Technical Overview

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Single Cell and Microplastic Analysis by ICP-MS with Automated Micro-Flow Sample Introduction

Multi-element analysis of yeast cells and polystyrene microbeads using an Agilent 8900 ICP-QQQ method

Introduction

Single cell ICP-MS (scICP-MS) is increasingly seen as a powerful and fast tool for the measurement of elements in individual cells, mainly due to the high sensitivity and selectivity of ICP-MS (*1*). Analysis is performed in the same way as single nanoparticle (spICP-MS) analysis, which has become a well-established technique for the analysis of nanoparticles and particles (*2*).

For successful scICP-MS or spICP-MS analysis, the ICP-MS must be operated in fast Time Resolved Analysis (TRA) mode. Suspension solutions containing cells or particles are introduced directly into the ICP through a nebulizer where they are decomposed, atomized, and ionized. The ion plume is detected within 1 ms, which is faster than the signal integration time used in conventional ICP-MS measurements (10–100 ms). To measure the signals from individual single cells or single particles, the fast TRA mode of Agilent single quadrupole ICP-MS or Agilent triple quadrupole ICP-MS (ICP-QQQ) uses an integration time of 0.1 ms.

scICP-MS studies require dedicated sample introduction systems for ICP-MS, as conventional ICP-MS sample introduction systems are not suitable for the measurement of cells. Cells must not be degraded during nebulization and the spray chamber needs to have a high transport efficiency to accommodate larger particles such as cells. To overcome these challenges, specially designed nebulizers and spray chambers have been developed for scICP-MS measurements.

To better understand the impact of plastic contamination on ecosystems and human health, there is increasing interest in characterizing microplastics (MPs), in terms of size, number of particles, concentration, and elemental content in environmental and food samples. As the particle sizes of MPs are relatively large (in the order of µm to mm), the analysis of MPs by ICP-MS also requires a non-standard sample introduction system to transport the particles to the plasma.

Instrumentation

Samples containing cells and MPs were introduced to the Agilent 8900 ICP-QQQ using an ESI microFAST Single Cell (sc) Autosampler (Elemental Scientific Inc., Omaha, NE, USA). The microFAST comprises a complete system that includes the autosampler, a CytoNeb nebulizer, a CytoSpray spray chamber, and a one-piece torch. The specially designed nebulizer and spray chamber ensure high transport efficiency of large particles to the ICP, while the backpressure of the CytoNeb is low enough not to disrupt the integrity of the cells. The ESI micro-sampling system is also compatible with the Agilent 7850 ICP-MS (with fast TRA) and the Agilent 7900 ICP-MS.

Advantages of combining the ESI micro-sampling system with an Agilent ICP-MS or ICP-QQQ include:

- Provision of a fully automated, microflow technique for easy and efficient analysis of single cells or particles.
- High sensitivity and wide dynamic range of Agilent ICP-MS enable measurement of small-to-large cells or particles.
- A short ICP-MS dwell time (0.1 ms) ensures a higher particle signal-to-ion (noise) background ratio (S/N).
- Multi-element analysis enabled by the Agilent Rapid Multi-Element Nanoparticle Analysis Mode module within the Agilent ICP-MS MassHunter software.
- The dedicated software facilitates the measurement of an unrestricted number of analytes in cells or particles.

ICP-MS modes of analysis

Metals in cells are often measured using a conventional “bulk” ICP-MS analysis method following acid digestion of the cells. This bulk method provides the mean concentration results based on the total amount of metals in many types of cells after cell lysis, not the metal content of a single type of intact cells. For the determination of the elemental and metallic nanoparticle (NP) content of a single type of intact cell, a scICP-MS method is needed.

The mechanisms of conventional ICP-MS analysis of sample solutions, spICP-MS analysis of nanoparticles, and scICP-MS analysis of single cells is shown in Figure 1. Dissolved analyte ions produce a continuous signal, while the analyte ions present in a single particle or single cell generate a short signal that can be detected as a sharp peak using fast TRA mode.

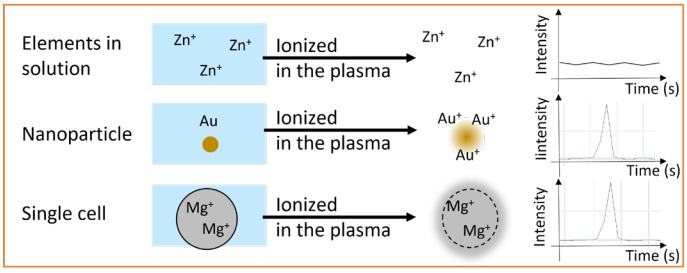

Figure 1. Mechanism of ICP-MS detection of metallic ions in an ionic solution (top), in nanoparticles (middle), and in single cells (bottom).

Analysis of yeast cells using scICP-MS

Commercially available dried yeast and selenium-enriched yeast (Se-yeast) were suspended in de-ionized (DI) water. The samples were introduced to the 8900 ICP-QQQ using the microFAST Single Cell autosampler. The 8900 was equipped with the ESI single cell sample introduction kit. A platinum (Pt) NP reference material (RM) containing 50 nm diameter NPs was used to calculate the nebulization efficiency of the method. The nebulization efficiency was estimated as >80%, which is higher than is achievable using conventional ICP-MS sample introduction systems (5~8%). The typical operating conditions of the instrumentation are shown in Table 1.

Table 1. Typical operating parameters of the ESI microFAST autosampler and Agilent 8900 ICP-QQQ for scICP-MS analysis of yeast cells.

ESI microFAST Single Cell Autosampler	
Sample flow rate (µL/min)	10
Loop size (µL)	100
Nebulization efficiency (%)	> 80
Agilent 8900 ICP-QQQ	
Plasma power (W)	1550
Sampling depth (mm)	8
Nebulizer gas flow rate (L/min)	0.63
Makeup gas flow rate (L/min)	0.20

Fast analysis times using dedicated software

All the calculations needed for the multi-element NP analysis of a single cell (or particle) can be performed by the integrated Rapid Multi-Element Nanoparticle Analysis module software. The multi-element mode software is included in the Single Nanoparticle Application Module, which is an option for ICP-MS MassHunter.

As shown in Figure 2, the software collects data sequentially for an unrestricted number of analytes in a single sample analysis, using optimum conditions for the measurement of each individual element. Compared to single-element spICP-MS analysis, the multi-element NP method reduces analytical run times and minimizes the risk of contamination from sample carryover.

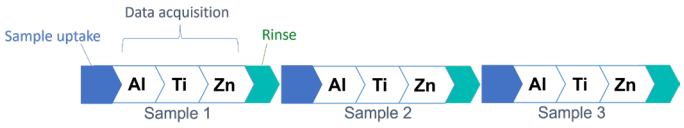

Figure 2. Sequential analysis of samples using multi-element spICP-MS method using the Rapid Multi-Element Nanoparticle Analysis module for Agilent ICP-MS MassHunter software.

Carbon (C), phosphorus (P), iron (Fe), and selenium (Se) were measured in the yeast and Se-yeast samples by scICP-MS. Figure 3 shows the TRA data for Se in both samples. Only Se-yeast showed clear peaks for Se. The signal distribution plots for C, P, Fe, and Se in both yeast samples are shown in Figure 4. C, P, and Fe were detected in the single yeast cells and all four elements were clearly distinguishable from the background in single cells of the Se-yeast sample.

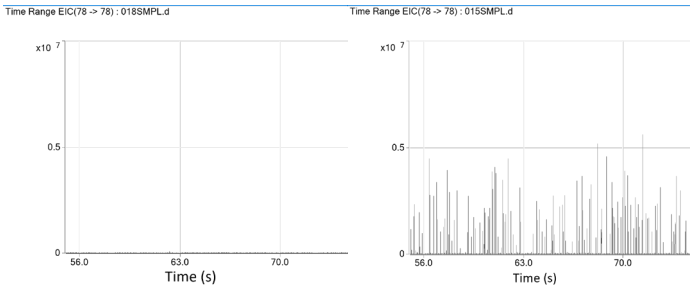

Figure 3. Se signal obtained by scICP-MS method for yeast (left) and Se-yeast (right).

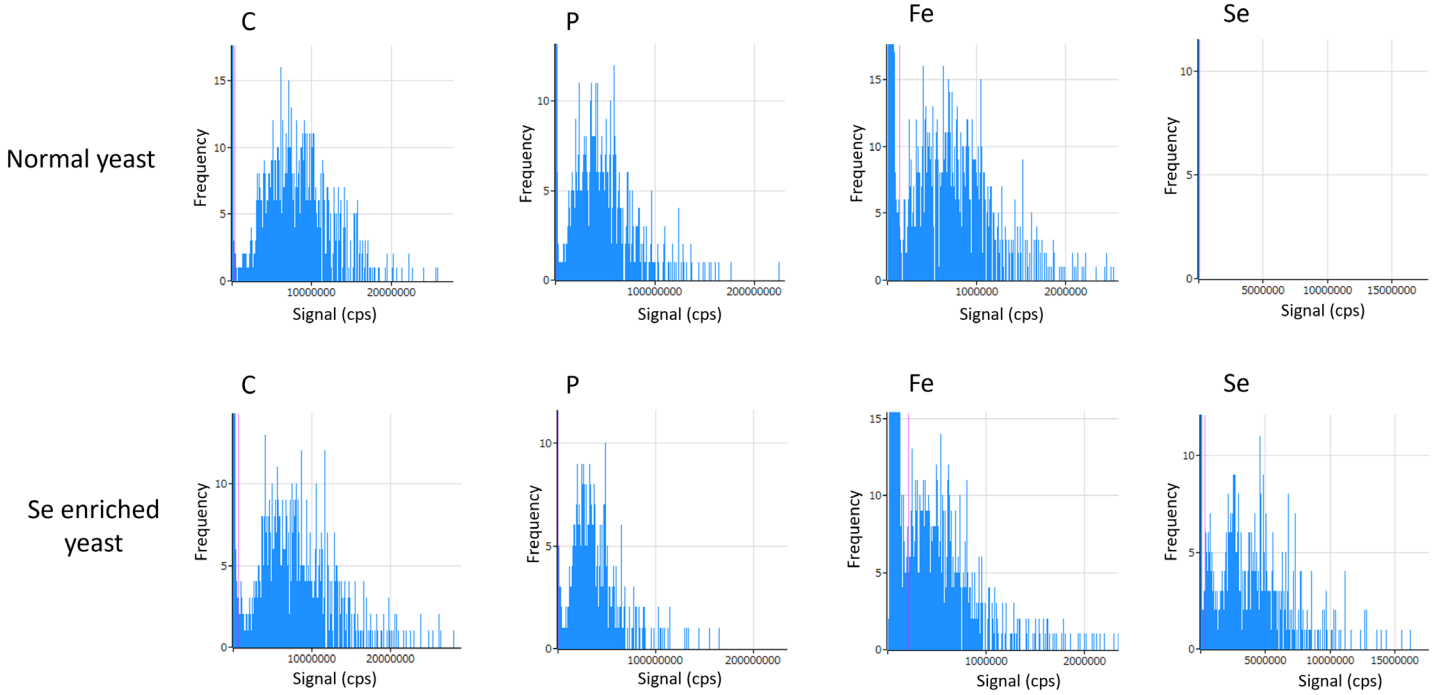

Figure 4. Signal distribution for C, P, Fe, and Se in yeast (top) and Se-yeast (bottom).

Table 2 shows the number of detected cells containing the four elements in two samples of yeast and Se-yeast, respectively. The measurement time was 20 s for each element. There was good agreement in the number of detected cells that contained the four elements, confirming the feasibility of the scICP-MS method for the measurement of multiple elements in single cells.

Table 2. Number of detected cells of yeast and Se-yeast that contained C, P, Fe, and Se (20 s measurement for each element).

	Number of Detected Cells			
Sample	**C**	**P**	**Fe**	**Se**
Yeast - 1	1186	1022	1100	0
Yeast - 2	1096	937	1028	0
Se enriched yeast - 1	1003	1183	1032	829
Se enriched yeast - 2	1097	1234	1059	803

Analysis of microplastics by spICP-MS

For the study of MPs by spICP-MS, polystyrene (PS) microbeads were used to represent microplastics. Three PS suspensions of 1, 2, and 5 µm microbeads were obtained from Sigma Aldrich (St. Louis, MI, USA). A mixed solution containing 1, 2, and 5 µm PS microbeads was prepared in DI water. The same instrumentation setup and operating parameters (Table 1) used for the scICP-MS analysis of yeast was applied to the determination of the mixed PS microbead sample.

The size distribution plot of ^{13}C in the PS microbeads mixture is shown in Figure 5. The data shows a clear separation between 1, 2, and 5 µm PS microbeads signals, confirming the feasibility of the spICP-MS technique for the detection of the elemental content of MPs.

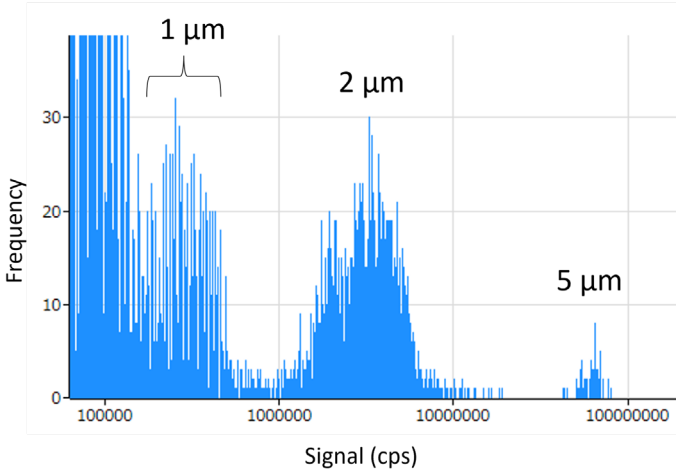

Figure 5. Signal distribution of ^{13}C in a mixed sample containing 1, 2, and 5 µm PS microbeads.

Solutions containing 1, 2, and 5 µm PS microbeads at 0.1, 1, and 5 ppm were prepared in triplicate by diluting the PS suspensions in DI water before analysis of ^{13}C using spICP-MS. Table 3 shows the number of detected MPs in a 20 s measurement, measured particle concentration, and measured median size for each size of MP in the nine PS solutions.

There was good agreement of the measured particle concentration for each size of MP in the triplicate PS solutions, especially for the 2 and 5 µm PS microbead samples. Also, the measured median size results agreed with the nominal sizes of the PS microbeads.

The results demonstrate the effectiveness of the 8900 ICP-QQQ method coupled with the microFAST autosampler for the detection of MPs down to 1 µm. The detection capability of the 8900 ICP-QQQ would complement the more well-known laser direct infrared (LDIR) technique, which has an MP lower size limit of around 20 µm (*3*).

Table 3. Number of detected MPs, measured particle concentration, and median size of 1, 2, and 5 µm PS microbead samples by spICP-MS.

Polystyrene Sample	Number of Detected Particles	Particle Concentration (particles/L)	Median Size (µm)
1 µm 0.1 ppm - 1	2366	4.3×10^8	0.95
1 µm 0.1 ppm - 2	1616	2.9×10^8	1.04
1 µm 0.1 ppm - 3	2100	3.8×10^8	0.97
2 µm 1 ppm - 1	1733	3.1×10^8	1.9
2 µm 1 ppm - 2	1763	3.2×10^8	1.9
2 µm 1 ppm - 3	1947	3.5×10^8	1.9
5 µm 20 ppm - 1	197	3.5×10^7	5.2
5 µm 20 ppm - 2	181	3.3×10^7	5.1
5 µm 20 ppm - 3	211	3.8×10^7	5.2

Conclusion

The ESI microFAST Single Cell Autosampler and sample introduction system combined seamlessly with the Agilent 8900 ICP-QQQ, providing a fully automated solution for the elemental analysis of single cells or microplastics (MPs).

Due to the high sensitivity and wide dynamic range of Agilent ICP-MS, the elemental content of small to large cells or particles can be measured using a single cell (sc-) or single particle (sp-) ICP-MS method. Proof of performance data was provided for the measurement of C, P, Fe, and Se in single yeast cells and the determination of ^{13}C in 1, 2, and 5 µm polystyrene microbeads.

All the calculations needed for the multi-element analysis of a single cell or MPs were automatically performed by the Nanoparticle Analysis Mode module in the Agilent ICP-MS MassHunter software, providing a fully integrated method.

References

1. Tanaka, Y., Ogra, Y., Kubota, T., Evaluation of the Elemental Content of a Single Cell using Fast Time-Resolved Analysis (TRA) ICP-MS, Agilent publication, 5994-4460EN
2. Resano, M., *et al.*, Living in a transient world: ICP-MS reinvented via time-resolved analysis for monitoring single events, *Chem. Sci.*, **2022**, 13, 4436
3. Alwan, W., Robey, D., Characterization of Microplastics in Environmental Samples by Laser Direct Infrared Imagining and User-Generated Libraries, Agilent publication, 5994-4822EN

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