UTILIZING HYDROGELS TO PREVENT SILVER NANOPARTICLES AGGLOMERATION IN MARINE ENVIRONMENTS FOR *IN VIVO* ENERGETIC MEASUREMENTS IN ROTIFER *BRACHIONUS PLICATILIS*

GARCIA, T.O.[1,2]; ALE, A.[3]; DESIMONE, M.F. [1,2,4]; MONSERRAT, J.M.[1,2]

[1] Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brasil.
[2] Programa de Pós-graduação em Ciências Fisiológicas, Instituto de Ciências Biológicas (ICB), FURG.
[3] Cátedra de Toxicología, Farmacología y Bioquímica Legal (FBCB-UNL), CONICET, Santa Fe, Argentina.
[4] Universidad de Buenos Aires (UBA), CONICET, Buenos Aires, Argentina.

INTRODUCTION: Silver nanoparticles (AgNPs) are widely used for their antibacterial properties and applications in pharmaceuticals and textiles. However, their use raises environmental concerns regarding their impact on non-target organisms. Properties, such as colloidal stability and surface charge, influence the environmental fate of AgNPs. This study examined the metabolic responses of the marine rotifer *Brachionus plicatilis* after exposure to AgNPs embedded in a hydrogel matrix (AgNPs-gel) or as free particles. **OBJECTIVE:** To evaluate *in vivo* energetic conditions in *B. plicatilis* after a 24-h exposure to AgNPs-gel or AgNPs (0, 2, and 20 ug/L). **MATERIAL AND METHODS:** AgNPs were synthesized through a chemical method with a 2:1 $NaBH_4$:$AgNO_3$ ratio, and sodium citrate as stabilizer. Complete characterization (TEM, DLS, and zeta potential) was performed in ultrapure and saline water (26 ppt). The AgNPs-gel was prepared using a mixture of tetraethyl orthosilicate, ultrapure water, HCl, and phosphate buffer (pH 7.8). Bioassays were performed in incubators (25 ºC) with constant aeration at a density of ~700 rotifers/mL, and metabolism was measured using a simple resazurin reagent-based method. **RESULTS AND CONCLUSIONS:** AgNPs were successfully synthesized, achieving a higher nanoparticle yield than the conventional 1:1 reagent ratio. DLS measurements showed that AgNPs were ~50 nm in ultrapure water but aggregated up to ~800 nm in saline media. Zeta potential analysis revealed a surface charge of -40.7 mV in ultrapure water and -27 mV in saline water, indicating reduced colloidal stability in marine conditions. Exposure to the AgNPs-gel caused a dose-dependent decrease in the metabolic activity of *B. plicatilis*, whereas direct exposure to AgNPs did not affect metabolism. These findings reveal the critical role of nanoparticle formulations in shaping the biological effects. Specifically, embedding AgNPs within hydrogels appears to alter their bioavailability and subsequent toxicity, likely by controlling nanoparticle agglomeration, potentially influencing their stability and release. Furthermore, our study provides a robust *in vivo* evaluation of the effects of AgNPs at environmentally relevant concentrations, while maintaining colloidal stability through hydrogel encapsulation. This approach, which also offers enhanced ease of manipulation in experimental setups, presents a promising avenue for future marine nanoparticle studies, seeking their effects in saline environments.

Keywords: nanotoxicology; rotifers; metabolism; resazurin.