



# Osmolality Monitoring using Cedex<sup>®</sup> Bio & Bio HT Analyzers

## Easy and convenient calculation from standard parameters

The osmolality of a solution is often determined by measurement of the freezing point depression using a special instrument.

For determination of the osmolality and other common parameters in cell cultures and growth media, the inconvenience of a separate osmometer device in addition to a Cedex Instrument can be avoided, because culture media share common characteristics which allow to calculate the osmolality with a good accuracy using the sodium, glucose and glutamine concentrations only. An application for the Cedex Bio Analyzers provides automated calculation of the osmolality according to an empirical formula.

### Calculated osmolality in culture media

The Cedex formula uses three specific parameters which significantly contribute to the osmolality. The influence of further components is estimated by empirical factors:

$$\text{Osmo} = (2 \times [\text{Na}^+] + 1.3 \times [\text{Glc}] + [\text{Gln}]) \times 1.003$$

Unit of osmolality: mOsm/kg,

Units of sodium (Na<sup>+</sup>), glucose (Glc), glutamine (Gln): mmol/L

### Background:

Na<sup>+</sup> is the most relevant cation in cell culture media, and anions (e.g. chloride, lactate, glutamate) are included by the factor 2. All other components (e.g. K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, amino acids, proteins) contribute to < 5 % in total and are included by the other factors. The formula is the result of a biostatistical evaluation

of the concentrations of potentially relevant components and the cryoscopic osmolality values of 5,820 cell culture samples derived from 30 different media formulations.

### Option for customization of the formula:

In case of an uncommon media formulation including a special additive with a relevant contribution to the osmolality of > 3 %, there is the option to simply adapt the factor in the formula according to the typical concentration of this additive (note that all types of anionic additives are already included by the factor 2 on the almost exclusive cation Na<sup>+</sup>).

### Sample requirements for accurate calculation results

The osmolality calculation formula is applicable if samples fulfill the following conditions, which are typical for cell culture media:

- Highest concentrated ingredient is sodium chloride (NaCl) (e.g. physiologic 0.9 % = 154 mmol/L → 308 mOsm/L).
- pH is in the range of 5 to 9.
- There is no other non-ionic or cationic ingredient with a concentration >10 mmol/L besides Glc and Gln.

For chemically defined cell culture media, which are commonly used in current bioprocessing, high accuracy of the calculated osmolality values has been observed in most cases.

Culture media with a substantial content of undefined components like peptone and media formulations for bacteria and yeast are often too complex and this formula may be not applicable.

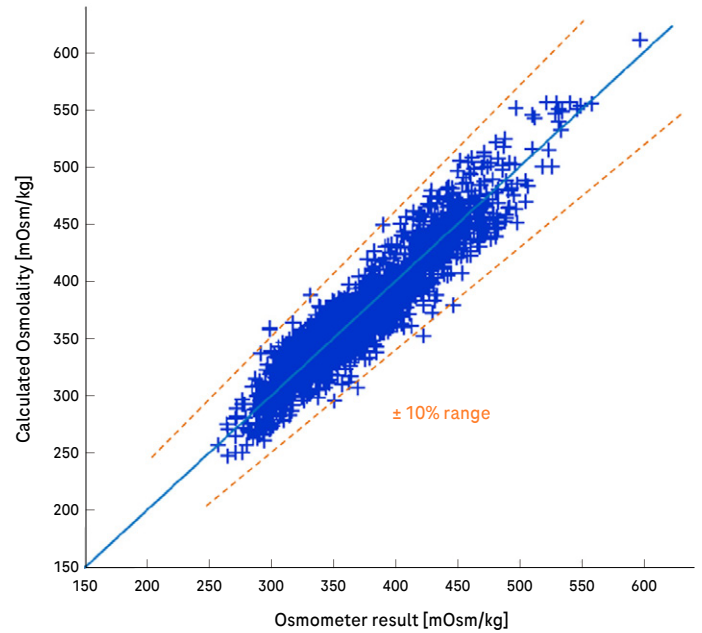
### Accuracy compared to freezing point determination

Samples of fresh culture media and a greater number of samples taken during cell culturing have been analyzed with an osmometer instrument using the freezing point depression method (Osmomat 3000 from Gonotec GmbH, Germany) and also using the osmolality calculation of the Cedex Bio HT Analyzer based on the concentrations of Na<sup>+</sup>, Glc and Gln (Evaluation data of Roche Pharma, Germany).

The study included 30 different culture media with commercially available variations of RPMI 1640, DMEM and Ham's F-12, as well as special formulations, most of them designed for CHO cell cultures. 5,820 samples were taken from cell cultures of different strains and scales of 2 L up to 1000 L culture volume.

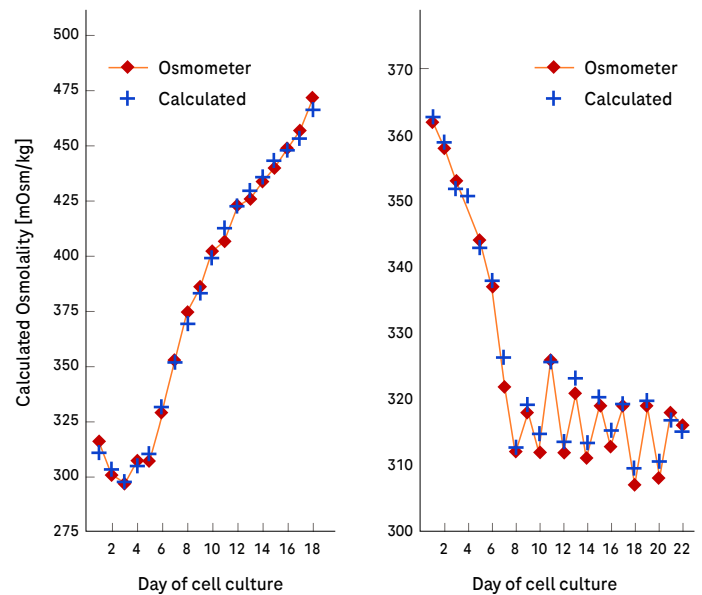
The results show a good equivalence of the calculated osmolality to the osmometer values. Among the tested samples, 98 % of the calculated values are within  $\pm 10\%$  of the osmometer result, and 76 % are within a  $\pm 5\%$  range.

Samples taken from a fermentation process over time (similar media composition) show a very good correlation between the osmometer results and the Cedex calculated osmolality over the whole process.



01

**Accuracy of the calculated Osmolality.** Comparison of the osmolality determination using an osmometer instrument versus Cedex calculation based on the concentrations of Na<sup>+</sup>, Glc and Gln.



02

**Osmolality during cell culture.** Osmolality was monitored during cell culture processing, comparing osmometer values to the Cedex calculation based on the concentrations of Na<sup>+</sup>, Glc and Gln. Two examples of CHO cell cultures with different processing principles are shown (fermenter scales of 1000 L and 250 L).

## Setup of the automated osmolality application

With an "Osmolality" test setting, the analyzer can automatically run the determination of Na<sup>+</sup>, Glc and Gln and provide the calculated osmolality result.

Note: When using the standard setting of "OSM2B", the concentration units of the involved tests for Na<sup>+</sup>, Glc and Gln must be set to "mmol/L". If "mg/L" is preferred as unit for the specific tests, refer to next page how to adapt.

### Installation on a Cedex Bio Analyzer::

The Osmolality profile "OSM2B" (code 925) can be installed on the instrument manually using the function "Add Ratio" in the instrument software, with the following settings:

<b>Code</b>	925
<b>Short Name</b>	OSM2B
<b>Long Name</b>	Osmolality 2 Bio
<b>Application A</b>	NAB
<b>Application B</b>	GLN2B
<b>Application C</b>	GLC2B
<b>Application D</b>	None
<b>Coefficient w</b>	2
<b>Coefficient x</b>	1
<b>Coefficient y</b>	1.3
<b>Coefficient z</b>	1.003
<b>Formula</b>	$(A*w+B*x+C*y)*z$
<b>Factor</b>	1
<b>Offset</b>	0
<b>Laboratory Correlation</b>	Off
<b>Valuation</b>	No valuation
<b>Low Used</b>	Off
<b>High Used</b>	Off
<b>Low Limit</b>	0
<b>High Limit</b>	0
<b>Standard Unit</b>	mOsmol
<b>Laboratory Unit</b>	mOsmol
<b>Result Conversion Factor</b>	1
<b>Displayed Unit</b>	Standard unit
<b>Dec. Position</b>	2

### Installation on a Cedex Bio HT Analyzer::

The Osmolality ratio setting "OSM2B" (code 0-909) can be downloaded from Roche support portal eLabDoc: [documentation.roche.com](https://documentation.roche.com)

File name TAS file "Cedex Bio HT ickv5038" (or higher):  
<https://go.roche.com/cedextestapplicationsoftware>



Alternatively, the ratio setting can be installed on the instrument manually using the function "Add Ratio" with the parameters shown for the Cedex Bio Analyzer (note: GLN3B is used as Application B).

### Options for adaption of the calculation::

For both analyzers, additional ratio settings with individually adapted parameters can be entered with another code number and name:

1. If there are higher concentrations (> 3 % of total osmolality) of special media additives which are not related to Na<sup>+</sup>, Glc or Gln, then "Coefficient z" can be increased according to the empiric correlation to osmometer results as a reference.
2. If Na<sup>+</sup>, Glc or Gln shall be displayed in units of "mg/L", then change the test-specific factors:  
Na<sup>+</sup> "Coefficient w" = 0.087  
Glc "Coefficient y" = 0.00721  
Gln "Coefficient x" = 0.00684
3. If concentrations above the test range are expected, then the corresponding D-protocols with higher ranges can be included in the profile:  
Na<sup>+</sup> > 275 mmol/L (> 6.3 g/L): "Application A" = NAD  
Gln > 10 mmol/L (> 1.5 g/L): "Application B" = GLN2D  
Glc > 40 mmol/L (> 7.5 g/L): "Application C" = GLC2D

## Ordering information

For determination of glucose, glutamine and sodium the following products are required in addition to the Cedex instrument with the general system reagents and accessories:

Product	Pack size	Catalog Number
Glucose Bio <sup>1</sup>	2 x 100 tests	06 343 732 001
Glucose Bio HT <sup>1</sup>	800 tests	06 608 418 001
Glutamine V2 Bio <sup>2</sup>	4 x 50 tests	07 395 655 001
Glutamine V2 Bio HT <sup>2</sup>	200 tests	07 395 612 001
Sodium Bio <sup>2</sup>	4 x 50 tests	08 881 863 001
Sodium Bio HT <sup>2</sup>	200 tests	08 881 871 001

### Regulatory Disclaimer

<sup>1</sup> For use in quality control/manufacturing process only.

<sup>2</sup> For quality control/manufacturing of IVD/medical devices/pharmaceutical products only.

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