



Characterization of Modified Biotherapeutics by FTIR and Raman Spectroscopy

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Abstract: Vibrational spectroscopic techniques provide the foundation to investigate the structures, hydrogen-bonding, orientation, and interactions of side chains (as aromatics, free sulfhydryl, and disulfide bonds) in proteins that play key role to their biostability and bioactivity. These techniques are used for characterization of protein pharmaceuticals in native and/or denatured states, to assess conformation changes, protein–protein interactions, aggregation, fragmentation, and also for protein drug product characterization.

In our study, we used FTIR and Raman spectroscopy to characterize chemically modified monoclonal antibody preparations, specifically antibodies conjugated with different bifunctional chelating agents that were prepared in order to be subsequently labeled with radioisotopes, in solution, in freeze-dried state and after reconstitution.

Based on the frequencies assigned for amide bands, the investigated formulations contain the highest percentage of β -sheet conformation (antiparallel and parallel), followed by α -helices in the structure. Significant changes in comparison with FTIR and Raman spectra of native antibody upon applied processes of conjugation and freeze-drying were not observed. In the experimental IR (in the region $2000\text{--}500\text{ cm}^{-1}$) and Raman spectra ($2000\text{--}400\text{ cm}^{-1}$ region) we observed retaining of native structure of the antibody and modified antibody and no obvious aggregation.

Our investigation has provided characterization of the formulations using FTIR and Raman spectroscopy, thus enabling insight in the structural changes of conformation and the stability of preparation in dissolved and solid (freeze-dried) state. In addition, these techniques were suitable for conformation assessment of the protein in solid state and in reconstituted solution. These results create good foundation for further studies of this kind in order to characterize protein-based biotherapeutics that have similar structure properties.