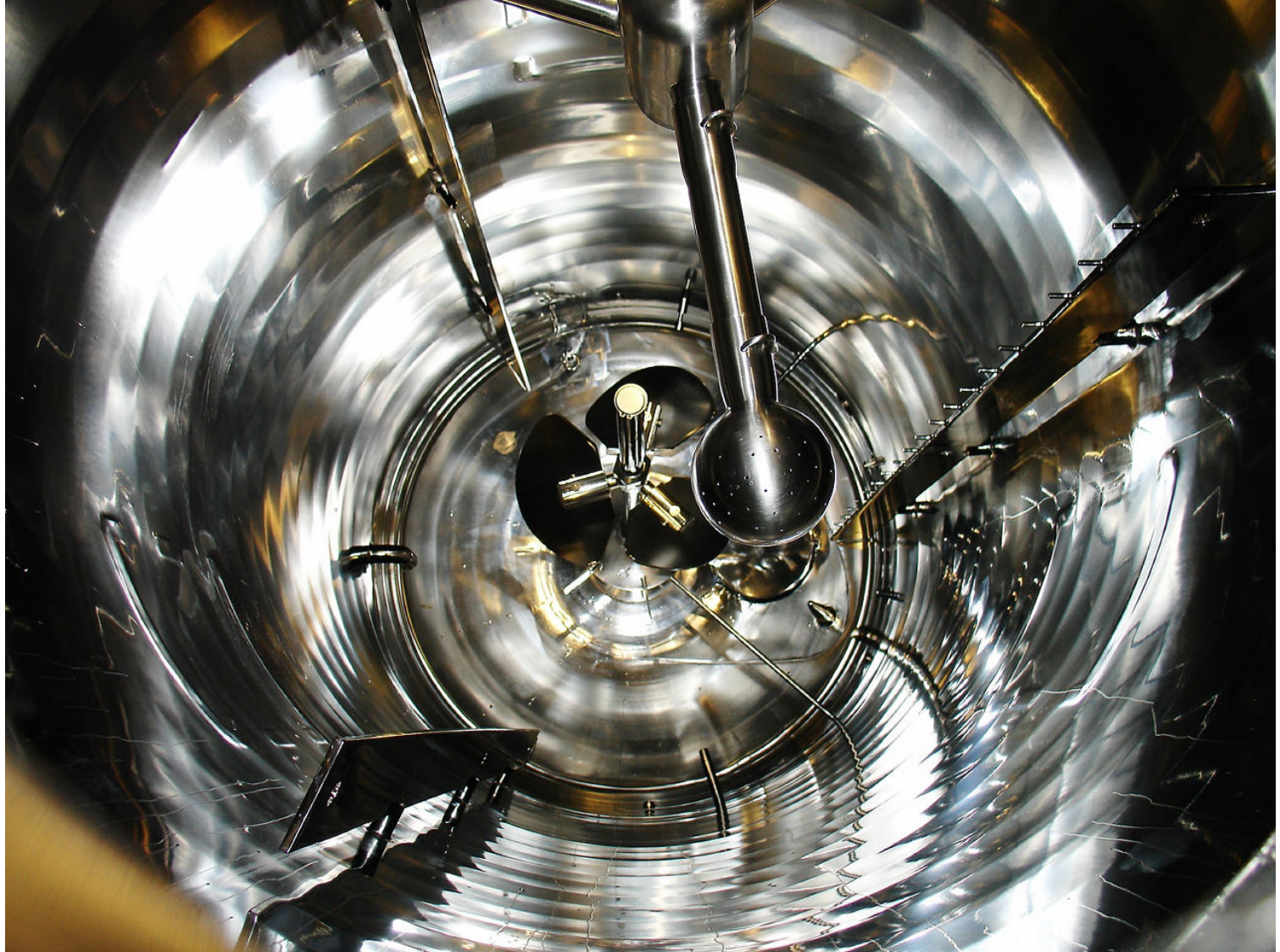




Application Note AN-NIR-099

# Quality Control of fermentation broths

## Multiparameter determination within one minute

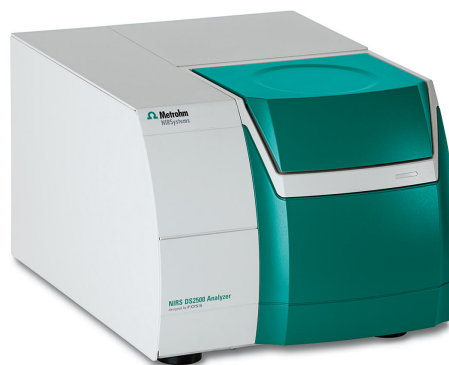
Cell fermentation processes are a reliable production method for small molecules and protein-based active pharmaceutical ingredients (APIs), allowing pharmaceutical companies to optimize the production process and reduce time to market. The fermentation process requires monitoring of many different parameters 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.

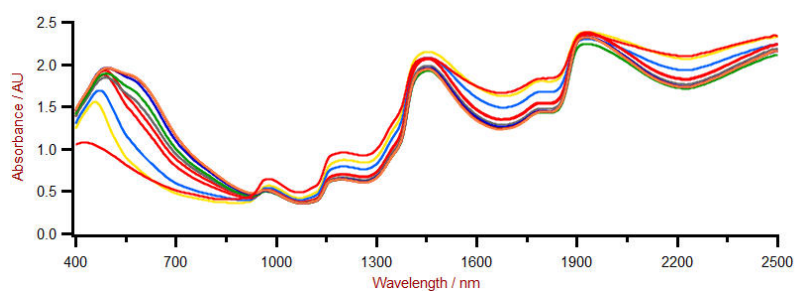
**Table 1.** Hardware and software equipment overview

| 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     |

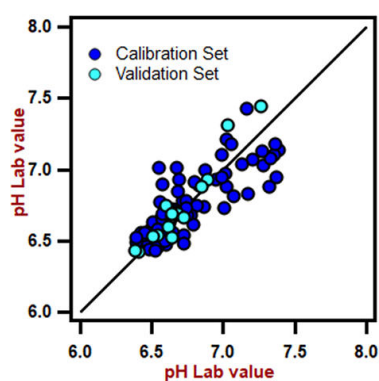
## RESULT

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**.



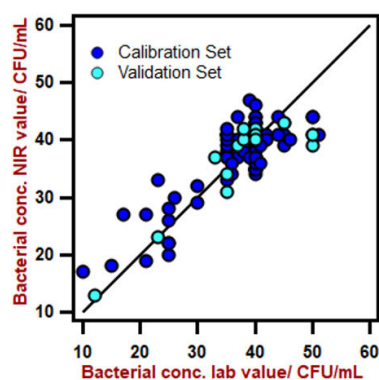
**Figure 2.** Vis-NIR spectra of fermentation broth samples taken at different fermentation times and analyzed on a DS2500 Solid Analyzer.



**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.

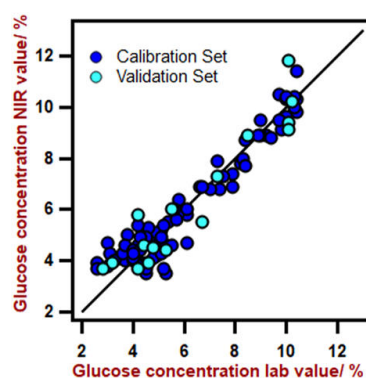
| Figures of merit                   | Value  |
|------------------------------------|--------|
| $R^2$                              | 0.6461 |
| Standard error of calibration      | 0.1645 |
| Standard error of cross-validation | 0.1686 |
| Standard error of validation       | 0.0997 |



**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.

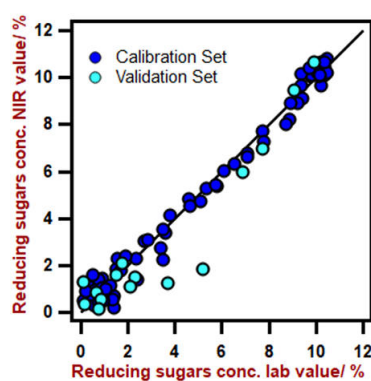
| Figures of merit                   | Value         |
|------------------------------------|---------------|
| $R^2$                              | 0.7086        |
| Standard error of calibration      | 4.6884 CFU/mL |
| Standard error of cross-validation | 4.7429 CFU/mL |
| Standard error of validation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

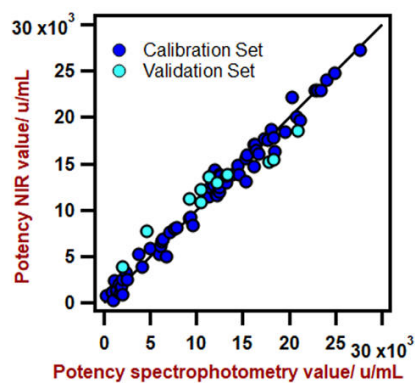
| Figures of merit                   | Value   |
|------------------------------------|---------|
| $R^2$                              | 0.9165  |
| Standard error of calibration      | 0.6938% |
| Standard error of cross-validation | 0.7896% |
| Standard error of validation       | 0.8628% |



**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.

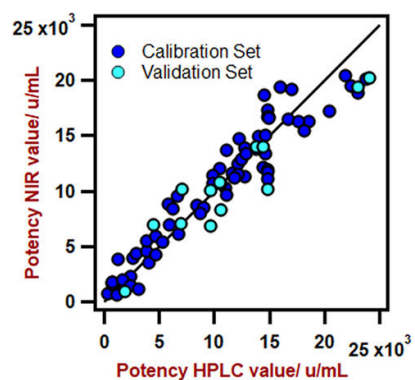
| Figures of merit                   | Value   |
|------------------------------------|---------|
| $R^2$                              | 0.9863  |
| Standard error of calibration      | 0.4767% |
| Standard error of cross-validation | 0.6821% |
| Standard error of validation       | 1.2429% |



**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 | 1 hour (sample preparation) + 20 minutes (HPLC + PCR) |

Internal reference: AW NIR CN-0017-112021

## CONTACT

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### DS2500 Solid Analyzer

**Robuste Nahinfrarotspektroskopie für die Qualitätskontrolle im Labor sowie im Produktionsumfeld.**

Der DS2500 Analyzer ist die bewährte, flexible Lösung für die Routineanalytik von Feststoffen, Cremes und optional auch Flüssigkeiten entlang der gesamten Produktionskette. Das robuste Design macht den DS2500 Analyzer unempfindlich gegen Staub, Feuchtigkeit, Vibrationen sowie Temperaturschwankungen und damit hervorragend geeignet für den Einsatz im rauen Produktionsumfeld.

Der DS2500 deckt den gesamten Spektralbereich von 400 bis 2500 nm ab und liefert in weniger als einer Minute genaue und reproduzierbare Ergebnisse. Der DS2500 Analyzer erfüllt die Anforderungen der pharmazeutischen Industrie und unterstützt durch die einfache Bedienung die Anwender in ihren täglichen Routineaufgaben.

Durch perfekt auf das Gerät abgestimmtes Zubehör werden bei jedem noch so herausfordernder Probentyp, wie z.B. grobkörnige Feststoffe wie Granulate oder halb fest-flüssige Proben wie Cremes, bestmögliche Ergebnisse erzielt. Bei Messungen von Feststoffen kann die Produktivität gesteigert werden durch Einsatz des MultiSample Cups, welches automatisierte Messungen in Serie von bis zu 9 Proben ermöglicht.





#### NIRS Transflexionsgefäß, optisch plan

optisch planes Transflexionsgefäß für die spektrale Vermessung von Flüssigkeiten. Kombinierbar mit folgenden Geräten:

- NIRS DS2500 Analyzer (**Bestellnummer: 2.922.0010**)
- NIRS XDS MaserLab Analyzer (**Bestellnummer: 2.921.1310**)
- NIRS XDS MultiVial Analyzer (**Bestellnummer: 2.921.1120**)
- NIRS XDS RapidContent Analyzer (**Bestellnummer: 2.921.1110**)
- NIRS XDS RapidContent Analyzer - Solids (**Bestellnummer: 2.921.1210**)



#### DS2500 Halter

Halter für die Verwendung mit:

- Kleinen Probengefäße (**6.7402.030**)
- DS2500 Iris (**6.7425.100**)



## Vision Air 2.0 Complete

Vision Air - Universelle Spektroskopie Software.

Vision Air Complete ist eine moderne und einfach zu bedienende Softwarelösung für den Einsatz im regulierten Umfeld.

Die Vorteile von Vision Air im Überblick:

- Individuelle Softwareanwendungen mit angepassten Nutzeroberflächen gewährleisten eine intuitive und einfache Bedienung
- Einfache Erstellung und Wartung von Arbeitsvorschriften
- SQL Datenbank für ein sicheres und einfaches Datenmanagement

Die Version Vision Air Complete (66072208) beinhaltet alle Anwendungen für die Qualitätssicherung mittels Vis-NIR Spektroskopie:

- Anwendung für das Instrumenten- und Datenmanagement
- Anwendung für die Methodenentwicklung
- Anwendung für die Routineanalyse

Weitere Vision Air Complete Lösungen:

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