Application Note AN-NIR-099

Quality Control of fermentation broths

Multiparameter determination within one minute

Cell fermentation processes are a reliable production method for small molecules and protein-based active pharmaceutical ingredients (APIs), allowing pharmaceutical companies to optimize the production process and reduce time to market. The fermentation process requires monitoring of many different parameters to ensure optimal production. These quality parameters include (but are not limited to) pH, bacterial content, potency, glucose, and concentration of reducing sugars. Traditional laboratory analysis takes a significant amount of time and requires different analytical techniques to monitor these quality parameters in the fermentation process.

Near-infrared spectroscopy (NIRS) offers a faster and more cost-efficient alternative to traditional methods for the determination of critical parameters in fermentation broths at any stage of the fermentation process.

EXPERIMENTAL EQUIPMENT

Fermentation broth samples taken at different fermentation times were measured in reflection mode with the Metrohm DS2500 Solid Analyzer. Because the samples were dark in color (yellow-brown), they were measured without needing to use the gold reflector and required no sample preparation. The Metrohm software package Vision Air Complete was used for all data acquisition and prediction model development.

Figure 1. DS2500 Solid Analyzer.

Table 1. Hardware and software equipment overview

Equipment	Metrohm number
DS2500 Solid Analyzer	2.922.0010
NIRS transflection vessel	6.7401.000
NIRS Mini Sample Cup Holder for DS2500	6.7430.040
Vision Air 2.0 Complete	6.6072.208

RESULT

The obtained Vis-NIR spectra (Figure 2) were used to create prediction models for quantification of the bacterial, glucose, and reducing sugars concentration, as well as the pH and potency. The quality of the prediction models was evaluated using the correlation diagram, which displays a high correlation between the Vis-NIR prediction and the reference values. The respective figures of merit (FOM) display the expected precision of a prediction during routine analysis. Potency (Figures 7 and 8) was measured with two different laboratory methods as described in Table 8.

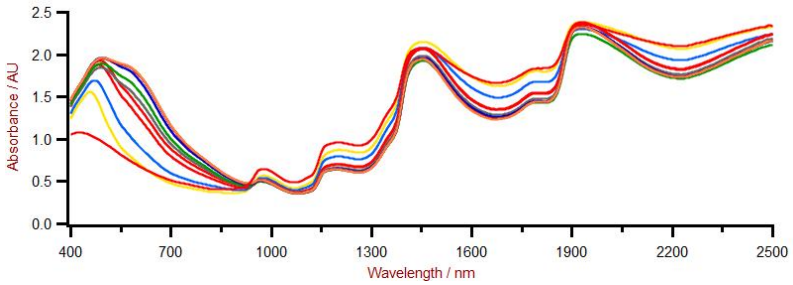

Figure 2. Vis-NIR spectra of fermentation broth samples taken at different fermentation times and analyzed on a DS2500 Solid Analyzer.

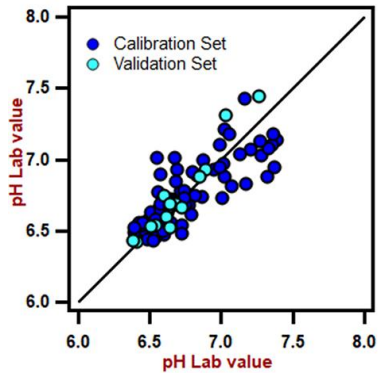

Figure 3. Correlation diagram for the prediction of pH in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using a pH meter.

Table 2. Figures of merit for the prediction of pH in fermentation broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.6461
Standard error of calibration	0.1645
Standard error of cross-validation	0.1686
Standard error of validation	0.0997

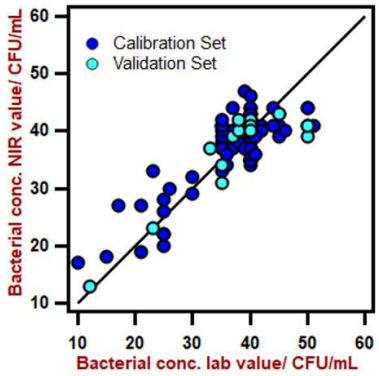

Figure 4. Correlation diagram for the prediction of bacterial concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

Table 3. Figures of merit for the prediction of bacterial concentration in fermentation broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.7086
Standard error of calibration	4.6884 CFU/mL
Standard error of cross-validation	4.7429 CFU/mL
Standard error of validation	5.0916 CFU/mL

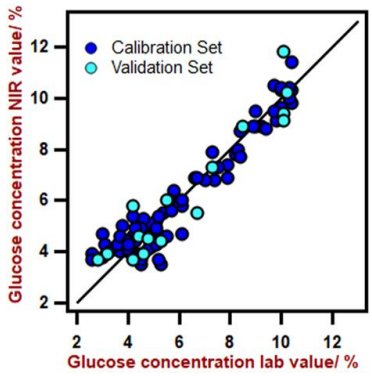

Figure 5. Correlation diagram for the prediction of glucose concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

Table 4. Figures of merit for the prediction of glucose content in fermentatoin broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.9165
Standard error of calibration	0.6938%
Standard error of cross-validation	0.7896%
Standard errror of validation	0.8628%

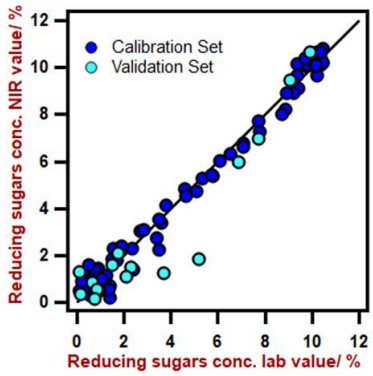

Figure 6. Correlation diagram for the prediction of reducing sugars in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

Table 5. Figures of merit for the prediction of sugars content in fermentation broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.9863
Standard error of calibration	0.4767%
Standard error of cross-validation	0.6821%
Standard error of validation	1.2429%

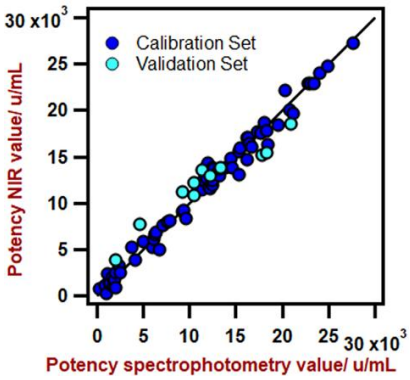

Figure 7. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

Table 6. Figures of merit for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.9083
Standard error of calibration	2295 u/mL
Standard error of cross-validation	2968 u/mL
Standard error of validation	2089 u/mL

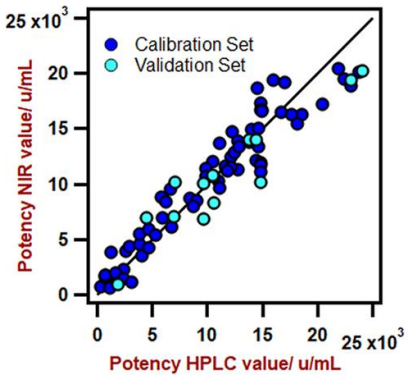

Figure 8. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC + PCR.

Table 7. Figures of merit for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer.

Figures of merit	Value
R^2	0.9156
Standard error of calibration	1913 u/mL
Standard error of cross-validation	2172 u/mL
Standard error of validation	1168 u/mL

CONCLUSION

This application note demonstrates the feasibility to determine key parameters of the quality control of the fermentation process with NIR spectroscopy. The main advantages of Vis-NIR spectroscopy over wet chemical methods are that running costs are significantly lower and time-to-result is significantly reduced. Additionally, no chemicals are required, and the technique is non-destructive to the samples.

Table 8. Time to result overview for the different quality parameters

Parameter	Method	Time to result
pH	pH Meter	3–5 minutes
Bacterial concentration	UV-Vis	8 hours (sample preparation) + 1 minute (UV-Vis)
Glucose and reducing sugars concentration	HPLC	30–45 minutes
Potency	UV-Vis	7minutes (sample preparation) + 1 minute (UV-Vis)
Potency	HPLC + PCR	1hour (sample preparation) + 20 minutes (HPLC + PCR)

Internal reference: AW NIR CN-0017-112021

CONTACT

Metrohm Brasil
Rua Minerva, 161
05007-030 São Paulo
metrohm@metrohm.com.br