Multi-Wavelength UV/DUV Photometry for Biomolecule Concentration Measurement in GMP and Non-GMP Testing

The Kemtrak SPECTRA industrial process photometer utilizes multi-wavelength ultraviolet (UV) and deep ultraviolet (DUV) technology for the continuous measurement of biomolecule concentrations in bioprocessing. With up to 10 simultaneous discrete wavelengths available, SPECTRA delivers real-time, accurate results with exceptional system-to-system, operator-to-operator, and site-to-site consistency.

- **No Dilutions**: Measure highly concentrated samples without pretreatment
- **Instant Results**: Real-time data acquisition
- **No Sampling**: Non-destructive inline measurement
- **No Consumables**: No reagents or moving parts
- **Low Cost of Ownership**: Ultra-low maintenance requirements
- **Zero Dead Volume**: Optimized for process integrity and hygiene
- **NIST Traceable**: Simplified verification and regulatory compliance.

Unlike wavelength process photometers that provide a single value, the SPECTRA unit simultaneously measures across a broad spectrum of wavelengths. According to Beer-Lambert Law, the molar absorption coefficient is wavelength-dependent; SPECTRA leverages this fact to continuously monitor concentration without the need for sample dilution or complex mechanical components.

The result is an instantaneous validated measurement. The absence of moving parts drastically reduces the risk of measurement error while keeping maintenance requirements and operational costs to a minimum.

Biomolecule Concentration Measurement

Due to its specificity, non-destructive nature, and rapid measurement speed, multi-wavelength UV/DUV spectroscopy is the preferred technique for biomolecule concentration measurement (Singh et al. 2021; Carvalho et al. 2025).

Biomolecules such as proteins, peptides, monoclonal antibodies, mRNA/DNA, and amino acids absorb light uniquely at specific UV/DUV wavelengths. Proteins predominately absorb at 280 nm and below 220 nm due to the aromatic side chains of tryptophan, tyrosine, and phenylalanine (Antosiewicz and Shugar 2016). The ratio of

Kemtrak SPECTRA Multiwavelength Photometer

wavelengths over this region can be used to characterize or "fingerprint" the protein for fractionation, identification, quality control and real time release monitoring (Carvalho et al. 2025; Farag et al. 2022; Schmid (2008); He et al. 2019).

RNA predominantly absorbs at 260 nm, which is the basis for quantifying its concentration and assessing purity due to the conjugated double bonds in its nucleotide bases. The ratio of absorbance at 260 nm to absorbance at 280 nm (A260/A280) helps determine RNA purity; a ratio around 2.0-2.1 indicates high purity, while lower values suggest protein contamination (Wilfinger et al. 1997; Imbeaud 2005; Gomes 2021).

The Requirement for Ultra High Protein Concentration Monitoring in Biotechnology

Accurately quantifying DNA or protein levels in cell extracts is a cornerstone of modern bioprocessing. As the industry moves toward sophisticated monoclonal antibody (mAb) therapies and enhanced production technologies, the demand for real-time online monitoring has never been greater. This shift has necessitated new methods for measuring exceptionally high protein concentrations, often reaching between 40 and 500 mg/mL, and sometimes even higher.

In a typical chromatography setup, 215 nm and 280 nm UV sensors are the gold standard for detecting trace amounts of protein with high precision, making these wavelengths ideal for determining fraction collection windows. However, for the ultra-high concentrations required in advanced biomanufacturing, even with a significantly shortened optical path length, the instrument will often go off-scale at these wavelengths.

To accurately measure ultra-high concentrations, the simplest solution is to utilize a second wavelength. This approach ensures the photometer operates within its ideal linear range and provides reliable data throughout the entire elution process.

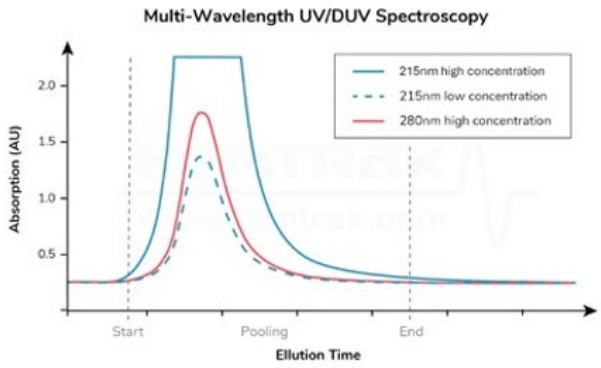

Fig. 1) Application of Multi-Wavelength Technology for measuring ultra-high protein concentrations to ensure accurate control of chromatographic elution. In this example, 215 nm is employed for high-sensitivity start/stop control of fractionation, while 280 nm is used to calculate the total eluted volume.

Understanding Absorbance, Optical Density and its Measurement

Absorbance is defined by the Beer-Lambert Law, which establishes a direct, linear relationship between the amount of light a substance absorbs and its physical properties:

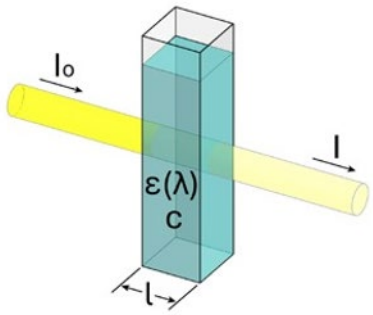

Fig. 2) The Beer-Lambert law defines absorbance as being proportional to both concentration and optical path length at a specific wavelength.

- *A (Absorbance): Measured using a photometer or a spectrophotometer*
- *ε (λ) (Molar Absorptivity): Substance-specific constant that quantifies strength of absorption at the specific wavelength.*
- *l (Pathlength): Distance the light travels through the sample.*
- *c (Concentration): Conc. of the absorbing substance.*

In bioprocessing, “optical density” (OD) and “absorbance” are frequently used interchangeably to describe the same logarithmic measurement of light loss, though they have technical distinctions depending upon context. Kemtrak, and other manufacturers, use OD to define absorption normalized to a 1 cm path length.

$$OD = A / l$$

This allows for direct comparison between different sensor configurations without having to know the optical pathlength.

The Ideal Operating Range

Absorbance is typically measured in Absorbance Units (AU). Due to electronic and optical design limitations, measurements for most standard instruments should ideally fall between 0.01 AU and 2.0 AU for maximum precision.

Below 0.01 AU, instrument noise or zero drift can disproportionately affect accuracy as the signal strength approaches the detection limit. Conversely, above 2.0 AU, where only 1% of light reaches the detector, the analyzer’s response often deviates from the linear range. At these higher optical densities, the signal becomes weak, and stray light begins to ‘pollute’ the reading, causing a negative deviation from values calculated using the Beer-Lambert Law.

Optimizing the Measurement for Process Applications

The utility of the Beer-Lambert Law lies in its flexibility: by adjusting the wavelength (which determines the molar absorption coefficient, ε (λ)), pathlength (l), or sample concentration (c), the Absorbance (A) can be maintained within the analyzer’s ideal operating range. This flexibility allows a photometer to routinely measure substances across a very wide range of concentrations, from ultra-high protein concentrations exceeding 500 mg/mL down to trace levels (0.001 mg/mL).

- **Concentration (c)**: In laboratory settings, concentration is the most common variable. Samples are typically diluted to ensure they fall within the analyzer’s design specifications. However, for continuous in-line applications, sample dilution is generally impractical or impossible.
- **Pathlength (l)**: While laboratory benches typically use a 1 cm cuvette, selecting an optimized optical pathlength is standard practice for process photometers. However, working with narrow pathlengths (less than 0.5 mm) is often avoided. Small gaps are difficult to fill, prone to clogging, and subject to surface forces that impede flow and trap air bubbles. Insitu pathlength adjustment introduces mechanical complexity and uncertainty regarding the exact pathlength value, which directly leads to concentration calculation errors. To ensure data integrity, it is preferable to use pathlengths sized specifically to keep the target absorbance within the detector’s linear dynamic range.

- **Wavelength / Molar Absorption Coefficient (ε (λ))**: Because the molar absorption coefficient is wavelength-dependent, selecting an alternative measurement wavelength is the simplest and most effective method for in-line applications. By choosing a wavelength where the substance absorbs less strongly, high-concentration samples can be measured accurately and repeatably without the need for dilution or problematic ultra-short pathlengths.

The Requirement for Wide Dynamic Range in Biotechnology

In modern bioprocessing, it is essential to utilize analyzers capable of covering a very wide range of concentrations, from trace levels during purification to high-concentration formulations.

Challenges with Fixed-Wavelength Photometers

Due to the simplicity and robustness of these devices, fixed-wavelength photometers are commonly used for real-time protein monitoring in production environments. However, fixed wavelength sensors face limitations regarding dynamic range when high sample concentrations are present. Typically, these instruments are configured for high concentrations at the expense of sensitivity at lower levels. In post-column API (Active Pharmaceutical Ingredient) detection, this imbalance often results in sub-optimal fractionation and lost yield.

Challenges with Variable Pathlength Spectroscopy

Variable pathlength systems mechanically adjust the optical path to maintain absorbance within the photometer's linear range. However, these systems present several drawbacks for modern facilities:

- **Discontinuous Data**: Readings are not truly real-time, often requiring up to 30 seconds per reading.
- **Mechanical Risks**: Moving parts are subject to mechanical tolerances and wear, necessitating frequent maintenance. When integrated into a flow path, these units can increase hold-up volumes and create process disturbances.
- **Safety Concerns**: Moving glass optical components may cause damage and introduce glass fragments into the process medium.
- **Validation Hurdles**: The most significant disadvantage is that these devices often require removal from the process line for validation, leading to potential process disruption and increased contamination risk.

Advantages of Multi-Wavelength Technology

Multiwavelength spectroscopy was introduced to overcome limitations at higher concentrations due to saturation of the signal and nonlinearity of the Beer-Lambert law (Chen, Z et al. 2018, Vanderlinde 1982).

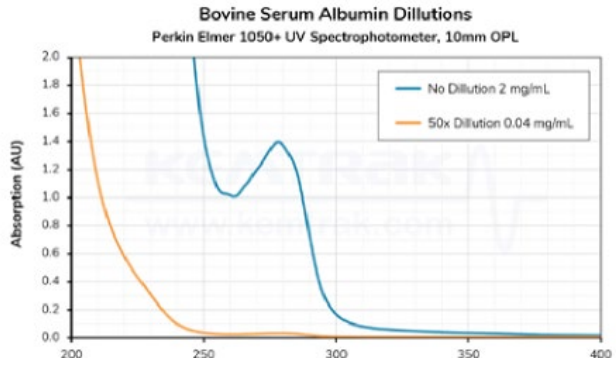

Fig. 3) Absorption spectrum of Bovine Serum Albumin (BSA) standard, 20 mg/mL (Thermo Scientific Product Number: 23210, Lot: XA341285) using a Perkin Elmer Lambda 1050+ UV/Vis/NIR laboratory spectrophotometer. High concentrations (20 mg/mL) can be measured above 250nm or below 250nm after a 50x dilution.

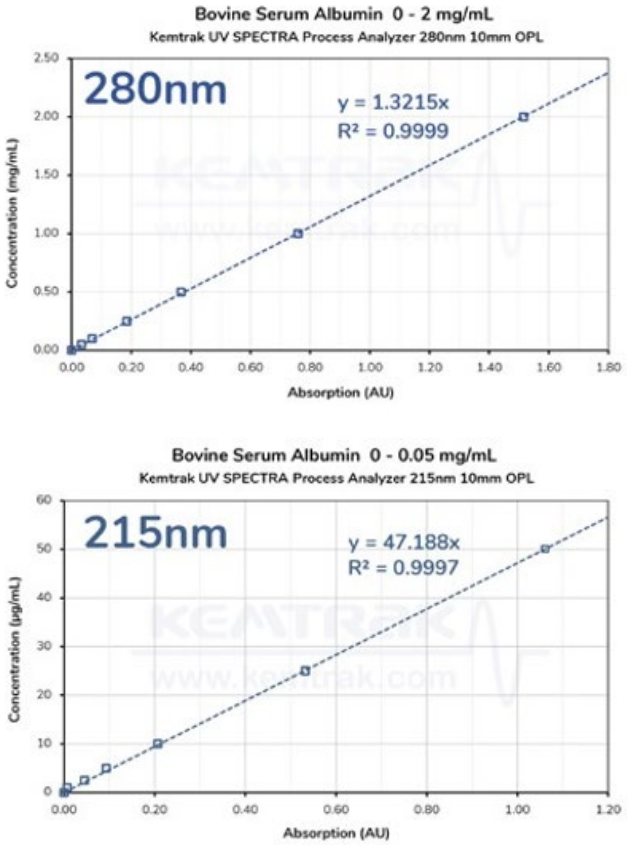

Fig. 4) The Kemtrak SPECTRA is capable of continuous multi-wavelength measurement of Bovine Serum Albumin (BSA) across a wide range, from concentrated (20 mg/mL) to trace (0.001 mg/mL) levels. By monitoring two wavelengths simultaneously, 215 nm and 280 nm, using a fixed 10 mm optical pathlength, the system maintains high precision. Notably, the r2 value exceeds 0.999, demonstrating a linear response between absorbance and concentration across the entire measurement range.

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The Kemtrak SPECTRA is an industrial process photometer that utilizes a diode array spectrophotometer with fixed optical pathlength and offers a superior alternative through simplicity and reliability:

- **Instantaneous Measurement:** With no moving parts, data acquisition is real-time and continuous.
- **In-Line Validation:** The analyzer can be validated in-situ using NIST-traceable standards, eliminating the need to breach the process line. This ensures compliance without the risk of contamination.
- **Operational Efficiency:** By eliminating mechanical failure points, the system minimizes maintenance requirements and lowers the total cost of ownership.
- **Uncompromised Accuracy:** Measurements are validated to traceable standards, ensuring the highest level of confidence in both GMP and non-GMP environments

Overview of Spectrophotometer/Photometer Design Architectures

Spectrophotometers are available in several distinct designs, each optimized for specific environments:

Scanning Spectrophotometer

This instrument utilizes a movable diffraction grating to isolate and measure wavelengths sequentially. While a laboratory workhorse capable of linearity up to 3 AU and offering high spectral resolution and sensitivity, it is generally unsuitable for continuous process applications. The reliance on precision moving parts leads to slower data acquisition speeds and high maintenance requirements, making it difficult to implement in 24/7 manufacturing environments.

Fixed-Wavelength (Filter) Photometer

This device uses physical optical filters to isolate one or more specific wavelengths, providing a rugged, cost-effective solution for continuous in-line analysis.

- Traditional Models: When using broad-spectrum light sources, spectral resolution is often limited by stray light (unwanted wavelengths leaking through the filter). This leakage can cause non-linear absorption, typically limiting the effective operating range between 1 and 2 AU.
- Modern LED Models: LED-based photometers significantly reduce stray light by using narrow-band light sources. This enables linear operation over a much wider range, often comparable to high-end laboratory spectrophotometers, while maintaining the durability of a solid-state design.

Diode Array Photometer (Kemtrak SPECTRA)

This system employs a fixed grating and an array of photodiodes to measure the entire spectrum simultaneously. This architecture enables extremely fast data capture and high reliability due to the total absence of moving parts. Diode array systems can be susceptible to higher stray light levels and lower spectral resolution compared to scanning models, and are therefore typically operated below 2.0 AU for peak accuracy, they are the only choice for real-time process monitoring where instantaneous, multi-wavelength data is required.

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SOUTH FORK INSTRUMENTS
info@southforkinstruments.com

3845 Buffalo Road
Auburn, CA 95602

CALL US AT: (925) 461-5059