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Research paper

Stabilization of IgG by supercritical fluid drying: Optimization of formulation and process parameters

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Abstract

The aim of this study was to stabilize human serum immunoglobulin G (IgG) by a supercritical fluid (SCF) drying process. Solutions containing IgG (20 mg/ml) and trehalose or hydroxypropyl-β-cyclodextrin in a 1:4 (w/w) ratio were sprayed into a SCF phase consisting of CO_2 and ethanol at 100 bar and 37 °C. Initially, a set of drying conditions previously developed to successfully stabilize lysozyme and myogobin formulations was used [N. Jovanović, A. Bouchard, G.W. Hofland, G.J. Witkamp, D.J.A. Crommelin, W. Jiskoot, Eur. J. Pharm. Sci. 27 (2006) 336–345]. Dried formulations were analyzed by Karl Fisher titration, scanning electron microscopy, X-ray powder diffraction, and modulated DSC. Protein structure in the solid-state was studied by FTIR and after reconstitution by UV/Vis, circular dichroism and fluorescence spectroscopy, GPC and SDS–PAGE. When IgG was dried under the above-mentioned conditions, substantial amounts of insoluble aggregates were formed. Addition of buffer helped to reduce the fraction of insoluble material but not of soluble aggregates. Full stabilization could be achieved by adjusting the process conditions: drying without ethanol while keeping the other conditions the same, or drying with ethanol at a temperature below the critical point (20 °C). In conclusion, it is possible to stabilize human IgG by SCF drying provided that the formulation and process conditions are tailored to meet the specific requirements of the protein.

Keywords: Hydroxypropyl-β-cyclodextrin; Human serum immunoglobulin G; Protein stability; Supercritical fluid drying; Trehalose

1. Introduction

A common approach to stabilize pharmaceutical proteins is to lyophilize them [1]. Although many commercially available protein drugs are freeze-dried formulations, this technique has some disadvantages: it is time- and energy-consuming, may induce process-induced degradation during the freezing and drying phases [1–3], and is less suitable when protein particles with a well-defined, narrow size distribution are required, e.g., for pulmonary protein delivery [4,5]. Supercritical fluid (SCF) drying has been advocated as an attractive alternative for freeze-drying because it avoids these limitations [5]. However, the scarce literature on SCF drying of proteins is mainly devoted to particle engineering with no or limited investigation of protein integrity or storage stability [5–8], describes the drying of pure, unformulated proteins [6–8], or reports incomplete protein stabilization [6,9]. Nesta et al. showed the possibility to obtain SCF dried immunoglobulin G (IgG), formulated with sucrose [9]. However, only one formulation was tested, there was no information on storage stability, and the authors acknowledged the need for process improvement.

Recently we compared sucrose and trehalose as stabilizers for lysozyme and myoglobin in SCF drying and showed

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the possibility to produce homogeneous protein-sugar powders with SCF drying [10]. Furthermore, we have been able to stabilize lysozyme and myoglobin (unpublished data) using a 1:4 (w/w) protein-to-excipient ratio and trehalose or hydroxypropyl-β-cyclodextrin (HP-β-CD) as excipients.

The aim of this work was to study whether the process and formulation conditions that resulted in stable myoglobin and lysozyme powders can be extrapolated to a more complex and pharmaceutically relevant protein, human serum IgG. The results demonstrate that IgG is sensitive to process-induced aggregation and therefore required formulation and process optimization to be fully stabilized.

2. Materials and methods

2.1. Materials

Polyclonal human serum IgG (lot no.: 144401001) was a kind gift from Sanquin (Amsterdam, The Netherlands). Trehalose (crystalline, dihydrate from *Saccharomyces cerevisiae*) was acquired from Sigma–Aldrich (Steinheim, Germany) and HP-β-CD was purchased from Fluka (Buchs, Switzerland). Aqueous solutions were prepared in reverse-osmosis water. Technical grade ethanol (100%) was used and carbon dioxide (grade 3.5) was purchased from Hoek Loos (Schiedam, The Netherlands). All other chemicals were obtained from different commercial suppliers and were of analytical grade, unless mentioned otherwise.

2.2. Drying of protein formulations

IgG solutions were dried in a type SFP4 (Separex, Champigneulles, France) supercritical drying apparatus as described previously [11]. IgG (final concentration 20 mg/ml) with trehalose or HP-β-CD (80 mg/ml) was dissolved in water or in buffer. As buffer, sodium phosphate (10 mM, pH 5.0 or pH 6.2), sodium citrate (10 mM, pH 6.2) or sodium acetate (10 mM, pH 5.0) was used. Protein solutions were initially sprayed into supercritical CO_2 (SC-CO_2) which contained ethanol to enhance the solubility of water in SC-CO_2 [5,12].

In the first set of experiments, the same conditions were used as reported earlier [11]: flow rates of 0.5 ml/min for protein solution, 250 g/min for the supercritical CO_2 (SC-CO_2) and 25 ml/min for the ethanol. The pressure and temperature were maintained at 100 bar and 37 °C. This set of conditions is further in the text called "conditions 1a". In the second set of experiments, variations on the standard conditions (conditions 1a) were made, as indicated in Table 1. Briefly, conditions marked as 1 and 2 included drying with ethanol, whereas during SCF drying under conditions 3, no ethanol was used. Two temperatures were tested for all conditions, 37 °C (in the supercritical region) and 20 °C (below the critical point).

After completion of the spraying process, the vessel was flushed for 16 min with SC-CO_2 before depressurization and product recovery. The dry powder was recovered from the filter on the bottom of the vessel and stored under nitrogen in air-tight containers. Part of the powders was analyzed within 3 days after drying and the rest was stored at 4 and 40 °C for 4 weeks to estimate storage stability. For the analysis of rehydrated protein, prior to analysis powders were reconstituted in water and solutions were centrifuged for 5 min at 10,000 rpm to remove undissolved material [9].

To calculate the amount of completely reconstituted protein, the protein concentrations in the solutions before centrifugation and in the corresponding supernatants were determined by the Peterson protein assay [13].

2.3. Physical properties of SCF dried powders

2.3.1. Residual water content

Samples (ca. 30 mg, accurately weighed) of the SCF dried formulations were dispersed in methanol and the residual water content of the formulations was measured by the Karl–Fischer method using a Metrohm 756 KF instrument (Metrohm, Herisau, Switzerland) as described in the manufacturer's manual. Methanol was used as a blank.

2.3.2. Scanning electron microscopy (SEM)

SEM (JSM-5400, Jeol, Peabody, USA) images were used to examine the morphology of the dried particles. Conductive double-sided tape was used to fix the particles to the specimen holder before covering them with a thin layer of gold.

2.3.3. Modulated differential scanning calorimetry (MDSC)

MDSC was performed on a Q-1000 calorimeter (TA instruments, New Castle, Delaware, USA). Dry powder samples (5–10 mg) in sealed aluminium pans were heated from 0 to 170 °C at a rate of 1 °C/min and a modulation of ±1 °C/30 s.

2.3.4. X-ray powder diffraction (XRPD)

XRPD was performed using a D8 Discover X-ray diffractometer with a general area detector diffraction system (Bruker AXS, Madison, USA). The incident Cu radiation of 1.54 Å was used with a sample-to-detector distance of 6 cm. An incident beam with a cross section of 0.5 mm was used and dried sample powders were prepared in a 0.5-mm thick holder. Background (air scattering) was measured for the same sampling time, typically 30 min, and subtracted from each measurement. Data were integrated along the χ angle intensity versus scattering angle (2θ) plots were made. Diffraction profiles were used to confirm the amorphous or crystalline state of the powders.

2.4. Protein characterization

2.4.1. FTIR

Protein structure in the solid state was studied by FTIR spectroscopy using a Bio-Rad FTS6000 FTIR spectrome-

Table 1
Process conditions used for SCF drying of IgG formulations

Process conditions	Flow rates			Temperature (°C)	Pressure (bar)
	Protein flow rate (ml/min)	Ethanol flow rate (ml/min)	CO_2 flow rate (g/min)		
Conditions 1a	0.5	25	250	37	100
Conditions 1b	0.5	25	250	20	100
Conditions 2a	0.5	25	416	37	100
Conditions 2b	0.5	25	416	20	100
Conditions 3a	0.5	0	500	37	100
Conditions 3b	0.5	0	500	20	100

ter with Win-IR Pro software, version 2.95 (Cambridge, USA). KBr pellets were prepared by mixing 5–10 mg of SCF dried formulation with approximately 150 mg of spectroscopy grade KBr and pressing the mixture into a pellet (diameter 13 mm) at a pressure of 260 MPa. The number of scans per experiment was set to 256, the scan speed to 0.16 cm/s, and the resolution to 2 cm^{-1}. The spectra were corrected for water vapour and smoothed with a 7-point Savitsky–Golay smoothing function. The second derivative spectra were smoothed using a 7-point Savitsky–Golay smoothing function and subsequently inverted. The amide I region of the spectra (1720–1600 cm^{-1}) was used for analyzing protein secondary structure. To compare spectra and to quantify changes in protein secondary structure, 2nd derivative spectra were truncated between 1720 and 1600 cm^{-1}, baseline-corrected, and area-normalized to 1.00 [14].

2.4.2. Circular dichroism (CD) and fluorescence spectroscopy

Far-UV and near-UV CD as well as fluorescence spectroscopy were used to check protein conformation. Far-UV (195–260 nm) circular dichroism (CD) spectroscopy was performed at room temperature in a 0.02-cm quartz cuvette and near-UV/Vis CD (250–320 nm) in a 1-cm quartz cuvette with a dual beam DSM 1000 CD spectrometer (On-Line Instrument Systems, Bogart, GA, USA). The subtractive double-grating monochromator was equipped with a fixed disk, holographic gratings (2400 lines/mm, blaze wavelength 230 nm) and 1.24 mm slits. Each measurement was the average of at least 5 repeated scans.

Intrinsic tryptophan fluorescence emission spectra were measured on a Fluorolog fluorimeter (Jobin Yvon–Horriba, Edison, NJ, USA). The excitation wavelength was set at 295 nm and emission scans were made from 300 to 450 nm. The excitation and emission slits were set at 5 nm. Measurements were carried out at room temperature in a quartz cuvette (1 cm path length). Concentrations of analyzed solutions were adjusted to $A_{280nm} < 0.1$. Spectra were normalized for the concentration differences.

2.4.3. UV/Vis spectroscopy

Absorption spectra between 240 and 350 nm were acquired at room temperature using a $\lambda 2$ UV/Vis spectrophotometer (Perkin-Elmer, Ueberlingen, Germany). Optical density (OD) above 320 nm was taken as an indication for the presence of aggregates.

2.4.4. Gel permeation chromatography (GPC)

A BioSep-SEC-S 3000 300 × 7.8 mm column (Phenomenex®, USA) was used for GPC analysis. The mobile phase was prepared by dissolving 17.9 g Na_2HPO_4, 1.0 g NaN_3 and 46 g NaCl in 2 l water and adjusting the pH to 7.0, and was passed through a 0.2 μm filter prior to use. The mobile phase was delivered to the column at a flow rate of 0.5 ml/min by a Waters 600 controller equipped with an autosampler (model 717, Waters). Chromatographs were recorded with an absorbance detector (Waters 2487, dual λ absorbance detector). IgG standards with concentration range 2.5–12.5 mg/ml were prepared from reference material with known concentration (IgG from Sanquin). Sample concentration was ca. 10 mg/ml and the injection volume was 20 μl. A calibration curve was made based on the area under the curve (AUC) of standards and linear regression analysis ($R^2 = 0.99987$). Monomer, dimer, aggregate and fragment contents are expressed as a percentage of total AUC.

2.4.5. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was done as described earlier [11].

3. Results and discussion

3.1. Formulation optimization

When aqueous (non-buffered) IgG solutions containing either trehalose or HP-β-CD were SCF dried following the drying conditions (marked as conditions 1a in Table 1) and formulation compositions used previously for lysozyme and myoglobin [11], the recovery of soluble protein was incomplete (see Table 2), especially for IgG formulated with HP-β-CD. Moreover, GPC showed that the soluble fraction of both non-buffered formulations contained 19–21% soluble aggregates.

One of the reasons for this incomplete recovery of SCF dried IgG formulations could be acid-instability of IgG [15,16]. Precipitation of monoclonal IgG has been reported after incubation at low pH and readjustment to neutral pH [16]. A certain pH drop (pH 3–4), depending on protein

Table 2
Recovered percentage of soluble protein for different IgG formulations dried under conditions 1a[a]

Excipient	Buffer	pH of reconstituted solutions	Percentage of dissolved protein (%)
Trehalose	Non-buffered	6.0	**92.2–92.4**[b]
	Phosphate pH 5.0	5.0	**99.5 ± 2.0**
	Phosphate pH 6.2	6.2	**100.6–103.2**[b]
HP-β-CD	Non-buffered	6.8	**61.1 ± 4.7**
	Phosphate pH 5.0	5.0	**98.1 ± 0.8**
	Phosphate pH 6.2	6.2	**98.2 ± 1.5**
	Citrate pH 6.2	6.2	**94.8–104.0**[b]
	Acetate pH 5.0	10.1	**50.9–53.0**[b]

[a] 25 ml/min ethanol, 250 g/min CO_2, 37 °C, see Table 1.
[b] Lower–upper value of two batches; for all other formulations, results are expressed as average value ± SD of three batches.

concentration, pressure and temperature in the precipitation vessel, can be expected for non-buffered solutions during the drying process [17]. To support our hypothesis, the sensitivity of IgG to low pH was tested under atmospheric conditions. IgG solutions at different pHs (2.0, 4.0, 5.0 and 6.2) were incubated for 1 h at 37 °C and UV scans were taken. None of the solutions showed an increase in scattering (OD intensity > 320 nm) (data not shown). However, after readjustment of the pHs to pH 6.2, scattering was observed for solutions incubated at pH 2.0 and pH 4.0 (see Fig. 1). These results demonstrate that pH stabilization is required during SCF drying of IgG formulations. Therefore, several buffers often used in other drying techniques were tested, whereas SCF drying conditions were kept the same (conditions 1a).

Addition of phosphate buffer helped in decreasing the percentage of insoluble protein (Table 2). No difference was seen between the effect of phosphate buffer pH 5.0 and pH 6.2. Similar results were also obtained with citrate buffer pH 5.0, indicating that the pH rather than the buffer species was responsible for the stabilizing effect. Acetate buffer failed to protect the protein in SCF drying, as acetic acid is dissolved in CO_2 and removed from the drying vessel together with CO_2 during the SCF drying, leaving an alkaline dried powder.

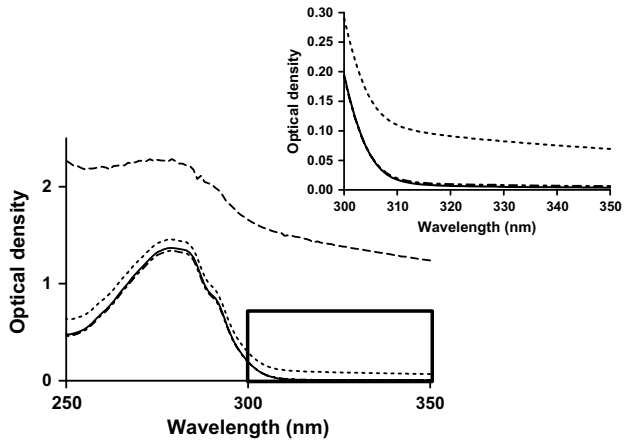

Fig. 1. UV scans of IgG solutions incubated at 37 °C for 1 h at different pHs after readjustment of pH to pH 6.2. Solid line: untreated reference solution (pH 6.2); dashed line: solution incubated at pH 2.0; dotted line: IgG solution incubated at pH 4.0; dashed–dotted line: IgG solution incubated at pH 5.0. Inset represents enlargement of 300–350 nm region.

Since phosphate buffers were shown to positively affect protein recovery, for further work only IgG formulations (with trehalose or HP-β-CD) in phosphate buffer pH 6.2 were used. These formulations were amorphous as determined by XRPD and MDSC, and contained smooth, spherical particles (Fig. 2) with comparable water contents (2.6 ± 1.1%; $n = 4$). Protein structure in the dry powders was checked by FTIR. As reported earlier [18], IgG shows two characteristic bands at 1640 and 1690 cm^{-1}, reflecting the presence of β-sheet structure. As seen from Fig. 3, the IgG formulation with HP-β-CD showed less β-sheet structure when compared to the IgG-trehalose formulation (peak at 1640 cm^{-1}), but there is an increase in the shoulder at 1653 cm^{-1}. This has not been reported as a typical peak for IgG. This region (1650–1658 cm^{-1}) is usually assigned to α-helical structure but the 1650–1655 cm^{-1} region could represent unordered structures as well [19].

The formulations were investigated further after reconstitution for preservation of the protein conformation. FTIR could not discriminate between the different reconstituted formulations (results not shown). Fig. 4a and b show the far-UV and near-UV CD spectra of the trehalose-containing and HP-β-CD-containing IgG formulations to be comparable to those of the reference IgG solution. Also fluorescence spectroscopy showed no major differences between freshly prepared and the SCF dried IgG formulations (Fig. 5). The fluorescence emission maximum was at 337–338 nm in all cases. Only small differences in signal intensity were seen, which may be due to minor conformational changes or experimental errors in the protein concentration assay. So, although some structural differences in the solid state were observed between the trehalose-containing and HP-β-CD-containing IgG formulations, this did not measurably affect the protein structure in solution. Still, although both formulations showed excellent recovery of soluble protein (Table 2) and preservation of structure in solution (Figs. 4 and 5), GPC showed that the percentage of soluble dimers and larger aggregates (oligomers) had significantly increased as compared to the

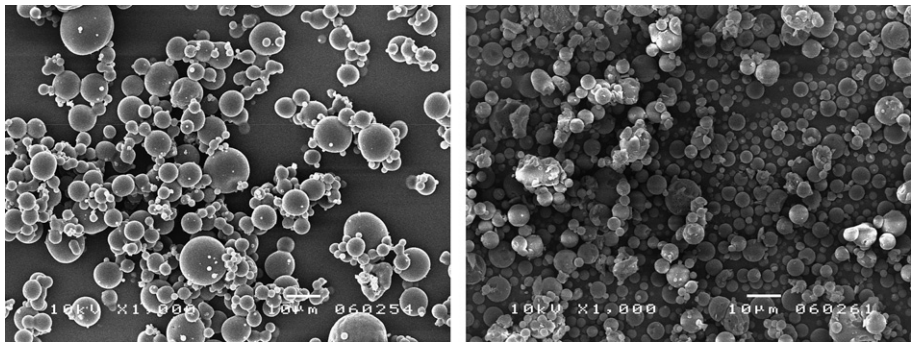

Fig. 2. SEM pictures of SCF dried IgG formulations (conditions 1a, phosphate buffer pH 6.2) with trehalose (left) and HP-β-CD (right) as excipients.

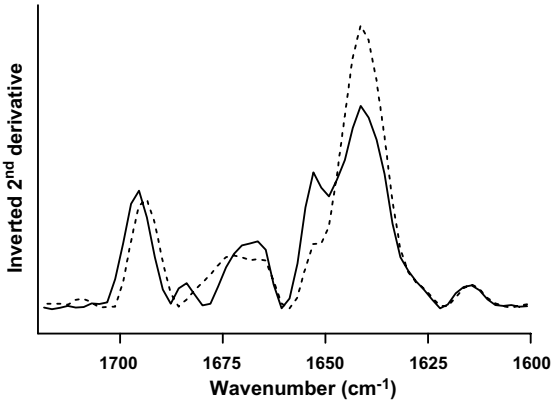

Fig. 3. Second derivative FTIR spectra of SCF dried IgG formulations (buffered with 10 mM phosphate, pH 6.2, conditions 1a). Solid line: SCF dried IgG HP-β-CD formulation; dotted line: SCF dried IgG trehalose formulation.

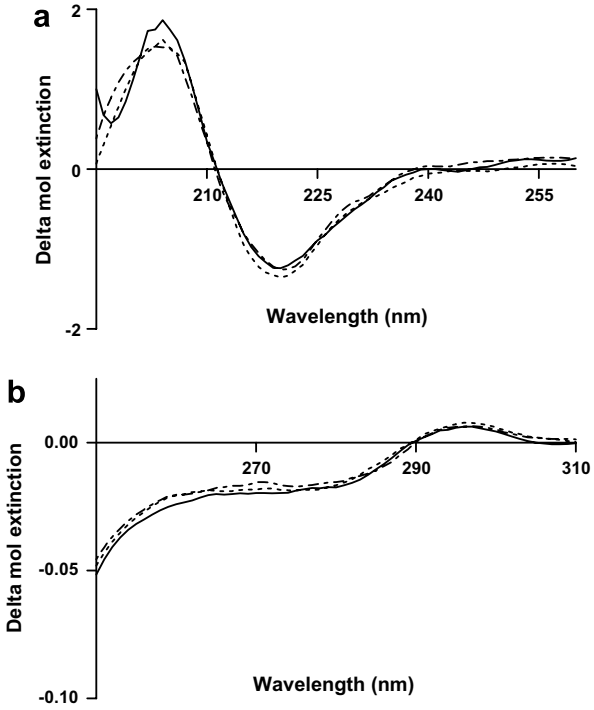

Fig. 4. CD spectra of SCF dried IgG formulations (buffered with 10 mM phosphate, pH 6.2, conditions 1a) compared to reference IgG. Solid line: reference IgG; dotted line: SCF dried IgG HP-β-CD formulation; dashed line: SCF dried IgG trehalose formulation. (a) Far-UV CD spectra (path length 0.02 cm, protein concentration 0.5 mg/ml). (b) Near-UV CD spectra (path length 1.0 cm, protein concentration 0.5 mg/ml).

starting material (Table 3, conditions 1a). The presence of aggregates was confirmed by UV spectroscopy: UV spectra of SCF dried formulations showed some scattering (OD significantly above baseline levels at wavelengths >320 nm, Fig. 6). Since SDS–PAGE did not show any difference between SCF dried formulations and reference IgG under non-reducing (single band with an apparent MW of ca. 150 kDa) and reducing conditions (double band with apparent MW of ca. 50 kDa and 25 kDa), we conclude that the aggregates were non-covalent in nature. Furthermore, the aggregates probably consisted of native like protein units, because CD and fluorescence scans did not show significant conformational changes when compared to the reference, non-dried IgG (Figs. 4 and 5), despite the high oligomer content (12–17%) seen in GPC.

3.2. Process optimization

In the previous section, we showed that the addition of a buffer to the IgG formulations prevented process-induced formation of insoluble aggregates. However, properly buffered formulations still contained soluble aggregates. The next step was to address potentially critical process parameter(s) for protein stabilization in SCF drying. Recently we showed that SCF dried lysozyme formulations contained high amounts of residual ethanol (ca. 8%) when dried under conditions 1a (manuscript in preparation). Although this did not influence lysozyme stability after drying, for larger and more complex proteins such as IgG, it can be expected that ethanol presence could have more impact on stability [20]. Another process parameter to be considered is the working temperature (37 °C). Although incubation of IgG at 37 °C for 50 min (i.e., the duration of the spraying process) did not cause aggregation (authors' observation), the combination of elevated temperature, the presence of ethanol and the spraying process might induce aggregation. Therefore, several drying conditions

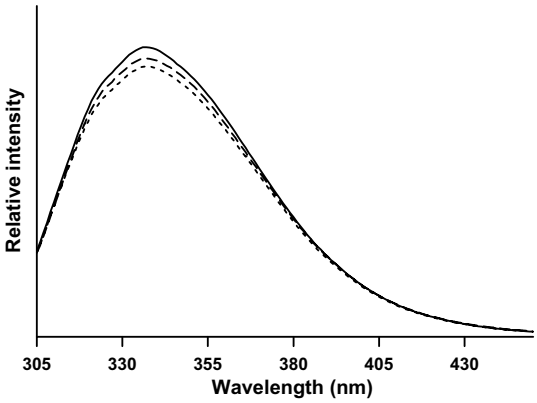

Fig. 5. Fluorescence spectra of SCF dried IgG formulations (buffered with 10 mM phosphate, pH 6.2, conditions 1a) compared to the reference IgG. Solid line: reference IgG; dotted line: SCF dried IgG HP-β-CD formulation; dashed line: SCF dried IgG trehalose formulation. Protein concentration ca. 0.05 mg/ml.

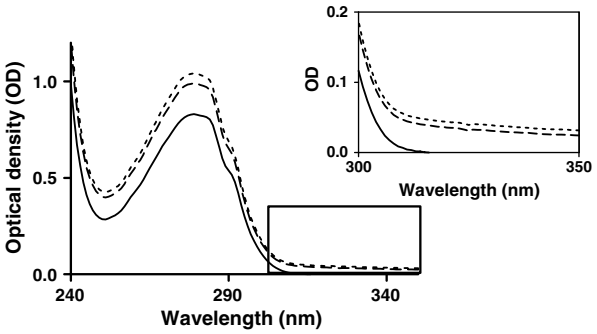

Fig. 6. UV spectra of SCF dried IgG formulations (buffered with 10 mM phosphate, pH 6.2, conditions 1a) compared to the reference IgG. Solid line: reference IgG; dotted line: SCF dried IgG HP-β-CD formulation; dashed line: SCF dried IgG trehalose formulation. Inset represents enlargement of 300–350 nm region. Protein concentration ca. 0.5 mg/ml.

were tested for the phosphate buffered (pH 6.2) IgG formulations. The solutions were dried at 37 and 20 °C, with different ethanol fractions in the SCF drying medium (see Table 1 for drying conditions).

All formulations showed complete recovery of soluble protein after reconstitution (not shown). The GPC results for the HP-β-CD-containing IgG formulations dried under the different conditions are shown in Table 3. Based on GPC, the best formulations were obtained when no ethanol was used at either 37 or 20 °C. Interestingly, ethanol has been used to improve the solubility of water into SC-CO_2 and thereby facilitate the drying process [12], but we showed that it was possible to successfully SCF dry IgG without ethanol (Table 3, conditions 3a). These formula-

Table 3
GPC analysis of the soluble fraction of the SCF dried IgG formulations[a]

IgG formulation	GPC results (% of total AUC)			
	Monomers	Dimers	Oligomers	Fragments
IgG reference[b]	94.7 ± 0.2	1.3 ± 0.5	0	4.0 ± 0.4
Freeze-dried				
HP-β-CD	90.6–90.7	3.9–5.3	0.3–0.6	3.4–5.1
Trehalose	89.9–91.1	4.4–4.6	0.6–1.0	3.2–5.1
SCF dried				
Conditions 1a (37 °C)				
HP-β-CD[b]	76.6 ± 7.0	6.4 ± 0.8	12.7 ± 7.3	4.0 ± 0.2
Trehalose[b]	70.9 ± 5.7	7.1 ± 1.1	17.1 ± 6.3	4.9 ± 0.5
Conditions 1b (20 °C)				
HP-β-CD	89.5–89.7	4.9–5.0	1.1–1.2	4.2–4.3
Trehalose	88.7–89.1	5.4–5.5	0.3–0.4	5.2–5.4
Conditions 2a (37 °C)				
HP-β-CD	66.5–67.0	6.4–6.5	22.4–22.6	4.0–4.6
Trehalose	67.0–67.1	6.5–6.6	21.6–21.7	4.6–4.8
Conditions 2b (20 °C)				
HP-β-CD	88.5–89.0	4.6–5.1	1.4–1.5	4.9–5.1
Trehalose	90.0–90.1	4.2–4.3	0.9–1.0	4.6–4.8
Conditions 3a (37 °C)				
HP-β-CD	90.7–90.9	4.7–4.8	0	4.4–4.5
Trehalose	89.7–89.8	4.5–4.6	0.4–0.5	5.2–5.3
Conditions 3b (20 °C)				
HP-β-CD	91.5–91.6	4.2–4.4	0	4.0–4.3
Trehalose	n.a.	n.a.	n.a.	n.a.

n.a., not analyzed.
[a] All IgG formulations were buffered with 10 mM phosphate, pH 6.2.
[b] Average value ± SD ($n = 3$); for all other formulations results are expressed as lower–upper limit of two repeats per formulation.

tions did not show increased aggregation (oligomer content) as compared to reference IgG (<0.5%). There was some increase in dimer content (4–5%) when compared to reference IgG (ca. 1%), whereas the content of fragments (4–5%) was unchanged. When ethanol was used at 37 °C in any ratio to CO_2, the soluble aggregate content was significantly higher (>12%; see Table 3, conditions 1a and 2a). Interestingly, when IgG solutions were dried at 20 °C (subcritical conditions), ethanol did not seem to have a severe negative effect on protein structure. In that case the amount of aggregates was ca. 1% (for conditions 1b and 2b, see Table 3).

Trehalose formulations gave similar results as HP-β-CD formulations (Table 3). Both formulations were also checked for protein conformation and no differences were seen in CD and fluorescence spectra when compared to reference IgG (data not shown).

There could be a few explanations for the less detrimental effect of ethanol on protein structure at lower temperature (20 °C) when compared to drying at 37 °C. Firstly, the protein could have been exposed to lower ethanol concentrations during drying at 20 °C. According to a modeling study by Martin et al. [21], at higher operating temperatures more ethanol and CO_2 are condensing into the aqueous droplets during particle formation than at lower temperatures. Secondly, at lower temperatures the density of SCF fluid is higher, which might result in a significant change in the course of the drying process, leading to decreased entrance of ethanol into the droplets and therefore less exposure of protein to ethanol [8]. Finally, a direct temperature effect could also be an explanation since IgG is not very stable in the presence of ethanol at ambient or elevated temperatures [20,22]. For this reason, Cohn fractionation of blood proteins is done at −5 to 0 °C [22].

Storage stability of the non-aggregated formulations (dried with conditions 3a, no ethanol in the SCF process) was estimated after 4 weeks storage at refrigerated conditions (4 °C) and elevated temperature (40 °C). No change in solubility or increase in amount of aggregation was seen for the HP-β-CD-containing and trehalose-containing formulations stored under these conditions (Fig. 7a and b). Moreover, CD and fluorescence spectra showed no evidence of conformational changes of the IgG for the stored formulations (results not shown).

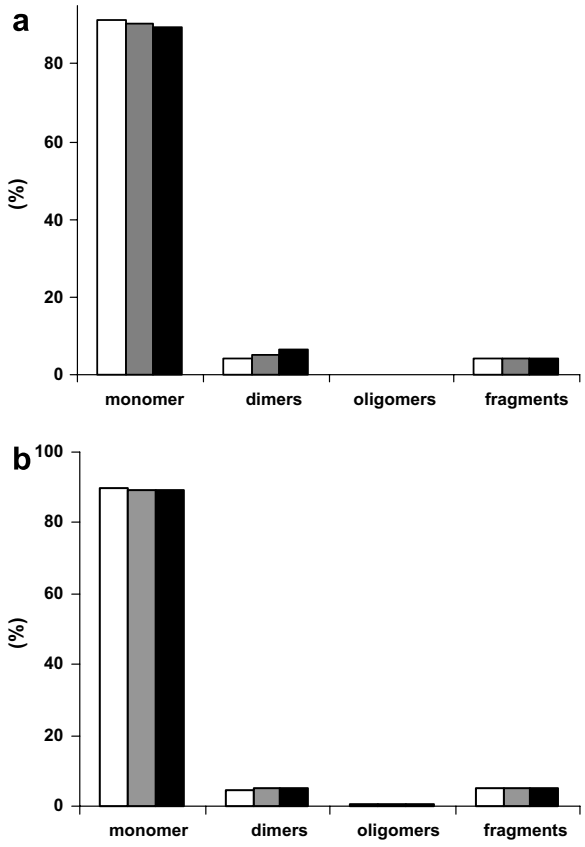

Fig. 7. GPC results of SCF dried IgG formulations containing (a) HP-β-CD or (b) trehalose (conditions 3a): freshly prepared (white bars), stored for 4 weeks at 4 °C (grey bars) or 40 °C (black bars). *Y*-axis represents percentage of total AUC.

4. Conclusions

In conclusion, the results of the present study show that stable, aggregate-free IgG formulations can be obtained by SCF drying. For SCF drying of IgG formulated with trehalose and HP-β-CD, usage of a buffer and avoidance of ethanol turned out to be critical for protein stabilization. Like for freeze-drying, for SCF drying some general guidelines should be followed, but this is clearly not sufficient: the process and the formulation should be adjusted to the properties of the protein. General recommendations for protein stabilization by SCF drying include the use of buffers, stabilizers such as carbohydrates, avoidance of the use of ethanol, and/or subcritical drying at lower temperatures. When following these guidelines and selecting appropriate process/formulation parameters, SCF (or subcritical) drying can be a valuable process for making stable microparticulate protein powders.

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