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Evaluating Integrated Analytics for Single-Use Mini Bioreactors

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Introduction

In the past decade, single-use automated micro bioreactors have been widely adopted in biopharma facilities for scaling-down mammalian and microbial cell processes for production of biologics (1,2,3). This has been proven to significantly increase speed and throughput of cell line and process development (4) with results that are more reproducible than those taken using shake flasks as scale down models (5).

While the use of automated scale-down bioreactors increases throughput in clone/strain screening as well as testing of culture conditions and process parameters, it also leads to a rise in the number of samples that need to be taken and analyzed. Key assays typically run by bioprocess scientists include: off-line pH checks, viable cell density (VCD), viability, and metabolites including glucose, lactate, glutamine, and glutamate. These measurements can be used for process control and monitoring, to calculate feed additions and determine optimum time for harvest. Currently, Sartorius estimates that from the 300+ Ambr® 15 Cell Culture automated microscale bioreactor systems installed, four million samples are generated globally every day. Figure 1 highlights areas where manual operations have traditionally caused bottlenecks in the overall workflow when running these assays.

Overcoming these bottlenecks requires analytic devices such as pH modules, cell counters and metabolite readers which have fast cycle times, and the ability to run outside working hours, reacting to adjust events in the bioreactor, while allowing for automated data transfer and advanced control strategies which also consider sample volume.

This poster discusses the work to evaluate different integrated systems with automated single-use micro bioreactor cultures and includes a comparison of manual versus automated sampling as well as assessing different types of automated glucose control stategies using cell count and glucose measurements.

Figure 1: Workflow overview for key process control assays

Assay	Sampling	Sample Transfer	Assay	Data Transfer	Calculate	Adjust
Shake Flask						
Ambr® 15 pH						
Ambr® 15 VCD						
Ambr® 15 Glucose						
Ambr® 15 Metabolite						

Manual Automated

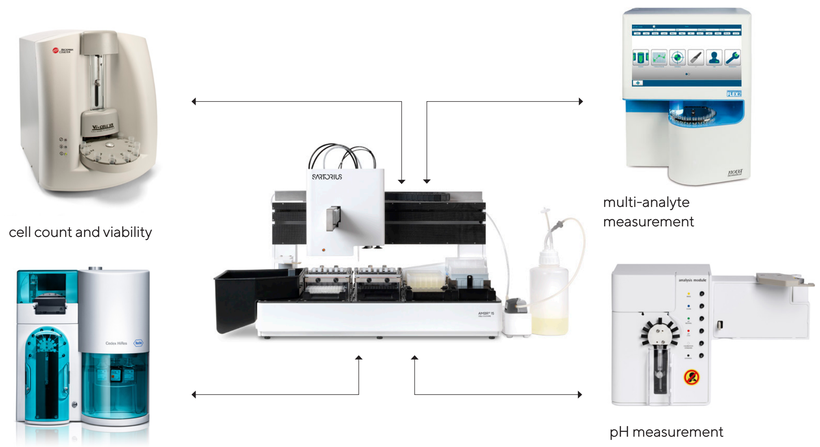
Integrated Analytics

The Ambr® 15 Cell Culture system can be used with a suite of integrated analytics (Figure 2) for automated sampling, analysis and data transfer to the Ambr® software. These include the Vi-CELL™ Cell Viability Analyzer (Beckman Coulter Life Sciences) and Cedex HiRes Analyzer (Roche CustomBiotech) for cell count and viability, the Ambr® Analysis Module (Sartorius) for pH and the BioProfile® FLEX2™ Automated Cell Culture Analyzer (Nova Biomedical) for multi-analyte measurement.

Integration of analyzers with Ambr® 15 extends the capability to automate sampling, analysis and data transfer of results into the Ambr® software

- automated pH checks and calibration
- automated cell counting
- automated measurement of multiple analytes

Figure 2: Integrated analyzers for the Ambr® 15



Integrated pH Analytics

Automated pH measurement and control is performed in the Ambr® 15 using a pH reader robot in the workstation and pH sensor spots in the bioreactor vessels. Initial single point pH calibration is needed at the start of the process and it is recommended to regularly check the pH measurements through the process and where necessary to adjust values to compensate for pH drift. This is required when using any type of pH control, whether single-use technology or standard glass electrodes. In benchtop bioreactors off-line samples would have to be taken for routine pH checks, in the Ambr® 15 Cell Culture system this is not necessary with the integrated analysis module, where initial calibration and routine checks can all be automated.

The analysis module (Figure 3) directly connects to the Ambr® 15 Cell Culture system and will fit within a standard biosafety cabinet. The small sample volumes used (60 µL) reduces the impact on the overall culture volume but still allows automated pH measurement checks within the 4-9 pH range as well as direct feedback of the pH to the Ambr® software to allow for automatic pH offset values to be applied if needed. The analysis module uses custom liquid handling scripts to limit sample degassing which are known to cause errors in pH readings. This script allows the Ambr® liquid handler to withdraw a small volume of head space gas, then the sample volume followed by another small volume of head space gas, preventing sample degassing, and generating pH readings that are accurate to within 0.01 pH unit.

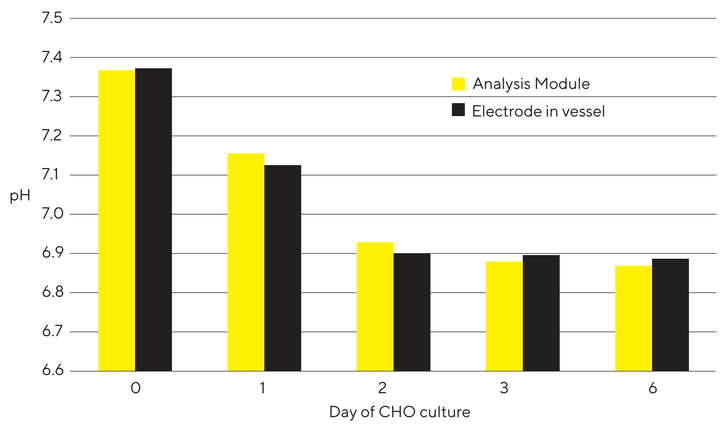
To validate the analysis module for at-line automated pH analysis, we measured the pH of CHO cell samples taken from 24 Ambr® 15 bioreactors at day 0, 1, 3 and 6 using the Ambr® 15 Analysis Module and a manual pH probe (Mettler Toledo) inserted into each bioreactor (see Figure 8).

Figure 3: Ambr® Analysis Module integrated with the Ambr® 15 system

Working range	1.0 - 9.0 pH
Sample volume	60 µL
Cycle time per read	90 s
Resolution	0.01 pH
Calibration buffer accuracy	± 0.01 pH
Minimum sample temperature	ambient +3°C

- Fully integrated at-line pH assay
- Fits into and alongside Ambr® 15 in standard biosafety cabinets
- Close coupling and custom liquid handling to prevent sample degassing

Figure 8: Comparison of pH measurements from CHO cells cultured for 6 days in single-use bioreactors using the Ambr® 15 analysis module (yellow) and a manual pH electrode (black).



Integrated Metabolite Analytics:

The BioProfile® FLEX2™ Automated Cell Culture Analyzer (Nova Biomedical) directly connects to the Ambr® 15 system via an External Sampling Module (ESM) (Figure 4). Analysis types are defined on the FLEX2 and these are transferred directly to the Ambr® software, which dictates the types of assays to be executed and the dilution ratios. The Ambr® liquid handler withdraws a sample from the bioreactor to the sample cup which is routed to the FLEX2 via the external sampling module (ESM). A 1:2 dilution ratio is available for the cell counter module and for diluting chemical analytes a range of dilution ratios are available. Once the FLEX2 analysis is done, the data generated is transferred directly back to the Ambr® software. The software then tracks and processes the data. If required, the Ambr® software can be programmed to perform in-run calculations e.g. doubling time, growth rate and feed addition volumes.

Not only is there a substantial time saving when moving from off-line to automated at-line sampling and analysis, the operator also benefits from a reduction in data transfer efforts and removes the risk of introducing errors from incorrect data entries.

The powerful combination of Ambr® 15 Cell Culture system with FLEX2 enables fully integrated automatic collection of up to 16 cell culture parameters, including total and viable cell density, cell diameter, pH, pCO_2, pO_2, glucose, lactate, glutamine, glutamate, ammonium, Na^+, K^+, Ca^{++} and osmolality, which can be sampled and measured within a cycle time of 6–7 minutes.

To validate Ambr® 15 with integrated FLEX2 as an at-line automated method of metabolite analysis, we measured lactate and glucose in CHO cell samples taken automatically from the integrated system and compared them to samples taken with the Ambr® 15 liquid handler that were then manually transferred to the FLEX2 (See Figure 9).

Furthermore we assessed the use of Ambr® 15 with integrated FLEX2 for automated glucose control in collaboration with the Massachusetts Institute of Technology (MIT). CHO cell cultures were set up in the Ambr® 15 in quadruplicate testing six different feed control strategies including automated feedback and feed forward glucose control (Figures 6 and 7). We measured cell density, glucose and lactate from each of the 24 Ambr® 15 bioreactors daily over a 12 day culture run.

Figure 4: BioProfile® FLEX2™ Automated Cell Culture Analyzer integrated with the Ambr® 15 system.

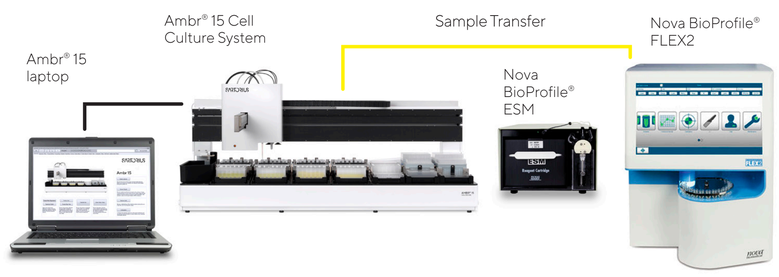
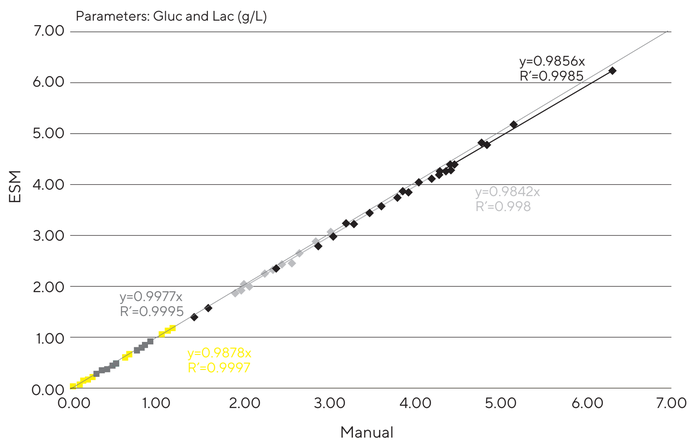


Figure 9: Comparison of glucose (black) and lactate (yellow) measurements from manual and automated samples of CHO cells cultured in single-use bioreactors.



Case Study:
Automated Glucose Control Strategies

- Collaboration between MIT, NOVA Biomedical, and Sartorius
- CHO culture with different feeding conditions
- Automated sampling and glucose control using Ambr® 15 with integrated FLEX2

Figure 6: Automated sampling and glucose control using Ambr® 15 integrated with FLEX2

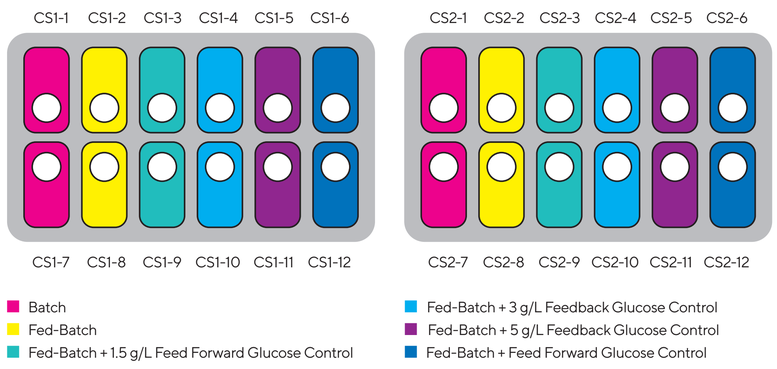
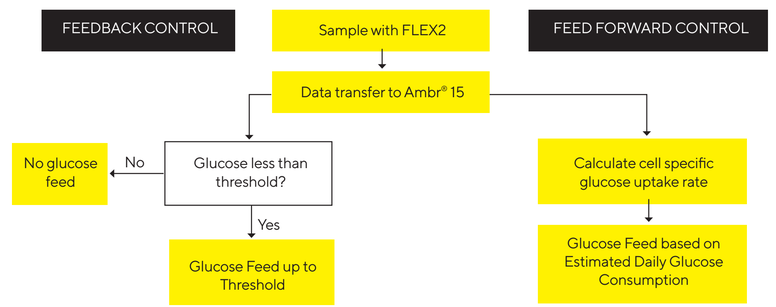


Figure 7: Automated feed back and feed forward glucose control strategies



Integrated pH Analytics

The pH measurement of CHO cell cultures were compared using the Ambr® 15 analytics module and a manual pH probe. The results (Figure 8) demonstrate that over the 6 days monitored, the pH changed over a range of 6.8 to 7.4 and the measurements between the two methods differed by 0.01-0.02 pH units across this pH range showing that these methods provide comparable results.

Analysis module performance

- R&D test culture in Royston – CHO cells
- N = 24 bioreactors
- ~ 10^6 cells/mL at Day 6
- Reference measurement – Mettler electrode inserted into Ambr® 15 vessel

Integrated Metabolite Analytics

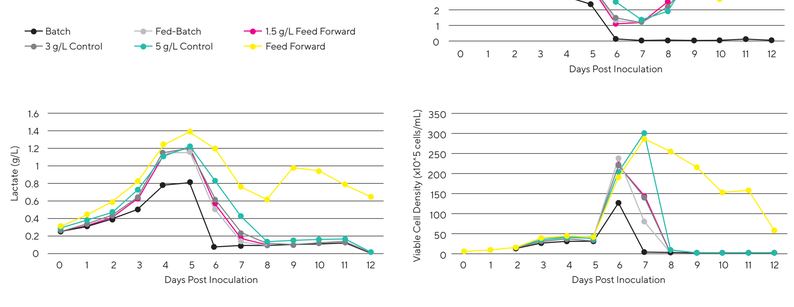
The glucose and lactate measurement of CHO cell cultures were compared using the integrated and manual FLEX2 analysis methods. The results (Figure 9) demonstrate that lactate and glucose concentration changed over a range of 0-1.5 g/L and lactate concentration over a range of 1.5 to 6 g/L and there was a strong correlation of measurements between manual and automated samples over these wide concentration ranges.

Automated Glucose Control Strategies

The results (Figure 10) demonstrate that automated sampling and data transfer can allow walk-away glucose control of bioreactors which can be monitored and controlled from a remote desktop location. Increasing the feed concentration in feedback control was shown to increase the peak viable cell densities achieved. Applying feed forward control achieved similar peak cell density but furthermore significantly prolonged the culture duration. The average cell specific glucose consumption rate was calculated as 9.13 x 10^-12g/cell which is consistent with values reported in literature. Therefore, instead of using glucose consumption constants from scientific papers, scientists could use the integrated analytics to automatically calculate these (and other values such as doubling time and growth rate) directly during a run and compare the trends from run to run.

- Automated sampling and data transfer allowed for walk-away glucose control
- Glucose control strategies were identified that led to higher peak cell densities and prolonged culture duration
- Average cell specific glucose consumption was 9.13 x 10^-12g/cell which is consistent with values reported in literature

Figure 10: Glucose, lactate and cell density measurements from FLEX2 automated samples of CHO cells cultured in single-use bioreactors using different glucose control stratagies.



Conclusion

The studies showed that CHO cells cultured in single-use micro bioreactors integrated to the analysis module generated pH measurements comparable to those produced using manual pH sensors proving that at-line pH measurement checks can be fully automated, replacing the need for an operator to perform off-line pH checks manually.

CHO cells cultured in micro bioreactors integrated to a BioProfile FLEX2 produced similar glucose and lactate measurements to those analyzed using a manual sample transfer. Using the integrated analytics, higher cell densities and prolonged culture durations were achieved with automated feed forward and feed back glucose control. Furthermore it was demonstrated through automated sampling and data transfer that walk-away glucose control could be fully realized.

In conclusion, integrating the Ambr® Analysis Module and the FLEX2 with Ambr® 15 microbioreactors provides accurate and consistent measurements. Using these integrated analytics could save scientists time by allowing monitoring and feedback outside of working hours or from different locations, this in turn could enable rapid process optimization to cost-effectively manufacture biologics at scale.

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