APPENDIX I

PRIMARY CARACTERIZATION OF NANOPARTICLES

Primary characterization of nanoparticles was performed in MilliQ water using the techniques of Delayed Light Scattering (DLS) and the Electric Scanning Microscope (TEM) in the laboratory of Department of Materials, University of Oxford (nanoFATE project partner).

Silver nanoparticles with average diameter of 5 nm (nominal diameter 2-8 nm) were provided by the Polish industry "Amepox" in stabilized aqueous solution at concentration 1 ppm. Silver nanoparticles with nominal diameter of 50 nm were provided by Czech industry "Nanotrade" and supplied in powder form. For the latter, a concentration of approximately 1 mg Ag/mL was dispersed in deionized water. Both solutions were subjected to sonication in low power US bath for 30 seconds before use.

A drop of each solution was then deposited on a carbon-coated Cu TEM grid and dried at room temperature for several hours before TEM examination.

TEM analysis allows the observation of particles morphology and aggregates (Fig 1A and 2A).

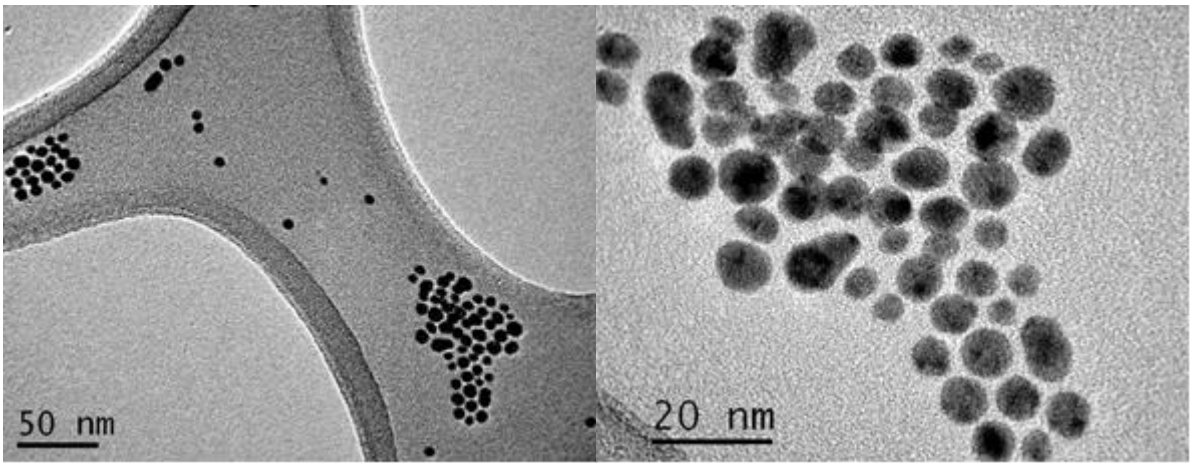


***Fig 1A** – Transmission Electron Microscopy of Ag nanoparticles 5nm. Primary particles are 2-8 nm in size, frequently as separate particles and even in aggregates well separated with a diameter of about 100nm*

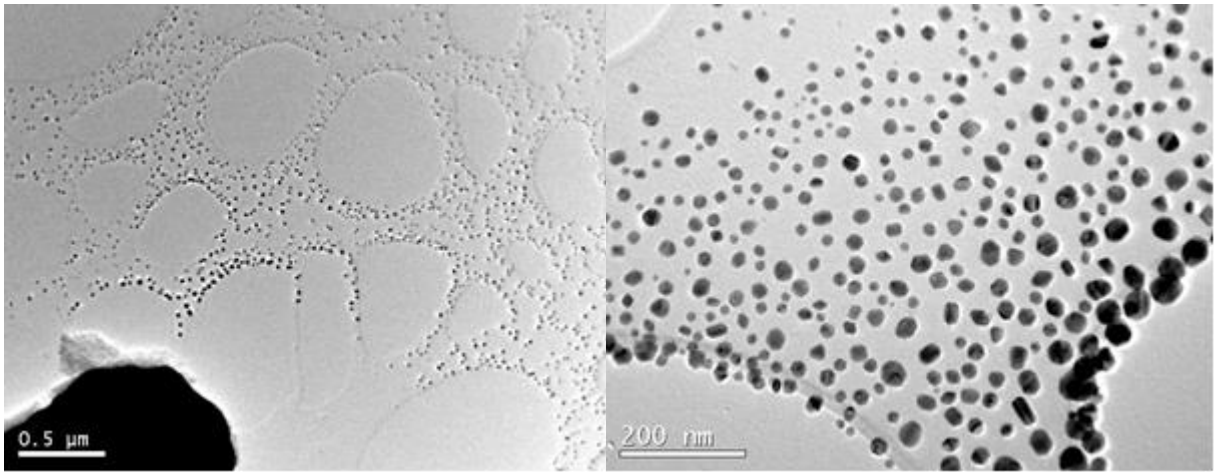


***Fig 2A** – Transmission Electron Microscopy of Ag nanoparticles 50nm. Primary particles are 30-60 nm in size, almost all in micron sized aggregates*

DLS technique, instead, is a method that is generally used for determine the profile of the distribution of small particles in suspension. This allow us to characterizing the size and state of aggregation of nanoparticles added in the experiment (Fig 3A, 4A).

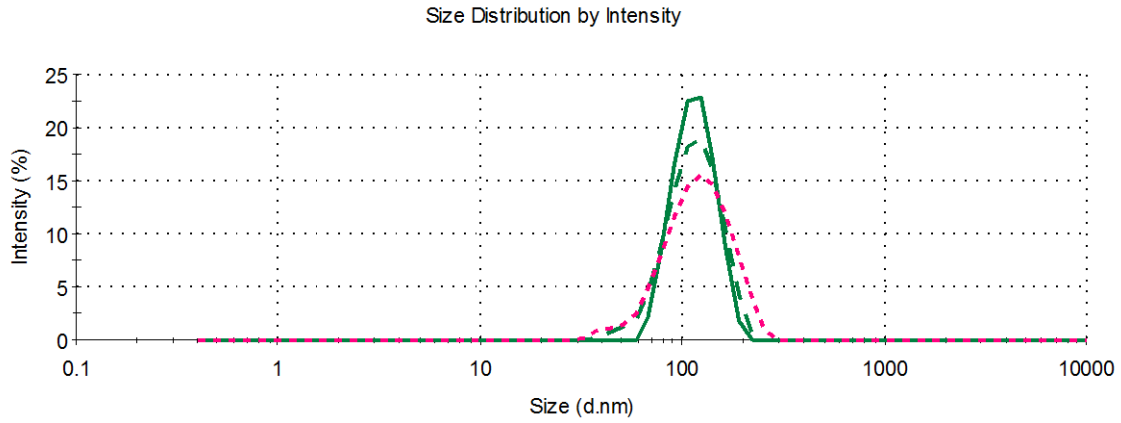


***Fig 3A** – Particle size distribution of Ag nanoparticles 5nm measured by Dynamic Light Scattering. The measurement results of three technical replicates are shown*

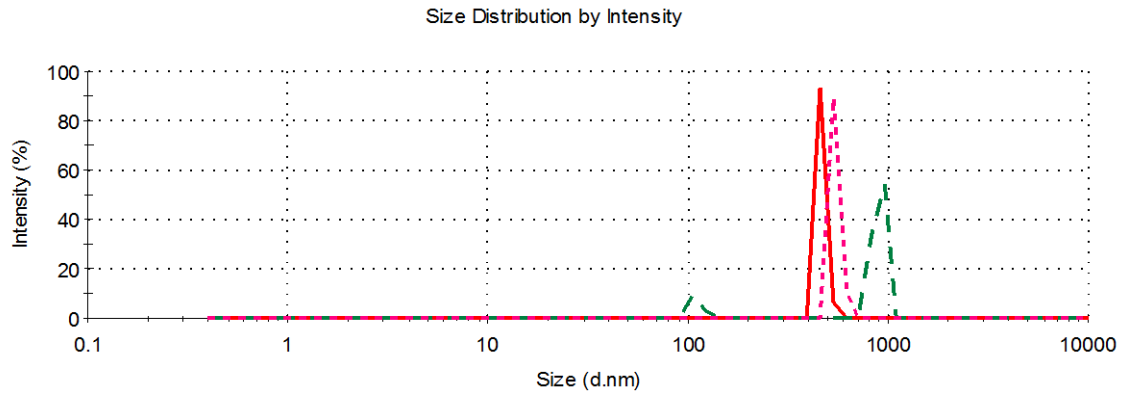


***Fig 4A** – Particle size distribution of Ag nanoparticles 50nm measured by Dynamic Light Scattering. The measurement results of three technical replicates are shown*

INTERACTION OF NANOPARTICLES WITH ARTIFICIAL SEAWATER

Preliminary experiments were conducted to observe the behavior of nanoparticles in artificial seawater. The experiment was carried out at the University of Gothenburg (Department of Chemistry, a nanoFATE project partner) using Dynamic Light Scattering to observe aggregation of particles across time.

Only 5nm nanoparticles were analyzed since the precipitation of larger 50 nm particles was too fast. For the experiment three concentrations compatible with DLS limit of sensitivity (1 mg Ag/L, 5 mg Ag/L and 10 mg Ag/L) were chosen.

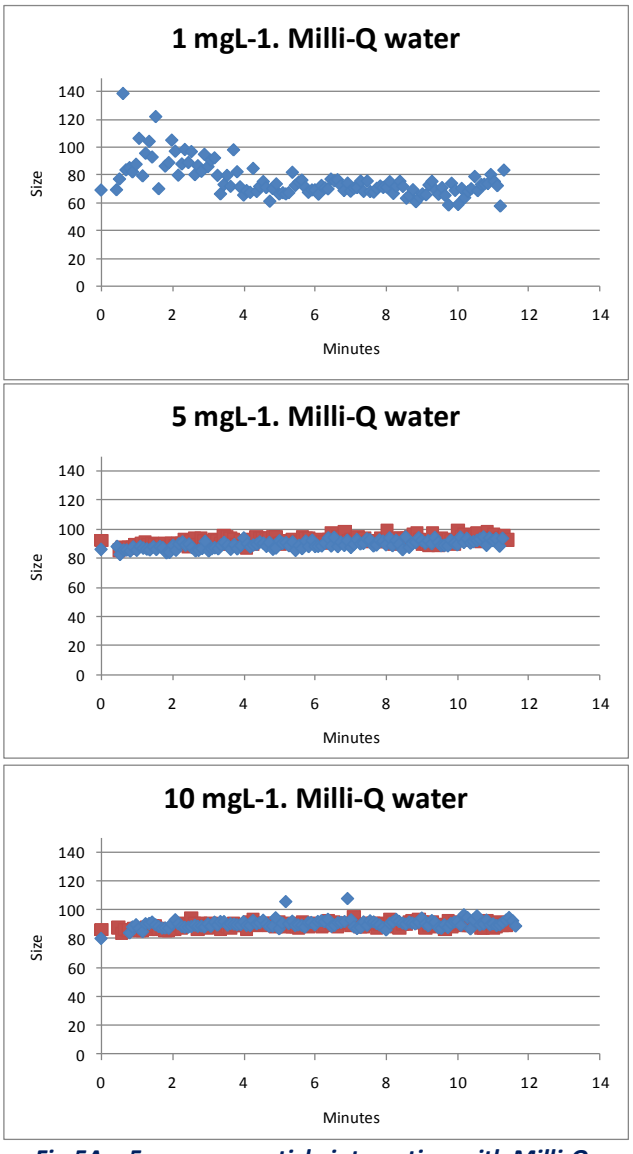
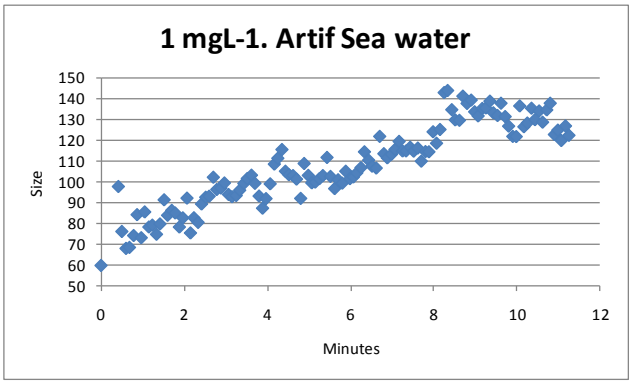
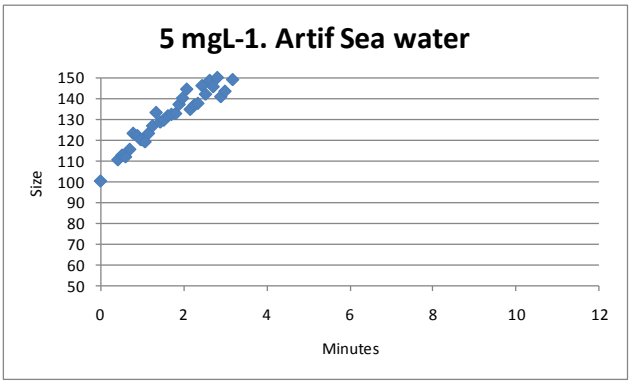


***Fig 5A** – 5nm nanoparticle interaction with Milli-Q water at different concentrations*

The particles show nearly instantaneous aggregation to form aggregates of a size of about 80-100nm (as shown also by previous analyzes, see Figures 1A, 3A). The size remains constant across the observation time (10 min).

In seawater, instead, aggregation was instantaneous and dose-dependent, giving rise to micron-size agglomerates





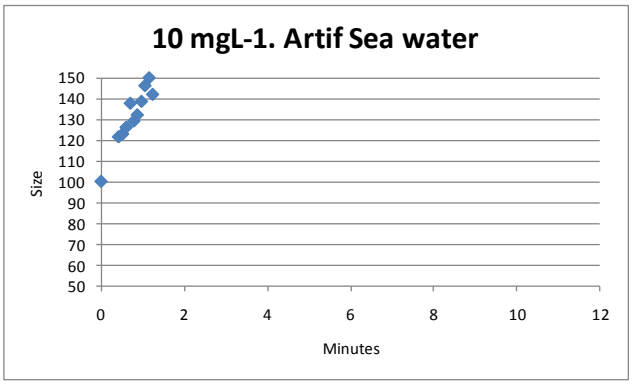


***Fig 6A** – 5nm nanoparticle interaction with Artificial Seawater at different concentrations in function of time*