SCF Application #527

Precipitation of Protein Powders into a Compressed Antisolvent

Introduction

The pharmaceutical and health care industries are seeking new methods of administering protein powders. Controlled release systems are typically implantable devices or injectable microparticulate systems that can dispense appropriate amounts of peptides or proteins into the biological environment. Such systems are becoming increasingly popular because they can reduce injection frequency, decrease the total administered protein dose, and can increase patient compliance for chronic indications.

Since controlled release systems are made up of protein or peptide particles dispersed within a polymeric matrix, it is important to regulate the particle size. Ideally, protein particles should be in the 1- to 5-µm size range. Small particles allow a higher percentage of solids to be incorporated, and thus distributed more uniformly within the polymeric matrix.

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 distribution, or rely on large volumes of organic solvent.

The precipitation by compressed antisolvent (PCA) process is an alternative technique that produces dry, biologically active microparticulate powders of proteins, enzymes, and peptides. In the PCA method, a solid (such as a protein) is dissolved in a organic solvent.

This liquid is then continuously sprayed in small amounts into a vessel filled with supercritical CO_2. As the liquid dissolves in the SC-CO_2 nano sized particles of proteins are produced.

This application will describe a method for reducing the particle size of insulin using supercritical CO_2.

Equipment

- ✓ Applied Separations' Helix Supercritical System
- ✓ Modifier Pump

Materials

- ✓ Insulin (bovine, Zn, low endotoxin)
- ✓ CO_2-bone dry grade (99.8 %)
- ✓ DMSO (Dimethylsulfoxide)
- ✓ DMFA (N,N,-dimethylformamide)

Method

Prepare a 15 mg/mL DMSO/insulin solution and a 5 mg/mL DMFA/insulin solution. Add a few drops of 1 N HCL to the DMFA/insulin solution in order to fully solubilize the protein. Next, fill crystallization vessel with SC-CO_2. Spray organic solvent containing solute into supercritical fluid. The organic solvent will dissolve into the SC-CO_2, causing supersaturation and protein precipitation. The supercritical fluid containing the dissolved solvent is carried to a separator vessel. As the vessel is depressurized, the solvent becomes a liquid and SC-CO_2 can be vented.

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Stop the organic solvent/protein solution pump and continue the supercritical CO_2 flow to insure protein is solvent free.

PCA Conditions

Crystallizer Vessel:

Pressure:	86 BAR
Temperature:	35 ºC
Solvent Flow Rate:	0.3 mL/min (liquid)
Solvent Nozzle:	30 micron diameter, .24mm thickness
CO_2 Flow Rate:	8.0 L/min
Time:	
Crystallization:	40 minutes
SC-CO_2 Drying:	120 minutes

Separator Vessel:

Pressure:	35 BAR
Temperature:	35°C

Analysis

Examine the size of the protein particles by scanning electron microscopy (SEM).

Conclusion

PCA processing with supercritical antisolvent produces biologically active, dry, and fine powders of proteins, enzymes, and peptides quickly and uniformly. When applied to insulin, PCA produced particles between the 2- to 3-µm rage, outperforming conventional methods.

References

Yeo, S.; Lim, G.; Debenedetti, P.; Bernstein,H. "Formation of Microparticulate Protein Powders Using a Supercritical Fluid Antisolvent." *Biotechnology and Bioengineering*. 41 (1993) '341-346.

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