Variable Pathlength Spectroscopy for GLP-1 Concentration Analysis: Improving Analytical Efficiency Through UV Platform Standardization

Application Note

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Abstract

As antibody formulations increase in concentration, traditional UV-Vis spectroscopy encounters several operational limitations that can negatively impact laboratory productivity and data quality. Variable Pathlength Spectroscopy is the modern analytical alternative designed to overcome these limitations while simplifying laboratory workflows.

Variable Pathlength Spectroscopy has been widely adopted for protein concentration analysis in monoclonal antibody manufacturing, and the same analytical procedures are increasingly applicable to the rapidly growing field of GLP-1 therapeutics. The commercial success of GLP-1 receptor agonists has accelerated investment in peptide manufacturing capacity, creating many of the same analytical and operational challenges historically encountered in monoclonal antibody production. Biopharmaceutical manufacturers require accurate concentration measurements to monitor purification performance, optimize formulation processes, support analytical testing, and demonstrate product quality throughout development and commercial manufacturing. As production volumes increase and manufacturing timelines become more compressed, laboratories are seeking platform technologies that deliver rapid, reliable, and standardized concentration measurements across diverse biologic modalities.

This paper discusses the advantages provided by Variable Pathlength Spectroscopy and presents a brief feasibility study for GLP-1 concentration analysis.

Introduction

Glucagon-like peptide-1 (GLP-1) therapeutics are a rapidly growing modality in biopharmaceutical manufacturing. While GLP-1 receptor agonists differ structurally from monoclonal antibodies, their manufacturing processes rely on many of the same quality control principles. Analytical workflows must provide precise concentration measurements to support process development, in-process monitoring, product release, and stability testing, all while maintaining compliance with current Good Manufacturing Practice (cGMP) requirements. Traditional fixed-pathlength UV methods can introduce bottlenecks through sample dilution, manual calculations, and analyst-dependent variability—challenges that become increasingly significant in high-throughput manufacturing environments. Variable Pathlength Spectroscopy addresses these limitations by eliminating routine dilution steps, automating data analysis, and providing a robust, transferable analytical platform capable of supporting both established monoclonal antibody processes and the expanding portfolio of peptide-based therapeutics, including GLP-1 products. This convergence highlights the opportunity to leverage proven analytical methodologies across multiple classes of biologics, improving laboratory efficiency while maintaining the accuracy and data integrity required for modern biopharmaceutical manufacturing.

Growth of High-Concentration Biotherapeutics and GLP-1 Medicines

While monoclonal antibodies continue to dominate the biologics market, GLP-1 products have emerged as one of the fastest-growing therapeutic classes worldwide. Initially developed for the treatment of type 2 diabetes, GLP-1 therapeutics are now widely prescribed for obesity and chronic weight management. Continued clinical development has expanded their investigation into cardiovascular disease, chronic kidney disease, metabolic dysfunction-associated steatohepatitis (MASH), obstructive sleep apnea, and other chronic metabolic disorders. This broadening therapeutic landscape has driven unprecedented demand for large-scale biologics manufacturing.

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Unlike traditional small-molecule pharmaceuticals, GLP-1 therapeutics are peptide or protein-based biologics that require highly controlled manufacturing processes. Although these molecules differ structurally from monoclonal antibodies, they share many of the same analytical requirements throughout development and manufacturing, including accurate protein concentration measurements during purification, formulation, quality control testing, and product release.

Commercial demand for GLP-1 products has accelerated investments in manufacturing capacity worldwide. Major manufacturers, including Novo Nordisk, Eli Lilly, Amgen, Pfizer, AstraZeneca, Roche, and Boehringer Ingelheim, have announced significant expansions of biologics manufacturing facilities and contract manufacturing partnerships to increase production capacity for both marketed and next-generation incretin therapies.

The scale of these manufacturing investments has intensified the need for robust analytical technologies capable of supporting higher sample throughput while maintaining regulatory compliance and data integrity. Regulatory agencies require manufacturers to demonstrate that therapeutic products consistently meet predefined specifications for identity, purity, potency, and concentration before release for clinical or commercial use. Analytical methods that can eliminate unnecessary sample preparation and standardize workflows across manufacturing sites are increasingly valuable in both monoclonal antibody and peptide therapeutic production.

Analytical Challenges Associated with High-Concentration Drug Products

Product concentration influences virtually every stage of biopharmaceutical manufacturing. During downstream purification, accurate concentration measurements ensure chromatography columns are loaded within validated operating ranges. Overloading purification media can reduce product recovery and compromise impurity clearance, while underloading decreases manufacturing efficiency and increases production costs. Thus, concentration measurements are essential for formulation development, product release, stability assessment, and therapeutic dose determination.

The trend toward higher-concentration biologic formulations places additional demands on traditional analytical methods. Concentrated samples frequently exceed the linear absorbance range of conventional fixed-pathlength UV spectrometers, requiring multiple dilution steps prior to analysis, increasing analyst workload and introducing opportunities for pipetting error, sample handling variability, and transcription mistakes. Within GMP quality control laboratories, where hundreds of protein concentration measurements may be performed each week, these additional steps can significantly reduce laboratory productivity while increasing the likelihood of repeat analyses.

Additionally, most GLP-1 products are made with multiple options for dosing concentrations depending on the patient. However, many assays require standardized sample concentrations to maintain method performance and operate within validated analytical ranges. This constraint can create a need to develop and validate multiple methods for the same drug product lines.

Commercial biopharmaceutical manufacturing requires analytical technologies that simplify protein concentration analysis, reduce workflow complexity, and maintain the accuracy, precision, robustness, and regulatory compliance expected of GMP quality control laboratories.

Conventional UV-Visible Spectroscopy

Protein concentration is traditionally determined using UV absorbance at 280 nm, where aromatic amino acids such as tryptophan and tyrosine exhibit strong absorbance. Measurements are calculated using the Beer-Lambert law:

$$A = \varepsilon l c$$

where

- A is the absorbance
- ε is the extinction coefficient
- l is the optical pathlength
- c is the protein concentration

When the extinction coefficient and pathlength remain constant, absorbance is directly proportional to concentration.

Limitations of Fixed-Pathlength Measurements

Micro-volume spectrophotometers and conventional UV spectroscopy exhibit several inherent limitations. The most significant limitation of fixed-pathlength UV methods is that they rely on a single-point absorbance measurement. Because only one absorbance value is collected, there is no direct assessment of whether the measurement falls within the linear region of the Beer-Lambert relationship. As concentrations increase, absorbance values approach the instrument's upper detection limit where stray light, detector saturation, and optical nonlinearities begin to affect measurement accuracy. To compensate, analysts must dilute samples until absorbance falls within the validated operating range of the instrument. These dilution steps increase assay complexity and introduce

opportunities for pipetting errors, analyst-to-analyst variability, transcription mistakes, and sample handling errors. In GMP quality control laboratories, where consistency and data integrity are paramount, these additional manual steps often become the largest contributors to measurement uncertainty, rather than imperfections in the instrument itself.

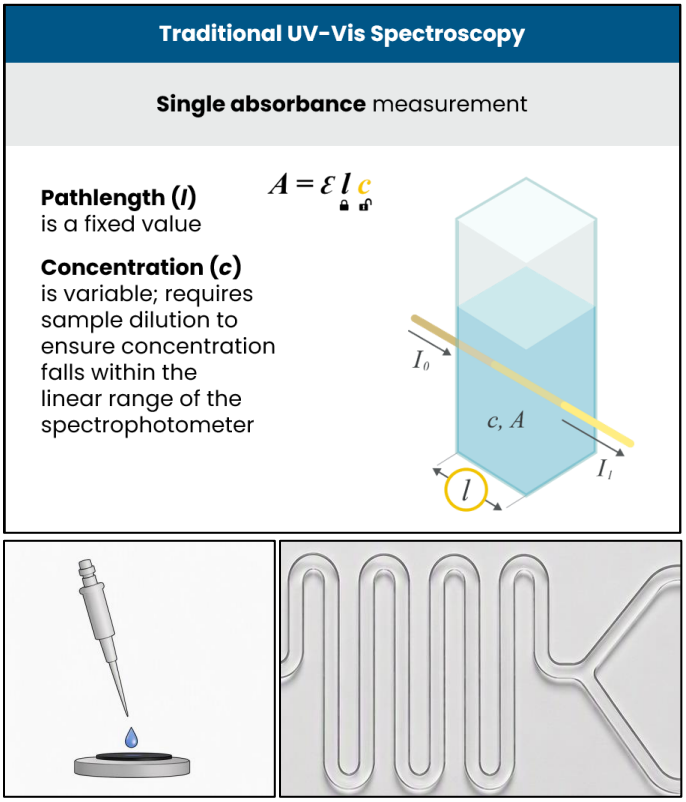

Figure 1. Conventional analytical methods exhibit inherent risk and complexity.

Variable Pathlength Spectroscopy

The Slope Spectroscopy® method fundamentally changes this analytical approach. Rather than relying on a single absorbance measurement, the analytical instrument automatically collects multiple absorbance measurements across a range of optical pathlengths, generating a dense absorbance-versus-pathlength dataset for each sample. Protein concentration is calculated from the slope of the linear regression according to the Beer-Lambert law, $A = \varepsilon lc$, rearranged as:

$$\frac{A}{l} = \varepsilon c$$

The slope m, defined as $m = \frac{A}{l}$, is then directly proportional to protein concentration.

This multi-point approach offers several analytical advantages. First, it verifies Beer-Lambert linearity during every measurement, providing an inherent quality check that is unavailable with traditional fixed-pathlength instruments. Second, because the instrument automatically selects pathlengths that maintain absorbance within the linear region, concentrated samples can typically be analyzed without dilution, dramatically reducing sample preparation and operator-dependent variability. Finally, the use of multiple data points improves measurement robustness by minimizing the influence of random noise or isolated measurement artifacts.

From an operational perspective, the differences become even more significant. Micro-volume spectrophotometers and conventional UV spectroscopy workflows often require blank measurements, serial sample dilutions, manual concentration calculations, and secondary review of calculations to satisfy GMP documentation requirements. In contrast, Slope Spectroscopy automates concentration determination, eliminates routine dilution steps, and generates standardized analytical reports directly from the instrument software. The result is a simplified, high-throughput workflow that improves reproducibility, enhances data integrity, and reduces laboratory turnaround time.

Table 1. Analytical methods comparison

Microvolume Spectrophotometer / Traditional UV Spectroscopy	Repligen's Slope Spectroscopy (Variable Pathlength) Method
Single absorbance measurement	Multiple absorbance measurements across several pathlengths
Fixed (or limited selectable) pathlength	Continuously optimized pathlength
Frequently requires sample dilution	Samples measured directly
No verification of Beer-Lambert linearity	Linearity verified during every measurement
Greater dependence on analyst technique	Minimal operator influence
Manual calculations and review often required	Automated concentration calculation and reporting
Higher risk of dilution and pipetting error	Reduced sample preparation error
Narrow practical dynamic range	Broad dynamic range (approximately 0.01 to 300+ mg/mL)
Better suited for research use and low-throughput testing	Optimized for GMP QC, manufacturing support, and high-throughput environments

These advantages become increasingly valuable as manufacturers expand production of high-concentration biologics—including monoclonal antibodies and GLP-1 therapeutics—where large sample volumes, compressed manufacturing timelines, and stringent regulatory expectations demand analytical methods that are both efficient and highly reliable.

ASTM E169-16 (reapproved 2022) establishes general practices for quantitative UV-Vis spectrophotometric analysis, including recommendations for sample preparation, calibration, measurement, and data analysis to ensure accurate and reproducible results. Notably, the standard recognizes automated pathlength adjustment as a means of extending the analytical range, stating:

> *To avoid the dilution step, the instrument may contain an automatic system which will allow adjustment of the path length of the measurement cell to optimize the measured absorbance.*

The growing acceptance of variable pathlength measurements within industry standards reflects an important evolution in UV-Vis spectroscopy. By recognizing automated pathlength adjustment as a means of optimizing absorbance and eliminating unnecessary dilution steps, these standards provide a scientific foundation for analytical approaches such as Slope Spectroscopy that leverage variable optical pathlengths to improve accuracy, precision, and workflow efficiency.

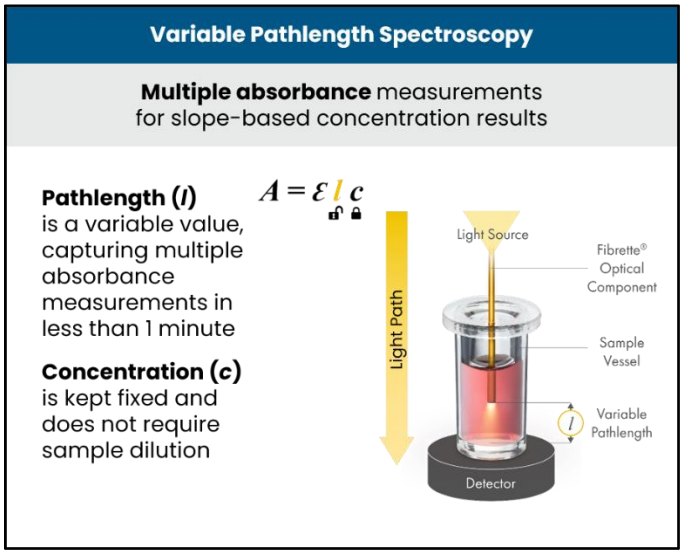

Figure 2. Variable pathlength method illustration

Workflow Comparison

Conventional Workflow

Protein concentration analysis using conventional fixed-pathlength UV spectroscopy has long served as the industry standard for release and in-process testing within GMP Quality Control (QC) laboratories. While this methodology is well established and widely accepted, the analytical workflow requires numerous manual steps to ensure compliance with regulatory expectations for accuracy, precision, and data integrity. Each manual operation represents a potential source of analytical variability, including pipetting inaccuracies, dilution errors, transcription mistakes, and calculation discrepancies. Although these risks are mitigated through procedural controls and independent verification, they increase analyst workload and lengthen assay turnaround time.

A typical GMP workflow using conventional UV spectroscopy includes:

1. Instrument qualification and system suitability verification
2. Background (blank) measurement
3. Assay control or reference standard analysis
4. Sample preparation in triplicate
5. Serial dilution of samples to achieve an acceptable absorbance range
6. UV absorbance measurements
7. Manual concentration calculations incorporating dilution factors and extinction coefficients
8. Independent verification of calculations as part of GMP data review
9. Documentation, quality review, and final reporting

Variable Pathlength Spectroscopy Workflow

Variable Pathlength Spectroscopy allows highly concentrated protein samples to be analyzed directly in their native formulation, eliminating the need for manual dilution while maintaining analytical accuracy across a broad concentration range.

The streamlined GMP workflow using Variable Pathlength Spectroscopy consists of:

1. System suitability test
2. Direct sample analysis without dilution

The system automatically calculates concentration and reports the result electronically. By removing dilution and manual calculation steps, Variable Pathlength Spectroscopy significantly reduces analyst intervention while simplifying method execution. Automated data processing minimizes transcription

and calculation errors, improves result consistency between operators, and reduces overall assay variability.

Workflow Impact on GMP Operations
The simplified workflow offered by Variable Pathlength Spectroscopy provides measurable operational advantages within regulated biopharmaceutical laboratories. Reducing the number of manual processing steps not only improves laboratory efficiency but also supports key GMP objectives related to process robustness, data integrity, and analytical reproducibility.

Beyond reducing assay time, elimination of dilution significantly decreases cumulative analytical error associated with pipetting precision and dilution factor calculations. These improvements become increasingly important during late-stage process development and commercial manufacturing, where protein concentrations routinely exceed 150–250 mg/mL for monoclonal antibodies and continue to increase for next-generation biologics.

In practice, this simplification of the workflow reduces analyst hands-on time to 2 minutes per sample using the PATsmart™ SoloVPE® PLUS System, approximately a 45-minute reduction compared to conventional workflows. For laboratories processing approximately 100 samples each week, this equates to an estimated 8 hours saved per day or 30 laboratory days recovered annually. Additionally, because measurements are performed using consistent automated workflow, analytical methods can be transferred more easily between laboratories and manufacturing sites.

Feasibility Study

To demonstrate the SoloVPE PLUS System's ability to accurately quantify high-concentration peptide therapeutics, a test was performed using a commercially available tirzepatide injection (Zepbound, Eli Lilly and Company). Tirzepatide is a synthetic 39-amino acid peptide formulated in an aqueous buffer containing excipients including sodium chloride and phosphate buffer components to maintain isotonicity and pH.

Materials and Methods

- PATsmart SoloVPE PLUS instrument [IN-VPE-SOLO-P]
- Cary 60 UV-Vis Spectrophotometer [IN-CARY60]
- Fibrette® Optical Components [OF0002-P50]
- SoloVPE Plastic Sample Vessels [OC0009-P50]
- Stock lab supplies (pipettes, tips, etc.)
- 15 mg/mL tirzepatide (Zepbound, Eli Lilly)

Samples were analyzed directly from the commercial drug product without dilution, allowing assessment of Variable Pathlength Spectroscopy under conditions representative of final drug product testing.

Results and Discussion

The SoloVPE PLUS System first collected a series of absorbance spectra at discrete optical pathlengths. Spectra were acquired only within the instrument's validated linear response region, ensuring compliance with the Beer-Lambert law throughout the regression dataset. By restricting analysis to linear absorbance values, the variable pathlength method eliminates non-linearity associated with detector saturation while maximizing analytical accuracy, precision, and dynamic range for high-concentration biopharmaceutical samples.

Figure 3 illustrates the absorbance vs. pathlength regression at 280 nm used for concentration determination by Slope Spectroscopy. Individual absorbance values are collected at the same wavelength but different, discrete pathlengths within the validated linear response region. The data points exhibit a highly linear relationship, demonstrating adherence to the Beer-Lambert law. The concentration is calculated from the slope of the linear regression rather than a single absorbance reading.

Measurement data of the GLP-1 sample is given in Table 2. The readings demonstrated superb accuracy, precision, and linearity.

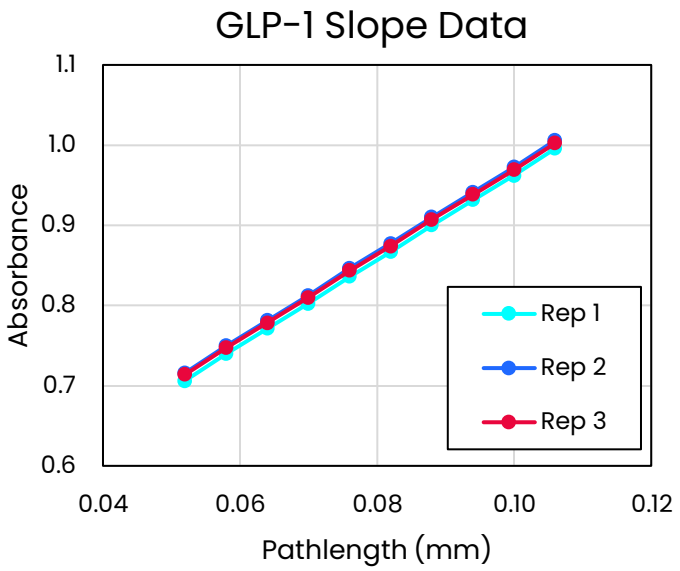

Figure 3. SoloVPE PLUS absorbance vs. pathlength data

Table 2. SoloVPE PLUS data: GLP-1 sample

Rep	Wavelength	EC [$cm^{-1}(mg/mL)^{-1}$]	Slope (Abs/mm)	Concentration (mg/mL)	R^2	Data Points
1	280 nm	3.560	5.3438	15.010	0.9999	10
2	280 nm	3.560	5.3487	15.024	0.9999	10
3	280 nm	3.560	5.3281	14.966	0.9999	10
Average Over Three Replicates			**5.3402**	**15.001**	**0.9999**	**—**

Conclusion

Slope Spectroscopy has represented a significant advancement in protein concentration analysis for monoclonal antibody manufacturing for nearly 20 years. By eliminating routine sample dilution, automating concentration calculations, and expanding the measurable concentration range, variable pathlength technology addresses many of the practical limitations associated with traditional UV spectroscopy.

Expanding into modalities such as the GLP market applies the same techniques and extends the same benefits that have become the gold standard, allowing for continued expansion and adoption of this platform. For GMP quality control laboratories, these improvements translate into enhanced analytical consistency, improved data integrity, reduced analyst intervention, and substantially increased laboratory throughput. As biologics continue to increase in complexity and concentration, Variable Pathlength Spectroscopy offers a scalable analytical platform capable of supporting modern biopharmaceutical manufacturing while maintaining the rigorous standards required for regulatory compliance.

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