



Safety Evaluation of TiO₂ Nanoparticle-Based Sunscreen UV Filters on the Development and the Immunological State of the Sea Urchin *Paracentrotus lividus*

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Characterization of the TiO₂ NPs Constituting the UV Filters and Degree of Dispersibility in the Pre-dispersant Mediums

The primary particle size corresponds to the larger dimension of the elementary TiO₂ crystallites constituting the UV filter. They are generally assembled in larger aggregates existing in the dry powder form. Those aggregates can be partly fractionated when the UV filters are dispersed in a liquid. Scanning Electron Microscopy was used to determine the primary particle size of the TiO₂ NP powder (not declared by the suppliers) used as UV filters for sunscreen formulation, and compared to P25 TiO₂ NPs used as non-coated nano-TiO₂ reference. Results are shown in Figure S1, and the sizes (larger dimension) obtained from the SEM images are reported as nm in Table S1. The P25 TiO₂ NPs showed a non-uniform spherical morphology (Figure S1a) with a diameter of 22.7 ± 4.3 nm, in accordance with what declared by the supplier (compare Table 1 and Table 1S). The TiO₂ NPs coated with a hydrophilic layer of silica (T-AVO) showed a more spherical elongated shape than P25 TiO₂ NPs (Figure S1b) and a primary particle size of 51.0 ± 10.6 nm, whereas both TiO₂ NPs coated with hydrophobic layers, respectively dimethicone (T-Lite) and stearic acid (T-S), showed a rod-like shape (Figure S1c, S1d), and a primary particle sizes of 58.3 ± 10.7 and 64.4 ± 11.4 .

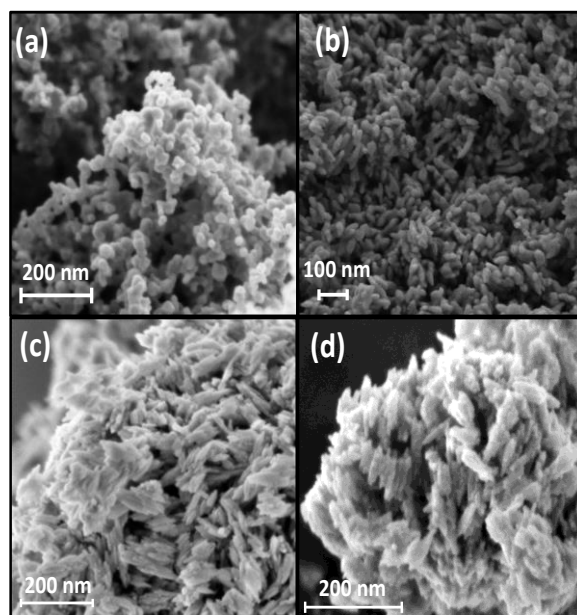


Figure S1. High-resolution scanning electron microscopy (HR-SEM) analysis of the pristine TiO₂-based NPs. (a) P25 TiO₂; (b) T-AVO, (c) T-Lite and (d) T-S.

The dispersion state of the TiO₂ NPs in the water and in the cosmetic oil was evaluated using Dynamic Light Scattering (DLS). The average size measurement obtained for each NPs is reported in intensity weight (Table S1). The T-AVO average size in water was 1543.7 ± 208.6 nm, three times larger than P25 TiO₂ NPs (552.6 ± 5.8 nm, average size). This could be due to stronger interparticle attractions between the T-AVO NPs than between the P25 NPs, already existing in the original powder form. The spherical/elongated shape of the former may favour this aggregation, due to a larger surface area of interaction between the NPs.

Table S1. Comparison of primary particle size and hydrodynamic aggregate size of the TiO₂ NPs. Primary particle size measurements of each dry powder was determined by SEM. Hydrodynamic size measurement was determined by DLS after dispersion in water (P25 TiO₂ and T-AVO NPs, 125 mg/L) or cosmetic oil (T-S and T-Lite NPs, 25 g/L).

TiO ₂ NPs	Primary particle size (nm)	Hydrodynamic size (nm)
P25 TiO ₂	22.7 ± 4.3	552.6 ± 5.8
T-AVO	51.0 ± 10.6	1543.7 ± 208.6
T-Lite	58.3 ± 10.7	173.9 ± 63.5
T-S	64.4 ± 11.4	857.3 ± 131.7

The average T-Lite NPs size in the cosmetic oil was 173.9 ± 63.5 , whereas the T-S NPs resulted in an average size five times larger, indicating a more pronounced aggregation (857.3 ± 131.7 nm, average size). This results from the respective affinities of the UV filter surface coatings with the oil components. The higher affinity of the T-Lite with the oil favours its dispersion, while the lower affinity of the T-S with the oil favours the homo-aggregation of the particles that minimize the surface area of interaction with the solvent [1].

It is unclear how the dispersion states of the different UV filters measured here in the aqueous or oil cosmetic medium are altered after dispersion and dilution in the culture medium. NP fate is then characterized by co-evolving size and concentration of the suspended NPs. Such mechanism is challenging to track *in situ* in the exposure medium because the NP concentration decreases close to zero while the composition of the complex medium can lead to multiple scenarios of interaction such as hetero-aggregation [2,3]. This was not investigated here. However, aggregation and sedimentation

will surely occur in artificial seawater for the hydrophilic P25 TiO₂ and T-AVO NPs pre-dispersed in water because salt-induced aggregation is a well-known effect in such system [4]. The sea urchin coelomic fluid (blood equivalent) resembles natural seawater and contains functional biomacromolecules that interact with the TiO₂NPs to form a biomolecule coating, which may lead to a higher stability in suspension or an increased aggregation [5].

The T-S and T-Lite hydrophobic filters remained mostly concentrated in the oil phase over the duration of the exposure, floating at the culture medium surface and limiting the interaction of the NPs with embryos to the area close to the oil/water interface. However, the migration of the NPs from the oil to the water may be facilitated by the amphiphilic components of the cosmetic oil, e.g. surfactant molecules. For T-S and T-Lite UV filters, further aggregation is not expected, as we assume that these hydrophobic filters remain in the oil phase during the exposure without entering in contact with the aqueous culture medium. For these reasons, we assume that the average sizes measured by DLS and reported for the hydrophobic UV filters dispersed in cosmetic oil are mostly preserved during the embryo exposition, while those returned with the hydrophilic UV filters pre-dispersed in water are totally different, because particle aggregation is expected.

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