Characterization of Modified Biotherapeutics by FTIR and Raman Spectroscopy

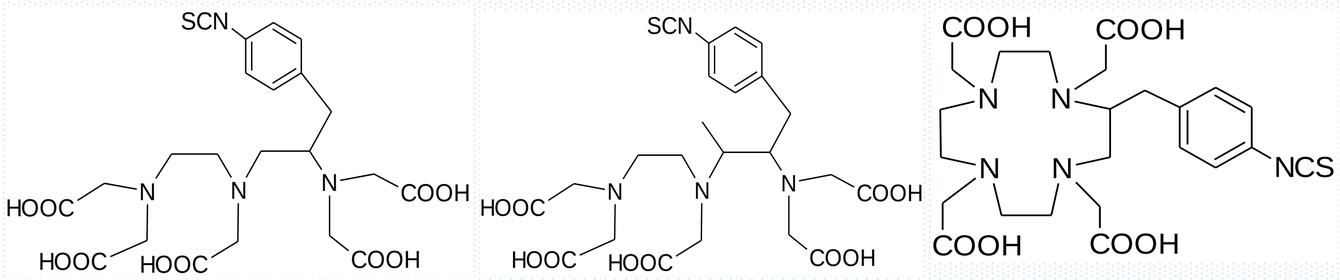
Darinka Gjorgieva Ackova[1], Katarina Smilkov[1], Petre Makreski[2]

[1] Department of Applied Pharmacy, Division of Pharmacy, Faculty of Medical Sciences, Goce Delčev University, 2000 Štip, Republic of North Macedonia
[2] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, 1000 Skopje, Republic of North Macedonia

e-mail: darinka.gjorgieva@ugd.edu.mk

Background: 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.

Materials and methods: 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.



***p*-SCN-Bn-DOTA** *(2-(4-izothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)*

***p*-SCN-Bn-DTPA** *(2-(4-izothiocyanatobenzyl)-diethylenetriamine- pentaacetic acid)*

1B4M-DTPA *(2-(4-izothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid)*

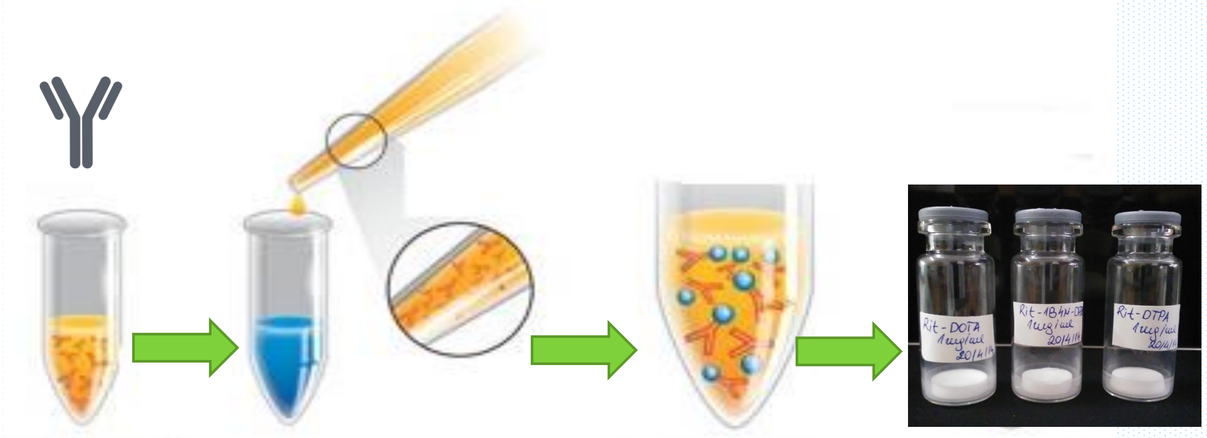


Fig. 1: Diagram of preparation of lyophilized formulations of modified monoclonal antibody preparations

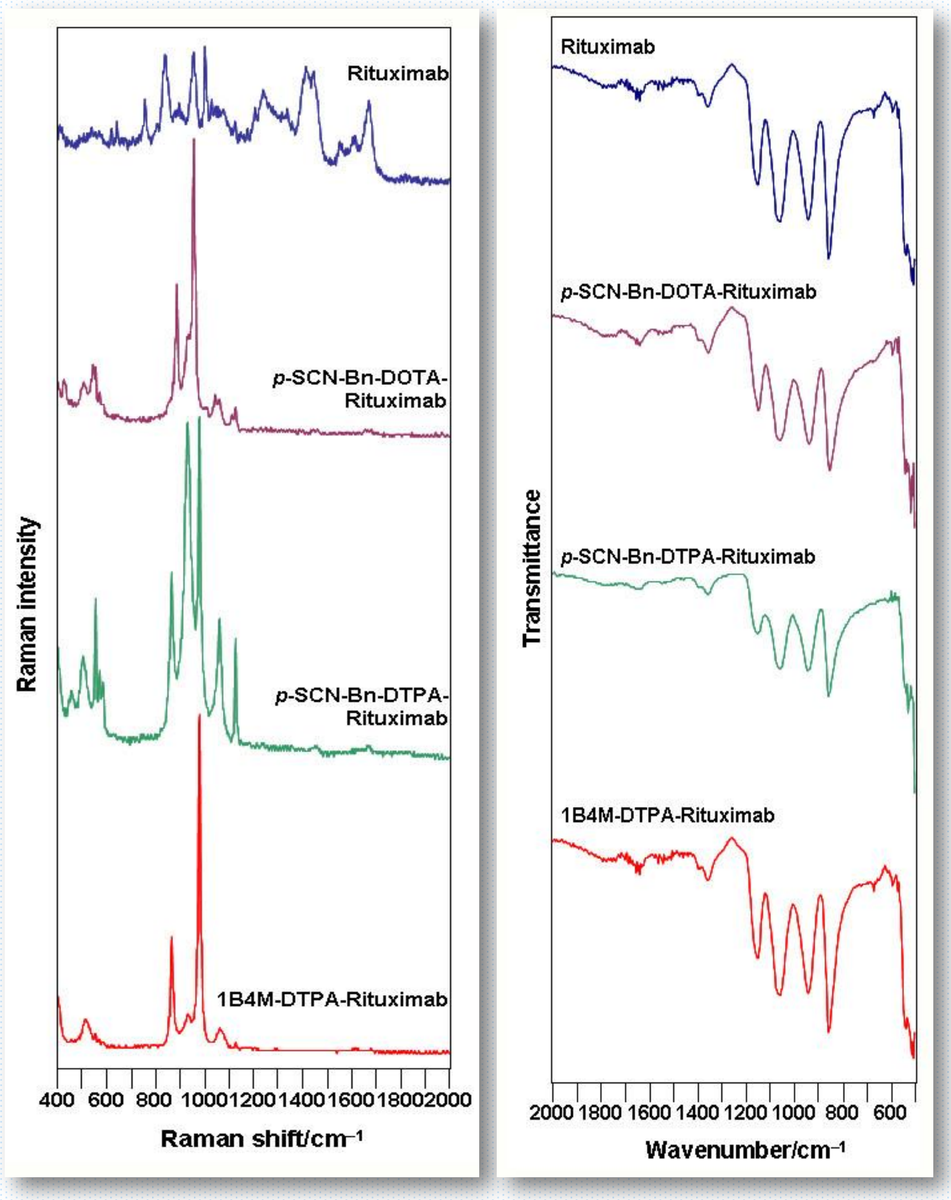


Fig. 2: Raman and ATR-IR spectra of rituximab and three types of experimental complexes

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. 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 2000-500 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.

CONCLUSION

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 preparations 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 of formulations in order to characterize protein-based biotherapeutics that have similar structure properties.

Training School: Advanced spectroscopic analytical methods and new technologies in drug delivery; Faculty Of Pharmacy, University Of Helsinki, Helsinki, Finland; 23-25 October, 2019