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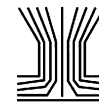
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Dust Generator for Inhalation Studies with Limited Amounts of Archived Particulate Matter

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A novel design for a dry-aerosol generator that efficiently produces a well-dispersed dust suspension using small quantities of a PM_{2.5}-enriched powder sample is described. The motivation to develop a highly efficient dry-aerosol particle generator was to facilitate collaborative projects that combine *in vitro* cell culture experiments and multiday inhalation exposures using a single batch of well-characterized particles. Premixing of the test particles with larger diameter glass beads permits delivery of aerosol concentrations from 100–1000 µg/m³ to an exposure chamber using only milligram quantities of the test powder per hour. Examination of exposure chamber filter samples by scanning electron microscopy showed well-dispersed particles of the test powder free of glass spheres or fragments. Data are presented from experiments using coal fly ash as the test powder to illustrate the system performance.

INTRODUCTION

Particulate matter found in ambient, domestic, and occupational environments has been associated with a wide range of adverse health effects, but the mechanisms by which specific particle types induce biological responses remain elusive. Inhalation toxicology studies require reproducible exposures to controlled concentrations of particles. Particle sources for such experiments include real-time concentrated ambient particles

(Sioutas et al. 1996; Sioutas and Koutrakis 1996; Gong et al. 2000), generation of particles in laboratory sources connected to an exposure chamber (Tesfaigzi et al. 2002; Yu et al. 2002; Veranth et al. 2003; Zhou et al. 2003), and resuspension of previously collected fine particles. Examples of inhalation studies using archived materials include purchased manufactured materials such as TiO₂ (Baggs et al. 1997) or carbon black (Donaldson 1999), dust collected by air pollution control devices (Kadiiska et al. 1997), and filter samples collected from source emissions or ambient air (Costa and Dreher 1997; Mitkus et al. 2002). Laboratory-generated or modified particles can be selected to have characteristics suitable for testing a specific mechanistic hypothesis (Ball et al. 2000). Working with archived powders has the advantage of allowing replication of multiple biological assays using a homogeneous lot of well-characterized material. However, using archived powders for inhalation studies creates the need to resuspend and disperse the material while minimizing artifacts from sample handling. Frequently, only small quantities of the archived material are available, making inhalation studies infeasible when using commercially available dust generators that require gram quantities of test material per hour.

The motivation to develop a highly efficient dry-aerosol particle generator was to facilitate collaborative studies that combine *in vitro* cell culture studies with multiday inhalation studies using a single batch of well-characterized particles. The primary source powder was PM_{2.5}-enriched coal fly ash, but other materials were also tested. Methods used to generate the fine-mode particle samples are time and labor intensive (Veranth et al. 2000b), so the supply was limited. The criteria for inhalation exposures with archived powders are (1) to produce a steady aerosol flow to the exposure chamber, (2) to create an aerosol concentration that is relevant to environmental and occupational exposures, and (3) to disperse particles to achieve a size distribution similar to the source emission.

Reviews of aerosol generators and dust delivery to inhalation chambers have been provided by Hinds (1982) and Wong (1999). Solid particles can be suspended in a liquid, nebulized,

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and subsequently dried prior to inhalation exposure. However, this approach produces both single particles and multiparticle clusters that distort the size distribution. The use of a liquid carrier may also introduce other undesirable artifacts such as creating new particles enriched in soluble components of the original powder. Aerosols can also be generated directly from dry powders. Alternatives include rotating turntable feeders, rotating scrapers, and fluidized bed particle generators. Commercial dry-powder aerosol generators are available from BGI Inc. (Waltham, MA, USA), TSI Inc. (St Paul, MN, USA), In-Tox Products (Moriarty, NM, USA) and Topas GmbH (Dresden, Germany). A second, more problematic limitation of many dry-powder aerosol generators is that they require significant amounts of feed material, usually on the order of grams/hour. For example, Raabe et al. (1979) used a combination of a Wright dust feeder and a cyclone separator to produce a coal fly ash aerosol smaller than $5\ \mu\text{m}$ aerodynamic diameter for a long-term animal exposure. However, this study used 75 kg of size-classified fine particles extracted from 3000 kg of electrostatic precipitator fly ash.

An inhalation study at ambient-to-occupational concentrations theoretically requires only milligrams of particles per hour to supply a reasonable aerosol flow to the exposure chamber. Marple (1978) developed a fluidized bed dry aerosol generator that could operate with 50 mg/h of feed material. Other designs that have successfully resuspended small quantities of powder include an improved turntable design (Reist and Taylor 2000) that operated as low as 0.1 mg/min, and a shock and expansion tube design that was able to resuspend $50\ \mu\text{g}$ batches of 325 nm latex particles (Rajathurai et al. 1990).

The dust generator described in this article was developed to reduce the test powder consumption compared to existing dry-powder aerosol generator designs. The fluidized bed dust generator was then used as part of an ongoing coal fly ash toxicology study.

EQUIPMENT AND METHODS

A photograph of the dry powder aerosol generator and exposure chamber is shown in Figure 1, and Figure 2 provides a schematic of the system. Major subassemblies are the rotary belt feed mechanism, the fluidized bed, the cyclone separator, and the dilution control system. Tubes containing one animal each are connected to the ports in the chamber on the left, and the remaining ports are either plugged or used for instrument connections.

The particle path through the system is described first. A $d < 10\ \mu\text{m}$ test powder is premixed with $100\ \mu\text{m}$, glass beads (Ballotini Glass Beads, Potters Industries, Valley Forge, PA, USA; www.pottersbeads.com) and placed in the glass burette feed hopper. The stopcock valve releases the mixture onto a rotating felt belt that conveys and discharges the mixture at a controlled rate into the fluidized bed. The fluidized bed consists of a 15 ml conical test tube with a 3.1 mm ID brass tube for the

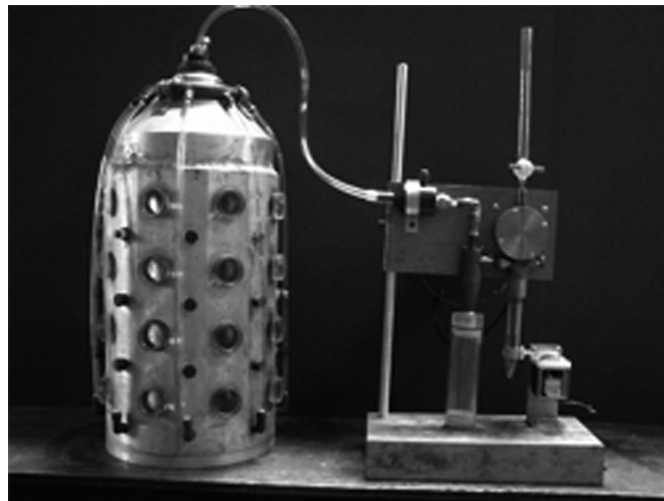


Figure 1. The assembled dry powder aerosol generator (right) connected to a nose-only exposure chamber (left).

feed input. The fluidizing air is added with a separate 1.5 mm ID tube placed at the bottom of the cone. The aerosol and excess beads are removed through a 3.1 mm brass discharge tube positioned 29 mm above the tube bottom. This maintains the steady-state fluidized bed volume at 2.5 ml. The bed material represents slightly more than 1 ml. The conical tube is mechanically vibrated by a DC motor with an eccentric cam drive mechanism. The vigorous agitation combined with air flow through the fluidized bed releases particles of the test powder from the glass beads. The discharge mixture passes through a cyclone, which readily separates the suspended test particles from the glass beads due to the large difference in aerodynamic diameter. The overflow of glass beads with some test powder still attached is collected in a container below the cyclone. The test aerosol is diluted with filtered air, passes through a Kr-85 charge neutralizer, and flows into the nose-only inhalation chamber.

The gas-phase control system meters air flow and sets pressure. The filtered, compressed air supply is split between the variable area flow meter (rotameter) measuring the fluidizing and conveying air, which is delivered below the stationary glass bead bed surface, and the rotameter controlling the dilution air, which is delivered downstream of the cyclone. The diluted aerosol enters the top of the inhalation chamber and is exhausted from the bottom. The nose-only exposure chamber has ports allocated to pressure measurement, online aerosol concentration measurement, and filter sampling. The exposure chamber is evacuated through a vacuum rotameter and filter (not shown), and is maintained at slightly negative pressure.

For the inhalation exposure experiments, the particle concentration in the inhalation chamber was monitored by a continuous-reading light-scattering dust concentration monitor. A 1.2 lpm sample is extracted from one of the bottom row ports in the inhalation chamber. The feeder settings were adjusted as needed to achieve the target time-averaged concentration for the exposure.

LVDG (Low Volume Dust Generator)

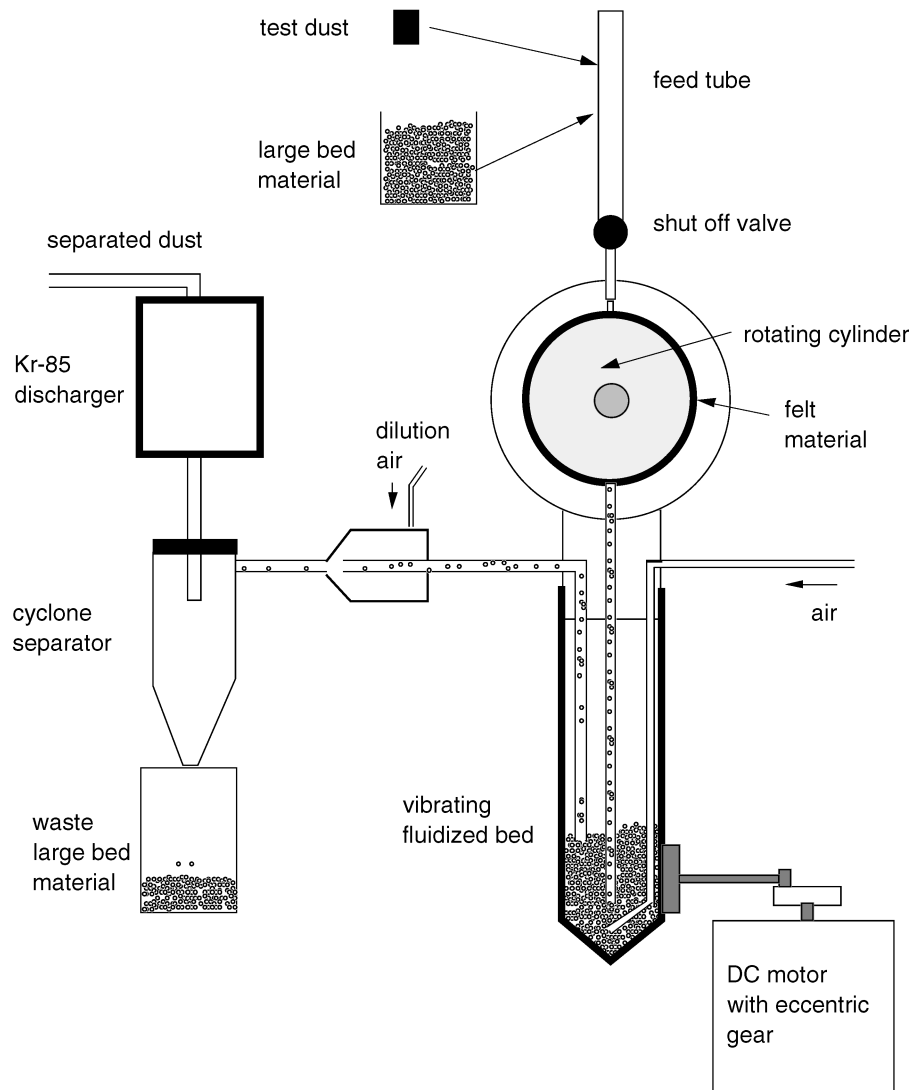


Figure 2. Schematic flow diagram of the aerosol generator system.

Both a DustTrak (TSI Inc., St Paul, MN, USA) and a Grimm Series 1.108 Aerosol Spectrometer (GRIMM Aerosol Technik GmbH, Douglasville, GA, USA) were used to monitor particle concentration. Both of these instruments use proprietary algorithms to convert light scattering into particle concentration. The Grimm instrument was operated in the environmental mode to produce the mass concentration versus time data and in the 16-channel particle count mode to produce the size distribution. Grimm data are reported based on the default C-factor of 1.0.

The weight on a filter sample collected from the exposure chamber was used to calculate the calibration factor needed to convert the default light-scattering instrument output to ac-

tual mass concentration during the exposure studies. The filter sample was collected at 3 lpm on a 25 mm Pallflex EMFAB TX40HI20-WW filter using an InTox filter housing connected to a diaphragm pump and a bellows-type dry gas meter.

The purchased glass beads were repeatedly washed in distilled water by stirring while the container was immersed in an ultrasonic cleaner. This removed any contamination that would confound the inhalation experiment. The beads were thoroughly dried by heating and were sifted through a screen to insure a free-flowing material. Weighed amounts of beads and the test powder were then mixed by tumbling.

System operation consists of adjusting particle and air flows to achieve the desired exposure conditions. The fluidizing air is

Table 1
Specifications and settings

Test-powder-to-glass-bead ratio	0.02–1%
Glass bead feed rate	1–5 g/h
Rotary feeder speed	0.1–2 rpm
Fluidized bed volume	2–3 cm ³
Fluidizing and conveying air	<1 lpm
Dilution air	5–10 lpm
Inhalation chamber vacuum	20–30 mm H ₂ O
Filter sample flow	2 lpm
GRIMM aerosol monitor flow	1.2 lpm
DustTrak aerosol monitor flow	1.7 lpm

first adjusted to achieve a bubbling condition in the stationary glass bead bed, then mechanical agitation is started, followed by starting the powder feed. Table 1 lists typical flow rates.

The desired operating condition is a steady state where the flow of test powder into the fluidized bed matches the resuspended aerosol flow at the target mass concentration. This is achieved by (1) choosing the mixture ratio of test powder to glass beads, typically ranging from 1/100 to 1/2000 by mass; and (2) adjusting the speed of the rotating drum to control the feed rate of the mixture to the fluidized bed. Dilution air flow is set to maintain a sufficient fresh air flow through the exposure chamber for breathing, but it can be increased as needed to provide additional control of aerosol concentration.

The system was used for a three-day inhalation experiment with PM_{2.5}-enriched coal fly ash. The test material consisted of several grams of size-fractionated powder that was being shared between multiple laboratories. The PM_{2.5} powder was prepared from fly ash collected by the electrostatic precipitator at a power plant burning Utah bituminous coal. Veranth et al. (2000b) describe the method used for aerodynamic separation of a PM₁₀- or PM_{2.5}-enriched fraction from mixed-size samples of environmentally relevant dusts and powders.

Electron microscopy was used to examine the feed materials, the inhalation chamber aerosol, and the excess beads collected in the cyclone. Inhalation chamber aerosol samples for electron microscope imaging were collected on polycarbonate membrane filters (Gelman type GTTP), and an appropriate loading for imaging was obtained with 1 min sampling. The clean glass beads, the feed mixture, and of the cyclone-collected material were dispersed for imaging by gently blowing particles from a capillary pipette onto a polycarbonate membrane. Samples were gold-coated and examined using a Hitachi S-3000N or a Hitachi S-2460N scanning electron microscope in secondary electron mode. Multiple digital images were made at each magnification. A calibration standard was used to verify the SEM display scale bar. Particle size distributions were obtained using NIH Image software to measure 500–1000 particles on a series of images taken with a structured grid pattern.

RESULTS

A representative example of the dry powder feeder system performance is shown as a graph of particle concentration in the inhalation chamber over a 6 h period of equipment testing with coal fly ash (Figure 3). The initial period of system setup (from 11:30 to 13:00) shows that the response to air flow and glass bead feed rate adjustments is rapid. The subsequent period (from 13:30 to 15:30) shows steady-state operation at a reasonably constant aerosol concentration with no adjustments. The final 1 h period shows an approximately exponential decay of the dust aerosol concentration after feed of the glass bead mixture to the vibrating bed was stopped.

When planning the coal fly ash aerosol exposure study, concerns included whether the fluidized bed generator would produce well-dispersed particles, whether the aerosol would contain fragments from the glass beads, and whether the resuspension would grind ash particle to create finer material than was in the source powder. Figure 4a shows coal fly ash collected directly on a filter from the exit of a pulverized coal fired furnace. Melting of the mineral matter in the coal results in spherical particles that occur as individual particles or small clusters. Coal fly ash also contains unburned carbon in the form of char and soot aggregates (Veranth et al. 2000a). Figure 4b shows aerosol deposited on a filter sampling from the exposure chamber. The coal ash particles are spherical and there are no irregular fragments, indicating that there was no mechanical shattering of larger ash particles. Note that producing the source powder by inertial collection of a specific size fraction has resulted in loss of the smallest particles seen in Figure 4a. Figure 4c shows the clean glass beads, and Figure 4d shows the feed mixture with particles of coal fly ash adhering to the glass beads. Filter pores appear as black holes in the higher magnification images. There was also no indication of shattering of the glass beads in the entrained material collected by the cyclone (data not shown). Based on finding no glass

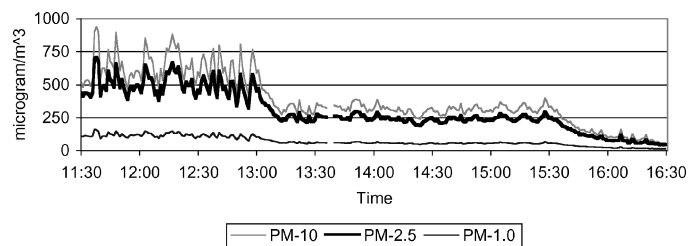


Figure 3. Aerosol concentration from a continuous period of equipment testing shows PM₁₀ (top), PM_{2.5} (middle), and PM₁ (bottom) as measured by the Grimm Aerosol Spectrometer in the environmental mode. The initial period shows the rapid response of the aerosol concentration to adjustments in air flow and feed rate. At 13:00 h the set point was reduced and the subsequent period represents steady-state operation. At 15:30 the feeding of glass beads with coal fly ash was stopped with the final time period showing the decay in concentration as particles are removed from the fluidized bed.

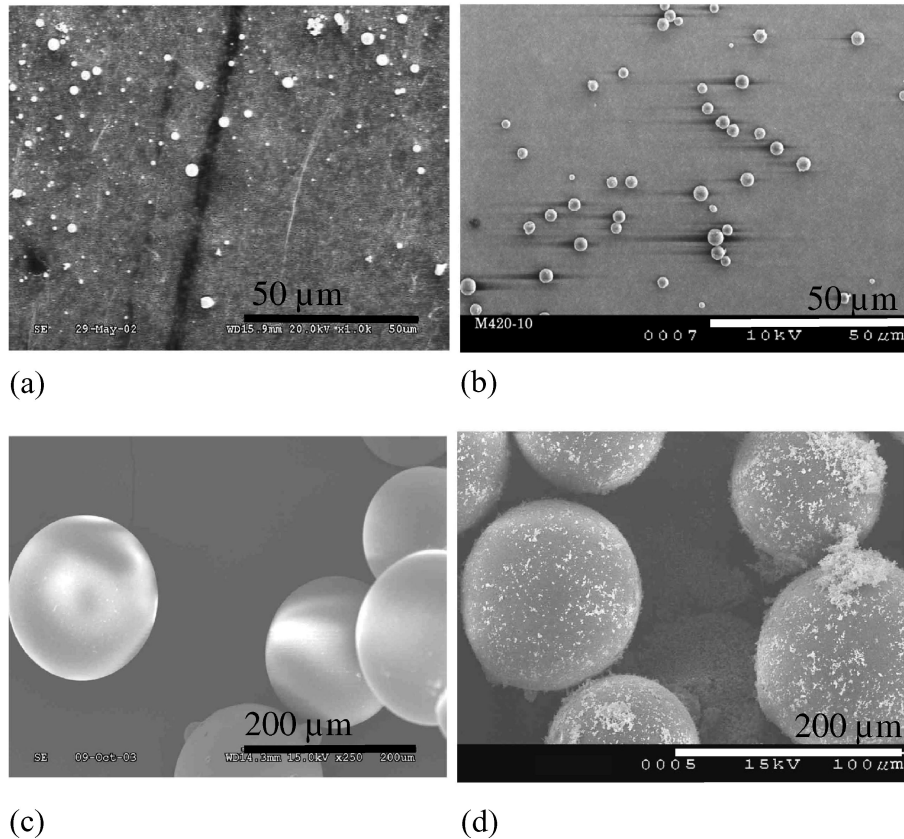


Figure 4. SEM images. (a) Typical coal fly ash directly collected on a filter at the exit of a pulverized-coal-fired furnace. (b) Resuspended coal fly ash sampled from the exposure chamber. (c) Washed glass beads. (d) Feed mixture of glass beads and coal fly ash. Scale bars provide a size reference.

sphere fragments, and calculating from the number of images examined and the volume of air flowing through the filter, the maximum number of fragments is less than $1/\text{cm}^3$.

Gravimetric calibration of the real-time laser light-scattering mass concentration demonstrated reasonable agreement between actual mass and the manufacturer's default calibration. For the coal fly ash, the total mass concentration calculated from a filter sample was 1.75 times the PM_{10} mass concentration that was calculated by the instrument algorithm using the default density factor.

Figure 5 shows the particle volume distribution determined by SEM for the original coal fly ash powder and for the resuspended exposure chamber aerosol collected on a filter, and compares these physical size measurements to the exposure chamber aerosol as measured by the Grimm particle spectrometer. The source powder and the resuspended aerosol size distributions are similar, and the difference is within the uncertainty of the technique. The on-line light-scattering instrument shows smaller particle size than the physical diameter measured by electron microscopy. The difference is increased if the physical particle diameter is converted to aerodynamic diameter using a typical particle density of $2.1\text{--}2.2\text{ g/cm}^3$. The instrument manufacturer states that the size distribution in the number count

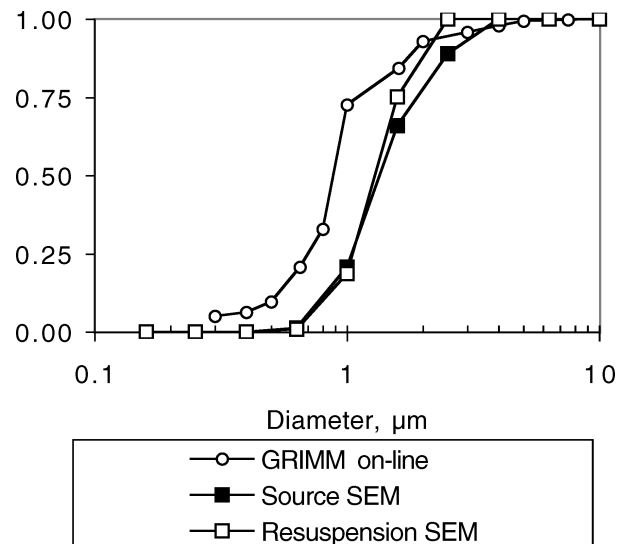


Figure 5. Cumulative particle volume versus physical size for the source powder and for the resuspended coal fly ash aerosol as measured by electron microscopy of filter samples, and the size distribution in the exposure chamber measured by a light-scattering particle counter and converted to volume distribution.

Table 2

Material balance on the inhalation exposure experiment

Total PM _{2.5} particles used to prepare glass bead mixtures	226 mg	
Animal exposure duration	3 da × 6 h/da	
Calculated PM _{2.5} particles fed into the fluidized bed during the animal exposure periods	68 mg	
Dust concentration in the inhalation chamber	700 μg/m ³	Note 1
Air flow through the inhalation chamber	10 lpm	
Calculated particle mass into the inhalation chamber during the 18 h exposure time	8 mg	
Particles into inhalation chamber/ particles into fluidized bed	12%	Note 2

Note 1: This calculation uses the time-integrated exposure concentration obtained from filter weight.

Note 2: PM_{2.5} material remaining on the entrained glass beads collected by the cyclone was not measured.

mode is based on calibration with a monodisperse latex test aerosol, and that a single conversion factor is used to correct for index of refraction and particle density when the instrument's algorithm reports mass concentration (Thomas Petry, personal communication).

Table 2 presents the material balance on the PM_{2.5} particles during a three-day animal exposure study to illustrate that this device efficiently uses small amounts of the test powder. The difference between total fluidized bed input and the theoretical amount of particles delivered to the inhalation chamber during the exposure experiment includes startup and shutdown periods, line losses, and material remaining on the glass beads. The entrained beads collected by the cyclone are intermediate in color between washed beads and the feed mix, and particles of the test powder were observed by electron microscopy on the surface of the cyclone-collected beads. In a confirming test, the beads recovered from the cyclone were recycled to the feed and additional aerosol was generated.

The dry powder generator was tested with alternative feed materials. Coarse-fraction coal fly ash (2.5–10 μm) and kaolin clay worked well, indicating that the technique of premixing particles with glass beads is suitable for mineral dusts. A test with submicron carbon was less successful. The beads became coated with carbon and virtually no aerosol was generated, indicating that the fluidized bed cannot be used with sticky or cohesive materials.

DISCUSSION

The results show that the fluidized-bed generator can efficiently produce an aerosol containing well-dispersed particles of a test powder with minimal aggregate clusters or bed mate-

rial fragments. The on-line particle spectrometer provided useful data for maintaining constant aerosol concentration, but the indicated particle size differed from the physical diameter. The mass collected on the filter was also higher than the mass calculated from the aerosol size spectrometer. A sample-specific calibration is needed to reconcile continuous-reading light-scattering instrument data with the actual mass concentration and the physical and aerodynamic particle size distribution.

The dry powder aerosol generator described here is an improvement over previous technology for dry aerosol generation. The results demonstrate the feasibility of conducting inhalation experiments with small quantities of archived test particles.

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