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Author: Verena Wahl Otto Scheibelhofer Ulrich Roessl Stefan

Leitgeb Thomas De Beer Johannes Khinast

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ACCEPTED MANUSCRIPT

1 The influence of residual water on the secondary structure and

2 crystallinity of freeze-dried fibrinogen

- 3 Verena Wahl^{a,b}, Otto Scheibelhofer^a, Ulrich Roessl^{a,c}, Stefan Leitgeb^a, Thomas De Beer^d,
- 4 Johannes Khinast^{a,b}*
- 5 ^a Research Center Pharmaceutical Engineering, Graz, Austria
- 6 b Institute for Process and Particle Engineering, Graz, University of Technology, Austria
- ⁷ Institute for Biotechnology and Biochemical Engineering, Graz, University of Technology, Austria
- 8 d Laboratory of Pharmaceutical Process Analytical Technology, Ghent University, Belgium

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Abstract

- 11 The purpose of this work was to investigate the influence of water content on the secondary
- 12 structure of a freeze-dried protein (fibrinogen) after a storage period of two weeks. To that
- end, Attenuated Reflectance Fourier Transformed Infrared (ATR-FTIR) and Raman spectra
- 14 were generated and evaluated and the crystalline state of the fibrinogen bulks was
- determined via X-ray diffraction. First, a PCA (principal component analysis) of the spectral
- 16 data was performed. While the α -helix and β -turn contents were increasing with the
- 17 increasing water content, the β-sheet content was decreasing. A partial least squares (PLS)
- 18 model was developed to correlate the mid-infrared and Raman spectral changes with the
- 19 degree of crystallinity. The obtained R² value of 0.953 confirmed a correlation between
- 20 changes in the secondary structure and crystallinity of the samples. The results
- 21 demonstrated that the combined ATR-FTIR and Raman approach could be used to predict
- 22 the crystalline state in freeze-dried fibrinogen products.
- 23 **Keywords:** solid-state protein; secondary structure; ATR-FTIR spectroscopy; Raman
- 24 spectroscopy; relative crystallinity; PLS
- 25 *Corresponding author. Institute for Process and Particle Engineering; University of
- 26 Technology Graz, Inffeldgasse 13, A 8010 Graz, Austria, Tel.: +43 316 30400, Fax.: +43 (0)
- 27 316 /873-30402

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29 E-mail address: khinast@tugraz.at

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