APPLICATION NOTE

nirrin

ACCURATE SIMULTANEOUS QUANTITATION OF HIGH CONCENTRATION PROTEIN TITER AND POLYSORBATE

INTRODUCTION

In high-concentration monoclonal antibody (mAb) formulations, which are increasingly common for subcutaneous administration to improve patient compliance, the role of polysorbate 80 (PS80) becomes even more critical to ensure stability and prevent aggregation. For these high-concentration formulations, typically above 50 mg/mL, the use of polysorbate 80 is carefully optimized based on extensive formulation and stability testing. Measuring polysorbate in monoclonal antibody (mAb) formulations presents several analytical challenges due to the nature of polysorbate and the complexity of mAb formulations.

Here, our high precision tunable laser system demonstrates the ability to measure both protein titer and PS80 at formulation-relevant concentrations with the following highlights:

- **Simple workflow:** current analytical methods struggle with complicated workflows requiring dilution, sample prep and complex analysis
- **Wide dynamic range:** current methods struggle with protein across a wide range, requiring variable path lengths to accommodate, or lack sensitivity for the low-levels of polysorbate
- **Accuracy with matrix interference:** high concentration product can pose a problem for measuring polysorbate with current methods

THE WORKFLOW: TIME-TO-INSIGHT IN LESS THAN A MINUTE

Currently, two workflows are required for measuring both protein titer and PS80. For protein measurement, UV-Vis is the gold standard and can take up to 5 minutes per sample with instrument setup, sample loading and analysis. For polysorbate, a measurement can take hours to days. This can require method development and optimization, and requires high levels of expertise to ensure accurate, repeatable results. Our tunable laser system provides simultaneous measurements of protein titer and polysorbate from the same 15uL drop. No sample prep or dilution necessary, and no complex analysis or Ph.D. required.

1 PIPETTE 15 µL
of sample (no dilution or preparation)

5 SECONDS

2 CLOSE THE COVER
to kick off your run

10 SECONDS

3 REVIEW YOUR DATA
quantitative results on each of your excipients

45 SECONDS

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TUNABLE LASER FIXED-PATHLENGTH NEAR-INFRARED (NIR): BEST-IN-CLASS DYNAMIC RANGE

The system employs an advanced tunable laser in the NIR region of the electromagnetic spectrum. With optimized laser power and proprietary optics, the system achieves a linear dynamic range (R^2=1.0 for BSA, R^2=0.9994 for mAb) for all high concentration formulations, all with a fixed pathlength.

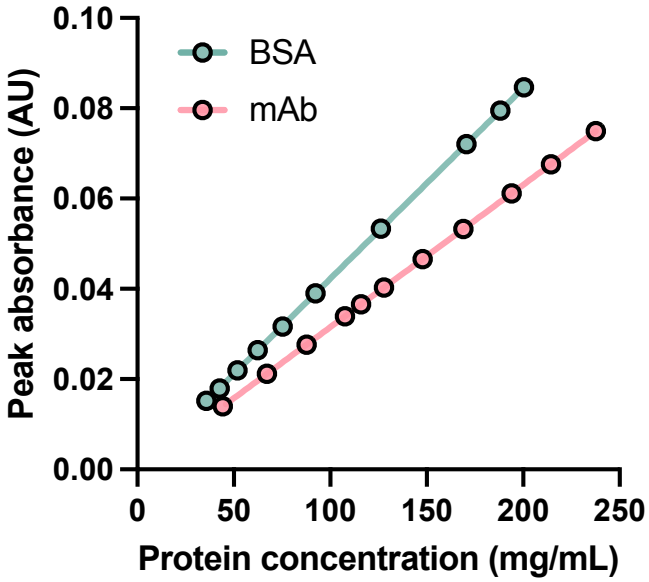

Figure 1. Linear dynamic range across low-to-high BSA (R^2 = 1.0) and mAb (R^2 = 0.9994) concentrations

ACCURATE POLYSORBATE 80 IN A mAb MATRIX

With a wide tuning range and exquisite signal-to-noise ratio, the system enables measurement of both mAb and PS80, simultaneously. The effects of diluting a 50mg/mL solution of mAb are observed as concentration of PS80 is increased from 0.01-1% (w/v).

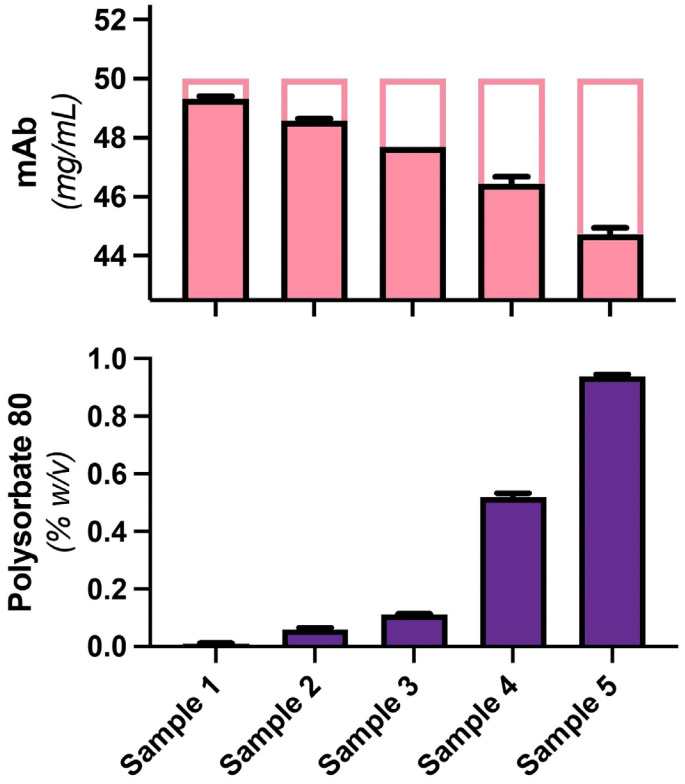

Figure 2. Simultaneous measurement of mAb and Polysorbate 80 from a single 15uL sample

DISCUSSION

Here it has been demonstrated that the high precision tunable laser system provides rapid results without sample dilution up to high concentration of protein titer with an expansive dynamic range and low noise floor. Furthermore, it is possible to also simultaneously measure surfactants, like Polysorbate 80 shown here, in these high concentration formulations. This represents a quantum leap in bioprocessing analytical instrumentation. It gives the technician the combined power of UV-Vis and HPLC without the limitations e.g., limited dynamic range, dilution and sample prep, assay development, etc. Implementing our tunable laser system will save time and money, while illuminating critical process insights to mitigate process and user-related risks.