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Measuring nanoparticles size distribution in food and consumer products: a review

L. Calzolai*, D. Gilliland and F. Rossi

European Commission – DG Joint Research Centre, Institute for Health and Consumer Protection, Ispra (VA), Italy

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Nanoparticles are already used in several consumer products including food, food packaging and cosmetics, and their detection and measurement in food represent a particularly difficult challenge. In order to fill the void in the official definition of what constitutes a nanomaterial, the European Commission published in October 2011 its recommendation on the definition of 'nanomaterial'. This will have an impact in many different areas of legislation, such as the European Cosmetic Products Regulation, where the current definitions of nanomaterial will come under discussion regarding how they should be adapted in light of this new definition. This new definition calls for the measurement of the number-based particle size distribution in the 1–100 nm size range of all the primary particles present in the sample independently of whether they are in a free, unbound state or as part of an aggregate/agglomerate. This definition does present great technical challenges for those who must develop valid and compatible measuring methods. This review will give an overview of the current state of the art, focusing particularly on the suitability of the most used techniques for the size measurement of nanoparticles when addressing this new definition of nanomaterials. The problems to be overcome in measuring nanoparticles in food and consumer products will be illustrated with some practical examples. Finally, a possible way forward (based on the combination of different measuring techniques) for solving this challenging analytical problem is illustrated.

Keywords: method validation; metals; packaging; water; beverages

Introduction

Nanotechnology is having a large socio-economic impact in many fields of industrial activity: a large number of consumer products containing nanomaterials are already on the market and there are great expectations for the role that nanoparticles-based materials could play in fields as disparate as medical diagnostics and photovoltaic cells. At the moment nanomaterials are also used in a wide range of different applications in the area of consumer products, i.e. potentially coming into direct contact with the general public. The use of nanotechnology-based ingredients additives and contact materials is expected to grow in the near future (Tiede et al. 2008) and in some countries nanomaterials are already used in alimentary supplements and food packaging (Chaudhry et al. 2008).

These very fast developments have spurred scientific and regulatory activities aimed at maximising the benefits of products containing nanomaterials while minimising their potential toxic effects. For example the Organisation for Economic Co-operation and Development (OECD) has set up an international collaboration through the Working Party on Manufactured Nanomaterials (http://www.oecd.org/sti/nano) to advise upon emerging policy issues related to the responsible development of nanotechnology.

The testing activities that have been performed by different research groups and governmental organisations up to now have been developed using a variety of definitions of 'nanomaterial', since an official definition did not exist. Until now nanomaterials have been regarded as materials (Borm et al. 2006) with at least one dimension below 100 nm (Loevestam et al. 2011).

In this context, the European Commission (2011) published its recommendation on the definition of nanomaterials. The recommended definition states that:

> Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm.

Thus, to detect (and measure) the presence of nanomaterials in food according to this definition requires analytical methods able to determine the number size distribution of particles at least in the 1–100 nm size range.

*Corresponding author. Email: luigi.calzolai@jrc.ec.europa.eu

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Table 1. Metal and metal oxide compounds in food and food-contact materials.

Material	Classification (E number)	Uses
Titanium dioxide	E171	Colouring agent (white)
Iron oxides	E172	Colouring agent (various colours)
Metallic silver	E174	Labelling colour for food packaging
Metallic gold	E175	Colour
Silicon dioxide	E551	Anticaking agent

This paper will address the known (or proposed) uses of nanomaterials in the food sector and give an overview of the available analytical techniques that could be used to measure the number size distribution of nanoparticles in food. Finally it will critically review the pros and cons of the various techniques and propose a framework for developing an integrated approach to the development of a robust analytical platform for the detection of nanoparticles in food and consumer products. This activity is of particular importance and urgent in view of the recent European Cosmetic Products Regulation that requires labelling nanomaterials in the list of ingredients (European Parliament and European Council 2009), and the regulation on food information to consumers (Regulation EU No. 1169/2011).

Overview on nanoparticles in food, food packaging and cosmetics

Information on the use of nanomaterials in consumer products including food and food-related products at the moment is somewhat sketchy and hypothetical as, up to now, there are no legal requirements for reporting such information to consumers and/or regulatory authorities. The year 2013 will see entering into force the new European regulation EC 1223/2009 on cosmetic products that requires the labelling of ingredients present as nanomaterial. The application of nanotechnologies in the food industry, and its implication in terms of risks and regulation, has been recently reviewed by Cushen et al. (2011). They concluded that progress in the field may be stifled by lack of governance and potential risk.

The use of nanomaterials in the food industry can be divided in three broad categories: food, food additives and food packaging with various products for each category already on the market (Blasco and Picò 2011). For example, a recent market research report on 'Nano-enabled packaging for the food and beverage industry' (Innovative Research and Products Inc. 2009) estimated that the total nano-enabled food and beverage packaging market to have been US$4.2 billion in 2009 and predicted an annual growth rate of around 11% for the next years. Another very recent review on the detection of organic nanoparticles in food by Peters et al. (2011) points out the use of nanotechnology for food-processing applications, such as the ceramic NanoGlaze in cookware to prevent food sticking. Recognising the developments in the field and the possible applications of nanotechnology to the food sector the European Food Safety Authority (EFSA) published in 2011 the first practical guidance for assessing nano applications in food and feed.

Some metal and metal oxides compounds approved as food additives are also produced in nano forms and are currently used in different consumer products. Table 1 reports some of these compounds with their E-number classification, properties and possible use in the food sector. Titanium dioxide, silicon oxide and metallic silver are probably the most widely used nanoparticles currently on the market. Although there is little reported evidence of the deliberate use and presence of nanoparticles in commercially available food and food packaging, there are several food-grade nanoparticle products on the market (such as food-grade silica) and thus their presence in some alimentary products can be considered as being likely. However, they are also used in cosmetics, where titanium dioxide particles smaller than 100 nm in size are used as transparent sunscreen blockers in contrast to 'normal' white titanium dioxide.

Techniques to measure nanoparticles size

The characterisation of nanomaterials and nanoparticles is not a trivial task, especially in the case of materials in complex matrices (such as food) and below the 100 nm size limit. At the moment, to the best of our knowledge, there is no single technique able satisfactorily and routinely to measure the number particle size distribution of objects in the 1–100 nm size range. In practice the only method that is technically capable of addressing the need to count and size particles in both free and agglomerated states is transmission electron microscopy. Unfortunately this method has a major disadvantage in terms of cost and complexity and in practice it is unlikely to be suitable for undertaking numerous routine measurements.

Based on a recent literature survey – searching peer-reviewed articles on Science Direct (http://www.sciencedirect.com) combining nanoparticle and sizing technique – the most cited techniques used for measuring the size of nanoparticles are: electron microscopy (cited in around 85% of the cases), laser light-scattering (around 10% of citations), and field flow fractionation, centrifugation techniques, particle tracing analysis (combined all together in around 4% of cases). These techniques, together with an emerging technique called single-particle ICP-MS, show the potential to partially address the measurement of the number size distribution of nanoparticles and will be discussed in the following sections.

Electron microscopy

Performing a search on the techniques used for the size characterisation of nanomaterials using peer-reviewed literature (SciFinder database at http://www.scifinder.cas.org) indicates that the most cited techniques are electron microscopy based: either scanning electron microscopy (SEM) or transmission electron microscopy (TEM). These techniques, while very accurate, require some sample preparation: samples have to be adsorbed on a grid surface and any volatile solvent has to be removed to permit their introduction into the high-vacuum environment common to the majority of SEM and TEM instruments (Dudkiewicz et al. 2011). The problem of sample drying can potentially be reduced by the use of so-called 'environmental' SEM/TEM systems which are more tolerant of water or solvent containing materials (Lorenz et al. 2010).

These solutions, although feasible, use complex and expensive equipment that also requires highly specialised technical support. On the positive side, electron microscopy techniques can handle samples containing mixtures of nanoparticles not only of different sizes, but also of different shapes – a characteristic which is not measurable by most other methods. Furthermore, electron microscopy provides true particle size measurements (see example in Figure 1), while optical, chromatographic and centrifugal methods determine the apparent hydrodynamic size. This difference, between true and hydrodynamic diameter, must be recognised and taken into consideration as it will lead to an overestimate of particle size with respect to true particle size for spherical particles. In the case of non-spherical particles, the non-imaging methods cannot provide any reliable measure of size without detailed prior knowledge of particle shape data which can only be determined by electron microscopy.

For this situation there are already available specialised image analysis software which can be adapted partially to automate the labour-intensive process of measuring the size of the relatively large number of particles needed for meaningful statistical analysis of the results (Tiede, Tear, et al. 2009). Time, complexity and cost may prohibit the routine use of electron microscopy for determining nanoparticle size distributions, but the technique may instead provide the means to produce well-characterised standards which could then assist in calibrating the more accessible optical or chromatographic method.

Laser light-scattering techniques

Of the techniques that can directly work with liquid samples, the most widespread methods are based on laser light-scattering techniques: the most used are either known as photon correlation spectroscopy (PCS) or dynamic light-scattering (DLS) (Brar and Verma 2011). These techniques are quite simple to use, fast, relatively low cost, but while performing well when dealing with samples of nanoparticles of a single size (monodispersed) they can give misleading results when used to analyse samples containing particles of different sizes. For example, the authors have recently shown that using dynamic light scattering to measure a mixture of gold nanoparticles of 5, 15 and 45 nm (with a 350:15:1 number of particles ratio) practically shows only the presence of gold particles of 45 nm (Calzolai et al. 2011). These data highlight that while this technique can be applied to both diluted and concentrated solutions (Kaszuba et al. 2007), it has a limited ability to resolve mixtures of particles of different sizes. This inability to detect the presence of smaller particles among bigger ones is due to the fact that the scattering intensity depends by the sixth power of the particle radius (Berne and Pecora 2000; Gun'ko et al. 2003)

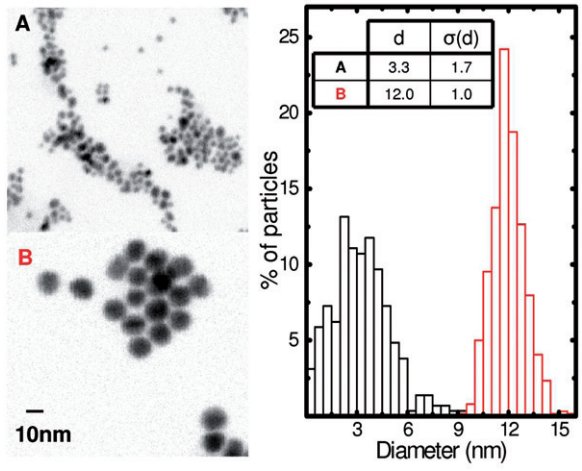

Figure 1. Scanning electron microscopy (SEM) analysis of two samples of citrate-stabilised gold nanoparticles of different sizes: (A) SEM micrograph of 3 nm gold nanoparticles; (B) SEM micrograph of 12 nm gold nanoparticles; and (C) particle size distribution obtained by semiautomatic image analysis of sample A (left) and B (right).

and thus larger particles tend to cover the signal coming from smaller ones. In practice, this limits the size resolution to the point where reliable separation of particles can be achieved only when there is a factor of 3–4 difference in size.

A laser diffraction-related technique, particle tracking analysis (PTA), exploits two important physical phenomena of (nano)particle behaviour when suspended in a liquid: the ability of individual particles to scatter light and the characteristic movement of particles produced by the effect of Brownian motion. A specially designed optical cell containing a dilute solution of particles is illuminated with a laser light source and, using an optical microscope, the pin-points of light scattered by the rapidly moving particles or aggregates are observed and recorded using a highly sensitive video camera. The video image obtained can be analysed so as to allow particles to be located, individually identified, and their movements and trajectories followed on a frame-by-frame basis. Since the diffusion of each individual particle depends only on liquid viscosity, temperature and particle hydrodynamic diameter, it is possible, through the Stokes–Einstein equation, to calculate the particle size. When the movements of a statistically relevant number of particles in a population are evaluated over a suitable lapse of time, reliable statistics for particle number–size distributions can be obtained. Since this technique calculates particle size on a particle-by-particle basis, it is effective in overcoming the inherent weaknesses of the DLS and SLS methods when confronted with mixtures of relatively similarly sized particles. This method has a number of important advantages including relatively low instrument cost and high sensitivity which can detect nanoparticles at concentrations as low as low as 10^6 particle / cm^3 (Gallego-Urrea et al. 2011).

These advantages are unfortunately contrasted by a number of important disadvantages when considering its application to the detection of nanoparticles as specified in the recommended definition. The first disadvantage is that the process of image analysis requires a significant input from the operator, and as such may be subject to accidental biasing towards larger or smaller particles. The second, and probably most important, disadvantage of this technique is that it has a fundamental limitation on the lowest particle size that can be detected. The scattering of light by a particle in solution depends on a number of factors including the wavelength of the scattering light, the refractive indices of the particle and liquid and, most importantly, the size of the particles. In practice the effective lower limit is dependent on a combination of these factors, but detecting particles below 20 nm becomes problematic for many materials other than those with high refractive indexes such as gold or TiO_2. It should be noted that this limitation has been reduced by recent advances in laser diode technology which have resulted in the availability of powerful, stable near-UV light sources which can be used as an alternative to the red light sources more commonly used in this technology. The use of shorter wavelength light permits more efficient detection of small particles but reaching the 1 nm limit in the definition remains beyond the capabilities of this technology.

Centrifugation-based techniques

Other interesting techniques, even if not very widely cited in the peer-reviewed literature, are centrifugal particle sedimentation (CPS) and analytical ultracentrifugation (AUC). The major feature of these techniques compared with the previously mentioned optical methods is that they are more effective in dealing with particle size mixtures. In the case of CPS and AUC the principle of operation is dependent on the fact that particles generally have a density which is different (usually higher) than that of the liquid in which they are suspended and consequently gravity or centrifugal forces will tend to make them sink or float in the liquid (Kamack 1951; Coelfen 2004). In CPS and AUC, particles suspended in a liquid are subjected to a centrifugal force induced by the acceleration of a rotor that tends to move the particles through a disc or column of liquid in the direction of the force. Since this force is proportional to particle mass (and consequently also to density), the larger particles sediment faster than smaller ones (provided the mass of the particle is greater than the displaced mass of the solvent). The buoyant force (governed by the Archimedes' principle) and frictional force act in opposite direction to the centrifugal force, impeding sedimentation. The frictional force is generated by movement of the solute through the solvent according to the hydrodynamic treatment of viscous drag and is proportional to a frictional coefficient and the solute terminal velocity. The three forces come into balance very quickly (within approximately 10^{-6} s) and the particle achieves terminal velocity that is related to particle size. This process of sedimentation can be modelled by a theoretical treatment of thermodynamic and hydrodynamic principles which, when combined with experimentally determined particle sedimentation times, can be used to determine particle size.

These two techniques offer two great advantages when compared with the DLS optical methods. The first advantage is the much better size resolution such that particle size can be determined with an accuracy better than 2–3 nm in the range of interest for the definition. The second advantage is that as the particles sediment, they are effectively separated in size before passing though a (usually optical) detector which can potentially be calibrated to give a quantified measure

of the amount of material of any given size passing through the field of the detector (Carney et al. 2011). Unfortunately these methods also have some important limitations that must be considered. While both methods operate on basically similar principles, the equipment used for AUC permits access to much higher centrifugal forces (>100,000 *g*) which allows the more rapid sedimentation of small particles. This difference becomes critical when considering small, low-density particles, which in the case of CPS may sediment very slowly or in extreme cases may not sediment at all. In practice, this may limit CPS to a minimum size of 3–5 nm for dense particles such as gold ($\rho = 19.3\,g/cm^3$) and as much as 20 nm for amorphous silica ($\rho = 1.8$–$2.2\,g/cm^3$). This limitation may be overcome in AUC by the higher centrifugal forces, but the accuracy of this method may be compromised by another complication that is common to both methods: as previously described, the sedimentation process depends on the mass of the particles, therefore on their size and density. Thus, without a reasonable knowledge of particle density, it is not possible to calculate accurately the size from sedimentation data. This problem becomes particularly relevant in any sample where there is the formation of aggregates since even in the case of primary particles with known density but with a tendency to aggregate, it will be difficult to know the exact density of those aggregates. This problem is further exasperated when it is necessary to consider the potential presence of agglomerates, which are also relevant to the definition.

Single-particle ICP-MS

Ion-coupled plasma mass spectrometry (ICP-MS) is an ideal method for measuring traces of inorganic elements. Operated in the 'single particle' mode (SP-ICP-MS), the technique allows the detection of individual nanoparticles without prior digestion. SP-ICP-MS is particularly interesting and is the object of intensive development. It consists in measuring the signal emitted by the vaporisation of the nanoparticles in the inductive plasma with a time resolution of typically 10–20 ms. Instead of an average background proportional to the concentration of the element present in the sample, sharp peaks are analysed, whose intensity depends on the size of the nanoparticle in the sample. A size distribution can thus in theory be reconstructed. The technique has been developed and modelled and it was found that the signal depends mostly on the fraction of NP reaching the plasma zone, a parameter difficult to access and setup. The technique has been recently applied successfully to the analysis of silver nanoparticles in wastewater (Mitrano et al. 2012), but there are still problems to be resolved and it is not clear at this stage how general the use of SP-ICP-MS could become.

Field flow fractionation

The final method that will be considered is field flow fractionation (FFF), which is a one-phase chromatography technique (schematically shown in Figure 2). The flow and sample are confined within a channel consisting of two plates separated by a spacer foil. The upper channel plate is impermeable, while the bottom channel plate is permeable and made of a porous frit material covered with an ultra-filtration membrane that retains the sample in the channel. A syringe pump is connected to the lower channel plate and when operated it can pull liquid through the dialysis membrane and out of the flow channel.

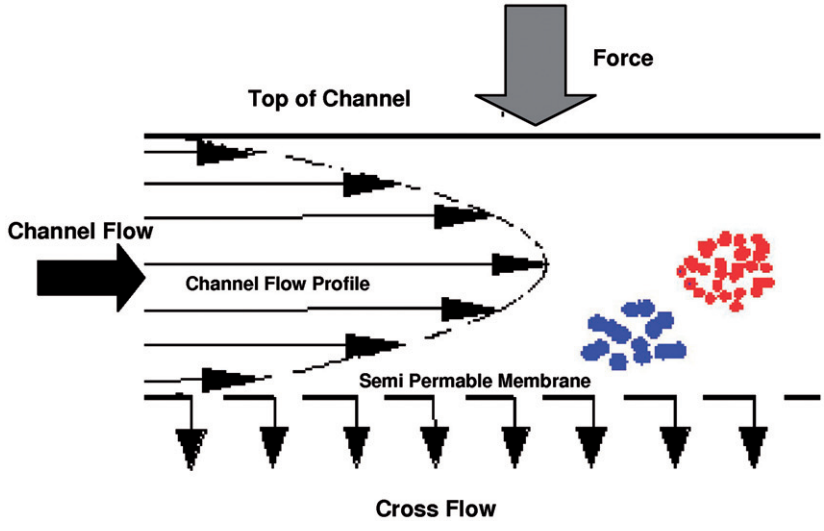

Figure 2. Schematic view of a flow field flow separation channel showing smaller particles moving closer to the centre of the channel in a higher velocity zone and thus existing before larger particles.

Table 2. Qualitative evaluation of the relative advantages and disadvantages for different techniques to measure the size of nanoparticles in the 1–100 nm size range.

	SEM	TEM	FFF	CPS	PTA	AUC	DLS	SP-ICP-MS
Minimum size	++	+++	+++	+	+	++	+++	+
Dynamic range	+++	++	++	+++	+	++	+++	++
Accuracy of measure	++	++	+	+	+	++	+	+
Suitable for mixtures	+	+	++	++	++	++	−	++
In-situ measure	−	−	+	+	++	+	++	++
Ease of use	−	−	+	++	+	+	++	+
Cost	−	−	++	++	++	+	+++	+

Notes: Different properties are evaluated as: excellent (+++), good (++), fair (+) and insufficient (−).
AUC, analytical ultracentrifugation; CPS, centrifugal particle sedimentation; DLS, dynamic light-scattering; FFF, field flow fractionation; PTA, particle tracking analysis; SEM, scanning electron microscopy; SP-ICP-MS, single particle inductively coupled plasma; TEM, transmission electron microscopy.

A laminar flow of the liquid within the flow channel produces a parabolic flow profile in which the stream moves slower closer to the boundary edges than it does at the centre of the channel flow. When the syringe pump creates a perpendicular force field (cross-flow) across the flowing, laminar stream, the analytes are driven towards the boundary layer or 'accumulation wall' of the channel.

Diffusion associated with Brownian motion, in turn, tends to counteract this motion. Particles with a smaller mean hydrodynamic diameter, which have higher diffusion rates, tend to reach an equilibrium position closer to the centre of the channel, where the longitudinal flow is faster. Thus, the velocity gradient flowing inside the channel separates the different sizes of particles, with smaller particles eluting before the larger ones (Giddings et al. 1976). The flow of liquid eluted from the separation channel can then be introduced into any one of a variety of different instrument for the detection and characterisation of the size-separated particles.

Since the FFF technique is a size separation method that can be coupled to a wide variety of possible particle detectors, the detection limit depends mainly on two factors: (1) the detector chosen and (2) any loss of materials that can occur by irreversible absorption of particles onto the dialysis membrane. Common detection methods are UV absorption, refractive index, multi-angle light-scattering (MALS) as well as fluorescence. In the case of inorganic nanoparticles, the method may be combined with on-line ICP-MS, which can provide direct quantification of particle quantity as function of size. In this case, the combination of FFF with ICP-MS would seem to be one of the most promising routes for high sensitivity detection and quantification of nanoparticles according to the recommended definition. The FFF is a highly promising technique (von der Kammer et al. 2011): it can separate and measure complex mixtures containing NP of different sizes, it can separate particles down to 1 nm size, it has an excellent dynamic range, and the various components can be recovered for further analysis. This possibility of recovering the size-separated components offers an important opportunity for further characterisation of the nanoparticles, such as for example of the NP–protein complexes as in the case of biological systems (Laera et al. 2011).

FFF suffers from a limited precision in measuring the absolute size of NP if the measurement is determined on the basis of the particle elution time from the separation channel. Fortunately, this limitation of FFF can be largely overcome if the technique is used in combination with other sizing techniques such as light scattering. In this case, the light-scattering methods can be more reliably used for particle sizing since the FFF ensures that they are presented with solutions of near monodispersed particles rather than complex mixture of sizes.

One of the main limitations of FFF is that the separation process does not distinguish between nanoparticles and aggregates of the same size nor can it distinguish particles with different shapes which may have the same mean hydrodynamic diameter. These considerations suggest the need to use different techniques sensitive to different NM properties (for instance FFF sensitive to the hydrodynamic diameter, and CPS sensitive to size and density). In addition, the interaction of nanoparticles with the dialysis membrane could cause problems, and care should be taken into optimising the experimental conditions, such as the material of the membrane and eventual addition of surfactants to the cross-flow buffer.

Comparison of methods

A qualitative comparison of the different techniques is shown in Table 2, which reports the suitability of the different techniques with respect to several parameters that the authors consider critical for measuring the number size distribution as dictated by the new definition of nanomaterials. Table 2 indicates that

electron microscopy-based techniques (SEM/TEM) are probably the most accurate and universally applicable techniques but they suffer from the need of extensive sample preparation that can potentially introduce bias in measuring the particle size distribution relative to the starting sample. They are also quite expensive to acquire and run and are not amenable even to medium levels of throughput. Centrifugal particle sedimentation (and to a lesser extent analytical ultracentrifugation) could represent a lower-cost alternative to electron microscopy techniques. Unfortunately CPS, in the case of low-density particles, may not be able to detect particles much smaller than 20 nm and the different components cannot be recovered after separation.

FFF is a promising alternative, especially if used in combination with sizing techniques such as light-scattering methods.

It must be noted that most of the existing work has been performed on pristine nanoparticles in 'simple' matrices, such as water and water plus buffer systems. The challenge in the case of real systems is much more complex due to the huge variety and composition of food matrices in which nanoparticles could be embedded and on the large range of concentrations that need to be analysed.

Analysis of nanomaterials in food products and cosmetics

Several reviews have covered both the available analytical techniques for identifying and characterising NP in food (Luykx et al. 2008) and in complex matrices in general (Tiede et al. 2008). Very recent reviews have also covered the first examples of applications to the analysis of NP in food matrices both in general (Blasco and Picò 2011) or targeted towards the use of specific techniques such as flow FFF (von der Kammer et al. 2011), particle tracking analysis (Gallego-Urrea et al. 2011) and electron microscopy (Dudkiewicz et al. 2011). These reviews provide an excellent overview on the use of the different techniques to detect nanoparticles in complex matrices and we refer to those references for an in-depth analysis of each technique. The general review of Blasco and Picò (2011) reports in their Table 4 some examples of applications for detection and characterisation on nanomaterial in food and food-like matrices. The reported examples used a large variety of different experimental techniques, and in most cases they were limited to simple detection thus underlining the inherent difficulty of measuring the size of NP in food matrices.

Among the different techniques, FFF (coupled to various detectors for NP sizing and identification) seems to emerge as one of the few techniques (probably together with TEM) able to measure the size distribution in the case of samples containing nanoparticles of different sizes in the 1–100 nm range, even if the limited number of examples suggests a cautious approach. A detailed analysis of the use of FFF for the characterisation of NPs in different matrices was carried out by von der Kammer et al. (2011); they concluded that while there are already several examples of using FFF for NPs in environmental samples, the literature dealing with NPs in food samples is relatively scarce. There are examples of the use of FFF for the analysis of milk suspensions (Saeseaw et al. 2005) or liposomes (Hupfeld et al. 2009).

In one excellent work, Schmidt et al. (2011) coupled FFF with light-scattering detection (DLS and MALS), and with ICP-MS, to measure quantitatively the size distribution of mixtures of gold and polystyrene nanoparticles (with diameters in the 10–100 nm size range) in both water and biological samples. In particular, rats were exposed to mixtures of gold NP of 10 and 60 nm, and their livers were subsequently analysed to detect the presence of gold NP. Even with extensive sample treatment (homogenised liver were treated with tetramethyl ammonium hydroxide), the separation of AuNPs by FFF was not possible due to the association of AuNP with undissolved biological tissues. This example just shows the challenges in the sample treatment that will be encountered in the development of methods for detecting NP in solid food matrices.

The literature survey on the detection and characterisation of NM in food shows that, up to now, a very limited number experimental studies have actually been reported and that these publications each adopt different experimental approaches and techniques applied to a large variety of materials in different food matrices. Almost all the published literature related to NP in food has been mostly concerned with the detection and characterisation of the nanomaterial and we did not try to address the much more challenging task of determining the number size distribution of the primary particles, including those present in the form of agglomerates/aggregates. The few examples that successfully tackled the measurement of the particle size distribution did so using simplified systems such as mixtures of gold nanoparticles of different sizes in water by using FFF for separation and light-scattering and electron microscopy for size measurement (Calzolai et al. 2011; Schmidt et al. 2011). Using a similar analytical platform, it was possible to determine the partial particle size distribution of titanium dioxide in sunscreen lotions (Contado and Pagnoni 2008; Samontha et al. 2011).

One recurring theme in the available literature is the need for extensive, carefully executed and documented sample preparation. In fact, irrespective of the analytical technique used, a proper measurement of

nanoparticle size usually requires a simplification of the matrix into which nanoparticles are embedded. To this end two approaches can be used: either extracting the NPs from the embedding matrix or removing (or at least simplifying) the complex matrix. If any sample preparation treatment is used, it will be necessary to control that the treatment method does not modify the original particle size distribution.

Another challenge is posed by the need to distinguish between engineered nanoparticles that have been added on purpose to the food material and naturally occurring nanoparticles. Different types of food naturally contain particles below the 100 nm size, e.g. milk contains lactoglobulin proteins, the properties of which clearly fit the definition of nanomaterials. In most cases the techniques used to measure the size of particles cannot provide unambiguous data about their chemical identity. In this case some other techniques will be needed to discriminate between engineered nanoparticles and naturally occurring nanoparticles.

Measuring particle size distribution in complex matrices

The available, peer-reviewed, experimental work described above was mainly attempting to detect and characterise NP in complex matrices. The task at hand to fulfil the requirement of the European Union definition of what constitutes a nanomaterial is much more challenging than that: it effectively requires the measurement of the size distribution in terms of the number of primary particles, including those present in agglomerates, across the whole particle size range from 1 nm upwards. Given these stringent requirements there are few techniques apart from electron microscopy that can directly size and count particles and none which can effectively deal with counting primary particles within agglomerates. If the simplified situation of a sample free from agglomerates is considered, then one possible technique for direct sizing/counting could be single-particle detection using ICP-AES and ICP-MS. These techniques have been used for direct analysis of individual nanoparticles (Suzuki et al. 2011) and cells. Very recently single-particle ICP-MS has been used to measure the number concentration of silver nanoparticles of different sizes (Laborda et al. 2011).

The overall picture that emerges from reviewing the available techniques for measuring particle size distribution is that, most probably, simple techniques will not work.

Further challenges for full method development

It is likely that many sample types will not be suitable for instrumental analysis without some degree of pre-treatment. While varying degrees of pre-treatment could be envisioned, it is likely that as a minimum most samples will be subjected to varying degrees of ultrasonic treatment to homogenise the sample and to ensure the breaking up of any loosely agglomerated material. In principle this should be quite simple, but experimental studies show that the re-dispersion of even simple powders of nanomaterials can give very different results depending on the treatment used. This can be illustrated in the examples in Figure 3, which shows the results obtained from re-dispersion of nano-ZnO powders using centrifugal particle sedimentation. When nano-ZnO is added to pure water and treated

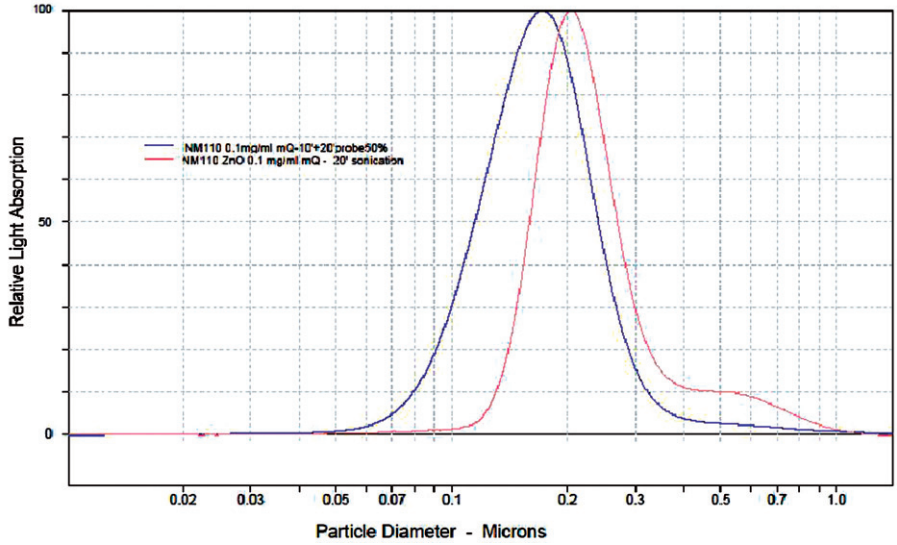

Figure 3. Size distribution obtained by CPS analysis of a ZnO sample dissolved in water and treated with ultrasonic probe for varying time periods of 20 s (right curve) or 30 s (left curve).

with an ultrasonic probe for only 20 min, the CPS data shows the sample as being effectively non-nano, while a relatively minor increase in ultrasonic treatment to 30 min shows a clear shift of the size distribution to smaller values with easily detectable sub-100 nm material which, on a number basis, may well require this material to be reclassified as being nano.

In another example shown in Figure 4, CeO_2 nanopowder was re-dispersed in water by firstly vortexing and then using ultrasonic treatment for increasing amounts of time. In this case it can be seen that while simple vortexing can produce a nano-dispersion, it is also clear that brief short and medium (10 min and 10 + 20 min) treatments can further reduce the mean particle size. On the contrary longer ultrasonic treatment (10 + 20 + 40 min) actually begins to produce deleterious results with a clear increase in the apparent mean particle size, larger even that after simple vortexing. This behaviour is likely due to the onset of particle fusion caused by an excessively high total input of ultrasonic energy. While this effect is not unknown, it serves in this case to illustrate that even an apparently simple single-step process, ultrasonic homogenisation, must be carefully evaluated and optimised to ensure that it is assisting the measurement process and not altering the results to the point of producing a false-negative result.

These two examples, although very simple experimentally, serve to illustrate that in addition to the challenges of developing the instrumental measuring methods, much effort will be required to define material pre-treatment procedures without artificially distorting the original size distribution of the particles.

Outlook

The ideal fit-for-purpose analytical method should have the following characteristics:

- Be sensitive enough to measure a low concentration and be able to analyse the size distribution and properties of NP in addition to concentration.
- Minimise sample disturbance to ensure that laboratory analyses reflect the unperturbed environmental state.
- Be non-destructive.
- Be able to analyse samples of diverse elemental compositions and samples containing more than one type of nanoparticle.
- Be able to provide a wide size-separation range.

A possible solution towards an analytical platform able to measure the number particle size distribution in complex mixtures or matrices is the use of a multi-step approach (Tiede, Hassellöv, et al. 2009) where different building blocks are used in a sequential order. These building blocks would be: size separation, followed by size measurement, and particle quantification and identification (Figure 5). For the size separation step

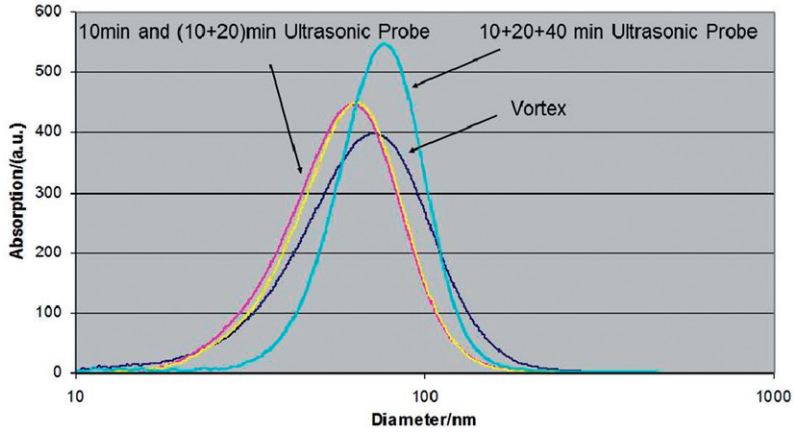

Figure 4. CPS analysis of a CeO_2 sample re-dissolved using simple vortexing (blue line) or a combination of vortexing and ultrasonic treatment for 10 s (yellow line), 30 s (pink line) and 70 s (cyan line).

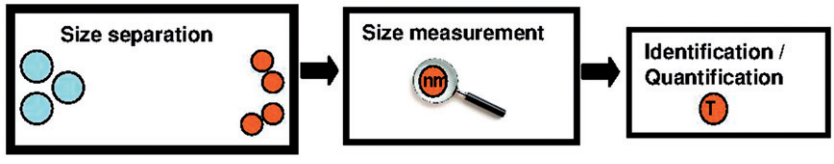

Figure 5. Building blocks for an analytical platform to measure the number size distribution of nanoparticles.

different techniques able to separate particles based on size could be used, such as: size exclusion chromatography, hydrodynamic chromatography, field flow fractionation, electrophoresis and ion mobility techniques. The measurement of size on the nanometer scale could be performed by either dynamic light scattering or static light scattering. Finally for the particle identification some possible techniques that have been already used for the identification of NP are: ion-coupled plasma mass spectrometry, atomic emission spectroscopy and electro-spray ionisation mass spectrometry.

The combination of some of these techniques has already been tested and has given quite promising results. For example, by combining FFF with DLS we were able to separate and correctly quantify the relative number of particles of a mixture of gold nanoparticles of 5, 15 and 45 nm (Calzolai et al. 2011). Directly coupling FFF with MALS detection and ICP-MS Schmidt et al. (2009) measured the migration of nanoclay nanoparticles from a biopolymer composite to the ethanol liquid phase used as food simulant.

An important factor in accelerating the development of methods for detecting specific nanomaterials in food (and other complex matrices) would definitely be the availability of standard reference materials, ideally matrix based. In addition, the whole process would be helped by the availability of databases with information about the chemical composition, main characteristics and uses of nanomaterials present in consumer products.

Conclusions

All the instrumental techniques providing size distributions have advantages and drawbacks. At the moment there is no single technique that can by itself provide a robust analytical method, especially considering the need to measure the number size distribution of nanoparticles introduced by the definition of nanomaterials. The most likely solution to this problem will be to use combinations of instruments, each with a different physical principle of operation, and in this way complement the weaknesses of each instrument with the strengths of another.

Finally, the association of separation and size measurement, and particle identification analysis is particularly promising, by alleviating the difficulties inherent to size distribution measurement alone. In any case, one should not underestimate the measurement of nanoparticle size particularly when complex media are involved and requiring appropriate sample preparation, i.e. separation of nanomaterials from the matrix.

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