FREEZE DRIED KIT FORMULATION OF TRASTUZUMAB IMMUNOCONJUGATES

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Introduction

Trastuzumab:

- Humanized IgG1 monoclonal antibody.
- Selective and specific therapy (HER2 positive breast cancer).
- Conjugation with another dugs, toxins and radioisotopes.
- Synthetized many stable conjugates with BFCA (p-SCN-Bn-DTPA, p-SCN-Bn-DOTA, 1B4M-DTPA) for further labeling with radioisotopes

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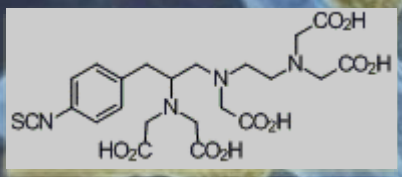

Fig. 1 p-SCN-Bn-DTPA

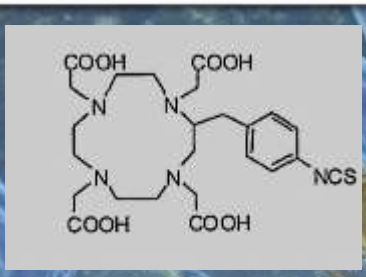

Fig. 2 p-SCN-Bn-DOTA

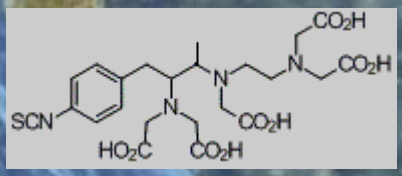

Fig. 3 1B4M-DTPA

Material and methods

- Trastuzimab - purified from Herceptin® with six cycles of ultrafiltration (0.1M PBS, pH=8).
- Mixing the antibody with 10 mg/ml solution of BFCAs in different ratio (p-SCN-Bn-DTPA – 1:10; 1:20; 1:50, 1B4M-DTPA – 1:10; 1:20; 1:50, p-SCN-Bn-DOTA – 1:20)
- 18 hours incubation on 4°C with gentle shaking.
- Immunoconjugates - purified with six cycles of ultrafiltration and adjusted to concentration of 1 mg/ml.
- Trastuzumab immunoconjugates - lyophilized to solid states with Labconco Free Zone Stoppering Tray Dryer.

Results and discussion

- The binding is via thiourea linkage between amine groups of lysine residues of trastuzumab and isothiocyanato groups of chelators.
- Obtained stable, homogeneous cakes with flat surfaces.
- After reconstitution with 0,9 % NaCl → clear to opalescent solutions without presence of visible solid particles and colloids.

Conclusion

After complete freeze drying the vials were closed and kept at 4°C for following examinations and protein characterization with SDS-PAGE, IR, RAMAN, MALDI-TOF and for further labeling with radioisotopes (Lu-177 and Y-90).