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THE ROYAL SOCIETY

Overview of methods for analysing single ultrafine particles

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Increasing awareness that structures and attributes on a nanometre scale within aerosol particles may play a significant role in determining their behaviour has highlighted the need for suitable single ultrafine particle analysis methods. By adopting technologies developed within complementary disciplines, together with the development of aerosol-specific methods, a basis for characterizing single sub-100 nm (ultrafine) particles and features in terms of size, morphology, topology, composition, structure and physicochemical properties is established. Size, morphology and surface properties are readily characterized in the scanning transmission electron microscope (STEM), while high-resolution transmission electron microscopy (HRTEM) allows structural information on particles and atomic clusters to sub-0.2 nm resolution. Electron energy loss spectroscopy (EELS) and X-ray emission in the STEM allow the chemical analysis of particles and particle regions down to nanometre diameters. Scanning probe microscopy offers the possibility of analysing nanometre-diameter particles under ambient conditions, thus getting away from some of the constraints imposed by electron microscopy. Imaging methods such as atomic force microscopy and near-field scanning optical microscopy (NSOM) offer novel and exciting possibilities for the characterization of specific aerosols. Developments in aerosol mass spectrometry are providing the means for chemically characterizing size-segregated ultrafine particles down to 10 nm in diameter on-line. By taking a multi-disciplinary approach, the compilation and development of complementary tools allowing both routine and in-depth analysis of individual ultrafine particles is possible.

Keywords: ultrafine; aerosol; single-particle analysis; particle collection; electron microscopy; scanning probe microscopy

1. Introduction

An aerosol is a complex material state that lies between a gas or vapour and a bulk material. At each extreme, analysis is simplified by compositional, and to a certain extent structural, homogeneity. However, an aerosol may consist of many orders of magnitude of discrete particles, each having the possibility of slightly different physical and chemical properties. The relevance of each particle's nature within the aerosol will depend on context, and in most systems some degree of simplification is possible. For instance, the motion in a gas of an aerosol consisting of chemically and structurally diverse particles with similar aerodynamic properties may be characterized by

relatively few collective parameters. On the other hand, understanding interactions with the aerosol at a physical, biological or chemical level will require a more complex characterization. In aerosols where there is wide variation in particle size, shape, structure, composition, etc., and where the relevance of these parameters in determining aerosol behaviour is not well understood, the use of collective attributes such as gravimetric mass particle size distribution or overall chemical composition may not explain observed phenomena adequately. Within such systems, characterization on a particle-by-particle basis should be considered as the first step to understanding interaction mechanisms and simplifying monitoring requirements.

The investigation of single ultrafine particles is not a new or original venture. Commercial interest in the activity of nanometre-sized particles within heterogeneous catalysts, the role of ultrafine particles in determining microstructure within materials, development of quantum microdot technology, together with a more general fascination with the unique properties of nanometre-sized particles and atomic clusters, have collectively led to the application and development of a range of methods able to characterize individual particles in detail. However, few of these methods have found application in the analysis of environmental aerosols. This is perhaps understandable, given the complexity of most analysis methods, together with the hitherto relatively simple requirements of environmental aerosol analysis. However, data relating to the impact of fine (typically less than 5–10 μm) and ultrafine (typically smaller than 100 nm) aerosol particles on biological systems are becoming increasingly difficult to reconcile with simple mass-based analyses. Both epidemiology and toxicology studies indicate that biological response is mediated by factors other than mass and composition, although the nature of the underlying factors is by no means clear (Dockery *et al.* 1993; Oberdörster 1996; Donaldson *et al.* 1998). Published data in these fields alone justify a multi-disciplinary approach to environmental aerosol characterization, bringing methods and expertise from a variety of disciplines to bear on the problem of determining the role of specific particle attributes in initiating and mediating biological responses. However, given the unique nature of nanometre-sized particles, distinct from either the molecular or bulk state, it is likely that the application of ultrafine single-particle analysis methods to environmental aerosols will also shed light on aerosol interaction dynamics within other systems.

2. Single ultrafine particle analysis methods

Numerous methods have been applied to the analysis of single aerosol particles and have been well documented in a number of sources (Grasserbauer 1983; Fletcher & Small 1993; Ortner *et al.* 1998; De Bock & Van Grieken 1999). The vast majority of available methods are limited by spatial resolution and/or detection limits, and tend to be more applicable to the analysis of particles 0.5 μm to 1 μm in diameter and above. This includes many of the particle beam techniques such as particle-induced X-ray emissions (PIXE), electron probe micro analysis (EPMA) and secondary ion mass spectrometry (SIMS) (Maynard 1993). Electron microscopy has been used to characterize sub-100 nm diameter particles since the early days of its development (Drummond 1950), and for some time was considered the only method for investigating single particles in the nanometre region. Over the past decade, development of the resolution and analytical capabilities of the electron microscope has further increased its applicability to the study of ultrafine particles. The development of

scanning force microscopes such as the scanning tunnelling microscope and atomic force microscope (AFM) have further added to the available instrumentation for nanometre particle analysis. Although still at a relatively early stage of development, methods involving mass spectrometry of vaporized and ionized particles are beginning to allow the size-related compositional analysis of single ultrafine particles *in situ*. These three technologies—electron microscopy, scanning probe microscopy, and particle vaporization-ionization/mass spectrometry—form the core of current single ultrafine particle analysis capabilities.

(*a*) *Collection methods*

Although *in situ* single-particle analysis methods allow direct sampling of an aerosol with little or no preparation, the more versatile off-line methods such as electron microscopy require the aerosol to be collected and presented in an appropriate manner. Suitable collection methods vary according to the size and nature of the particles under investigation. An applicable method must allow the particles to be presented as a homogeneous uniform deposit, while not altering the relevant particle characteristics significantly. Analysis of relatively large particles in the scanning electron microscope or environmental scanning electron microscope can be achieved with relatively little preparation, from particles collected onto a variety of substrates. At the opposite end of the spectrum, nanometre-diameter particles to be analysed in the transmission electron microscope or scanning transmission electron microscope must be presented without contaminants on a suitably thin electron-transparent support. Re-suspension (usually in liquid) and deposition of aerosols onto a suitable substrate has been a common approach used in the past for particle analysis, but the modification of aerosol particles from their native state is an inherent problem (Bérubé *et al*. 1999).

Inertial collection methods such as gravitational settling and centrifugal collection are suitable for relatively massive particles (e.g. greater than 1–10 μm in diameter), but are impractical to implement for ultrafine particles. Inertial deposition in impactors is achieved by increasing particle momentum in a high velocity air flow, and enabling inertial deposition onto a substrate by rapidly changing the flow direction. Use of low pressure stages in cascade impactors allows the collection of particles as small as 50 nm in devices such as the electrical low pressure impactor (Keskinen *et al*. 1992). Recent developments in nozzle design have led to hypersonic impactors capable of collecting particles down to 50 nm (Hering & Stolzenburg 1995), and focusing impactors capable in principle of operating below 10 nm (de Juan *et al*. 1998). However, deposition forces are necessarily high, leading to the possibility of particle damage. Aerosol samples collected by impaction are generally restricted to a small region of the substrate, thus increasing the probability of particle coincidence, and may be non-uniform with respect to particle size.

Electrostatic deposition allows relatively high deposition velocities, particularly at high particle charge-to-mass ratios. Where particles are unlikely to be damaged by the charging mechanism used or the electric fields encountered, relatively gentle and uniform deposition is possible. Assuming that particles are charged to their theoretical charge limit, electrostatic deposition velocities are relatively independent of particle size (Hinds 1999). However, this limit is difficult to achieve under practical sampling conditions. Under conditions where positive and negative ions may

freely attach to aerosol particles, a charge equilibrium is reached that is highly size-dependent (characterized by a Boltzmann distribution). The fraction of nanometre-sized particles having a minimum of one charge drops off rapidly with decreasing size, leading to a dramatic fall in deposition velocity. Diffusional or photoelectric charging can be used to increase the average particle charge at small diameters, and as a general rule of thumb electrostatic precipitation can be used effectively for particles larger than 20 nm in diameter.

Below 10–20 nm, diffusion begins to dominate other deposition mechanisms. For particles smaller than 10 nm diffusion is ideally suited to obtaining uniform particle deposits on a range of sampler substrates, although samples will be highly biased towards smaller particles, and are unlikely to contain a significant fraction of particles larger than 20–30 nm.

Thermophoresis, the movement of aerosol particles in the presence of a temperature gradient, has the advantage that for a given particle composition, deposition velocity is constant below a size of *ca.* 100 nm (Talbot *et al.* 1980). Achievable deposition velocities are relatively low, but deposition is gentle and unlikely to influence the physical nature of the particles (although the thermal field may be detrimental to some temperature-sensitive particles). The technique has been used widely in the past; the Green and Watson thermophoretic precipitator formed a mainstay of occupational health aerosol sampling for many years in the mid-1900s (Watson 1937, 1958). Implementation of thermophoresis in a uniform temperature gradient between two horizontal surfaces has enabled uniform deposits of discrete particles from below 5 nm to nearly 1 μm directly on to transmission electron microscope support grids (Maynard 1995*b*).

(*b*) *Electron microscopy*

Electron microscopy is perhaps the most versatile tool for the analysis of single ultrafine aerosol particles. Scanning electron microscopes (SEMs) are routinely used for the analysis of micrometre-sized particles and above. Particles may be presented on a variety of substrates, provided they lie on the surface of the substrate, and are easily differentiable from it. Samples must be conducting to prevent localized charging, and this is achieved either by coating them with gold or carbon, or by using a conducting substrate. The latter leads to a deterioration in the imaging capabilities unless the particles themselves are sufficiently conducting. Samples are imaged by scanning a finely focused electron beam in a raster across their surface, and using the detection of resulting emissions such as backscattered or secondary emission electrons to modulate the intensity of a synchronized raster shown on a display device. In this manner, an image of the sample's surface is formed. Resolution is primarily a function of electron beam diameter and the area from which detected electrons are scattered or emitted, and approaches the diameter of the electron beam for secondary electron imaging. Low-energy secondary electron emissions are restricted to the sample's surface and allow detailed morphological imaging. Similarly, Auger electron emissions occur from the top few nanometres of the sample and may be used for surface layer elemental analysis. Current scanning Auger microscopy applications tend to have relatively poor lateral resolution, but may be adaptable to the surface analysis of ultrafine particles. Standard SEMs generally use a relatively low brightness tungsten electron source that provides insufficient beam current to

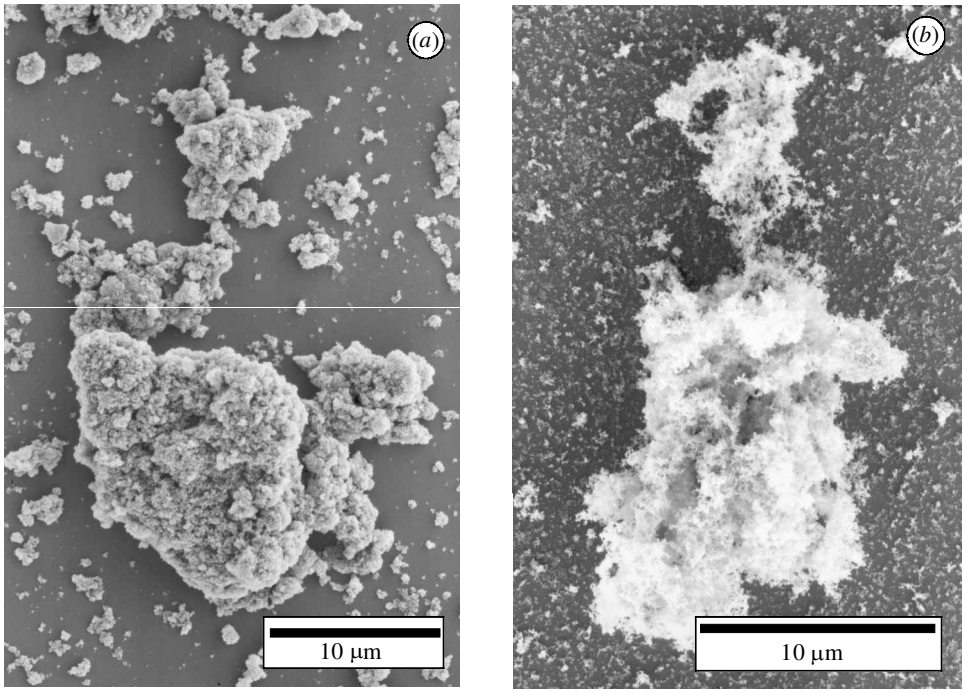

Figure 1. Images of diesel exhaust particles taken in the FEG-SEM following precipitation (*a*) from liquid, following ultrasonic agitation, and (*b*) following direct deposition onto a SEM substrate. Reproduced with permission from Bérubé *et al.* (1999). © 1999 Elsevier Science Ltd.

obtain images with a resolution much below 50–100 nm. However, brighter sources such as LaB_6 filaments allow higher resolution imaging, and SEMs equipped with high brightness, high coherence field emission electron guns (FEG-SEMs) are able to image to a resolution of below 5–10 nm (Takasu *et al.* 1993; De Hosson *et al.* 1998; Van Cleempoel *et al.* 1998; Bérubé *et al.* 1999). The use of a bright electron source has the additional advantage of allowing imaging at lower accelerating voltages, thus reducing charging within poorly conducting samples. FEG-SEMs are able to provide size and surface-structure information on deposited nanometre particles, provided that there is sufficient contrast between the features of interest and the background. Bérubé *et al.* (1999) used the FEG-SEM to compare the morphology of diesel exhaust particles impacted directly onto a substrate with that of similar particles collected on a filter and deposited from an aqueous suspension onto a suitable substrate. The indirect collection method was found to alter the morphology and the size distribution of the particles significantly (figure 1). The effect of moisture on diesel exhaust particles has also been studied directly in the environmental SEM (ESEM) (Huang *et al.* 1994). A gas/vapour chamber above the sample in the ESEM allows sample analysis in a range of environments other than vacuum (see Donald & Thiel 1999). The possibilities of aerosol analysis in a 'natural' state before the removal of volatiles is clearly attractive, although the presence of the gas/vapour chamber within the ESEM currently restricts spatial resolution to *ca.* 100 nm at best (although this is dependent on the sample, and conditions within the microscope). Huang *et al.* were able to observe directly the alteration in morphology of diesel

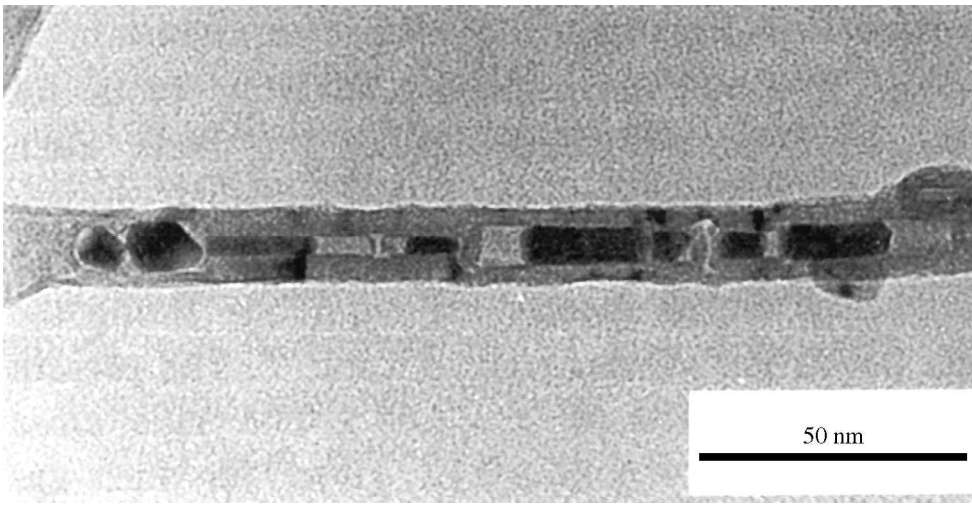

Figure 2. HRTEM imaging of rare earth dicarbide crystals encapsulated in carbon nanocages. Reproduced with permission from Yosida (1997). © 1997 Elsevier Science BV.

particles through a water condensation–evaporation cycle, as a function of particle sulphur content.

Higher spatial resolution is possible using transmission electron microscopy (TEM). Thin samples are mounted onto an electron-transparent substrate (usually a carbon film a few nanometres thick), which in most cases is held on a 3 mm diameter metal support grid. Spatial resolution is a function of electron wavelength (determined by the accelerating voltage) and spherical and chromatic aberration within the microscope, although chromatic aberration can be minimized with the use of stable high-coherence electron sources. High-resolution TEM (HRTEM) is extensively used at resolutions below 0.2 nm to investigate the properties and nature of atomic clusters. For instance, Tanaka *et al.* (1993) have studied the behaviour of sub-nanometre tungsten clusters on a MgO film, using a 200 kV HRTEM. The application of HRTEM to studying internal particle structure is illustrated by Yosida's analysis of rare earth dicarbide crystals encapsulated in carbon nanocages of the order of 10–30 nm in diameter (Yosida 1997; figure 2). The relatively high proportion of surface atoms associated with nanometre particles and atom clusters has a profound effect on their reactivity and physicochemical behaviour in some cases (explaining their widespread use in heterogeneous catalysts). By providing insight into the atomic structure of such particles, HRTEM is able to contribute to the understanding of how particle behaviour in this size range differs from the bulk and free molecular regimes (Thölen 1990; Jefferson & Tilley 1999).

The scanning transmission electron microscope (STEM) offers an alternative configuration of transmission electron microscopy, and with it an extended range of analytical methods. In the STEM, as in the SEM, a finely focused electron beam is scanned across a raster on the specimen. Resultant signals used to image the specimen include the intensity of the transmitted beam, secondary electron emissions and elastically scattered electrons. TEMs are usually configurable as STEMs, although there is inevitably a degree of compromise with the electron optics, resulting in marginally reduced imaging and analysis capabilities. Spatial resolution in

a dedicated STEM is typically better than 1 nm, and may approach *ca.* 0.3 nm in a high-resolution system. Resolution is limited by spherical aberration within the microscope, although current approaches to reducing spherical aberration (Krivanek *et al.* 1997) will allow significantly increased spatial resolution.

Imaging aerosol particles within the electron microscope, together with appropriate image analysis methods, provides a powerful tool for gaining information on particle size, morphology and structure. However, the analytical capabilities of the electron microscope extend far beyond imaging. Many analytical methods are highly specialized, and are only applicable to particle analysis in specific situations. However, a small number of methods are generally applicable to aerosol particles, and deserve inclusion here.

Selected area electron diffraction (SAED) within the TEM and STEM allows atomic order information within areas from tens of nanometres in diameter upwards. The method has been used to aid the identification of individual asbestos fibre types for some years, and has been used as an additional source of information for ambient aerosol identification in some instances (Sturges *et al.* 1989; Pósfai *et al.* 1994). Its application to ultrafine aerosol particle analysis is possibly more relevant to investigating the atomic arrangement within nanometre-sized particles and structural features, as this begins to have a significant effect on particle behaviour. Its applicability to ultrafine particles has been demonstrated in many investigations into metal and metal oxide ultrafine particle characteristics, usually within the context of heterogeneous catalysts. Structural information from a smaller specimen area is possible using convergent beam electron diffraction (CBED) in the STEM (Humphreys 1999). The area of analysis is defined by the electron beam width, allowing crystallographic information from particles, or regions of particle a few nanometres in diameter.

The use of X-ray emissions within the electron microscope is perhaps the most widely applied form of analytical electron microscopy within aerosol science (De Bock & Van Grieken 1999). Electrons interacting with the specimen excite inner shell atomic electrons, and the decay of these excited states leads to the emission of X-rays with energies characteristic of the element. Energy dispersive X-ray analysis (EDX) allows the quantification of elemental species of atomic number 6 (carbon) and above in the SEM, ESEM, TEM and STEM, although many detectors using a thin silicon protective window are limited to the detection of elements of atomic number 14 (silicon) and above. Analysis in the SEM is not ideal for ultrafine particles, as X-ray emissions from the holding substrate rapidly obscure those from particles under analysis. For the same reason, spatial resolution within the SEM is relatively low (of the order of 0.5–1 μm). Spatial resolution in the STEM and TEM approaches the electron beam width when using thin substrates or arranging for samples to be over a hole on the substrate. Sensitivity to high Z elements is sufficient for the identification of major elemental species in nanometre-diameter particles.

The sensitivity of EDX analysis in the TEM and STEM is limited by the relatively low detection efficiency for X-ray emissions. However, each core electron excitation within the specimen results in a corresponding energy loss within the electron beam. By extracting energy loss information from the beam using an energy-dispersive spectrometer, increased sensitivity to core electron excitations is achievable. Electron energy loss spectroscopy (EELS) within the STEM (and TEM in some configurations) is perhaps the most powerful analysis technique available for analysing single particles within the electron microscope. By recording and analysing the electron

energy loss spectrum, details of specific inelastic interactions, and thus sample composition and structure, can be investigated. Energy losses below 50–100 eV are dominated by bulk electron excitations (plasmons) within the sample. At higher-energy losses, energy loss is characterized by atomic core electron excitations, appearing as 'edges' on a decreasing background. The position, amplitude and shape of each edge contain information on atomic core electron excitations, and the chemical environment surrounding the atom. The energy loss at which the edge occurs is related to the atomic electron transition, allowing identification of elemental components (Brown 1999).

Dedicated STEM/EELS systems are currently able to achieve an energy resolution of *ca.* 0.3 eV over a range of losses of up to 2 kV (Brown 1999). Serial detection systems scan the spectrum over a single detector, to build up a record of energy loss over a specific loss interval. Although such systems are effective, sample acquisition times can be long, restricting the speed of analysis, and increasing the risk of specimen damage within the electron beam. Parallel acquisition systems (parallel EELS or PEELS) allow the simultaneous collection of data over a range of energy losses, and are more suited to the analysis of single aerosol particles (Maynard 1995*a*). The analysis area is characterized by the electron beam width, and in principle a spatial resolution approaching that of the beam width is possible. Elemental analysis is possible in principle for most elements (Ahn & Krivanek 1983), although in practice quantification is most applicable to the lighter elements with atomic numbers greater than 3. Quantification using higher-energy edges is compromised by a complex edge shape in many cases. However, the edge structure contains valuable, if difficult to interpret, information on the chemical environment of an element. For instance, Sánchez López *et al.* (1998) have demonstrated the use of EELS near edge structure (ELNES) to distinguish the partitioning between Al and Al_2O_3 in passivated aluminium nanometre-sized particles (figure 3).

Although EELS spectra contain a wealth of information, analysis is not as straightforward as methods such as EDX. Limitations on the energy loss range that can be analysed at any one time and complexities in interpreting data, together with the difficulties of detecting edges against the background energy loss, result in EELS not being directly applicable to routine analysis using currently available systems. Most applications of EELS are to specimens where the constituent elements are known, and it is rare to see the method applied to a sample of unknown composition. However, the successful application of PEELS to the analysis of ambient aerosol particles has been demonstrated by detecting edges using a difference method to eliminate the background, and then quantifying elemental composition from each edge (Maynard 1995*a*). Semi-quantitative elemental analysis of particles down to 5 nm in diameter indicated a practicable relative mass detection limit of *ca.* 1–2% for elements as light as oxygen, with qualitative detection being possible at lower concentrations. Comparison of the results with EDX demonstrated the superior detection efficiency of PEELS for low Z elements, although there were clear advantages in using both methods for identifying and analysing higher Z components (figure 4).

Although the electron microscope is a versatile tool for the analysis of single ultrafine aerosol particles, it has a number of limitations. The high vacuum environment (up to 10^{-11} Torr) and high current density electron beam used in the majority of microscopes has implications for the preparation of samples, and their stability under analysis. To maintain the high vacuum in a TEM or STEM, samples must be free

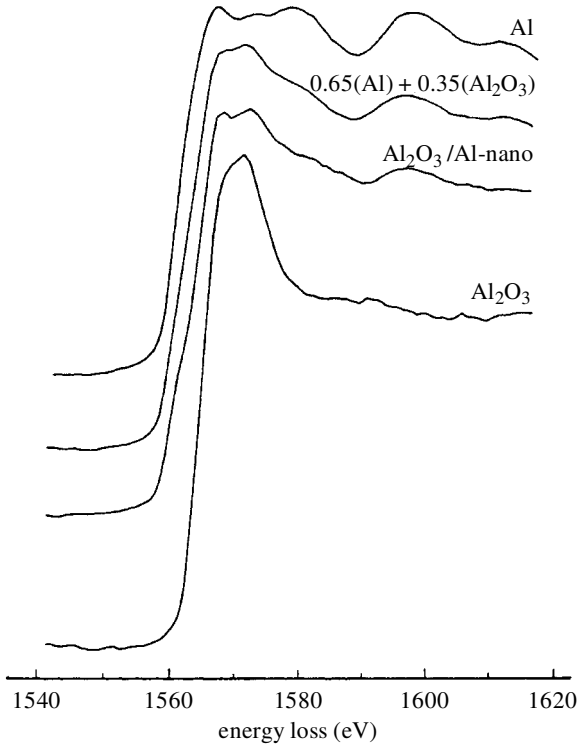

Figure 3. EELS spectra of the Al K edge taken from Al, Al_2O_3, and passivated ultrafine Al particles, demonstrating the use of near-edge structure to investigate chemical environment. Reproduced with permission from Sánchez López *et al.* (1998). © 1998 Elsevier Science Ltd.

from volatile species that will degrade the vacuum. Removal of such 'contaminants' is commonly carried out by heating the sample in a vacuum, and thus particles that contain volatile components, or change structure or chemistry at elevated temperatures, are likely to be damaged prior to imaging and analysis. Once in the microscope, susceptible materials may be easily damaged within the electron beam, particularly if the beam is held in the same place for EELS or EDX analysis for an appreciable length of time. Analysis in the TEM and STEM is also time consuming, unlike many emerging SEM systems where automation has led to increasingly rapid analysis of simple specimens. Whether the same degree of automation is possible in the transmission microscope has yet to be seen, and will undoubtedly depend on the commercial demand for such systems. EELS spectra are complex to interpret, and do not lend themselves to automated analysis. However, the use of novel edge detection and quantification methods, together with high capacity, rapid data acquisition systems, may lead to viable systems (Hunt & Williams 1991; Kundmann & Krivanek 1991; Maynard 1995*a*).

(*c*) *Scanning probe microscopy (SPM)*

The development of SPM methods has led to further techniques for imaging nanometre-sized particles. All methods are typified by a fine probe that is scanned in a raster across a surface. Probe position above (or on) the surface is controlled

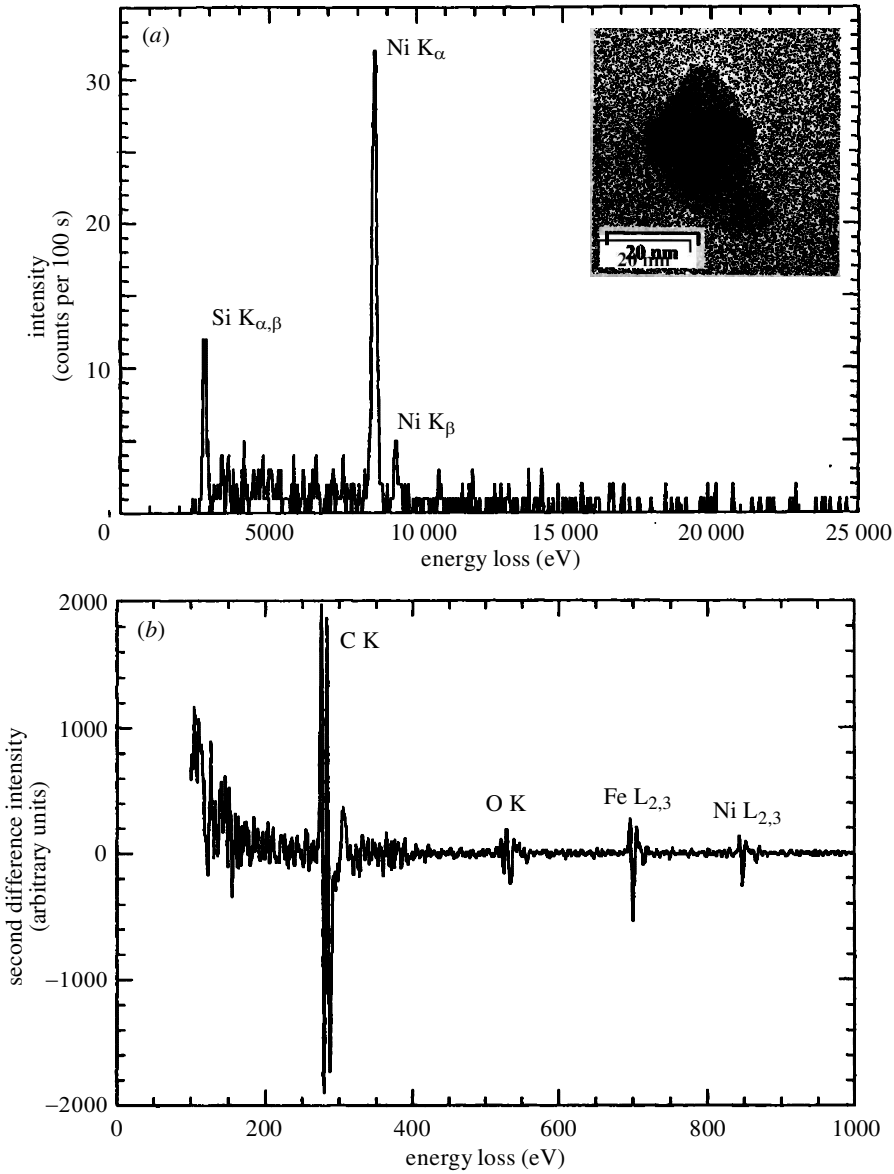

Figure 4. Comparison of double difference PEELS spectra and EDX spectra for identifying elemental species in an ultrafine ambient particle. Si and Ni peaks in the EDX spectrum are attributable to the Ni support grid, and the EDX detector Si window. © 1995 Elsevier Science Ltd.

by a range of feedback signals which are also used to provide image contrast on the associated display raster. Initial SPM development used the electron tunnelling current between a conducting specimen and probe suspended a few angstroms above its surface to map topographic features at angstrom resolution (scanning tunnelling microscopy (STM)). Later developments led to the use of Van der Waals forces between the specimen and the probe (atomic force microscopy (AFM)), allowing

imaging of non-conducting specimens. While a gap of *ca.* 10 Å is maintained between the probe and specimen in STM, AFM may be carried out with the probe in contact with the specimen, or separated by up to several tens of angstroms. The use of further feedback mechanisms has led to a number of SPM imaging methods, including magnetic force microscopy, lateral force microscopy, shear force microscopy and near field scanning optical microscopy. All methods can be operated in a range of environments, including atmospheric conditions, liquid immersion and vacuum.

Of all the available SPM methods, AFM is perhaps the most applicable to aerosol analysis, as high-resolution imaging is possible in air, and there are relatively few limitations on the type of sample imaged. However, the clear advantages it has over electron microscopy methods, such as rapid sample analysis, minimal sample preparation, and analysis under ambient conditions, are somewhat balanced by a lack of clarity concerning image interpretation and applicability. Friedbacher *et al.* (1995) have successfully applied AFM to the analysis of ultrafine environmental particles collected on a polyester foil using a low pressure cascade impactor. The substrate was found to have a suitably flat surface (root mean square roughness of 1 nm over 4 μm^2) to allow the identification and sizing of sub-30 nm particles. The AFM-derived size distribution agreed well with that expected from the impactor stage cut-off. However, the presence of large-diameter particles with very little height in samples indicated that there was some degree of particle modification subsequent to sampling, bringing into question the direct interpretation of aerosol size distribution from the AFM data. The assumption was made that these particles were the result of droplet deposition followed by evaporation, leaving a residue. Interestingly, the ability to differentiate by height gave the analysis method an advantage over TEM imaging, where differentiation between droplet residues and solid particles isn't always straightforward. Although it is likely that these particles resulted from a loss of volatile components, Köllinsperger *et al.* (1999) were able to demonstrate that the AFM may be used to image environmental particles prior to the loss of volatiles. They were also able to demonstrate the use of automated image analysis in the AFM with environmental particles, allowing rapid characterization of the aerosol size distribution. However, the samples analysed were from the lower stages of a cascade impactor, and thus did not contain large particles that may have caused complications. Cohen *et al.* (2000) have used the AFM to detect and size ultrafine acid particles deposited onto an iron film a few nanometres thick. The reaction between the acid component of the particles and the ion substrate was found to lead to distinctive raised features around the deposition site, with an overall reaction site diameter several times that of the original particle (in many ways the technique is similar to the use of Liesegang rings described by Podzimek & Podzimek (1999)). By detecting and sizing these features using an AFM, Cohen *et al.* were able to rapidly analyse the number and size distribution of 100 nm diameter sulphuric acid-coated carbon particles.

Although SPM can resolve horizontal and vertical details to fractions of a nanometre, it is unable to deal with large changes in vertical profile occurring over a few nanometres. Köllinsperger *et al.* (1997) estimated errors arising from convolutions between the scanning tip and the relatively sharp vertical gradients at the edges of nanometre-sized particles to be of the order of 10%. There is also some concern over the degree to which scanning probe analysis alters the distribution of particles on a substrate. Friedbacher found no alteration of the distribution of environmental particles on a polyester substrate after repeated scans. However, Schleicher *et al.*

(1993) reported the removal of *ca.* 8 nm diameter silver particles from highly oriented pyrolytic graphite during STM analysis. Cohen *et al.* also reported the removal of particles while using AFM in contact mode.

Near-field scanning optical microscopy (NSOM or SNOM) is an SPM technique that has some potential benefits for the analysis of ultrafine particles. Conventional optical microscopy is limited to a theoretical spatial resolution of $\lambda/2$. However, if a specimen is illuminated through a sub-wavelength sized aperture held to within a few angstroms of its surface (the near-field), spatial resolution approaching the diameter of the aperture is possible (Synge 1928). By using SPM methods to scan a fine aperture over a sample, optical imaging with a resolution below 100 nm can be achieved. The aperture is usually formed at the tip of a drawn glass fibre coated with aluminium to form a light pipe, and is held a few angstroms from the specimen using non-contact AFM or shear force microscopy feedback methods (Pohl *et al.* 1984; Betzig *et al.* 1991, 1992). Although resolution does not extend far into the ultrafine region, the possibilities for applying optical analysis and detection methods to isolated nanometre diameter particles are of interest.

(*d*) *Laser desorption/ionization of ultrafine particles*

Mass spectrometry (MS) of vaporized then ionized single particles has gained increasing recognition over the past few years as a viable method for analysing the size-resolved compositional make-up of aerosols in near real-time. The aerosol is first formed into a particle beam and transported to a high-vacuum region (*ca.* 10^{-4} Torr), using a series of differentially pumped orifices (see, for example, Liu *et al.* 1995*a*, *b*). Particle acceleration can be related to aerodynamic diameter in the expanding flow fields, and time-of-flight measurements may be used to size particles larger than *ca.* 0.3 μm. Formation of a particle beam in vacuum is followed by particle vaporization and ionization, and detection of ions in a mass spectrometer (Prather *et al.* 1994). Flash vaporization on a resistively heated surface may be used for ion formation, but has limitations at small particle sizes. Laser desorption/ionization (LDI) of individual particles is an alternative vaporization method that is finding increasing use in single particle mass spectrometry (Johnson & Wexler 1995). In a typical system, particles entering the final analysis zone within the instrument are detected using scattered light pulses from a continuous wave laser. These are used to trigger the firing of a second high-energy laser, which vaporizes them in flight. A commercial aerosol time-of-flight mass spectrometer (ATOFMS) is now available, based in the work of Prather *et al.*, that allows single particle size and compositional measurements down to 0.3 μm diameter (TSI Inc. Model 3800 ATOFMS).

Although LDI and MS are in principle applicable to particles of nanometre diameters, the use of optical scattering to trigger vaporization becomes impractical for particles smaller than 0.3 μm. Reents *et al.* (1995) have developed a system capable of analysing particles as small as 20 nm in diameter by using a laser pulsed at between 10 and 30 Hz, independently of the presence of particles. However, the reduction in particle size is at the expense of detection frequency. Reents *et al.* were interested in monitoring contaminant particles in the semiconductor industry. Carson *et al.* (1997) extended the technique down to 12 nm diameter particles for the analysis of size-selected aerosol particles. Size differentiation was on the basis of electrical mobility, using a differential mobility analyser (DMA). Analysis of sodium chloride,

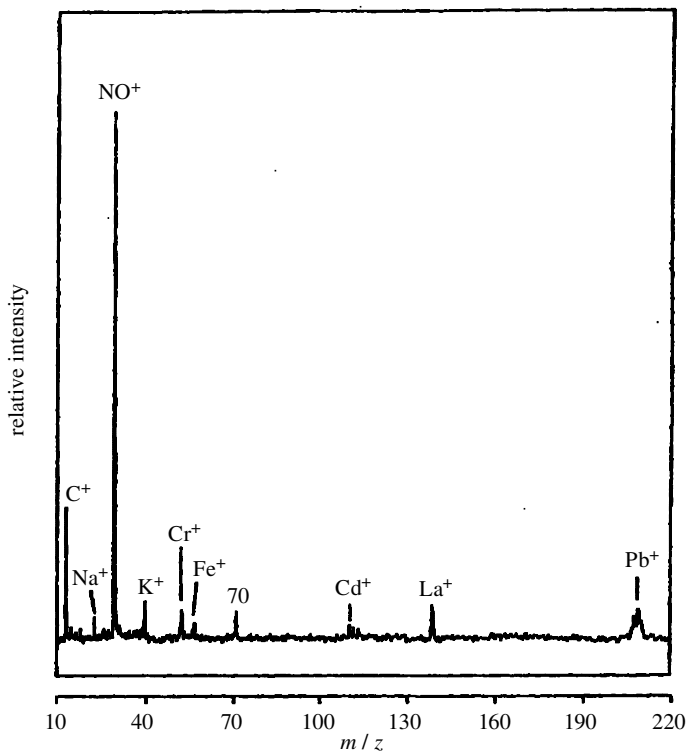

Figure 5. Mass spectrometry of an individual 60 nm particle doped with *ca.* 1% concentrations of a number of metal species, and vaporized using LDI. Reproduced with permission from Zhaozhu *et al.* (1998). © 1998 American Chemical Society.

ammonium nitrate, potassium chloride and anthracene particles demonstrated that chemical speciation is feasible for nanometre-sized single particles, and that positive ion and free electron production tends to dominate for ultrafine particles with the UV excimer laser used. Ion peak area relative to particle mass increased for smaller particles, with the implication that higher ion yields were being observed at smaller particle sizes. Zhaozhu *et al.* (1998) developed the instrument used by Carson *et al.* and carried out a feasibility study into the analysis of single multi-component ultrafine aerosol particles. Analysis of NaCl/KCl particles of *ca.* 50 nm diameter indicated that detection of a relative mass of KCl of *ca.* 0.06% was possible in a single particle (corresponding to *ca.* 10^{-20} g KCl in the particle). Analysis of 60 nm particles containing traces of several metal salts indicated that detection at mole fractions *ca.* 1% is possible for metal species (corresponding to an absolute mass of the order of 10^{-17} g for each metal), and that for some species the detection limit may be significantly lower (figure 5).

3. Summary

Single-particle analysis has rarely been a valid surrogate for collective particles analysis; perhaps even more so in the case of ultrafine particles, where characterized particles may represent a small fraction of a per cent of a given aerosol. However,

in many cases the role of individual particle properties must be understood prior to the selection of appropriate collective analysis methods, and this is where the ability to characterize an aerosol at the single particle level is invaluable. Electron microscopy is perhaps the most generally applicable method. Size and morphology are readily characterized in the FEGSEM, TEM and STEM. HRTEM allows structural information on particles and atomic clusters to sub-0.2 nm resolution, while EELS and EDX analysis in the STEM allow the chemical analysis of particles down to nanometre diameters. By combining analysis methods, investigation of particle size, shape, structure, composition and surface properties is in principle possible. However, the analysis environment is harsh, and only suited to robust particles with low volatility. Analysis in the ESEM overcomes some of the analysis environment restrictions and allows in principle the characterization of particles with a significant volatile component, although its application is currently restricted to particles larger than *ca.* 100 nm. SPM offers the possibility of analysing nanometre-diameter particles under ambient conditions, thus getting away from some of the constraints imposed by electron microscopy. Imaging methods such as AFM and NSOM offer novel and exciting possibilities for the characterization of specific aerosols. For instance, the use of NSOM to identify, size and count fluorescently tagged ultrafine particles would seem applicable to identifying particle transport and deposition characteristics within biological systems. While SPM is currently limited in the information that can be obtained from ultrafine aerosol samples, the uniqueness of the information available should allow it to be developed as a complementary tool to electron microscopy. While electron microscopy and SPM are confined to the analysis of collected samples, and are constrained by the limitations of the collection and preparation systems used, developments in aerosol mass spectrometry are providing the means for chemically characterizing size-segregated ultrafine particles on-line. Current technology allows the speciation of individual particles *ca.* 10 nm in diameter, and as this is reduced still further, the resulting methods should provide invaluable complementary data to off-line methods.

By adopting technologies developed within complementary disciplines, together with the development of aerosol-specific methods, it is possible to develop a basis for characterizing single sub-100 nm particles and features in terms of size, morphology, topology, composition, structure and physicochemical properties. The methods available provide complementary means to characterize single ambient particles in depth. Currently, with few exceptions, they are complex, time-consuming to use, and in many cases still at a developmental stage. As such they are not ideally suited to the routine analysis of aerosols. However, by adopting a multi-disciplinary approach, the potential is there to develop complementary tools that will provide routine and detailed information on the particles that influence the environment we live and work in.

4. Disclaimer

Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC).

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T. Benham (*Volvo Technical Development, Sweden*). With respect to the picture of particles prepared by two different techniques (figure 1), how is it possible to identify which one is the correct representation of the particles?

A. D. Maynard. Intuitively, the particles with the least preparation—in this case those simply collected via impaction on a substrate, with no further processing other than being given a conductive coating—will be most representative of the airborne particles. In this case, we also know a great deal about what we expect the particles to look like (agglomerates of very small primary particles) from a large body of published data, and so we can be reasonably confident that the impacted particles are the closest representation of the airborne particles.

M. S. Bingley (*Cobham, UK*). I would like to remind people that greater resolution can be obtained with immersion objectives with numerical apertures of 1.524 instead of the usual 'schoolboy' objective, 1.25 NA × 100 that is supplied to scientists. I have use one of these, so they really do exist!

The use of mono-brom-naphthalein immersion fluid enables numerical apertures of 1.6 to be realized. The late Horace Dall, master lens and instrument maker, made the front element of an immersion objective out of diamond and reached an aperture of 1.9 NA. These techniques might provide more information on ultrafine particles. The microscope makers should be pressed to make some decent lenses again!

A. D. Maynard. The resolving power of an optical objective lens is $X = 0.61\lambda/\mathrm{NA}$, where X is the distance between two just-resolved points, λ is the illumination light

wavelength and NA is the lens numerical aperture. Thus with a numerical aperture of 1.6 and using illuminating light with a wavelength of 400 nm, it is in principle possible to achieve a resolution of 150 nm (a 1.25 NA objective would raise the resolution limit to 200 nm). These limits are theoretical limits, and in practice, will be dependent on a number of other factors, including illuminating conditions and specimen contrast. Although this resolution limit is sufficient to observe fine details on larger sub-micrometre particles, it is insufficient for the detailed analysis of particle smaller than 100 nm in diameter.

C. V. Howard (*Fetaltoxico-Pathology, University of Liverpool, UK*). Have you considered the use of partial vacuum electron microscopy? For example, Mike Gorringe in Oxford, among others, has been showing video images of catalyst particles in motion on a substrate by using an environmental cell. Could this approach be used in your study?

A. D. Maynard. The use of partial vacuum electron microscopy (environmental SEM) is particularly attractive to the study of ultrafine particles that may have an appreciable mass of volatile material, and have a physical structure that changes with the loss of volatile material. However, there is a trade-off within the ESEM between resolution and gas pressure, that renders this type of analysis somewhat difficult. Current ESEMs are able to image at a resolution of *ca.* 100 nm at pressures of a few pascals.