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Detection and characterization of engineered nanoparticles in food and the environment Â□ a review

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Detection and characterization of engineered nanoparticles in food and the environment – a review

Nanotechnology is a fast growing market and it is expected that increasingly more products will contain some sort of nanomaterial in the future. So far, little is known about the occurrence, fate and toxicity of nanoparticles. The limitations in our knowledge are partly due to the lack of methods for the detection and characterisation of engineered nanoparticles in complex media i.e. water, soil or food. This review provides an overview of the characteristics of nanoparticles that could affect nanoparticle behaviour and toxicity as well as techniques available for determining these. Important properties comprise size, shape, surface properties, aggregation state, solubility, structure and chemical make up. Methods are available that have been developed for natural nanomaterials or engineered nanomaterials in simple media which could be optimized to provide the necessary information. These include microscopy, chromatography, spectroscopy, centrifugation as well as filtration and related techniques. A combination of these is often required. There are a number of challenges that will arise when analysing environmental and food materials including extraction challenges, the presence of analytical artefacts caused by sample preparation, the problems of distinction between natural and engineered nanoparticles and the lack of reference materials. Work in the future should focus on addressing these challenges.

Keywords: Nanoparticles, nanomaterials, food, environment, analysis, characterization, detection

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Introduction & background

Nanomaterials are commonly regarded as materials with at least one dimension below 100 nm (Borm et al. 2006), although there is no official definition yet. They include nanofilms and coatings (< 100 nm in 1 dimension), nanotubes and wires (< 100 nm in 2 dimensions) and nanoparticles (< 100 nm in 3 dimensions) (Hochella 2002). Nanoparticles can occur naturally (e.g. in ashes, as soil particles or bio molecules), be produced unintentionally (e.g. in Diesel exhausts) or be intentionally engineered. This review will mainly focus on engineered or manufactured nanoparticles (ENPs).

As a consequence of their size, nanoparticles show different physico-chemical properties compared to their respective bulk material. These include changes in optical properties, which can cause changes in colour (e.g. gold colloids appear as deep red), thermal behaviour, material strength, solubility, conductivity and (photo) catalytic activity (Kamat 2002; Hochella 2002; Burleson et al. 2004). Nanoparticles are effectively a bridge between atomic or molecular structures and bulk materials (Henglein 1993). For example nanoparticles made of semi conducting materials and with a size between ~ 1 - 10 nm (corresponding to the diameter of around 10 to 50 atoms) are small enough to show quantum effects (quantization of electronic energy levels) and are typically called quantum dots (Rao et al. 2002). Probably the most significant influence on the behaviour of nanoparticles however is the change in surface to volume ratio (Banfield and Zhang 2001). The volume decreases with size but the proportion of atoms at the particle surface increases and therefore the surface properties can dominate the properties of the bulk material (Waychunas 2001). Furthermore, the

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structure and properties of the surfaces of nanoparticles are substantially modified over that of the surfaces of the same materials in bulk form because of the proportionally high curvature of the nanoparticle surfaces, more surface defects and edges as well as the presence of highly catalytically active sites (Madden and Hochella 2005). Additionally, targeted change in surface properties of ENPs can be achieved by coating or functionalisation of nanoparticles.

The potential benefits of engineered nanomaterials have been recognized for a long time but it has not been until recently that the step from research to manufacture and use has been made. Engineered nanomaterials are now being manufactured in ever increasing quantities and they are finding application in a wide range of products and sectors including medicines, cosmetics, clothing, engineering, electronics, and environmental protection (Ponder et al. 2001; Obare and Meyer 2004). Current applications range from antibacterial wound dressings and clothing, through to reinforced tennis rackets to advanced transparent sun protection.

In the food sector the uses of nanotechnology-derived food ingredients, additives, supplements and contact materials are expected to grow rapidly. Chaudhry et al. (2007) claim that worldwide over 200 companies are conducting R&D into the use of nanotechnology in either agriculture, engineering, processing, packaging or delivery of food and nutritional supplements. Food safety will also potentially benefit with the introduction of nano-based detectors, sensors and labelling (Weiss et al. 2006). In some countries nanomaterials are already applied in food supplements and food packaging

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both nanoclays as diffusion barriers and nano-silver as antimicrobial agents (Sanguansri and Augustin 2006; Chaudhry et al. in press; Corporate watch 2007; table 1).

Table 1. Examples for applications of nanomaterials in consumer products.

The proliferation of nanotechnology has prompted discussions over the safety of these materials to human health and the environment. It is almost inevitable that humans will be exposed to engineered nanoparticles e.g. due to migration of nanoparticles from food packaging into food, as well as the application of creams directly to the skin. In addition, the unintended (e.g. waste, wastewater, sludge) and intended (e.g. groundwater remediation) release of nanoparticles to the environment may lead to indirect human exposure (e.g. via drinking water, food chain).

The pulmonary toxicity of airborne particles (mostly referred to as ultrafine particles < 10 µm) has been well studied and it is known that toxicity is strongly related to particle size (Brown et al. 2001; Hasegawa et al. 2004; Geiser et al. 2005; Frampton et al. 2006). However, the toxicity of engineered nanoparticles and their effects on human health, as well as their environmental fate and impact in water and soil is still widely unknown (Burleson et al. 2004), although some studies suggest (eco-) toxicity. It has been reported that different types of nanoparticles can cause cytotoxicity and cross-cellular layers (Shiohara et al. 2004; Koch et al. 2005; Chen and von Mikecz 2005; Hardman 2006; Brunner et al. 2006) as well as accumulate in tissue (BullardDillard et al. 1996). Further toxicity of fullerenes and TiO_2 nanoparticles to daphnia, large mouth bass and other aquatic species has been found (Oberdorster 2004; Oberdorster et al. 2006; Lovern

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and Klaper 2006), whereas Yang & Watts (2005) discovered phytotoxicty of alumina nanoparticles (Yang and Watts 2005). Fullerenes, silver and other nanoparticles have also shown antibacterial behavior e.g. in health care applications and in aquatic environments (Sondi and Salopek-Sondi 2004; Oberdorster et al. 2006; Lyon et al. 2006; see table 2).

Table 2. Examples for nanoparticle (eco-) toxicity and other effects.

Even in cases where nanoparticles do not show any acute toxicity, the question of long-term effects, bioaccumulation and the impact on food webs remains. Engineered nanoparticles may also affect the toxicity of other substances, since natural nanomaterials are known to act as nanovectors for contaminants (Mccarthy and Zachara 1989; Kersting et al. 1999; Lyven et al. 2003; Lamelas and Slaveykova 2007). For example a study with carp showed enhanced cadmium bioaccumulation in the presence of TiO_2 nanoparticles (Zhang et al. 2007).

Therefore it is crucial that we begin to understand the behaviour of engineered nanoparticles in food materials, consumer products and environmental matrices as well as their toxicity to humans and the environment. In order to do this, it is essential that we have access to robust analytical methodologies for detecting and characterising engineered nanoparticles in a range of matrix types.

This paper therefore provides an overview of the different analytical techniques available for the detection as well as physical and chemical characterization of

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engineered nanoparticles in product formulations, environmental matrices and food materials. As limited work has been done to date on the detection and characterization of engineered nanoparticles in food, the review draws heavily upon studies reporting characterization of nanoparticles in raw products and environmental matrices where much more information is available (e.g. Walther 2003; Lead and Wilkinson 2006; Wigginton et al. 2007a). Possible future directions of ENP analysis and characterisation in biological, environmental or food samples are identified and areas of further work are recommended.

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Nanoparticle properties & their analysis

The potential toxicity and behaviour of nanoparticles will be affected by a wide range of factors including particle number and mass concentration; surface area, charge, chemistry and reactivity; size and size distribution; state of aggregation; elemental composition as well as structure and shape (Borm et al. 2006; Chau et al. 2007); table 3). Therefore when analysing nanoparticles in different matrices, it is not only the composition and concentration that will need to be determined but also the physical and chemical properties of the engineered nanoparticles within the sample and the chemical characteristics of any capping/functional layer on the particle surface.

Table 3. Nanoparticle properties and their importance for measurement.

The analytical techniques should be sensitive enough to measure low concentrations as small particles normally represent only a small part of the total mass. The techniques should also minimise sample disturbance to ensure that laboratory analyses reflect the unperturbed environmental state (Chen and Buffle 1996; Gimbert et al. 2007b). A range of analytical techniques is available for providing information on concentration and properties; these include microscopy approaches, chromatography, centrifugation and filtration, spectroscopic and related techniques (table 4). In the following sections, a selection of these methods will be discussed that are potentially suitable for nanoparticle characterisation and literature examples will be used to demonstrate the application of different techniques to complex media.

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Overview of analytical methods applicable to nanoparticle analysis

A wide range of methods is available for the detection and characterization of nanoparticles, a choice of different approaches are described below and a summary of the information generated by different techniques and their application to complex media is given in tables 4 and 5 respectively.

Table 4. Nanoparticle properties and examples of analytical methods potentially suitable for their measurement.

Table 5. Overview of discussed analytical methods suitable for nanoparticle characterization in alphabetical order with literature examples for their application in complex media.

Microscopy and microscopy related techniques

Microscopy-based methods are available that could be used in the detection and characterization of engineered nanoparticles. These methods include optical approaches including confocal microscopy as well as electron and scanning probe microscopy.

The typical dimensions of nanoparticles are below the diffraction limit of visible light, so that they are outside of the range for optical microscopy. However, near-field scanning optical microscopy (NSOM) – a scanning probe microscopy (SPM) technique - obtains with a spatial resolution of ~ 50 – 100 nm much better resolutions than

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conventional optical microscopes. This is achieved through the use of a sub-wavelength diameter aperture. NSOM may therefore be suitable for optical imaging of nanoparticle aggregates (Maynard 2000).

The diffraction of light is also the limiting factor for confocal microscopy. However, using confocal laser scanning microscopy (CLSM), resolutions of up to 200 nm can be achieved and tiny fluorescent objects can often be located more precisely than the resolution limit. Another feature of a CLSM is the high-resolution optical imaging of thick specimen (optical sectioning). Naturally fluorescent samples or samples treated with fluorescent dyes are detectable. Confocal microscopy has only recently been applied in colloid characterisation and has been combined with fluorescence correlation spectroscopy (FCS) to characterise fluorescent species in complex systems (Lead et al. 2000b; Prasad et al. 2007).

The most popular tools for the visualization of engineered nanoparticles though are electron and scanning probe microscopes. Depending on the technique, resolutions down to the sub-nanometer range can be achieved. Using atomic force microscopy (AFM), scanning electron (SEM) and transmission electron microscopy (TEM) nanoparticles can not only be visualized, but also properties like the state of aggregation, dispersion, sorption, size, structure and shape can be observed (Mavrocordatos et al. 2004). For comparison, Figure 1 shows TiO_2 and ZnO nanoparticles imaged by SEM, TEM and AFM.

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Figure 1. ZnO (1st row) and TiO_2 (2nd row) nanoparticles suspended in distilled water, allowed to dry and imaged in order from left to right by SEM, AFM and TEM. Initial sizes as stated by the manufacturer (Sigma Aldrich, UK): 50 – 70 nm for ZnO particles and 5 – 10 nm for TiO_2 particles.

In TEM, electrons are transmitted through a specimen (therefore the specimen has to be very thin) to obtain an image whereas in a SEM scattered electrons are detected at the sample interface for imaging. In general imaging of lighter atoms in an electron microscope is more difficult as they scatter electrons less efficiently.

Analytical (mostly spectroscopic) tools can be coupled to electron microscopes for additional elemental composition analysis generally known as analytical electron microscopy (AEM). For example, energy dispersive X-ray spectroscopy (EDS), can be combined with SEM and TEM and permits a clear determination of the composition of elements heavier than oxygen, Quantitative analysis however, leads generally to ~ 20 % uncertainty (Mavrocordatos et al. 2004).

Electron energy loss spectroscopy (EELS) is based on the loss of energy of the incident electron through the specimen. Thus, elements can be discriminated. This technique can only be used with TEM and quantitative analysis has uncertainties as low as 10 % (Mavrocordatos et al. 2004). Selected area electron diffraction (SAED) can also be combined with TEM and provides information on crystalline properties of particles (Mavrocordatos et al. 2004).

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Electron microscopy is usually a destructive method meaning that the same sample cannot be analyzed twice or by another method for validation. Other disadvantages of electron microscopes are charging effects caused by accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. This can normally be overcome by using sample coating made of a conducting material, but this can result in a loss of information. Also biological samples often need treatment, like heavy metal staining, for better contrast.

For biological samples, a scanning transmission electron microscope (STEM) belonging to the group of TEMs, can be of use. Dark-field microscopy with a STEM allows high contrasts and therefore imaging of biological samples without staining. In combination with diffraction and spectroscopic techniques STEMs can also provide images and chemical data for nanomaterials with a sub nanometer spatial resolution (Liu 2005). Utsunomiya and Ewing (2003) successfully applied high-angle annular dark field scanning transmission electron microscopy, scanning transmission electron microscopy-energy dispersive X-ray spectrometry, and energy-filtered transmission electron microscopy to the characterization of heavy metals on airborne particulates (Utsunomiya and Ewing 2003).

X-ray microscopy (XRM) can provide spatial resolution (down to ~30 nm, limited by the X-ray beam focusing optics) imaging of a specimen in the aqueous state without the need for sample preparation e.g. fixation, staining, sectioning (Jearanaikoon and braham-Peskir 2005; Thieme et al. 2007). X-ray microscopy can also be combined with computer tomography to enable 3D imaging (Thieme et al. 2003). A variation of the

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XRM is the scanning transmission X-ray microscopy (STXM), which has been used for example to characterize metallic Fe particles for remediation purposes (Nurmi et al. 2005).

The major limitation of conventional electron microscopes like transmission electron and scanning electron microscopes is however, that they have to be operated under vacuum conditions. This means no liquid samples can be introduced to the sample chamber and sample preparation (dehydration, cryo-fixation or embedding) is necessary, which leads in general to sample alteration and dehydration artifacts (Mavrocordatos et al. 2007).

There has therefore been a lot of effort to improve sample preparation techniques for electron microscope imaging in order to limit artifacts. For example, Lonsdale et al. (1999) applied high pressure freezing and freeze substitution to image barley aleurone protoplasts by transmission electron microscopy (TEM) (Lonsdale et al. 1999). This method preserves the cellular fine structure and antigenicity of proteins better than conventional chemical fixation and dehydration techniques. Another possibility is the use of a cryo-TEM, which enables imaging of frozen samples on a cold specimen stage and microscope. This has the advantage of preserving and visualizing structures that would be lost or altered by other sample preparation methods. Wang et al. (2004) employed this method to image Fe(III)-doped TiO_2 nanoparticles (2 - 4 nm) in an aqueous environment with a special sample holder (Wang et al. 2004). Mavrocordatos & Perret (1998) embedded iron-rich particles (30 - 200 nm) in resin and then sectioned these samples for visualization by TEM and EELS (Mavrocordatos and Perret 1998).

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However, none of these preparative techniques can fully avoid artifacts caused by sample drying or preparation. As imaging of nanoparticles in their original state is crucial for nanoparticle research other methods are required. One possibility to image nanoparticles under more natural conditions is to use an environmental scanning electron microscope (ESEM). In an ESEM the gun and lenses of the microscope are under vacuum conditions as in a conventional SEM, but due to a detector that is able to operate under higher pressure and multiple pressure limiting apertures to separate the sample chamber from the column, the sample chamber itself can be operated at around 10-50 Torr. Therefore, samples can theoretically be imaged in their natural state without modification or preparation under variable pressure and humidity, theoretically up to 100 %. Additionally the gas ionization in the ESEM sample chamber eliminates the charging artifacts and therefore materials do not have to be coated with a conducting material anymore. Other advantages of an ESEM are that the detector is insensitive to light and fluorescence or cathodoluminescence does not disturb imaging. ESEM still allows X-ray data, e.g. from EDS, to be obtained. However, an ESEM cannot achieve real atmospheric pressure and only the top surface of a specimen can be imaged, which in the case of a liquid sample is the water surface. The contrast is increasingly poor with increasing humidity and there is the possibility of specimen drifting. Also a loss in resolution from ~ 10 nm up to ~ 100 nm is unavoidable.

Doucet et al. (2005) compared the performance of an environmental and a conventional scanning electron microscope (ESEM and SEM respectively) for the imaging of natural aquatic particles and colloids. Analyzing river estuary samples they found that the

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conventional SEM provides sharper images and lower resolution limits, but produces more imaging artifacts due to the drying of the sample. ESEM samples retain to some extent their morphological structures without the need of sample preparation, but image interpretation and imaging itself is more complex. Also it has been stated that the maximum relative humidity at which imaging could be performed was 75 %, as at 100 % layers of free water over the sample made colloid visualization impossible. Sizing of colloids revealed technique-dependent differences. Hence they suggest that ESEM and SEM should be used as complementary techniques, but are in favor of the ESEM for imaging colloids and colloid aggregation (Doucet et al. 2005a).

Redwood et al. (2005) applied an ESEM to analyze and quantify humic substances (Suwannee river humic acid, 100 mg/L) as a function of humidity and pH (3.3 – 9.8). They concluded that the ESEM is an important complementary technique to other analytical methods for probing changes in colloid structure as a function of hydration state, however, they also concluded that at present non-perturbed samples cannot be imaged (Redwood et al. 2005).

The technique of WetSTEM allows transmission observations of wet samples in an ESEM under annular dark-field imaging conditions down to a few tens of nm. Combining elements of TEM and ESEM, samples that are fully submerged can be imaged. The imaging is achieved by placing a TEM grid with the sample on a TEM sample holder. This holder is placed in the ESEM chamber allowing transmission imaging under non-vacuum conditions (Bogner et al. 2005).

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An alternative to the ESEM methods described above is the use of a WetSEM™ capsule as a specimen holder, in which the sample is added and the holder is then sealed. These capsules have been developed by the QuantomiX Company for imaging of samples in a conventional SEM under hydrated conditions. There are two different types of WetSEM capsules on the market suitable for conventional SEM with a back-scattered electron detector: one for imaging in liquids and another for imaging of solid but wet materials (e.g. biological samples, food or soil). With this technique *in situ* imaging of nanoparticles in natural media is possible. The capsule separates the sample from the vacuum chamber of the microscope and a membrane in the capsule allows electrons to pass into the sample thus enabling imaging under atmospheric pressure. It is possible to conduct semi-quantitative and qualitative elemental analysis with these capsules provided that the microscope is equipped with an energy dispersive x-ray spectrometer (Thiberge et al. 2004a; Thiberge et al. 2004b; Joy and Joy 2006; Timp et al. 2007). Limitations are a loss of resolution and the sensitivity of the membrane to radiation damage. Also objects have to be close to the membrane to be visible. Thiberge et al. (2004) describe in detail the theory, characteristics, limitations and possible applications of WetSEM capsules using a conventional SEM and an ESEM (Thiberge et al 2004a; Thiberge et al 2004b).

Imaging under fully liquid conditions is also possible using atomic force microscopy (AFM). The AFM belongs to the family of scanning probe microscopes (SPMs) (Balnois et al. 2007). An oscillating cantilever is scanning over the specimen surface and electrostatic forces (down to 10^{-12} N) are measured between the tip and the surface. An AFM can achieve 3D surface profiles from these force measurements with height

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resolutions of ~ 0.5 nm. The main advantage of an AFM is that it images sub-nanometer structures under wet or moist conditions. Although under liquid conditions particles not fixed to a substrate will float around and eventually stick to the cantilever, which leads to imaging artefacts, both as smearing effects and changes in the cantilever oscillation properties as the tip gains weight. This smearing effect could be minimized by using a non-contact scanning mode where the tip is not touching the particles but only feel its forces (Balnois et al. 2007).

The main limitation of AFM for nanoparticle visualization is that the geometry of the tip is often larger than the particles being probed and this leads to errors in the onset and offset of a particle topography on a scan, resulting in severe overestimations of the lateral dimensions of the nanoparticles. Therefore accurate size measurements should only be taken on the height (Z-axis) of the particles and the lateral dimensions only used with great caution. Furthermore AFM for environmental or food related samples is limited in the ability to obtain qualitative or quantitative information of the sample composition. Although, the force patterns that emerge can also help in identifying the nature of individual atoms, this technique is called chemical force microscopy, short CFM (Sugimoto et al. 2007; Shluger and Trevethan 2007). This recent development could lead to a vast progress in AFM application to more complex samples. Scanning tunneling microscopy (STM) is another type of scanning probe microscopy based on quantum electronic properties where a conducting tip is oscillating close to the surface and if it comes in close contact with a metallic or semiconducting component of the surface then electrons can be allowed to “tunnel” over the gap to the surface. STM has

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been applied to environmental samples to image redox properties of microbial enzymes (Wigginton et al. 2007b).

AFM has been used to characterize natural colloidal matter. For example Lead et al. (2005) analyzed natural aquatic colloids by AFM and colloid structure was found to vary as a function of pH. Mica slides were dipped for 30 min into filtrated samples rinsed with distilled water and allowed to dry prior to imaging in tapping mode. It has been stated that it is not known whether imaging under ambient humidity or liquid water produces better results. *A priori*, imaging under liquid water appears to provide ideal experimental conditions. However, atmospheric humidity retains colloid-bound water, helping to maintain structure, and AFM tips exposed to organic matter in solution soon become coated in the organic matter, potentially affecting the veracity of the images. This is also a possibility in imaging after air-drying. Recommendation is given as a complementary tool and comparison between TEM and AFM using different sample preparation methods indicate similar morphologies (Lead et al. 2005). Balnois et al. (1999) employed tapping mode AFM for the analysis of humic acid on mica. They found that aggregation might be related to the hydrophobicity of the sample. No aggregates were observed for relatively hydrophilic humic acids (Suwanee river) at pH 3 to 10, but aggregates were seen for peat humic acid at low pH and high ionic strength. A comparison between AFM, Fluorescence Correlation Spectroscopy, Field-Flow Fractionation and Pulsed Field Gradient-NMR was carried out on a reference fulvic acid sample (Lead et al. 2000a). It consistently showed that AFM resulted in smaller particle sizes measurements compared to the other techniques even after considering AFM is a number average method while the others in the study were mass average methods. This

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underestimation of the size of the fulvic acid was thought to be due to drying or other substrate effects during the AFM procedure.

Although an AFM is operated under ambient conditions, samples still have to be applied to a specimen holder, which can cause alterations and the sample application has to be done carefully. A range of sample preparation techniques have been reported by Balnois and Wilkinson (2002). These include drop deposition, adsorption, ultracentrifugation and they have successfully been applied in the characterization of environmental biopolymers (e.g. humic substances, polysaccharides) by AFM (Balnois and Wilkinson 2002). Bickmore et al. (1999) developed methods (including electrostatic attraction and adhesion based) to fix clay minerals to a substrate to allow imaging in aqueous suspensions by AFM (Bickmore et al. 1999). Further information about the application of AFM to environmental colloids can be obtained from the review by Maurice (1996). He describes the AFM as powerful tool to image environmental colloids and surfaces in air or immersed in water at sub-nanometer-scale resolution with examples of applications and limitations (Maurice 1996). Very recently a review has also been published relating the application of AFM to nanotechnology in food science (Yang et al. 2007).

From the above, it is clear that using a combination of microscopic techniques we can not only visualize nanoparticles but also generate useful data on the size, size distribution and other measurable properties (Jose-Yacaman et al. 2001; Biberthaler et al. 2003; Rabinski and Thomas 2004; Chuklanov et al. 2006; Baatz et al. 2006). However, it needs to be recognized that the image analysis of the microscope outputs is

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as crucial as imaging itself. Only small amounts of samples can be analyzed by microscopic techniques and this has an impact on the statistical significance of the results. The average particle size is a number average and size distribution obtained by image analysis depends on the number of particles measured. Since there are often fewer larger particles it is important to count and measure enough particles to obtain good counting statistics on these size fractions. The same issues need to be considered when measuring ENPs in food or environmental samples in the presence of high concentrations of natural nanomaterials. It may therefore be necessary to measure millions or billions of particles to generate reliable data. Therefore it is essential to develop automation and image analysis procedures. Also the image contrast can have an influence on the visible size of the particles as well as light element particle coatings that can be invisible and therefore lead to controversial or incomparable results.

Chromatography and related techniques

Techniques based on or related to chromatography can be used for the separation of nanoparticles in samples. These techniques are mostly fast, sensitive (detector-dependent) and non-destructive, so that samples are available for further analysis. Although some chromatographic tools allow a range of solvents to be used, samples usually cannot be run in their original media, which can cause sample alteration and sample solvent interaction. By attaching traditional analytical tools (e.g. ICP-MS, DLS) as detectors to size separation techniques, it is not only possible to quantify different nanoparticles in food, water, biota and soil but also to characterise or elementally analyse them.

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The best known technique for size separation is size exclusion chromatography (SEC). A size exclusion column is packed with porous beads as the stationary phase. The pores of the column retain particles depending on their size and shape. This method has been applied to the size characterization of quantum dots, single walled carbon nanotubes and polystyrene nanoparticles (e.g. Krueger et al. 2005; Ziegler et al. 2005; Huang et al. 2005). Size exclusion chromatography has good separation efficiency. Major disadvantages of (size exclusion) chromatography are the possible interactions of the solute with the solid phase (Lead and Wilkinson 2006) and the limited size separation range of the columns, which may not allow covering the size range of both the primary nanoparticles and their aggregates. Methods employed to overcome the problem of solid phase interactions include the addition of capping agents to the mobile phase and the recycling of the analyte. SEC has been successfully combined with a range of detection techniques to not only monitor the size fractionation of the particles but also to characterize them. For example, Song et al. (2004) used voltammetric detection for gold nanoparticles separation and Helfrich et al. (2006) employed ICP-MS as multi-element detection method, whereas Porsch et al. (2005) worked with multi angle laser light scattering (MALLS) (Song et al. 2004; Porsch et al. 2005; Helfrich et al. 2006).

Unlike SEC, in Capillary electrophoresis (CE) there are no solid phase interactions. CE allows the separation of particles in different solution based on the charge and size distribution of the components. However, as separation is not only based on size, data interpretation is more complex. Also mobile phase interactions cannot be excluded. Lin et al. (2007) used CE for the sizing of engineered Au and Au/Ag nanoparticles and

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Schmitt-Kopplin & Junkers (2003) have used CE in the characterization of humic substances and other natural organic matter.

Hydrodynamic chromatography (HDC) separates particles based on their hydrodynamic radius. A HDC column is packed with non-porous beads building up flow channels, in which particles are separated by flow velocity and the velocity gradient across the particle. Therefore larger particles elute faster from the column than smaller ones (Mcgowan and Langhorst 1982). The non-porous beads considerably reduce the risk of solid phase interactions compared to the porous packaging in a SEC column. Available HDC columns show size separation ranges from 5 nm up to 1200 nm depending on the column length, whereas the size separation range of a SEC column is dominated by its pore size distribution. The wider particle size separation range of HDC allows a whole range of nanoparticles to be sized in different media and is particularly helpful in allowing a better understanding of formation of aggregates. HDC has been connected to the most common UV/Vis detector for the size characterization of (fluorescent) nanoparticles, colloidal suspensions and biomolecules (Williams et al. 2002; Chmela et al. 2002; Blom et al. 2003), but also to dynamic light scattering (DLS) to size separate lipid nanocapsules (Yegin and Lamprecht 2006). A major limitation of HDC is the poor peak resolution.

A highly promising technique for the size separation of ENPs in complex natural samples is field flow fractionation (FFF) techniques (Giddings 1993; Beckett and Hart 1993; Schimpf et al. 2000; Hassellöv et al. 2007). It is similar to chromatographic techniques, but separation is solely based on physical separation in an open channel

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without relying on a stationary phase. The particles are separated based on how they are affected by an applied field. The field controls the particle transport velocity by positioning them in different average laminar flow vectors in a thin channel. The field can be a centrifugal force (Sedimentation FFF) or a hydrodynamic flow perpendicular to the separation flow (Flow FFF). FFF is able to fractionate particles in a range of 1 nm - 1 µm in brownian mode.

FFF instruments can be coupled to online or offline detection and characterization, which in addition to size distributions allows analysis and visualisation of the fractionated samples by electron microscopy (Baalousha et al. 2005a). FFF can also be coupled to a range of sensitive and multi-element techniques such as multi angle laser light scattering (MALLS) and ICP-MS (Hassellov et al. 1999b; Kammer et al. 2005). FFF coupling techniques have been successfully applied in geochemistry and natural colloid research as well as studies into the behaviour of engineered nanoparticles. Applications range from colloids in fresh and marine water to size separation of soil suspensions (Ranville et al. 1999; Hassellov et al. 1999a; Hassellov et al. 1999b; Chen and Beckett 2001; Lyven et al. 2003; Siepmann et al. 2004; von der Kammer et al. 2004; von der Kammer et al. 2005; Stolpe et al. 2005; Kammer et al. 2005; Baalousha et al. 2005a; Graff and Frazier 2006; Lead and Wilkinson 2006; Gimbert et al. 2006; Peng et al. 2006; Baalousha et al. 2006a; Baalousha et al. 2006b; Baalousha and Lead 2007). Also single walled carbon nanotubes have been length separated by Dielectrophoresis FFF (Peng et al. 2006) and many engineered nanoparticles such as SiO_2, metals, metal oxides, carbon black etc (Schimpf et al. 2000).

The limitations of FFF techniques are membrane or accumulation wall interactions, the continuous re-equilibration in the channel (for trace constituent studies), and the need

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(in some circumstances) of pre-concentration, additional concentration of sample during equilibration and increasing possibility of aggregation in the channel (Beckett and Hart 1993; Hassellöv et al. 2007).

In theory any aqueous or non-aqueous phase of any ionic strength and a pH between 2 – 11 can be used as carrier. This gives versatility in terms of selecting carrier composition to favor colloidal stability, in order to minimize wall and membrane interactions and particle-particle interactions.

Stegeman et al. (1994) compared the resolving power and separation time in thermal field flow fractionation (TFFF), hydrodynamic chromatography, and size exclusion chromatography for the size separation of polymers and concluded that TFFF theoretically has the best separation potential because of the high selectivity, but this may not be able to be exploited in practice due to the technical requirements. On the other hand SEC was found to be the fastest method for low molecular masses (Stegeman et al. 1994). In general FFF and HDC has a wider dynamic size range than SEC, while SEC has higher separation efficiency (less peak broadening). SEC also suffers from more sample perturbations than FFF and HDC.

Centrifugation and filtration techniques

Centrifugation and filtration techniques are well-established tools for the preparative size fractionation of samples. These are low-cost, high speed and high volume techniques. Ultracentrifugation (UC) e.g. is a centrifuge system that is capable of very high spinning speeds for accelerations up to 1 000 000G. There are two different types of ultracentrifugation: analytical and preparative UC. In an analytical ultracentrifuge

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(ANUC) a sample can be monitored in real time through an optical detection system using ultraviolet light absorption and/or interference optical refractive index sensitive systems. This allows the operator to observe the evolution of the sample concentration versus the axis of rotation profile as a result of the applied centrifugal field. This is for sedimentation velocity and sedimentation equilibrium experiments (gross shape of macromolecules, conformational changes in macromolecules and size distribution). Preparative ultracentrifugation has been used for pelleting of fine particulate fractions, for gradient separations (Bootz et al. 2004), and for harvesting aquatic colloids and nanoparticles on TEM and AFM substrates (Mavrocordatos et al. 2007; Balnois et al. 2007).

Traditional membrane filtration allows the fractionation of particle sizes between 0.2 – 1 µm (Lead and Wilkinson 2006). Comparative data obtained for soil suspensions, for filtration and SdFFF indicates that membrane filtration can both over and underestimate smaller size fractions due to clogging as well as electrostatic interactions (Gimbert et al. 2005). Microfiltration with pore sizes > 0.1 µm is a simple and common method, although exhibiting many artifacts caused by e.g. filter cake formation and concentration polarization (Morrison and Benoit 2001). Ultrafiltration is applicable for large sample volumes, however, with decreasing pore sizes, common filtration artifacts are even more likely. For the separation of nanoparticles and ions nanofiltration with pore sizes of 0.5 or 1 nm can be used.

Cross flow filtration (CFF) or tangential filtration recirculates the samples and therefore reduces clogging, concentration polarization and other artifacts caused by traditional

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dead end filtration (Lead and Wilkinson 2006). It has become the standard method for separating colloids and particles. Its use has been evaluated against AFM by Liu & Lead (2006). The method has been applied to fluorescence investigations of colloidal organic matter and dissolved organic matter in lake and river water (Liu et al. 2007) as well as in seawater (Guo et al. 2000). Electrically assisted cross flow filtration has also been used for the separation of nanoparticles (Sung et al. 2007). Doucet et al. (2004) evaluated cross flow ultrafiltration (CFUF) for the size fractionation of freshwater colloids and particles (1 nm – 1 µm) by AFM and SEM and concluded that CFUF is not fully quantitative and separation is not always based on size alone. Amounts of large colloids might be overestimated and fractionation is not always consistent with the nominal pore size of the membranes. These conclusions have to be treated with some caution as the validation techniques used (i.e. AFM and SEM) have their limitations (Doucet et al. 2004).

Spectroscopic & related techniques

A wide range of spectroscopic methods is available for nanoparticle analysis and characterization. Scattering techniques that are useful for nanoparticle characterization include light scattering techniques like static (SLS) and dynamic light scattering (DLS) as well as neutron scattering such as small angle neutron scattering (SANS).

DLS or photon correlation spectroscopy (PCS) is particularly useful for sizing nanoparticles and determining their state of aggregation in suspensions. DLS provides fast *in situ* and real time sizing (Ledin et al. 1994), but also has considerable limitations.

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For example, interferences can be caused by a range of possible artifact sources such as dust particles, which will have a great influence on the scattering intensity compared to smaller particles and therefore on the sizing result. Also data obtained from samples containing particles with heterogeneous size distributions is difficult to interpret. DLS is solely quantitative and unless the sample content is known or pure, size fractions cannot be related to particles of a specific composition. (e.g. Bootz et al. 2004).

Static light scattering also known as multi angle (laser) light scattering (MAL(L)S gives information of particle structure and in combination with dynamic light scattering or FFF particle shape can be determined.

SANS can be used on solid or liquid samples. For example Diallo et al. (2005) have applied SANS for the characterization of Suwannee River fulvic acid aggregates in aqueous solutions (Diallo et al. 2005).

Small angle X-ray scattering (SAXS) is an analytical X-ray application technique to investigate the structural characterization of solid and fluid materials in the nanometer range. Monodisperse and polydisperse systems can be studied. In monodisperse systems size, shape and structure determination is possible whereas in polydisperse systems only the size distribution can be calculated.

Laser-induced breakdown detection (LIBD) is a laser based technique featuring extremely low detection limits, which is able to analyze the size and concentration of colloids depending on the measured breakdown probability (BP). LIBD is therefore a highly promising tool for nanoparticle characterization, although it cannot distinguish

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between different types of particles and is in need of particle specific size calibration (Bundschuh et al. 2001a; Bundschuh et al. 2001b).

Other laser-based techniques include Raman spectroscopy and laser-induced fluorescence (LIF). Instruments are now available combining these techniques, allowing the atomic, molecular and structural characterization of a specimen as well as a better understanding of physical properties.

UV/Vis and infrared spectroscopy offer the possibility to characterise nanoparticles, especially quantum dots and organic based nanoaprticles like fullerenes and carbon nanotubes. Fourier transformation infrared (FTIR) and UV/Vis spectroscopy have been used to compare aqueous colloidal suspensions of C_{60} (Andrievsky et al. 2002). Pesika et al. (2003) also used UV spectroscopy to study the relationship between absorbance spectra and particle size distributions for quantum-sized nanocrystals.

Nuclear magnetic resonance (NMR) is a powerful technique providing information on the dynamics and three-dimensional structure of a solid compound or a suspension. Carter et al. (2005) characterized air and water stable silica nanoparticles by NMR and Valentini et al. (2004) used diffusion NMR spectroscopy for the characterization of the size and interactions of colloidal matter (Valentini et al. 2004; Carter et al. 2005). Lead et al (2000) used pulsed field gradient NMR to measure the diffusion coefficients of fulvic acids (Lead et al. 2000a).

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X-ray spectroscopy comprises i.e. X-ray photoelectron (XPS), X-ray fluorescence (XRF) as well as X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD). XPS is highly surface specific due to the short range of the photoelectrons that are excited from the solid sample and therefore XPS could be useful to characterize nanoparticle surfaces and coatings respectively. X-ray diffraction is non-destructive and can reveal information about the crystallographic structure, elemental composition of natural and manufactured materials. Nurmi et al. (2005) used this technique as well as XPS for the characterization of zero-valent Fe nanoparticles for use in remediation (Nurmi et al. 2005). X-ray fluorescence (XRF) spectroscopy is also non-destructive and can be used to identify and determine the concentrations of elements present in solid, powdered and liquid samples. XRF can be subdivided into wavelength separation (WDXRF) and energy dispersive XRF (EDXRF).

X-ray absorption (XAS) and emission spectroscopy is used in chemistry and material sciences to determine elemental composition and chemical bonding.

Other potentially suitable spectroscopic techniques for nanoaprticle characterisation include electron paramagnetic resonance (EPR), Moessbauer, Auger electron (AES) and 3D fluorescence excitation-emission matrix spectroscopy (EEM). Mössbauer spectroscopy provides information about chemical, physical and magnetic properties by analyzing the resonant absorption of characteristic energy gamma-rays known as the Mössbauer effect. Liu et al. (2007) and Lead et al. (2006) applied 3D fluorescence excitation-emission matrix (EEM) spectrophotometry for the fluorescence investigation of colloidal organic matter and dissolved organic matter in lake and river water (Lead et al. 2006; Liu et al. 2007). Electron paramagnetic resonance spectroscopy can be applied

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for particle surface reactivity analysis. EPR is a sensitive, specific method for studying organic and inorganic radicals formed in chemical reactions and the reactions themselves similar to NMR. Auger electron spectroscopy is also commonly used in the surface characterization of nanostructures. Quantitative bulk analysis by AES is described i.e. by Powell & Seah (1980).

Mass spectrometry

Mass spectrometers consist of an ion source, a mass analyzer, and a detector system. Two ionization techniques often used with liquid and solid biological samples include electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). Inductively coupled plasma (ICP) sources are mainly used for metal analysis. Mass analyzers (e.g. ion trap, quadrupole or time-of-flight) cover different mass to charge ranges, differ in the mass accuracy, and the achievable resolution. Most of the available analyzers are compatible with electrospray ionization, whereas MALDI is not usually coupled to a quadrupole analyzer.

Mass spectrometry (MS) approaches such as MALDI, laser induced fluorescence (LIF), ion trap (IT) mass spectrometry have been applied for the analysis of fluorescently labeled nanoparticles (Peng et al. 2003; Cai et al. 2003).

In the case of ICP-MS, samples cannot only be injected directly into the ion source but also via combined techniques like HPLC. An increasingly popular combination in this respect is FFF-ICP-MS, which allows the size separation of the sample with quantitative and elemental analysis of the obtained size fractions. This development is highly promising for nanoparticle analysis as particles can be simultaneously sized and

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analyzed in their original environment (Ranville et al. 1999; Hassellov et al. 1999a; Hassellov et al. 1999b; Lyven et al. 2003; von der Kammer et al. 2004; Bolea et al. 2006; Baalousha et al. 2006a).

Whereas conventional mass spectrometry (MS) is applicable for identifying unknown compounds and their mass concentrations as well as their isotopic composition, single particle mass spectrometry (SPMS) has also the ability to size single particles. MS techniques have also been used in aerosol characterization, including aerosol time-of-flight mass spectrometer (ATOF-MS). An ATOF-MS consists of an aerosol introduction interface; a light scattering region for sizing and a TOF-MS. Suess and Prather (1999) published a review on the topic of mass spectrometry of aerosols. They describe tools for offline MS of aerosols like LAMMS, SIMS and ICP-MS, tools for online MS like surface/thermal ionization MS (SIMP, DIMS, CAART, PAMS) and laser desorption/ionization MS (ATOFMS, PALMS, RSMS, LAMPAS). More applied examples are described by Janzen et al. (2002) who compared the sizing of nanoparticles with SPMS and TEM (Janzen et al. 2002). Lee et al. (2005) used SPMS to characterize the size and composition of polydisperse aerosol nanoparticles (Lee et al. 2005). They estimated the particle size by laser ablation/ionization time-of-flight single-particle mass spectrometer and validated their results by differential mobility analysis (DMA). In situ characterization of size and elemental composition of individual aerosol particles in real time was performed by Prather et al. (1994) with the help of an ATOF-MS (Prather et al. 1994). For the sizing and analysis of aerosol nanoparticles a DMA has also been coupled to an ICP-MS (Okada et al. 2002).

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Other techniques

Particle counters for number concentrations. The electrical sensing zone method counts and sizes particles by detecting changes in electrical conductance as particles suspended in a weak electrolyte solution are drawn through a small aperture. The technique has been successfully applied to the size and surface charge characterization of nanoparticles using a carbon nanotube-based coulter counter (Ito et al. 2003). Condensation particle counter (CPC) measurements can also provide data on the number and concentration of individual particles by growing the particles through a condensing process using various operating liquids like alcohol and water.

DMA for sizing aerosols. A differential mobility analyzer (DMA) can be used to determine the size distribution of sub-micrometer aerosol particles. Particles are firstly charged and then their electrical mobility is measured as a function of their charge and size. After sizing the particles are still suspended in air and are ready for further analysis (McMurry et al. 1996; Weber et al. 1996; Okada et al. 2002).

SMPS for sizing and number concentration determination. A scanning mobility particle sizer (SMPS) consists of a DMA and a CPC. First particles are separated by their electrical mobility in the DMA. Then the size fractionations enter a CPC which determines the particle concentration at that size.

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BET method for surface area determination. The very common Brunauer Emmett Teller (BET) method enables the determination of the specific surface area of solids and therefore also nanoparticles by gas adsorption (Brunauer et al. 1938).

Thermogravimetry and differential thermo analysis (TG-DTA). DTA can be applied for phase changes and other thermal processes like the determination of the melting point. In combination TG-DTA is useful for investigating the thermal stability and decomposition, dehydration, oxidation as well as the determination of volatile content and other compositional analysis. Thermogravimetry in combination with a mass spectrometer can be used for surface analysis. Surface molecules are removed by heating and afterwards analysed by MS.

Electrophoretic mobility and the zeta potential. Electrophoresis is used for studying properties of dispersed particles in particular for measuring the zeta potential. The zeta potential is a measure of the overall charge a particle acquires in a specific medium and gives an indication of the potential stability of a colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other which leads to higher stability than if the particle charge is near neutral. The zeta potential is a measure of the net charge and there may be significant charge heterogeneities that can still lead to aggregation even though the net zeta potential is suggesting otherwise. Information about the aggregation state of a nanoparticle dispersion is highly valuable for nanoparticle fate and behavior studies. As an example the electrophoretic mobility of silica spheres dispersions suspended in water at different concentrations and salinities has been studied by Reiber et al. (2007) (Reiber et al. 2007).

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Nanomaterial analysis in food and biological samples

As previously discussed when measuring nanoparticles in different media, it will not just be necessary to generate data on concentrations but also it is likely that information will be required on the size distribution and properties of the particles. No one technique can provide all this information so a range of analytical techniques will be required. Moreover, whilst a range of methods have been shown to be applicable to analysis of nanoparticles, it is likely that the current methods do not fulfill all the data requirements.

As shown in the previous section many analytical tools are theoretically suitable for the characterization of nanoparticles ranging from electron microscopy to dynamic light scattering to flow field fractionation techniques but only a few of these are applicable to the analysis of more complex samples. The requirement for analysis of engineered nanoparticles in natural and food related samples will differ quite strongly from their analysis in pure or neutral media (e.g. air, distilled water). In complex media it will be essential to analyze samples of diverse elemental compositions and samples containing more than one type of nanoparticle. Many techniques are destructive or if not, application of some sample preparation methods can lead to artifacts. In addition natural samples will be hetero-dispersed and for measuring size distributions instruments providing a wide size separation range from ideally 1 nm to up to several µm are needed. There are many methods available for the sizing of particles, but very few if any of them is applicable to the entire size range. In the next section some of these challenges are discussed in more detail.

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Bulk vs single particle analysis

An issue with some of the methods (discussed in the previous chapter) is their application range. Existing techniques have to be divided between tools suitable for analysing individual particles (depending on the particle size) or the bulk material. Classic composition and mass based tools are readily applicable for the bulk material, however elemental analysis of single particles in a dilute environment has only recently become available (e.g. aerosol mass spectrometry). Whereas standard tools for elemental composition and mass concentration are limited by their limit of detection (LOD), techniques able to characterize individual particles face spatial limitations. Especially particle sizing techniques are restricted by their size separation range. Figure 2 illustrates the size range of selected methods for particle sizing.

Figure 2. Sizing methods and their size range for nanoparticle measurement. Adapted from (Lead and Wilkinson 2006) and (Gimbert et al. 2007b).

Sizing artefacts and the lack of reference materials

The limitations of each analytical method for nanoparticle characterization can lead to confusing inconsistent results and therefore to inaccurate predictions of material properties and structure (Carter et al. 2005). For example it is still almost impossible to determine the absolute size of particles. Correct size measurements are difficult, which often lead to artifacts depending on the applied tool and the medium the particles are

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analyzed in. For example organic coatings that are not visible in the electron microscope (due to light elements like carbon) can lead to errors in sizing, especially when compared to sizing tools that measure the hydrodynamic radius of particles like FFF or DLS. It has been reported that the average size and size distribution of nanoparticles can significantly vary when comparing results from different techniques such as electron microscopy, dynamic light scattering, CFF and ultracentrifugation (Bootz et al. 2004). The lack of consistent reference materials and standards further exacerbates this problem (Lead and Wilkinson 2006). Nanoparticle sizing standards as well as standardized methods for sampling and measurement are therefore urgently required in order to overcome the problem of inconsistent data (Borm et al. 2006). To our knowledge standardized nanoparticles are not yet available and researchers have to rely on commercially available, often not well-characterized nanoparticles.

Sample preparation

Depending on the technique, to analyse natural samples, sample preparation and/or digestion is often required. As nanoparticles can and do change structure and composition in response to their environment, results obtained for pre-treated or digested samples can often be very different from if the particles were characterised *in situ* (Burleson et al. 2004). These artefacts in analysis can be avoided by using techniques that either do not require or which reduce sample preparation to a minimum. The complexity data obtained for some techniques (e.g. NMR, CE) for samples in their original state can make the analysis and interpretation of data rather difficult.

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If sample preparation cannot be avoided, a careful record of sampling and preparation steps is essential to track artifacts. The nature of nanoparticles can also change over time, for example aggregation can increase or decrease and particles could dissolve. A lot of effort has been put into the development of sample preparation methods that improve the conservation of the original state of the sample. Especially in the microscopy area, achievements have been made in sample preparation ranging from gel trapping techniques for imaging emulsions under the SEM (Paunov et al. 2007) to high pressure freezing and freeze drying for imaging biological specimen under the TEM (Lonsdale et al. 1999; Bootz et al. 2004). Fixation methods for imaging clay minerals and particles in aqueous solutions under the AFM have also been developed (Bickmore et al. 1999).

Natural vs. engineered nanoparticles

At the moment it is very difficult to distinguish between particles of engineered origin and particles of a natural or other sources (Burleson et al. 2004). A way has to be found to differentiate between natural occurring and engineered nanoparticles. This will allow the concentrations of engineered nanoparticles in consumer products and the environment to be determined, as it is currently not known how many engineered nanoparticles will actually reach the environment or be bioavailable. Therefore selective detection methods need to be developed. Another solution to this problem could be nanomaterial labeling. Suggestions range from fluorescent and radioactive labeling for carbon based nanoparticles, to isotopic enrichment or depletion of metal-based nanoparticles. Also special particle coatings or entrapment of rare elements in nanotubes

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or fullerenes could be used to enable the detection of these distinctive chemical characteristics after an experimental study. Gulson and Wong (2006) published a paper on the possibilities of isotopic labeling and tracking of metal and metal oxide nanoparticles for nanotechnology research (Gulson and Wong 2006). Isotopic labelling of carbon nanotubes and fullerenes has already been performed. For example ^{13}C isotope carbon nanotubes are available and ^{14}C C_{60}s have been synthesized with subsequent uptake and toxicity studies (Scrivens et al. 1994b; BullardDillard et al. 1996).

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Conclusions & recommendations for future work

Analytical methods are required to reliably detect and characterise nanoparticles and their properties in the media in which humans and ecosystems are exposed to them. This includes air, soil and water as well as food and consumer products. These methods have to be also applicable for nanoparticle characterisation in toxicological and ecotoxicological testing. Only then can an appropriate risk assessment for nanoparticles be performed and the properties that are truly of risk can be identified and regulated or used in standard tests respectively (SCENIHR 2005).

These techniques have to a) be able to deal with heterogeneous samples b) to minimize sample alteration to avoid artefacts and c) provide as much information as possible because most characterization techniques are destructive and therefore samples often cannot be analyzed twice or by more than one technique. An ideal analytical instrument would allow simultaneous determination of all physico-chemical properties of a nanoparticle and obtain them by real-time sampling, as many of these nanoparticles are transient in nature (Prather et al. 1994). Whilst a wide range of tools is available, the existing tools do not fulfil all desirable criteria and they all have their limitations when considering their application for food and natural samples. Therefore, until new tools have been developed, existing tools have to be used and combined in such a way that the data obtained can be validated. Analysis of the unperturbed sample or further analysis of the size fractionations is preferred. Complementary analytical tools should be applied and care be taken with sample preparation.

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This review demonstrated that promising developments have been made in nanoparticle analysis; however, further developments are essential to overcome the deficiencies in this area. Especially *in situ* analysis as well as routine and reliable techniques to improve size determination, size distribution of particles and other nanoparticle properties are of great importance.

Nanotoxicology and nanoecotoxicology are still in their fledgling stages and risk assessments are practically non-existent especially in the food sector. Therefore progress in nanoparticle testing (in vivo and in vitro) is urgently needed to secure consumer safety including the development of standard testing materials and testing guidelines. In addition to toxicity studies, different uptake paths have to be studied including dermal, oral and intestinal as well as nanoparticle accumulation and long-term effects. Other effects of nanoparticle uptake could be the interaction with other (toxic) substances and their mobilisation or dislocation etc not only in the human body but also already in the consumer product. The environmental fate and behaviour of nanoparticles as well as their bioavailability is widely unknown and therefore also their potential impact on the food web and their persistence. Also their effect on other substances has to be examined e.g. whether contaminant transport in the environment could be facilitated through adsorption to nanoparticles, whether nanoparticles enhance contaminant uptake or have a negative impact on bacteria useful for natural remediation etc. Further, data on environmental and exposure concentrations are not available. To increase the current knowledge about nanoparticle and related issues developments in these mentioned analytical fields will be crucial.

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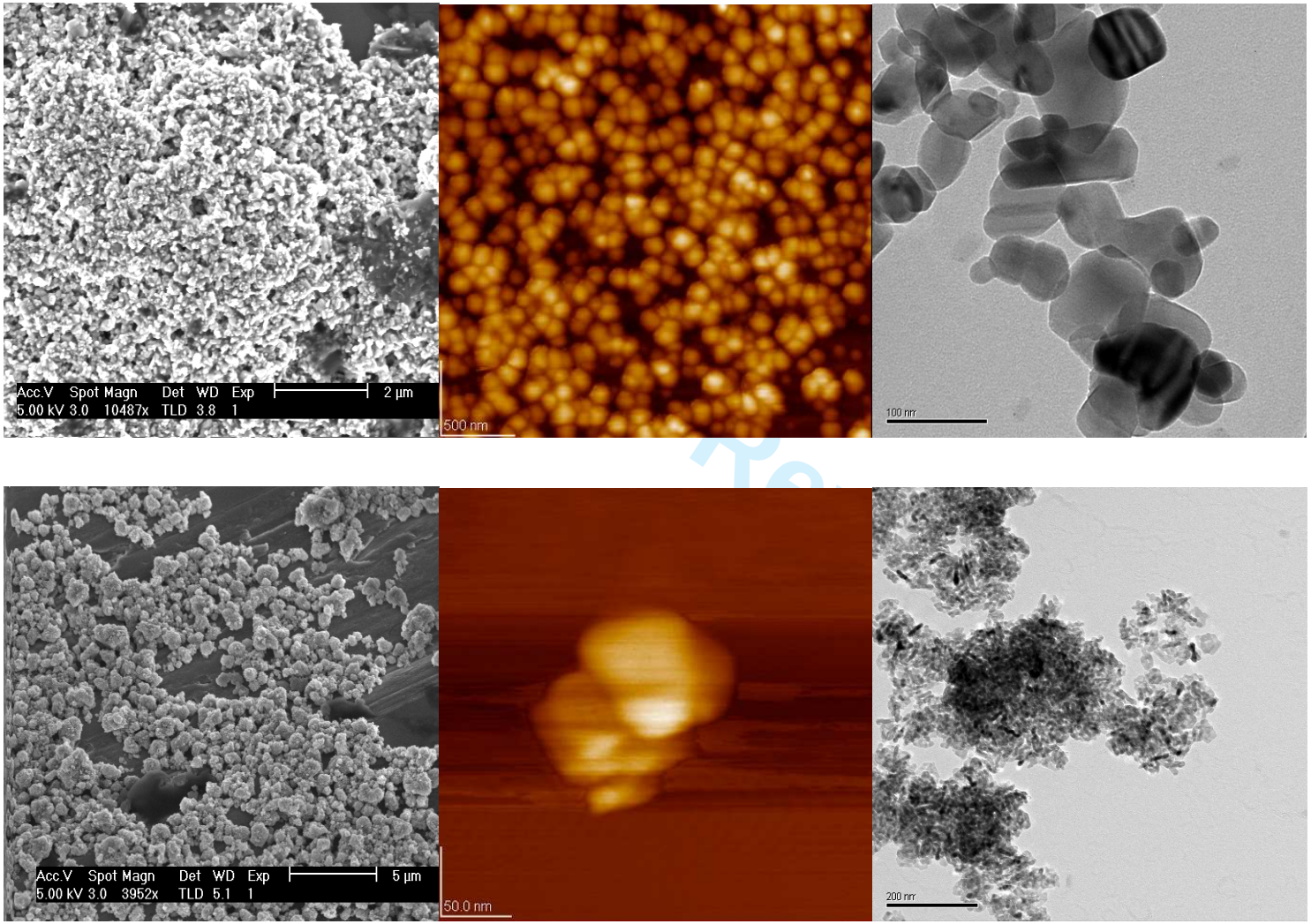
Acc.V Spot Magn Det WD Exp 2 µm
5.00 kV 3.0 10487x TLD 3.8 1
500 nm
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Acc.V Spot Magn Det WD Exp 5 µm
5.00 kV 3.0 3952x TLD 5.1 1
50.0 nm
200 nm

Figure 1. ZnO (1st row) and TiO_2 (2nd row) nanoparticles suspended in distilled water, allowed to dry and imaged in order from left to right by SEM, AFM and TEM. Initial sizes as stated by the manufacturer (Sigma Aldrich, UK): 50 – 70 nm for ZnO particles and 5 – 10 nm for TiO_2 particles.

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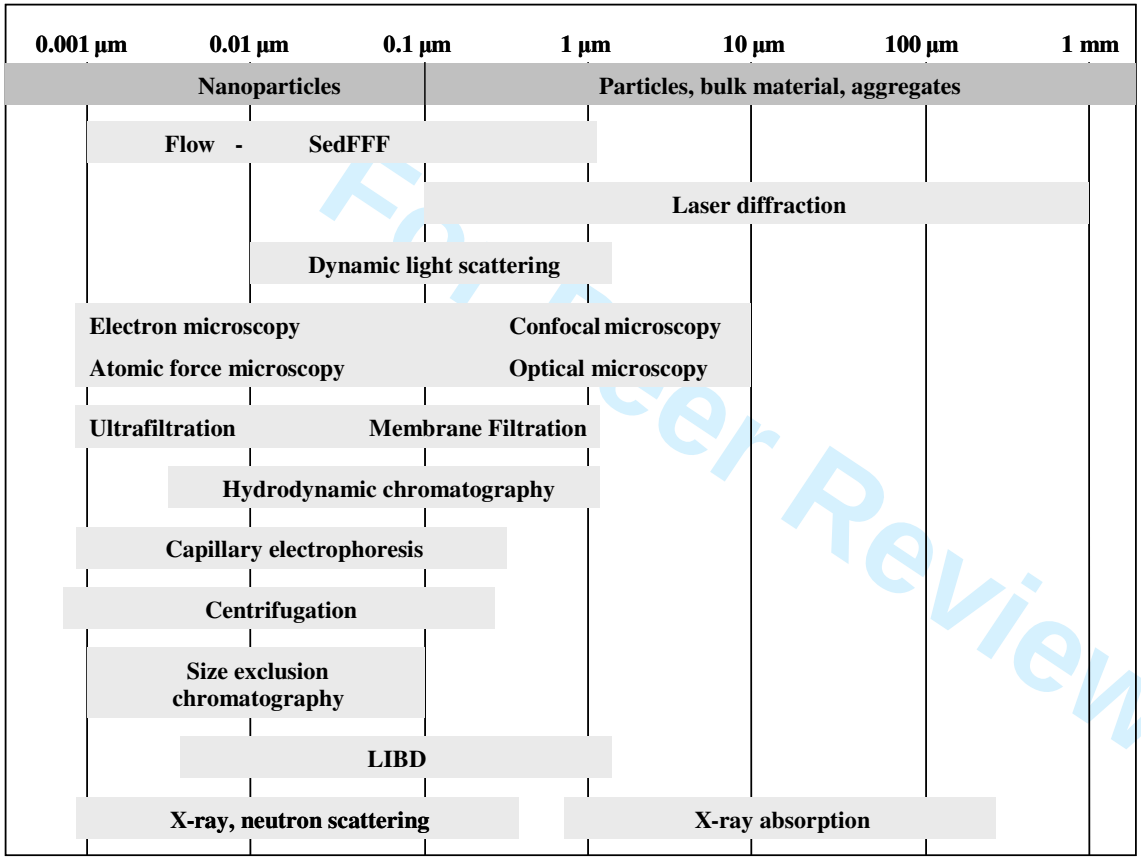

Figure 2. Sizing methods and their size range for nanoparticle measurement. Adapted from Lead & Wilkinson (2006) and Gimbert et al. (2007b).

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Table 1. Examples for applications of nanomaterials in consumer products.

Application	Nanotype	Reference
Imperm® food & beverage packaging by Nanocor®	Nanoclay composite	Chaudhry et al. 2007
Novasol® food supplement by Aquanova®	Soy isoflavones	Chaudhry et al. 2007
Nanotea® nano delivery system by Become Industry & Trade Co. Ltd.	Selenium	Chaudhry et al. 2007
Boots® Soltan® facial sun defense cream – containing Optisol® by Oxonica® Ltd	Manganese-doped TiO_2	Corporate watch 2007 (Internet)
Leorex® skin care cosmetics by GlobalMed®	Silica	Corporate watch 2007 (Internet)
Fullerene C_{60} day & night cream by Zelens®	Fullerene C_{60}	Corporate watch 2007 (Internet)
Envirox™ fuel borne catalyst by Oxonica® Ltd	Cerium oxide	Corporate watch 2007 (Internet)
Acticoat® wound dressings by Smith & Nephew	Silver	Corporate watch 2007 (Internet)
NanoCluster™ delivery system for food products by RBC Life Sciences Inc.®/USA	Nanopowder of unknown composition	Chaudhry et al. 2007
Aegis® OX oxygen scavenging barrier resin for PET bottles by Honeywell	Polymerized nanocomposite	Chaudhry et al. 2007
Various clothing lines by Brooks Brothers, manufacturer Nanotex	Nano fibre	Corporate watch 2007 (Internet)
Various washing machines by Samsung, manufacturer Nanogist	Silver	Corporate watch 2007 (Internet)
Various refrigerators by Daewoo, manufacturer Nanogist	Silver	Corporate watch 2007 (Internet)

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Table 2. Examples for nanoparticle (eco-) toxicity and other effects.

Toxicity study	Nanotype	Reference
In vitro cytotoxicity of oxide nanoparticles	SiO_2, Fe_2O_3, TiO_2, ZnO, $Ca_3(PO_4)_2$, CeO_2, ZrO_2	Brunner et al. 2006
Tissue sites of uptake of ^{14}C-labeled C_{60}	C_{60}	BullardDillard et al. 1996
Cytotoxicity of quantum dots	Quantum dots	Shiohara et al. 2004, Hardman 2006
Transport of surface-modified nanoparticles through cell monolayers	Amino-CLIO	Koch et al. 2005
Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles	SiO_2	Chen & von Mikecz 2005
Manufactured nanomaterials (Fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass	C_{60}	Oberdorster 2004
Daphnia magna mortality when exposed to titanium dioxide and fullerene (C_{60}) nanoparticles	C_{60}, TiO_2	Lovern & Klaper 2006
Phytotoxicity of alumina nanoparticles	Alumina	Yang & Watts 2005
Silver nanoparticles as antimicrobial agent	Silver	Sondi & Salopek-Sondi 2004
Antibacterial activity of fullerene water suspensions	C_{60}	Lyon et al. 2006

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Table 3. Properties likely to influence nanoparticle behaviour and toxicology.

Property	Importance of measurement
Aggregation state	Nanoparticles that have a tendency to aggregate and are bigger than 100 nm in their aggregated state are not classed as nanoparticles
Elemental composition	Different particle composition leads to different behaviour/impact, e.g. Cd vs Fe
Mass concentration	Normally increased contaminant concentration leads to increase in toxicity/impact, this is not always applicable for nanoparticles
Particle number concentration	Nanoparticles have low mass concentrations, but show high percentage of total particle numbers
Shape	Different particle shapes (e.g. spherical, tubular) can posses different affinities or accessibilities e.g. transport through membranes into cells, different antibacterial behaviour
Size & size distribution	Nanoparticles are defined and classed by their size and size is one of the primary properties describing transport behaviour
Solubility	Soluble nanoparticles; once dissolved cannot be classed as nanoparticles (e.g. ZnO vs Zn^{2+})
Speciation	Different species can have different behaviour, toxicity, impact (e.g. C_{60} vs C_{70}, ENP complexes with natural organic matter or oxidation state)
Structure	The structure can have an influence on stability or behaviour (e.g. rutile or anastase as possible crystal

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	structures of TiO_2)
Surface area (& porosity)	Increase in surface area increases reactivity and sorption behaviour
Surface charge	Surface charge has an influence on particle stability especially in dispersions
Surface chemistry	Coatings can consist of different chemical compositions and influence particle behaviour or toxicity (e.g. Quantum dots with CdSe core and ZnS shell)

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Table 4. Nanoparticle properties (see table 3) and examples of analytical methods potentially suitable for their measurement.

Nanoparticle properties	Microscopy and related techniques	Chromatography and related techniques	Centrifugation and filtration techniques	Spectroscopic and related techniques	Other techniques
Aggregation	e.g. STEM, TEM, SEM, AFM. STM		e.g. ANUC	e.g. XRD, SANS	e.g. Zeta potential
Chemical composition	AEM, CFM			e.g. NMR, XPS, Auger, AES, AAS, MS, XRD, EBSD	
Mass concentration	AEM, CFM	√		√	e.g. Gravimetry, thermal analysis
Particle number concentration					e.g. Particle counter, CPC
Shape	e.g. STEM, TEM, SEM, AFM. STM	e.g. FlFFF-SLS, SedFFF-DLS	e.g. UC		
Size	e.g. STEM, TEM, SEM, AFM, STM	√			e.g. DMA
Size distribution	e.g. STEM, TEM, SEM, AFM, STM	e.g. FFF, HDC, SEC	e.g. CFF, UC, CFUF	e.g. SPMS, SAXS	e.g. UCPC, SMPS
Dissolution			Dialysis, CFUF		Voltammetry, diffusive

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				gradients in thin films
Speciation		e.g. SEC-ICP-MS	e.g. XAFS, XRD	e.g. Titration
Structure	e.g. STEM, TEM, SEM, AFM, STM		e.g. XRD, SANS	
Surface area (& porosity)				e.g. BET
Surface charge		e.g. CE		e.g. Zeta potential
Surface chemistry	AEM, CFM		e.g. XPS, Auger, SERS	

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Table 5. Overview of discussed analytical methods suitable for nanoparticle characterization in alphabetical order with literature examples for their application in complex media.

Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
3D fluorescence excitation-emission matrix	EEM	ppb		Complex data interpretation	Probing chemical structure / functional groups		Fluorescent characteristics of colloidal organic matter filtrates	Liu et al. 2007
Aerosol time of flight mass spectrometry	ATOFMS	3 nm - µm particle size	Analysis of individual particles Real time measurement	Not fully quantitative	Sizing Elemental composition		Single particle analysis Aerosols	Prather et al. 1994 Suess and Prather 1999 Angelino et al. 2001

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Analytical Electron Microscopy (EDX&EELS)	AEM	~ 0.5 nm	e.g. EELS also applicable for light elements (<Zn)	e.g. EDX only applicable for heavier elements	Elemental composition (Semi-) quantitative analysis	TEM SEM STEM	Combination of electron microscopy with AEM techniques like EELS and EDS	Mavrocordatos and Perret 1998 Leppard et al. 2004 Luther W 2004 Gilbert et al. 2004

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Atomic force microscopy	AFM	~ 0.1 nm	Dry, moist or liquid samples, ambient environment 3D surface profiles, sub nanometer topography resolution	Overestimations of lateral dimensions, artefacts due to movement of particles (smearing) and particles adhering to the tip	Sizing Electrical and mechanical properties Visualization		Force measurement between sample and tip CFM = chemical force microscopy, Quantum electronic mapping: STM=scanning tunnelling microscopy	Lead et al. 2005 Friedbacher et al. 1995 Maurice 1996 Bickmore et al. 1999 Balnois et al. 1999 Balnois and Wilkinson 2002 Yang et al. 2007 Wigginton et al. 2007
Auger Electron Spectroscopy	AES	~ 1 – 2 nm			Surface composition Surface topography Oxidation state	SEM	Extremely surface sensitive technique	Powell CJ 1980 Liu 2005

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Brunauer Emmett Teller	BET	Thousands of m^2/g			Total surface area Porosity			Brunauer et al. 1938 Nurmi et al. 2005
Capillary electrophoresis	CE		Sensitive, fast, & separation by charge	Mobile phase interactions, complex data interpretation, need of standard material	Electrophoretic mobility Sizing Separation of ionic species by charge and frictional forces	UV/Vis Fluo MS		Schmitt-Kopplin and Junkers 2003 Chan et al. 2007 Lin et al. 2007

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Centrifugation		For a given density and spherical particles: what is the size ranges for a certain number of g	Low surface effects	Aggregation can be induced by differential settling velocity (heavier, larger particles bump into slower settling velocities)	Settling rates, buoyant mass, for known density: equivalent spherical volume, size separation		e.g. differential centrifugation	Lead et al. 1999 Novak et al. 2001 Bootz et al. 2004 Lyon et al. 2006
Condensation particle counter	CPC				Number concentration	DMA		Luther W 2004 Flagan and Ginley 2006

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Cross flow ultrafiltration	CFUF	1 nm – 1 µm	Higher speed, higher volume, less concentration polarisation and clogging than piston filtration or stirred cells	Potential alterations, due to increased particle concentrations, turbulent flows, extensive surface exposure Not well defined size fractionation	Separation based on size & surface charge			Guo et al. 2000 Doucet et al. 2004 Doucet et al. 2005b Liu and Lead 2006 Sung et al. 2007
Cryo transmission electron microscopy	Cryo-TEM		Imaging of liquid & biological specimen	Sample alteration	Sizing Visualization	EDS	Special sample holder needed	Guo et al. 2000 Tang et al. 2004

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Differential mobility analyzer	DMA	3 nm - µm particles	In combination with a wide range of techniques	For water necessary to form an aerosol that is dried in which can cause sample changes	Sizing	ES CPC ICP-OES ICP-MS ATOF-MS	Also as tandem differential mobility analyzer (TDMA)	McMurry et al. 1996 Weber et al. 1996 Cass et al. 2000 Seol et al. 2001 Okada et al. 2002 Luther W 2004 Flagan and Ginley 2006 Naono et al. 2006

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Dynamic light scattering (photon correlation spectroscopy or quasi elastic light scattering)	DLS (PCS, QELS)	3 nm - µm particles	In situ measurement Rapid and simple analysis, useful to follow aggregation processes,	Difficult to interpret results based on intensity weighted sizes. Aggregates dust particles can ruin the measurements on nanoparticles Multiple scattering and particle interactions in high concentrations, limited capability on polydisperse samples.	Intensity weighted diffusion coefficient. can be calculated to a z-average hydrodynamic diameter or distribution			Huve et al. 1994 Bootz et al. 2004 Lecoanet et al. 2004 Lecoanet and Wiesner 2004 Brant et al. 2005a Phenrat et al. 2007 Viguie et al. 2007

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Electrophoretic mobility	EM	>3nm	Minimum perturbing, rapid and simple measurement	Interpretation of the zeta potential in relation to surface potential	Net Zeta potential (potentialat a slipping plane in the electric double layer of the particle)	DLS	Dependence of electrolyte solution	Ryan et al. 2000 Lecoanet et al. 2004 Brant et al. 2005b Chen and Elimelech 2007 Reiber et al. 2007
Electro-zone sensing					Sizing Number concentration Surface charge			Ito et al. 2003

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Environmental scanning electron microscope	ESEM	30-50 nm	No sample preparation No charging effects Variable temperature & pressure Imaging of hydrated samples	Loss in resolution Contrasting Atmospheric pressure & imaging under fully wet conditions not possible	Sizing Elemental composition Visualization	EDS	Semi-in situ measurements	Bogner et al. 2005 Redwood et al. 2005 Doucet et al. 2005a De Momi and Lead 2006

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Field flow fractionation	FFF	Flow FFF 1 nm – 1 µm Sed FFF: 50nm-1µm	Size range, mild fractionation, direct relation between retention time and size, versatility in carrier composition	Optimization of carrier composition demands experience, membrane interactions, dilution, concentration gradients	Size distributions (Flow FFF: diffusion coefficient and hydrodynamic diameter, Sed FFF: buoyant mass and equivalent spherical diameter) Size separation	On-line: UV/Vis DRI MALLS ICP-MS FLD LIBS Off-line: TEM-EDS AFM		Beckett and Hart 1993 Schimpf et al. 2000 Hassellöv et al. 2007 von der Kammer et al. 2004 Rameshwar et al. 2006 Lyven et al. 1997 Hassellov et al. 1999 Siripinyanond et al. 2002 Lyven et al. 2003 Gimbert et al. 2003 Stolpe et al. 2005 Baalousha et al. 2005a Gimbert et al. 2006 Baalousha et al. 2006 Baalousha and Lead 2007 (Gimbert et al. 2007

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Filtration			Fast Low cost	Clogging	Size separation			Kang and Shah 1997 Lau et al. 2004 Marani et al. 2004 Hett A 2004
Fluorescence correlation spectroscopy (Confocal microscopy)	FCS	~ 200 nm	Dilute samples in small volumes No multiple scattering	Only fluorescent samples	Diffusion coefficient, hydrodynamic diameter, Concentration	Fluorescence labelling		Kuyper et al. 2006b Kuyper et al. 2006a Pinheiro et al. 2007 Lead et al. 2000

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
High performance liquid chromatography	HPLC			Mobile phase interactions Size separation range limited by column	Sizing Separation Purification Quantification	UV/Vis ICP-MS Voltammetry Amperometry		Scrivens et al. 1994 Sivamohan et al. 1999 Song et al. 2003 Song et al. 2004 Giusti et al. 2005
Hydrodynamic chromatography	HDC	5 – 1200 nm		Mobile phase interactions	Sizing Size separation	UV/Vis ICP-MS		Blom et al. 2003 Williams et al. 2002 Yegin and Lamprecht 2006
Laser induced break down detection	LIBD		Highly sensitive	No elemental information	Size Concentration			Bundschuh et al. 2001a Bundschuh et al. 2001b
Membrane filtration		Mainly 0.2 & 0.4 µm filtration steps	High speed, high volume fractionation	Broad pore size distribution. Filtration artefacts	Size separation			Akthakul et al. 2005 Howell et al. 2006

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Moessbauer spectroscopy	Moessbauer				Oxidation state Phase identification Magnetic properties		Bulk	Burleson et al. 2004
Near-Field Scanning Optical Microscopy	NSOM	~ 30 nm	Optical imaging	Spatial resolution	Sizing Chemical bonding Visualization		Thin samples ~ 200 nm	Maynard 2000

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Nuclear magnetic resonance spectroscopy and Pulsed field gradient NMR	NMR		Suitable for colloidal matter in liquid or solid state	Lack of available standards	PFG-NMR: diffusion coefficient hydrodynamic diameter, Structure of coating & particles Elemental composition			Valentini et al. 2004 Luther W 2004 Carter et al. 2005
Raman spectroscopy	Raman		Compatible with aqueous suspensions & wet nanoparticle samples	Parameter effects	Oxidation state Structure Sizing		Vibrational spectroscopy Bulk	Li Bassi et al. 2005

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Scanning electron microscopy	SEM	1 nm – 1 µm	High resolution	High vacuum Sample preparation Contrasting Charging effects	Sizing	Auger EDS		Paunov et al. 2007
Scanning mobility particle sizer	SMPS				Size distribution Sizing Number concentration			Hasegawa et al. 2004 Luther W 2004 Lenggoro et al. 2007

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Scanning transmission electron microscopy	STEM	< 0.1 nm	Analysis of low concentrations (ppm)		Sizing Shape Structure Visualization	XRD HAADF CEND ADF TAD AEM CBED		Utsunomiya and Ewing 2003 Liu 2005 Bogner et al. 2005
Scanning Transmission X-ray Microscopy	STXM	30 nm	No sample preparation, liquid conditions		Sizing Shape Visualization			Leppard et al. 2004 Nurmi et al. 2005 Thieme et al. 2007

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Secondary ion mass spectrometry	SIMS		Atomic composition of layers from 1 – 3 nm	Sample preparation Offline technique Destructive	Elemental composition Surface properties			Kim et al. 1999 Borm et al. 2006
Single particle mass spectrometer	SPMS				Sizing Elemental composition			Janzen et al. 2002 Cai et al. 2002 Lee et al. 2005

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Size exclusion chromatography	SEC		Good separation efficiency, simple	Unwanted solvent & column interactions Limited size separation range	Separation Sizing	DRI FL PDA UV/Vis ICP-MS		Huve et al. 1994 Zhou et al. 2000 Novak et al. 2001 Zhao et al. 2001 Wilcoxon and Provencio 2005 Krueger et al. 2005 Wang et al. 2006 Helfrich et al. 2006 Bolea et al. 006

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Small angle neutron scattering	SANS		Analysis in liquids		Charge density Structure in dependence of pH, ionic strength, solute concentration			Diallo et al. 2005
Static light scattering	SLS				Molecular weight Root mean square radius of gyration	SEC FFF DLS		Baalousha et al. 2005b Baalousha et al. 2005a
Thermo-gravimetric analysis	TGA				Oxidation state		Bulk analysis	Pang et al. 1993

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Time-of-flight mass spectrometry	TOF-MS	ppb-ppt			Mass/charge ratio Elemental composition	Other TOF-MS variations: LAI MALDI NAMS	Aerosols Macromolecules like polymers	Reents et al. 1995 Lou et al. 2000 Bauer et al. 2004 Wang and Johnston 2006
Transmission electron microscopy	TEM	> 0.1 nm	High resolution	Sample preparation High vacuum Contrasting	Sizing Shape Visualization Structure	EELS EDS		Mavrocordatos and Perret 1998 Wilkinson et al. 1999 Mavrocordatos et al. 2004

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Ultracentrifugation (analytical/ preparative)		Size range: 100 Da to 10GDa (molar mass from calibrations)	Acceleration: up to 1,000,000 G (9,800km/s2)	Differential settling rates can induce aggregation	Sedimentation velocity Sedimentation equilibrium Shape and molar mass Size distribution			Bootz et al. 2004
UV/Vis spectroscopy	UV/Vis		In situ	Insensitive	Quantitative Concentration, some structure or size information can be derived			Pesika et al. 2003

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
Wet scanning electron microscopy	WetSEM	Low contrast samples: ~ 100 nm High contrast samples: ~ 10 nm	Imaging under fully wet conditions	Loss in resolution Sensitive membrane	Sizing Shape Visualization	EDS	Wet imaging	Timp et al. 2007
Wet scanning transmission electron microscopy	WetSTEM		Imaging in liquids		Sizing Shape Visualization		Transmission observations in ESEM	Bogner et al. 2005
X-ray absorption spectroscopy	XAS	ppm			Oxidation state Elemental composition Structure		Includes EXAFS and XANES Bulk	Venkateswarlu et al. 2005 Arcon et al. 2005

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
X-ray diffraction	XRD	1 – 3 wt%			Structure Sizing		Especially for crystalline nanoparticles Bulk	Zhang et al. 2003 Guzman et al. 2006
X-ray fluorescence spectroscopy	XRF				Solid state speciation Quantitative bulk analysis Isotope ratios Morphology		Aerosols	Ortner et al. 1998
X-ray microscopy	XRM	~ 30 nm		Radiation damage	Sizing Shape Visualization			Jearanaikoon and Braham-Peskir 2005

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Method	Acronym	Spatial resolution or LOD	Advantages	Disadvantages	Information	Possible combination	Comments	Examples of application/References
X-ray photoelectron spectroscopy	XPS	~ 1 µm	Atomic composition of layers from 1 – 10 nm		Shape Sizing Elemental composition Oxidation state		Extremely surface sensitive technique	Schrick et al. 2004 Nurmi et al. 2005

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