Application Note
Clinical research

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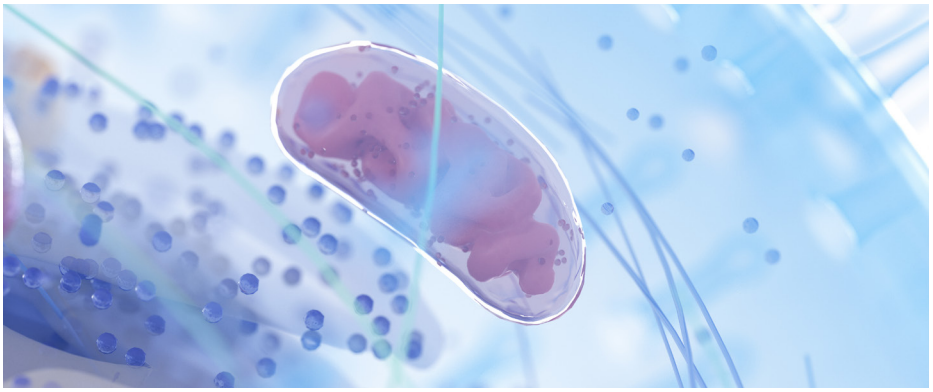
Evaluation of the Elemental Content of a Single Cell using Fast Time-Resolved Analysis (TRA) ICP-MS

Determination of Mg, P, Fe, and Zn in a single yeast, green alga, and red blood cell by Agilent 8900 ICP-QQQ



Authors

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Introduction

Advances in ICP-MS technology mean that elemental signals from single particle-like materials such as nanoparticles and living cells can now be detected, measured, and reported more easily. Known as single particle (SP-) or single-cell (SC-) ICP-MS, these techniques are of interest in a range of industries and fields of study, including semiconductor, environmental, foods, and clinical research. For successful SP/SC-ICP-MS analysis, the ICP-MS must be operated in fast time resolved analysis (TRA) mode. Suspension solutions containing particles or cells 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 much faster than the signal integration time used in conventional ICP-MS measurements (10–100 ms). To measure the signals from individual single particles or single cells, 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.

In this study, a precise quantification method was developed using SC-ICP-MS for the determination of biologically important elements, Mg, P, Fe, and Zn, in a single yeast, green alga, and red blood cell (RBC). To validate the accuracy of the SC-ICP-MS method, which measures intact cells, data was also obtained using a conventional "bulk" ICP-MS analysis method following acid digestion of the cells. The acid digestion step destroys the individual cell structure, so the reported metal concentration results are derived from the mean values measured from thousands of cells. Compared with bulk analysis, SC-ICP-MS requires significantly fewer cells owing to the enhanced signal-to-noise ratio (S/N) of the signal in fast TRA mode. Requiring fewer cells for a successful analysis is a major advantage of the technique, especially in applications such as biological and clinical research where sample volumes may be limited.

When samples with a complex matrix are analyzed by ICP-MS, matrix-based polyatomic interferences are more likely to form that affect the accurate measurement of some analytes. For example, in biological samples, phosphorus (^{31}P) can be affected by high background noise at *m/z* 31 arising from interferences such as $^{12}C^{18}OH^+$, $^{14}N^{16}OH^+$, $^{15}N_2H^+$, $^{15}N^{16}O^+$, and $^{13}C^{18}O^+$. Also, P has a high first ionization potential (IP) of 10.5 eV. The high IP means that a relatively low number of ions is formed in the argon plasma compared to elements with an IP between ~6 and 8 eV, leading to lower sensitivity.

The Agilent 8900 ICP-QQQ is suitable for SC-ICP-MS studies due to its high sensitivity, low background, and interference removal capabilities. The 8900 is a tandem MS instrument that uses MS/MS mode for reliable reaction chemistry in the collision/reaction cell (CRC) to resolve polyatomic ion interferences on analytes such as P. Using MS/MS, the instrument can measure analytes 'on-mass' or in 'mass-shift' mode, as explained elsewhere (*1, 2*). A mass-shift method with O_2 cell gas is often used to measure ^{31}P via its oxide ion, $^{31}P^{16}O^+$, at *m/z* 47 (*3*). MS/MS methods provide a reliable and reproducible way to avoid polyatomic ion interferences on analytes of interest.

To simplify SC-ICP-MS analysis using Agilent ICP-MS or ICP-QQQ instrumentation, Agilent has developed the Single Nanoparticle Application Module (p/n G5714A) for Agilent ICP-MS MassHunter instrument control software. The Agilent particle detection technique, which has been patented in the United States, provides clear signal distribution data even in biological samples that have a high ionic background arising from the cell-suspended solution.

In this study, SC-ICP-MS was evaluated as a potential technique for metabolism studies of essential elements in fungus, plant, and animal cells. It is an important area of research, as many elements are essential for cell health, and an imbalance, deficiency, or excess of certain metals may disrupt natural cell processes.

Experimental

Cell samples and chemicals

Three kinds of cells of approximately 1 to 5 µm in diameter were investigated, as shown in Figure 1. Commercially available dried yeast (*Saccharomyces cerevisiae*) was bought from Sala Akita Shirakami Corporation (Akita, Japan). Green unicellular alga, *C. reinhardtii* (NIES-3379) was obtained from Microbial Culture Collection at the National Institute for Environmental Studies (Tsukuba, Japan). Red blood cells were collected from 11-week-old male Wistar rats (Japan SLC Inc.). The cell samples were washed and suspended in 0.9% NaCl solution before analysis by SC-ICP-MS.

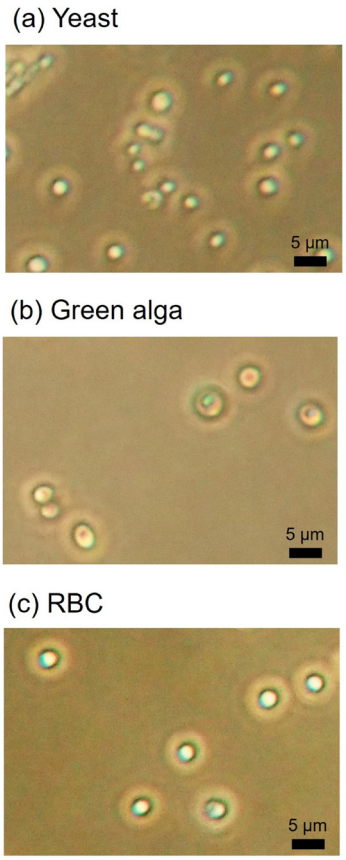

Figure 1. Microscopic images of three types of cell suspended in 0.9% NaCl solution.

Ionic standards were prepared by mixing single element standards for Mg, Si, P, Fe, and Zn. Details are given in Table 1. To matrix match the standards and samples, the 0.9% NaCl solution was added to each ionic standard.

Table 1. Details of the element standards and calibration range.

Element	Ionic Standard Concentration for SC-ICP-MS (ppb)	Calibration Range for Bulk Concentration Analysis (ppm)	Supplier
Mg	10	0–15	Kanto Chemical
Si	100	-	Kanto Chemical
P	1000	0–20	FUJIFILM Wako Pure Chemical
Fe	10	0–5	Kanto Chemical
Zn	10	0–1	Kanto Chemical

A SiO_2 nanoparticle certified reference material (Merck Sigma Aldrich) with a nominal internal particle diameter of 200 nm was diluted to 2 ppb in ultrapure water. The SiO_2 nanoparticle and ionic Si solutions were used to estimate nebulization efficiency.

Instrumentation

For the SC-ICP-MS study, single cell samples were introduced into an 8900 ICP-QQQ using an MVX-7100 autosampler (Teledyne Cetac Technologies, Omaha, NE, USA), as shown in Figure 2. The 8900 was fitted with a MicroMist glass nebulizer and a Single-Cell Sample Introduction System (Glass Expansion, Victoria, Australia) total consumption spray chamber.

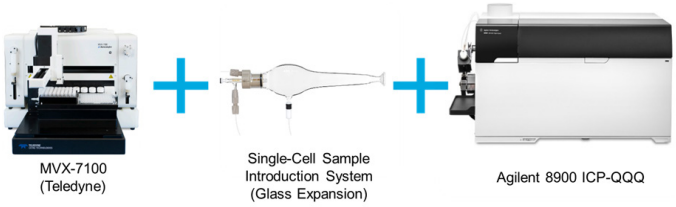

Figure 2. Configuration of SC-ICP-MS system.

For bulk concentration analysis, digested cell samples were introduced into an Agilent 8800 ICP-QQQ equipped with a standard sample introduction system. The method parameter settings of the two ICP-QQQ instruments are shown in Table 2. The same cell gases were used in both methods. Mg and Zn were measured in no gas mode. ^{31}P was measured as the $^{31}P^{16}O$ product ion by operating the ICP-QQQ in O_2 mass-shift mode, and Fe and Si were measured on-mass in H_2 mode.

Table 2. ICP-QQQ operating conditions for the SC-ICP-MS and conventional bulk cell ICP-MS methods.

Parameter	Method					
	SC-ICP-MS			Bulk Concentration Analysis by ICP-MS		
ICP-QQQ Instrument Model	8900			8800		
RF Power (W)	1600			1550		
Nebulizer Gas/Make-up Gas (L/min)	0.65/0.20			1.0/0.3		
Cell Gas Mode	No gas	O_2	H_2	No gas	O_2	H_2
Cell Gas (mL/min)	-	0.38	5.5	-	0.38	5.5
Analytes*	^{24}Mg, ^{66}Zn	^{31}P**	^{56}Fe, ^{28}Si	^{24}Mg, ^{66}Zn	^{31}P**	^{56}Fe
Measurement Mode	Single particle			Full quantitative		
Integration Time (ms)	0.1			100		
Nebulization Efficiency (%)	~55			Not applicable		

**All elements were measured in MS/MS mode. **MS/MS mass-shift mode with O_2 cell gas; the quadrupole mass filters, Q1 and Q2, were set to m/z 31 and 47, respectively.*

Sample preparation

The sample preparation procedure is described in detail elsewhere (*4*). The number of cells for the three cell types were counted using a Bürker-Türk hemocytometer. The measurements were used in the calculations of the bulk concentration analysis section of this note.

Determination of the elemental content of a single cell

Four essential elements (Mg, P, Fe, and Zn) were measured in a single cell using SC-ICP-MS. The mass of each analyte element (m) in a single cell was obtained using the following equation (eq. 1).

$$m = \frac{I_{\text{Cell}}}{(I_{\text{Std}} - I_{\text{Blk}})} \cdot f \cdot \text{C}_{\text{Std}} \cdot \text{v} \quad \text{(eq. 1)}$$

I_{Cell}, I_{Std}, and I_{Blk} represent signal intensity of a single cell, ionic standard, and 0.9% NaCl blank solution, respectively. f, C_{Std}, and v denote nebulization/transport efficiency of the sample aerosol, concentration of ionic standard solution, and sample flow rate (0.015 mL/min), respectively.

Nebulization/transport efficiency (f) was calculated from the signal intensities of ^{28}Si measured in both an ionic Si solution and SiO_2 nanoparticle suspension solution using equation (eq. 2). I_{Si} and I_{Silica} represent signal intensity of ionic Si solution and intensity of transient signals for SiO_2, respectively. Mass of Si (m_{Silica}) in a single SiO_2 nanoparticle was calculated from the radius (100 nm), density (2.63 g/cm^3), and mass fraction (Si/SiO_2~28/60) of the SiO_2 nanoparticle solution. C_{Si} is the concentration of ionic Si solution (100 ng/mL). In summary, the transport efficiency is calculated by comparing the sensitivity factor of ^{28}Si between the ionic solution ($(I_{Si}-I_{Blk})/C_{Si}\cdot v$) and the SiO_2 nanoparticle (I_{Silica}/m_{Silica}) solution.

$$f = \frac{\text{m}_{\text{Silica}}}{\text{C}_{\text{Si}} \cdot \text{v}} \cdot \frac{(I_{\text{Si}} - I_{\text{Blk}})}{I_{\text{Silica}}} \quad \text{(eq. 2)}$$

The Single Nanoparticle Application Module of the ICP-MS MassHunter software automatically performed all calculations.

Bulk concentration analysis

A small aliquot (0.1 mL) of yeast, green alga, and RBC suspended in NaCl solution was digested with 0.5 mL HNO_3 (60%). The cell samples were prepared in a glass test tube, which was heated on a hot plate at 100 °C. The digested cell samples were diluted with Milli-Q water before elemental analysis by ICP-MS. The elemental concentrations were determined from external calibration curves. The elemental content per cell was calculated as follows:

ICP-MS determined concentration (fg/mL))/counted cells (cell/mL)

Results and discussion

Transient signals for Mg, P, Fe, and Zn were detected in yeast, green alga, and RBC using the 8900 ICP-QQQ operating in fast TRA mode. Typical signal profiles are shown in Figure 3, using Fe and P as examples. A cell suspension solution with a cell density of ~10^6 cells/mL provided enough signal for detection.

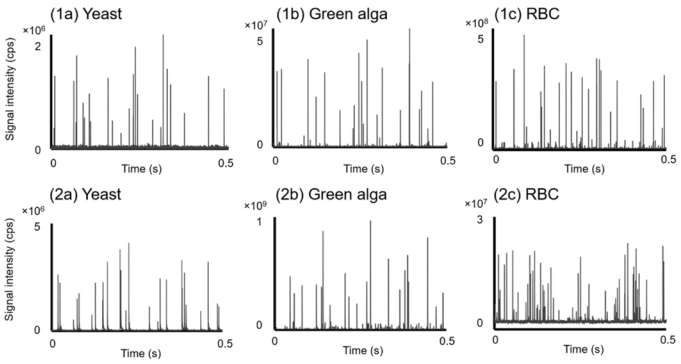

Figure 3. Signal profiles of Fe (top, 1a to 1c) and P (bottom, 2a to 2c) in the three types of cell obtained by ICP-QQQ in fast TRA mode.

The mass (fg/cell) of each element in a single cell was automatically calculated by the ICP-MS MassHunter software. The results can be displayed in a histogram of mass per cell against frequency of that mass. Figure 4 shows the Fe and P content of a single cell. Mass distributions of Fe and P in each of the three cells can be clearly distinguished from the background noise on the *Y-axis*.

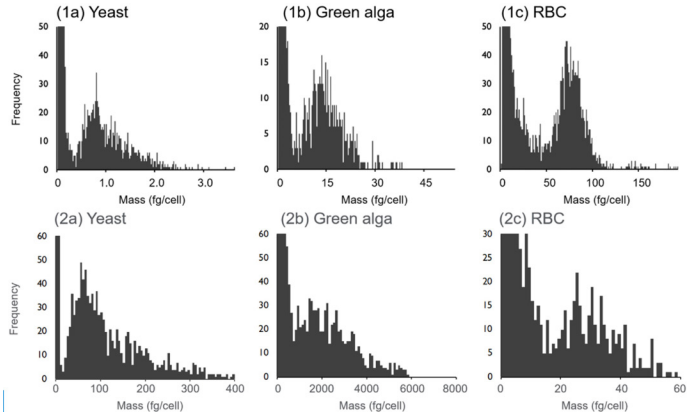

Figure 4. Histograms for the mass of Fe (top, 1a to 1c) and P (bottom, 2a to 2c) in a single cell.

A comparison of single cell (SC-ICP-MS, blue bars) and bulk cell (ICP-MS, yellow bars) results for Mg, P, Fe, and Zn is shown in Figure 5. The SC-ICP-MS results show that the average contents of each element varied significantly among the three types of cell.

Green alga was characterized by the higher Mg content than for yeast and RBC, possibly due to the presence of chlorophyll. Mg is required for photosynthesis in the cell. P and Zn in RBC were lower than in other two types of cells, since P and Zn are highly contained in the cell nucleus, and RBCs are enucleated during its differentiation. The higher Fe content of 70 fg in RBC may be due to the presence of hemoglobin.

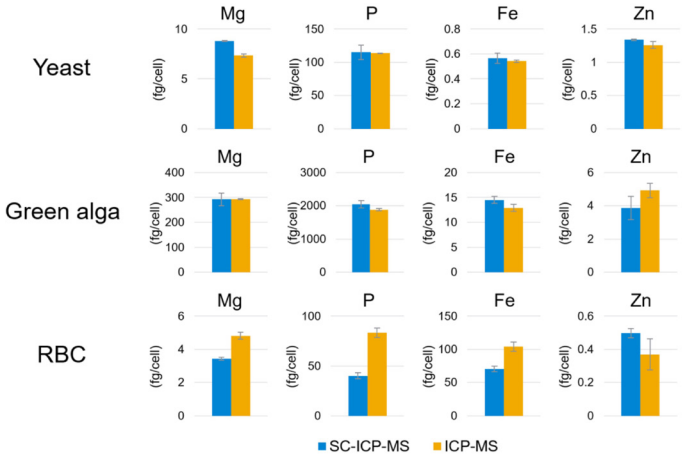

Figure 5. Elemental content of yeast (top), green alga (middle), and RBC (bottom).

For yeast and green alga, there was general agreement between the results obtained by SC-ICP-MS and the bulk analysis method (Figure 5) (*4*). In the RBC, the results obtained by SC-ICP-MS for P, Fe, and Zn were lower than the results obtained by bulk analysis. However, the SC-ICP-MS result for Fe in RBC was consistent with reference data (66.5 fg/cell) calculated using the certified Mean Cell Hemoglobin (MCH) value (*5*), as shown in Figure 6. Possibly the data from the bulk analysis included a contribution from elements in lysed RBC and/or serum in suspension, resulting in an over-estimation of the results for a single RBC. The confirmation of the Fe data suggests that SC-ICP-MS provides more accurate results than the conventional acid digestion bulk cell method for the determination of the elemental content of single cells.

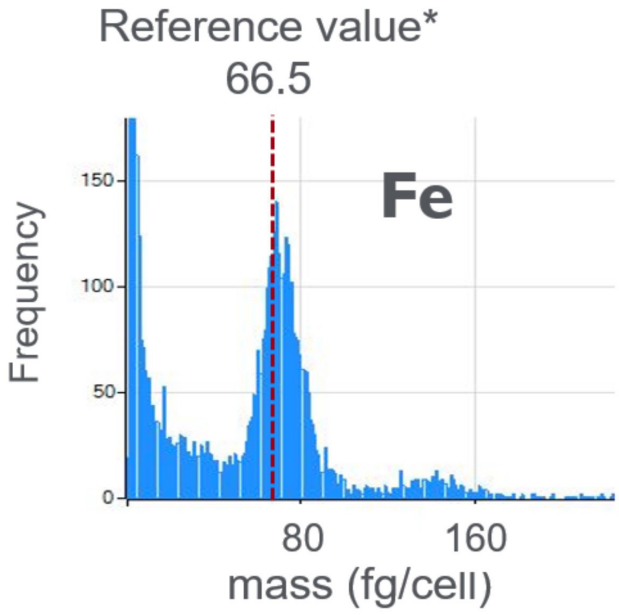

Figure 6. Fe mass in RBC measured by SC-ICP-MS.

**The reference value was calculated from the certified mean hemoglobin content in RBC for an 11-week-old male Wistar rat provided by the vendor.*

Conclusion

The high sensitivity, low background, advanced interference removal capabilities, and fast TRA mode of the Agilent 8900 ICP-QQQ enabled the multi-element analysis of a single yeast, green alga, and red blood cell using SC-ICP-MS. All the calculations needed for the analysis of a single cell were automatically performed by the integrated Single Nanoparticle Application Module data analysis software for ICP-MS MassHunter.

The results for Mg, P, Fe, and Zn in yeast and green alga obtained by SC-ICP-MS and conventional bulk ICP-MS analysis were in good agreement, verifying the SC-ICP-MS method. The accuracy of the result for Fe in RBC by SC-ICP-MS was confirmed against a calculated reference value. Since SC-ICP-MS provided a more reliable result for Fe than conventional ICP-MS, the results for Mg, P, and Zn were also likely to be more accurate. By measuring the content of intact RBCs, SC-ICP-MS avoids contamination from elements originating from lysed RBC and/or serum in suspension, leading to more accurate results.

The study demonstrates the potential of SC-ICP-MS as a useful and powerful technique for the quantitative analysis of the elemental content of a single cell.

References

1. E. Bolea-Fernandez, L. Balcaen, M. Resano, F. Vanhaecke. Overcoming spectral overlap via inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS). A tutorial review, *J. Anal. At. Spectrom.*, **2017**, 32, 1660–1679, https://doi.org/10.1039/C7JA00010C
2. E. McCurdy, G. Woods, N. Sugiyama. Method Development with ICP-MS/MS: Tools and Techniques to Ensure Accurate Results in Reaction Mode, *Spectroscopy*, 2019 (9):20–27, http://www.spectroscopyonline.com/method-development-icp-msms-tools-and-techniques-ensure-accurate-results-reaction-mode
3. K. Nakano, Ultralow level determination of phosphorus, sulfur, silicon, and chlorine using the Agilent 8900 ICP-QQQ, Agilent publication, 5991-6852EN
4. Y. K. Tanaka, R. Iida, S. Takada, T. Kubota, M. Yamanaka, N. Sugiyama, Y. Abdelnour, Y. Ogra, Quantitative Elemental Analysis of a Single Cell by Using Inductively Coupled Plasma Mass Spectrometry in Fast Time-Resolved Analysis Mode. *Chem Bio Chem*, **2020**, 21 (22), 3266–3272, https://doi.org/10.1002/cbic.202000358
5. Japan SLC Inc., Wistar rat mean corpuscular haemoglobin (MCH), accessed October 2021, http://www.jslc.co.jp/pdf/data/2007/wistar2007.pdf

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