

## Colloidal Stability has a crucial role for nanomaterials toxicity testing *in-vitro*: nZVI-algae colloidal system as case study

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### Abstract

Aggregation is one among those parameters focusing the attention in Nanotoxicology due to its methodological implications [1, 2]. Aggregation is a physical symptom of a more general physicochemical condition of colloidal particles, that is, colloidal stability. A destabilized colloidal system may tend to reduce its net surface energy by self-aggregation, but also by hetero-aggregation which may involve biological interfaces. In this regards, colloidal stability may have an important role as driver of ENM bioactivity. In the present study, a physical speciation phenomenon of nZVI nanoparticles, we called *colloidal singularity* [3], was found when generating a dose gradient of zero-valent iron nanoparticles (nZVI) in algal culture medium. The *colloidal singularity* consisted of an exceptionally stable ENM suspension with particles in their primary size (4 - 12 nm) occurring within a tight dose range (0.1-0.5 mg/L). Outside this range, nZVI suspensions aggregated. Interestingly, nZVI exhibited toxicity to the algal model organism, except in the 0.1-0.5 mg/L dose range. Stability analyses, TEM images and FTIR revealed that nZVI toxicity was mediated by nZVI-alga interaction, and that the increased colloidal stability of nZVI suspensions in the 0.1-0.5 mg/L dose range prevented nZVI-algae interaction and subsequent toxicity. Furthermore, *in-situ* destabilization of this particular suspension using a classical flocculating agent  $Al_2(SO_4)_3$  resulted in toxicity. These observations demonstrate that colloidal stability has a major role in nZVI toxicity and that may be carefully taken in to account when performing safety assessment of nanomaterials beyond the initial considerations for stock sample preparation.

### References

- [1]. Handy, R. D.; Cornelis, G.; Fernandes, T.; Tsyusko, O.; Decho, A.; Sabo-Attwood, T.; Metcalfe, C.; Steevens, J. A.; Klaine, S. J.; Koelmans, A. A.; Horne, N., *Environ Toxicol Chem* (2012), **31** (1), 15-31. DOI 10.1002/etc.706.
- [2]. Schrurs, F.; Lison, D., *Nat Nanotechnol* (2012), **7** (9), 546-8. DOI n nano.2012.148 [pii]10.1038/nano.2012.148.
- [3]. Gonzalo, S.; Llana, V.; Pulido-Reyes, G.; Fernandez-Pinas, F.; Bonzongo, J. C.; Leganes, F.; Rosal, R.; Garcia-Calvo, E.; Rodea-Palomares, I., *PloS one* (2014), **9** (10), e109645. DOI 10.1371/journal.pone.0109645.