DEVELOPMENTS AND APPLICATIONS OF CHEMICAL CHARACTERIZATION OF BIOPHARMACEUTICALS

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Background: Understanding the behaviour and function of biomolecules at the molecular level is the key to the discovery and development of new drugs, as well as diagnostic techniques. The characterization of biological drugs, where therapeutic monoclonal antibodies (mAbs) are placed, poses many challenges compared to those of low-molecular mass drugs because of their inherent complexity due to their protein nature. Achievements in this field of science have changed the way that drugs are being designed and developed nowadays.

Materials and methods: Vibrational spectroscopy techniques, Fourier Transform Infrared (FTIR) Spectroscopy and Raman Spectroscopy (RS) have been applied and helped to determine the secondary structure and possible interactions and modifications of complex protein molecules, as well as protein-ligand complexes.

FT-IR spectroscopy measurements were conducted on PARAGON 1000 (Perkin Elmer) spectrophotometer in the spectral range 2000–500 cm^{-1} and the plates were scanned three times to minimize the influence of spotting variance. After spectral acquisition, data manipulation was performed with the Grams_32 software (Thermo Scientific).

The room temperature Raman spectra (2000–400 cm^{-1}) were recorded on micro-Raman multichannel spectrometer Horiba JobinYvon LabRam 300 Infinity. The Raman effect was obtained using 632.8 nm line from a He:Ne laser. An Olympus MPlanN confocal microscope with ×100 objective for magnification was selected. The spectral resolution was set to 4 cm^{-1}. The acquisition time and the accumulation number were set to 10 s and 10 scans, respectively.

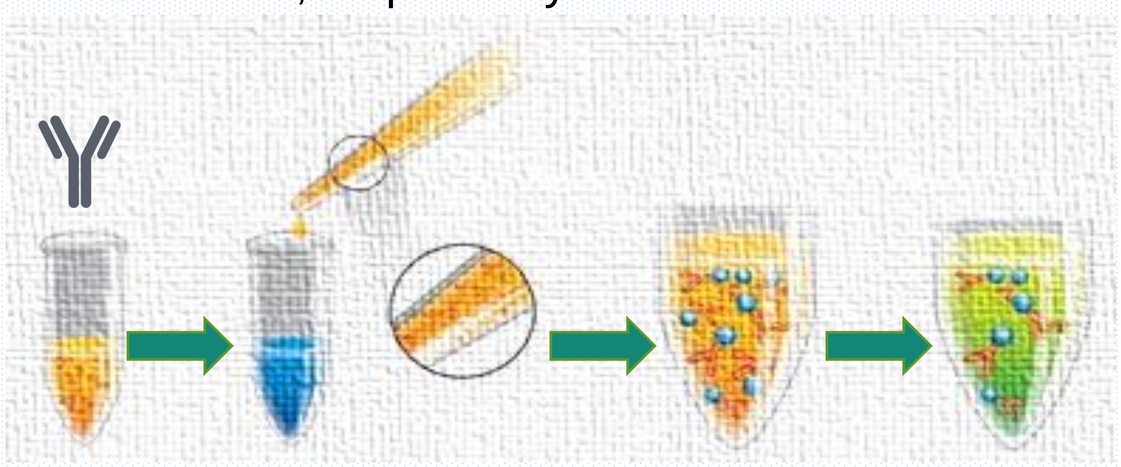

Fig. 1: Diagram of preparation of formulations of monoclonal antibody preparations

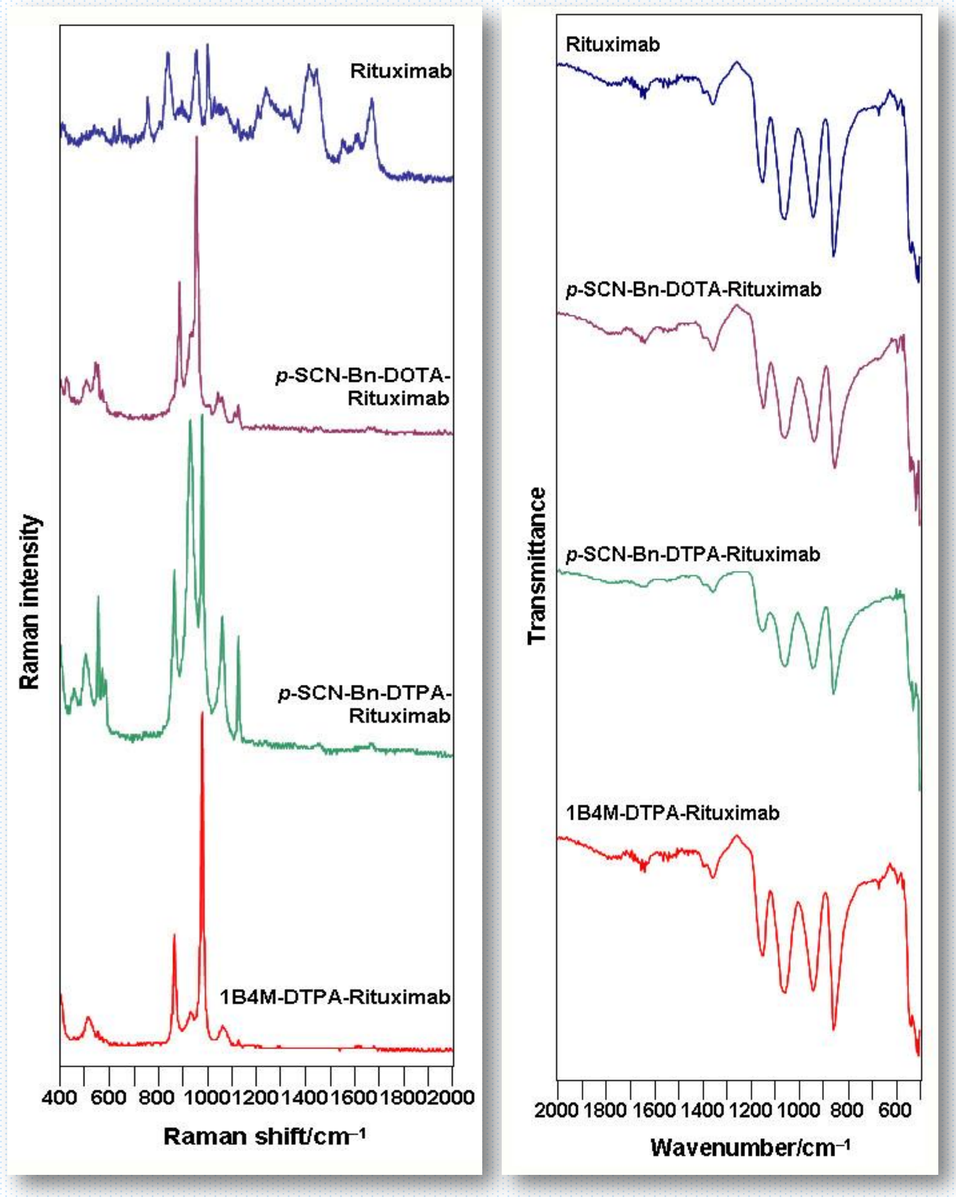

Fig. 2: Raman and ATR-IR spectra of rituximab and three types of experimental complexes

Results: 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. In the experimental IR (in the region 2000-500 cm^{-1}) and Raman spectra (2000–400 cm^{-1} region) we observed retaining of native structure of the antibody and modified antibody and no obvious aggregation in the formulations.

Our investigation has demonstrated the use of these tools to understand protein-ligand interactions in important immune-complexes with previously no available structural information.

CONCLUSION

The approach presented here has significant potential for analyzing the structure, stability and possible toxicity of biotherapeutics as well as any other biological molecules which are used as therapeutic/diagnostic agents.

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