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Indoor illumination: A possible pitfall in toxicological assessment of photo-active nanomaterials



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ABSTRACT

Standardization of the experimental protocols used in the hazard assessment of nanomaterials (NMs) is strongly required to reduce inconsistency among data deriving by different laboratories. The parameters that are known to modify the toxic response of cells to NMs are in fact higher than for soluble toxicants. Among them illumination, that may induce activation of some semiconducting NMs, has been poorly investigated.

The present study, conducted within the FP7 EU project SETNanoMetro, has been designed to assess the effect of indoor illumination on the oxidative potential and dispersion degree of nano-TiO₂. The generation of Reactive Oxygen Species (ROS) by four nanometric anatase or rutile-anatase TiO₂ specimens under ordinary laboratory illumination has been evaluated by means of Electronic Paramagnetic Resonance (EPR) spectroscopy, while their ability to damage DNA has been measured by agarose gel electrophoresis using plasmid DNA as model. The effect of illumination on nanoparticles dispersion has been evaluated by Dynamic Light Scattering (DLS). The results show the occurrence of photo-activation of TiO₂ under indoor illumination that leads to the generation of ROS and slight plasmid DNA damage. Furthermore, significant differences in the amount of ROS generated were found for small variation of the intensity of the illumination. A small effect on the size distribution of TiO₂ agglomerates in water was observed.

The present findings suggest that illumination should be included among the parameters that have to be controlled during toxicological assessment of photo-active nanomaterials.

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1. Introduction

The knowledge of the hazard is a fundamental pre-requisite to reduce the risk associated to the exposure to chemicals. The current European regulation (REACH), places responsibility on industry to provide safety information on the substances. As consequence, test method standardization for hazard assessment is strongly needed [1,2]. In the case of nanomaterials (NMs) standardization is a particularly relevant issue. Numerous specific and non-specific factors have been shown to influence the results of toxicological testing [3], such as: i) the degree of dispersion of the NM, that varies depending upon the media used [4–6], ii) the

real dose, that, oppositely to molecular substances may not correspond to the nominal one [7,8], iii) the presence of contaminants in the materials like bacterial lipopolysaccharides (LPS) [9] or metal ions [10] iv) the occurrence of artefacts due to adsorption of reagents or intrinsic absorbance/fluorescence of the material [3,11,12]. Much less explored is the effect of illumination during the preparation of samples and NM exposure in toxicological testing. Except in the case in which a specific illumination is necessary due to the kind of endpoint evaluated, incubation of cells in *in vitro* tests is performed in the dark. On the other hand, the preparation of the NMs and the administration to cells is performed in laboratories illuminated by artificial or natural light. During these steps, photo-activation of semiconductors materials like ZnO, CeO₂, NiO or TiO₂ may occur since indoor natural illumination and some artificial light (e.g. halogen lamps) contains UV radiation.

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Among them titanium dioxide (TiO₂) and TiO₂-based materials are the most widespread NMs [13,14], being used for several purposes, e.g. as UV blockers in sunscreens and plastics [15,16]. TiO₂ is a powerful photo-catalyst. When illuminated with UV light it generates at its surface a high amount of reactive species, a property that finds application in several fields, like in water and air remediation [17] or in the production of self-cleaning coatings and textiles [18].

The adsorption of photons with energies higher or equal to the TiO₂ band gap (>3.2 eV for anatase) results in electrons to be excited in the conduction band (e⁻_{CB}) leading to the formation of a positive hole in the valence band (h⁺_{VB}). These charge carriers can recombine each other or migrate at the surface where they react with electron donors or acceptors that diffuse close to the surface [19]. For example, by reacting with water and oxygen, hydroxyl radicals (HO[•]), superoxide radicals (O₂^{•-}), singlet oxygen (¹O₂), hydroperoxyl radicals (•OOH) and hydrogen peroxide (H₂O₂) are formed. The generation of such oxygenated radicals and molecules, commonly called Reactive Oxygen Species (ROS), affects the ability of TiO₂ to interact with cells by increasing its oxidative potential, i.e. the ability to induce an oxidative burst [20,21].

The role of particle-derived ROS in the in the photo-toxicity of TiO₂ is well established [22,23]. On the other hand, the toxicity of non-illuminated TiO₂ is not expected to be related to them [24]. Nevertheless, several studies performed in the absence of specific illumination reported TiO₂-induced effects related to the occurrence of oxidative burst [25,26]. Whether it is a consequence of ROS generated by light-activated TiO₂ or of cell-derived ROS is not clear since in most of the studies the illumination condition used during NMs handling is not described.

Another well-known property of TiO₂ is the superhydrophilicity: under irradiation with UV light the abundance of hydrophilic groups at the surface of TiO₂ (Ti-OH) increases, an effect that is reversed in the dark [27]. Superhydrophilicity may affect the agglomeration degree of TiO₂ suspensions in water. In fact, particle agglomeration may occur in colloidal suspensions when particles exhibit a low osmotic repulsion. In this case attractive van der Waals forces and entropy driven surface dehydration prevail leading to agglomeration [28]. This largely depends on the thickness of the Stern layer around particles [29] that in turn depends upon particles surface chemistry, in particular the abundance and type of charged groups.

Indeed, a light-induced disaggregation of TiO₂ nanoparticles under UV light was previously reported [30].

The present study is aimed to assess the effect of indoor illumination on the oxidative potential and dispersion degree of nano-TiO₂. Four samples of fully characterized nano-TiO₂ in the anatase or anatase-rutile forms, the most photo-active ones, has been selected and analyzed for their ability to generate ROS in different illumination conditions by using a set of EPR-based tests previously proposed as integrated protocol for the assessment of biological-relevant photo-activity of TiO₂ [31]. The ability to damage DNA was also tested. Finally, the effect on the NM dispersion, evaluated by means of Dynamic Light Scattering (DLS) analysis was investigated.

2. Methods

2.1. TiO₂ samples

Four types of titania NMs were considered, three commercial materials (i.e. P25 by Evonik Industries, Germany; SX001 by Solaronix, Switzerland; and PC105 by Cristal, Saudi Arabia) and one lab-made TiO₂ prepared *via* hydrothermal synthesis, and then coded as UT001. Details of preparation, structural and

morphological characterization of the specimens are in Ref. [32]. In brief, UT001 was obtained by forced hydrolysis of an aqueous solution of Ti(Teoah)₂ complex (Teoa = triethanolamine; initial pH 10), carried out by hydrothermal treatment at 453 K for 90 h. Before the use, each material was suspended in water and then processed according to the following procedure in order to remove organic and inorganic impurities adsorbed onto TiO₂ NPs: i) dialysis against ultrapure water (MilliQ, Millipore) using a Spectra/Por dialysis membrane tubing (MWCO 8–12 kD or MWCO 12–14 kD); final pH of the permeated liquid in the 5–6 range, Cl⁻ and SO₄²⁻ <1 ppm (by ion chromatography); ii) freeze-drying; iii) re-suspension in milli-Q water; iv) irradiation for 48 h of the suspension in contact with air, added of 10 ml of H₂O₂ (30%), under UV light using a medium pressure mercury lamp (emission max at 360 nm), ca. 50 W/m² in the range 290–400 nm; followed by dialysis and free-drying as steps i) and ii). Step iv) ensures a complete photo-degradation of organic impurities adsorbed onto TiO₂ NPs that can change their surface properties and reactivity of TiO₂. H₂O₂ is used as electron scavenger to speed up impurities degradation.

2.2. Surface area measurements

The specific surface area (SSA_{BET}) of the powders was measured by adsorption of N₂ at 77 K, applying the BET model for the analysis of results

2.3. X-ray diffraction (XRD)

X-ray diffraction (XRD) pattern of the powders were recorded with an Analytical X'Pert Pro equipped with an X'Celerator detector powder diffractometer using Cu K α radiation generated at 40 kV and 40 mA. The instrument was configured with 1/2° divergence and receiving slits. A quartz sample holder was used. The 2 θ range was from 20° to 80° with a step size (°2 θ) of 0.05 and a counting time of 3 s.

2.4. Morphological characterization

TEM images were obtained with a Jeol 3010 instrument, operated at 300 kV. For the observation, powders were contacted in dry form with standard Cu grids coated with a lacey carbon film, and then introduced in the microscope. To evaluate the presence of aggregates the samples were also analyzed by Dynamic Light Scattering in 200 mM ammonia solution, in order to maximize the particles electrostatic repulsion, after 30 min sonication and adjusting the concentration depending on the sample characteristics. The Dynamic Light Scattering system used was an ALV (Langen Germany), NIBS model (non invasive backscattering) with fixed scattering angle (173°). Through the Stokes-Einstein equation the hydrodynamic radius r_H of the agglomerates/aggregates were obtained.

2.5. Diffuse reflectance UV–vis spectroscopy

The optical behaviour of the powders in the UV–vis range was investigated by electronic absorption spectroscopy in the diffuse reflectance mode. Spectra were acquired with a Cary 5000 instrument (Varian), equipped with an integrating sphere coated with Spectralon[®], also used as reference. In order to avoid side effects due to differences in particle packing, the powder cell provided by Varian was used, allowing pressing a sample toward the optically pure quartz window constituting the front part of the cell. Proper amounts of powders were used, resulting in layers of ca. 3 mm in thickness, thus reaching the usual condition for correct measurements in the diffuse reflectance mode [33].

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