COLD LABELED TRASTUZUMAB-*p*-SCN-Bn-DTPA AND TRASTUZUMAB-*p*-SCN-Bn-1B4M-DTPA CONJUGATES– PREPARATION AND SPECTROSCOPIC ANALYSIS

Sterjova M[1,2], Džodić P[2], Makreski P[3], Janevik-Ivanovska E[1]

[1] University 'Goce Delčev', Faculty of Medical Sciences, str. "Krste Misirkov" No. 10-A, 2000 Štip, Republic of Macedonia

[2] University of Niš, Faculty of Medicine, Department of Pharmacy, str. dr Zoran Đindić 81, 18000 Niš, Serbia

[3] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, 1000 Skopje, Republic of Macedonia

INTRODUCTION

The importance of immunoconjugates in treatment of various cancers was motivation for us to formulate a stable cold labeled trastuzumab conjugates with two bifunctional chelators (BFCAs) (*p*-SCN-Bn-1B4M-DTPA (2-(4-isothiocyanatobenzyl)-6-methyl-diethylene-triaminepentaacetic acid and *p*-SCN-Bn-DTPA (2-(4-izothiocyanatobenzyl)-diethylenetriaminepentaacetic acid)). The labeling with non-radioactive $LuCl_3$ and YCl_3 is important to determine the possible physicochemical changes in the structure of immunoconjugates after metal binding. ATR-IR (Attenuated total reflectance-infrared) and Raman spectroscopy as powerful and non-destructive techniques are appropriate for verification of possible secondary structure chances of trastuzumab after conjugation and labeling.

MATERIAL AND METHODS

Anti-HER2/neu monoclonal antibody trastuzumab was conjugated with p-SCN-Bn-DTPA, *p*-SCN-Bn-1B4M-DTPA in ratio of 1:10 and 1:50 and lyophilized to solid state. The freeze dried conjugates were labeled with cold $LuCl_3$ and YCl_3. The retained secondary structure of the antibody was proven by spectroscopic analysis with ATR-IR and Raman spectroscopy and compared with purified trastuzumab from commercial product Herceptin®.

RESULTS

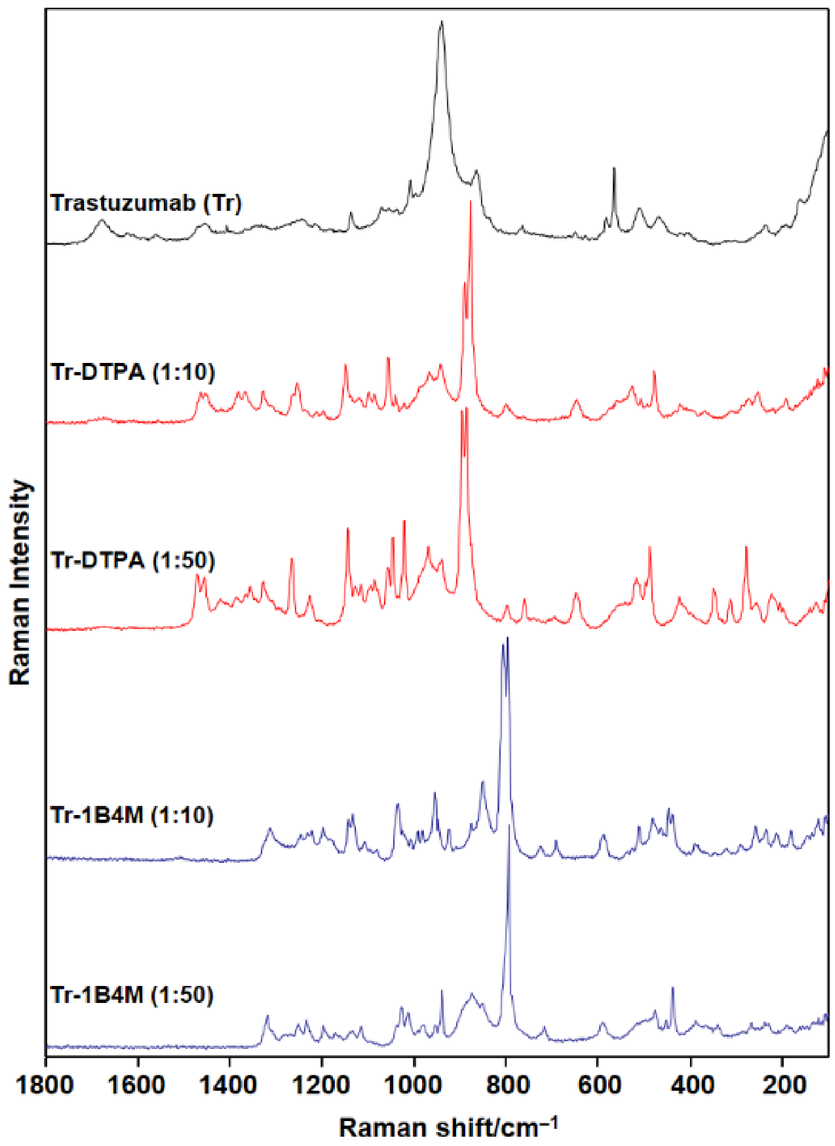

Fig. 1 IR spectra

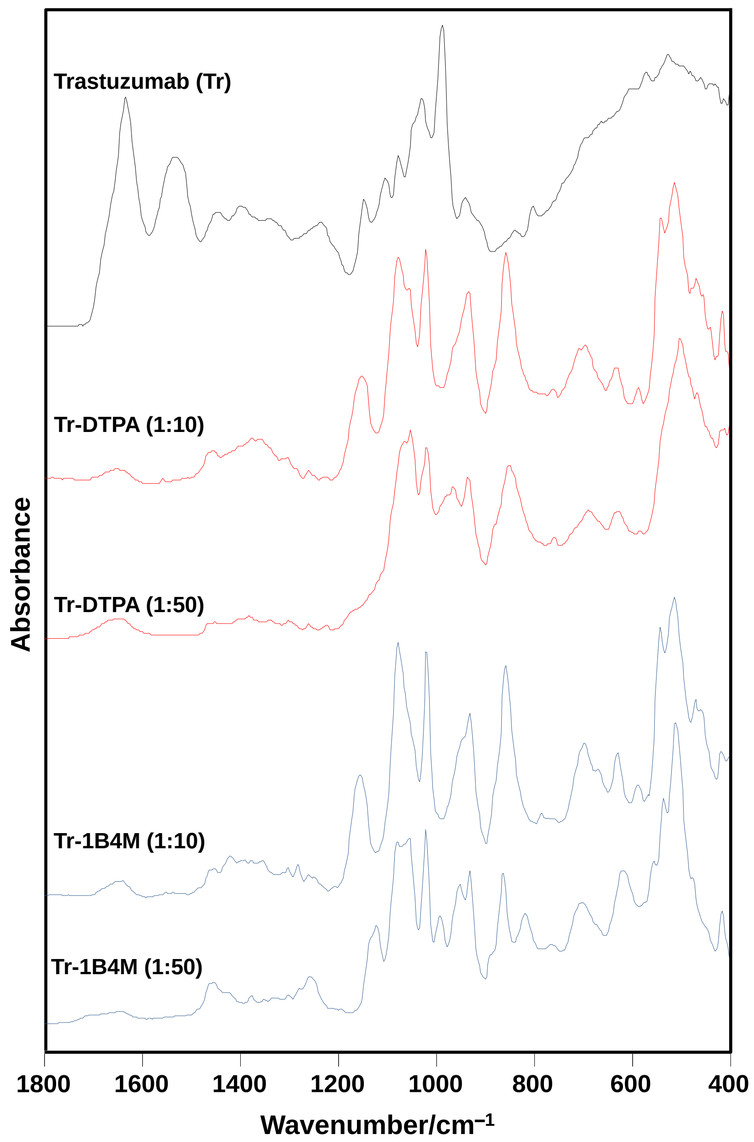

Fig. 2 Raman spectra

Characteristic Raman bands						
S-S	**Tyr**	**Trp**	**Phe**	**Indol ring**	**Amid band I**	**Amide band III**
400-700 cm^{-1}	647cm^{-1}, 760-790 cm^{-1}	757 cm^{-1}, 878 cm^{-1}, 1337 cm^{-1}	1004-1060 cm^{-1}, 1610 cm^{-1}	1560 cm^{-1}	1668-1688 cm^{-1}	1235-1260 cm^{-1}

Characteristic IR bands				
Amide band I	**Amide band II**	**Amide band III**	**Amide band IV and V**	**Amid band VI**
1640-1645 cm^{-1}	1480-1575 cm^{-1}	1233-1300 cm^{-1}	620-810 cm^{-1}	500-595 cm^{-1}

Table. 1 Characetristic Raman andIR bands

CONCLUSION

No significant changes in antibody structure after cold labeling gives us a hope for further radiolabeling of immunoconjugates with $^{177}LuCl_3$ and $^{90}YCl_3$ and development of radioimmunotherapeutics and diagnostic products active against HER2 positive breast tumors.