Replacement of the Cedex Analyzers' ISE module by photometric tests

Improved convenience and performance for control of Na^+ and K^+

In cell culture and microbial fermentation, the most important cations for the living cells are sodium (Na^+) and potassium (K^+). Reliable control of the Na^+ and K^+ concentrations within narrow operating ranges is important to gain healthy and fast growing cultures and to obtain high yields of bio-products.

A common method for determination of these ions is the use of ion-selective electrodes (ISE). However, ISE are susceptible for various interferences and require frequent maintenance and continuous recalibration in short intervals.

Therefore, the Cedex Analyzers are now using the proven format of enzyme-based assays with photometric detection which also work for Na^+ and K^+, making the ISE module obsolete. There is no need for the periodic ISE maintenance any more, and Na^+ and K^+ are determined in the same way as the other analytes.

For use in quality control/manufacturing process only.

Advantages of the ISE replacement

- Fully automated assays with high robustness against interferences, no need for sample pretreatment
- Easy workflow integration of the convenient photometric tests for Na^+ and K^+
- Wide measuring ranges for Na^+ and K^+, option for automated on-board dilution
- No need for time consuming maintenance of the ISE module, and no need for extra ISE reagents
- Smaller footprint of the Cedex Bio Analyzer without ISE module saves benchtop space

Comparison ISE ⇔ Photometric

Protocol	ISE (past)	Photometric (now)
Daily maintenance	≈ 20 min	not needed
Calibration	daily	stable for several weeks or months
Measuring ranges in mmol/L		
Na^+:	20 – 250	50 – 27,500
K^+:	0.2 - 30	2.0 – 3,000
Instrument dimensions (h/d/w, weight)		
Cedex Bio:	48/55/72 cm, 39 kg	48/55/60 cm, 33 kg
Cedex Bio HT:	75/66/315 cm, 210 kg, no change of housing	

Method comparison for Na^+/ K^+

In the past, the Cedex Analyzers used ion-selective electrodes (ISE) for determination of sodium and potassium, measuring an electrical potential of a membrane sensor in contact to the sample, compared to another sensor in a reference solution.

ISE sensors require continuous maintenance for correct function and frequent recalibration to compensate fluctuations and drift. Furthermore, there is a considerable risk of interferences of various substances affecting the affinity of these cations to the membrane (e.g. other cations, chelators, lipophilic compounds).

The photometric tests for Na^+ and K^+ are designed as more convenient alternatives. The photometric detection format fits perfectly into the analyzer concept. No special maintenance is required, and the assay is robust against interferences.

Results of the photometric assays perfectly match to ISE results. Replacement of ISE testing by the photometric assays is easy and should not require revalidation of established procedures.

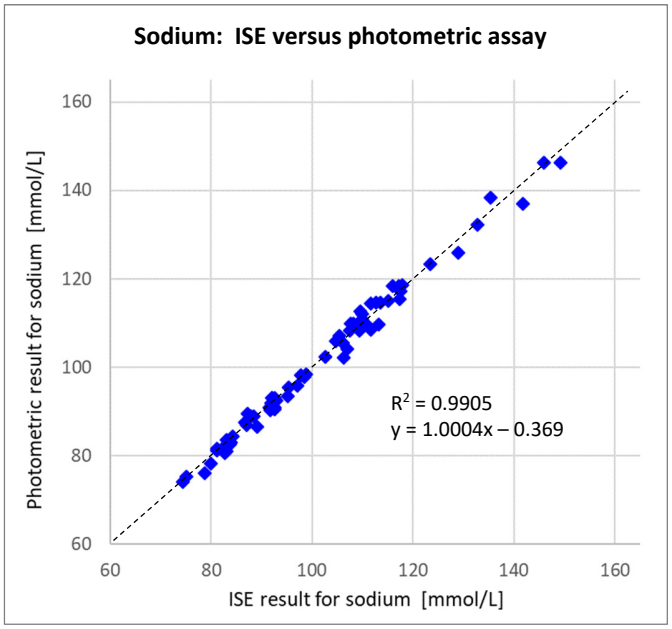

Figure 1: 70 samples of fermentation supernatants of various culture media were tested for sodium on a Cedex Bio HT Analyzer using the ion-selective electrode and the photometric sodium assay for comparison. The differences are all within ±4%, with an average difference close to zero (ISE 0.3% higher than enzymatic), confirming the equivalence of the two methods.
(Data generated with culture samples of Roche Pharma)

High accuracy

Over wide test ranges, the concentrations of Na^+ and K^+ can be determined accurately in various culture media formulations.

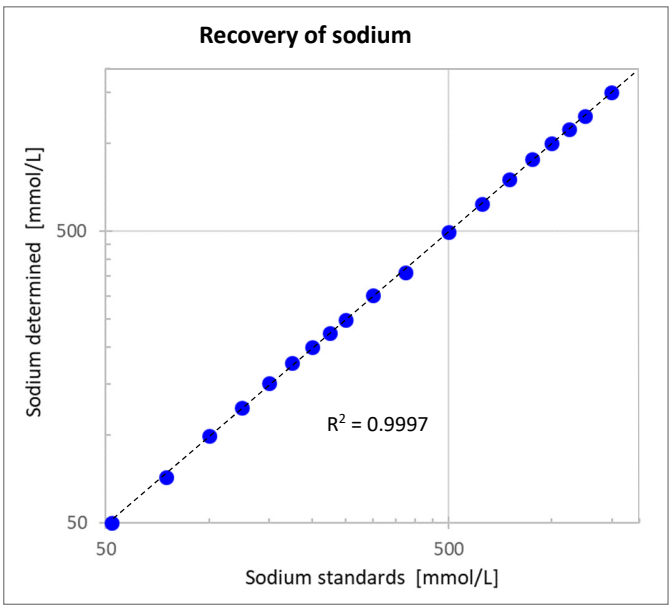

Figure 2: Sodium was determined in a row of standards with increasing NaCl concentrations spiked to a CHO cell culture medium using the photometric sodium assay on a Cedex Bio HT Analyzer. The results show a good linearity of the test and all standards are found with deviations < 2.5% from the target concentrations.
(Evalulation data of Roche Diagnostics)

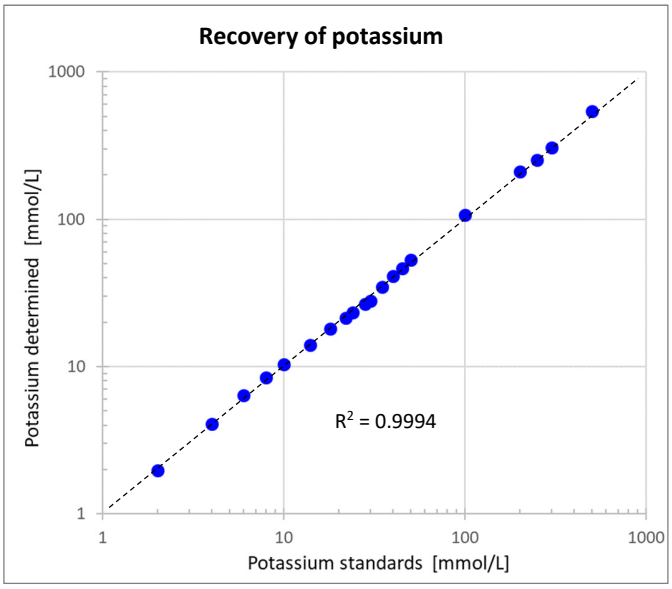

Figure 3: Potassium was determined in a row of standards with increasing KCl concentrations spiked to a CHO cell culture medium using the photometric potassium assay on a Cedex Bio HT Analyzer. The results show a good linearity of the test and all standards are found with deviations < 6% from the target concentrations.
(Evalulation data of Roche Diagnostics)

Principle of the photometric assays

While the ISE is based on an electrophysical method with a potentiometric measurement against a reference electrode, the photometric method makes use of the specific Na^+ or K^+ dependent activity of an enzyme, determined by the enzymatic conversion of a substrate which can be monitored by a change in the photometric absorption.

The enzyme ß-galactosidase (ß-Gal) in a Na^+ depleted solution shows no enzymatic activity on the substrate o-nitro-phenyl-ß-D-galactopyranoside (ONPG). If a Na^+ containing sample is added, the enzyme gets active and produces o-nitro-phenolate (ONP) with a rate depending on the Na^+ concentration. The formation of the yellow-colored ONP can be measured photometrically and the increase of the signal correlates to the Na^+ concentration of the sample.

$$\text{ONPG} \xrightarrow{\text{ß-Gal} + \mathbf{Na^+}} \text{ONP} + \text{Galactose}$$

In a similar way, the K^+ concentration is determined using the K^+ dependent activity of pyruvate kinase (PK) on the substrate phosphoenolpyruvate (PEP) generating pyruvate, and in a following reaction with lactate dehydrogenase (LDH) the NADH is oxidized to NAD^+ which can be measured photometrically.

$$\text{PEP} + \text{ADP} \xrightarrow{\text{PK} + \mathbf{K^+}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

Ordering information

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

Product	Pack size	Cat. no.
For sodium testing:		
Sodium Bio [1]	4 x 50 tests	08 881 863 001
Sodium Bio HT [1]	200 tests	08 881 871 001
Calibrator F Bio [2]	6 x 1 mL	08 377 987 001
Control F Level 1 Bio [2]	6 x 1 mL	08 377 995 001
Control F Level 2 Bio [2]	6 x 1 mL	08 378 002 001
Control F Level 3 Bio [2]	6 x 1 mL	08 378 029 001
For potassium testing:		
Potassium Bio [2]	4 x 50 tests	08 881 367 001
Potassium Bio HT [2]	165 tests	08 881 731 001
Calibrator K Bio [2]	6 x 1 mL	09 336 699 001
Control F Level 1 Bio [2]	6 x 1 mL	08 377 995 001
Control F Level 2 Bio [2]	6 x 1 mL	08 378 002 001
Control F Level 3 Bio [2]	6 x 1 mL	08 378 029 001

Regulatory disclaimer

[1] For quality control/manufacturing of IVD/medical devices/ pharmaceutical products only.

[2] For use in quality control/manufacturing process only.

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