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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.

Conflicts of interest: The authors have no conflict of interest to disclose.

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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).