SCF 528

Precipitation of Protein Powders by Gas Anti-Solvent (GAS)

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Introduction

The pharmaceutical and health care industries are seeking new methods of administering protein powders. Whether delivered in encapsulated formations, aerosol delivery, or implanted controlled release systems, it is essential to regulate the particle size of these proteins. Ideally, protein particles should be in the 1- to 5-µm size range in order to allow for predictable release kinetics. At this size, there is also an increase in the bioavailability of the protein and the required dose can be minimized.

Conventional methods for reducing the particle size of proteins and peptides include spray drying, milling, fluid energy grinding, lyophilization, and using miscible organic antisolvents. Unfortunately, these processes can also inactivate or denature proteins, produce small final yields, lead to electrostatically charged powders, produce particles with a broad size distribution, or rely on large volumes of organic solvent.

The gas antisolvent (GAS) process, is an alternative batch precipitation technique that produces biologically active microparticulate powders of peptides and proteins. In the GAS method a solid (such as a protein) is dissolved in an organic solvent. Next, a supercritical fluid miscible with the organic solvent is added as an antisolvent.

The supercritical fluid expands the volume of the organic solvent dramatically, reducing its density and causing the dissolved protein or drug to precipitate.

Using certain conditions, it is possible to control particle characteristics such as size, porosity, shape, and residual solvent concentrations.

This application describes the precipitation of insulin and lysozyme by the GAS method to produce nano-sized particles that retain their chemical activity, are free flowing, non-aggregated, and have a narrow particle size distribution.

Equipment

- ✓ Applied Separations' Helix Supercritical System

Materials

- ✓ Insulin
- ✓ Lysozyme (hen egg white from Sigma)
- ✓ DMSO (Dimethylsulfoxide)
- ✓ Methanol
- ✓ CO_2 (liquid) 99.6% pure

Method

Dissolve lysozyme in DMSO or insulin in methanol. Partially fill the crystallizer vessel allowing room for the solvent to expand in volume. Then, pump supercritical CO_2 into the

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crystallizer vessel. The supercritical CO_2 dissolves into the organic solvent; expanding the solvent volume and lowering the strength of the organic solvent. At this point, super-saturation and solute precipitation occurs.

Discharge the expanded solvent through a filter in the crystallizer vessel, trapping the particles. Residual solvent is then separated from the decompressed CO_2 gas in the separator. After all the solvent is discharged from the crystallizer, the protein powder is flushed dynamically with supercritical CO_2 until dry.

Helix Conditions

Crystallizer Vessel:

Crystallization :

Temperature:	35 °C
Rate of CO_2 Pressurization:	2.0 Bar/min
Final Pressure:	80 Bar

Drying :

Temperature:	33°C
CO_2 Pressure:	80 Bar
CO_2 Quantity:	5 Crystallizer Vessel volumes

Analysis of Particle Size

Scanning electron microscopy.

Conclusion

Biologically active, dry, stable, and discrete protein powders are produced at ambient temperatures using supercritical carbon dioxide as an antisolvent (GAS). The average diameter of the lysozyme particles precipitated from DMSO ranged from between 0.1µm and 1.5µm. Also, discrete insulin nanoparticles between 0.1µm and 0.6µm were formed. These proteins were free of residual solvents and were non-agglomerated.

References

Thiering, R.; Dehghani, F.; Dillow, A.; and Foster, N. "The influence of operating conditions on the dense gas precipitation of model proteins." *J Chemical Technology and Biotechnologyl* **75**:29-41 (2000).