Visualizing 'Invisible Fluctuations' on the Manufacturing Site
—Optimizing Process Control through Raman Spectroscopy—

Takashi Kinoshita

Yutaro Hirose

Shohei Miyamoto

Hirotaka Yabushita

In pharmaceutical manufacturing, product quality is strongly influenced by process conditions and process progression, making real-time monitoring essential to continuously observe and control the internal process state without interrupting operation. This is particularly important in biopharmaceutical cell culture, where variability is high and cellular metabolic and nutritional states directly determine final product quality and yield. Raman spectroscopy enables direct online measurement of key metabolites in bioreactors --such as glucose and lactate --because it is non-destructive and non-contact, requires little to no sampling or sample preparation, and is relatively insensitive to water interference. When combined with chemometrics (multivariate analysis such as Partial Least Squares, PLS), it can estimate metabolite concentrations and cell states with high accuracy, enabling applications such as automated nutrient feed control and quality prediction. Looking ahead, further advances are expected toward implementing more sophisticated Process Analytical Technology (PAT) by integrating AI and digital twins.

Keywords
Raman spectroscopy, process monitoring, biopharmaceuticals, pharmaceuticals, chemometrics

Introduction

To ensure a stable supply of high-quality and low-cost pharmaceuticals, innovation in analytical technologies for quality control is indispensable. The U.S. Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), which promotes harmonisation of pharmaceutical regulations across Japan, the United States, and Europe, strongly call for innovation in scientifically evidence-based quality and manufacturing management systems. Rather than testing only the final product as in conventional approaches, the concept of "Quality by Design (QbD)," in which the manufacturing process itself is designed and appropriately managed and controlled so that the intended quality is consistently achieved, is becoming increasingly important for ensuring product quality.

In this article, we introduce real-time monitoring of pharmaceutical manufacturing processes using Raman spectroscopy, covering topics from the principles of Raman spectroscopy to specific measurement case examples.

1. Fundamentals of Raman Spectroscopy

Raman spectroscopy has the advantage of requiring no sample pretreatment and being well suited to in situ observation, thereby offering high ease of measurement. In the following, we describe the principles of Raman spectroscopy, and the types of Raman spectroscopic instruments used in manufacturing processes.

1.1. Principle

When incident light interacts with matter, inelastically scattered light is generated (Figure 1a). This inelastic scattered light has a wavelength different from that of the excitation light and is referred to as Raman-scattered light. Because the wavelength of Raman-scattered light is shifted from the excitation wavelength by an amount corresponding to the molecular vibrational energy of the material being measured, it is used to obtain information on molecular structure and compositional characteristics (Figure 1b). Among the scattered light produced upon irradiation, scattered light having the same wavelength as the excitation light is also obtained; this is referred to as Rayleigh-scattered light. Because Rayleigh scattering is approximately 10^6 times more efficient than Raman scattering, it acts as background relative to Raman scattering and thus constitutes an inhibiting factor for compositional evaluation by Raman spectroscopy. Therefore, it is necessary to remove Rayleigh scattering using an optical filter and allow only Raman scattering to pass through[1].

1.2. Types

General-purpose Raman spectrometers can be broadly classified into a macro *type, intended for measurements over regions on the order of millimeters, and a micro type, which is integrated with an optical microscope to* measure local structures on the order of micrometers. Many macro-type instruments are portable and compact and are used, for example, for incoming inspection of raw materials. Transmission Raman spectroscopy, which is used for the quantification of active pharmaceutical ingredients (APIs) in tablets as described later, as well as probe Raman instrumentation for process monitoring, also fall into this category. By contrast, micro-type systems are characterized by high spatial and wavenumber resolution and the capability for imaging, and they are used mainly

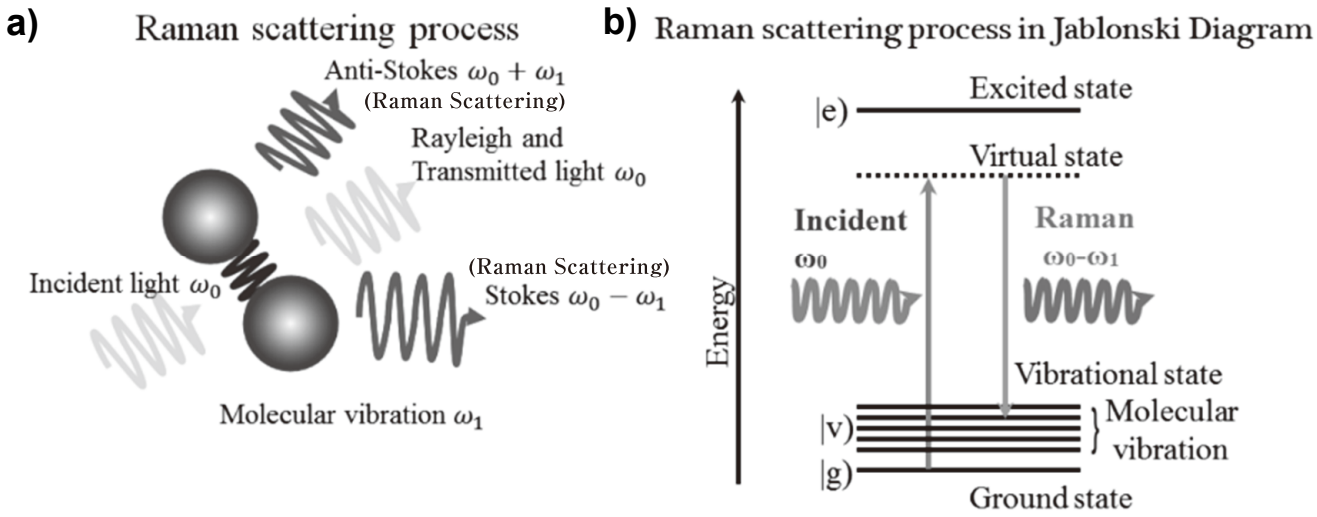

Figure 1 Conceptual diagram of molecular vibration (a), explanatory diagram of the Raman effect (b).

in pharmaceutical manufacturing development and quality control. Below, we describe two macro-type Raman analytical instruments that are primarily used in pharmaceutical production.

1.2.1. Transmission Raman

In transmission Raman spectroscopy, a laser is irradiated onto one side of a sample, and among the Raman-scattered light generated as the light undergoes diffuse reflection within the sample, the light transmitted through the sample is measured. Accordingly, an average Raman spectrum from the entire sample can be obtained. In addition, because Raman spectroscopy can provide sharp peaks and thus offers high chemical discriminative capability, it is less susceptible to the effects of changes in physical parameters (e.g., granule size and tablet thickness). For these reasons, transmission Raman spectroscopy has attracted attention as a method for quantitative determination of components in bulk samples.

1.2.2. Probe Raman

In recent years, with the shift toward continuous manufacturing of pharmaceutical tablets, there has been increasing demand to monitor their crystallinity and concentration. Compared with conventional methods, Raman spectroscopy—being non-destructive, non-contact, and non-invasive, enabling sample evaluation without pretreatment—has attracted attention as a technique that can be integrated into production processes for in-process evaluation. To incorporate a Raman spectrometer into a manufacturing line, a fiber-optic probe is used as the light-delivery/collection interface, and it is connected to a spectrometer and a detector. By adopting a fiber-optic configuration, remote measurements become possible, for example by separating the light source, spectrometer, and instrument control unit from the probe placed near the sample by several hundred meters. Probe designs include those that measure by direct contact with powders or solutions, as well as those that enable non-contact measurements through an observation window by using an objective lens at the probe tip. The use of probe Raman facilitates discrimination in-process among different polymorphic forms during manufacturing, detection of hydration states and phase transitions, and characterization of processes such as kneading (mixing) and drying.

2. Real-Time Monitoring Technologies Using Raman Spectroscopy

2.1. Advantages of Applying Raman Spectroscopy to Continuous Manufacturing Processes

Raman spectroscopy has recently become an important means of real-time monitoring in continuous manufacturing processes, as an analytical technique capable of obtaining chemical-structure information at the molecular level in a non-destructive and non-contact manner. Whereas laboratory analysis tends to focus on single measurements and short-term operation, continuous manufacturing sites require more advanced operation, including long-term stable operation of instruments and analytical methods, data reliability, and multipoint monitoring that provides an overview of the entire process. A key feature of Ramans instrumentation is that it requires little to no sample pretreatment and can be applied to samples in diverse states, including liquids, solids, and gases. Moreover, because water exhibits weak Raman scattering, Raman spectroscopy has the advantage of being readily applicable to processes that use water, such as those in the pharmaceutical and chemical industries. Against this background, Raman systems are rapidly becoming widespread in the field of process analysis.

2.2. Status Monitoring and Anomaly Detection to Support Continuous and Stable Instrument Operation

To operate Raman spectroscopic instrumentations stably over long periods in continuous manufacturing environments, it is essential not only to ensure instrument robustness but also to implement a real-time monitoring system that continuously tracks fluctuations in the instrument's internal status and the external environment. This enables early detection of potential failures or performance degradation, thereby allowing preventive maintenance and rapid response.

2.2.1. Specific Examples of Monitoring Parameters

Internal temperature of the instrument enclosure: Because excessively high or low temperatures can contribute to deterioration of electronic and optical components, the temperature should be continuously monitored using temperature sensors.

Laser status: The oscillation current, voltage, power, wavelength, and related parameters are measured in real time, and warnings are issued when abnormalities are detected.

Temperature of the spectrometer and detector: To prevent expansion of the spectrometer and optical components

due to temperature fluctuations, as well as increases in detector dark current, cooling mechanisms and temperature-control systems are implemented.

2.2.2. Anomaly Detection and Alert System

By monitoring these parameters, abnormalities in the instrument and changes in the external environment can be detected immediately. When an abnormality occurs, the system outputs alerts or error messages and is designed so that operators can respond promptly. This enables minimization of downtime and maintenance of data reliability.

2.3. Instrument and Data Protection Through Redundant Design

In continuous manufacturing processes, instrument failures can have a major impact on production efficiency and quality. Accordingly, Raman spectroscopic instrumentation incorporates redundant designs that assume the possibility of failures and component degradation, thereby enhancing system fault tolerance.

2.3.1. Instrument Redundancy (Example: Lasers)

Semiconductor lasers, which serve as the primary light source, inevitably undergo aging-related degradation due to heat and light exposure. Therefore, two lasers are installed, and automatic switching is performed at fixed intervals, enabling continued operation with the other laser even if one fails.

2.3.2. Redundancy for Data Protection (Example: Storage)

Loss of measurement data affects traceability and quality assurance. The instrument's hard disks are redundantly configured via mirroring (RAID configuration), thereby reducing the risk of data loss and system downtime in the event of disk failure. This improves data integrity and ensures the safety of critical process data.

2.4. Ensuring Long-Term Spectral Stability

In real-time monitoring, it is important to suppress the effects of day-to-day environmental changes and instrument aging on spectra and to continue acquiring data of equivalent quality over extended periods.

2.4.1. Temperature Control of Optical Components

Optical devices such as the laser, spectrometer, and detector are sensitive to changes in ambient temperature, and temperature fluctuations directly lead to spectral fluctuations. For example, temperature-induced changes in the emission wavelength of a semiconductor laser and thermal expansion of components in the spectrometer optics can shift the wavelength axis of Raman spectra. In addition, increases in detector temperature affect the S/N ratio of spectra. To maximize the performance of each component, it is desirable that the laser, spectrometer, and detector can each be independently regulated at their respective optimal temperatures.

2.4.2. Online Automatic Calibration Technology

Process Raman spectrometers are equipped with an internal reference light source, such as a noble-gas lamp, and include a function that periodically measures emission lines to automatically calibrate the spectrometer's wavelength axis. In addition, calibration of the laser emission wavelength is performed using known peaks of the sample, thereby enabling long-term stable management of both the spectrometer and the laser. As a result, highly accurate spectral data can be obtained without being affected by environmental changes or instrument aging.

2.5. Visualization of the Entire Process Through a Multipoint Measurement System

In continuous manufacturing, there is a strong need to manage multiple processes and multiple locations. In Raman spectroscopy, incorporating a multipoint measurement scheme makes it possible to grasp the status of the entire process and to further enhance quality control.

2.5.1. Multipoint Measurement Using a Multiplexer

In Raman spectroscopy, multipoint measurement can be achieved with a single instrument by using a multiplexer to switch among multiple measurement channels. This reduces instrument costs while enabling real-time understanding of the status of the overall process.

2.5.2. Operational Benefits of Multipoint Measurement

Multipoint measurement enables fine-grained monitoring of quality variations at each process and location, thereby allowing early detection of process abnormalities and process optimization. It also enables centralized monitoring of the entire production line and contributes to strengthening traceability and quality assurance.

3. Spectral Analysis Methods (Chemometrics)

3.1. Information Contained in Raman Spectra and the Importance of Analysis

Raman spectra directly and indirectly contain diverse information, including the concentrations of chemical components in a sample and physical properties (e.g., boiling point and density), as well as sensory attributes such as color and odor, mechanical strength, and other characteristics. In process monitoring, it is important to extract such information from spectra and utilize it for process control and quality control. However, Raman spectra constitute multidimensional data comprising hundreds to thousands of intensity values, and extracting the information of interest requires advanced data processing and analytical techniques.

3.2. Development of Calibration Models

3.2.1. Workflow for Building Calibration Models

A calibration model is a mathematical model that relates spectral data to calibration targets (e.g., component concentrations). Model development proceeds through the following steps: data collection, preprocessing, regression model construction, and validation.

3.2.2. Data Collection Methods

Measurement of process samples: Samples from the production line are measured using a Raman spectrometer, and, in parallel, target reference values for calibration (e.g., component concentrations) are obtained by offline analysis.

Data acquisition using design of experiments: To improve the generality and robustness of the calibration model, another approach is to prepare in-house samples with known concentrations and use them as model-building data. A method known as Design of Experiments (DoE) is employed, in which factors and levels are set in a planned manner to construct the model with as few experiments as possible.

Dataset splitting: The collected data are split into training data and test data in order to appropriately evaluate generalization performance on unseen data. The training data are used for hyperparameter selection and model fitting, whereas the test data are used to estimate the final generalization error.

3.2.3. Spectral Data Preprocessing Techniques

In addition to Raman-scattered light, spectral data include fluorescence, Rayleigh-scattered light, instrument-derived noise, and other components. To develop a high-accuracy model, preprocessing of spectral data to appropriately remove or reduce these effects is indispensable.

Wavenumber selection: Only wavenumber regions that are highly sensitive to the calibration target are selected. Effective wavenumber regions are explored using methods such as MWPLS, thereby improving model accuracy[2],[3].
Baseline correction: Flexible baseline estimation methods, such as the ALS method[4], are used to remove complex baselines. Polynomial correction, MSC, and derivative processing using a Savitzky–Golay filter[5] are also employed.

Normalization: To remove the influence of laser-intensity fluctuations, normalization is performed using the spectral area or the intensity of a specific peak.

Noise reduction and correction for interfering components: Methods such as binning, Savitzky–Golay filtering, and the GLSW method[6] are used to reduce random noise and suppress the influence of interfering components. In recent years, end-to-end preprocessing using deep learning has also been investigated[7],[8].

3.2.4. Construction of Regression Models

Regression algorithms are applied to the preprocessed spectral data (X) and the calibration target values (y) to build calibration models. In spectral analysis, PLS is widely used.

PLS: Spectral X, which exhibits strong collinearity, is projected onto a latent space that maximizes covariance with y, and y is regressed using a small number of variables. The dimensionality of the latent space (K) is optimized by cross-validation (CV). PLS includes PLS1, which corresponds to a single calibration target, and PLS2, which corresponds to multiple calibration targets.

Cross-validation (CV): The training data are divided into multiple subsets, and models are built and evaluated while holding out a portion of the data. The dimensionality of the latent variables is determined using RMSECV (the Root Mean Squared Error of Cross-Validation) as an index, thereby preventing overfitting.

Outlier removal: Abnormal data are detected and removed using distributions such as the Q statistic and Hotelling's

T2. This improves model accuracy and robustness.

Other regression methods: Machine-learning methods based on decision trees, such as GBM (gradient boosting machines), as well as nonlinear regression using deep learning, have also been actively studied in recent years.

3.2.5. Model Validation Methods

The developed calibration model is applied to a test dataset that was not used for training, and the prediction error, RMSEP, is evaluated. If RMSEP is confirmed to fall within a pre-specified acceptable range, generalization performance to unseen data is ensured.

3.2.6. Model Transfer and Calibration Techniques

Because developing calibration models entails substantial cost, reuse of models is desirable. In general, even instruments of the same model often do not exhibit identical spectral characteristics. Therefore, model reuse is achieved by applying techniques such as CCD channel sensitivity calibration using a standard light source[9], SBC (Slope/Bias Correction), PDS (Piecewise Direct Standardization), and TOP (Transfer by Orthogonal Projection), which transform the data to enable model transfer[10], [11].

3.2.7. Continuous Model Maintenance

Because calibration models are built based on training data, prediction accuracy may deteriorate when process conditions or sample composition change. It is important in practice to periodically compare offline analytical results for samples with the values predicted by the Raman spectrometer and, as needed, to update the model by adding or modifying training data. This approach enables maintenance of accuracy and reliability in long-term process monitoring.

3.3. Evolution of Analytical Methods and Future Perspectives

3.3.1. Utilization of Deep Learning and Machine Learning

In addition to conventional linear regression models such as PLS, deep learning and decision-tree-based machine-learning methods have recently begun to be applied to the analysis of Raman spectroscopic data[12],[13]. These methods are well suited to capturing nonlinearity and complex correlations and may enable end-to-end automation of the multi-step preprocessing traditionally required.

3.3.2. Improving Robustness and Generality

To maintain high-accuracy predictions even under diverse process conditions and sample variability, there is a growing need to improve the robustness and generality of analytical methods. Through technological innovations in noise reduction, outlier detection, and wavenumber selection the scope of Raman spectroscopy applications continues to expand.

4. Case Studies

4.1. Management of Active Pharmaceutical Ingredients Using Transmission Raman

For the quantification of pharmaceutical active ingredients, analysis is typically performed by dissolving tablets and then applying high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC). Analysis by transmission Raman enables quantitative determination of the drug substance comparable to that achieved by UPLC, using a method in which tablets or powders are measured without pretreatment and without the use of organic solvents (Figure 2). In addition, measurement is completed within a few seconds per tablet, providing high throughput. Furthermore, transmission Raman is useful not only for quantifying drug substances but also for determining the contents of crystalline polymorphs[14],[15], amorphous forms[16], and cocrystals[17].

Ohashi et al. reported that a calibration model for a drug developed using transmission Raman is robust to variations in process parameters in wet granulation and tableting processes, and that it can be applied to non-destructive analysis of tablets even at low drug concentrations[18]. Superiority over existing technologies such as

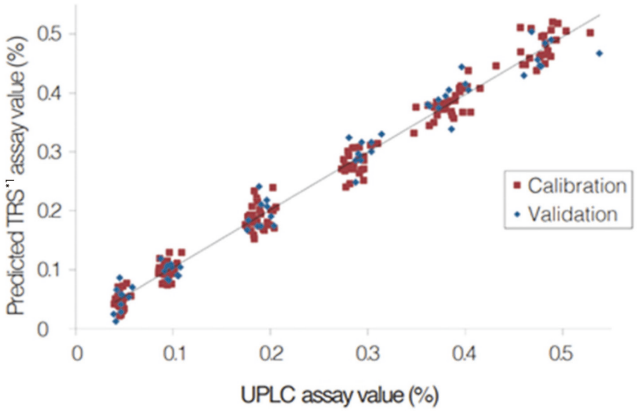

Figure 2 Correlation between Raman spectra and UPLC for tablets with different active ingredient concentrations (Provided by Professor Fukami, Meiji Pharmaceutical University).

*1 Transmission Raman spectroscopy

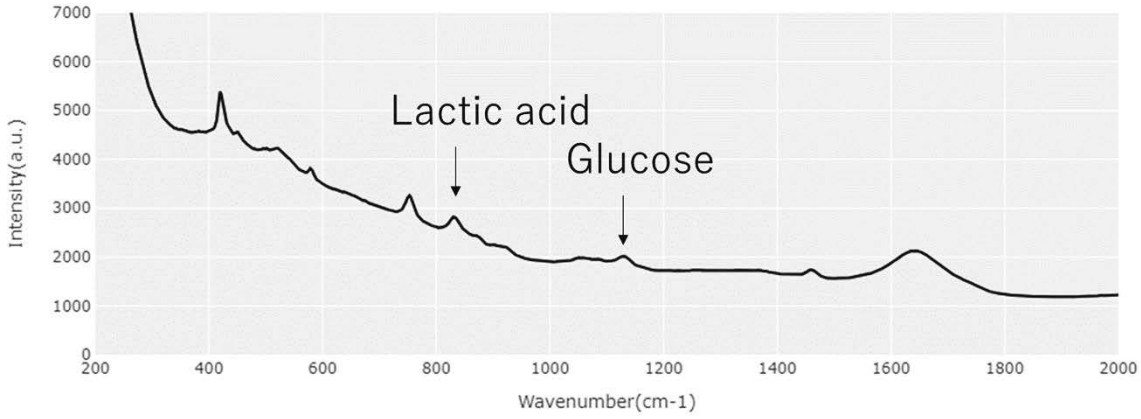

Figure 3 Raman Spectra of Cell Culture Medium.

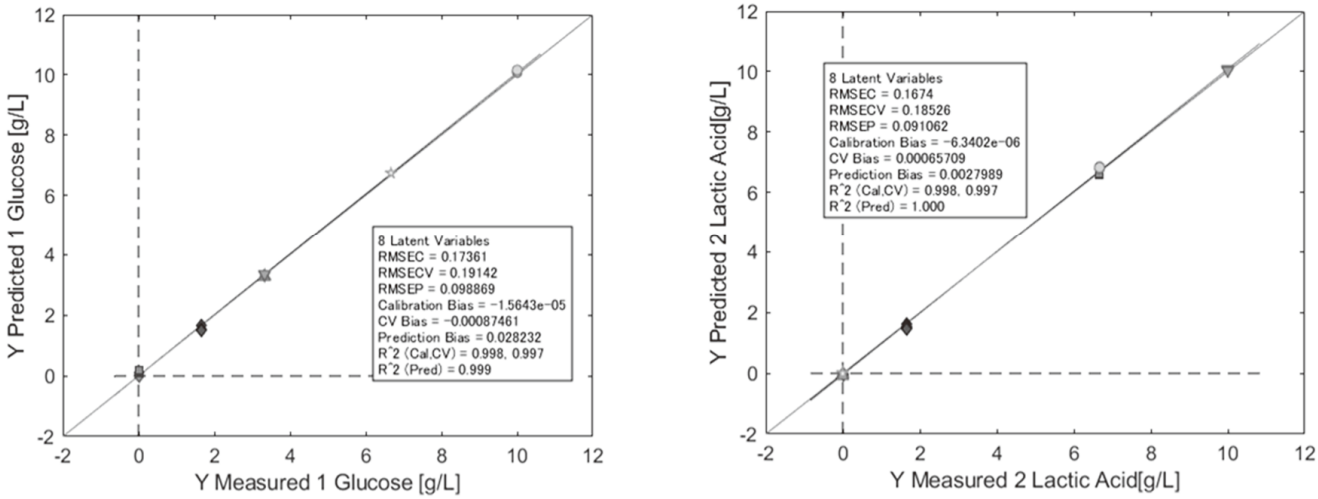

Figure 4 Prediction of Glucose and Lactic Acid concentrations from Raman Spectra using Chemometrics.

near-infrared spectroscopy has been demonstrated, and the use of transmission Raman as a process analytical technology (PAT) tool is expected to contribute to improving the robustness of continuous manufacturing.

4.2. Culture Process Monitoring

Monoclonal antibodies (mAbs) as therapeutic antibodies accounted for the majority of the biopharmaceutical industry in 2019, representing 70% of total sales. As demand for mAb products increases year by year and biosimilars[*2] are introduced, competition among industrial pharmaceutical companies is intensifying, with emphasis placed on maximizing production volume while reducing both manufacturing and development costs and shortening manufacturing lead times

In recent years, substantial improvements have been observed in bioreactor cell density, product yield, and process efficiency. Intensification and optimization of upstream processes are mainly associated with cell line development and media optimization, and in recent years, new strategies based on PAT and QbD have gained popularity.

*2 Biosimilar: Unlike generics (follow-on drugs) for low-molecule pharmaceuticals, a biosimilar is a follow-on version of a biopharmaceutical with a complex molecular structure.

Because it is difficult to produce an identical structure, “similarity” to and “equivalence” with the reference (originator) product are demonstrated through rigorous testing.

In cell-culture operations, particularly during cell proliferation, appropriate management of glucose feeding is critical. As shown in Figure 3, components such as lactate and glucose can be spectrally separated and assigned even in mixed solutions. Accordingly, by applying the techniques introduced in 3. Spectral Analysis Methods (Chemometrics), the concentrations of these components in culture media can be determined[18],[19] (Figure 4). If the glucose concentration is not properly maintained, the culture-medium environment deteriorates, leading to a decline in product quality. Similarly, excessive accumulation of lactate can also degrade the culture environment and may result in reduced cell growth and productivity. The introduction of PAT that combines Raman spectroscopy with chemometrics is considered to promote process

intensification and to be useful for shortening the development timeline of pharmaceuticals and enabling stable manufacturing.

Indeed, according to a report by Gibbons et al.[19], in batch cultures in which glucose concentration was controlled using Raman spectroscopy, the accuracy of glucose feeding improved, cell growth rate and viability increased, and the final product titer increased. They further reported that glycation was substantially reduced in some batches, resulting in improved product quality. These results demonstrate the effectiveness of process monitoring by Raman spectroscopy in cell culture.

Conclusion

In this article, we discussed practical considerations for applying Raman spectroscopy to real-time process monitoring, as well as data-analysis techniques. We also presented examples in which these approaches have been implemented in practice, namely, management of active pharmaceutical ingredients and monitoring of culture processes.

As a vibrational spectroscopic technique that is non-destructive and non-invasive and provides rich information, Raman spectroscopy is expected to continue to develop. In particular, recent years have seen rapid advances not only in laser, spectrometer, and detector technologies but also in data-analysis methods, including machine learning. By combining the various measures for stable long-term operation introduced in this article with these technological advances, we anticipate that Raman spectroscopy will be broadly applied to diverse industrial processes, including those involving previously uncharacterized composite materials and complex biological samples.

* Editorial note: This content is based on HORIBA's investigation at the year of publication unless otherwise stated.

References

[1] HORIBA, Ltd., " What is Raman Spectroscopy?" https://www.horiba.com/jpn/scientific/technologies/raman-imaging-and-spectroscopy/raman-spectroscopy/

[2] R. Hara et al., Development of Raman Calibration Model Without Culture Data for In-Line Analysis of Metabolites in Cell Culture Media, *Applied Spectroscopy*, 77(5), 521-533(2023)

[3] J.H. Jiang, R.J. Berry, H.W. Siesler, Y. Ozaki. Wavelength Interval Selection in Multicomponent Spectral Analysis by Moving Window Partial Least-Squares Regression with Applications to Mid-Infrared and Near-Infrared spectroscopic Data, *Analytical Chemistry*, 74(14), 3555–3565(2002)

[4] Gallagher, NB,“ Fitting Smooth Curves Part III: Baselining with an Asymmetric Least-Squares Algorithm,” [white paper] Eigenvector Research, Inc., www.eigenvector.com

[5] Gallagher, NB,“ Savitzky-Golay Smoothing and Differentiation Filter,” [white paper] Eigenvector Research, Inc., www.eigenvector.com

[6] Gallagher, NB et al., Generalized Weighting to Account for Sampling Artifacts, [white paper] Eigenvector Research, www.eigenvector.com

[7] Corner C. Horgan et al., High-Throughput Molecular Imaging via Deep-Learning-Enabled Raman Spectroscopy, *Analytical Chemistry*, 93, 15850-15860(2021)

[8] Hao He et al, Noise learning of instruments for high-contrast, high-resolution and fast hyperspectral microscopy and nanoscopy, *Nature communications*, 15, 754(2024)

[9] KRISTIN J. FROST, Calibration of Raman Spectrometer Instrument Response Function with Luminescence Standards: An Update, *Applied Spectroscopy*, 52(12), 1614-1618(1998)

[10] J.J.Workman Jr., A Review of Calibration Transfer Practices and Instrument Differences in Spectroscopy, Applied Spectroscopy, 72(3), 340-365(2018)

[11] L. Salguero-Chaparro et al., Calibration transfer of intact olive NIR spectra between a pre-dispersive instrument and a portable spectrometer, *Computers and Electronics in Agriculture* 96, 202–208(2013)

[12] Weng et al., Deep learning networks for the recognition and quantitation of surface-enhanced Raman spectroscopy, *Analyst*, 145, 4827-4835(2020) (in Japanese)

[13] Gao et al., Machine learning algorithms for rapid estimation of holocellulose content of poplar clones based on Raman spectroscopy, *Carbohydrate Polymers*, 292, 119635(2022)

[14] 柏木ら, 医薬品固形製剤の生産におけるラマン分光法の活用, *製剤機械技術学会誌*, 27(2), 42-51(2018) (in Japanese)

[15] Griffen JA et al., Rapid quantification of low level polymorph content in a solid dose form using transmission Raman spectroscopy, *J.Pharma. Biomed. Anal.*, 128, 35-45(2016)

[16] Terada H et al., Quantitation of trace amorphous solifenacin succinate in pharmaceutical formulations by transmission Raman spectroscopy, Int. *J.Pharm.*, 565, 325-332(2019)

[17] Koide T et al., Quantification of a cocrystal and its dissociated compounds in solid dosage form using transmission Raman spectroscopy, *J.Pharma. Biomed. Anal.*, 177, 112886(2020)

[18] Ohashi R et al., Effects of wet granulation process variables on the quantitative assay model of transmission Raman spectroscopy for pharmaceutical tablets, Euro. *J.Pharma. Biopharma.* 191,276-289(2023)

[19] L. Gibbons et al., An assessment of the impact of Raman based glucose feedback control on CHO cell bioreactor process development, *Biotechnol. Prog*, 39(5), e3371(2023)

Takashi Kinoshita
Biomedical Science Solution Dept.,
Bio & Healthcare Technology Division,
HORIBA, Ltd.

Yutaro Hirose
Bio & Health Care Field Dept.,
Sales Promotion Center,
Sales Division,
HORIBA, Ltd.

Shohei Miyamoto
IoT & Data Analytics Dept.,
Design Engineering Center,
R&D Division,
HORIBA, Ltd.

Hirotaka Yabushita
Data Science Manager
HORIBA Instruments Incorporated (HII)