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

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

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

21
22 **Keywords:** Nanoparticles, nanomaterials, food, environment, analysis, characterization,
23 detection
24

25 Introduction & background

26

27 Nanomaterials are commonly regarded as materials with at least one dimension below
28 100 nm (Borm et al. 2006), although there is no official definition yet. They include
29 nanofilms and coatings (< 100 nm in 1 dimension), nanotubes and wires (< 100 nm in 2
30 dimensions) and nanoparticles (< 100 nm in 3 dimensions) (Hochella 2002).
31 Nanoparticles can occur naturally (e.g. in ashes, as soil particles or bio molecules), be
32 produced unintentionally (e.g. in Diesel exhausts) or be intentionally engineered. This
33 review will mainly focus on engineered or manufactured nanoparticles (ENPs).

34

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

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4 49 structure and properties of the surfaces of nanoparticles are substantially modified over
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7 50 that of the surfaces of the same materials in bulk form because of the proportionally
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10 51 high curvature of the nanoparticle surfaces, more surface defects and edges as well as
11
12 52 the presence of highly catalytically active sites (Madden and Hochella 2005).
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14 53 Additionally, targeted change in surface properties of ENPs can be achieved by coating
15
16 54 or functionalisation of nanoparticles.
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20
21 56 The potential benefits of engineered nanomaterials have been recognized for a long time
22
23 57 but it has not been until recently that the step from research to manufacture and use has
24
25
26 58 been made. Engineered nanomaterials are now being manufactured in ever increasing
27
28 59 quantities and they are finding application in a wide range of products and sectors
29
30
31 60 including medicines, cosmetics, clothing, engineering, electronics, and environmental
32
33 61 protection (Ponder et al. 2001; Obare and Meyer 2004). Current applications range from
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35 62 antibacterial wound dressings and clothing, through to reinforced tennis rackets to
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37 63 advanced transparent sun protection.
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42 65 In the food sector the uses of nanotechnology-derived food ingredients, additives,
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44 66 supplements and contact materials are expected to grow rapidly. Chaudhry et al. (2007)
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46
47 67 claim that worldwide over 200 companies are conducting R&D into the use of
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50 68 nanotechnology in either agriculture, engineering, processing, packaging or delivery of
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52 69 food and nutritional supplements. Food safety will also potentially benefit with the
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54 70 introduction of nano-based detectors, sensors and labelling (Weiss et al. 2006). In some
55
56 71 countries nanomaterials are already applied in food supplements and food packaging
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5 72 both nanoclays as diffusion barriers and nano-silver as antimicrobial agents (Sanguansri
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7 73 and Augustin 2006; Chaudhry et al. in press; Corporate watch 2007; table 1).
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11 75 *Table 1. Examples for applications of nanomaterials in consumer products.*
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16 77 The proliferation of nanotechnology has prompted discussions over the safety of these
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18 78 materials to human health and the environment. It is almost inevitable that humans will
19
20 79 be exposed to engineered nanoparticles e.g. due to migration of nanoparticles from food
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22 80 packaging into food, as well as the application of creams directly to the skin. In
23
24 81 addition, the unintended (e.g. waste, wastewater, sludge) and intended (e.g. groundwater
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26 82 remediation) release of nanoparticles to the environment may lead to indirect human
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28 83 exposure (e.g. via drinking water, food chain).
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35 85 The pulmonary toxicity of airborne particles (mostly referred to as ultrafine particles <
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37 86 10 µm) has been well studied and it is known that toxicity is strongly related to particle
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39 87 size (Brown et al. 2001; Hasegawa et al. 2004; Geiser et al. 2005; Frampton et al. 2006).
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41 88 However, the toxicity of engineered nanoparticles and their effects on human health, as
42
43 89 well as their environmental fate and impact in water and soil is still widely unknown
44
45 90 (Burleson et al. 2004), although some studies suggest (eco-) toxicity. It has been
46
47 91 reported that different types of nanoparticles can cause cytotoxicity and cross-cellular
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49 92 layers (Shiohara et al. 2004; Koch et al. 2005; Chen and von Mikecz 2005; Hardman
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51 93 2006; Brunner et al. 2006) as well as accumulate in tissue (BullardDillard et al. 1996).
52
53 94 Further toxicity of fullerenes and TiO₂ nanoparticles to daphnia, large mouth bass and
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55 95 other aquatic species has been found (Oberdorster 2004; Oberdorster et al. 2006; Lovern
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96 and Klaper 2006), whereas Yang & Watts (2005) discovered phytotoxicity of alumina
97 nanoparticles (Yang and Watts 2005). Fullerenes, silver and other nanoparticles have
98 also shown antibacterial behavior e.g. in health care applications and in aquatic
99 environments (Sondi and Salopek-Sondi 2004; Oberdorster et al. 2006; Lyon et al.
100 2006; see table 2).

101

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

103

104 Even in cases where nanoparticles do not show any acute toxicity, the question of long-
105 term effects, bioaccumulation and the impact on food webs remains. Engineered
106 nanoparticles may also affect the toxicity of other substances, since natural
107 nanomaterials are known to act as nanovectors for contaminants (Mccarthy and Zachara
108 1989; Kersting et al. 1999; Lyven et al. 2003; Lamelas and Slaveykova 2007). For
109 example a study with carp showed enhanced cadmium bioaccumulation in the presence
110 of TiO₂ nanoparticles (Zhang et al. 2007).

111

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

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

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5 120 engineered nanoparticles in product formulations, environmental matrices and food
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7 121 materials. As limited work has been done to date on the detection and characterization
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9 122 of engineered nanoparticles in food, the review draws heavily upon studies reporting
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11 123 characterization of nanoparticles in raw products and environmental matrices where
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14 124 much more information is available (e.g. Walther 2003; Lead and Wilkinson 2006;
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16 125 Wigginton et al. 2007a). Possible future directions of ENP analysis and characterisation
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18 126 in biological, environmental or food samples are identified and areas of further work are
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21 127 recommended.
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5 128 **Nanoparticle properties & their analysis**
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9 130 The potential toxicity and behaviour of nanoparticles will be affected by a wide range of
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11 131 factors including particle number and mass concentration; surface area, charge,
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13 132 chemistry and reactivity; size and size distribution; state of aggregation; elemental
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15 133 composition as well as structure and shape (Borm et al. 2006; Chau et al. 2007); table
16
17 134 3). Therefore when analysing nanoparticles in different matrices, it is not only the
18
19 135 composition and concentration that will need to be determined but also the physical and
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21 136 chemical properties of the engineered nanoparticles within the sample and the chemical
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23 137 characteristics of any capping/functional layer on the particle surface.
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30 139 *Table 3. Nanoparticle properties and their importance for measurement.*
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35 141 The analytical techniques should be sensitive enough to measure low concentrations as
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37 142 small particles normally represent only a small part of the total mass. The techniques
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39 143 should also minimise sample disturbance to ensure that laboratory analyses reflect the
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41 144 unperturbed environmental state (Chen and Buffle 1996; Gimbert et al. 2007b). A range
42
43 145 of analytical techniques is available for providing information on concentration and
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45 146 properties; these include microscopy approaches, chromatography, centrifugation and
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47 147 filtration, spectroscopic and related techniques (table 4). In the following sections, a
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49 148 selection of these methods will be discussed that are potentially suitable for nanoparticle
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51 149 characterisation and literature examples will be used to demonstrate the application of
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53 150 different techniques to complex media.
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5 152 **Overview of analytical methods applicable to nanoparticle analysis**
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9 154 A wide range of methods is available for the detection and characterization of
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11 155 nanoparticles, a choice of different approaches are described below and a summary of
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13 156 the information generated by different techniques and their application to complex
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15 157 media is given in tables 4 and 5 respectively.
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21 159 *Table 4. Nanoparticle properties and examples of analytical methods potentially*
22
23 160 *suitable for their measurement.*
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28 162 *Table 5. Overview of discussed analytical methods suitable for nanoparticle*
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30 163 *characterization in alphabetical order with literature examples for their application in*
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32 164 *complex media.*
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37 166 *Microscopy and microscopy related techniques*
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42 168 Microscopy-based methods are available that could be used in the detection and
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44 169 characterization of engineered nanoparticles. These methods include optical approaches
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46 170 including confocal microscopy as well as electron and scanning probe microscopy.
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51 172 The typical dimensions of nanoparticles are below the diffraction limit of visible light,
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53 173 so that they are outside of the range for optical microscopy. However, near-field
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55 174 scanning optical microscopy (NSOM) – a scanning probe microscopy (SPM) technique
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57 175 - obtains with a spatial resolution of ~ 50 – 100 nm much better resolutions than
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4 176 conventional optical microscopes. This is achieved through the use of a sub-wavelength
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6 177 diameter aperture. NSOM may therefore be suitable for optical imaging of nanoparticle
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9 178 aggregates (Maynard 2000).
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14 180 The diffraction of light is also the limiting factor for confocal microscopy. However,
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16 181 using confocal laser scanning microscopy (CLSM), resolutions of up to 200 nm can be
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18 182 achieved and tiny fluorescent objects can often be located more precisely than the
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21 183 resolution limit. Another feature of a CLSM is the high-resolution optical imaging of
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23 184 thick specimen (optical sectioning). Naturally fluorescent samples or samples treated
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25 185 with fluorescent dyes are detectable. Confocal microscopy has only recently been
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27
28 186 applied in colloid characterisation and has been combined with fluorescence correlation
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31 187 spectroscopy (FCS) to characterise fluorescent species in complex systems (Lead et al.
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33 188 2000b; Prasad et al. 2007).
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37 190 The most popular tools for the visualization of engineered nanoparticles though are
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39 191 electron and scanning probe microscopes. Depending on the technique, resolutions
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41
42 192 down to the sub-nanometer range can be achieved. Using atomic force microscopy
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44 193 (AFM), scanning electron (SEM) and transmission electron microscopy (TEM)
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47 194 nanoparticles can not only be visualized, but also properties like the state of
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49 195 aggregation, dispersion, sorption, size, structure and shape can be observed
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51
52 196 (Mavrocordatos et al. 2004). For comparison, Figure 1 shows TiO₂ and ZnO
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54 197 nanoparticles imaged by SEM, TEM and AFM.
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5 199 *Figure 1. ZnO (1st row) and TiO₂ (2nd row) nanoparticles suspended in distilled water,*
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7 200 *allowed to dry and imaged in order from left to right by SEM, AFM and TEM. Initial*
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9 201 *sizes as stated by the manufacturer (Sigma Aldrich, UK): 50 – 70 nm for ZnO particles*
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11 202 *and 5 – 10 nm for TiO₂ particles.*
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16 204 In TEM, electrons are transmitted through a specimen (therefore the specimen has to be
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18 205 very thin) to obtain an image whereas in a SEM scattered electrons are detected at the
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20 206 sample interface for imaging. In general imaging of lighter atoms in an electron
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22 207 microscope is more difficult as they scatter electrons less efficiently.
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28 209 Analytical (mostly spectroscopic) tools can be coupled to electron microscopes for
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30 210 additional elemental composition analysis generally known as analytical electron
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32 211 microscopy (AEM). For example, energy dispersive X-ray spectroscopy (EDS), can be
33
34 212 combined with SEM and TEM and permits a clear determination of the composition of
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36 213 elements heavier than oxygen, Quantitative analysis however, leads generally to ~ 20 %
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38 214 uncertainty (Mavrocordatos et al. 2004).
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42 215 Electron energy loss spectroscopy (EELS) is based on the loss of energy of the incident
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44 216 electron through the specimen. Thus, elements can be discriminated. This technique can
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46 217 only be used with TEM and quantitative analysis has uncertainties as low as 10 %
47
48 218 (Mavrocordatos et al. 2004). Selected area electron diffraction (SAED) can also be
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50 219 combined with TEM and provides information on crystalline properties of particles
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52 220 (Mavrocordatos et al. 2004).
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4 222 Electron microscopy is usually a destructive method meaning that the same sample
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6
7 223 cannot be analyzed twice or by another method for validation. Other disadvantages of
8
9 224 electron microscopes are charging effects caused by accumulation of static electric
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11 225 fields at the specimen due to the electron irradiation required during imaging. This can
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13
14 226 normally be overcome by using sample coating made of a conducting material, but this
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16 227 can result in a loss of information. Also biological samples often need treatment, like
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18 228 heavy metal staining, for better contrast.
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23 230 For biological samples, a scanning transmission electron microscope (STEM) belonging
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26 231 to the group of TEMs, can be of use. Dark-field microscopy with a STEM allows high
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28 232 contrasts and therefore imaging of biological samples without staining. In combination
29
30 233 with diffraction and spectroscopic techniques STEMs can also provide images and
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32
33 234 chemical data for nanomaterials with a sub nanometer spatial resolution (Liu 2005).
34
35 235 Utsunomiya and Ewing (2003) successfully applied high-angle annular dark field
36
37 236 scanning transmission electron microscopy, scanning transmission electron microscopy-
38
39 237 energy dispersive X-ray spectrometry, and energy-filtered transmission electron
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42 238 microscopy to the characterization of heavy metals on airborne particulates
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45 239 (Utsunomiya and Ewing 2003).
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49 241 X-ray microscopy (XRM) can provide spatial resolution (down to ~30 nm, limited by
50
51 242 the X-ray beam focusing optics) imaging of a specimen in the aqueous state without the
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53 243 need for sample preparation e.g. fixation, staining, sectioning (Jearanaikoon and
54
55 244 braham-Peskir 2005; Thieme et al. 2007). X-ray microscopy can also be combined with
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58 245 computer tomography to enable 3D imaging (Thieme et al. 2003). A variation of the
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4 246 XRM is the scanning transmission X-ray microscopy (STXM), which has been used for
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7 247 example to characterize metallic Fe particles for remediation purposes (Nurmi et al.
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9 248 2005).

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11 249
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14 250 The major limitation of conventional electron microscopes like transmission electron
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16 251 and scanning electron microscopes is however, that they have to be operated under
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18 252 vacuum conditions. This means no liquid samples can be introduced to the sample
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21 253 chamber and sample preparation (dehydration, cryo-fixation or embedding) is
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23 254 necessary, which leads in general to sample alteration and dehydration artifacts
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25
26 255 (Mavrocordatos et al. 2007).

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30 257 There has therefore been a lot of effort to improve sample preparation techniques for
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32 258 electron microscope imaging in order to limit artifacts. For example, Lonsdale et al.
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34 259 (1999) applied high pressure freezing and freeze substitution to image barley aleurone
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37 260 protoplasts by transmission electron microscopy (TEM) (Lonsdale et al. 1999). This
38
39 261 method preserves the cellular fine structure and antigenicity of proteins better than
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41 262 conventional chemical fixation and dehydration techniques. Another possibility is the
42
43 263 use of a cryo-TEM, which enables imaging of frozen samples on a cold specimen stage
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46 264 and microscope. This has the advantage of preserving and visualizing structures that
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49 265 would be lost or altered by other sample preparation methods. Wang et al. (2004)
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51 266 employed this method to image Fe(III)-doped TiO₂ nanoparticles (2 - 4 nm) in an
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53 267 aqueous environment with a special sample holder (Wang et al. 2004). Mavrocordatos
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55 268 & Perret (1998) embedded iron-rich particles (30 - 200 nm) in resin and then sectioned
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57
58 269 these samples for visualization by TEM and EELS (Mavrocordatos and Perret 1998).
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7 271 However, none of these preparative techniques can fully avoid artifacts caused by
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9 272 sample drying or preparation. As imaging of nanoparticles in their original state is
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11 273 crucial for nanoparticle research other methods are required. One possibility to image
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13 274 nanoparticles under more natural conditions is to use an environmental scanning
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15 275 electron microscope (ESEM). In an ESEM the gun and lenses of the microscope are
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17 276 under vacuum conditions as in a conventional SEM, but due to a detector that is able to
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19 277 operate under higher pressure and multiple pressure limiting apertures to separate the
20
21 278 sample chamber from the column, the sample chamber itself can be operated at around
22
23 279 10-50 Torr. Therefore, samples can theoretically be imaged in their natural state without
24
25 280 modification or preparation under variable pressure and humidity, theoretically up to
26
27 281 100 %. Additionally the gas ionization in the ESEM sample chamber eliminates the
28
29 282 charging artifacts and therefore materials do not have to be coated with a conducting
30
31 283 material anymore. Other advantages of an ESEM are that the detector is insensitive to
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33 284 light and fluorescence or cathodoluminescence does not disturb imaging. ESEM still
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35 285 allows X-ray data, e.g. from EDS, to be obtained. However, an ESEM cannot achieve
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37 286 real atmospheric pressure and only the top surface of a specimen can be imaged, which
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39 287 in the case of a liquid sample is the water surface. The contrast is increasingly poor with
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41 288 increasing humidity and there is the possibility of specimen drifting. Also a loss in
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43 289 resolution from ~ 10 nm up to ~ 100 nm is unavoidable.

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53 291 Doucet et al. (2005) compared the performance of an environmental and a conventional
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55 292 scanning electron microscope (ESEM and SEM respectively) for the imaging of natural
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57 293 aquatic particles and colloids. Analyzing river estuary samples they found that the
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4 294 conventional SEM provides sharper images and lower resolution limits, but produces
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6 295 more imaging artifacts due to the drying of the sample. ESEM samples retain to some
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9 296 extent their morphological structures without the need of sample preparation, but image
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11 297 interpretation and imaging itself is more complex. Also it has been stated that the
12
13 298 maximum relative humidity at which imaging could be performed was 75 %, as at 100
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15 299 % layers of free water over the sample made colloid visualization impossible. Sizing of
16
17
18 300 colloids revealed technique-dependent differences. Hence they suggest that ESEM and
19
20 301 SEM should be used as complementary techniques, but are in favor of the ESEM for
21
22 302 imaging colloids and colloid aggregation (Doucet et al. 2005a).

23
24
25 303 Redwood et al. (2005) applied an ESEM to analyze and quantify humic substances
26
27 304 (Suwannee river humic acid, 100 mg/L) as a function of humidity and pH (3.3 – 9.8).
28
29 305 They concluded that the ESEM is an important complementary technique to other
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31 306 analytical methods for probing changes in colloid structure as a function of hydration
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33 307 state, however, they also concluded that at present non-perturbed samples cannot be
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35 308 imaged (Redwood et al. 2005).

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41 310 The technique of WetSTEM allows transmission observations of wet samples in an
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43 311 ESEM under annular dark-field imaging conditions down to a few tens of nm.
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45 312 Combining elements of TEM and ESEM, samples that are fully submerged can be
46
47 313 imaged. The imaging is achieved by placing a TEM grid with the sample on a TEM
48
49 314 sample holder. This holder is placed in the ESEM chamber allowing transmission
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51 315 imaging under non-vacuum conditions (Bogner et al. 2005).

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4 317 An alternative to the ESEM methods described above is the use of a WetSEM™
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6 318 capsule as a specimen holder, in which the sample is added and the holder is then
7
8 319 sealed. These capsules have been developed by the QuantomiX Company for imaging
9
10 320 of samples in a conventional SEM under hydrated conditions. There are two different
11
12 321 types of WetSEM capsules on the market suitable for conventional SEM with a back-
13
14 322 scattered electron detector: one for imaging in liquids and another for imaging of solid
15
16 323 but wet materials (e.g. biological samples, food or soil). With this technique *in situ*
17
18 324 imaging of nanoparticles in natural media is possible. The capsule separates the sample
19
20 325 from the vacuum chamber of the microscope and a membrane in the capsule allows
21
22 326 electrons to pass into the sample thus enabling imaging under atmospheric pressure. It is
23
24 327 possible to conduct semi-quantitative and qualitative elemental analysis with these
25
26 328 capsules provided that the microscope is equipped with an energy dispersive x-ray
27
28 329 spectrometer (Thiberge et al. 2004a; Thiberge et al. 2004b; Joy and Joy 2006; Timp et
29
30 330 al. 2007). Limitations are a loss of resolution and the sensitivity of the membrane to
31
32 331 radiation damage. Also objects have to be close to the membrane to be visible. Thiberge
33
34 332 et al. (2004) describe in detail the theory, characteristics, limitations and possible
35
36 333 applications of WetSEM capsules using a conventional SEM and an ESEM (Thiberge et
37
38 334 al 2004a; Thiberge et al 2004b).
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42 336 Imaging under fully liquid conditions is also possible using atomic force microscopy
43
44 337 (AFM). The AFM belongs to the family of scanning probe microscopes (SPMs)
45
46 338 (Balnois et al. 2007). An oscillating cantilever is scanning over the specimen surface
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48 339 and electrostatic forces (down to 10^{-12} N) are measured between the tip and the surface.
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50 340 An AFM can achieve 3D surface profiles from these force measurements with height
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4 341 resolutions of ~ 0.5 nm. The main advantage of an AFM is that it images sub-nanometer
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6 342 structures under wet or moist conditions. Although under liquid conditions particles not
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9 343 fixed to a substrate will float around and eventually stick to the cantilever, which leads
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11 344 to imaging artefacts, both as smearing effects and changes in the cantilever oscillation
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13 345 properties as the tip gains weight. This smearing effect could be minimized by using a
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15 346 non-contact scanning mode where the tip is not touching the particles but only feel its
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17 347 forces (Balnois et al. 2007).
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23 349 The main limitation of AFM for nanoparticle visualization is that the geometry of the
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25 350 tip is often larger than the particles being probed and this leads to errors in the onset and
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27 351 offset of a particle topography on a scan, resulting in severe overestimations of the
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29 352 lateral dimensions of the nanoparticles. Therefore accurate size measurements should
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31 353 only be taken on the height (Z-axis) of the particles and the lateral dimensions only used
32
33 354 with great caution. Furthermore AFM for environmental or food related samples is
34
35 355 limited in the ability to obtain qualitative or quantitative information of the sample
36
37 356 composition. Although, the force patterns that emerge can also help in identifying the
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39 357 nature of individual atoms, this technique is called chemical force microscopy, short
40
41 358 CFM (Sugimoto et al. 2007; Shluger and Trevethan 2007). This recent development
42
43 359 could lead to a vast progress in AFM application to more complex samples. Scanning
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45 360 tunneling microscopy (STM) is another type of scanning probe microscopy based on
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47 361 quantum electronic properties where a conducting tip is oscillating close to the surface
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49 362 and if it comes in close contact with a metallic or semiconducting component of the
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51 363 surface then electrons can be allowed to “tunnel” over the gap to the surface. STM has
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4 364 been applied to environmental samples to image redox properties of microbial enzymes
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6 365 (Wigginton et al. 2007b).
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11 367 AFM has been used to characterize natural colloidal matter. For example Lead et al.
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13 368 (2005) analyzed natural aquatic colloids by AFM and colloid structure was found to
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15 369 vary as a function of pH. Mica slides were dipped for 30 min into filtrated samples
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17 370 rinsed with distilled water and allowed to dry prior to imaging in tapping mode. It has
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19 371 been stated that it is not known whether imaging under ambient humidity or liquid
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21 372 water produces better results. *A priori*, imaging under liquid water appears to provide
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23 373 ideal experimental conditions. However, atmospheric humidity retains colloid-bound
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25 374 water, helping to maintain structure, and AFM tips exposed to organic matter in solution
26
27 375 soon become coated in the organic matter, potentially affecting the veracity of the
28
29 376 images. This is also a possibility in imaging after air-drying. Recommendation is given
30
31 377 as a complementary tool and comparison between TEM and AFM using different
32
33 378 sample preparation methods indicate similar morphologies (Lead et al. 2005). Balnois et
34
35 379 al. (1999) employed tapping mode AFM for the analysis of humic acid on mica. They
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37 380 found that aggregation might be related to the hydrophobicity of the sample. No
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39 381 aggregates were observed for relatively hydrophilic humic acids (Suwanee river) at pH
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41 382 3 to 10, but aggregates were seen for peat humic acid at low pH and high ionic strength.
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43 383 A comparison between AFM, Fluorescence Correlation Spectroscopy, Field-Flow
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45 384 Fractionation and Pulsed Field Gradient-NMR was carried out on a reference fulvic acid
46
47 385 sample (Lead et al. 2000a). It consistently showed that AFM resulted in smaller particle
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49 386 sizes measurements compared to the other techniques even after considering AFM is a
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51 387 number average method while the others in the study were mass average methods. This
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4 388 underestimation of the size of the fulvic acid was thought to be due to drying or other
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6 389 substrate effects during the AFM procedure.
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11 391 Although an AFM is operated under ambient conditions, samples still have to be
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13 392 applied to a specimen holder, which can cause alterations and the sample application
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15 393 has to be done carefully. A range of sample preparation techniques have been reported
16
17 394 by Balnois and Wilkinson (2002). These include drop deposition, adsorption,
18
19 395 ultracentrifugation and they have successfully been applied in the characterization of
20
21 396 environmental biopolymers (e.g. humic substances, polysaccharides) by AFM (Balnois
22
23 397 and Wilkinson 2002). Bickmore et al. (1999) developed methods (including
24
25 398 electrostatic attraction and adhesion based) to fix clay minerals to a substrate to allow
26
27 399 imaging in aqueous suspensions by AFM (Bickmore et al. 1999). Further information
28
29 400 about the application of AFM to environmental colloids can be obtained from the
30
31 401 review by Maurice (1996). He describes the AFM as powerful tool to image
32
33 402 environmental colloids and surfaces in air or immersed in water at sub-nanometer-scale
34
35 403 resolution with examples of applications and limitations (Maurice 1996). Very recently
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37 404 a review has also been published relating the application of AFM to nanotechnology in
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39 405 food science (Yang et al. 2007).
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50 407 From the above, it is clear that using a combination of microscopic techniques we can
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52 408 not only visualize nanoparticles but also generate useful data on the size, size
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54 409 distribution and other measurable properties (Jose-Yacaman et al. 2001; Biberthaler et
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56 410 al. 2003; Rabinski and Thomas 2004; Chuklanov et al. 2006; Baatz et al. 2006).
57
58 411 However, it needs to be recognized that the image analysis of the microscope outputs is
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4 412 as crucial as imaging itself. Only small amounts of samples can be analyzed by
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6 413 microscopic techniques and this has an impact on the statistical significance of the
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8 414 results. The average particle size is a number average and size distribution obtained by
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10 415 image analysis depends on the number of particles measured. Since there are often
11
12 416 fewer larger particles it is important to count and measure enough particles to obtain
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14 417 good counting statistics on these size fractions. The same issues need to be considered
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16 418 when measuring ENPs in food or environmental samples in the presence of high
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18 419 concentrations of natural nanomaterials. It may therefore be necessary to measure
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20 420 millions or billions of particles to generate reliable data. Therefore it is essential to
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22 421 develop automation and image analysis procedures. Also the image contrast can have an
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24 422 influence on the visible size of the particles as well as light element particle coatings
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26 423 that can be invisible and therefore lead to controversial or incomparable results.
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35 425 *Chromatography and related techniques*
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39 427 Techniques based on or related to chromatography can be used for the separation of
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41 428 nanoparticles in samples. These techniques are mostly fast, sensitive (detector-
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43 429 dependent) and non-destructive, so that samples are available for further analysis.
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45 430 Although some chromatographic tools allow a range of solvents to be used, samples
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47 431 usually cannot be run in their original media, which can cause sample alteration and
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49 432 sample solvent interaction. By attaching traditional analytical tools (e.g. ICP-MS, DLS)
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51 433 as detectors to size separation techniques, it is not only possible to quantify different
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53 434 nanoparticles in food, water, biota and soil but also to characterise or elementally
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55 435 analyse them.
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7 437 The best known technique for size separation is size exclusion chromatography (SEC).

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9 438 A size exclusion column is packed with porous beads as the stationary phase. The pores

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11 439 of the column retain particles depending on their size and shape. This method has been

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13 440 applied to the size characterization of quantum dots, single walled carbon nanotubes and

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15 441 polystyrene nanoparticles (e.g. Krueger et al. 2005; Ziegler et al. 2005; Huang et al.

16
17 442 2005). Size exclusion chromatography has good separation efficiency. Major

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19 443 disadvantages of (size exclusion) chromatography are the possible interactions of the

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21 444 solute with the solid phase (Lead and Wilkinson 2006) and the limited size separation

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23 445 range of the columns, which may not allow covering the size range of both the primary

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25 446 nanoparticles and their aggregates. Methods employed to overcome the problem of solid

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27 447 phase interactions include the addition of capping agents to the mobile phase and the

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29 448 recycling of the analyte. SEC has been successfully combined with a range of detection

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31 449 techniques to not only monitor the size fractionation of the particles but also to

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33 450 characterize them. For example, Song et al. (2004) used voltammetric detection for gold

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35 451 nanoparticles separation and Helfrich et al. (2006) employed ICP-MS as multi-element

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37 452 detection method, whereas Porsch et al. (2005) worked with multi angle laser light

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39 453 scattering (MALLS) (Song et al. 2004; Porsch et al. 2005; Helfrich et al. 2006).

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43 455 Unlike SEC, in Capillary electrophoresis (CE) there are no solid phase interactions. CE

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45 456 allows the separation of particles in different solution based on the charge and size

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47 457 distribution of the components. However, as separation is not only based on size, data

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49 458 interpretation is more complex. Also mobile phase interactions cannot be excluded. Lin

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51 459 et al. (2007) used CE for the sizing of engineered Au and Au/Ag nanoparticles and

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4 460 Schmitt-Kopplin & Junkers (2003) have used CE in the characterization of humic
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6 461 substances and other natural organic matter.
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11 463 Hydrodynamic chromatography (HDC) separates particles based on their hydrodynamic
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14 464 radius. A HDC column is packed with non-porous beads building up flow channels, in
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16 465 which particles are separated by flow velocity and the velocity gradient across the
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19 466 particle. Therefore larger particles elute faster from the column than smaller ones
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21 467 (Mcgowan and Langhorst 1982). The non-porous beads considerably reduce the risk of
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23 468 solid phase interactions compared to the porous packaging in a SEC column. Available
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26 469 HDC columns show size separation ranges from 5 nm up to 1200 nm depending on the
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28 470 column length, whereas the size separation range of a SEC column is dominated by its
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31 471 pore size distribution. The wider particle size separation range of HDC allows a whole
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33 472 range of nanoparticles to be sized in different media and is particularly helpful in
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35 473 allowing a better understanding of formation of aggregates. HDC has been connected to
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37 474 the most common UV/Vis detector for the size characterization of (fluorescent)
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40 475 nanoparticles, colloidal suspensions and biomolecules (Williams et al. 2002; Chmela et
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42 476 al. 2002; Blom et al. 2003), but also to dynamic light scattering (DLS) to size separate
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45 477 lipid nanocapsules (Yegin and Lamprecht 2006). A major limitation of HDC is the poor
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47 478 peak resolution.
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52 480 A highly promising technique for the size separation of ENPs in complex natural
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54 481 samples is field flow fractionation (FFF) techniques (Giddings 1993; Beckett and Hart
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56 482 1993; Schimpf et al. 2000; Hassellöv et al. 2007). It is similar to chromatographic
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59 483 techniques, but separation is solely based on physical separation in an open channel
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4 484 without relying on a stationary phase. The particles are separated based on how they are
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7 485 affected by an applied field. The field controls the particle transport velocity by
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9 486 positioning them in different average laminar flow vectors in a thin channel. The field
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11 487 can be a centrifugal force (Sedimentation FFF) or a hydrodynamic flow perpendicular to
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13 488 the separation flow (Flow FFF). FFF is able to fractionate particles in a range of 1 nm -
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15
16 489 1 μm in brownian mode.

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18 490 FFF instruments can be coupled to online or offline detection and characterization,
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21 491 which in addition to size distributions allows analysis and visualisation of the
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23 492 fractionated samples by electron microscopy (Baalousha et al. 2005a). FFF can also be
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26 493 coupled to a range of sensitive and multi-element techniques such as multi angle laser
27
28 494 light scattering (MALLS) and ICP-MS (Hasselov et al. 1999b; Kammer et al. 2005).
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30 495 FFF coupling techniques have been successfully applied in geochemistry and natural
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32 496 colloid research as well as studies into the behaviour of engineered nanoparticles.
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34 497 Applications range from colloids in fresh and marine water to size separation of soil
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36 498 suspensions (Ranville et al. 1999; Hasselov et al. 1999a; Hasselov et al. 1999b; Chen
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38 499 and Beckett 2001; Lyven et al. 2003; Siepmann et al. 2004; von der Kammer et al.
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40 500 2004; von der Kammer et al. 2005; Stolpe et al. 2005; Kammer et al. 2005; Baalousha et
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42 501 al. 2005a; Graff and Frazier 2006; Lead and Wilkinson 2006; Gimbert et al. 2006; Peng
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44 502 et al. 2006; Baalousha et al. 2006a; Baalousha et al. 2006b; Baalousha and Lead 2007).
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46
47 503 Also single walled carbon nanotubes have been length separated by Dielectrophoresis
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49 504 FFF (Peng et al. 2006) and many engineered nanoparticles such as SiO_2 , metals, metal
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51 505 oxides, carbon black etc (Schimpf et al. 2000).

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54 506 The limitations of FFF techniques are membrane or accumulation wall interactions, the
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57 507 continuous re-equilibration in the channel (for trace constituent studies), and the need
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4 508 (in some circumstances) of pre-concentration, additional concentration of sample during
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7 509 equilibration and increasing possibility of aggregation in the channel (Beckett and Hart
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9 510 1993; Hassellöv et al. 2007).

11 511 In theory any aqueous or non-aqueous phase of any ionic strength and a pH between 2 –
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13 512 11 can be used as carrier. This gives versatility in terms of selecting carrier composition
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15 513 to favor colloidal stability, in order to minimize wall and membrane interactions and
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17 514 particle-particle interactions.

20 515 Stegeman et al. (1994) compared the resolving power and separation time in thermal
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22 516 field flow fractionation (TFFF), hydrodynamic chromatography, and size exclusion
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24 517 chromatography for the size separation of polymers and concluded that TFFF
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26 518 theoretically has the best separation potential because of the high selectivity, but this
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28 519 may not be able to be exploited in practice due to the technical requirements. On the
29
30 520 other hand SEC was found to be the fastest method for low molecular masses (Stegeman
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32 521 et al. 1994). In general FFF and HDC has a wider dynamic size range than SEC, while
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34 522 SEC has higher separation efficiency (less peak broadening). SEC also suffers from
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36 523 more sample perturbations than FFF and HDC.
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44 525 *Centrifugation and filtration techniques*

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49 527 Centrifugation and filtration techniques are well-established tools for the preparative
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51 528 size fractionation of samples. These are low-cost, high speed and high volume
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53 529 techniques. Ultracentrifugation (UC) e.g. is a centrifuge system that is capable of very
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55 530 high spinning speeds for accelerations up to 1 000 000G. There are two different types
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57 531 of ultracentrifugation: analytical and preparative UC. In an analytical ultracentrifuge
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5 532 (ANUC) a sample can be monitored in real time through an optical detection system
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7 533 using ultraviolet light absorption and/or interference optical refractive index sensitive
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9 534 systems. This allows the operator to observe the evolution of the sample concentration
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11 535 versus the axis of rotation profile as a result of the applied centrifugal field. This is for
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13 536 sedimentation velocity and sedimentation equilibrium experiments (gross shape of
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15 537 macromolecules, conformational changes in macromolecules and size distribution).
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18 538 Preparative ultracentrifugation has been used for pelleting of fine particulate fractions,
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20 539 for gradient separations (Bootz et al. 2004), and for harvesting aquatic colloids and
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22 540 nanoparticles on TEM and AFM substrates (Mavrocordatos et al. 2007; Balnois et al.
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24 541 2007).
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30 543 Traditional membrane filtration allows the fractionation of particle sizes between 0.2 –
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32 544 1 μm (Lead and Wilkinson 2006). Comparative data obtained for soil suspensions, for
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34 545 filtration and SdFFF indicates that membrane filtration can both over and underestimate
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36 546 smaller size fractions due to clogging as well as electrostatic interactions (Gimbert et al.
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38 547 2005). Microfiltration with pore sizes $> 0.1 \mu\text{m}$ is a simple and common method,
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40 548 although exhibiting many artifacts caused by e.g. filter cake formation and
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42 549 concentration polarization (Morrison and Benoit 2001). Ultrafiltration is applicable for
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44 550 large sample volumes, however, with decreasing pore sizes, common filtration artifacts
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46 551 are even more likely. For the separation of nanoparticles and ions nanofiltration with
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48 552 pore sizes of 0.5 or 1 nm can be used.
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56 554 Cross flow filtration (CFF) or tangential filtration recirculates the samples and therefore
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58 555 reduces clogging, concentration polarization and other artifacts caused by traditional
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4 556 dead end filtration (Lead and Wilkinson 2006). It has become the standard method for
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7 557 separating colloids and particles. Its use has been evaluated against AFM by Liu & Lead
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9 558 (2006). The method has been applied to fluorescence investigations of colloidal organic
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11 559 matter and dissolved organic matter in lake and river water (Liu et al. 2007) as well as
12
13 560 in seawater (Guo et al. 2000). Electrically assisted cross flow filtration has also been
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15 561 used for the separation of nanoparticles (Sung et al. 2007). Doucet et al. (2004)
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17 562 evaluated cross flow ultrafiltration (CFUF) for the size fractionation of freshwater
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19 563 colloids and particles (1 nm – 1 µm) by AFM and SEM and concluded that CFUF is not
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21 564 fully quantitative and separation is not always based on size alone. Amounts of large
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23 565 colloids might be overestimated and fractionation is not always consistent with the
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25 566 nominal pore size of the membranes. These conclusions have to be treated with some
26
27 567 caution as the validation techniques used (i.e. AFM and SEM) have their limitations
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29 568 (Doucet et al. 2004).

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31 570 *Spectroscopic & related techniques*

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33 572 A wide range of spectroscopic methods is available for nanoparticle analysis and
34 573 characterization. Scattering techniques that are useful for nanoparticle characterization
35 574 include light scattering techniques like static (SLS) and dynamic light scattering (DLS)
36 575 as well as neutron scattering such as small angle neutron scattering (SANS).

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38 577 DLS or photon correlation spectroscopy (PCS) is particularly useful for sizing
39 578 nanoparticles and determining their state of aggregation in suspensions. DLS provides
40 579 fast *in situ* and real time sizing (Ledin et al. 1994), but also has considerable limitations.

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4 580 For example, interferences can be caused by a range of possible artifact sources such as
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7 581 dust particles, which will have a great influence on the scattering intensity compared to
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9 582 smaller particles and therefore on the sizing result. Also data obtained from samples
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11 583 containing particles with heterogeneous size distributions is difficult to interpret. DLS is
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13 584 solely quantitative and unless the sample content is known or pure, size fractions cannot
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15 585 be related to particles of a specific composition. (e.g. Bootz et al. 2004).

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18 586 Static light scattering also known as multi angle (laser) light scattering (MAL(L)S gives
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21 587 information of particle structure and in combination with dynamic light scattering or
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23 588 FFF particle shape can be determined.

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26 589 SANS can be used on solid or liquid samples. For example Diallo et al. (2005) have
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28 590 applied SANS for the characterization of Suwannee River fulvic acid aggregates in
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30 591 aqueous solutions (Diallo et al. 2005).

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35 593 Small angle X-ray scattering (SAXS) is an analytical X-ray application technique to
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37 594 investigate the structural characterization of solid and fluid materials in the nanometer
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39 595 range. Monodisperse and polydisperse systems can be studied. In monodisperse systems
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41 596 size, shape and structure determination is possible whereas in polydisperse systems only
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43 597 the size distribution can be calculated.

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49 599 Laser-induced breakdown detection (LIBD) is a laser based technique featuring
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51 600 extremely low detection limits, which is able to analyze the size and concentration of
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53 601 colloids depending on the measured breakdown probability (BP). LIBD is therefore a
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55 602 highly promising tool for nanoparticle characterization, although it cannot distinguish
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4 603 between different types of particles and is in need of particle specific size calibration
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7 604 (Bundschuh et al. 2001a; Bundschuh et al. 2001b).
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11 606 Other laser-based techniques include Raman spectroscopy and laser-induced
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13 607 fluorescence (LIF). Instruments are now available combining these techniques, allowing
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15 608 the atomic, molecular and structural characterization of a specimen as well as a better
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17 609 understanding of physical properties.
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23 611 UV/Vis and infrared spectroscopy offer the possibility to characterise nanoparticles,
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25 612 especially quantum dots and organic based nanoparticles like fullerenes and carbon
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27 613 nanotubes. Fourier transformation infrared (FTIR) and UV/Vis spectroscopy have been
28
29 614 used to compare aqueous colloidal suspensions of C₆₀ (Andrievsky et al. 2002). Pesika
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31 615 et al. (2003) also used UV spectroscopy to study the relationship between absorbance
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33 616 spectra and particle size distributions for quantum-sized nanocrystals.
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39 618 Nuclear magnetic resonance (NMR) is a powerful technique providing information on
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41 619 the dynamics and three-dimensional structure of a solid compound or a suspension.
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43 620 Carter et al. (2005) characterized air and water stable silica nanoparticles by NMR and
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45 621 Valentini et al. (2004) used diffusion NMR spectroscopy for the characterization of the
46
47 622 size and interactions of colloidal matter (Valentini et al. 2004; Carter et al. 2005). Lead
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49 623 et al (2000) used pulsed field gradient NMR to measure the diffusion coefficients of
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51 624 fulvic acids (Lead et al. 2000a).
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5 626 X-ray spectroscopy comprises i.e. X-ray photoelectron (XPS), X-ray fluorescence
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7 627 (XRF) as well as X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD).
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9 628 XPS is highly surface specific due to the short range of the photoelectrons that are
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11 629 excited from the solid sample and therefore XPS could be useful to characterize
12
13 630 nanoparticle surfaces and coatings respectively. X-ray diffraction is non-destructive and
14
15 631 can reveal information about the crystallographic structure, elemental composition of
16
17 632 natural and manufactured materials. Nurmi et al. (2005) used this technique as well as
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19 633 XPS for the characterization of zero-valent Fe nanoparticles for use in remediation
20
21 634 (Nurmi et al. 2005). X-ray fluorescence (XRF) spectroscopy is also non-destructive and
22
23 635 can be used to identify and determine the concentrations of elements present in solid,
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25 636 powdered and liquid samples. XRF can be subdivided into wavelength separation
26
27 637 (WDXRF) and energy dispersive XRF (EDXRF).
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29 638 X-ray absorption (XAS) and emission spectroscopy is used in chemistry and material
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31 639 sciences to determine elemental composition and chemical bonding.
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33 640
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35 641 Other potentially suitable spectroscopic techniques for nanoparticle characterisation
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37 642 include electron paramagnetic resonance (EPR), Moessbauer, Auger electron (AES) and
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39 643 3D fluorescence excitation-emission matrix spectroscopy (EEM). Mössbauer
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41 644 spectroscopy provides information about chemical, physical and magnetic properties by
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43 645 analyzing the resonant absorption of characteristic energy gamma-rays known as the
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45 646 Mössbauer effect. Liu et al. (2007) and Lead et al. (2006) applied 3D fluorescence
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47 647 excitation-emission matrix (EEM) spectrophotometry for the fluorescence investigation
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49 648 of colloidal organic matter and dissolved organic matter in lake and river water (Lead et
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51 649 al. 2006; Liu et al. 2007). Electron paramagnetic resonance spectroscopy can be applied
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4 650 for particle surface reactivity analysis. EPR is a sensitive, specific method for studying
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6 651 organic and inorganic radicals formed in chemical reactions and the reactions
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8 652 themselves similar to NMR. Auger electron spectroscopy is also commonly used in the
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10 653 surface characterization of nanostructures. Quantitative bulk analysis by AES is
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12 654 described i.e. by Powell & Seah (1980).

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18 656 *Mass spectrometry*

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23 658 Mass spectrometers consist of an ion source, a mass analyzer, and a detector system.

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25 659 Two ionization techniques often used with liquid and solid biological samples include
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27 660 electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).

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29 661 Inductively coupled plasma (ICP) sources are mainly used for metal analysis. Mass
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31 662 analyzers (e.g. ion trap, quadrupole or time-of-flight) cover different mass to charge
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33 663 ranges, differ in the mass accuracy, and the achievable resolution. Most of the available
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35 664 analyzers are compatible with electrospray ionization, whereas MALDI is not usually
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37 665 coupled to a quadrupole analyzer.

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39 666 Mass spectrometry (MS) approaches such as MALDI, laser induced fluorescence (LIF),
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41 667 ion trap (IT) mass spectrometry have been applied for the analysis of fluorescently
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43 668 labeled nanoparticles (Peng et al. 2003; Cai et al. 2003).

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45 669 In the case of ICP-MS, samples cannot only be injected directly into the ion source but
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47 670 also via combined techniques like HPLC. An increasingly popular combination in this
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49 671 respect is FFF-ICP-MS, which allows the size separation of the sample with quantitative
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51 672 and elemental analysis of the obtained size fractions. This development is highly
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53 673 promising for nanoparticle analysis as particles can be simultaneously sized and
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4 674 analyzed in their original environment (Ranville et al. 1999; Hasselov et al. 1999a;
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6 675 Hasselov et al. 1999b; Lyven et al. 2003; von der Kammer et al. 2004; Bolea et al.
7
8 676 2006; Baalousha et al. 2006a).

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14 678 Whereas conventional mass spectrometry (MS) is applicable for identifying unknown
15
16 679 compounds and their mass concentrations as well as their isotopic composition, single
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18 680 particle mass spectrometry (SPMS) has also the ability to size single particles. MS
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20 681 techniques have also been used in aerosol characterization, including aerosol time-of-
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22 682 flight mass spectrometer (ATOF-MS). An ATOF-MS consists of an aerosol
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24 683 introduction interface; a light scattering region for sizing and a TOF-MS. Suess and
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26 684 Prather (1999) published a review on the topic of mass spectrometry of aerosols. They
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28 685 describe tools for offline MS of aerosols like LAMMS, SIMS and ICP-MS, tools for
29
30 686 online MS like surface/thermal ionization MS (SIMP, DIMS, CAART, PAMS) and
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32 687 laser desorption/ionization MS (ATOFMS, PALMS, RSMS, LAMPAS). More applied
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34 688 examples are described by Janzen et al. (2002) who compared the sizing of
35
36 689 nanoparticles with SPMS and TEM (Janzen et al. 2002). Lee et al. (2005) used SPMS to
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38 690 characterize the size and composition of polydisperse aerosol nanoparticles (Lee et al.
39
40 691 2005). They estimated the particle size by laser ablation/ionization time-of-flight single-
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42 692 particle mass spectrometer and validated their results by differential mobility analysis
43
44 693 (DMA). In situ characterization of size and elemental composition of individual aerosol
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46 694 particles in real time was performed by Prather et al. (1994) with the help of an ATOF-
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48 695 MS (Prather et al. 1994). For the sizing and analysis of aerosol nanoparticles a DMA
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50 696 has also been coupled to an ICP-MS (Okada et al. 2002).

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4 698 *Other techniques*
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9 700 *Particle counters for number concentrations.* The electrical sensing zone method counts
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11 701 and sizes particles by detecting changes in electrical conductance as particles suspended
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13 702 in a weak electrolyte solution are drawn through a small aperture. The technique has
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15 703 been successfully applied to the size and surface charge characterization of
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17 704 nanoparticles using a carbon nanotube-based coulter counter (Ito et al. 2003).
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19 705 Condensation particle counter (CPC) measurements can also provide data on the
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21 706 number and concentration of individual particles by growing the particles through a
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23 707 condensing process using various operating liquids like alcohol and water.
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30 709 *DMA for sizing aerosols.* A differential mobility analyzer (DMA) can be used to
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32 710 determine the size distribution of sub-micrometer aerosol particles. Particles are firstly
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34 711 charged and then their electrical mobility is measured as a function of their charge and
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36 712 size. After sizing the particles are still suspended in air and are ready for further analysis
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38 713 (McMurry et al. 1996; Weber et al. 1996; Okada et al. 2002).
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44 715 *SMPS for sizing and number concentration determination.* A scanning mobility particle
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46 716 sizer (SMPS) consists of a DMA and a CPC. First particles are separated by their
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48 717 electrical mobility in the DMA. Then the size fractionations enter a CPC which
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50 718 determines the particle concentration at that size.
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5 720 *BET method for surface area determination.* The very common Brunauer Emmett Teller
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7 721 (BET) method enables the determination of the specific surface area of solids and
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9 722 therefore also nanoparticles by gas adsorption (Brunauer et al. 1938).
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14 724 *Thermogravimetry and differential thermo analysis (TG-DTA).* DTA can be applied for
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16 725 phase changes and other thermal processes like the determination of the melting point.
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18 726 In combination TG-DTA is useful for investigating the thermal stability and
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20 727 decomposition, dehydration, oxidation as well as the determination of volatile content
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22 728 and other compositional analysis. Thermogravimetry in combination with a mass
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24 729 spectrometer can be used for surface analysis. Surface molecules are removed by
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26 730 heating and afterwards analysed by MS.
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33 732 *Electrophoretic mobility and the zeta potential.* Electrophoresis is used for studying
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35 733 properties of dispersed particles in particular for measuring the zeta potential. The zeta
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37 734 potential is a measure of the overall charge a particle acquires in a specific medium and
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39 735 gives an indication of the potential stability of a colloidal system. If all the particles
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41 736 have a large negative or positive zeta potential they will repel each other which leads to
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43 737 higher stability than if the particle charge is near neutral. The zeta potential is a measure
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45 738 of the net charge and there may be significant charge heterogeneities that can still lead
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47 739 to aggregation even though the net zeta potential is suggesting otherwise. Information
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49 740 about the aggregation state of a nanoparticle dispersion is highly valuable for
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52 741 nanoparticle fate and behavior studies. As an example the electrophoretic mobility of
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54 742 silica spheres dispersions suspended in water at different concentrations and salinities
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57 743 has been studied by Reiber et al. (2007) (Reiber et al. 2007).
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4 744 **Nanomaterial analysis in food and biological samples**
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9 746 As previously discussed when measuring nanoparticles in different media, it will not
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11 747 just be necessary to generate data on concentrations but also it is likely that information
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13 748 will be required on the size distribution and properties of the particles. No one technique
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15 749 can provide all this information so a range of analytical techniques will be required.
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18 750 Moreover, whilst a range of methods have been shown to be applicable to analysis of
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21 751 nanoparticles, it is likely that the current methods do not fulfill all the data
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23 752 requirements.
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28 754 As shown in the previous section many analytical tools are theoretically suitable for the
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30 755 characterization of nanoparticles ranging from electron microscopy to dynamic light
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32 756 scattering to flow field fractionation techniques but only a few of these are applicable to
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34 757 the analysis of more complex samples. The requirement for analysis of engineered
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36 758 nanoparticles in natural and food related samples will differ quite strongly from their
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38 759 analysis in pure or neutral media (e.g. air, distilled water). In complex media it will be
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41 760 essential to analyze samples of diverse elemental compositions and samples containing
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43 761 more than one type of nanoparticle. Many techniques are destructive or if not,
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45 762 application of some sample preparation methods can lead to artifacts. In addition natural
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47 763 samples will be hetero-dispersed and for measuring size distributions instruments
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49 764 providing a wide size separation range from ideally 1 nm to up to several μm are
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52 765 needed. There are many methods available for the sizing of particles, but very few if
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54 766 any of them is applicable to the entire size range. In the next section some of these
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57 767 challenges are discussed in more detail.
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7 769 *Bulk vs single particle analysis*

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11 771 An issue with some of the methods (discussed in the previous chapter) is their
12 772 application range. Existing techniques have to be divided between tools suitable for
13 773 analysing individual particles (depending on the particle size) or the bulk material.
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16 774 Classic composition and mass based tools are readily applicable for the bulk material,
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18 775 however elemental analysis of single particles in a dilute environment has only recently
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21 776 become available (e.g. aerosol mass spectrometry). Whereas standard tools for
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24 777 elemental composition and mass concentration are limited by their limit of detection
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27 778 (LOD), techniques able to characterize individual particles face spatial limitations.
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30 779 Especially particle sizing techniques are restricted by their size separation range. Figure
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32 780 2 illustrates the size range of selected methods for particle sizing.

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37 782 *Figure 2. Sizing methods and their size range for nanoparticle measurement. Adapted*
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39 783 *from (Lead and Wilkinson 2006) and (Gimbert et al. 2007b).*

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44 785 *Sizing artefacts and the lack of reference materials*

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49 787 The limitations of each analytical method for nanoparticle characterization can lead to
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52 788 confusing inconsistent results and therefore to inaccurate predictions of material
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54 789 properties and structure (Carter et al. 2005). For example it is still almost impossible to
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56 790 determine the absolute size of particles. Correct size measurements are difficult, which
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59 791 often lead to artifacts depending on the applied tool and the medium the particles are
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4 792 analyzed in. For example organic coatings that are not visible in the electron microscope
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6 793 (due to light elements like carbon) can lead to errors in sizing, especially when
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9 794 compared to sizing tools that measure the hydrodynamic radius of particles like FFF or
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11 795 DLS. It has been reported that the average size and size distribution of nanoparticles can
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13 796 significantly vary when comparing results from different techniques such as electron
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15 797 microscopy, dynamic light scattering, CFF and ultracentrifugation (Bootz et al. 2004).
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18 798 The lack of consistent reference materials and standards further exacerbates this
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20 799 problem (Lead and Wilkinson 2006). Nanoparticle sizing standards as well as
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22 800 standardized methods for sampling and measurement are therefore urgently required in
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24 801 order to overcome the problem of inconsistent data (Borm et al. 2006). To our
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26 802 knowledge standardized nanoparticles are not yet available and researchers have to rely
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28 803 on commercially available, often not well-characterized nanoparticles.
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35 805 *Sample preparation*

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40 807 Depending on the technique, to analyse natural samples, sample preparation and/or
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42 808 digestion is often required. As nanoparticles can and do change structure and
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44 809 composition in response to their environment, results obtained for pre-treated or
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46 810 digested samples can often be very different from if the particles were characterised *in*
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48 811 *situ* (Burlinson et al. 2004). These artefacts in analysis can be avoided by using
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50 812 techniques that either do not require or which reduce sample preparation to a minimum.
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53 813 The complexity data obtained for some techniques (e.g. NMR, CE) for samples in their
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55 814 original state can make the analysis and interpretation of data rather difficult.
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4 815 If sample preparation cannot be avoided, a careful record of sampling and preparation
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6 816 steps is essential to track artifacts. The nature of nanoparticles can also change over
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8 817 time, for example aggregation can increase or decrease and particles could dissolve. A
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10 818 lot of effort has been put into the development of sample preparation methods that
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12 819 improve the conservation of the original state of the sample. Especially in the
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14 820 microscopy area, achievements have been made in sample preparation ranging from gel
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16 821 trapping techniques for imaging emulsions under the SEM (Paunov et al. 2007) to high
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18 822 pressure freezing and freeze drying for imaging biological specimen under the TEM
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20 823 (Lonsdale et al. 1999; Bootz et al. 2004). Fixation methods for imaging clay minerals
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22 824 and particles in aqueous solutions under the AFM have also been developed (Bickmore
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24 825 et al. 1999).

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32 827 *Natural vs. engineered nanoparticles*

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37 829 At the moment it is very difficult to distinguish between particles of engineered origin
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39 830 and particles of a natural or other sources (Burleson et al. 2004). A way has to be found
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41 831 to differentiate between natural occurring and engineered nanoparticles. This will allow
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43 832 the concentrations of engineered nanoparticles in consumer products and the
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45 833 environment to be determined, as it is currently not known how many engineered
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47 834 nanoparticles will actually reach the environment or be bioavailable. Therefore selective
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49 835 detection methods need to be developed. Another solution to this problem could be
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51 836 nanomaterial labeling. Suggestions range from fluorescent and radioactive labeling for
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53 837 carbon based nanoparticles, to isotopic enrichment or depletion of metal-based
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55 838 nanoparticles. Also special particle coatings or entrapment of rare elements in nanotubes
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4 839 or fullerenes could be used to enable the detection of these distinctive chemical
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7 840 characteristics after an experimental study. Gulson and Wong (2006) published a paper
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9 841 on the possibilities of isotopic labeling and tracking of metal and metal oxide
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11 842 nanoparticles for nanotechnology research (Gulson and Wong 2006). Isotopic labelling
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13 843 of carbon nanotubes and fullerenes has already been performed. For example ^{13}C
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15 844 isotope carbon nanotubes are available and ^{14}C C_{60}s have been synthesized with
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18 845 subsequent uptake and toxicity studies (Scrivens et al. 1994b; BullardDillard et al.
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21 846 1996).
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5 847 **Conclusions & recommendations for future work**
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9 849 Analytical methods are required to reliably detect and characterise nanoparticles and
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11 850 their properties in the media in which humans and ecosystems are exposed to them. This
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13 851 includes air, soil and water as well as food and consumer products. These methods have
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15 852 to be also applicable for nanoparticle characterisation in toxicological and
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17 853 ecotoxicological testing. Only then can an appropriate risk assessment for nanoparticles
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19 854 be performed and the properties that are truly of risk can be identified and regulated or
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21 855 used in standard tests respectively (SCENIHR 2005).
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28 857 These techniques have to a) be able to deal with heterogeneous samples b) to minimize
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30 858 sample alteration to avoid artefacts and c) provide as much information as possible
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32 859 because most characterization techniques are destructive and therefore samples often
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34 860 cannot be analyzed twice or by more than one technique. An ideal analytical instrument
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36 861 would allow simultaneous determination of all physico-chemical properties of a
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38 862 nanoparticle and obtain them by real-time sampling, as many of these nanoparticles are
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40 863 transient in nature (Prather et al. 1994). Whilst a wide range of tools is available, the
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42 864 existing tools do not fulfil all desirable criteria and they all have their limitations when
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44 865 considering their application for food and natural samples. Therefore, until new tools
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46 866 have been developed, existing tools have to be used and combined in such a way that
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48 867 the data obtained can be validated. Analysis of the unperturbed sample or further
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50 868 analysis of the size fractionations is preferred. Complementary analytical tools should
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52 869 be applied and care be taken with sample preparation.
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5 870 This review demonstrated that promising developments have been made in nanoparticle
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7 871 analysis; however, further developments are essential to overcome the deficiencies in
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9 872 this area. Especially *in situ* analysis as well as routine and reliable techniques to
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11 873 improve size determination, size distribution of particles and other nanoparticle
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13 874 properties are of great importance.

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18 876 Nanotoxicology and nanoecotoxicology are still in their fledgling stages and risk
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20 877 assessments are practically non-existent especially in the food sector. Therefore
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22 878 progress in nanoparticle testing (in vivo and in vitro) is urgently needed to secure
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24 879 consumer safety including the development of standard testing materials and testing
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26 880 guidelines. In addition to toxicity studies, different uptake paths have to be studied
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28 881 including dermal, oral and intestinal as well as nanoparticle accumulation and long-term
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30 882 effects. Other effects of nanoparticle uptake could be the interaction with other (toxic)
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32 883 substances and their mobilisation or dislocation etc not only in the human body but also
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34 884 already in the consumer product. The environmental fate and behaviour of nanoparticles
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36 885 as well as their bioavailability is widely unknown and therefore also their potential
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38 886 impact on the food web and their persistence. Also their effect on other substances has
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40 887 to be examined e.g. whether contaminant transport in the environment could be
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42 888 facilitated through adsorption to nanoparticles, whether nanoparticles enhance
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44 889 contaminant uptake or have a negative impact on bacteria useful for natural remediation
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46 890 etc. Further, data on environmental and exposure concentrations are not available. To
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48 891 increase the current knowledge about nanoparticle and related issues developments in
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50 892 these mentioned analytical fields will be crucial.
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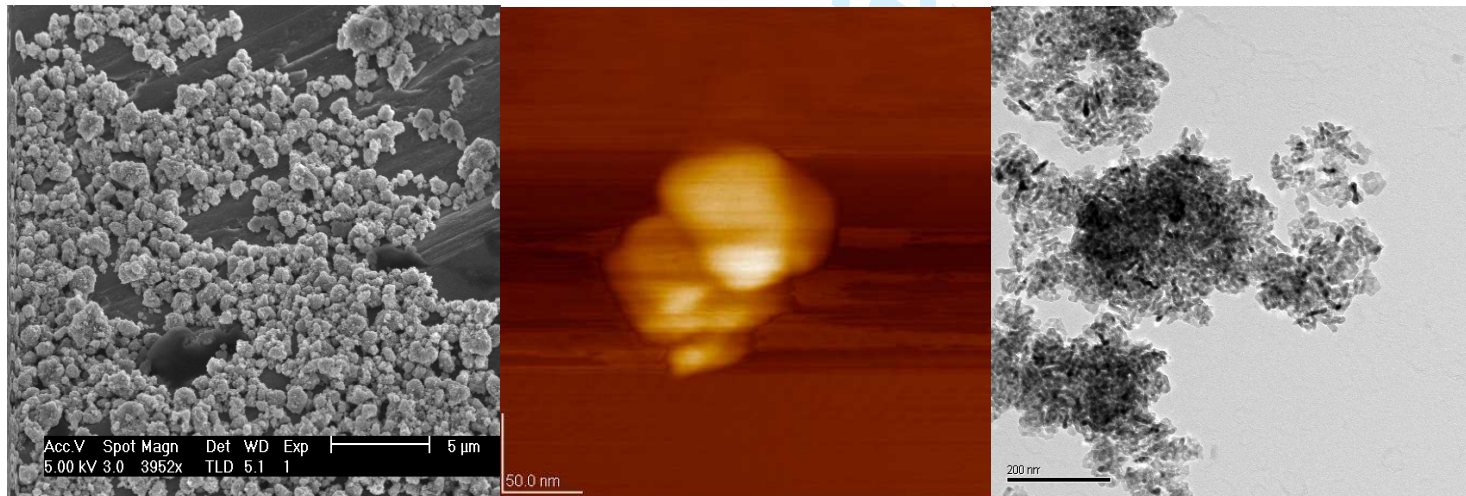
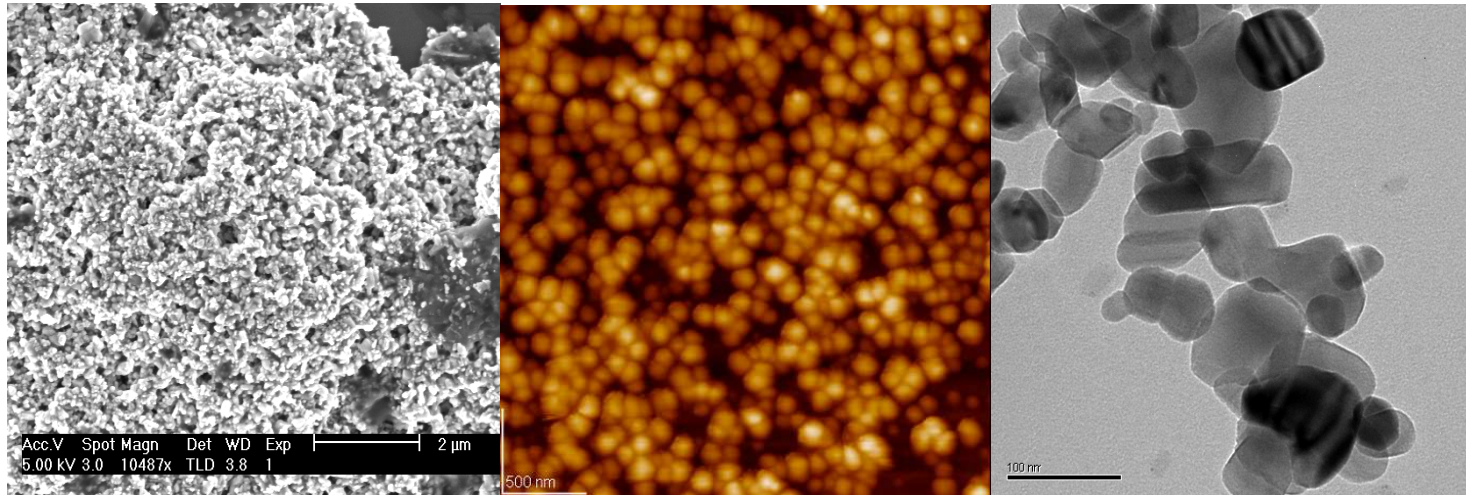
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4 Figure 1. ZnO (1st row) and TiO₂ (2nd row) nanoparticles suspended in distilled water, allowed to dry and imaged in order from left to right
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7 by SEM, AFM and TEM. Initial sizes as stated by the manufacturer (Sigma Aldrich, UK): 50 – 70 nm for ZnO particles and 5 – 10 nm for
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9 TiO₂ particles.
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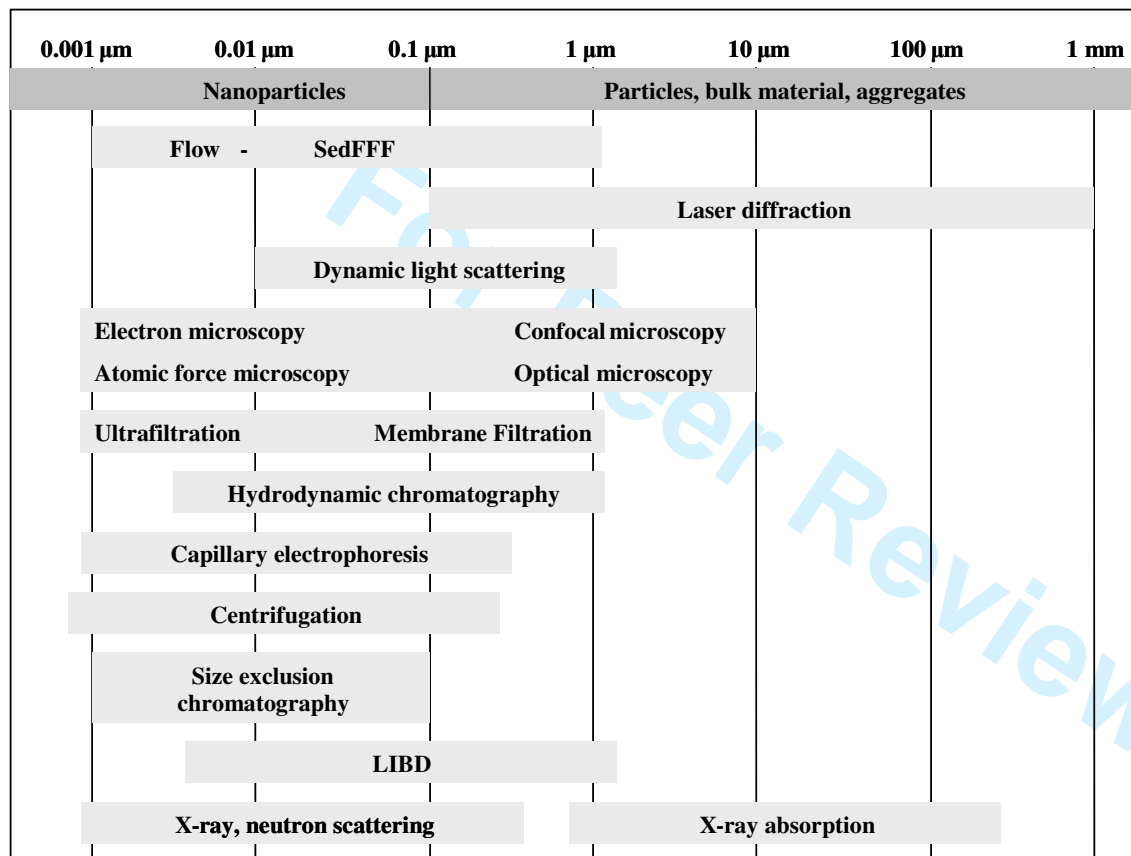


Figure 2. Sizing methods and their size range for nanoparticle measurement. Adapted from Lead & Wilkinson (2006) and Gimbert et al. (2007b).

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)

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

Toxicity study	Nanotype	Reference
In vitro cytotoxicity of oxide nanoparticles	SiO ₂ , Fe ₂ O 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

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 possess 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 anatase as possible crystal

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5 structures of TiO₂)
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7 Surface area

Increase in surface area increases reactivity and sorption behaviour

8 (& porosity)

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10 Surface charge

Surface charge has an influence on particle stability especially in dispersions

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13 Surface chemistry

Coatings can consist of different chemical compositions and influence particle behaviour or toxicity

14 (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. FIFFF-SLS, SedFFFDLS	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	

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

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	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			Brunauer et al. 1938
Capillary electrophoresis	CE		Sensitive, fast, & separation by charge	Mobile phase interactions, complex data interpretation, need of standard material	Electrophoretic mobility Sizing	UV/Vis Fluo MS		Schmitt-Kopplin and Junkers 2003 Chan et al. 2007 Lin et al. 2007

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	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

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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5 Method	6 Acronym	7 Spatial resolution or 8 LOD	9 Advantages	10 Disadvantages	11 Information	12 Possible combination	13 Comments	14 Examples of application/References
15 Dynamic light scattering (photon correlation spectroscopy or quasi elastic light scattering)	16 DLS (PCS, QELS)	17 3 nm - μm particles	18 In situ measurement 19 Rapid and simple analysis, 20 useful to follow aggregation processes,	21 Difficult to interpret results based on intensity weighted sizes. 22 Aggregates dust particles can ruin the measurements on nanoparticles 23 Multiple scattering and particle interactions in high concentrations, 24 limited capability on polydisperse samples.	25 Intensity weighted diffusion coefficient. can be calculated to a z-average hydrodynamic diameter or distribution			26 Huve et al. 1994 27 Bootz et al. 2004 28 Lecoanet et al. 2004 29 Lecoanet and Wiesner 2004 30 Brant et al. 2005a 31 Phenrat et al. 2007 32 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 (potential at 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

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 Hasselov 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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5 Method	6 Acronym	7 Spatial 8 resolution or 9 LOD	10 Advantages	11 Disadvantages	12 Information	13 Possible 14 combination	15 Comments	16 Examples of 17 application/References
18 Filtration			19 Fast 20 Low cost	21 Clogging	22 Size separation			23 Kang and Shah 1997 24 Lau et al. 2004 25 Marani et al. 2004 26 Hett A 2004
27 Fluorescence 28 correlation 29 spectroscopy 30 (Confocal 31 microscopy)	32 FCS	33 ~ 200 nm	34 Dilute samples 35 in small 36 volumes 37 No multiple 38 scattering	39 Only fluorescent 40 samples	41 Diffusion 42 coefficient, 43 hydrodynamic 44 diameter, 45 Concentration	46 Fluorescence 47 labelling		48 Kuyper et al. 2006b 49 Kuyper et al. 2006a 50 Pinheiro et al. 2007 51 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	Sizing	UV/Vis		Scrivens et al. 1994
				Size separation range limited by column	Purification	Voltammetry		Sivamohan et al. 1999
					Quantification	Amperometry		Song et al. 2003
								Song et al. 2004
								Giusti et al. 2005
Hydrodynamic chromatography	HDC	5 – 1200 nm		Mobile phase interactions	Sizing	UV/Vis		Blom et al. 2003
					Size separation	ICP-MS		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

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

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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5 Method	6 Acronym	7 Spatial 8 resolution or 9 LOD	10 Advantages	11 Disadvantages	12 Information	13 Possible 14 combination	15 Comments	16 Examples of 17 application/References
18 Size exclusion 19 chromatography	20 SEC		21 Good separation 22 efficiency, 23 simple	24 Unwanted solvent 25 & column 26 interactions 27 Limited size 28 separation range	29 Separation 30 Sizing	31 DRI 32 FL 33 PDA 34 UV/Vis 35 ICP-MS		36 Huve et al. 1994 37 Zhou et al. 2000 38 Novak et al. 2001 39 Zhao et al. 2001 40 Wilcoxon and Provencio 2005 41 Krueger et al. 2005 42 Wang et al. 2006 43 Helfrich et al. 2006 44 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

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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5 Method	6 Acronym	7 Spatial resolution or 8 LOD	9 Advantages	10 Disadvantages	11 Information	12 Possible combination	13 Comments	14 Examples of application/References
15 Ultracentrifugation 16 (analytical/ 17 preparative)		18 Size range: 19 100 Da to 20 10GDa 21 (molar mass 22 from 23 calibrations)	24 Acceleration: up 25 to 1,000,000 G 26 (9,800km/s ²)	27 Differential 28 settling rates can 29 induce aggregation	30 Sedimentation 31 velocity 32 Sedimentation 33 equilibrium 34 Shape and molar 35 mass 36 Size distribution			37 Bootz et al. 2004
38 UV/Vis 39 spectroscopy	40 UV/Vis		41 In situ	42 Insensitive	43 Quantitative 44 Concentration, 45 some structure or 46 size information 47 can be derived			48 Pesika et al. 2003

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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5 Method	6 Acronym	7 Spatial resolution or 8 LOD	9 Advantages	10 Disadvantages	11 Information	12 Possible combination	13 Comments	14 Examples of application/References
15 X-ray diffraction	16 XRD	17 1 – 3 wt%			18 Structure		19 Especially for	20 Zhang et al. 2003
					21 Sizing		22 crystalline	23 Guzman et al. 2006
							24 nanoparticles	
							25 Bulk	
26 X-ray fluorescence spectroscopy	27 XRF				28 Solid state		29 Aerosols	30 Ortner et al. 1998
					31 speciation			
					32 Quantitative bulk analysis			
					33 Isotope ratios			
					34 Morphology			
35 X-ray microscopy	36 XRM	37 ~ 30 nm		38 Radiation damage	39 Sizing			40 Jearanaikoon and Braham-Peskir
					41 Shape			42 2005
					43 Visualization			

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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 sensitive surface technique	Schrack et al. 2004 Nurmi et al. 2005