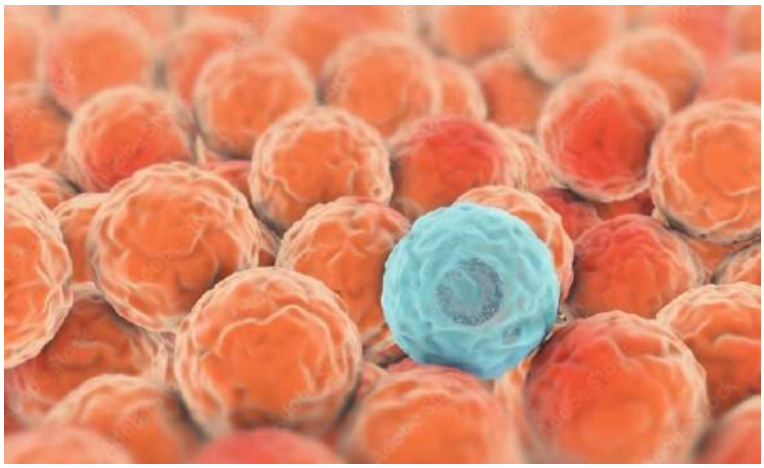
Application Note

Use of ICP-MS MassNeb® nebulizer in total content and single particle/cell measurements



17/11/2022

Authors

Mario Corte-Rodríguez[1], Jörg Bettmer[1], María Montes Bayón[1], Braulio Gañán Riesco[2], Marcos Barrera Parrilla[2].

[1] Group of Mass Spectrometry and Biomedical Analysis (EMAB). University of Oviedo. cortemario@uniovi.es

[2] Ingeniatrics. marcos.barrera@ingeniatrics.com

1. Introduction

During the last years, the number of applications regarding the elemental analysis of single cells and particles by ICP-MS (SC/SP-ICP-MS) has significantly increased. The extremely high sensitivity of current ICP-MS systems has made this type of analysis possible. However, the efficiency of the transport of the cells from the sample introduction system to the plasma is still an issue of concern, since low values of transport efficiency may skew or even completely hamper the single cell analysis.

Despite some others have been proposed, the most common sample introduction systems for single cell analysis still rely on pneumatic nebulization systems[1]. However, the traditional combinations of concentric nebulizers and filtering spray chambers, are nowadays being replaced by dedicated sample introduction systems for single cell analysis that combine the use of low-flow (in the microliters/minute range) pneumatic nebulizers and total consumption spray chambers, some of them using sheath gas flows that prevent sample deposition and therefore increase the transport efficiency of the samples into the plasma.

This report shows the possibilities of the MassNeb® pneumatic nebulizer as an all-in-one solution for both total elemental analysis and single cell/particle analysis applications by ICP-MS. The high versatility of this nebulizer allows its use at very low sample flow rates in combination with total consumption systems for maximized transport efficiency, as well as at high sample flow rates for total elemental analysis, providing better sensitivity than traditional nebulizers

2. Experimental

Instrumentation

All measurements were performed in a Thermo Scientific™ iCAP™ TQ ICP-MS. The instrument was equipped with different sample introduction systems for comparison.

The standard configuration for total ion analysis was based on the use of a regular cyclonic spray chamber and a MicroMist concentric nebulizer optimized for sample flow rates in the range of 0.4 mL/min.

The single-cell standard configuration was achieved by using the Single Cell Sample Introduction System (SCIS) from Glass Expansion, which includes a specific microflow concentric Micromist nebulizer, optimized for sample flow rates in the range of 10 µL/min, and a dedicated total consumption spray chamber using a sheath Ar gas flow.

Both standard configurations, were compared with their equivalent combination with the MassNeb® nebulizer.

Samples

For the evaluation of the total elemental content, a multielement solution containing Li, Co, In, U, Ce and Ba was used for the preparation of calibration curves for each element in a range between 10 ppt and 1.5 ppb in 2% HNO3.

The performance of the different systems in single particle analysis was comparatively evaluated by the measurement of 30 nm gold nanoparticles in a quality control material produced by LGC (QCLGC5050), which is certified in nanoparticle size and number. In order to evaluate the system performance for single-cell analysis, first a suspension of polystyrene beads was used. These beads are around 3 µm diameter and loaded with natural-abundance europium. They serve as a synthetic approach to the transport of cells. They are provided as a suspension of $3.3 \cdot 10^5$ beads/mL and were diluted ten times to a final working concentration of $3.3 \cdot 10^4$ beads/mL

However, in order to corroborate these results with a real biological material, SELM1 certified reference material was used. SELM1 consists of lyophilized selenized yeast and has been previously characterized by our research group in terms of selenium content at the single cell level[2]. This material was resuspended in water to produce a single-cell suspension of relatively stable and resistant yeast cells and diluted to a final concentration around $5 \cdot 10^4$ cell/mL.

3. Results and discussion

Total elemental analysis

Typical conditions for total elemental analysis include the use of concentric nebulizers and spray chambers at relatively high sample flow rates (i.e. from 0.1 to 1.0 mL/min).

In order to address the performance of the MassNeb® nebulizer in comparison with traditional systems, the cyclonic spray chamber was either fitted with the conventional MicroMist nebulizer or the MassNeb®, while keeping the sample flow rate constant at 0.4 mL/min. However, each combination was evaluated in their optimal conditions, obtained after applying the interface autotuning algorithm of the iCAP TQ ICP-MS, which includes the optimization of sample gas flow, torch position and voltages of different extraction lenses. The results are shown in Figure 1 and Table I.

		Sensitivity (cps/ppb)·10^{-3}		
	m/z	MicroMist	MassNeb®	Sensitivity change (%)
Li	7	98	137	+40.1
As*	75	9	13	+43.0
Se*	80	2	3	+31.1
In	115	239	277	+15.8
Ba	138	185	209	+12.9
Ce	140	269	302	+12.1
U	238	313	293	-6.4

Table I. Elemental sensitivity obtained for the elements present in the multielement solution in a comparison between the standard sample introduction system and the use of the MassNeb® nebulizer. Last column shows, in percentage, the change in sensitivity from MicroMist to MassNeb®

*As and Se were measured in their most abundant isotopes (78As+ and 80Se+) using the triple quadrupole mode and O2 as reaction gas to measure the mass transition to the oxide formation (78As16O+ and 80Se16O+, respectively).

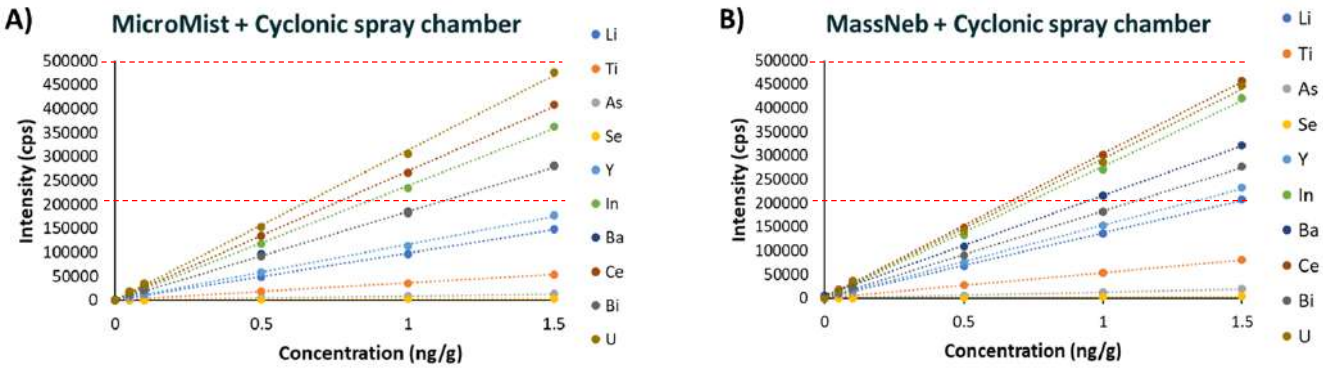


Figure 1. Calibration curves obtained for a multielement standard containing lithium, titanium, arsenic, selenium, yttrium, indium, barium, cerium, bismuth and uranium using the standard cyclonic spray chamber with A) the standard MicroMist nebulizer and B) the MassNeb® nebulizer.

As can be observed, mainly in Table I, at total ion measurement conditions (sample flow rate of 0.4 mL/min and cyclonic spray chamber), the sensitivity for low m/z is increased in up to 40% when using the MassNeb® nebulizer, while this increase is above 10% for higher masses. At high m/z ($^{238}U^{+}$), the sensitivity is not increased by MassNeb®.

Although the explanation for this effect may be complex, the increase in sensitivity at low m/z ratios seems to be due to lower mass discrimination effects in the ICP-MS by increasing the ion sensitivity for light elements while keeping constant the sensitivity for heavy elements.

Single cell/Single particle analysis with MassNeb® and cyclonic spray chamber

At the sight of promising results with the combination of the MassNeb® nebulizer and the conventional cyclonic spray chamber for high sample flow rates, the transport efficiency of this combination was compared with the one obtained with the conventional MicroMist nebulizer.

Although such combinations, with cyclonic spray chambers, usually report very low transport efficiencies (well below 10%), being therefore not the best option for this kind of analysis, this is the most useful and inexpensive approach for those laboratories where specific single cell/single particle sample introduction systems are not available.

Figure 2 below, shows a comparison in the transport efficiency obtained for 30 nm standard gold nanoparticles and europium-loaded polystyrene beads between the MicroMist nebulizer and MassNeb®, both in combination with the cyclonic spray chamber. For both standards the values for transport efficiency are quite low (below 6% in all cases). However, the transport efficiency obtained using the MassNeb® is between 1.5 and 3.5 times higher than the one achieved with the traditional MicroMist nebulizer.

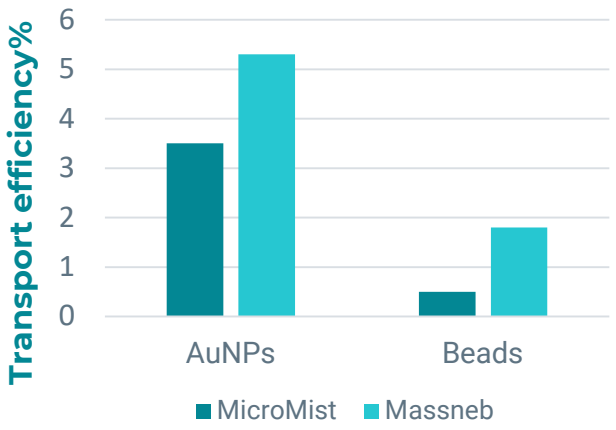


Figure 2. Comparison of transport efficiencies obtained with the MicroMist (blue bars) and MassNeb® (orange bars) nebulizers, with 0.4 mL/min sample flow rate.

In order to optimize the analysis time per sample and decrease memory effects, especially for single cell/particle applications, the washout time of both systems was compared. As shown in Table II, the washout times of the MassNeb® nebulizer were slightly shorter than these for the MicroMist nebulizer. The washout times were calculated as the time needed for the initial signal intensity to be decreased by 90% with a 2% HNO3 washing solution.

	Washout time (s)	
	MicroMist	**MassNeb®**
$^{7}Li^{+}$	8.98	8.56
$^{59}Co^{+}$	8.97	9.37
$^{115}In^{+}$	9.37	7.74
$^{238}U^{+}$	9.38	7.74

Table II. Washout times obtained with the MicroMist vs. MassNeb® nebulizers.

Single cell/particle analysis using dedicated sample introduction system

The SCSIS is one of the most efficient commercial systems for single-cell analysis, which has also shown a good performance for single-particle analysis. In this work, its performance for both particles and cells analysis was compared with this obtained when replacing the nebulizer included in this sample introduction kit (a microflow MicroMist nebulizer) by the MassNeb®.

The elemental sensitivities achieved with both systems are shown in Table III. In this case, the sensitivity increase given by the MassNeb® is much more significant, varying from 55% for ^{115}In to over 80% for ^{80}Se. Interestingly, the increase in sensitivity now does not show a dependence on the m/z and is more constant (except the one observed for As) across the different m/z values).

		Sensitivity (cps/ppb)·10^{-3}		
	m/z	**SCSIS**	**MassNeb®**	**Sensitivity change (%)**
Li	7	41	67	+63
As*	75	2.5	2.9	+14
Se*	80	0.8	1.4	+82
In	115	83	129	+56
Ba	138	9	16	+76
Ce	140	91	143	+57
U	238	105	166	+59

Table III. Elemental sensitivity obtained for the elements present in the multielement solution in a comparison between the Single Cell Sample Introduction System (SCSIS) and the use of the MassNeb® nebulizer with the same spray chamber. Last column shows, in percentage, the change in sensitivity from MicroMist to MassNeb®

*As and Se were measured in their most abundant isotopes ($^{78}As^{+}$ and $^{80}Se^{+}$) using the triple quadrupole mode and O_2 as reaction gas to measure the mass transition to the oxide formation ($^{78}As^{16}O^{+}$ and $^{80}Se^{16}O^{+}$, respectively).

The transport efficiency was also evaluated in this dedicated spray chamber, making the comparison between the microflow MicroMist nebulizer and the MassNeb®. In this case, the transport efficiency was calculated for standard 30 nm gold nanoparticles, europium-dopped polystyrene microparticles and selenized yeast reference material SELM1. The values for the transport efficiency are summarized in Table IV and show an increase of the transport efficiency for all three materials.

	Transport efficiency (%)	
	MicroMist	MassNeb®
AuNPs	45	66
Eu-beads	37	41
Yeast cells	30	38

Table IV. Transport efficiencies obtained with the combination of the SCSIS spray chamber and either the standard MicroMist for this system or the MassNeb®.

The transport efficiency is, as expected, slightly higher with both systems for the smallest particles (gold nanoparticles), decreasing with the increasing particle size and complexity. However, the transport efficiency obtained with MassNeb® is almost 50% higher for nanoparticles and almost 30% higher for yeast cells.

4. Conclusions

The tested MassNeb® nebulizer improves elemental sensitivity and transport efficiency of both reference systems: cyclonic and SCIS total consumption spray chambers.

The difference is especially remarkable for the elemental sensitivity when the nebulizers are compared in single-cell analysis mode (with the SCIS spray chamber), being increased in up to 80%. Furthermore, during the comparison tests, a higher versatility of MassNeb® has been proven, since a single MassNeb® nebulizer works at optimum performance under totally different flowrate conditions, covering what until now required at least, two different conventional nebulizers with different flowrate specifications.

During these studies, additional advantages have been identified regarding the practical use of MassNeb®. The most evident was the high resistance of the MassNeb®, made of plastic material, in comparison to the fragile conventional glass nebulizers. Additionally, no clogging problems were observed during its use. It is also remarkable that the gas backpressure of the MassNeb® is very low, especially when compared to the microflow MicroMist nebulizer used in the SCIS, that limits the nebulizer gas flow rate to 0.5 L/min, whereas it can be increased over 1 L/min in the MassNeb®. This allows the sheath gas flow in the SCIS spray chamber to be decreased down to very low values, that can be easily controlled by the built-in mass flow controllers of any ICP-MS.

References

[1] M. Corte-Rodríguez, R. Álvarez-Fernández, P. García-Cancela, M. Montes-Bayón, J. Bettmer, Single cell ICP-MS using on line sample introduction systems: Current developments and remaining challenges, Trends Anal. Chem. 132 (2020). https://doi.org/10.1016/j.trac.2020.116042.

[2] J.S.F. Pereira, R. Álvarez-Fernández García, M. Corte-Rodríguez, A. Manteca, J. Bettmer, K.L. LeBlanc, Z. Mester, M. Montes-Bayón, Towards single cell ICP-MS normalized quantitative experiments using certified selenized yeast, Talanta. 252 (2022). https://doi.org/10.1016/j.talanta.2022.123786.

Ingeniatrics
Nebulizers and Nozzles

P.I. Parque Plata
C/ Camino Mozárabe, 41
41900 Camas, Seville
(Spain)

(+34) 954 08 1214
info@ingeniatrics.com

www.Ingeniatrics.com