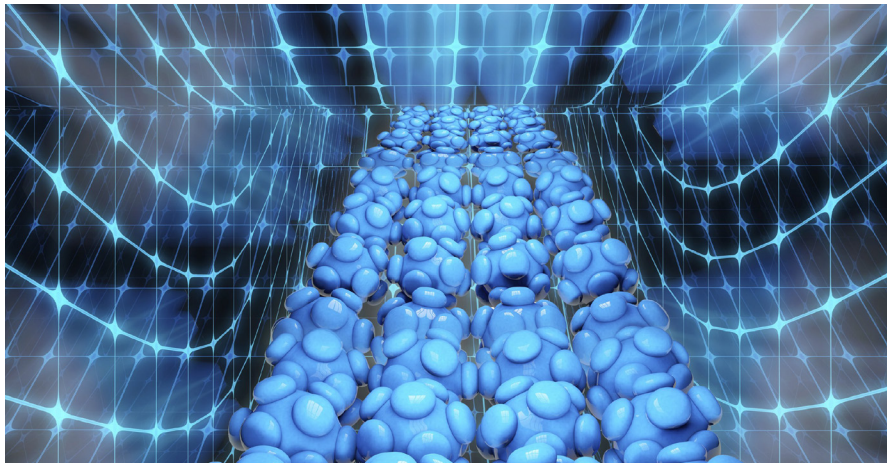
Characterization of nanoparticles in aqueous samples by ICP-MS

White paper



Authors

Steve Wilbur, Michiko Yamanaka, and Sebastien Sannac

Agilent Technologies

Abstract

ICP-MS has become the technique of choice for detection and characterization of nanoparticles in solution. Compared with other techniques, ICP-MS is unique in its ability to provide information on nanoparticle size, size distribution, elemental composition, and number concentration in a single, rapid analysis. In addition, only ICP-MS can simultaneously determine the concentration of dissolved analyte in the sample.

ICP-MS can be used in two different modes, either in single particle mode to characterize individual particles, or coupled to a separation technique such as field-flow fractionation or capillary electrophoresis to characterize bulk samples. Both techniques have benefits and limitations, but are complementary when used together.

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Introduction

Nanoparticles are microscopic particles, either naturally occurring or manmade (engineered), of any shape with dimensions in the 10^{-9} m to 10^{-7} m range (IUPAC). The use of engineered nanoparticles to enhance the performance or properties of products ranging from semiconductor materials to foods, drugs, cosmetics, and consumer goods is increasing at a rapid rate. Because of the novel physical and chemical characteristics of these materials, much remains unknown of their environmental fate and toxicological properties. As a result, there is growing need for a rapid, accurate, sensitive technique for characterizing and quantifying nanoparticles in a wide range of sample types. ICP-MS has demonstrated the ability to meet these requirements through the recent implementation of some application-specific enhancements to both hardware and software.

The main benefits of ICP-MS for the detection, characterization, and quantification of nanoparticles are related to its high sensitivity and specificity, which can provide additional information compared to other techniques such as dynamic light scattering. ICP-MS is also fast and requires little sample preparation compared to techniques such as scanning or transmission electron microscopy (SEM, TEM), atomic force microscopy (AFM), or separation techniques such as differential centrifugation [1].

However, ICP-MS analysis of nanoparticles is not without its unique challenges. Characterization of nanoparticles using ICP-MS detection can be achieved via one of two different strategies, each with its own benefits and challenges.

Table 1. Comparison of pros and cons of single particle ICP-MS versus hyphenated ICP-MS for nanoparticle characterization, modified from Heithmar, 2011 [2].

Single Particle ICP-MS	Hyphenated ICP-MS
Determines the particle number concentration (particles/mL) and the mass of metal in individual particles and size distribution	Determines total metal concentration as a function of particle size fraction
Does not provide direct information on particle shape or diameter	Minimum particle size is not limited by ICP-MS sensitivity
Minimum particle size is limited by ICP-MS sensitivity, background and dissolved (ionic) element content	Does not provide direct information on number of particles or characteristics of individual particles

Single particle ICP-MS

Using ICP-MS to directly detect and quantify the signal generated from the atomization and ionization of a single particle introduced to the plasma

This is commonly termed "single particle mode". In theory, single particle mode is simple. A particle suspended in a nebulized liquid is carried to the plasma contained within a droplet of liquid aerosol. That droplet is sequentially desolvated and its contents atomized and ionized, creating a plume of ions that enter the mass spectrometer where they are separated by mass/charge (m/z) and detected (Figure 1) using a time resolved analysis (TRA) acquisition. If the sample is sufficiently dilute with respect to the number of nanoparticles in solution, then no more than one particle will enter the plasma at a time. The resulting plume of ions, measured as a discrete signal pulse, is proportional to the mass of the selected analyte elements in the original particle. If the masses and densities of the elemental constituents of the particle are known along with the elemental response factor based on an ionic calibration standard, then the theoretical size of the particle, calculated as a sphere, can be determined. If the transport efficiency from the nebulizer to the plasma is also known, then the particle number concentration can be determined [3].

However, several additional assumptions need to be made:

- Data analysis capability to date assumes spherical particle shape
- The particle is solid, not hollow
- The total elemental composition is known or can be determined
- The elemental components are uniformly distributed throughout the particle, i.e., the particle is not constructed in layers
- The elemental determination is free from interferences

- The particle is completely and consistently atomized and ionized in the plasma, free from matrix effects (the ionization efficiency matches that of the ionic calibration standard solution and reference material)

- The nebulization efficiency can be accurately determined and remains constant between reference materials and samples

Some of these assumptions can be accepted with their known limitations, such as the assumption that the particle is spherical. For many types of particle this is generally true. For nonspherical particles (tubes, needles, stars etc.), knowing the particle mass and volume is still useful, with the understanding that the calculated spherical volume equivalent diameter is just that, an assumption. Validation of particle shape by electron microscopy or another method is a useful ancillary technique when the particle shape is unknown.

While not all of these assumptions can be validated, several can be addressed and minimized or eliminated by careful technique and ICP-MS instrument selection and optimization. The controllable assumptions are:

- Freedom from interferences can be achieved for most elements by using helium collision mode. This is universal for most polyatomic interferences and therefore can be applied generically to the determination of nanoparticles containing more than one metal. However, determination of more than one element will increase data acquisition time and introduce a mass jump settling time, reducing the number of scans acquired for a single nanoparticle event. Some of the elements that commonly occur in engineered nanoparticles, such as silicon and titanium, are subject to intense polyatomic interferences that cannot be completely removed by conventional quadrupole ICP-MS instruments. In this case the MS/MS capability of triple quadrupole ICP-MS should be used.

- Complete and consistent atomization and ionization require high temperature and highly robust plasma conditions. Plasma robustness can be easily determined by monitoring the dissociation of refractory oxides in the plasma, measured as the ratio of CeO^+/Ce^+. A highly robust plasma is evidenced by CeO^+/Ce^+ of 1% or less.

Combining an initial separation technique, FFF (typically asymmetric flow-field flow fractionation (AF4) as depicted in Figure 2) or capillary electrophoresis (CE), with detection by single particle mode shows potential to increase the available information from a single analysis.

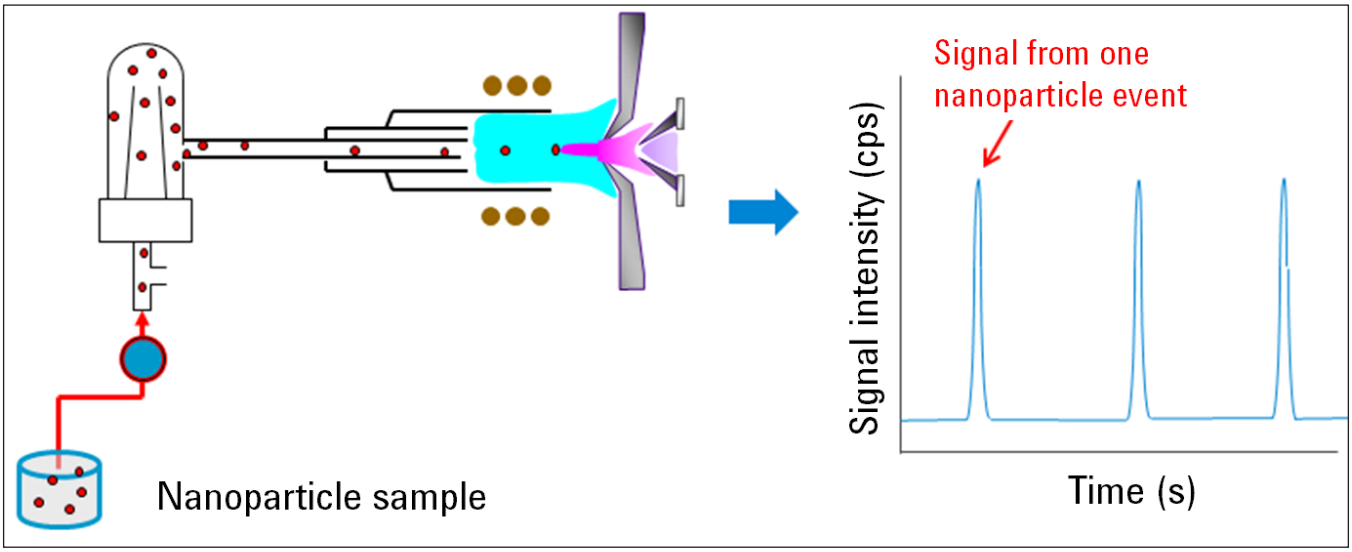

Figure 1. Schematic diagram of particle transport from sample to ICP-MS interface and resulting time-resolved signal generated by the mass spectrometer.

Hyphenated ICP-MS

Using ICP-MS as a sensitive, element-specific detector after an online separation step as part of a hyphenated system

Most commonly, field-flow fractionation is used as the separation step because it is well suited to the mass and size range of nanoparticles. Field-flow fractionation (FFF) is a developing online fractionation technique that enables the separation of macromolecules, colloids, nano- and microparticles according to size, chemical composition or density with excellent resolution over a size range from a few nanometers up to several microns. Many of the limitations of other separation techniques, including degradation, filtration, decreased resolution or unwanted adsorption, can be overcome by FFF. The FFF principle was developed by Calvin Giddings in 1966. In FFF, separation is performed in an empty channel by applying an external separation field perpendicular to the sample flow. Different sized particles diffuse back against this field at different rates (smallest, fastest) which leads to separation of the particles in the different flow layers (Figure 2). The parabolic profile of the laminar solution flow means that the different layers travel at different velocities, and as a result, the analyte molecules or particles emerge sequentially (smallest first). Since the FFF technique allows the characterization of a sample inside an empty channel without using a stationary phase, a source of bias when using other separation techniques is eliminated [4].

Recently, the use of capillary electrophoresis (CE) coupled to ICP-MS has been demonstrated to separate a range of very small metallic nanoparticles in dietary supplements and other complex matrices with excellent resolution [5,6]. CE-ICP-MS has the potential benefits of much shorter run times than FFF, higher resolution, and smaller sample size requirements.

It is expected that improvements to these and other hyphenated separation techniques will be developed, which may reduce some of the limitations of current systems.

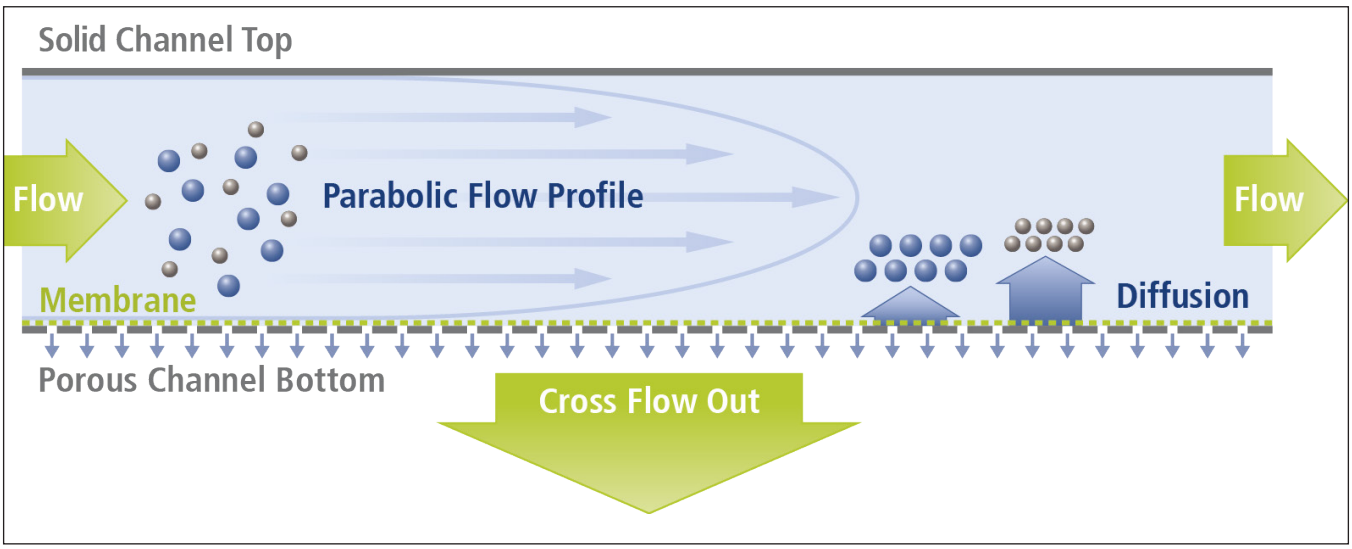

Figure 2. Schematic diagram of a field-flow-fractionation channel. (Diagram courtesy of PostNova Analytics)

Minimum detectable particle diameter (single particle mode)

Within the limitations of these assumptions, the minimum particle diameter that can be determined by single particle ICP-MS is strictly limited by signal to background. In the case of single particle analysis, the background is composed of instrument noise, spectral interferences, and uniquely, dissolved ion concentration. For example, silver nanoparticles may partially dissolve in the matrix, depending on the matrix composition and sample preparation resulting in a solution that contains dissolved as well as particulate silver (Figure 3). Therefore, it is necessary to compensate for any on-mass background before the mass of the particle can be determined. Also, it is often necessary to quantify the concentration of dissolved, ionic metals in the sample to fully understand the dynamics of the nanoparticles in the system under investigation.

In the simple case of a spherical nanoparticle composed entirely of a single element, such as silver or gold, the volume (v) of the particle is simply the total mass of the background-corrected ion plume divided by the density of the element. The total mass determination is limited by the sensitivity of the instrument. This is even more critical when using very short integration times.

Since the volume of a sphere is equal to $4/3\ \pi r^3$, and the particle volume can be calculated from the particle mass, m_p provided by the ICP-MS, divided by the density, ρ, the spherical volume equivalent diameter, d is given by the equation below:

$$d = \sqrt[3]{\frac{6 * m_p}{\pi * \rho}}$$

Because the volume (mass) of a sphere is related to the cube of its diameter, a reduction by half in particle diameter, for example from 60 nm to 30 nm, results in an 8x reduction in mass and therefore signal. A 15 nm particle will generate only 1/64 the signal of a 60 nm particle. This rapidly decreasing signal with particle size, especially when using very short integration times or in the presence of high background, is the main limitation of single particle mode for analysis of small (< 10 µm) nanoparticles. The highest possible sensitivity and lowest possible background are critical to this analysis.

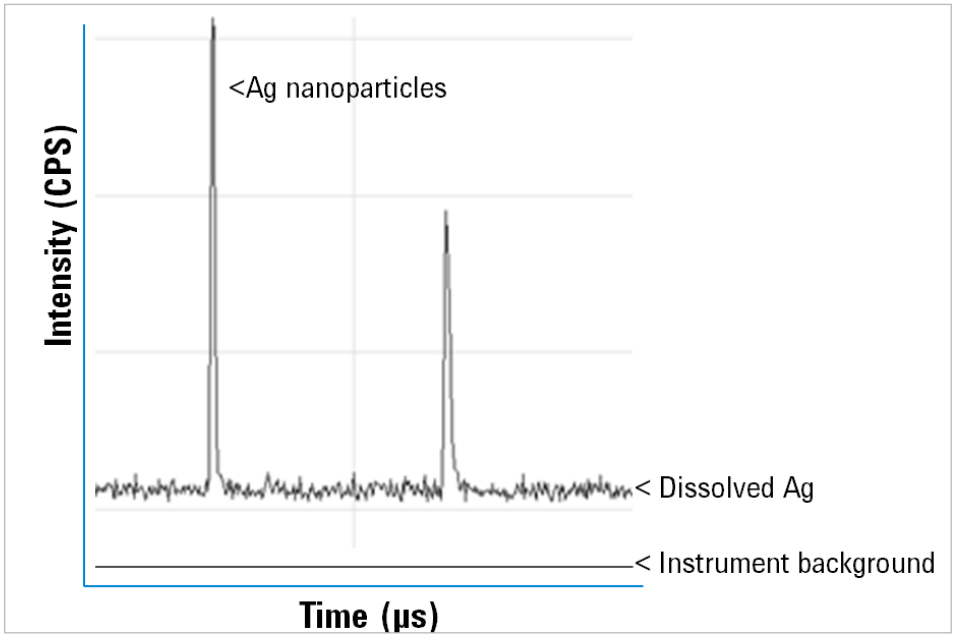

Figure 3. Expanded view of signal from two silver nanoparticle events. The baseline between particles, which is due to dissolved Ag, must be subtracted before calculation of nanoparticle mass.

Effect of integration (dwell) time

Several authors have stated that reducing integration time enables the detection of smaller particles because, while the total background counts over the dwell period are reduced proportionally, the signal from the particle plume is not reduced [2,8]. This results in higher signal-to-noise. However. this is only true if the entire plume of ions is contained within a single dwell period. If the dwell period is smaller than the duration of the plume, which is typically around 500 µs, then analyte signal is also reduced with decreasing dwell time. In addition, at the very short integration times that are sometimes quoted (as low as 10 µs), counting statistics can increase the error of the determination significantly. For example, the difference between 0 counts and 1 count at 10 µs dwell time is equivalent to a difference of 100,000 cps. For these reasons, it is important to maintain statistically valid count rates by using the highest possible sensitivity (signal-to-noise), and an appropriate integration time.

Additionally, when dwell times shorter than the particle plume duration are employed with integration of the total signal over several dwell periods, especially for small particles, negative particle size bias can result. This is caused by inability to detect the small signal at both ends of the Gaussian distribution (Figure 6). In general, integration times between 100 µs and 10 ms have been shown to be optimum [7,8]. The biggest limitation to dwell times that are too long (longer than the plume duration), in addition to increased background signal, is the possibility of more than one particle being counted within a single dwell period (Figures 4 and 5). This will result in positive bias of the particle size distribution and negative bias in the particle number concentration. For this reason, it is important that the number of particles entering the plasma per unit time is controlled by appropriate sample flow rate and/or dilution. Ideally around 1 in 10 dwell periods should contain a single particle, with the remaining dwell periods allowing accurate measurement of the background signal. For particle concentrations of 250,000 particles/mL at 300 µL/min flow, integration times of 50 µs to 1 ms resulted in accurate and consistent particle size calculation for 60 nm Au particles [8].

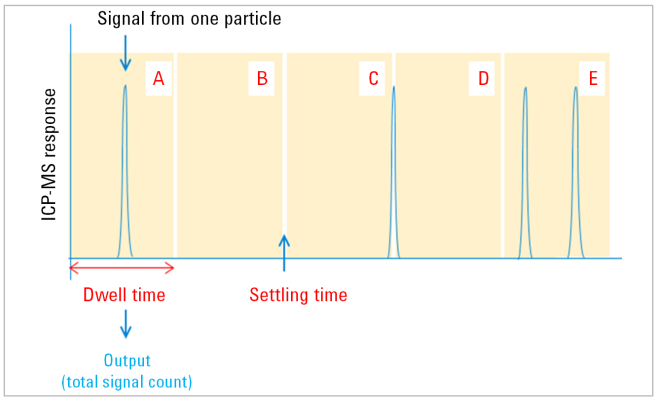

Figure 4. Dwell time is much longer than particle plume duration. A – ideal situation, entire single particle is captured within the integrated signal time. B – acceptable, no particles were present during this dwell time. C – Must be avoided, particle is split between two dwell times. E – Must be avoided, two particles within a single dwell time will result in double the signal of a single particle.

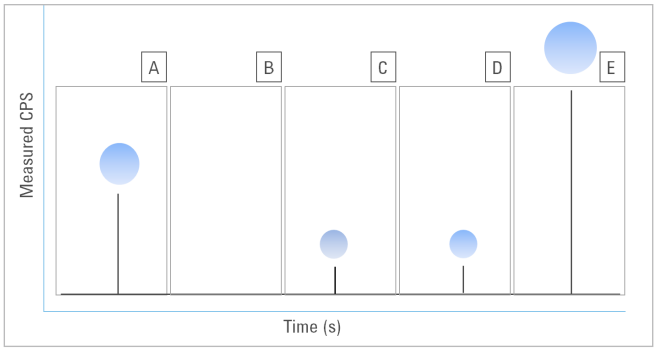

Figure 5. Resulting mass spectrum (integrated signal over each dwell time) and approximate particle sizes for example in Figure 4

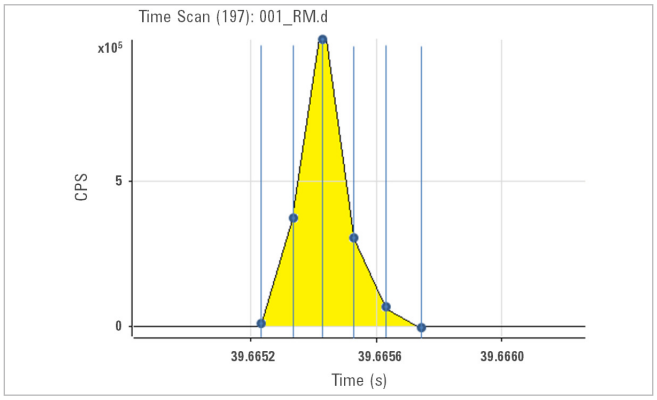

Figure 6. Single nanoparticle event acquired using very fast time resolved analysis (TRA) mode with 100 µs dwell time and no settling time. Shaded area represents total ion count summed over five dwell periods. The small area in the rightmost dwell period could be lost in the baseline noise under less than ideal conditions.

Special requirements for single particle data analysis

Single particle data analysis presents some difficult challenges. First, due to the very short integration times typically used (50 to 100 µs), a very large number of data points are generated. At 100 µs dwell time, 10,000 data points (time and intensity for each mass) per second are created. Managing and processing such large data files with reasonable speed are critical. Early researchers typically saved the data in CSV format for export into Microsoft Excel for processing. However, limitations on the maximum number of lines supported by Microsoft Excel limited the data file size that could be processed. More recently, dedicated data analysis software integrated into MassHunter software removes this limitation on file size, permitting higher frequency acquisition and longer analysis times if needed.

Data analysis for single nanoparticles, whether performed in Microsoft Excel, or in integrated instrument software, follows the same process:

1. Data are acquired and stored in a TRA data file, with each data point representing the measured intensity of the monitored mass(es) during the integration period. The raw TRA data, when plotted as intensity (CPS) versus time, are shown in Figure 7a.
2. The average background is determined by evaluating the signal between nanoparticle peaks and subtracted from the raw data. In this way, the total signal generated for each nanoparticle is due to the particle alone, and not to any dissolved metal or instrument background signals.
3. If the dwell times are shorter than the particle event duration, the signal for each dwell period within the particle peak is integrated, creating a total signal for the particle.
4. Nebulization efficiency is calculated by comparing measured number of particles with known number of particles in a reference material. Alternatively, nebulization efficiency can be calculated from a reference material of known particle size.
5. The background-corrected, integrated data is sorted by response and plotted as shown in Figure 7b. Since the intensity of each integrated signal is proportional to the particle mass, a relative distribution of particle sizes can easily be visualized.
6. The total number of particles is calculated from the number of detected particles corrected for the nebulization efficiency.
7. The response distribution is converted to a size distribution profile using the corrected response, the elemental response factor for the measured element as determined by an ionic calibration standard, and the density of the measured element. The assumptions about spherical particle shape and elemental homogeneity are applied (Figure 7c).

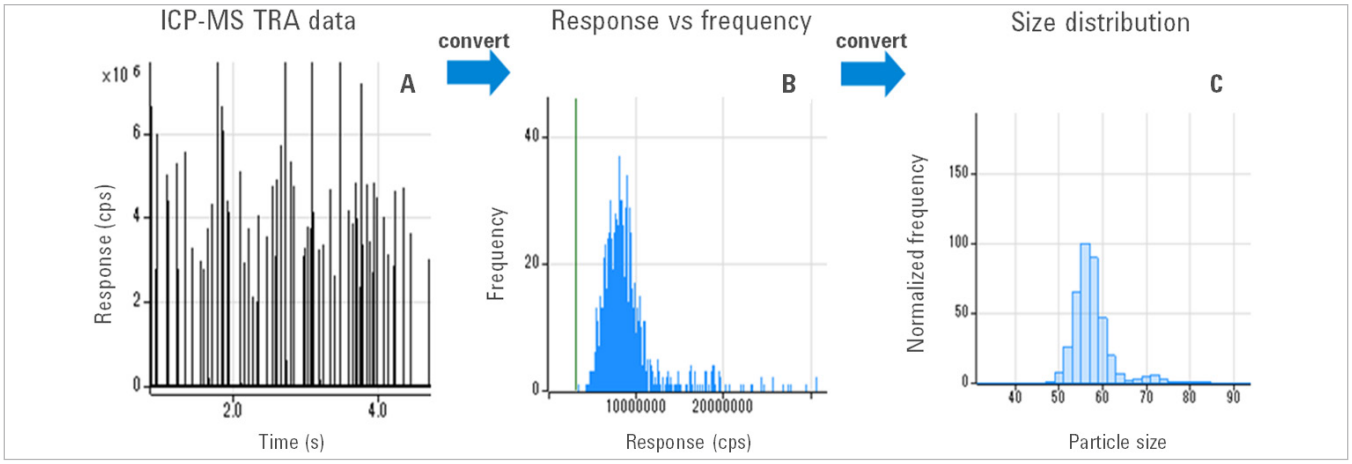

Figure 7. A. TRA CPS data, B. TRA data sorted by response per dwell time (after background subtraction), C. Calculated size distribution for a population of like-sized particles

A final report in the MassHunter Single Nanoparticle Analysis Module is shown in Figure 8. Tabulated results for all standards, reference materials, and samples are presented in the easy-to-read, interactive "Batch at a Glance" format common to all MassHunter data analysis modules. Detailed graphical results for individual samples can be viewed by selecting the sample from the table. Powerful manual data optimization and validation tools are available to ensure the best possible results are finally printed or archived.

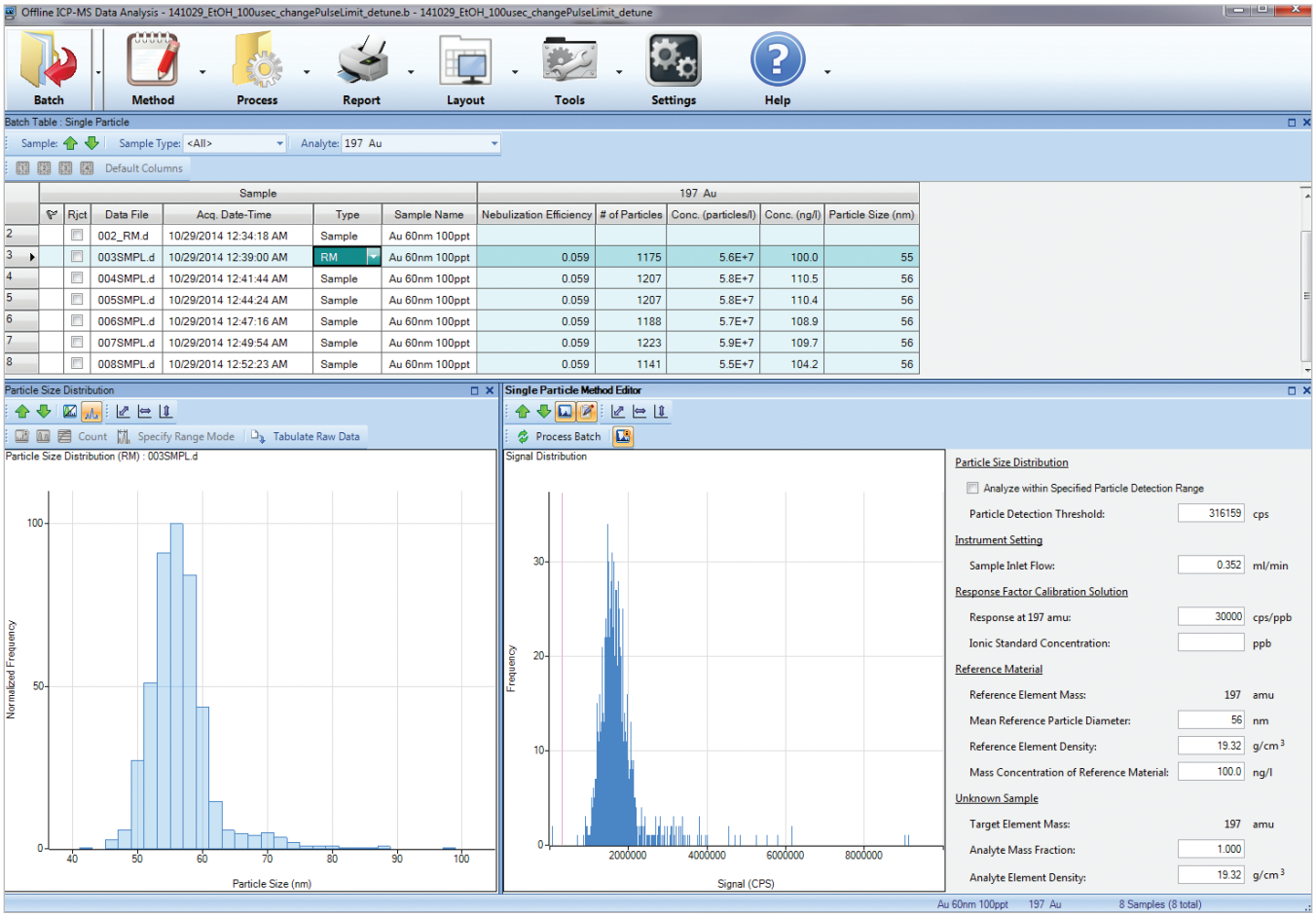

Figure 8. Data view from the Agilent MassHunter Single Nanoparticle Analysis Module. Tabular results for all samples are displayed in the Batch at a Glance table at the top of the screen. Graphical results for the selected sample (highlighted in table) are displayed below, including particle size distribution, signal distribution, and method parameters. Raw TRA data can also be displayed graphically. This single particle method editor pane permits interactive, graphical evaluation and optimization of results and can be applied to a single sample or the entire batch at the click of a button.

Conclusion

ICP-MS is an excellent tool for the determination and characterization of nanoparticles in aqueous samples, and provides, in a single analysis, a range of particle and sample information that is generally not available with other techniques.

When coupled to a separation technique such as field-flow fractionation or capillary electrophoresis, ICP-MS can provide particle size information for particle populations composed of different sized particles of variable composition within a sample. Total metal concentration within each particle size population is also provided. As long as sufficient numbers of particles are present, with appropriate separation resolution, there is no lower limit to the size of particles that can be determined. Additionally, there is no practical limit on the number of elements that can be determined within a particle population. When used in single particle mode, ICP-MS can measure the ions created from a single nanoparticle in the sample, to determine its size (mass), size distribution, number concentration, and chemical composition. Since these techniques provide somewhat different information, the choice of which technique is best will depend on the characteristics of the sample and the information desired. These two complementary techniques can be combined when neither technique can provide the desired information alone.

In both cases, the most critical performance characteristics of the ICP-MS for single nanoparticle analysis are:

- High sensitivity, especially when measuring small particles in single particle mode
- Low background signal, especially from polyatomic interferences that can contribute to baseline signal, limiting the minimum particle size that can be detected
- Ability to scan fast enough to capture the signal from a single nanoparticle without risk of multiple particles being detected within a single dwell period and without excessive integrated signal from ionic background (single particle mode)
- Minimum or no settling time between scans (single particle mode)
- Robust plasma ($CeO^+/Ce^+ < 1\%$) to ensure complete atomization and ionization of nanoparticles and reduce matrix-effect differences between samples and standards
- Integrated data analysis software capable of quickly and automatically performing the complex calculations required, while permitting simple, visual optimization and validation of results (single particle mode)

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