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Quantitative characterization of aggregated and agglomerated titanium dioxide nanomaterials by transmission electron microscopy

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ABSTRACT

The physical properties of TiO_2 nanomaterials are determined quantitatively using a method that combines imaging by transmission electron microscopy (TEM) with semi-automatic particle detection and analysis. The method is applied on four powdered TiO_2 nanomaterials, NM-102, NM-103, NM-104 and NM-105, dispersed in distilled water.

Qualitative analysis shows that the stability and polydispersity of the dispersed nanomaterials are influenced by the material from which the vial used for dispersion, is made. In glass vials, the uncoated nanomaterials, NM-102 and NM-105, precipitate immediately after sonication, while the coated nanomaterials, NM-103 and NM-104, remain stable in dispersion. In polypropylene vials, stable dispersions are obtained for all nanomaterials. It is shown that the vial material alters the pH of the dispersions, which in turn influences the agglomeration state of the nanomaterials.

Quantitative analysis of stable dispersions, based on TEM imaging combined with semi-automatic image analysis, results in number-based distributions of characteristic parameters, measuring the size, shape and surface topology of the unbound, aggregated and agglomerated TiO_2 particles. Iterative curve fitting is applied to the number-based distributions of selected parameters and allows objective comparison of the distributions based on the properties of the fitted curves. Using this method, it is shown that the size, the shape and the surface properties of NM-102 and NM-105 and of the coated nanomaterials, NM-103 and NM-104, are significantly different. The physical characteristics of NM-103 and NM-104 are similar. This supports the validity of the method as these are in fact the same material with a different coating.

1. Introduction

The latest technological developments allow manufacturing of titanium dioxide (TiO_2) materials with dimensions in the nanometer scale. These TiO_2 nanomaterials (NM) consist of unbound, aggregated and/or agglomerated particles with one or more external dimensions in the size range of 1 to 100 nm. Due to their specific properties, a number of new applications utilizing TiO_2 NM have recently been developed. Nano-sized TiO_2 is nowadays used in cosmetics and skin care products as a pigment, sunscreen or a thickener [1]. In addition, TiO_2 NM are applied in coatings and as photo catalysts in air and water cleaning [2]. The use of TiO_2 particles as food additive (E171) is approved in the European Union [3,4]. New methods remain to be developed, however, to show which fraction of this additive is present as NM as defined by the European Commission (EC) definition [5].

A different behavior of nano-sized TiO_2 compared to the bulk material has been observed and raises concerns about the general human and environmental exposure [6–10]. This different behavior can often be attributed to the increased surface-to-volume ratio. For example, Xiong et al. [11] show that a higher specific surface area of similar sized TiO_2 particles results in higher cytotoxicity and phototoxicity. Several other factors determine the activity of TiO_2 NM such as the particle size, the crystallinity and the morphology [9]. Monticone et al. [12] report changes in the electronic band structure with TiO_2 nanoparticle size. Additionally, Sayes et al. [13] demonstrate that the phase of the TiO_2 NM can play an important role as well. They show that the cytotoxicity of anatase TiO_2 is significantly higher than that of rutile TiO_2 for human dermal fibroblasts and human lung epithelial cells.

For safety testing [14] and to evaluate the risk of the application of manufactured NM in food [15], the Organization for Economic Co-operation and Development (OECD) and the European Food Safety Authority (EFSA) emphasize the need for a detailed characterization of manufactured NM by appropriate, validated testing methods. Such testing methods should include, as relevant, the characterization of properties of the constituent primary particles such as mean diameter and volume specific surface area (VSSA). These primary particle properties

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allow defining a NM as proposed by the EC [5]. In addition, the testing methods should measure physical properties and toxicology of aggregates and agglomerates [14–17]. The interaction between a NM and a biological system depends on the characteristics of these aggregated and agglomerated particles, such as size, morphology, surface topology, coating and charge [18–21]. It is, however, not evident to extend the conventional methods for biological and toxicological testing of bulk materials to manufactured NM [16].

In this paper, a testing method is proposed, which provides a detailed physical characterization of TiO_2 NM based on parameters that describe the unbound, aggregated and agglomerated particles of the NM. The method is applied on a range of powdered TiO_2 NM, dispersed in distilled water. The effects of the sample preparation conditions, such as the material from which the vial is made, the sonication time, the charge of the grid and the dilution, on the stability and polydispersity of the NM are examined. The possibility of characterizing the NM by a combination of conventional imaging by transmission electron microscopy (TEM) and semi-automatic image analysis, as reported earlier for synthetic amorphous silica NM [22], is explored. The method results in number-based distributions of characteristic parameters, measuring the size, shape and surface topology of the unbound particles and of the aggregates and agglomerates. Iterative curve fitting is applied to the number-based distributions of selected parameters. This approach allows objective comparison of the distributions based on the properties of the fitted curves.

2. Materials and methods

2.1. Nanomaterials

TiO_2 NM samples are obtained from the NM repository of the EC Joint Research Centre, Institute for Health and Consumer Protection (JRC-IHCP, Ispra, Italy), as dry powders in glass vials, sealed under argon atmosphere. The following NM are investigated: NM-102 (anatase, uncoated), NM-103 (rutile, hydrophobic coating), NM-104 (rutile, hydrophilic coating) and NM-105 (approximately 14% rutile and 86% anatase, uncoated). NM-103 and NM-104 have an Al_2O_3 surface coating. In addition, NM-103 is covered with a polymethylsiloxane layer (dimethicone) to render it hydrophobic, while NM-104 is treated with glycerin to render it hydrophilic. Specific material characteristics are summarized in [23–25].

2.2. Sample preparation

TiO_2 NM samples are prepared by applying a modified generic NANOGENOTOX dispersion protocol [26]. The original NANOGENOTOX protocol includes prewetting powders with ethanol, and dispersing the powders in distilled water containing 0.05 wt.% bovine serum albumin (BSA) at a concentration of 2.56 mg/ml, followed by sonication. In this work, ethanol pre-wetting of the NM is omitted. In addition, BSA is not added because it adsorbs onto the surface of the nanoparticles and influences agglomeration [24]. The dispersions are sonicated for 16 min using a Vibracell™ 75041 ultrasonifier (750 W, 20 kHz, Fisher Bioblock Scientific, Aalst, Belgium) equipped with a 13 mm horn (CV33) at 40% amplitude. This setup results in an average horn power of 25 W and a sample specific energy of 2.4 ± 0.2 GJ/m^3. During sonication, the samples are cooled to prevent excessive heating. To obtain a suitable amount of material on the EM-grid for quantitative analysis, after sonication, dispersions of NM-103 and NM-104 are diluted 5 times with distilled water to obtain a concentration of 0.51 mg/ml. The dispersions of NM-102 and NM-105 remain undiluted at a concentration of 2.56 mg/ml. The effect of the recipient on the stability and the polydispersity of the NM is examined by preparing samples of all NM in both glass vials (Wheaton Science Products, Millville, New Jersey, distributed by Fisher Scientific, nr. 10560503-X500) and polypropylene (PP) vials (VWR International B.V., Amsterdam, The Netherlands, nr. 216-2694). The pH of the dispersions is measured before and after sonication. The dispersed NM are brought on pioloform- and carbon-coated, 400 mesh copper grids (Agar Scientific, Essex, England) by the grid-on-drop method [27].

2.3. Imaging

The samples are imaged in bright-field TEM mode using a Tecnai G^2 Spirit electron microscope (FEI, Eindhoven, The Netherlands) with Biotwin lens configuration operating at 120 kV. The methodology described by De Temmerman et al. [22] is followed. To avoid subjectivity in the selection of particles, images are recorded at positions predefined by the microscope stage, and evenly distributed over the entire EM-grid area. Per sample, ten images are recorded with a 4×4 k Eagle CCD camera (FEI) using TEM imaging and analysis (TIA) software (FEI) at a magnification suitable for analysis. Appendix A presents the selected magnifications for each NM, together with the corresponding pixel size and field of view. To obtain a maximum traceability of information, each image is stored in a dedicated database integrated in iTEM software (Olympus, Münster, Germany), together with the administrative information, the sample preparation conditions and the imaging conditions, as described in [22].

2.4. Image analysis and data processing

Threshold-based detection of the NM is applied using iTEM software. This approach includes optimizing the contrast and brightness of the image, applying a 10×10 smoothing filter, enclosing the particles in a region of interest (ROI) and setting the threshold to separate particles from the background based on their gray values. Particles consisting of less than 150 pixels, particles on the border of the ROI and structures with an aberrant morphology, judged to be artifacts based on visual inspection, are omitted from analysis. Twenty three characteristic parameters, which are described in Appendix B, are measured for each particle. The data collected for each characteristic parameter is represented as a number-based distribution using SigmaPlot software (Systat, Cosinus Computing, Drunen, The Netherlands). The analysis conditions and the results of the analysis are stored in the iTEM database and are linked to the original image.

The characteristic parameters are grouped into classes by examination of the correlation matrix. To characterize the NM in detail, at least one representative parameter is selected from each of the classes. The correlation matrix and classes are further described in Section 3.4.

To compare number-based distributions of different materials and experiments, iterative curve fitting is applied by using Fityk 0.9.8 software [28]. The Levenberg–Marquardt algorithm is used to fit a predefined model to the number-based distributions of the representative parameters of the classes. To assess whether the model is appropriate, the goodness-of-fit is evaluated based on the weighted sum of squared residual (WSSR) value. The uncertainties (standard errors) associated with the fitted model, are computed as described in [29]. These standard errors indicate how precisely the height, width, mode and asymmetry of the distribution of a characteristic parameter are estimated by the model.

3. Results

3.1. Sample preparation effects

After 16 min of sonication, stable dispersions of samples NM-103 and NM-104 are obtained, regardless whether the preparation is performed in glass vials or in PP vials. In addition, TEM inspection shows that particles are homogeneously distributed over the EM-grid surface and that after dilution, the particles are well separated with only occasional overlap.