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Ecotoxicological studies of micro- and nanosized barium titanate on aquatic photosynthetic microorganisms

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ABSTRACT

The interaction between live organisms and micro- or nanosized materials has become a current focus in toxicology. As nanosized barium titanate has gained momentum lately in the medical field, the aims of the present work are: (i) to assess BT toxicity and its mechanisms on the aquatic environment, using two photosynthetic organisms (*Anabaena flos-aquae*, a colonial cyanobacteria, and *Euglena gracilis*, a flagellated euglenoid); (ii) to study and correlate the physicochemical properties of BT with its toxic profile; (iii) to compare the BT behavior (and Ba^{2+} released ions) and the toxic profile in synthetic (Bold's Basal, BB, or Mineral Medium, MM) and natural culture media (Seine River Water, SRW); and (iv) to address whether size (micro, BT MP, or nano, BT NP) is an issue in BT particles toxicity. Responses such as growth inhibition, cell viability, superoxide dismutase (SOD) activity, adenosine-5-triphosphate (ATP) content and photosynthetic efficiency were evaluated. The main conclusions are: (i) BT have statistically significant toxic effects on *E. gracilis* growth and viability even in small concentrations (1 $\mu g\,mL^{-1}$), for both media and since the first 24 h; on the contrary of on *A. flos-aquae*, to whom the effects were noticeable only for the higher concentrations (after 96 h: $\geq$75 $\mu g\,mL^{-1}$ for BT NP and =100 $\mu g\,mL^{-1}$ for BT MP, in BB; and $\geq$75 $\mu g\,mL^{-1}$ for both materials in SRW), in spite of the viability being affected in all concentrations; (ii) the BT behaviors in synthetic and natural culture media were slightly different, being the toxic effects more pronounced when grown in SRW – in this case, a worse physiological state of the organisms in SRW can occur and account for the lower resistance, probably linked to a paucity of nutrients or even a synergistic effect with a contaminant from the river; and (iii) the effects seem to be mediated by induced stress without a direct contact in *A. flos-aquae* and by direct endocytosis in *E. gracilis*, but in both organisms the contact with both BT MP and BT NP increased SOD activity and decreased photosynthetic efficiency and intracellular ATP content; and (iv) size does not seem to be an issue in BT particles toxicity since micro- and nano-particles produced significant toxic for the model-organisms.

1. Introduction

The interaction between live organisms and micro- or nanosized materials has become a current focus in toxicology – or nanotoxicology, as it is been currently called (Dukhin et al., 2010). This is due, to a large extent, to the fact that smaller particles can be more toxic than bulk materials with the same composition, mostly because of their increased specific surface area and reactivity (Pang et al., 2012). However, a thorough understanding of the threats these materials pose to the environment is not completely available yet (Brayner et al., 2010).

Nanosized barium titanate (BT) has gained momentum lately in several fields, being a promising future biological nanocarrier for proteins (Ciofani et al., 2010a), as an enhancer of the uptake of low molecular weight drugs such as doxorubin (Ciofani et al., 2010b), as a biomarker, through the bioconjugation of its nanocrystals with immunoglobulin G (IgG) antibodies for imaging probes (Hsieh et al.,

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2010), as well as in the design of bone graft material (Ball et al., 2013). In the meantime, in vivo long-term biocompatibility experiments and studies on its impact on the environment have not yet been performed, to the best of the authors' knowledge. Therefore, it is a material with high future biomedical applications, but with scarce safety data.

For these investigations, physico-chemistry is paramount when trying to understand the fate and behavior of the particles on the environment (Brayner et al., 2010), to the extent that characteristics such as size, state of dispersion, surface charge, shape, chemical composition, surface area, and surface chemistry play important roles in the uptake and distribution of the particles within live organisms (Oberdörster et al., 2005a,b; Powers et al., 2006). Also, it is paramount that the particle characterization is performed in relevant media (Jiang et al., 2009), i.e., those where the ecotoxicological experiments will take place, wherein potential physicochemical changes (e.g., agglomeration/aggregation and surface charge variation) can occur with the particles in different solutions – which, in turn, can have direct impact on the toxicological responses (Powers et al., 2007). Since BT presents a perovskite structure, it is expected to show thermodynamic instability and reactivity in aqueous environment (Lee, 1998), one of the reasons why not only a natural culture media was used in this study, but also a synthetic one, with a high content of nutrients. Actually, different dispersions of BT either in aqueous or in non-aqueous media have already been studied (Khastgir and Adachi, 2000; Paik et al., 1998; Bergstrm et al., 1997; Lee, 1998, 1999; Wang et al., 2000), but none of them covered all of the characterizations listed here or were performed in biorelevant media for toxicological assays.

In this study, we used two different test organisms: a colonial cyanobacteria (*Anabaena flos-aquae*) and a flagellated unicellular euglenoid (*Euglena gracilis*), which are quite different morphologically and metabolically. This can be helpful to provide a spectrum of actions of the particles, although both are representatives of the first aquatic trophic level (producers). The use of primary producers as biological indicators is important because they are situated at the base of the food chain and any change in the dynamics of their communities can affect higher trophic levels of the ecosystem. Typically, they are also quite sensitive to changes in the environment and have relatively short life cycles, which allows the observation of toxic effects in several generations (Cleuvers and Weyers, 2003; Costa et al., 2008). Yet, the aquatic toxicity model was chosen because these ecosystems are the main enclosures of contaminants, whether they are coming from direct waste into water bodies through the discharge of effluents or released into the air or deposited in the soil (Kendall et al., 2001).

Thus, the aims of the present work are: (i) to assess BT toxicity and its mechanisms in two photosynthetic organisms (*A. flos-aquae* and *E. gracilis*); (ii) to study and correlate the physicochemical properties of BT with its toxic profile; (iii) to compare the BT behavior and the toxic profile in synthetic (Bold's Basal, BB, or Mineral Medium, MM) and natural culture media (Seine River Water, SRW); and (iv) to address whether size is an issue in toxicity of BT particles.

2. Materials and methods

2.1. The subjects of the study: BT particles and test organisms

2.1.1. BT characterization

Two different lots of commercial BT powders were obtained from Sigma–Aldrich, namely BT MP (barium titanate microparticle) (CAT no. 338842, lot MKBD3182V, <2 μm) and BT NP (barium titanate nanoparticle) (CAT no. 467634, lot MKBF7837V, <100 nm and cubic crystalline phase).

The X-ray diffraction (XRD) patterns of the powders were recorded with a X'pert Pro diffractometer (PANalytical), equipped with a multichannel X'celerator detector, and using the Co Kα radiation (= 1.790307 Å), in the 2θ range 5–120°, with a scan step of 0.05° for 5 s. The sample holder used was a Si monocrystal. Morphological observation of powders by scanning electron microscopy (SEM) was done using a Zeiss Supra 40 microscope equipped with an in-lens detector. Low excitation voltage (2.5 kV) and a small working distance (3 mm) were used, so that the charging effects were minimal to the point that the metallization of powders was not necessary, and then true features were not masked. Transmission electron microscopy (TEM) images were obtained using a JEOL 100CX-II microscope operating with an accelerating voltage of 100 kV. The specimens were prepared by ultrasonically dispersing the powders in ethanol prior to deposition on SEM mounts and carbon-coated TEM grids. The specific surface area (Sg) and pore size were measured by the collection of nitrogen (N_2) adsorption–desorption isotherms on a Gemini V 2380 system (Mic America, Inc.) at 77 K after the sample had been dried at 160 °C for 1 h. The Brunauer–Emmett–Teller (BET) surface area was calculated from the linear part of the BET plot. The pore size distribution was obtained from a QUANTACHROME-Autoscan 33 mercury porosimeter.

The hydrodynamic size and the surface charge/interparticle forces (zeta potential, ζ) of the dispersions of BT powders (100 μg mL^{-1}, or 4.28×10^{-7} M, – in BB medium and SRW) were characterized using a ZetaSizer Nano ZS (Malvern Instruments Inc.) utilizing dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively. Effects of pH variation in the media (3, 5, 7, 9, 12, adjusted with HNO_3 or NaOH in different molarities) were evaluated, as well as the degree of aggregation as a function of time (0, 24, 48, 72 and 96 h). The dispersions were sonicated (100 W, 5 min) daily before analysis in order to disperse agglomerates and so that it could be inferred that increased hydrodynamic sizes are due to aggregation. Response surfaces were built up in order to evaluate the roles each factor plays at the aggregation state or the surface charge of the BT dispersions. A 5^2 factorial experimental design (two factors, time and pH, with 5 levels of variations each) was used according to Montgomery (2012) and Polonini et al. (2011), which provide the theoretical background for the graphical representation used (see Supplementary Material). The factors and their levels (codified levels in parenthesis, and real ones outside them) were X_1: time [(−1) 0 h, (−0.5) 24 h, (0) 48 h, (0.5) 72 h, (1) 96 h] and X_2: pH [(−0.88) 3, (−0.44) 5, (0) 7, (0.44) 9, (0.88) 12].

The degree of dissolution of the BT powders as a function of time (1, 2, 4, 8, 24, 48, 72 and 96 h) within the media (BB medium and SRW) was evaluated following a protocol proposed by Sivry et al. (2014). Briefly, from a stock solution (100 μg mL^{-1}, final volume = 100 mL) prepared at time 0, aliquots (3.5 mL) were withdrawn at the specified time intervals and ultra-filtered using 3 kDa filters (Microsep Advance Centrifugal Device, Pall Corporation), placed in a centrifuge (EBA 8, Hettich) for 1 h, and then added with 50 μL of saturated HNO_3 (with no trace of Ba^{2+}). All solutions were immediately frozen until elemental analysis (Ba^{2+}) was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6200, Thermo Scientific). Detection limit was set as 1.0 ppb. The remaining aqueous dispersion (72 mL) was centrifuged at 20,000 × *g* (Sorvall Lynx 6000, Thermo Scientific) and the media was completely evaporated in an oven at 50 °C. The dry residues were then analyzed by XRD and X-ray photoelectron spectroscopy (XPS) (Thermo VG Escalab 250, using Al Kα of 1486.6 eV, 15 kV, 150 W) for evaluation of the chemical/surface changes that might possibly have occurred.

All reagents were analytical grade, and ultrapure water (18.2 MΩ cm resistivity at 25 °C and <10 ppb total organic carbon) was obtained with an Elga Pure-Lab Classic UV.