Supplementary Material of

Raman marker bands for secondary structure changes of frozen therapeutic monoclonal antibody formulations during thawing

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Specific Raman spectroscopy bands for adipic acid buffer (see Figure 1, A) and for the objective glass slide (see Figure 1, B) were determined in order to distinguish those bands from the sample. Bands for adipic acid buffer are determined to be 923 cm^{-1} (ν(C'-C)) and 2934 cm^{-1} (ν(CH))(1) (see Figure 1, A, red boxes).

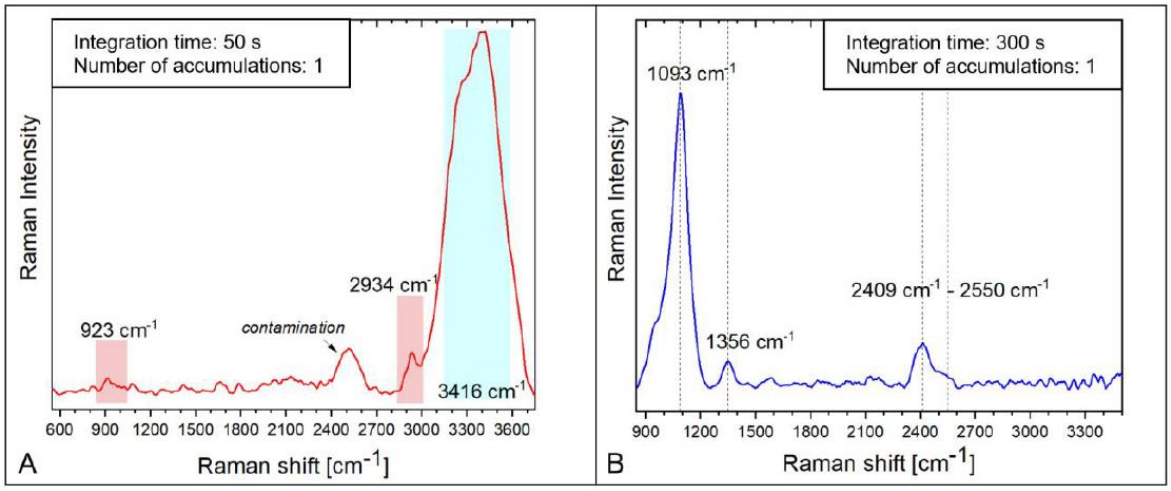

Figure 1: (A) Single spectrum of adipic acid buffer at 10°C. Blue boxes and red boxes indicate Raman spectroscopy bands assigned to water and buffer components. (B) Single spectrum of objective glass slide (SiO2) recorded at -50°C.

The bands positioned at and 3416 cm^{-1} (O-H-stretch) is assigned to liquid water(2) (see Figure 1, A, blue box). The band at 2513 cm^{-1} is of unknown origin and is explained as contamination of the glass slide. The bands at 1093 cm^{-1} and 1356 cm^{-1} are observed when focusing directly onto the glass slide which can be assigned to SiO2(3) (see Figure 1, B).

Additionally, Raman spectra were recorded of frozen mab formulation 1 buffer and mab formulation 3 buffer (see Figure 2). Spectra of buffer formulation 3 show a higher signal/noise ratio since the overall Raman intensity is lower (see counts in Figure 2). Peaks that can be assigned to the buffer components are highlighted with a grey box. For the mab formulation 1 buffer bands at 930 cm^{-1} (ν(C'-C), 1050 - 1070 $^{-1}$ (Si-O2), 1420 cm^{-1} - 1450 cm^{-1} (CH def) were repeatably detected. Buffer formulation with 42 mg/ml mannitol showed additionally a band at 880 cm^{-1}. Fortunately, none of the bands concur with the bands which are characteristic for proteins in the amide III or amide I region.

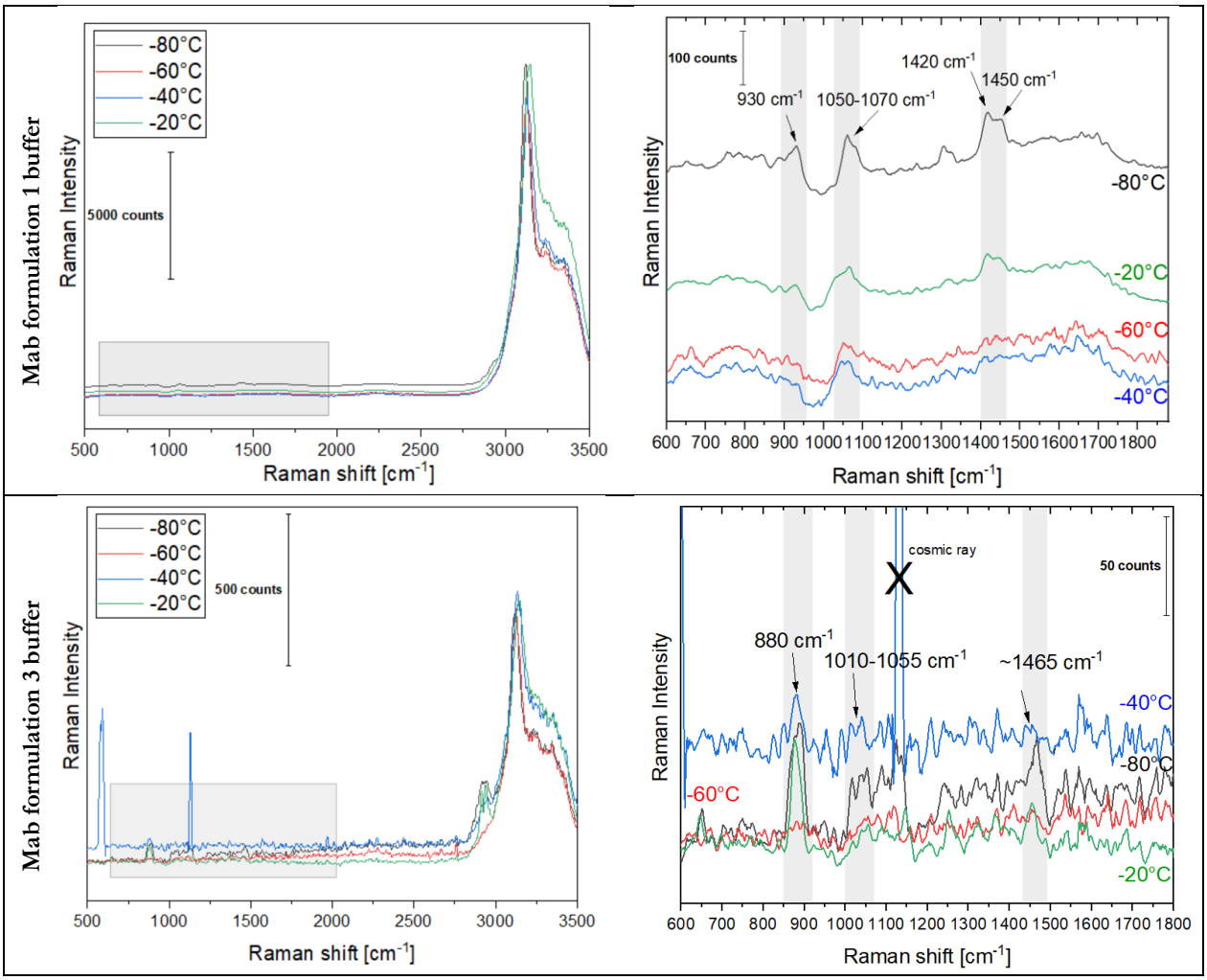

Figure 2: Raman spectra of frozen mab formulation 1 buffer, without mannitol (upper row) and mab formulation 3 buffer, containing mannitol (lower row). Spectra were cooled to -80°C at a rate of 20°C/min and heated to -60°C, -40°C and -20°C at a rate of 20°C/min. The grey shaded areas in the top right panel mark bands assigned to adipic acid, the grey shaded areas in the bottom right panel mark Raman bands for mannitol.

Upon potential dehydration of droplet, a phase separation was noticed causing crystals to remain upon heating (see red cross in Figure 3, left). In this case, mainly protein is detected in the crystals (see Figure 3, right).

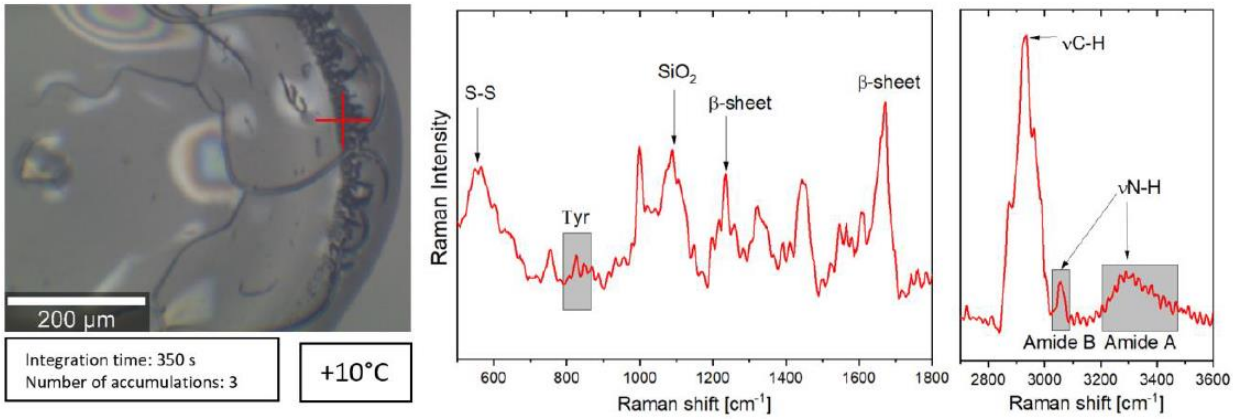

Figure 3: (left) Microscopic image of dehydrated mAb formulation recorded at 10°C after 1 FT cycle. The red cross marks the point of measuring with (right) corresponding Raman spectra.

The mAb within the crystalline structure shows the native secondary structure (antiparallel β-sheet) but a significantly lower tyrosine ratio of 0.82 which indicates π–π interactions of stacked phenol rings in crystal phase(4). Additionally, only the N-H vibrational bands of protein but not the O-H stretching band at 3416 cm^{-1} is visible indicating dehydration (see Figure 3, left). An elevated intensity of the 550 cm^{-1} band assigned to disulfide bonds is noticed (see Figure 3, middle).

References:

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