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Advanced analytical techniques for the measurement of nanomaterials in complex samples: a comparison

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RESEARCH ARTICLE

Abstract

To solve the various analytical challenges related to the measurement of nanomaterials in complex matrices new advanced analytical techniques must be developed. In this study an interlaboratory exercise was organised to compare the capabilities and limitations of newly developed techniques with classical techniques. Classical techniques involved were: (1) dynamic light scattering (DLS); (2) transmission electron microscopy (TEM); and (3) scanning electron microscopy (SEM). The newly developed techniques were: (1) single-particle inductively coupled mass spectrometry (sp-ICPMS); (2) nano tracking analysis (NTA); (3) differential centrifugal sedimentation (DCS); and (4) particle induced x-ray emission (PIXE). The results show that methods based on completely different physical principles, such as TEM, DLS, DCS, sp-ICPMS and PIXE, can produce similar results for pure suspensions of metallic nanoparticles. Electron microscopy (TEM and SEM) and sp-ICPMS were more accurate for size determination than NTA, DLS and DCS, while sp-ICPMS and PIXE were most accurate in the determination of mass concentrations of nanoparticles in pure suspensions. If an organic matrix was present the detection and sizing of nanoparticles became more difficult, especially for microscopic techniques, while light scattering methods like DLS and DCS detected multiple particle sizes. sp-ICPMS and NTA showed accurate results for the determination of particle size in chicken digest as well as in 10% DMEM, while only sp-ICPMS showed accurate results for mass concentrations in these complex matrices.

Keywords: analytical methods, interlaboratory exercise, nanoparticles

1. Introduction

Nanomaterials have unique functional properties and are therefore being used in many industries. An increasing number of consumer products that contain nanomaterials can already be found on the market (Chaudry, 2008; Gruére, 2011; Woodrow Wilson International Center for Scholars, 2012). These include electronics, household and cleaning products, paints and coatings, sport products and textiles, cosmetics and personal care products, food products and food packaging materials. Only a limited number of nanomaterials are known to be used in food, food additives, food supplements and food packaging applications (Chaudry, 2008). Silver is by volume not the most used material, but it is the fastest growing nanomaterial application in food, food supplements and food packaging as an antimicrobial, as it is in refrigerators and food storage boxes. Shrink films, cutting boards and storage boxes containing silver nanoparticles (NPs) are offered on the internet and it is not unthinkable that silver NPs migrate from these materials into foodstuffs. Patents also mention the possible use of silver to prepare antibacterial wheat flour (Park *et al.*, 2006) while, more recently, silver has been studied as an alternative for antibiotics used in the poultry production (Pineda *et al.*, 2012).

While nanoparticles, or nanomaterials consisting of such particles, are generally considered to be particles with at least one dimension below 100 nanometres, this size limit is fairly arbitrary. There has also been debate whether concentrations of nanomaterials should be expressed on a mass basis or a particle-number basis. In this respect, the European Commission has adopted a recommendation for the definition of nanomaterials,

Commission Recommendation 2011/696/EU (EC, 2011). According to this recommendation a 'nanomaterial' is:

> 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 are in the size range 1-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

It is expected that this definition will be used primarily to identify materials for which special provisions might apply (e.g. for ingredient labelling or risk assessment). However, there are various scientific-technical challenges related to the measurement of materials in the implementation of the recommended nanomaterial definition. Particular the requirement of measuring the constituent particles inside aggregates, the difficulty to convert experimentally measured signals into accurate number-based size distributions, and to detect and count particles at the lower size range of the definition, i.e. smaller than 10 nm. Analytical methods able to identify materials as nanomaterials according to the recommended nanomaterial definition are in development, however, most current methods have a detection limit higher than 1 nm or a lower sensitivity for smaller particles. As a consequence, they can only be used for a positive test to prove that a material is a nanomaterial, but not for a negative test to prove that a material is not a nanomaterial. Presently, none of the current available methods can determine for all kinds of potential nanomaterials whether they fulfil the definition or not (Linsinger *et al*., 2013).

Classical techniques for the detection and characterisation of nanomaterials are: (1) dynamic light scattering (DLS); (2) transmission electron microscopy (TEM); and (3) scanning electron microscopy (SEM). These techniques have proven their value in the analysis of pure particles or suspensions thereof, but it is uncertain how well they will perform in case of the analysis of nanoparticles in complex matrices. More recent developed techniques are: (1) single-particle inductively coupled mass spectrometry (sp-ICPMS); (2) nano tracking analysis (NTA); differential centrifugal sedimentation (DCS); and (4) particle induced x-ray emission (PIXE). Two of these techniques, NTA and DCS, are already commercially available, while sp-ICPMS and PIXE are quickly developing now more results are published. A brief description of these new and advanced analytical techniques that are applied in this study is given below.

sp-ICPMS uses a standard ICP-MS for the detection of single particles in suspensions and was first published by McCarthy and Degueldre (McCarty and Degueldre, 1993). More recently, sp-ICPMS has been described as a tool for the determination of NPs, practically as well as in various applications (Laborda *et al.*, 2011; Pace *et al.*, 2011). In sp-ICPMS, metal- or metal oxide based NPs are introduced into the ICP-MS producing a plume of metal ions in the plasma torch. This plume is detected as a signal spike in the mass spectrometer and allows the determination of the mass of the metal in each NPs. Based on the particles mass and an assumed spherical particle shape, the particle size is estimated. Adequate time resolution and a low particle density are required to ensure that each signal originates from one particle only, hence the name 'single particle' ICP-MS. As a consequence, samples generally have to be diluted which also results in a reduction of interferences from complex matrices.

NTA is a method for sizing particles in liquids by correlating the rate of the Brownian motion to particle size (Vasco *et al.*, 2010). The technique is used in conjunction with a microscope and a laser unit that together allow the detection of the scattering of small particles in liquid suspension. The scattered light is captured using digital imaging over multiple frames and software is used to track the motion of each particle from frame to frame. The rate of particle movement is related to a sphere equivalent hydrodynamic radius as calculated through the Stokes-Einstein equation. The technique calculates particle size on a particle-by particle basis and allows the determination of a size distribution profile of particles with a diameter of approximately 20-1000 nm in liquid suspension.

DCS or analytical centrifugation is used for particle size characterisation of materials 5 to >1000 nm, depending on the density of the materials. For high density materials smaller particle sizes can be separated. Particle size distributions are measured using a spinning disc with a sucrose gradient to separate particles on the basis of size. The system can separate particles that differ in size by as little as 5%, including separations in complex matrices such as plasma or cell culture media. Different from other particle sizing techniques, particles are actually separated first and then measured using a light scattering technique. The analysis time depends on the range of sizes that is being analysed and the density of the particles being measured. For nanomaterials, analysis times are in the range of 15-30 minutes (Monopoli *et al.*, 2011; Walczyk *et al*., 2010).

PIXE is a technique that historically has been used to quantify trace elements in materials, like traces of metal in archeological artifacts (Demortier, 1988), or more recently trace detection of nanomaterials in rat lungs and faeces (Lozano *et al.*, 2012, 2013). PIXE can be used to detect trace metallic contaminants such as nanomaterials

in liquids without sample preparation. PIXE is based on exciting electronic levels of the atoms, by means of an ion beam, producing X-rays. These X-rays are characteristic and proportional to elements present in the sample, thus allowing identification and quantification of the elemental composition of the measured target (both, the nanomaterial and its containing matrix) in a single measurement. PIXE possess no restrictions of nanomaterial size, achieving mg/l levels of sensitivity in the sample. Typical run time is 2-5 minutes per sample. A stage for non-vacuum PIXE has been designed. It is convenient for measuring the nanomaterial content directly from a droplet of the liquid.

In this study two interlaboratory exercises were organised to determine the different capabilities and limitations of the newly developed and more classical techniques especially in the detection and characterisation of nanoparticles in complex matrices. In total 10 laboratories participated in the interlaboratory exercise and at the end results of 8 laboratories were received. All laboratories involved in the interlaboratory exercise used their own protocols and methods except for those applying the sp-ICPMS method. For this method RIKILT (Wageningen, the Netherlands) supplied the procedure. A few participants used more than one technique resulting in 15 sets of measurement results. The results of the different techniques were compared.

2. Materials and methods

Materials

Spherical, monodisperse gold (Au) nanoparticles of 30 nm and 60 nm nominal diameter (RM-8012 and RM-8013) were obtained from NIST (NIST, Boulder, CO, USA) as a citrate stabilised suspension with a concentration of 50 mg/l. Spherical, monodisperse silver nanoparticles of 60 nm (SKU-AGCB60) were obtained from nanoComposix (San Diego, CA, USA) as a citrate stabilised suspension with a concentration 1 mg/ml. Dulbecco's modified eagle medium (DMEM), a growth medium for cells, was obtained from Lonza (Verviers, Belgium). Chicken digest was prepared by enzymatic digestion. Briefly, the digestion consisted of a sonication of chicken meat in a digestion buffer followed by addition of the enzyme and incubation for 3 hours at 37 °C.

Preparation of interlaboratory materials

For the first interlaboratory exercise an aqueous suspension of Au nanoparticles with particle sizes of 30 and 60 nm and a mass concentration of 1 mg/l was used. Two NIST reference materials, RM-8012 and RM-8013, were used for this purpose since these RMs are intended for evaluating and qualifying instrument or method performance. Each RM consists of a citrate stabilised aqueous suspension of gold nanoparticles in sealed pre-scored amber glass ampoules sterilised by gamma irradiation.

The RM's contain spherical primary particles (monomers) and a small percentage of clusters of monomers. RM-8012 contains particles with a nominal size of 30 nm and an Au mass concentration of 48.2 mg/l, RM8013 contains particles with a nominal size of 60 nm and an Au mass concentration of 51.9 mg/l. Because stability tests showed that the shelf-life of sub mg/l dilutions of nanoparticle suspensions in Milli-Q water is limited, dilutions of both RM's were prepared in 2 mM sodium citrate solutions. A blank sample, consisting of an organic dye in 2 mM sodium citrate, was included to detect false positives. For calibration purposes a suspension of RM-8013 with known concentrations was provided as a calibration standard. The following materials and samples were prepared:

1. Standard particle suspension Au, 60 nm, concentration 1 mg/l, prepared by 50 times dilution of RM-8013 in 2 mM sodium citrate solution.
2. Sample 1.1, a red coloured blank, prepared by dilution of an organic (red) dye in a 2 mM sodium citrate solution.
3. Sample 1.2, a suspension of 30 nm Au particles, concentration 0.8 mg/l, prepared by 60 times dilution of RM8012 in 2 mM sodium citrate solution.
4. Sample 1.3, a suspension of 60 nm Au particles, concentration 2.5 mg/l, prepared by 20 times dilution of RM8013 in 2 mM sodium citrate solution.

Table 1 gives an overview of the properties of the samples and the particles in the samples.

For the second interlaboratory exercise, silver (Ag) nanoparticles were used, not in aqueous suspensions but in complex matrices. Samples consisted of suspensions of Ag nanoparticles in chicken digest and in DMEM, a growth medium for cell lines used for *in vitro* toxicity studies. A blank sample consisting of 2 mM citrate solution was included to detect false positives. Suspensions of standard materials are citrate stabilised. The following 5 materials were prepared:

1. Standard particle suspension Au, 60 nm, concentration 1 mg/l, prepared by 50 times dilution of RM-8013 in 2 mM sodium citrate solution. This material was added as a link to the first interlaboratory exercise.
2. Standard particle suspension Ag, 60 nm, concentration 1 mg/l, prepared by 1000 times dilution of SKU-AGCB60 in 2 mM sodium citrate solution. This material was used for calibration purposes.
3. Sample 2.1, a suspension of 60 nm Ag particles, concentration 2.0 mg/l, prepared by 500 times dilution of SKU-AGCB60 in the chicken digest.
4. Sample 2.2, a suspension of 60 nm Ag particles, concentration 0.8 mg/l, prepared by 1,250 times dilution of SKU-AGCB60 in 10% DMEM growth medium in water.
5. Sample 2.3, a blank 2mM sodium citrate solution.

Table 1. Properties of the Au suspensions used in the interlaboratory exercise. Data of the RM's provided by NIST, uncertainties are single standard deviations.

	Sample 1	Sample 2	Sample 3
Diameter atomic force microscopy (nm)	not applicable	24.9±1.1	55.4±0.3
Diameter scanning electron microscopy (nm)	not applicable	26.9±0.1	54.9±0.4
Diameter transmission electron microscopy (nm)	not applicable	27.6±2.1	56.0±0.5
Diameter differential mobility analysis (nm)	not applicable	28.4±1.1	56.3±1.5
Diameter dynamic light scattering (nm)	not applicable	28.6±0.9	56.6±1.4
Diameter small-angle X-ray scattering (nm)	not applicable	24.9±1.2	53.2±5.3
Median particle diameter sp-ICPMS (nm)[1]	not detected	30.0±1.2	59.5±2.0
Mass concentration (mg/l)[1]	<0.1	0.96±0.02	2.60±0.06
pH of solution	7.0	7.0±0.3	7.3±0.3
Zeta potential	not applicable	-33.6±6.9	-37.6±3.0
Appearance	red pink solution	red pink solution	red pink solution
Stabiliser	2 mM citrate	2 mM citrate	2 mM citrate

[1] Results obtained by a single laboratory before the start of the study.
sp-ICPMS = single-particle inductively coupled mass spectrometry.

Table 2 gives an overview of the properties of the samples and the particles in the samples.

Homogeneity and stability of interlaboratory materials

All standard particle suspension and sample suspensions were stored in 4 ml amber, screw-capped glass vials and labelled. The homogeneity of the suspensions was determined by random selection of six vials of each composition and analysis with ICPMS and sp-ICPMS. The standard deviation of the median particle diameters of the Au nanomaterials was <2% for both sizes while the Au particle and mass concentrations varied <5%, demonstrating sufficient homogeneity. For the Ag nanomaterials, total-Ag mass and particle concentrations varied <5% and <10%, respectively, demonstrating sufficient homogeneity. The stability of the materials was determined measuring the materials in time using sp-ICPMS. Citrate stabilised Au suspensions with mass concentrations of 1 mg/l are stable for at least 6 weeks when stored in the dark. Median particle diameter and total Au mass concentration remained unchanged during this period. The stability of the Ag nanomaterials in chicken digest was determined as part of a method validation. The study showed that size and particle concentrations of 60 nm Ag nanomaterials in chicken digests at mass concentrations of 1 mg/l or higher are stable for a period of 6 weeks.

Analytical methods

A short description of the essential parameters of the applied methods and sample preparation procedures are given below.

sp-ICPMS was performed on standard ICP-MS equipment set to acquire data with a dwell time of 3 ms. Element specific measurements are performed at m/z 192 for gold and at m/z 107 for silver during a run time of 60 s. The

Table 2. Properties of the Ag suspensions used in the interlaboratory study. Data of the nanomaterials as provided by nanoComposix (San Diego, CA, USA), uncertainties are single standard deviations.

	Sample 1	Sample 2	Sample 3
Diameter transmission electron microscopy (nm)	57.4±4.0	57.4±4.0	not applicable
Matrix	chicken digest	10% DMEM	none
Mass concentration (mg/l)	2.0±0.1	0.8±0.05	<0.1
Zeta Potential (mV)	-47.9	-47.9	not applicable
Appearance	translucent liquid	light pink liquid	clear solution
Stabiliser	not applicable	not applicable	2 mM citrate

raw data are exported to a spreadsheet program as a CSV file to calculate particle size and size distribution and the number and mass concentration. Samples are diluted by a factor of 10,000 and introduced continuously into the ICP-MS system at a sample flow of 0.5 ml/min. Further details about the calibration and calculation procedure can be found elsewhere (Peters *et al.*, 2014).

TEM analysis was performed with instruments operating at 100-120 kV. Samples were used undiluted and prepared by drop deposition on carbon coated Cu grids. Samples were air-dried before imaging and typically 100 particles were measured to determine size. One participant used automated image analysis software to obtain quantitative results.

SEM analysis was performed on a Jeol JAMP-9500F Field Emission Auger Microprobe (Jeol, Tokyo, Japan). Samples were used undiluted, deposited and air-dried on smooth silicon wafers. Particle size was determined from 10 or more particles.

NTA analysis was performed using a NanoSight LM10 equipped with a 40 mW, 640 nm laser (NanoSight Limited, Amesbury, UK). Samples were analysed undiluted at ambient laboratory temperature (at that time 25-27 °C) and four 60 s videos were recorded for each sample to determine size and particle concentration. Typically, the camera shutter was set at 0.27 ms, the gain at 0, and the frame collection frequency at 25. The video data was analysed using NTA 2.3 software (NanoSight Limited) with the blur size set at 9×9, detection threshold at 10, and the minimum track length at 10 frames.

DLS was performed with different types of DLS instruments, all equipped with disposable sizing cuvettes and a laser with a wavelength in the range of 550-650 nm. The instruments used different angles for measuring scattered light and dedicated analysis software is used. Only one of the used instruments, a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK), was capable of measuring the zeta potential. For size measurements the undiluted samples were transferred to a disposable sizing cuvette, while for surface charge measurements the undiluted sample was transferred to the Malvern zeta cell. Typically, samples were measured five times at 20 °C following an equilibration period of 1-5 min.

DCS analyses were performed using a DC24000 line-start DCS system from CPS Instruments Inc. (Prairieville, LA, USA). The sample was used as received and 0.2 ml of sample was collected with a syringe needle from the sample vial just prior to the measurement. The DCS settings are as follows; disc speed, 22,000 rpm; sucrose gradient fluid, 8-2% (w/v) with a total volume of 12.8 ml calibration standard, 226 nm polyvinylchloride particle.

PIXE measurements were performed with a linear particle accelerator (HVEE, Amersfoort, the Netherlands). Droplets of the undiluted samples are air-dried on concave sample holders. Typical run time is 2-5 minutes per sample. Calibration of the detection is achieved using an external low intensity radioactive source (ORTEC, Oak Ridge, TN, USA) and a certified lead-glass (BCR126A; IRMM, Geel, Belgium). Data is processed with GUPIXWIN software (University of Guelph, Guelph, Canada) taking into account X-ray absorption.

Data collection

All participants were informed about the shipping dates of samples and were requested to perform the analysis within 3 weeks. Participants that were using sp-ICPMS also received a standard operating procedure and a calculation sheet to process the raw data. All participants received a form to fill out the data and return the results. The analysis were generally performed within the given timeframe and the results were reported back to the coordinator. The results are summarised in Table 3 and 4 and all partners were asked to verify the data presented in the tables.

3. Results and discussion

First interlaboratory exercise

The interlaboratory exercise was performed to compare the capabilities of different newly developed, advanced analytical techniques and classical techniques to detect and characterise nanomaterials in pure suspensions and complex matrices. Stepwise the complexity of the samples to be analysed was increased, starting from Au nanomaterials in citrate buffer (first exercise) to Ag nanomaterials in complex organic mixtures, e.g. a chicken digest and a buffer containing proteins (second exercise). The participants were asked to determine the presence of nanoparticles, the particle size, the mass concentration, and if possible the zeta potential. The methods that were applied by the participants were briefly described in the introduction and method section. The results have been made anonymous and are summarised in Table 3 (first exercise) and Table 4 (second exercise).

Sample 1.1 in the first exercise did not contain Au nanoparticles but a citrate solutions to which an organic pink red dye was added to resemble the presence of Au nanoparticles. None of the participants using sp-ICPMS, TEM or SEM reported the presence of particles in this sample, except for participant 2 who reported a particle concentration of 0.01 mg/l of a 60 nm Au particle. However, taking into account the usual dilution factor applied in sp-ICPMS, this result is close to the concentration detection limit and is probably noise. The light scattering methods cannot distinguish between organic and inorganic

Table 3. Results of the first interlaboratory exercise.

	Technique	Sample 1.1			Sample 1.2			Sample 1.3		
		Particle size (nm)	Concentration (mg/l)	Zeta potential (mV)	Particle size (nm)	Concentration (mg/l)	Zeta potential (mV)	Particle size (nm)	Concentration (mg/l)	Zeta potential (mV)
Composition prepared materials		-	-	-	30	0.8	-33.6	60	2.5	-37.6
Participant 1	sp-ICPMS	nd	nd	-	32±2	0.8±0.07	-	61±2	2.2±0.17	-
Participant 2	sp-ICPMS	60	0.01	-	31	1.08	-	60	2.5	-
Participant 3	sp-ICPMS	nd	nd	-	30	0.7	-	60	1.8	-
Participant 2	TEM	nd	nd	-	31.1±3.5	0.2	-	58.3±5.9	1.5	-
Participant 4	TEM	nd	-	-	207/27	-	-	56	-	-
Participant 5	SEM	nd	-	-	36±3	-	-	57±9	-	-
Participant 2	NTA	112±53	0.7	-	35±6	1.0	-	65±7	10	-
Participant 4	NTA	nd	-	-	46.7	-	-	75.8	-	-
Participant 8	DLS	105	-	-	31	-	-	59.4	-	-
Participant 4	DLS	nd	-	-11.7±0.8	35	-	-12.5±1.4	59.1	-	-31.0±3.5
Participant 5	DLS	nd	-	-	306	-	-	50	-	-
Participant 7	DLS	164	-	-18.7	32.7	-	-8.7	37.8	-	-15.6
Participant 8	DCS	76.7	-	-	22	-	-	48.4 / 62	-	-
Participant 9	DCS	132.4	-	-	32.5	-	-	70	-	-
Participant 9	PIXE	-	nd	-	-	0.84	-	-	2.68	-

DCS = differential centrifugal sedimentation; DLS = dynamic light scattering; sp-ICPMS = single-particle inductively coupled mass spectrometry; NTA = nano tracking analysis; PIXE = particle induced x-ray emission; SEM = scanning electron microscopy; TEM = transmission electron microscopy; - = not applicable; nd = not detected.

nanoparticles and for that reason may detect particles. The particle mass concentration measured with NTA in sample 1.1 by participant 2, i.e. 0.7 mg/l in Table 3, is however unlikely. Participant 2 reported that this may be a result of scattering of electron-lucent regions in the prepared sample. This was based on the results that this participant achieved with TEM and the fact that the scattering intensity of the observed relatively large particles (112 nm) was much lower than what would be expected from Au particles.

Measuring the particle size of the 30 and 60 nm Au nanoparticle in a citrate buffer (sample 1.2 and 1.3 in Table 3) resulted in particle sizes ranging from 22 to 306 nm with a median size of 33 nm for the 30 nm Au particle, and in particle sizes ranging from 38 to 75.8 nm with a median of 59 nm for the 60 nm Au particle. While most techniques show comparable results, those determined with light scattering methods, NTA, DLS and DCS seem to deviate most from the reference value. Particle sizes determined by TEM, SEM and sp-ICPMS in general were very similar and matched the particle sizes of the nanoparticles in the diluted reference material in samples 1.2 and 1.3. A typical TEM image of sample 1.3 is shown in Figure 1A. As a comparison, Figure 1B shows the TEM image of the standard particle suspension that was sent with the samples and contains the same Au nanoparticles as sample 1.3. The particle sizes determined with sp-ICPMS and reported by three participants using this technique show a good agreement and accuracy. However, it should be mentioned that the accuracy depends on the shape of the nanoparticle because the processing of the sp-ICPMS data assumes a spherical particle shape. While the latter is the case in this study, the accuracy will decrease for particles that are not spherical. Figure 1A and B shows the size distribution obtained with sp-ICPMS for samples 1.2 (30 nm Au particle) and 1.3 (60 nm Au particle). TEM and DLS particle sizes reported by participant 4 show good agreement for both particle sizes, whereas the results of the NTA analysis of the same participant indicates larger particle sizes. While this deviation may be due to the different measurement principle of NTA, it can also be a consequence of sample dilution prior to NTA analysis. Participant 4 observed that further dilution of the samples with deionised water before NTA analysis resulted in a shift to larger particle size, probably due to particle agglomeration. Since the materials were stabilised in 2 mM sodium citrate, dilution with deionised water may result in a decreased stabilization and particle agglomeration. Since participants were not aware of the exact composition of the dispersion media,

Table 4. Results of the second interlaboratory exercise.

	Technique	Sample 2.1		Sample 2.2		Sample 2.3	
		Particle size (nm)	Concentration (mg/l)	Particle size (nm)	Concentration (mg/l)	Particle size (nm)	Concentration (mg/l)
Composition prepared materials		60	2	60	0.8	-	-
Participant 1	sp-ICPMS	61±2	2.0±0.1	60±2	0.8±0.05	nd	nd
Participant 2	sp-ICPMS	60	0.5±0.1	58±2	0.7±0.1	nd	nd
Participant 3	sp-ICPMS	89	2.1	64	1.6	nd	nd
Participant 2	TEM	nd	nd	60.2±4.9	nd	30.7±4.7	nd
Participant 4	TEM	83±6	-	9.5±2.2	-	nd	-
Participant 5	SEM	nd	-	nd	-	nd	-
Participant 2	NTA	55±19	60.5	67±8	3.08	136±62	0.58
Participant 4	NTA	nd	-	nd	-	nd	-
Participant 8	DLS	74/451	-	145/754	-	55/1,230	-
Participant 4	DLS	92	-	10	-	nd	-
Participant 5	DLS	80-2,000	-	104	-	nd	-
Participant 7	DLS	241	-	88.1	-	376	-
Participant 8	DCS	83	-	33/42	-	nd	-
Participant 9	DCS	94±0.1	-	91±3	-	92.5±1.0	-
Participant 9	PIXE	-	3.0±1.2	-	9.4±3.2	-	0.85±0.44

DCS = differential centrifugal sedimentation; DLS = dynamic light scattering; sp-ICPMS = single-particle inductively coupled mass spectrometry; NTA = nano tracking analysis; PIXE = particle induced x-ray emission; SEM = scanning electron microscopy; TEM = transmission electron microscopy; - = not applicable; nd = not detected.

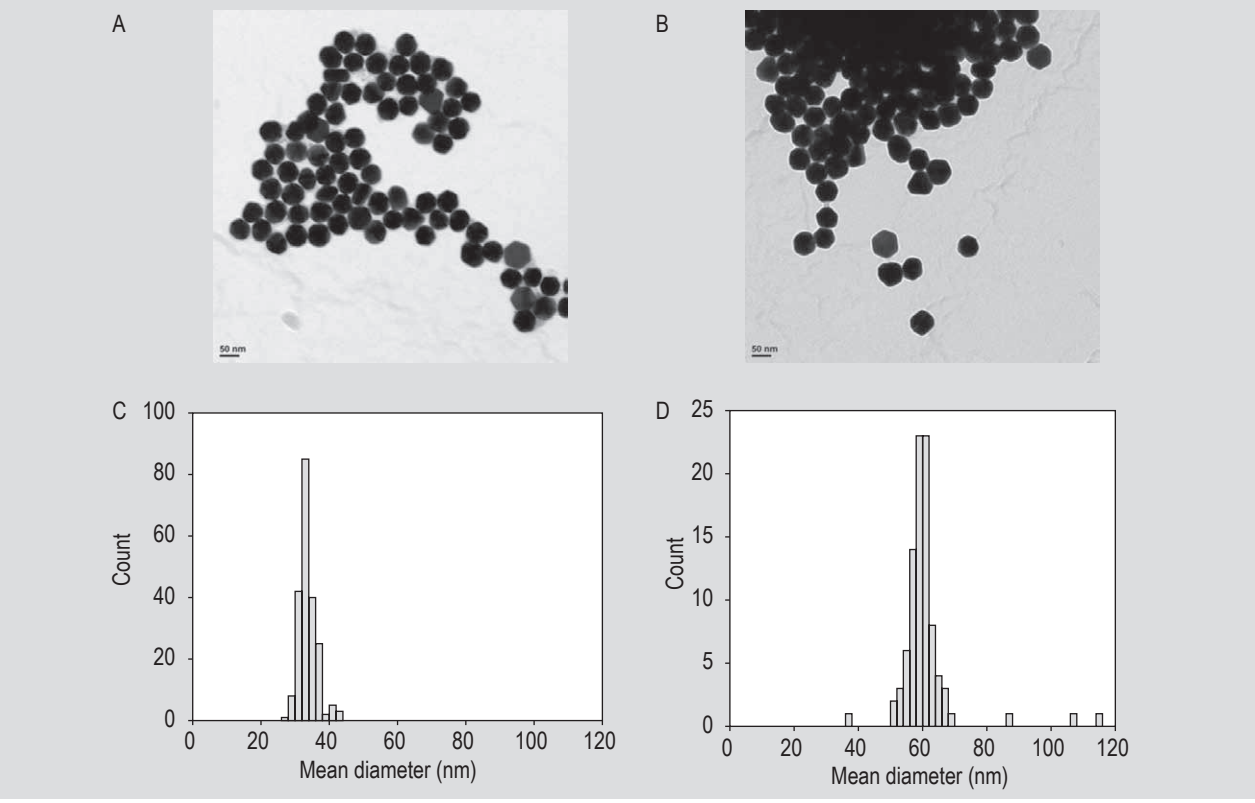

Figure 1. Transmission electron microscopy images of (A) sample 1.3 and (B) the standard particle suspension, and single-particle inductively coupled mass spectrometry size distributions of (C) sample 1.2 and (D) sample 1.3.

appropriate dilutions could not be made to carry out NTA analysis at lower particle concentrations.

The results in Table 3 show that sp-ICPMS is the most effective method in determining the mass concentrations of the Au nanoparticle suspensions. Participant 2 has estimated the mass concentration in samples 1.2 and 1.3 from the TEM and NTA measurements which however let to an under- and overestimation of the concentrations. Particle concentrations can be estimated from TEM and NTA data but only if a statistically representative number of particles are observed. Ideally, the number of observed particles on a TEM grid should be about 1000 μm^{-2}, which for dispersions corresponds to a particle concentration of 10^{12} l^{-1}. If the average particle is determined the mass concentration in the sample can be estimated. PIXE, used by participant 9, cannot determine the nanoparticle size but can measure the element composition of the particles and the overall mass concentration. The concentrations determined for samples 1.2 and 1.3 were both close to the expected mass concentrations.

The zeta potential was determined by only two participants and was determined in the undiluted sample in both cases. The zeta potentials range from -8.7 to -31.0. Only the latter value is in the range of the expected zeta potential of -33.6 and -37.6 for samples 1.2 and 1.3. It should be noted that the initial 50 fold dilutions of the RM materials may have affected the zeta potential. In the blank sample 1.1, which contained no nanoparticles zeta potentials of -11.7 and -18.7 mV were measured by the same participants and methods.

Second interlaboratory exercise

In the second round of the interlaboratory exercise, 60 nm Ag nanoparticles were analysed in the presence of complex organic matrices. Sample 2.1 consisted of a chicken meat digests to which 60 nm Ag particles were added in a concentration of 2.0 mg/l, while sample 2.2 consisted of DMEM, a growth medium for cell lines to which 60 nm Ag particles were added in a concentration of 0.8 mg/l. As in the first exercise, a blank sample, sample 2.3, was included. The results of the participants in the second exercise are presented in table 4 and it is immediately clear that the differences between the results of participants and methods has increased as compared to the first exercise. Since the chicken digest as well as the DMEM growth medium will contain proteins, lipids and other large organic molecules, agglomerates or particles, light scattering methods such as NTA, DLS and DCS will detect different sized particles. This is illustrated by the DLS graph in Figure 2 that clearly shows the presence of two distinct particles in sample 2.1 (chicken digest). The first peak at 92 nm was contributed to Ag nanoparticles which is in agreement with the results reported with TEM, DLS and DCS, while the second peak at 396 nm most probably is a complex of matrix components. DCS also observes more and larger complexes in sample 2.2 (10% DMEM) as illustrated by Figure 3. Participant 9 reported two peaks, a major peak at 91.3 nm (as reported in Table 4), and minor peaks at 43.8, 53.5, 170 and 500 nm.

The results for sp-ICPMS and the NTA measurements closely resembles the particle size of the Ag nanoparticles that were added to the sample matrices. For sp-ICPMS this is no surprise since the ICP-MS is tuned to observe only silver. Therefore, the size of the original silver particle will be found, even if the silver particle has become part of a larger organic complex, or has been coated by a layer of proteins. In addition, samples are typically diluted by a factor of 10,000 prior to analysis and this reduces the influence of the matrix on the measurement. The observation that NTA reports a similar particle size as sp-ICPMS is unexpected but may be explained by the fact that silver particles scatter harder than organic particles and are therefore more visible in the image analysis. Another unexpected finding is the overestimation of the particle size as determined by TEM in

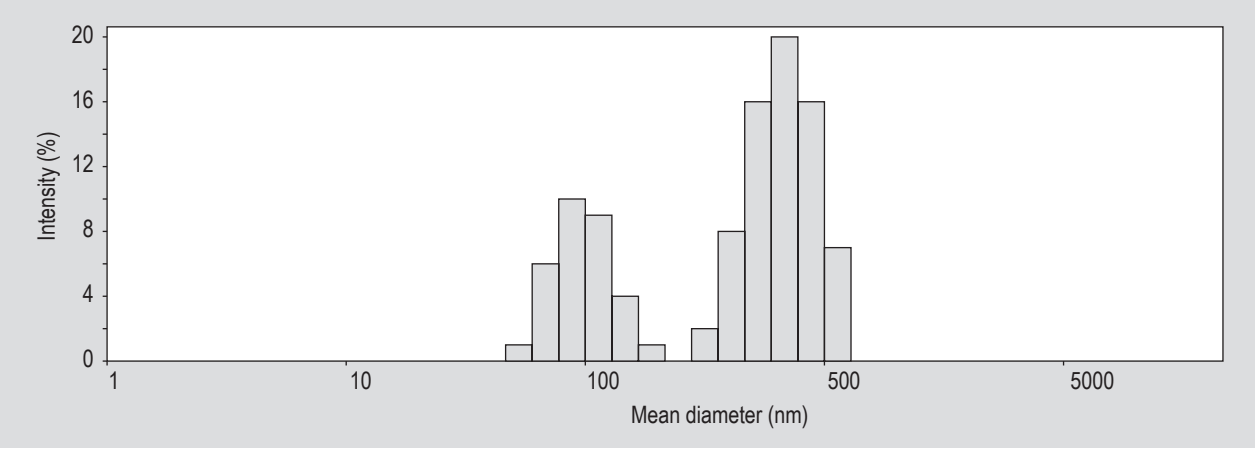

Figure 2. Dynamic light scattering measurements of sample 1 (chicken digest) showing two particles, a smaller with diameter 92 nm, and a larger particle with a diameter of 396 nm.

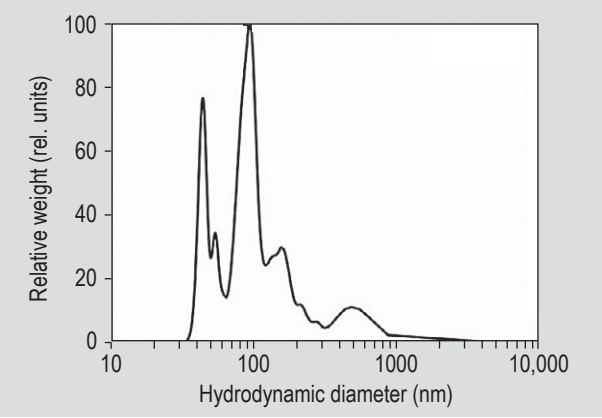

Figure 3. Dynamic light scattering 'chromatogram' of sample 2 showing a major peak at 91.3 nm and a number of additional peaks with other sizes.

sample 2.1. Since the density of the silver particles is much higher than that of the organic matrix, it was expected that the silver particles could still be accurately measured. However, in the TEM measurement of sample 2.2 an underestimation of the particle size is observed, but this may be due to the low concentrations of the nanoparticles. With SEM no particles could be observed at all in any of the samples. It is clear that following standard sample preparation techniques, particles can no longer be observed with these microscopic techniques due to the matrix and relatively low concentrations and that therefore some form of sample preparation is needed to improve this.

As with size, the determination of the mass concentration of the Ag nanoparticles in the samples is more difficult in the second exercise. While concentrations were reported for TEM and NTA analysis in the first exercise, during the second exercise no results were reported (TEM) or reported results that deviated strongly (NTA). TEM analysis can give quantitative results but only if a large enough number of particles are observed on the EM-grid. However, due to the presence of a matrix, particles are difficult to detect, and therefore no reliable concentrations can be determined. As in the first exercise, sp-ICPMS produces reasonable accurate results for mass concentration although at least two of the six results deviate by a factor of more than 2. PIXE reports a reasonable result for sample 2.1 but deviates strongly for sample 2.2 and sample 2.3. The fact that silver is found in the blank sample may indicate a blank problem.

4. Conclusions

The results show that methods with principally different physical principle such as microscopy methods, light scattering methods, sp-ICPMS and PIXE, can produce similar results when clean suspensions of metallic nanoparticles are measured. For 30 and 60 nm gold nanoparticles the median particle sizes found with the different methods are close to the expected sizes. Sizes reported by electron microscopic and sp-ICPMS seem to be a little more accurate than those determined by NTA, DLS and DCS. Mass concentrations of nanoparticles in the suspensions were accurately determined with sp-ICPMS and PIXE, while results from TEM and NTA deviated by a factor of 2. If an organic matrix is present the detection and sizing of nanoparticles becomes more difficult. With light scattering methods like DLS and DCS multiple peaks are observed resulting in a large variation in the results. With TEM and SEM it is difficult to detect nanoparticles due to the matrix and sample purification methods are required to improve their performance. sp-ICPMS and NTA show accurate results for the determination of particle size in chicken digest as well as in 10% DMEM. NTA, PIXE and sp-ICPMS were used to determine the mass concentration of the Ag nanoparticles in the samples. Of these, only sp-ICPMS showed reasonable accurate results.

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