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

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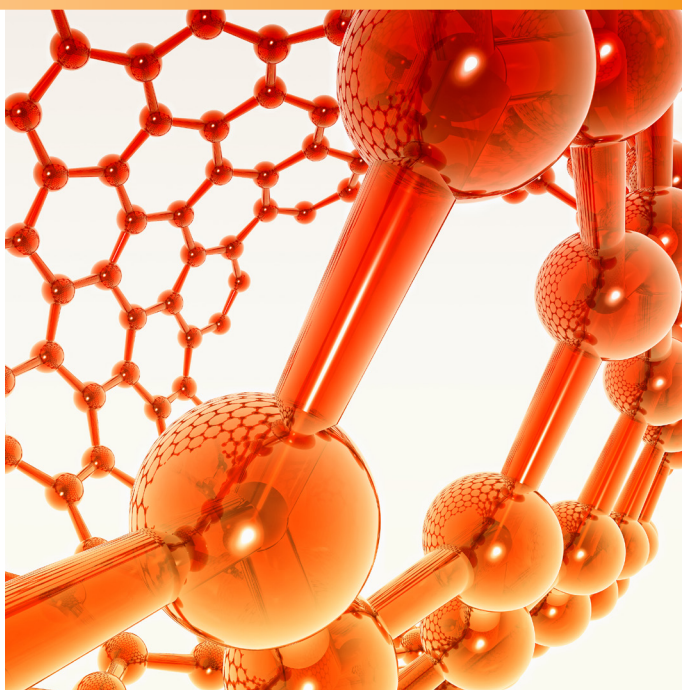
Key Words
Nanoparticles, spICP-MS, ICP-MS, Qtegra ISDS Software, AF4-ICP-MS

Goal

To demonstrate the analysis of nanomaterials using spICP-MS and highlight the features and options included in the dedicated evaluation module npQuant available for the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software.



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:

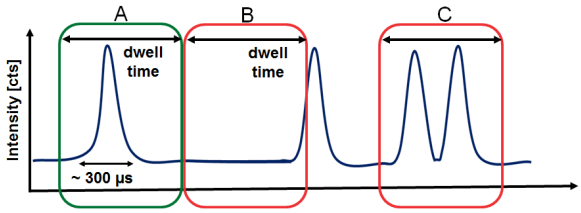

Figure 1. Correlation between observed signals in spICP-MS and potential formation of artifacts.

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.

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, Ti_{O2} 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 Qtegra 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 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 familar with Qtegra ISDS can easily get acquainted with the new task of analyzing nanoparticles.

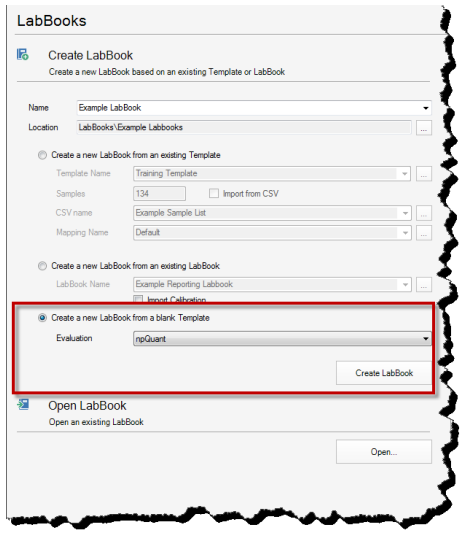

Figure 2. LabBook Editor after installation of npQuant.

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) the same automated scheduling of LabBooks so that the available instrument time can be exploited efficiently.

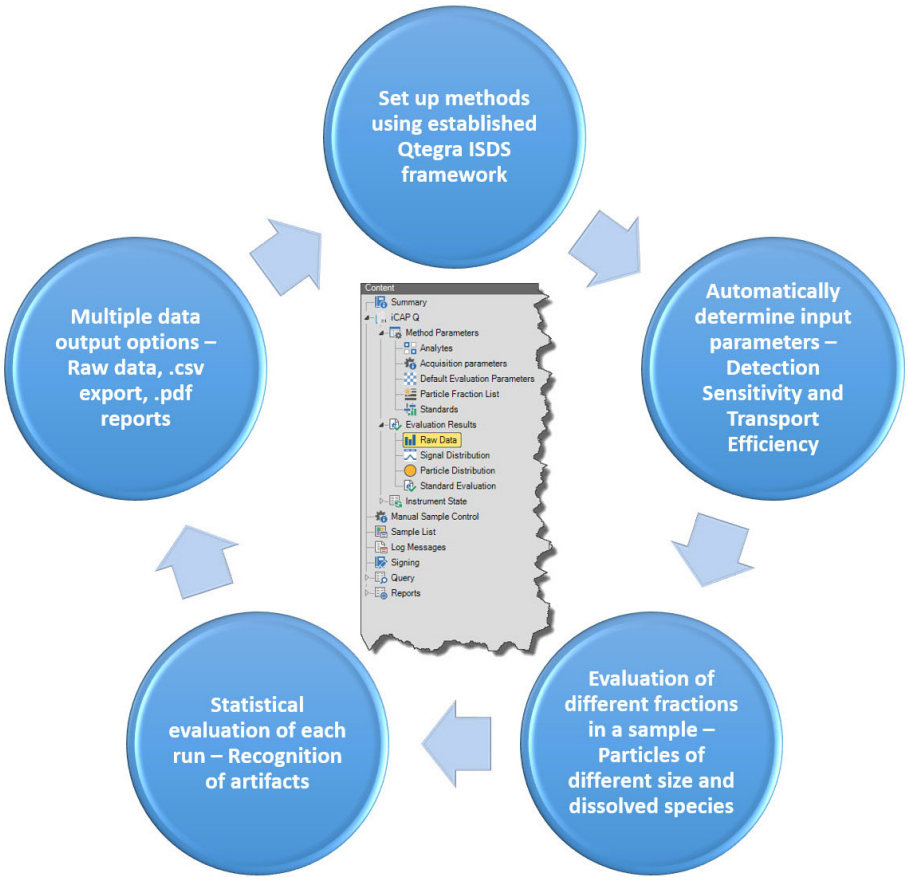

Figure 3. Overview of the npQuant evaluation method.

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. ng·L^{-1}.

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.

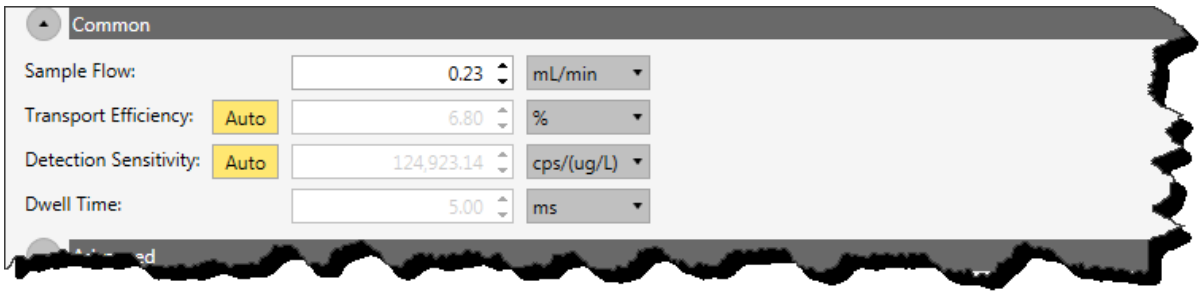

Figure 4. Default Evaluation Parameters View with automated determination of key input parameters.

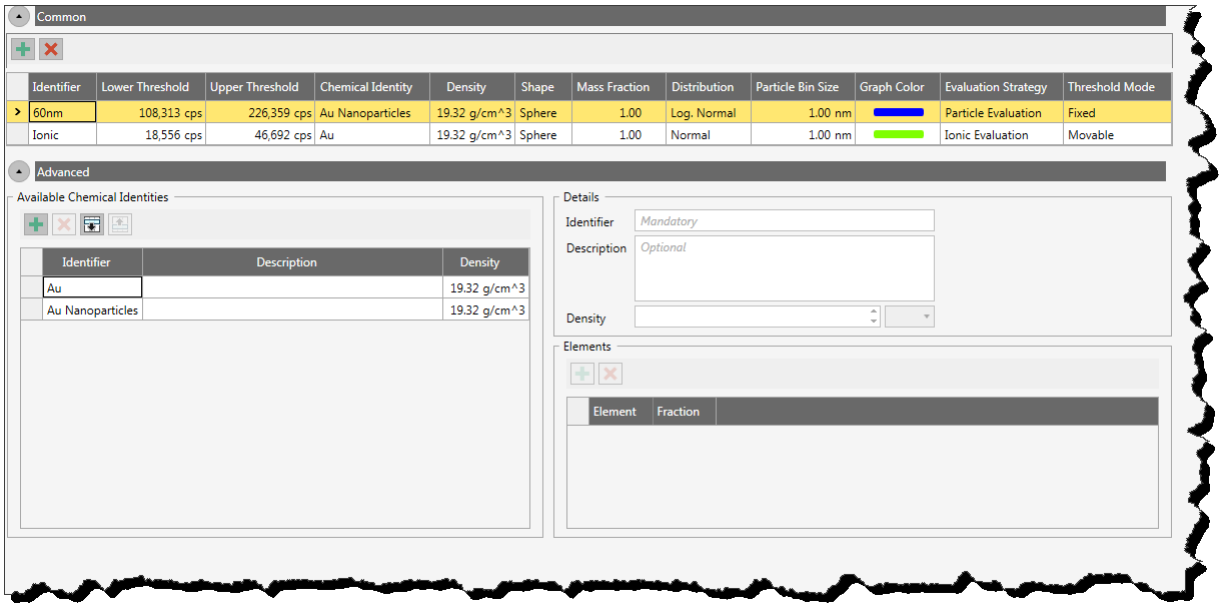

Figure 5. Definition of threshold values and material specific parameters using the Editor.

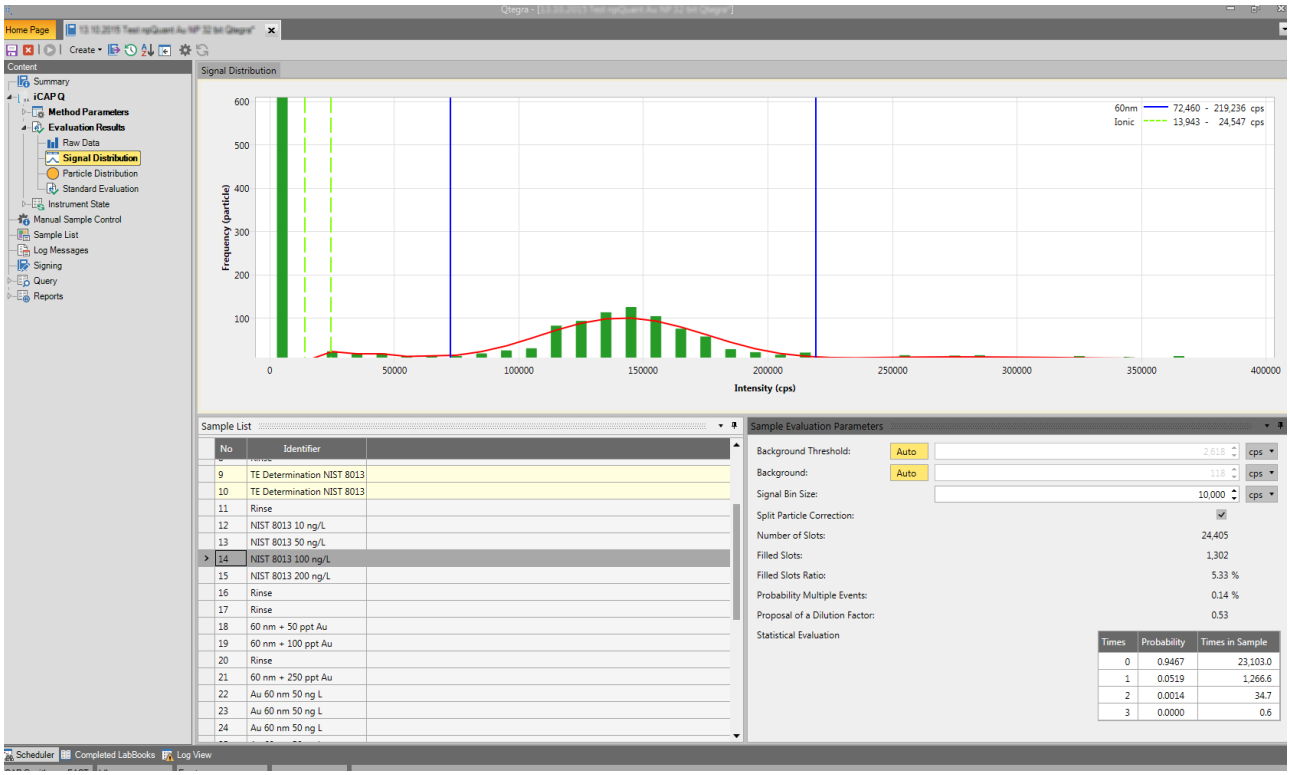

Figure 6. Signal Distribution View to identify and select nanoparticle derived signals.

Data Evaluation

Once the data acquisition is complete, npQuant 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.

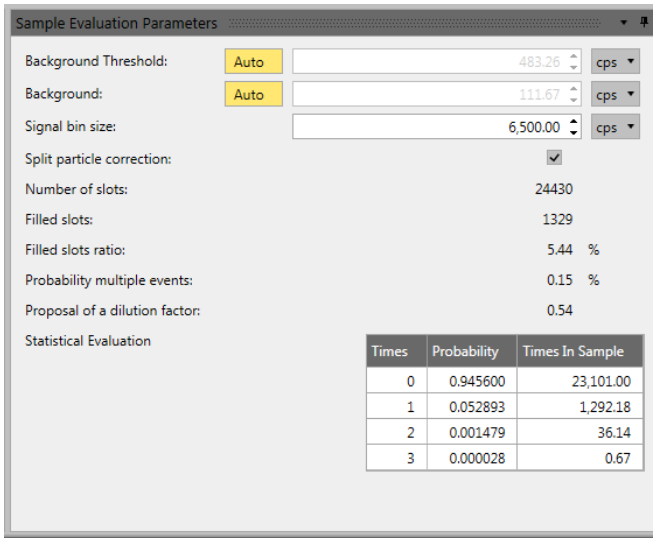

Figure 7. Statistical Data Evaluation available for each sample including estimation of optimum dilution factor.

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.

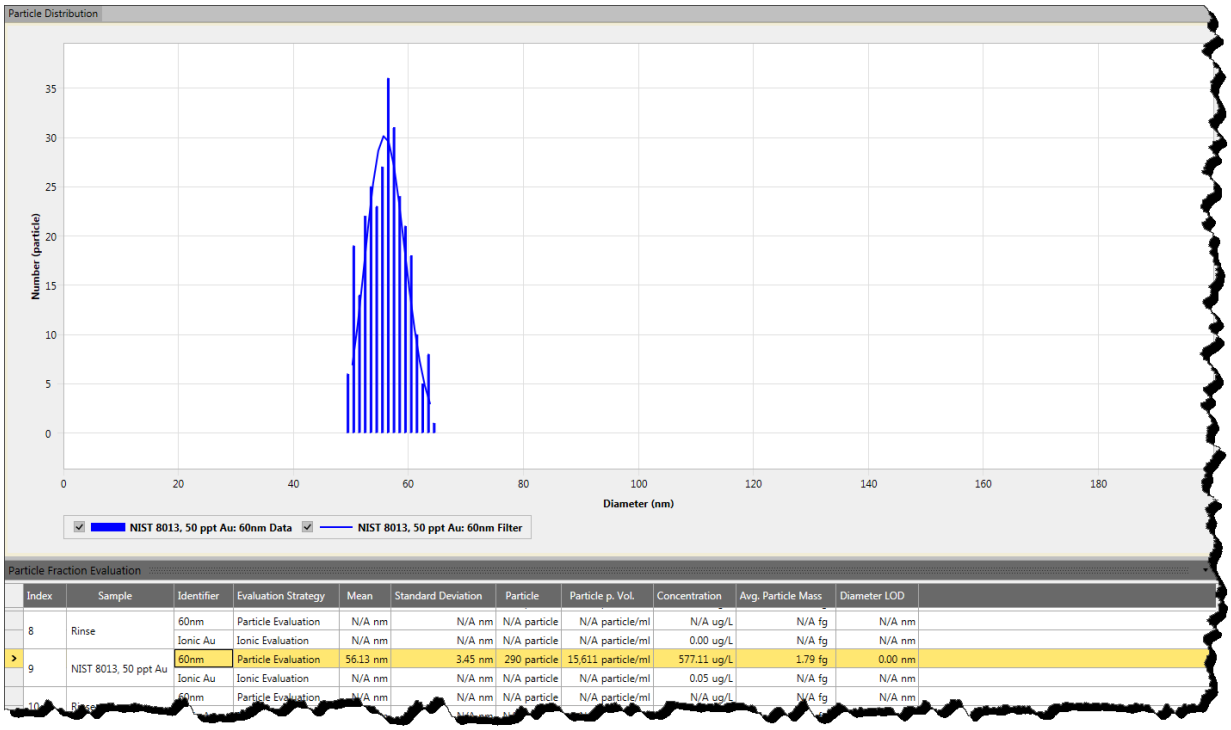

Figure 8. Particle Distribution View for result output.

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.

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	0.60%	> 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.

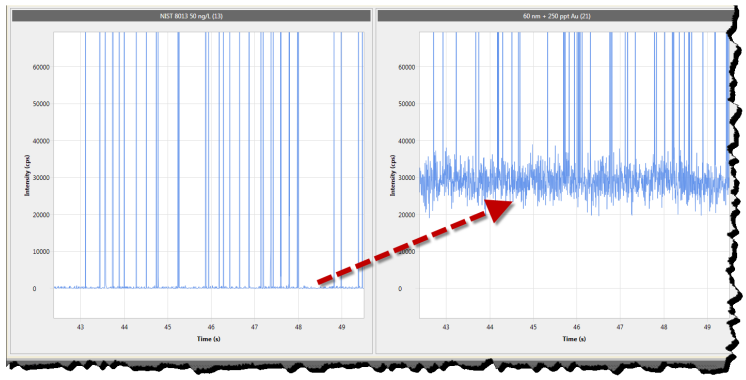

Figure 9. Comparison of background intensity for samples containing Au nanoparticles and 0 and 0.25 µg · L^{-1}.

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

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

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

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

References

1. EU commission Recommendation of 18 October 2011 on the definition of nanomaterial
2. Pace et al., *Anal. Chem. 83* (**2011**), 9361-9369
3. Liu et al., *Anal. Chem. 86* (**2014**), 3405-3414

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