Methods to Assess Personal Exposures to Airborne Metallic Nanoparticles

Final Progress Report

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Contents

List of Terms and Abbreviations

APS – aerodynamic particle sizer

C_{in}, C_{out} – particle number concentration entering and exiting the size separator, respectively

d_{16}, d_{50}, and d_{84} – particle size associated with 16%, 50%, and 84%, respectively

ICP – inductively coupled plasma

ICRP – International Commission on Radiological Protection

IPM – inhalable particulate matter

GSD – geometric standard deviation

LOD – limit of detection

LOQ – limit of quantitation

MCE – mixed cellulose ester

NMAM – NIOSH Manual of Analytical Methods

NPM – nanoparticulate matter

NRD – nanoparticle respiratory deposition

PVC – polyvinyl chloride

SMPS – scanning mobility particle sizer

Sr – coefficient of variation

SSE – sum of the squares error

TEM – transmission electron microscope

TiO2 – titanium dioxide

Abstract

When inhaled, nanoparticles can elicit adverse cardiopulmonary health outcomes and their toxicity, particularly for metals and metal oxides, can be substantially greater than that of larger particles of the same composition. No standard measurement methods are available to quantitatively assess personal exposures to engineered nanoparticles, and without such methods, the extent to which workers are at risk from inhalation of nanoparticles is unknown. The laboratory work carried out in this research standardized and validated measurement methods needed to quantify personal exposure to a range of metallic nanoparticles that are commonly incorporated into commercial products. We developed an innovative nanoparticle respiratory deposition (NRD) sampler to collect nanoparticles specifically (separate from the larger particles in workplace air) and in a manner that reflects their deposition in the respiratory tract (Aim 1). We also developed and validated methods to analyze the content of metal in the nanoparticles collected with the NRD sampler and common respirable samplers (Aim 2). These sampling and analysis methods are easily adaptable by industrial hygiene practitioners to provide the system needed to assess worker exposure to metal nanoparticles. They represent enabling tools to study potential epidemiological relationships among metallic nanoparticle exposures and adverse acute health effects.

Section 1

Significant Findings

Under Aim 1, we developed a novel sampler—the Nanoparticle Respiratory Dose (NRD) sampler (**Fig. 1-1**). The lightweight (~60 g) NRD sampler consists of three primary components assembled in series: a 25-mm respirable aluminum cyclone, an impaction stage and a diffusion stage. Air is drawn through the cyclone, which removes particles larger than the respirable sampler criterion and transports the respirable fraction to the impaction stage, where particles larger than 300 nm are removed. In the diffusion stage, the remaining airborne nanoparticles diffuse to and are collected onto nylon mesh screens with an efficiency closely matching particle deposition in the human respiratory tract.

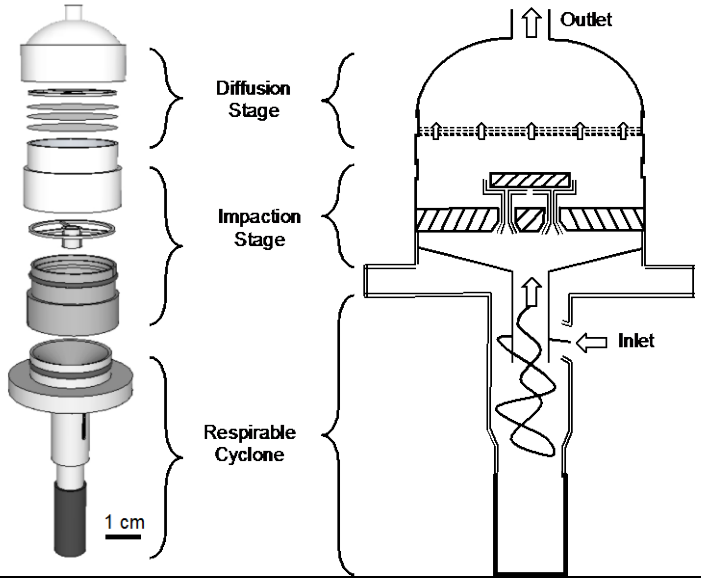

Fig. 1-1. The components and schematic drawing with airflow paths of the Nanoparticle respiratory dose (NRD) sampler.

Central to our new sampling device is a new sampling criterion for nanoparticles—the nano-particulate matter criterion (NPM, **Fig. 1-2**). This criterion is analogous to other sampling criteria (i.e., respirable, thoracic, and inhalable) in that it ties sampler performance with respiratory deposition. Rather than attempting to collect nanoparticles with 100% efficiency, the sampler was designed to collect nanoparticles with efficiency matching how they deposit in the respiratory tract to provide a physiologic relevance to sampler's performance. The NPM provides the target collection efficiency, by particle size, for the sampler.

To facilitate the development of media used in the diffusion stage of the NRD, we adapted a theoretical single-fiber efficiency expression developed for wire-mesh screens to estimate collection efficiency of 11–300 nm particles for nylon mesh screens. Nylon mesh screens, unlike metal screens, are attractive as a collection substrate for nanoparticles because they can be digested or ashed prior to chemical analysis. This work showed that particle morphology for metallic particles has limited influence on collection efficiency. This model is now available for other researchers to design diffusion-based samplers.

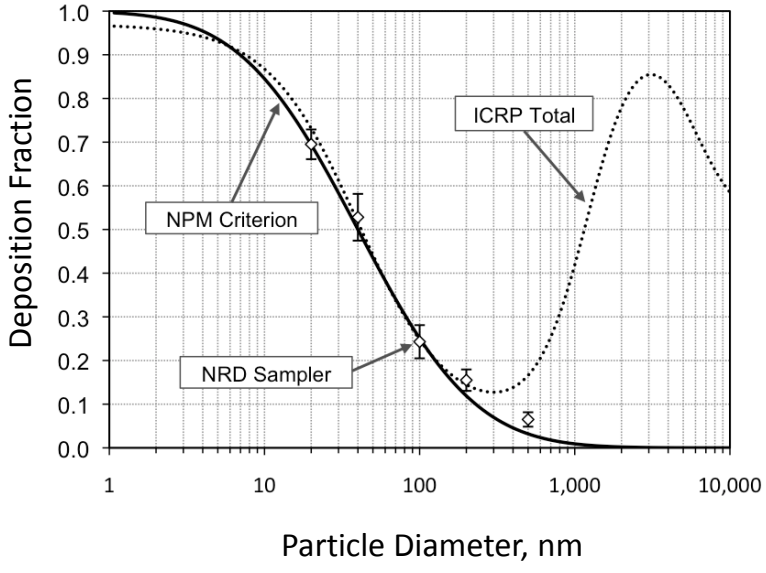

Fig. 1-2. Nanoparticulate matter (NPM) sampling criterion, International Commission on Radiological Protection (ICRP) total respiratory deposition and effective deposition on the diffusion stage of the NRD sampler.

Under Aim 2, we evaluated various analytical methods to assess the mass concentration of engineered metallic nanoparticles collected on nylon mesh screens and polyvinyl chloride (PVC)

filters. Most importantly, this work identified the need to modify traditional digestion methods for metals analysis (provided in NIOSH Manual of Analytical Methods, NMAM, Method 7300) to obtain adequate recoveries (>70%) for titanium dioxide nanomaterials. This work resulted in a new microwave method combined with hot sulfuric acid to adequately digest TiO_2.

Using the new microwave digestion method, we found that nylon mesh screens contain substantial quantities of titanium, presumably as titanium dioxide used to make the screens white. The background quantity of titanium varies by lot and is present at approximately 1/20 of the target recommended exposure limit for TiO_2. Recovery tests showed that the nylon mesh screens can be used to reliably assess TiO_2 when concentrations are near the recommended exposure limit. This finding does not discount their use as collection media when assessing TiO_2 exposures but does motivate the need to identify alternative substrates for this purpose. Further blank and recovery tests confirmed that the nylon mesh screens are suitable for a wide range of metals.

In contrast, PVC filters—recommended by NIOSH when used in conjunction with a respirable sampler for TiO_2 assessments—were found to contain low background levels of titanium when analyzed with the microwave digestion method. Further tests confirmed that the modified microwave method is required to achieve adequate recovery for titanium dioxide nanomaterial.

Translation of Findings

The immediate application of our work is for sampling of metal-based nanoparticles in the workplace. Current strategies recommended by NIOSH to assess exposures rely on sampling with respirable samplers followed by analysis with expensive and not well-defined electron microscopy for particle sizing (>$300 per sample). With the chemical analysis methods we have developed, the NRD sampler can be used to directly assess exposures to nanoparticles of a specific composition apart from other airborne particles for roughly $30 per sample.

A patent application has been submitted for the NRD sampler, and the sampler can now be made using injection molding, made possible with further financial assistance from the University of Iowa Research Foundation in partnership with Zefon International. The sampler can now be produced for less than $1 compared to >$600 when machined by traditional methods. As a result of this development, Zefon International licensed the technology from the University of Iowa in fall of 2013.

We have disseminated our work at national conferences. Our work has also been highlighted on the web, including a review of the NRD sampler at: http://www.nanowerk.com/spotlight/spotid=22308.php. Our work conducted under Aim 1 has been published in two articles in peer-reviewed scientific journals, and we are preparing two manuscripts for publication on the work conducted in Aim 2.

Outcomes/ Impact

Our contribution is significant because the cost-effective, streamlined methods that we developed are available for exposure assessment of metal and metal-oxide particles in a wide range of industries. Analogous to NMAMs, these new methods enable direct investigation of the relationship of adverse health effects with nanoparticle exposures. When applied to the nanotechnology industry, they may promote long-term, commercial sustainability by having routine methods to document exposures. Moreover, these methods will be adaptable to environmental sampling, such as stack testing or community monitoring, and may be developed further to include assessment of non-metal based exposures. Consequently, our methods will be applicable to lowering morbidity and mortality from inhalation exposure to airborne particle contaminants in a variety of settings where nanoparticle exposures may occur.

Our findings also provided avenues for future research, which we are now pursuing as part of an R01 Award through NIOSH. First, we need to identify other types of diffusion media to detect metals at lower limits of quantitation. Second, further methods development is needed to speed up analysis and achieve lower limits of quantitation. Third, the sampler must be field tested under various workplace settings to determine its ability to function as designed.

Section 2

Background

Metallic nanoparticles—particles smaller than 100 nm—are in widespread commercial use in products, from metal oxides (e.g., titanium dioxide, TiO_2) in cosmetics and paints to antibacterial silver nanoparticles in clothing. The toxicity of these nano-sized metal and metal oxide particles can be substantially greater than that of larger particles of the same composition. However, no standardized methods are available to assess their presence in the workplace. Neither can the mass of nanoparticles be separated from that of larger particles collected with traditional 8-hr, filter-based personal samplers (e.g., respirable samplers). The National Institute of Occupational Health and Safety (NIOSH) recommends mapping of the workplace with direct-reading nanoparticle monitors followed by identification of particle types with transmission electron microscopy (TEM). In more specific guidance, they recommend that the exposure to nanoparticle TiO2 be quantified from two simultaneously collected respirable samples with a complicated combination of gravimetric, metals, and TEM analysis. Mapping provides only a brief snapshot of the workplace and may not accurately reflect personal exposures because nanoparticles tend to rapidly decrease in concentration away from a source. In addition, analysis of particles by TEM is neither standardized (therefore subject to great uncertainty) nor automated (therefore costly, at approximately $300 per sample). The lack of sampling methodologies specific to nanoparticles hinders development of practical and effective personal exposure assessment strategies. Consequently, the extent to which workers are exposed to nanoparticles, particularly metals and metal oxides, is unknown and the effectiveness of control measures is difficult to evaluate.

TiO_2 is commercially produced as a non-fading, white pigment used in paint, plastic, and paper. The dominant form of TiO_2 used in pigment is in the rutile phase, a particular crystal structure, with particles typically in the 250 nm to 1000 nm size range. Recently, nanoparticle (size <100 nm), anatase-phase TiO_2 has become popular for use in sunscreens and other skin care products. The permissible exposure limit for TiO_2 is 15 mg/m3 total dust. In 2011 in a current intelligence bulletin (CIB), NIOSH recommended an exposure limit of 2.4 mg/m3 for fine TiO_2 and 0.3 mg/m3 for ultrafine (i.e., engineered nanoscale) TiO_2 based on evidence that TiO_2 toxicity is related to particle surface area, which increases with decreasing particle size.

NIOSH recommends a multi-tiered exposure assessment to distinguish concentrations of nanoparticle TiO_2 separately from fine-mode TiO_2 and other background aerosols (NIOSH, 2011). The first step is to gravimetrically measure respirable mass concentration according to NIOSH's Manual of Analytical Methods (NMAM) method 0600 in duplicate, with one sampler using a polyvinylchloride (PVC) filter and another using a mixed cellulose ester (MCE) filter. No further action is required if respirable mass concentration is less than 0.3 mg m^{-3}, the recommended exposure limit for nanoparticle TiO_2. If measurements exceed this limit, the PVC filter is analyzed chemically by inductively coupled plasma (ICP) NMAM method 7300 to determine the respirable mass concentration attributed to TiO_2 alone, which if <0.3 mg m^{-3} no further action is required. The MCE filter is analyzed by transmission electron

microscopy to determine the percent TiO_2 by particle size. With microscopic size information, the mass concentration is adjusted to reflect only that mass attributed to fine and ultrafine TiO_2.

Method 7300, elements by inductively coupled plasma (ICP), is a popular method used to determine the airborne concentration of a wide variety of metals. The method specifies the use of a respirable sampler to collect airborne particles onto a polyvinyl chloride (PVC) or mixed cellulose ester (MCE) filter. The metals in the sample and the filter are dissolved with an ashing acid (concentrated nitric acid/concentrated perchloric acid in a ratio of 4:1), repeatedly added and evaporated at elevated temperature until the extraction solution is clear. The solution is nebulized and the resulting aerosol is transported to plasma, where it is atomized and excited and/or ionized by the plasma. The excited atoms or ions emit their characteristic radiation which is collected by a spectrometer that sorts the radiation by wavelength. A calibration curve is prepared and used to calibrate the spectrometer according to manufacturer recommendations.

Issue 3 of NIOSH method 7300, Tables 3 and 4 include precision and recovery data at concentrations estimated to be 3x and 10x the instrumental limits of detection (LOD) for numerous metals. PVC filters are described as having good results for the metals evaluated. NIOSH method 7300 Table 4, Measurement Procedures and Data Polyvinyl Chloride Filter (5.0 um) lists the estimated LOD for Ti as 0.05 ug/filter and 2.0 ng/mL. Recovery data presented includes at 3xLOD of 0.78 the recovery is 101% and percent RSD of 9.46. At 10x LOD of 3.18 the recovery is 92.4 and percent RSD of 5.5. Several metals are identified as qualitative only due to low recovery and suggest the digestion technique used may not be the most appropriate, including Ca, La, Mg, Sb, Sn, Sr, W, Zr. Some species of specific elements will not be completely solubilized by the procedure and refers to other sample digestion techniques, although TiO_2 is not listed as one of these elements. Recovery of TiO_2 from PVC or MCE filters does not appear to have been evaluated to our knowledge.

The solubility of TiO_2 makes it difficult to transfer into solution. Titanium dioxide is described in the Merck Index as a white powder with a melting point of 1855^{o}C, insoluble in water, hydrochloric acid, nitric acid or dilute sulfuric acid but is soluble in hot concentrated sulfuric acid and hydrofluoric acid (HF). Use of hot concentrated sulfuric acid and HF are a concern to chemists. Use of concentrated sulfuric acid in non-teflon ICP OES nebulizer is expected to cause degradation of nebulizer if introduced repeatedly. The occupational hazards of HF, an extremely strong inorganic acid, that can significantly burn and continue to cause severe health complications including possible death if not neutralized. Researchers have used used 7.4M H_2SO_4 and 30% hydrogen peroxide and 18 mol l-1 H_2SO_4 at 250^{o}C followed by dilution to 5.9 mol l-1 of $H2_SO_4$ to adequately digest TiO_2 prior to analysis by ICP.

Specific Aims

As written in the original proposal, the specific aims of the R21 were:

Aim 1. Develop the NPD sampler to collect nanoparticles. We will conduct laboratory tests to design the components of the NPD sampler, and then evaluate the ability of the sampler to collect particles of varying size, composition, and morphology with an efficiency that reflects nanoparticle deposition in the respiratory tract.

Aim 2. Develop and validate an analysis method for detecting metallic nanoparticles collected with the NPD sampler. Laboratory tests will be conducted to optimize an analysis method to detect TiO_2 nanoparticles by ICP-MS. We will then establish method limits of detection (LOD) and quantification (LOQ) and precision for nanoparticles of commercial interest— TiO_2, Ag, CuO, ZnO. We expect analysis to cost roughly $30 per sample, including substrates used in the NPD sampler.

Methodology, Results, and Discussion

Aim 1. Develop the NPD sampler to collect nanoparticles.

Aim 1: Study #1. Refine the design of the impactor and diffusion stages

Impactor. Various impactor designs were assembled and tested. Pressure drop was measured as the difference between the pressure entering and exiting the impactor stage. For collection efficiency tests, we generated an aerosol composed of ammonium fluorescein, with a number median diameter of ~300 nm and geometric standard deviation of ~2, by nebulizing a solution of 0.1 N ammonium hydroxide with fluorescein dissolved in it. This aerosol was passed through a diffusion dryer to dry it and then through a charge neutralizer to render it electrically neutral. We measured the aerosol concentration by size after it passed through the impactor, $C_{out,I}$, and through a bypass tube, $C_{in,I}$, with an APS (Model 3321, TSI, Inc., Shoreview, MN) and an SMPS (Model 3936, TSI, Inc., Shoreview, MN).

The collection efficiency of the impactor was calculated for each size bin of the SMPS and APS as: $E_I = 1 - (C_{out,I} / C_{in,I})$. Three replicates were made for each collection efficiency by size measurement. We altered the geometry of the impactor (number of holes, hole diameter, and distance of the hole exit to the impaction substrate) until the collection efficiency curve had a d50 cutoff diameter of 300 nm +/- 50 nm and a fairly sharp cutoff curve expressed as a geometric standard deviation, $GSD = (d_{84}/d_{16})^{1/2}$, of less than 1.2. The results of the final design are provided under Aim 1: Study #2.

Diffusion Stage. We adapted a theory for stainless steel screens to use with nylon mesh screens. This research was published as follows: **Cena, L. G., Ku, B. K., & Peters, T. M. (2012). Particle Collection Efficiency for Nylon Mesh Screens. Aerosol Science and Technology, 46(2), 214-221.**

Briefly, nylon mesh screens, unlike metal screens, are attractive as a collection substrate for nanoparticles because they can be digested or ashed prior to chemical analysis. A theoretical single-fiber efficiency expression developed for wire-mesh screens was evaluated for estimating the collection efficiency of 11–300 nm particles for nylon mesh screens. Pressure drop across the screens, the effect of particle morphology (spherical and highly fractal-like) on collection efficiency, and single-fiber efficiency were evaluated experimentally for three pore sizes (60, 100, and 180 µm) at three flow rates (2.5, 4, and 6 Lpm). The pressure drop across the screens was found to increase linearly with superficial velocity. The collection efficiency of the screens was found to vary by less than 4% regardless of particle morphology. Single-fiber efficiency calculated from experimental data was in good agreement with that estimated from theory for particles between 40 and 150 nm but deviated from theory for particles outside this size range. New coefficients for the single-fiber efficiency model were identified that minimized the sum of square error (SSE) between the values estimated with the model and those determined experimentally. Compared to the original theory, the SSE calculated using the modified theory was at least one order of

magnitude lower for all screens and flow rates with the exception of the 60-µm pore screens at 2.5 Lpm, where the decrease was threefold. These results were used to design the diffusion stage of the NRD sampler.

Aim 1: Study #2. Evaluate the complete sampler design

We evaluated the fully assembled NRD sampler (respirable cyclone, impactor stage, diffusion stage, and filter) using laboratory procedures that are more rigorous and time consuming than the real-time sizing methods applied above. This work is fully described in the manuscript: **Cena, L. G., Anthony, R., & Peters, T. M. (2011). A Personal Nanoparticle Respiratory Deposition (NRD) Sampler. Environmental Science & Technology, 45, 6483-6490.**

Target Sampler Collection Curve. The first part of this work was to develop a target size selection curve. Particle deposition in all regions of the respiratory tract is shown as the dashed line in **Fig. 2-1**. Deposition of particles measured experimentally under a wide variety of conditions generally follows the respiratory deposition curve for the average adult under light exercise and nose-breathing conditions presented by the ICRP. For this reason, the ICRP curve was used to develop the NPM sampling criterion. The region of interest for the NPM curve was all particles smaller than 300 nm, the minimum deposition for sub-micrometer particles.

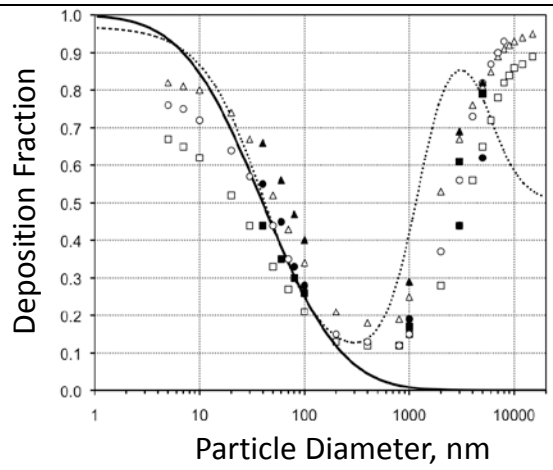

Fig. 2-1. Total deposition fraction vs. particle diameter (aerodynamic). — is the NPM criterion; ···· is deposition in all regions of the respiratory tract as defined by the ICRP. All other symbols are deposition fraction in the normal lung.

We have defined NPM fraction, for a given particle diameter, as the fraction of those particles smaller than 300 nm that, when inhaled, can deposit in the respiratory system. Therefore, the NPM fraction is a subset of the inhalable particulate matter (IPM) collection efficiency, defined as:

$$IPM(d)=0.5[1+\exp(-0.06\,d)] \text{ for } (0 < d \leq 100\ \mu m), \quad (1)$$

where d is the particle diameter in µm (9). The collection efficiency for NPM is given by:

$$NPM(d)=IPM(d)[1-F(x)], \quad (2)$$

where F(x) is the cumulative probability density function of the standardized normal variable x,

$$x = \frac{\ln(d/\Gamma)}{\ln(\Sigma)}, \quad (3)$$

with Γ = 0.04 µm (40 nm), and Σ = 3.9. The mathematical form for this criterion is the same as that used for the thoracic and respirable criteria. The value for Γ represents the particle size associated with 50%

deposition, or d50 cutoff diameter. This value was selected as the particle size smaller than 300 nm associated with 50% deposition as defined by the ICRP curve. Examining **Fig. 2-1**, we find that in this region d50 = 40 nm. The value for Σ was fitted by minimizing the sum of squares error between the ICRP total deposition curve and the NPM equation for particles smaller than 300 nm. This minimization was carried out in an MS Excel (Microsoft Corp., Redmond, WA) spreadsheet using the solver function.

The resulting NPM sampling criterion is shown by the solid line in **Fig. 2-1**. This criterion provided a rational target for the development of the NRD sampler and ties its performance to the physiologically relevant fractional deposition of nanoparticles in all regions of the respiratory tract. The shallow shape of the collection efficiency curve matched the collection efficiency performance form associated with diffusion techniques (rather than the sharp curve associated with impaction). This shallow target curve allowed the sampler to rely on diffusion-based collection with a pressure drop compatible with conventional occupational hygiene belt-mounted sampling pumps.

Description of the NRD Sampler. The NRD sampler consists of three primary components assembled in series: a 25-mm respirable aluminum cyclone (Model 225-01-01, SKC Inc., Eighty Four, PA), an impaction stage and a diffusion stage (**Fig. 1-1**). Air is drawn through the cyclone, which removes particles larger than the respirable sampler criterion and transports the respirable fraction to the impaction stage, where particles larger than 300 nm are removed. In the diffusion stage, the remaining airborne nanoparticles diffuse to and are collected onto eight hydrophilic nylon mesh screens with 11 µm pore size and 6% porosity (Model NY1102500, Millipore Inc., Billerica, MA) with an efficiency closely matching the NPM sampling criterion.

The sampler is lightweight (~60 g), fits in a standard lapel mount (Model 225-1, SKC Inc., Eighty Four, PA) and operates at an airflow rate of 2.5 Lpm with a pressure drop of 3.54 kPa (14.2 in. H2O). The sampler can be used with a commercially available belt-mounted sampling pump for the duration of a work-shift (e.g., AirCheck 2000, SKC Inc., Eighty Four, PA).

The impaction stage was designed to achieve a d50 of approximately 300 nm at a flow rate of 2.5 Lpm. The impaction plate requires the application of a thin layer of vacuum grease prior to operation. The theory developed under Aim 1: Study #1 was used to determine the number and mesh size of diffusion screens necessary to match the NPM sampling criterion. The diffusion screens are tightly held in place in the diffusion stage by an aluminum ring with three spokes. Tests of the collection efficiencies of the impactor, both clean and pre-loaded, and the mesh screen diffusion collector were performed in series.

Effectiveness of the impaction stage of the NRD Sampler. The collection efficiency curve of the impaction stage is shown in **Fig. 2-2**. The minimum collection efficiency (8% ± 3%) was observed for particles with a diameter near 100 nm. For particles progressively smaller than this minimum value, collection efficiency gradually increased to 26% (± 7%) for 15 nm particles. This increase in efficiency was attributed to diffusion losses that may occur throughout the impactor stage. A similar increase in efficiency due to diffusion was observed in the smallest stages of a recently developed, high flow rate (40 Lpm), portable nanosampler consisting of four impaction stages and an impaction filter. For particles larger than 100 nm, the collection efficiency of the NRD sampler impaction stage rapidly increased to

96% (± 6%) for 550 nm particles. Particles in this size range carry sufficient inertia to impact upon the greased impaction plate where they were trapped.

The characteristic cut-off diameter (d50) of the impactor stage was measured to be 295 nm, and the GSD or collection efficiency sharpness was 1.53. This curve is sufficiently sharp to remove particles larger than the target cutoff diameter (300 nm) from the airstream. The square root of Stokes number at the 50% collection efficiency was 0.32 and the pressure drop across the stage was 2.49 kPa. The Reynolds number of the impactor nozzles was 2212, within the desired range of 500 < Re < 3000, where the efficiency curve is at its sharpest.

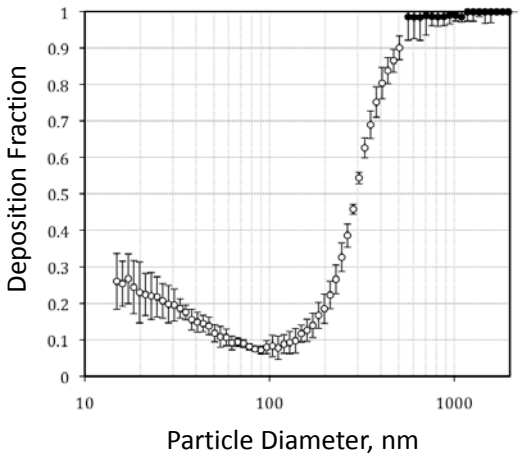

Fig. 2-2. Collection efficiency by size of the impaction stage. Open symbols represent SMPS data; closed symbols represent APS data. Particle diameter is mobility equivalent diameter.

The results of the loading tests performed on the impaction stage are summarized in Table 2. When compared to tests with no prior loading, the effect of particle loading on collection efficiency was negligible, as the mean ± 1 standard deviation overlap for all values in **Table 2-1**. The results of the two-way ANOVA confirmed that there is not a significant difference in efficiency between loadings (p-value = 0.257). The p-value for the interaction term between particle size and loading was close to significant (p-value = 0.063) at a 5% alpha level, indicating that with different loadings the efficiency varies at different particle sizes. The greatest reduction of the impactor's collection efficiency was observed at 15 nm, the smallest particle size tested, where efficiency after loading experienced the greatest decrease from an efficiency of 0.26 without prior loading to 0.11 after 13.6 mg/m3×hr loading. The lower efficiency after loading is attributed primarily to greater uncertainty in this size channel because of lower particle counts. One-way ANOVA was performed on efficiency at the three loading levels for the 15-nm particles showing no significant difference (p-value = 0.102).

Loading of particles on the impactor plate yielded minimal effects (4% to 6% difference) at the largest particle diameters (>300 nm), where particle bounce had greater potential to affect the performance of the subsequent diffusion stage in the NRD sampler. Bounce of particles larger than 300 nm from the impaction plate would cause particles with substantially greater mass than nanoparticles to pass through the impactor and collect on the diffusion screens. This phenomenon would results in a positive sampling bias. The cut-off diameter of the impactor was not substantially shifted after loading (d50 = 265 nm for 13.6 mg/m3×hr loading and 275 nm for 21.5 mg/m3×hr loading). The test dust used in the loading tests contained considerable mass (10 µm volume median diameter) above the d50 of the cyclone of 4 µm, which passed through the cyclone and contributed to the loading of the impaction plate. This indicates that the impaction substrate can handle worst-case particle loadings without experiencing substantial shifts in the way that the impactor performs.

TABLE 2-1: Effects of loading on collection efficiency of the impaction stage.

Particle Diameter [nm]	Prior Impactor Loading [mg/m³×hr]		
	0	13.6	21.5
	Collection Efficiency (StDev)		
15	0.26 (0.08)	0.11 (0.08)	0.15 (0.06)
50	0.12 (0.02)	0.08 (0.04)	0.07 (0.02)
80	0.07 (0.01)	0.07 (0.04)	0.04 (0.01)
100	0.08 (0.03)	0.09 (0.02)	0.06 (0.02)
300	0.54 (0.02)	0.57 (0.01)	0.57 (0.08)
500	0.90 (0.03)	0.89 (0.01)	0.86 (0.07)
800	0.98 (0.02)	0.97 (0.01)	0.92 (0.08)
1000	0.99 (0.01)	0.97 (0.01)	0.93 (0.07)

Effective Deposition to the Screens of the NRD Sampler. The direct measurement of effective deposition of particles to the eight screens of the NRD sampler is shown in **Fig. 1-2** (open symbols). Deposition was lowest (6% ± 2%) for 500 nm particles, where the impactor efficiency was at its maximum, and gradually increased with decreasing particle size. This figure shows that the deposition to the screens was in agreement with the NPM sampling criterion (solid line), within uncertainty, for all points with the exception of the 200 and 500 nm particles where it matched within < 4%. This means that particles deposited on the screens can be analyzed to determine the concentration of nanoparticles that would deposit in the respiratory system.

Aim 1: Study #3. Evaluate the NRD sampler with metal aerosols of varying morphology

Lastly for the development of the NRD sampler, we determined the extent to which nanoparticle collection efficiency to the substrates of the diffusion stage changes as a function of particle morphology. Using the experimental set up shown in **Fig. 2-3**, silver (Ag) particles of varying morphology

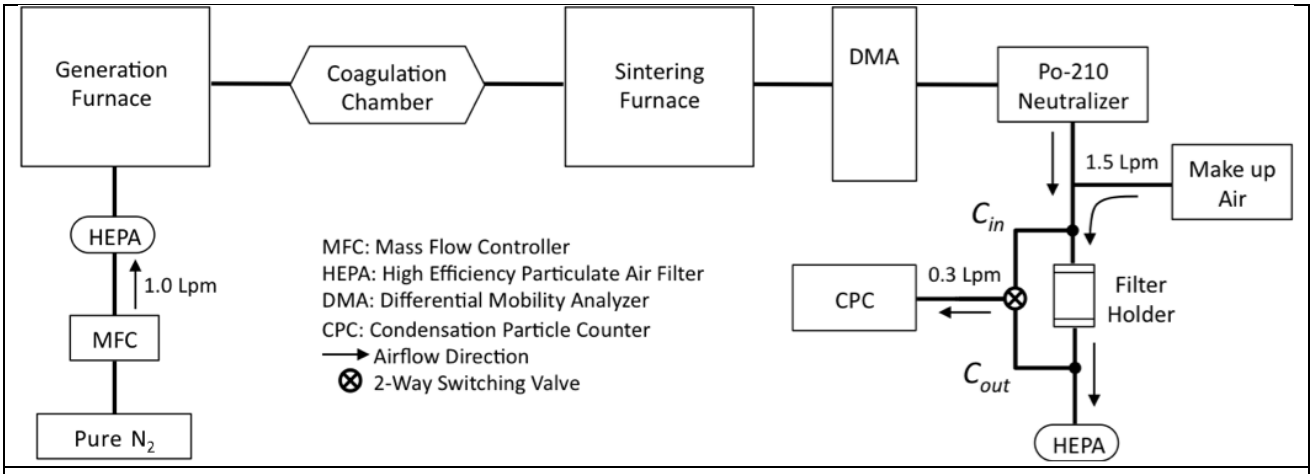

Fig. 2-3. Collection efficiency by size of the impaction stage. Open symbols represent SMPS data; closed symbols represent APS data. Particle diameter is mobility equivalent diameter.

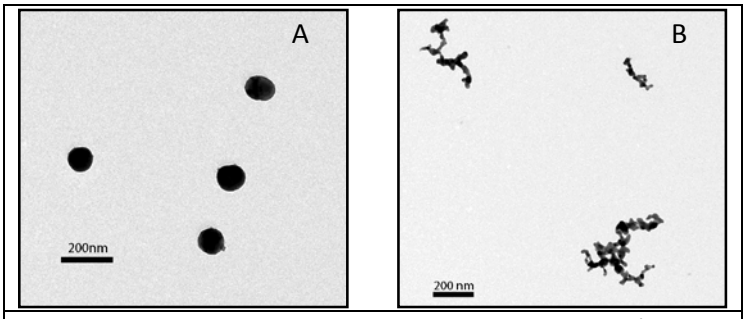

Fig. 2-4. Silver nanoparticles with sintering at 600˚C (A) and without sintering (B).

were generated using two horizontal tube furnaces in series. The first furnace (Lindberg/Blue, Laboratory Tube Furnace STF55433C) was operated at 1200˚C with pure nitrogen (purity level 99.999%) at an airflow of 1.0 Lpm passed over silver wire (purity level 99.9%) placed in a ceramic boat. A digital mass flow controller (Model 0154, Brooks Instrument, Hatfield, PA) was used to maintain a constant nitrogen flow rate. The aerosol leaving the first furnace was passed through a coagulation chamber (with residence time of about 40 seconds) where the aerosol cooled to form chain agglomerates with an open structure (**Fig. 2-4, B**). For highly fractal tests, the second furnace (Blue TF55035A, Blue M, New Columbia, PA) was operated at room temperature (furnace off) for highly fractal tests. Alternatively for spherical particle tests, the second furnace was operated or at 600˚C to fully sinter the agglomerates so that they would form spheres when cooled (**Fig. 2-4, A**).

A differential mobility analyzer (DMA -Model 3081, TSI Inc., Shoreview, MN) was used to select the particles with mobility diameters of 15, 40 and 100 nm. A polonium-210 radioactive source (Model 2U500, NRD LLC, Grand Island, NY) was used to neutralize the aerosol before it was passed through a stack of either five or twenty NY60 nylon net screens at 2.5 Lpm. New screens were passed under a Po-210 source (Model 2U500, NDR LLC, Grand Island, NY) for 10 seconds and loaded in the filter holder before each test. A condensation particle counter (Model 3022A, TSI Inc, Shoreview, MN) was used to measure monodisperse aerosol concentration for 120 seconds before (Cin) and after (Cout) passing through the screens. This measurement procedure was repeated three times. Collection efficiency was calculated as E=1-(average Cout/average Cin)

The results of the morphology tests are summarized in **Table 2-2**. For each mobility size, morphology affected efficiency by less than 4 percent. No specific morphology was found to affect overall efficiency more than another. For the 15 nm aerosol, highly fractal particles had lower collection efficiency (34.3%) than spherical particles (37.9%). For 40 and 100 nm mobility sizes the spherical particles had lower collection efficiency (43.9% and 23.4% respectively) while highly fractal particles had higher collection efficiency (45.6% and 24.0% respectively). Highly fractal particles have larger interception length than spherical particles, however, when compared to the screen fiber diameter (33 μm) the

Table 2-2. Effects of Particle Morphology on Collection Efficiency of NY60 Screens

dp [nm]	Number of Screens	Collection Efficiency (Standard Deviation) %	
		Spherical	Highly Fractal
15	5	37.9 (0.6)	34.3 (0.7)
40	20	43.9 (0.1)	45.6 (0.5)
100	20	23.4 (0.3)	24.0 (0.2)

particles studied in these tests are so small (≤100 nm) that interception may not play a significant role in their collection.

Aim 2. Develop and validate an analysis method for detecting metallic nanoparticle collected with the NRD sampler

The primary objective of Aim 2 was to develop and validate a method to determine the metals content of nanoparticles collected on the substrate in the diffusion stage of the NRD sampler. A secondary objective is to determine the ability of this method to determine the fraction of metals attributed to nanoparticles within a respirable sample.

Aim 2: Study #1. Evaluate collection substrates of the NRD sampler for interferences

Laboratory tests were conducted to evaluate background metals content of the media, recovery of the analyte (i.e., Ti from TiO_2 particles) from the media, and stability of the sample extract.

TiO_2 Experiments. Twenty-five blank samples were prepared by placing 8 nylon screens into a digestion vessel. Blanks for three different batches were prepared. Spikes were prepared by placing 8 nylon screens were placed into a digestion vessel. A solution of titanium dioxide in distilled water was deposited onto the filter to prepare spikes with 36 µg or 360 µg of titanium dioxide. The spiking solution was then analyzed immediately (not dried spikes) or allowed to dry (dried spikes) and then analyzed. The 36-µg spike was selected to assure detection at 1/10 of the NIOSH recommended exposure limit of nanoparticle titanium dioxide.

For each condition, 25 spikes were prepared for each of two digestion methods: Traditional Method 7300; Modified Microwave Digestion Method. Using the *traditional Method 7300*, 3.0 mL of concentrated nitric acid was added and the digestion vessel placed in a heatblock at 95^{o}C until the sample volume reduced to about 0.5 mL. The digestion vessel was removed from heat and allowed to cool. Another 3.0 mL of concentrated nitric acid was added to the digestion vessel and returned to the heatblock until sample reduced to near dryness. Sample was removed from heat block and allowed to cool. Sample was brought to a final volume of 25 mL with 5% nitric acid (v/v) and 5% hydrochloric acid (v/v).

For the Modified Microwave Method, 4 mL of concentrated sulfuric acid and 2.5 mL of nitric acid were added to each digestion vessel. The vessel was then placed in a microwave digester, where the temperature was increased to 190^{o}C over 15 minutes, held for 15 minutes, and then cooled to ambient temperature. The contents were then transferred to a 50-mL plastic digestion tube and brought to a 25-mL volume with de-ionized water. Secondary dilutions were prepared with de-ionized water.

As shown in **Table 2-3**, the nylon filters spiked with titanium dioxide nanoparticles yielded an average recovery of 13% for titanium dioxide (as Ti) using the Traditional Method 7300. Using the Modified Microwave Method, recoveries were poor until the higher spike value was used. With the higher spike, recovery was 92%.

Table 2-3. Comparison of recoveries of TiO_2 for the Traditional NIOSH 7300 and Modified Microwave Method

Spike application to filter	Level of spike ug	8 Screens	Method 7300		Microwave Method	
			% Recovery	Sr	% Recovery	Sr
Not dried	36	Y	12.6	0.45	32	0.48
dried	36	Y			16.5	0.57
not dried	360	Y			75	0.18
Direct, not applicable	26	N			92	0.18

As shown in **Table 2-4**, the nylon screens used in the NRD sampler were found to contain titanium dioxide. Several lot numbers of filters were used and the level of titanium dioxide on the filters was significantly greater than the desired reporting limit.

Table 2-4. Analysis of blank NRD media (8 Nylon mesh screens) analyzed by Modified Microwave Method.

Lot Number	**Mean Mass TiO_2, µg**	**StDev TiO_2, µg**	**CV, %**
1 RONA88520	217	18	8.3
2 ROPA12501	421	30	7.1
3 R2DA01938	428	29	7.0

Experiments Conducted with Metals Other Than TiO_2. Spikes were prepared by placing 8 nylon screens were placed into a digestion vessel and spiking solutions containing metals as shown in **Table 2-5** were applied by pipet. Selection of concentrations was based on 1/10 TLV of the metal. Samples were analyzed by the Traditional Method 7300 as described above.

Recovery and an estimated LOD for select metals is presented in **Table 2-6**. Recovery for most metals were acceptable, except for chromium and nickel. Nickel may be present as an artifact or some other contamination. NIOSH 7300 addresses chromium as requiring special treatment for recovery and thus the sample preparation method was not optimized for chromium recovery. These results show that the many different metals can be analyzed when the nylon screens are used as a collection media.

Table 2-5. Spike levels and rationale.

Element	Lot # of standard	TLV mg/m3	Spike Value, ug/sample	Notes
Al	16-122AL	1	24	
Co	17-99CO	0.02	0.88	
Cr	CL5-10CR	0.5 metal	12	TLV as metal
Fe	AF1Y55FE	1 as salts	250	TLV as salts
Mn	16-65MN	0.2	5	
Mo	16-167MO	0.5 soluble	12	TLV as soluble
Ni	16-170NI	0.1 soluble	2.5	TLV as soluble
Ti	1034414	NIOSH REL	5	
V	17-18V	0.05	1.2	
Zn	17-871ZN	2	50	

NIOSH Manual of Analytical Methods Chapter C discusses the practice of analytical standards bracketing the expected concentration of the samples (eg. To determine if exposure standards are being met or if element is present). A concentration at 1/50th of the TLV was selected as a target value for the spike level as an initial evaluation of the determination of element detection. The exception was Titanium where 1/10 of the NIOSH REL for titanium dioxide was selected.

Table 2-6. Results NIOSH method 7300 with liquid spikes.

		Blank				Recovery	
Element	Analysis LOQ [a]	Mean Mass, µg	St. Dev. Mass, µg	CV, %	Est. LOD 3*STD	Spike Level, µg	Recovery, % (CV, %)
Aluminum	2.5	1.5	0.42	29	1.3	24	92 (1)
Cobalt	1.25	0.005	0.013	279	0.038	0.88	75 (5)
Chromium	0.5	0.59	0.22	36	0.65	12	19 (2)
Iron	0.5	1.02	0.46	45	1.37	250	90 (1)
Magnesium	0.5	1.13	0.037	3.2	0.11	5	93 (1)
Molybdenum	1.25	ND	ND	ND	ND	12	93(1)
Nickel	1.25	ND	ND	ND	ND	2.5	620(1)
Titanium	1.25	1.71	0.234	14	0.70	5	88(1)
Vanadium	1.25	0.001	0.0032	316	0.009	1.2	95(1)
Zinc	0.5	1.633	0.297	18	0.892	50	87(1)

[a] single laboratory limit of quantitation.

ND – not detected

Aim 2: Study #2. Develop and evaluate the analysis method

The original objective of this study was to develop and evaluate the overall system of sampling, digestion, and analytic methods for assessing TiO_2 with the NRD sampler. Given that the nylon mesh screens were found to contain titanium, the scope of this study was modified to evaluate the ability to detect TiO_2 using PVC filters. These filters are recommended for use in conjunction with respirable sampling by NIOSH to detect TiO_2.

The filters used in this study were 37 mm, 5.0-µm-pore PVC filters (SKC Part # 225-8-01-1, lot # T21015-2171. lot # T21380, T21271 and T217661). Titanium dioxide was dispensed onto the filters at a level of 36 ug titanium dioxide by placing 150 uL of titanium dioxide solution prepared with 12.18 mg titanium dioxide (Nano Scale Corporation Titanium Dioxide Nanoactive Part # AC012-0100-00NS, Lot # 12-0272) in 50 mL distilled water. Samples were analyzed by traditional NIOSH 7300 and using the modified microwave digestion method described in the previous section.

As shown in **Table 2-7**, the titanium dioxide found in the PVC filters was 0.54 ug/filter, far less than found on 8 nylon mesh screens (see **Table 2-4**). We are finishing the analysis of samples so that we will have 25 filters analyzed for the modified microwave digestion method.

Table 2-7. Summary of analysis of blank filters.

Digestion Method	N	Mass of TiO_2, µg/filter		Coefficient of Variation, %	Estimated Limit of Detection, µg/filter
		Mean	Standard Deviation		
Traditional NIOSH 7300	25	0.05	0.01	17	0.03
Modified Microwave Method	1	0.54			

LOD defined as 3 * standard deviation of the blank

As shown in **Table 2-8**, recoveries were low with for the traditional NIOSH Method 7300 (50%) compared to those with the modified microwave method (73%). These results demonstrate that more aggressive digestion is needed to ensure that the TiO_2 goes into solution and are consistent with the results found for the NRD samplers.

Table 2-8. Summary of Titanium dioxide recovery by method (n = 25 for each cell)

Matrix	Matrix digestion method	Recovery, %	Coefficient of Variation, %
PVC Filter + Spike	NIOSH 7300	50	10
Spike Solution Only	NIOSH 7300	50	9.3
PVC Filter + Spike	Modified Microwave Method	73	4.6

Blank-PVC-std 0.068 ug/sample Titanium dioxide
Blank-PVC-mod 0.54 ug/sample Titanium dioxide

Conclusions

The lightweight, personal NRD sampler was developed to selectively collect particles smaller than 300 nm similar to their typical deposition in the respiratory tract. The pressure drop of the NRD sampler is sufficiently low to permit its operation with conventional, belt-mounted sampling pumps. With chemical analysis of the diffusion media, the NRD sampler can be used to directly assess exposures to nanoparticles of a specific composition apart from other airborne particles.

Future work will include field tests of the NRD sampler to distinguish nanoparticles apart from background aerosols. Practical considerations will be assessed, including sample duration, frequency of impaction plate cleaning and re-greasing, the effects of tipping the cyclone-based sampler, and increases of pressure drop across the diffusion stage with mass loading. In addition, analytical techniques require further elaboration, including nylon mesh digestion and detection limit determination.

Publications Resulting From This Work

Cena, L.G., Ku, B K., & Peters, T.M. (2012) Particle Collection Efficiency for Nylon Mesh Screens. Aerosol Science and Technology, 46(2), 214-221.

Cena, L.G., Anthony, T.R., & Peters, T.M. (2011) A Personal Nanoparticle Respiratory Deposition (NRD) Sampler. Environmental Science & Technology, 45, 6483-6490.

Kostle, P.A., Anthony, T.R., & Peters, T.M. (in preparation) Analysis Methods to Detect Metallic Nanoparticles Using a Novel Respiratory Deposition Sampler. Journal of Occupational and Environmental Hygiene.

Kostle, P.A., Anthony, T.R., & Peters, T.M. (in preparation) Analysis of Polyvinyl Chloride Filters to Detect Titanium Dioxide in Air. Journal of Occupational and Environmental Hygiene.