

PHARMA

NMR Coupled With Multivariate Data Analysis For Monitoring The Degradation Of A Formulated Therapeutic Monoclonal Antibody

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Innovation with Integrity

Monoclonal antibodies (mAbs) represent a large class of therapeutic biological compounds that are effective against a wide variety of pathologies. The higher order structure (HOS) of a mAb is directly related to its biological activity and can be probed by NMR. In this white paper, we show the ability of NMR (using both 1D ¹H diffusion filtered and 2D ¹H-¹³C AFHMQC experiments) coupled with multivariate data analysis (PCA and PLS regression) to effectively monitor the degradation of a formulated mAb-like protein and quantify related critical quality attributes (CQAs), i.e., potency, purity and impurities (aggregates and fragments).

A PCA analysis performed on undegraded, photo stressed and thermally stressed samples is shown in Figure 1. Two axes clearly emerge from this analysis, corresponding to the two different modes of degradation.

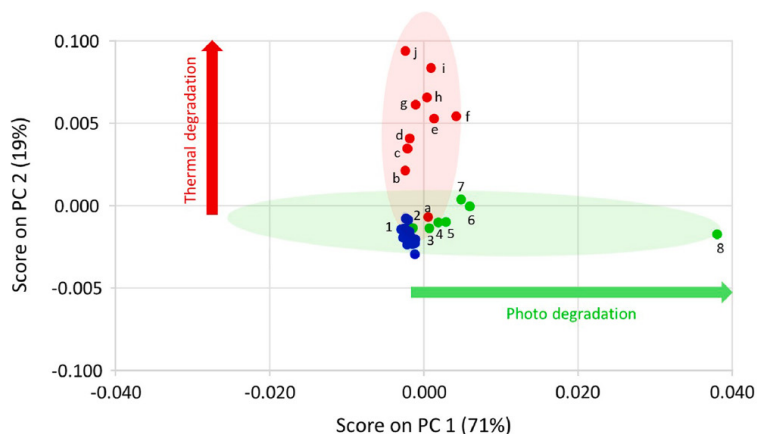


Figure 1: PCA score plot based on 1D NMR spectra. Undegraded samples are represented in blue, photo stressed samples in green and thermally stressed samples in red.

¹ Further details can be found in the original publication: C. Schaefer et al, International Journal of Pharmaceutics 667 (2024) 124894

Predictive PLS regression models based on size exclusion chromatography (SEC) data can also be computed to quantify the purity and protein-related impurities (aggregates, fragments I and total fragments). The results of the PLS models obtained with 1D NMR data are shown in Figure 2. A very good agreement is found between the reference and predicted values for the four CQAs.

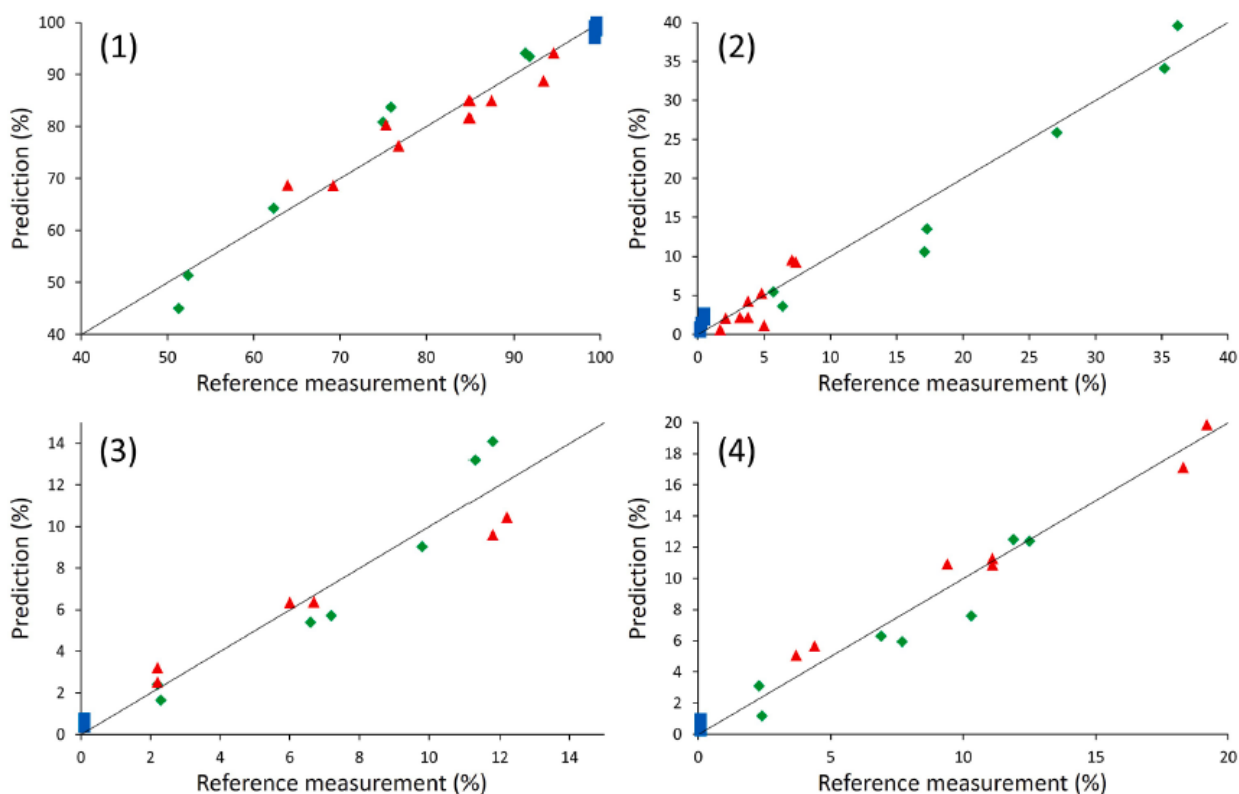


Figure 2: Calibration of PLS models of purity (1), aggregates (2), fragments I (3) and total fragments (4) based on 1D ¹H NMR spectra. The undegraded samples are represented by blue squares, photo stressed samples by green diamonds and thermally stressed samples by red triangles

In order to obtain a first evaluation of the predictive qualities of the PLS models, the CQAs of verification samples coming from different batches and that were not used during the model development, were predicted with these models. Figure 3 shows again a good agreement between the predictions of the four models and the reference method values (SEC) for the verification samples.

In summary, in this white paper we have shown the power of NMR to model the CQAs of a mAb-like protein. Furthermore, the methodology based on 1D NMR data is fast and efficient and could be used as a credible alternative to vibrational spectroscopy for the rapid analysis of pure and formulated mAbs.

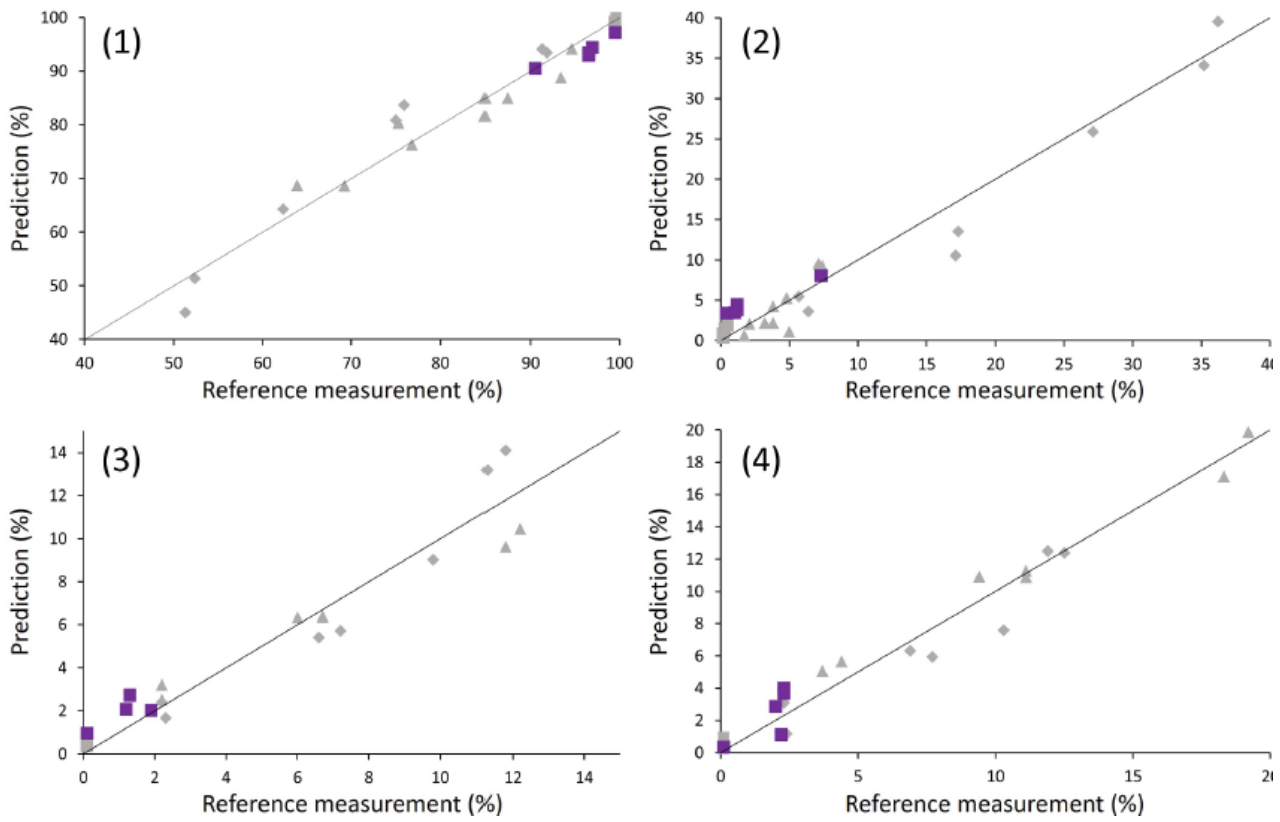


Figure 3: Verification of PLS models of purity (1), aggregates (2), fragments I (3) and total fragments (4) based on 1D ¹H NMR spectra. The grey squares, triangles and diamonds represent the calibration samples. The purple squares represent the verification samples

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