BIOTECHNOLOGY

The Impact of Drying Method and Formulation on the Physical Properties and Stability of Methionyl Human Growth Hormone in the Amorphous Solid State

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ABSTRACT: The objective of this work was to investigate the impact of drying method and formulation on the physical stability (aggregation) and selected important physical properties of dried methionyl human growth hormone (Met-hGH) formulations. Solutions of Met-hGH with different stabilizers were dried by different methods (freeze drying, spray drying, and film drying), with and without surfactant. Properties of the dried powders included powder morphology, specific surface area (SSA), protein surface coverage, thermal analysis, and protein secondary structure. Storage stability of Met-hGH in different formulations was also studied at 50°C and at 60°C for 3 months. The dried powders displayed different morphologies, depending mainly on the method of drying and on the presence or absence of surfactant. Film dried powders had the lowest SSA (~0.03 m^2/g) and the lowest total protein surface accumulation (~0.003%). Surfactant caused a reduction in the SSA of both spray dried and freeze dried powders. Spray dried powders showed greater protein surface coverage and SSA relative to the same formulations dried by other means. Greater in-process perturbations of protein secondary structure were observed with polymer excipients. Formulation impacted physical stability. In general, low molecular weight stabilizers provided better stability. For example, the aggregation rate at 50°C of Met-hGH in a freeze dried trehalose-based formulation was approximately four times smaller than the corresponding Ficoll-70-based formulation. Drying method also influenced physical stability. In general, the film dried preparations studied showed superior stability to preparations dried by other methods, especially those formulations employing low molecular weight stabilizers.
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Keywords: freeze drying; spray drying; film drying; human growth hormone (hGH); composition heterogeneity; electron spectroscopy for chemical analysis (ESCA); glass transition temperature (T_g); particle morphology; protein aggregation; protein secondary structure; specific surface area

INTRODUCTION

Freeze drying is widely used for the preparation of protein pharmaceuticals for parenteral administration,[1–3] while spray drying is widely used for pulmonary delivery of protein pharmaceuticals.[4,5]

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Other drying methods, such as spray coating,[6] supercritical fluid technology,[7] and spray freeze drying[8] have also been used to dry protein solutions. Drying by different methods subjects the protein to different thermal histories and different "stresses," resulting in potential differences in physical properties, such as moisture content, particle size, particle morphology, powder density, specific surface area (SSA), surface composition, and thermal properties.[6,9–12]

It is becoming accepted[9,13–19] that there may be significant separation of stabilizer from protein during drying without actual phase separation. That is, the surface region becomes rich in protein, and the interior therefore becomes richer in stabilizer, resulting in an uneven distribution of chemical components throughout the dried particle.[9,13–19] Such a phenomenon is referred to as "composition heterogeneity" or "chemical heterogeneity." Phase separation, on the other hand, refers to the formation of two or more distinct phases. Examples of phase separation include the crystallization of one or more components (such as a buffer component and/or a bulking agent) from the amorphous phase,[20,21] or the formation of two different amorphous phases (as has been documented with PEG/dextran mixtures).[22] Composition heterogeneity has been documented using special surface analysis techniques, such as electron spectroscopy for chemical analysis (ESCA).[9,13–19] Phase separation in amorphous systems has been documented by techniques such as electron microscopy[22–24] and differential scanning calorimetry.[25,26]

Composition heterogeneity perhaps occurs as a result of differences in diffusion coefficient between formulation components in solution and/or because of differences in surfactant properties of the components.[9,13–19] As might be expected, the inclusion of a surfactant in the formulation significantly moderates the accumulation of protein at the surface of spray dried samples.[9,13,14,17–19] Development of composition heterogeneity from a solution initially uniform in composition clearly requires molecular mobility. That is, the system must possess sufficient molecular mobility to support the mutual diffusion needed to create separation of components. Composition heterogeneity could arise during freezing for a freeze drying process and during much of the drying process for a spray dried material. However, once the material is well below the glass transition temperature, it seems unlikely that sufficient translational mobility would exist to allow further separation of components on the time scale of the process. Moreover, although there is spatial variation in composition in freeze dried materials, it does appear that composition variation in spray dried materials is typically an order of magnitude larger.[9,18] Since one normally finds that the stability of a given protein improves as the weight ratio of stabilizer to protein increases, it follows that the separation of components might have adverse stability consequences. That is, the protein near the surface would be much less stable than what the overall composition would suggest, and the protein in the interior may not gain sufficient stability to compensate. However, the implications of such component separation on storage stability are poorly understood.

It is becoming well known that stability of proteins in amorphous pharmaceuticals shows a log-linear dependence on composition.[27–31] Assuming that the measured or observed rate constant for degradation (k_{obs}) is a summation of contribution of stability from both surface and bulk protein, we may write:

$$k_{obs} = k_S F_{PS} + k_B F_{PB} \quad (1)$$

where k_S is the rate constant for decomposition of surface protein, k_B is the rate constant for decomposition of bulk protein, F_{PS} is the fraction of the surface protein and F_{PB} is the fraction of bulk protein. Note that "surface protein" as used in Eq. (1) refers to the fraction of total protein which is within the first 50 Å outer shell thickness, as measured by ESCA. As described by Eq. (1), an increase in "surface protein" increases the first term on the right hand side of Eq. (1) and decreases the magnitude of the second term. Numerical calculations show that when the rate constant shows a log-linear dependence on composition, the net effect of composition heterogeneity is an increase in the magnitude of k_{obs}. That is, the net effect decreases stability relative to what one would expect if no separation of components occurred. This effect is expected to be more significant for formulations rich in stabilizer.[32]

A few studies have shown that formulations with a high SSA had inferior storage stability relative to the same formulations with lower SSA, as prepared by other drying methods.[9,33] For example, Sane et al.[33] observed that the storage stability of a freeze dried antibody formulation was superior to the stability of the same formulation prepared by spray drying. Protein secondary structure was the same in both formulations, but the SSA of the spray dried formulation was