BioTech Pharma

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In-line inspection of protein production using advanced UV-Vis spectroscopic measuring techniques

Introduction

UV-visible (UV-Vis) spectroscopy is an essential tool in biotechnology which is particularly crucial for monitoring the different steps of the downstream processes, notably for quantifying products such as proteins or oligonucleotides present at diverse concentrations in various media. Its versatility makes it suitable for a wide range of applications, from highly-concentrated solutions to more diluted ones.

Frequently cited in Pharmacopeia monographs for the quality control of numerous products, UV-Vis spectroscopy complies with the standards and good practices strictly defined in USP 857. Despite its immense potential, this technique requires precise optimization and customization. One crucial aspect of this process is the selection of the most suitable measuring method, particularly for in-line analysis.

Measure up

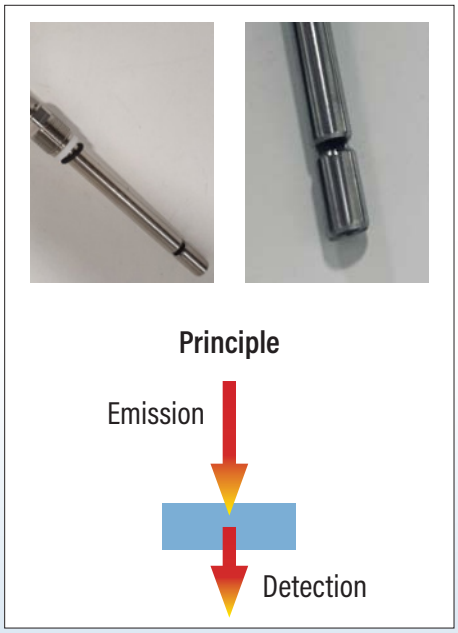
In-line measurement of protein concentration using probes

1- Transmission/Transflection probe

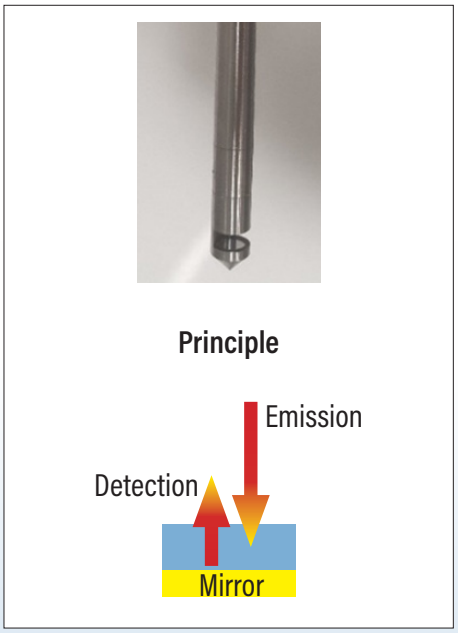
Transmission and transflection probes operate according to different principles. A transmission probe directs a light beam through a sample, measuring the quantity of light which passes through and emerges on the other side. This method is effective for transparent or translucent samples, enabling accurate determination of the absorbance and concentration according to the amount of light absorbed by the sample.

A transflection probe, meanwhile, also initially directs light through the sample, but includes a reflective surface at the tip of the probe. This surface redirects part of the transmitted light back through the sample, thus increasing the length of the optical path and improving detection sensitivity. This design is particularly useful for opaque or highly-diffusing samples, for which direct transmission measurements may be less effective.

Transmission probe



Transflexion probe

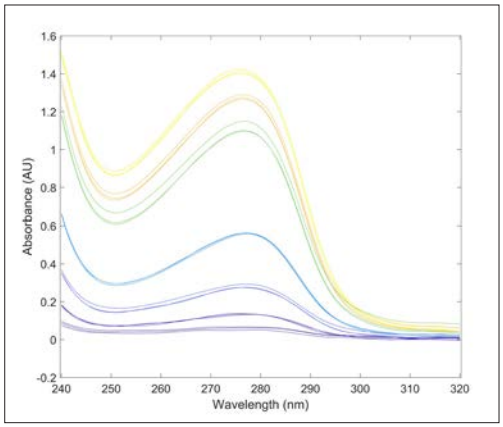


For solutions at standard concentrations prepared in tanks or reactors, transmission or transflection probes are preferred because of their accuracy, ruggedness and flexibility. The possibility of adjusting the optical path length provided by these probes makes it possible to adapt it to different concentration ranges, thus offering appreciable flexibility for biotechnological applications.

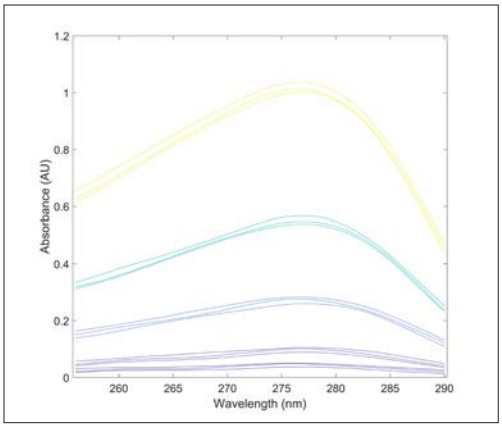
Case Study 1: BSA quantification

Samples of bovine serum albumin (BSA) were prepared at various concentrations ranging from 0.05 mg/mL to 3 mg/mL and measured using a transflection probe with two optical paths of 10 mm and 20 mm. A PLS (Partial Least Squares) quantification model was then constructed for each optical path used.

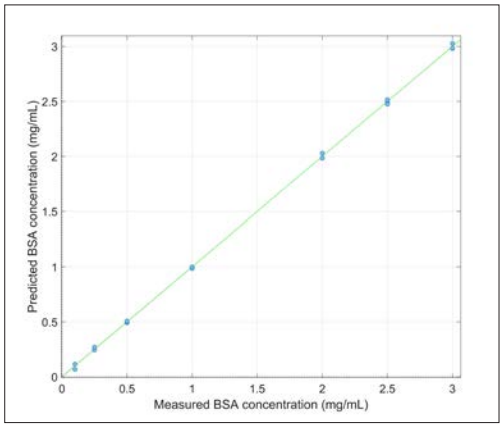
10 mm optical path length
Concentration range: **0.1-3 mg/mL**

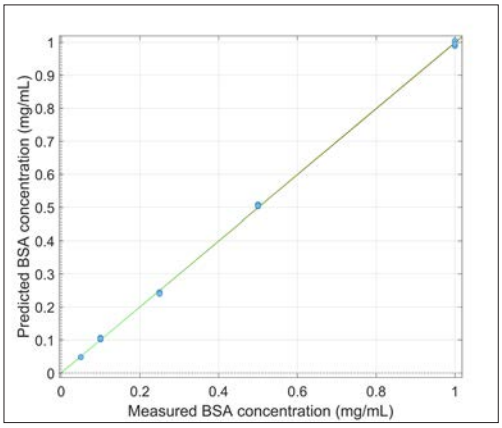


20 mm optical path length
Concentration range: **0.05-1 mg/mL**



PLS models





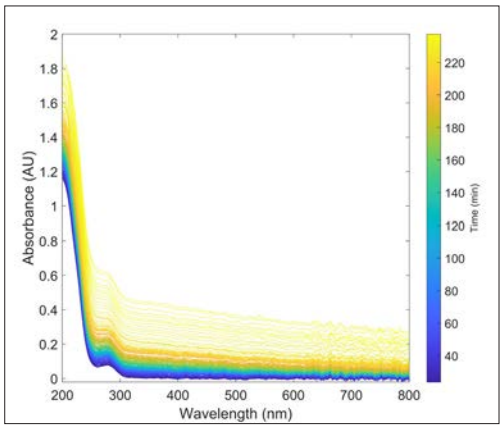
Case Study 2: Monitoring of IgG protein aggregation

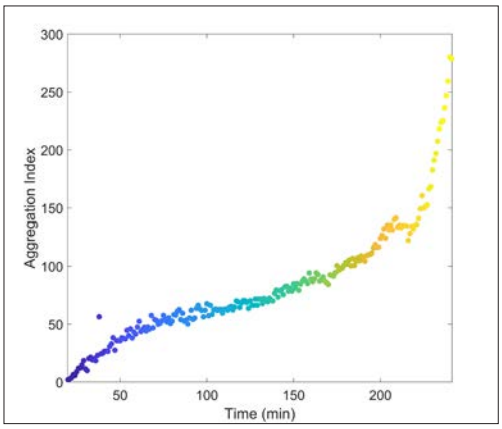
To ensure that biopharmaceuticals are stable and safe, the proteins must be produced in their native form. Protein aggregation is therefore one of the most important parameters to control during the processes. In-line measurement is an essential tool for detecting the conditions liable to favor such aggregation.

In the context of this study, we monitored the evolution of an immunoglobulin G (IgG) solution at a concentration of 0.5 mg/mL over a period of 3 hours and 40 minutes, while increasing the temperature by 5 °C every 15 minutes up to 75 °C. The spectra were acquired with a transflection probe equipped with a 2 mm optical path.

The aggregation index (AI) is calculated from the absorbance spectra by using the following formula:

$$AI = \left(\frac{A_{350}}{A_{280}\text{-}A_{350}} \right) x100$$





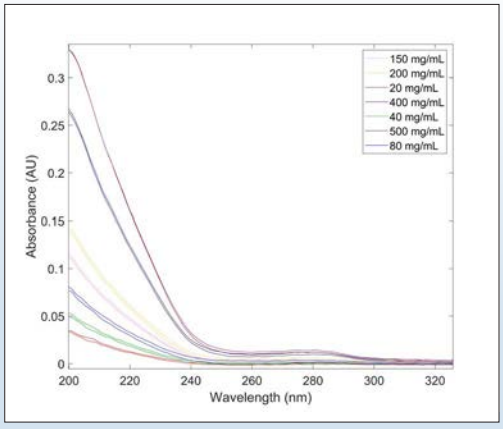
This index is a semi-quantitative indicator of the protein aggregation rate. An aggregation index below 10, as observed at the beginning of the experiment, corresponds to solutions with an insignificant number of aggregates. In function of the thermal stress induced, an increase in the aggregation index is then observed, indicating that aggregates have formed.

2- ATR (Attenuated Total Reflectance) probe

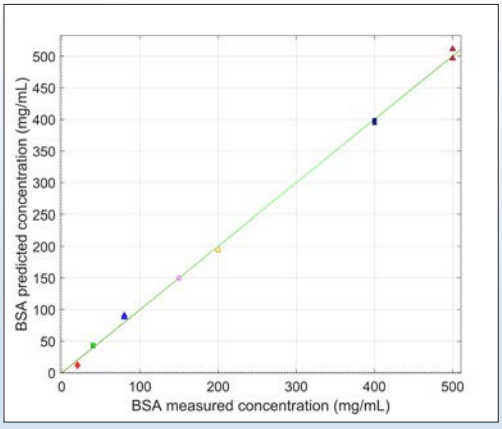
Highly-concentrated media are critical for several reasons. The main concern is the high cost per volume, giving the product significant value. Furthermore, concentrated media may generate aggregates, crystallization or instability. In the UV range, these media demonstrate very high absorbance and a standard transmission configuration is not usually capable of providing accurate concentration data. One good solution involves using ATR (Attenuated Total Reflectance) probes.

The ATR measuring principle is based on the use of a crystal with a high refractive index placed in contact with the sample. When light passes through the crystal and reaches the contact surface, it undergoes total internal reflection.

A small portion of that light, called the «evanescent wave», slightly penetrates the sample and interacts with it. These probes can measure an optical path length of just a few microns thanks to penetration by the evanescent light at the point of reflection. An example of this is shown below for high BSA concentrations (20 mg/mL to 500 mg/mL).



PLS model



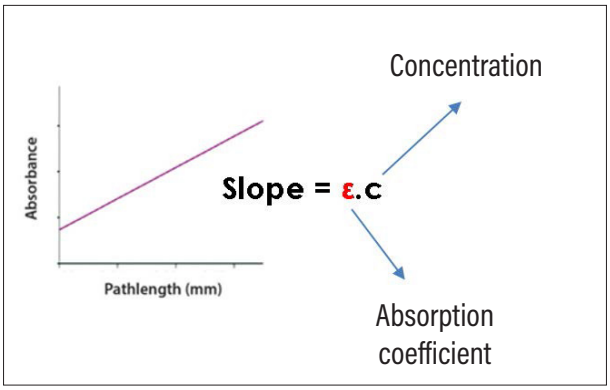
In-line measurement of protein concentration using a flow cell

In processes requiring measurements in a continuous flow, the use of a flow cell instead of traditional probes is an effective solution. These flow cells have adjustable optical path lengths to optimize the signal/noise ratio and provide flexibility for the range of concentrations to be analyzed, thus guaranteeing reliable measurements even in dynamic environments.

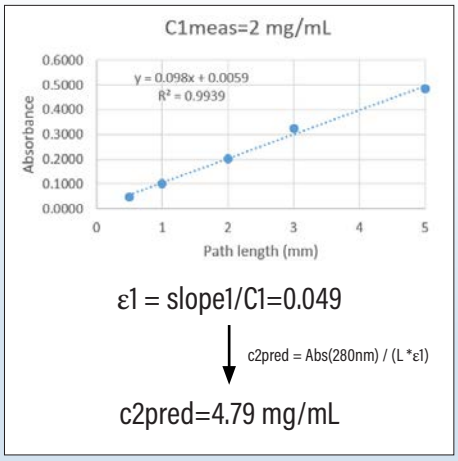
To validate the cleaning, a longer optical path is an advantage to reach the levels of detection in ppm for proteins or cleaning agents. In some applications, several optical path lengths (Multi-path length or MPL) may be combined, as in slope spectroscopy or in processes with significant variations in concentration. To generate robust measurements compliant with the GMP directives, new flow cells have been developed which are capable of measuring three different optical paths simultaneously. In some cases, one channel may even integrate a near infrared (NIR) measurement, allowing the analyzer to capture both electronic and vibrational information.

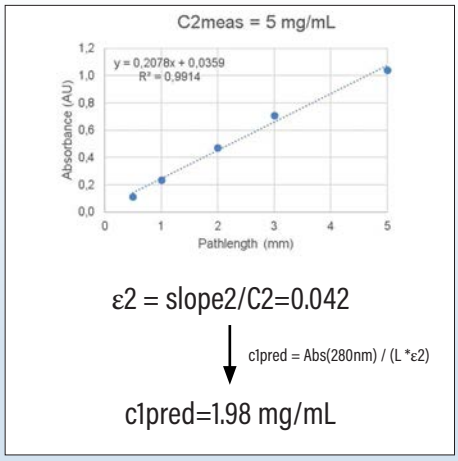
Case Study 1: Approach without calibration for protein quantification

We vary the optical path length (L) and then plot Abs = f(L). The slope of the line is equal to C (known). From this, we determine the extinction coefficient ε, which will then be used to quantify new samples of unknown concentration via C = Abs / (L * ε). This method allows for more precise quantification of concentration (with a well-calculated ε and multiple data points), rather than relying on a single data point using absolute absorbance.



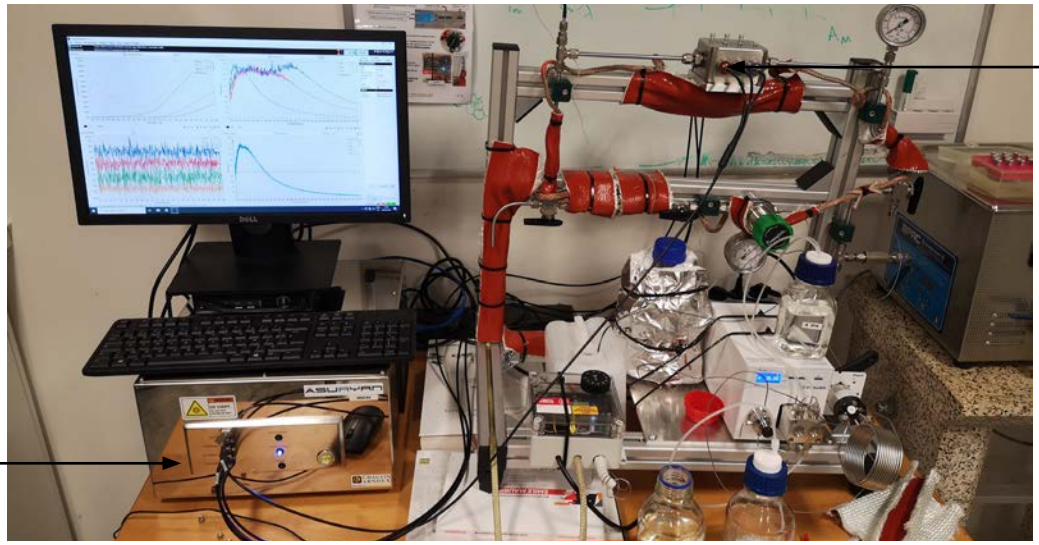
To determine the extinction coefficient (ε) for BSA and predict its concentration, measurements were performed at two BSA concentrations (C1 = 2 mg/mL and C2 = 5 mg/mL) with Indatech's MPL flow cell, using 5 optical path lengths from 0.5 mm to 5 mm.



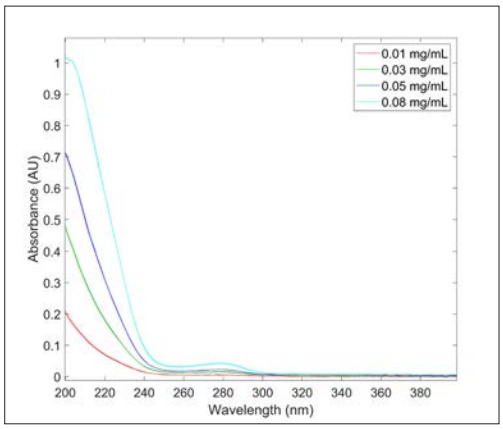


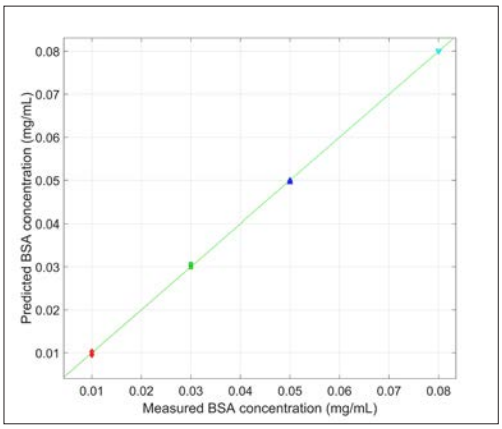
Case Study 2: MPL approach for a wide range of protein concentrations

Various BSA samples at different concentrations, ranging from 0.01 to 100 mg/mL, were measured using a flow unit (25 mL/min, 10 bar) with an MPL flow cell connected to the Asuryan UV-Vis system. Three different optical path lengths were used: 10 mm, 3 mm and 0.5 mm.

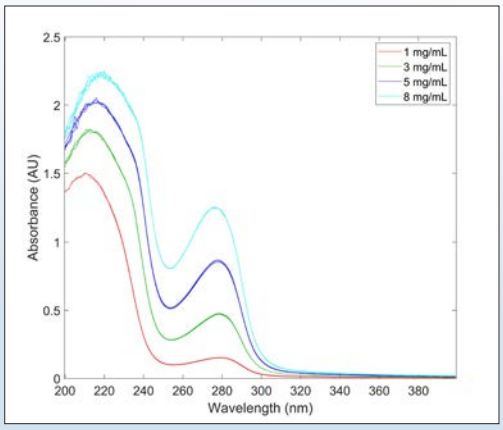


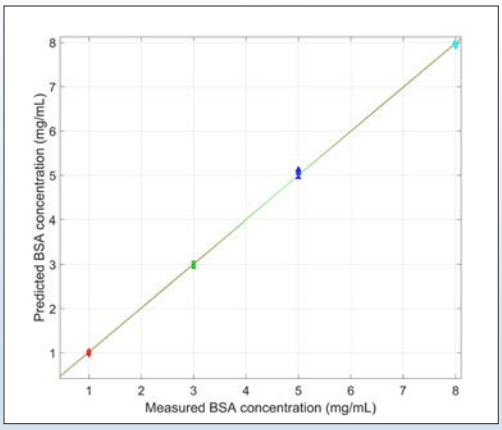
Channel 1 of the MPL flow cell: 10 mm optical path length
Concentration range: **0.01 to 0.08 mg/mL**



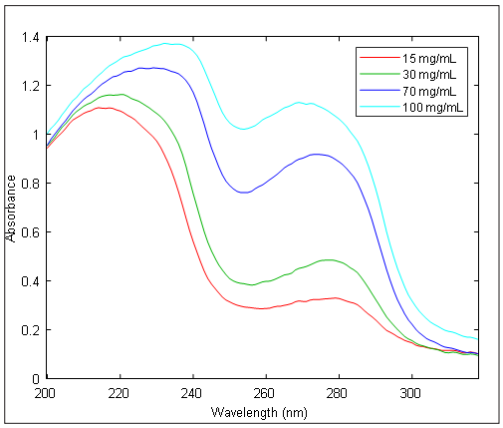


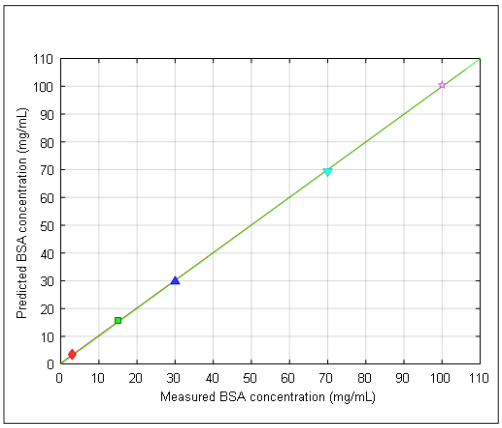
Channel 2 of the MPL flow cell: 3 mm optical path length
Concentration range: **1 to 8 mg/mL**





Channel 3 of the MPL flow cell: 0.5 mm optical path length
Concentration range **15 to 100 mg/mL**

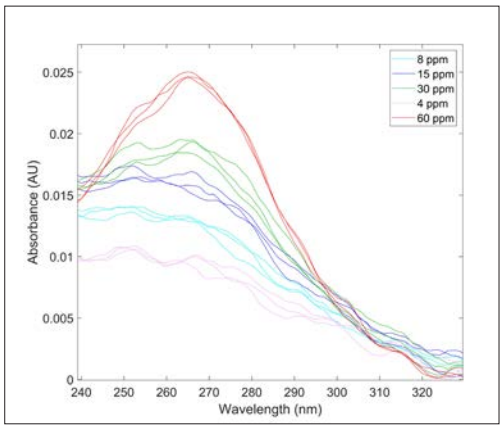


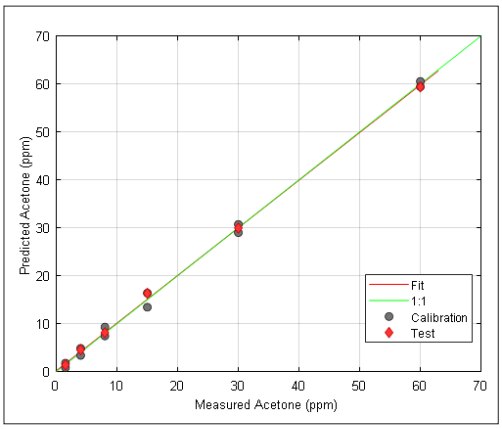


Case Study 3: Detection of traces of acetone used as a cleaning agent

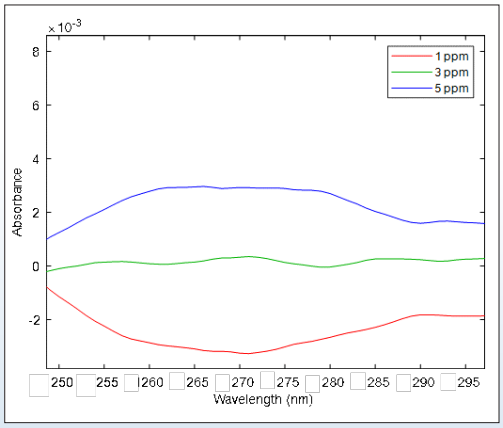
In this proof of concept, we are aiming to determine the effectiveness of a Cleaning In Place (CIP) protocol by quantifying the residual concentration of acetone in the water. After measuring the protein, acetone was used to clean the flow unit. Using two optical path lengths (10 mm and 20 mm) for the flow cell (with a 60 mm maximum option to detect very weak traces), the residual acetone will be measured accurately to ensure that the CIP process complies with the required cleanliness standards.

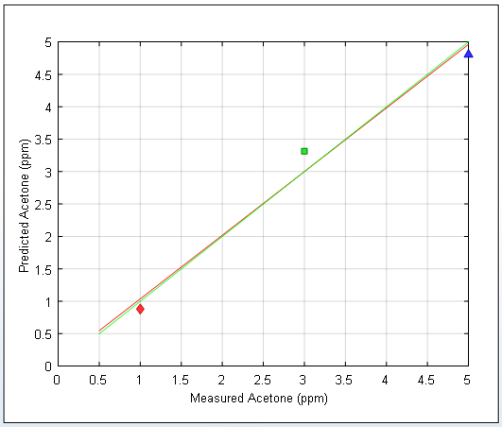
Channel 1 of the MPL flow cell: 10 mm optical path length
Concentration range: **4-60 ppm**





Channels 2 and 3 of the MPL flow cell: 20 mm optical path length (2x10)
Concentration range: **1-5 ppm**





By increasing the optical path length, it is possible to detect very low concentrations. In this way, traces as low as 1 ppm can easily be quantified in-line using the MPL flow cell.

Conclusion

The study examined various methods for quantifying and qualifying proteins, as well as detecting trace contaminants by means of UV-visible spectroscopy, studying both the traditional techniques using immersion probes and innovative methods involving flow cells.

The transmission/transflection probe and the ATR probe proved effective on various protein solution concentration ranges, highlighting their versatility in biotech applications. In particular, the ATR probe was outstanding in highly-concentrated media where standard transmission configurations often encounter difficulties due to the proteins' high absorption characteristics.

The MPL flow cell appeared to be a robust tool for in-line measurements in continuous-flow processes. This cell offers adjustable optical path lengths, optimizing the signal/noise ratios and guaranteeing reliable measurements in dynamic environments. Using several optical path lengths, the flow cell allowed accurate quantification across a wide range of concentrations, from trace levels to high concentrations of BSA or acetone. This capability is essential to fulfill the strict regulatory requirements and validate cleaning processes in the biotech industry.

In conclusion, UV-visible spectroscopy continues to play a central role in the biotech industry for precise quantification of proteins and sensitive detection of contaminants. Its diverse applications and evolving methodologies ensure high product quality and efficient processes in biotechnological operations.

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