



***p*-SCN-Bn-DOTA-TRASTUZUMAB IMMUNOCONJUGATES AND VIBRATIONAL SPECTROSCOPY EXAMINATION**

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Introduction

Attenuated total reflectance-infrared (ATR-IR) and Raman spectroscopy, as vibrational techniques, provide reliable molecular structure information and are significant for determination of any of changes in the secondary structure. The aim of this study is structural examination of non-labeled conjugated antibody with *p*-SCN-Bn-DOTA 2-(4-izothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) in differ molar ratio by applying of these two techniques.

Material and methods

Freeze dried kit of immunoconjugates were produced after conjugation of antibody with the 10- and 50-fold molar excess of *p*-SCN-Bn-DOTA. The lyophilized non-labeled samples were used for further physical-chemical characterization with ATR-IR and Raman spectroscopy, by directly applying under the laser beam of the instrument. The spectra of naked antibody purified from commercial product Herceptin® was used for comparison with the collected spectra of conjugated samples.

Results and discussion

The ATR-IR and Raman spectra of conjugated antibody correspond with the spectra of naked antibody. The presence of characteristic amide I band at ~ 1670 cm^{-1} and amide III band (1230 - 1300 cm^{-1}) in Raman spectra and amide I (1700 - 1600 cm^{-1}), amide II (1480 - 1575 cm^{-1}) and amide III bands (1255 - 1244 cm^{-1}) in ATR-IR spectra have showed retained IgG1 structure of the antibody principally composed of β -sheets.

Conclusion

The minor difference in the ATR-IR and Raman spectra between immunoconjugates and native trastuzumab showed that only insignificant changes occurred in the protein's structure as a result of chelator binding. The retained β -sheet structure of the antibody supports the opportunity for further development of anti-HER2 positive breast cancer radioimmunotherapeutics and diagnostic products.