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Nanoparticle Compendium



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Introduction

Nanoparticles have existed since the dawn of time, but the term nanoparticle has only been coined fairly recently, having emerged in the 1990s. The **International Organization for Standardization** (ISO) defines nanoparticles as particles that have at least one dimension (i.e. length, width or diameter) which is between 1 and 100 nanometers in size. They come in a variety of shapes, including spheres, nanotubes, nanowires, quantum dots and fullerenes. Nanoparticles exist naturally in the environment, in, for example, volcanic ash, smoke from forest fires, clouds and ocean spray. They are also found in car exhaust and cigarette smoke.

With the advent of nanotechnology in the early 1980s, an increasing amount of so-called engineered nanomaterials (ENMs) that have novel physical, thermal, optical and biological properties have been created and are finding their way into the environment. Today, there are a myriad of ENMs being manufactured for a wide range of medical, cosmetic, environmental and energy applications (see **UnderstandingNano.com**). Perhaps the most well-known application of nanoparticles is in **sunscreen formulations**, where the absorption and light scattering properties of ZnO and TiO₂ particles prevent harmful UV radiation from reaching the skin. ENMs are of concern as it has been identified that, as particle size decreases, some metal-based nanoparticles in particular (e.g. Ag, Au, and Cu particles) show increased toxicity compared to the same material in bulk form. In addition, this type of nanoparticle has been shown in laboratory studies to interact with components inside mammalian cells, initiating inflammatory responses and causing destruction of mitochondria, leading to apoptosis (programmed cell death) or necrosis (un-programmed cell death).

Inevitably, a proportion of ENMs end up in our water supplies and food. This has sparked concern about the possible toxicity of some of these materials to humans and now there are moves to introduce legislation relating to the production, use, and disposal of such materials. More details about this are described in the blog **Nanoparticles: A Storm in a Teacup or Something to Worry About?**, referred to in the resources section at the end of this compendium.

In recent years, the technique of single particle (sp) ICP-MS to detect, characterize and quantify metal and metal oxide nanoparticles, such as Au, Ag and TiO₂ particles has evolved. With advances in software, tools have now been developed that enable analysts to gain a clearer insight into nanoparticle composition and size distribution to aid in assessing the environmental and potential health impact of these entities.

This compendium provides an overview of the capabilities of the Thermo Scientific™ family of ICP-MS instruments and the npQuant nanoparticle evaluation software plug-in for characterizing and quantifying metal and metal oxide nanoparticles.

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Characterization of Nanoparticles using ICP-MS: Advantages and Challenges For Nanoparticles in Food.

Michal Godula¹, Daniel Kutscher², Shona McSheehy Ducos², Lothar Rottmann², Jörg Bettmer³, Katrin Loeschner⁴, Erik H.Larsen⁴

1: Thermo Fisher Scientific, Prague, Czech Republic

2: Thermo Fisher Scientific, Bremen, Germany

3: University of Oviedo, Oviedo, Spain

4: Technical University of Denmark, National Food Institute, Soeburg, Denmark

Overview

Purpose: Demonstrate the potential of FFF-ICP-MS and spICP-MS for the characterization of nanoparticles.

Methods: FFF was coupled to Q-ICP-MS, with liquid flows driven using a Thermo Scientific™ Dionex™ ICS-5000+ Reagent Free™ HPIC™ system. For spICP-MS, samples were directly aspirated.

Results: FFF was shown to separate Ag and Au nanoparticles based on their diameters. spICP-MS was used to determine the average diameter of Ag nanoparticles.

Introduction

The need for nanoparticle (NP) characterization has exploded in recent years due to the ever increasing use of engineered nanoparticles (EN) in various industries and the consequent studies that investigate the environmental and consumer risk.

Of the methods developed with this goal in mind, Field Flow Fractionation (FFF) coupled to ICP-MS has proved to be one of the most promising. FFF has a separation principle based on the differing mobilities of different particle sizes in a laminar liquid flow contained in a channel between two plates. Smaller particles flow faster through the channel, enabling a separation based on size. FFF is compatible with particle sizes in the low nm to low μm range and is thus perfectly suited to NP separation. Another promising approach for NP characterization is spICP-MS. Through direct analysis of an appropriately diluted solution containing NPs, the NPs can be counted. If the NPs consist of just one element, peak height, proportional to the size of the NP can be used to determine the NP diameter.

Although both strategies benefit from the high sensitivity of Q-ICP-MS detection, the single particle approach limit of particle size detection is actually governed by signal-to-noise ratio. The more sensitive an instrument, the smaller the particle it can detect.

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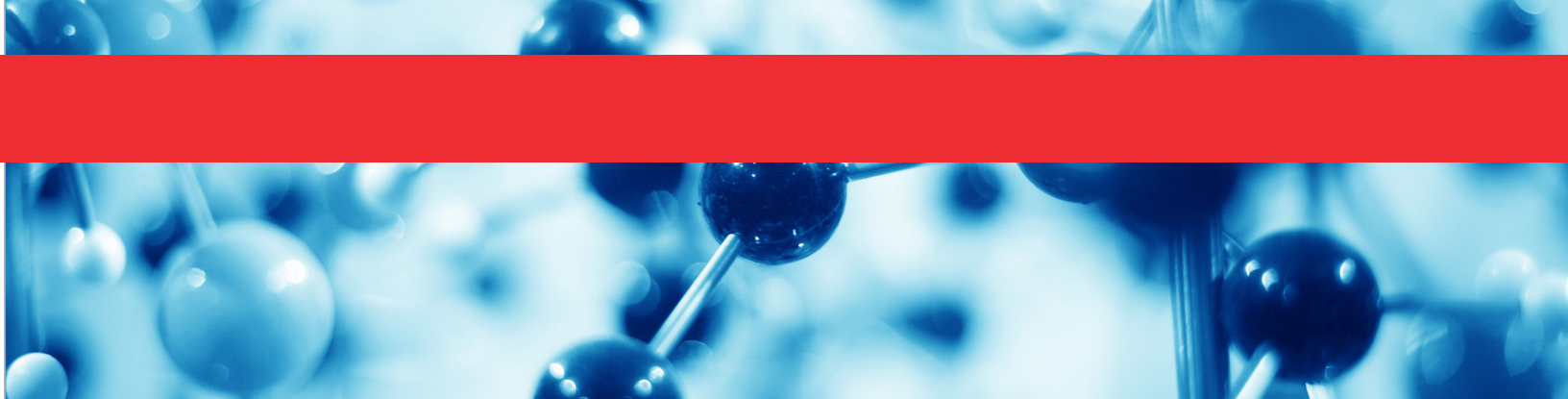
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Methods

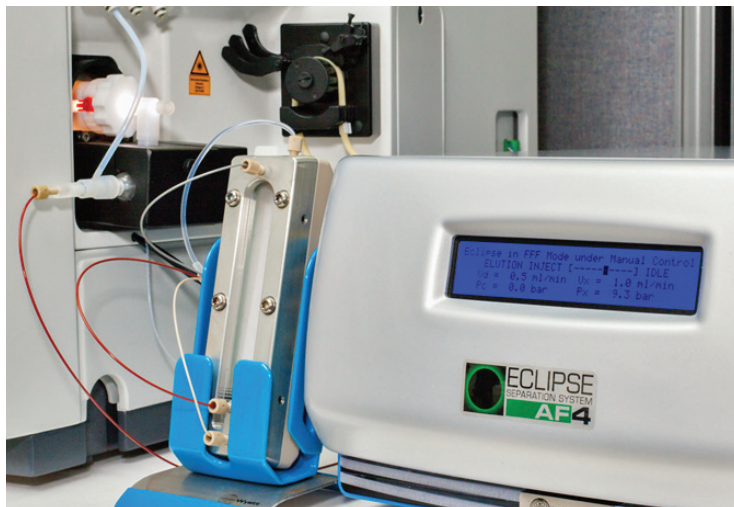
Sample Preparation

In general NP standards were diluted in water and sonicated for 5 to 15 minutes just prior to analysis.

Field Flow Fractionation

A Wyatt Technology™ Eclipse® AF4 Field Flow Fractionation system equipped with a short channel (SC) was coupled to a Thermo Scientific™ iCAP™ Qc ICP-MS (Fig. 1). Mobile phase was delivered to the Eclipse AF4 system chassis using the ICS-5000+ ion chromatography system and injections were performed using the sample loop of the AS-AP ICS-5000+ autosampler. The Eclipse system chassis splits the flow appropriately with a series of specially configured valves.

Figure 1. Wyatt Technology Eclipse AF4 FFF system with SC coupled to a Thermo Scientific iCAP Qc ICP-MS.



The FFF membrane used and the separation parameters are shown in Table 1.

Table 1. Field Flow Fractionation Conditions.

	Gold NPs		Silver NPs	
Membrane	10 kD RC*		10 kD RC*	
Mobile Phase	Water		0.5 mM Amm. Carbonate	
Injection vol. (µL)	20		20	
Detector flow (mL/min)	0.5		1.0	
Focus flow (mL/min)	1.5		1.0	
FFF Protocol	Time	Vx	Time	Vx
Elution	1	0	2	0
Inject	1	0	1	0
Focus and Inject	5	0	2	0
Focus	1	0	5	0
Elution	5	1.5	40	1.0
Elution	15	1.5	5	0
Elution	5	0.5	-	-
Elution and Inject	10	0	5	0

*RC – Regenerated Cellulose

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ICP-MS

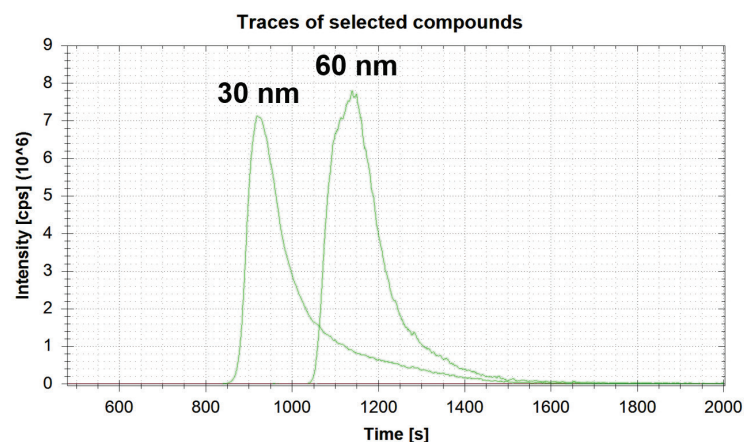
A Thermo Scientific iCAP Qc ICP-MS was used for all experiments. Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software was used throughout for iCAP Q ICP-MS control and all data acquisition. Thermo Scientific™ Chromeleon™ software was used to drive the Wyatt Eclipse AF4 FFF and ICS-5000 + HPIC systems in a single method. ISIS™ (Intelligent Separation Improvement Software) was used to optimize the FFF separation conditions.

Methods

Field Flow Fractionation

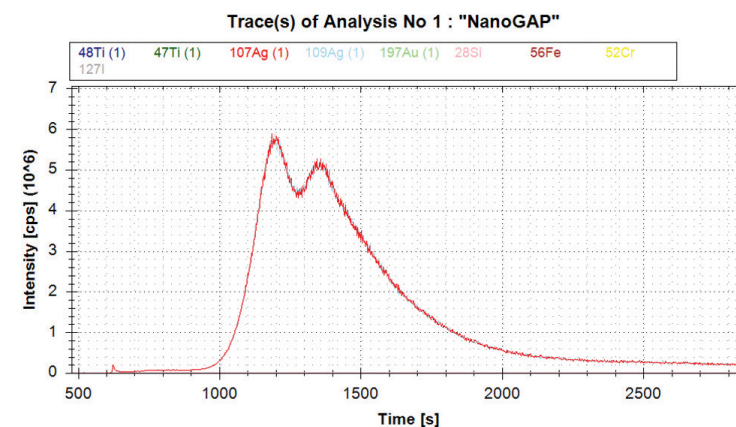
Certified gold NPs with 30 nm (NIST 8012) and 60 nm (NIST 8013) diameters were used to evaluate the NP separation potential of FFF. Table 1 summarizes the channel, membrane and conditions used. Figure 2 shows elution profiles of 30 nm and 60 nm gold NPs.

Figure 2. ¹⁹⁷Au FFF-ICP-MS fractograms of 30 nm and 60 nm gold NPs.



A slightly different FFF elution program was used to separate PVP stabilized silver NPs (Nanogap Spain) The nominal size of the NPs is 42 ± 10 nm but a doublet profile (Fig. 3) clearly indicates a mixture of sizes (or shapes). The fractogram profile is in excellent agreement with a separate study that comprehensively optimized the FFF parameters for this sample¹.

Figure 3. FFF-ICP-MS fractogram of Nanogap® AgNPs.



Single Particle ICP-MS

The underlying principle of spICP-MS lies in the fact that the size of the NP is directly proportional to the intensity of the single particle event (SPE). Smaller NPs (40 nm) generate lower intensity SPEs than the larger NPs (100 nm). The frequency in which SPE are observed is related to the number of particles in solution and thus the concentration of the nanoparticles.

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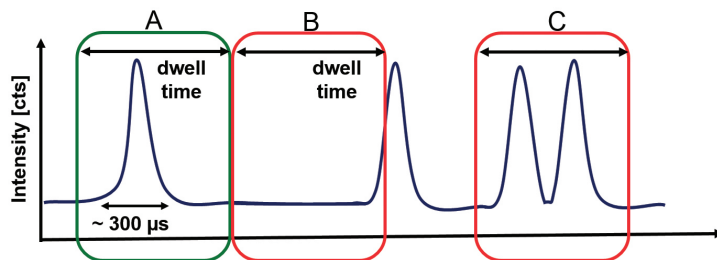
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The sample was diluted until it was within a suitable range of particles per volume and directly aspirated into the plasma. Data from fast scanning across a single isotope in a predetermined time window (e.g. 3 ms dwell time for 60 s) was exported into a calculation spreadsheet, where the nanoparticle size distribution and concentration was calculated.

The correlation between dwell time and particle events is illustrated in Figure 4. A typical nanoparticle event has a duration of 300 μ s.

- Only one nanoparticle event should be monitored per data point (A).
- Short dwell times lead to incomplete registration of particle events (underestimation of particle size, B).
- Long dwell times lead to registration of two (or more) particle events (overestimation of particle size, C).

Figure 4: Relation between single particle event and dwell time.



Analysis of Nanoparticles in Food²

Nanoparticle characterization in food faces many challenges due to the presence of complex matrix and the lack of fully developed and validated sample preparation protocols. An approach based on enzymatic extraction, followed by fractionation using FFF and final nanoparticle characterization with spICP-MS was investigated for a chicken meat paste.

Chicken meat paste was spiked with AgNPs and extracted with Proteinase K 1:5 (60 μ g enzyme /mg tissue) with incubation for 40 minutes in a water bath (37°C). The extracted sample was analyzed using a similar FFF-ICP-MS setup to that described above and fractions were collected. The observations from the FFF analysis indicated that a significant proportion of approximately 80% of the nanofraction was recovered from the FFF analysis and that there was a formation of additional peaks in comparison to a standard of AgNPs (or pristine AgNPs). Additionally, the presence of the partially degraded meat matrix had an influence on the elution behavior of the AgNPs following FFF separation. Earlier elution (approx. 2 mins) was observed in comparison to the pristine AgNPs.

Five FFF fractions were collected and analyzed by spICP-MS: Two of the fractions are shown in Figure 5.

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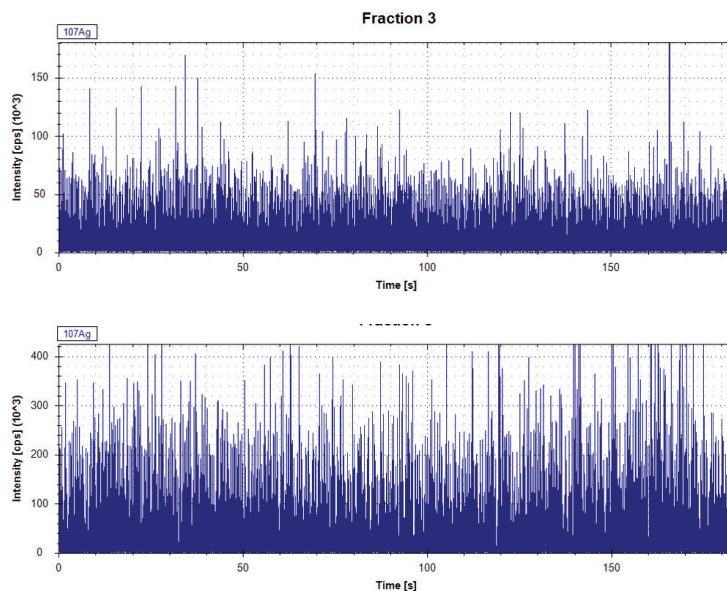
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Figure 5. Analysis of FFF fractions of chicken meat paste spiked with AgNPs with spICP-MS.



Fraction 3 was collected in the rising slope of the FFF nanoparticle peak at an elution time of 9 minutes and fraction 5 was collected in the tailed elution of the nanoparticle peak at an elution time of 26 minutes. The fractions were found to have mean particle diameters (MPD) of 30 and 43 nm respectively. The obtained number-based particle size distributions for each fraction present more accurate size information than the mass concentration-based size distribution based on FFF calibration. Additionally, an earlier fraction was found to contain non-nano Ag which was most likely ionic Ag bound to organic constituents of the enzymatic meat digestate.

This demonstrated that additional information can be obtained by combination of single particle ICP MS with a separation method like FFF in comparison to bulk analysis, especially for NP analysis in complex matrices.

Conclusion

- Both FFF-ICP-MS and spICP-MS bring analytical advantages to the characterization of NPs and act as complementary techniques.
- The integrated FFF-ICP-MS package is fully automated with bi-directional control and emergency shut-off features.
- The completely metal free FFF/IC system operates with a single pump and offers a switch option that allows the user to quickly change from FFF to IC.
- The high base sensitivity and low backgrounds of the iCAP Q ICP-MS offer a particular advantage in spICP-MS.

References

1. K. Loeschner et al., J. Chromat. A 1272 116-25.
2. K. Loeschner et al., Anal. Bioanal. Chem. 405 8185-8195.

Acknowledgements

We would like to thank the team at Wyatt Technology Europe, Dernbach, Germany, for both technical and application support.

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Nanoparticle Characterization Via Single Particle Inductively Coupled Plasma – Mass Spectrometry (spICP-MS) Using a Dedicated Plug-in for Qtegra ISDS Software.

Daniel Kutscher, Julian D. Wills, Shona McSheehy Ducos
Thermo Fisher Scientific, Bremen, Germany

Introduction

Due to their unique properties, nanomaterials have found their way into many everyday consumer products. In some cases, the use of nanomaterials is openly declared for marketing purposes, for example, the use of Ag nanoparticles to inhibit bacterial growth. In other cases however, the use of nanomaterials is not obvious from the product labeling. Despite their growing prevalence, the potential adverse effects of nanomaterials on human health and the environment is still not fully understood. Recently this has been acknowledged through the development of nanomaterial definitions by regulatory authorities e.g. the European Commission.¹

Common approaches used to characterize nanomaterials include microscopy based techniques e.g. transmission electron microscopy (TEM), and optical properties methods e.g. dynamic light scattering, (DLS). Fractionation or separation of different particle sizes within one sample prior to detection can be accomplished using asymmetric flow field flow fractionation (AF4) for example, or alternative chromatographic techniques such as hydrodynamic chromatography (HDC).

All of the aforementioned techniques, however, have advantages and drawbacks. Whereas techniques based on microscopy only allow the sampling of a small amount of particles, optical techniques may be limited in detection sensitivity with samples derived from environmental sources. Furthermore, not all techniques are able to directly deliver a number based size distribution, which is mandatory to meet the current definition of a nanomaterial.

Single particle ICP-MS (spICP-MS) has found its niche in the portfolio of techniques available to characterize nanoparticles, both in terms of their size distribution, as well as the number of particles with a given size present in a sample. Indeed, a low number of particles is a prerequisite for spICP-MS.

Implementation of a new data acquisition and evaluation strategy for spICP-MS, must be straightforward to be successful. Intuitive workflows should guide the user through the critical stages of defining method parameters and data evaluation, whilst integrating seamlessly into daily operation, especially in high-throughput laboratories analyzing environmental and food/beverage related samples.

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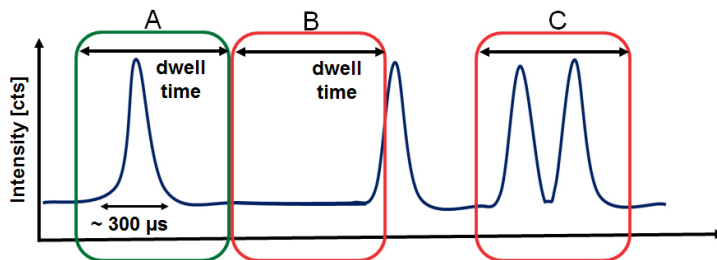
Method

The spICP-MS technique is used to characterize a nanomaterial both in terms of particle size and number distribution. In short, every particle that enters the plasma source will be completely atomized and ionized, so that a plume of ions will travel to the detector and cause a short transient signal with a duration of approximately 300-700 μs . If this signal is recorded with short enough dwell times (typically 1-10 ms), signals corresponding to single nanoparticles can be recorded and evaluated individually.

This implies, however, that only one particle at a time enters the plasma, as multiple particles would be counted as one and hence increase the estimated particle size. Nevertheless, under optimized conditions, spICP-MS is capable of collecting information on a sufficiently high number of particles (up to a few thousand) in a short time e.g. 60s acquisition time.

The chosen dwell time together with the sample dilution are critical parameters in spICP-MS, as artefacts can occur that lead to incorrect results. Figure 1 shows the correlation between dwell time and artefact formation in spICP-MS:

Figure 1. Correlation between observed signals in spICP-MS and potential formation of artifacts.



The following event types may be observed:

Ideal Particle Events (A)

A nanoparticle signal is observed in one single measurement slot. The signal intensity can be used to calculate the mass of element in the particle.

Split Particle Events (B)

A nanoparticle signal is observed in two adjacent measurement slots. The extent of split particle events depends on the nanoparticle pulse duration and the applied dwell time, and can be reduced by using longer dwell times.

Double or Multiple Particle Events (C)

Two or more particles are observed in one measurement slot, leading to an overestimation of the particle size. The occurrence of such events can be estimated using Poisson statistics and may be reduced by sample dilution.

The use of dwell times of less than 1 ms enables the full resolution of each particle derived signal. Due to the short duration of the signals, however, the number of available data points can be insufficient for accurate peak integration. Furthermore, the statistical fluctuation of the background signal may compromise the attainable measurement precision.

The instrument response in ICP-MS is directly proportional to the number of ions arriving at the detection system in a given time. Therefore, the mass of the particle can be calculated for each particle derived signal if key parameters are known.

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In a second step, the particle mass can be converted into the corresponding volume, and hence external dimensions can be calculated. This strategy requires exact knowledge of key parameters and uses the following assumptions:

- Sample uptake rate, detection sensitivity and the transport efficiency of the nebulizer are known. These parameters are used to determine the particle mass.
- The density of the particle material e.g. Au, Ag, TiO₂ etc. is equal to the density of the bulk material.
- The external dimensions e.g. radius can be calculated, assuming the particle shape is spherical.
- The particle number concentration can be assessed by counting the observed number of individual signals.

Instrumentation

In this work, a Thermo Scientific™ iCAP™ Qc ICP-MS was used for all experiments. However, any other ICP-MS operated using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software could be used as well. The instrument was fitted with a PFA-100 self-aspirating nebulizer. The exact sample uptake rate of the nebulizer was determined daily.

A standard Ni based skimmer cone was used, but in order to increase the attainable detection sensitivity, a high sensitivity skimmer cone insert was used (PN 1311880). The instrument was tuned daily for best detection sensitivity on the targeted analyte e.g. Au or Ag. Since no spectral interferences were expected on m/z 107 (Ag) and 197 (Au), the STDS mode was used with the QCell™ operating as an ion guide. The dwell time was set between 3 and 10 ms.

Gold and silver nanoparticles with nominal diameters of 30 and 60 (Au, NIST reference materials 8012 and 8013) and 20, 40 and 60 nm (Ag) were used for instrument calibration and measurements. According to the manufacturer, batch-to-batch variation can be expected.

Software

Qtegra ISDS is a software platform for laboratories tasked with the analysis of elements and isotopes. The modular concept of the software uses dedicated plug-ins to control analytical instruments such as the Thermo Scientific range of ICP-MS instruments, as well as peripheral devices. This includes, for example, autosamplers from Elemental Scientific and Teledyne CETAC Technologies. Different evaluation modules can be used to acquire and evaluate data, such as total element quantification, speciation analysis or laser ablation.

The npQuant evaluation method is dedicated to the analysis of nanoparticles using the spICP-MS mode. It is compatible with Qtegra ISDS version 2.6 SP1 (and all subsequently released versions) and can be installed separately.

Figure 2 shows the LabBook Editor after the installation of npQuant. It enables the creation of templates and LabBooks in order to acquire and evaluate data sets according to the criteria established for spICP-MS.

The npQuant evaluation method uses the well-established Qtegra ISDS software architecture (Figure 3) so that users familiar with Qtegra ISDS can easily get acquainted with the new task of analyzing nanoparticles.

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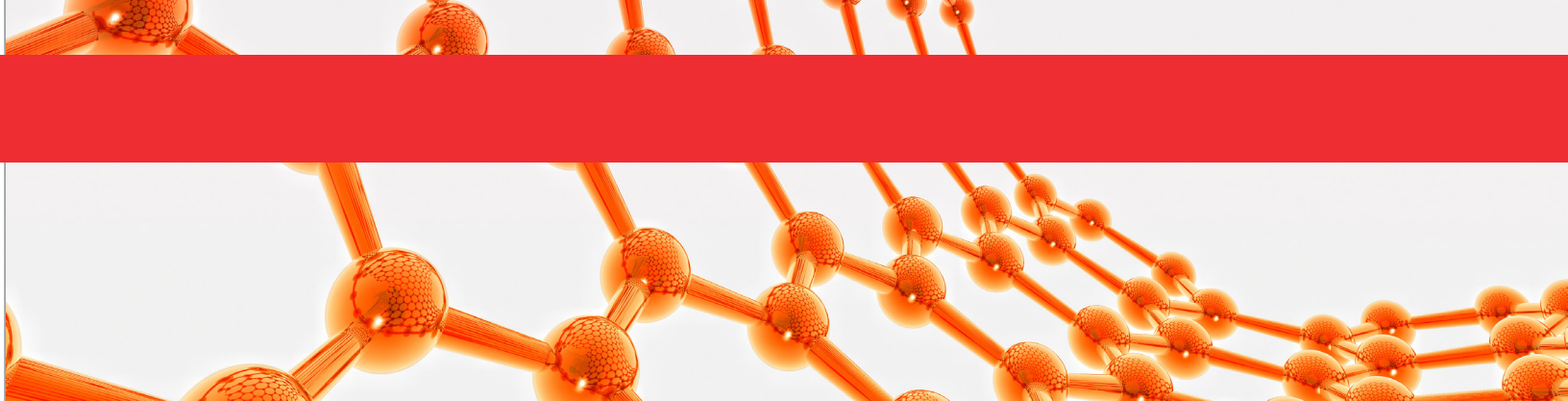
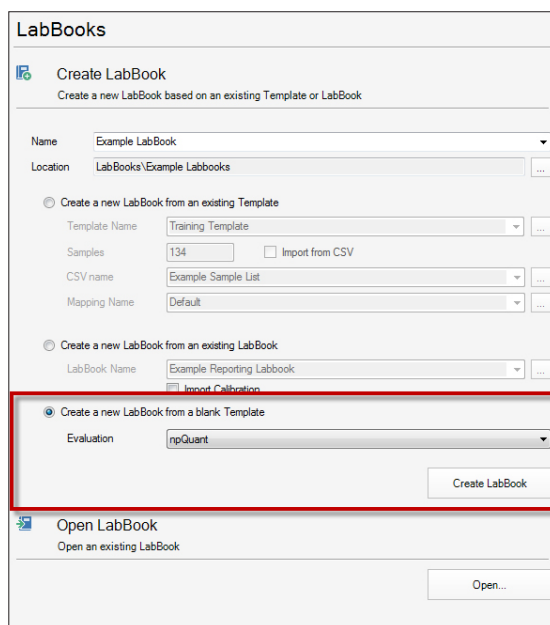


Figure 2. LabBook Editor after installation of npQuant.



The Qtegra ISDS architecture easily accommodates a mix of LabBooks using different evaluation methods e.g. total element quantification (with eQuant) and nanoparticle evaluation (with npQuant) using the same automated scheduling of LabBooks so that the available instrument time can be exploited efficiently.

Figure 3. Overview of the npQuant evaluation method.



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Method Definition

In order to reliably define key parameters for data evaluation, npQuant mode automatically determines the detection sensitivity and the transport efficiency (Pace et al., 2011) via a measurement with appropriate standards. In case there is no suitable standard available, the value can be typed in manually.

npQuant mode is able to evaluate different nanoparticle fractions within a batch of samples, but also within a single sample. At the same time, npQuant mode can determine the concentration of dissolved ions in a sample:

Particle Evaluation

All signals are evaluated according to the spICP-MS evaluation algorithms, resulting in a particle size- and number distribution.

Ionic Evaluation

All signals are averaged and evaluated against the slope of the calibration curve, the result is a concentration in, e.g. $\text{ng}\cdot\text{L}^{-1}$.

Figure 4. Default Evaluation Parameters View with automated determination of key input parameters.

Common			
Sample Flow:		0.23	mL/min
Transport Efficiency:	Auto	6.80	%
Detection Sensitivity:	Auto	124,923.14	cps/(ug/L)
Dwell Time:		5.00	ms

For each fraction, the user must provide a lower and a higher threshold value to select the signal range to be used for data evaluation. Both threshold values can also be modified graphically once data acquisition is in progress or completed.

Fractions can use “Fixed” and “Movable” threshold values:

- *Fixed* threshold values can only be altered for the entire batch affecting every sample, e.g. to correctly delimit a size range.
- *Movable* threshold values can be altered per sample to reflect varying signal intensities, such as changing dissolved ionic background.

Material specific information such as density and mass fraction of the detected element in the particle can be edited, so that complex composite materials can also be analyzed. Chemical identities can be simply exported to and imported from other Templates/LabBooks.

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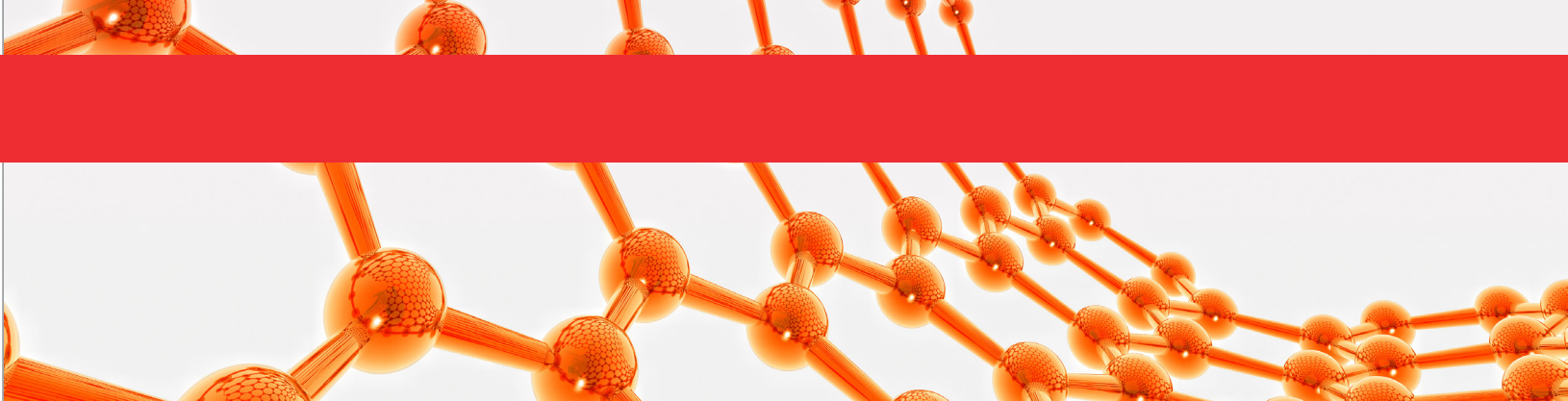


Figure 5. Definition of threshold values and material specific parameters using the Editor.

Common

Identifier	Lower Threshold	Upper Threshold	Chemical Identity	Density	Shape	Mass Fraction	Distribution	Particle Bin Size	Graph Color	Evaluation Strategy	Threshold Mode
> 60nm	108,313 cps	226,359 cps	Au Nanoparticles	19.32 g/cm ³	Sphere	1.00	Log. Normal	1.00 nm	Blue	Particle Evaluation	Fixed
Ionic	18,556 cps	46,692 cps	Au	19.32 g/cm ³	Sphere	1.00	Normal	1.00 nm	Green	Ionic Evaluation	Movable

Advanced

Available Chemical Identities

Identifier	Description	Density
Au		19.32 g/cm ³
Au Nanoparticles		19.32 g/cm ³

Details

Identifier: Mandatory

Description: Optional

Density: [Dropdown]

Elements

Element	Fraction



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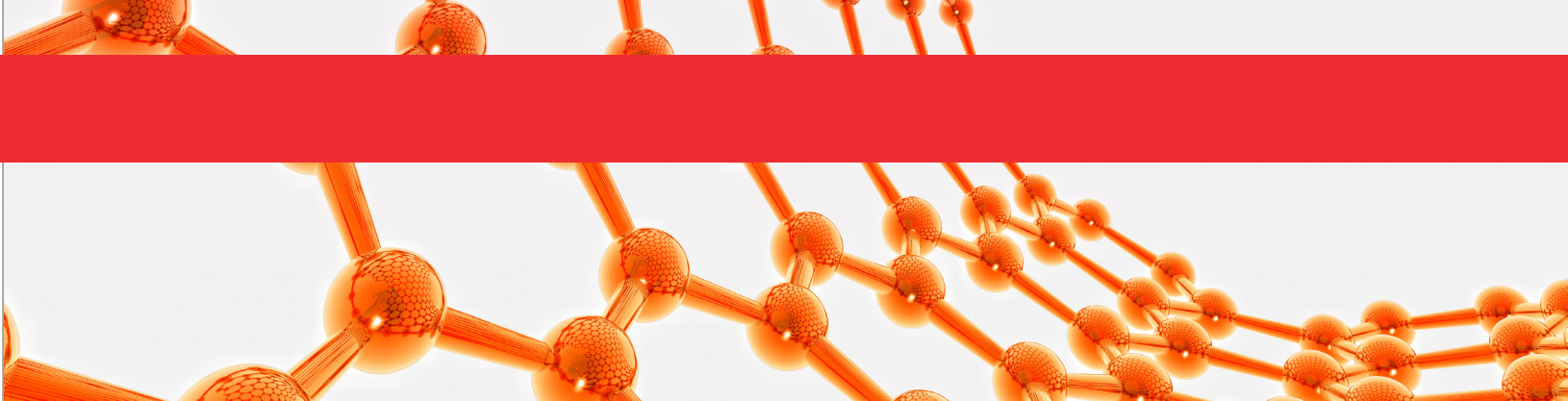


Figure 6. Signal Distribution View to identify and select nanoparticle derived signals.

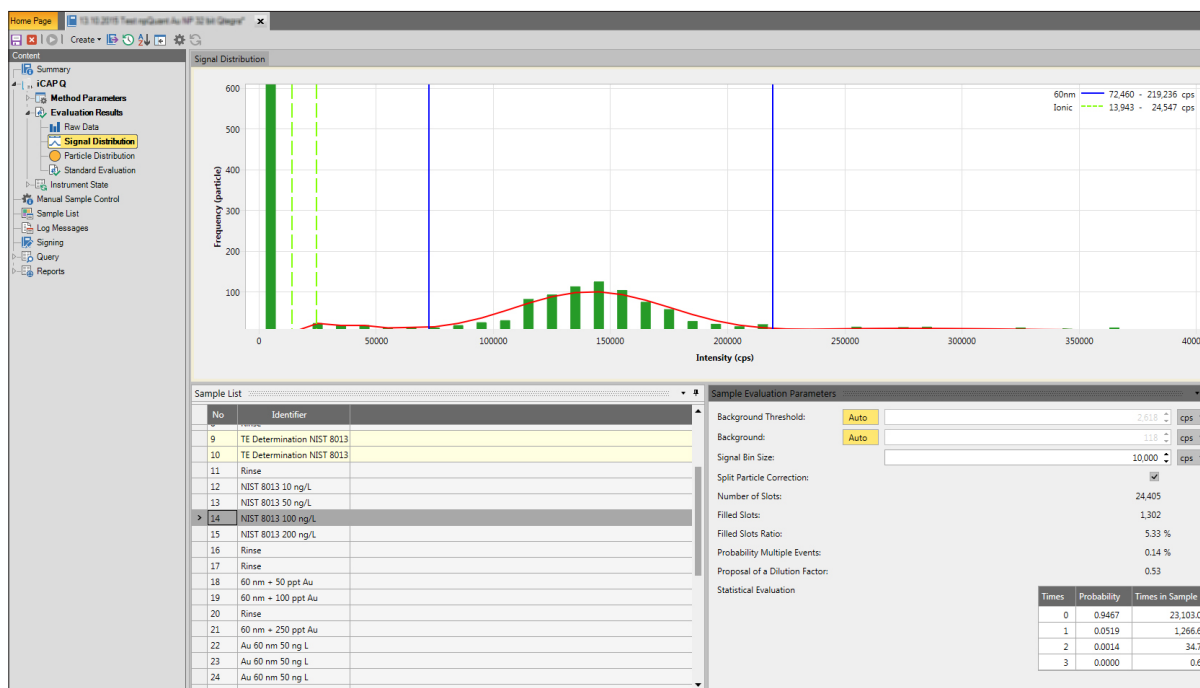


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Data Evaluation

Once the data acquisition is complete, npQuant mode is able to perform all data handling and calculates the particle size distribution as well as the particle number concentration in unknown samples.

In order to correctly recognize particle fractions, the Signal Distribution View displays the data as a histogram (# of signals with a given intensity vs. intensity) together with the corresponding threshold values for each particle fraction. The limiting bars can be moved easily, using the cursor.

The occurrence of double or multiple particle events in a run is assessed through the total number of signals above the background level, resulting in the filled slot ratio. Subsequently, based on a user definable value for the filled slot ratio (default value 10%), a dilution factor is proposed to reflect either, that the number of signals was too high and the occurrence of double particle events is likely, or the sample could be analyzed at a lower dilution in order to increase the number of detected particle signals used for data evaluation. Finally, the total number of observed signals is virtually redistributed over the available number of slots using Poisson statistics, so that the extent of double (or multiple) particle signals can be compared.

Figure 7. Statistical Data Evaluation available for each sample including estimation of optimum dilution factor.

Sample Evaluation Parameters		
Background Threshold:	Auto	483.26 cps
Background:	Auto	111.67 cps
Signal bin size:		6,500.00 cps
Split particle correction:	<input checked="" type="checkbox"/>	
Number of slots:		24430
Filled slots:		1329
Filled slots ratio:		5.44 %
Probability multiple events:		0.15 %
Proposal of a dilution factor:		0.54
Statistical Evaluation		
Times	Probability	Times In Sample
0	0.945600	23,101.00
1	0.052893	1,292.18
2	0.001479	36.14
3	0.000028	0.67

Split particle events can be corrected for, using the split particle correction function. The principle of this function is described in Pace et al., 2011.

Finally, all signals in the selected intensity range are converted into particle diameters and their number is counted in order to generate a particle size distribution plot and estimation of the particle number concentration.



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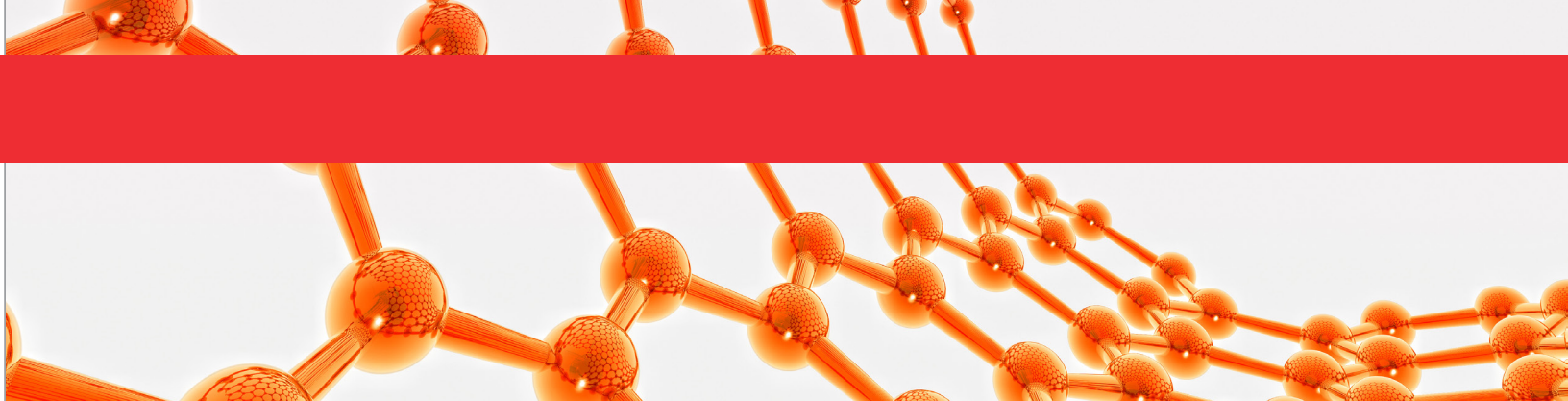
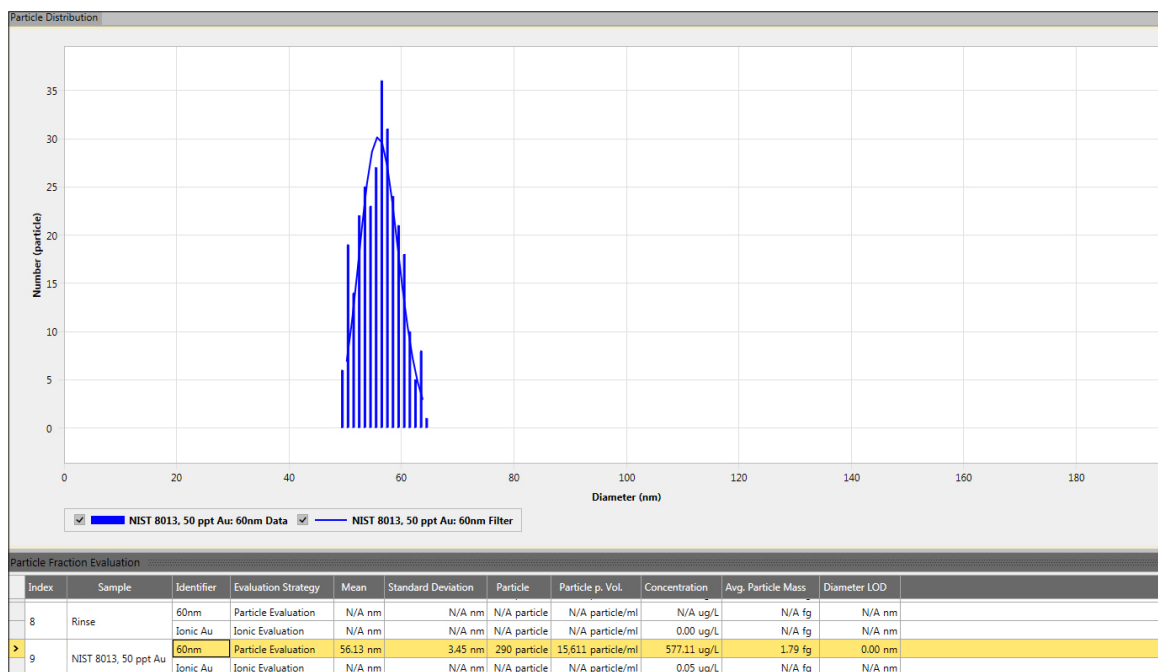


Figure 8. Particle Distribution View for result output.



The final generated results are shown in the Particle Distribution View, (Figure 8). Together with the particle distribution, the size average and the particle number concentration are also shown for particle fractions. At the same time the mass concentration of the element is calculated to allow comparison of the results. The concentration of dissolved species is displayed for fractions evaluated following the ionic evaluation strategy.



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Results

In order to demonstrate the features of npQuant mode, different samples containing the aforementioned particles were analyzed. The detection sensitivity was calibrated using single element standard solutions, whereas the transport efficiency was calibrated using NIST CRM 8013 (Au nanoparticles with 60 nm nominal diameter). The results obtained are described below.

Analysis of Au Nanoparticles

The same nanoparticles (NIST 8013) were analyzed at different dilutions (10, 50, 100 and 200 ng · L⁻¹). For each sample, the particle size and number concentration was calculated and is shown in Table 1.

As expected, the particle number concentration increases linearly, but the estimated size of the particles does not change. The filled slots ratio indicates that the particle number is still in an acceptable range and further sample dilution is not required. Under these conditions, the probability for multiple particle events is less than 1%, as can be also seen in the table.

Table 1. Results of the analysis of NIST 8013 nanoparticles in different concentrations.

Concentration [ng · L ⁻¹]	Size [nm]	Number Concentration [# · L ⁻¹]	Filled slots Ratio [%]	Multiple Events Probability [%]
10	55.0	3,564	0.06%	> 0.01
50	54.8	18,649	3.00%	0.05
100	55.2	33,647	5.33%	0.14
200	55.0	64,734	10.35%	0.55

Analysis of Nanoparticles Together with Dissolved Species

In a second experiment, Au nanoparticles were mixed with dissolved gold. While the amount of nanoparticles was kept constant, the amount of dissolved gold was increased between 0.05 and 0.5 µg · L⁻¹. The background signal was increased from 200 CPS (no Au added) to approximately 30,000 CPS (0.25 µg · L⁻¹ of Au added) and was evaluated using the ionic evaluation strategy for the corresponding fraction. Figure 9 shows the corresponding raw data.

Through setting the corresponding threshold values and evaluating both fractions (nanoparticles and dissolved background) separately, however, the correct results are obtained as can be seen in Table 2. At the same time, the result of the particle evaluation (size and number concentration of the Au nanoparticles) remains unaffected through the presence of dissolved Au in the sample solution.

Analysis of Particle Mixtures

A mixture of silver nanoparticles with nominal diameters of 20, 40 and 60 nm was analyzed qualitatively with the objective to estimate the particle size. By correctly setting the corresponding threshold values for all fractions, the particles could be evaluated separately revealing the average particle diameter for each fraction.

The determined particle sizes (20 ± 4 nm, 32 ± 4 nm and 47 ± 3 nm) corresponds well to the expected size taking into account the potential variability of the parameter.

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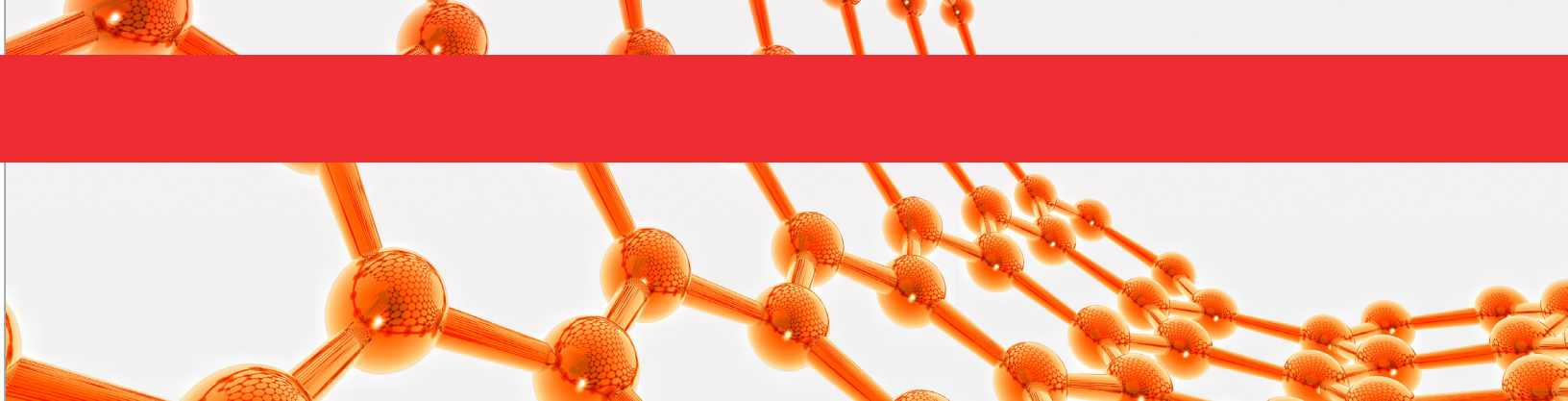


Figure 9. Comparison of background intensity for samples containing Au nanoparticles and 0 and 0.25 $\mu\text{g} \cdot \text{L}^{-1}$ dissolved Au.

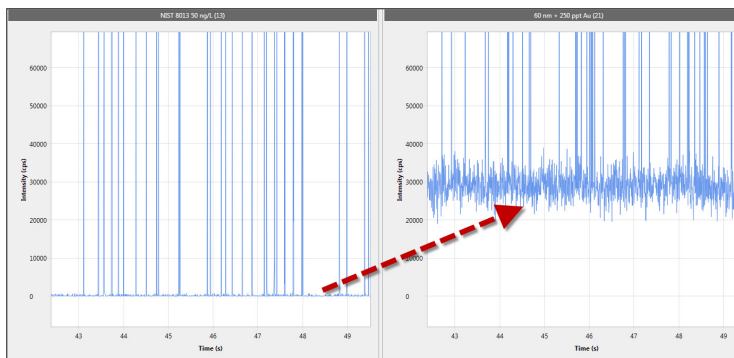


Table 2. Results of the simultaneous determination of nanoparticle size, number concentration and ionic concentration.

Sample	Result Particle Evaluation	Result Ionic Evaluation
60 nm	54.7 nm	0.00 $\mu\text{g} \cdot \text{L}^{-1}$
	11,620 # $\cdot \text{mL}^{-1}$	
60 nm + 0.05 $\mu\text{g} \cdot \text{L}^{-1}$	55.8 nm	0.06 $\mu\text{g} \cdot \text{L}^{-1}$
	12,260 # $\cdot \text{mL}^{-1}$	
60 nm + 0.1 $\mu\text{g} \cdot \text{L}^{-1}$	57.4 nm	0.12 $\mu\text{g} \cdot \text{L}^{-1}$
	11,087 # $\cdot \text{mL}^{-1}$	
60 nm + 0.5 $\mu\text{g} \cdot \text{L}^{-1}$	58.6 nm	0.58 $\mu\text{g} \cdot \text{L}^{-1}$
	11,849 # $\cdot \text{mL}^{-1}$	

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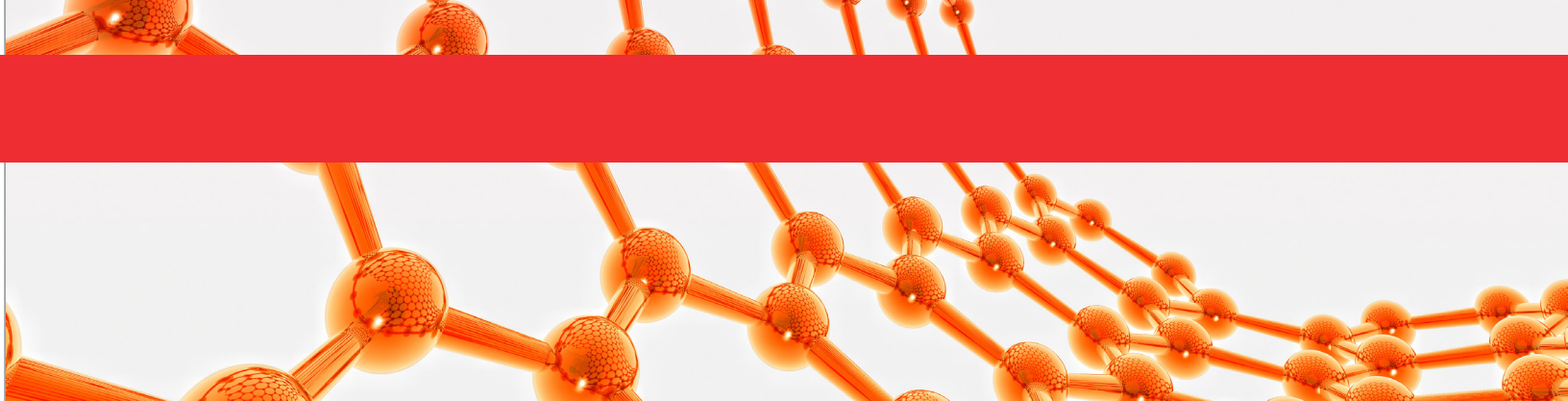


Figure 10. Statistical Data Evaluation available for each sample including estimation of dilution factor.

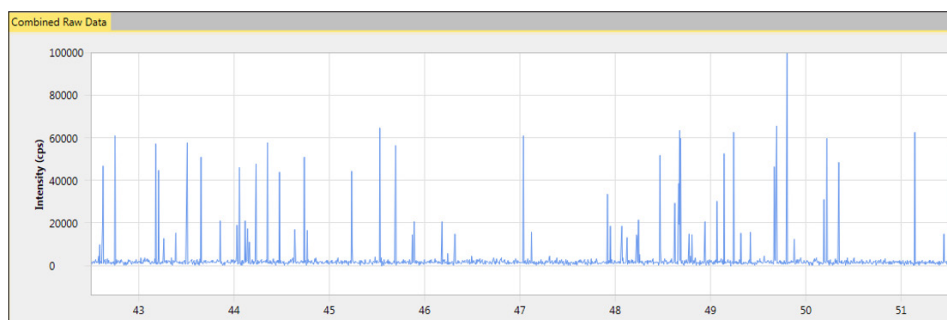


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Conclusion

The analysis of a nanomaterial is pushed ever further into the realm of routine analysis, following the direction of the regulatory authorities. The new npQuant evaluation module for Qtegra ISDS software enables the user to easily start this new task and offers key functionalities such as;

- Workflow based approach to method set up and data evaluation for ease of use
- Automated determination of key input parameters
- Tools to effectively judge the data quality and recognize/eliminate artefacts
- Seamless integration with existing workflows in routine analysis

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Verification of the Calculation Procedures in the npQuant Evaluation Module for Qtegra Intelligent Scientific Data Solution Software.

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Application Brief

The analysis of nanoparticles using inductively coupled plasma-mass spectrometry (ICP-MS) operated in the so-called single particle (sp) mode has gained increasing attention during recent years. In comparison to other common analytical methods (e.g. TEM, DLS etc.) it has several advantages that explain the growing role of this technique in the common method portfolio for nanoparticle analysis¹.

Above all, spICP-MS is experimentally simple to perform, since no special peripheral devices are required. Furthermore, it allows a sufficiently high number of particles to be probed in a short period of time (e.g. 500 particles in 60s). Last but not least, spICP-MS works at a low particle concentration range ($2\text{--}5 \times 10^4$ particles mL^{-1}), so that often, no pre-concentration steps are required for real samples, e.g. from environmental studies.

For correct results, however, it is necessary to determine special instrumental parameters, such as sample flow and transport efficiency to the plasma. For data evaluation, conversion of raw data into a particle size, distribution and number concentration can be accomplished through spreadsheet calculations, however, detailed knowledge on the different calculation steps is required to obtain accurate results.

In order to facilitate the use of spICP-MS in routine analysis, a dedicated software solution, npQuant, has been developed as an additional plug-in to the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software². This note highlights the correct calculation of results through verification against a well established spreadsheet calculation tool, namely the Single Particle Calculation Tool (SPC) created by the experts at RIKILT, Wageningen UR, The Netherlands. This spreadsheet is available and downloadable via the internet⁴ and is a widely accepted tool for performing spICP-MS calculations.

Method

A typical sample analysis workflow for the analysis of nanoparticles using a single quadrupole ICP-MS (Thermo Scientific™ iCAP™ Qc ICP-MS) operated in the single particle mode (spICP-MS) was performed. A well characterized, spherical, monodisperse certified reference material based on citrate stabilized gold nanoparticles with a nominal diameter of 60 nm (NIST 8013) was used for sample preparation. In brief, the reference particles were sonicated for 10 min in an ultrasonic bath and then diluted to obtain a final concentration of 50 ng L^{-1} .

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The acquired dataset was evaluated using both the npQuant evaluation module for Qtegra ISDS and the validated RIKILT Single Particle Calculation Tool (SPC). Relevant parameters potentially affecting the calculation (including sample flow, detection sensitivity and threshold values) were taken into account in order to evaluate the data under identical conditions. The calculations covered in this note included the determination of the transport efficiency and the calculation of particle size and number concentration. The transport efficiency parameter is especially crucial for the correct calculation of any results and needs to be determined carefully. As in the SPC tool, the npQuant plug-in uses an automated procedure based on the measurement of a particle containing standard solution.



Results

In order to obtain correct results using spICP-MS, it is crucial to determine the transport efficiency of the ICP-MS system. Briefly, this parameter reflects the fraction of sample that actually reaches the plasma with respect to the amount delivered to the nebulizer. Both, the npQuant plug-in and the SPC allow the determination of the transport efficiency based on a particle measurement, if a suitable standard with known particle size and number concentration is available. Transport efficiency can be assessed either through comparison of the expected and observed number of particles in a run, or the expected and observed particle size or mass. In a larger batch (50 unknown samples plus the required standards, 59 samples in total), 10 independent determinations of this parameter were performed overall. The transport efficiency determined through both of the above mentioned strategies was compared to the result obtained with the SPC tool. The results are summarized in Table 1.

As can be seen from the results, both ways of calculating the transport efficiency with the npQuant module agree with each other and furthermore, as expected, the transport efficiency also agrees well with the value determined using the SPC as a reference calculation tool. It is also evident that the assessment of the transport efficiency using the particle mass shows less variations in a larger batch as it is not as dependent on the conditions of the sample solution (particles may agglomerate over time).



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Table 1. Comparison of transport efficiency determined using the npQuant plug-in evaluation module and the SPC (N=10).

	npQuant – Particle number	npQuant – Particle mass	SPC
Average [%]	4.1	4.3	4.6
SD	0.3	0.04	0.3
RSD [%]	7.3	0.9	6.5

Particle Determination

In a similar way, the particle size and number determination was verified against the SPC. The reference particles were analyzed (6 repetitions), and the data was processed using both the npQuant module and the SPC. The average of the calculated particle size and the detected number of particle signals (which is subsequently converted into the number concentration in the sample) are shown in Table 2. Please note that the number of detected particles is slightly lower for the npQuant module, as, in contrast to the SPC, an upper threshold is also applied in addition to the lower threshold in order to discriminate a given signal range for evaluation.

The particle solution was analyzed under optimized concentration conditions (approx. 50 ng L⁻¹ for NIST 8013).

Both particle size and particle number concentration determined, using either the npQuant module or the SPC, did not show any significant variation when a t-test was applied (P>0.05). The values determined are virtually identical taking into account the aforementioned difference in data collection.

Table 2. Comparison of particle size and detected particle number.

Repetition		SPC	npQuant
1	Size [nm]	52	53
	# · mL⁻¹	21.800	21.000
2	Size [nm]	53	54
	# · mL⁻¹	23.200	22.900
3	Size [nm]	54	54
	# · mL⁻¹	24.300	23.500
4	Size [nm]	54	54
	# · mL⁻¹	24.000	23.300
5	Size [nm]	53	54
	# · mL⁻¹	26.200	25.800
6	Size [nm]	53	54
	# · mL⁻¹	21.700	21.900



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Conclusion

The performance of the npQuant Plug-in for Qtegra ISDS software was successfully evaluated against an external and validated calculation spreadsheet for spICP-MS. The verification procedures comprised both the automated determination of the transport efficiency input parameter, and the correct calculation of the particle size and determined number of particle derived signals in a data set.

Acknowledgement

S. Böhme, C. Cascio, H. Marvin and M. van der Lee from RIKILT – Institute of Food Safety (WUR, The Netherlands) are acknowledged for conducting the experimental work and scientific exchange. This work has been carried out within the Nanodefine Project (European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 604347).

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Expanding the Detectable Nanoparticle Size Range by High Resolution ICP-MS

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Overview

Purpose: To overcome the challenge of detecting smaller nanoparticle sizes with high absolute sensitivity of high resolution (HR) ICP-MS using the spICP-MS approach.

Methods: Samples containing nanoparticles were analyzed directly with a Thermo Scientific™ ELEMENT XR™ HR-ICP-MS by single particle ICP-MS (spICP-MS).

Results: The high sensitivity of the ELEMENT XR HR-ICP-MS and the Thermo Scientific™ ELEMENT 2™ HR-ICP-MS enables detection of smaller nanoparticles.

Introduction

The small size and high surface to mass ratio of nanoparticles compared to ionic species of the same analyte, has a strong influence upon the analytes' physiochemical and toxicokinetic properties. Smaller silver nanoparticles are shown to have more significant effects than larger nanoparticles or AgNO_3 [1]. Therefore, there is a trend for single particle ICP-MS towards reducing the smallest detectable nanoparticle size. This is however, hampered by the sensitivity and background of most instrumentation.

The smaller the nanoparticles which have to be detected, the higher sensitivity of the ICP-MS required to be able to detect these small particles.

Therefore, the ELEMENT XR HR-ICP-MS instrument with its high inherent sensitivity and low dark-noise was used in this study to demonstrate its capabilities to detect small nanoparticles.

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Methods

Sample Preparation

After sonication, samples were diluted in 2 mM sodium citrate. The diluted samples were sonicated again before analysis.

Sample Analysis

Samples were analyzed directly by the ELEMENT XR HR-ICP-MS using the conditions shown in Table 1.

The sample flow and nebulization efficiency were determined gravimetrically. The detection sensitivity was determined with an ionic silver standard.

Table 1. ELEMENT XR HR-ICP-MS parameters for Ag nanoparticle determination.

Parameter	Value
RF Power	1250 W
Sample Gas Flow	1.2 L/min
Nebulizer	Quartz, Self Aspirating
Spray Chamber	Cyclonic
Isotope Monitored	^{109}Ag
Detection Mode	Triple
Dwell Time	3 ms (0.001 s sample time, 4% mass window)
Sample Flow	0.14 mL/min
Nebulization Efficiency	0.25
Detection Sensitivity	1.07×10^6 cps/($\mu\text{g/L Ag}$)



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Results

The time resolved intensities of a solution containing 1 ng/L of 20 nm silver nanoparticles are shown in Figure 1. The nanoparticle 'events' are significantly above the background signal caused by dissolved silver.

Figure 1. Time resolved intensities of 20 nm Ag nanoparticles (1 ng/L).

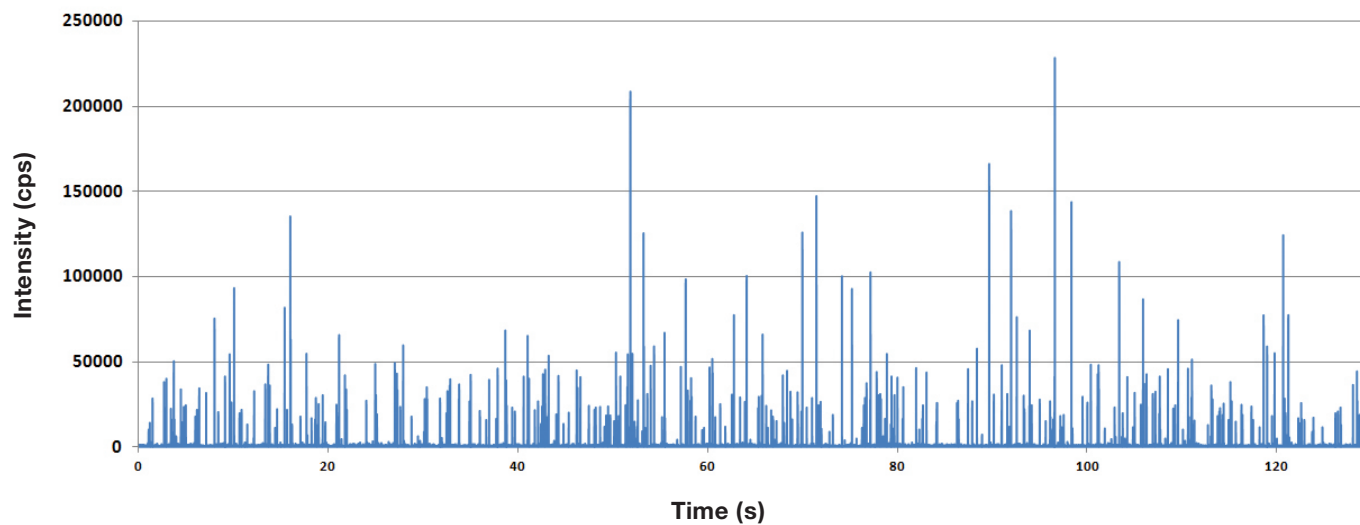


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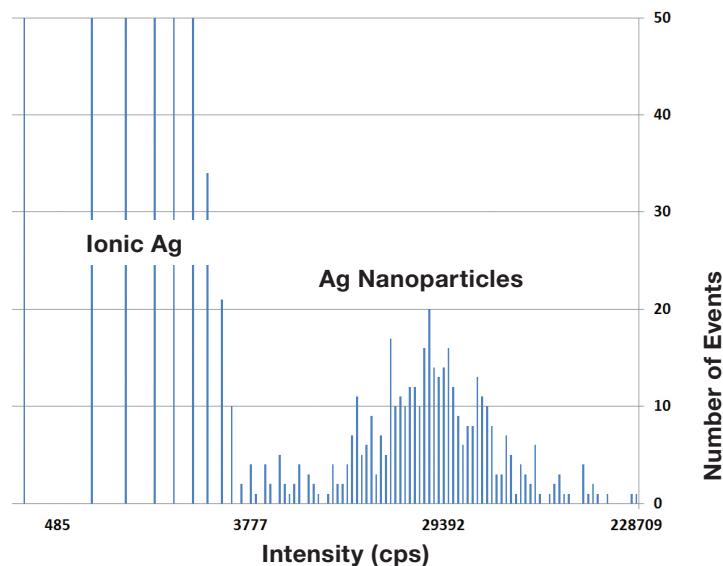
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Figure 2 shows the histogram for the signal distribution which is calculated from the time resolved intensities. Calculations were made using an in-house developed spreadsheet. A more comprehensive tool for single particle calculation has been developed by RIKILT Wageningen UR [2, 3].

Figure 2. Signal distribution (20 nm Ag nanoparticles).



The nanoparticles (right side of Figure 3) are well separated from the ionic Ag signals (left side). The fact that there is a wide valley between the ionic Ag signal and the 20 nm Ag particles shows that even smaller than 20 nm nanoparticles would have been able to be detected. The threshold for particle detection was visually determined in this figure to be 5000 cps.

Figure 3. Calculated Ag nanoparticle size distribution.

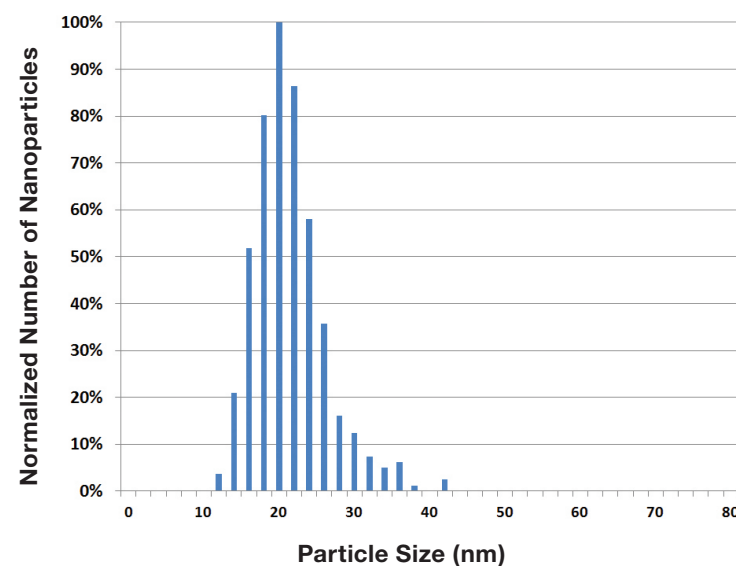


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With this threshold of 5000 cps and the silver density of 10.49 g/mL the nanoparticle size distribution shown in Figure 3 was calculated. A 20 nm Ag particle equals 42 attogram silver, which results in an intensity of 26 000 cps. Within the 3 ms dwell time this means that 78 counts were registered at the detector within that time. A 10 nm particle would result in 1/8 of this count rate. The results calculated from this dataset are shown in Table 2. The calculated particle size and the concentration calculated agree well with the expected values.

Table 2. Calculated results for a 1 ng/L solution of 20 nm Ag nanoparticles.

Parameter	Value
Particle Size	20 nm \pm 5 nm
Number of Particles Detected	395
Particle Number Concentration	1.1 x 10 ⁷ Particles/L
Ag Concentration of Particles	1 ng/L

Conclusion

- For the determination of small nanoparticles it is important to have an ICP-MS with the highest sensitivity.
- The high sensitivity of the ELEMENT 2 and ELEMENT XR HR-ICP-MS enables good detection of silver nanoparticles with 20 nm diameter and below.
- Hassellöv and coworkers have shown that 6.4 nm gold nanoparticles can be detected with the ELEMENT 2 using 0.1 ms time resolution [5].

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