Inductively Coupled Plasma Mass Spectrometry for the Analysis of Metal Content in Single Chinese Hamster Ovary Cells

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ABSTRACT

Single-cell inductively coupled plasma mass spectrometry (ICP-MS) determines the mass of a given element incorporated at the single-cell level. This novel technique, from sample preparation to data acquisition, was developed for Chinese Hamster Ovary (CHO) K1 cell lines to explore intracellular elemental content. After establishing system suitability with nanoparticles and selenized yeast cells as reference materials, CHO cells were purified from the growth media and individually introduced into the ICP-MS. Subsequent quantitation of elements (metals and non-metals) in individual cells can be achieved using this technique. Due to its relevance in biological systems, iron level in single CHO cells was the focus of this study. Results from the quantitation at the single-cell level were comparable to a recently developed bulk-cell quantitation approach using conventional ICP-MS.

INTRODUCTION

Elements, particularly metals, can heavily influence protein production for therapeutic applications (e.g., growth, viability, yield, and protein quality). ICP-MS has been extensively used to determine elemental contents in cell culture media, both in fresh and spent media. However, exploring intracellular elemental content to establish potential links between growth media and cell culture performance was previously impossible.

ICP-MS can be operated in multiple modes: conventional mode (optimal for fresh/spent media) or the novel single-cell mode (capable of intracellular investigation). In single-cell mode, the elemental content of individual cells (as opposed to bulk-cell analyses) can be determined, and information about the distribution of elements within a cell population could also be obtained. Multiple studies have shown the successful use of single-cell ICP-MS for cell types such as yeast, alga, and red blood cells [1]. However, its application for CHO cells, commonly used to produce therapeutic proteins, has not been explored. This study aims to develop a single-cell analysis method, from CHO cell preparation to data acquisition and processing, to determine metal content in individual cells. Such information may provide insights to appropriately tailor cell culture media for better control of cellular metal uptake.

MATERIALS AND METHODS

Reference Materials

Gold nanospheres (100 nm, nanoComposix, California, USA), europium embedded calibration beads (3 µm, Fluidigm, California, USA), and selenized yeast cells (~ 5 µm, SELM-1, National Research Council of Canada, Ottawa, Canada) were used as reference materials to check the performance and suitability of the ICP-MS system for single-cell analysis.

CHO Cell Preparation

Two CHO-K1 cell lines were passaged and maintained in Gibco™ Easy-Adapt™ CHO-K1 CD Medium (Thermo Fisher Scientific, Massachusetts, USA). The cells were then washed three times with Tris-buffered saline (TBS), and cell viability was measured by Vi-CELL (Beckman Coulter, Indianapolis, USA) prior to bulk- and single-cell analyses.

Average cellular elemental content (bulk-cell) analysis

TBS washed cells were vacuum-dried and microwave-digested in concentrated nitric acid. The elemental content of the digested solutions was determined by conventional ICP-MS. Together with the pre-determined cell number and cell mass, elemental values were used to calculate the average elemental contents per cell.

Single-cell ICP-MS System Setup

A single-cell sample introduction system (Glass Expansion, Melbourne, Australia) was connected to a Thermo Scientific™ iCAP™ TQ ICP-MS (Bremen, Germany) for single-cell analyses. Dilute cell suspensions were delivered into the ICP-MS system through a syringe pump.

Data Acquisition and Analysis

Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ Software was used to acquire transient signal (5 ms dwell time, time resolved acquisition mode). Kinetic energy discrimination (KED) mode was used to quantify ^{57}Fe, while triple quadrupole (TQ)-O_2 mode was used to quantify ^{31}P (as $^{31}P^{16}O$) and ^{80}Se (as $^{80}Se^{16}O$). An in-house application was used for the automatic processing of the exported Qtegra data. Advanced functions, such as consecutive cell signal detection and correction, were also incorporated in this application.

Table 1. Single-Cell ICP-MS Parameters

Parameter	Value
Injector	Quartz, 2.0 mm ID
Nebulizer Flow	0.5 L/min
Additional Gas Flow	0.4 L/min
Sample Flow Rate	10 µL/min
RF Power	1550 W
Interface	Ni sample and skimmer cones High-sensitivity skimmer cone insert
CRC Conditions	0.3 mL/min O2 in TQ-O2 mode 4.2 mL/min He in KED mode
Dwell Time	5 ms
Total Analysis time per sample	6 min

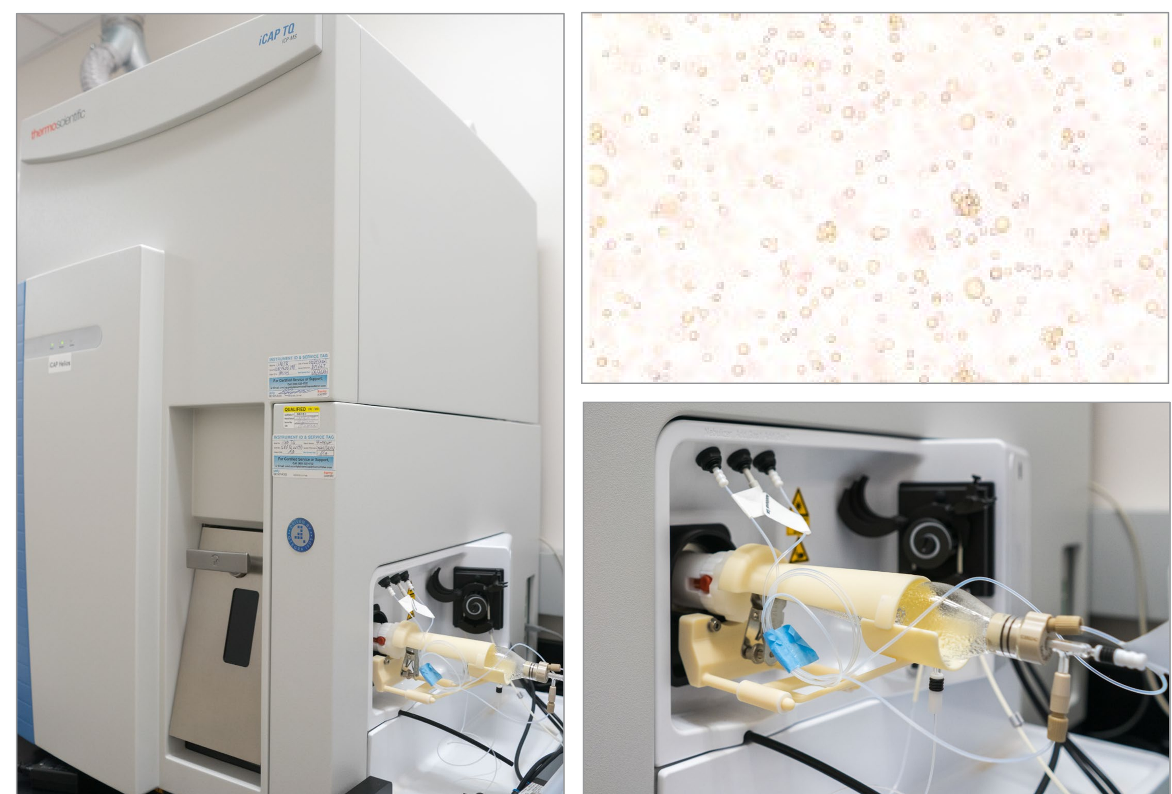

Figure 1. (Left) iCAP-TQ ICP-MS used in the study. (Bottom-right) Close-up of the single-cell sample introduction system with custom nebulizer and spray chamber. (Top-right) Light microscope image of CHO cells in TBS suspension.

RESULTS

CHO Cell Preparation

The viability of both CHO cell types was assessed after the three TBS washes (Table 2).

Table 2. Cell viability of CHO cells at 2–8 °C after TBS washing

Condition	Viability of CHO K1 line 1, %			Viability of CHO K1 line 2, %		
	0 h	1 h	2 h	0 h	1 h	2 h
Easy-Adapt CHO K1 Medium	99.8			99.2		
TBS – 3 washed	98.2	97.6	97.0	95.9	95.1	95.1

- Both CHO cell types remained viable after three TBS washes
- Washed cells still maintained their viability at 2-8 ºC

Elemental levels in the TBS-washed supernatant were measured to evaluate removal of backgrounds derived from the growth medium (Table 3).

Table 3. Representative elemental concentration in the TBS washed supernatant

Condition	CHO K1 line 1 (mg/L)		CHO K1 line 2 (mg/L)	
	Phosphorus	Iron	Phosphorus	Iron
TBS – 3 washed	<0.545	<0.006	<0.545	<0.006

- Elemental levels in the supernatants after three TBS washer were reduced several orders of magnitude below the limit of quantitation levels and were therefore suitable for single-cell analysis.

The established cell preparation protocol produced viable washed CHO cells. All potential elemental contaminants from the media had been removed, resulting in low background for analysis at the single-cell level.

Performance Verification of the Single-Cell ICP-MS System

Performance of the single-cell setup was confirmed with the analyses of nano- and micro-particles (data not shown), as well as individual yeast cells (SELM-1) (Figure 2 and Table 4).

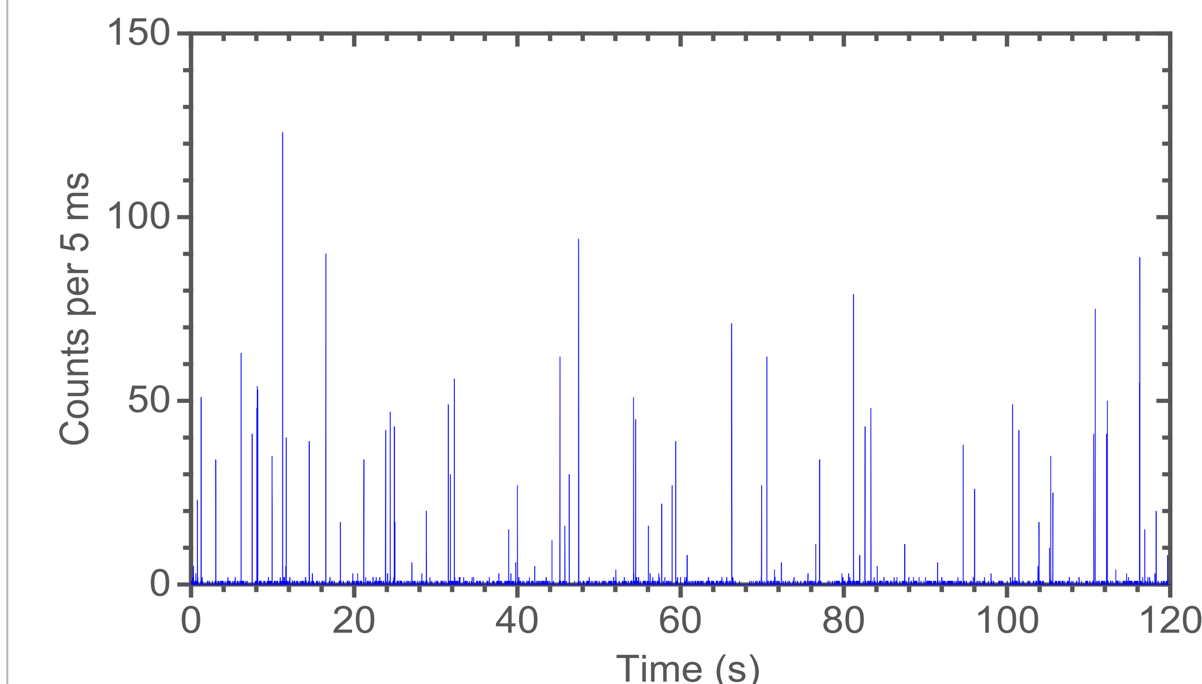

Figure 2. Single-cell ICP-MS signal of ^{80}Se (as $^{80}Se^{16}O$) from selenized yeast cells

- Background signal ∝ Concentration of dissolved species
- Number of events (spikes) ∝ Cell number concentration
- Signal intensity of each spike ∝ Mass of element per cell

Table 4. Quantitation results for selenium and phosphorus in selenized yeast cells

	Selenium (fg per cell)		Phosphorus (fg per cell)	
	Average	Range	Average	Range
Reference #2	26.5	1.1–94.7	55.3	8.3–201
This study	24.5 ± 2.1	5.3–130	72.6 ± 8.0	15.3–570

Selenium and phosphorus content in the selenized yeast cells were comparable with previously published values (Table 3), confirming the system suitability of the single-cell apparatus.

Quantitation of Iron in Single CHO Cells

Single-cell iron signals were acquired (shown for CHO K1 line 2), and distribution levels in individual cells were determined (Figure 3).

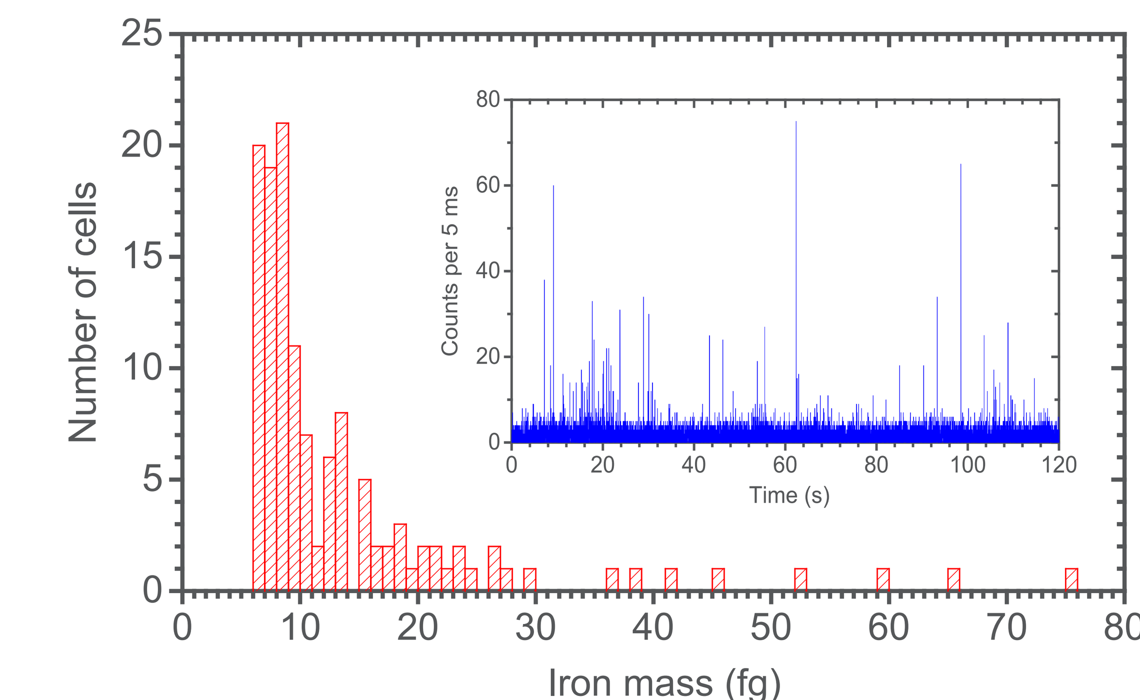

Figure 3. Distribution of iron in individual CHO K1 line 2 cells. Inset shows single-cell ICP-MS signal of ^{57}Fe

- Femtogram levels of iron were determined for individual CHO cells

Iron levels determined for individual CHO cells by single cell were compared to average levels determined through bulk cell analysis (Table 5).

Table 5. Single- and bulk-cell analyses comparison

Type of analysis	Average iron (fg per cell) in CHO K1 line 1	Average iron (fg per cell) in CHO K1 line 2
Single-cell (~100–400 cells)	16.3 ± 1.2	15.5 ± 1.9
Bulk-cell (~10^8–10^9 cells)	23.3	11.4

The levels of iron were comparable between single-cell and bulk-cell approaches with low fg/cell concentrations determined for both cell lines.

CONCLUSIONS

- A protocol for the preparation of viable CHO cells for single-cell analysis was established.
- Single-cell capabilities for the iCAP TQ ICP-MS were demonstrated with commercially available reference materials.
- A method for estimating the average cellular elemental concentrations through bulk-cell analysis was developed.
- Single-cell ICP-MS provides more detailed information on the distribution of iron in individual CHO cells (fg/single cell) as compared to bulk-cell analysis (average fg/cell).

REFERENCES

1. Theiner, Sarah et al., 2020, Single-cell analysis by use of ICP-MS, *Journal of Analytical Atomic Spectrometry.*

2. Álvarez-Fernández García et al., 2019. Addressing the presence of biogenic selenium nanoparticles in yeast cells: analytical strategies based on ICP-TQ-MS, *Analyst.*

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