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Research Article

A Comparative Study to Evaluate the Effect of Different Carbohydrates on the Stability of Immunoglobulin G during Lyophilization and Following Storage

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ABSTRACT

Background: Although the stabilizing effects of cyclodextrins (CDs) on the liquid protein formulations have been proven, there is no comprehensive data on evaluation of their effects on the lyophilized antibody powders. In this study, the influence of two CD derivatives namely beta-cyclodextrin (βCD) and hydroxypropyl beta-cyclodextrin (HPβCD) was compared with trehalose and mannitol regarding the molecular and thermodynamic stability of lyophilized IgG formulations as well as its biological activity.

Methods: Sugars were separately added to IgG solutions and lyophilization process was conducted. In each group of carbohydrates, the formulations with lowest amounts of aggregates were examined regarding the biological activity. The storage stability of selected formulations was subsequently determined following 1 and 3 month of storage at 45 °C.

Results: Trehalose and HPβCD in the ratios of 80% showed the most stabilizing effects by control of aggregated forms in the orders of 1.02% and 0.83%, respectively. Also, it was shown that trehalose and HPβCD could incomparably preserve IgG activity in values of 100% and 96.5%. The results of DSC and SEM analysis confirmed the existence of crystalline parts in mannitol and βCD formulations of antibody. During the storage time, the lowest rate constant of aggregation was observed in formulations containing trehalose 80% (0.16/month). All prepared formulations were beta-dominant and no fragmentation was detected.

Conclusion: Molecular, thermodynamic and biological stability of lyophilized IgG was more desirable in the presence of trehalose and HPβCD in comparison to mannitol and βCD.

Introduction

Therapeutic antibodies are considered as the fastest-growing class of proteins which are successfully administered in diagnosis and treatment of various diseases namely cancer, autoimmune disorders, inflammation, allergy and infectious diseases.[1,2]

Several antibodies have been formulated in the liquid dosage forms whereas these proteins are prone to stress factors. Consequently, high-cost cold chains should be ensured for maintaining liquid dosage forms.[3,4] Moreover, temperature fluctuation and shear stresses are the most common problems may occur during shipping and storage which directly affect the stability of liquid biopharmaceuticals.[5] Therefore, formulation of antibodies with preserved stability during preparation, transportation and shelf time storage is of crucial importance.[6]

During the past two decades, numerous attempts have been devoted to develop dried pharmaceutical formulations and several drying techniques such as freeze-drying, spray-drying, spray-freeze drying and foam-drying have been evaluated to overcome this issue.[7] Amongst the mentioned techniques, freeze-drying or lyophilization is the most popular and commonly used method in pharmaceutical industry.[8] Ice crystallization and increasing the concentration of solute lead to major challenges in proteins such as aggregation, fragmentation and

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conformational alterations.[9]

Pharmaceutical excipients, particularly sugars, enhance the stability of proteins during both lyophilization process and/or storage time.[10,11] Two hypotheses have been suggested to explain the protective effects of sugars. The first opinion describes the ability of sugar molecules to form hydrogen bonds, like water molecules, in the dry state.[12] Another one discusses formation of amorphous, rigid and neutral matrix by sugars that decreases the molecular mobility of antibodies during drying.[13]

Mannitol is a well known sugar that has been repeatedly applied as a bulking agent to create elegant cake and several studies have confirmed that amorphous mannitol can stabilize lyophilized proteins.[14,15] Additionally, trehalose as a non-reducing disaccharide has been proved a prominent stabilizer due to its ability to form effective hydrogen bonds with proteins in the absence of water.[16]

Cyclodextrins (CDs) as circular oligosaccharides are the other category of sugars which are proposed to decrease aggregation of proteins.[17] Some reports have claimed that hydroxypropyl beta cyclodextrin (HPβCD) could enhance short-term stability of lyophilized proteins even more than trehalose, sucrose and dextran.[18,19] The impact of CDs as aggregation inhibitors for enzymes and various proteins has been thoroughly studied in liquid state;[20,21] however, very few studies have investigated the effect of CDs on the stabilization of lyophilized antibodies.

The aim of the present study was to assess the impact of a parent CD (βCD) and HPβCD on the stability of lyophilized-IgG as a model antibody. Furthermore the efficacy of these derivatives was compared with mannitol and trehalose regarding antibody' secondary structure, biological activity, particle properties and amount of induced aggregates immediately following process and upon storage time.

Material and methods

Materials

Human polyclonal antibody-IgG (~150 kDa) was supplied by Kedrion (Italy). Potassium phosphate dibasic, trehalose dehydrate, mannitol, disodium sulfate and coomassie brilliant blue were purchased from Merck (Germany). HPβCD was provided by Acros Organics (Belgium) and βCD was obtained from Sigma Aldrich (USA). Ladder for SDS-PAGE was purchased from CinnaGen (Iran).

Preparation of antibody solutions and freeze-drying

Primary aqueous solutions of antibody were prepared through dialysis of human IgG to a final protein concentration of 50 mg/mL. Sugars namely trehalose, mannitol, βCD and HPβCD were separately added to IgG solutions. All prepared solutions were passed through a 0.22 µm filter just before freeze drying process.

The prepared solutions were frozen at -20 ºC for 24 h. Lyophilization cycle was conducted applying lab scale freeze-dryer (Christ, Germany) by primary drying at -50 ºC and 0.01 mbar vacuum for 24 h as well as secondary drying up to 25 ºC and 0.01 mbar for 24 h.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were provided using a Nicolet Magna spectrometer (USA) at room temperature. About 2 mg of lyophilized powders were mixed with 200 mg KBr and then compacted under pressure of 6-7 T, applying a carver press. The amide I region (1600–1700 cm^{-1}) of spectra was analyzed using Jasco Spectra Manager® software (Japan) and curve fitting was performed by a mixed Gaussian/Lorentzian function.

Size exclusion high performance liquid chromatography (SEC-HPLC)

SEC-HPLC was employed to quantify the induced soluble aggregates. The chromatographic system consisted of a pump (Jasco, USA), a UV detector (Jasco, USA) at 280 nm and a TSK 3000 SWXL column (7.8mmx30cm, Tosoh Biosep, Germany). The mobile phase included 0.1 M sodium sulfate and 0.1 M disodium hydrogen phosphate di-hydrate which was adjusted to pH of 6.8 by phosphoric acid 85%. Flow rate of 0.5 mL/min was applied to inject 20 µL of filtered solution containing 2.5 mg/mL of IgG solution. Experiments were performed in triplicate.

Scanning electron microscopy (SEM)

The surface morphology and particle size of selected lyophilized samples were evaluated by scanning electron microscopy (Philips, The Netherlands). Powders were attached on carbon tape on the aluminum SEM stub. Then samples were sputtered with gold and the related images were taken at an accelerating voltage of 25 kV.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Non-reduced SDS-PAGE analyses was used to evaluate the integrity of lyophilized antibody following the process. The 10% polyacrylamide gels were prepared to load 50 µg of IgG, based on the Laemmle method. Samples were incubated with N-ethylmaleimide (NEM) to inhibit the reduction before loading. The applied conditions for running gels were 20 mA for 2.5 at 100 V. Coomassie blue solution of 0.1% was used to stain the gels. Molecular weight markers included 11,17, 25, 35, 48, 63, 75, 100, 135 and 180 KDa as standards.

Enzyme-linked immune sorbent assay (ELISA)

ELISA was employed utilizing polycolonal anti-IgG antibody. ELISA has been proved as a method with high sensitivity and specificity to determine the total IgG in preliminary tests. Nunc Maxisorp microwell plates (Denmark) were applied for running tests.

Differential scanning calorimetry (DSC)

Thermal behavior of the lyophilized formulations was analyzed using DSC instrument (Mettler Toledo, Switzerland). About 10 mg of lyophilized powders and pure excipients were transferred in aluminum pans to create sealed samples with sample encapsulation press. The applied temperature protocol was designed from 10 ºC and heated to 300 ºC with a scanning rate of 10 ºC/min.

Storage stability

In order to assess the stability of lyophilized formulations with/without excipient, sealed glass vials were stored under accelerated conditions, 40±1ºC for 1 and 3 months, separately. Powders were subsequently analyzed by SEC-HPLC to be evaluated in terms of formation of soluble aggregates during storage time. The kinetic of aggregation for each formulation was also calculated.

Results and Discussion

Various stresses in freezing step and dehydration phases are considered as the main challenges during lyophilization process.[22] Carbohydrates are the most applicable stabilizers of lyophilized peptides and proteins. Among them, trehalose and HPβCD have been repeatedly studied as protein stabilizers.[23,24] On the other hand, application of mannitol and βCD for stabilizing the lyophilized proteins is a matter of controversy.[25] In the current research work, the lyoprotection efficacy of four carbohydrates was compared with each other to provide stable lyophilized antibody formulation. The effects of excipients on the particle properties, molecular, structural and biological stability of IgG (as a model antibody) were monitored after lyophilization process as well as storage period.

Stability of IgG immediately following lyophilization

SEC-HPLC

The amount of induced soluble aggregates following freeze-drying process was evaluated using SEC-HPLC and the results are demonstrated in Table 1. Pure antibody contained 10.92% soluble aggregates following the process. Carbohydrates showed different efficacies in reduction of antibody aggregation. Comparison between various ratios of carbohydrates is schematically illustrated in Figure 1. Analysis of IgG/ trehalose and IgG/ HPβCD formulations revealed that increasing the amount of excipients was associated with decrease in aggregation level. To further elucidate, the least amount of aggregates was detected in formulations containing 80% trehalose and HPβCD (1.02 and 0.83%, respectively). However for mannitol and βCD, the results were different. The most stable formulations of antibody were resulted in the presence of 60% mannitol and 70% βCD (Table 1).

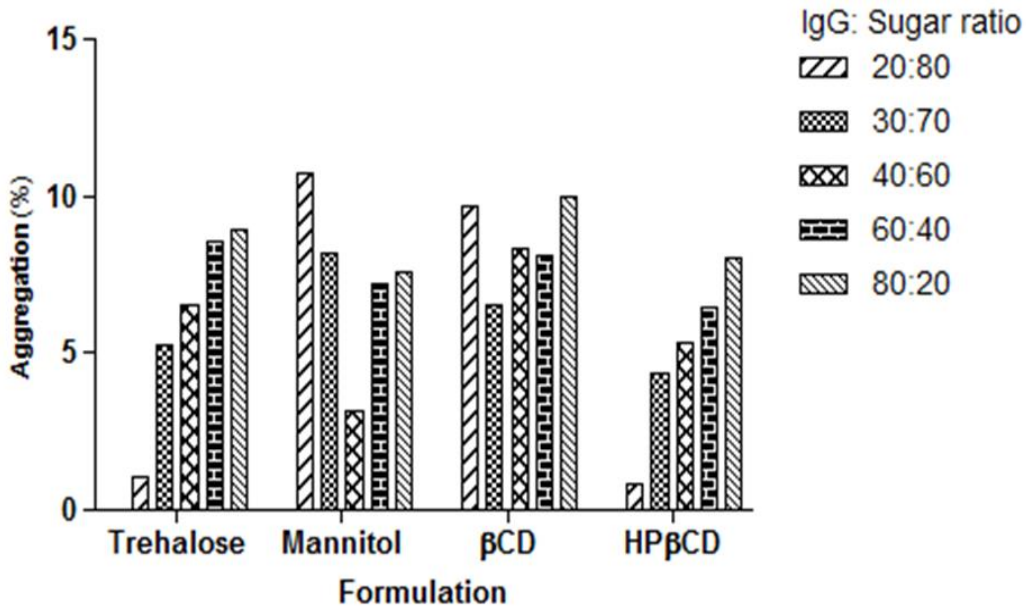

Figure 1. Comparison of the amount of aggregation following lyophilization process.

Table 1. Amounts of IgG aggregation (%) in the presence of carbohydrates following freeze- drying.

IgG: Sugar ratio	Carbohydrate			
	Trehalose	Mannitol	βCD	HPβCD
20:80	1.02	10.71	9.70	0.83
30:70	5.27	8.17	6.54	4.38
40:60	6.49	3.17	8.35	5.34
60:40	8.52	7.22	8.08	6.45
80:20	8.95	7.55	9.95	8.03

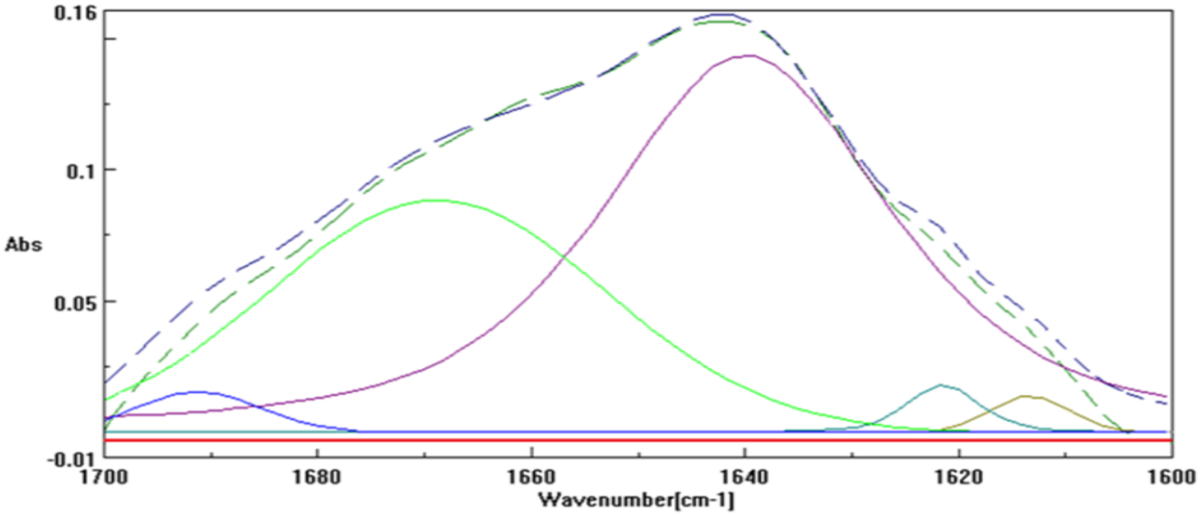

Figure 2. FTIR-spectra of spray-dried pure-IgG: The original and fitted trace spectra (dashed lines), the resulted fitted- curves (solid lines).

Sugars protect proteins against aggregation through various mechanisms namely stabilization by vitrification, glass dynamics and water substitution mechanisms.[26] The higher efficacy of trehalose and HPβCD in suppressing IgG aggregation would be rationally the result of their ability to form more effective hydrogen bonds with protein in the absence of water. Based on some previous studies, HPβCD was less efficacious in comparison to trehalose in protection of lyophilized peptides and proteins.[27,28]

However in the current study the amount of induced aggregates immediately following process was lower in the presence of 80% HPβCD. Likewise, the result of an earlier investigation demonstrated that either HPβCD or trehalose at same concentration could equally stabilize IgG monoclonal antibody within supercritical fluid processing.[29]

It could be assumed that less protection efficacy of mannitol and βCD for antibody could be attributed to the partial crystallization and less solubility, respectively.[30]

FTIR- spectroscopy

FTIR analysis is considered as a frequently applied method to study the secondary conformation of peptides and proteins. Based on several performed investigations, IgG is a beta- dominant protein (more than 66% beta- sheet content).[31] The band components at the wavelengths of 1639, 1691, 1613 and 1621cm^{-1} are related to beta-sheet conformation (Figure 2). The calculated beta-sheet content of antibody formulations is presented in Table 2.

Table 2. Beta- sheet percentages (%) of IgG in the presence of carbohydrates.

IgG: Sugar ratio	Carbohydrate			
	Trehalose	Mannitol	βCD	HPβCD
20:80	72.54	54.32	68.53	69.22
30:70	71.29	61.23	67.52	68.43
40:60	71.61	68.60	67.29	67.98
60:40	70.42	64.19	66.95	66.87
80:20	70.38	62.27	66.81	66.56

Pure antibody was composed of 66.28% beta-sheet. As it is shown, all the formulations in the prepared ratios protected the native conformation of antibody in comparison to pure protein. The only exception was mannitol which stabilized IgG structure just at antibody:sugar ratio of 40:60. Partial crystallization of mannitol during freeze drying prevents it from formation of effective hydrogen bonds with antibody.

ELISA assay

Formulations containing the minimum amount of aggregation and preserved conformation were selected for ELISA assay. The results showed that in the presence of carbohydrates (at appropriate ratios) the activity of antibody was better protected in comparison to the pure antibody (Figure 3). Trehalose and HPβCD (at IgG:excipient ratio of 20:80) significantly preserved antibody activity (remained activity of 100% and 96.50%, respectively). These findings are in agreement with the report of Esteves et al. that demonstrated perfect protection of enzyme activity of lyophilized immunoconjugates by trehalose.[32]

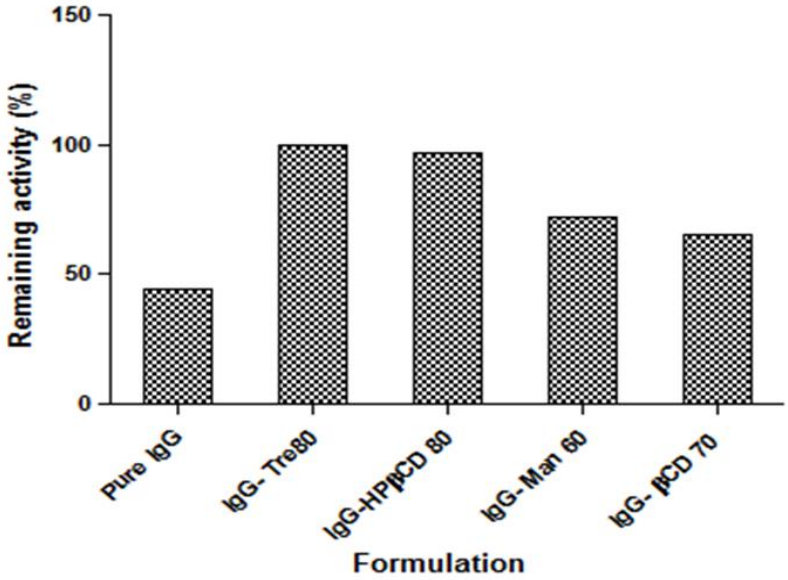

Figure 3. Comparison of remained biological activity of selected formulations.

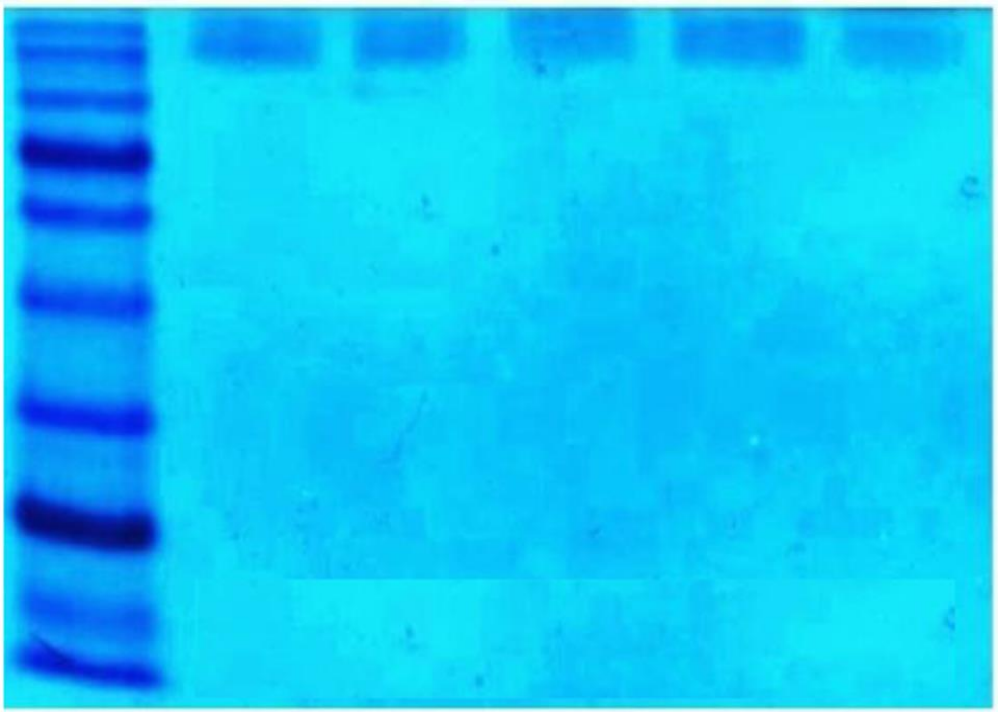

Figure 4. SDS- PAGE analysis of selected lyophilized formulations. From left to right, lane1: marker. Lane 2: Pure-IgG, lane 3: IgG-trehalose 80%, lane 4: IgG-mannitol 60%, lane 5: IgG-CD 70%, lane 6: IgG-HPβCD 80%.

SDS-PAGE analysis

Selected formulations were characterized by SDS-PAGE regarding the fragmentation and the result is demonstrated in Figure 4. The observed band at 150 KDa is indicative of intact IgG and as it is clear, all lyophilized formulations were structurally preserved in the presence of applied carbohydrates similar to that of pure antibody. These results proved that neither lyophilization condition nor presence of carbohydrates degraded IgG molecules and there was no sign of fragmentation in the downward regions.

Stability of IgG following 1 and 3 month of storage

Although short-term stability of lyophilized proteins is considered as the key element in preparation of appropriate formulations, desired storage stability is also crucial for predicting protein shelf life as well as selecting the best excipients. Since aggregation is considered as the major stress factor which influences antibody' stability,[33] the selected formulations with appropriate short-term stability profile were characterized considering amount of aggregation following 1 and 3 month of storage at 40 ºC (Table 3). As it is shown, pure antibody consisted of 14.81 and 47.24% soluble aggregate following 1 and 3 month of storage at 40 °C. The existence of all carbohydrates significantly protected antibody against aggregation during storage. Formulations containing HPβCD and trehalose showed the minimum amount of aggregation, while βCD provided the least stabilizing effect on IgG lyophilized powder.

The rate of aggregation was separately modeled by both zero and first-order kinetics. The kinetic of aggregation reaction in all formulations was fitted to first-order model. As it is demonstrated in Table 3, the rate constant of aggregation related to pure IgG was 0.22/month. This parameter was decreased to 0.16 and 0.17/month in the presence of trehalose and HPβCD, respectively.

Thermal behavior of lyophilized IgG powder

DSC analysis was performed to investigate the powders amorphous/crystalline state. The results are exhibited in Figure 5. IgG-trehalose remained fully amorphous (Figure 5a), while pure trehalose consisted of two endothermic peaks at 190 and 220 °C indicative of the crystalline part of the powder (Figure 5f). IgG-βCD formulation composed of a minor endotherm at 110 °C (Figure 5b), similar to that one for pure βCD (Figure 5g). Combination of IgG and HPβCD (Figure 5c) as well as pure HPβCD (Figure 5h) were completely amorphous in nature without any observable endotherms. IgG-mannitol mixture had a clear endotherm at 170 °C which showed the crystalline pattern of this composition (Figure 5d). It is also worth noting that pure mannitol was mainly crystalline with sharp endotherm at 175 °C (Figure 5e). Pure IgG was remained amorphous following freeze drying as was previously reported (Figure 5i).

Some studies revealed the ability of mannitol to stabilize lyophilized antibodies.[34] Furthermore, addition of mannitol to spray-dried humanized anti-IgE monoclonal antibody effectively decreased aggregate level.[35] The proposed mechanism for these studies was related to the amorphous nature of mannitol. On the contrary, some other studies proved that crystal formation during lyophilization of proteins is regarded as the major cause of destabilization by mannitol.[36] Present study confirmed the fact that crystallization of mannitol not only decrease its short-term stabilizing effect but also increase the rate of aggregation following storage compared to other carbohydrates.

Morphology of lyophilized IgG powder

The morphology of stable formulations was visualized through scanning electron microscopy. Pure IgG was composed of similarly pin-like structures with smooth edges (Figure 6a). Powders displayed different morphologies depending on the carbohydrate content. Trehalose containing powders composed of thin plates with unordered structure which confirmed the amorphous structure of these particles (Figure 6b).

Table 3. Kinetic of aggregation related to selected IgG formulations.

Formulation	Aggregation Following process (%)	Aggregation After 1mo at 40 °C (%)	Aggregation After 3mo at 40 °C (%)	Zero-order reaction R^2	First-order reaction R^2	Suggested order	Rate constant of aggregation (1/month)
Pure IgG	10.92	14.81	47.24	0.945	0.982	1	0.22
IgG-Tre 80%	1.02	1.36	3.15	0.966	0.993	1	0.16
IgG-Man 60%	3.17	5.21	13.06	0.981	0.999	1	0.20
IgG-βCD 70%	6.54	8.43	23.14	0.948	0.979	1	0.19
IgG-HPβCD 80%	0.83	0.95	2.69	0.925	0.948	1	0.17

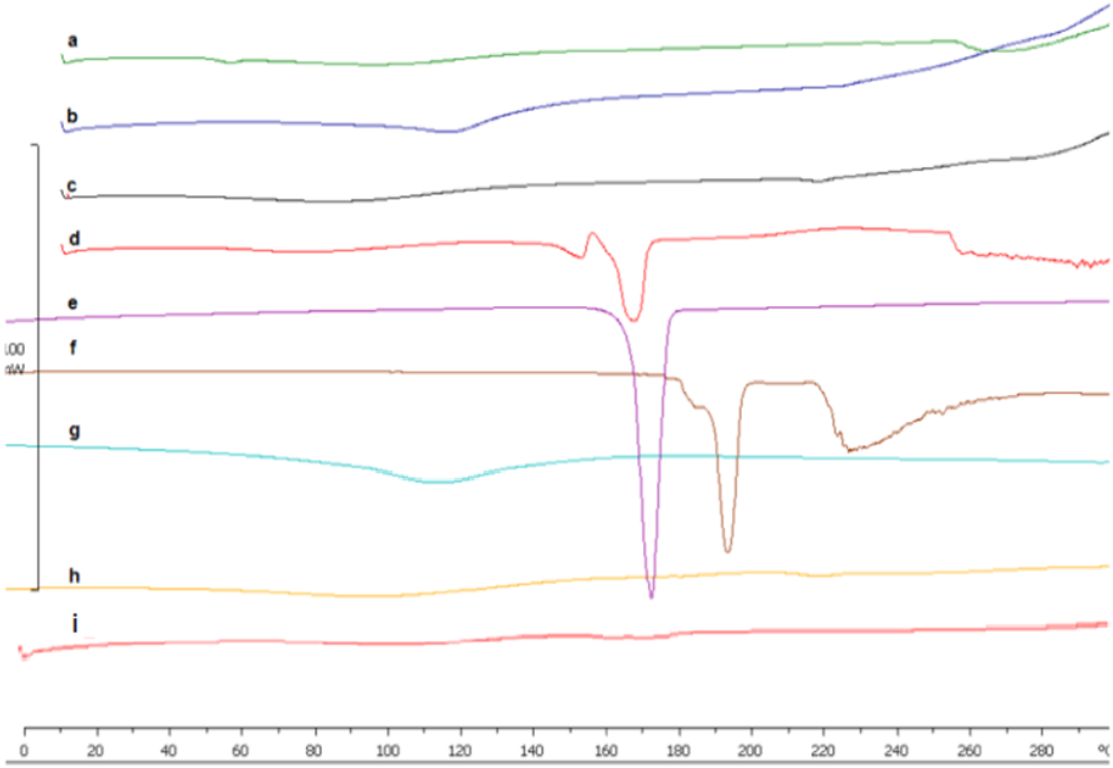

Figure 5. Thermograms of excipients and lyophilized formulations; a: IgG-trehalose 80%, b: IgG-βCD 70%, c: IgG-HPβCD 80%, d: IgG-mannitol 60%, e: mannitol, f: trehalose, g: βCD, h: HPβCD, i: Pure-IgG.

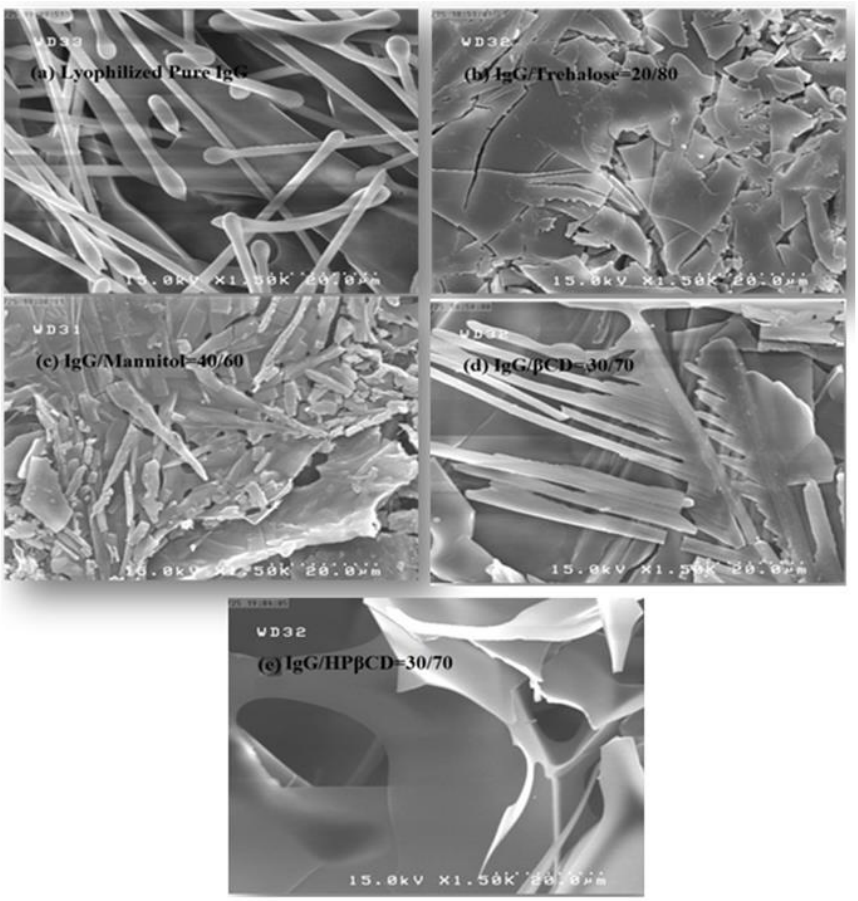

Figure 6. SEM photograph of lyophilized powders. a: pure-IgG, b: IgG-trehalose 80%, c: IgG-mannitol 60%, d: IgG-βCD 70%, e: IgG-HPβCD 80%.

In the case of IgG-mannitol formulation, the morphology exhibited a regular thin plates and flaky structure which is in agreement with DSC data (Figure 6c). Presence of βCD caused the formation of large thick plates with unordered morphology (Figure 6d). IgG powder consisting of HPβCD presented amorphous appearance which did not follow any defined shape (Figure 6e).

Conclusion

The influence of mannitol and trehalose was compared with two CDs derivatives namely HPβCD and βCD on the molecular, structural and biological activity of lyophilized IgG. All carbohydrates preserved native conformation of antibody. Although the presence of 80% HPβCD incomparably stabilized IgG molecules, the best biological activity as well as the least rate constant aggregation was observed in trehalose containing formulation. Inability of mannitol in suppressing aggregation was attributed to its crystallization. Both solubility problem and partial crystallization were accounted for least stabilization of βCD.

Conflict of interests

The authors declare that have no competing interests.

Acknowledgement

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Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Breen ED, Curley JG, Overcashier DE, Hsu CC, Shire SJ. Effect of Moisture on the Stability of a Lyophilized Humanized Monoclonal Antibody Formulation. Pharm Res. 2001;18(9):1345-53.
2. Wang W, Singh S, Zeng DL, King K, Nema S. Antibody structure, instability, and formulation. J Pharm Sci. 2007;96(1):1-26. doi:10.1002/jps.20727
3. Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. Int J Pharm. 1999;185(2):129-88. doi:10.1016/s0378-5173(99)00152-0
4. Arakawa T, Prestrelski SJ, Kenney WC, Carpenter JF. Factors affecting short-term and long-term stabilities of proteins. Adv Drug Deliv Rev. 2001;46(1-3):307-26. doi:10.1016/s0169-409x(00)00144-7
5. Carpenter J, Pikal M, Chang B, Randolph T. Rational Design of Stable Lyophilized Protein Formulations: Some Practical Advice. Pharm Res. 1997;14(8):969-75.
6. Manning MC, Patel K, Borchardt RT. Stability of protein pharmaceuticals. Pharm Res. 1989;6(11):903-18.
7. Abdul-Fattah AM, Kalonia DS, Pikal MJ. The challenge of drying method selection for protein pharmaceuticals: product quality implications. J Pharm Sci. 2007;96(8):1886-916. doi:10.1002/jps.20842
8. Schwegman JJ, Hardwick LM, Akers MJ. Practical formulation and process development of freeze-dried products. Pharm Dev Technol. 2005;10(2):151-73. doi:10.1081/pdt-200056308
9. Cleland JL, Powell MF, Shire SJ. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation. Crit Rev Ther Drug Carrier Syst. 1993;10(4):307-77.
10. Andya JD, Hsu CC, Shire SJ. Mechanisms of aggregate formation and carbohydrate excipient stabilization of lyophilized humanized monoclonal antibody formulations. AAPS PharmSci. 2003;5(2):21-31. doi:10.1208/ps050210
11. Wang B, Tchessalov S, Warne NW, Pikal MJ. Impact of sucrose level on storage stability of proteins in freeze-dried solids: I. Correlation of protein-sugar interaction with native structure preservation. J Pharm Sci. 2009;98(9):3131-44. doi:10.1002/jps.21621
12. Allison SD, Chang B, Randolph TW, Carpenter JF. Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. Arch Biochem Biophys. 1999;365(2):289-98. doi:10.1006/abbi.1999.1175
13. Wang W. Protein aggregation and its inhibition in biopharmaceutics. Int J Pharm. 2005;289(1-2):1-30. doi:10.1016/j.ijpharm.2004.11.014
14. Meyer JD, Nayar R, Manning MC. Impact of bulking agents on the stability of a lyophilized monoclonal antibody. Eur J Pharm Sci. 2009;38(1):29-38. doi:10.1016/j.ejps.2009.05.008
15. Izutsu K-i, Yoshioka S, Terao T. Decreased Protein-Stabilizing Effects of Cryoprotectants Due to Crystallization. Pharm Res. 1993;10(8):1232-7.
16. Duddu SP, Dal Monte PR. Effect of glass transition temperature on the stability of lyophilized formulations containing a chimeric therapeutic monoclonal antibody. Pharm Res. 1997;14(5):591-5. doi:10.1023/A:1012144810067
17. Otzen DE, Knudsen BR, Aachmann F, Larsen KL, Wimmer R. Structural basis for cyclodextrins' suppression of human growth hormone aggregation. Protein Sci. 2002;11(7):1779-87.
18. Brewster ME, Hora MS, Simpkins JW, Bodor N. Use of 2-hydroxypropyl-beta-cyclodextrin as

a solubilizing and stabilizing excipient for protein drugs. Pharm Res. 1991;8(6):792-5. doi:10.1023/A:1015870521744
19. Karuppiah N, Sharma A. Cyclodextrins as protein folding aids. Biochem Biophys Res Commun. 1995;211(1):60-6. doi:10.1006/bbrc.1995.1778
20. Couthon F, Clottes E, Vial C. Refolding of SDS- and thermally denatured MM-creatine kinase using cyclodextrins. Biochem Biophys Res Commun. 1996;227(3):854-60. doi:10.1006/bbrc.1996.1596
21. Pinotsis N, Leonidas DD, Chrysina ED, Oikonomakos NG, Mavridis IM. The binding of beta- and gamma-cyclodextrins to glycogen phosphorylase b: kinetic and crystallographic studies. Protein Sci. 2003;12(9):1914-24. doi:10.1110/ps.03149503
22. Cao W, Krishnan S, Ricci MS, Shih LY, Liu D, Gu JH, et al. Rational design of lyophilized high concentration protein formulations-mitigating the challenge of slow reconstitution with multidisciplinary strategies. Eur J Pharm Biopharm. 2013;85(2):287-93. doi:10.1016/j.ejpb.2013.05.001
23. Hedoux A, Paccou L, Achir S, Guinet Y. Mechanism of protein stabilization by trehalose during freeze-drying analyzed by in situ micro-raman spectroscopy. J Pharm Sci. 2013;102(8):2484-94. doi:10.1002/jps.23638
24. Morlock M, Koll H, Winter G, Kissel T. Microencapsulation of rh-erythropoietin, using biodegradable poly(d,l-lactide-co-glycolide): protein stability and the effects of stabilizing excipients. Eur J Pharm Biopharm. 1997;43(1):29-36. doi:10.1016/s0939-6411(96)00017-3
25. Davis ME, Brewster ME. Cyclodextrin-based pharmaceutics: past, present and future. Nature reviews Drug discovery. 2004;3(12):1023-35. doi:10.1038/nrd1576
26. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. J Pharm Sci. 2004;93(6):1390-402. doi:10.1002/jps.20079
27. Lee T-H, Lin S-Y. Additives affecting thermal stability of salmon calcitonin in aqueous solution and structural similarity in lyophilized solid form. Process Biochem. 2011;46(11):2163-9. doi:10.1016/j.procbio.2011.08.017
28. Maury M, Murphy K, Kumar S, Mauerer A, Lee G. Spray-drying of proteins: effects of sorbitol and trehalose on aggregation and FT-IR amide I spectrum of an immunoglobulin G. Eur J Pharm Biopharm. 2005;59(2):251-61. doi:10.1016/j.ejpb.2004.07.010
29. Jovanovic N, Bouchard A, Hofland GW, Witkamp GJ, Crommelin DJ, Jiskoot W. Stabilization of IgG by supercritical fluid drying: optimization of formulation and process parameters. Eur J Pharm Biopharm. 2008;68(2):183-90. doi:10.1016/j.ejpb.2007.05.001
30. Izutsu K, Kojima S. Excipient crystallinity and its protein-structure-stabilizing effect during freeze-drying. J Pharm Pharmacol. 2002;54(8):1033-9. doi:10.1211/002235702320266172
31. Faghihi H, Vatanara A, Najafabadi AR, Ramezani V, Gilani K. The use of amino acids to prepare physically and conformationally stable spray-dried IgG with enhanced aerosol performance. Int J Pharm. 2014;466(1-2):163-71. doi:10.1016/j.ijpharm.2014.03.020
32. Esteves M, Quintilio W, Sato R, Raw I, Soares de Araujo P, Bueno da Costa M. Stabilisation of immunoconjugates by trehalose. Biotechnol Lett. 2000;22(5):417-20. doi:10.1023/A:1005605616356
33. Miller MA, Rodrigues MA, Glass MA, Singh SK, Johnston KP, Maynard JA. Frozen-state storage stability of a monoclonal antibody: Aggregation is impacted by freezing rate and solute distribution. J Pharm Sci. 2013;102(4):1194-208. doi:10.1002/jps.23473
34. Cleland JL, Lam X, Kendrick B, Yang J, Yang TH, Overcashier D, et al. A specific molar ratio of stabilizer to protein is required for storage stability of a lyophilized monoclonal antibody. J Pharm Sci. 2001;90(3):310-21. doi:10.1002/1520-6017(200103)90:3<310::aid-jps6>3.3.co;2-i
35. Andya JD, Maa YF, Costantino HR, Nguyen PA, Dasovich N, Sweeney TD, et al. The effect of formulation excipients on protein stability and aerosol performance of spray-dried powders of a recombinant humanized anti-IgE monoclonal antibody. Pharm Res. 1999;16(3):350-8. doi:10.1023/A:1018805232453
36. Al-Hussein A, Gieseler H. The effect of mannitol crystallization in mannitol-sucrose systems on LDH stability during freeze-drying. J Pharm Sci. 2012;101(7):2534-44. doi:10.1002/jps.23173