ESI

Extended Introduction

For inorganic analysis of elements in life science applications, inductively coupled plasma mass spectrometry (ICP-MS) excels by extremely low limits of detection for most metals down to ppt-level (ng L^{-1} relative) or femto-gram (fg) level absolute, outstanding accuracy and thus ultimate quantification capabilities by application of simple calibration strategies, extremely wide linear dynamic range of up to 12 orders of magnitude and a very high multi-element coverage so that most elements of the periodic table can be detected. Concerning the elemental analysis of single cells (for more detail see a review[i,ii]), first applications have been described already in literature[iii,iv] but it is still hesitating. Therefore it is our intention to demonstrate that the sensitivity of ICP-MS is already more than sufficient to measure elements at cellular dimensions.

A new impetus to bio-inorganic applications of ICP-MS is given by the new discipline metallomics[i] and ionomics. In this new discipline metals are the main target of research and the interaction of metals with and their function in biomolecules is investigated, whereas in the second approach the number of elements has been extended to nonmetals as well. A prominent example for the latter discipline is the determination of the plant ionome by Salt and co-workers.[iii]

Living cells are composed by a large variety of different elements, with C, N, O and H being the most abundant ones. Many of the trace elements in cells are essential, because they are needed as co-factors in metalloproteins to facilitate for example enzymatic reactions or electron transport (i.e. Zn, Mn, Cu, Fe), or are even constituents of aminoacids (such as S, Se) or play an important role as essential constituents of energy transferring components or are involved in posttranslational modifications (i.e. P). Deficiency of trace elements can be source of many diseases whereas a too high dose can become toxic for many organisms, thus transport, intracellular storage, and intracellular regulation to prevent over-accumulation of trace elements are important processes often studied in cell cultures. A very popular model cell system often investigated to study ionomics, which is the uptake, metabolism, and homeostatic control of essential elements, is *Saccharomyces cerevisiae* (yeast). 13 elements (Ca, Co, Cu, Fe, K, Mg, Mn, Ni, P, Se, Na, S, and Zn) in this cell type have been assayed after digestion by use of ICP-AES[iv] in 4,385 mutant strains to identify genes that control the yeast ionome. 212 mutant strains were identified with changes in the levels of multiple elements. But due to the digestion step, the information about individual cells was completely lost.

Yeast is often used commercially as a food supplement. For instance, enrichment of Cr in yeast [v] was used as a supplement for broiler production [vi] and of Fe [vii] in case of the improvement of lambs' growth.[viii] Se is an important essential element and the main supply of this element is coming mainly from natural sources, for instance food (for more details see the review of Polatajko *et al.*)[ix]. Due to the Clark study[x] and the fact that Se intake by nutrition in certain geographical areas is low, the use of Se supplements has become increasingly popular. Selenium supplements, in particular selenized yeast, are currently one of the most commonly used mineral supplements. Due to the high interest in selenium speciation a selenized yeast reference material (SELM 1) is already commercially available and has been analytically characterized by Mester *et al.*[xi], but still improved extraction methods are developed most recently for selenium containing amino acids[xii,xiii] or novel metabolites are identified in body liquid.[xiv] Recently, Rampler *et al.*[xv] have implemented a dedicated sample preparation and speciation method based on LC-ICP-MS to investigate the optimum fermentation process for a yeast strain using different selenite feed condition. Although the interest in selenium speciation is rather high, little work has been performed about the uptake by and detection of selenium in single yeast cells.

Fermentation

Saccharomyces cerevisiae cells were provided by Biomin (Tulln, Austria) (1 mL containing ~10^6 cells). Cells were grown in complex medium (containing sulfur) under aerobe batch conditions at pH=4.5 and 32°C. The starting dissolved oxygen was 100 %. Fed-batch phase with selenium feed and synthetic minimal medium (Carbon source was Saccharose) was initiated (after ~24 h) when the fermentation decreased to 20 % dissolved oxygen. The growth rate of the yeast cell was set to 0.2 µ h^{-1}. Fed-Batch fermentation was stopped as whole selenite feed was consumed (70-72 h) reaching a final volume of 1.2 L. After fermentation several 1 mL aliquots were collected and stored at -20°C for further reprocessing.

Cell number determination

The cell counting experiments via flow cytometry were conducted at the Biomin Research Center. An Accuri C6 flow cytometer (BD Biosciences, Erembodegem, Belgium) was used and different yeast cell dilutions were determined. Yeast cell staining was conducted using thiozole orange to count viable cells and propidium iodide dyes for dead cell counting. After freezing, the total cell number was $7.28 \cdot 10^8$ per mL accounting for 40 % dead or damaged cells and 60 % viable cells.

Data acquisition and processing

Data acquisition settings and raw data evaluation is discussed in the ESI (Table I, II), because it had been already extensively been discussed in our previous paper.

A fast scan mode of the sector field instrument was used throughout this investigation. This mode is based on a so called E-scan by which the acceleration voltage and the voltages of the electrostatic analyser are changed simultaneously, the fastest scan mode available for this instrument. The shortest time resolution (which we define here as integration time) to measure a single data point is 100 µs (in the pulse counting mode) and 1 ms (in the analogue mode). We would emphasize that there are no delays in the detection and processing of data during one run. This is discussed in more detail in results and discussion.

In the fast scan mode all measurements were carried out by acquiring the signals from a single isotope. The following isotopes were selected for this investigation: ^{23}Na, ^{24}Mg, ^{57}Fe, ^{63}Cu, ^{65}Cu, ^{74}Se and ^{115}In and they have been measured in the pulse counting mode of the secondary electron multiplier (SEM), whereas ^{64}Zn, ^{66}Zn, ^{77}Se and ^{82}Se have been measured in the analogue mode. Blanks have always been measured in the pulse counting mode. Pulse counting and analogue mode are cross calibrated manually before analysis so that the analogue signal is always converted into count rates (cps). After each measurement cycle (one run) which will be discussed in more detail in the result and discussion section, the raw data were exported. It should be mentioned that the instrumental software automatically calculates from the measured signals I (raw counts) a count rate i (cps): $i = I / t$, where t is the integration time (s).

In our study, for all measurements presented in this investigation element intensities have been measured for droplets with the shorter time resolution of 100 µs or in the pulse counting mode or with 1 ms in the pulse counting and the analogue mode.

The raw data obtained with ICP-MS software (ELEMENT software 3.0.) were exported as csv files and processed using Excel software. A background correction can be applied for all intensity measurements (signal and blank). The background was measured in the interval between adjacent signals from single droplets and the mean value was subtracted from the droplet intensity. For integration times of one ms it sometimes happens that a droplet is measured at the end of the integration time interval so that the intensity is split into two integration intervals. Due to this reason, always signals from adjacent integration intervals are integrated after blank and background correction.

For all elements investigated 1,000 (if not mentioned otherwise) droplets are measured and the results were plotted as a histogram, which is nothing else than the number of events

measured in a pre-defined intensity interval (which is chosen arbitrarily). From all droplet events mean intensities and corresponding relative standard deviations (RSD) were calculated.

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Table I (in ESI) : Pulse sequence for triple pulse mode operation of the micro droplet generator (µDG) system

Parameter	Value		
Dispenser setting			
Nozzle diameter	50 µm		
	P1	P2	P3
Driver voltage	44 V	75〜80 V	28 V
Pulse duration	12 µs	4 µs	11〜13 µs
Delay	3 µs	5 µs	0 µs
Droplet generation frequency	50Hz		
Droplet characteristic			
Droplet diameter	23 µm		
Droplet volume	6.7 pL		
Droplet velocity	〜 3.2 m s^{-1}		

Table II (in ESI) : Operating conditions of a micro droplet generator (μDG) coupled to ICP-SFMS in the fast scanning mode (E-scan)

Parameter	Value
Plasma and gas settings	
Generator forward power	850 W
Plasma gas flow rate	16 L min^{-1} Ar
Auxiliary gas flow rate	1.0 L min^{-1} Ar
Desolvation gas flow rate	0.6 L min^{-1} He
Transport gas flow rate	0.7 L min^{-1} Ar
Instrument setting	
Mass resolution ($m/\Delta m$)	400
Scanning mode	Fast scanning mode (E-scan)
Mass window	20%
Samples per peak	200 for 20% mass window
Integration time (Detection mode)	0.1 ms (pulse counting mode) or 1 ms (pulse counting or analogue mode)
Isotopes monitored	^{24}Mg, ^{25}Mg, ^{57}Fe, ^{63}Cu, ^{65}Cu, ^{64}Zn, ^{66}Zn, ^{74}Se, ^{77}Se, ^{82}Se, ^{115}In

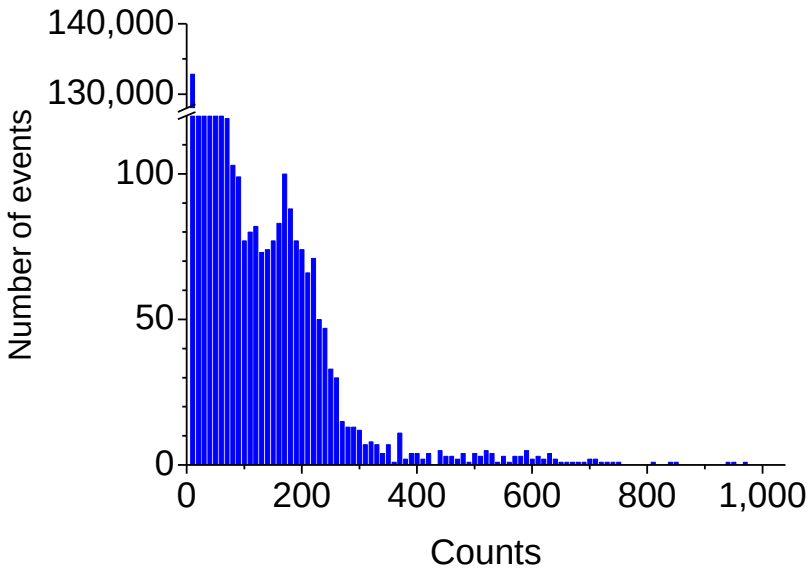

Fig. I a (ESI): Histogram of measured $^{24}Mg^{+}$ ion signal intensities. (0.01 cell/drop; sample integration time: 1 ms; counting mode. Histogram calculated for intensity intervals of 10 counts. Number of runs: 2000).

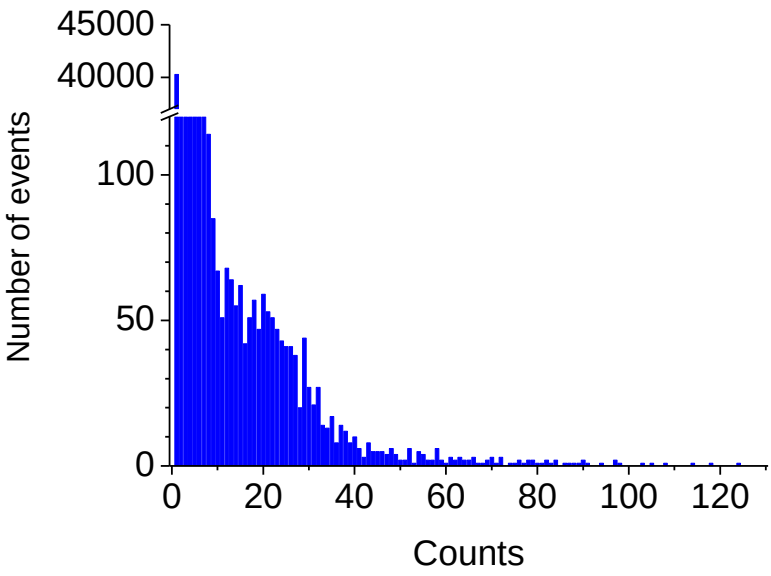

Fig. I b (ESI) Histogram of measured $^{25}Mg^{+}$ ion signal intensities. (0.01 cell/drop; sample integration time: 1 ms; counting mode. Histogram calculated for intensity intervals of 1 counts. Number of runs: 2000).

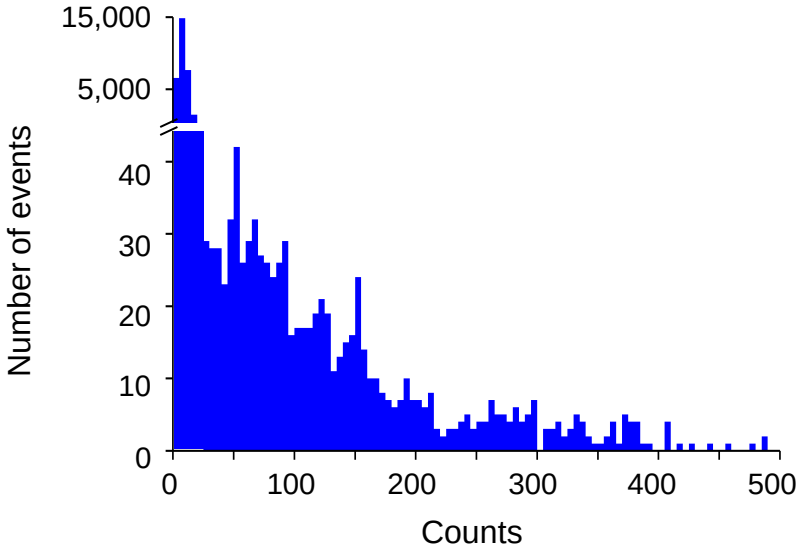

Fig. II (ESI): Histogram of measured $^{57}Fe^{+}$ ion signal intensities. (0.05 cell/drop; sample integration time: 1 ms; counting mode. Histogram calculated for intensity intervals of 5 counts. Number of runs: 400).