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## Index

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### Regular article

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## Comparative Evaluation of micro-particles of different materials by M-DIN ICP-MS

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### Abstract

In this study, artificial microparticles were used as pseudo-cells to select samples that can be used as substitutes for micro-droplet injection nebulizer (M-DIN) ICP-MS standards. The microparticles were selected on the basis that they should be comparable in size to human cells, have a small deviation in volume, and have different elemental contents and pyrolysis properties. Polymer latex particles, magnetic latex particles, borosilicate glass particles, and straight polymethyl methacrylate particles were used. As a result, we succeeded in detecting the contained elements in polymer latex, magnetic latex and polymethyl methacrylate particles. In addition, focusing on Mg contained in all particles, we compared the correlation between the mass signal intensity by droplet ICP-MS and the average elemental content in a particle.

**Keywords:** ICP-MS, Single-cell analysis, Micro-droplet injection system, Desolvation system, Micro particles, Elemental analysis

**Statements about COI:** The authors declare no conflict of interest associated with this manuscript.

### Introduction

With the progress of basic research in regenerative medicine, including that on iPS cells, single-cell analysis of genomes and proteins has been the focus of considerable interest. This has led to significant progress in the development of highly sensitive single-cell analysis techniques for proteins, DNA, and other macromolecules. In the field of single-cell trace element analysis, Matsuyama et al. performed X-ray fluorescence (XRF) analysis in single cells using Spring-8, and successfully detected P, S, Cl, Ca, Fe, Cu, Zn, and Au [1]. However, because this analytical method uses a large synchrotron radiation facility, it is limited to researchers with access, and there are issues regarding opportunities for use, cost, and throughput of analysis. Inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS), which use ICP as excitation and ionization sources are currently widely used for trace element analysis [2,3]. Starting with the determination of calcium in animal cells (10-20  $\mu\text{m}$ ) [4]

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using ICP-AES in 1994 [5], single-cell analysis using ICP-AES and ICP-MS has been widely performed [6-10]. The possibility of conducting highly sensitive single-cell analysis will allow researchers to elucidate the differentiation mechanism of iPS cells, construct a reliable differentiation induction method, help clarify the properties of cancer stem cells, and elucidate Alzheimer's and Parkinson's diseases (believed to be caused by the loss of cell function due to cytotoxicity caused by the abnormal uptake of ultra-trace elements). However, the conventional sample introduction system (i.e., pneumatic nebulizer and spray chamber) in this analysis method atomizes the solution sample with a carrier gas, so the solution sample is introduced to the plasma in a conical diffuse state. The solution sample is ejected drop-by-drop and introduced into the plasma in droplet form, resulting in 100% sample introduction efficiency into the plasma, which is the same as the direct sample injection nebulizer. This method allows the solution sample to be introduced into the plasma without being diffused into the plasma, and thus does not significantly lower the ambient plasma temperature.

We have developed a micro-droplet injection system (M-DIS) to achieve high sensitivity, high efficiency analysis, and individual analysis of trace samples. M-DIS introduces a sample of microdroplets (diameter, 30-70  $\mu\text{m}$ ) into the central axis of the plasma by applying a pulse voltage to a piezo element and injecting the suspension samples one-by-one. Therefore, compressing the samples temporally and spatially enables the highly sensitive analysis of very small amounts of samples [9-15], and individual analysis is possible by injecting a droplet containing a specific cell. By applying this introduction system to ICP-AES and ICP-MS and investigating the analytical characteristics, it was found that an adequate sensitivity for single-cell analysis could not be obtained [14]. Notably, the volume of the droplet (18 fg) is more than 1,000 times larger than the droplet volume (6.5 ag) introduced by conventional nebulizers. Therefore, we developed a desolvation device for introducing droplet samples, in which the solvent in the droplet is heated and evaporated before plasma introduction, and is then cooled and removed [15,16]. This system was applied to ICP-AES capable of simultaneous multi-element analysis, and single-cell analysis was performed using unicellular algae as a sample. Based on the results, we succeeded in simultaneously analyzing multiple elements, such as Ca, Mg, and Fe, which are contained in 11 to 80 fg per cell with a diameter of 3 to 8  $\mu\text{m}$  [17]. To quantitatively evaluate the distribution of trace element content among individual cells, it is necessary to consider the quantitative accuracy and performance of this analysis system. For this purpose, analyzing standard materials with a certain elemental value quantitatively is pertinent. However, there are no suitable standard materials for evaluating this system. In this study, artificial microparticles will be used as pseudo-cells to select samples to replace M-DIN ICP-MS standards. The microparticles are selected on the basis that they are comparable in size to human cells, have a small deviation in volume, and have different elemental content and pyrolysis properties. Polymer latex particles, magnetic latex particles, borosilicate glass particles, and polymethyl methacrylate straight particles shall be used. Focusing on the elements contained in all particles, the correlation between the mass signal intensity by droplet ICP-MS and the average elemental content in the particles will be compared.

## Experimental

### Micro-particle sample preparation

A variety of commercially available microparticles are used as reference materials for biological cells. The candidate reference materials used in this study are listed in **Table 1**. From among these materials, several microparticles were selected based on their size being equivalent to human cells, small volume deviation, elemental content, and thermal degradability. Materials included polymer latex particles (Corefront Corporation, Tokyo, Japan), magnetic latex particles (Corefront Corporation, Tokyo, Japan), borosilicate glass particles (Thermo Fisher Scientific Corporation, Yokohama, Japan), polymethyl methacrylate (Cospheric LLC, California, USA), and others. Particle washing was performed by adding ultrapure water (Direct-Q UV 3, Millipore, Bedford, MA, USA) to the selected particles and centrifuging (4000 x g, 3 min) three times, discarding the supernatant liquid. After washing, the particle samples were dried to powder form. Powdered polymer latex particles, magnetic latex particles, and polymethyl methacrylate particles were treated with 5 mL of nitric acid, 4 mL of sulfuric acid, and 1 mL of hydrogen peroxide solution. Only borosilicate glass was treated with 10 mL of nitric acid and 0.5 mL of hydrofluoric acid and diluted to 50 mL with ultrapure water. Impurities other than particles were then removed with an 8  $\mu\text{m}$  diameter filter (Millipore, Tokyo, Japan), stirred with a vortex mixer for 10 s, introduced into a cell counter (OneCell Counter, BMS, Tokyo, Japan), and counted with an optical microscope (ECLIPSE Ti2-E, Nikon, Tokyo, Japan) and counted the number of particles with an optical microscope (ECLIPSE Ti2-E). The sample concentration was adjusted so that each of the 40 dispensed droplets contained one particle and each droplet contained no more than two particles.

**Table 1.** | Comparison of properties for each particle (melting point, composition, and absolute amount).

No.	Material	Melting point/°C	Composition	Absolute amount/fg
(a)	Polymer latex	240	$[\text{CH}_2\text{CH}(\text{C}_6\text{H}_5)]_n$	Mg : 0.83, Fe : 0.18
(b)	Magnetic latex	—	$[\text{CH}_2\text{CH}(\text{C}_6\text{H}_5)]_n$ $\text{Fe}_3\text{O}_4$	Fe : 120, Mg : 0.62
(c)	Polymethyl methacrylate	160	$(\text{C}_5\text{O}_2\text{H}_8)_n$	Mg : 0.032
(d)	Borosilicate glass	846	$\text{SiO}_2, \text{Na}_2\text{O}, \text{CaO}$ $\text{MgO}, \text{Al}_2\text{O}_3, \text{K}_2\text{O}$ $\text{FeO}, \text{Fe}_2\text{O}_3, \text{B}_2\text{O}_3$	Ca : 1300, Al : 590, Na : 560 B : 560, Mg : 42, K : 16 Ti : 27, Fe : 12, Sr : 12 Zr : 5.0, Ba : 2.1, Mn : 0.37

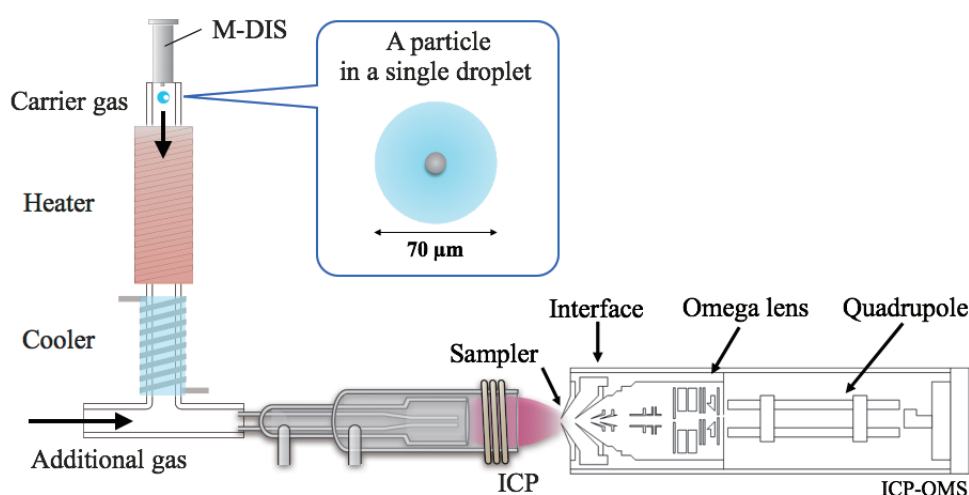
### Elemental analysis of micro particles using M-DIS-ICP-MS

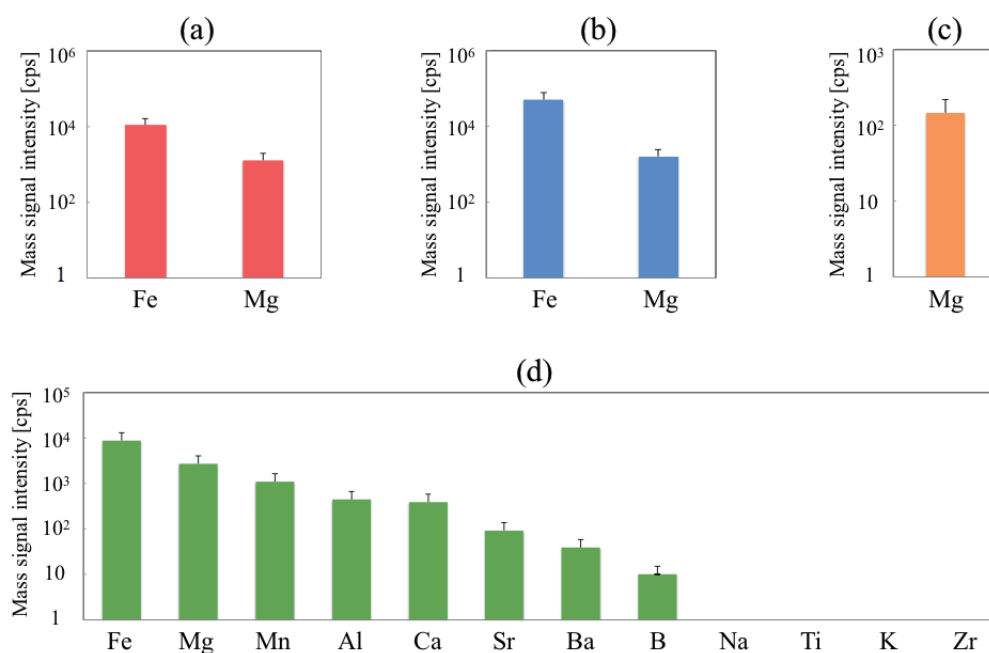
The droplet sample introduction apparatus shown in **Figure 1** was applied to an ICP mass spectrometer (7500ce, Agilent Technologies, Tokyo, Japan) for the mass spectrometry of the prepared samples. Typical operating parameters for the ICP-MS were as follows: incident rf power was 1500 W, outer Ar gas flow rate: 15 L/min, intermediate Ar gas flow rate: 1.5 L/min, carrier Ar gas flow rate: 1.5 L/min and make-up Ar gas flow rate: 1100 mL/min.

## Results and Discussion

### Candidate selection of micro particles for standard sample

Various commercially available microparticles were used to select particles that could serve as standards for this analysis system. Quantitative analysis was performed using the calibration curve method, and the average absolute elemental content per particle was calculated using the number concentration of each particle from the measured concentration. The calculated average elemental content per particle and other properties are listed in **Table 1**. Polymethyl methacrylate particles had a large volume deviation, but were chosen for an investigation into the analytical properties owing to differences in pyrolysis properties

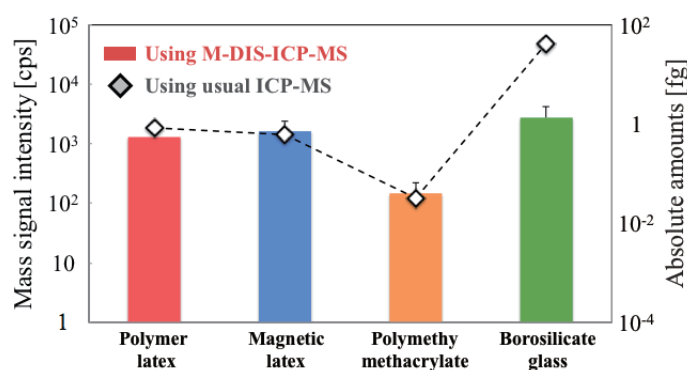
**Figure 1.** | Schematic representation of the Micro-droplet Injection System Inductively Coupled Plasma Mass Spectrometry (M-DIS-ICP-MS) set up.



**Figure 2.** | Single particle analysis of each particle by droplet ICP-MS  
(a) Polymer latex, (b) Magnetic latex, (c) Polymethy methacrylate and (d) Borosilicate glass

other than polymer and silica-based properties. First, each particle was introduced into the solution using a wet ashing method, and the average elemental content in a single particle was determined by ICP optical emission spectrometry using the usual solution spray-introduction method. As shown in **Table 1**, elements such as iron and magnesium were mixed in addition to the component elements of the sample.

Then, the droplet sample introduction method was applied to the ICP MS to perform a single-particle analysis of each particle. One droplet was ejected every 10 ms and trace element analysis was performed. The results are shown in **Figure 2**. Two types of samples were used: one with the number density of each microparticle adjusted and the other with only ultrapure water containing no microparticles. **Figure 2** shows the difference between the signal intensity of the sample containing microparticles and that of ultrapure water. One droplet per 10 ms was used for trace element analysis measurement. This figure shows that all of the elements listed in **Table 1** were detected in the polymer latex, magnetic latex, and polymethyl methacrylate particles. Sodium (Na), titanium (Ti), potassium (K), and zirconium (Zr) were not detected in the borosilicate glass particles. This may be because of the low amount of elements contained in the particles (Zr: 5.0 fg) and the spectral interference with elements such as potassium and sodium in ultrapure water. In addition, because solid microparticles were used as the sample in this experiment, extra energy was required for the melting process to ionize the sample. Therefore, a low melting point is important for detecting



**Figure 3.** | Comparison of Mg signal intensity with average elemental content in each sample.

all elements in the sample. Therefore, we compared the signal intensity of the magnesium contained in all particles with the average elemental content. **Figure 3** shows the results, confirming the correlation between the signal intensity of Mg and the average elemental content of the polymer latex, magnetic latex, and polymethyl methacrylate particles. In contrast, the signal intensity of Mg of the borosilicate glass particles was lower than the average elemental content. This may be because of the higher melting point of the borosilicate glass particles compared to the other samples, which did not sufficiently ionize the sample. This indicates that samples with a high melting point, such as borosilicate glass particles, are unsuitable as a reference material for this analysis system. We believe that it is important to select a sample with a low melting point, excellent pyrolyzability, and traceability of the particle size and elemental addition rate as a reference material.

## Conclusion

In this study, the accuracy of droplet ICP-MS was evaluated using fine particles. Fine particles were selected to be of a size equivalent to a human cell, with small deviations in volume, constituent elemental differences, and thermal degradability. Polymer latex particles, magnetic latex particles, borosilicate glass particles, and polymethyl methacrylate particles were used for the particles. First, each particle was analyzed and a sample was selected to serve as a substitute for the reference material for this analyzer. Focusing on Mg contained in all particles, we compared the correlation between the signal intensity of Mg obtained by droplet ICP-MS and the average elemental content in the particles. As a result, the signal intensity of Mg in borosilicate glass particles could not be obtained as expected from the average elemental content. It is important to select a sample with a low melting point, excellent pyrolyzability, and traceability of particle size and elemental addition rate as a candidate reference material for our analysis system.

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