

Evaluating Integrated Analytics for Single-use Mini Bioreactors

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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.¹⁻³ Using these types of small-scale bioreactors has been proven to significantly increase speed and throughput of cell line and process development⁴ with results that are more reproducible than using shake flasks as scale down models.⁵

Whilst the use of automated scale-down bioreactors does increase throughput in clone or 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 Stedim Biotech estimates that from the hundreds of ambr® 15 automated microscale bioreactor systems installed, over four million samples are generated globally every day and Figure 1 highlights areas where manual operations have traditionally caused bottlenecks in the overall workflow when running these assays.

Overcoming these bottlenecks requires integrated analytic devices such as pH modules, cell counters, and metabolite readers to provide accurate and consistent measurements. These systems need to have fast cycle times, the ability to run outside working hours and the

capability to react and adjust to events in the bioreactor. They have to allow for automatic data transfer and advanced control strategies, while also taking into consideration sample volume.

This article 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 strategies using cell count and glucose measurements.

Materials and Method

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 Analyser for cell count and viability (Roche Diagnostics), the ambr 15 analysis module (Sartorius Stedim Biotech) for pH and the BioProfile® FLEX2™ Automated Cell Culture Analyzer (Nova Biomedical) for multi-analyte measurement.

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

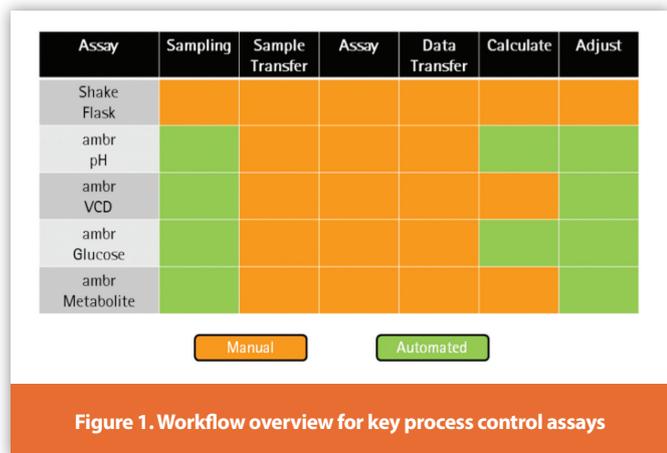


Figure 1. Workflow overview for key process control assays

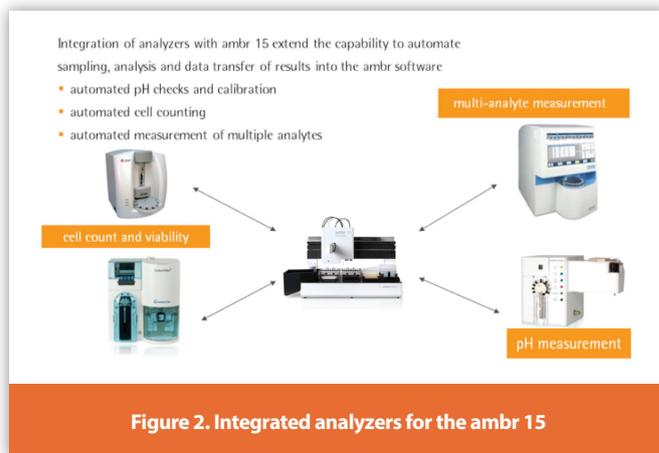


Figure 2. Integrated analyzers for the ambr 15

it is recommended to regularly check the pH measurements through the process and where necessary to adjust values to compensate for pH drift. This requirement is recommended when using any type of pH control, whether single-use sensors or standard glass electrodes. Majority of benchtop bioreactors require these off-line pH sample to be taken manually. In the ambr 15 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 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.

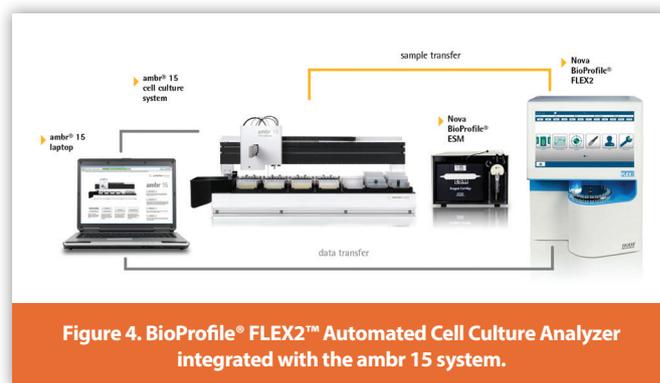
- Working range 4.0-9.0 pH
- Sample volume 60 µL
- Cycle time per read 90 s
- Resolution 0.01 pH
- Cal. buffer accuracy ± 0.01 pH
- Min. sample temp. ambient +3°C
- Fully integrated at-line pH assay
- Fits onto and alongside ambr 15 in standard biosafety cabinets
- Close coupling and custom liquid handling to prevent sample degassing



Figure 3. ambr analysis module integrated with the ambr 15 system

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.

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 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, for example doubling time, growth rate and feed addition volumes. Not only is there a substantial time savings when moving from off-line to automated at-line sampling and analysis, scientists also benefit from a reduction in data transfer efforts and this 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, pCO₂, pO₂, glucose, lactate, glutamine, glutamate, ammonium, Na⁺, K⁺, Ca⁺⁺ and osmolality, which can be sampled and measured within a cycle time of 6–7 minutes. Detection limits of the different parameters analyzed are detailed in Figure 5.

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.

Furthermore, we assessed the use of ambr 15 with integrated FLEX2 for automated glucose control in collaboration with Nova Biomedical and 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

Chemistry Module				
Parameter	Units	Linear Range Low End	Linear Range High End	User Selected Dilution Ratio
Gluc	mmol/L	0.05*	3.00*	Dil. Ratio = 1:0.5*
		0.10	6.00	Dil. Ratio = 1:1
		0.20	12.00	Dil. Ratio = 1:2
Gluc	µL	0.05*	3.00*	Dil. Ratio = 1:0.5*
		0.10	6.00	Dil. Ratio = 1:1
		0.20	12.00	Dil. Ratio = 1:2
Lac	µL	0.05*	3.00*	Dil. Ratio = 1:0.5*
		0.10	6.00	Dil. Ratio = 1:1
		0.20	12.00	Dil. Ratio = 1:2
NH ₄ ⁺	mmol/L	0.20	25.00	Range = user selected Dil. Ratio
Na ⁺	mmol/L	40.0	300.0	Range = user selected Dil. Ratio
K ⁺	mmol/L	1.00	100.00	Range = user selected Dil. Ratio
Ca ⁺⁺	mmol/L	0.10	10.00	Range = user selected Dil. Ratio

Range depends on selected dilution ratio. Note ranges denoted with an asterisk (*) are not currently available and are intended for future releases and as such are included for reference only.

pH/Gas Module			
Parameter	Units	Linear Range Low End	Linear Range High End
pH	-	6.000	8.000
pO ₂	mmHg	3.0	500.0
pCO ₂	mmHg	3.0	300.0

Cell Density Module			
Parameter	Units	Linear Range Low End	Linear Range High End
Total and Viable Cell Density	1 x 10 ⁶ cells/mL	0.10	35.00
1:2 Dilution (actually 1:2.5)	cells/mL	0.20	80.00
Viability	%	0.0	100.0
Live cell diameter	microns	4	70

Dormometer Module
Operational range of 0 to 2,000 mOsm/kg (per Advanced Instruments Specifications)

- Sample types are created on the FLEX2 – user can select which panels to run as well as dilution ratios (if applicable)
- For the Chemistry Module, the dilution ratios are configured when creating sample types on the FLEX2 and any of the available ranges can be selected.
- For the Cell Density Module, only the 1:2 option is available when integrated to the ambr and using the ESM.
- All dilutions are done within the FLEX2 using buffers from the respective reagent packs for the different modules.
- There are pumps and flow paths within the FLEX2 that does any necessary dilutions and directs the samples to the various sensor cards.

Figure 5. FLEX2 Assay Panel with Detection Limits

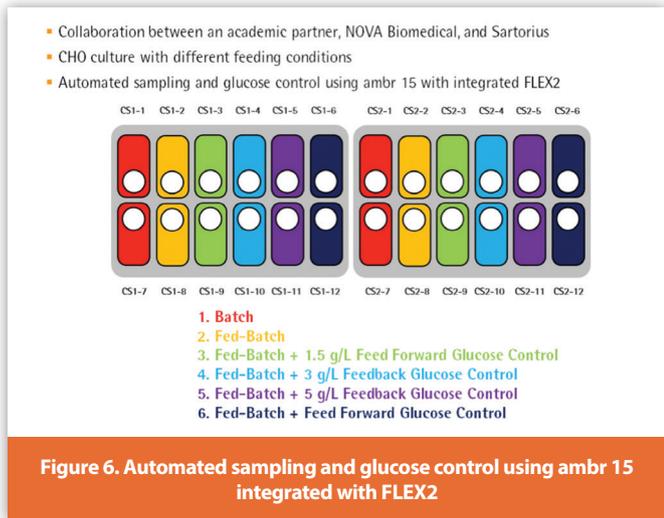


Figure 6. Automated sampling and glucose control using ambr 15 integrated with FLEX2

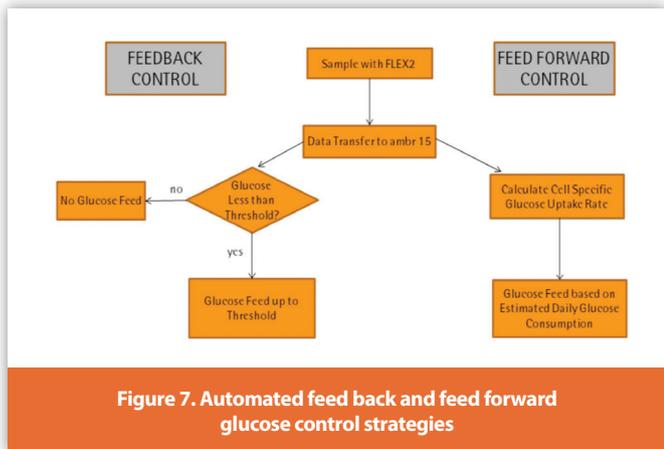


Figure 7. Automated feed back and feed forward glucose control strategies

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.

Results

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.

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 the lactate concentration ranged from 0-1.5 g/L and the glucose ranged from 0-6 g/L. There

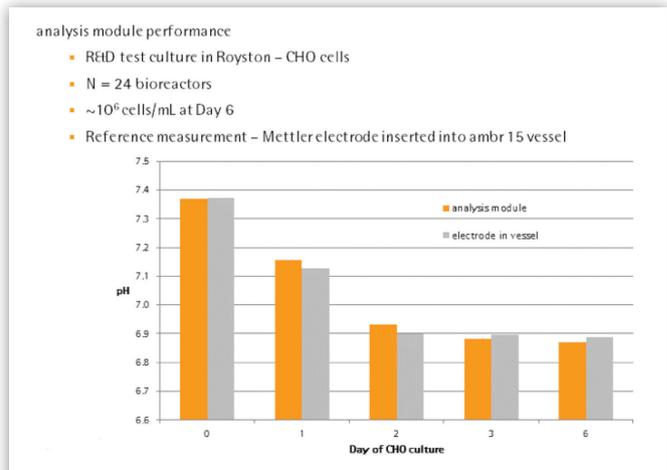


Figure 8. Comparison of pH measurements from CHO cells cultured for 6 days in single-use bioreactors using the ambr 15 analysis module (yellow) and a manual pH electrode (grey).

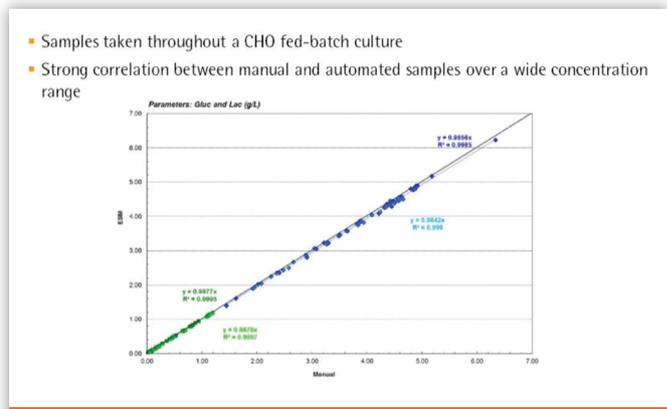


Figure 9. Comparison of glucose (blue) and lactate (green) measurements from manual and automated samples of CHO cells cultured in single-use bioreactors.

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. When comparing the growth curves of the different glucose strategies, the highest cell density and prolonged culture duration was achieved with the automated feed forward control. The average cell specific glucose consumption rate was calculated as 9.13×10^{-12} g/cell which is consistent with values reported in the 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.

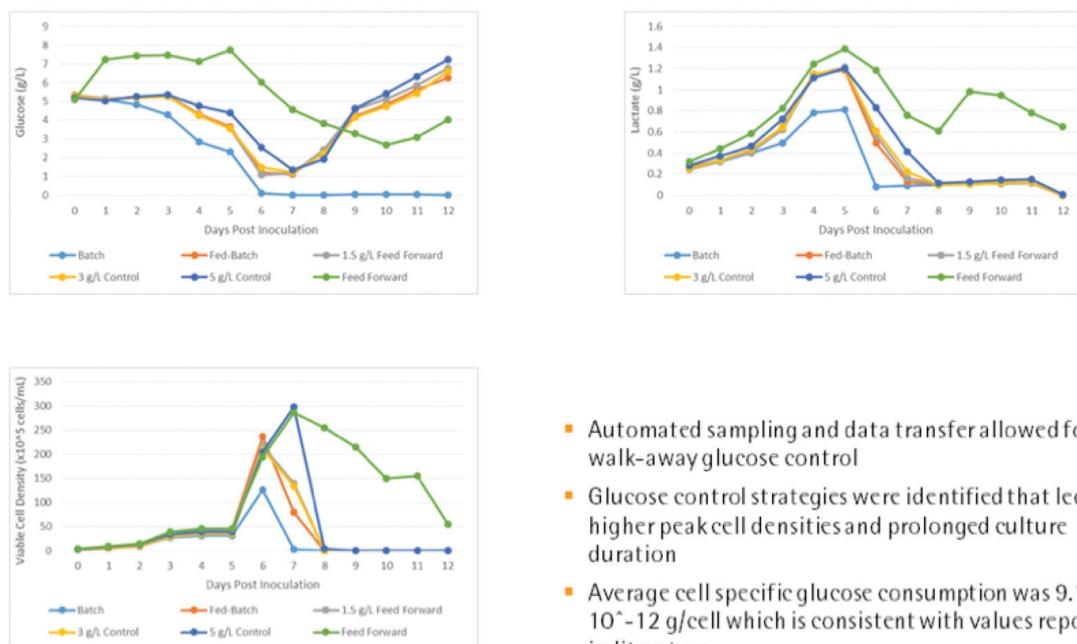


Figure 10. Glucose, lactate and cell density measurements from FLEX2 automated samples of CHO cells cultured in single-use bioreactors using different glucose control strategies.

- 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 9.13×10^{-12} g/cell which is consistent with values reported in literature

Conclusion

We evaluated the application of integrated analytics with single-use micro bioreactor technology. 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 in this type of bioreactor and replaces the need for a scientist 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. Additionally, using the integrated analytics, higher cell densities and prolonged culture durations were achieved with automated feed forward glucose control. Furthermore, it was demonstrated through automated sampling and data transfer that walk-away glucose control could be fully realised.

In conclusion, integrating the ambr analytics module and the FLEX2 with ambr 15 micro bioreactors 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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