Nanoparticle Sampling and Analysis
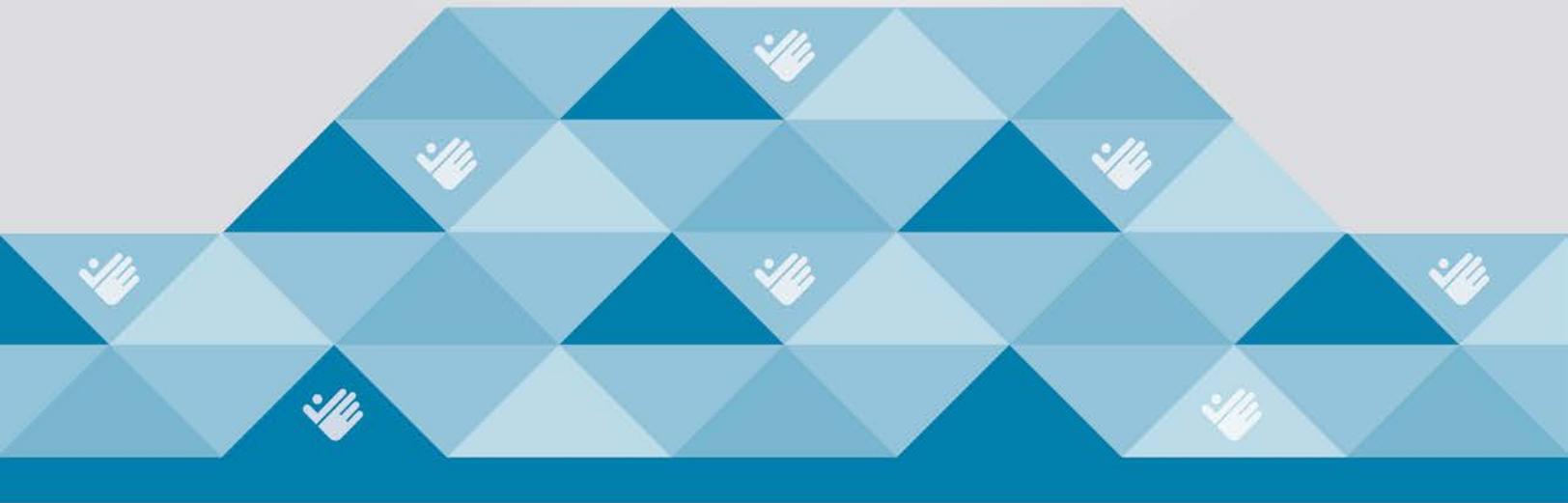
Fact Sheet Sponsored by the
AIHA® Nanotechnology Working Group

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General Overview

Nanoparticles are typically described as having at least one dimension in the size range of approximately 1 to 100 nanometers, although they are often present as larger aggregates or agglomerates. Sampling of nanoparticles is often similar to sampling for other particles. But because much is still unknown about which properties of engineered (i.e., intentionally produced) nanoparticles contribute to health hazards, it can be difficult to know what specific parameters to test for.

The hazards associated with an engineered nanoparticle (ENP) may be different from the hazards of the non-nanoscale version of the same material. It is important to collect data on these materials to gain a better understanding of how they behave and what, if any, potential health risks they may pose.

Each sampling scenario for ENPs can be unique, so good communication between all parties involved is important to ensure quality data that have value to the end user. Some of the typical challenges encountered when sampling for ENPs include:

- It is difficult to differentiate ENPs from "background" levels of ambient (incidental, not purposefully engineered and manufactured) nanoparticles, which can be ubiquitous in the work environment.
- Work activities involving ENPs may be periodic and short in duration.
- Optimizing procedures to achieve low detection limits is challenging, especially for mass-based methods.
- Readily available reference materials or standards are lacking for particular ENPs of interest.
- In most cases, sparse guidance is available for acceptable levels of ENPs.

It is important to carefully consider up front what questions need to be answered, in order to determine the best sampling and analytical approach. The ENP supplier (technical contact or product steward) may be able to provide useful information, such as agglomeration tendency or presence of surface modifications. Samples should not be collected without first having an understanding of the approaches to be used, the capabilities and limitations of equipment and methods, and how the data will be interpreted.

Some sampling and analytical methods are used only to determine particle concentrations. Others are used primarily for particle characterization. For most nanoparticle sampling scenarios, a combination of methods will give the best overall picture. A tiered approach is often used for airborne particle monitoring, progressing from methods that are easier but less informative to methods that are more complex and resource intensive but that provide more in-depth information.

The use of multi-metric particle concentration measurements (e.g., some combination of mass, count, surface area, and size distribution) may be useful when the most appropriate metric has not been determined. It is always a good idea for the industrial hygienist and the analytical laboratory to discuss the target analyte and sampling scenario beforehand to determine the goals and objectives of sampling, as well as the advantages and disadvantages (and uncertainties) of the various sampling and analytical approaches.

Example Scenarios for ENPs in Products or Processes

Listed below are example ENP scenarios and possible sampling and analytical approaches. These examples are only for illustration of sampling and analytical considerations. Specific techniques are described in more detail at the end of this fact sheet in the Sampling and Analytical Toolbox section.

Are there ENPs in this product?

Each type of bulk sample is unique. Finding and identifying ENPs in a material often requires removing as much of the non-ENP matrix material as possible prior to analysis. This is easier for some samples than others. Looking for and quantifying titanium dioxide nanoparticles in toothpaste would likely involve gravimetrically tracking removal of the organic matrix materials such as glycerol prior to analysis. Once the sample has been reduced to a powder, it is more easily prepped for analysis to determine the concentration of ENPs in the initial sample.

One analytical approach is to deposit the sample onto a filter or other substrate (after proper preparation) for analysis by scanning electron microscopy (SEM), transmission electron microscopy (TEM), or scanning transmission electron microscopy (STEM) in order to detect, identify (for example determining elemental composition), and quantify any ENPs present. A particle-size distribution analysis can be performed, as well as other measurements such as circularity to characterize particle shape.

Analysis for "any and all" nanoparticles in a sample is more complex than analysis for a specific target analyte (e.g., titanium dioxide nanoparticles in toothpaste). Remember that naturally occurring or incidental nanoscale particles are often present that may not be the engineered nanoparticles of interest.

Are ENPs released from the product during use?

Product testing for ENP release is unique to the product, the nanoparticles, work practices, engineering controls, and the end-use scenario. Examples include looking for silver nanoparticles released from antibacterial fabrics, or titanium or zinc oxide nanoparticles from sunscreens during spray application.

There are numerous ways to conduct such studies. One approach is to use an environmental chamber to collect air samples during simulated product use with subsequent surface sampling of the chamber. The advantage of using a chamber is that the experiment is controlled, with better containment of possibly hazardous materials and fewer variables such as background contamination from incidental nanoparticles.

My company makes a fine powder product (e.g., pharmaceuticals, activated carbon). How fine are the powders/granules and what is the active surface area?

For surface area measurements of fine powders, a BET (Brunauer, Emmett and Teller) analysis can be performed. This technique determines active surface area by measuring the amount of gas (typically nitrogen) that can be adsorbed onto a sample as a function of relative pressure and temperature.

Atomic force microscopy (AFM) is also a good choice due to its unique ability to size particles in three dimensions (including height) for a much more accurate estimation of surface area. With this technique, particle size, shape, and size distributions can be performed.

A microscopic analysis (SEM/TEM/STEM) with energy dispersive X-ray spectroscopy (EDS) provides morphological data as well as data on sizing and elemental composition.

Are existing engineering controls sufficient for worker handling of ENPs?

It may be possible to identify potential ENP release points via "walk-through" observation and knowledge of the process. To confirm observations or identify ENP release via sampling, a tiered approach is typically recommended, beginning with direct-reading instruments and supplemented as needed with filter-based samples for additional analyses.

The direct-reading instruments can help in identifying sources of particle emissions and temporal variability. The filter-based analyses can provide data on physical and chemical properties such as particle size, shape, surface area, agglomeration state, and composition.

Air-monitoring projects should be carefully designed to consider background sources, levels and characteristics of any ENPs present, sampling instrument, and method capabilities and limitations. One limitation of air sampling is the variability that can occur due to electrostatic charge and humidity levels depending on the ENP involved. If a source of ENP release is discovered, changes to the process or engineering controls should be considered to reduce the potential for exposure.

To examine the effectiveness of an engineering control used to provide worker protection (e.g., a laboratory hood), a traditional approach involves comparing the concentration in the worker breathing zone to an occupational exposure limit (OEL) relevant for that material. However, the standard tools —OEL, validated sampling and analytical methods, and standard approach to data interpretation— to complete quantitative worker exposure assessments for nanomaterials rarely exist. There are, however, assessment methods that can help evaluate performance of engineering controls for the handling of ENPs.

For example, data interpretation may involve comparison of measurements made inside and outside a laboratory hood, or outside the hood before and after a process. In addition, the use of traditional methods for evaluating hood containment, such as tracer gas release, can also be employed. Other options include an ALARA (as low as reasonably achievable) approach, or a straightforward, "only non-detect is acceptable" strategy.

Direct-reading instruments such as particle counters can be a good first line of testing, because they give real-time concentration data. Depending on the target nanoparticle size, shape, agglomeration state, and so on, the IH can employ aerosol photometers, optical particle counters, and condensation particle counters, or a combination of these. Because these techniques are nonspecific and cannot distinguish between single particles and agglomerates, particle counters are typically used in conjunction with other techniques such as TEM, SEM, or STEM analysis.

Personal or area samples — collected on filters or directly onto TEM grids using thermophoretic or electrostatic precipitators for microscopic analysis — can shed light on what the particle counters are measuring. In addition, these analyses can provide elemental composition data for the particles present and information on particle-size distributions, shape, etc.

Regardless of the sampling and analytical approaches selected, the IH must consider the presence of background particles, concentrations of which can be significant and highly variable and can impact the proper interpretation of sample results. The various approaches to measuring background particles depend on the sampling environment and other factors.

My company handles and produces carbon nanotubes (CNTs). What is the concentration of CNTs in the air during the use/production cycle?

CNTs are one of the few nanomaterials for which an OEL has been published and a sampling and analytical approach suggested. NIOSH has adopted a recommended exposure limit (REL) of 1 microgram per cubic meter ($\mu g/m3$) of respirable elemental carbon (EC) as an 8-hour time-weighted average (TWA). Analysis is performed by the NIOSH 5040 method.

Unfortunately this mass-based elemental carbon REL is an imprecise and relatively insensitive metric. Moreover, in some work environments background particles can contribute significantly to the resulting mass measurement. To address this, background samples for EC can be collected outdoors or at air intakes of the facility, in addition to other indoor locations where CNT exposure is unlikely.

Any background levels can be subtracted from the EC worker measurements to determine whether the worker exposure exceeds the REL. Mass results are often augmented with a count-based or particle speciation analysis by TEM with EDS, which provides better sensitivity to low levels of CNTs as well as confirmatory visual, morphological, and chemical data on CNTs that are present.

I am an ENP producer/user and have cleaned the facility (such as prior to changing processes or after a release). How can effectiveness of the cleaning process be verified?

Particle counters can provide instant feedback for air samples. Obtaining background-level measurements before cleanup is a good idea. Simultaneous sampling in both control areas and active areas is another approach to generate background information for comparison.

Air sampling for TEM, SEM, or STEM analysis can provide higher quality data, including separate concentration determination of single ENPs vs. agglomerates and characterization by elemental composition, diameters, circularity, etc.

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Surface sampling in conjunction with air sampling gives a more complete picture of the potential for exposure. Wipe samples can assist in the evaluation of general housekeeping and determine whether migration of ENPs is occurring outside of the production area, an event that would increase the potential for dermal, oral, or respiratory exposure.

As long as the ENP has a specific and unique chemical composition not found in the background, analysis of a clearance wipe or adhesive lift sample may provide valuable information in determining cleaning efficacy.

Sampling and Analytical Toolbox

Listed below are some of the many techniques that can be applied to various engineered nanoparticle (ENP) characterization or concentration measurement scenarios. Each has pros and cons. The choice of sampling and analytical options will depend on many factors including the particles and properties of interest, target nanoparticle size, sample matrix, cost, and required turnaround time.

Some of these techniques, such as the use of condensation particle counters for airborne particles, are matrix specific. Others, such as the microscopy techniques, are more flexible and can lend themselves to analysis of particles captured from air, suspended in water, and even embedded in solid materials.

DIRECT-READING REAL-TIME MONITORS (MATRIX: AIR)

These instruments are an attractive option: their real-time monitoring of nanoparticles in air provides both immediate feedback on the sources of emissions and data on the performance of engineering controls, if any, that are in place. Some of these instruments are count based while others are mass based. Because of the limitations of these instruments (outlined below), they are often used in conjunction

with other techniques such as conventional filter-based sampling.

Optical particle counters are limited in that they can detect and quantify particles in air only in the 300 nm to 20 μm range, which is, generally speaking, outside the defined nanoparticle range. However, since nanoparticles very often are present as large agglomerates, these instruments have definite value. They are often used in conjunction with condensation particle counters, allowing for better size classification of all particles present.

Condensation particle counters can detect and quantify particles in the air in the 10 nm to 1 μm range, which makes them a good tool for nanoparticle screening.

Specialized direct-reading instruments measure various parameters of particles in air. Examples include the scanning or differential mobility particle sizer (SMPS or DMPS), which provides a number concentration by size; diffusion chargers (active surface area concentration); mass-based aerosol photometers (nephelometers); nanoparticle surface area monitors; and Electrical Low Pressure Impactors (ELPIs), etc.

Direct-Reading Real-Time Monitor Limitations

- These devices are not particle specific. They provide number concentrations. Because they can also measure diameters, they can provide some information about the level of agglomeration.
- Optical-based instruments measure the optical diameters of particles rather than a physical property that may be related to particle transport which could, help determine the chance for respiratory deposition.
- Instrument readings may include contributions from incidental nanoparticles such as welding fumes, outdoor diesel exhaust, propane (such as forklift) exhaust, and gas-powered heaters as well as ambient environmental particles. It may be advisable, therefore, to run simultaneous background samplers or to compare results before, during, and after a process involving ENPs.
- Nanoparticles very often occur as agglomerates or groups of particles. Real-time monitors cannot distinguish groups of nanoparticles from individual particles. Particle counters will count them as one large particle, resulting in false negatives or underreporting of nanoscale particles. This can be a serious limitation. The use of these types of instruments with fibrous particles like CNTs presents an additional challenge because fibrous particles are not spherical in shape, contrary to the way that many of these instruments are calibrated.
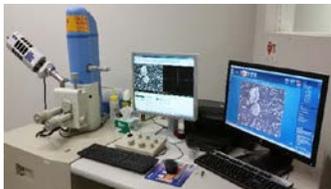
FILTER-BASED MICROSCOPY METHODS (MATRIX: AIR, SOLID, AND LIQUID)

Though they do not supply real-time information, these lab-based analyses can provide more detailed information of the particles present. Simply being able to visualize the particles is the most obvious advantage that the microscopic methods provide. Concentration, size, shape, and chemical composition are other possible parameters. The two main disadvantages of these methods (and others) are that: in most cases the methodologies are not yet fully standardized for ENPs, and the collection efficiencies of the filters themselves have not been fully determined.

Scanning Electron Microscopy (SEM)

Although it does not possess the magnification power of TEM or STEM (see below), this approach can provide excellent detail of surface morphology and particle-size distribution as well as elemental analysis by energy dispersive spectroscopy (EDS) or wavelength dispersive spectroscopy (WDS).

Transmission Electron Microscopy (TEM)

TEM is the ultimate tool for resolving power, having magnification and resolution capabilities beyond AFM and SEM. It has the ability to perform elemental analysis on even the smallest individual nanoparticles with energy dispersive spectroscopy (EDS).

Scanning Transmission Electron Microscopy (STEM)

This microscope combines all the power of TEM (including EDS) with the ability to provide scanned surface images like the SEM. In many ways, this is the ideal solution for high-resolution ENP analysis.

OTHER METHODS

### Thermo-Optical Analysis for Carbon Nanotubes (Matrix: Air)

NIOSH method 5040 is most typically used to measure the level of soot in workplace environments. With this method, one can determine the levels of organic carbon (OC), elemental carbon (EC), and total carbon (TC). The amount of EC can relate to the presence of carbon nanotubes. The current NIOSH REL is 1 microgram EC per cubic meter ($\mu g/m^3$) of air as an 8-hour TWA.

This is a relatively insensitive method; moreover, other types of EC, such as diesel soot or carbon black, can lead to false positives for CNTs. Other analytical techniques such as transmission electron microscopy (TEM) are often used to verify the presence or absence of CNTs.

Inductively Coupled Plasma (ICP) (Matrix: Air)

NIOSH methods 7300 and 7303 for metals can be used to determine compliance with the NIOSH REL for titanium dioxide: 2.4 mg/cubic meter for fine TiO_2, 0.3 mg/cubic meter ultrafine (nano-scale) TiO_2 as a Time Weighted Average (TWA) concentration for up to 10 hours per day during a 40 hour work week.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Matrix: Air)
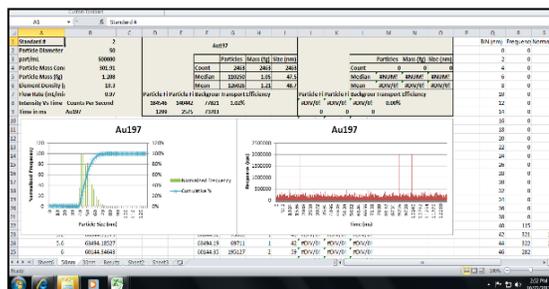
ICP-MS enables single-particle count with identification for insoluble particles in suspension. ICP-MS in time-resolved mode makes it possible to collect the intensity for a single particle as it is vaporized and atomized in the plasma. Each measured data point can be correlated to the size and mass fraction of the particle.

Dynamic Light Scattering (DLS) with Zeta Potential (Matrix: Bulk as Powders, Liquids)

This technique is also known as photon correlation spectroscopy (PCS). It can be used to determine the size distribution profile of small particles *in suspension*. It determines particle hydrodynamic diameter by measuring the diffusion speed of particles or molecules undergoing Brownian motion. This technique can detect particles down to a single nanometer.

DLS has limitations, however. Like other types of particle sizers and counters, it cannot distinguish particle types or composition, nor can it distinguish agglomerates of nanoparticles from other large particles. This can lead to under or over reporting. The predominant factor for determining whether a dispersion of nanoparticles will agglomerate is the magnitude of the electrostatic repulsion/attraction (zeta potential). Knowing the zeta potential adds value to the corresponding DLS result.

Thermogravimetric Analysis (TGA) (Matrix: Solid, Liquid)

For many applications (e.g., medical) or for quality control for ENP producers, it is extremely important to know the purity and exact composition of nanoparticles. Microscale TGA is uniquely suited to handle the small sample size of ENPs to determine composition purity, including the presence of surface coatings. TGA can also be used to determine the amount of surface-bound ligand coverage on gold nanoparticles for instance.

TGA measures a sample's weight change as it is heated (or cooled) in a temperature range from -196°C to >1000°C. This is useful in determining the composition of materials including, for example, the presence of residual catalyst. This technique can quantify water, solvent, plasticizer, decarboxylation, pyrolysis, oxidation, decomposition, weight percentage filler, and weight percentage ash.

Spectroscopy (Fourier Transform Infrared (FTIR/Raman) (Matrix: Solid or Liquid)

Absorption/transmission spectroscopy measures how a sample absorbs light at each wavelength. As light passes through the sample, the resulting spectrum represents the molecular absorption and transmission to create a molecular fingerprint of the sample.

### Atomic Force Microscopy (AFM) (Matrix: Powder)

Sample preparation for powders involves the stable attachment of particles onto a very smooth substrate (typically a mica sheet). AFM's unique ability to size particles in three dimensions (including height) provides impressive morphological detail and a much more accurate estimation of surface area than other microscopy methods. With this technique, particle size, shape, and size distributions can be determined.

Small-Angle X-ray Scattering (SAXS) (Matrix: Solid or Liquid)

This specialized X-ray diffraction technique measures diffuse X-ray scattering at a very low angle, caused by electron density fluctuations in the material under study. The angle is inversely proportional to the size of the particles. This technique can provide good particle-size distribution data, but only over very narrow ranges.
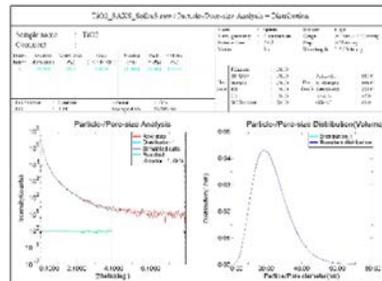
BET/Surface Area (Brunauer, Emmett and Teller) (Matrix: Powder)

The surface area of a powder can affect its behavior in many applications including pharmaceuticals. The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface area of the test powder. The determination is usually carried out at the temperature of liquid nitrogen.

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Acknowledgments