Application Note AN-NIR-099

发酵液的质量控制

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.

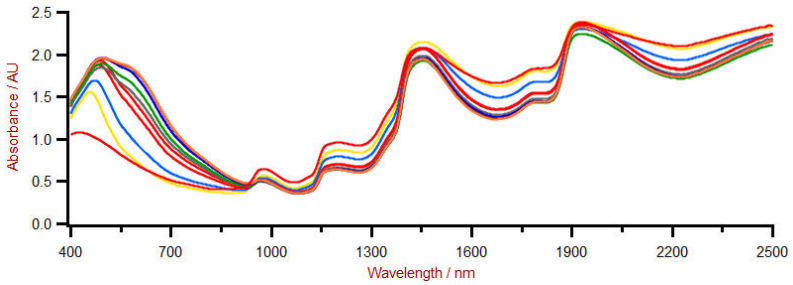

Figure 2. Vis-NIR spectra of fermentation broth samples taken at different fermentation times and analyzed on a DS2500 Solid Analyzer.

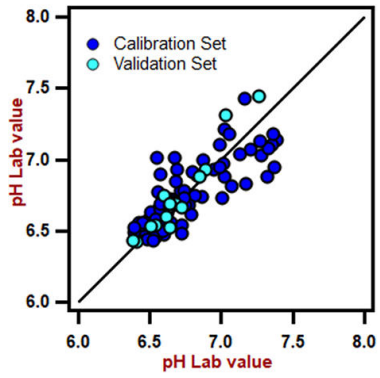

Figure 3. Correlation diagram for the prediction of pH in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using a pH meter.

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

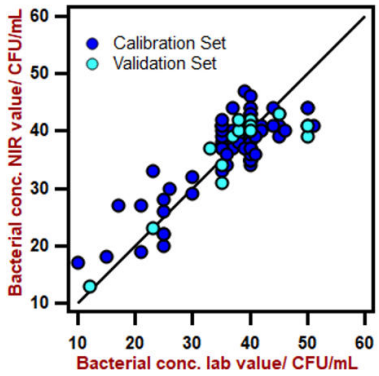

Figure 4. Correlation diagram for the prediction of bacterial concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

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

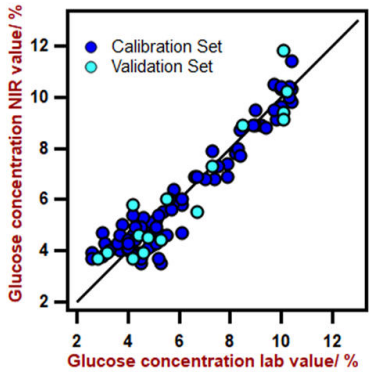

Figure 5. Correlation diagram for the prediction of glucose concentration in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

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%

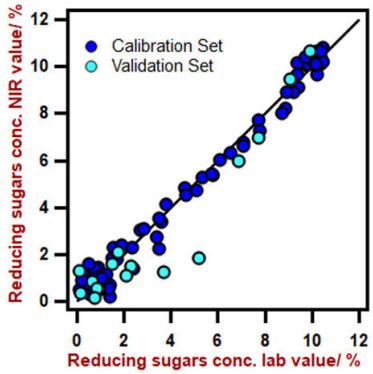

Figure 6. Correlation diagram for the prediction of reducing sugars in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC.

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%

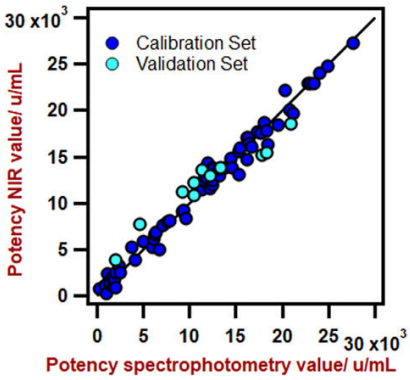

Figure 7. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using UV-Vis spectrophotometry.

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

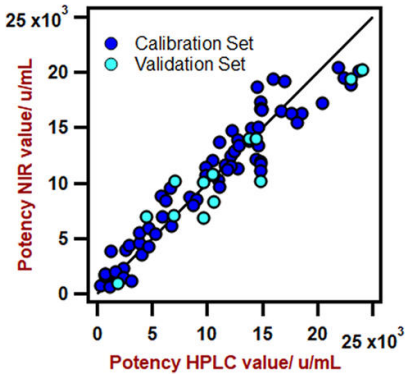

Figure 8. Correlation diagram for the prediction of potency in fermentation broth using a DS2500 Solid Analyzer. The lab value was evaluated using HPLC + PCR.

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

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DS2500 Solid Analyzer

坚固结实的近红外光谱仪,用于生产环境和试验室中的质量检查。

DS2500 分析仪是经过验证的灵活解决方案,用于对整个生产链过程中的固体、乳膏和液体进行常规分析。其坚固结实的设计使 DS2500 Analyzer 分析仪不受灰尘、湿度、振动和温度波动的影响,因此非常适用于在恶劣的生产环境中使用。

DS2500 涵盖了从 400 到 2500 nm 的整个光谱范围,并能在不到一分钟时间内提供准确和可再现的结果。DS2500 Analyzer 满足制药行业的要求,并由于操作简便而能帮助用户完成其日常工作任务。

由于与设备匹配,附件可以承受任何具有挑战性的样品类型,例如:粒料之类的粗颗粒固体或乳膏之类的半固体样品,可获得结果。测量固体的时候,使用 MultiSample Cup 可以提高生产率,可以自动批量测量多 9 个样品。

NIRS

对液体进行光谱测量的光学平面透射反射器皿。可与下列设备组合使用:

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

DS2500

本支架使用时搭配:

- 小样品容器 (6.7402.030)
- DS2500 Iris (6.7425.100)

Vision Air 2.0 Complete

Vision Air – 通用的光谱分析软件。

Vision Air Complete 是用于监管规范环境的先进易用的软件解决方案。

Vision Air 优点一览:

- 便捷的软件应用和经过适配的用户界面保证了直观简单的操作方式
- 操作过程的创建与维护方式简单
- SQL 数据库,可安全且简单地管理数据

Vision Air Complete (66072208) 版本包含所有用于可见近红外光谱分析质量保证过程的应用:

- 仪器和数据管理应用
- 方法开发应用
- 常规分析应用

其它 Vision Air Complete 解决方案:

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