Doctoral Dissertation

Doctoral Program in Chemical Engineering (37th Cycle)

Spectroscopic Techniques as PAT monitoring Tools for the Development of Biopharmaceutical Product Processes

By

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Monitoring the drug product CQAs impacted by the process steps involved in process development and manufacturing is fundamental to guarantee the product quality. As mentioned above, the implementation of PAT in pharmaceutical development aims to improve product quality, reduce costs, enhance process understanding, and promote a culture of continuous improvement and innovation according to QbD approach.

In this framework, NIR and Raman spectroscopy serve as potent, non-invasive tools suitable for characterizing the DP process development and expediting the technology transfer to manufacturing sites. As such, at-line and in-line applications have been devised and presented in this Thesis, with the goal of significantly enhancing process comprehension.

Chapter 2 delineates the working principles of the most prominent PAT tools in pharmaceutical field, spectroscopy techniques, specifically NIR and Raman spectroscopy.

Chapter 3 provides details on the equipment, experimental setup, and procedures used in this Thesis.

Chapter 4 and Chapter 5 pertain to the qualitative and quantitative at-line applications of Raman spectroscopy, with the objective of predicting various protein-related CQAs at different stages of the manufacturing process, including compounding, filtration, and visual inspection of a liquid mAb product. Specifically, assessments were conducted on high molecular weights, low molecular weights, and protein/excipient contents. Furthermore, the chapter delves into the application of the developed model to the freeze-dried mAb, demonstrating that the freeze-drying process did not impact the aggregation level.

Chapter 6 focuses on the quantification of RM following the freeze-drying process, which constitutes one of the final stages in manufacturing solid products. A key enhancement involved the implementation of a neural network to minimize the experimental effort required for model generalization. Initially, the model developed for a specific surrogate product was tested on various products to assess its robustness and its performances were compared with a linear model. Subsequently, a significant innovation in this approach was the evaluation of the model developed for the surrogate to predict the RM of an actual drug product. Thus, minimal experimental effort and cost are required to develop these methods.

Chapter 7 explores the at-line and in-line application of the models developed off-line. Specifically, this chapter discusses the use of NIR spectroscopy for the freeze-drying process monitoring, highlighting its potential to enhance process understanding and control. By concentrating on the drying phases, NIR spectroscopy facilitated insights into physicochemical changes occurring in the formulation and helped identify deviations from standard operating conditions that could affect product quality. The need for a forecasting procedure to estimate the behavior of new cycles based

on limited observations is acknowledged and currently under investigation. Moreover, the residual moisture trend and the endpoint of drying phases was assessed using PLS sensor and compared with Pirani-Baratron methods, demonstrating the reliability of the PLS model in monitoring the drying phases in real-time. The second part of the chapter presents two in-line Raman applications for monitoring protein content during filtration and dilution processes. The results obtained from the PLS model aligned well with the expected behavior of the drug product under investigation. These in-line applications are valuable for process characterization, serving as alternatives to traditional methods and providing real-time data availability.

Finally, Chapter 8 is focused on remarkable conclusions and perspectives for future developments of this work.