Challenges in the Identification of Engineered Nanomaterials in Foods

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ABSTRACT

Engineered nanoparticles and/or nanomaterials (ENs) could be present in food as ingredients or additives, but also as contaminants from the environment or from food contact materials. Several methods have been developed to detect ENs in simple matrices. However, a number of challenges arise when analysing food and beverages for ENs. In contrast to simple matrices, food has a complex composition, is hetero-dispersed and may contain more than one type of ENs. When analysing ENs in foods, it is therefore of utmost importance to determine in addition to the particle-size distribution the chemical composition as well as the physical and chemical properties of the ENs within the sample. No single technique can provide all relevant information. Therefore, a range of analytical techniques is required for detection and characterisation of nanomaterials in foods. However, many analytical techniques are destructive and therefore a certain sample cannot be analysed twice or by more than one technique. Furthermore, sample preparation methods very often lead to artefacts or changes of the ENs. The nature of ENs can also change over time; e.g., particles could interact to form agglomerates or particles could dissolve. Additional problems arise because it is almost impossible to distinguish between natural and engineered nanomaterials and food-grade reference material is often lacking. In addition, the analytical techniques should be sensitive enough to measure low concentrations, as ENs in a food represent only a small part of the total mass. Furthermore different analytical methods such as SEM, SLS, DLS, BET or hydrodynamic chromatography lead generally to different size distributions and average particle sizes. To illustrate some of the difficulties arising when analysing food for the presence of nanoparticles light scattering techniques were used as an example to analyse simple particle systems.

Keywords: nanomaterials; nanoparticles; detection; characterisation; laser light scattering analysis; food

INTRODUCTION

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which is expected to impact almost all areas of daily life. Since the 1980's electronics has been a leading commercial driver for nanotechnology R&D, but other areas including materials, biotechnology and energy are of significant and growing importance. Over the last few years applications of nanotechnology to materials that are determined for direct contact with humans and animals have become more apparent. Pharmaceutical products, dietary supplements/nutraceuticals and food are some examples. In food nanomaterials could be found according to its proper use as ingredient or additive but they might also appear as contaminants from the environment or from food contact materials. Therefore the development of analytical methods for the characterisation and/or detection of these materials is essential.

In spite of the complexity of the problem (determination of size, structure, surface properties, material interactions, etc.) analytical methods for the characterisation of isolated (mostly inorganic) nanomaterials are available. An overview on several analytical techniques is given in table 1. Detailed descriptions of the different techniques can be found in the respective literature [1-8]. The techniques listed in table 1 are suitable for routine characterisation of isolated nanomaterials, and in some cases for nanomaterials, which are dispersed in simple homogeneous media. However, when characterising particle mixtures in heterogeneous food matrices or in physiological media significant problems may arise. In addition, several of the above mentioned methods have to be applied for a comprehensive sample characterisation.

Intentionally added nanoparticles in food are mostly lipid based products, however also proteins and polysaccharide as well as some inorganic particles could be found. The detection of these engineered nanoparticles along with other particles which are part of the food matrix causes a number of difficulties.

Table 1. Analytical Methods for nanomaterial characterisation.

Techniques	**Methods**	**Acronyms**
imaging techniques	Atomic Force Microscopy	AFM
	3 D Atom Probe Field Ion Microscopy	3D APFIM
	Confocal Laser Scanning Electron Microscopy	CLSM
	(Environmental) Scanning Electron Mikroscopy	(E)SEM
	PhotoEmission Electron Microscopy	PEEM
	(Scanning) Transmission Electron Microscopy	(S)TEM
	Scanning Tunneling Microscopy	STM (RTM)
spectroscopy	Auger Electron Spectroscopy	AES
	Electron Energy Loss Spectroscopy	EELS
	Energy Dispersive X-Ray Spectroscopy	EDXS
	Electron Paramagnetic Resonance	EPR (ESR)
	Acoustic /Electroacoustic Spectroscopy	ESA
	Raman Spectroscopy	IR / Raman
	Positron Annihilation Spectroscopy	PAS
	X-Ray Photoelectron Spectroscopy/Ultraviolet Photoelectron Spectroscopy	XPS/UPS
diffraction techniques	Dynamic Light Scattering	DLS
	Low Energy Electron Diffraction	LEED
	Small Angle-/ Wide Angle- X-ray Scattering	SAXS/ WAXS
	Static Light Scattering	SLS
	X-Ray Diffraction	XRD
other techniques	surface area analysis (Brunauer Emmett Teller)	BET
	centrifugation/ultra-centrifugation	
	Field Flow Fractionation	FFF
	Size Exclusion Chromatography	SEC
	Superconducting Quantum Interference Device Magnetometry	SQUID
	X-Ray Reflectivity	XRR

In order to detect and characterise nanomaterials in food, a sample preparation is inevitable. Sample preparation is dependent on both, the analytical method used and the nanomaterial present in the sample. The simplest preparation methods are obviously a pure dispersion/dilution of the food sample or an enrichment of the particles to be detected. In most cases, however, isolation of the nanomaterial from the food matrix prior to characterisation is needed. Common preparation techniques to separate nanoparticles from food matrices are centrifugation, filtration, field-flow-fractionation (FFF) or hydrodynamic chromatography (HDC). Very detailed descriptions of analytical and preparation techniques can be found in the reviews of Tiede [8], and Luykx [5]. For the discussion of the results obtained it should be always kept in mind that every sample preparation changes the environment of the nanoparticles and this could lead to the formation of agglomerates/aggregates or to modification of the surface of the nanoparticle itself. Furthermore, different analytical methods result in different particle sizes of the nanoparticles to be analysed. Dynamic light scattering (DLS), for example, delivers the hydrodynamic diameter whereas Scanning Electron Microscopy

(SEM) determines the “electro-optical” diameter. Some of these problems have already been discussed by Bonnaire [9] and Coupland [10].

Milk, a relative simple food matrix, was chosen here to illustrate the problems in detecting and characterising nanomaterials in food materials. Furthermore colloidal SiO_2 was added to milk at various concentrations and considered as a model system for studying the problematic related with the detection of EN in food.

MATERIALS & METHODS

Commercially available UHT-milk (skimmed 0.1 % fat and semi-skimmed 1.5 % fat) as well as a commercially available 50 %-colloidal alkaline stabilised amorphous silica formulation were used for particle size measurements. For the SLS and DLS measurements the following refractive indices (RI) were used: water: 1.33, milk fat droplets: 1.456 (real part), 0 (imaginary part (absorption)), casein micelles: 1.57 (real part), $6*10^{-8}$ (imaginary part).

Static light scattering (SLS)
Particle size distributions and mean particle diameters were measured using SLS (Mastersizer 2000, Malvern Instruments Ltd., UK). The equipment allows size determinations in the range of 20 nm to 2000 µm. Samples were injected into the Hydro 2000G device filled with water. Measurements were conducted at ambient temperature (22 °C). The calculation models “general purpose” and “calculation sensitivity normal” were used for “spherical particles”. Stirrer and pump intensity were 500 rpm and 1250 rpm respectively. The software (version 5.60) calculates the best fit between the experimental measurements and the predictions made using light scattering theory (Mie theory). Each particle size distribution was calculated from the measurement of three samples and each sample was measured three times for 10 seconds.

Dynamic light scattering (DLS)
Measurements of the samples were performed on a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) with a detection angle of 173° at 25 °C. The maximum size range of this instrument is specified from 0.3 nm to 10 µm (sample dependent). Volume and number size distributions were calculated by converting the intensity size distributions using Mie theory. Samples were diluted with water to a suitable concentration prior to the determination of the size distribution. For viscosity a value of 0.8872 mPas was used.

RESULTS & DISCUSSION

Description of the size distribution

Particle size distributions can be described as volume, surface or number size distributions. One important characteristic value of particle size distributions is the median or D_{50} value. The D_{50} value is defined as the 50th percentile of the particle size distribution with regard to volume, surface or number that is 50 % of the particles are of this value or less. In contrast to the number distribution, the volume distribution rates larger particles more than smaller ones

Figure 1 shows the particle size distributions of semi-skimmed milk (1.5 % fat) measured by SLS (RI = 1.57 - 0) using the above mentioned size distribution types. The bimodal volume size distribution shows a peak for the casein micelles and a smaller peak for the bigger fat globules. The fat droplet peak disappears completely in the number size distribution. Table 2 shows the D_{50} values of the volume, surface and number size distributions of skimmed and semi-skimmed milk obtained by SLS using different RIs.

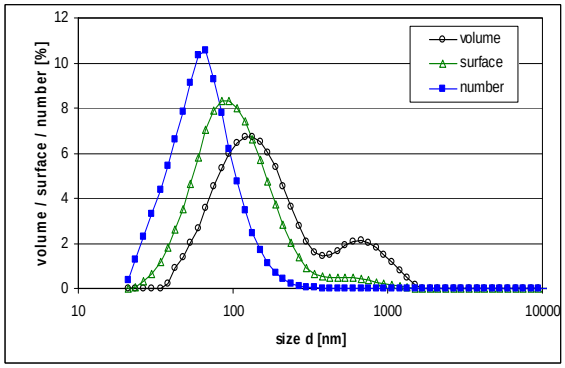

Figure 1. Particle size distributions of semi-skimmed milk (1.5 % fat) using different distribution types.

	semi-skimmed milk 1.5 % fat		**skimmed milk 0.1 % fat**	
Refractive Index (real)	1.456	1.57	1.456	1.57
Refractive Index (imag.)	0	$6*10^{-8}$	0	$6*10^{-8}$
D_{50} (volume)	151 +/- 0.001	151 +/- 0.003	122 +/- 0.003	125 +/- 0.003
D_{50} (surface)	99 +/- 0.000	97 +/- 0.000	96 +/- 0.004	102 +/- 0.005
D_{50} (number)	63 +/- 0.000	63 +/- 0.000	65 +/- 0.003	72 +/- 0.005

Table 2. D_{50} values (volume, surface, number) for semi- and skimmed milk obtained by SLS.

To convert the scattered light intensity distribution into a size distribution, the optical properties (relative and imaginary RI) of all components of the respective mixtures are needed. However, this approach is not feasible in practice. In general, only the RIs of the principal component of the mixture or a mean RI is used. Furthermore, nanoparticles in food may consist of different materials whose RIs might be unknown. In the case of milk the following RIs could be found in the literature: casein micelles: RI = 1.57, RI_{imag} = $6*10^{-8}$; milk fat droplets: RI = 1.456, R_{Iimag} = 0 resp. 0.01 [11-13]. Conversion of the scattered light intensity distribution into a size distribution using the two different sets of refractive indices did not have any significant effect on the calculated D_{50}-value.

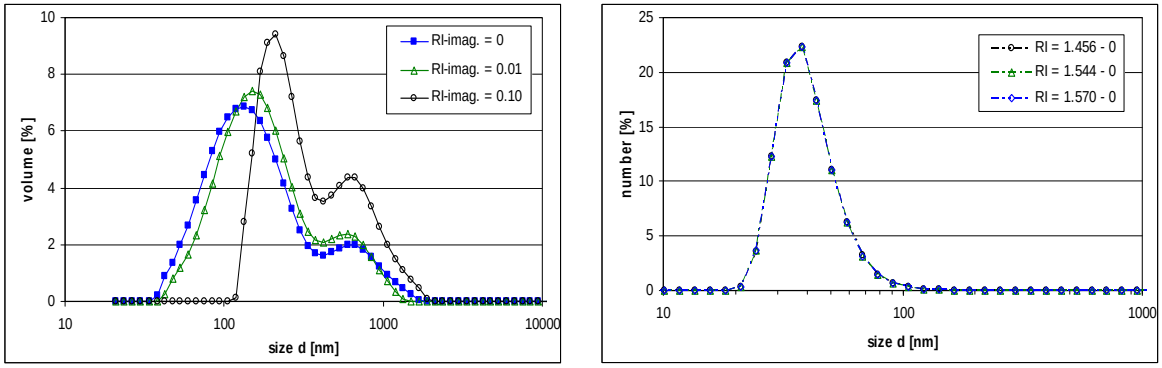

Figure 2. (a) Volume particle size distribution (SLS) of semi-skimmed milk (1.5 % fat) for different values for the imaginary part of the refractive index; (b) Number particle size distributions (DLS) of a colloidal silica dispersion for different values for the real part of the refractive index.

The effect of the imaginary part of RI on the volume size distribution of milk is shown in figure 2a. Using RI_{imag} = 0.01 results in almost the same volume size distribution as for an absorptive value of 0. With RI_{imag} = 0.1 a significant shift to larger particles was observed. This example demonstrates the need to use the correct RI in order to obtain a reliable result. The dependence of the number size distribution on the real part of the RI of a colloidal silica dispersion measured by DLS is shown in Figure 2b. No significant change in the number size distribution was observed with RI of 1.456, 1.544 and 1.570.

Effect of nanoparticle concentration

A colloidal silica dispersion was mixed with skimmed milk (0.1 % fat) in different concentrations and size distribution was determined by DLS. Increasing silica concentration from 0 to 2.5 % results in a significant shift of the number size distribution to smaller sizes (figure 4). Using the two different sets of RI of fat globules and casein micelles respectively, did not show significant differences in the calculated number size distributions (figure 4a and b.) However, even with 2.5 % silica dispersed in milk no bimodal size distribution was observed. Therefore, a clear identification of the silica nanoparticles in milk was not achieved.

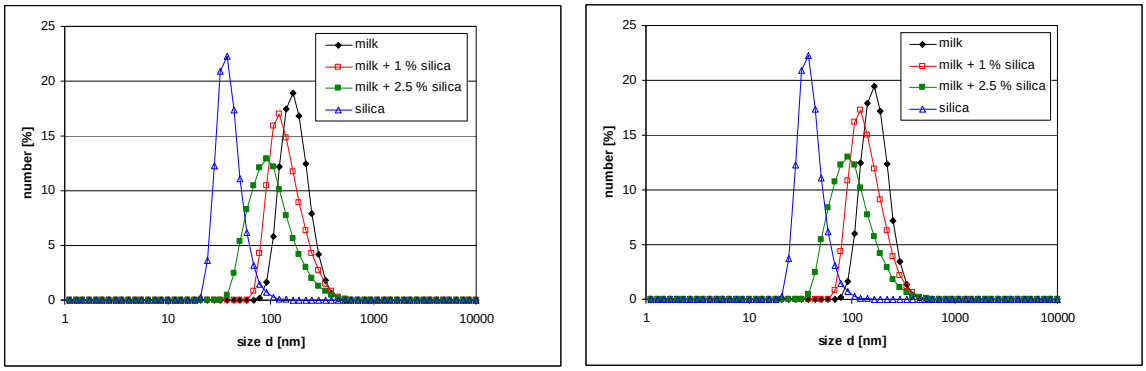

Figure 4 (a). Number size distributions of different silica concentrations in skimmed milk (refractive index 1.456 - 0).**(b).** Number size distributions of different silica concentrations in skimmed milk (refractive index 1.57 - 0).

Comparison between SLS and DLS

Figure 5 illustrates the number size distribution of a colloidal silica dispersion in water measured by SLS (Mastersizer 2000) and DLS (Zetasizer Nano ZS). Significant differences are evident. The number size distribution obtained by DLS is shifted towards smaller particles compared to the distribution obtained by SLS. The D_{50} values were determined to be 38 nm (DLS) and 82 nm (SLS), respectively. This could be due to the different measurement principles of the two systems and the fact that SLS technique used does not allows size determinations below 20 nm, whereas the threshold for DLS was specified to be 0.3 nm.

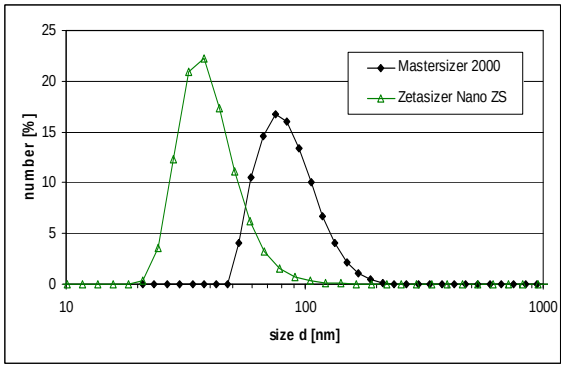

Figure 5. Number size distribution of a colloidal silica dispersion measured by SLS and DLS

CONCLUSION

Many analytical tools are theoretically suitable for the characterisation of nanomaterials in simple matrices. However, only a few methods are applicable to the analysis of more complex samples such as food. Electron microscopy is still seen by some as the “gold standard”, as it provides reassuringly direct visual images of the particles (aggregation state, shape, composition etc.). The disadvantages of electron microscopy are: the technique needs elaborate sample preparation protocols, it is a destructive method, electron microscopes have

to be operated under vacuum conditions and only small amounts of samples can be analysed and this has an impact on the statistical significance of the results. In case of the described light scattering methods the quality of the results depends not only on sample preparation, but also on the knowledge of the properties of the sample to be analysed (RI, viscosity etc.) and instrument settings (selection of mathematical/analysis model). The particle size distribution of a defined nanomaterial added to a food matrix is furthermore dependent on its concentration and on the food matrix itself (e.g. water, milk, juice). In addition, some general questions need to be addressed. It is still unknown if the nanomaterials we are identifying with our analytical tools are the same as the nanomaterials we are exposed to. To answer this question minimal sample preparation is recommended and matrix effects need to be addressed. Furthermore, agreement upon the unit (e.g. mass, particle number) used for measurement is needed. Since different analytical tools result in different size distributions respectively average particle sizes, the question about the possibility to correlate between different size measurement techniques came up. In addition, it is almost impossible to to distinguish natural from engineered nanomaterials. Identification and characterisation of nanomaterials in food is furthermore hampered by the in general low amount of nanoparticles present in a food, the presence of more than one type of nanomaterial, the necessity of determining a wide range of parameters (size, shape, composition, charge etc.) and the lack of standardised reference materials as well as standardised methods for sampling and measurement.

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