Simplifying Progress

High Productivity and Process Economy in GxP Applications with the Octet® Platform

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Abstract

Utilizing the label-free optical technique of Bio-Layer Interferometry (BLI), the Octet® BLI platform provides real time analysis of biomolecular interactions. It relies on the robust and easy to use Dip and Read format, which provides faster time to results relative to technologies like ELISA and SPR. It also operates in a fluidics-free format thereby minimizing the complexity in analyte detection by fluidics-based technologies like SPR or Grating Coupled Interferometry (GCI). It provides highthroughput analysis, with the capability of analyzing up to 96 samples simultaneously, thereby increasing analytical productivity. It has a high tolerance to a diverse array of sample types, making it compatible with in-process testing during drug substance manufacturing. In addition, samples analyzed on an Octet® system can be reused for other analysis, minimizing depletion of precious samples and maximizing process economy. In this poster, we will describe multiple ways in which the Octet® platform is well suited for GxP and QC applications.

Introduction to BLI technology

BLI is an optical technique that analyzes the interference pattern of white light reflected from two surfaces – a layer of immobilized protein on a biosensor tip and an internal reference layer (Figure 1A). Any change in the number of molecules bound to the biosensor tip causes a shift in the light interference pattern, which can be measured in real time (Figure 1A and 1B). The binding between a ligand immobilized on the biosensor surface and an analyte in solution produces an increase in optical thickness measured as a wavelength shift, Δλ (Figure 1C)

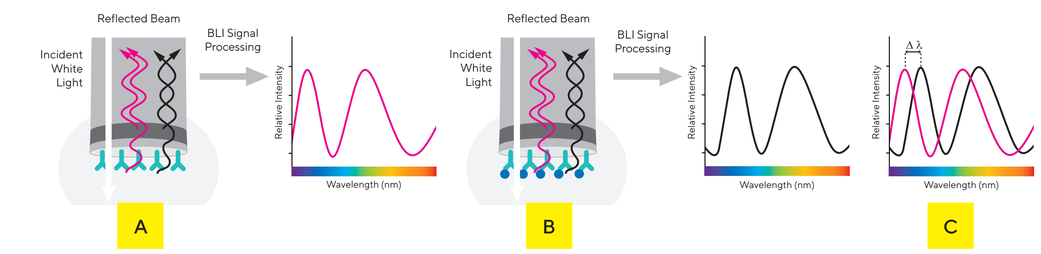

Figure 1. Schematic overview of the proprietary Octet® BLI technolgy, a label-free optical technique based on fluidic-free format in SLAS standard microtiter plates. Relative intensity of the light reflection pattern from the two surfaces on the biosensor (A & B). Octet® sytems with BLI technology measure the difference in the reflected light's wavelength (Δλ) between the two surfaces (C).

GMP Compliant Octet® Platforms and Services

Multiple models of the Octet® instruments (Figure 2) are available for use in GMP compliant labs*:

Octet® R2 – Grows with your biomolecule characterization needs. Simultaneous reading of up to two samples in 96 well plates.

Octet® R4– The perfect balance between cost and throughput with given leeway. Simultaneous reading of up to four samples in 96 well plates.

Octet® R8 – Unmatched flexibility and versatility in protein analysis. Simultaneous reading of up to eight samples in 96 well plates. Evaporation cover included for longer runs.

Octet ® RH16 - Automation compatible – For high throughput small and large molecule characterization. Simultaneous reading of up to 16 samples in 96 or 384 well plates.

Octet® RH96 – Automation compatible – For high throughput small and large molecule characterization. Simultaneous reading of up to 96 samples in 96 or 384 well plates.

* All systems listed are GMP compliant with IQOQ and are compatible with CFR software. The R8 and RH16 have enhanced GMP compliance with additional performance qualification (PQ) and IQOQ kits.

Octet® R2 System | **Octet® R4 System** | **Octet® R8 System** | **Octet® RH16 System** | **Octet® RH96 System**

Figure 2. Octet® BLI range of instruments.

In order to meet the quality standards required of a GMP compliant lab the Octet® Systems are provided with all essential requirements (Figure 3) stated below:

- Octet® 21 CFR Part 11 software
- IQOQ & PQ packages
- Software validation support
- Biosensor validation support services
- Technical support assistance

Comprehensive GxP packages including the Octet® instrument and accessories are available for seamless integration into GxP workflows. Each GxP package contains:

Octet® system of your choice – Five systems to choose from:

Installation and Operational Qualification (IQOQ) Services – This comes with IQOQ Kits providing documented verification that the Octet® instrument operates as intended at the user site by inspecting the critical components. It is installed per Sartorius' guidelines by certified personnel.

Performance Qualification (PQ) Kits – Specific PQ Kits are available for Octet® R8 and Octet® RH16 providing rapid and convenient methods to verify performance of your Octet® instrument. PQ can be performed using one or both of two tightly controlled kinetics (PQ-K) and quantitation (PQ-Q) assays.

Octet® CFR Software – Using software that complies with 21 CFR Part 11 guidelines is a regulatory requirement for using any analytical instruments in a GxP environment. Octet® CFR software complies with this requirement by including features such as controlled user access, comprehensive audit trails, customized locked reports and electronic signatures.

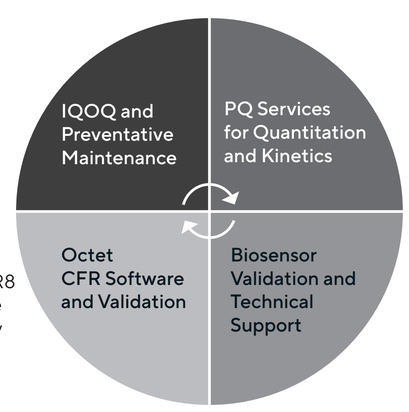

Figure 3. GxP Compliance.

Software Validation Package Support – The customer-run Octet® Software Validation Package provides the most comprehensive documentation and tools available to validate the Octet® CFR software features. It trims validation time to just three days using documentation that mirrors the validation process in a regulated laboratory.

Biosensor Validation Service – This service enables Octet® system users to sample multiple lots of a biosensor during assay qualification and validation, and reserve a well-characterized lot for purchase.

Faster Time to Results with Easy to Use Dip and Read Format

The Octet® BLI platform provides a convenient and reliable method for measuring antibody and protein concentrations. The simple fluidic-free Dip and Read approach enables streamlined workflows and rapid quantitation of up to 96 samples in as little as 5 minutes on the Octet® RH96 system or 96 samples in < 30 minutes for the Octet® RH16 system. Samples can be analyzed in cell culture supernatant or in complex media, thus eliminating the need for time consuming for purification and minimizing the potential for valuable sample loss during this process. We estimate that Octet® instruments provide increased sample analysis capacity over ELISA and most SPR instruments, which can lead to an increase in productivity and to significant FTE cost savings, enhanced productivity and labor efficiency in lot release and in-process testing of biologics in GxP laboratories. View Octet® GxP Whitepaper[1] for further details.

In a typical quantitation assay, biosensors coated with capture molecules are dipped into samples in the sample plate and the on-rate is measured in real time. The measured on-rate is then used to determine the concentration of the target protein against a standard curve with signals from known analyte concentrations. In quantitation assays, precision, linearity and accuracy are key parameters that must be demonstrated in order for the assay to be validated for use in manufacturing. In addition, the limit of quantitation (LOQ) should be determined to increase confidence in the assay's performance. As an example, the Octet® BLI technology was used to determine method precision and accuracy in the quantitation of recombinant insulin.[2] High Precision Streptavidin biosensors (SAX) were coated with anti-insulin antibody and used to bind purified insulin samples. Known insulin sample concentrations were prepared in Sartorius' 1X Kinetics Buffer at concentrations ranging from 0–50 µg/mL and used in binding assays (Figure 4A) to generate a standard/reference curve (Figure 4B). Three samples (25, 6.25 and 1.56 µg/mL) of insulin were also spiked in 1X Kinetics Buffer and treated as unknown samples. Samples were performed in triplicate and data analyzed using the initial slope binding rate analysis mode in Octet® Data Analysis software.

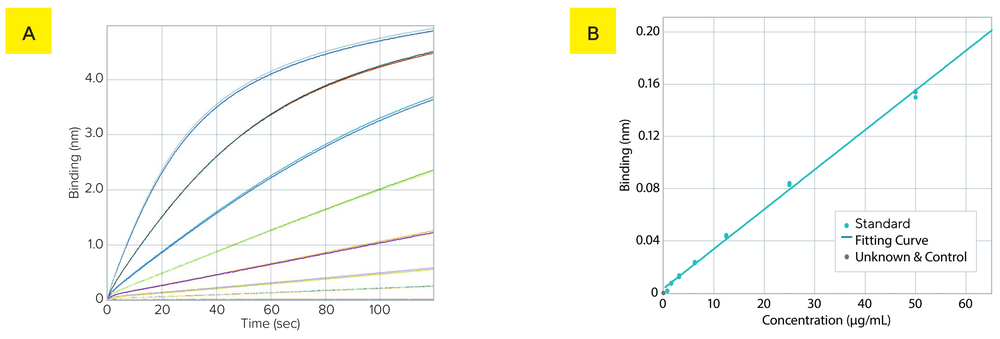

Figure 4: Binding of known concentrations of recombinant insulin (A) used to generate a standard/reference curve (B) for the quantitation of recombinant insulin. SAX biosensors loaded with an anti-insulin antibody were used for the studies.

The standard samples exhibited concentration % CVs of < 3% (Table 1), while the unknown samples exhibited concentration % CVs below 2% indicating excellent precision. Dose recovery for the unknown samples was found to be within 102–106%, indicating excellent method accuracy (Table 2). A similar approach can be used for other recombinant proteins.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. CV
50.0	1.02	50	2.39
25.0	0.5	25	1.88
12.5	0.1872	12.5	1.24
6.25	0.0611	6.2	1.48
3.13	0.0189	3.1	0.35
1.56	0.0058	1.6	2.83
0.78	0.0012	0.8	0.28
0.0	0.0001	0.0	0.0

Table 1: Insulin quantification. Standard samples exhibit excellent precision at < 3% CV.

Conc. µg/mL	Binding rate, nm/s	Conc. avg, µg/mL	Conc. CV	% Recovery
25.0	0.5182	25.9	1.41	104
6.25	0.064	6.4	0.16	102
1.56	0.0066	1.7	1.4	106

Table 2: Insulin quantification. Unknown samples, n=3, exhibit excellent precision and accuracy.

Reproducible Potency Assays and Lot-to-Lot Comparison During Drug Substance Manufacturing

Ligand binding kinetics assays are increasingly finding use as batch lot release methods, especially in potency assays. In these binding kinetics studies, the interaction is often assessed through the measurement of either the affinity of the analyte/drug product to a receptor or ligand immobilized on the biosensor surface or by monitoring the drug product's binding response signals as a function of concentration and comparing it to a control reference product for relative potency assesments.[3] Sartorius offers ready-to-use biosensors such as Ni-NTA and FAB2G biosensors for the capture of different panels of Fc gamma receptors that can in turn be used to bind drug products for potency assesment.[4] In all cases, reproducibility measured through precision and accuracy are key metrics. Octet® BLI systems are highly suitable for these assays as they decrease method development time significantly compared to SPR and ELISA. An overlay of replicate data (Figure 5) for the binding of an FcRn molecule to an IgG, with the IgG captured onto Anti-Human Fab-CH1 (FAB2G) biosensors shows excellent reproducibility.[5]

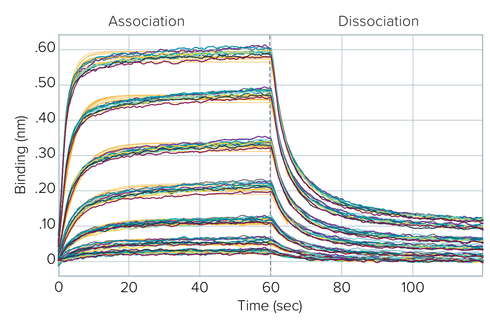

Figure 5: Overlay of several replicates of FcRn-IgG interactions on the Octet® BLI platform using FAB2G biosensors.

Stability and Forced Degradation Studies

The Octet® BLI platform can also be used for developing stability-indicating assays, and are suitable for measuring and distinguishing between fully-functional drug products and those whose binding activities have been affected by degradation. In one study, it was used to distinguish between native and deactivated antigen and showed reduced binding activity of the deactivated antigen[6], hence proving the assay was stability indicating. In another study[7] (Figure 6), the affinity of an IgG1 to an Fc gamma receptor IIIa molecule was shown to decrease with increasing percent deglycosylation, further indicating that Octet® BLI systems are suitable for use in developing stability-indicating methods.

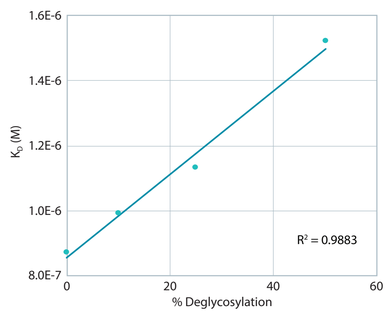

Figure 6: Affinity analysis of the binding of an IgG1 to an Fc gamma receptor as a function of deglycosylation.

References

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Conclusion

Sartorius offers Octet® BLI system users complete qualification, validation, and support solutions that ensure compliance in the regulatory space, and allow rapid development, optimization and validation of assay methods for various applications in GLP and QC laboratories.