Raman spectroscopic methods in bioprocessing: Two-dimension correlation analysis of pH-induced transitions in phosphorylated amino acids

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Many proteins undergo post-translational modifications (PTM) by the attachment to amino acids of other biochemical functional groups such as phosphate, carbohydrates, lipids etc. An understanding of these PTM's is extremely important for bioprocessing and structural analysis as they result in changes to physical and chemical properties, conformation, stability and activity, ultimately determining the function of the protein. The therapeutic activity of biopharmaceuticals can depend on specific PTMs and therefore effective monitoring of PTM of proteins is needed to confirm safety and quality of the final product. Raman and Raman optical activity (ROA) has been shown to be extremely informative in the study of glycosylation, with spectra of glycoproteins providing bands that originate from both the polypeptide and carbohydrate components giving information about the structure of both components [1]. Despite this success only limited investigations have been carried out on phosphorylation, possibly due to spectral intensities of peptide phosphate groups being weak in comparison to aromatic amino acid side chain bands. However, previous Raman studies have shown that by changing the pH of the solution of phospho-peptides dramatic changes in Raman bands originating from phosphate stretch are observed [2]. We present Raman and ROA spectra revealing differences between the phosphorylated and non-phosphorylated amino acids serine and threonine monomers and pH-dependent Raman and ROA spectral sets monitoring large changes in bands associated with phosphate stretch.

Two-dimensional (2D) correlation analysis is a cross-correlation approach which improves visualisation of a perturbation-induced spectral set by spreading the data over a second dimension [3]. The further 2D correlation technique of moving window analysis directly relates spectral variations to the perturbation and thereby identifies distinct phases of change [4]. With the application of moving window analysis, trends in behaviour of spectral variations in both the Raman and ROA spectral sets monitoring pH-induced transitions in the phosphorylated serine monomers have been determined. The sensitivity of the ROA data to changes not only to the phosphate stretch but also to possible interactions with the solvation shells of the monomers can also be observed through the comparison of the Raman and ROA moving window plots.

References:

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