EFFECT OF CRYOPROTECTANT MANNITOL IN FREEZE-DRYING OF RITUXIMAB IMMUNOCONJUGATES

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INTRODUCTION

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) - emmiting 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. The main aim of this work was the design of freeze-dried immunoconjugate kit, specifically in decision whether to add or not a cryoprotective and bulking agent, mannitol.

METHODS

Rituximab, commercially available, was purified and 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). The immunoconjugates were freeze-dried, using Labconco Free Zone Stoppering Tray Dryer (USA), with and without addition of 10 mg/mL mannitol, using previously determined protocol, presented in Fig. 1. As a repesentative, the immunoconjugate of rituximab with 1B4M-DTPA was used in furhter experiment. The freeze-dried sample was 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). For comparison, purified rituximab was used.

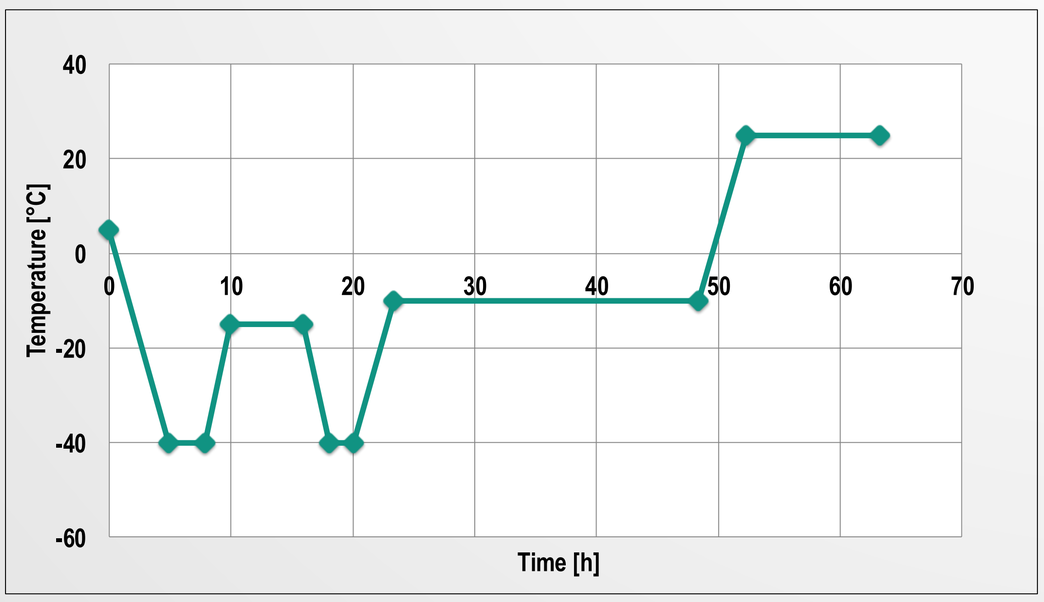

Fig. 1: Graphical representation of temperature variations of the employed freeze-drying protocol.

RESULTS AND DISCUSSION

The results, given as chromatographic profile of the eluted fractions at 280nm, using reconstituted rituximab-1B4M-DTPA immunoconjugate, as presented in Fig. 2 (B), revealed no significant difference in the profiles of the samples containing mannitol vs. samples that did not contain mannitol. In addition, the obtained chromatographic profile of the immunoconjugate, was comparable to the chromatographic profile of purified rituximab (Fig. 2 (A)), and all tested samples eluted in the fourth fraction. The chromatograms did not reveal elution of higher Mw species, which imply presence of aggregates, nor significant amount of low Mw species, that contribute to antibody fragments, in both samples. Therefore, the effects of the added cryoprotectant to the integrity of the antibody are of minimal/no significance.

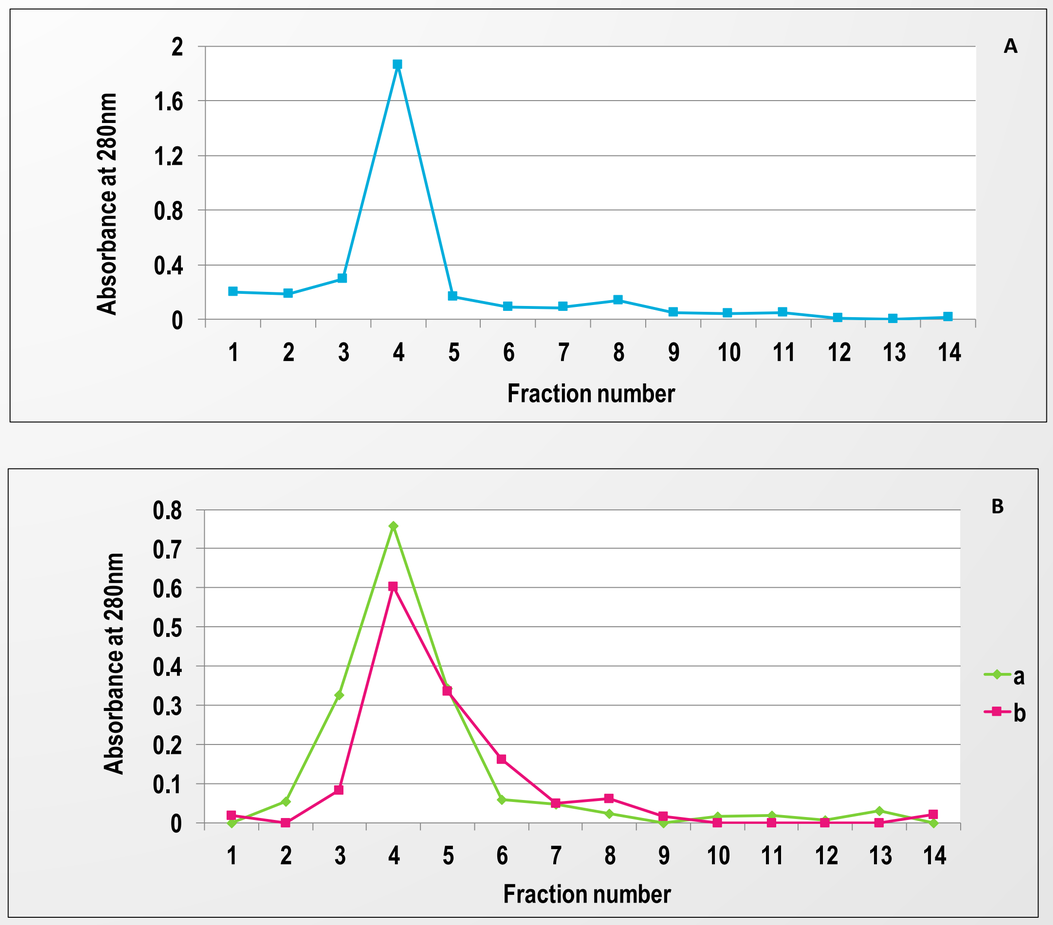

Fig. 2: (A) Chromatographic profile of liquid, purified rituximab at 280nm. (B) Chromatographic profile of the eluted fractions at 280nm of the freeze-dried rituximab-1B4M-DTPA immunoconjugate after reconstitution: a) sample without mannitol, b) sample with 10 mg/mL mannitol.

CONCLUSION

Although mannitol is frequently used in protein pharmaceutical formulation as cryoprotective agent, in this particular case, no significant differences in the chromatographic profiles of the tested samples were observed. The obtained results provided a basis for further experiments in assessing long- term stability and optimizing radiolabeling experiments with the samples, without mannitol in formulation.

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