Automated control of glucose concentration in CHO cell cultures via an advanced control system

As a solution to stably and efficiently drive the cultivation process of biopharmaceutical production, we have developed an advanced, integrated control system that both senses and predicts future state of glucose consumption. the basic nutrient source of cells, to a constant feed forward predicted value. The system has its own mathematical prediction model, using in-line measurements of glucose and lactate concentration by fourier transformed near-infrared (FT-NIR) spectroscopy and in-line measurements of viable cell density (VCD) by capacitance measurement as input values to estimate the specific rate of cells and future glucose concentration consumption. The system functions to automatically control dosing of glucose that maintains glucose at the user defined setpoint. It is expected to improve productivity and quality by finding the optimal glucose concentration set point for the cell line as well as increasing the reproducibility between batches compared to cultures where glucose is administered intermittently by conventional sampling.

This application note presents the results of controlling glucose concentration in CHO cell cultures to constant values with high accuracy.
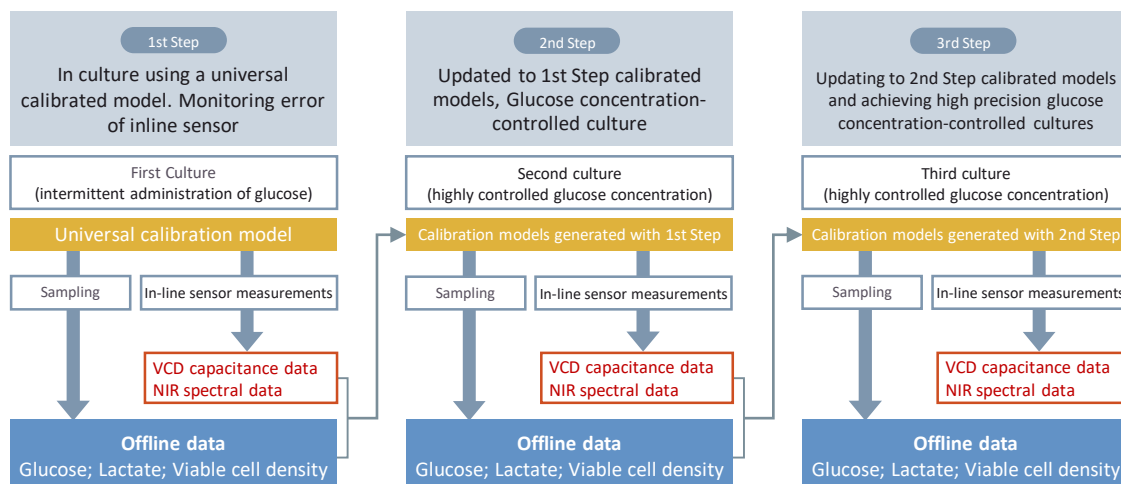
Features of the advanced control system

■ Major functions of the unique prediction model

This reactor employs model-predictive control (MPC: Model Predictive Control). In the model prediction control, the consumption rate at each time point is calculated, and the glucose concentration is predicted after a certain time, so that the glucose concentration becomes the set concentration at that time point. At that time, glucose levels were measured again to verify the deviation from the previous prediction and to calibrate it, thereby improving the accuracy of glucose measurement itself. This predictive control mechanism enables smooth control without hunting, as is seen with conventional PID control.
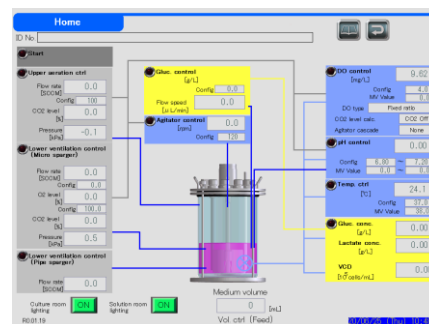
Method for creating an in-line sensor calibration model for use in advanced controlled cultures

1st Step	2nd Step	3rd Step
In culture using a universal calibrated model. Monitoring error of inline sensor	Updated to 1st Step calibrated models, Glucose concentration-controlled culture	Updating to 2nd Step calibrated models and achieving high precision glucose concentration-controlled cultures
First Culture (intermittent administration of glucose)	Second culture (highly controlled glucose concentration)	Third culture (highly controlled glucose concentration)
Universal calibration model	Calibration models generated with 1st Step	Calibration models generated with 2nd Step
Sampling / In-line sensor measurements	Sampling / In-line sensor measurements	Sampling / In-line sensor measurements
VCD capacitance data NIR spectral data	VCD capacitance data NIR spectral data	VCD capacitance data NIR spectral data
Offline data Glucose; Lactate; Viable cell density	Offline data Glucose; Lactate; Viable cell density	Offline data Glucose; Lactate; Viable cell density

■ Calibration model for glucose/lactate densitometry

Cultivate 1st Step using a universal calibration model generated from near-infrared (NIR) spectral data of off-line spiked samples containing glucose or lactate. We then generate 1st Step calibrated models from spectral data in culture and offline data from sampling, where glucose is administered intermittently during culture and a wide range of concentrations has been shifted. This model is then used to perform a highly controlled culture of 2nd Step glucose levels, and to generate a 2nd Step calibration model generated from spectral data and offline data from sampling. This calibration model provides high-precision glucose concentration control.

### ■ Calibration models for measuring live cellularity (VCD: viable cell density)

In each 1st Step, 2nd Step culture, we acquire several frequencies of capacitance data and offline data of raw cell densities to create a calibration model. It is recommended to use 2nd Step calibrated models.

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Validation of glucose concentration control accuracy in CHO cell cultures

To demonstrate that 3 Step cultivation process allows high-accuracy control of the level of glue, CHO-cells were cultivated in two media: BalanCD CHO and CD FortiCHO. Daily sampling was performed in each culture, and offline measurements (glucose, lactate, and viable cell density) were measured and matched to spectral data to create a tuned sensor calibration model.

Culture with Forti medium

FortiCHO medium contains ingredients that correspond to feed agents and can be cultured without feeding during lab scale fed batch bioreactor runs. There is a large deviation between in-line and off-line glucose measurements in the 1st Step, but the error between in-line and off-line measurements was reduced in the 2nd and 3rd Steps, and glucose levels were maintained in the vicinity of 2g/L.
The value of the error RMSEP (root mean squared error of prediction) to assess the deviation between the in-line and off-line measurements also decreased from 0.859 (2nd step) to 0.351 (3rd step). This indicates that the accuracy was increased to a range of approximately ± 1g per liter (0.351 x 3 = 1.053).

1st Step	2nd Step	3rd Step

RMSEP=1.81 | RMSEP=0.859 | RMSEP=0.351
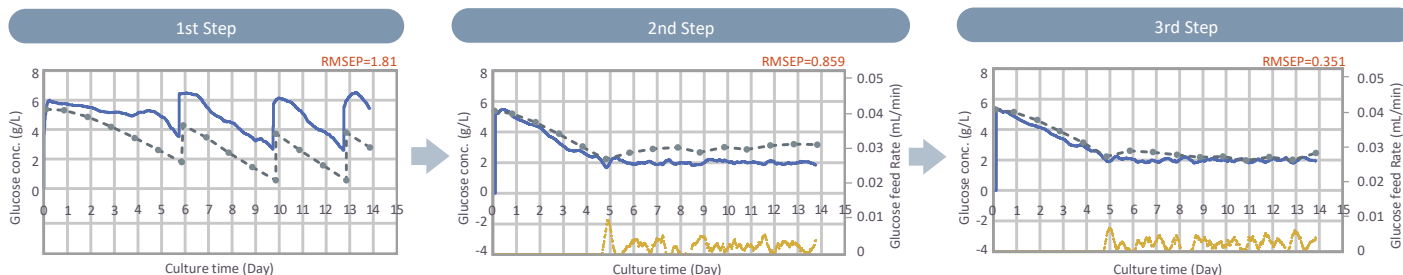
Culture with Balan medium

Cultures using BalanCD CHO media require regular dosing of the feed. In the 1st Step, glucose was administered intermittently, and the sampling was performed once a day plus one sampling 30 min later when the feed was administered and cultured for 14 days. In the 2nd and 3rd Steps, we updated the calibrated models and performed high-level control so that glucose concentrations were 2g per liter.
The error between the in-line and off-line measurements, RMSEP, decreased to 0.287 in 3rd Step, indicating that the accuracy increased to within ± 1g per liter of measurement error (0.287 x 3 = 0.861).

1st Step	2nd Step	3rd Step

RMSEP=5.427 | RMSEP=0.306 | RMSEP=0.287
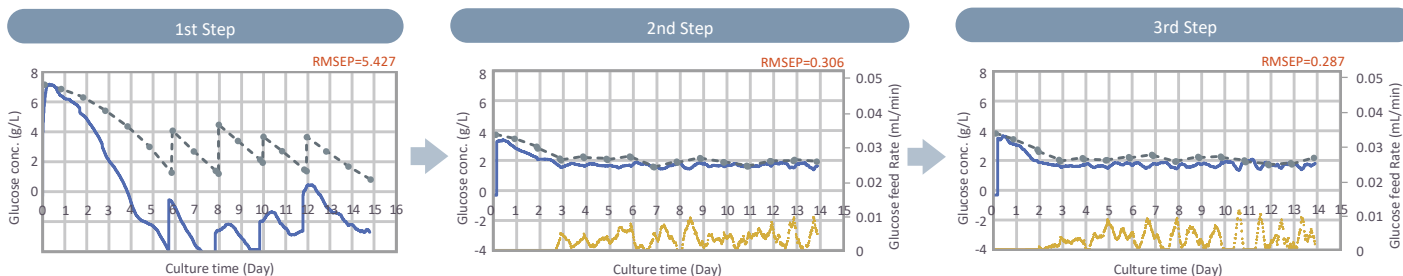
Culture conditions		Instruments for analysis
Cells: IgG-producing CHO cells (DG44)	pH: 6.8~7.2	■ Analysis of media components
Cell-density: 5.0 x 10^5 cells/mL	Dissolved oxygen: 4.0 mg/mL	Cedex Bio; Roche, (631-24711)
Media: FortiCHO/BalanCD CHO (1.4L)	Agitation rate: 60 rpm	■ Live cell density analysis
	Temperature: 37°C	Vi-Cell XR; BECKMAN COULTER, (383721)

Legend:
— Glucose measurement
— Automated glucose dose
- ● - Offline measurements

※ During intermittent incubation periods when Balan medium is used
Administration of feed (sampling before and after administration)

> Prediction models generated from offline calibrated model data and from two cultures have shown that high precision glucose concentration measurements and control can be achieved. Using Forti medium but also cell culture using Balan medium can be employed to control with high precision of glucose concentration in culture by optimization of calibration model performed in three steps.

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