's ability to accurately reproduce NP aggregation.

Additionally, to minimize further aggregation during sample transportation from the field to the laboratory, various stabilizers were tested. Dilution of silver nanoparticles with 1mg/L concentration in the solution with increased pH at 12, resulted in the stability of nanoparticles in the mixture while applying vertical rotation at 95 rpm up to 4 days. The successful implementation of this method not only enables in situ assessment of NP
homoaggregation but also provides a foundation for studying heteroaggregation processes, particularly interactions between NPs and naturally occurring particles such as clays and iron oxides. To further enhance sensitivity at lower NP concentrations and to differentiate between various aggregate types, future studies will integrate single-particle inductively coupled plasma time-of-flight mass spectrometry, offering improved resolution and compositional analysis.