Real-time monitoring of CHO cell cultures with dual inline sensors

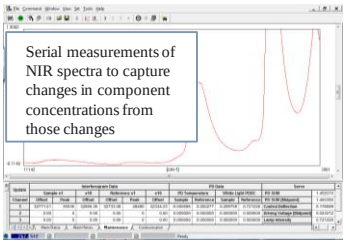
The active ingredients of biopharmaceuticals often have complex and unstable molecular structures compared with chemical synthetic drugs, and quality control is particularly important. In 2004, the U.S. FDA proposed monitoring of the manufacturing process of pharmaceuticals using PAT: Process Analytical Technology (Process Analytical Technique) in order to improve the quality assurance of pharmaceuticals (Ref. 1, 2).
We have developed two types of in-line sensor systems using our long-cultivated near-infrared spectroscopy techniques and a mathematical model software technique to achieve real-time monitoring of glucose and lactate and live cell density. This application note presents the characteristics of these in-line sensor systems and the results of their monitoring in CHO cell cultures.

※1:FDA, Pharmaceutical cGMPs for the 21st century - A risk-based approach; Final Report, (September 2004).
※2:FDA, Guidance for industry:PAT - A framework for innovative pharmaceutical development, manufacturing and quality assurance, (September 2004).

Features of the two inline sensors

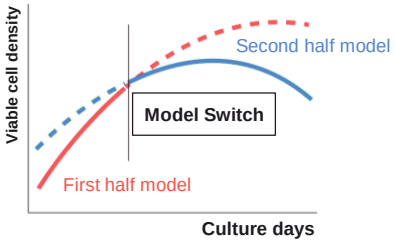
In-line sensor system for glucose concentration/lactate concentration by fourier transformed near-infrared (FT-NIR) spectroscopy techniques

The near-infrared spectrum of the culture solution is acquired, and the concentration of glucose and lactate contained in the culture solution are calculated using a calibration model with multivariate analysis.
An improved system for cell culture of our near-infrared spectroscopic analyzer NR800 achieves high-precision spectrum acquisition. Additionally, the unique calibration model software allows rapid generation of a calibration model that is compatible with individual cell cultures.



In-line sensor system using software technology to calculate live cell density from capacitance measurements

Capacity of the culture medium is obtained, and the density of live cells in the culture medium is calculated using a calibration model.
Our software employs a single-frequency calibrated model from the early stage of culture to the proliferative phase and a multivariate calibrated model from the stationary phase to the late stage of culture that appropriately switches these algorithms. This technology enables stable monitoring of live cell density throughout the culture period. Additionally, the unique calibration model creation software allows for rapid generation of the calibration model.



How to create a calibration model

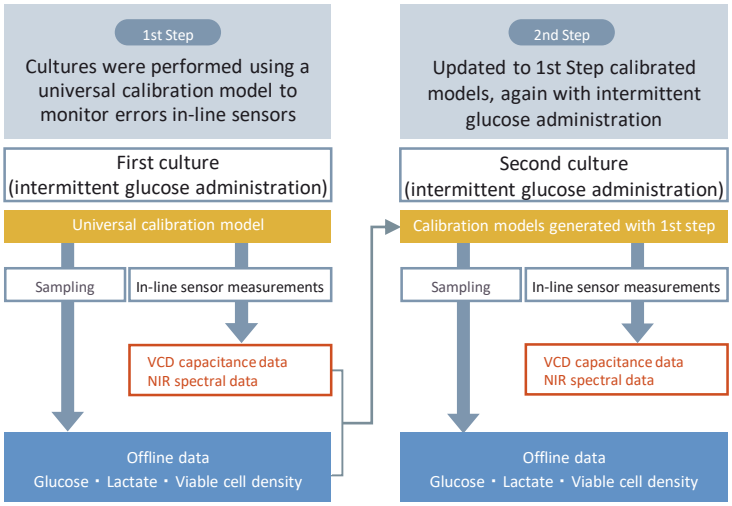
Calibration model for glucose/lactate densitometry

Culture of 1st Step is performed using a universal calibration model generated with spectral data from offline spiked samples containing glucose and lactate, provided by Yokokawa, and the calibration model is updated by spectral data from 1st Step cultures and offline data from sampling.

Calibration model for measuring viable cell density

1st Step cultures acquire several frequencies of capacitance data and sampling offline data of live cell densities to create a calibration model.

In two cultures, precise measurements can be achieved, and even if the culture condition changes, a minimal task from 1st Step to 2nd Step allows for high-precision measurements with new calibration models.



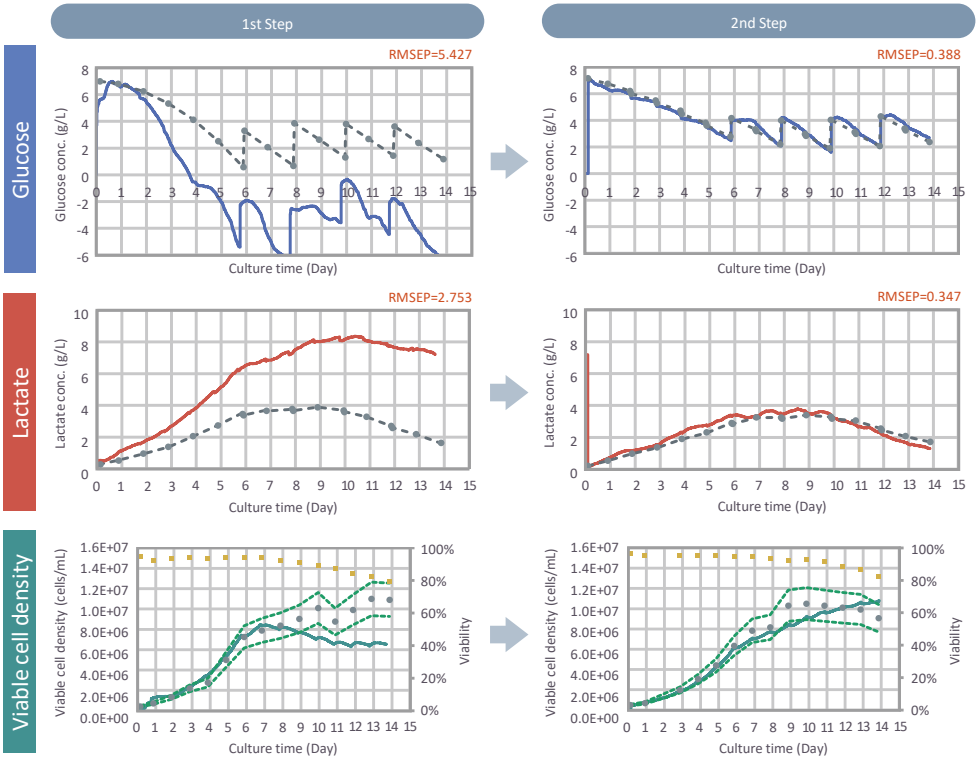
Verification of the monitoring accuracy of in-line sensors in CHO cell culture

Improving the accuracy of monitoring glucose, lactate, and live cell densities by culturing 2 steps

To show that the accuracy of monitoring indices such as glucose, lactate, and viable cell counts can be improved by applying the calibrated models derived from 1st Step cultures to 2nd Step cultures, cultures were performed under the following conditions:

1st Step In 1st Step, feed medium was administered once a day (glucose was also added depending on the day), and sampling (each time before and after feeding) was performed, and offline data were acquired to match the spectral data at that time, creating the calibration models.

2nd Step This calibrated model was placed in the reactor and 2nd Step cultures were performed as in 1st Step to calculate the error RMSEP (root mean squared error of prediction) between the in-line measurements and the offline data for glucose and lactate. In 2nd Step, RMSEP value was 0.388/0.347, which indicates that the error in glucose measurements nearly fell within the range of ± 1g/L of the set concentration ($3\sigma = 1 \rightarrow \sigma = 0.33$).



Culture condition

Cells: IgG-producing CHO cells (DG44)
Cell-density: 5.0 x 10^5 cells/mL
Media: BalanCD CHO medium (1.4L)
pH:6.8~7.2
Dissolved oxygen: 4.0 mg/mL
Agitation rate: 60 rpm
Temperature: 37°C
Perform intermittent Feed

Measure Instruments

- Analysis of Media components
  Cedex Bio; Roche, (631-24711)
- Viable cell density
  Vi-Cell XR; BECKMAN COULTER, (383721)

- Glucose measurement
- Lactate measurement
- Viable cell density
- Offline measurement
- Values ±15% of offline measurement
- Viable cell percent

Compared with the universal calibrated model alone, the updated calibrated model with additional culture data shows that glucose, lactate, and live cell densities during the culture period can be monitored accurately in real time.
This system can provide more data and update the calibration model, and we can also expect further accuracy improvements. Thus, using our system allows high-precision monitoring of cell cultures, which is expected to advance the stabilization and efficiency of the culture process.

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