

## Production of nanoplastics for biodegradation and ecotoxicological studies

C. Jonqua<sup>1</sup>, K. Vin<sup>1</sup>, A. L. Parvin<sup>2</sup>, V. Coma<sup>1</sup>, Y. Boulard<sup>3</sup>, J-P. Renault<sup>3</sup>, S. Pin<sup>3</sup>, S. Devineau<sup>4</sup>,

E. Lallemand<sup>3</sup>, C. Fischer<sup>5</sup>, M. Baudrimont<sup>2</sup>, E. Grau<sup>1</sup>, <u>O. Sandre<sup>1</sup></u>

<sup>1</sup>Laboratoire de Chimie des Polymères Organiques – CNRS, Université de Bordeaux, Bordeaux-INP, 33600 Pessac
<sup>2</sup>Environnements et Paléoenvironnements OCéaniques – Université de Bordeaux, CNRS, OASU, 33120 Arcachon
<sup>3</sup>Institute for Integrative Biology of the Cell – Université Paris-Saclay, CNRS, CEA Saclay, 91191 Gif-sur-Yvette
<sup>4</sup>Unité de Biologie Fonctionnelle et Adaptative – CNRS, Université Paris Cité, 75205 Paris 13
<sup>5</sup>Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université Paris-Saclay, 91000 Evry

olivier.sandre@enscbp.fr

## <u>Abstract</u>

We developed a methodology to obtain surfactant-free suspensions of polymer nanoparticles in water for ecotoxicological and biodegradation studies. Biosourced poly(lactic acid) (PLA) and oil-based polystyrene (PS) are commodity polymers used in many applications like food packaging, while polycaryophyllene (PCAR) is a biosourced polymer interesting for cosmetics formulation. The nanoprecipitation method consists in quickly adding a large proportion of aqueous medium into a polymer solution in a water-miscible solvent. Control of the parameters (initial concentration, stirring and evaporation rates...) allowed obtaining nanoscale particles with good repeatability. In previous work, we used nanoprecipitation to prepare additive-free mode PS nanoplastics that were compared in terms of toxicological effects (from macroscopic to molecular responses) to environmental nanoplastics (*i.e.* cryomilled from plastic debris) on a freshwater bivalve species.<sup>1</sup> In this work, we evaluated the stability of these surfactant-free nanoparticles in deionized water. Negative zeta potential stabilizing the particles in suspension was ascribed to HCO3<sup>-</sup> anions adsorption at the hydrophobic polymer-water interface, arising from the dissolution of CO<sub>2</sub> from air, as reported in literature.<sup>2</sup> While there was no significant change in hydrodynamic size of the particles for PLA and PCAR, thereby confirming their temporal stability, this was not the case for PS.

In a second part of this study, we developed a biodegradation method to compare micro- and nano plastics biodegradability. Namely, we assessed the biodegradation conditions following the OECD 301F guidelines. Colloidal stability of model nanoplastics in the mineral water used as growth medium for the BOD assay was estimated by DLS and zetametry. We did not find significant change compared with the samples prepared by nanoprecipitation in DI water in the first part. First preliminary biodegradability results show that nanoparticles degrade at a lower rate than microparticles, suggesting that the biodegradation depends on the inability of the inoculated microorganisms to detect nanosized particles as compared to microparticles, on which they can adhere and produce their biofilm. This 'micro vs. nano' effect will be further studied through biotechnological analysis (qPCR assays) of bacterial populations of the biofilms as planned by the interdisciplinary consortium of the ANR project ELIMINATORS.

<u>References:</u> 1/ A. Arini, S. Muller, V. Coma, E. Grau, O. Sandre, M. Baudrimont, Env. Sci.: Nano 2023 10(5), 1352; 2/X. Yan, M. Delgado, J. Aubry, O. Gribelin, A. Stocco, F. Boisson-Da Cruz, J. Bernard, F. Ganachaud, J. Phys. Chem. Lett. 2018, 9, 96