



ATR-IR AND RAMAN SPECTROSCOPIC CHARACTERIZATION OF *p*-SCN-Bn-DTPA AND *p*-SCN-Bn-1B4M-DTPA TRASTUZUMAB CONJUGATES

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INTRODUCTION

As powerful and non-destructive techniques, Attenuated total reflectance-infrared (ATR-IR) and Raman spectroscopy provide valuable molecular structure information and are convenient for verification of any changes in the native state of proteins. The purpose of this experimental work is to determine the secondary structure of trastuzumab after conjugation with (*p*-SCN-Bn-1B4M-DTPA (2-(4-isothiocyanatobenzyl)-6-methyl-diethylene-triaminepentaacetic acid) and *p*-SCN-Bn-DTPA (2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid)) by applying of these two techniques.

MATERIAL AND METHODS

The 10- and 50-fold molar excess of chelators were conjugated with pure trastuzumab and freeze dried to solid state. Vibrational spectroscopy (Raman and ATR-IR) was applied for further physico-chemical examinations of lyophilized non-labeled samples by directly applying under the laser beam of the instrument. The spectra of conjugated samples were compared with naked trastuzumab purified from commercial product Herceptin®.

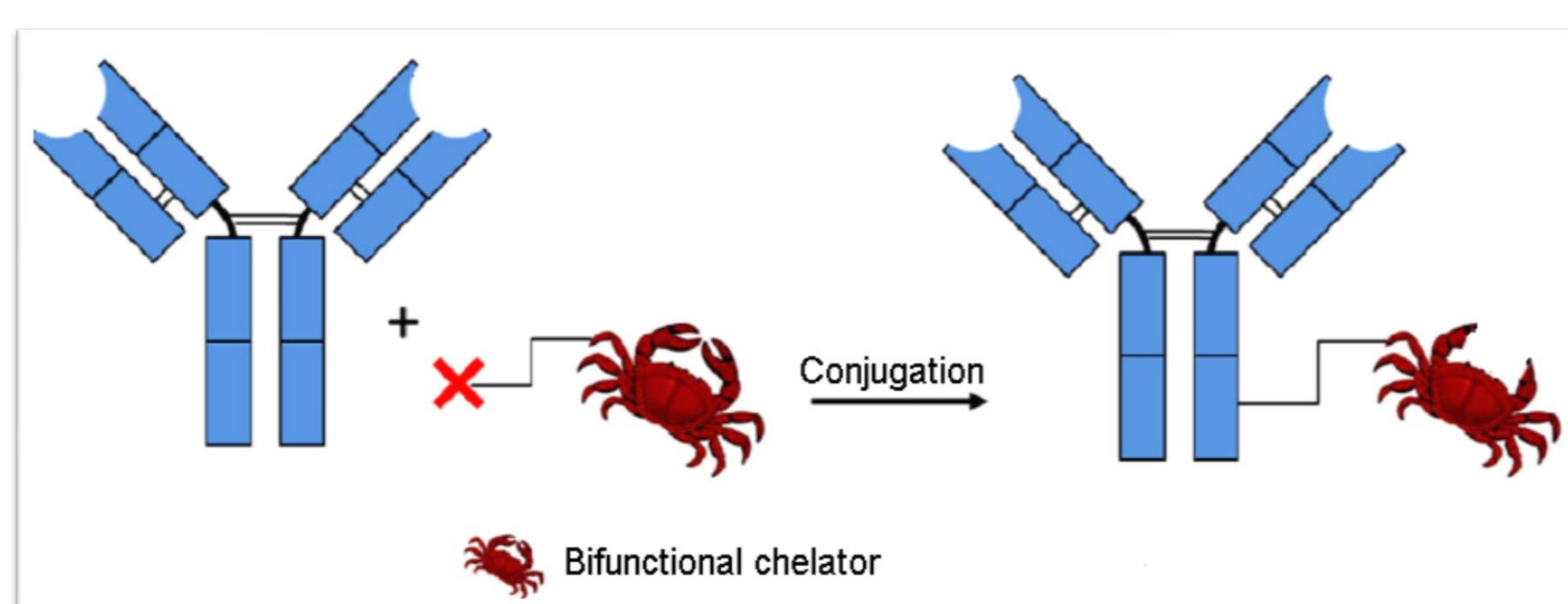


Fig. 1 Conjugation of antibodies with BFC

RESULTS

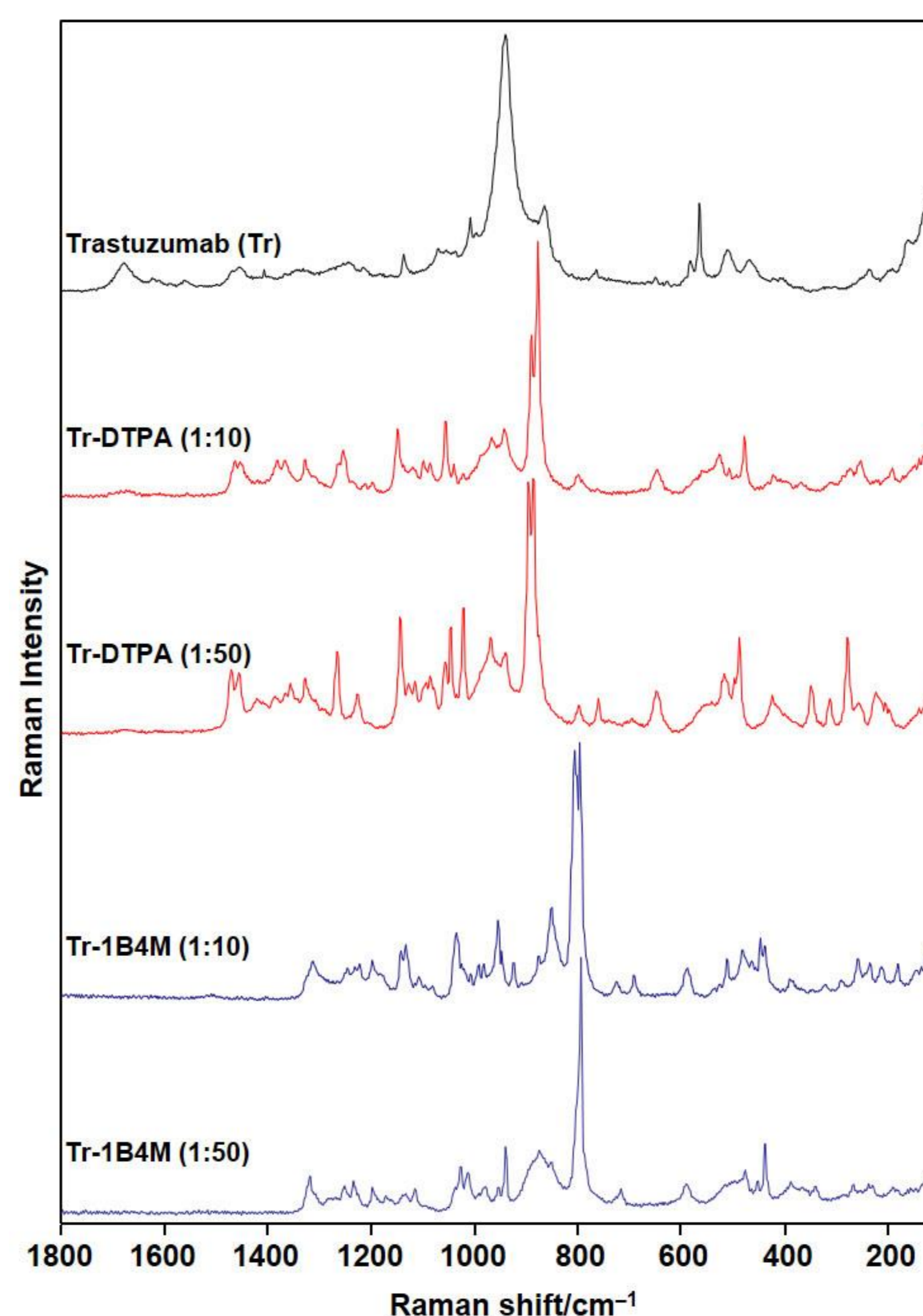


Fig. 2 Raman spectra

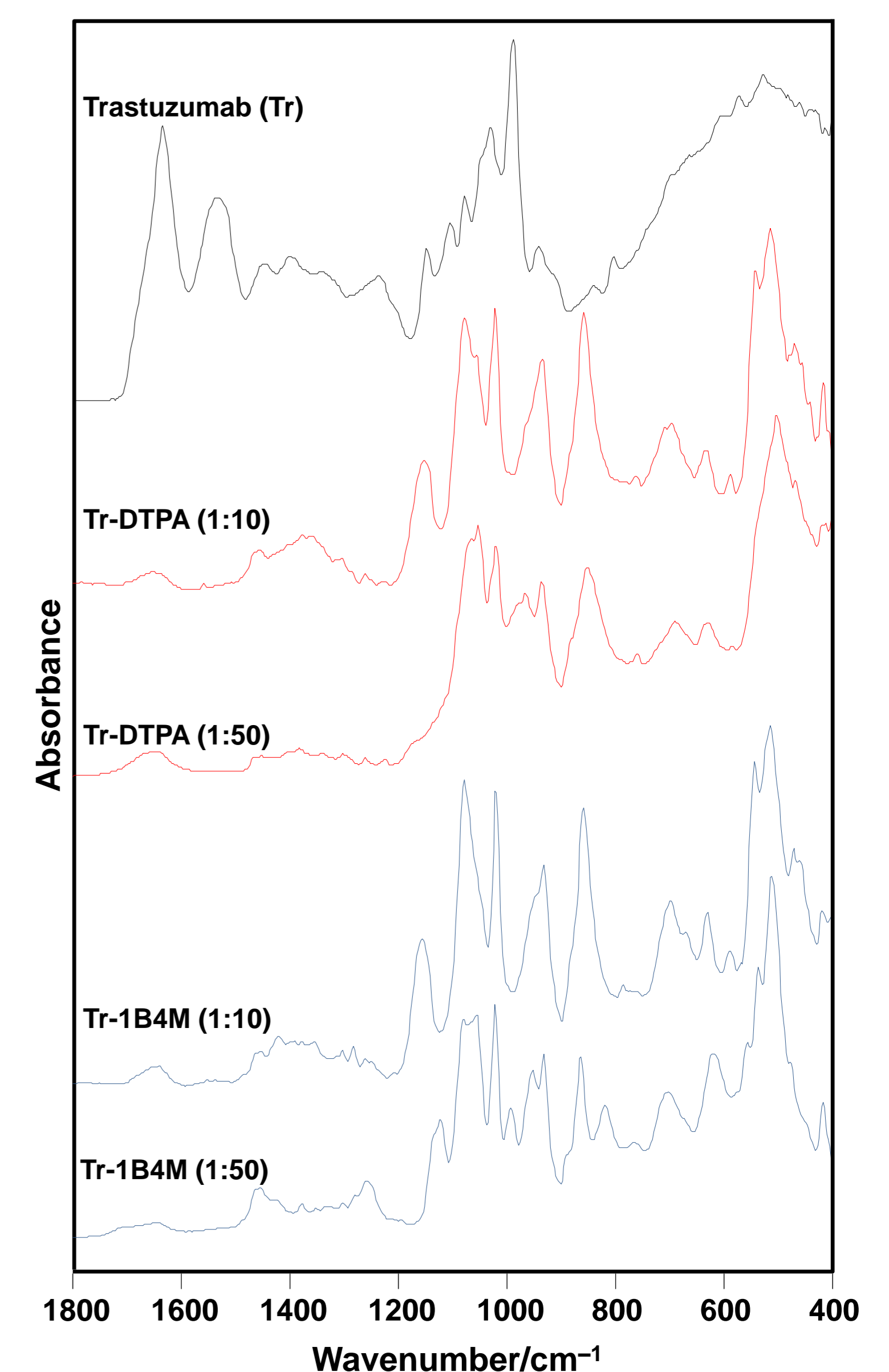


Fig. 3 IR spectra

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 Characteristic Raman and IR bands

CONCLUSION

No integrity changes, physicochemical and structural modifications of trastuzumab after conjugation approve retained β -sheet structure of the antibody and support the opportunity for further development of radioimmunotherapeutics and diagnostic products active against HER2 positive breast tumors.