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Rapid Communication

Significant Drying Time Reduction Using Microwave-Assisted Freeze-Drying for a Monoclonal Antibody

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ABSTRACT

Microwave-assisted freeze-drying (MFD) is a rapid drying process well known in food technology. However, little is known about its application to biologicals. In this study, we investigated the applicability and feasibility of this technology to different monoclonal antibody formulations and the influence on the resulting product properties. Moreover, one of our main objectives was to study if significant reductions in drying times could be achieved. In addition, the effect of the drying process on the accelerated stability of a sucrose-based antibody formulation at 40°C and 25°C over 12 weeks was investigated. MFD resulted in drying time reduction >75%. For all model formulations, cake appearance and solid state properties were found to be comparable to standard lyophilized products. These formulations covered a wider range of lyophilization excipients comprising sucrose and trehalose, semi-crystalline forming solids like mannitol:sucrose mixtures and others like arginine phosphate and a mixture of 2-hydroxypropyl-β-cyclodextrin with sucrose. Moreover, comparable low changes in relative monomer content, the relative amount of soluble aggregates and cumulative particles ≥1 μm per mL were observed over 12 weeks of storage, regardless of the drying technology. This makes MFD a promising innovative alternative for the rapid production of freeze-dried biologicals while maintaining product quality.

Introduction

Long process times are a typical shortcoming of conventional freeze-drying (CFD).[1,2] One approach to reduce drying time is microwave-assisted freeze-drying (MFD), which is a well-known process in food technology for high-value goods needing significantly shorter process times while maintaining overall quality of the product, that is, color, taste, texture, and shape.[3-5] Especially in the field of food processing, microwave (MW) radiation has versatile applications like cooking, drying, or preservation of food products.[6] Owing to its ability to allow for a rapid heat transfer and because of the volumetric and selective heating of dielectric material, it has major advantages over other conventional drying techniques. In contrast to heating via convection or conduction, MWs as electromagnetic waves directly interact with dielectric materials such as permanent dipoles, for example, water or disaccharides, or ions, for example, buffer salts.[7] In particular, drying at the typical industrial frequency of 2.45 GHz is mainly driven by the interaction of permanent dipoles and MWs.[8] The capability of a material to absorb MWs and thereby to convert electric field energy into thermal energy by molecular interactions with the electromagnetic field is characterized by the frequency-depending absolute complex permittivity.[9] More detailed information on physical principles of MW heating can be found elsewhere.[6,7,9,10] As typical lyophilization formulations used for biologicals are based on aqueous systems containing polar stabilizers, for example, sucrose or trehalose, buffer salts and the active pharmaceutical ingredient,[11] suitability for a microwave-assisted drying process is likely. Therefore, MFD also raised interest in pharmaceutical applications recently. As reported by Robert Evans[12] at the CPPR conference in 2014, microwave-assisted drying could be applied to both

Abbreviations used: CFD, conventional freeze-drying; HPW, highly purified water; HP-β-CD, 2-Hydroxypropyl-β-cyclodextrin; mAb, monoclonal antibody; MFD, microwave-assisted freeze-drying; MW, microwave; rM, residual moisture content; HP-SEC, high-performance size exclusion chromatography; SSA, specific surface area; XRD, X-ray diffraction.

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monoclonal antibody (mAb) and vaccine formulations. In preliminary data, they found similar aggregation rates as obtained by high-performance size exclusion chromatography (HP-SEC) for 2 monoclonal antibodies and no severe loss in antigen potency for different vaccine formulations. Based on that, an international patent is pending, claiming the formulation and production of thermostable dried vaccine formulations using MW vacuum drying.[13] The inventors claim for shortened drying processes by MFD. However, high sugar concentrations of 17.5% (w/w) up to 60% (w/w) in combination with vaccines were investigated. Therefore, our aim is to have a closer look on the applicability of MFD to various pharmaceutically relevant formulations of an mAb with a different experimental setup. In this article, we show the successful implementation of MFD for excipient concentrations of 10% (w/V) or 1% (w/V). In addition, a main focus will be on maintenance of product quality while shortening the drying time significantly. Moreover, we study the accelerated stability of a sucrose-based IgG antibody formulation over 3 months either produced by CFD or MFD.

Materials and Methods

Materials

A monoclonal IgG type 1 antibody was kindly provided by Boehringer Ingelheim Pharma GmbH & Co. KG (Ingelheim am Rhein, Germany).

D(+)-Sucrose and L-Arginine were purchased from Sigma-Aldrich (Steinheim, Germany). D(+)-Trehalose dihydrate and D(−)-Mannitol were obtained from VWR International BVBA (Leuven, Belgium). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) (Cavasol® W7 HP; Wacker Chemie AG, Burghausen, Germany) was a kind gift of PARI GmbH (Starnberg, Germany). L-Histidine monohydrochloride monohydrate and L-Histidine were purchased from Alfa Aesar (Karlsruhe, Germany). Di-sodium hydrogen phosphate dihydrate and sodium dihydrogen phosphate dihydrate were obtained from AppliChem (Darmstadt, Germany). Sodium chloride was purchased from Bernd Kraft (Duisburg, Germany). Tween 80®, ortho-Phosphoric acid, and sodium hydroxide were obtained from Merck KGaA (Darmstadt, Germany). For the preparation of buffers and stock solutions, highly purified water (HPW; Purelab Plus; USF Elga, Germany) was used.

All excipients had at least analytical grade and were used without further purification.

Preparation of Formulations

The mAb was concentrated prior to dialysis by using Vivaspin 20 with polyethersulfone membrane (molecular weight cutoff 30,000 Da; Sartorius AG, Goettingen, Germany) and then subsequently dialyzed for 24 h using dialysis membranes Spectra/Por® (molecular weight cutoff 6000-8000 Da; Spectrum Laboratories Inc., Compton, CA). After dialysis, concentration of mAb was measured with a NanoDrop™ 2000 UV photometer (Thermo Scientific, Wilmington, DE) at 280 nm using an extinction coefficient of $\varepsilon^{0.1\%} = 1.49$ g/100 mL^{-1} cm^{-1}. For preparation of the final formulations, excipient stock solutions ranging from 1% to 25% (w/V) prepared in 10-mM histidine buffer (pH 6.0) were mixed with the dialyzed mAb solution in 10-mM histidine buffer (pH 6.0) in a way that final formulations contained either 3- or 5-mg/mL mAb, 0.02% (w/V) tween 80® and either 10% (w/V) sucrose or trehalose or HP-β-CD:sucrose 1:1 or mannitol:sucrose 4:1. For the low stabilizer containing formulation, the same procedure was applied but ending up with a lower final sucrose concentration of 1% (w/V), 3 mg/mL mAb, and 0.02% (w/V) tween 80®. The arginine phosphate formulation contained 10% (w/V) of arginine phosphate to which 5 mg/mL of mAb dialyzed in 10-mM arginine phosphate (pH 6.0) and 0.02% (w/V) tween 80® prepared in 10-mM arginine phosphate (pH 6.0) were added. A tabular overview of the used formulations could be found in the Supplementary Data (Table S1). The formulation for the accelerated stability study contained 5 mg/mL mAb and 10% (w/V) sucrose as aforementioned. All formulations were filtered using 0.2-μm Cellulose Acetate Membrane syringe filters (VWR International, Radnor, PA) prior to filling of the vials. 2.3 mL of each formulation was filled in 10R tubing vials (MGlas AG, Muennerstadt, Germany) and semi-stoppered with lyophilization stoppers (FluroTec® rubber stopper; West Pharmaceuticals, Eschweiler, Germany). The vial population for CFD was arranged on a lyophilization tray and surrounded with 1 row of 10% (w/V) sucrose-shielding vials.

Freeze-Drying Process

All samples were frozen in the same freezing step using a Christ ε2-6D laboratory scale freeze-dryer (Martin Christ, Osterode am Harz, Germany) with equilibration at −5°C for 1 h followed by ramping down the shelf with 1 K/min to −60°C setpoint. The frozen samples were subjected to one of the following drying protocols:

Conventional Freeze-Drying

Primary drying was carried out at a pressure of 0.1 mbar and a shelf temperature of −20°C. T-type thermocouples were used to determine primary drying time. Secondary drying was carried out at 0.05 mbar applying a 0.05 K/min ramp to 0°C and subsequently, a ramp of 0.2 K/min to 20°C which was held for 6 h. After completion of the drying, samples were stoppered at approximately 600 mbar in a nitrogen atmosphere and kept refrigerated until analysis.

Microwave-Assisted Freeze-Drying

Drying was conducted on a modified laboratory scale Püschner μWaveVac 0250fd vacuum dryer (Püschner GmbH + Co KG, Schwanewede, Germany)[14,15] equipped with a 2 kW/2450 MHz magnetron, a condenser (−80°C) and a vacuum system comprising a root pump and a rotary vane pump. The tuner, which was located between the magnetron and water load, was adjusted that way that approximately 1/10 of the generated MWs went into the product chamber. Frozen samples, which were frozen as previously described and which were transported on dry ice, were loaded on the precooled rotating sample tray. Drying was carried out at a pressure of 0.008 to 0.03 mbar as measured by Pirani gauge and at a radiated MW power between 20 W and 110 W as measured by a HOMER™ impedance analyzer (S-TEAM Lab, Bratislava, Slovak Republic) until constant mass was reached. For process monitoring, a glass fiber temperature measurement probe (TS2; Weidmann Technologies Deutschland GmbH, Dresden, Germany) and a balance to determine total weight loss were used. Samples were stoppered externally in a glove bag flushed with dry nitrogen and kept refrigerated until analysis.

Residual Moisture Content

Karl Fischer titration was used to determine residual water content after freeze-drying. Between 10 and 30 mg of sample aliquots were prepared in a glove box filled with pressurized air with a relative humidity of less than 10%, filled into 2R vials and stoppered. The samples were then placed in an oven with 100°C to enable fast extraction of water. The headspace moisture is transported into a coulometric Karl Fischer titrator (Aqua 40.00; Elektrochemie Halle, Halle [Saale], Germany). Results are calculated in relative water content (w/w).

Specific Surface Area

Specific surface area (SSA) of dried samples was determined using Brunauer–Emmet–Teller krypton gas adsorption in a liquid nitrogen bath at 77.3 K (Autosorb 1; Quantachrome, Odelzhausen, Germany). Approximately 80-140 mg of a sample was gently crushed with a spatula and weighed into glass tubes. Before measurement, an outgassing step was performed for at least 6 h at room temperature. A 9-point gas adsorption curve was measured, covering a p/p_0 ratio of approximately 0.05-0.25. Data evaluation was performed according to the multipoint Brunauer–Emmet–Teller method fit of the Autosorb 1 software.

X-Ray Powder Diffraction

To determine the solid state of the lyophilizates, an X-ray diffraction (XRD) 3000 TT diffractometer (Seifert, Ahrensburg, Germany) was used. The device is equipped with a copper anode (40 kV, 30 mA) and has a wavelength of 0.154178 nm. The scintillation detector voltage was 1000 V. The samples were placed on the copper sample holder and analyzed in the range of 5-45° 2-theta with steps of 0.05° 2-theta.

Reconstitution of Lyophilizates

The lyophilized cakes were reconstituted by the addition of HPW. The HPW volume for each formulation was calculated to match the volume of the water removed during freeze-drying.

High-Performance Size Exclusion Chromatography

To determine relative monomer content and the relative amount of soluble aggregates, HP-SEC was used. Separation was performed on a Waters 2695 Separation module (Waters GmbH, Eschborn, Germany) with a Tosoh TSKgel G3000 SWxl column (Tosoh Bioscience, Griesheim, Germany) using a Waters 2487 Dual λ Absorbance Detector (Waters GmbH) at 214 and 280 nm. 10 µL of reconstituted formulation with a final concentration of either 3 or 5 mg/mL was injected and separated using a 50-mM PBS running buffer containing 300-mM sodium chloride (pH 7.0) with a flow rate of 0.7 mL/min. Samples were measured as triplicates with 3 individual injections. Data integration of relative areas was performed using Chromeleon 6.80 (Thermo Scientific).

Light Obscuration

Subvisible particles were determined using a PAMAS SVSS-35 particle counter (PAMAS–Partikelmess-und Analysesysteme GmbH, Rutesheim, Germany) equipped with an HCB-LD- 25/25 sensor, which has a detection limit of approximately 120,000 particles ≥1 µm per mL. The rinsing volume was 0.5 mL and was followed by 3 measurements of 0.3 mL. Before and between samples, the system was rinsed with HPW until less than 30 particles/mL ≥1 µm, and no particles larger than 10 µm were present. Data collection was carried out using PAMAS PMA software and particle diameters in the range of ≥1 to 200 µm were determined. All results are given in cumulative particles per milliliter.

Turbidity

The turbidity of samples was measured as scattered laser light ($\lambda = 860$ nm) detected at an angle of 90° using a Hach Lange Nephla nephelometer (Hach Lange GmbH, Düsseldorf, Germany). Sample of 2.0 mL was gently pipetted in turbidity glass cuvette free of particles with a flat bottom and placed into the device. The result is given in FNU (Formazin nephelometric units).

Results and Discussion

Applicability to Different Formulations

Cake Appearance

With both MW-assisted and CFD, elegant and look alike cakes could be produced (Fig. 1). Only for the low concentration stabilizer formulation with 1% (w/V) sucrose (Fig. 1b) and the ionic arginine phosphate formulation (Fig. 1f), shrinkage of the cake was observable in both processes.

Process Time Reduction

MFD resulted in a significantly shorter drying process as shown in Figure 2. The conventional process (Fig. 2a) was finished after approximately 77 h. Since primary drying was kept longer to allow for complete sublimation in all investigated formulations, the actual drying time in CFD could, therefore, be reduced by about 18 h. In addition, the 16 h for the freezing step should be kept out of consideration since both CFD and MFD samples underwent the same freezing procedure. Consequently, the adjusted total drying time, that is, primary and secondary drying time, for the conventional drying is estimated to be approximately 43 h (Fig. 2a curly brackets b + c). In contrast, the drying procedure using MFD (Fig. 2b) yielded in a total drying time of nearly 10 h, which is a reduction by 77% in overall drying time. Although a standardized nonoptimized CFD cycle was used, still differences in a large order of magnitude were achieved for the process time reduction using MFD.

Residual Moisture and SSA

For both drying technologies, low residual moisture content (rM) was found independent of the excipient system (Fig. 3a, bars). Only the arginine phosphate formulation showed higher residual moisture of 2.3% (w/w) after MFD. This could be related to the smaller SSA, which indicates a microcollapse compared to the conventionally dried product (Fig. 3a, symbols). Overall, MFD products exhibited low rMs while the lyophilization-specific porous cake structure represented by the SSA was maintained. For some of the MFD samples, a higher variance could be determined, especially in the low concentration sucrose formulation, the 10% trehalose and arginine phosphate formulation. This was also observable in a 100% analysis for solid state properties of a full batch of a sucrose-based formulation produced by MFD (Supplementary Data, Fig. S1). It is assumed that this is on the one hand caused by the indirect setup of the magnetron to the product chamber via a water load which could favor an inhomogeneous field distribution. On the other hand, the sample handling in the used MFD laboratory setup was not yet optimal. This is due to the fact that first samples were frozen externally and kept deep-frozen until processing and second that dried samples were stoppered externally in a dry nitrogen-flushed glove bag.

X-Ray Diffraction

Regardless of the drying approach, the same solid state was found for all formulations as determined by XRD (Fig. 3b) and confirmed by DSC (Supplementary Data, Fig. S2). Only mannitol:sucrose 4:1 formulations showed a semicrystalline structure indicating mainly the formation of δ-mannitol by characteristic peaks at 9.7°, 20.4°, and 24.6° (Fig. 3b, arrow 1). A detailed

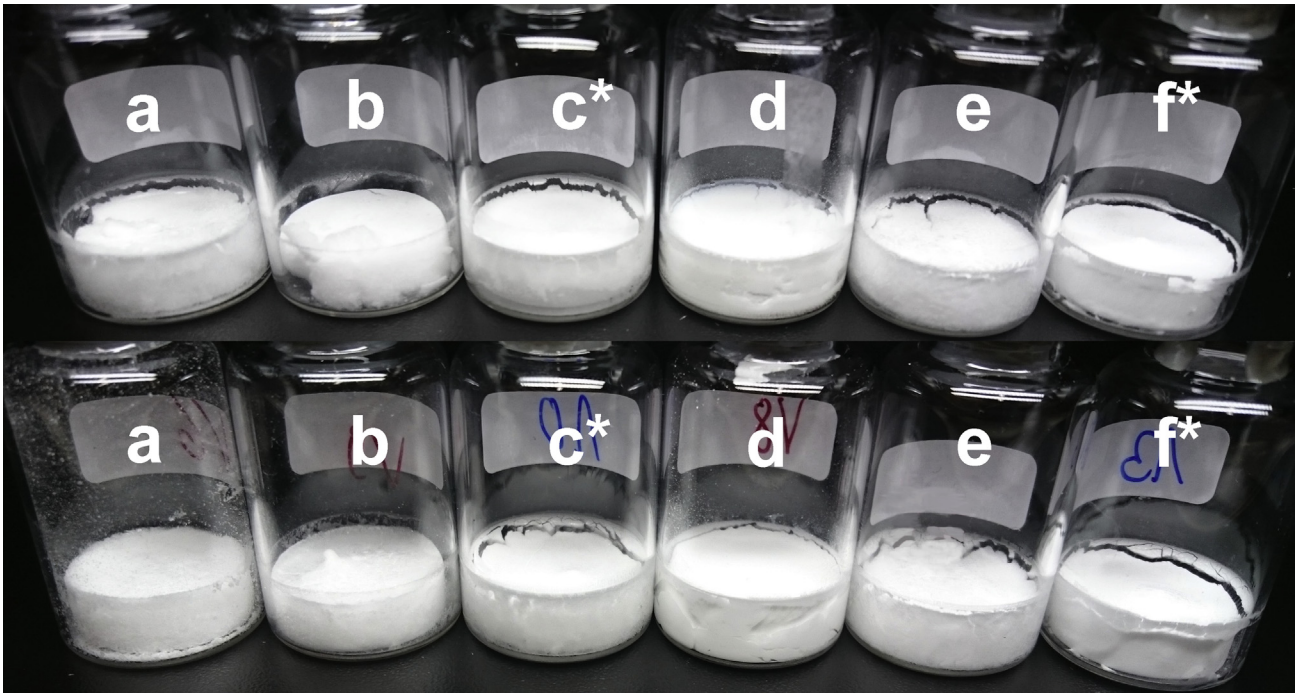

Figure 1. Representative photographs of differently dried formulations. Small letters represent the excipient used: (a) 10% sucrose; (b) 1% sucrose; (c) 10% trehalose; (d) 10% mannitol:sucrose 4:1; (e) 10% HP-β-CD:sucrose 1:1; and (f), arginine phosphate. Top picture: CFD products; bottom picture: MFD samples. The asterisk indicates that these formulations contained 5-mg/mL mAb and were dried in a separate but similar run to the one shown in Figure 2.

comparison of XRD diffractograms for this formulation is shown in Supplementary Data (Fig. S3). Indications for the formation of mannitol hemihydrate (Fig. 3b, arrow 2) were found. The crystal modification of mannitol hemihydrate is unfavored because of its destabilizing effect on the dried product.[16,17] However, it was found to be present in conventionally and MFD samples. Application of a thermal treatment will be taken into consideration to crystallize mannitol in its favored anhydrous crystalline modifications. All other formulations, independent of the drying approach were found to be fully amorphous.

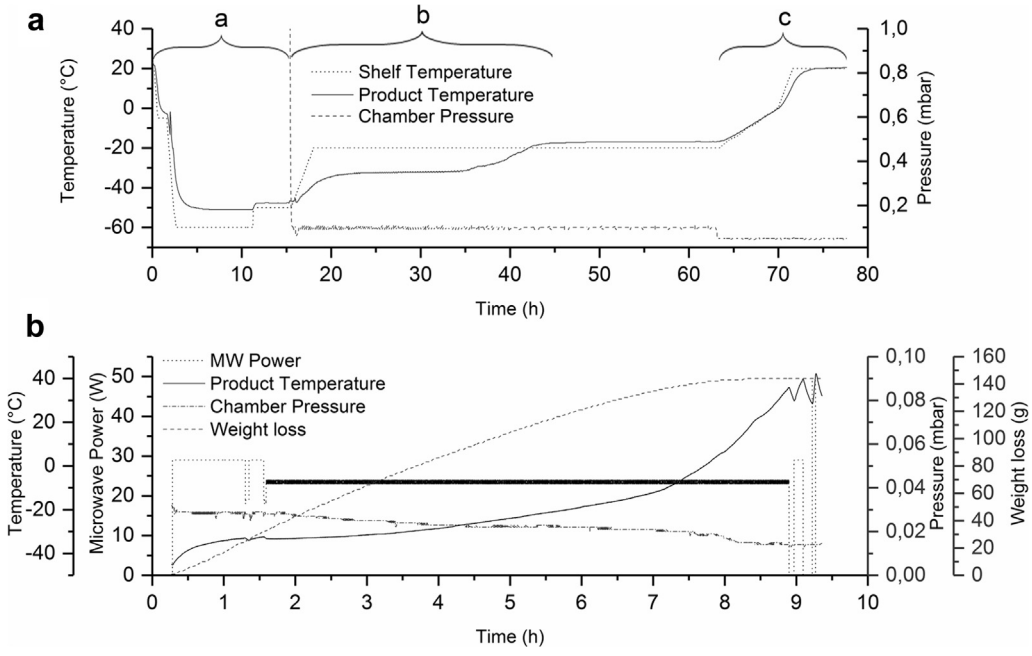

Figure 2. (a) Thermocouple and pressure readouts of the CFD cycle. Shelf temperature setpoint is represented by dotted line, thermocouple readout is depicted with a solid line. The coarse dotted line represents the chamber pressure setpoint. Curly bracket "a" tags the freezing step which is the same for both batches, whereas curly brackets "b" and "c" represent the actual drying time of primary and secondary drying. (b) Glass fiber (solid line), chamber pressure (dash dot-line), MW power (dotted line), and balance readings (dashed line) of the MFD-cycle. Product temperature measurement was performed in either case in the 10% sucrose formulation.

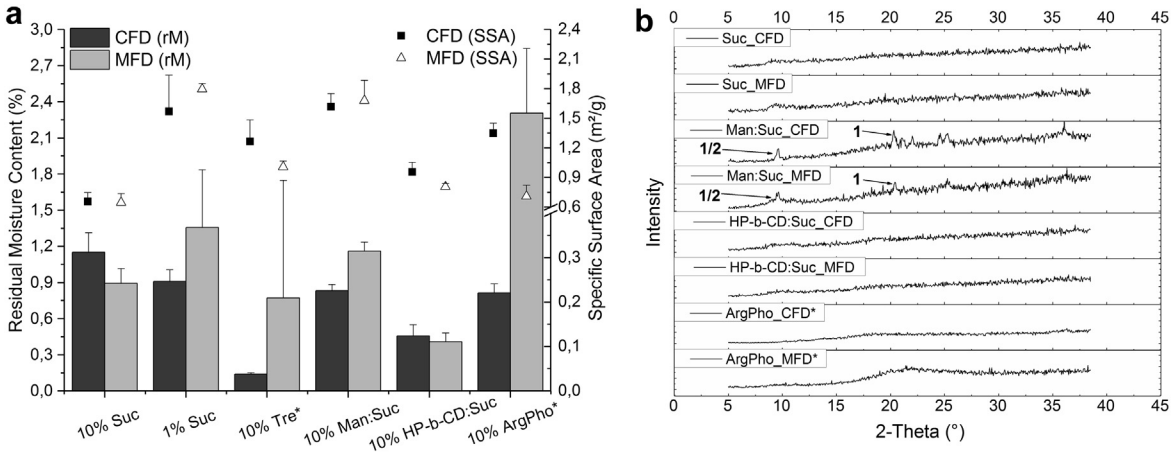

Figure 3. (a) rM (bars) and SSA (symbols) for all 6 formulations and the 2 respective drying protocols (black: CFD; light gray/white triangles: MFD). (b) X-ray diffractograms of exemplary formulations. "1" represents δ-Mannitol peaks at 9.7° and 20.4°; "2" indicates the overlapping peak of mannitol hemihydrate at 9.6°. Characteristic 2-theta values have been taken from literature.[14] The asterisk indicates that these formulations contained 5-mg/mL mAb and were dried in a separate but similar run to the one shown in Figure 2. The shown values represent the mean of measurements from 3 different vials. Error bars indicate the standard deviation of the mean. Suc, 10% sucrose; Man:Suc, 10% mannitol:sucrose 4:1-mixture; HP-b-CD:Suc, 10% HP-β-CD:sucrose 1:1-mixture; ArgPho, 10% arginine phosphate.

Accelerated Stability Study

To see the influence of the respective drying method on protein stability, a 10% sucrose formulation with 5 mg/mL mAb produced either by CFD or MFD was stored at 25°C and 40°C for 12 weeks. The drying processes which were adjusted to the formulation took 41 h 5 min and 10 h 15 min for CFD and MFD, respectively. Although the MFD procedure decreased drying times by 75%, rMs after freeze-drying were determined to be similar as 1.1 ± 0.13% for CFD and 1.0 ± 0.5% for MFD samples. Over 12 weeks of storage, no changes in solid state properties as SSA, rM, glass transition temperature, and solid state were observable (data are not shown). Concerning relative monomer content (Fig. 4a) and the relative amount of soluble species (Figs. 4b and 4c), no changes were observed over 12 weeks of storage for both CFD and MFD even at 40°C. This is confirmed by cumulative subvisible particle counts (Fig. 4d symbols) and

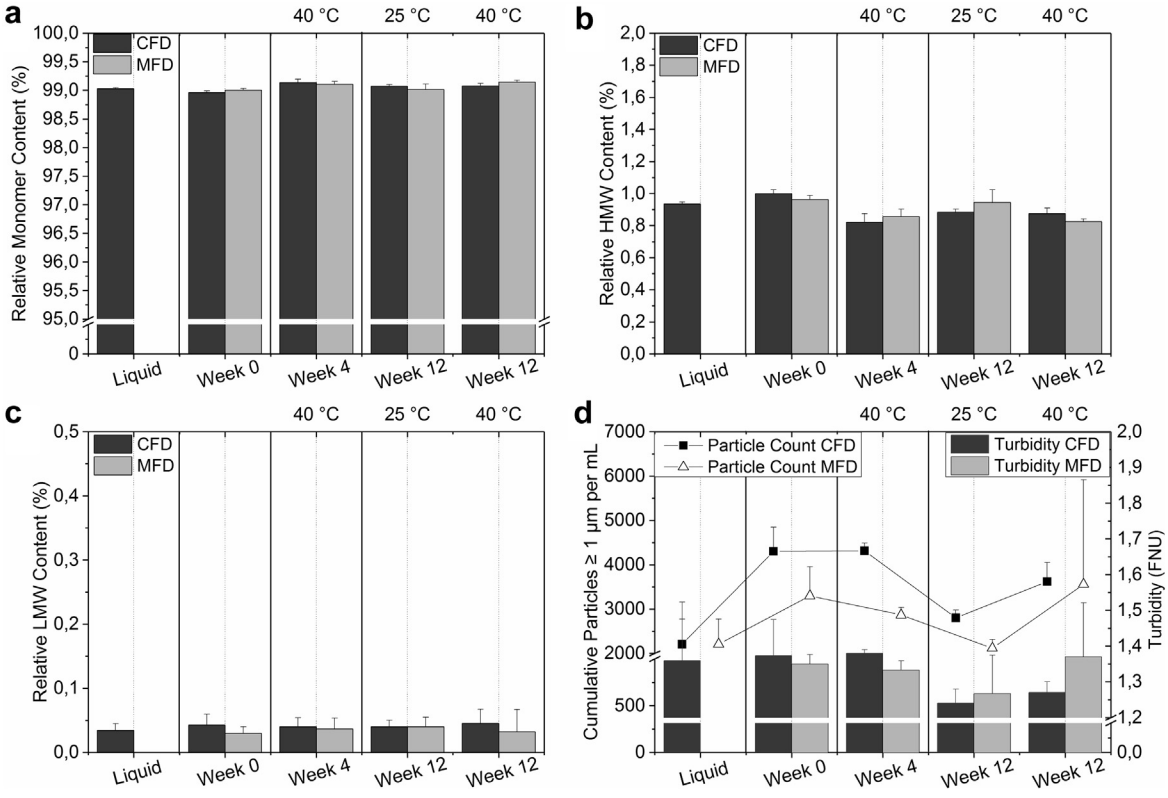

Figure 4. (a) Relative monomer content of a sucrose-based mAb formulation stored for 12 weeks as determined by HP-SEC; (b) and (c) represent soluble high-molecular-weight and low-molecular-weight species, respectively. (d) Development of cumulative particles ≥1 µm/mL (symbols) and turbidity (bars) over 12 weeks. The shown values represent the mean of measurements from 3 different vials. Error bars indicate the standard deviation of the mean.

turbidity (Fig. 4d bars) which show no clear trend and stay at comparably low levels over storage time.

Conclusion and Outlook

With this study, we can confirm that MFD is able to shorten the freeze-drying process by more than 75%. Moreover, it is applicable to various relevant antibody formulations while obtaining elegant look alike cakes and similar solid state properties. The ionic stabilizer system arginine phosphate showed a higher variance in solid state properties and therefore needs to be studied further. Yet, our results show that after 12 weeks of accelerated stability study, no differences between conventionally and MW-assisted freeze-dried products were observable. Neither in solid state properties nor in protein-related properties like soluble aggregates and subvisible particles, differences occurred for a sucrose-based antibody formulation at 2 different accelerated storage temperatures. It should be noted that alternative setups are currently under development since MFD processes are more difficult to control compared to CFD processes. In addition, typical challenges related to the application of MWs in freeze-drying processes should be taken into account like the potential emergence of cold plasma and the potential induction of batch inhomogeneity due to cold and hot spots.

However, MFD has the potential to become a highly promising alternative to the conventional approach. We were able to confirm its potential to significantly shorten drying times while maintaining product quality, which would be also the main requirement for continuous processing. This, in fact, makes MFD a promising concept for continuous pharmaceutical freeze-drying in future.

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References

1. Patel SM, Doen T, Pikal MJ. Determination of end point of primary drying in freeze-drying process control. *AAPS PharmSciTech*. 2010;11(1):73-84.
2. Walters RH, Bhatnagar B, Tchessalov S, Izutsu KI, Tsumoto K, Ohtake S. Next-generation drying technologies for pharmaceutical applications. *J Pharm Sci*. 2014;103(9):2673-2695.
3. Duan X, Zhang M, Mujumdar AS, Wang S. Microwave freeze drying of sea cucumber (Stichopus japonicus). *J Food Eng*. 2010;96(4):491-497.
4. Jiang H, Zhang M, Liu Y, Mujumdar AS, Liu H. The energy consumption and color analysis of freeze/microwave freeze banana chips. *Food Bioprod Process*. 2013;91(4):464-472.
5. Duan X, Zhang M, Mujumdar AS. Studies on the microwave freeze drying technique and sterilization characteristics of cabbage. *Dry Technol*. 2007;25(10):1725-1731.
6. Chandrasekaran S, Ramanathan S, Basak T. Microwave food processing—a review. *Food Res Int*. 2013;52(1):243-261.
7. Metaxas AC. Microwave heating. *IEE Power Eng J*. 1991;5(5):237-247.
8. Ryynänen S. The electromagnetic properties of food materials: a review of the basic principles. *J Food Eng*. 1995;26:409-429.
9. Barba AA, D'Amore M. Relevance of dielectric properties in microwave assisted processes. In: Costanzo PS, ed. *Microwave Materials Characterization*. Rijeka: InTech; 2012:91-118.
10. Meredith RJ. *Engineers' Handbook of Industrial Microwave Heating*. London: The Institution of Engineering and Technology; 2007.
11. Franks F. Freeze-drying of bioproducts: putting principles into practice. *Eur J Pharm Biopharm*. 1998;45(3):221-229.
12. Evans R. *Applications of Microwave Vacuum Drying and Lyospheres to Freeze-drying of Vaccines and Biologics at CPPR: Freeze-drying of Pharmaceuticals and Biologicals*. Garmisch-Partenkrichen, Germany: CPPR; 2014.
13. Bhambhani A, Evans RK, Sinacola J, Jones M. World Intellectual Property Organization Patent Application No. WO 2015/057541 A1. Method of Obtaining Thermostable Dried Vaccine Formulations. Published: April 23, 2015.
14. Püschner GmbH+Co KG. Püschner vacuum drying/freeze-drying. Available at: http://www.pueschner.com/en/products/vacuum-drying-freeze-drying. Accessed July 10, 2018.
15. Püschner GmbH+Co KG. Good Manufacturing practise (GMP) for industrial microwave plants. Available at: http://www.pueschner.com/en/microwave-technology/gmp-microwaves. Accessed July 10, 2018.
16. Yu L, Milton N, Groleau EG, Mishra DS, Vansickle RE. Existence of a mannitol hydrate during freeze-drying and practical implications. *J Pharm Sci*. 1999;88(2):196-198.
17. Mehta M, Bhardwaj SP, Suryanarayanan R. Controlling the physical form of mannitol in freeze-dried systems. *Eur J Pharm Biopharm*. 2013;85(2):207-213.