

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.

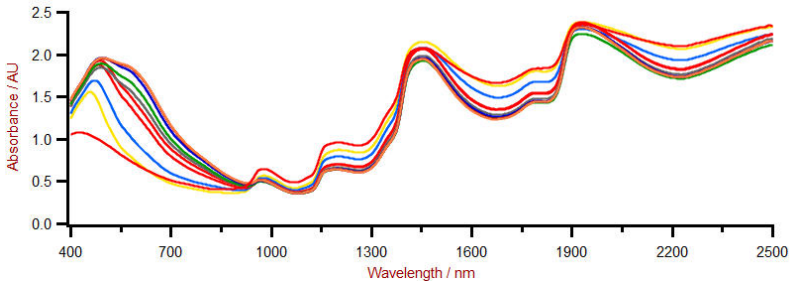


Figure 2. Vis-NIR spectra of fermentation broth samples taken at different fermentation times and analyzed on a DS2500 Solid Analyzer.

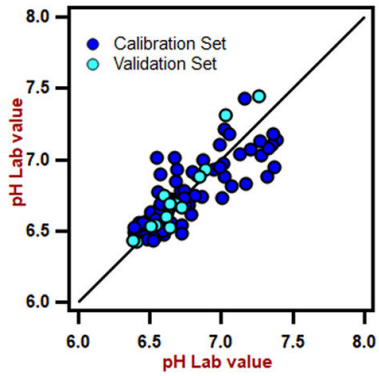


Figure 3. Correlation diagram for the prediction of pH in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using a pH meter.

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

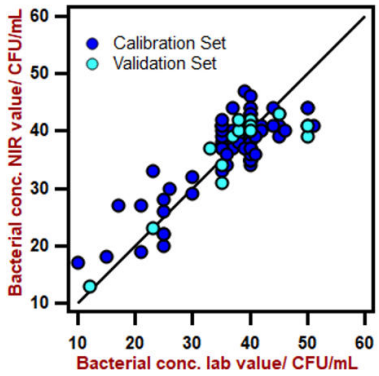


Figure 4. Correlation diagram for the prediction of bacterial concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

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

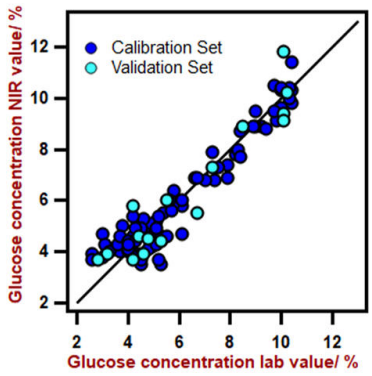


Figure 5. Correlation diagram for the prediction of glucose concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

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%

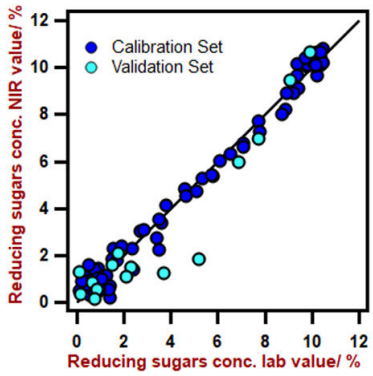


Figure 6. Correlation diagram for the prediction of reducing sugars in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

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%

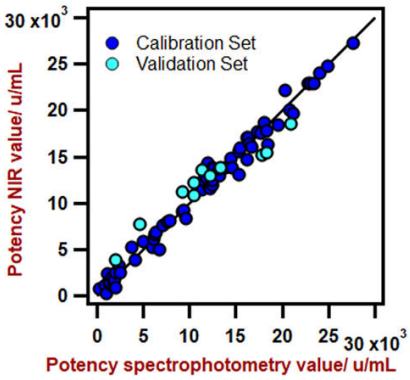


Figure 7. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

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

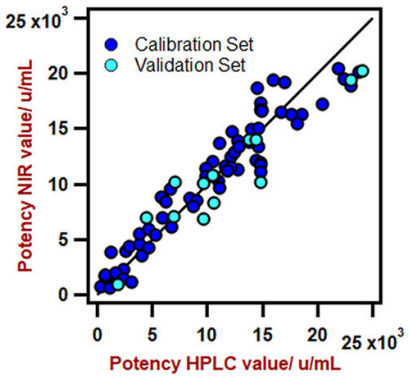


Figure 8. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC + PCR.

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 UK & Ireland
Evenwood Close
WA71LZ Runcorn
info@metrohm.co.uk



DS2500 Solid Analyzer

Robust near-infrared spectroscopy for quality control, not only in laboratories but also in production environments.

The DS2500 Analyzer is the tried and tested, flexible solution for routine analysis of solids, creams, and optionally also liquids along the entire production chain. Its robust design makes the DS2500 Analyzer resistant to dust, moisture, vibrations, and temperature fluctuations, which means that it is eminently suited for use in harsh production environments.

The DS2500 covers the full spectral range from 400 to 2500 nm and delivers accurate, reproducible results in less than one minute. The DS2500 Analyzer meets the demands of the pharmaceutical industry and supports users in their day-to-day routine tasks thanks to its simple operation.

Thanks to accessories tailored perfectly to the instrument, optimum results are achieved with every sample type, no matter how challenging it is, e.g. coarse-grained solids such as granulates or semi-solid samples such as creams. The MultiSample Cup can help improve productivity when measuring solids, as it enables automated measurements of series containing up to 9 samples.



NIRS transflection vessel, optically flat

Optically flat transflection vessel for the spectral measurement of liquids. Can be used in combination with the following instruments:

- NIRS DS2500 Analyzer (**order number: 2.922.0010**)
- NIRS XDS MasterLab Analyzer (**order number: 2.921.1310**)
- NIRS XDS MultiVial Analyzer (**order number: 2.921.1120**)
- NIRS XDS RapidContent Analyzer (**order number: 2.921.1110**)
- NIRS XDS RapidContent Analyzer - Solids (**order number: 2.921.1210**)



DS2500 Holder

Holder for use with:

- Small sample vessels (**6.7402.030**)
- DS2500 Iris (**6.7425.100**)

Vision Air 2.0 Complete

Vision Air - Universal spectroscopy software.

Vision Air Complete is a modern and simple-to-operate software solution for use in a regulated environment.

Overview of the advantages of Vision Air:

- Individual software applications with adapted user interfaces ensure intuitive and simple operation
- Simple creation and maintenance of operating procedures
- SQL database for secure and simple data management

The Vision Air Complete version (66072208) includes all applications for quality assurance using Vis-NIR spectroscopy:

- Application for instrument and data management
- Application for method development
- Application for routine analysis

Additional Vision Air Complete solutions:

- 66072207 (Vision Air Network Complete)
- 66072209 (Vision Air Pharma Complete)
- 66072210 (Vision Air Pharma Network Complete)