QUALITATIVE AND QUANTITATIVE ANALYSIS OF N-GLYCANS PART OF PHARMACEUTICAL PROTEINS BY FT-IR SPECTROSCOPY

Sabrina Hamla¹, Pierre-Yves Sacré¹, Allison Derenne², Erik Goormaghtigh², Philippe Hubert¹, Eric Ziemons¹

¹University of Liege (ULiege), CIRM, Vibra-Santé HUB, Laboratory of Pharmaceutical Analytical Chemistry, ULiege, Avenue Hippocrate 15, 4000 Liege, Belgium

²Université libre de Bruxelles (ULB), Center for Structural Biology and Bioinformatics, Laboratory for the Structure and Function of Biological Membranes, Campus Plaine CP206/02, 1050 Brussels, Belgium

Glycosylation is a post-translational modification of extracellular proteins and some intracellular proteins. It consists in covalently linking a glycan that is a polymer of monosaccharides to a peptide chain by N-glycosylation or O-glycosylation, the latter being less frequent [1]. This process has an impact on the stability and activity of therapeutic proteins, hence the need for regulatory authorities to characterize the composition and structure of glycoproteins [2]. Actually, most of the existing analysis methods are long and expensive, consisting of a time-consuming step of sample preparation followed by a separation technique (HPLC, UHPLC, …) coupled to fluorescence and/or mass spectrometry detector. In this framework, the use of FT-IR spectroscopy, which is a fast, simple and non-destructive technique could be an interesting analytical tool.

First, the qualitative aspect of FT-IR spectroscopy was evaluated. Six spectra per protein were recorded in ATR mode over the spectral range 4000 - 800 cm⁻¹ for each of the 16 therapeutic antibodies investigated. Further analyses were performed on the specific spectral range (1200-865 cm⁻¹) related to the glycan part. The spectra were assigned to the following classes depending on their category of N-glycans: “high mannose”, “complex” and “hybrids”. Then, three metric algorithms (Pearson’s correlation coefficient, Euclidean distance and spectral angle mapping (SAM)) were applied to assign new spectra to the corresponding classes.

In a second step, a first estimation of the quantitative performances of FT-IR spectroscopy to predict monosaccharides content were evaluated by partial least squares (PLS) and support vector machine (SVM) regression models. The regression algorithms were applied using the PLS-Toolbox running in the Matlab® environment. SVM model showed better results compared to PLS one in terms of R² of calibration (1 vs 0.98), R² of cross-validation (0.99 vs 0.98) and RMSECV (0.15 vs 0.37) for example for the sialic acid content determination over a calibration range from 0.05 to 12.5mg/mL.

To conclude, FT-IR spectroscopy combined with the SVM model seems to provide promising results, regarding qualitative and quantitative analysis of the glycosylation part of the samples.

References:
