Monitoring the glycosylation status of proteins using Raman spectroscopy

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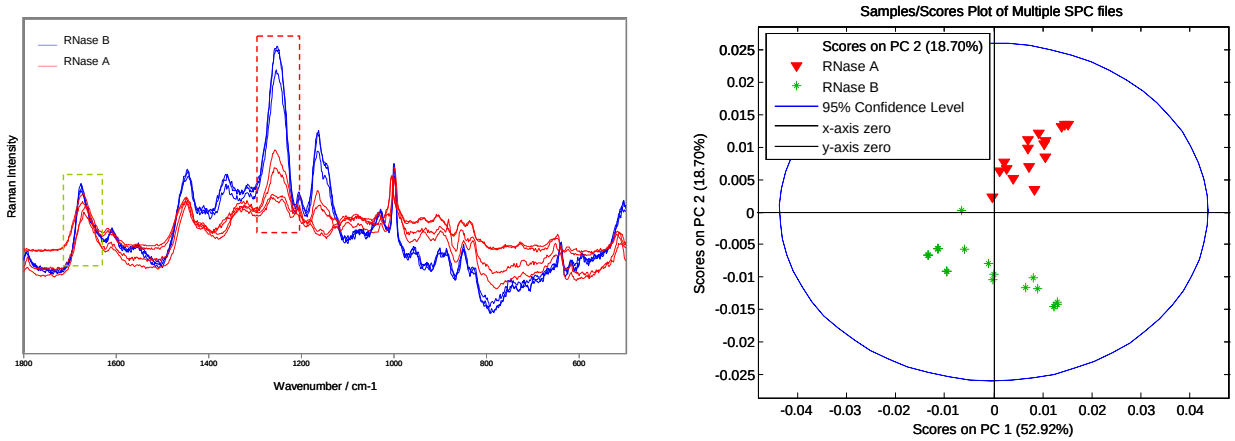
Protein based pharmaceuticals are becoming increasingly popular therapeutic agents with over 500 different proteins under development, many of which are glycoproteins [1]. It is therefore essential that process analytical technologies are developed, not only for the characterisation of biopharmaceuticals but also for the monitoring of the bioprocesses which produce such drugs. A major area of interest in this field is the detection and quantification of post-translational modifications (PTMs) to therapeutic proteins. The most common PTM is glycosylation [2], and this can affect the stability, immunogenicity and pharmacokinetics of a drug. Raman spectroscopy is an ideal technique to be investigated for the on-line monitoring of bioprocesses as it is non-destructive, inexpensive, rapid and quantitative, and its confocal nature makes it possible to focus through transparent vessels [3].

Raman spectroscopy and principal components analysis (PCA) have been used to distinguish successfully between a non-glycosylated protein (RNase A) and its glycosylated equivalent (RNase B) (Figure 1). We are currently working on a model for the quantification of glycosylation of the RNase protein using partial least squares regression (PLSR) in conjunction with Raman spectroscopic data. We have also demonstrated the ability to detect glycoproteins which have been deglycosylated by both enzymatic and chemical methods. Raman spectroscopy has also been used combination with fluorescence spectroscopy and FT-IR to monitor the chemically induced unfolding of RNase proteins, confirming that the presence of a sugar group increases the stability of a protein.

Figure 1:(A) Raman spectra of RNase A and B showing differences in both amide I and III bands, and (B) PCA plot (PC1 *vs*. PC2) of RNase data showing RNase A and B spectra resolved into two separate clusters.

A B



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