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Experimental Determination of Ultrafine TiO_2 Deagglomeration in a Surrogate Pulmonary Surfactant: Preliminary Results

A. D. MAYNARD

National Institute of Occupational Safety and Health, Cincinnati, Ohio, USA

Although a number of studies have demonstrated an association between the surface area of low-solubility particles and biological response within the respiratory system, the use of agglomerated particles has led to ambiguities over the interpretation of results in many cases. A clear understanding of the role of particle size and total available surface area requires some knowledge of the degree of deagglomeration that takes place following deposition in the lungs. Samples of ultrafine TiO_2 (primary particle diameter ~20 nm) have been suspended in a surrogate pulmonary surfactant, and the size distribution of the suspended particles was measured using transmission electron microscopy. Comparison with airborne particle size distributions indicates a shift in modal diameter from ~300 nm to ~100 nm following suspension in the surfactant. There was no indication of particle deagglomeration to primary particles. It is hypothesized that the manufacturing process of materials such as ultrafine TiO_2 leads to the formation of primary agglomerates—clusters of primary particles held together by partial sintering—and that these represent the limit of deagglomeration following lung deposition.

Keywords: pulmonary surfactant; titanium dioxide; ultrafines

INTRODUCTION

A particle size- or surface area-mediated response to relatively insoluble particles within the respiratory system has been established by a number of studies. Investigations using discrete ultrafine (defined here as particles <100 nm in diameter) polytetrafluoroethylene (PTFE) particles have indicated that the response is associated with particle size (Oberdörster *et al.*, 1995), while investigations with insoluble particles approaching micrometre diameters have indicated a surface area-mediated response (Lison *et al.*, 1997; Tran *et al.*, 2000). However the majority of investigations have relied on agglomerates of fine insoluble particles, such as TiO_2 and carbon black, and in these cases the relationship between primary particle diameter, agglomerate size and particulate has led to ambiguities in interpreting results.

Materials such as ultrafine TiO_2 are generated from the gas phase, leading to the primary particles generated being a few nanometres in diameter. These rapidly coagulate, forming larger particles (primary agglomerates), followed by agglomerate–agglomerate coagulation resulting in agglomerates hundreds to thousands of nanometres in diameter. Following inhalation and deposition in the respiratory system, particle number, effective size and available surface area will depend on the degree of particle deagglomeration that occurs. Published toxicology data indicate that deagglomeration may occur within some ultrafine agglomerates following deposition and that deagglomeration is material specific (Oberdörster, 1996), although the extent to which it occurs and the resulting effective particle size and surface area are unclear. This investigation was therefore aimed at developing a method to predict the nature of particle deagglomeration in the lungs. Deagglomeration was investigated using transmission electron microscopy (TEM) of particles following suspension in a pulmonary surfactant surrogate. Ultrafine TiO_2 powder (P25 TiO_2; Degussa, Germany) was used as the test material.

MATERIALS AND METHODS

Dipalmitoylphosphatidycholine (DPPC) was used as the pulmonary surfactant surrogate. A micelle suspension of 5 mg DPPC in 1 ml deionized and ultrafiltered water (NANOPure, Barnstead, USA) was prepared by placing the materials in an ultrasonic bath for ~3 h. A sample of 0.25 mg ultrafine TiO_2 powder with an approximate primary particle diameter of 20 nm was added to 0.4 ml of the

DPPC suspension and the mixture was shaken. The suspension was then further diluted in NANOPure water at a dilution ratio of 1:312 DPPC suspension to water. The dilution ratio was calculated to give a residual DPPC layer <20 nm thick (corresponding to the primary particle diameter) when a droplet was applied to a TEM specimen support grid and dried.

Carbon-coated nickel TEM support grids were rendered hydrophilic by etching in a low temperature plasma oven operated at 65 W for 30 s. These were then held firmly on a magnetic substrate whilst 20 µl of the suspension was pipetted onto them and allowed to dry. Post-etching of the grids in the low temperature plasma oven was attempted to remove the residual layer of DPPC. However, it was found that the carbon film was insufficiently robust to survive a second etching process. Fortunately, the DPPC film was sufficiently electron transparent to allow the detection and sizing of TiO_2 particles down to 20 nm in diameter without further specimen preparation.

Deposited TiO_2 particles were viewed in a JEM1220 TEM (JEOL, Japan) and images were collected using a CCD camera. As particles were widely dispersed over the substrate, the sample was scanned visually and each particle detected was imaged (up to ~100 images/sample). Following collection, each image was corrected for CCD camera background intensity inhomogeneity, thresholded manually to form a binary image and features known not to be associated with TiO_2 particles removed. The area of each discrete particle was calculated and the equivalent sphere projected area diameter derived. It was estimated that the error associated with calculating the projected area diameter though image processing was no more than ±5%. For comparison purposes the above procedure was also carried out for a suspension of 0.25 mg TiO_2 in NANOPure water and for a DPPC suspension with no TiO_2 added.

Measurements of the aerosolized TiO_2 powder size distribution were made to give an indication of the agglomerate size distribution following mechanical separation. The powder was aerosolized in a two-component fluidized bed and the size distribution measured between 5 nm and 20 µm using a parallel array of two TSI Scanning Mobility Particle Sizers (SMPS) and a TSI 3320 Aerodynamic Particle Sizer (APS) (TSI Inc., USA). The aerosolized TiO_2 was also collected directly onto a TEM support grid using a point-to-plane electrostatic precipitator operated at 1 l/min and a corona discharge current of 4 µA.

RESULTS

The approach of diluting the particulate suspension followed by deposition onto a TEM grid appeared to work successfully for the DPPC suspension. The residual DPPC layer was sufficiently thin to allow particles as small as the primary TiO_2 particle size to be identified and imaged and dispersion across the grid appeared to be relatively uniform. There were isolated regions where both particles and DPPC were present in greater abundance, indicating some isolated non-uniformity in the deposit. However, there was no direct evidence of particle restructuring during the deposition process. Inspection of the DPPC sample prepared without TiO_2 showed no indication of features that could be interpreted as TiO_2 particles. Examination of the TiO_2/water sample again showed a uniform deposit across the TEM grid. However, agglomerates were generally much larger within this sample and there was some indication of restructuring following deposition (characterized by closely associated clusters of large and small agglomerates indicative of break-up during water evaporation).

Figure 1 plots the particle size distributions of TiO_2 agglomerates from the DPPC and water suspensions and within the aerosol. No evidence is seen of significant numbers of isolated primary particles existing following suspension of the TiO_2 in the surfactant. Comparison between the SMPS/APS measured size distribution and the TiO_2/DPPC size distribution indicates a shift in sub-micrometre mode from 333 to 115 nm between the aerosol and the suspension. This difference is not reflected in the comparison between the electrostatic precipitator/TEM and DPPC suspension distributions. The TiO_2 size distribution following suspension in water is markedly different from the DPPC suspension distribution.

Figure 2 presents the results of collecting the aerosol using the electrostatic precipitator at 0.3 l/min and a corona discharge current of 1 µA, after first passing the aerosol through a 0.8 µm cut inertial impactor. TEM analysis of the resulting distribution was carried out at two magnifications and modified to ignore closely positioned groups of three or more particles (clumps) on the sample. In this instance, there is closer agreement between the two aerosol size distribution measurements.

DISCUSSION

Measurement of agglomerate deagglomeration within a surfactant suspension is confounded by the lack of an absolute measure of particle size prior to deposition. This investigation was therefore limited to developing a method allowing the size of suspended particles to be analysed and a qualitative comparison between two alternative analyses of particle size: that in a mechanically generated aerosol and that in a water suspension.

Consideration of the TiO_2/DPPC data alone indicates that the agglomerates do not deagglomerate into isolated primary particles within the surfactant, but

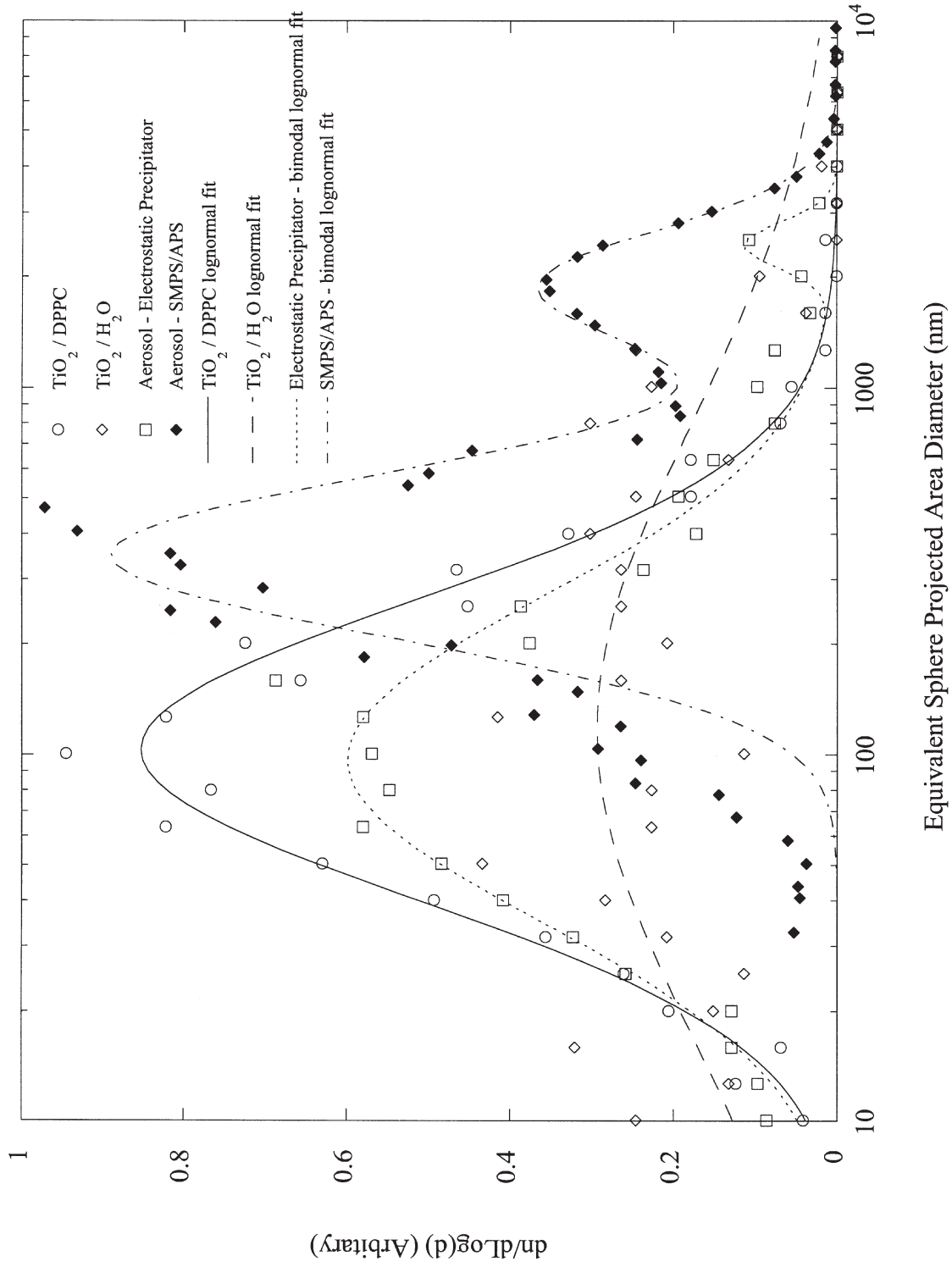

Fig. 1. Measured aerosol distributions in surfactant, water and aerosol. Size distributions measured by TEM have been normalized by the number of fields of view imaged.

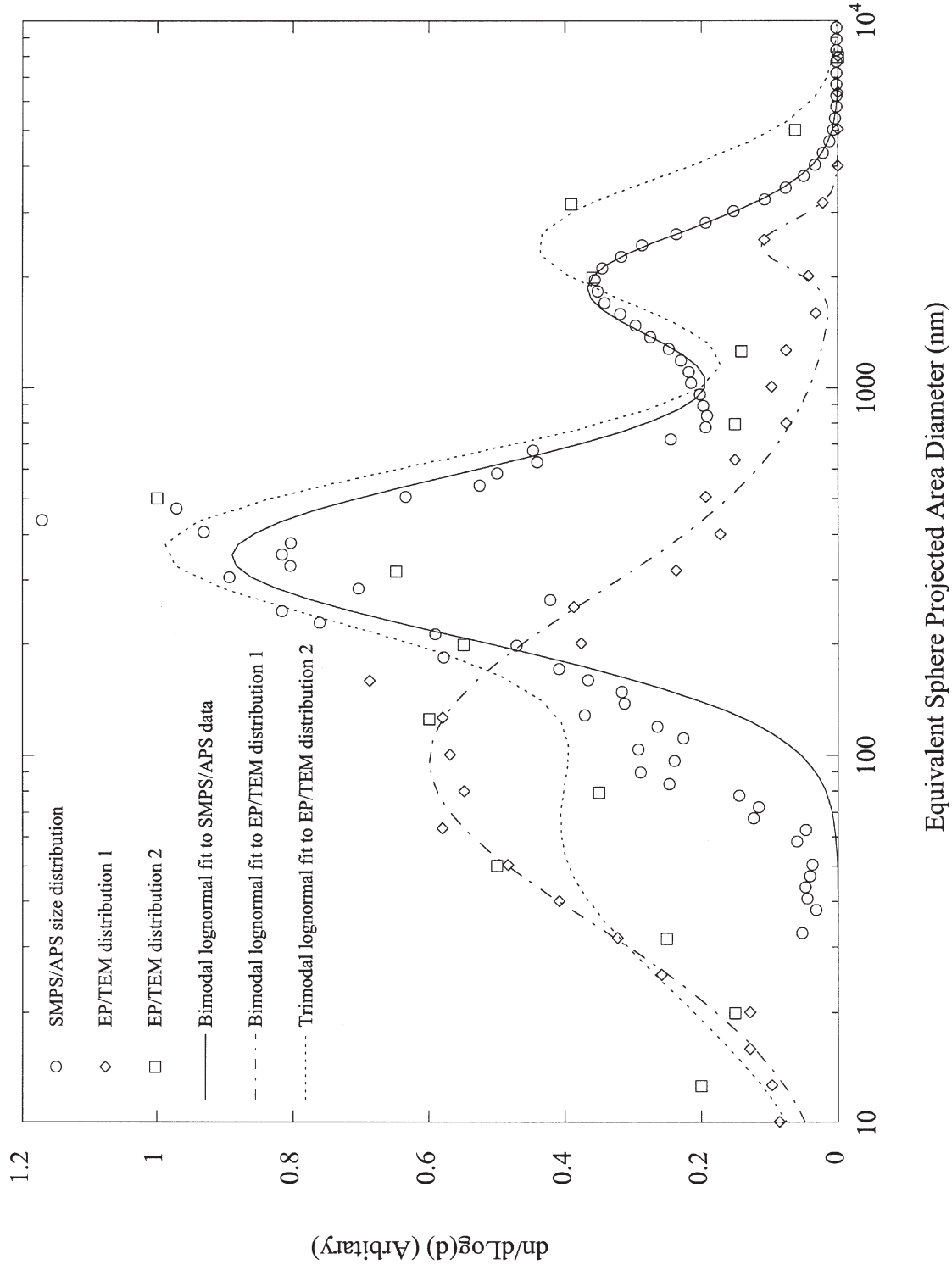

Fig. 2. Measured aerosol distributions using an SMPS/APS combination and an electrostatic precipitator (EP) with TEM analysis. Electrostatic precipitator distribution 2 was obtained at a lower flow and corona discharge current than distribution 1, and closely positioned groups of three or more particles within the sample were not included in the TEM analysis.

rather tend to a stable distribution with a projected area count median diameter (CMD) of ~115 nm. Comparison with the water suspension shows a slightly larger modal particle size, but a much broader size distribution. Visual inspection indicates the sample to be dominated by super-micrometre agglomerates, with evidence of some large agglomerate break-up into large numbers of much smaller particles during specimen preparation, indicating that the measured size distribution was artificially biased towards small particles. However, it was unclear from this investigation whether the size of the agglomerates following suspension in water reflected the size distribution in the dry powder or whether further agglomeration was occurring within the suspension.

Comparison with the aerosol size distribution is confounded by significant differences between the electrostatic precipitator and SMPS/APS aerosol size distributions. Previously published data have demonstrated a close correspondence between mobility diameter and projected area diameter for fractal-like agglomerates (Rogak *et al.*, 1993). This relationship was verified by preselecting TiO_2 agglomerates with an electrical mobility diameter of 300 nm in a differential mobility analyser, sampling them using the electrostatic precipitator and sizing them by TEM. Agreement was found between electrical mobility diameter and TEM-derived projected area diameter to within ±10%. The differences between the two aerosol size distribution measurements cannot therefore be interpreted in terms of differences in measurement method. A plausible hypothesis is that the electrostatic precipitator distribution in Fig. 1 is artificially biased towards small particles by the break-up of large agglomerates in the sampling process. A feasible mechanism is that very large agglomerates are removed from the electrostatic precipitator substrate by shear forces in the air flow and/or charging effects in the strong electric field, and that in the process smaller agglomerates break away and remain attached to the substrate. The electrostatic precipitator collected aerosol size distribution shown in Fig. 2 was collected with reduced flow shear forces and electrostatic forces. TEM analysis did not include closely associated groups of three or more particles on the substrate, which were thought to arise from large particle detachment. The resulting size distribution shows a good agreement between the precipitator and the SMPS/APS combination, although there is still evidence for a mode below 100 nm. This is consistent with the large agglomerates being composed of partially sintered primary agglomerates with a CMD projected area diameter of ~100 nm, held together by van der Waals forces. The contact force between large agglomerates and the substrate surface would be comparable to that between primary agglomerates, leading to some of the primary agglomerates remaining on the substrate following separation.

The remarkably close agreement between the TiO_2/DPPC and electrostatic precipitator distributions in Fig. 1 indicates that a similar process is occurring within the surfactant. Both distributions support the hypothesis that van der Waals forces between primary agglomerates are being disrupted, leaving a size distribution dominated by these agglomerates. The lack of isolated primary particles in either distribution indicates that the bonds between these particles in the primary agglomerates are sufficiently strong to not be overcome by mechanical separation or surfactant action, supporting the hypothesis that particles within the primary agglomerates are partially sintered. If these hypotheses are valid, it is unlikely that the TiO_2 agglomerates will undergo further restructuring than has been observed, following deposition in the lungs.

CONCLUSIONS

Interpretation of lung exposure studies using agglomerates of ultrafine particles is dependent on an understanding of how those particles restructure following deposition in the pulmonary surfactant. A test method has been developed that allows the size distribution of agglomerates following suspension in a surfactant to be measured. Using the method with agglomerates of ultrafine TiO_2, it has been shown that particles tend to a preferred equivalent sphere projected area diameter distribution with a CMD of ~115 nm when suspended in a micelle suspension of DPPC. There was no evidence of significant deagglomeration to single primary particles. Comparison with aerosol size distributions of the same powder indicates the CMD of the suspended particles to be significantly smaller than that within the aerosol. The data support the hypothesis that the ultrafine TiO_2 powder constitutes tightly bound partially sintered agglomerates of primary particles (primary agglomerates) and large agglomerates comprised of many primary agglomerates, and that following deposition in surfactant restructuring occurs, leading to free primary agglomerates dominating the size distribution. Further research is required to establish the primary agglomerate hypothesis for ultrafine TiO_2 and other ultrafine agglomerates such as carbon black, fumed silica and ultrafine aluminium oxide.

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