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In-line Fourier-Transform Infrared Spectroscopy as a Versatile Process Analytical Technology for Preparative Protein Chromatography

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Abstract

Fourier-Transform Infrared Spectroscopy (FTIR) is a well-established spectroscopic method in the analysis of small molecules and protein secondary structure. However, FTIR is not commonly applied for in-line monitoring of protein chromatography. Here, the potential of in-line FTIR as a Process Analytical Technology (PAT) in downstream processing was investigated in three case studies addressing the limits of currently applied spectroscopic PAT methods. A first case study exploited the secondary structural differences of monoclonal antibodies (mAbs) and lysozyme to selectively quantify the two proteins with Partial Least Squares Regression (PLS) giving Root Mean Square Errors of Cross Validation (RMSECV) of 2.42 g/l and 1.67 g/l, respectively. The corresponding Q^2 values are 0.92 and, respectively, 0.99, indicating robust models in the calibration range. Second, a process separating lysozyme and PEGylated lysozyme species was monitored giving an estimate of the PEGylation degree of currently eluting species with RMSECV of 2.35 g/l for lysozyme and 1.24 g/l for PEG with Q^2 of 0.96 and 0.94, respectively. Finally, Triton X-100 was added to a feed of lysozyme as a typical process-related impurity. It was shown that the species could be selectively quantified from the FTIR 3D field without PLS calibration. In summary, the proposed PAT tool has the potential to be used as a versatile option for monitoring protein chromatography. It may help to achieve a more complete implementation of the PAT initiative by mitigating limitations of currently used techniques.

Keywords: chromatography, proteins, process analytical technology (PAT), fourier-transform infrared spectroscopy (FTIR), downstream processing

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