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Research paper

Stabilization of IgG1 in spray-dried powders for inhalation

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Abstract

The protein stabilizing capabilities of spray-dried IgG1/mannitol formulations were evaluated. The storage stability was tested at different residual moisture levels prepared by vacuum-drying or equilibration prior to storage. Vacuum-drying at 32 °C/0.1 mbar for 24 h reduced the moisture level below 1%, constituting an optimal basis for improved storage stability. The crystalline IgG1/mannitol powders with a weight ratio of 20/80 up to 40/60 failed to prevent the antibody aggregation as assessed by size exclusion chromatography during storage. Ratios of 60/40 up to 80/20 IgG1/mannitol provided superior stability of the antibody and the powders could be produced with high yields. The lower the residual moisture, the better was the stabilizing capability. An amount of 20% mannitol provided the best stabilization. Storage stability of 60/40, 70/30, and 80/20 IgG1/mannitol formulations over one year was adequate at 2–8 °C and 25 °C. Closed storage (sealed in vials) at 40 °C/75% RH and open storage at 25 °C/60% RH revealed that the stability still required optimization. The lower the protein content, the better was the powder flowability. The aerodynamic properties of powders spray-dried with 10% solids content were inadequate, as the particle size ranged between 5.1 and 7.2 μm and the fine particle fraction accounted for only 4–11%. Reduction of the solids content to 2.5% did improve the aerodynamic properties as the mass mean aerodynamic diameter was reduced to 3.6 μm and the fine particle fraction was increased to about 14%. The reduction of the solids content did not influence the storage stability significantly. Also spray-drying at higher temperatures had no significant impact on the storage stability, despite a higher tendency to form amorphous systems. In order to improve the storage stability and to maintain the good flowability of 70/30 IgG1/mannitol powder or to keep the storage stability but to improve the flowability of the 80/20 IgG1/mannitol powder, mannitol was partially substituted by a second excipient such as trehalose, sucrose, glycine, lactose, lactosucrose, or dextran 1. Differences in the stabilizing capability were noticeable upon closed storage at 40 °C/75% RH and open powder storage. Protein stabilization was improved by the addition of glycine but trehalose and sucrose were most effective in preventing aggregation, which can be primarily attributed to the water replacement properties of the sugars. The addition of another excipient, isoleucine had positive effects on both flowability and protein stability.

Keywords: Spray-drying; Antibody; Powder inhalation; Protein stability

1. Introduction

For many years, the development of non-parenteral routes for the administration of proteins and peptides has been promoted. The most promising non-parenteral route seems to be the aerosol delivery [1–3]. Recombinant human deoxyribonuclease (rhDNase) has been the first protein for inhalative therapy [4]. Inhalation delivery systems for the pulmonary delivery of biomolecules encompass nebulizer [5], pressurized metered dose inhalers [6], and dry powder inhalers [7,8]. The use of dry powder inhalers has a couple of advantages because the device can be designed to be small and relatively inexpensive, and no propellants are required [4]. Furthermore, the proteins may be stabilized in the dried state by the addition of glass-forming stabilizers such as trehalose or sucrose. Proteins in liquid formulations are generally at a greater risk of chemical and physical degradation [9,10].

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The inhalative application can be used for a local treatment of pulmonary diseases such as asthma or for the systemic therapy with biomolecules as the lung epithelium is adequately permeable and accessible [11–13]. In the area of systemic therapy, insulin was the first protein approved for inhalation therapy (Exubera®), but has recently been taken off the market due to low sales [14,15]. In the case of antibodies there is a special interest in the local treatment of chronic lung diseases such as asthma and chronic obstructive bronchitis, since the potential of antibodies in the treatment of chronic inflammation has been demonstrated, e.g. for Infliximab and numerous monoclonal antibodies [16–18]. The development of dry powder formulations for inhalation of this IgE-antibody was addressed in various publications [19–23]. Yet, Fahy et al. revealed that the inhalation of nebulized anti-IgE was inefficient due to the inability to reach high concentration levels in the tissue compartments [24].

Spray-drying is frequently used for preparing protein powders for inhalation, because the spray-drying process can be designed in order to produce protein containing powders of high stability [2,7,13,19–21]. The achieved particles have a suitable particle size, in the range of 1–7 μm, as well as an appropriate density and shape for inhalation and deposition in the lungs [25,26]. The goal of this study was to develop a stable formulation for an IgG1 based on mannitol as stabilizing excipient as used in the literature [19–23]. The powders also had to provide good yield of approx. 70% or more, adequate inhalative properties as well as good flowability to enable dispensing into unit doses. The effect of the residual moisture in the powders was to be evaluated and different pre-treatments as well as storage scenarios were considered. Promising candidates had to be studied in further details and an additional optimization by a second excipient was to be evaluated.

2. Materials and methods

2.1. Materials

A humanized monoclonal antibody (IgG1) was provided by Boehringer Ingelheim Pharma GmbH & Co. KG. The initial aqueous immunoglobulin solution was dialyzed to a solution containing 95.0–100.0 mg/ml antibody, 1.6 mM glycine, and 25 mM histidine. The pH value was adjusted to 6.3. The antibody solution contained between 0.3% and 0.9% of aggregates after diafiltration. During the storage at 2–8 °C the stability (formation of aggregates) of the antibody solution was tested in regular periods by SE-HPLC. The immunoglobulin solution was stored at 2–8 °C and was stable for at least 2 years. Mannitol was obtained from Caesar & Lorentz GmbH (Hildem, Germany), Sucrose from Sigma (Steinheim, Germany), L-Isoleucin and Trehalose from Fluka (Taufkirchen, Germany), and Lactosucrose from Hayashibara Shoji Inc. (Okoyama, Japan).

2.2. Methods

2.2.1. Preparation of solutions for spray-drying

The stabilizing excipients were dissolved in demineralized water (pH ~7.5) and added to the antibody solution to obtain the desired ratio for spray-drying. The total solid content (ts) of the spray solutions was 10% or 2.5% (w/v).

2.2.2. Spray-drying

Spray-drying was performed with a Büchi Mini Spray Dryer B – 290 (Büchi Labortechnik, Flawil, Switzerland) at controlled ambient conditions of 25 °C/50% RH. For the spray-drying process the inlet and outlet temperatures (T_{in}/T_{out}) were kept at either 90/50 °C or 130/75 °C. The drying air volumetric flow rate was set at 600 l/min, the liquid feed flow rate at 3 ml/min, and the atomizing air volumetric flow rate at 670 l/min. The resulting aspirator flow rate was 35 m^3/h. The spray solutions (50–200 ml) were atomized with a water-cooled two-fluid nozzle (0.7 mm liquid orifice internal diameter) using compressed air from the in-house supply. After spray-drying, the powders were collected through a high-efficiency cyclone in a glass container, transferred in a glass vial, and stored in a desiccator with a relative humidity of 20% at ambient temperature. Formulations were sprayed only once due to the enormous quantities of protein required.

2.2.3. Vacuum-drying

For vacuum-drying of the powders, a vacuum dryer VTS-2 (Memmert, Schwabach, Germany) at 0.1 mbar and 32 °C for 24 h was used (drying procedure: vd1). Subsequently, the vials were closed by a rubber stopper under nitrogen and sealed in aluminium bags. Another portion of the powder was vacuum-dried at 20–25 °C and 300 mbar for 3 days according to Maa et al. [22] (drying procedure: vd2), and the vials were closed under ambient conditions without nitrogen degassing and sealed in aluminium bags.

2.2.4. Size exclusion high-performance liquid chromatography (SE-HPLC)

Size exclusion chromatography was used to determine the amount of soluble protein aggregates in the spray solutions and in the spray-dried powders after dissolution. Analysis was performed on a Hewlett–Packard HP 1090 or a HP 1100 chromatograph (Agilent Technology, Waldbronn, Germany) with a TSK3000SWXL column (300 × 7.8 mm, Tosoh Biosep, Stuttgart, Germany) and UV-detection at 280 nm ($n = 3$). The mobile phase consisted of 0.1 M disodium hydrogen phosphate dihydrate and 0.1 M sodium sulfate and was adjusted with orthophosphoric acid 85% to pH 6.8. The flow rate was 0.5 ml/min and the injection volume was 25 μl with a pro-

tein concentration of about 2–10 mg/ml. The chromatogram showed three separate peaks. For integration the HP Chemstation Version 9 from Agilent was used. Samples were analyzed in triplicate (SD not given in figures and tables for clarity reasons).

2.2.5. Differential scanning calorimetry (DSC)

DSC experiments were performed with 8–12 mg of powder sealed in closed aluminium pans (DSC 204 Phoenix, Netzsch, Selb, Germany). Samples were cooled to −20 °C, heated to 100 °C, again cooled to −20 °C and reheated to 140 °C at 10 K/min. The glass transition temperature (T_g) was determined as the midpoint of the phase transition ($n = 2$).

2.2.6. Modulated differential scanning calorimetry (MDSC)

Modulated differential scanning calorimetry was performed with 8–12 mg of powder sealed in closed aluminium pans (DSC 822e Mettler Toledo, Giessen, Germany). The measurements were recorded with an amplitude of ±3 K, a period of 42 s, and a heating rate of 1 K/min during heating from −20 °C to 150 °C. The glass transition temperature (T_g) was determined as the midpoint of the phase transition ($n = 2$).

2.2.7. Scanning electron microscopy (SEM)

The particle morphology was determined with a scanning electron microscope (DSM 962 or SUPRA 55VP, Zeiss, Oberkochen, Germany). Samples were fixed with self-adhesive carbon tape on aluminium stubs and sputtered with gold at 20 mA/5 kV for 90 s.

2.2.8. X-ray powder diffraction (XRD)

Powders were investigated with a X-ray diffractometer (XRD 3000 TT, Seifert, Ahrensberg, Germany) at a scanning rate of 0.05° (2°θ) from 5° to 40° (Cu-Kα radiation $\lambda = 0.15418$ nm, 40 kV, 30 mA). For relative humidity controlled experiments a X'Pert Pro MPD (Panalytical, Kassel, Germany) in combination with a temperature and humidity chamber was used.

2.2.9. Time-of-flight measurement (TOF) measurements

The aerodynamic properties of the powders were determined based on time-of-flight by using the Aerodynamic Particle Sizer®APS 3321 (TSI Inc., MN, USA) with an impactor inlet. According to USP 25 4 l air with a pressure drop of 4 kPa was drawn through the device. The powder was emitted at 39 l/min for 6.15 s. The baffle plate was coated with Brij® 35 solution in order to prevent the bouncing-off of particles. After the measurement the capsules were weighed in order to determine the emitted dose, with reference to the initial powder content in the capsule prior to the measurement ($n = 2$).

2.2.10. Andersen Cascade Impactor (ACI) analysis

Selected powders were analyzed for the mass median aerodynamic particle size and the fine particle fraction with an Andersen Cascade Impactor (1 ACFM Eight-Stage Non-Viable Sampler Series 20-800, Thermo Andersen, Smyrna, GA, USA). The eight-stage device with cut-off diameters of 9.9 (pre-separator), 9.0, 5.8, 4.7, 3.3, 2.0, 1.0, 0.7, 0.4, and 0.22 μm (filter) was operated at 39.3 l/min with emission of 12–16 mg powder filled in hard gelatine capsules inserted in the Handihaler® over 6.15 s. A total of 4 l air was passed through the impactor at a pressure drop of 4 kPa and an absolute pressure ratio of $P2/P3 \leqslant 0.5$. The amount of deposited material on each stage was determined by weighing the individual stages on a balance with 0.01 mg accuracy.

2.2.11. Dynamic vapour sorption (DVS) measurements

About 50 mg powder was weighed on the Dynamic vapour sorption apparatus (DVS 2/2085, Porotech, Hofheim, Germany) and exposed to a continuous flow (200 ml/min) of carrier gas (dry air) from 0% to 90% RH at 10% RH increments at 25 °C.

2.2.12. Karl-Fischer titration

The moisture content of powders was determined by Karl-Fischer titration (KF 373, Metrohm GmbH & Co., Filderstadt, Germany) using Hydranal TM Composite as a titration reagent. The powders were dissolved in 1.0 ml anhydrous methanol inside a nitrogen-filled glove box and centrifuged for 5 min before injecting 500 μl into the titration cell. In addition, a Karl-Fischer titrator Aqua 40,00 with a head-space module oven was used (Analytik Jena AG, Jena, Germany), heating the powders up to 80 °C ($n = 3$).

2.2.13. Determination of the powder flowability

The powder flowability was evaluated macroscopically by comparison to standard powders with a defined flowability expressed as flow index value (F_i) (Table 1). The flow index value was determined with a Powder Flow Analyzer (Micro Stable Systems, Godalming, United Kingdom) based on the measurement of energy profiles. For these measurements, the standard powder samples were pre-consolidated by moving the blade twice through the powder at a velocity of 1.74 mm/s downwards with −41.5 rpm clockwise rotation, and the AUC of the force vs. distance curve was determined as the blade was moved upwards at 1.74 mm/s at 41.5 rpm counterclockwise rotating. Six measurements were performed in a row and F_i mean and standard deviation calculated.

2.2.14. Storage stability

For closed storage the powders were pre-treated by vacuum-drying (see Section 2.2.3) or equilibrated at 22 °C/50% RH for 24 h (eq). These pre-treated samples were transferred into glass vials, stoppered, sealed in aluminium bags, stored at 2–8 °C, 25 °C/60% RH, or 40 °C/75% RH, and analyzed after 1, 4, 15, and 52 weeks. For open storage the powders were exposed to 25 °C/30% RH or 25 °C/60%

Table 1
Reference substances with defined flowability for the evaluation of the flowability of spray-dried powders (F_i determined with the powder flow analyzer)

Reference number for the determination of the flowability	Substance	F_i
1	Tablettose®	9.2
2	Pharmatose DCL11®	12.7
3	Compactrol®	13.0
4	Pharmatose DCL40®	15.2
5	Starch 1500	21.8
6	Mannitol/HSA, spray-dried	26.6
7	Corn starch, dried	36.5
8	Lactose/HSA, spray-dried	38.1
9	Mannitol, spray-dried	44.2
10	Lactose, fine	55.3
11	Lactose, spray-dried	121.4

RH in open glass vials, adjusted by supersaturated salt solutions. Samples were taken after 1, 4, 15, and 25 weeks.

3. Results and discussion

3.1. Characteristics of antibody 1/mannitol formulations after spray-drying

Spray-drying was performed at T_{in}/T_{out} 90/50 °C to achieve adequate product recovery and stability. Since the analysis of antibody/mannitol solutions revealed ratio-dependent aggregation, understanding IgG1 aggregation in the dry state formed an important part of the study [27–29]. For the IgG1/mannitol powders 30/70, 40/60, 60/40, and 70/30 the product recovery was slightly below 70% (Table 2). The formulation 50/50 IgG1/mannitol showed a pronounced decrease in yield down to 1% which can be attributed to powder sticking to the walls of the drying chamber and the cyclone, as the "sticky point" [30] is exceeded by the spray-drying temperature. X-ray diffractograms demonstrated that powders up to a ratio of 70/30 IgG1/mannitol contained crystalline mannitol, mainly the δ-polymorph (Table 2). With increasing protein amount, the amorphous mannitol fraction increased and a completely amorphous formulation was obtained at the ratio 80/20. Crystallization is more favourable at higher initial mannitol concentrations and as critical supersaturation is reached early in the evaporation process as the crystals have time to grow during the process [31].

Table 2
Properties of IgG1/mannitol powders 20/80–100/0 after spray-drying at T_{in}/T_{out} 90/50 °C

IgG1/mannitol	Yield (%)	Mannitol crystallinity	Residual moisture (%)	Aggregates in spray solutions (%)	Aggregates in spray-dried powders (%)
20/80	84	δ, α	3.0	2.6	8.3
30/70	69	δ	4.9	4.1	5.1
40/60	69	β, δ	5.0	3.0	5.0
50/50	1	Not determinable due to insufficient yield			
60/40	67	δ	5.4	1.1	1.1
70/30	67	δ	7.0	0.8	0.8
80/20	87	Amorphous	6.7	0.8	0.8
100/0	90	Amorphous	9.1	0.8	2.7

Conventional DSC scans showed no thermal events between −10 and 100 °C for the powders, which is partially due to the strong internal heterogeneity of protein molecules [32]. Pure proteins typically have a high glass transition temperature (~100–200 °C), whereas amorphous mannitol is characterized by a T_g of 13 °C in the dry state [33]. But also recrystallization events of amorphous mannitol could not be assigned. Maury could not detect a T_g for an amorphous IgG/sorbitol powder (70/30), but assumed a T_g of ⩽10 °C, as dry sorbitol displays a T_g of −2 °C [34,35]. Modulated DSC (MDSC) provides an opportunity to determine the T_g more clearly and accurately [36,37]. For 80/20 IgG1/mannitol only marginal glass transition and mannitol recrystallization events could be detected (Fig. 1a). In comparison, a 70/30 IgG1/trehalose powder showed a clear T_g at 48 °C and crystallization (Fig. 1b).

A mannitol content of 60–80% induced high amounts of aggregates already in the solutions prior to spray-drying (Table 2). The drying process promoted further monomer loss up to 8.3% (Table 2). The stability of the pure antibody was also negatively affected by spray-drying and aggregates ascended from 0.8% to 2.7%. In contrast, the addition of 20–

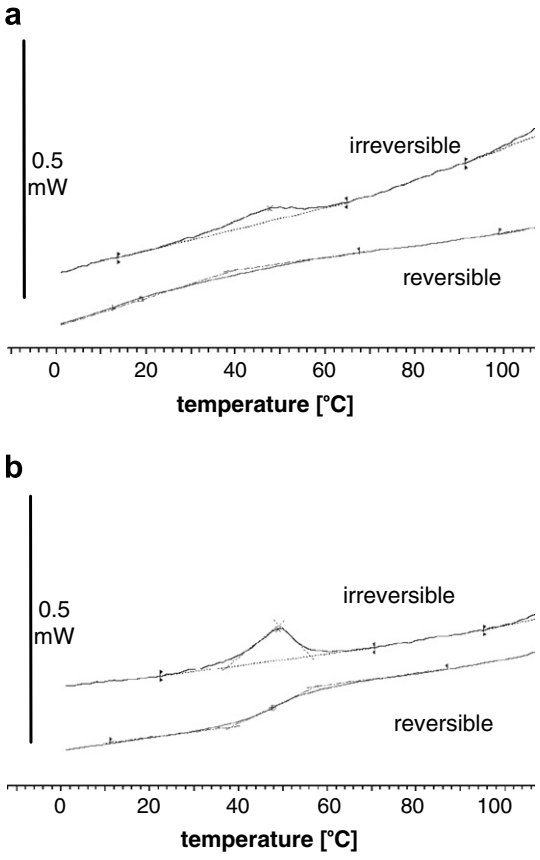

Fig. 1. Determination of the T_g with MDSC of (a) IgG1/mannitol 80/20 and (b) IgG1/trehalose 70/30 spray-dried at T_{in}/T_{out} 90/50 °C.

40% mannitol provided good protection during spray-drying. The antibody formulations exhibited advanced stability compared to the aggregate formation of 4–5% up to 17.3% for a different IgG1 spray-dried with mannitol or sorbitol [19,35]. Possible reasons for this difference compared to the literature may be the process parameters, such as atomizing air-flow rate and temperature, the stability of the antibody molecule itself, or the formulation conditions such as pH, ionic strength, or the quality of the starting material. In order to test whether the spray-drying temperature was responsible for the differences in aggregation, as claimed by Maury [35], 130/75 °C conditions were applied. These higher temperatures induced only a marginal change (Table 3). Even spray-drying of the pure antibody at T_{in}/T_{out} of 170–185/95 °C led to only 3.7% aggregates, as shown by Fuhrherr [38]. Consequently, the spray-drying temperature has only a minor influence on the IgG1 aggregation. Higher spray-drying temperatures were claimed to reduce the product recovery [30], which is not the case for the prepared IgG1/mannitol powders (Table 3). The residual moisture was clearly dependent on the protein content and ranged from 3.0% to 9.1% for the powders spray-dried at T_{in}/T_{out} 90/50 °C (Table 2). However, a reduction of the residual moisture by 0.5–1.5% was found at T_{in}/T_{out} of 130/75 °C (Table 3), which is in overall agreement with previous reports [39–41]. The reduced moisture content may be advantageous for the protein stability during storage and the protein stability may additionally be improved by using higher spray-drying temperatures, as the formation of amorphous powders is promoted [23]. The formulation IgG1/mannitol 70/30, only partially amorphous at T_{in}/T_{out} of 90/50 °C, showed no reflections in the X-ray diffractograms that referred to crystalline mannitol when spray-dried at 130/75 °C. But the system is highly prone to recrystallization as can be seen in X-ray diffractograms recorded at different relative humidities at 25 °C which demonstrated mannitol cystallization as δ-polymorph already at 20% RH. This could entail reduced protein stability due to a lack of stabilization in the glassy matrix [42–44]. The crystalline structure did not change further upon exposure to higher humidity as described for spray-dried salmon calcitonin/mannitol powder 70/30 [31].

Table 3
Properties of IgG1/mannitol powders 60/40–100/0 after spray-drying at T_{in}/T_{out} 130/75 °C

IgG1/mannitol	Yield (%)	Mannitol crystallinity	Residual moisture (%)	Aggregates (%)
60/40	72	δ	4.9	1.4
70/30	84	Amorphous	5.6	1.0
80/20	75	Amorphous	6.2	1.0
100/0	76	Amorphous	8.2	2.6

SEM pictures of 60/40 IgG1/mannitol spray-dried at T_{in}/T_{out} of 90/50 °C and 130/75 °C displayed donut-like particles with crystalline appearing structures on the surface (not shown). The 70/30 powders also exhibited a donut-like shape of the particles. In contrast to the powder spray-dried at 130/75 °C, the powder obtained at 90/50 °C showed a few structures, reminiscent of crystalline material (Fig. 2a and b) which corresponds to the crystalline mannitol detected by XRD. The 80/20 IgG1/mannitol

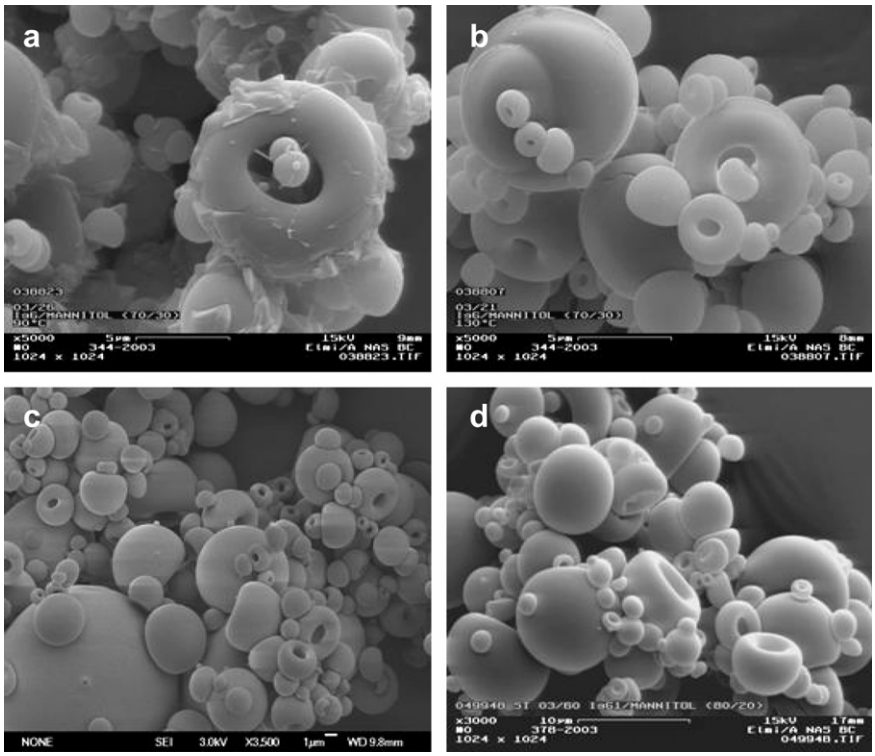

Fig. 2. SEM pictures of IgG1/mannitol (a) 70/30 spray-dried at T_{in}/T_{out} 90/50 °C, (b) 70/30 spray-dried at T_{in}/T_{out} 130/75 °C, (c) 80/20 spray-dried at T_{in}/T_{out} 90/50 °C, (d) 80/20 spray-dried at T_{in}/T_{out} 130/75 °C.

formulations did not reveal temperature related differences in the SEM (Fig. 2c and d). Powders with a mannitol content of 60–80% showed superior flowability compared to the powders with 20–30% mannitol with the formulation 60/40 showing the best flowability (Table 4). This might be due to the crystalline structures on the surface acting as spacers reducing particle interactions. This might also explain the better flowability of the 70/30 powder spray-dried at T_{in}/T_{out} 90/50 °C as compared to spray-dried at higher temperature or the 80/20 IgG1/mannitol powders.

Via the drying rate, T_{out} can affect the particle shape, the particle size, and ultimately the aerodynamic properties [45]. However, the mass mean aerodynamic diameter (MMAD) of the powder 60/40 IgG1/mannitol was not affected by the spray-drying temperature (7.2–7.4 μm) (Table 4). With increasing protein content the particle size decreased. Also the emitted dose which is directly related to the flowability of the powders is affected as the more cohesive formulation 80/20 IgG1/mannitol revealed the lowest emitted dose (<70%) (Table 4). For inhalation, the aerodynamic particle size should range between 2 and 5 μm [1,46] and to achieve a therapeutic effect, high doses of immunoglobulin have to be delivered [47]. The overall high MMAD was reflected in a low fine particle fraction (FPF) of only 5–10% (Table 4). In order to reduce the MMAD and to rise the FPF, the atomizing air-flow rate can be increased, the liquid feed be reduced or the total solid content of the spray solutions can be lowered [48]. As higher atomizing air-flow rates cause an increase in air–liquid interface and can foster protein aggregation [49,50] a lower total solid content was tested. At 2.5% total solids all powders from 70/30 up to 100/0 IgG1/mannitol were amorphous, and yield, residual moisture and aggregate level were comparable to that of the 10% total solid samples. In contrast, the MMAD decreased to an appropriate size of 3–4 μm [47] and the FPF increased slightly to 14%. The emitted dose remained unaffected.

3.2. Storage stability of IgG1/mannitol powders

Besides antibody stabilization during the spray-drying process, adequate storage stability is of importance. The powders were pre-treated prior to storage by vacuum-drying either for 1 day at 32 °C/0.1 mbar (vd1) or for 3 days at 25 °C/200 mbar (vd2) to decrease the residual moisture content [51,22]. Alternatively, the powders were equilibrated (eq) for 24 h at controlled elevated humidity conditions of 22 °C/50% RH.

Table 4
Properties of IgG1/mannitol powders 60/40–100/0 spray-dried at T_{in}/T_{out} 90/50 °C and 130/75 °C

IgG1/ mannitol	T_{in}/T_{out} (°C)	MMAD (μm)	Flowability	ED (%)	FPF (%)
60/40	90/50	7.2	6.5	85.0	4.3
	130/75	7.4	6.5	85.2	7.3
70/30	90/50	6.1	7.0	78.5	9.1
	130/75	6.3	7.5	80.8	9.8
80/20	90/50	5.1	10	69.5	11.0
	130/75	5.5	10	66.2	10.1

3.2.1. Storage stability of pre-treated IgG1/mannitol powders

Analysis of the influence of mannitol on the antibody stability in solution [27] and after spray-drying (see Section 3.1) demonstrated that high amounts of mannitol promoted IgG1 aggregation. The vd1 process resulted in a pronounced increase in aggregates' levels in 30/70 and 40/60 IgG1/mannitol powders, whereas water removal exerted only a minor effect on the other spray-dried formulations (Fig. 3). The moisture content decreased below 1% for all formulations providing a good basis for improving the storage stability. The monomer loss was further enhanced after utilizing vd2 (Fig. 3). The residual moisture varied between 2% and 5%. Equilibration for 24 h at 50% RH resulted in 8.1–10.4% water content and 60/40 IgG1/mannitol powders formed 3.2% aggregates, whereas the formulations 70/30 and 80/20 stayed below 2% (Fig. 3). Furthermore, equilibration promoted mannitol crystallization especially in the 60% and 70% mannitol-containing powders as could be shown by XRD. Hageman postulated that a water content of 6–8% corresponds to a monolayer covering highly active sorption sites of the protein and that further hydration induces denaturation [52]. Although the samples were sealed in aluminium bags, vacuum-dried samples took up moisture over 52 weeks (vd1: 1.1–2.7%; vd2: 3.2–5.5% for the powders 20/80 – 40/60 and to 7.5–10.3% for the powders 60/40–100/0 IgG1/mannitol), whereas the equilibrated samples lost some water (eq: 7.5–9%).

The study demonstrated that the pure antibody formed 5.0% aggregates at 2–8 °C and 10.3% at 25 °C/60% RH, respectively, after vd1 (Table 5). After vd2 the IgG1 showed slightly reduced storage stability at 2–8 °C and 25 °C/60% RH. But after vd2 and storage at 40 °C/75% RH the amount of aggregates was doubled (30.2%) compared to the vd1-treated material. Upon storage the pow-

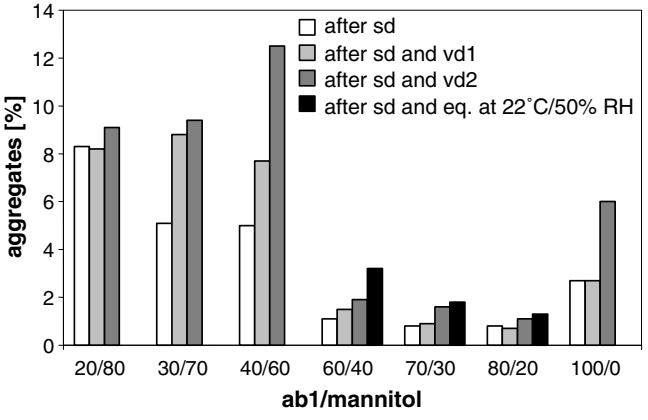

Fig. 3. Influence of pre-treatment on the aggregation of IgG1/mannitol powders 20/80–100/0 spray-dried at T_{in}/T_{out} of 90/50 °C, ts 10%.

Table 5
Aggregates (%) in IgG1/mannitol powders after spray-drying at T_{in}/T_{out} of 90/50 °C and after 52 weeks storage at 2–8 °C, 25 °C/60% RH, and 40 °C/75% RH

IgG1/ mannitol	Pre-treatment	52 weeks, 2–8 °C	52 weeks, 25 °C/ 60% RH	52 weeks, 40 °C/75% RH
20/80	Vd1	11.9	13.3	18.0
	Vd2	12.9	21.8	35.8
30/70	Vd1	12.2	11.7	19.6
	Vd2	11.8	19.5	31.4
40/60	Vd1	8.0	7.7	17.6
	Vd2	8.7	10.7	27.1
60/40	Vd1	1.8	3.6	10.1
	Vd2	2.9	4.9	21.4
	Equ.	4.4	14.2	28.0
70/30	Vd1	1.8	3.1	10.5
	Vd2	2.9	5.3	22.2
	Equ.	3.1	12.8	31.8
80/20	Vd1	1.9	2.8	10.0
	Vd2	1.9	3.5	18.0
	Equ.	1.9	9.1	26.1
100/0	Vd1	5.0	10.3	15.0
	Vd2	6.6	12.9	30.2

ders with 60–80% mannitol formed high aggregate levels which increased with higher storage temperature and residual moisture. This can be explained by the damage of the antibody that is already in the spray solutions leading to further rise in aggregates during storage, which might be enhanced by the partially crystalline nature of the mannitol in these powders [34]. Furthermore, water absorption in the crystalline regions is mainly confined to the surface [53] and the water uptake in these partially crystalline powders especially takes place in the amorphous phase further enhancing denaturation of the unprotected antibody.

As seen in protein stability in solution and process stability formulations with a ratio of 60/40–80/20 IgG1/mannitol provided superior storage stability (Table 5). Costantino et al. found a similar effect for recombinant human growth hormone [54]. The aggregate levels stayed below 2% after 52 weeks storage at 2–8 °C. Although the formulations 60/40 and 70/30 revealed mannitol crystallinity – consistent over 52 weeks – they provided similar antibody stabilization after vd1 as the completely amorphous 80/20 powder. Upon storage at 25 and 40 °C the amount of aggregates increased up to about 3% and 10%, respectively, for the powders with 20–40% mannitol due to the increased storage temperature [12].

After vd2 stability was reduced as compared to samples pre-treated by vd1 as molecular mobility is increased [55,56]. The amorphous phase of the powder containing 80% IgG1 showed a superior stabilizing potency compared to the other formulations (Table 5). After 1 year only 1.9% aggregates were measured at 2–8 °C, 3.5% at 25 °C/60% RH, and 18.0% at 40 °C/75%. There was only marginal difference in protein aggregation detectable for the formulations 60/40 and 70/30 IgG1/mannitol. Upon storage at 40 °C none of the powders inhibited aggregate formation to an acceptable degree.

After 1 year storage at 2–8 °C the aggregate level of the formulation 80/20 equilibrated at 50% RH was still <2% despite a water content of 9%. But with increasing storage temperature the stability was drastically declined. In the formulations 60/40 and 70/30 IgG1/mannitol, the higher residual moisture content led to 14.2% and 12.8%, respectively, at 25 °C/60% RH and 28.0% and 31.8%, respectively, at 40 °C/75% RH.

3.2.2. Storage stability of powders spray-dried at T_{in}/T_{out} of 130/75 °C

As already discussed, higher spray-drying temperatures promote the formation of amorphous mannitol which is considered to be beneficial for protein stability [20,50]. However, storage stability of the 70/30 and 80/20 IgG1/mannitol powders spray-dried at T_{in}/T_{out} of 90/50 at 25 °C was not further improved by spray-drying at 130/75 °C due to the low overall moisture content despite differences in mannitol crystallinity and residual moisture

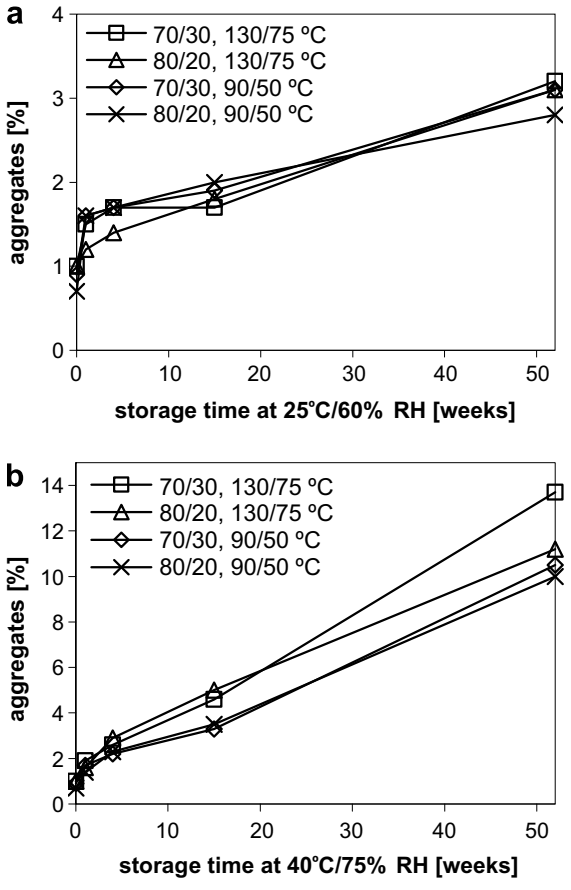

Fig. 4. Comparison of the stability of IgG1/mannitol formulations 70/30 and 80/20 spray-dried at T_{in}/T_{out} of 90/50 °C and 130/75 °C and treated by vd1 during 52 weeks closed storage at (a) 25 °C/60% RH and (b) 40 °C/75% RH.

(Fig. 4a). At 40 °C/75% RH the monomer loss reached 10–15% after 52 weeks (Fig. 4b). The powders 80/20 IgG1/mannitol spray-dried at different temperatures were comparable. Within the formulations 70/30 the difference accounted for 3%. The effect might be traced back to the higher residual moisture of the powder 70/30 IgG1/mannitol spray-dried at T_{in}/T_{out} of 130/75 °C at the beginning of the storage, but over the course of time the residual moisture converged. Another potential explanation is that the thermal stress could have damaged the protein during the process, but did not result in immediate aggregation after spray-drying [57]. Using spectroscopic techniques the protein secondary structure can be determined [58,59]. Based on infrared spectroscopy and circular dichroism, it was found that the overall secondary protein structure of antibodies is basically not affected [27,28].

3.2.3. Storage stability of IgG1/mannitol powders at 25 °C/30% RH and 25 °C/60% RH open storage

Vd1 proved to be an excellent method to reduce the moisture content and to improve the storage stability. However during manufacturing, processes such as filling the antibody powders would be prone to moisture absorption. The relative humidity of the environment can typically be controlled at 30% RH at which the powder would gain 6–8% moisture as can be seen from the absorption isotherm recorded with DVS (Fig. 5). Consequently, powders with 2.5% and 10% total solids prepared at T_{in}/T_{out} of 130/75 °C were tested for their storage stability without previous vacuum-drying at higher residual moisture (25 °C/30% RH and 25 °C/60% RH). After 25 weeks of open storage at 25 °C/30% RH only 3.5–4.5% aggregates were found in the 70/30 and 80/20 IgG1/mannitol powders (Fig. 6a). The residual moisture content was adjusted to 5.1–5.3% for the powders 80/20 IgG1/mannitol and to 4.2–4.3% for the formulations 70/30. The latter showed mannitol crystallization in contrast to 80/20, explaining the different moisture levels. Nevertheless, protein-stabilizing efficiency was comparable. At 25 °C/60% RH the residual moisture content was adjusted to about 10% for all powders. The 80/20 IgG1/mannitol powders and the 70/30 spray-dried with 2.5% total solids formed about 15% aggregates (Fig. 6b). However, the formulation 70/30 spray-dried with 10% total solids revealed slightly higher aggregation levels of 18%. This difference might be due to a different course of drying or varying microstructures [31].

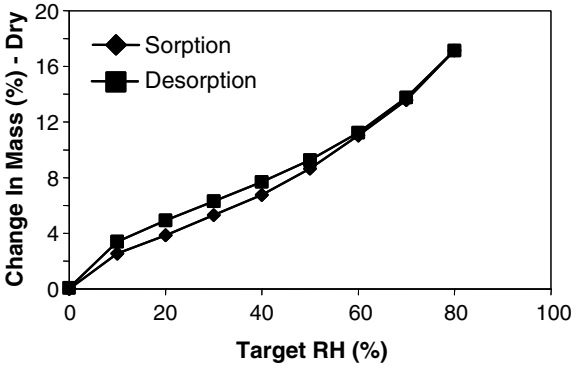

Fig. 5. Water absorption isotherm of the formulation 70/30 IgG1/mannitol spray-dried at T_{in}/T_{out} of 130/75 °C.

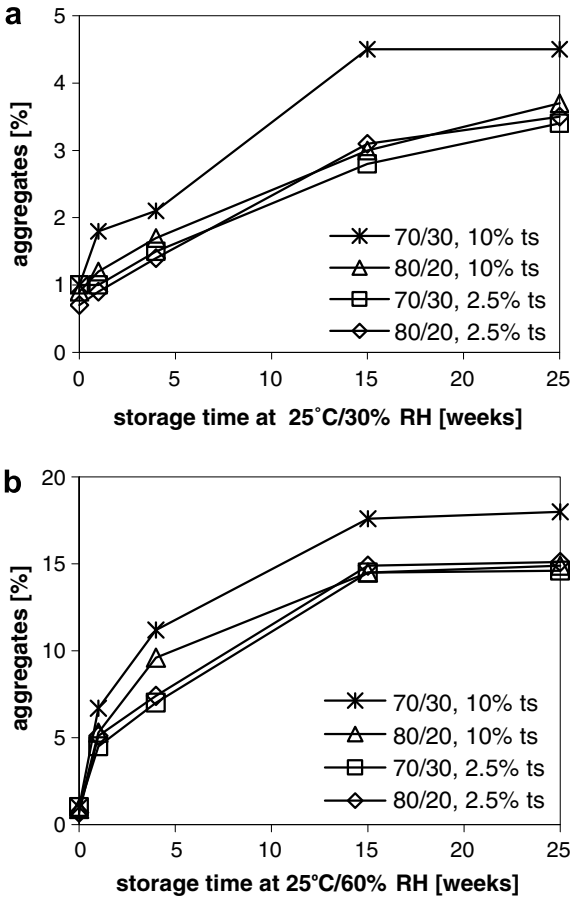

Fig. 6. Comparison of the stability of IgG1/mannitol powders 70/30 and 80/20 spray-dried at T_{in}/T_{out} of 130/75 °C with a ts of 2.5% and 10% during 25 weeks storage at (a) 25 °C/30% RH and (b) 25 °C/60% RH.

3.3. Variations based on the 70/30 IgG1/mannitol formulation

The previous storage stability tests revealed that the IgG1/mannitol powders were effective in stabilizing the antibody at low residual moisture contents and at moderate storage conditions. But at higher residual moisture level and open storage at 60% RH the antibody stability is insufficient, and makes further improvement necessary. Two approaches were pursued by the addition of a second stabilizer, either enhancing the stability of 70/30 IgG1/mannitol powder or improving the flowability of 80/20 IgG1/mannitol material. Mannitol powders with 30% mannitol displayed the tendency to crystallize during spray-drying or storage which results in reduced stabilizing capabilities. Mannitol crystallization can be inhibited by the presence of proteins or other excipients [60]. To ensure long-term stability the powders should be stored well below their T_g [36]. But amorphous mannitol powders display a very

low T_g and therefore the addition of excipients with a high T_g appears appropriate.

Trehalose being fully amorphous after spray-drying [61] is suitable, due to its high glass transition temperature of 120 °C in the dry state [62], its good water replacement character, and its chemical inertness [35]. Its major disadvantage is the formation of fused and sticky agglomerates [57,50], resulting in powders with poor flowability. Another well-known stabilizing excipient is sucrose, which appears to be even more effective in inhibiting protein unfolding than trehalose through excellent water replacement [63]. However, sucrose is rather hygroscopic [57], which can negatively affect protein stability. As further secondary excipients lactose, glycine, lactosucrose, and dextran 1 were utilized. Lactose was an obvious candidate, because it is usually employed as a carrier for preparing powder blends with micronized drugs for inhalation [64] and was applied as stabilizing spray-drying excipient [19,65]. However, at relative humidities >17%, moisture uptake can lead to lactose crystallization [66] and lactose is as a reducing sugar and can therefore induce protein glycation [19,63,64]. Glycine was chosen as second excipient, since it protected rhIL-11 in combination with trehalose during spray-drying and storage [50] and in combination with mannitol stabilized lyophilized human growth hormone [67]. Another promising excipient is lactosucrose, a non-reducing trisaccharide, built of fructose, glucose, and galactose. It was first applied by Fuhrherr for the spray-drying and showed promising protein stabilization at IgG concentrations of 10% [38]. Dextran 1 was applied due to reported stabilizing effects of [38,68,69].

3.3.1. Physicochemical characteristics of variations based on the 70/30 IgG1/mannitol

The effect of a second major excipient on the different powder characteristics such as yield, glass transition temperature, protein stability, and residual moisture is summarized in Table 6. Overall, the recovery was in the range of 75–80% and for only a few preparations below 70%. All formulations prevented antibody aggregation during spray-drying. Only the lactosucrose formulations were slightly less effective in stabilizing the protein during the process. All powders apart from the glycine formulations were amorphous and T_g values were detectable with standard DSC. The X-ray reflections of IgG1/mannitol/glycine formulations indicated the presence of stable γ-glycine and δ-mannitol for the ratios 70/20/10 and 70/25/5 [70]. The powder 70/15/15 revealed reflections of γ-glycine. But a high amount of amorphous phase existed, since T_g values were measurable for the glycine formulations. The partially crystalline structure of the glycine powders was associated with a slightly lower residual moisture level (4.2–5.0%) as compared to the other powders. The residual moisture of the remaining powders ranged from 5.0% to 6.5%. Addition of a second excipient caused a significant increase of the T_g. Apart from the glycine powder 70/25/5 with a T_g of 14.2 °C, which correlates approximately with the T_g values of the IgG1/mannitol powders, the T_g ranged from 35.2 to 61.0 °C. Typically, the T_g increased with an increasing amount of second excipient. Furthermore, the ratio of mannitol to second excipient and the residual moisture level influenced the T_g. Interestingly, the exchange of trehalose against sucrose induced an increase in the T_g by 4–10 °C

Table 6
Characteristics of IgG1/mannitol/second excipient powders spray-dried at T_{in}/T_{out} of 130/75°, 10% ts (nd, not determinable)

IgG1/mannitol/	Ratio	Yield (%)	T_g (°C)	Residual moisture (%)	Aggregates (%)	MMAD (μm)	Flowability	ED (%)	FPF (%)
–	70/30/0	80.0	nd	5.6	1.0	6.3	7.5	80.8	10.1
Trehalose	70/15/15	78.0	40.7	5.6	1.2	5.0	11	64.0	11.4
	70/20/10	78.0	35.2	5.8	1.1	5.8	10	82.8	12.2
	70/25/5	77.0	36.4	6.5	1.4	5.1	10	74.2	11.7
Sucrose	70/15/15	64.0	50.3	5.3	1.0	4.2	9	65.5	11.9
	70/20/10	74.0	41.5	5.5	1.2	4.4	8	61.6	15.8
	70/25/5	76.0	39.1	5.6	1.0	5.5	8	89.6	12.8
Lactose	70/15/15	80.0	55.9	6.4	1.0	5.2	9	51.6	12.5
	70/20/10	82.0	54.6	5.7	1.3	4.7	8	70.4	10.2
	70/25/5	78.0	48.6	5.3	1.0	4.6	8	76.6	11.1
Glycine	70/15/15	66.0	53.8	5.0	1.3	5.2	7	84.1	12.2
	70/20/10	64.0	39.3	4.2	1.2	6.5	6.5	95.0	13.4
	70/25/5	65.0	14.1	4.8	1.2	6.3	6.5	86.4	14.6
Lactosucrose	70/15/15	70.0	61.0	5.1	1.6	4.9	8	77.0	14.2
	70/20/10	74.0	54.2	5.9	1.5	5.1	8	72.9	13.5
	70/25/5	76.0	50.4	5.5	1.6	5.9	7	86.6	13.7
Dextran 1	70/15/15	76.0	62.4	5.3	1.1	5.2	7	74.7	14.4
	70/20/10	82.0	58.6	5.8	1.3	6.5	7	69.7	13.1
	70/25/5	89.0	41.0	5.1	1.1	6.3	7	85.9	10.9

for the ratios 70/15/15 and 70/20/10 IgG1/mannitol/sucrose. Fitzner observed an increase in T_g with the exchange of trehalose against sucrose in protein formulations as well [50]. At first sight, trehalose powders were supposed to provide a higher T_g than sucrose due to a higher T_g of the pure amorphous substance. As the T_g correlates reciprocally with the residual moisture, difference in the T_g might be attributed to the lower moisture level of the sucrose powders compared to the trehalose powders. The different powders with trehalose, sucrose, glycine, lactosucrose, and dextran 1 revealed all a moisture uptake of 7–8% upon exposure to about 40% RH in DVS. Upon exposure to 50–70% RH, the investigated powders showed a weight loss indicating mannitol crystallization. Fig. 7 shows the DVS isotherm of IgG1/mannitol/trehalose formulation 70/20/10 as an example. The powders absorbed about 13% water upon increasing to 60% RH and at 70% RH showed a distinct weight loss indicating mannitol crystallization [66]. Crystallization was confirmed by XRD recorded at different relative humidities. The water absorption isotherm of the IgG1/mannitol/sucrose formulation 70/20/10 revealed as well a moisture uptake with increasing relative humidity. At approximately 50% RH crystallization occurred which was confirmed by XRD. As already supposed the glycine powders contained a large portion of amorphous phase, despite crystalline reflections as shown by XRD. The amorphous phase could be detected by DVS which recorded weight loss reflecting crystallization upon exposure to 60% RH.

Microscopy of the spray-dried powders revealed, apart from the glycine-containing formulations, smooth particle surfaces. In agreement to the literature, during the drying process the particles underwent indentation and received a donut-shaped appearance [50,38,21]. The MMAD of the powders ranged from 4.2 to 6.5 μm, except for a larger MMAD found for the dextran- and glycine-containing powders (Table 6). These differences might be due to particle shape, agglomerates, and different aerodynamic behaviour. Overall, the diameter appeared to tend towards slightly smaller values than that of the IgG1/mannitol formulation 70/30 (6.3 μm). The emitted dose was overall similar with 70–90%. Only formulations with poor flowability comprising sucrose, trehalose, and lactose, revealed an emitted dose of below 70%. The fine particle fraction of the second excipient-containing powders ranged from 10% to 16%, which was slightly better than that of the IgG1/mannitol powder 70/30, but should be further increased by the reduction of the total solid. The flowability of the powders lies within a narrow range, but differences were noticeable. The glycine- and dextran 1-containing powders exhibited a slightly improved flowability. In contrast, sucrose, trehalose, and lactose powders showed worse flowability related to the formation of sticky and cohesive aggregates.

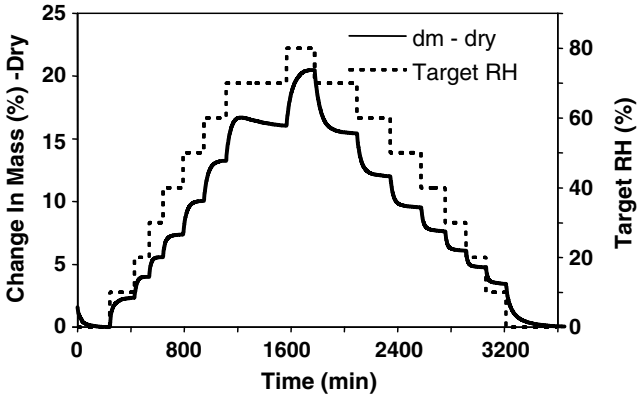

Fig. 7. Absorption isotherm of IgG1/mannitol/trehalose powder 70/20/10 spray-dried at 130/75 °C.

3.3.2. Storage stability of antibody 1/mannitol/second excipient powders based on the 70/30 IgG1/mannitol

Our goal was to preserve the good flowability and to improve the storage stability of the formulation 70/30 IgG1/mannitol. Consequently, the effect of the added second excipient on storage stability was tested. Vd1 reduced the residual moisture to about 1.8–3% which was higher than that for the IgG1/mannitol powders. After storage for 52 weeks at 2–8 °C, no significant differences in aggregation were found for the different powders (Table 7), as crystallization was prevented and the storage temperature was below T_g. Storage at 25 °C/60% RH and at 40 °C/75% RH revealed strong variation in the stabilizing efficiency of the second excipients. Dextran 1 provided the least protein stability and exerted a clear destabilizing effect at 40 °C storage. Fuhrherr already mentioned that dextran 1 amount <40% (m/m) failed to prevent protein aggregation, because it is less effective in hydrogen bonding [38]. The lactose powders revealed as well poor stability at 40 °C. Moreover, they displayed a brown discoloration indicating Maillard reactions [54]. Insufficient stabilization was also provided by lactosucrose. Up to 20% aggregates after 52 weeks at 40 °C/75% RH were measured. Fuhrherr found stabilization at low antibody levels by lactosucrose, but with a higher protein content the amount of aggregates also increased [38]. In contrast, formulations with glycine showed better stability at 40 °C, although the powder contained already crystalline glycine and mannitol structures after spray-drying. The amount of aggregates was slightly lower than the amount of aggregates formed in the IgG1/mannitol powder 70/30 (13.7%). The most stable protein formulations were achieved by the addition of sucrose and trehalose. Especially the powders 70/20/10, 70/25/5 IgG1/mannitol/trehalose, and 70/15/15 IgG1/mannitol/sucrose enhanced the IgG1 stability at 40 °C compared to the formulation 70/30. The stabilizing effect seems not to be related to a high T_g, since the storage temperature was close to T_g and other excipients like dextran 1 provided higher T_g values. Therefore, the differences in protein stabilization can be rather ascribed to distinct interactions of the IgG1 with the excipients by water replacement, which is provided best by trehalose and sucrose.

Table 7
Aggregates (%) in IgG1/mannitol/second excipient powders spray-dried at T_{in}/T_{out} of 130/75 °C and pre-treated by vd1 after 52 weeks closed storage at 2–8 °C, 25 °C/60% RH, and 40 °C/75% RH and after 25 weeks open storage at 25 °C/30% and 60% RH

IgG1/mannitol/	Ratio	2–8 °C	25 °C/30% RH	25 °C/60% RH	25 °C/60% RH	40 °C/75% RH
–	70/30/0	1.9	4.5	18.0	3.2	13.7
Trehalose	70/15/15	1.7	2.6	7.7	3.1	10.5
	70/20/10	1.7	3.1	8.2	2.4	7.8
	70/25/5	2.0	3.6	11.6	2.8	8.0
Sucrose	70/15/15	1.8	2.8	6.5	2.6	8.8
	70/20/10	1.8	3.0	10.9	2.6	11.1
	70/25/5	1.8	3.3	10.7	2.7	10.2
Lactose	70/15/15	1.8	5.1	28.5	3.1	29.8
	70/20/10	2.1	8.0	30.8	2.9	45.0
	70/25/5	2.0	9.5	30.0	3.0	59.2
Glycine	70/15/15	2.0	4.3	16.1	3.0	10.5
	70/20/10	1.8	3.8	15.6	2.7	9.0
	70/25/5	1.7	3.5	15.0	2.8	7.3
Lactosucrose	70/15/15	1.8	3.2	21.6	3.0	31.3
	70/20/10	2.1	3.8	22.0	3.2	28.2
	70/25/5	2.0	4.4	20.6	3.1	27.5
Dextran 1	70/15/15	1.9	9.0	55.3	3.7	48.8
	70/20/10	1.8	4.6	45.5	3.6	47.5
	70/25/5	2.0	6.7	37.3	3.5	45.2

Upon open storage at 25 °C/30% all antibody formulations comprising a secondary compound showed crystallization of mannitol within 15 weeks. Crystallization was detected for the formulations 70/20/10, 70/25/5 IgG1/mannitol/sucrose, and trehalose as well as for dextran- and glycine-containing formulations. The lactose and lactosucrose containing powders remained amorphous. The residual moisture levels of all powders were comparable (4.0–5.5% after 25 weeks). The aggregate levels remained below 4% for all formulations apart from the powders 70/15/15 IgG1/mannitol/glycine and 70/25/5 IgG1/mannitol/lactosucrose, and the dextran 1-containing powders (Table 7). In comparison, the IgG1/mannitol 70/30 powder showed a monomer loss of 4.5%. The ratios 70/15/15 IgG1/mannitol/sucrose and trehalose provided the best stability with less than 3% aggregates. Both remained amorphous upon storage over 15 weeks. Although lactose- and lactosucrose-containing powders did not reveal mannitol crystallization, they did not provide an improved stability. As some of the trehalose- and sucrose-containing formulations showed mannitol crystallization during storage, but a better stability than lactose, lactosucrose, glycine, and dextran1, the results again point to water replacement as the reason for enhanced protein stability.

At 60% RH the dextran 1- and lactose-containing powders failed to stabilize the antibody and about 20–55% aggregates were formed, corresponding to the results after storage at 40 °C/75% RH (Table 7). Both excipients led to a brown discoloration of the powders upon 25 weeks storage. In contrast, sucrose- and trehalose-containing powders provided superior inhibition of IgG1 aggregation and only 6.5–11.6% aggregates were measured. At 60% RH all powders showed mannitol crystallization. The improved stability consequently seems to derive mostly from good water replacement provided by sucrose and trehalose. This is supported by the fact that the stability decreased from formulations with 15% sucrose or trehalose to powders with only 5%. Thus, the formulations 70/20/10 IgG1/mannitol/trehalose and 70/15/15 IgG1/mannitol/sucrose provided the best storage stability during 52 weeks at 40 °C/75% RH. But these sucrose- and trehalose-containing powders revealed worse flowability compared to the glycine- and dextran 1-containing powders.

3.4. Variations based on the 80/20 IgG1/mannitol formulation

The second approach to achieve the optimal formulation with both adequate stability and good flow properties was to improve the flowability based on the formulation 80/20 IgG1/mannitol by the addition of a hydrophobic amino acid. Amino acids are well known as excipients in protein formulations for providing additional stability in the liquid and the solid state [71]. The addition of amino acids such as leucine as a carrier material has been proven to ameliorate flowability and aerodynamic performance of a salbutamol sulphate-containing dry powder [72], which can be attributed to the hydrophobic alkyl side chain of leucine. The improvement of the aerodynamic properties upon the addition of leucine is also reflected by a change in the particle morphology [73,74]. In our group, isoleucine as similar hydrophobic amino acid has shown a significant improvement of the powder flowability of 10% protein containing formulations [38]. Consequently, an isoleucine content of 6.6–10% was added to the 80/20 IgG1/mannitol formulation and the solutions were spray-dried with a total

solid content of 10% at T_{in}/T_{out} of 130/75 °C. The 80/20-based powders exhibited a yield in the range of 70–80% with a residual moisture of approx. 5%, respectively. The addition of isoleucine had a positive effect on the glass transition temperature which increased from about 18 °C to over 45 °C. This suggests that the protein stability especially during storage might be improved. The process stability of the antibody 1 was not affected by the addition of isoleucine. Isoleucine provided a significant improvement of the aerodynamic parameters. Emitted dose and fine particle fraction increased to approximately 90% and 20%, respectively. The effect arises from the surface enrichment of the hydrophobic amino acid and the change of the particle morphology [38]. Furthermore, the flowability was significantly improved for the isoleucine-containing powders from 10 to 6. Powders with 80% antibody and 20% mannitol showed spherical, donut-like morphology with a smooth surface. In contrast, the formulations 80/13.3/6.6 and 80/10/10 IgG1/mannitol/isoleucine displayed spherical and donut-shaped particles with structured surfaces. Isoleucine did neither positively nor negatively affect the antibody stability during storage at 30% RH for 25 weeks (Fig. 8). Considering the 25 weeks storage at 60% RH, isoleucine provided even improved IgG1 stability compared to the pure mannitol powder. In contrast to the formulation

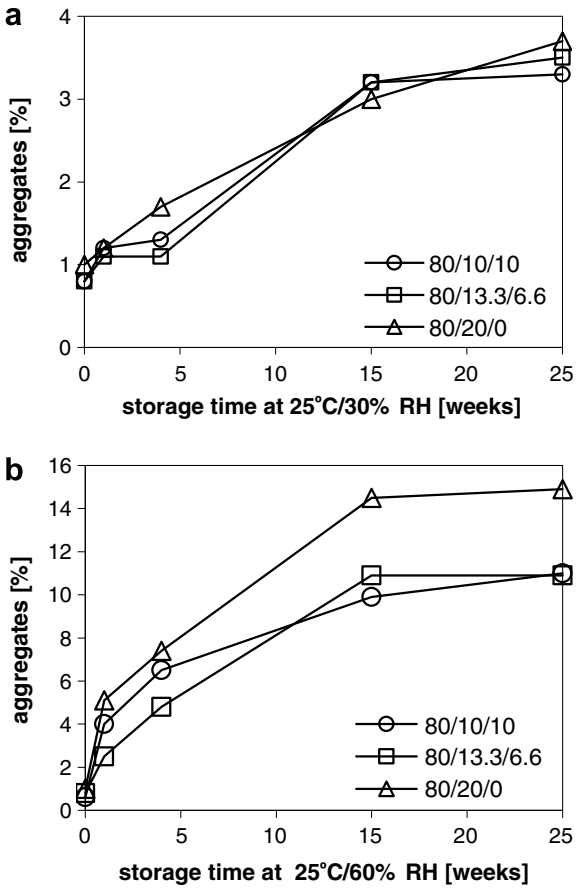

Fig. 8. Stability of the IgG1/mannitol/isoleucine formulations 80/10/10 and 80/13.3/6.6 during 25 weeks storage at (a) 25 °C/30% RH and (b) 25 °C/60% RH.

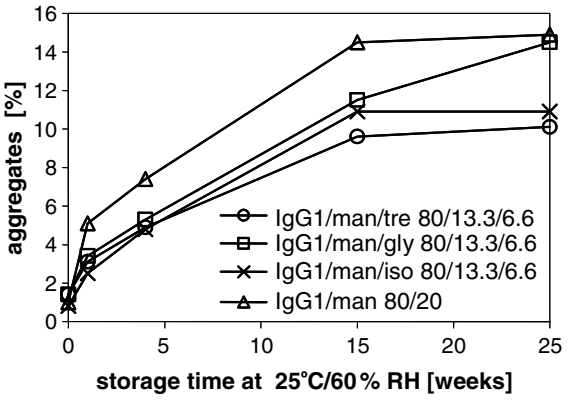

Fig. 9. Stability of IgG1/mannitol/(glycine, trehalose, or isoleucine) formulations 80/13.3/6.6 spray-dried at T_{in}/T_{out} of 130/75 °C during 25 weeks storage at 25 °C/60%.

80/20 IgG1/mannitol, which formed crystalline mannitol, the isoleucine-containing powders remained amorphous over the storage time at 60% RH.

Overall, the addition of isoleucine was suitable to improve the aerodynamic and the flow properties of the formulation 80/20 IgG1/mannitol and provided additionally enhanced antibody stability. For a better understanding of the reason for the superior stability, the formulations 80/13.3/6.6 IgG1/mannitol/glycine or trehalose were spray-dried. The trehalose-containing formulations formed cohesive and sticky powder particles with low yield. In contrast, spray-drying of glycine-containing formulations led to a yield of 74%. The residual moisture level was 5.5% and 6.1%, respectively and both formulations stabilized the antibody during the process with only 1.4% aggregates formed. During storage at 25 °C/30% RH no differences in the stabilizing potency between the three formulations became apparent. Upon storage at 25 °C/60% RH, the addition of glycine did not improve the storage stability compared to the pure mannitol formulation (Fig. 9). In contrast, the addition of trehalose or isoleucine turned out to be beneficial since the aggregate formation was only approx. 10% after 25 weeks. The mannitol crystallization was suppressed in all three powders. Despite maintaining an amorphous matrix, glycine could not provide improved stability compared to the formulation 80/20 IgG1/mannitol. Consequently, stabilization by water replacement is the major mechanism which is best provided by trehalose. But also isoleucine seems to provide a beneficial interaction with the protein and additionally results in strongly improved flow properties.

4. Conclusions

In conclusion, the reduction of the residual moisture content in IgG1/mannitol formulations by vacuum-drying at 32 °C/0.1 mbar provided an excellent method to significantly increase the storage stability of spray-dried powders [51]. Especially the formulations 60/40, 70/30 and 80/20

IgG1/mannitol showed a very good inhibition of aggregate formation upon storage for 52 weeks at 2–8 °C and 25 °C based on aggregate formation analyzed by SEC. Overall, the stability of the antibody compositions depended strongly on the residual moisture, the storage temperature, and the relative humidity, which influenced the protein stability by changing the molecular mobility of the system [56]. With higher mannitol content, the flowability of the powders improved markedly. Adequate particle size for inhalation can be achieved with 2.5% total solid in the spray solution. Excipient combinations can provide an additional improvement in stability and flow properties and indicated that water replacement is a major factor in protein stabilization compared to storage below T_g. Trehalose and sucrose proved to be very efficient. Isoleucin presents a very good alternative as additional excipient for combinations as it significantly improves aerodynamic and flow properties but it also leads to antibody stabilization.

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