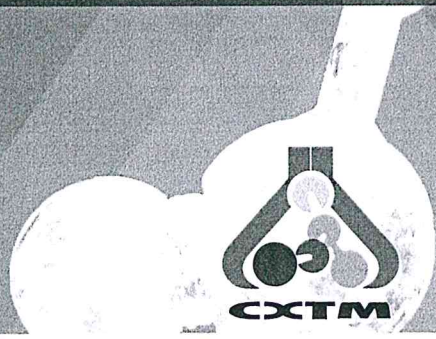
24th Congress of Chemists and Technologists of Macedonia

BOOK of ABSTRACTS



11-14 September 2016
Ohrid, Republic of Macedonia

Сојуз на хемичарите и технолозите на Македонија
Society of Chemists and Technologists of Macedonia

XXIV Congress
with international participation

BOOK OF ABSTRACTS

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Metropol Lake Resort

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MPCE 014

EFFECT OF CRYOPROTECTANT MANNITOL IN FREEZE-DRYING OF RITUXIMAB IMMUNOCONJUGATES

Katarina Smilkov[1], Darinka Gjorgieva Ackova[1], Emilija Janevik-Ivanovska[1], Icko Gjorgoski[2]

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The chimeric anti-CD-20 antibody, rituximab is among standard treatment regimes in treating B-cell Non-Hodgkin's lymphoma. The possibility of conjugation and subsequent radiolabeling with beta (gamma) - emitting radioisotopes can provide an added value to this therapeutical, thus increasing the possibility of targeted action to mature, malignant B-cells. Furthermore, designing a "ready to label", freeze-dried immunoconjugate holds much advantages both in preserving the antibody stability and in the preparation and labeling with radioisotopes.

Design of freeze-dried immunoconjugate kit was the main aim of this work, specifically in decision whether to add or not a cryoprotective and bulking agent, mannitol. For this purpose, rituximab, previously conjugated with three different bifunctional chelating agents, *p*-SCN-Bn-DOTA, *p*-SCN-Bn-DTPA and 1B4M-DTPA, adjusted to concentration of 1 mg/mL in 0.1M PBS (pH=8.0), with and without 10 mg/mL mannitol was freeze-dried, using Labconco Free Zone Stoppering Tray Dryer (USA). The immunoconjugate was gradually frozen, from 5°C to -40 °C, at a rate of 0.40 °C/min, with retention time (RT) 3 h, followed by annealing step at -15 °C with RT of 6 h and again cooling to -40 °C with RT of two hours. The primary drying was conducted at -10 °C, with heating rate of 0.15 °C/min, and a RT of 25 hours, and the secondary drying at a temperature of 25 °C, with a heating rate of 0.15 °C/min, and RT of 11 hours. The freeze-dried samples were reconstituted with 0.9% NaCl, separated in Sephadex G25 column and resulted fractions analysed for absorbance at 280nm, using UV spectrophotometer (Jenway UV/VIS spectrophotometer 6715). The results, given as chromatographic profile of eluted fractions at 280nm, revealed no significant difference in profiles of samples containing mannitol vs. samples that did not contain mannitol. This result gave us the basis for further experiments in assessing long- term stability and optimizing radiolabeling experiments with the samples without mannitol in formulation.

Key words: rituximab immunoconjugates, cryoprotectants, mannitol

n.b.: Manuscripts submitted to this Congress were not subjected to language or other corrections, except in some extreme cases. Authors are fully responsible for the content of their Abstracts.

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