

Application of Raman spectroscopy for estimating critical quality attributes in protein biotherapeutics

Nitika Nitika, Anurag S. Rathore*

Department of Chemical Engineering, Indian Institute of Technology Delhi, New Delhi, India

**Corresponding author Email: asrathore@biotechcmz.com*

Abstract:

Quality of biotherapeutic products is of paramount importance for ensuring patient safety. Analytical tools that can facilitate rapid quality assessment of the therapeutic product at the point of care are very much in demand. In this article, we apply chemometrics based analysis of Raman spectra towards quantitative prediction of protein aggregation in lyophilized biotherapeutic products (erythropoietin and human growth hormone) and charge variant concentration in liquid monoclonal Antibodies (mAbs). To detect percentage aggregate, thermally induced protein aggregation was monitored by size exclusion chromatography as well as Raman spectroscopy with a 785 nm wavelength laser. In case of charge variant determination, cation exchange chromatography was correlated with Raman spectroscopy with a 785 nm wavelength laser. Partial least square (PLS) regression was used to analyze the Raman spectra and create a model for quantitative determination of aggregates and charge variant. Satisfactory performance was observed with both aggregate and charge variant determination. EPO and HGH percentage aggregate determination model had R^2 of 0.91 and 0.94, cross-validation correlation coefficient of 0.85 and 0.89, and Root Mean Square Error computed from cross calibration (RMSE_{cv}) of 5.25 and 1.92, respectively. For charge variant determination, different PLS models were made to determine acidic, main and total protein, R^2 of all the models was above 0.92 with RMSE_{cv} less than 0.4. This study indicates fast and accurate use of Raman spectroscopy for determination of Critical Quality Attributes (CQA) of biotherapeutics.