Detection and Identification: Instrumentation and Calibration for Air/Liquid/Surface-borne Nanoscale Particles

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Abstract. Nanoscale particles can be found in the air-borne, liquid-borne and surface-borne dispersed phases. Measurement techniques for nanoscale particles in all three dispersed phases are needed for the environmental, health and safety studies of nanomaterials. We present our studies on connecting the nanoparticle measurements in different phases to enhance the characterization capability. Microscopy analysis for particle morphology can be performed by depositing air-borne or liquid-borne nanoparticles on surfaces. Detection limit and measurement resolution of the liquid-borne nanoparticles can be enhanced by aerosolizing them and taking advantage of the well-developed air-borne particle analyzers. Sampling electrically classified air-borne virus particles with a gelatin filter provides higher collection efficiency than a liquid impinger.

1. Introduction
Nanoscale particles, e.g., engineered nanoparticles, soot agglomerates from engines, and viruses, exist in the air-borne, liquid-borne and surface-borne dispersed phases [1]. It is necessary to develop instrumentation and measurement techniques for all three dispersed phases in order to gain better understanding of the life cycle of these nanoscale particles. For example, engineered nanoparticles often are produced in flame synthesis in the air-borne phase. They are coated on various surfaces to enhance performance of nano-products in the surface-borne phase. Upon disposal of these nano-products, the coated nanoparticles may be released in the waste streams in the liquid-borne phase. Physico-chemical characterization of nanoscale particles in these phases are therefore important in the environmental, health and safety studies of nanomaterials in support of sustainable nanotechnology [2, 3].

Each characterization method for the nanoscale particles in different phases has its own applicable size and concentration ranges. Decision on the use of a specific method will depend on the properties of the particles of interest. Particles are best characterized at their original phase, with minimum handling or perturbation. For example, nanoscale particles in liquid-borne phase will form aggregates when dried or pH changes. However, transferring the particles from one phase to another, sometimes, allows additional information of the particles to be obtained. In this paper, we will describe some of the experimental techniques we have developed to connect nanoscale particle measurements in three dispersed phases. By aerosolizing liquid-borne nanoparticles into the air-borne phase, the particle sizing resolution improves significantly because the air-borne size analyzer, the Scanning Mobility Particle Sizer (SMPS), has sizing resolution significantly better than the liquid-borne Nanoparticle
Tracking Analysis (NTA) or the Dynamic Light Scattering (DLS) techniques. By depositing air-borne or liquid-borne nanoparticles on surfaces, they can be inspected by the Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Spectrometry (EDS) and Atomic Force Microscopy (AFM) for particle morphology and chemical compositions. While viruses are often transmitted in the air-borne phase, most of the virological tools are based on liquid-phase analysis. It is therefore particularly important to develop tools that can disperse and deposit nanoparticles in all three phases. We will demonstrate that by correlating nanoscale particles dispersed in different phases, it is possible to obtain accurate calibration of instruments for particle size and concentration.

2. Liquid-borne/surface-borne correlation

Liquid-borne nanoscale particles are encountered when they are manufactured and stored in liquid phase, e.g. CMP slurry, or released to the water stream in the environment. Common commercialized methods for sizing liquid-borne nanoparticles include: Field-Flow Fractionation (FFF), Dynamic and Static Light Scattering (DLS and SLS), Laser Particle Sizing (LPS) and Nanoparticle Tracking Analysis (NTA) [4-6]. It should be noted that these methods use different principles, namely the particle diffusion and light scattering properties, to measure the equivalent particle size distribution. One should be careful when comparing the size measured by different methods.

In addition to various liquid-borne nanoparticle characterization techniques, microscopy methods can further provide valuable information of the nanoparticles. In order to perform microscopy analysis, nanoscale particles in liquid-borne phase need to be transferred onto a surface. Common microscopy techniques, having different resolutions, include: SEM, Environmental-SEM, which allows the sample to be measured under variable pressure and humidity, TEM and AFM. From microscopy analysis, the shape, structure and area-equivalent size of the particles can be determined [4]. Additionally, electron microscopy methods allow the determination of the elemental composition of the particles if an EDS is included. Nanoscale particles in liquid-borne phase are often sensitive to physical (e.g., mixing) or chemical (e.g., pH, ionic strength) disturbances. The primary disadvantage of microscopy analysis is that the sample preparation procedures require drying the nanoparticles and transferring them to a surface. This may cause particle aggregation and change in particle size distribution [4]. The solute in the liquid phase may also precipitate onto the particle surface, affecting the image quality. Secondly, microscopy analysis usually has a limited sample size. It is time consuming to obtain and analyze sufficient images in order to have a statistically significant analysis [7].

The nanoscale particles do not always exist in spherical form. As a single value of particle size cannot completely represent the non-spherical particles, any equivalent particle size can be different from another. Researchers have been studying the correlations between various particle size representations [8-10]. Different particle size representations may have different significance. For example, the hydrodynamic size, which relates the drag experienced by the particles, will determine the inertial and diffusion behaviors. On the other hand, the geometric size may be more relevant for particle filtration by interception mechanism [11].
Beads mill has been used in industrial processing for grinding and dispersing agglomerated particles into primary particles in submicron size range [12]. We used a beads mill to disperse liquid-borne Titanium oxide (TiO$_2$) aggregated particles. By controlling the milling time, particles of different degree of agglomeration can be obtained. Figure 1 shows example images of the aggregated particles collected onto a 50 nm-rated Nuclepore filter for milling times at 0, 1 10 and 60 min. We first characterized the geometric properties of the aggregated particles by analyzing the SEM images. The image analysis software, ImageJ, was used to determine the projected-area equivalent diameter of the particles. We also used the NTA, (LM10, NanoSight Ltd.) to characterize the hydrodynamic properties of the particles. Table 1 lists the statistics of the analysis. Figure 2 shows the comparison of hydrodynamic and area equivalent size distributions of the TiO$_2$ aggregates.

![Figure 1. TiO$_2$ aggregate particles collected at different milling times.](image)

**Table 1.** Statistics of the Nanoparticle Tracking Analysis and microscopy analysis for TiO$_2$ aggregate particles.

| Time  | 50$^{th}$ percentile diameter (nm) | # of particles analyzed from SEM |
|-------|-----------------------------------|---------------------------------|
|       | Hydrodynamic | Area equivalent |                               |
| 0 min | 220          | 180             | 650                            |
| 1 min | 149          | 170             | 901                            |
| 10 min| 65           | 58              | 509                            |
| 60 min| 44           | 35              | 80                             |
Figure 2. Cumulative size distribution of TiO\textsubscript{2} aggregates based on projected-area equivalent diameter and hydrodynamic diameter

It has been shown that the drag of fractal cluster scales with the radius of gyration and orientationally averaged projected area-equivalent size in the continuum and free molecular regimes, respectively [8]. Zhang et al. [10] also suggested the Smoluchowski radius can represent the drag of any non-spherical particles in the continuum regime. Binder et al. [9] reported that the mean drag on aggregates (with $D_{f}=1.85$) can be related to the projected area in the continuum regime with a power law with 15% variation, thus giving a rough estimate of the hydrodynamic properties from projected area. In the example shown, we have demonstrated a combined liquid and surface characterization method for determining the hydrodynamic size and projected area of aggregate particles and their correlation. Overall, surface-borne microscopy characterization provides supplementary information on particle properties, namely projected area, shape and structure, chemical composition etc, which enhance the understanding on the nanoscale particles under study.

3. Air-borne/liquid-borne correlation
In the previous section, the use of various liquid-borne particle instruments and microscopy methods to analyzed nanoscale particles in liquid-borne phase have been discussed. Since liquid particle instruments, primarily rely on the light scattering from nanoparticles, they are limited due to the low scattered light intensity of the particles and small refractive-index contrast between particles and the liquid medium [13]. While microscopy analysis can offer additional information of the particles, the technique is not able to provide fast-respond
measurements. Aerosol instruments, on the other hand, are considered to be better developed compared to those for liquid-borne particles [14]. Therefore, researchers have been exploring aerosol techniques for characterizing nanoscale particles in liquid-borne phase. Aerosolizing liquid-borne nanoparticles with an atomizer or nebulizer is relatively simple and inexpensive. After aerosolization and subsequent evaporation of solvent in the droplets, we can size and count the air-borne particles by a SMPS [15].

The challenge for this method is to preserving the original size distribution as in the liquid-borne phase. It needs to produce single particles which are agglomeration-free and avoids the influence from the impurities (soluble or insoluble) present in the liquid phase. The nonvolatile surfactant, sometimes necessary to stabilize the nanoparticles in liquid-borne phase, is also considered as part of the impurities. After evaporation of the volatile solvent, the nonvolatile impurities can either form smaller residue particles, which interfere with the particles of interest on the size distribution plot, or coat onto the particles of interest, increasing the size and changing the chemical nature of them. To overcome these problems, Lenggoro et al. [16] applied electrospray operating in the cone-jet mode, instead of an atomizer, to disperse liquid-borne nanoparticles into air-borne phase. Since the electrospray is capable of generating finer droplets compared to conventional atomizers, one can ensure that only one nanoscale particle is contained in a droplet, avoiding particle aggregation. The small droplets also minimize the effects of nonvolatile impurities after solvent evaporation. Lenggoro et al. [17] further demonstrated that a tubular furnace can evaporate residue particles and coatings on the nanoparticles of interest, leading to size measurement with better accuracy. Park et al. [18] also suggested that the effect of impurities can be suppressed by reverse osmosis process under high pressure. The aerosolization method to analyze nanoscale particles in liquid-borne phase has been applied to monitoring water quality and primary marine aerosol formation in seawater [13] and studying filtration of liquid-borne nanoparticles [14, 19]. Reversing the aerosolization process, it is sometimes advantageous to transfer air-borne particles to liquid-borne phase. Greenwald et al. [20] reported a technique to measure the insoluble fraction of ambient air-borne particles, by sending them to an aqueous medium and measuring them with a liquid particle counter. Also, air-borne viruses are usually transferred to liquid medium for virological analysis.

The nanoparticle nebulizer (TSI Inc.) has an improved design for dispersing droplets smaller than the conventional atomizers or nebulizers, making it suitable for the purpose of characterizing nanoscale particles by the aerosolization method. We have compared the size distribution of gold nanoparticles measured by 1) a nanoparticle nebulizer-SMPS system and 2) NTA (NanoSight LM10). The samples include monodisperse 80, 50 and 30 nm gold particles (Ted Pella, Inc.) and a mixture of them with equal number concentration. The concentration limit for the two methods should be noted. SMPS gives results with good statistics with particle concentration > 1E9 #/ml, while the NTA has a concentration window between 1E8 and 1E9 #/ml. Therefore, proper dilution was needed.

Figure 3 shows the size distribution of 30 nm gold particles measured with SMPS and NTA. On the SMPS size distribution, the first and second peaks correspond to the residue and gold particles, respectively. Residue particles have significantly higher concentration compared to the gold particles. However, since the residue particles are small (mode diameter at 10 nm), they do not interfere with the gold particles on the size distribution. In many cases, they can
be removed by using a diffusion screen or by electrostatic classification using a Differential Mobility Analyzer (DMA). Figure 4 shows the size distributions of mixed 80, 50 and 30 nm gold particles with equal number concentration. On the SMPS plot, while the residue particles are present in high concentration, they are kept in small size, not interfering with the particles of interest. The peaks at 30, 50, 80 nm can be distinctly resolved. The NTA plot, which bases on particle light scattering, shows a biased measurement towards the larger particles. From the comparison on the two measurements, it is obvious that SMPS gives more accurate representation for nanoparticles with polydisperse distribution. However, one drawback is that it usually requires higher particle concentration to give results with good statistics.

**Figure 3.** Size distributions of 30 nm gold particles measured with SMPS and NTA. The SMPS measurement is represented with resolution of 32 channels/decade. NTA measurement is represented with resolution of 4nm/channel.
Figure 4. Size distributions of mixed 80, 50 and 30 nm gold particles with equal number concentration measured with SMPS and NTA. The SMPS measurement is represented with resolution of 32 channels/decade. NTA measurement is represented with resolution of 4nm/channel.

4. Air-borne/surface-borne correlation
As in the case for liquid-borne nanoscale particles, additional analyses can be performed by depositing nanoparticles from air-borne phase to surface-borne phase, or vice versa. Researchers have been using microscopy tools for characterizing air-borne nanoparticles collected onto a surface [11, 21-22]. Another example is the calibration of Condensation Particle Counters (CPC). Liu and Pui [23] introduced singly charged air-borne particles into an electrometer current sensor, where particles are removed by a filter, and related the particle concentration to the electrical current reading, air flowrate and elementary unit of charge. Tapered Element Oscillating Microbalance (TEOM) is a device for near real-time particle mass measurement by depositing the particles onto a surface and relating the mass to the change in vibrating frequency [24]. Yook et al. [25] reported a method to deposit the monodisperse air-borne particles onto a surface with controlled number and spot size. Their work has found application in surface inspection tool characterization. In the remaining section, we describe how depositing air-borne virus particles onto a gelatin filter can enhance collection efficiency with easy recovery process.

Virus aerosols, also known as air-borne viruses, are assemblies of solid particles or liquid droplets suspended in a gaseous medium that carry viruses. They are raising more and more public concern due to their involvement in infectious disease transmission [26]. For example,
respiratory viruses such as influenza virus have been detected in ambient aerosols [27] as well as human expiratory particles [28]. The physical sizes of most viruses typically range from 25 to 300 nm. Liquid impingers are commonly used for virus aerosol sampling, which transfer air-borne particles to liquid-borne phase. However, they have $d_{50\%}$ (particle size with 50% collection efficiency) at around 300 nm and their physical collection efficiency decreases rapidly to 10% at 100 nm [29]. Therefore, they have low physical collection efficiency for nanoscale particles. In addition, liquid impingers provide little information on the particle size of the collected virus aerosol samples, which governs virus transport in air, its deposition in the respiratory tract, and its control by filtration [30]. Although size-segregated samplers such as cascade impactors can also be used [31], they provide poor size resolution for submicron particles.

Recently, we developed a DMA and gelatin filter based sampling system to characterized laboratory-generated virus aerosols [32]. A schematic diagram of the experiment is shown in Figure 5. Virus aerosol particles generated using a Collison nebulizer were first size-classified (100-450 nm) by a DMA and then collected onto a piece of gelatin filter. The use of a DMA enables the measurement of submicron airborne virus with high size resolution. Gelatin filters have excellent physical collection efficiency, e.g. >96% for airborne MS2 [33] and high biological collection efficiency, e.g. help preserve virus infectivity of the collected virus aerosol [34-35]. More importantly, after virus aerosol sampling, the collected virus can be recovered easily by dissolving the gelatin filter in a liquid medium for subsequent virus infectivity assay. Therefore, there is no additional step needed to extract the collected virus from filters. The virus extraction efficiency from filters depends on virus-fiber surface interaction [36] and may be difficult to quantify. Compared with traditional virus aerosol sampling methods, the novel DMA-gelatin filter sampling method transfers airborne particles to surface-borne particles with high collection efficiency, high size resolution, and easy virus recovery.

![Figure 5. Schematic diagram of the experimental setup for the characterization of virus aerosols.](image-url)
We used the DMA and gelatin filter based sampling system to measure four types of viruses, namely MS2 bacteriophage (MS2), transmissible gastroenteritis virus (TGEV), swine influenza virus (SIV), and avian influenza virus (AIV). Virus aerosol size distribution measured by real-time instruments such as SMPS does not necessarily represent virus infectivity distribution among polydisperse particles. We defined a particle size distribution particularly for particles carrying infectious virus, which was known as infectious virus size distribution, $IV(D_p)$ and was calculated as [32]:

$$IV(D_p) \equiv \frac{dC_{IV}(D_p)}{d \log_{10} D_p} \approx \frac{C_{IV,gel}(D_p)V_{gel}}{Q_{gel}t[\Delta \log_{10} D_p] f_{+1}(D_p)P(D_p)}$$

where $C_{IV}$ is the concentration of airborne infectious virus, $C_{IV,gel}$ is the concentration of infectious virus recovered in the effluent from the gelatin filter, $V_{gel}$ is the volume of eluent used to dissolve the gelatin filter, $Q_{gel}$ is the air sampling flow rate through the gelatin filter, $t$ is the sampling time, $\Delta \log_{10} D_p$ is the logarithm of the width of the size interval of the DMA, $f_{+1}$ is the fraction of singly positively charged particles, and $P$ is penetration of particles through the DMA. For the four types of viruses tested, the infectious virus size distributions generally followed particle volume distribution rather than particle number distribution (Figure 6). The results show that there is strong correlation between airborne virus infectivity and particle size, suggesting the importance of particle size in viral disease transmission via the aerosol route.

**Figure 6.** Normalized infectious virus size distribution, particle number distribution, and particle volume distribution for airborne MS2, TGEV, SIV, and AIV.
5. Summary
Nanoscale particles exist in air or liquid phases or on surfaces by deposition. Techniques for physico-chemical characterization of nanoparticles in these phases are therefore important in the environmental, health and safety studies of nanomaterials in support of sustainable nanotechnology. We describe experimental techniques that enhance characterization capability by transferring the nanoparticles from one dispersed phase to another. Figure 7 summarizes our work in bridging nanoscale particle measurements in different dispersed phases. Aerosolizing liquid-borne nanoparticles and measuring them with a SMPS improves the sizing resolution of liquid-borne particle measurements. By correlating particle concentrations in the air-borne and surface-borne phases, accurate calibration of both air-borne and liquid-borne instruments over a wide range of particle concentration may be achieved. Depositing air-borne and liquid-borne nanoparticles on a surface for microscopy analysis enables more complete physical morphology and chemical analysis. We also demonstrated that the DMA-gelatin filter system is a sampling method that has high collection efficiency and allows easy recovery for air-borne viruses.

Figure 7. Relation of the measurements of nanoscale particles in air-borne, liquid-borne and surface-borne dispersed phases.

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