Application note
#2022-11-22

Glucose monitoring and control using Siemantec Feeding module®, LIS®, M-online system®

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Abstract

Critical process parameters (e.g. glucose, glutamine, lactate, etc.) must be monitored and controlled in each biological process to achieve the desired product quality and yield in an efficient manner. At present, although offline sampling can be used for real-time calculation and regulation, there is still a lack of faster and more accurate ways to make the results controlled by analysts better meet the needs of the actual fermentation and culture process. This paper illustrates the combination of three methods, namely Sieman M-online automatic sampling and online analysis system, automatic calculation control feeding module and Sieman analysis parameter information collection and management system-LIS, to achieve online and real-time reliable data monitoring and control. In this paper, a metabolic analysis control experiment during CHO-K1 culture was used to demonstrate the monitoring and control of glucose concentration.

Introduce

In some biological pharmaceutical companies and biotechnology companies, the purpose of using DoE to conduct bioprocess development and process characterization research is to obtain a stable and efficient process to produce high-quality and high-yield products. In order to achieve this purpose, the relevant normative guidelines also give the concept of ctitical process parameters (CPPs), key process parameters (kPPs) or critical material attributes (CMAs), which have a significant impact on process performance and product quality. In the concept of QbD, Critical process parameters (CPPs) are used throughout the entire drug product life cycle, from development, scale-up to validation, involving each process operation. ICH Q8 document also mentions that "CPPs need to be identified and controlled to achieve the desired quality attribute objectives of the product", so CPPs are also commonly recommended for the development and production process and quality control assurance. Real-time online parameter monitoring and control, and analysis of the results can better optimize and control the culture process. As shown in Figure 1, Sieman M-online can monitor a variety of CPPs, and multiple analysis results can be obtained online and in real time through LIS software. The test results of M-online can be fed back to the feeding control module to control the related CPPs. With the help of Sieman's new technology of online analysis and control, we can support customers to understand the changes of biological culture process more deeply, and finally obtain stable and efficient biological culture control process.

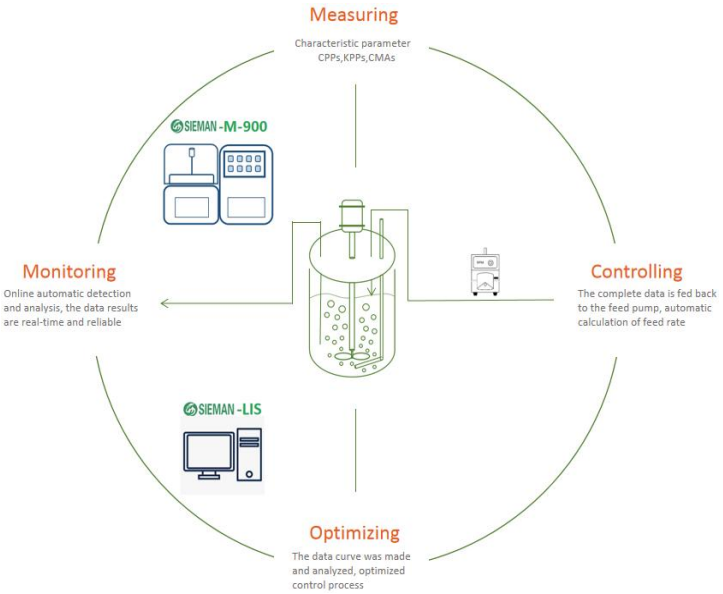

Figure 1: Sieman M-online analyzer + analysis software LIS+ sampling and feeding module, accurate and efficient process monitoring and control design. In this cycle, the characterization of CPPs, kPPs, and CMAs is effectively monitored, and these parameters can be analyzed, optimized, and controlled.

The commonly used reference method for CQA analysis is HPLC method or enzyme reaction analysis method, and enzyme analysis method includes immobilized enzyme membrane electrode method, liquid enzyme reaction optical method, etc. These methods are generally off-line analysis, and there are few online methods to monitor and control the biological culture process in real time. In order to control the culture process more conveniently and accurately, Sieman Technology has launched the M-online biochemical analysis system for biological process based on the principle of immobilized enzyme membrane electrode method as the gold standard, as shown in Figure 1, combined with Sieman LIS (Laboratory Information system software), It can perform instrument status view, sample test, data view, data statistical analysis, data curve drawing, sample test and other operations. Combined with the sampling and feeding module, it includes the feeding pump system of automatic sampling device and data transmission control. An online sampling and analysis control solution is provided to the user. The following test study of the combined use of M-online, Sieman LIS, and the sampling feeding module is presented as an example of the CHO-K1 culture.

Equipment of the system

The modules of Sieman M-online+LIS online sampling analysis, quantitative sampling and feeding system are detailed as follows:

1.M-online analysis control module: it can use dilution mode to determine biochemical and ion parameters of samples; The sampling volume, sampling frequency, final concentration of feeding control and feeding pump communication information of the corresponding channel can be set.

2. Sampling and quantitative module (the module will be integrated in AP-100 later) :

Including multi-channel automatic sampler + quantitative module,

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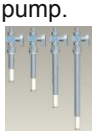
Sampling probe rod + filter ceramic membrane, linkage control of the feed pump.



3.Siemantec LIS: view data, automatically draw curves to analyze the biological culture process.

Closed loop control system, LIS software support online viewing and analysis

Samples filtered by ceramic membrane, examined online.

Automatic sampling, according to Settings.

Automatic feeding, according to setting calculation.

Figure 2: The required infrastructure for the laboratory consists of two main things: the laboratory hardware and the software for the control system. The applied hardware includes the bioreactor system (including pump, balance, etc.), M-online is equipped with multiple channels, which can support online quantitative sampling and test control of multiple bioreactors, and can take dilution function for different samples. The filtration test results of sampling probe and nano-ceramic membrane are also basically consistent with the centrifugal test results. The path and control direction of the working process data and culture samples are identified by blue lines and blue arrows in the figure. At the software level, only LIS is needed, which can master the communication between devices and control actions, and analyze the detection results.

The instrument automatically collected samples aseptically from the biological culture tank and imported them into the test area. The substance to be tested contained in the sample generates an electrical signal at a specific electrode. The instrument calculates the concentration of the substance by collecting the electrical signal on the electrode. Before sample measurement, the instrument calibrates a standard of known concentration, and the voltage value of the standard is a measure of the concentration of the substance to be measured. The unknown concentration can be obtained by comparison with the electrical signal of the standard. After each measurement, the instrument automatically cleaned each electrode, after which the next test could be carried out.

Due to the feeding module, after the concentration of the specified parameters is measured, the feeding module automatically and accurately performs the feeding according to the concentration value set by the user. Ensure that the concentration of the specified parameters is continuously maintained at the set value, thus achieving a fully automatic closed-loop control.

Study case - Objectives

To demonstrate the monitoring of Glucose, Lactate, Glutamate and Ammonium ions by M-online in CHO-K1 cell culture process and the application of glucose online sampling data for feeding control.

Material and Method

Test Setup

The setup consists of a 3L bioreactor (SciVario-twin-DR03, effpendorf), a sampling probe rod with a ceramic membrane (nano-ceramic membrane is continuously sonicated with pure water twice for 0.5h each time) is inserted into the reactor's preset hole, installed, sterilized and cooled, connected to the quantitative module and the automatic sampler, and is connected to the automatic sampler. Then connect to the Sieman M-online bioprocess biochemistry analyzer. The reactor itself is equipped with pH and DO probes, temperature electrodes, stirring device, liquid level electrode, water cooler (exhaust gas cooler), heating blanket, and feed pump and provides air, O2, and CO2 to control dissolved O2 and CO2.
Cells counted using --- Countstar-IC1000 cell counter.
Sterile operations --- SW-CJ-1FD ultra-clean table.

CHO-K1 culture parameter setting and process

In this study, the application of M-online monitoring was demonstrated by using the CHO-K1 Fed-Batch process (adding Feed, Gluc and antifoam C emulsion). pO2 was controlled at 40%, pH at 7.10±0.1 and temp at 37℃, stir according to P/V= 5-10, initial volume V=2.1L, and cascade control by effpendorf software. Automated sampling and analysis was performed every 1 hour (mid-early stage) or every 4 hours (mid-late stage) using M-online.

CHO-K1 cells were GS systems without the addition of glutamine. pH control was controlled using a 7.5% sodium bicarbonate solution to automatically adjust alkalinity. The basal medium was OPM VEGA medium, Hyclone Cell boost 7a and 7b were used as feeds. The cells were cultured for 9 days. 1% 7a and 0.1% 7b of the culture volume were added on Day 2, and 2% 7a and 0.2% 7b of the culture volume were added on Day 3-8, added separately at one time. The initial seeding density was 2.5×10^5cells/mL. Glucose concentration was controlled at 4g/L, and M-online set sampling and glucose supplement controls as follows:

Cycle of monitoring	Monitoring parameters	Sampling channel	Sampling interval	Sampling volume	Duration of sampling
Day2~4	Gluc, Lac, Glu, NH4+	Channel 1	60min	5000uL	150s
Cycle of monitoring	**Monitoring parameters**	**Sampling channel**	**Sampling interval**	**Sampling volume**	**Duration of sampling**
Day5~9	Gluc, Lac, Glu	Channel 1	240min	5000uL	250s

Data use	Volume in tank	Control concentration	Added Feed concentration	Pump tube specification	Feed pump address
Channel 1	2.1L	4g/L	130g/L	16# , clockwise	01

The culture process was counted and centrifuged daily, and the centrifuged samples were tested and compared with the online data.
Note: Cell boost 7a contains glucose, so the Gluc concentration will rise after each stream.

Result

Glucose, lactate, glutamate, and ammonium were analyzed by M-online during the CHO adding feeding phase.
If the analyte exceeded the test range, it was automatically diluted by M-online, which was important for both glucose at the beginning of the fed-Batch and lactate later in the fed-Batch. If the analyte is within the test range, then no dilution function is required and the data can be used for control.
During the fed-Batch, the trend of the above analytes was monitored by automatic sampling and analysis every 1 hour or every 4 hours according to the Settings. In addition, manual samples were drawn every 24 hours as a reference. The results of the two measurements were in good agreement, demonstrating the reliability of the automated quantitative sampling system (Figure 3).
In different batches, the number of channels selected will affect the minimum sampling interval. The minimum sampling interval is 15 minutes for one channel, 20 minutes for two channels, 30 minutes for three channels, and 40 minutes for four channels.
During the whole process of automatic culture sampling, there was no bacterial infection, and under the high-frequency sampling (Figure. 5) condition of well-growing cells (Figure. 4), the sampling probe rod could still take out the samples for analysis normally (the higher cell density in the later stage could prolong the sampling time to ensure the sample removal).

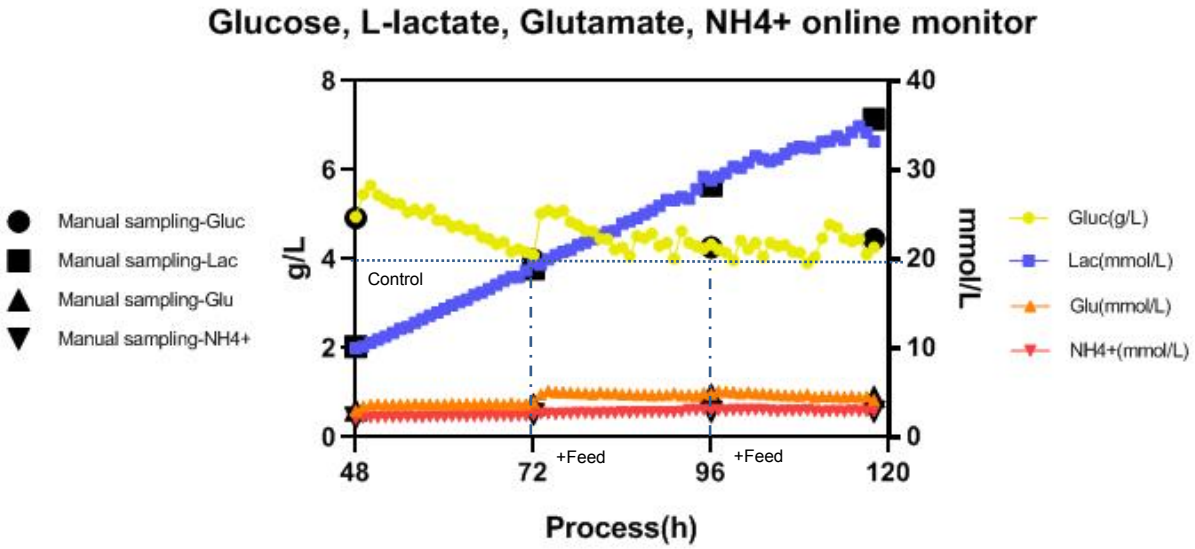


Figure 3: Results are shown for monitoring glucose (yellow), lactate (blue), glutamate (orange), and ammonium ions (red) during CHO Fed-Batch culture. Online data corresponding to manually drawn samples are shown in black. As shown in the glucose control 4g/L, the error of automatic addition of glucose is within positive 10% and will not be lower than 4g/L. The results of manual sampling and automatic sampling have good consistency. A slight increase in the glucose concentration after each addition of a glucose-containing Feed 7a is normal.

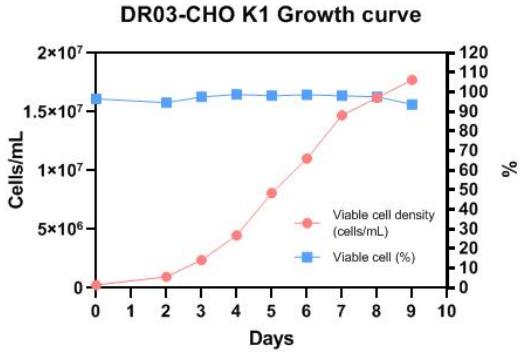


Figure 4: At Day7 (168h) to Day9 (216h), cell density was shown in the figure with VCD≥1.5×10^7cells/mL.

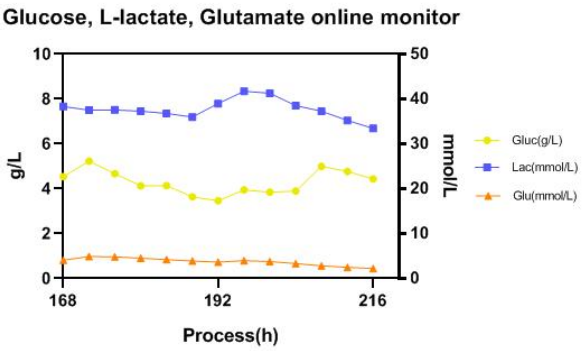


Figure 5: Sampling every 4 h from 168h to 216h can still be normal automatic sampling (the shorter the sampling interval, the more accurate the control).

Conclusion

Through the on-line sampling processing of nano-ceramic membrane, sampling probe pole, multi-channel automatic sampler and quantitative module, M-online and LIS software, the connection of bioreactor with biosensing analyzer and feeding pump system was successfully established. The Fed -Batch culture process of CHO-K1 was demonstrated to prove that the M-online can be used as an effective tool for substrate, metabolite, and fed -batch control.

In the demonstrated application, the sampling and analysis interval used is 60 minutes. The current minimum sampling interval for a single channel is 15 minutes. The more channels, the longer the time, and the multi-channel sampling interval can be further shortened with the introduction of AP-100 automated sampling dedicated analyzers that integrate individual components.

In addition, the precision calculation of M-online system software makes it possible to control the target concentration of specific analytes. When the sampling time is short enough, the control concentration error can be controlled within 3%, and even if the automatic sampling time is 4 hours, the control concentration error can be controlled within 10%. In the future, the control will be more refined through algorithm optimization.

In summary, the introduction of the M-online biosensing analyzer system greatly facilitated the process control and metabolic analysis, making the culture process more smoothly. Therefore, the M-online system, which combines online sampling, analysis and feeding, is the preferred experimental instrument for users of microbial or cell culture.

Key Results

- Substrates, metabolites, and ions were successfully monitored.
- Optional up to 4 channels of online sampling analysis, minimum 15 minutes of single channel high sampling frequency (including sampling, analysis and LIS recording, calculation---M-online analysis results feed back to the feeding pump).
- Glucose concentration can be controlled by the combination and interaction of the M-online system and the linked feeding pump.
- M-online Sieman ® online analysis results can be consistent with the off-line sampling centrifugal analysis results.
- M-online Sieman ® quantitative module with on-off control function, the sampling process is free of bacterial phenomenon.
- M-online Sieman ® uses a good nano-ceramic membrane to perform stable and continuous automatic sampling of high-density cells during culture.

References

[1] Guideline IHT (2011). "Development and manufacture of drug substances (chemical entities and biotechnological/biological entities) Q11", London: European medicines agency
[2] Kroll, P. et al (2017), "Model- Based Methods in the Biopharmaceutical Process Lifecycle", Pharmaceutical Research, 34 (12), 2596 – 2613

Contact information

For more information about our products and services, please visit our CN website:

www.siemantec.com

Our English website is under development.

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