Demonstrating chemometric model transferability for 5 mammalian cell lines and 5 media types using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer to monitor upstream bioprocesses.

Authors

Juan Villa, Matthew Zustiak, David Ramirez, Jon Kruger, David Kuntz, Lin Zhang, Nimesh Khadka, Kevin Broadbelt and Sue Woods
(Thermo Fisher Scientific)

MarqMetrix All-In-One Process Raman Analyzer.

Background

Cultured cells are used to manufacture biologics such as vaccines, therapeutic proteins, and cell therapy products. As the need for biologic therapies and vaccines increases, there is increasing interest in establishing more efficient cell culture processes by developing cell cultures for higher yield and enhancing cell growth and scalability. Protein therapeutics (including monoclonal antibodies [mAbs], peptides, and recombinant proteins) represent the largest group of new products in development by the biopharmaceutical industry. Mammalian expression systems are generally the preferred platform for manufacturing biopharmaceuticals, as these cell lines can produce large, complex proteins with post-translational modifications (PTMs), most notably glycosylation, like those produced in humans[1,2,3].

In recent years, process Raman spectroscopy has gained popularity as a process analytical technology (PAT) tool that enables real-time monitoring and control of critical bioprocessing parameters that are key to the successful production of therapeutic drugs. Successful production implies high process efficiency, high and consistent product quality, and minimized manufacturing costs[1]. Implementing Process Analytical Technology (PAT) tools in biopharmaceutical manufacturing continues to receive much interest because of PAT tools' potential to allow rapid development and increased access to therapeutics and existing medications without compromising high quality[5,6].

Raman spectroscopy is a laser-based method for generating a chemical fingerprint of a sample[5,6]. Analysis with Raman spectroscopy in bioprocess monitoring can measure numerous variables in a non-destructive manner, *in situ*, and with low interference from water. These variables include nutrient feed, metabolites, growth profiles, product levels, and product quality attributes[4,5]. The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer is designed to offer rapid, accurate, reliable, and real-time identification and quantification in both upstream and downstream bioprocesses. An immersed Raman optical sensor provides real-time process information about the critical process parameters (CPPs), unlike traditional monitoring systems, which require manual sampling and offline analysis[4,5].

This application note describes the integration of the MarqMetrix All-In-One Process Raman Analyzer with various bioreactors to perform in-line measurement of CPPs in various production processes, using multiple representative cell lines used today in bioproduction. This integrated system uses continuously generated spectral data acquired throughout cell culture runs to develop accurate prediction models for several parameters and metabolites.

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Materials and methods

Cell Culture and Feeding Strategy

Five different mammalian cell line cultures were grown in various bench and pilot scale bioreactors. The cell lines used were CHO-M, CHO-K1, NistCHO, ExpiCHO, and Freedom HEK-293. For each experiment, the bioreactor was inoculated at 0.6×10^6 cells/mL. Various bioreactor types used different initial working volumes of 1.5 L, 2.5 L, and 350 L for the Hyperforma (Thermo Fisher), Biostat (Sartorius), and DynaDrive (Thermo Fisher) reactors, respectively, using the basal media listed in the table below (see table 1). Additional settings included a temperature setpoint of 36.5 °C, a pH setpoint of 7.0 +/- 0.2, and a dissolved oxygen setpoint of 40%. The pH was controlled by CO_2 and sodium carbonate addition for high-end and low-end pH control, respectively. The cells were grown in fed-batch or perfusion mode for approximately 14 days. The fed-batch cultures were fed using a proprietary feeding regimen, and the perfusion cultures used a proprietary cell-specific perfusion rate to maintain nutrient levels. All bioreactors were shrouded to protect from stray light.

Each bioreactor was configured with a Raman optical immersion probe for in-line real time measurements that were collected using 3-4 units. The MarqMetrix All-In-One Process Raman Analyzer can provide measurements of CPPs like glucose, glutamine, glutamate, and lactate, as well as total and viable cell densities. This work was supported by prior fed-batch experiments using ExpiCHO cells cultured in a Stable production medium with Efficient feed C. Model training data included orthogonal, offline measurements to build the models, the models confidently followed the normal batch trajectory, but could be further improved with additional spiking studies for future development [8,9]. In the current study, offline samples were taken daily to measure viable cell density (VCD) and viability using a ViCell-blu (Bechman Coulter) as well as glucose and lactate using the Bioprofile Flex 2 (Nova Biomedical) device.

MarqMetrix All-In-One Process Raman Analyzer Measurements

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer System, with the optical Bioreactor ball probe of the analyzer directly immersed in the bioreactors. Each Raman spectrum resulted from an average of 20 measurements with an integration/exposure time of 3000 milliseconds and laser power set at 450 mW. The total acquisition time per data spectrum was 2 minutes (1 min dark spectrum correction and 1 min sample spectrum) with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and offline instrument analysis to align the online and offline results.

Chemometric model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzer instruments, probes, and 15 bioreactors were used to create models. The training datasets were collected from 45 samples per bioreactor to create each chemometric model. All data were reviewed before building the models. In addition, an algorithm was implemented to remove data spikes in the spectra caused by cosmic rays. The spectral region of interest was selected, and 5 SPC files were averaged; therefore, each data point corresponds to 10 min acquisition time. The spectra were pre-processed to remove the baseline, maximize signal-to-noise, and correct for path length differences. Partial Least Squares (PLS) models were created for each property of interest, and leave-out-one-run cross-validation was performed to test the optimization of each model. Properties of interest included glucose, lactate, titer, TCD, VCD, and other common metabolites generated during the bioreactor culture run.

Runs	Cell Line	Initial Media	Feed Media	Reactor Size & Model	Run Mode
1	CHO-M	BalanCD CHO Growth A + Glutamine	Cell Boost 7A/7B	500 L DynaDrive	Fed-Batch
2	CHO-K1	Stable Production Media (SPM) + Pluronic + Glutamine	EFC 2X	3 L Hyperforma	Fed-Batch
3	ExpiCHO	HipCHO + Pluronic + Glutamine	HipCHO + Pluronic + Glutamine	5 L Biostat	Perfusion
4	NistCHO	Ex-Cell Advanced CHO Fed-Batch medium	EX-CELL® Advanced™ Feed	3 L Hyperforma	Fed-Batch
5	HEK-293	Expi293 expression media	Expi293 expression media	5 L Biostat	Perfusion

Table 1. Cell lines and media used in cell culture experiment.

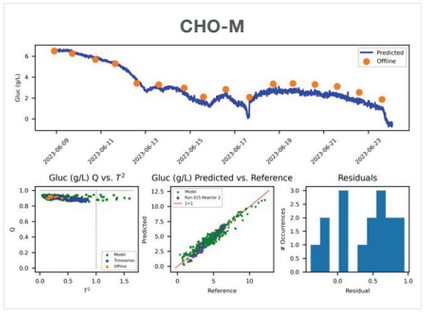
Results

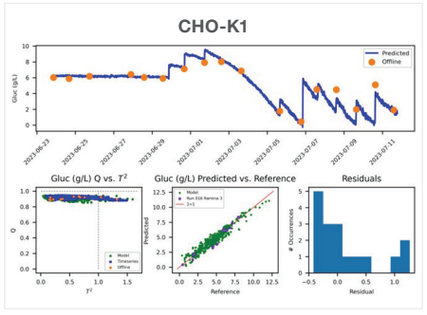
This work applied continuous in-line Raman spectroscopy to fed-batch and perfusion cell culture processes. The in-line spectral data were correlated to the offline analytical data acquired for parameters of interest. Using Raman spectroscopy to monitor process parameters first requires a chemometric model built with an externally calibrated data set (i.e., independent offline data). Bioreactor samples were collected daily and analyzed for comparison to assess the accuracy of the MarqMetrix All-In-One Process Raman Analyzer predicted values. The root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP) were calculated for each parameter. The RMSEP values reported in the figures below represent the average prediction error across each run. The RMSEP tests the model against "new" data that the model has not seen. Outlier diagnostic information (spectral residual and distance to model) were also recorded and used to qualify predictions. The coefficient of variation, R^2, was recorded for each PLS model. The R^2 value determines the Y variable's variation, which the model predictors (X variables) can explain.

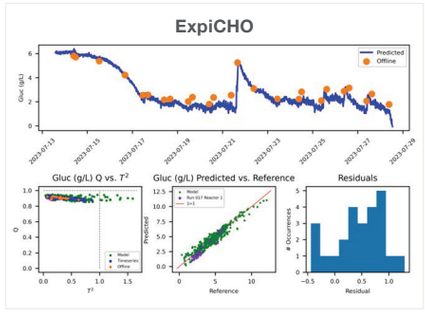
This study combined 15 independent datasets from bioreactor runs utilizing ExpiCHO cells to train a large chemometric model. This model was then applied to the spectral data obtained during the 5 bioreactor runs with the different cell and media types. The data indicate that the ExpiCHO model was able to accurately predict numerous CPPs in the bioreactor runs using alternate cell lines. The transferability of this model is evident from the high correlation between model predictions and offline analysis for multiple metabolites and parameters (as shown in figure tables). This demonstrates the applicability of our versatile model solution to provide accurate predictions across various cell and media types.

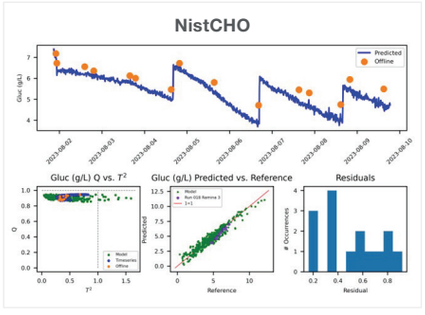
Measurements and average prediction errors utilizing the single model developed from the independent runs are shown for the parameters predicted across the cell lines in Figures A through E. The predicted values are in excellent agreement with the reference values. Overall, the errors of the predicted values are within 4–10% but are also very consistent between the different cell lines. Thus, the MarqMetrix All-In-One Process Raman Analyzer is shown to provide an accurate and reliable in-line prediction for the parameters monitored during upstream processing.

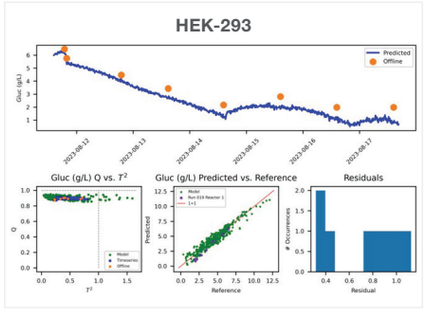
Figure 1A. Glucose Model Transferability Across 5 Cell Lines in Bioreactor Cell Culture runs.









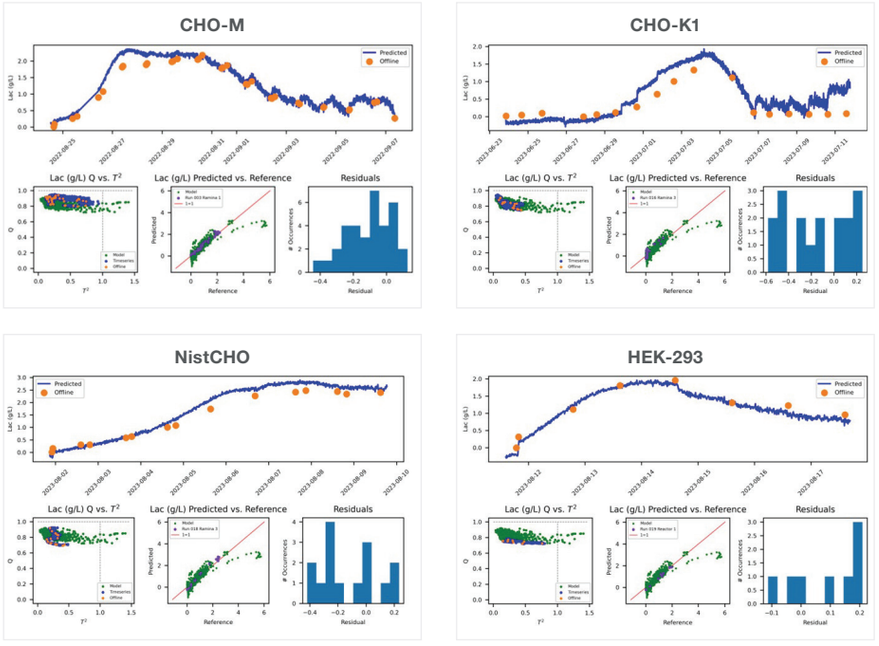


Correlation of Model Prediction with Offline Data

Cell Line : Media	Metabolite Predicted	R^2 Predicted	RMSEP
CHO-M : BalanCD CHO Growth A + Glutamine	Glucose (g/L)	0.96	0.54
CHO-K1 : Stable Production Media (SPM) + Pluronic + Glutamine	Glucose (g/L)	0.97	0.54
ExpiCHO : HipCHO + Pluronic + Glutamine	Glucose (g/L)	0.95	0.55
NistCHO : Ex-CELL Advanced CHO Fed Batch medium	Glucose (g/L)	0.97	0.56
HEK293 : Expi293 expression media	Glucose (g/L)	0.99	0.78

Figure 1. (A–E). Chemometric Model Plots: Comparison of Raman Models vs. Offline Analytical Data for Important Bioreactor Parameters in 4 different bioproduction cell lines. The 1st plot is time series predictions; the 2nd plot shows how well the new run fits in with existing data; the 3rd plot is a regression line; and the 4th plot shows the difference between the reference and the prediction and summary statistics. The data demonstrate excellent model transferability for different CPPs monitored in the other cell types across bioreactor runs. The calibration models for predicting the various parameters were built with an independent dataset comprising over 15 bioreactor runs, all using ExpiCHO cells.

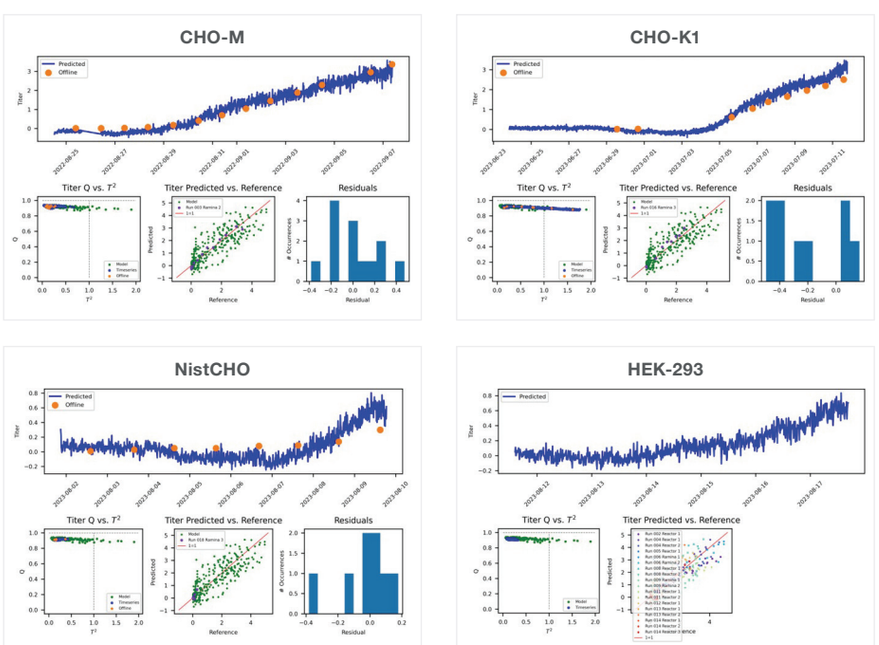
Figure 1B. Lactate Model Transferability Across 5 Cell Lines in Bioreactor Cell Culture runs.



Correlation of Model Prediction with Offline Data

Cell Line : Media	Metabolite Predicted	R^2 Predicted	RMSEP
CHO-M : BalanCD CHO Growth A + Glutamine	Lactate (g/L)	0.97	0.17
CHO-K1 : Stable Production Media (SPM) + Pluronic + Glutamine	Lactate (g/L)	0.81	0.31
ExpiCHO : HipCHO + Pluronic + Glutamine	Lactate (g/L)	0.93	0.19
NistCHO : Ex-CELL Advanced CHO Fed Batch medium	Lactate (g/L)	0.99	0.23
HEK293 : Expi293 expression media	Lactate (g/L)	0.98	0.14

Figure 1C. Titer Model Transferability Across 5 Cell Lines in Bioreactor Cell Culture runs.



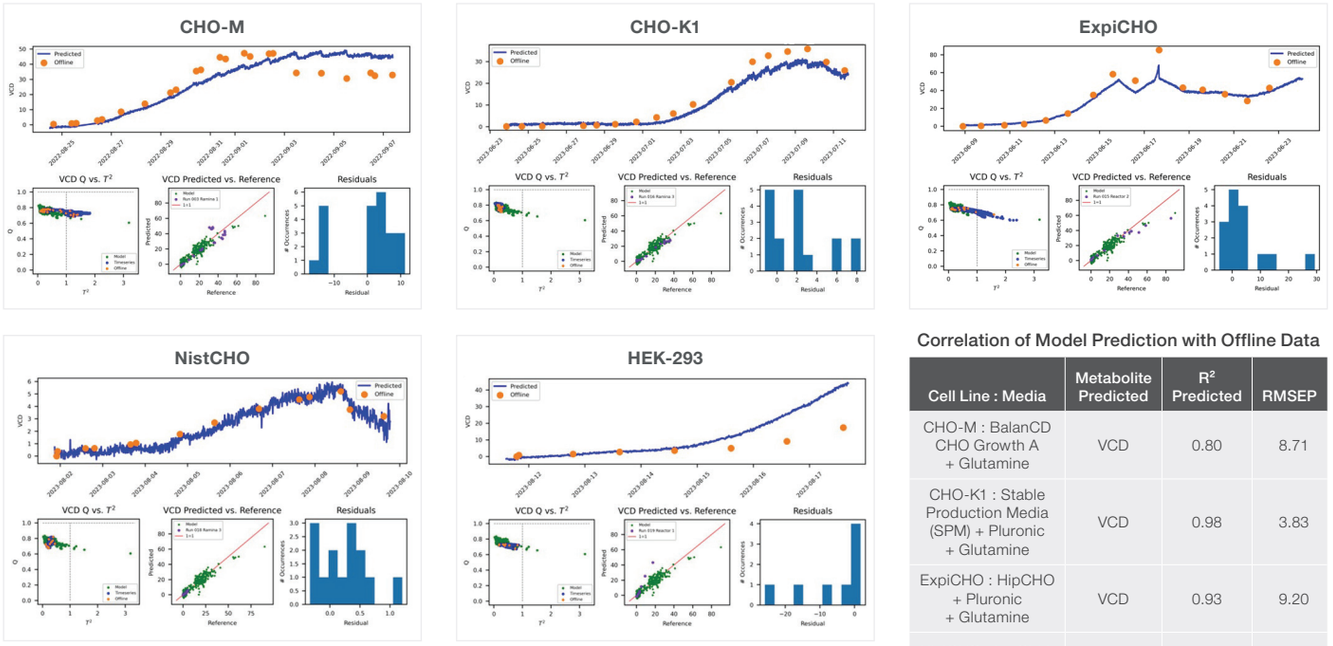
Correlation of Model Prediction with Offline Data

Cell Line : Media	Metabolite Predicted	R^2 Predicted	RMSEP
CHO-M : BalanCD CHO Growth A + Glutamine	Titer	0.96	0.22
CHO-K1 : Stable Production Media (SPM) + Pluronic + Glutamine	Titer	0.98	0.31
ExpiCHO : HipCHO + Pluronic + Glutamine	Titer	0.77	0.96
NistCHO : Ex-CELL Advanced CHO Fed Batch medium	Titer	0.84	0.16
HEK293 : Expi293 expression media	*Titer	N/A	N/A

*HEK293T cells does not produce mAbs (titer).

Figure 1. (A–E). Chemometric Model Plots: Comparison of Raman Models vs. Offline Analytical Data for Important Bioreactor Parameters in 4 different bioproduction cell lines. The 1st plot is time series predictions; the 2nd plot shows how well the new run fits in with existing data; the 3rd plot is a regression line; and the 4th plot shows the difference between the reference and the prediction and summary statistics. The data demonstrate excellent model transferability for different CPPs monitored in the other cell types across bioreactor runs. The calibration models for predicting the various parameters were built with an independent dataset comprising over 15 bioreactor runs, all using ExpiCHO cells.

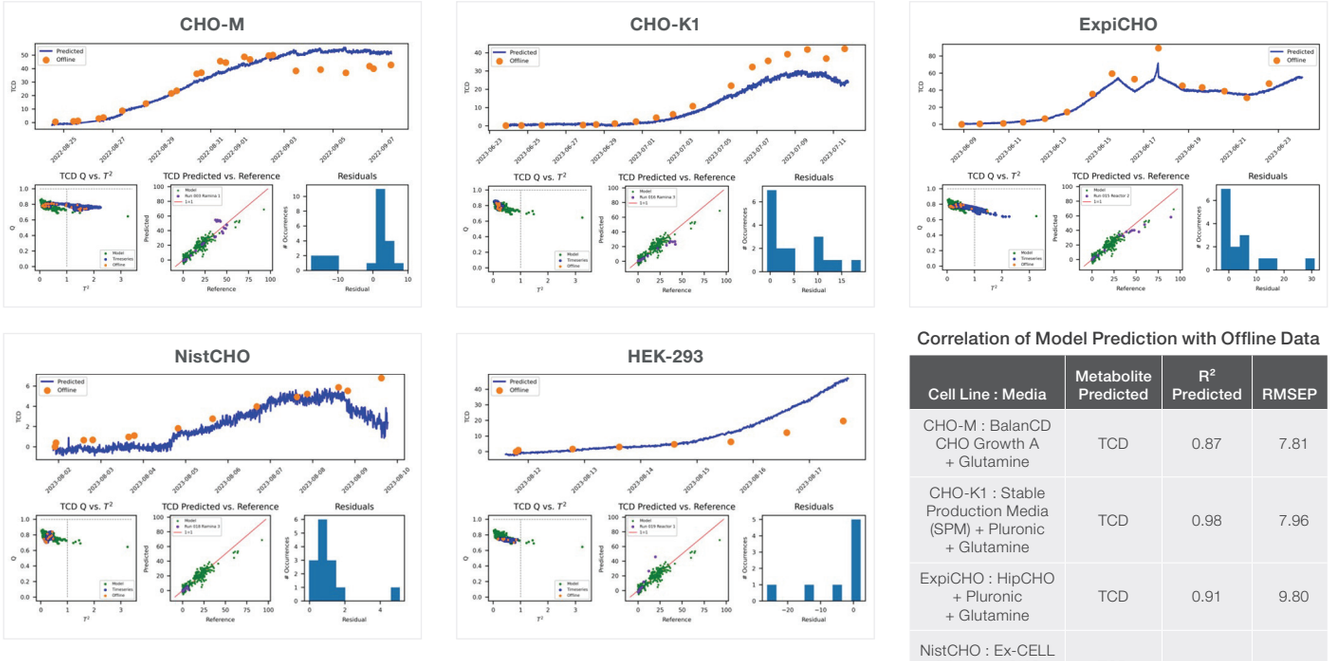
Figure 1D. VCD Model Transferability Across 5 Cell Lines in Bioreactor Cell Culture runs.



Correlation of Model Prediction with Offline Data

Cell Line : Media	Metabolite Predicted	R^2 Predicted	RMSEP
CHO-M : BalanCD CHO Growth A + Glutamine	VCD	0.80	8.71
CHO-K1 : Stable Production Media (SPM) + Pluronic + Glutamine	VCD	0.98	3.83
ExpiCHO : HipCHO + Pluronic + Glutamine	VCD	0.93	9.20
NistCHO : Ex-CELL Advanced CHO Fed Batch medium	VCD	0.95	0.47
HEK293 : Expi293 expression media	VCD	0.99	11.08

Figure 1E. TCD Model Transferability Across 5 Cell Lines in Bioreactor Cell Culture runs.



Correlation of Model Prediction with Offline Data

Cell Line : Media	Metabolite Predicted	R^2 Predicted	RMSEP
CHO-M : BalanCD CHO Growth A + Glutamine	TCD	0.87	7.81
CHO-K1 : Stable Production Media (SPM) + Pluronic + Glutamine	TCD	0.98	7.96
ExpiCHO : HipCHO + Pluronic + Glutamine	TCD	0.91	9.80
NistCHO : Ex-CELL Advanced CHO Fed Batch medium	TCD	0.73	1.64
HEK293 : Expi293 expression media	TCD	0.99	10.81

Figure 1. (A–E). Chemometric Model Plots: Comparison of Raman Models vs. Offline Analytical Data for Important Bioreactor Parameters in 4 different bioproduction cell lines. The 1st plot is time series predictions; the 2nd plot shows how well the new run fits in with existing data; the 3rd plot is a regression line; and the 4th plot shows the difference between the reference and the prediction and summary statistics. The data demonstrate excellent model transferability for different CPPs monitored in the other cell types across bioreactor runs. The calibration models for predicting the various parameters were built with an independent dataset comprising over 15 bioreactor runs, all using ExpiCHO cells.

Conclusion

The MarqMetrix All-In-One Process Raman Analyzer is a rapid, reliable, and easy-to-use instrument for in-line monitoring of real-time process parameters in upstream bioprocesses, with an accuracy highly comparable to the reference values from off-line instrumentation. The MarqMetrix All-In-One Process Analyzer provides a Raman-based PAT solution for monitoring metabolites and additional critical process parameters. This allows for the instant implementation of a PAT tool that can be used across a wide range of media and cell culture processes to produce biotherapeutics. As demonstrated here, using appropriate spectral data and preprocessing, a Raman-based model for predicting different variables is feasible. It is directly applicable for monitoring other processes, independent of the cell type or media used in the culture process. The MarqMetrix All-In-One Process Raman Analyzer methodology discussed in this paper may be similarly applied to other applications in upstream bioprocessing.

Figure 2. MarqMetrix All-In-One Process Raman Analyzer with optical MarqMetrix Bioprocess probe immersed in a 5L glass bioreactor.

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