Freeze Drying of L-Arginine/Sucrose-Based Protein Formulations, Part I: Influence of Formulation and Arginine Counter Ion on the Critical Formulation Temperature, Product Performance and Protein Stability

PETER STÄRTZEL,[1] HENNING GIESELER,[1] MARGIT GIESELER,[1] AHMAD M. ABDUL-FATTAH,[2] MICHAEL ADLER,[2] HANNS-CHRISTIAN MAHLER,[2] PIERRE GOLDBACH[2]

[1]GILYOS GmbH, Friedrich-Bergius Ring 15, Wuerzburg 97076, Germany

[2]F. Hoffmann-La Roche Ltd., Pharmaceutical Development & Supplies, Pharma Technical Development Biologics EU, Basel 4070, Switzerland

Received 22 January 2015; revised 30 March 2015; accepted 27 April 2015

Published online 20 May 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24501

ABSTRACT: The objective of this study was to investigate product performance of freeze dried L-arginine/sucrose-based formulations under variation of excipient weight ratios, L-arginine counter ions and formulation pH as a matrix to stabilize a therapeutic monoclonal antibody (MAb) during freeze drying and shelf life. Protein and placebo formulations were lyophilized at aggressive primary drying conditions and key attributes of the freeze dried solids were correlated to their thermal properties and critical formulation temperature. Stability (physical) during processing and long-term storage of the MAb in different formulations was assessed by SE-HPLC. Thermal properties of the mixtures were greatly affected by the type of L-arginine counter ion. High glass transition temperatures were achieved by adding multivalent acids, whereas the temperature values significantly decreased in the presence of chloride ions. All mixtures were stable during freeze drying, but storage stability varied for the different preparations and counter ions. For L-arginine-based formulations, the protein was most stable in the presence of chloride ion, showing no obvious correlation to estimated global mobility of the glass. Besides drying behavior and thermal properties of the freeze dried solids, the counter ion of L-arginine must be considered relevant for protein shelf life stability. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2345–2358, 2015

Keywords: protein formulation; freeze drying/lyophilization; amorphous solids; glass transition; solid-state stability; protein aggregation; calorimetry (DSC); L-arginine

INTRODUCTION

Freeze drying is commonly used in the pharmaceutical industry to improve protein stability for molecules that are not stable in aqueous solution.[1–4] However, some proteins are inactivated during the drying process if not appropriately formulated. Therefore, excipients, for example stabilizers, buffers, and surfactants are added to stabilize the protein during the various stresses exerted by freezing and drying as well as long-term storage.[2,5,6] In the solid state, proteins are protected thermodynamically by replacement of water molecules[2,7,8] and kinetically by suppression of global motion (also referred to as α-relaxation) within the glass.[9,10] α-Relaxation mainly occurs because of the translational and rotational motion of the molecules and therefore strongly affects the diffusion of reactive species, potentially leading to protein aggregation.[11] Both concepts have been used to explain protein stability in the lyophilized state,[12,13] although in some cases neither concept was found conclusive to the data.[14,15] Some of this divergence was attributed to the presence of motions which occur in a shorter time scale and are therefore referred to as fast dynamics or β-relaxations.[11,16,17] Global motions are typically characterized by "structural relaxation time" (denoted as τ)[18–20] as both degradation (chemical and physical) and structural relaxation require a certain degree of molecular mobility within the glass. Thus, it is suggested that instability-causing processes, such as protein aggregation, are coupled to the structural relaxation time.[18] Results are typically compared under application of the parameter β, a stretched exponential constant (τ^{β}).[18]

Similar to sugars, various amino acids and their salts (e.g., L-arginine hydrochloride)[21,22] also showed lyo- and cryoprotective effects.[23] However, effective protection can only be achieved if the amino acid remains in the amorphous state. Addition of various acids was reported to affect the crystallinity and the glass transition temperature of vacuum dried and freeze dried L-arginine.[24] Izutsu et al.[25] found that mixing L-arginine and a hydroxyl di- or tricarboxylic acid led to amorphous solids with a glass transition temperature of the maximally freeze concentrated solute (T_g') significantly higher than those of the individual solute solutions (i.e., the pure acid and L-arginine base). Especially multivalent acids such as citric and L-tartaric acid raised the T_g' as well as the T_g of the freeze dried solids. This was attributed to the formation of an intense interactive network between the involved molecules.[25] Increase of the relevant glass transition temperatures (T_g' and T_g) may allow application of a higher product temperature during primary drying, and decrease molecular mobility in the freeze dried solids which can provide enhanced formulation robustness and may also improve protein stability in the freeze dried state.[26] However, the assessment of greater protein stability at higher glass

Correspondence to: Pierre Goldbach (Telephone: +41-61-6885242; Fax: +41-61-68-88689; E-mail: pierre.goldbach@roche.com)

Journal of Pharmaceutical Sciences, Vol. 104, 2345–2358 (2015)
© 2015 Wiley Periodicals, Inc. and the American Pharmacists Association

transition temperatures of the freeze dried excipient matrix is based on the assumption that stability in the solid state is governed by global mobility.[26] However, so far, little information is available on stability of freeze dried therapeutic proteins as a function of the varying physical properties obtained for different types of L-arginine counter ions.[25,26] Moreover, L-arginine and acid combinations have been used to assist recovery of chemically unfolded proteins or recombinant proteins expressed in inclusion bodies,[27–29] due to the ability of L-arginine to suppress aggregation of folding intermediates in the liquid state.[30] This unique feature may render L-arginine a versatile excipient for protein freeze drying, as an L-arginine-containing formulation may also stabilize the protein in solution prior to freeze drying and after reconstitution.[25,31] In the present study, L-arginine was included into sucrose-based formulations at varying concentrations. Sucrose was used as a second excipient in the light of its frequent application as a lyoprotectant in pharmaceutical freeze drying.[2,32]

Although analysis of L-arginine and acid combinations showed that the L-arginine counter ion influences the physical properties of the amorphous amino acid, no data have been published on such combinations included into a multi-excipients formulation. However, the presence of additional excipients may significantly alter interactions between molecules which may lead to properties of L-arginine different from those as a single component. Therefore, investigating L-arginine as a part of a typical pharmaceutical, multi-component formulation may be considered essential as a basis for assessment of its stabilizing effect on proteins. The main purpose of this study was to investigate how protein stability in various L-arginine/sucrose-based formulations (including buffer and surfactant) is affected by the type of counter ion during freeze drying and during storage. Long-term stability with regard to protein aggregation was studied for a recombinant humanized monoclonal IgG1 antibody over a maximum period of 180 days at different storage conditions ranging from 5°C to 40°C. Results obtained through this study also provide insight into the effect of the counter ion on drying performance, product appearance and characteristic product attributes (i.e., residual moisture, reconstitution time (RT), and physicochemical properties) for active and placebo formulations.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade and used as supplied. Sucrose from Ferro Pfanstiehl Inc. (Waukegan, Illinois) was used during all experiments. L-Arginine base, L-histidine base, and citric acid monohydrate were purchased from Ajinomoto Foods Europe SAS (Hamburg, Germany); polysorbate 20 was obtained from Croda GmbH (Nettetal, Germany). Hydrochloric acid 25% (v/v) and succinic acid (cryst.) were acquired from Merck KGaA (Darmstadt, Germany). Phosphoric acid 85% was purchased from Carl-Roth (Karlsruhe, Germany). Water for injection (WFI) was used for preparation of all formulations. Solutions were sterile filtered through a 0.22 μm membrane filter before use (placebo formulations: PES membrane, MILLEX-GP50; Millipore Corporation, Bedford, Massachusetts; active formulations: PVDF membrane, SteriCup™; Millipore Corporation, Bedford, Massachusetts). Twenty milliliters clear glass tubing vials (Fiolax, 20 mm) were purchased from SCHOTT (Müllheim, Germany) and 20 mm D777–1 Lyo Stoppers were acquired from Daikyo Seito Ltd. (Tokyo, Japan). Purified monoclonal antibody (MAb) was supplied as 76.4 mg/mL stock solution in 20 mM histidine buffer (histidine/histidine-hydrochloride) pH 6.0 by F. Hoffmann-La Roche (Basel, Switzerland). The MAb used in this study was a recombinant humanized monoclonal IgG1 antibody with an approximate molecular weight of 149 kDa and an isoelectric point (p*I*) of 7.8.

Methods

Preparation of Product Solutions

Solutions containing 104 mg/mL (304 mM) L-arginine base were titrated to a pH of 6.0 ± 0.3 with citric, phosphoric, succinic, or hydrochloric acid to investigate the physicochemical characteristics of L-arginine as a single solute in presence of different counter ions. All pH measurements were executed using a calibrated pH-meter (SCHOTT Instruments Lab 870 with Mettler-Toledo InLab Micro Pro pH electrode). For placebo formulations, the calculated mass of excipients was dissolved in WFI as tabulated in Table 1 (F01–F08). The mixing ratio of L-arginine to sucrose was 4:1 and 1:4 (by weight). Both L-arginine and L-histidine (buffer) were added in form of the base, and solutions were then titrated to a pH of 6.0 ± 0.3 using the aforementioned acids (cf. Table 1). For investigating the effect of pH (discussed below), several mixtures were adjusted to pH setpoints other than 6.0 (i.e., 5.0 and 7.0) while maintaining accuracy of pH adjustment and the compounding procedure. Polysorbate 20 was added using a 4% (w/v) stock solution (final concentration 0.02%, w/v), and the total solid content of 130 mg/mL in solution (excluding buffer and surfactant) was adjusted by diluting with WFI. Histidine buffer concentration was 20 mM after dilution. Formulations with a higher

Table 1. Overview of Formulation Compositions

Name of Formulation	Composition[a] L-Arginine Base (mg/mL)	Sucrose (mg/mL)	MAb (mg/mL)	Acid[b]
F01	26	104	–	Hydrochloric
F02	104	26	–	Hydrochloric
F03	26	104	–	Citric
F04	104	26	–	Citric
F05	26	104	–	Phosphoric
F06	104	26	–	Phosphoric
F07	26	104	–	Succinic
F08	104	26	–	Succinic
F09	16	64	50	Hydrochloric
F10	64	16	50	Hydrochloric
F11	16	64	50	Citric
F12	64	16	50	Citric
F13	16	64	50	Phosphoric
F14	64	16	50	Phosphoric
F15	16	64	50	Succinic
F16	64	16	50	Succinic
F17	–	80	50	–

[a]All formulations contained 20 mM L-histidine as a buffer which was added in form of the base (for F17: histidine was added as histidine/histidine–HCl, 20 mM to obtain a pH of 6.0) and 0.02% (w/v) polysorbate 20 as a surfactant. pH of the formulations was adjusted to 6.0 ± 0.3 using the respective acid.
[b]Acid used for pH adjustment.

Download English Version:

https://daneshyari.com/en/article/2484530

Download Persian Version:

https://daneshyari.com/article/2484530

Daneshyari.com