Evaluation of Analytical Methods for Nanomaterials in Water

KWR Watercycle Research Institute

BTO

Evaluation of Analytical Methods for Nanomaterials in Water

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Summary

Understanding the risks of nanotechnology for the environment is considered a crucial constraint for the societal acceptance of its many potential innovative applications. Currently, insight into factors governing environmental and human health risks of engineered nanoparticles (ENP) and their behaviour in the water cycle is largely lacking, despite the fact that the application of ENP is growing tremendously and there is a rapidly growing body of scientific literature on effects of nanomaterials. Quantification and characterisation of nanomaterials is essential to assess their occurrence and distribution in the aqueous environment. However, determination of the occurrence and state (*i.e.* homo- or hetero-aggregated, freely dissolved, sorbed) of nanomaterials in (aqueous) environmental matrices is an analytical challenge that necessitates complex chemical- and physical detection techniques. A first step in meeting this challenge is an inventory of the available analytical techniques. A previous report B2013.202(s) names the available analytical techniques for organic and inorganic nanomaterials of GWRC members and associated research institutes. The current report will summarise and evaluate the results of an interlaboratory study. Several laboratories have received samples containing nanomaterials, and analysed these samples using available techniques. The data will be used to evaluate the current state of nanoparticle analysis within this group.

The most important findings in a nutshell are:

1   A combination of various techniques is necessary to fully characterise samples containing ENPs.
2   Imaging tools are useful in pure samples. However, they experienced difficulties when the samples contain other particles than the one to be measured (e.g. environmental samples).
3   For C_{60} and its derivatives MS is recommended because only MS can provide information about the chemical composition.
4   A "proper" round robin for the different techniques used in this study is advisable to verify the obtained data and determine the variability and robustness of the techniques applied in this study.

Contents

1 Introduction

1.1   Background

The GWRC working group Nanotechnology and Nanomaterials (NTNM) has developed a set of project proposals during the Monte Verità workshop (May 2011). At the Board meeting in Sydney, the project 'Evaluation of Analytical Methods for Nanomaterials in Water' was selected as priority project. The objectives of the project include:

- Preparation of an overview of the present state of the science of analytical methods for organic and inorganic nanomaterials in water;
- Survey of the methods used by the GWRC members and affiliated organisations;
- **Evaluation/comparison of available methods (interlaboratory study).**

This report will focus on the last objective. Several activities were agreed on to get an overview of the current analytical techniques and possibilities. Firstly all members and affiliated organisations were asked to give an account of their analytical equipment. Based on that information they were asked if they are willing to analyse several samples containing ENPs. The data will be used to assess the current analytical possibilities.

The following activities were accomplished.

- Selection of target materials (C_{60}, Ag, Au & TiO_2) and methods for the evaluation (member's choice). The three most important nanoparticle classes are represented, the metals, the metal oxides and the organic ones.
- Samples containing Ag, Au, C_{60} and TiO_2 were prepared by KWR.
- Members and organisations were asked if they are capable to participate
- Samples with unknown concentration and sizes of nanomaterials were sent to the participants

Those partners who wanted to take part in this practical survey were asked to state which nanoparticles could be analysed using their in house methods. Based on this information the appropriate sample were sent by KWR to the laboratories for analysis. Therefore not every laboratory received all samples. In Table 1 it is shown which laboratory participates, where they are from and which sample(s) they requested.

1.2   Aim of this project

The aim of this project is to compare the data returned from the partners and evaluate briefly the current analytical situation. It is the intention to **(i)** draw up an inventory of the laboratories and their analytical equipment, **(ii)** acquire knowledge about parameters can be measured by which partner**, (iii)** set-up an interlaboratory study based on that information and **(iv)** to compare the data returned from the partners.

Table 1 – List of participants and the requested samples.

Participant	C$_{60}$ (fullerenes)	TiO2	Ag	Au
EAWAG (Switzerland)	X	X	X	X
PUB (Singapore)		X	X	X
Vitens (The Netherlands)			X	X
TZW (Germany)		X	X	X
KWR (The Netherlands)	X	X	X	X
UvA (The Netherlands)	X			

2 Results and conclusion

The following chapter will show the data reported by the different institutes and a brief conclusion.

2.1   Equipment partners

Table 2 shows the analytical techniques used by the partner and the nanoparticles that were analysed with it. In BTO report B2013.202(s) "Inventory of analytical methods for engineered nanomaterials of GWRC members and associates" additional and more detailed information on the equipment and techniques is given.

Table 2: Equipment used per partner and the particles they were able to measure.

Participant	Equipment	Nanoparticles
EAWAG	TEM	Ag, Au, TiO_2, C_{60}
PUB	ICP-MS, DLS, TEM	Ag, Au, TiO_2
VITENS	ICP-MS	Ag, Au
TZW	ICP-MS/OES, LIBD	Ag, Au, TiO_2
KWR	FFF, ICP-MS, MS	Ag, Au, TiO_2, C_{60}
UvA	MS	C_{60}

TEM = transmission electron microscopy, DLS = dynamic light scattering, LIBD = laser induced breakdown spectroscopy

2.2   Materials

Table 3 and Figure 1 show the specifications – if known - of the samples, that were sent to the partners.

Table 3: Nanomaterial samples: Specifications as stated by manufacturer.

Sample	Size particles (average)	Total Concentration	Concentration in NP	Particle number	Shape
Ag 70 PVP[a]	69.9 (4.6) nm	0.2 mg/L	n/a	$1.1*10^8$ NP/mL	Sphere
Au 60 PVP[a]	56.8 (8.2) nm	0.5 mg/L	n/a	$2.7*10^8$ NP/mL	Sphere
C_{60}[b]	0.97 nm – n/a	n/a	n/a	n/a	n/a
TiO_2[b]	n/a	n/a	n/a	n/a	n/a
n/a = not available, PVP = polyvinylpyrrolidone a) data received from manufacturer b) these samples were not identified before.					

Ag and Au nanoparticles were obtained from NanoComposix (San Diego, USA). C_{60} was supplied by Sigma-Aldrich (Zwijndrecht, The Netherlands). TiO_2 particles were prepared from sun screen Eucerin KIDS 50[+].
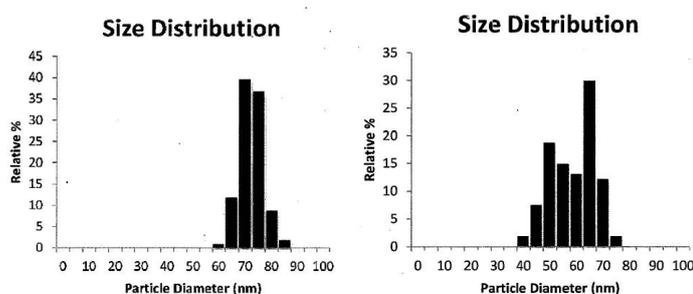
Figure 1: Size distribution for the stock solutions of silver 70 nm PVP (left) and gold 60 PVP (right) as stated by the manufacturer.

2.3   Preparation

Ag und Au solutions were made by dilution of the stock solutions in MilliQ water.

The C_{60} solution was made as follows. About 5 mg of C_{60} were added to 500 mL of MilliQ water. This mixtures was stirred vigorously for 3 weeks. The meanwhile yellow solution was filtered to remove non-dissolved C_{60}.

To receive the TiO_2 sample, 1 g of sun screen was added to 10 mL of MilliQ water. This mixture was first shaken and then placed in a ultrasonic bath for 5 min. The supernatant was removed and used for analysis.

2.4   Shipment

The samples were shipped by mail. Samples were not sent at a specific temperature. Samples were not frozen.

2.5   Data comparison

Table 4 shows the received data corresponding to the parameters that the participants were asked to measure. As each technique delivers different information on the sample, the participants only had to fill in the data they could obtain.

Table 4: Data reported by the participants.

Participant (Technique)/Nanopart.	Size particles (average)	Total Concentration	Concentration in NP	Particle number	Shape
EAWAG (TEM)					
Ag	64 nm			$3.05*10^7$ NP/mL	spheres
Au	57 nm			$7.09*10^7$ NP/mL	spheres
TiO2	20 nm				rods, cluster
PUB (SP–ICP–MS)					
Ag	55 nm	0.068 mg/L	0.02 mg/L	$2.15*10^7$ NP/mL	
Au	52 nm	0.565 mg/L	0.565 mg/L	$4.07*10^8$ NP/mL	
PUB (DLS)					
Ag	66 nm				
Au	109.6 nm				
TiO2	127.2 nm				
PUB (TEM)					
Au	50 nm				spheres
TiO2	20 nm				rods,cluster
Vitens (SP–ICP–MS)					
Ag	55 nm	0.130 mg/L		$4.62*10^7$ NP/mL	
Au	49 nm	0.197 mg/L		$2.79*10^8$ NP/L	
TZW (ICP–MS)					
Ag		0.162 mg/L			
Au		0.56 mg/L			
TZW (ICP–OES)					
TiO2		0.557 mg/L			

Participant (Technique)/Nanopart.	Size particles (average)	Total Concentration	Concentration in NP	Particle number	Shape
TZW (LIBD) TiO2	25 nm NB 326 nm MB		0.8 mg/L	$4.21*10^9$ NP/mL	
KWR (FFF+UV) Ag Au	 70 nm 60 nm				
KWR (FFF+MALS) TiO2 C60	TiO_2 (60-500nm, range), C_{60} (60 – 440 nm, range) 60 – 500 nm 60 – 440 nm				
KWR (MS) C60		164.7 (±14.2) µg/L			
UvA (MS)		248.9 (±16.1) µg/L			

TEM = transmission electron microscopy, DLS = dynamic light scattering, LIBD = laser induced breakdown detection, MALS = Multi angle light scattering, FFF = Field Flow Fractionation, NB = number based, MB = mass based
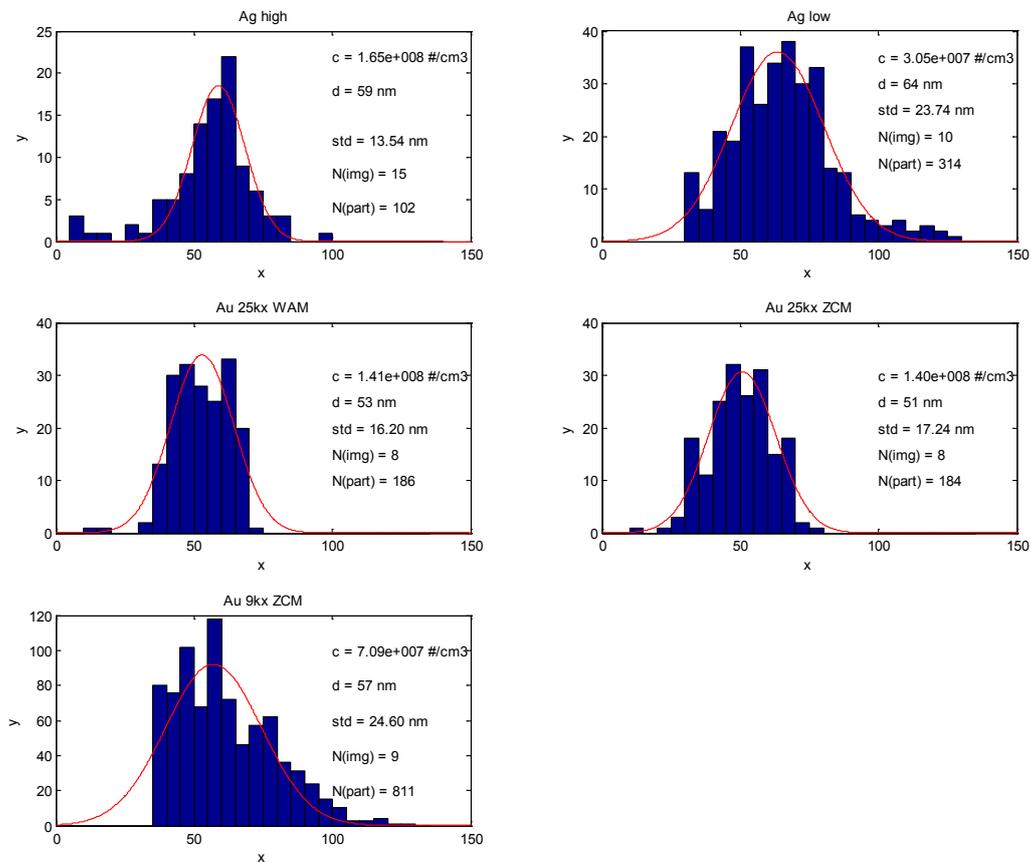
Figure 2: Size distribution calculated from TEM images by EAWAG.
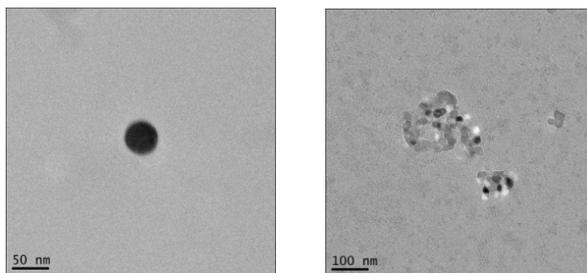
Figure 3:TEM images from PUB of a gold nanoparticle (left) and TiO$_2$ clusters (right).

2.6   Evaluation

Literature shows that the characterisation and analysis of organic and inorganic nanoparticles is complex [1-3]. In that perspective, it can be said that the results (Table 4, Figure 2 and 3) of the various labs are rather comparable.

2.6.1 Silver and gold

Au and Ag data were in most of the cases nearly coincident. The size distribution for silver was found to be between 55 nm – 70 nm and for gold 49 - 60 nm. Sole exception was the measurement of Au with DLS (109.6 nm).

TEM analysis performed by EAWAG (Figure 2) results in a slightly different size distribution then the one reported by the manufacturer (Figure 1). However, as different parameters, such as the number of images analysed, have an influence on the calculated size distribution as shown by EAWAG, this might be the explanation for the variation. Specific information on the method used by the manufacturer is not available.

Also total concentrations showed acceptable discrepancies. In case of silver a factor of 2 separated the highest and the lowest concentration measured. In case of gold we are dealing with a factor of 2.5. Concerning the amount of particles, the measurements are comparable too. The highest and lowest amount of silver nanoparticles measured differ only by a factor of two. For gold it is a factor of about 8. This might be attributed to the use of TEM in case of the lowest number of particles. Two groups used SP-ICP-MS to determine the particle number and here the difference amounts to 1.5. All these results are almost identical to the data that was reported by manufacturer (see Table 3). Also the spherical shape of the particles was confirmed (for gold see Figure 3, silver not shown).

Important information gained from the measurement of Ag and Au is the fact that Au samples do not contain anything else than nanoparticular matter, whereas in case of silver a considerable amount of silver was present in form of dissolved ions Ag^+ (about 60%). This was reported by PUB. Furthermore, it is noteworthy that the sample that was sent to Singapore ended up in America first due to a mistake made by the shipping company. Nevertheless, the results reported by PUB are comparable to the other results. This unintentional stability experiment showed that it seems to be possible to send at least this type of samples to other laboratories without risk of (complete) decomposition. Nevertheless, it cannot be excluded that the high amount of silver ions are a consequence of the long travel of that specific sample.

2.6.2 Titanium dioxide

Primary TiO_2 nanoparticles were found to be around 20 nm analysed with TEM by two institutes and one institute reported them to vary between 20 and 500 nm using LIBD. TEM showed that the particles are rods (Figure 3). Also it was obvious from the imaging analysis that TiO_2 particles formed aggregates. That would explain the size distribution (60 – 500 nm) measured by means of FFF/MALS and the mean size of 127 nm by DLS. Total concentration was reported to be 0.557 mg/L with ICP-OES and the concentration in nanoparticles to be 0.8 mg/L. As TiO_2 is a stable compound that does not dissolve easily, these two numbers should be the same. The variation can probably be ascribed to the use of different techniques.

2.6.3 Fullerenes (C_{60})

The C_{60} sample was tried to be analysed by one partner by means of TEM. However, the particles could visually not be separated from other particles in the solution. Apparently, bacteria were found in the sample. When the contamination occurred is unclear. The size distribution of the C_{60} clusters determined by FFF/MALS was found to be 60 – 440 nm. The concentration of the sample was found to be 164 and 248 µg/L by two institutes. The difference in concentration can probably partly be attributed to two different extraction techniques used. At KWR C_{60} was extracted by adding NaCl to the water matrix whereas at the UvA $Mg(ClO_4)_2$ was added. An explanation can be that the addition of the magnesium salt does allow for a better extraction of the C_{60} fullerenes with toluene. Therefore, a higher concentration could be measured. Furthermore, at the UvA only one extraction experiment was performed, whereas at KWR three fractions of the same sample were extracted independently.

2.6.4 Lessons learnt

The lesson that can be drawn from this practical survey is, that a combination of several techniques is necessary to acquire all the information about a nanoparticle sample. Also contamination of samples or highly complex matrices can interfere with some techniques. E.g. it is difficult to analyse C_{60} samples with TEM to visually characterize their size and shape when bacteria are present. Here MS is a powerful tool as it specifically can look for C_{60} and determine concentrations [4, 5]. However, only the monomeric form can be analysed as the rigorous extraction and solution in organic solvents disrupts the aggregates. Here FFF and TEM are alternatives that enable to study the aggregation state and the size of the C_{60} aggregates.

Furthermore it is evident, that e.g. SP-ICP-MS is very useful when it comes to determine the concentration and amount of nanoparticles. However, the exact nature and size of the particles cannot be obtained by this technique. Complimentary tools are necessary to get the extra information. The same holds true for other analysis methods. FFF is capable to separate particles of different size. Additional information can only be gained when supporting detectors that physically characterise the particles are linked to it. But even the combination of FFF for size characterization and analytical detectors that characterise elemental composition and concentrations do not provide information on the shape and the exact nature of the particle. For that purpose imaging techniques are required such as TEM. At present no institute has the facilities to measure all the characteristics of a nanoparticle. This on its own underlines the necessity for several laboratories to work together. But even if everything could be measured in one laboratory, an interlaboratory measurement is advisable to compare results.

The good news of this survey is that the results presented by the labs coincide with the data from manufacturer and/or they can be compared among themselves. This suggests that the application of these techniques for the chemical and physical characterisation of the particles is possible and that a more extensive study (Round robin) could be set up.

2.6.5 Most important findings of this survey:

- A combination of various techniques is necessary to fully characterise samples containing ENPs.
- Imaging tools are useful in pure samples. However, they experienced difficulties when the samples contain other particles than the one to be measured (e.g. environmental samples).
- For C_{60} and its derivatives MS is recommended because only MS can provide information about the chemical composition.
- A "proper" round robin for the different techniques used in this study is advisable to verify the obtained data and determine the variability and robustness of the techniques applied in this study.

3 Acknowledgment

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