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Nanomaterial synthesis and characterization for toxicological studies: TiO_2 case study

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ABSTRACT

In recent years it has become apparent that the novel properties of nanomaterials may predispose them to a hitherto unknown potential for toxicity. A number of recent toxicological studies of nanomaterials exist, but these appear to be fragmented and often contradictory. Such discrepancies may be, at least in part, due to poor description of the nanomaterial or incomplete characterization, including failure to recognise impurities, surface modifications or other important physicochemical aspects of the nanomaterial. Here we make a case for the importance of good quality, well-characterized nanomaterials for future toxicological studies, combined with reliable synthesis protocols, and we present our efforts to generate such materials. The model system for which we present results is TiO_2 nanoparticles, currently used in a variety of commercial products.

KEYWORDS: nanomaterials, nanoparticles, anatase, rutile, TiO_2, synthesis, toxicology, nanoform.

Introduction

NANOTECHNOLOGY involves the manipulation of materials and the creation of devices at the nm-scale. These processes often produce nanomaterials (nanoparticles and other nanostructures, such as tubes, rods, tripods) with one or more dimensions of 100 nm or less, synthesized to have special characteristics for dedicated applications. Nanotechnology has been described as the next technological revolution, but has also prompted concerns about the potential of nanomaterials to harm humans or the biosphere. One of the first authoritative reports to emerge was published by the Royal Society and the Royal Academy of Engineering (The Royal Society, 2004) and was followed by a number of governmental and intergovernmental studies from around the world, e.g. the European Commission (SCENIHR, 2005) and United States Environmental Protection Agency – USEPA (USEPA, 2007).

The main concern when considering the potential toxicity of nanomaterials is that the novel properties, present exclusively when the material is nano-sized and which could not have been foreseen based on bulk properties, may bring along novel potential for toxicity. Such toxicity may remain unnoticed, because it is likely to be due to physicochemical properties currently not included in standard toxicity screening tests (Oberdörster *et al.*, 2005). A further concern is that the rapid expansion of nanotechnology means there is a great number of nanomaterials to assess, many of which are already in industrial production (see for example: www.nanotechproject.org/consumerproducts). Because of the wide variety

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in physicochemical properties amongst different nanomaterials and their potential complex individual structures (see Fig. 1) it is not possible at present to predict which ones may cause harm.

Ecotoxicological studies with engineered nanomaterials have recently begun to emerge, whereas a number of studies on nanoparticle toxicity to humans, laboratory animals and *in vitro* studies already exist. A lot of the literature, however, appears to have produced contrasting conclusions, even for similar nanoparticles. Although it is expected that not all nanoparticles are hazardous, some of the inconsistencies may be the result of poor experimental protocols, insufficient characterization and the fact that the investigations may have been too limited to produce comprehensive results.

Until the mechanistic associations between nanomaterial characteristics and their toxicity are understood, comprehensive characterization of the nanomaterial used in toxicity studies is essential, so that specific properties can be linked to (or eliminated from) toxicity effects.

We make a case here that the development of reference nanoparticles for toxicological studies may be the best approach to allow standardization of the material used, and thus avoid material-induced uncertainties, and to guarantee that the nanoparticles have undergone thorough characterization. A methodology to develop such reference TiO_2 nanoparticles is discussed below.

Materials and methods

Synthesis of a range of TiO_2 nanoparticles was achieved by hydrolysis and oxidation of $TiCl_3$ using a number of adaptations of a published method (Cassaignon *et al.*, 2007). A $TiCl_3$ solution in 10–15% HCl was introduced to deionized water under vigorous stirring, producing a final solution strength of 0.15 M. The pH was adjusted using 2.5 M NaOH. The synthesis was carried out in air, either in a round bottom flask or a microwave vessel (MARS-X microwave system, XP-1500 vessels). The solids obtained were centrifuged, washed in acidic solution (HCl, pH = 1), followed by distilled water and dried at 60ºC overnight. All the chemicals used were from BDH Chemicals, without further processing or purification.

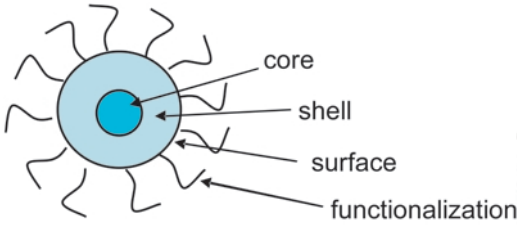

FIG. 1. A nanoparticle may consist of up to four distinct layers: (*a*) a core, which may be surrounded by; (*b*) a shell of a different composition; (*c*) a sub-nm surface layer, which, due to structural relaxation and chemical reactivity, will vary from the bulk; and finally (*d*) a surface functionalization, to give the nanoparticles specific surface properties (e.g. hydrophilicity, hydrophobicity).

The nanoparticles were characterized, using a variety of methods, to assess composition, size, surface area, purity, structure and crystallinity, but only selected XRD and TEM results are presented here. The XRD data were collected using a Nonius PDS 120 powder diffraction system consisting of an Inel curved, position sensitive detector (PSD). Measurements were made in reflection geometry with the sample surface at an angle of 7.5º to the incident beam (conditions: Cu-Kα_1, 45 kV and 32 mA). The TEM data were collected using a JEOL 2010, operating at 200 kV and a HITACHI H7100, operating at 100 kV. Sample preparation involved conventional mounting on a copper grid, using a drop of suspension, which was then allowed to dry at room temperature.

Results and discussion

There are many advantages in the dedicated synthesis of nanoparticles for toxicity studies. Firstly, synthesis allows tailoring the size and composition of the nanoparticles, as in the present study. The ability to produce a variety of sizes is important to allow rigorous links between size and toxicity to be tested and ultimately lead to a better mechanistic understanding of toxicity. An important consideration, however, when selecting synthesis protocols, is to chose a simple robust methodology and to ensure that it is flexible enough to allow synthesis across the widest possible range of sizes and production of phase-pure nanoparticles. If different methods are used to produce different-sized particles, there is a danger that variations in the synthesis method may introduce variations in the produced materials (e.g. different impurities, structural variation), which may in turn have an effect on toxicity. Synthesized particles should be produced

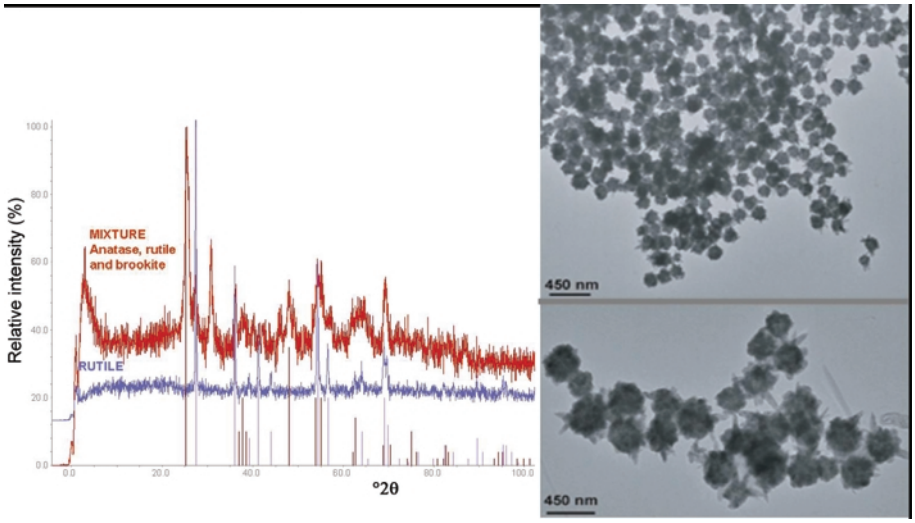

FIG. 2. XRD pattern and corresponding TEM images of TiO_2 nanoparticles formed at pH = 1.6 after 22 h at 78ºC, and rutile formed by annealing the sample at 900ºC.

using carefully selected chemicals for either synthesis or any further compositional manipulation of the particles (e.g. functionalization; see Fig. 1) and a wash protocol should be included after the synthesis to make sure all impurities are removed. A good control of particle-size range within each batch is also important if reliable links between toxicity and size are to be made. The produced nanoparticles should be dispersible in aqueous media. Once synthesis is complete, full characterization using a combination of physical and chemical methods in air and in appropriate solutions/solvents is essential.

The TiO_2 system is ideal to investigate for the purposes of generating a reference set of nanoparticles. This is because TiO_2 can be synthesized in various shapes (spheres, rods) and crystal forms (any one of three polymorphs or a mixture thereof) and its synthesis is well documented in the scientific literature. Furthermore, a number of toxicological studies using TiO_2 exist, some of which use a well-studied commercial TiO_2, uncoated nanosize Degussa P25, which, however, is well known to consist of mixed phases (anatase (70%) and rutile (30%)) (e.g. Long *et al.*, 2007).

Our synthesis focused firstly on identifying a robust methodology from the literature (Cassaignon *et al.*, 2007), which required simple chemicals with no inherent toxicity and had no need for capping agents to arrest particle growth. We then tested some of the synthesis variables, specifically pH, temperature and reactant concentration, to investigate how they affected size, shape and phase purity. When the synthesis was carried out at pH = 1.6 for 22 h and at 78ºC, star-shaped agglomerated particles were obtained (Fig. 2), each star being an auto-assembly of nanorods and nanospheres of mixed phases of

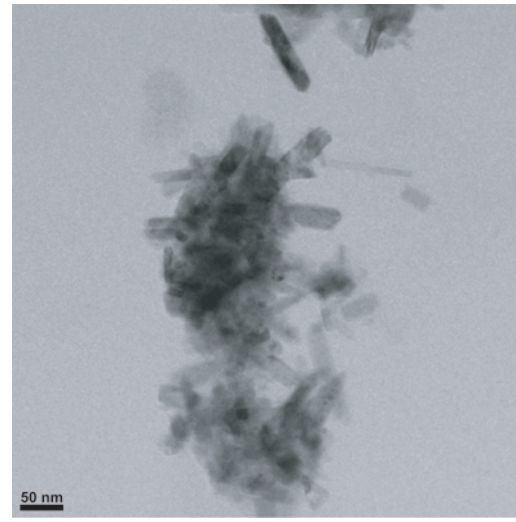

FIG. 3. TEM image of rod-shaped TiO_2, formed at pH = 1.5, after 24 h at 95ºC.

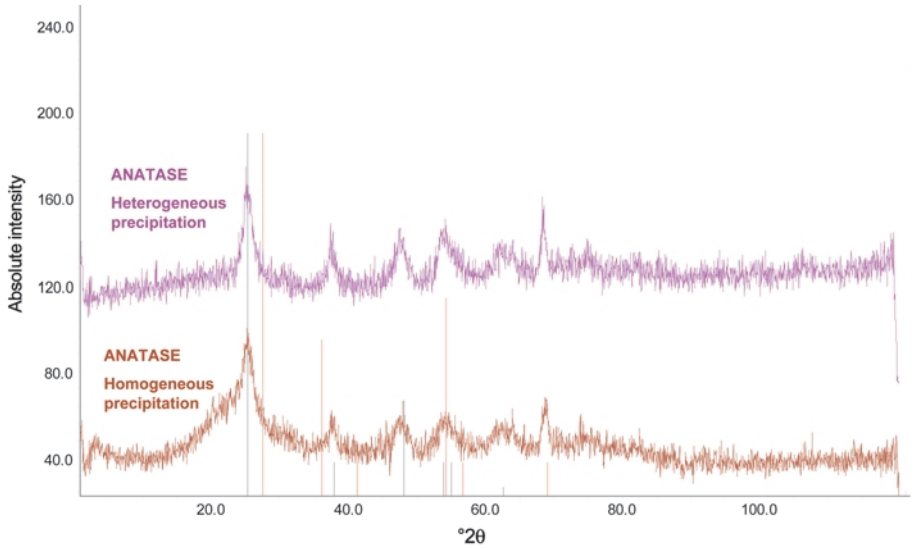

FIG. 4. XRD pattern of TiO_2 nanoparticles formed at pH = 1.5, T = 120ºC, P = 10 psi, heating time = 6 mins, constant T for 15 min through single (bottom) and double (top) synthesis cycle.

TiO_2. All three TiO_2 polymorphs (anatase, rutile, brookite) were identified by XRD, but the structures could be transformed to rutile by annealing at 900ºC (Fig. 2). However, the annealing process itself affected the structure of the aggregates, and this may in turn influence reactivity of the material, and hence potentially toxicity. The aggregate size was ~400 nm with individual particles <100 nm. We tested the method of Cassaignon *et al.* (2007) at high pH (6.7 and 12.4) and found that although the individual nanoparticles produced were smaller (<20 nm) they were also amorphous. We investigated the temperature effect further at pH = 1.5 and T = 95ºC (with 24 h of synthesis), we obtained nanorods of mixed rutile and anatase (50 nm) (Fig. 3), and by hydrothermal synthesis in a microwave system (pH = 1.5, T = 120ºC, P = 10 psi, heating time = 6 min, constant T for 15 min) we obtained 10 nm spheres of pure anatase (XRD in Fig. 4). We were able to grow these spheres by using the original 10 nm particles as seeds for further heterogeneous hydrothermal growth and this produced larger particles (up to 200 nm), which were also made of phase-pure anatase (Fig. 4); different solution concentrations were tested to optimize the growth process (data not shown). We are now following up this synthesis work with toxicological studies.

Conclusions

Further development of an existing simple and robust methodology for the synthesis of TiO_2 nanoparticles generated a series of well-characterised, good-quality particles of different sizes, shapes and structure, which can be used as reference materials in toxicological studies.

Acknowledgements

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