

## Osmolality Monitoring using Cedex Bio and Bio HT Analyzers

### *Easy and convenient calculation from standard parameters*

The osmolality of a solution is usually determined by measurement of the freezing point depression. This cryoscopic method is the current standard.

For determination of osmolality and other common parameters in samples of 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 medium

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

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

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<sup>-</sup>, lactate<sup>-</sup>, glutamate<sup>-</sup>) 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 factors are the result of a biostatistical evaluation of the concentrations of several components and the cryoscopic osmolality values of 5,820 cell culture samples derived from 30 different media formulations.

#### Option for custom optimization:

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 about 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 mostly used in recent bioprocessing, high accuracy of the calculated osmolality values has been observed in most cases.

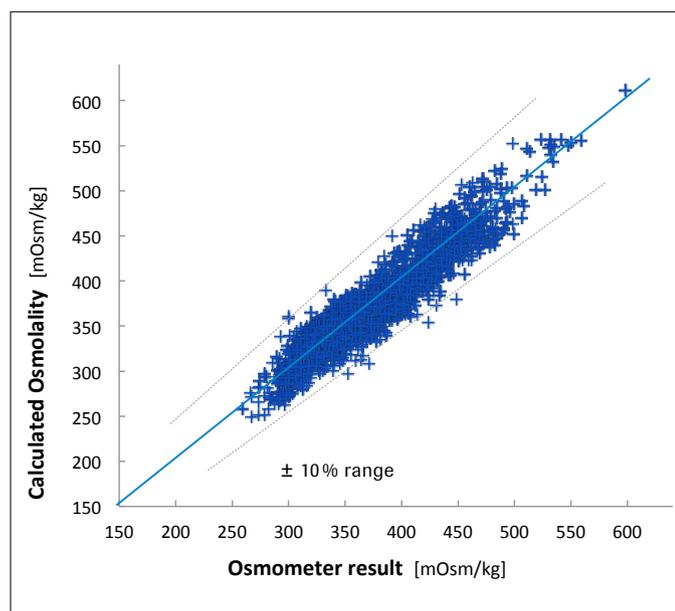
Culture media with a substantial content of undefined components like peptone and media formulations for bacteria and yeast are often too complex and this calculation may be not applicable.

### 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 (see formula on page 1).

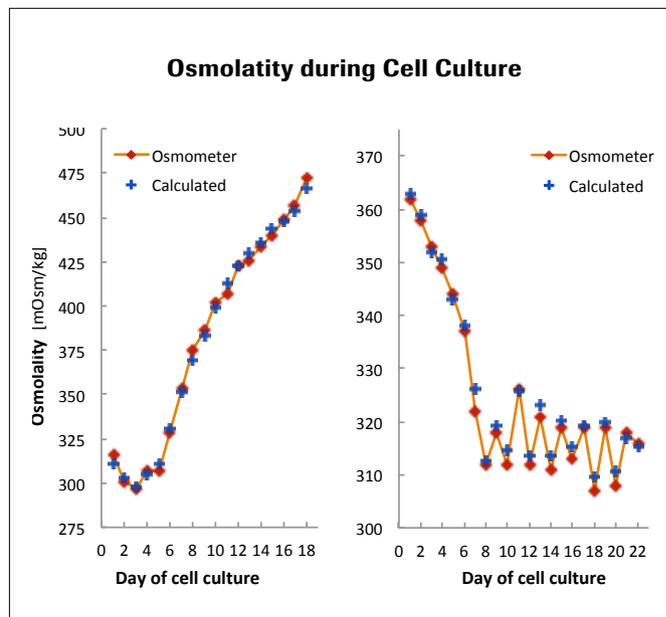
The study included 30 different culture media with commercially available variations of RPMI 1640, DMEM and Ham's F-12, as well as special formulations, most of them designed for CHO cell cultures. 5,820 samples were taken from cell cultures of different strains and scales of 2 L up to 1000 L culture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



**Figure 1:** Comparison of the osmolality determination using an osmometer instrument versus Cedex calculation based on the concentrations of Na<sup>+</sup>, Glc and Gln.

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.



**Figure 2:** 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 HT Analyzer:

The Osmolality profile “OSM2B” (code 0-909) can be installed on the instrument from the TAS file “ickv5035v2” (or higher version), which can be downloaded from the CustomBiotech internet website under following link:

[http://www.custombiotech.roche.com/home/Product\\_Details/INS\\_3590.html](http://www.custombiotech.roche.com/home/Product_Details/INS_3590.html)

### 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:

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

### Options for adaption of the calculation:

For both analyzers, there are options for individual adaption of the algorithm in the OSM2B profile settings:

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.
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 of Glc or Gln are expected above the test range, then the corresponding D-protocols with higher ranges can be included in the profile:  
Glc > 40 mmol/L (> 7.5 g/L): "Application C" = GLC2D  
Gln > 10 mmol/L (> 1.5 g/L): "Application B" = GLN2D

### Ordering information

For determination of sodium, glucose and glutamine, 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
ISE Sodium Electrode Bio <sup>(1)</sup>	1 piece	06 437 338 001
ISE Sodium Electrode Bio HT <sup>(1)</sup>	1 piece	06 664 237 001

### 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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