Generating Nano-Aerosols from TiO$_2$ (5 nm) Nanoparticles Showing Different Agglomeration States. Application to Toxicological Studies

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Generating Nano-Aerosols from TiO₂ (5 nm) Nanoparticles Showing Different Agglomeration States. Application to Toxicological Studies

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INTRODUCTION

Manufactured nanoparticles (NP) are known for their exceptional physicochemical properties, attributable to their nanoscale structure. It is anticipated that the nanometric properties will influence the biological behavior of these substances and, consequently, there is increasing concern associated with the growing use of nanostructured materials in the industrial workplace. NP inhalation is an important route of human exposure. Data on pulmonary effects related to human NP exposure are currently rare, but some useful information can be obtained from animal inhalation studies, which are more numerous.

Since the characteristics and properties of the NP present in the nano-aerosol can differ greatly from those of the bulk substances, a rigorous characterization of the inhaled aerosol is necessary. Significant efforts must be made to document the structure and size of the agglomerates formed in the aerosols, just as for other exposure metrics such as mass, number, volume, and surface. Consequently, research is required on the development and implementation of standardized methods for NP exposure assessments in the workplace and in experimental settings.

To our knowledge, 16 studies have addressed the pulmonary effects of TiO₂ NP during inhalation experiments in rodents. Several used dry powder dispersion techniques, NP generator/reactor, or nebulization of TiO₂ suspensions. Only three of these studies have reported generating aerosols with a count median diameter situated in the nanoscale (<100 nm). However, time exposure of these experiments were relatively short: 30 min, 1 hr, or 2 hr.
NP are very reactive and tend to agglomerate easily when in contact with each other, whether in the starting powder or in the generated aerosols. Agglomeration of NP is a quick process that depends on a number of factors, including concentration and primary particle size. As particle size decreases, the attractive force per unit mass becomes important, which favors agglomeration. It is also difficult to redisperse agglomerates consisting of small particles. Hence, it is actually a challenge to generate stable and reproducible aerosols composed of NP agglomerates (<100 nm) at concentrations and exposure times suitable for toxicological studies.

Thus, this study developed four different exposure scenarios using the same primary TiO2 NP to consistently generate nano-aerosols over 6 hr and produced nano-aerosols composed mainly of nanoscale (<100 nm) agglomerates. The two exposure concentrations selected for our study, 2 and 7 mg/m3, are based on previous animal inhalation studies. Hence, the originality of our study is derived from the first-time reporting the parameters associated with the sustained generation of TiO2 nano-aerosols at relatively high concentrations mainly composed of NP agglomerates having an aerodynamic diameter smaller than 100 nm. These nano-aerosols will be used in an animal inhalation experiment to eventually compare their relative toxicity.

METHODS

General Experimental Study Design

This study combined the use of current aerosol generation devices to obtain four exposure conditions from the same nanopowder. Nano-aerosols composed of large agglomerates (LA) (>100 nm) or small agglomerates (SA) (<100 nm) at 2 and 7 mg/m3 were produced, measured, and characterized for shape and structure. Nano-aerosol generation was performed for 6 hr in a cubic stainless steel 500-L rodent inhalation chamber adapted for nose-only exposures (Unifab, Kalamazoo, Mich.).

Nanoparticles

TiO2 NP, anatase, 5 nm, 200–220 m2/g specific surface area with a near spherical morphology (Nanostructured and Amorphous Materials Inc., Houston, Texas) were stored in a desiccator placed in a fume hood prior to use. These NP were characterized by transmission electron microscopy (TEM) (field emission gun [FEG] JEOL JEM-2100F) and X-ray diffraction (XRD) (X’Pert model; Philips, Lelyweg, Netherlands).

Nebulization of 5 nm TiO2

Generation of nano-aerosols composed of SA was achieved using a six-jet Collison device (BGI Inc., Waltham, Mass.) placed in parallel with a Delavan siphon spray nebulizer (Part number 30609-2 used with an adapter, part number DLN 17147; Delavan Spray Technologies, Goodrich Corp., Montreal, Canada) (Figure 1a). A 5 g/L NP suspension in distilled water (Milli-Q reference A+ system, water purification system with total oxidizable carbon indicator, Millipore Corp., Billerica, Mass.) was filtered on Whatman 41 filter paper (Piscatway, N.J.) to remove large agglomerates and sonicated for 10 min (Bransonic tabletop ultrasonic cleaner, model 5510; Branson, Danbury, Conn.). This suspension was poured in the Collison and Delavan devices. Since the filtered suspension did not allow reaching the targeted mass concentration, 2 g (2 mg/m3 experiment) or 7 g (7 mg/m3 experiment) of the TiO2 powder was added in the Collison device, which contained 450 ml of the original suspension. This new suspension was not sonicated. The suspension was agitated for the entire generation period using magnetic stirring plates placed under each nebulizer. A syringe pump was used at a rate between 5.1 and 8.4 ml/hr to gradually add suspension in the Collison device. Dual-element heating tapes (624 watts, 120 VAC; Cole-Parmer, Montreal, Canada) wrapped around a copper tube were used to dry the aerosol prior to its dispersion in the inhalation chamber.

The suspension used to generate the aerosol composed of SA at 2 mg/m3 was characterized by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). For TEM, the suspension samples were first allowed to settle for 1 hr. A droplet of sample was then placed on a 400-mesh TEM copper grid covered by a carbon film and left to dry under laminar flow conditions for 30 min. The electron microscope was operated at an accelerating voltage of 80 kV, (JEM 1200 EX; JEOL, Peabody, Mass.). Analysis was also performed using an NTA (LM20; NanoSight Ltd., Amsbury, U.K.) with a laser output of 30 mW at 650 nm, also on diluted (10/50) samples. Further information on the characterization techniques that were employed to characterize the NP can be found in Domingos et al.

Powder Dispersion of 5 nm TiO2

The 2 mg/m3 nano-aerosol composed of LA was generated using a Palas RBG-1000 device (Palas GmbH, Karlsruhe, Germany) placed online with a homemade Venturi flow ejector that disperses the aerosol (Figure 1b). The 7 mg/m3 nano-aerosol was produced using a Fluidized Bed 3400A device (TSI Inc., Shoreview, Minn.) (Figure 1c). Exposure concentrations were achieved by adjusting the various feed rates of the respective generators. No charge neutralization was performed.

All four aerosols were generated using compressed air. The compressed air going through the system first passed through a Donaldson high-efficiency industrial filter equipped with a coalescing filter (Model-DF 0070, Ultra-Filter Superplus; Donaldson Co., Inc., Bloomington, Minn.). The average temperature and relative humidity in the inhalation chamber were 23.1 ± 0.7°C and 37.5 ± 21% RH. For the 7 mg/m3 experiments, a fan was placed in the inhalation chamber. More details on airflow, generator pressure, and other experimental conditions related to the generation of nano-aerosols are found in Table I.

TiO2 Aerosol Sampling and Characterization

Air samples were collected throughout the experiment (6 hr) on cassettes (Sure Seal; SKC Inc., Eighty Four, Pa.) using 37-mm polyvinyl chloride (PVC) filters at a flow rate of
FIGURE 1. Experimental setups for the generation of nano-aerosols (A) Generation system for the nano-aerosols composed of SA at 2 and 7 mg/m³. Atomization of a TiO₂ suspension with a six-jet Collison and a Delavan. (B) Generation system for the nano-aerosol composed of LA at 2 mg/m³. Dry NP powder dispersion with a Palas and a Venturi. (C) Generation system for the nano-aerosol composed of LA at 7 mg/m³. Dry NP powder dispersion with a fluidized bed.
TABLE I. Experimental Conditions for Nano-Aerosol Generation

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Generation Techniques</th>
<th>Total Airflow Entering Chamber (m³/hr)</th>
<th>Generator Pressure (psi)</th>
<th>Specific Parameters</th>
<th>Reynolds Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 mg/m³ SA&lt;sup&gt;A&lt;/sup&gt;</td>
<td>Collison and Delavan</td>
<td>4.80</td>
<td>30</td>
<td>—</td>
<td>7646 (turbulent)</td>
</tr>
<tr>
<td>2 mg/m³ LA&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Palas and Venturi</td>
<td>4.77</td>
<td>58–60</td>
<td>Brush speed = 2 mm/hr and 1205 RPM</td>
<td>7591 (turbulent)</td>
</tr>
<tr>
<td>7 mg/m³ SA&lt;sup&gt;A&lt;/sup&gt;</td>
<td>Collison and Delavan</td>
<td>4.20</td>
<td>32</td>
<td>—</td>
<td>6693 (turbulent)</td>
</tr>
<tr>
<td>7 mg/m³ LA&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Fluidized bed</td>
<td>4.80</td>
<td>15</td>
<td>Bed speed = 40, bed flow = 10, bead purge = 2</td>
<td>7646 (turbulent)</td>
</tr>
</tbody>
</table>

<sup>A</sup>Nano-aerosol composed of small agglomerates.
<sup>B</sup>Nano-aerosol composed of large agglomerates.

4 L/min for gravimetric analysis. Filters were weighted on a model MX5 microbalance, (Mettler Toledo, Mississauga, Canada). The mass concentrations were followed and adjusted in real-time using a Model 8520 DustTrak Aerosol Monitor (TSI Inc.) previously calibrated with TiO₂ by comparison with the gravimetric method. The DustTrak recorded the mass concentration every minute and reported the min and max for each 6-hr experiment. Air samples were also collected at a flow rate of 1 L/min on pre-carbon coated Formvar copper grids glued onto 25-mm polycarbonate filters. The glue used was a current cyanoacrylate (Loctite superglue gel; Henkel, Boucherville, Canada) and applied only on a small spot at the periphery of the grid to prevent damage on removal. The sampling durations were 10 and 20 min for 7 and 2 mg/m³ nano-aerosols, respectively. Characterization (shape, agglomeration degree, and structure) of the nano-aerosols sampled on these grids was performed by TEM (Philips CM200 equipped with digital camera; AMTV600 2Kx2K, 80 kV; Corel Corp., Ontario, Canada). Numbers and particle size distributions were monitored in real-time with an electrical low pressure impactor (ELPI) (Dekati Ltd., Tampere, Finland), which was operated at a flow rate of 10 L/min in the filter stage configuration. The sintered impaction substrates were oiled to prevent or reduce particle bounce. NP/agglomerates were classified according to their aerodynamic diameter (7 to 10000 nm). Cumulative size distributions were acquired for the entire aerosol generation period (6 hr). Since the density and the shape of agglomerates change from an impactor stage to another, establishing a unique particle density for the entire aerosol was complex. For this reason a density of 3.5 g/cm³ was used. It was the closest value to TiO₂ bulk density, which is between 3.1 and 4.3 g/cm³. The ELPI was also used to determine the median aerodynamic diameter based on the number concentration (NMD). Air samples were all collected in the exposure chamber use in this study can be separated into two distinct zones. A mixing zone where two air jets (filtered air and the aerosol) from the supply vents collide, providing significant turbulence and mixing, and a breathing zone where the nanoparticle mass fraction is fairly uniform with no stagnant areas. The Reynolds number and the flow regime in the breathing zone are shown in Table I.

NP transport and diffusion in the exposure chamber were modeled to verify aerosol spatial uniformity as described and reported in Morency and Hallé. Briefly, based on the assumption that NP behave as a passive contaminant, the aerosol dispersion in a velocity field (airflow) was solved with an Eulerian model governed by the Reynolds averaged mass transport equation for the contaminant. Diffusional losses of airborne NP were taken into account by imposing a zero mass fraction at solid walls, and the Brownian diffusion coefficient was determined with the Friedlander model. Airflow was modeled by the three-dimensional Reynolds-averaged Navier-Stokes equations coupled to the k-ε turbulence model.

Both Brownian and turbulent diffusion are considered in the numerical model. Turbulence in the exposure chamber generates diffusion effects that are larger than Brownian diffusion. These turbulent diffusion effects are characterized by the turbulent Schmidt number in the concentration equation. However, the intensity of turbulence becomes negligible near solid walls; and the Brownian diffusion is the dominant mechanism in that region. The zero mass fractions imposed at solid walls assume that nanoparticles are trapped at the walls and neglect their rebound. The diffusive boundary layer is thinner than the momentum boundary layer because of the low diffusion coefficient values. Particular attention has been paid to node spacing near walls to make sure the diffusive boundary layer is captured correctly. The inhalation chamber use in this study can be separated into two distinct zones. A mixing zone where two air jets (filtered air and the aerosol) from the supply vents collide, providing significant turbulence and mixing, and a breathing zone where the nanoparticle mass fraction is fairly uniform with no stagnant areas. The Reynolds number and the flow regime in the breathing zone are shown in Table I.

RESULTS

Characterization of Initial Bulk NP

TEM analysis showed that the powder was composed mainly of 5 nm particles essentially present in the form of small agglomerates of 10 to 30 nm. Particles had a spherical or
rod-shaped morphology (Figure 2). TEM also revealed the presence of very large agglomerates, composed of multiple primary NP, reaching few nanometers to microns (Figure 2). Analysis by XRD revealed that the TiO$_2$ NP were predominantly in the anatase form and to a lesser degree (<10% volume) in the rutile form.

**Characterization of NP Suspension**

The NP suspension used to generate the nano-aerosol composed of SA via nebulization at 2 mg/m$^3$ was analyzed using two techniques. TEM analysis showed that the NP are present in the suspension as small agglomerates. For two samples of 50 particles analyzed, the average size of agglomerates was $n_1 = 116 \pm 46$ nm and $n_2 = 134 \pm 45$ nm. Analysis by NTA showed that the sample was fairly polydisperse, with sizes of agglomerates in the same order of magnitude as shown by TEM, 180 \pm 98 nm and 135 \pm 78 nm, for samples that had been diluted tenfold. A slightly larger sample dilution (50-fold) also gave similar values: 166 \pm 90 nm and 177 \pm 79 nm.

**Characterization of Nano-Aerosols**

Details on the size distributions of the four nano-aerosols generated are shown in Table II. The mass concentrations measured gravimetrically were within 2.1% of the targeted exposure concentrations of 2 and 7 mg/m$^3$. As measured with the ELPI, total particle number was higher in the aerosols composed of SA compared with the aerosols composed of LA for the same mass concentration. Total particle number would also be expected to increase with mass concentration for the same aerosol size distribution, which is observed for the aerosols composed of SA generated at 2 and 7 mg/m$^3$, using a wet generation system, but not for the aerosols composed of LA produced by dry dispersion techniques. As will be discussed below, size distribution profiles of the nano-aerosols (Figure 3) can explain this difference. NMAD (D$^{50}$) represents the aerodynamic diameters for which 50% of the particles present in the aerosol are smaller than this value. D$_{25}$, D$_{50}$, and D$_{75}$ were calculated from the cumulative size distributions based on the number concentrations (Figure 4) acquired by the ELPI for the four aerosols. The D$_{50}$ obtained for the nano-aerosols generated by nebulization were 30 and 31 nm for the 2 and 7 mg/m$^3$ exposure concentrations, respectively, whereas the D$_{50}$ values of 185 (2 mg/m$^3$) and 194 nm (7 mg/m$^3$) were obtained for the nano-aerosols generated by powder dispersion techniques (Table II). D$_{75}$–D$_{25}$ is the interquartile range and represents the size range where 50% of the particles are found. The interquartile ranges for the nano-aerosols composed of SA or LA did not overlap for a given concentration.

In this study, the nanometric size range has been defined as being between 1 and 100 nm. As shown in Table II, the percentage of agglomerates showing an aerodynamic diameter <100 nm was also estimated for each aerosol. For nano-aerosols generated by nebulization, a majority of the agglomerates, determined by number concentration, were in the nanometric size range, i.e., 86% (2 mg/m$^3$) and 91% (7 mg/m$^3$), whereas only 22% (2 mg/m$^3$) and 35% (7 mg/m$^3$) of the agglomerates were smaller than 100 nm for the powder dispersion technique. For this latter technique, agglomerates were mainly in the submicron range (100–1000 nm). Figure 5 shows representative TEM images of particle deposition patterns for the 7 mg/m$^3$ nano-aerosols composed of SA or LA, which are consistent with the aerosols’ size distribution determined with the ELPI, as will be discussed below. In addition, TEM images allowed the observation of qualitative differences in the structure of the agglomerates present in both nano-aerosols generated at 2 mg/m$^3$ (Figure 6). For a similar agglomerate size, the particles generated by nebulization show a more compact structure (Figure 6A) than those generated by powder dispersion (Figure 6B).
**TABLE II. Nano-Aerosol Measurements**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2 mg/m³ SA(^a)</th>
<th>2 mg/m³ LA(^b)</th>
<th>7 mg/m³ SA</th>
<th>7 mg/m³ LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass concentration(^c)</td>
<td>2.02</td>
<td>1.96</td>
<td>7.12</td>
<td>7.15</td>
</tr>
<tr>
<td>Min and max(^d) (mg/m³)</td>
<td>1.50 and 2.74</td>
<td>1.77 and 3.34</td>
<td>5.79 and 8.98</td>
<td>6.35 and 8.92</td>
</tr>
<tr>
<td>Total particle number(^e) (/cm³)</td>
<td>1 187 491</td>
<td>161 898</td>
<td>7 644 322</td>
<td>19 573</td>
</tr>
<tr>
<td>NMAD(^f) (nm)</td>
<td>30</td>
<td>185</td>
<td>31</td>
<td>194</td>
</tr>
<tr>
<td>D(<em>{25}) and D(</em>{75})(^g) (nm)</td>
<td>17 and 61</td>
<td>108 and 284</td>
<td>17 and 49</td>
<td>81 and 470</td>
</tr>
<tr>
<td>Fraction of NP agglomerates &lt;100 nm (%)</td>
<td>85.6</td>
<td>22.3</td>
<td>90.9</td>
<td>35.3</td>
</tr>
</tbody>
</table>

All measurements (cassettes, DustTrak, ELPI) were recorded over the entire 6-hr period.

\(^a\)Nano-aerosol composed of small agglomerates.

\(^b\)Nano-aerosol composed of large agglomerates.

\(^c\)Measured by weight measurement.

\(^d\)Measured with a DustTrak.

\(^e\)Measured with an ELPI.

\(^f\)Number median aerodynamic diameter, measured with an ELPI.

\(^g\)Aerodynamic diameters for which 25% (D\(_{25}\)), 50% (D\(_{50}\) or NMAD), or 75% (D\(_{75}\)) of the particles in the aerosol are smaller than this value.

**FIGURE 3.** Size distribution profiles of nano-aerosols measured by the ELPI size distribution profiles based on number collected over 6 hr measured by the ELPI for the (A) nano-aerosol composed of SA at 2 mg/m³ produced by a wet method using Collison and Delavan nebulizers; (B) nano-aerosol composed of LA at 2 mg/m³ produced by a dry dispersion technique using a Palas and a Venturi; (C) nano-aerosol composed of SA at 7 mg/m³ produced by a wet method using Collison and Delavan nebulizers; (D) nano-aerosol composed of LA at 7 mg/m³ produced by a dry dispersion technique using a Fluidized Bed.
Simulation results based on passive contaminant behavior for both SA and LA suggested that the mass fraction in the inhalation chamber, more specifically in the area normally corresponding to animal's breathing zone, was uniform with a standard deviation of 4%. Zhao and Wu (30) suggested an indicator determined by the product of nominal time ($\tau_n$) and relaxation time ($\tau_r$) to decide whether the airborne particles could be treated as a passive contaminant. According to Zhao and Wu, if this product ($\tau_n \times \tau_r$) is smaller than $2.8 \times 10^{-2}$ s$^2$, a passive scalar behavior can be assumed. Determination of this indicator for a TiO$_2$ spherical particle having an aerodynamic diameter of 1 $\mu$m gives a value of $1.26 \times 10^{-3}$ s$^2$, which is 22 times smaller than the upper limit value of Zhao and Wu.

**DISCUSSION**

This study reports the experimental conditions related to the generation and characterization of TiO$_2$ (5 nm) aerosols showing different agglomeration states (NMAD of 30 and 190 nm). This was done for two mass concentrations: 2 and 7 mg/m$^3$. The originality of our study is derived from the first time reporting the parameters associated with the sustained generation (6 hr) of TiO$_2$ nano-aerosols composed mainly of NP agglomerates having an aerodynamic diameter smaller than 100 nm. Indeed, for SA aerosols, 86% to 91% of the particles had diameters under 100 nm and were generated by atomizing a liquid suspension. The novelty of our study was achieved by combining different generating devices to obtain four different exposure conditions from the same nano-powder. These devices included the Palas, the Fluidized Bed, the Delavan, and the Collison. The latter is considered as the device offering the best characteristics for inhalation studies by generating aerosols showing stable numbers and mass concentrations over time,(31) which are important criteria for animal inhalation experiments.

In this study, considerable effort was invested in the generation, measurement, and characterization of nano-aerosols. Several experimental parameters such as the concentration and the filtration of TiO$_2$ suspension, the quantity of nano-powder placed in the nebulizers, the use of stirring plaques, the dilution airflow, and the adjustment of the various feed rates of the generators were optimized to generate the aerosols having the required characteristics of concentrations and size distributions. This also led to the selection of the combination of the Collison and Delavan nebulizers for the SA aerosols. Based on several generation experiments, we observed that the Collison generated stable relatively high mass concentrations as previously reported(23) and that the Delavan produced a large amount of small agglomerates as will be discussed below.

The nano-aerosols composed of LA were produced with two different dry-dust generators, a Palas and a Fluidized Bed. The Palas was used for the 2 mg/m$^3$ aerosol and the Fluidized Bed was chosen for the 7 mg/m$^3$ experiment because it showed more stability at higher mass concentrations. In addition, these two types of generators have already been used in rodent TiO$_2$ inhalation exposures and have resulted in aerosolization of large agglomerated TiO$_2$ NP,(4,15,17,18,31) Indeed, dry powder dispersion techniques usually result in greater agglomeration (>100 nm), since agglomerates of primary NP in the nano-powder are strongly bonded, thereby
FIGURE 5. Nano-TiO$_2$ agglomerates collected from the nano-aerosols at 7 mg/m$^3$. Images obtained by TEM with 2500× magnification. (A) Nano-TiO$_2$ agglomerates collected from the nano-aerosol composed of SA; (B) nano-TiO$_2$ agglomerates collected from the nano-aerosol composed of LA.

reducing the chance of dispersion of small agglomerates or single NP.$^{32,33}$ Furthermore, these techniques are subject to the creation of very high particle surface charges that can affect agglomeration, and the mechanical energy applied is often not sufficient to reduce cluster size in the powder. Since an increase in mass concentration implies an increase in agglomeration of particles,$^{4,34}$ the generation of aerosols mainly composed of SA (diameters <100 nm) at relatively high mass concentration constitutes a greater challenge.

To our knowledge, few studies have reported generating TiO$_2$ aerosols in the nano-size range, however, mass concentration or exposure time was not always reported.$^{16,17,21,31,32}$ Using a controlled gas-phase NP generator/reactor, Leppänen et al.$^{(21)}$ generated for 30 min an 8 mg/m$^3$ TiO$_2$ (20 nm) aerosol characterized by a size distribution having a geometric mean particle diameter of 91 nm (GSD: 1.6). Using an electrospay system, Kim et al.$^{(32)}$ generated a TiO$_2$ (25 nm) aerosol characterized by a peak at 60 nm for the size distribution, but no exposure time was reported. These authors also noted that mass concentration could be increased up to 20 mg/m$^3$ by adjusting the suspension concentration, but the results were not reported. Schmoll et al.$^{(31)}$ reported aerosolization of 5 nm TiO$_2$ NP with an electrospay and measured a geometric mean of 24 nm (GSD: 1.71) in aerosol (exposure time and mass
FIGURE 6. Nano-TiO$_2$ agglomerates collected from the nano-aerosols at 2 mg/m$^3$. Images obtained by TEM (A) with 45,000× magnification, typical tight or dense nano-TiO$_2$ agglomerate collected from the nano-aerosol composed of SA produced by nebulization; (B) with 33,000× magnification, typical nano-TiO$_2$ agglomerate with empty spaces collected from the nano-aerosol composed of LA produced by powder dispersion.

concentration not reported). In this same study, aerosolization of 21 nm TiO$_2$ NP under the same conditions resulted conversely in capillary clogging and thus failed to produce stable counts over time, showing a limitation related to electrospray.

In the context of toxicological studies, two other research groups have generated TiO$_2$ nano-aerosols using the nebulization technique. Ma-Hock et al.($^{18}$) used a two-component nebulizer (Schlick model 970) to produce TiO$_2$ (10–50 nm) aerosols and reported a median count distribution of 210 nm at 9.8 mg/m$^3$. Grassian et al.($^{23}$) used a Collison to generate a TiO$_2$ (5 nm) aerosol at a concentration of 7.22 mg/m$^3$ and reported a geometric mean of 119.5 nm (GSD: 1.56). While the nebulization technique and heating of particles theoretically favors the generation of ultrafine aerosols,($^{32,35}$) these authors did not succeed in generating aerosols composed mainly of particles with diameters smaller than 100 nm. An original aspect of our study is the addition of the Delavan in parallel with the Collison. The Collison enabled us to generate aerosols at relatively high mass concentration (e.g., 7 mg/m$^3$), while the use of the Delavan allowed the NMAD (31 nm) to be lowered by increasing the number of small NP agglomerates in the aerosol. The higher airflow that passes through the Delavan nebulizer helps to reduce the size of the droplets and might be a reason explaining this difference. Thus, the type and number of nebulizers used constitute important factors contributing to the ultrafine size distribution of the aerosols.

However, in Ma-Hock et al.($^{18}$) and Grassian et al.($^{23}$) a scanning mobility particle sizer (SMPS) was used to determine a mobility diameter; in our study, an ELPI was used to determine an aerodynamic diameter. These two types of diameters are not directly comparable since they are based on different sizing techniques and units. Previous studies($^{19,36,37}$) have compared these two devices in the characterization of the ultrafine particle size and number concentration in aerosols. Overall, these two devices showed good agreement and suggested that the aerodynamic diameters could be larger than the electrical mobility diameter.$^{(37)}$

As shown in Table II, nano-aerosols composed of SA had a higher number of particles per cubic centimeter compared with those composed of LA. This is consistent in that a greater number of small particles per cubic centimeter is expected compared with larger particles.$^{(9,15,18,22,23,34)}$ The total particle number would be expected to increase with mass concentration, which is observed for the nano-aerosols composed of SA generated at 2 and 7 mg/m$^3$ but not for the nano-aerosols composed of LA. Size distribution profiles of these nano-aerosols can explain this difference (Figures 3b,d). Despite
a similar median value, there were proportionally more LA in the 7 mg/m^3 aerosol than in the 2 mg/m^3 aerosol. Therefore, increasing the mass concentration from 2 to 7 mg/m^3 was achieved by changing the particle size distribution profile. The use of a Venturi in the 2 mg/m^3 experiment can explain this difference since this flow ejector device redesperses the nano-aerosol, resulting in a finer aerosol with a narrower size distribution (Figure 3b). These results, like those obtained in other studies, showed that TiO2 nano-aerosols can be highly polydisperse. This means that aerosol NMADs can be close in value, whereas their distribution profiles can be totally different. Therefore, a complete characterization of nano-aerosols must be considered, and the entire size distribution must be reported when attempting to better understand the influence of the size distribution of aerosols on their toxicity.

TEM provides two-dimensional images, and therefore, the comparison of physical diameters measured by TEM with aerodynamic diameters provided by the impactors is not direct. Multiple factors need to be considered to establish a correlation between these two different types of diameters. These factors are related to the physical characteristics of the particles, such as density and shape, and to aerodynamic properties, including the Cunningham slip factor. Establishing such a correlation is beyond the scope of this article; however, the deposition patterns obtained by TEM images representing the NP agglomerates present in the different aerosols, qualitatively support the results obtained with the ELPI (Figure 5).

As previously reported in Jiang et al., multiple factors can affect the dispersion characteristics of TiO2 suspensions. In our study, TEM characterization of the suspension showed small agglomerates in the range of 116 ± 46 nm. Thus, based on the measurements made on the stock solution used to generate the nano-aerosol composed of SA, it appears that the nebulizers applied sufficient energy to reduce the overall size of the agglomerates. It is well known that nebulization of an aqueous colloidal suspension results in aerosols in which particle physical characteristics (size, shape, and porosity) are dependent on multiple factors such as the size of the micelles in suspension and the concentration and condition of drying, which includes through a heated tube. Since the dry powder and the NP suspension both had small agglomerated structures, it seems that the capacity of the generation methods to de-agglomerate the NP is an important factor that affects the size distribution of an aerosol. In this manner, nebulization and dry powder dispersion represent interesting approaches with significant flexibility in NP generation characteristics.

In our study, generation of TiO2 nano-aerosols by dry-dust generators (Palas and Fluidized Bed) produced more agglomerated structures of TiO2 NP compared with nebulization (Collison and Delavan) of TiO2 suspensions. As observed by TEM, NP aggregates produced by nebulization (Figure 6a) were more compact than those produced by the dry-powder generator (Figure 6b), with the latter aggregates presenting more spaces between the primary NP. Thus, as mentioned above, we associate the difference observed in the structure of the NP aggregates to the aerosol generation technique.

Grassian et al. also observed that agglomerates of 21 nm TiO2 particles had more open void regions at the interface between particles (loose) than those formed from 5 nm particles (compact). They attributed this difference to the initial size of the NP. Indeed, a decrease in the size of NP implies an increase in the surface area per mass unit and leads to a higher surface-to-volume ratio, increasing the particle surface energy and, consequently, its ability to bond to other particles. In addition, geometrically, if an agglomerated structure is tightly packed, smaller spheres will have less void space. The interesting distinction between agglomerates from Grassian et al. and our study is between tight and loose packing of agglomerates of same size single particles. All together, these data suggest that the size and structure of the agglomerates may vary depending on the initial size of the NP, the method used to generate the aerosols, or in the work environment, according to the industrial processes. Most importantly, the biologically active surface of the NP structure will depend on the ability to mirror the characteristics of the specific aerosol exposure environment.

CONCLUSION

Using nebulization and dry nano-powder dispersion, it was possible to successfully generate stable 2 and 7 mg/m^3 TiO2 (5 nm) nano-aerosols over 6 hr showing different characteristics in their size distribution. Stable mass concentrations were generated by a wet method using Collison and Delavan nebulizers that resulted in aerosols composed of smaller agglomerates (<100 nm), while aerosols composed of larger agglomerates (>100 nm) were obtained by dry generation techniques using either a Palas dust feeder or a Fluidized Bed. The production of aerosols mainly composed of particle agglomerates with an aerodynamic diameter smaller than 100 nm (MNAD on the order of 30 nm) constitutes, to our knowledge, original data. Most significantly, this study highlights the needs of a qualitative and quantitative characterization of the NP and their agglomerates in aerosols. Overall, this study represents an important contribution to the current knowledge related to nano-aerosol generation and characterization techniques in nanotoxicology, more specifically, for animal exposure studies.

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REFERENCES


