

Determination of the Cellular Ion Concentration in *Saccharomyces cerevisiae* Using ICP-AES

Louise Thines¹, Anne Iserentant² and Pierre Morsomme^{1,*}

¹Louvain Institute of Biomolecular Science and Technology, UCLouvain, Belgium; ²Earth and Life Institute, UCLouvain, Belgium

*For correspondence: pierre.morsomme@uclouvain.be

[Abstract] The yeast *Saccharomyces cerevisiae* has been perceived over decades as a highly valuable model organism for the investigation of ion homeostasis. Indeed, many of the genes and biological systems that function in yeast ion homeostasis are conserved throughout unicellular eukaryotes to humans. In this context, measurement of the yeast cellular ionic content provides information regarding yeast response to gene deletion or exposure to chemicals for instance. We propose here a protocol that we tested for the analysis of 12 elements (Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , K^{+} , Mg^{2+} , Mn^{2+} , Na^{+} , Ni^{2+} , Zn^{2+}) in yeast using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). This technique enables determination of the cellular content of numerous ions from one biological sample.

Keywords: Ion content, Metal, ICP-AES, Yeast, *S. cerevisiae*

[Background] Yeast has been extensively used to study the ionic response after gene deletion, modification of the extracellular environment, or heterologous protein production for example. In this context, having methods to monitor the ionic status of yeast cells is of high interest. ICP-AES uses high-energy plasma from an inert gas like argon to burn analytes very rapidly. The color that is emitted from the analyte is indicative of the elements present, and the intensity of the spectral signal is indicative of the concentration of these elements. Compared with other techniques (for example spectrophotometric methods, atomic absorption spectrometry and atomic fluorescence spectrometry), ICP-AES is a multi-ion analysis method involving simple and fast procedure with relatively low detection limits (Dahlquist and Knoll, 1978). The method described here applies to concentration determination of Ba^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , K^{+} , Mg^{2+} , Mn^{2+} , Na^{+} , Ni^{2+} , and Zn^{2+} , with the possibility of measurement of numerous cellular ionic concentrations from one 100 ml yeast culture (Eide *et al.*, 2005; Thines *et al.*, 2018). While this protocol has been tested in our laboratory for these twelve ions, this protocol could most likely be transferred to other elements. Besides, although described here for whole-cell measurement of the yeast ionic content, this method could be transferred to other cell types or to isolated organelles by fractionation on sucrose gradient for instance.

Materials and Reagents

1. Petri dishes (Sigma-Aldrich, catalog number: P5606-400EA)
2. Toothpicks

3. 5 ml pipette (Gilson, catalog number: F123607)
4. 25 mm diameter Whatman® glass microfiber filters, grade GF/F (GE Healthcare, catalog number: 1825-025)
5. *Saccharomyces cerevisiae* strains to be analyzed (performed here on BY4741 or BY4742 *S. cerevisiae* strains)
6. Yeast extract KAT (Ohly, catalog number: OHLY® KAT)
7. Glucose (Merck, catalog number: 1083469029)
8. EGTA (Acros Organic, catalog number: 409910250)
9. HCl 37% (VWR, catalog number: 20255.29)
10. Suprapur® nitric acid 65% (Merck, catalog number: 100441)
11. Certipur® single-element standards for inductively coupled plasma spectroscopy (Merck, catalog number: depends on the ion to be analyzed)
12. Milli Q water
13. NaOH (Sigma-Aldrich, catalog number: S5881)
14. YD plates (see Recipes)
15. YD medium (see Recipes)
16. 1 mM EGTA (1 L) (see Recipes)

Equipment

1. 500 ml Erlenmeyer (VWR, catalog number: 734-1833)
2. 250 ml glass beakers (VWR, catalog number: 213-1124P)
3. 100 ml graduated cylinders (VWR, catalog number: 612-3836)
4. Haldenwanger™ porcelain crucibles (Thermo Fisher Scientific, catalog number: 12306507)
5. Analytical balance (Mettler Toledo, catalog number: AB104-S)
6. Filter holding manifold (Hoefer, catalog number: FH225V)
7. Laboratory oven (Utest, catalog number: UTD)
8. Desiccator (VWR, catalog number: 467-0071P)
9. Carbolite® muffle furnace (Sigma-Aldrich, catalog number: Z654973)
10. iCAP 6500 ICP-OES CID spectrometer (Thermo Fisher Scientific, discontinued)

Procedure

A. Sample preparation

1. Streak the *S. cerevisiae* strains to be analyzed on YD plates (Recipe 1). Incubate for two days at 28 °C.
2. Using a sterile toothpick, select individual colonies.
3. Re-suspend cells in 100 ml YD medium (in 500 ml Erlenmeyer) (Recipe 2) and grow them at 28 °C under agitation (120 rpm) to an OD₆₀₀ of 3 (OD₆₀₀ = 1 corresponds to a density of 1.25 x

10^7 cells/ml).

Note: Wash the 500 ml Erlenmeyer with 10% HCl before yeast growth to avoid any impact of the detergents used for washing the glassware on the ionic content measurement. Rinse them with Milli Q water and allow them to dry.

4. Filter the 100 ml culture using glass microfiber filters on the filter holding manifold at 4 °C.
5. Wash cells twice on the filter with 2 ml EGTA 1 mM (Recipe 3) at 4 °C.
6. Wash cells twice with 2 ml Milli Q water at 4 °C.
7. Immerse each filter in a heat-resistant porcelain crucible containing 10 ml Milli Q water for 10 min. Remove the filter from the crucible.

Note: Before introducing any sample in the porcelain crucibles, weigh them when empty for further determination of the mass of dry matter.

8. Place the porcelain crucibles in an oven at 95 °C overnight to dry the sample. Figure 1 summarizes the protocol Steps A4 to A8.
9. Place the porcelain crucibles in a desiccator for 8 h for further drying.
10. Weigh the porcelain crucibles for further determination of the mass of dry matter.

Note: Steps A4 to A8 can be carried out with 100 ml YD medium (without cells). This sample will be used as a blank for further measurement.

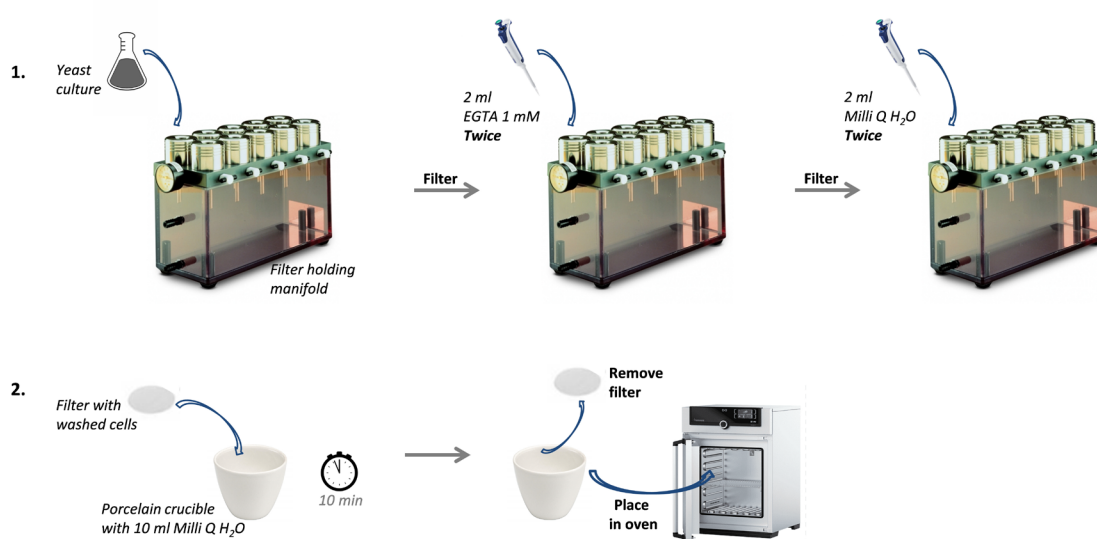


Figure 1. Experimental Steps A4 to A8. The 100 ml yeast culture is first filtered. Then, cells on the filter are washed with EGTA 1 mM (twice) and Milli Q water (twice). The filter with washed cells is subsequently immersed in a porcelain crucible containing 10 ml Milli Q water. Finally, the filter is removed and the porcelain crucible is placed in an oven for further drying.

B. Measurement of the ionic content

1. Place the samples in the muffle furnace at 500 °C for 4 h.
2. Resuspend the ashes in 6.5% nitric acid (0.5 ml nitric acid 65% + 9.5 ml Milli Q H₂O).
3. Dilute the ICP standards from Merck in a range of concentration that covers the expected ionic

concentration in the samples to be analyzed. Acidify the standard solutions to 0.5% nitric acid (from 65% nitric acid). The standard concentrations used for measurement of yeast ionic content in our study are mentioned in Table 1.

4. Measure the emitted light for each standard and sample. Typical instrument parameters are given in Table 2. Table 3 gathers the wavelengths and the viewing mode used for measurement of each cation.
5. Deduce the ionic content in the samples using the calibration curve established from the standards (see Data analysis).

Table 1. Concentrations of the standards (St) used for the calibration line

Element	St 0	St 1	St 2	St 3	St 4
Ba	0 ppm	0.5 ppm	1 ppm	2 ppm	4 ppm
Ca	0 ppm	2 ppm	4 ppm	10 ppm	20 ppm
Cd	0 ppb	5 ppb	10 ppb	20 ppb	40 ppb
Co	0 ppb	5 ppb	10 ppb	20 ppb	40 ppb
Cu	0 ppb	5 ppb	10 ppb	20 ppb	40 ppb
Fe	0 ppm	0.1 ppm	0.2 ppm	0.4 ppm	1 ppm
K	0 ppm	10 ppm	20 ppm	40 ppm	100 ppm
Mg	0 ppm	2 ppm	4 ppm	10 ppm	20 ppm
Mn	0 ppb	20 ppb	40 ppb	100 ppb	200 ppb
Na	0 ppm	10 ppm	20 ppm	40 ppm	100 ppm
Ni	0 ppb	5 ppb	10 ppb	20 ppb	40 ppb
Zn	0 ppb	200 ppb	400 ppb	1,000 ppb	2,000 ppb

Table 2. Typical instrument parameters used on the Thermo Scientific ICAP 6500 ICP OES

Pump rate	50 rpm
Nebulizer	concentric
Center tube	2 mm
Torch orientation	Duo (Axial and Radial view)
RF Power	1150 W
Coolant Flow	12 L/min
Auxiliary flow	0.6 L/min
Integration Time	15 s axial/20 s radial

Table 3. Wavelengths and viewing mode used for the twelve elements

Element	Wavelength(s) (nm)	Viewing mode
Ba	455.403	Radial
Ca	317.933	Radial
Cd	226.502; 228.802	Axial
Co	228.616; 230.786	Axial
Cu	324.754	Axial
Fe	259.940	Radial
K	766.490	Radial
Mg	279.553; 285.213	Radial
Mn	257.610	Axial
Na	589.592	Radial
Ni	216.556; 221.647; 231.604	Axial
Zn	202.548; 206.200; 213.560	Radial

Data analysis

The calibration curve of the emitted light as a function of the concentration of the standards is first modelled using linear regression. The validation parameter for a calibration line is a R^2 coefficient of minimum 0.999. An example of calibration line is provided in Figure 2.

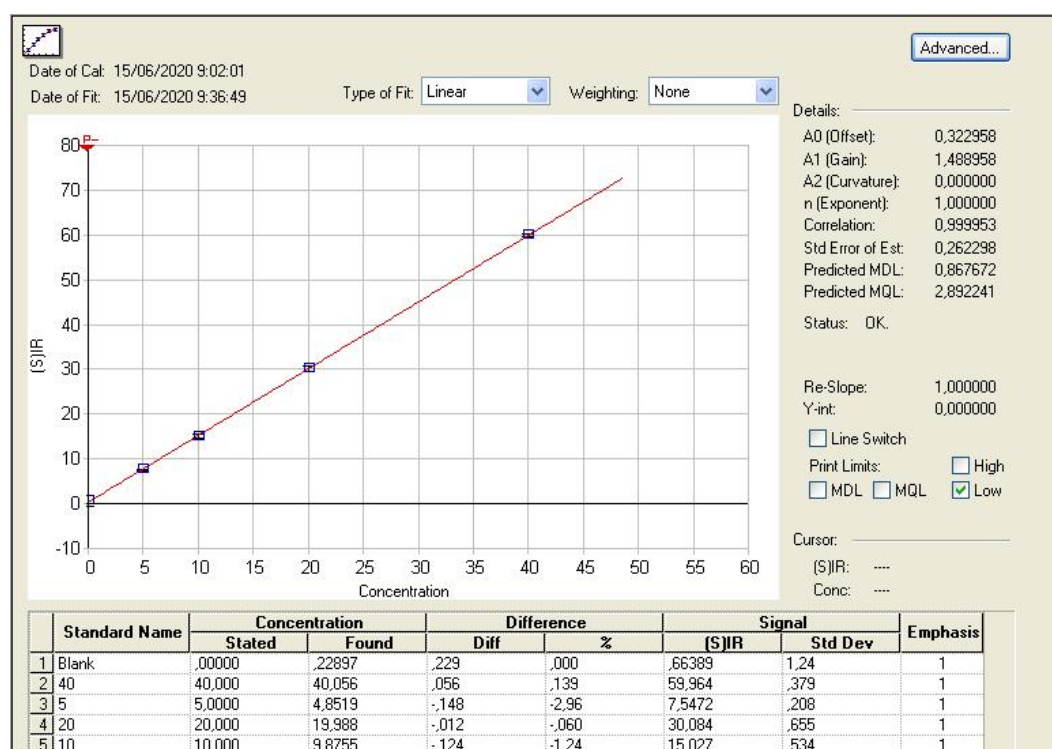


Figure 2. Calibration line of the emitted light according to the concentration of nickel (ppb) in the five standard solutions

The equation of the linear regression is determined as:

$$Emitted\ light = A * Concentration + B$$

with A the slope of the line and B its intercept. This equation is then used to deduce the concentration (in ppb or ppm) of the samples to be analyzed from the measurement of the intensity of the light emitted by the following equation:

$$Concentration = \frac{Emitted\ light - B}{A}$$

The ionic content obtained in ppm can then be converted into μmol of the ion per gram of yeast dry matter. To do so, first determine the mass of dry matter by subtracting the mass of the empty porcelain crucibles to that of the crucibles containing the cells after drying. Then convert the ionic concentration obtained in ppm to μmol of the ion per gram of dry matter according to the following formula:

$$\frac{\mu\text{mol}_{ion}}{g_{dry\ matter}} = \left(\frac{\frac{ppm_{ion}}{100}}{g_{dry\ matter}} \right) * 1000 * 1/MM_{ion}$$

With MM the molar mass of the ion in g/mol.

The limit of quantification of each element is mentioned in Table 4.

Table 4. Limit of quantification for the twelve elements

Element	Limit of quantification
Ba	0.01 ppm
Ca	0.01 ppm
Cd	0.6 ppb
Co	0.5 ppb
Cu	1 ppb
Fe	0.01 ppm
K	0.05 ppm
Mg	0.01 ppm
Mn	0.5 ppb
Na	0.05 ppm
Ni	2 ppb
Zn	2 ppb

Recipes

1. YD plates
 - 2 g (2% w/v) yeast extract KAT
 - 2 g (2% w/v) glucose
 - 2 g (2% w/v) agar
 - Adjust to 100 ml with Milli Q water and autoclave
 - Pour in Petri dishes
2. YD medium
 - 2 g (2% w/v) yeast extract KAT
 - 2 g (2% w/v) glucose
 - Adjust to 100 ml with Milli Q water and autoclave
3. 1 mM EGTA (1 L)
 - 0.3804 g EGTA
 - Adjust pH to 8.0 with NaOH

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Competing interests

The authors declare that they have no conflicts of interest with the contents of this article.

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