Generation of Nanoparticle Agglomerates and their Dispersion in Lung Serum Simulant or Water

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Abstract. Nanoparticles released into the atmosphere, due to their high diffusivity, will likely begin to agglomerate. The state of agglomeration upon inhalation and the potential to disperse back into nanoparticles may affect the toxicity of the inhaled material. In order to investigate particle dispersion, a system was set up to generate aggregates from agglomerates. Primary particles, composed of zinc, were generated using zinc rods in a spark generator (Palas GFG-1000, Karlsruhe, Germany). These particles formed agglomerates which were passed through a room temperature aging chamber or through a tube furnace (Carbolite HST, Derbyshire, UK). Agglomerate size was measured with a scanning mobility particle sizer (SMPS model 3936, TSI Inc., Shoreview, MN). When furnace temperature was set near the zinc coalescence temperature, instead of decreasing in size, agglomerate size increased up to 30%; a percentage increase duplicated with the room temperature aging chamber. Starting with an aerosol of primary zinc particles, equal concentrations of agglomerate and aggregate aerosol were produced. The extent of breakup and dispersion of agglomerates and aggregates to individual nanoparticles in lung serum simulant will be assessed using transmission electron microscopy.

1. Introduction

With the rapid emergence of nanotechnology and increased opportunity for exposure to nanomaterials, questions regarding the toxicity of nanoparticles and agglomerates continue to emerge as well. Initial studies investigating the toxicity of nanoparticles suggest that these particles possess enhanced toxicity beyond that of larger particles composed of the same species [1,2,3]. However, the question of nanoparticle toxicity is not as simple as examining individual particles only. Due to their high diffusivity, nanoparticles should exist as individual particles for only a short time and will agglomerate rapidly. Therefore, it is essential to understand the fate of these agglomerates upon inhalation to ascertain whether subsequent toxicological effects are attributable to the nanoparticle physical properties or is a function of their chemical composition. To date, studies on the degree of nanoparticle agglomeration have involved examining nanoparticle powders in solution [4] but not in the aerosol state.

In this study, we used zinc to generate agglomerates of constituent nanoparticles. Zinc is a relevant species to study as humans are exposed to it in different forms and from varying sources. For one, zinc is found bound to ligands in red meat and animal protein that facilitate its absorption [5]. Zinc has also been identified as a component of industrial emissions [6]. It is also detected in ambient air...
samples at higher levels than many other metals [7,8]. Exposure to zinc fumes in the workplace have shown a tendency to cause pulmonary inflammation [9, 6], thus making it an appropriate species to use for these initial agglomerate studies.

2. Experimental Procedure
Primary zinc nanoparticles were generated using zinc rods in a spark generator (GFG-1000, Karlsruhe, Germany). Before exiting the spark generator, these primary particles form agglomerates due to their high diffusivity. The degree of this agglomeration was controlled by adjusting the flow of dilution air both at the point of nanoparticle generation, as well as immediately after exiting the generator (Figure 1). Upon exiting the spark generator, agglomerates were sent into a glass tube for aging and dilution. Nanoparticles and agglomerates could then be sent through an aging volume for further agglomeration. Next, agglomerates were sent through a furnace (Carbolite HST, Derbyshire, UK) to be sintered to varying degrees by adjusting the furnace temperature, or sent through a bypass line for unsintered agglomerates. At this point, the agglomerates were characterized with a scanning mobility particle sizer (SMPS, model 3936, TSI Inc., Shoreview, MN) and collected on copper TEM grids using a nanometer aerosol sampler (NAS, model 3089, TSI Inc., Shoreview, MN) for TEM imaging.

![Figure 1: Experimental setup to produce agglomerated zinc nanoparticles. In this figure, NAS = nanometer aerosol sampler, and SMPS = scanning mobility particle sizer](image)

3. Imaging Agglomerate dispersion and translocation
In order to view agglomerates and the effect of immersion in a liquid, a drop of a biologically relevant fluid such as deionized (DI) water or lung serum simulant (LSS) [10] was then placed on the TEM grid. After allowing the liquid to evaporate, TEM images of the grids were again taken to examine the degree to which agglomerates that had been sintered to varying degrees had dispersed in the liquid.

Another method was also employed to exposed agglomerates to pure water. Zn agglomerates that had been collected on TEM grids and shadow coated were then exposed to water. Humidified air passed over the grids placed on a cooled surface (Figure 2). Moisture condensed onto the grid, wetting the surface without significant movement of liquid.
4. Results and Discussion

Initially, TEM grids, on which agglomerates that had been heated in a furnace to varying degrees had been deposited, were exposed to lung serum simulant (LSS) to observe its effect on the extent of agglomeration. An example of TEM images taken before and after exposure to LSS are shown in Figure 3. Comparing the images in Figure 3, it appears that the Zn agglomerates indeed dispersed following the application of LSS as evidenced by the lack of chain-like agglomerates in Figure 3b.

Figure 2: Schematic of DI water application method involving humidified air

Figure 3: TEM images of unsintered Zn agglomerates a) before and b) after exposure to LSS
One difficulty with this technique, however, was that upon evaporation, compounds dissolved in the LSS precipitated out, making it difficult to see the Zn nanoparticles or distinguish them from precipitated LSS components, as shown in Figure 4. Given this particular difficulty, application of DI water only to grids coated with Zn agglomerates was examined and is shown in Figure 5. The images in Figure 5 suggest that not only dispersion, but perhaps also dissolution of the agglomerates is occurring. In figure 5a, agglomerates are uniformly present across the grid, while in figure 5b, after application of DI water they are non-existent with what appears to be diffuse particle remnants from dissolution of the original agglomerates. While zinc is insoluble in water, it is slightly soluble in slightly acidic conditions. Since DI water is indeed slightly acidic (pH ~ 6.7), it is possible that these nanoparticles do dissolve to some extent.

Figure 4: TEM images a) before and b) after application of LSS. Zn particles are difficult to distinguish from precipitated LSS components.

Figure 5: TEM images of room temperature agglomerates a) before and b) after application of DI water.
Another possible explanation for what appeared to be dispersion and possible dissolution is the manner in which water was applied to the grids. Initially, this was done by placing a drop on each grid using a pipette. One thought was that perhaps the force of the water droplet from the pipette was forcing agglomerate dispersion to occur. An alternative method was developed in which water was condensed onto the grid. In order to detect the movement of deposited particles, the grids were first shadow coated (Figure 6), showing the shadows (in white) cast by the zinc agglomerates.

**Figure 6:** TEM images of Zn agglomerates that have been shadow coated

![Figure 6](image_url)

**Figure 7:** a) TEM images of shadow coated Zn agglomerates exposed to DI water for 1hr. Inside the circled area of each image is a large Zn agglomerate “unassociated” with any shadow.
Figure 7 shows an example of the results obtained when unsintered Zn agglomerates were shadow coated and exposed to DI water using the humidified air method. There are a few very important observations to be made regarding Figure 7. First, as shown in the circles drawn in Figure 7a, there are several instances of larger agglomerates that appear unassociated with any shadow, suggesting that they have moved, not necessarily dispersed, and certainly not dissolved, with the application of DI water. This can be contrasted with the images in Figure 5a, taken of agglomerates that hadn’t yet been exposed to DI water with each remaining with an associated shadow. It appears that mostly the larger agglomerates that moved. Also in Figures 7a and b as compared to Figure 5, is that smaller, more compact agglomerates appear not to move, instead remaining adjacent to their respectively cast shadow even after the application of DI water via the humidified air method. This is an interesting result that suggests a possible factor in the degree to which agglomerates are transported is both how they land and their shape factor, or how spherical versus spread out they are. For instance, it would be expected that long, branched agglomerates with high dynamic shape factors, elevated above the surface upon which they are deposited, experience a greater amount of torque for example from condensation of DI water onto the grid surface, making them more likely than smaller, more compact agglomerates to be moved by such a process.

![Figure 7b](image)

**Figure 7b** TEM image of shadow coated Zn agglomerates exposed to DI water for 3hrs.

These techniques of agglomerate generation and imaging require further refinement in order to be able to quantitate the size and number of the agglomerates before and after application of liquid. Agglomerates of nanoparticles that break up into individual nanoparticles may expose more respiratory tissues to nanoparticles, while larger agglomerates may be cleared via mucociliary action. On the other hand, there may be an agglomerate size or shape that is less likely to be broken up into its nanoparticle constituent parts. The transformation of agglomerates of nanoparticles in biological fluids requires further study to be able to assess the toxicological impact of nanoparticles.
References

[1] Donaldson K, Stone V, MacNee W. 1999 The toxicology of ultrafine particles. In: Particulate Matter: Properties and Effects Upon Health (Maynard RL, Howards CV, eds). Oxford: BIOS Scientific Publishers; 115-129.

[2] Ferin J, Oberdorster G, Penney D P. 1992 Pulmonary retention of ultrafine and fine particles in rats. American Journal of Respiratory Cell and Molecular Biology 6(5): 535-42.

[3] Li X Y, Gilmour P S, Donaldson K, MacNee W. 1996 Free radical activity and pro-inflammatory effects of particulate air pollution (PM10) in vivo and in vitro. Thorax 51:1216-1222.

[4] Grassian V H, O’Shaughnessy P T, Adamcakova-Dodd A, Pettibone J M, Thorne P S. 2007 Inhalation Exposure Study of Titanium Dioxide Nanoparticles with a Primary Particle Size of 2 to 5 nm. Environ Health Perspect 115(3):397-402.

[5] Vasto S, Mocchegiani E, Candore G, Listi F, Colonna-Romano G, Lio D, Malavolta M, Giacconi R, Cipriano C, Caruso C. 2006 Inflammation, genes and zinc in ageing and age-related diseases. Biogerontology 7:315-27.

[6] Kodavanti U P, Schladweiler M CJ, Ledbetter A D, Hauser R, Christiani D C, Samet J M, McGee J, Richards J H, Costa D L. 2002 Pulmonary and Systemic Effects of Zinc-Containing Emission Particles in Three Rat Strains: Multiple Exposure Scenarios. Toxicol Sci 70:73-85.

[7] Adamson I YR, Prieditis H, Hedgecock C, Vincent R. 2000 Zinc Is the Toxic Factor in the Lung Response to an Atmospheric Particulate Sample. Toxicol Appl Pharmacol 166:111-119.

[8] Balachandran S, Meena B R, Khillare P S. 2000 Particle size distribution and its elemental composition in the ambient air of Delhi. Environ Int 26(1-2):49-54.

[9] Fine J M, Gordon T, Chen L C, Kinney P, Falcone G, Sparer J, Becket W S. 2000 Characterization of clinical tolerance to inhaled zinc oxide in naïve subjects and sheet metal workers. J Occup Environ Med 42:1085-1091.

[10] Moss, O R 1979 Simulants of lung interstitial fluid. Health Physics, 36(3), 447-8.