Freeze-drying approach to enhance antibody stability

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INTRODUCTION

Preparations containing antibodies are more easily formulated in solution, but their stability is often impaired. Aqueous solutions are prone to numerous physical and chemical changes, such as denaturation, aggregation, adsorption, hydrolysis, deamidation, nonreducible cross-linking, formation/exchange of disulfide bonds, isomerization, fragmentation, Maillard-reaction, etc. Freeze-drying enables preservation of the desired characteristics of the product for a longer period of time, thus increasing its shelf life.
Part of the challenges associated with the first phase of the freeze-drying process can be addressed with its' corresponding design, i.e. defining the freezing rate, but also addition of bulking crystalline agents as mannitol, glycine or disaccharides (sucrose, trechalose), that can provide fine, microporous structure. Recent research had shown that slow freezing, which includes gradual cooling of the sample to a given temperature with a certain rate, and fast freezing, including direct introduction of the sample to a previously cooled freeze-dryer, are the most favored.

METHODS

Anti-CD-20 antibody (rituximab conjugated with 1B4M-DTPA) was freeze-dried with or without presence of cryoprotectant mannitol, using stoppering tray freeze dryer (Labconco Free Zone Stoppering Tray Dryer, USA), Fig 1. Two types of freeze-drying protocols were applied, Protocol I, with direct freezing to the initial temperature of -40 °C and Protocol II, with gradual freezing from 5 °C to -40 °C, at a rate of 0,40 °C/min. Appearance after reconstitution of the finished product and presence of aggregates at wavelength of 410nm were evaluated, as well as the IR and Raman spectra.

Fig. 1. Labconco Free Zone Stoppering Tray Dryer.

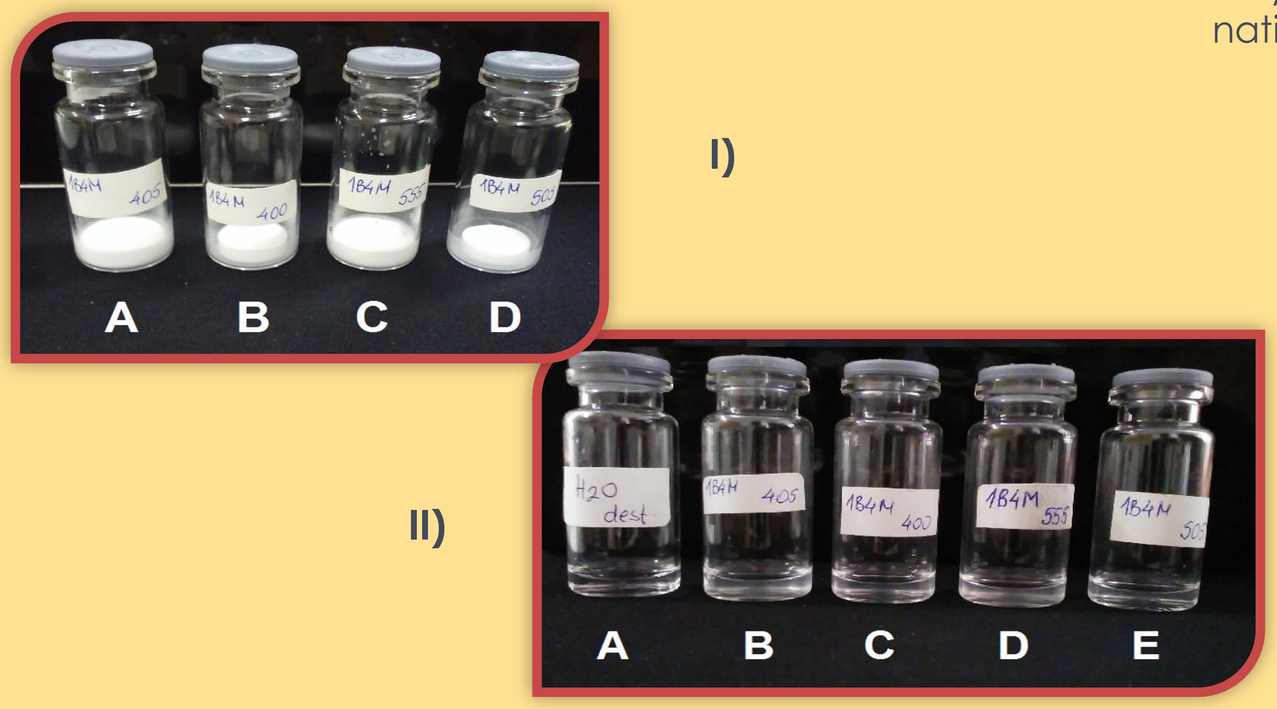

Fig. 2. **I)** Appearance of rituximab-DTPA-1B4M formulations after lyophilization: A) lyophilisate with 10 mg/mL mannitol, Protocol, I; B) lyophilisate without mannitol, Protocol I; C) lyophilisate with 10 mg/mL mannitol, Protocol II; D) lyophilisate without mannitol, Protocol II.
II) Appearance of the same batch after reconstitution.

Table 1. Reconstitution speed of the lyophilized formulations, appearance after reconstitution and absorbance at 410 nm.

Sample	Reconstitution time (s)	A_{410nm}	Clarity after reconstitution
Rituximab DTPA-1B4M without mannitol, Protocol I	25-40	0.219	Slightly opalescent
Rituximab DTPA-1B4M with 10 mg/mL mannitol, Protocol I	30-45	0.396	Moderately opalescent
Rituximab DTPA-1B4M without mannitol, Protocol II	30-45	0.225	Slightly opalescent
Rituximab DTPA-1B4M with 10 mg/mL mannitol, Protocol II	50-70	0.474	Moderately opalescent

RESULTS

The analysis of the results obtained showed that using gradual freezing from 5 °C to -40 °C, at a rate of 0,40 °C/min was more successful in maintaining the stability of the antibody during freeze-drying (Table 1). The appearance of the formulations after freeze-drying and reconstitution are given in Fig 2. The formulations that were freeze-dried using Protocol II gave clear solutions after reconstitution, no presence of aggregates (lower absorption at 410 nm) and IR and Raman spectra most similar to the spectra of the native conjugated form (results not shown).

CONCLUSION

The research performed, enabled selection of a freeze-drying protocol that was most successful in maintaining the stability of the conjugated Anti-CD-20 in terms of presence of aggregates and maintenance of antibody structure post-freeze-drying process.
The obtained results, are in further use for developing a formulation of ready-to label freeze-dried kits, intended for preclinical research for Non-Hodgkin's lymphoma therapy.

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