Use of Transmission Electron Microscopy for Analysis of Aerosol Particles and Strategies for Imaging Fragile Particles

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Abstract

For over 25 years, Transmission Electron Microscopy (TEM) has provided a method for the study of aerosol particles with sizes from below the optical diffraction limit to several microns, resolving the particles as well as smaller features. The wide use of this technique to study aerosol particles has contributed important insights about environmental aerosol particle samples and model atmospheric systems. TEM produces an image that is a 2D projection of aerosol particles

that have been impacted onto grids, and through associated techniques and spectroscopies, can contribute additional information such as the determination of elemental composition, crystal structure, and 3D particle structures. Soot, mineral dust, and organic/inorganic particles have all been analyzed using TEM and spectroscopic techniques. TEM, however, has limitations that are important to understand when interpreting data including the ability of the electron beam to damage and thereby change the structure and shape of particles, especially in the case of particles composed of organic compounds and salts. In this paper, we concentrate on the breadth of studies that have used TEM as the primary analysis technique. Another focus is on common issues with TEM and cryogenic – TEM. Insights for new users on best practices for fragile particles, that is, particles that are easily susceptible to damage from the electron beam, with this technique are discussed. Tips for readers on interpreting and evaluating the quality and accuracy of TEM data in the literature are also provided and explained.

Aerosol Particles & Atmospheric Chemistry

Aerosol particles are ubiquitous in the air, making their study important for understanding the Earth's climate as well as necessary for characterizing their implications for human health. As energy from the Sun enters Earth's atmosphere, it can interact with aerosol particles in two ways. When particles absorb or scatter energy, they have a direct effect on radiative forcing; whereas when a particle nucleates a cloud droplet and the cloud interacts with the Sun's energy, they have an indirect effect. The magnitude of the net cooling effect of aerosol particles is still highly uncertain, and additional studies are required to help reduce this uncertainty.[1]

Inhalation of aerosol particles is associated with many negative human health outcomes. A variety of medical conditions have been linked to high air pollution such as cardiovascular disease, respiratory disease, and cancer.[2-5] Depending on aerosol particle size, deposition can take place in the upper or lower respiratory tract, and particles < 100 nm in diameter can cross the blood-air barrier into the bloodstream.[6] A fraction of particles in the bloodstream can cross the blood-brain barrier and have been found in patients with dementia.[6] Of particular current concern is the role of aerosol particles in disease transmission, for example in superspreader events of SARS-CoV-2.[7] Because of the climate and health effects of aerosol particles, it is necessary to know their origins and transformations and how each type impacts the human body.[8] One way that the particles can be directly speciated is through transmission electron microscopy (TEM).

Several reviews have discussed the use of TEM for atmospheric chemistry.[9-13] Buseck et al. 2000 primarily focused on how mineral aerosol particles can be studied with TEM, whereas Buseck 2010 describes many types of aerosol particles that can be analyzed with TEM, some of the associated techniques, and how the information gained can be applied.[9, 12] Among others, Laskin et al. 2006 and 2019, Tang et al., and Ault and Axson all provide brief overviews of TEM and its relationship with other techniques.[10-11, 13-14] Similar to previous reviews, we give an overview of the types of particles on which TEM can be used. We then expand on associated analysis techniques, providing a more comprehensive overview of these techniques than in previous reviews. In contrast to previous reviews, our main interest is in the study of aerosol particles composed of organic compounds and salts, which are very fragile in the electron beam, that is, the particles are easily damaged by the electron beam. One impetus for this article is the fact that when these particles are presented in the literature, their images often show signs of damage, which limits the conclusions that can be drawn from these samples. We review our and other's work in this area and demonstrate explicitly the types of damage that can occur to these samples, and the origins of this damage. We intend this article for researchers who are interested in using TEM to study particles containing organic compounds and/or salts as well as readers of the atmospheric literature, in order that researchers can more easily identify and avoid damage to samples, and understand the impact of damage on their analysis. For readers of the atmospheric literature, a general overview of TEM and associated techniques with comparison to commonly used techniques in the atmospheric community is provided.

TEM Technique

TEM uses an electron beam to image samples. In brightfield imaging, the light regions of the image indicate where all the electrons are transmitted through the sample, whereas the darker regions are where some portion of the electrons are not able to pass through the sample. This typically leads to a light background with a dark object. The contrast of the object against the background is influenced by several factors such as the thickness of the object and the Z-number, or atomic number, of the elements in the sample. As the thickness or Z-number increases, the sample becomes darker in the image. Traditional TEM is performed under extremely high vacuum conditions, often reaching pressures as low as 10^{-12} torr.[15] An exception to this is environmental TEM (E-TEM) where samples can be kept at atmospheric pressure, while the rest of the instrument is under vacuum. E-TEM experiments are frequently performed using a sample holder that is a gas or liquid cell, allowing processes such as aerosol particle efflorescence or deliquescence to be observed in submicron particles.[16-18] These cells typically provide decreased resolution, however, when compared to standard TEM experiments because of the reduced mean free path of the incoming or transmitted electrons due to interactions with molecules in the air.[19] Additionally, many organic compounds (composed of primarily carbon, oxygen, and nitrogen) are unstable when exposed to a room temperature electron beam and will be damaged, and as a result, they cannot easily be studied with this technique. To minimize or delay damage to particles from the electron beam, a cryogenic holder, or a cryo-holder, can be used. A cryo-holder works with both a room temperature sample or a pre-frozen sample. Pre-frozen samples are prepared by cooling the holder down with liquid nitrogen and then inserting the sample into the holder while keeping it immersed in a liquid nitrogen bath. Then the holder can be inserted into the instrument. A room temperature sample can be placed in the holder, inserted into the instrument and then frozen or can be prepared in the same manner as a pre-frozen sample. The samples are then imaged using the same process used for non-cryo samples.

TEMs can be equipped with a variety of associated instruments for spectroscopy and diffraction. These include selected area electron diffraction, or SAED, which is used to determine the crystal lattice structure of nanoparticles. As the atoms in the sample interact with the electron beam, they scatter some electrons resulting in an electron diffraction pattern that can be analyzed for specific structural information. High angle-angular dark field (HAADF) images come from operating the instrument in scanning transmission electron microscopy (STEM) mode. The images from HAADF are commonly known as z-contrast images. In this technique, the detector is placed at an angle under the sample, allowing only the small amount of inelastically scattered electrons that are transmitted through the sample to reach the detector as the electron beam scans the sample. This type of imaging can cause increased contrast for some samples.[20] Energy dispersive x-ray spectroscopy (EDS or EDX), electron energy loss spectroscopy (EELS), and energy filtered transmission electron microscopy (EF-TEM) are all methods used to determine the elemental composition of a sample. These techniques can be qualitative or quantitative depending on the method of imaging, sample stability, and the elemental composition. EDS and EF-TEM can be done with or without STEM, but EELS typically is performed with the TEM in STEM mode. EDS can be used to analyze a single point or create an elemental map of the whole sample, but is not generally used quantitatively.[15] EF-TEM can provide elemental information as well as some chemical bonding information, but may damage the sample more than EDS or EELS.[20] EELS can be used to provide quantitative elemental data as well as occasionally provide chemical bonding information and has high spatial resolution.[21] Electron tomography is used to create a 3D image of the sample from many 2D images in TEM. By using a specialized holder, the TEM grid can be tilted into two directions, allowing a sample to be imaged at many different angles. These images are then combined to create a 3D image. Associated techniques and spectroscopies increase the variety of samples that can be studied with TEM and diversify the type of data that can be obtained for each sample.

Advantages of TEM for atmospheric chemistry research & examples

TEM has many advantages over other methods including ease of sample collection. Methods for collection include cascade impactors for size-selected samples, thermophoretic sampling, and nucleopore filtration sampling[22-23] Some sampling methods collect all particles sizes, while others collect a specific size range. The small size of many aerosol particles makes them ideal for TEM due to their thinness. If a sample is too thick, it will not be transparent to the electron beam, making it impossible to image directly with this technique. TEM allows for high through-put imaging. Around one hundred particles per hour can be imaged by hand, though particle density on the grid and necessary magnification may lower this number, compared to measurements of single digit numbers of particles per hour for atomic force microscopy (AFM). Additionally, a diverse size range can be studied with TEM. Submicron particles, which are generally difficult to study due to their size below the diffraction limit of light, can be imaged in addition to particles that are several microns in diameter, or until the sample becomes too thick. These are just a few of the advantages of TEM.

TEM is a powerful tool for the study of atmospheric aerosol particles. To know how and when to apply this tool, it is important to understand how TEM compares to other techniques that are commonly used in the field of atmospheric chemistry. One frequently used technique is the scanning mobility particle sizer (SMPS) which can provide real time data with very high throughput and without any substrate effects. The limitations of SMPS are that only sizing data is obtained and that the theory for SMPS is based on the assumption that the particles are spherical. Aerosol mass spectrometry (AMS) is frequently used to study the composition of aerosol particles and is used both online and offline with high throughput. Extensions to certain types of AMS have been made to give optical property data and differentiate core-shell structures from other morphologies.[24-26] Scanning electron microscopy (SEM) is used as an alternative to TEM and provides images showing the surface of the particles. SEM can be used on fragile samples with minimal damage due to low field electron emission detectors. AFM can provide high resolution sizing of the x, y, and z directions of an aerosol particle and does not require a sample to be under vacuum.[27-29] Optical microscopy provides an accessible method to study aerosol particles with control of factors such as temperature and humidity, and can be coupled to Raman or FTIR to provide information on functional group composition. Scanning transmission x-ray microscopy coupled with near-edge x-ray absorption fine structure (STXM-NEXAFS) is a highly powerful tool that accomplishes much of the same analysis of aerosol particles as TEM, but requires a synchrotron that is capable of generating tunable X-rays.[30-32] In some cases, TEM has advantages over these techniques. SMPS and AMS do not provide any of the morphology information that can be retrieved from TEM. Combined electrostatic mobility and aerodynamic diameter measurements have been used to characterize fractal from non-fractal particles.[33-34] Additionally, TEM can be used accurately on all particle shapes, unlike the SMPS, and can couple morphology information with some elemental data unlike most AMS instruments. When looking for the internal structure of an aerosol particle, TEM should be used in place of SEM. When compared to AFM, TEM collects data much more quickly and TEM can provide elemental analysis and direct internal structure information. For high-throughput characterization in which the morphology and elemental analysis of submicron particles is desired, TEM is preferred over other techniques. A variety of samples have been studied with TEM as detailed below.

The distinctive shape of some types of particles can give information on speciation. Soot and fly ash particles are often characterized by their fractal components and their different size spherules. Cubic crystals like sodium chloride can be differentiated from other salts and in general, some salts can be differentiated by their shape and crystal structure. Mineral dust is often irregularly shaped compared to nearly spherical organic/inorganic aerosol particles. Visual identification is often combined with elemental analysis for conformation. Below, we provide information on the types of aerosol particles that have been investigated with example studies that demonstrate the breadth of the TEM technique. While hundreds of papers studying aerosol particles have used TEM, those mentioned in this paper typically use TEM as a primary technique in the study.

Soot and heavy metal containing particles have been visually studied to understand their general structure, fractal dimension, and degree of aggregation. HAADF-STEM, high resolution TEM, and EDS have been used to characterize metals such as gold, iron, arsenic, and uranium in aerosol particles collected near a coal-fired power plant, developing a method for future characterization of heavy metals that are found as minority components in atmospheric nanoparticles. [35] These metals, which originated from the coal, are often incorporated in soot particles. The morphology of soot particles can be indicative of their source and their aging in the atmosphere.[36-39] There is a long-standing interest in the study of soot particles due to their existence in varying concentrations in urban environments, air pollution events, volcanic plumes, and biomass burning. Soot has been proposed as the second largest atmospheric warmer after CO_2 due to the presence of highly absorbing elemental carbon with additional warming coming from the lensing effect, where a non-absorbing, lower scattering coating can lead to enhanced absorption of an absorbing, higher scattering core.[40] An example of a soot particle is shown in Figure 1a, which has the characteristic overlapping spherules and is relatively spread out. This morphology is an indication that the soot particle is not yet highly aged. The particles that arise from different composition sources were characterized by Xiong and Friedlander, improving the understanding of the diverse nature of atmospheric aggregates such as soot.[41] Electron tomography has also been used on soot particles to better understand structure and aging.[42-43] A variety of sampling methods have been compared by Ouf et al. who showed that similar soot samples can be prepared using different methods, and confirmed that the collection method has minimal influence on the appearance and composition of the samples indicating that studies using different collection methods can be directly compared.[23] The lattice fringe structure of the soot particles has been investigated by Wentzel et al. to determine how artificial soot structure differs from that of a diesel engine.[44] The lattice fringe structure shows the spacing of the lattice planes of the crystalline sections of the sample. In China et al., TEM is used to investigate how soot becomes impacted in supercooled droplets and ice crystals in order to determine the impact of this type of aging on soot morphology.[45] The unique morphology of soot particles makes it easy to classify them in mixed ambient particle samples.

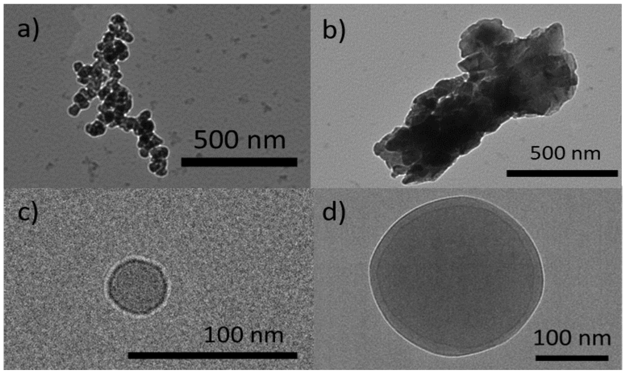

Figure 1: Particle type examples that include a) a fractal particle, b) a mineral dust particle, c)a homogenous organic/inorganic containing particle, and d) a core-shelled organic/inorganic particle.

Mineral dust aerosol is the second largest emission by mass into the atmosphere and is often composed of a mixture of mineral species. Figure 1b shows a mineral dust particle. Pósfai et al. characterized Middle East desert dust and found a range of different mineral types including smectites, illite, silicates (feldspars, quartz), and calcite.[18] To determine the types of minerals, TEM, SAED, and EDS were used, and to explore water uptake properties, E-TEM was used to study dust mixed with pollution. Half of the mineral particles were mixed with ammonium sulfate or soot, though most did not take up water until > 90 %RH. The abundance of mineral dust in the atmosphere makes for a large variety of these types of particles in the atmosphere.

The speciation of mixtures of particles is useful in determining the types and sources of pollution. Pósfai used TEM with EDS to study the composition of smoke from biomass burning in southern Africa that contained organic, inorganic, and soot particles.[46] The young smoke contained largely organic particles, aged smoke changed to contain high amounts of tar balls, and the haze afterward consisted of primarily sulfate and organic/sulfate particles.[46] In Li et al. the potassium and sulfur ratios calculated from EDS and SAED information provided insight into the difference in the haze composition in the presence and absence of biomass burning in Beijing.[47]

These studies provide important information about the variety of aerosol particles in ambient samples and how it varies with location and pollution.

Sea spray aerosol is the largest emission by mass into the troposphere, and consists of a range of salts, primarily sodium chloride, and organic compounds.[48] Aerosol particles created by marine aerosol reference tanks also investigated organic species found in marine environments of biological origin such as those studied by Patterson et al.[49] Pósfai et al. characterized the composition of Equatorial Pacific aerosol particles, and found NaCl as well as sulfates of sodium, calcium, magnesium, and potassium.[50] Los Angeles coastal aerosol was also investigated as part of the CalNex campaign.[51] Sodium containing aerosol from the CalNex campaign was found using EDS to often be highly reacted to form sulfur-containing compounds rather than chloride containing ones. E-TEM was used to understand the phase transitions of model sea spray aerosol.[52-53]These common salt particles are often internally mixed with other particles types that are transported toward the ocean.

Aerosol particles that contain organic compounds are less studied with TEM than other aerosol types due to their fragility in the electron beam. Mixed organic/inorganic aerosol is of interest due to its ubiquity in the environment, its importance in air pollution events and cloud droplet formation as well as the most common composition for newly formed particles. In addition, the composition of organic aerosol that results from the oxidation of volatile and semivolatile organic compounds is of continued interest. Lauraguais et al. used TEM to identify the structure of secondary organic aerosol particles and found both spherical and irregular particles, all of which were amorphous. [54] Our group has used cryogenic-TEM (cryo-TEM) extensively to determine the morphology of model organic/inorganic aerosol particles, as discussed below.[55-60] Figure 1 shows examples of a homogenous organic/inorganic particle (Figure 1c) and a core-shell organic/inorganic particle (Figure 1d). One of the commonly studied types of organic compounds is brown carbon. The formation pathways that produce brown carbon in air pollution and biomass burning are of current interest in the atmospheric chemistry community, as light-absorbing aerosol particles can limit ozone concentrations and impact radiative effects. This occurs when black and brown carbon particles absorb photons and prevent NO_2 from undergoing photolysis, which can reduce ozone production. To characterize the composition of brown carbon, Alexander et al. performed TEM and EELS on brown carbon spheres.[61] While imaging and analysis of organic compounds can be complicated due to their fragility and volatility, it provides important information about true sample morphology and composition. These fragile particles will be the primary focus below.

Our group has used TEM extensively to characterize the optical properties of mineral dust aerosol when combined with other techniques and to characterize the internal structure (morphology) of aerosol particles composed of organic compounds and salts. Initially, we used TEM to complement our measurements of the optical properties of mineral dust particles. Our technique for measuring aerosol optical properties is cavity ring-down spectroscopy, which often uses size-selected samples to constrain the effective refractive index of the particles of interest. Accurate and low dispersity size selection with a differential mobility analyzer requires the use of spherical particles. When non-spherical particles are used, the size distribution for a given mobility diameter of the sample has a larger dispersity. To characterize the sizes of particles selected, we used TEM for samples with aspect ratios near unity (calcite, hematite, quartz) and a combination of SEM and TEM for samples with aspect ratios that deviated from unity (aluminosilicate clay minerals).[62-65] We also worked with mixtures of different types of mineral dust particles, including Arizona test dust and NX illite.[65] The characterization of the size distribution then allowed us to model the optical properties retrieved from cavity ring-down spectroscopy through use of Mie theory or the discrete dipole approximation. The advantage of using TEM for these studies is that it is a high-throughput method, the shape of particles can be easily determined (with the caveat that SEM or another technique that is capable of both top down and side images is also needed when working with high aspect ratio samples), and we experienced no charging effects with our thin samples.

These initial studies led to work on two field studies. In Hasenkopf et al., aerosol particles from Ulaanbaatar, Mongolia were organized into broad classes: fractal (soot and fly ash), irregular (mineral dust), spherical (organic or organic/salt).[66] EDS was used to obtain the elemental content of a subset of particles, especially focusing on sulfur content as a function of season. Sulfur content was used as a marker for increased coal burning and was found to be higher during the colder months.[66] Alstadt et al. used TEM to characterize changes to ambient aerosol particle types as a university campus moved from coal to natural gas as a power plant fuel source, especially focusing on soot aerosol particles and soot mixing state.[22] As natural gas rose in usage, less soot was observed, and it was often more aged because it was no longer produced locally and was a result of long range transport, as judged based on morphology and mixing state.[22]

Concurrently to the studies of mineral dust aerosol particles, we began to use cryo-TEM to determine the morphology of aerosol particles composed of organic compounds and salts to understand previous results for the optical properties of these systems that were hypothesized to result from the internal structure of the particles.[67] These particles often undergo liquid-liquid phase separation.[68-69] We initially worked with individual dicarboxylic acids mixed with ammonium sulfate, where we found that liquid-liquid phase separation was inhibited at small particle sizes.[58, 70] We have subsequently used cryo-TEM extensively to characterize the size dependence of the phase separation. In particular, we have investigated the origins of the size dependence, specifically investigating the role of drying rate and the mechanism of phase separation on the size dependence.[56-57] We have worked with different systems including dicarboxylic acids/ammonium sulfate, polymer/salt, and polymer/polymer, as well as complex mixtures of organic compounds with ammonium sulfate.[57, 59, 70-71] We have recently expanded our preparation technique to vitrify aqueous aerosol particles to determine the relative humidity at which phase separation occurs.[60, 72] Through these studies, we have shown the origins and applicability of the size dependence of phase separation on atmospheric chemistry. TEM has allowed us to characterize the morphology of hundreds of particles for each study, allowing for accurate and comprehensive analysis.

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Secondary Characterization:

As mentioned above, TEM is powerful not just as an imaging technique, but also because of additional characterization that can be performed within the instrument. In particular, a host of associated spectroscopic and spectrometric techniques are commonly available on TEM instruments. Additionally, samples can be manipulated within the TEM holder, for example, by exposing to heat or purposeful radiation damage.

STEM takes the electron beam, condenses it to a point, and raster scans it across the sample which can increase image resolution.[15] This can provide additional contrast when imaging with HAADF which uses a detector placed at an angle to collect the scattered electrons, such as was done in the work of Utsunomiya and Ewing and by Patterson et al..[20, 35, 49] These techniques can be used on both fragile and stable samples. Additionally, EDS can be performed using STEM. In

EDS, the sample is exposed to X-rays, which excite core electrons. When higher energy electrons fill the holes created in the core levels of the atoms, X-ray radiation is emitted and detected. This light provides elemental information about the samples due to the characteristic signature of X-ray wavelengths that are emitted by atoms of a given element and has been used extensively in the study of atmospheric aerosol particles.[18, 20, 46-47, 49, 51, 73-95] This technique works best on high atomic weight elements and samples that are beam stable. Fragile samples are frequently destroyed before a full analysis can be obtained.

EF-TEM provides elemental information, is performed in regular microscope mode, and does not require the TEM to have STEM mode, with the added feature that it can be done with a range of filaments. EELS uses the inelastic scattering of electrons to obtain elemental information in spectral form and can also provide information about sample thickness.[20] This technique has been commonly used in place of EDS for samples with low atomic number elements.[46, 49, 79, 82, 84, 89, 96-98] Another method for particle identification is SAED. This technique is used to determine which samples are crystalline and which samples are not crystalline.[47, 73, 75-76, 89, 91, 94, 98-99] EF-TEM, EELS, EDS, and SAED are primarily used on electron beam stable samples, but qualitative information can, in some cases, be obtained for fragile samples. Electron tomography works by changing the angle that the sample is held within the instrument and taking pictures at many different angles. Then these 2D images can be combined to create a 3D image of the sample.[42-43, 81-82, 84] Tomography can be performed using a standard room temperature holder or using a cryo-tomography holder depending on the durability of the sample. Cryogenic-TEM is used on sensitive samples to improve their stability in the electron beam.[49, 56-60, 70] This method cools the samples to below -180°C which makes them more stable to the electron beam allowing them to be imaged for longer time periods without damage to the sample. For highly sensitive samples, this can be combined with a low dose of electrons by increasing the spot size or by using low-dose mode on the TEM.

E-TEM is useful for controlling the environment to which a TEM grid is exposed. Using this method, factors such as temperature and humidity are controlled. E-TEM allows for transitions such as efflorescence and deliquescence of submicron aerosol particles to be studied in addition to watching the water uptake of the particles.[52-53, 82, 100-103] Pósfai et al. used E-TEM to study water uptake of mineral dust particles, but found that the particles collected in Saudi Arabia did not uptake enough water to change the appearance of the particle.[18] Semeniuk et al. compared soot particles, tar ball particles, and organic particles as humidity was raised from 0% to 100% RH and then returned the particles back to 0% RH to understand the hygroscopicity of biomass burning aerosol particles.[77] Wise et al. studied NaCl containing particles from laboratory samples and samples collected in pollution plumes in coastal areas to understand the influence of pollution on the hygroscopicity of sea spray aerosol particles.[16] E-TEM provides important information on efflorescence and deliquescence for submicron and supermicron particle sizes. Note, however, that the extended exposure of the particle to the electron beam over the course of an experiment can cause damage to the aerosol particles, particularly to fragile samples.

Information can also be gained through unique treatment of the samples either inside the TEM instrument or in the grid collection process. Three examples stand out for how samples have been manipulated in the TEM to give additional information on particle chemical and physical properties. In Adachi et al., particles are heated between 25 and 600°C to understand how particles decompose with heating.[17] In Geng et al., bubbling due to sublimation, caused by radiation damage, is used as a method to help speciate particles. EDS is used as well to confirm composition.[104] In Hara et al., a chemical test incorporated onto the grids is used to speciate particles.[105] $BaCl_2$ grids were used for identification of sulfate, Ca-coated grids were used to identify acidic sulfate, and nitron coated grids were used to detect nitrate.[105] The use of Ca-coated grids to identify acidic sulfate has been performed repeatedly in the literature.[76, 85, 89, 105] These experiments typically by work by coating the grid with a chemical compound. The aerosol particles then interact with the coating on which they are impacted and produce crystallization rings which can be viewed using TEM. These forms of sample manipulation help to increase the data that can be obtained from a TEM.

Identifying Damage to Particles

Some samples are fragile in TEM due to the potential for damage from the electron beam. Damage comes from the scattering of electrons both elastically and inelastically. Elastic scattering causes electrostatic charging, atomic displacement, and electron beam sputtering.[106-107] Inelastic charging also causes electrostatic charging in addition to specimen heating, structural damage, mass loss, and deposition. [106] In samples such as NaCl this can initially be seen as degassing of the material.[108-109] If a sample is solely composed of metals, mineral dust, or soot, it will likely be stable for imaging. Any aerosol particles composed of organic compounds or salts can be damaged. As a result, damage can affect organic, brown carbon, and sea spray aerosol particles, as well as particles with organic coatings. One often unseen method of damage occurs through the evaporation of volatile organic compounds due to the vacuum conditions of the instrument. If the experimental data may be influenced due to this type of damage, in some cases E-TEM data may be compared to traditional TEM data to understand the impacts of volatility. Damage in organic particles often occurs as the particle shrinks due to the sublimation from exposure to the electron beam, whereas salt particles often appear to bubble.[108] In some cases, damage is used as a tool to speciate aerosol particles, more often, damaged particles are published and not acknowledged as damaged.[18, 41, 44, 47, 50, 54, 61, 85-86, 89, 96, 110-116] It is important to recognize damage to ensure that data from images are correctly interpreted and that accurate data are collected. If the damage occurs quickly, it can obscure the true morphology or size of the particle, as seen in the image of a particle composed of 3,3 – dimethylglutaric acid and ammonium sulfate in Figure 2. In Figure 2a the particle is shown in its undamaged form. Figure 2b shows the beginning signs of damage where there are slight circles appearing across the particles and the core looks larger and is less defined. Figure 2c shows more and more circles appearing and the presence of the core disappears more. Additionally, the particle in 2c is larger than the original particle in 2a because damage frequently causes changes in the size of particles. This 3,3-dimethylglutaric acid and ammonium sulfate particle is an example of why it can be misleading to assume the size or the morphology of a damaged particle. This particle was imaged using cryo-TEM, because standard (non-cryo) TEM would cause the particle to undergo damage faster than it could be imaged. When reading TEM literature, it is important to check for the appearance of texture due to bubbling in the particle such as found in Figure 2 b and c. Typically, the more a particle is covered with these features, the less accurate an analysis of the particle will be. In exceptional cases, the author should provide an explanation for why these features are not signs of damage or why they do not impede analysis. In cases when the composition of the particle is important, damage can make quantitative composition information difficult though some qualitative information may still be possible. While the amount of the components in the particle may change, the presence of some elements in the particle may still be detectable. Using cryo-imaging can help prevent or slow damage, as shown in Figure 3. When imaging ammonium sulfate particles using cryo-TEM, a user can easily obtain an undamaged image as shown in Figure 3a. When imaging this moderately beam stable compound with non-cryo TEM, damage occurs rapidly, which limits the usability of the image (Figure 3b).

Note that the particles imaged for this paper all show a bright white ring around them. This ring is the Fresnel fringe and is visible because the images are taken with the particle slightly under focused for improved contrast. This ring is not a part of the particle, and instead just surrounds the particle.

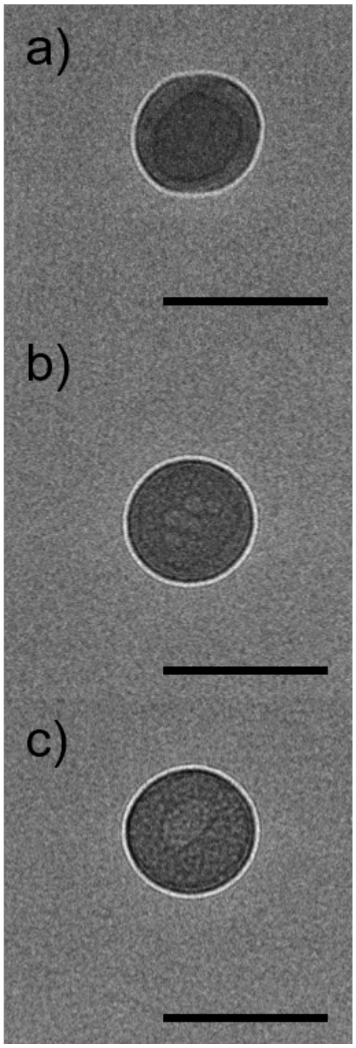

Figure 3: Electron beam damage to particles over time with estimated electron doses of a)7.34x10^6 electrons, b)103x10^6 electrons, and c)159x10^{6}electrons. All scale bars are 100 nm.

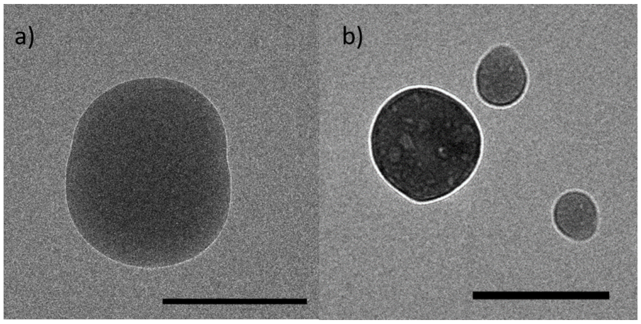

Figure 2: a)Ammonium sulfate that shows no damage due to cryogenic imaging while b)shows ammonium sulfate that is imaged without cryogenic conditions and particles show significant damage. Scale bars are 100 nm.

When looking at the morphology of phase separated particles it is important to know when small circles within the particle are indicative of damage and when they instead show inclusions within the particle. Inclusions can be a sign that the particle was not able to reach its equilibrium morphology and could cause the particle to have different optical properties than a core-shell particle. Because of these implications it is important not to confuse inclusions with damage. Figure 4 shows examples of both a diethylmalonic acid and sodium acetate beam damaged particle and some undamaged particles of the same composition that contain inclusions. Figure 4a shows more pitting, or small brighter spots, in the particle image which is often indicative of damage with the white arrow in the image pointing out an example of one of the brighter spots. Additionally, when imaging, the particle continually changed appearance as the particle was exposed to the electron beam which means the particle was undergoing damage. In the case of Figure 4b and 4c, the particles have some circles in them, but continual exposure to the beam did not change the size, location, or appearance of the circles. Since the amount of time it takes for a particle to become damaged was already known for particles of this composition, the inclusions are not signs of damage that occurred before the study occurred. Additionally, other signs of damage such as increased texture and constantly changing particle size were not observed. For a situation like this, we suggest the author include images showing a time lapse of the particles, which can help the reader be confident in an accurate assessment of the images. A constant appearance shows that the circles in the particle are inclusions and are not due to damage. Additionally, the particles in Figure 4b and 4c do not show the texture due to bubbling indicative of electron beam damage. Since homogenous, core shell, and partially engulfed particles can damage, an untrained user may falsely interpret damage as the particle itself having a more complex morphology.

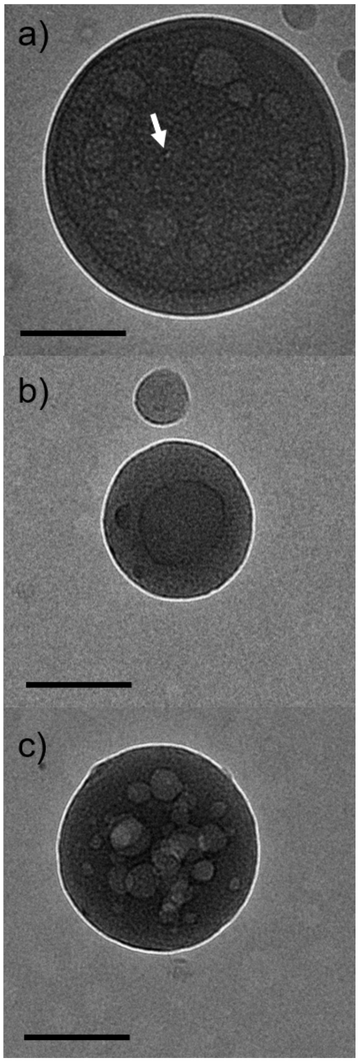

Figure 4: a) A damaged particle with the white arrow pointing to texture due to bubbling that is indicative of damage, b, c) Undamaged particles with inclusions. All scale bars are 100 nm.

Other common issues can occur in addition to beam damage of particles. These issues include the development of ice on cryo-TEM grids, wet impaction, and splattering (Figure 5). Ice is a common problem when using a cryo-holder in a standard TEM. The cryo-holder cools down to below -180°C, making it the coldest spot inside the TEM vacuum column. As a result, any moisture in the column moves preferentially to the sample and the ice shows in the images. Ice crystals can hide the morphology of a particle. The 2-methylglutaric acid and ammonium sulfate particles in pictures Figure 5a and 5b show large amounts of ice. Ice often starts as small crystals that cover the entire grid and cause a decrease in the contrast of the aerosol particles. Ice can be minimized by imaging on cool days with low humidity, storing samples in a desiccator, or using a TEM that has been designed for cryogenic imaging and contains special features such as a cryo-box. Whenever possible, it is best to avoid ice buildup on a sample since it may not only hide the morphology of a particle but also can hide the presence of small particles, or if the ice has grown sufficiently large, it can itself appear like one of the collected particles.

Wet impaction of particles can lead to samples easily icing over. It can also lead to excess frozen water sitting on the grids which will freeze and become visible when imaged cryogenically. Figure 5c shows an example of particles composed of poly(ethylene) glycol and dextran that still contained water when they were impacted. The particles are surrounded by large circles of frozen water and the morphology of the particles is hidden by the presence of frozen water. Additionally, when particles are impacted wet, they can splatter on the grid. Figure 5d shows the evidence of secondary organic aerosol (SOA) with an ammonium sulfate seed particles splattering on a grid when impacted on an angle with some light water splatter around them. In this case, the morphology and size of the particles were likely changed by their wet impaction.

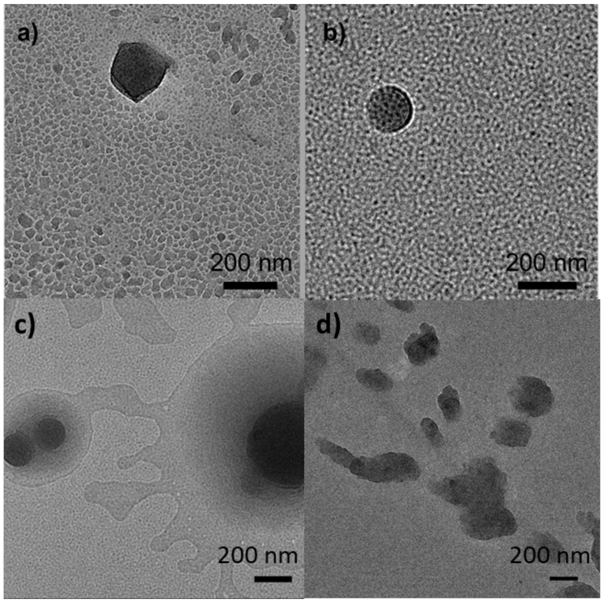

Figure 5: All images are examples of cryo-TEM with a and b showing large ice crystals. c shows an example of wet impaction and d shows examples of particle splatter that can occur on grids

In this section, a few of the pitfalls a TEM user may experience have been discussed as well as how a reader can spot them; other problems are possible and depend on the system of interest. These issues can have a significant impact on studies where the size or morphology of the particles is characterized. If a study is solely focused on the qualitative composition of a sample, then these limitations will be less important. However, qualitative studies will still be influenced by the rate that particles damage. In addition, the composition of a particle will influence its hygroscopicity which can impact the likelihood of wet impaction. While TEM is a versatile tool for atmospheric chemistry, it is important to remember its limitations, particularly for many of the easily damaged types of particles found in atmospheric and model atmospheric systems.

Conclusions & Best Practices for TEM Imaging

To summarize, TEM is a technique that can provide a 2D structure of submicron atmospheric aerosol particles by transmitting electrons through the sample. This technique provides images of samples below the optical diffraction limit and can be combined with techniques such as EDS, EF-TEM, EELS, SAED, and HAADF which provide additional information about features such as chemical composition and crystal structure. The studies of types of aerosol particles such as soot, mineral dust, salt, and organic/salt particles have been outlined. Studying fragile particles can result in damage and contribute to loss of data. This loss is often caused by the vacuum of the instrument, the electron beam, or from ice that develops on the samples. In this section we outline methods that both readers of TEM literature and users of TEM can use to increase the quality of TEM studies in atmospheric literature.

Best practices for TEM readers

To assess the quality of TEM literature data

- Check images for texture due to bubbling which is indicative of damaged particles.
- Look for signs of ice on the images or evidence of splattering which could be indicative of wet impaction. If these features exist in the images, the text should be searched for an explanation of the presence of the features. Is the use of the TEM image quantitative or qualitative? If quantitative data was collected, is there any signs that particle damage may be preventing accuracy?

Best Practices for new TEM users

To best use these TEM and associated techniques, it is necessary to understand the limitations of TEM such as beam damage or with cryo-TEM, the presence of ice. Some suggestions for ensuring proper use of the TEM on aerosol particles include:

- Initially, image a fragile material(e.g. ammonium sulfate) without causing damage. It is possible to image ammonium sulfate with standard (non-cryo) TEM without inducing damage. This step teaches a user about what damage can look like as well as providing practice on quick imaging for minimizing sublimation of beam sensitive samples.
- Work with laboratory samples and determine how much exposure is needed for damage to occur with each sample type. Using a laboratory sample can help determine what damage will look like and how much electron dosage the particle can withstand. This helps prevent waste on ambient samples because it will give an approximate starting point for minimizing damage.
- Use cryo-TEM when needed to avoid damage to organic compounds. Both a cryo-holder for a standard TEM and a cryo-TEM work well. Many organic compounds will still damage when using cryo-TEM, but with a decreased electron dosage it is possible to image these particles before they damage, giving a better understanding of the morphology of the particle.
- Recognize signs of damage and adjust interpretation of results appropriately. Damage typically looks like the sublimation or shape change of the particle while it is occurring, but may look different after the particle is completely destroyed. Sometimes it can be difficult to determine what is leftover particle residue and what is organic or water that remains on the grid. If in doubt, try to compare with a cryo-TEM sample.
- . Use low-dose TEM when searching for particles to minimize the number of electrons that interact with the sample. After a particle is selected, the TEM will increase the beam

intensity to take the image. This mode, widely available on TEM instruments, helps to minimize the amount of electron dosage while providing a good contrast photo.

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Notes

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