

icpTOF Quantistar Microdroplet Sample Introduction/Calibration System for Simple, Fast and Accurate Multi-Element Single- Particle and Single-Cell Analysis

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Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) is widely used for sensitive detection of individual particles and cells in different research fields. The main advantages of single particle (sp)-ICP-MS are high throughput, high specificity, and the ability to determine particle mass and particle number concentration (PNC) with exceptional detection limits. Here, particle refers to both solid nano- or submicron-particle and a biological cell. The icpTOF – TOFWERK's ICP-time-of-flight-MS - adds simultaneous multi-element detection capabilities to sp-ICP-MS and extends its application from single element to multi-element particles present in complex samples like space dust, soil, and surface waters [1-3].

Conventional Calibration Method

A calibration method for sp-ICP-MS was proposed by Pace et al. in 2011 [4] and is now widely accepted and used in different application fields including single-cell analysis. This method utilizes dissolved element standards to calibrate the instrument response. To attain the particle mass and PNC with this method the transport efficiency (TE) of the sample introduction system must be determined in addition to the calibration. This is done by measuring reference particles of either known size or known particle number concentration; often Au nanoparticles are used for this purpose. However, the PNC is rarely specified for particulate standards as it is unstable over time and depends strongly on handling conditions. Consequently, TE determination based on the known particle size (size-based method)

rather than PNC-based is more reliable and is, therefore, more often performed. In the size-based method, Au element standards must be measured in addition to Au nanoparticles. Despite its wide application, the conventional method has several drawbacks. First, it needs reference particles for the transport efficiency determination. Second, it requires matrix-matched samples and standards to generate accurate results. For instance, if a particle sample is dispensed in water, all standards should ideally be prepared in water. Some elements, however, are not stable at neutral pH. One prominent example is ionic Au which, when prepared in water, must be measured fresh and often separate from other element standards. Au also is prompt to generate significant memory effects, which requires extended washing times. In addition, the measurement of series of calibration standards (i.e., one series for analytes, one series for the TE determination) and standard particles make this method relatively time consuming. In summary, the method works accurately only if all aforementioned precautions are met.

Microdroplet Calibration Method

An alternative calibration method based on monodisperse discrete microdroplets of known volume has been developed and successfully applied for ICP-OES and ICP-MS analysis of NPs [5, 6]. Microdroplets can be used as

proxies for nanoparticles making this method standard-free. The method is simple and fast. Monodisperse microdroplets can be generated in several ways, but the piezo-driven inkjet printing technology is most frequently utilized. It produces picoliter droplets with drop-to-drop volume reproducibility of <1% at defined frequency. The droplets are ejected into a transfer line interfaced to the ICP-MS, in which they are dried and further transported into the plasma via a flow of He/Ar gas. The rest of the solvent is vaporized inside the ICP leaving behind a dry particulate salt residue consisting of all elements initially present in the element standard mixture solution. This dry particle is then atomized and ionized forming a cloud of ions, which is detected as a short transient signal, similar to the detection of a nanoparticle or a cell. The size of each droplet is optically measured a few milliseconds after its ejection to determine the volume and the absolute mass of all elements present in the droplets at a known standard concentration. By measuring different concentrations of element standards, a calibration curve can be constructed (Figure 3). In conclusion, a monodisperse microdroplet generator enables customized production of standard particles of any composition, mass and number, which are ideal for calibration in single particle and single-cell analysis.

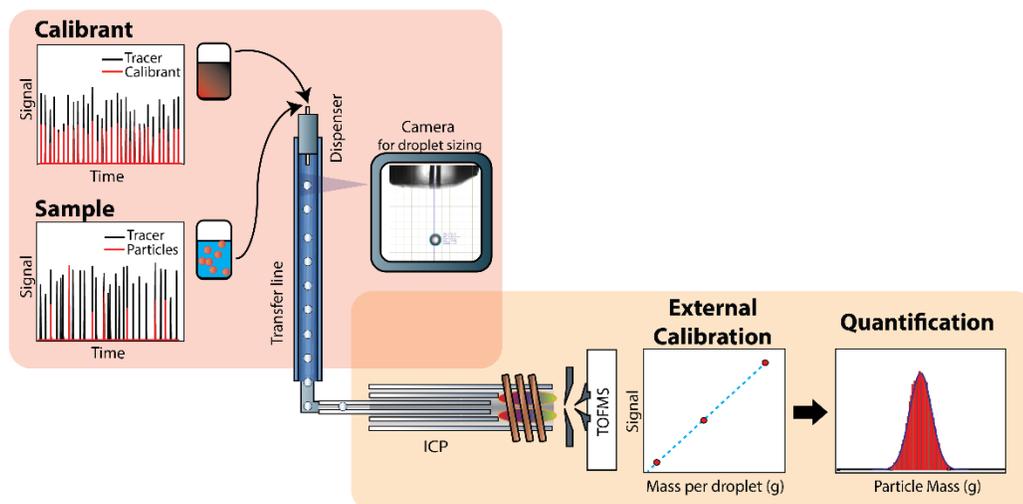


Figure 1. External calibration method. The particles or cells suspension (Sample) and the element standard solutions (Calibrant) are introduced subsequently into the ICP in the form of monodisperse discrete microdroplets generated by the Dispenser. The volume of each droplet is optically measured a few ms after its ejection. The droplets are transported via the transfer line into the ICP in the flow of He/Ar gas, and are subsequently vaporized, atomized, and ionized in the plasma, and detected as short transient signals. For calibrant droplets, the mass of each element per droplet is calculated from the droplet volume and its concentration. Calibration curves (isotope signal vs mass per droplet) are constructed by measuring calibrant solutions of increasing concentration. The isotope signal intensity of single particles or cells is converted to mass using the corresponding calibration curves. Particle number concentration is calculated from the total introduced sample volume, the number of counted events and the corresponding dilution factors.

Microdroplets can be used for both external calibration and online calibration.

In external calibration (Figure 1), the microdroplets are used for both calibration and sample introduction. External calibration with microdroplets has been extensively tested and was found to be suitable for systems with simple matrices. Although the method is straightforward, its applicability is hindered by its incapacity to account for changes in sensitivity due to matrix effects, unless standards are prepared in a similar medium as the samples.

In online calibration (Figure 2), the sample containing particles is aerosolized via a nebulizer/spray chamber and the microdroplets of a standard solution are ejected into the sample aerosol at the entry point to the plasma [7]. Droplets locally experience the same plasma conditions and generate the same response (ions/atoms) as sample particles, independent of the droplet matrix solution [8]. Therefore, this method allows to account for sensitivity changes caused by matrix effects. To distinguish signals of microdroplets from sample particle signals, a droplet tracer element, e.g. Cs, is added to the standard. The same tracer element is added to the

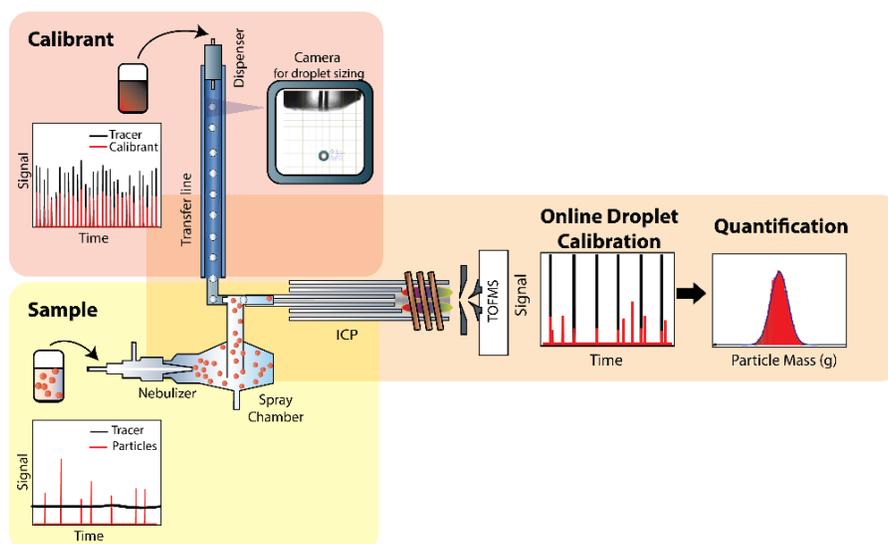


Figure 2. Online calibration method. The particles or cells suspension (Sample) is introduced into the ICP by a nebulizer/spray chamber. The element standard solutions (Calibrant) are introduced into the ICP in the form of monodisperse discrete microdroplets generated by the Dispenser. The volume of each droplet is optically measured a few ms after its ejection. The droplets are transported via the **transfer line** into the ICP in the flow of He gas and added to the sample aerosol. Particles and droplets are vaporized, atomized, and ionized in the plasma, and detected as short transient signals. The signals of calibrant droplets are distinguished from the sample particles based on a Tracer element. The mass of each element per droplet is calculated from the droplet volume and its concentration. Calibration curves (isotope signal vs mass per droplet) are constructed by adding calibrant droplets of increasing concentration to the sample aerosol. The isotope signal intensity of single particles/cells from the sample is converted to mass using the corresponding calibration curves. Particle number concentration is calculated from the number of counted events, tracer response from the calibrant droplets and the sample, and the corresponding dilution factor.

sample to determine PNC without the need for TE determination [9]. The online droplet calibration has been successfully applied for particle quantification in a range of different matrices, including beverages, phosphate buffer solution, and effluent/influent from wastewater treatment plants [3, 8, 10]. Despite the obvious advantages of the microdroplet calibration approaches over the standard calibration method, its realization is technically challenging. To date, microdroplet generators have only been used as a

research tool in few laboratories worldwide.

The icpTOF Quantistar

The icpTOF Quantistar was developed by TOFWERK in collaboration with the research group of Prof. D. Günther at ETH Zürich and the group of K.H. Selbmann at Bern University of Applied Science and is the first microdroplet-based sample introduction/calibration system designed to be used in routine applications.

The icpTOF Quantistar key features and advantages:

- generates stable monodisperse microdroplets at defined frequency
- provides controlled sample introduction with 100% transport efficiency
- measures the volume of each droplet to determine the absolute mass of element per droplet of a calibration solution
- provides large flexibility in elemental composition for calibration and is not limited by the availability of NPs reference material
- facilitates automatic workflows supporting two calibration methods
- provides automatic liquid handling, including rinsing, priming, and sample exchange
- can be used as standalone system or coupled with different liquid sample introduction systems

Application: Sizing of Pt Particles with the icpTOF Quantistar

Monodisperse Pt citrate capped nanoparticles of 70 nm nominal size were synthesized and characterized in the research group of M. Baalousha at the University of South Carolina. The median size of the NPs was previously reported to be 71 nm (measured by AFM), 73 nm (measured by TEM), and 71 nm (measured by DLS) [11]. Here, the NPs were sized using the icpTOF

Quantistar. The particles were dispersed in ultrahigh purity water and the external calibration method was chosen for the analysis. The calibration curve acquired with Pt solutions is shown in Figure 3. From the calibrated masses, the corresponding size was determined and the corresponding particle size distribution is presented in Figure 4 with a median size of 71 ± 5 nm which is in accordance with the previously reported results.

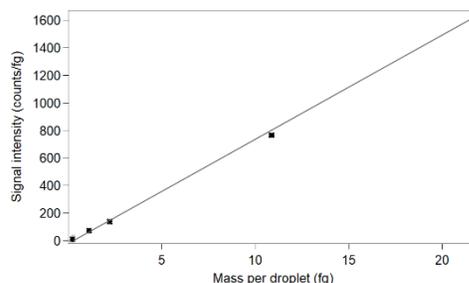


Figure 3. $^{195}\text{Pt}^+$ calibration curve acquired using Pt solutions prepared in 1% nitric acid at concentrations of $1\text{--}100\ \mu\text{g kg}^{-1}$. Mass of Pt per droplet was calculated from the measured droplet volume and the given concentration. The error represents the standard deviation of the Gaussian fit of signal intensity distribution of 10 000 individual droplets.

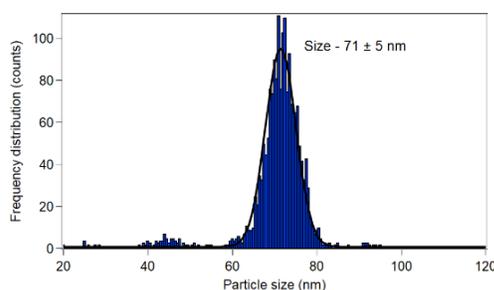


Figure 4. Size distribution of Pt particles fitted with the Gaussian function with a mean value of 71 ± 5 nm. The peak at 45 nm is most likely due to smaller particles present in the sample.

Conclusion

From its first application with ICP-OES through continued development of the technology, microdroplets show great potential for the quantification of nanoparticles and biological cells. Both the external calibration and the online calibration method using microdroplets yield accurate NPs quantification results in simple matrices, with the benefits of inherent matrix-matched capabilities of online calibration for more complex matrices. The Quantistar in combination with the icpTOF is a unique tool for innovative research in different research fields including environmental sciences, material sciences, biology, and toxicology.

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