Online test application
#2023-07-31

AP-100 Automated online analyzer
Application of glucose monitoring and control

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Abstract

Key process parameters (e.g., glucose, glutamine, lactate, etc.) must be monitored and controlled in each biological process in order 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 control, there is still a lack of faster and more accurate ways to make the results controlled by analysts more suitable for the needs of the actual fermentation culture process. This paper demonstrates the monitoring and control of fermentation medium parameters by AP-100 through on-line sampling experiment of the reactor. AP-100 integrates sampling module and software and hardware upgrade, and can integrate Sieman analysis parameter information collection management system LIS, which meets the requirements of 21 CFR PART 11 and cGMP. In this paper, the metabolic analysis and control experiment of CHO-K1 culture process was used to demonstrate the detection and control of glucose concentration.

Introduce

In some biological pharmaceutical companies, biotechnology and other companies, the purpose of using DoE for biological process development and process characterization research is to obtain a stable and efficient process to produce high-quality, high-yield products. In order to achieve this, the relevant specification guidelines also give concepts such as key process parameters (CPPs), critical process parameters (kPPs) or critical material properties (CMAs), which have a significant impact on process performance and product quality. In the QbD concept, Critical process parameters (CPPs) run through the entire product life cycle, from development, scale-up to validation, involving every process operation. ICH Q8 document also mentioned that

"CPPs need to be identified and controlled to make the product achieve the required quality attribute target", so CPPs is usually recommended for research and development production and quality control assurance. Real-time on-line parameter monitoring and control, and analysis of the results, can better optimize and control the cultivation process. As shown in Figure (Figure 1), Sieman AP-100 can monitor a variety of CPPs, and multiple analysis results can be obtained online and in real time through LIS software. The AP-100 test results can be fed back to the feeding control module to control the relevant CPPs. With the technology upgrade of Sieman online analysis control, customers can support a deeper understanding of the biological culture process changes, and finally obtain a stable and efficient biological culture control process.

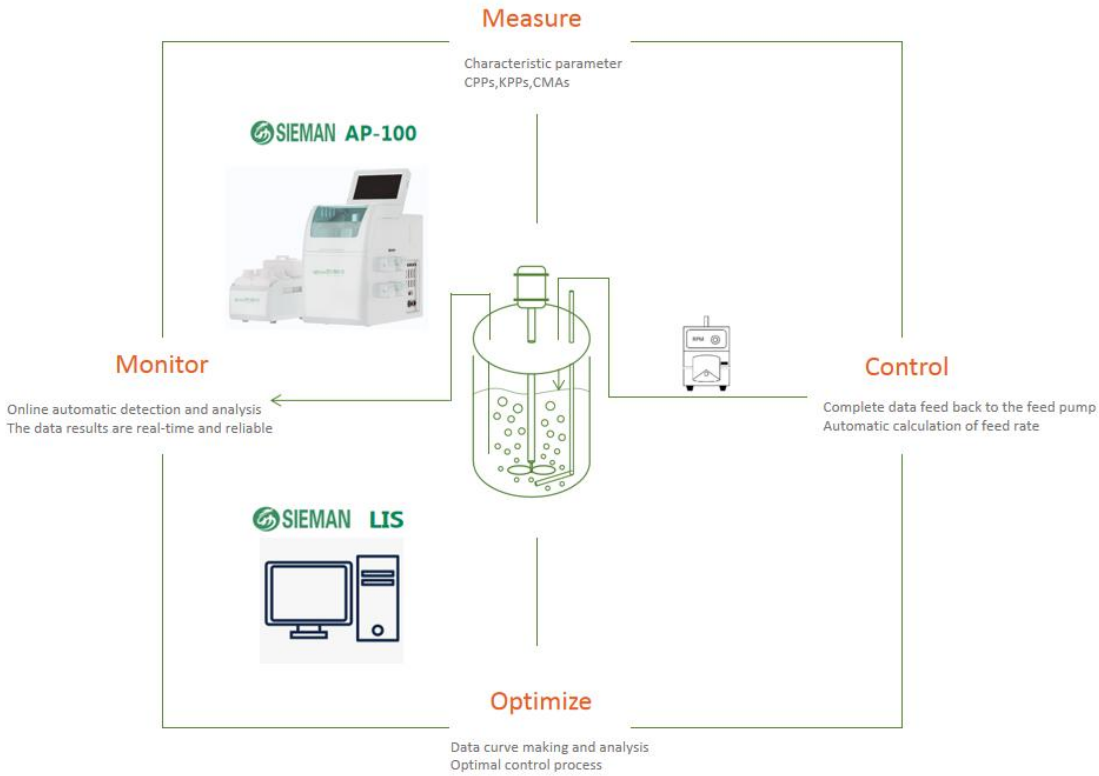


Figure 1: Sieman AP-100 online analyzer + analysis software LIS+ sampling and feeding module for accurate, efficient and stable process monitoring and control design. In this loop, the characterization of CPPs, kPPs, and CMAs can be effectively monitored, and these parameters can be analyzed, optimized, and controlled.

The most commonly used reference methods for CQA analysis are HPLC method or enzyme reaction analysis method, which includes immobilized enzyme membrane electrode method, liquid enzyme reaction optical method, etc. These methods are generally conducted off-line analysis, and there are few online methods for real-time monitoring, analysis and control of biological culture process. In order to more easily and accurately control the culture process, Sieman Technology has introduced a fully upgraded

biochemistry analysis system---AP-100, 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 view instrument status, sample test, data view, data statistical analysis, data curve drawing, sample testing and other operations; The combination of sampling module and data transmission control of the feed pump system module provides users with an online sampling analysis accurate control solution. The following simulation of CHO-K1 culture is taken as an example to introduce the glucose concentration test study of AP-100 combined with sampling module and feeding module.

System matching

Sieman AP-100+LIS online sampling analysis, quantitative sampling module, feeding system module is detailed as follows:

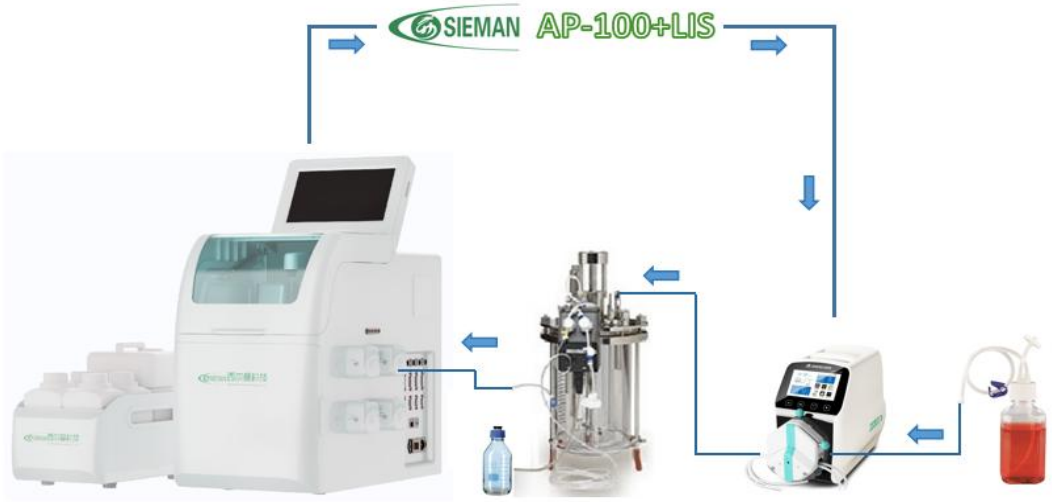
1. AP-100 analysis control module: It can use a variety of modes (original solution, dilution, intelligent, fast, accurate) to determine the biochemical and ion parameters of the sample; The sampling volume, sampling frequency, feeding control final concentration and feeding pump communication information of the corresponding channel can be set. Up to 4 bioreactors can be analyzed and controlled simultaneously.

2. Quantitative, feeding module; sampling rod + filter ceramic membrane, linkage control of the feed pump.



3.Sieman LIS system: View data and logs, automatically draw curves to analyze the biological culture process.

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



The samples were tested online Automatic sampling Automatically calculate and add feed

Figure 2: The infrastructure required for the laboratory consists of two main levels: the laboratory hardware and the software for the control system. The application hardware includes the bioreactor system (including pumps, weighing, etc.), the AP-100 itself is equipped with a multi-channel sampling module, which can support the online quantitative sampling test control of multiple bioreactors, and can decide whether to adopt dilution function according to different sample concentrations. The filtration test results of the sampling probe without membrane and with ceramic membrane are basically consistent with the centrifugal test results. The working process data and the path and control direction of the culture sample are marked by blue lines and blue arrows. At the software level, LIS can be used to master the data communication and control actions between devices, and analyze the detection results.

The instrument automatically collects samples aseptically from the bioreactor and pumps them to the test area. The substance contained in the sample to be measured produces an electrical signal at a specific electrode. The instrument calculates the concentration of the object to be measured by collecting the electrical signal on the electrode. Before sample testing, the instrument calibrates the standard of known concentration, and the voltage value of the standard is the scale to measure the concentration of the substance to be measured. The unknown concentration can be compared with the electrical signal of the standard. After each measurement, the instrument will automatically clean each electrode, and the next test can be carried out after the cleaning is completed.
Because of the feeding module, after measuring the concentration of the specified parameter, the feeding module automatically and accurately executes the feeding according to the user-set concentration value. Ensure that the concentration of the specified parameter is continuously maintained at the set value, thus achieving a fully automatic closed-loop control.

Study case -- Purpose

To demonstrate the glucose monitoring of AP-100 during CHO-K1 cell culture, the glucose concentration in the reactor was controlled by using the glucose online sampling data.

Materials and methods

Test setup

The setup consists of a 3L bioreactor (SciVario-twin-DR03, effpendorf), where the sampling probe rods with and without ceramic membrane are inserted into the reserved holes of the reactor (the length of the sampling hose with ceramic membrane is 1 m, and the length of the sampling hose without ceramic membrane is 5 m), sterilized and cooled. Connect to the dosing module, connect to the Sieman AP-100 on-line biochemistry analyzer, and connect the 200g/L glucose bottle to the feed pump module. The reactor is equipped with a pH probe, temperature electrode, stirring device, level electrode, cooling machine (tailpipe cooling condenser), heating blanket and pump. The dissolved oxygen electrode uses Sieman's self-developed optical DO electrode to provide compressed air,

O2 and CO2 to control dissolved oxygen and pH. Cell counts were performed using the Countstar-IC1000 cell counter, and aseptic procedures were performed on the SW-CJ-1FD ultra-clean table.

CHO-K1 culture parameter setting and process

This study demonstrated the application of AP-100 by using the CHO-K1 Fed-Batch process (adding Feed, Gluc, and silicon-based defoamer C emulsion). pO2 is controlled at 40%, pH value and temperature are controlled at 7.10±0.1 and 37℃ respectively, stirring is based on P/V=5~10, initial volume V=1.9L, and cascading control is used by effpendorf software by default. The AP-100 is used for automatic sampling and analysis every 4 hours (early middle stage) or every 2 hours (late stage).

CHO-K1 cells are GS system and glutamine is not required. pH control automatically adjusts alkalinity using 7.5% sodium bicarbonate solution. The basic medium was OPM Vega medium, and the supplement was Hyclone Cell boost 7a and 7b, which were cultured for 9 days. 7a of 1% culture volume and 7b of 0.1% culture volume were added on Day 2, and 7a of 2% culture volume and 7b of 0.2% culture volume were added on Day 3-8, all of which were added separately at one time. The initial inoculation density was 2.5×10^5cells/mL. Glucose concentration is controlled at 4g/L, AP-100 set sampling and glucose supplement control as follows:

Monitoring periods	Parameter	Sampling channel	Sampling interval	Sampling level	Liquid detection time	Sampling mode
Day2~9	Gluc	Channel 1	240→120 min	5	/	No photosensitive liquid detection

Monitoring periods	Parameter	Sampling channel	Sampling interval	Sampling level	Liquid detection time	Sampling mode
Day2~9	Gluc	Channel 2	240→120 min	5	60s→250s	Photosensitive liquid detection

Day7 changes the sampling interval from 240min to 120min, among them: Channel 1 is a sampling probe without ceramic membrane, the length of the sampling tube is 5 meters, and the sampling mode is No-liquid detection mode. Channel 2 is a sampling probe with selected ceramic membrane, the length of the sampling tube is 1 meter, the sampling method is photosensitive liquid detection, and the liquid detection time is 60s. Due to the high cell density in the late stage of culture, it is difficult to sample with ceramic membrane, so the liquid detection time is set to 250s in the late stage.

Data adoption	Tank volume	Control concentration	Feed concentration	Pump tube specification	Feed pump address
Channel 2	1.9L	4g/L	200g/L	16#，clockwise	03

Data collected using channel 2. During the culture process, off-line sampling and counting every day, centrifugal samples were taken for testing and compared with online data.

Note: Cell boost 7a contains glucose, so the glucose concentration will increase after each infusion.

Result

In the Fed-batch process of CHO culture, AP-100 was used to analyze glucose concentration, and the operation parameters of the reactor during the culture process were shown as the figure (Fig. 3). According to the Settings of Day2~7, J1 and J2 were automatically sampled and analyzed every 4 hours, and then the offline samples were taken as the control. The two measurement results showed good consistency. The changes and stability control of glucose during the process proved the reliability of online quantitative sampling measurement (Fig. 4 and Fig. 5).

The whole culture automatic sampling process was free of bacterial contamination, and the cells grew well (Fig. 6). Under the condition of high-frequency sampling (Fig. 7) at an interval of 2 hours from Day7 to 9, The 1 meter sampling tube with ceramic membrane sampling rod and the 5 meter sampling tube without ceramic membrane sampling rod can still normally take out samples for analysis (the increase of cell density in the later period can increase the sampling liquid detection time to ensure sample extraction).

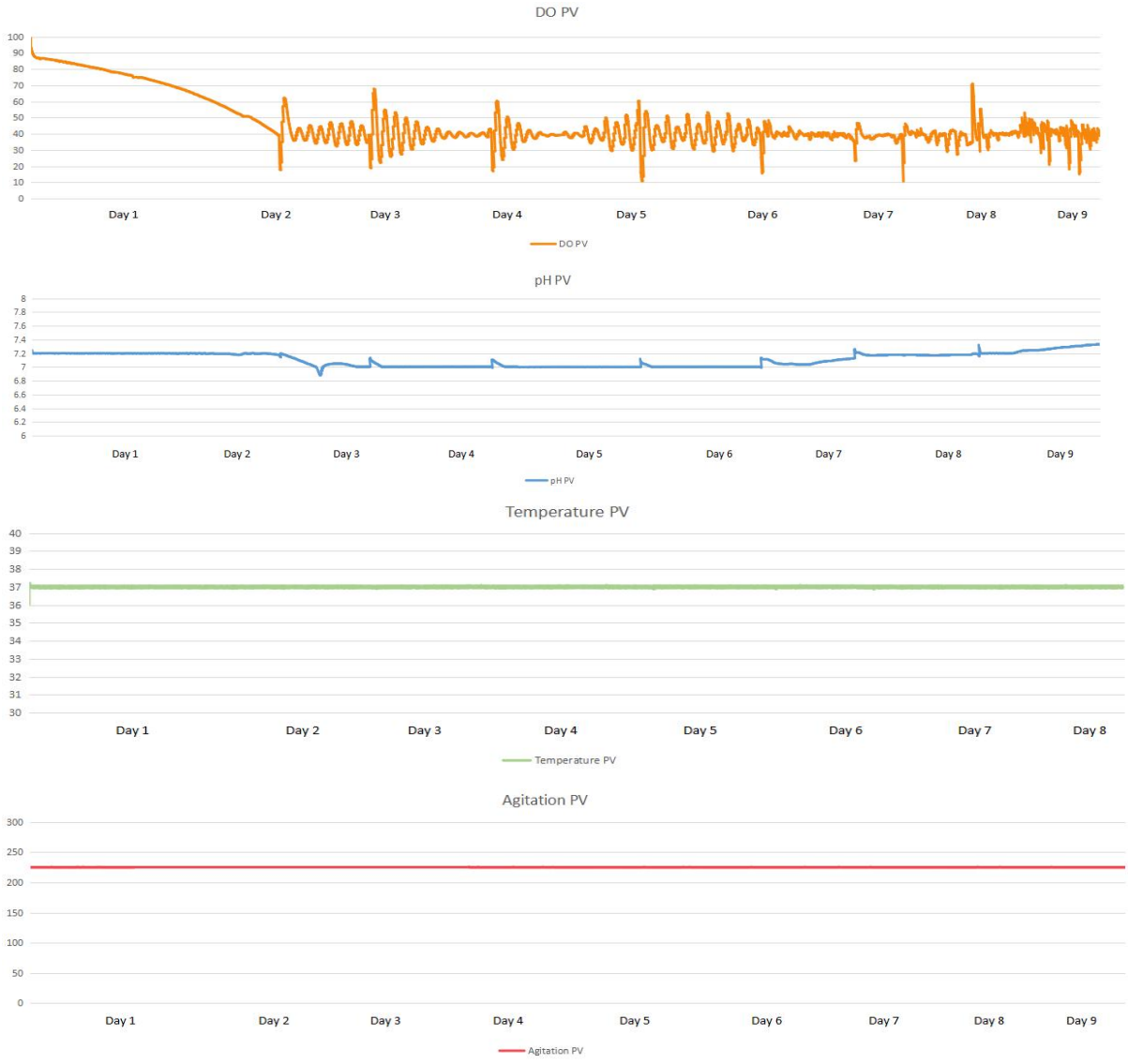


Figure 3: Shows the operation results of pH, DO, Temp and Stir monitored during CHO culture, all of which can be controlled normally.

AP-100 can accurately control Gluc concentration and show Gluc increase when Cellboost 7a is added

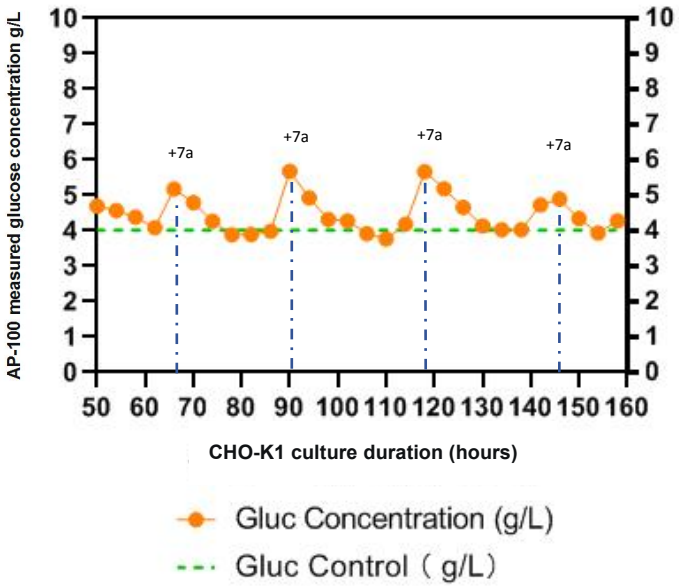


Figure 4: Shows the results of monitoring glucose (orange) in CHO-K1 during the Fed-Batch process. Since in addition to adding glucose, Cell boost 7a and 7b will be added once a day, and 7a contains glucose, so the glucose concentration will increase after each addition of 7a. As shown in the figure, glucose control 4g/L, automatic glucose addition error is less than 5%, and the control accuracy and stability are very high, which is also related to the comparison rule of CHO-K1 glucose consumption.

Date & Time	Cup No.	Sample No.	User	Gluc	NH4+	K+	Na+	Ca++
2023/8/2 18:44	J2	J2-20230802184404	sieman	4.69g/L	0.05g/L	0.25g/L	2.18g/L	0.01g/L
2023/8/2 18:47	J1	J1-20230802184741	sieman	4.83g/L	0.05g/L	0.25g/L	2.17g/L	0.01g/L
2023/8/2 22:44	J2	J2-20230802224411	sieman	4.56g/L	0.05g/L	0.25g/L	2.15g/L	0.01g/L
2023/8/2 22:47	J1	J1-20230802224743	sieman	4.56g/L	0.05g/L	0.25g/L	2.15g/L	0.01g/L
2023/8/3 2:44	J2	J2-20230803024412	sieman	4.38g/L	0.05g/L	0.25g/L	2.22g/L	0.01g/L
2023/8/3 2:47	J1	J1-20230803024744	sieman	4.40g/L	0.05g/L	0.24g/L	2.22g/L	0.01g/L
2023/8/3 6:45	J2	J2-20230803064521	sieman	3.69g/L	0.05g/L	0.24g/L	2.25g/L	0.01g/L
2023/8/3 6:48	J1	J1-20230803064851	sieman	3.44g/L	0.05g/L	0.24g/L	2.24g/L	0.01g/L
2023/8/3 8:01	J2	J2-20230803080151	sieman	4.08g/L	0.05g/L	0.25g/L	2.26g/L	0.01g/L
2023/8/3 8:05	J1	J1-20230803080528	sieman	4.00g/L	0.05g/L	0.24g/L	2.26g/L	0.01g/L
2023/8/3 11:20	J2	J2-20230803112053	sieman	5.17g/L	0.06g/L	0.28g/L	2.35g/L	0.01g/L
2023/8/3 11:24	J1	J1-20230803112430	sieman	5.29g/L	0.06g/L	0.28g/L	2.33g/L	0.01g/L
2023/8/3 15:20	J2	J2-20230803152059	sieman	4.79g/L	0.06g/L	0.27g/L	2.36g/L	0.01g/L
2023/8/3 15:24	J1	J1-20230803152431	sieman	4.78g/L	0.06g/L	0.26g/L	2.35g/L	0.01g/L

2023/8/3 20:39	J2	J2-20230803203931	sieman	4.26g/L	0.06g/L	0.27g/L	2.43g/L	0.01g/L
2023/8/3 20:43	J1	J1-20230803204303	sieman	4.23g/L	0.06g/L	0.26g/L	2.38g/L	0.01g/L
2023/8/4 0:39	J2	J2-20230804003931	sieman	3.88g/L	0.06g/L	0.25g/L	2.47g/L	0.01g/L
2023/8/4 0:43	J1	J1-20230804004306	sieman	4.03g/L	0.06g/L	0.25g/L	2.44g/L	0.01g/L
2023/8/4 4:39	J2	J2-20230804043933	sieman	3.89g/L	0.06g/L	0.25g/L	2.50g/L	0.01g/L
2023/8/4 4:43	J1	J1-20230804044303	sieman	3.82g/L	0.06g/L	0.24g/L	2.47g/L	0.01g/L
2023/8/4 8:39	J2	J2-20230804083929	sieman	3.98g/L	0.06g/L	0.24g/L	2.54g/L	0.01g/L
2023/8/4 8:43	J1	J1-20230804084305	sieman	4.18g/L	0.06g/L	0.24g/L	2.52g/L	0.01g/L
2023/8/4 10:18	S0	Off-line sample test	sieman	4.36g/L	0.06g/L	0.24g/L	2.54g/L	0.01g/L
2023/8/4 12:39	J2	J2-20230804123930	sieman	5.67g/L	0.07g/L	0.27g/L	2.62g/L	0.01g/L
2023/8/4 12:43	J1	J1-20230804124307	sieman	5.54g/L	0.07g/L	0.27g/L	2.59g/L	0.01g/L
2023/8/4 16:39	J2	J2-20230804163936	sieman	4.92g/L	0.07g/L	0.27g/L	2.64g/L	0.01g/L
2023/8/4 16:43	J1	J1-20230804164308	sieman	4.98g/L	0.07g/L	0.27g/L	2.61g/L	0.01g/L
2023/8/4 20:39	J2	J2-20230804203930	LIS-User	4.31g/L	0.07g/L	0.26g/L	2.67g/L	0.01g/L
2023/8/4 20:43	J1	J1-20230804204306	LIS-User	4.44g/L	0.07g/L	0.25g/L	2.63g/L	0.01g/L
2023/8/5 0:39	J2	J2-20230805003934	LIS-User	4.27g/L	0.07g/L	0.25g/L	2.69g/L	0.01g/L
2023/8/5 0:43	J1	J1-20230805004310	LIS-User	4.23g/L	0.07g/L	0.25g/L	2.66g/L	0.01g/L
2023/8/5 4:39	J2	J2-20230805043937	LIS-User	3.91g/L	0.07g/L	0.24g/L	2.72g/L	0.01g/L
2023/8/5 4:43	J1	J1-20230805044312	LIS-User	3.73g/L	0.07g/L	0.24g/L	2.69g/L	0.01g/L
2023/8/5 8:39	J2	J2-20230805083933	LIS-User	3.76g/L	0.07g/L	0.25g/L	2.79g/L	0.01g/L
2023/8/5 8:43	J1	J1-20230805084308	LIS-User	3.87g/L	0.07g/L	0.25g/L	2.76g/L	0.01g/L
2023/8/5 12:39	J2	J2-20230805123934	LIS-User	4.18g/L	0.07g/L	0.23g/L	2.79g/L	0.01g/L
2023/8/5 12:43	J1	J1-20230805124310	LIS-User	4.25g/L	0.07g/L	0.23g/L	2.74g/L	0.01g/L
2023/8/5 15:26	S0	Off-line sample test	sieman	4.23g/L	0.07g/L	0.23g/L	2.74g/L	0.01g/L
2023/8/5 16:39	J2	J2-20230805163940	sieman	5.66g/L	0.07g/L	0.26g/L	2.80g/L	0.01g/L
2023/8/5 16:43	J1	J1-20230805164313	sieman	5.49g/L	0.07g/L	0.26g/L	2.76g/L	0.01g/L
2023/8/5 20:39	J2	J2-20230805203941	sieman	5.18g/L	0.07g/L	0.25g/L	2.79g/L	0.01g/L
2023/8/5 20:43	J1	J1-20230805204314	sieman	5.01g/L	0.07g/L	0.25g/L	2.77g/L	0.01g/L
2023/8/6 0:39	J2	J2-20230806003942	sieman	4.65g/L	0.07g/L	0.25g/L	2.83g/L	0.01g/L
2023/8/6 0:43	J1	J1-20230806004314	sieman	4.54g/L	0.07g/L	0.25g/L	2.79g/L	0.01g/L
2023/8/6 4:39	J2	J2-20230806043948	sieman	4.13g/L	0.07g/L	0.25g/L	2.84g/L	0.01g/L
2023/8/6 4:43	J1	J1-20230806044318	sieman	4.20g/L	0.07g/L	0.24g/L	2.81g/L	0.01g/L
2023/8/6 8:39	J2	J2-20230806083949	sieman	4.02g/L	0.07g/L	0.25g/L	2.89g/L	0.01g/L
2023/8/6 8:43	J1	J1-20230806084314	sieman	4.10g/L	0.07g/L	0.25g/L	2.86g/L	0.01g/L
2023/8/6 12:39	J2	J2-20230806123950	sieman	4.02g/L	0.06g/L	0.24g/L	2.87g/L	0.01g/L
2023/8/6 12:43	J1	J1-20230806124317	sieman	3.92g/L	0.06g/L	0.23g/L	2.82g/L	0.01g/L
2023/8/6 15:06	S0	Off-line sample test	sieman	4.35g/L	0.06g/L	0.23g/L	2.83g/L	0.01g/L
2023/8/6 16:39	J2	J2-20230806163956	sieman	4.72g/L	0.06g/L	0.27g/L	2.86g/L	0.01g/L
2023/8/6 16:43	J1	J1-20230806164319	sieman	5.13g/L	0.06g/L	0.26g/L	2.83g/L	0.01g/L
2023/8/6 20:39	J2	J2-20230806203957	sieman	4.89g/L	0.06g/L	0.26g/L	2.88g/L	0.01g/L
2023/8/6 20:43	J1	J1-20230806204319	sieman	4.87g/L	0.06g/L	0.25g/L	2.84g/L	0.01g/L
2023/8/7 0:39	J2	J2-20230807003958	sieman	4.34g/L	0.06g/L	0.26g/L	2.89g/L	0.01g/L
2023/8/7 0:43	J1	J1-20230807004319	sieman	4.27g/L	0.06g/L	0.25g/L	2.85g/L	0.01g/L

2023/8/7 4:39	J2	J2-20230807043959	sieman	3.93g/L	0.06g/L	0.25g/L	2.86g/L	0.01g/L
2023/8/7 4:43	J1	J1-20230807044319	sieman	3.87g/L	0.06g/L	0.25g/L	2.84g/L	0.01g/L
2023/8/7 8:40	J2	J2-20230807084005	sieman	4.27g/L	0.06g/L	0.25g/L	2.91g/L	0.01g/L
2023/8/7 8:43	J1	J1-20230807084321	sieman	4.12g/L	0.05g/L	0.24g/L	2.89g/L	0.01g/L
2023/8/7 9:27	S0	Off-line sample test	sieman	4.04g/L	0.05g/L	0.23g/L	2.91g/L	0.01g/L
2023/8/7 10:40	J2	J2-20230807104005	sieman	5.64g/L	0.05g/L	0.29g/L	2.95g/L	0.01g/L
2023/8/7 10:43	J1	J1-20230807104317	sieman	5.50g/L	0.05g/L	0.28g/L	2.92g/L	0.01g/L
2023/8/7 12:40	J2	J2-20230807124016	sieman	5.17g/L	0.06g/L	0.29g/L	2.94g/L	0.01g/L
2023/8/7 12:43	J1	J1-20230807124318	sieman	5.03g/L	0.05g/L	0.28g/L	2.92g/L	0.01g/L
2023/8/7 14:40	J2	J2-20230807144037	sieman	4.84g/L	0.06g/L	0.29g/L	2.94g/L	0.01g/L
2023/8/7 14:43	J1	J1-20230807144320	sieman	4.66g/L	0.06g/L	0.27g/L	2.92g/L	0.01g/L
2023/8/7 16:43	J1	J1-20230807164323	sieman	4.37g/L	0.06g/L	0.27g/L	2.93g/L	0.01g/L
2023/8/7 19:15	J2	Detect time60s→250s	LIS-User	4.26g/L	0.06g/L	0.27g/L	2.91g/L	0.01g/L
2023/8/7 21:30	J2	J2-20230807213020	sieman	3.57g/L	0.05g/L	0.27g/L	2.90g/L	0.01g/L
2023/8/7 21:32	J1	J1-20230807213201	sieman	3.24g/L	0.05g/L	0.26g/L	2.84g/L	0.01g/L
2023/8/7 23:30	J2	J2-20230807233036	sieman	3.87g/L	0.05g/L	0.26g/L	2.92g/L	0.01g/L
2023/8/7 23:32	J1	J1-20230807233217	sieman	3.62g/L	0.05g/L	0.26g/L	2.89g/L	0.01g/L
2023/8/8 1:30	J2	J2-20230808013051	sieman	4.30g/L	0.05g/L	0.26g/L	2.91g/L	0.01g/L
2023/8/8 1:32	J1	J1-20230808013232	sieman	4.23g/L	0.05g/L	0.26g/L	2.89g/L	0.01g/L
2023/8/8 3:30	J2	J2-20230808033057	sieman	3.98g/L	0.05g/L	0.26g/L	2.89g/L	0.01g/L
2023/8/8 3:32	J1	J1-20230808033238	sieman	3.57g/L	0.05g/L	0.26g/L	2.88g/L	0.01g/L
2023/8/8 5:31	J1	J1-20230808053118	sieman	5.13g/L	0.05g/L	0.26g/L	2.90g/L	0.01g/L
2023/8/8 5:32	J2	J2-20230808053259	sieman	4.33g/L	0.05g/L	0.25g/L	2.89g/L	0.01g/L
2023/8/8 7:31	J2	J2-20230808073104	sieman	4.84g/L	0.05g/L	0.26g/L	2.94g/L	0.01g/L
2023/8/8 7:32	J1	J1-20230808073245	sieman	4.22g/L	0.05g/L	0.25g/L	2.93g/L	0.01g/L
2023/8/8 9:32	J1	J1-20230808093207	sieman	4.85g/L	0.05g/L	0.26g/L	2.94g/L	0.01g/L
2023/8/8 9:40	J2	J2-20230808094034	sieman	4.98g/L	0.05g/L	0.26g/L	2.91g/L	0.01g/L
2023/8/8 11:31	J1	J1-20230808113121	sieman	5.59g/L	0.05g/L	0.29g/L	2.97g/L	0.01g/L
2023/8/8 11:33	J2	J2-20230808113302	sieman	5.47g/L	0.05g/L	0.28g/L	2.94g/L	0.01g/L
2023/8/8 13:31	J1	J1-20230808133122	sieman	5.68g/L	0.05g/L	0.28g/L	2.95g/L	0.01g/L
2023/8/8 13:33	J2	J2-20230808133303	sieman	5.27g/L	0.05g/L	0.27g/L	2.93g/L	0.01g/L
2023/8/8 15:31	J1	J1-20230808153123	sieman	5.45g/L	0.05g/L	0.28g/L	2.94g/L	0.01g/L
2023/8/8 15:44	J2	J2-20230808154434	sieman	5.20g/L	0.05g/L	0.30g/L	2.98g/L	0.01g/L
2023/8/8 17:31	J1	J1-20230808173124	sieman	5.05g/L	0.05g/L	0.28g/L	2.94g/L	0.01g/L
2023/8/8 17:33	J2	J2-20230808173305	sieman	4.54g/L	0.05g/L	0.28g/L	2.93g/L	0.01g/L
2023/8/8 19:31	J1	J1-20230808193124	sieman	4.41g/L	0.05g/L	0.28g/L	2.99g/L	0.01g/L
2023/8/8 19:33	J2	J2-20230808193305	sieman	4.26g/L	0.05g/L	0.28g/L	2.98g/L	0.01g/L
2023/8/8 21:31	J1	J1-20230808213125	sieman	4.18g/L	0.05g/L	0.29g/L	3.06g/L	0.01g/L
2023/8/8 21:33	J2	J2-20230808213306	sieman	4.08g/L	0.05g/L	0.28g/L	3.03g/L	0.01g/L
2023/8/8 23:31	J1	J1-20230808233126	sieman	4.25g/L	0.05g/L	0.29g/L	2.98g/L	0.01g/L
2023/8/8 23:33	J2	J2-20230808233307	sieman	4.02g/L	0.05g/L	0.28g/L	2.96g/L	0.01g/L
2023/8/9 1:31	J1	J1-20230809013127	sieman	3.86g/L	0.05g/L	0.28g/L	2.96g/L	0.01g/L
2023/8/9 1:33	J2	J2-20230809013308	sieman	3.79g/L	0.05g/L	0.28g/L	2.95g/L	0.01g/L

2023/8/9 3:31	J1	J1-20230809033128	sieman	3.92g/L	0.05g/L	0.28g/L	2.95g/L	0.01g/L
2023/8/9 3:33	J2	J2-20230809033309	sieman	3.81g/L	0.05g/L	0.28g/L	2.93g/L	0.01g/L
2023/8/9 5:31	J1	J1-20230809053129	sieman	4.45g/L	0.05g/L	0.28g/L	2.94g/L	0.01g/L
2023/8/9 5:33	J2	J2-20230809053310	sieman	4.14g/L	0.05g/L	0.28g/L	2.92g/L	0.01g/L
2023/8/9 7:31	J1	J1-20230809073129	sieman	4.12g/L	0.05g/L	0.29g/L	2.94g/L	0.01g/L
2023/8/9 7:33	J2	J2-20230809073310	sieman	4.06g/L	0.05g/L	0.29g/L	2.92g/L	0.01g/L
2023/8/9 9:31	J1	J1-20230809093130	sieman	3.94g/L	0.05g/L	0.28g/L	2.93g/L	0.01g/L
2023/8/9 9:33	J2	J2-20230809093315	sieman	4.27g/L	0.05g/L	0.27g/L	2.88g/L	0.01g/L

Fig. 5: Both the 5-meter sampling tube without ceramic membrane sampling rod（J1） and the 1-meter sampling tube with ceramic membrane sampling rod（J2） can take out samples normally, and there is basically no difference in test results. The results of manual sampling of centrifugal sample S0 are in good agreement with those of automatic sampling.

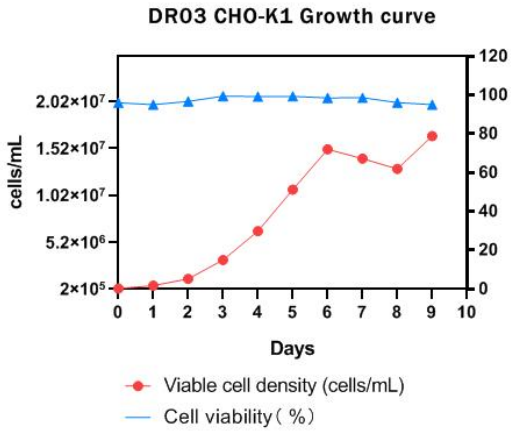


Figure 6: Cell density and viability from Day 1 to Day 9 were shown in the figure. The peak density of VCD was about 1.5×10^7cells/mL, and no bacterial contamination occurred during the process.

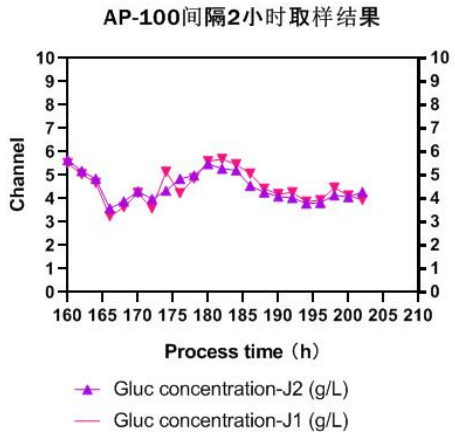


Figure 7: J1 and J2 channels can be sampled normally under the high-frequency sampling conditions of Day7~9 at an interval of 2 hours.

Conclusion

Through AP-100, nano-ceramic membrane, sampling probe and quantitative module online sampling and feeding processing, and LIS software management and control, the connection between bioreactor, biosensor analyzer and feeding pump system was successfully established. The process of CHO-K1 fed-batch culture was demonstrated, and the AP-100 analyzer was proved to be an effective tool for substrate, metabolite and feeding control.

In the demonstrated application, a sampling and analysis interval of 120 to 240 minutes is used, and the minimum sampling interval for a single channel is currently 15 minutes. In addition to the detection and control of glucose concentration, AP-100 can also be used to detect and control other parameters, including: lactate, sucrose, glutamate, glutamine, lysine, methanol, ethanol, glycerol, xylose, NH4+, K+, Na+, Ca++, etc.

In addition, through the precise calculation of the AP-100 system software, it is possible to control the target concentration of a specific analyte, and the concentration error can be controlled within 3% when the sampling time is short enough, even if the automatic sampling time is as long as 4h, the control concentration error can be controlled within 5% for the culture that is not consumed quickly.

In summary, the introduction of the AP-100 online biochemistry analyzer system greatly facilitates process control and metabolic flow analysis, making the culture process more smooth. Therefore, the AP-100 system, which combines online sampling with analysis and feeding, is the preferred laboratory instrument for microbial or cell culture users.

Key result

- Glucose and ion concentrations were successfully monitored.
- Up to 4 channels can be selected for online sampling analysis, with a minimum of 15 minutes of single channel high sampling frequency (including sampling, analysis and LIS recording, calculating the analysis results of the AP-100 to feed back to the feed pump).
- Glucose concentration can be controlled by the combination and interaction of the AP-100 system and the linked feed pump.
- AP-100 Sieman ® on-line analysis results are consistent with off-line sampling centrifuge analysis results.
- The AP-100 Sieman ® quantification module has an on-off control function, and the sampling process is free of bacterial contamination.
- AP-100 Sieman ® uses a good ceramic membrane for stable and automatic sampling of high density cells during culture.
- The AP-100 Sieman ® enables stable automatic sampling of high-density cell bioreactors at a distance of 5 meters using a sampling probe without ceramic membrane.

Reference

[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

AP-100 Online analyzer and its feed module: It is an online analyzer and its feed module under the Sieman registered brand. LIS software: Analyzer software under the Sieman registered brand. AP-100: For quality control/production process analysis/research experiments only.

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