Drug Development and Industrial Pharmacy, 27(10), 1003–1015 (2001)

REVIEW

Solution-Based Particle Formation of Pharmaceutical Powders by Supercritical or Compressed Fluid CO_2 and Cryogenic Spray-Freezing Technologies

True L. Rogers,[1] Keith P. Johnston,[2] and Robert O. Williams III[1,*]

[1]*College of Pharmacy,* [2]*Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712*

ABSTRACT

Micronization is an important procedure used in the pharmaceutical industry to reduce the particle size of active pharmaceutical ingredients (APIs). The spray-drying and milling techniques presently used to micronize drug substances cannot be used to process thermolabile or physically unstable drug substances. Therefore, new micronization techniques, including particle precipitation with supercritical or compressed fluid CO_2 and spray-freezing of drug solutions and suspensions into cryogenic gas to produce solid frozen microparticles, are currently being perfected for future use in the pharmaceutical industry. This review highlights the compressed gas and cryogenic liquid technologies being developed as potential solution-based particle formation technologies for drugs that cannot be processed by conventional micronization techniques.

Key Words: *ASES; Atomization; GAS; Micronization; Particle engineering; PCA; PIPS; RESAS; RESS; SAS; SEDS; Spray-freezing; Targeted drug delivery*

*Corresponding author. College of Pharmacy (Mailstop A 1920), University of Texas at Austin, Austin, TX 78712-1074. Fax: (512) 471-7474; E-mail: williro@mail.utexas.edu

INTRODUCTION

Small-particle engineering enables an active pharmaceutical ingredient (API) to be incorporated into a formulation for targeted drug delivery. Powder micronization can also be used to increase the dissolution rates of poorly water soluble drugs. Micronization procedures can modify particle size, porosity, and density, and the API may be mixed with pharmaceutical excipients using small-particle technologies to maximize delivery to the desired target for drug administration. Particle formation technologies may be classified as either mechanical micronization processes or solution-based phase separation processes. The most common mechanical micronization methods include ball and jet milling. However, friction generated during these milling processes may lead to either thermal or mechanical degradation of the API. Spray-drying is another common method used to micronize drug substances alone or with pharmaceutical excipients. This method requires extremely high temperatures, on the order of 150°C, to remove the solvent from the drug following atomization. The elevated temperatures may accelerate degradation of the active ingredient.

Relatively new solution-based particle formation techniques involve the use of conventional liquids, compressed gases, near-critical liquids, or supercritical fluids functioning as solvents, antisolvents, or cryogenic media for ultrarapid freezing. These techniques involve phase separation of solvent and API by evaporation, rapid expansion, change in solvent composition, or solidification by freezing. The spray configuration in many of these processes produces atomized droplets with high surface areas. Thus, phase separation and rapid nucleation result in small primary particles or highly porous microparticles.

The objective of this article is to provide a comprehensive review of (1) solution-based phase separation techniques involving gas antisolvent (GAS) precipitation, precipitation with a compressed antisolvent (PCA; aerosol solvent extraction system [ASES], supercritical antisolvent [SAS], and solution-enhanced dispersion by supercritical fluids [SEDS]), rapid expansion from supercritical solutions (RESS; and rapid expansion from supercritical to aqueous solutions [RESAS]), and precipitation from gas-saturated solutions (pressure-induced phase separation [PIPS]) and (2) formation of pharmaceutical powders by ultrarapid freezing techniques using halocarbon refrigerants and cryogenic gases or liquids.

Solution-based techniques require less particle handling and are often easier to scale up than conventional milling techniques. Reduced particle handling results in higher yields and simplifies cleaning and sterilization procedures. Furthermore, solution-based processes can be continuous or semicontinuous, unlike milling, which is typically a batch process.

SUPERCRITICAL SOLVENT AND ANTISOLVENT MICRONIZATION TECHNIQUES

The micronization techniques that utilize compressed fluids (namely, gaseous, liquid, or supercritical fluid carbon dioxide) as solvents and antisolvents involve precipitation of solutes primarily from an organic solution to produce amorphous or crystalline powders. Three types of supercritical precipitation techniques are (1) PCA, (2) precipitation by RESS, and (3) precipitation from gas-saturated solutions (1). Supercritical CO_2 is obtained by pressurizing and heating the CO_2 system to a minimum of 73.8 bar and 31.05°C, respectively (2). In PCA, the solubility of the CO_2 in the organic solvent must be sufficient at a given temperature and pressure to optimize API precipitation. At optimal conditions, the CO_2 can expand the solvent, thus lowering the solvent strength, resulting in precipitation of the solute into a micronized powder. In RESS, the solubility of the solute in the CO_2 must be sufficient at a given temperature and pressure to produce adequate yields of the micronized powder following RESS. API and solvent solubilities in the supercritical CO_2 can be altered by manipulating the temperature and pressure, and hence the density, of the compressed gas (2).

Precipitation with a Gaseous Antisolvent in the Batch Mode

Gas antisolvent (GAS) precipitation is a batch process that has been used to process explosives, low molecular weight organic compounds, proteins, and polymers (3). A diagrammatic representation of a GAS apparatus is shown in Fig. 1b. The GAS precipitation process involves the addition of CO_2 as an antisolvent to an organic solution containing

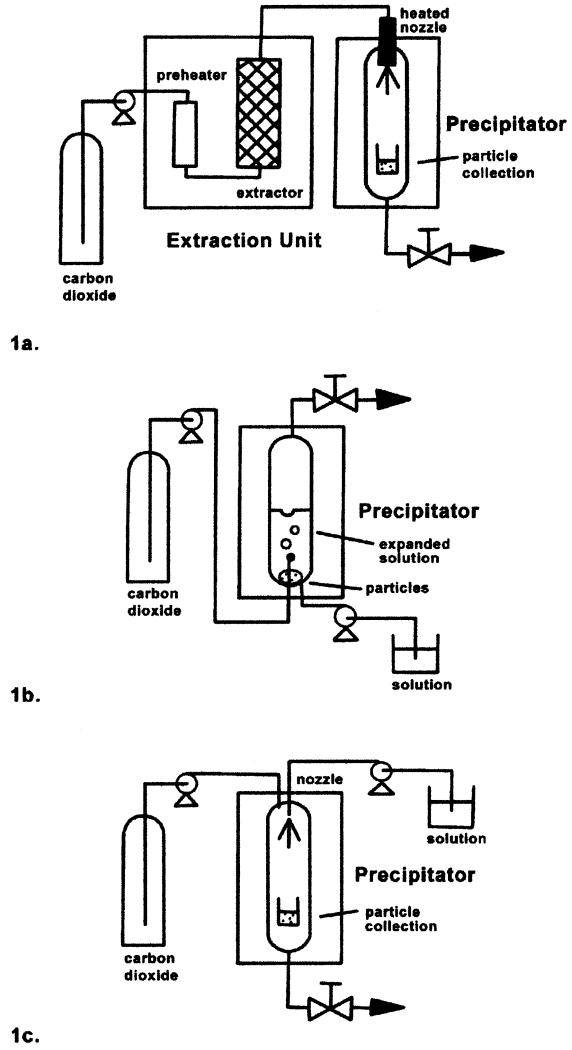

Figure 1. Schematic representations of the (a) RESS, (b) GAS, and (c) PCA/SAS/ASES processes. (From Ref. 5 with permission.)

an API and, if necessary, additional pharmaceutical excipients (4–9). CO_2, in the gaseous, liquid, or supercritical fluid states, must be significantly soluble in the solvent, and the API and excipients must not be soluble in the excess CO_2 phase above the solvent-rich phase. The CO_2 mole fraction in the solution can reach 50% or more. Once the gaseous antisolvent is dissolved into the organic solution, the solvent strength decreases significantly, precipitating the drug. If additional pharmaceutical excipients are present in the solution, the API will precipitate inside a matrix of the excipients. The precipitate is then flushed with fresh CO_2 to eliminate trace organic solvent (4–9).

A major challenge of the GAS precipitation process is the need to filter the precipitate from the organic solvent solution without particle growth and agglomeration. The rate of addition of the CO_2 to the organic solution influences the particle size. Both bimodal and unimodal particle size distributions have been formed in the same GAS-processed batch. If elevated temperatures are required to expand the organic solvent phase sufficiently, thermal degradation of the API can occur.

Precipitation with Compressed Liquid or Supercritical Fluid CO_2 as an Antisolvent: Semicontinuous Mode (PCA, ASES, SAS, SEDS)

In contrast to the GAS batch process, PCA is a semicontinuous technique. The two processes differ in terms of mass transfer pathways and atomization, as explained below. The PCA process is shown schematically in Fig. 1c. The organic feed solution containing the active ingredient is atomized into an excess flowing continuum of supercritical or liquid CO_2. The high surface area atomized droplets allow intimate contact with the excess antisolvent. In contrast to the unidirectional mass transfer of the CO_2 diffusion into the organic phase in the GAS process, API phase separation in PCA occurs by two-way mass transfer. The organic solvent diffuses into the CO_2 phase, and the CO_2 diffuses into the organic dispersed domains. Both rates are much faster than for conventional organic liquid antisolvents. The faster mass transfer rates result in more rapid nucleation of the API and smaller final particle sizes. The dry, micronized powder is available for collection following depressurization of the CO_2 (2,5,10).

Precipitation utilizing the PCA technique depends heavily on the efficiency of atomization of the organic solution into the supercritical antisolvent, with more intense atomization resulting in a higher surface area and more rapid two-direction mass transfer (2).

The Weber number N_{We} describes the degree of atomization of the feedstock solution into the supercritical antisolvent. The N_{We} is a dimensionless ratio of inertial forces to surface tension forces, given by

$$N_{We} = (\rho_A v^2 D)/\sigma$$

where ρ_A is the antisolvent density, v is the relative velocity, D is the droplet diameter, and σ is the

interfacial tension. The greater the value of the N_{We} for a given Reynolds number ($\rho vD/\mu$), the more intense the degree of atomization into smaller droplets (2,11). A detailed theoretical model of the spray process that describes mixing and precipitation has appeared recently (12).

A mass transfer pathway on a ternary phase diagram may be used to understand the separation into solute-rich and solute-lean phases. The phase behavior diagram of the PCA process is shown in Fig. 2. The solute and solvent are miscible, but CO_2 is only miscible with the solvent. For mass transfer pathways below the critical point (pathway AB), on the drug lean side of the phase diagram, drug-rich domains nucleate and grow in the metastable region after crossing the coexistence (binodal) curve. Often, more concentrated solutions result in larger particles. For pathways above the critical point (pathway EF), solvent voids are formed in a drug-rich phase.

Other names for the semicontinuous PCA process that have been utilized include aerosol solvent extraction system (ASES) (13,14), supercritical antisolvent (SAS) (15,16), and solution-enhanced dispersion by supercritical fluids (SEDS) (17–20). The diffusion coefficient of a particular organic solvent into compressed liquid or supercritical CO_2 is a function of temperature and density of the compressed antisolvent (21). As the density of CO_2 increases due to an increase in pressure, slower diffusion of the organic solvent can slow nucleation of the drug-rich phase and lead to larger particles.

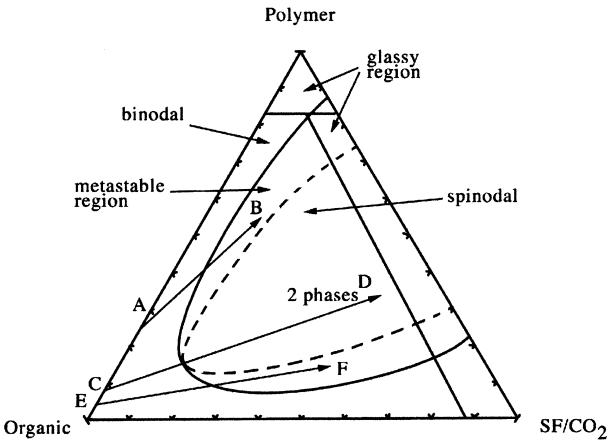

Figure 2. Phase solubility diagram showing different precipitation paths of the PCA process. (From Ref. 2 with permission.)

However, the atomization of the spray becomes more intense as this density increases, favoring faster nucleation and smaller particles. Both effects have been observed experimentally (22,23).

Figure 3 contains a drawing of a coaxial nozzle that is used in the PCA/SEDS precipitation technique. The organic solution is fed through one axis, and the prepressurized liquid or supercritical CO_2 is fed through the second axis. The two feed liquids meet in the mixing chamber prior to exiting the nozzle. The turbulence created in the mixing chamber by the collision of the high-speed CO_2 with the feedstock solution stream causes an intense atomization into primary feedstock microdroplets, followed by homogenization of the feedstock droplets and antisolvent (12). As a result, there is a highly efficient two-way mass transfer that results in rapid precipitation. The primary particle size of the powder can be manipulated by adjusting the relative velocity between the two streams (23). Examples of APIs that have been micronized using the PCA/SEDS precipitation process are salmeterol xinafoate (18,19), cromolyn sodium (20), and recombinant human immunoglobulin (24).

Although pharmaceutical protein and peptide powders have been precipitated using the GAS and PCA/SEDS/ASES/SAS processes (25), many organic solvents used to dissolve the API also denature proteins and peptides. Therefore, most of the PCA techniques may not lead to biologically active micronized protein powders. The PCA technique has been modified by Hanna and York to allow the dissolution of the pharmaceutical protein in an aqueous medium prior to spraying into CO_2 (26,27). Previous supercritical precipitation processes utilized only organic solvents due to the poor solubility of water in CO_2. In the modified PCA/SEDS process, a triaxial nozzle is used to introduce separately the aqueous solution containing the protein, the compressed CO_2, and an organic solvent, thus minimizing contact time between the protein and solvent. A schematic drawing of the triaxial nozzle is shown in Fig. 3.

The organic solvent dissolves the water from the aqueous protein solution. The water/organic solvent mixture then becomes miscible with the compressed antisolvent. Following depressurization of the system, the CO_2/organic solvent/water mixture evaporates, leaving the dry, micronized protein powder. This triaxial nozzle enables proteins to be micronized using a compressed antisolvent

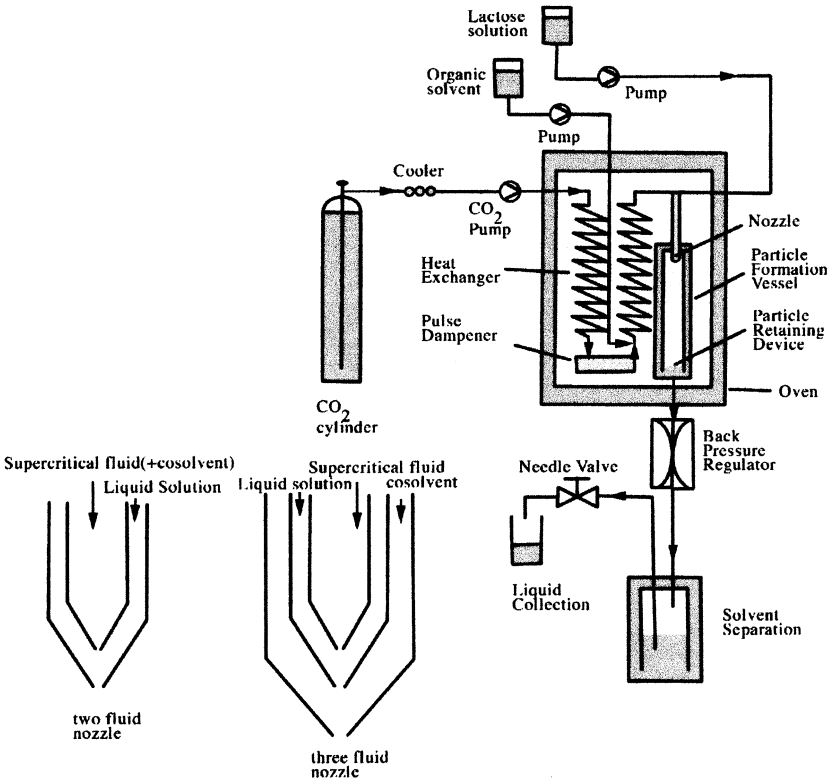

Figure 3. Schematic flow diagram of the SEDS process along with the cross section of the co- and triaxial fluid nozzles. (From Ref. 1 with permission.)

precipitation process while minimizing the extent of denaturation.

Lysozyme and trypsin are two proteins that have been successfully micronized using the modified PCA/SEDS process (27). Challenges of this process involve the low solubility of water in CO_2, the need for large quantities of organic solvent, optimization of the mixing of three flowing streams, and denaturation of the protein that can occur due to the exposure of the protein to the acidic CO_2 (pH 3) and the solvent-water mixture.

Young et al. used PCA to encapsulate the protein lysozyme, which was suspended in an organic solution containing poly (DL-lactide-*co*-glycolide) (PLGA) dissolved within dichloromethane (28). Following atomization of the suspension into excess CO_2, the PLGA precipitated onto the surface of the suspended lysozyme as the CO_2 expanded the dichloromethane. Because the protein was suspended and not dissolved in the organic solvent, denaturation may be expected to be much less severe.

Precipitation by Rapid Expansion from Supercritical Solutions

The RESS micronization technique is a precipitation process that has been used to produce powders from supercritical solutions of ceramics, polymers, and pharmaceutical steroids (5,29–31). In the RESS process, the solute is dissolved directly into the supercritical CO_2, and this solution is then atomized across a nozzle into a collection chamber, typically at atmospheric conditions. On expansion, the CO_2 rapidly evaporates, producing rapid nucleation of a fine micronized powder (32). Figure 1a illustrates a schematic representation of the RESS micronization process.

Parameters that can be modified to affect the resulting micronized powder particle size and morphology include pre- and postexpansion temperature and pressure, nozzle geometry, and solution concentration (5,29–31). Preexpansion temperature can be increased for high pressures to enable a

higher drug loading concentration in the supercritical solution prior to atomization. At a given temperature, the pressure of the supercritical CO_2 can be increased to raise the solvent density and API solubility. Postexpansion temperature can be increased to accelerate the evaporation rate of the supercritical CO_2 following atomization. The postexpansion pressure is typically at atmospheric conditions. Nozzle geometry can also be modified to achieve intense atomization. At a small nozzle length-to-diameter ratio, the pressure drop occurs closest to the free jet, resulting in micronized API particles.

The RESS micronization technique can be used to precipitate APIs alone, or it can be used to form a coprecipitate formulation of the drug embedded in a polymeric matrix. Tom et al. (15) found that an unacceptable coprecipitate was formed when using the RESS process to micronize lovastatin and DL-PLA (polylactic acid) due to the formation of needlelike crystals of drug protruding from the polymeric matrix instead of a molecularly homogeneous continuum. In another study, Kim et al. (33) discovered that higher preexpansion temperatures and pressures ($>114^{\circ}C$ and >190 bar, respectively) produced homogeneous naproxen/L-PLA microspheres following RESS processing, while lower system temperatures and preexpansion pressures allowed phase separation prior to precipitation. The requirement of elevated temperatures to produce homogeneous precipitates may enhance degradation of thermally labile drugs processed using the RESS technique. Another major disadvantage of the RESS process is the low solubility of most organic solids in supercritical CO_2 (1,34,35). Low drug loading into the supercritical CO_2 results in low production rates of powders.

A novel process, RESAS has been developed to reduce the coagulation rate in the free jet expansion of RESS (36). The supercritical solution is expanded through an orifice or tapered nozzle into an aqueous solution containing a stabilizer to minimize particle aggregation during free jet expansion. Young et al. (36) demonstrated the ability for Tween 80, a nonionic polysorbitan ester, to stabilize 400–700-nm cyclosporine particles produced by RESAS. If cyclosporine was sprayed directly into air without the aqueous surfactant solution, the particle size was several micrometers, which is typically observed in RESS.

Precipitation from Gas-Saturated Solutions

Precipitation from gas-saturated solutions is a supercritical micronization procedure that involves expansion through a nozzle, as in RESS. However, this precipitation process is used mainly for the micronization of polymeric powders. While relatively few organic compounds are soluble in CO_2, a number of polymers may be swollen with CO_2. Supercritical CO_2 is dispersed into a molten compound to create a gas-liquid suspension. The molten suspension is then atomized through a nozzle into a collection vessel at atmospheric conditions. The CO_2 evaporates, consequently cooling and solidifying the molten material into a porous powder (37). Nifedipine in combination with polyethylene glycol 4000 has been processed by the gas-saturated precipitation technique (38). Nifedipine was chosen as the model drug due to its relatively high solubility in supercritical CO_2 compared to other organic solids (34). Due to its high solubility, a more homogeneous dispersion could be produced. However, nifedipine is susceptible to thermal degradation at high temperatures (39), so the pressurized system must be kept at a sufficient temperature to achieve a homogeneous dispersion while minimizing API degradation.

The PIPS technique is similar to the precipitation from a gas-saturated solution process and almost identical to the RESS procedure. An API alone, or in combination with a polymer, is dissolved in supercritical CO_2, followed by atomization through a nozzle at atmospheric conditions (40). Following rapid nucleation and evaporation of the CO_2, a porous micronized powder consisting of the drug alone or embedded within a continuous polymeric network is produced (40). As with the RESS and gas-saturated precipitation processes, the applicability of the PIPS process can be limited by the low solubility of APIs and polymer excipients in supercritical CO_2.

CRYOGENIC SPRAY PROCESSES

Atmospheric Spray–Freeze-Drying

A relatively new cryogenic spray–freeze-drying process has been developed to produce a flowable micronized powder from an aqueous or cosolvent solution or suspension of an API and, if applicable, pharmaceutical excipients. This method involves

spraying the feedstock into a fluidized bed containing dry ice and predried, cryogenic air (41,42). Figure 4 illustrates the spray–freeze-drying apparatus developed by Mumenthaler and Leuenberger (41). The cryogenic air sublimes the frozen solvent(s) and carries the vapor into a recycling chamber, where it is condensed onto a solid refrigerant. The recycled air then reenters the cryogenic chamber for further sublimation. The frozen powder is fluidized to prevent coalescence of the individual particles during the drying step. Agitation of the powder also expedites the sublimation process, consequently reducing drying times to below those required for conventional lyophilization.

Mumenthaler and Leuenberger successfully spray–freeze-dried a number of APIs and pharmaceutical excipients, including mannitol, glycine, urea, glucose, $\alpha_1$1-bovine interferon, cyclosporin A, and S-adenosyl-L-methionine. In this study, cryogenic airflow rate, fluid bed temperature, and solids content of the spray-frozen solutions were manipulated to alter the sublimation rate of the frozen solvent. Increasing the cryogenic airflow rate while maintaining a constant fluid bed temperature at $-30^{\circ}C$ reduced the drying time to as low as 2 h, while increasing the temperature of the air to $-2^{\circ}C$ shortened the drying time to about 70 min. Increasing the solids content of the feed solutions to as high as 25% also accelerated sublimation rates. The interferon peptide activity was not affected following the spray–freeze-drying process; however, the S-adenosyl-L-methionine enzyme was denatured during the procedure. The researchers concluded that the protein denaturation occurred due to ice crystal growth and suggested that a sufficiently cold fluid bed temperature was needed to prevent eutectic coalescence and, consequently, ice crystallization.

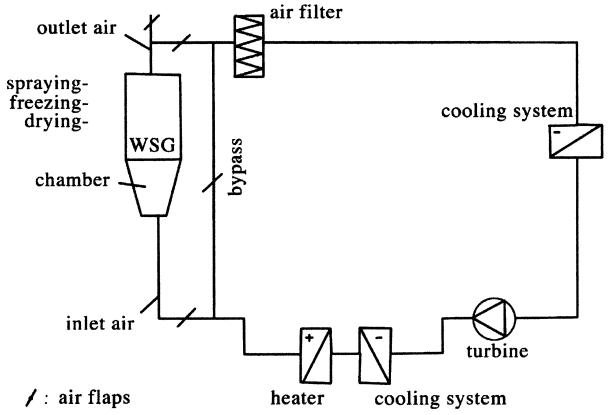

Figure 4. Schematic flow diagram of the atmospheric spray–freeze-drying apparatus. (From Ref. 41 with permission.)

Spray-Freezing into a Halocarbon Refrigerant Vapor

Proteins can be easily denatured during freezing due to phase separation of water and its soluble components, followed by ice crystal growth. Ultrarapid freezing of a solution or suspension containing a protein or peptide can prevent phase separation, thus minimizing ice crystal growth. As a result, the protein retains its activity following sublimation of the ice. One method of attaining ultrarapid freezing of a feedstock solution or suspension is to atomize the feedstock into the vapor phase above a boiling chlorofluorocarbon (CFC) or fluorocarbon refrigerant (43–47), as shown in Fig. 5. At atmospheric pressure, the refrigerants exist as liquids at temperatures on the order of $-20^{\circ}C$ to $-25^{\circ}C$. The atomized droplets fall into the refrigerant and are immediately frozen on contact with the cryogen. The frozen powder is then collected and lyophilized to remove the solvent.

Briggs and Maxwell developed a spray-freezing technique in which a feedstock solution or suspension is atomized through a nozzle situated 10–25 cm above the surface of a CFC refrigerant. The nozzle is at a sufficient height above the refrigerant to allow droplet formation prior to contact with the cryogenic medium (43–45). Briggs and Maxwell

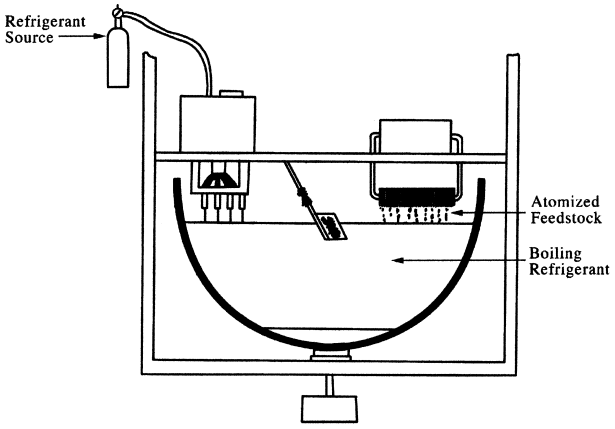

Figure 5. Schematic flow diagram showing atomization of an API feedstock solution or suspension onto a boiling halocarbon cryogen. (From Ref. 46 with permission.)

claim faster freezing rates by spray-freezing into the vapor phase above liquid halocarbons as compared to spraying into the vapor phase above liquid nitrogen. A vapor pocket is formed between the surface of the droplet and the cryogenic liquid on contact. Due to the greater temperature difference between the feedstock droplet and liquid nitrogen, which at atmospheric pressure exists in the liquid form at –194°C, a large vapor pocket is formed at the droplet/liquid nitrogen interface. At the droplet/halocarbon interface, the vapor pocket is smaller due to the decreased temperature difference between the feedstock and the cryogen. Briggs and Maxwell claim that the larger gas pocket associated with liquid nitrogen results in slower freezing rates compared to the freezing rates achieved using the CFC, Freon 12.

Several disadvantages are associated with using a CFC as the cryogenic refrigerant. In fact, the use of CFCs has been banned due to the deleterious effects of these compounds on the ozone layer (48). The relatively new ozone-friendly hydrofluoroalkane (HFA) refrigerants are expensive alternatives to the CFCs. However, both HFA 134a and HFA 227 are good solvents for a number of drugs, including the steroids and danazol (49). Thus, if an HFA refrigerant were used as the cryogenic medium, the HFA could solubilize the API and consequently decrease the potency of the powder formulation.

Adams et al. (46,47) developed a procedure similar to the technique of Briggs and Maxwell; atomization of the feed solution or suspension prior to contact with the halocarbon refrigerant is avoided. Instead, the solution is fed in a continuous stream onto the surface of the CFC/HFA refrigerant from an injection orifice 1–5 cm above the surface of the halocarbon. While particles produced by the Briggs and Maxwell procedure have diameters in the micrometer range, the continuous stream spray-freezing technique produces much larger particles, optimally between 0.84 and 1.68 mm. This procedure is not as useful in the pharmaceutical industry since relatively large particles are produced.

Spray-Freezing into a Halocarbon Refrigerant

In contrast to the methods discussed above, spray-freezing into a halocarbon refrigerant involves the atomization of a feed solution from a nozzle submerged beneath the surface of the CFC/HFA. Microdroplets are produced when the solution is atomized through a nozzle submerged beneath the surface of a halocarbon refrigerant compared to when the feedstock is atomized above the surface of the cryogen (50–52). Liquid-liquid collision between the feedstock and refrigerant results in an intense atomization that produces microdroplets, which have a much higher surface area than larger droplets atomized above the surface of the cryogen. Although the microdroplets with high surface area do not freeze immediately on atomization into the halocarbon refrigerant, the microparticles freeze much faster than larger droplets produced by atomization into halocarbon vapor. Following lyophilization, the micronized powders produced using this method consist of small, discreet microparticles.

Dunn et al. (50) developed a procedure by which the feedstock is atomized directly into a halocarbon refrigerant kept at a temperature slightly above the freezing point of the feedstock. This refrigerant serves to atomize the feed solution into microdroplets. A second, less-dense halocarbon refrigerant, which is immiscible with the refrigerant containing the atomized feedstock, serves as the cryogenic medium to freeze the atomized droplets as they cross the immiscible interface from the denser refrigerant into the less-dense refrigerant. Droplet solidification occurs because the less-dense refrigerant is kept at a temperature beneath the freezing point of the feedstock. The frozen microparticles float to the surface of the less-dense refrigerant and are collected and lyophilized.

In Fig. 6, a cross section of the refrigerant holding chamber and submerged nozzle are shown. This is a complex procedure because it is difficult to use two immiscible ozone-friendly refrigerants—one existing as a liquid above the freezing point of a particular feedstock and the other existing in liquid form below the freezing point of the feedstock and with neither as a solvent for the pharmaceutical compound being processed.

Buxton and Peach (51) developed another technique for spray-freezing into halocarbon similar to the representation in Fig. 6; the feedstock is atomized through a nozzle submerged into a single liquid refrigerant held at a temperature beneath the freezing point of the feedstock. The nozzle is heated to prevent the feedstock from freezing prior to atomization. As the feedstock is atomized, the droplets do not freeze immediately. Instead, they gradually freeze as they float to the surface of the refrigerant. Buxton and Peach chose trichloroethane,

trichloroethylene, dichloromethane, diethyl ether, and trichloromonofluoromethane as suitable refrigerants. However, these organic liquids are excellent solvents for many drugs, especially lipophilic compounds, and could extract the API from the feedstock prior to droplet freezing.

Spray-Freezing onto Liquid Nitrogen

To alleviate drug loss due to solvent extraction by halocarbon refrigerants, an inert cryogen with poor solvent capacity must be used. Lilakos (53) has developed a spray-freezing apparatus using liquid nitrogen as the cryogen. This process is used for the crystallization of preheated molten fats. The cryogen and molten fat liquids are fed separately into the cryogenic chamber via independent streams. Intense atomization of the fat stream as a result of the liquid–liquid impingement with the cryogen produces tiny fat droplets that solidify immediately following nitrogen evaporation, which occurs on contact with the molten fat. The solidified fat powder then falls to the bottom of the cryogenic vessel for collection. Since no solvents are used in this process, lyophilization is not required following powder solidification. However, this process cannot be used to micronize most APIs due to drug degradation that would occur during the melting of the feedstock.

Hebert and Healy (54) and Gombotz et al. (55) have developed two similar organic feedstock spray-freezing processes. The organic feedstock droplets pass first through gaseous nitrogen, then impact onto liquid nitrogen, followed by extraction of the frozen organic solvent by an antisolvent for the API. The organic feedstock is atomized into gaseous nitrogen as shown in Fig. 7. As the partially frozen droplets make contact with the liquid nitrogen, the particles are completely frozen. These frozen particles are then carried by the liquid nitrogen current into a vessel, in which the nitrogen evaporates, and the frozen particles are submerged in an organic antisolvent. As listed in Table 1, the antisolvent for the API extracts the organic solvent from the frozen droplets. The powder can then be removed from the antisolvent and dried. A significant disadvantage of

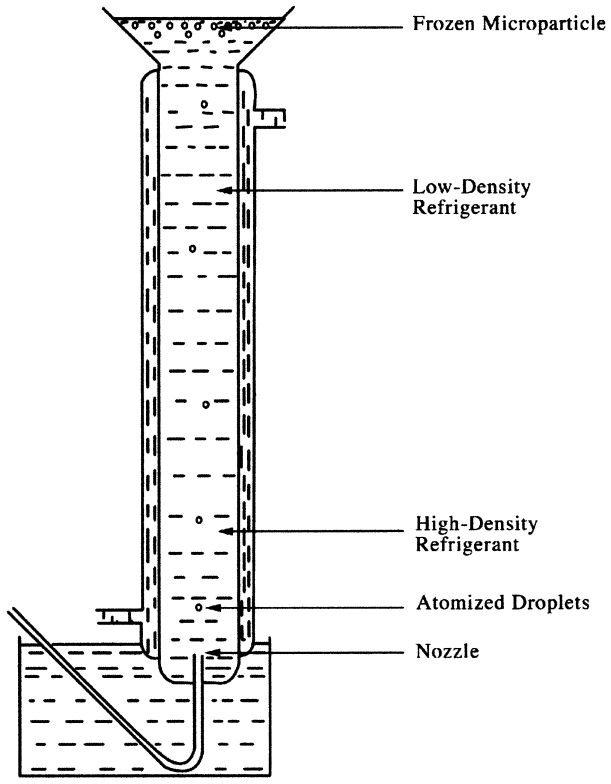

Figure 6. Schematic flow diagram showing atomization of an API feedstock solution or suspension beneath the surface of two immiscible halocarbon cryogens. (From Ref. 51 with permission.)

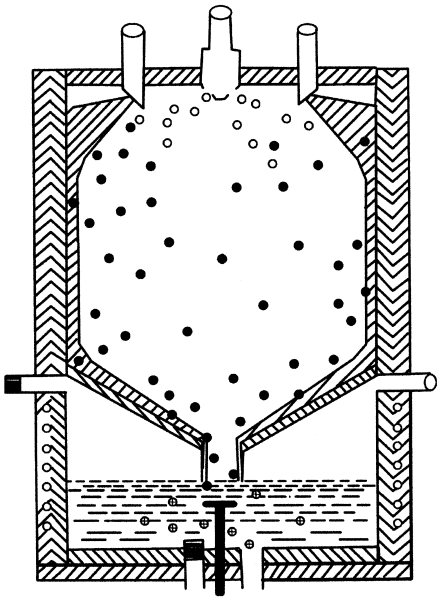

Figure 7. Schematic flow diagram showing spray-freezing of a feedstock onto flowing liquid nitrogen, followed by extraction of the organic solvent with an antisolvent. (From Ref. 54 with permission.)

Table 1

Comparison of the Micronization Techniques Using Either Compressed Fluids or Cryogenic Liquids

Process	API Load	Protein Denaturation/ Drug Degradation	Temperature Range (°C)	Organic Solvent Required	Pressurized System	Compressed Fluid as Solvent	Compressed Fluid as Antisolvent
GAS	Low	Yes	45 to 80	Yes	Yes	No	Yes
PCA/SAS/ASES/SEDS	Low	Yes	45 to 80	Yes	Yes	No	Yes
RESS/PIPS	Low	Yes	≥ 100	No	Yes	Yes	No
RESAS	Low	Yes	45–80	No	Yes	Yes	No
Spray-freezing into halocarbon refrigerant vapor	Low	No	−20 to −25	No	Yes	No	Yes
Spray-freezing into halocarbon refrigerant liquid	Low	No	−20 to −25	No	Yes	No	No
Spray-freezing into liquid nitrogen	High	No	−194	Yes (if antisolvent extraction is utilized)	No	No	No

ASES, aerosol solvent extraction system; GAS, gas antisolvent; PCA, precipitation with a compressed antisolvent; PIPS, pressure-induced phase separation; RESAS, rapid expansion from supercritical to aqueous solution; RESS, rapid expansion from supercritical solution; SEDS, solution-enhanced dispersion by supercritical fluids.

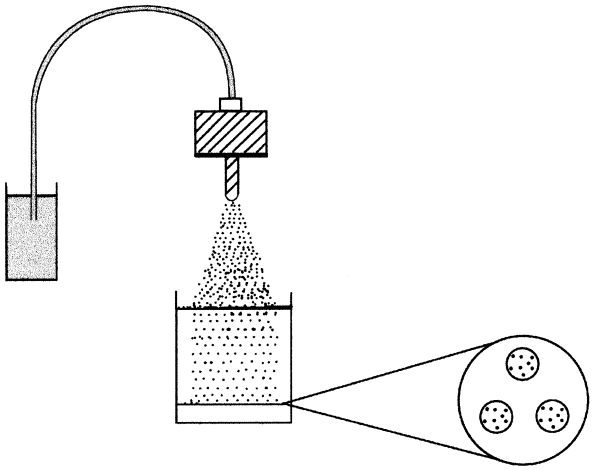

Figure 8. Schematic flow diagram showing spray-freezing of a feedstock solution or suspension onto stagnant liquid nitrogen. (From Ref. 55 with permission.)

this process is that complete removal of the trace organic antisolvent from the powder can be difficult. Another challenge of the antisolvent extraction step is that the API, along with the organic solvent, may also be extracted by the antisolvent; thus, the actual potency of the API in the dry powder may be significantly lower than the theoretical potency. Proteins and peptides can be difficult to process using these cryogenic spray-extraction techniques because these compounds can be denatured in the presence of the organic solvents.

Two procedures involving spray-freezing of a feedstock solution or suspension onto static liquid nitrogen have been developed by Gombotz et al. (56) and Gusman and Johnson (57). The frozen powder is collected and lyophilized to produce the dry, micronized powder. The setup of the spray-freezing apparatus is similar to that of the cryogenic spray-extraction processes involving liquid nitrogen; however, the liquid nitrogen is not circulated or agitated, and the frozen feedstock is trapped in the cryogenic chamber for collection, as shown in Fig. 8. Thus, product yields are extremely high with the static cryogenic spray-freezing techniques.

CONCLUSIONS

Several useful micronization techniques involving compressed and cryogenic gases that can generate dry powders comprised of either API alone or API in combination with pharmaceutical excipients (bulking agents, stability and absorption enhancers, etc.) were discussed. Compressed fluid micronization techniques are advantageous because CO_2 is an

environmentally safe antisolvent compared to the conventional organic antisolvents. However, low product yields and the chance for thermal degradation of APIs limit most of the supercritical micronization processes. In contrast, the cryogenic micronization processes produce high product yields, but require either lyophilization or antisolvent extraction to produce dry powder. Ultrarapid spray-freezing techniques are more applicable in protein and peptide micronization compared to most supercritical precipitation processes since aqueous-based systems can be used.

Ultimately, the physicochemical properties of the API and excipients being processed determine which micronization technique will produce the optimal pharmaceutical dry powder formulation.

ACKNOWLEDGMENT

True Rogers is supported by an American Foundation for Pharmaceutical Education (AFPE) Fellowship for the 2000–2001 fiscal year. We gratefully acknowledge Belinda Gonzalez-Lehmkuhle and Jason M. Vaughn for their assistance with the figures for this article.

REFERENCES

1. Palakodaty, S.; York, P. Phase Behavioral Effects on Particle Formation Processes Using Supercritical Fluids. Pharm. Res. **1999**, *16*, 976–985.
2. Dixon, D.J.; Johnston, J.P.; Bodmeier, R.A. Polymeric Materials Formed by Precipitation with a Compressed Fluid Antisolvent. AIChE J. **1993**, *39*, 127–139.
3. Reverchon, E. Supercritical Antisolvent Precipitation of Micro- and Nano-particles. J. Supercrit. Fluids **1999**, *15*, 1–21.
4. McHugh, M.A.; Krukonis, V.J. *Supercritical Fluid Extraction Principles and Practice,* 2nd Ed.; Butterworth-Heinemann: Woburn, MA, 1994.
5. Subramaniam, B.; Rajewski, R.A.; Snavely, K. Pharmaceutical Processing with Supercritical Carbon Dioxide. J. Pharm. Sci. **1997**, *86*, 885–890.
6. Kikic, I.; Sist, P. Applications of Supercritical Fluids to Pharmaceuticals: Controlled Drug Release Systems. *Second NATO ASI on Supercritical Fluids*, Kemer, Turkey, 1998.
7. Reverchon, E. Supercritical Antisolvent Precipitation: Its Application to Microparticle Generation and Products Fractionation. In *Proceedings of the Fifth Meeting on Supercritical Fluids. Materials and Natural Products Processing*; Nice, France, 1998; Vol. 1, 221–236.
8. Benedetti, L.; Bertucco, A.; Pallado, P. Production of Micronic Particles of Biocompatible Polymer Using Supercritical Carbon Dioxide. Biotechnol. Bioeng. **1997**, *53*, 232–237.
9. Bertucco, A.; Pallado, P.; Benedetti, L. Formation of Biocompatible Polymer Microspheres for Controlled Drug Delivery by a Supercritical Antisolvent Technique. In *Process Technology Proceedings 12, High Pressure Chemical Engineering*; Rohr, P.R.V., Trepp, C., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; 217–222.
10. Bodmeier, R.; Wang, J.; Dixon, D.J.; Mawson, S.; Johnston, K.P. Polymeric Microspheres Prepared by Spraying into Compressed Carbon Dioxide. Pharm. Res. **1995**, *12*, 1211–1217.
11. Sarkari, M.; Darrat, I.; Knutson, B.L. Generation of Microparticles Using CO_2 and CO_2-Philic Antisolvents. AIChE J. **2000**, *46*, 1850–1859.
12. Lengsfeld, C.S.; Delplanque, J.P.; Barocas, V.H.; Randolph, T.W. Mechanism Governing Microparticle Morphology During Precipitation by a Compressed Antisolvent: Atomization Versus Nucleation and Growth. J. Phys. Chem. B. **2000**, *104*, 2725–2735.
13. Engwicht, A.; Girreser, U.; Muller, B.W. Critical Properties of Lactide-*co*-glycolide Polymers for the Use in Microparticle Preparation by the Aerosol Solvent Extraction System. Int. J. Pharm. **1999**, *185*, 61–72.
14. Bleich, J.; Muller, B.W. Production of Drug Loaded Microparticles by the Use of Supercritical Gases with the Aerosol Solvent Extraction System (ASES) Process. J. Microencapsulation **1996**, *13*, 131–139.
15. Tom, J.W.; Lim, G.; Debenedetti, P.G.; Prud'homme, R.K. Applications of Supercritical Fluids in the Controlled Release of Drugs. ACS Symp. Ser. **1993**, *514*, 238–257.
16. Yeo, S.D.; Lim, G.; Debenedetti, P.G.; Bernstein, H. Formation of Microparticulate Protein Powders Using a Supercritical Fluid Antisolvent. Biotechnol. Bioeng. **1993**, *41*, 341–346.
17. Hanna, M.; York, P. Method and Apparatus for the Formation of Particles. Patent PCT/GB94/01426, 1994.
18. Hanna, M.; York, P. Particle Engineering by Supercritical Fluid Technologies for Powder Inhalation Drug Delivery. In *Proceedings of Respiratory Drug Delivery V*; Phoenix, AZ, 1996; 231–239.
19. Hanna, M.; York, P.; Shekunov, B.Y. Control of the Polymorphic Forms of a Drug Substance by Solution Enhanced Dispersion by Supercritical Fluids (SEDS). In *Proceedings of the Fifth Meeting on Supercritical Fluids. Materials and Natural Products Processing*; Nice, France, 1998; Vol. 1, 325–330.

20. Jaarmo, S.; Rantakyla, M.; Aaltonen, O. Particle Tailoring with Supercritical Fluids: Production of Amorphous Pharmaceutical Particles. In *Proceedings of the Fourth International Symposium on Supercritical Fluids*; Sendai, Japan, 1997; 263–266.
21. Sassiat, P.R.; Mourier, P.; Caude, M.H.; Rosset, R.H. Measurement of Diffusion Coefficients in Supercritical Carbon Dioxide and Correlation with the Equation of Wilke and Chang. Anal. Chem. **1987**, *59*, 1164–1170.
22. Randolph, T.W.; Randolph, A.D.; Mebes, M.; Yeung, S. Submicrometer Sized Biodegradable Particles of Poly (lactic Acid) via the Gas Antisolvent Spray Precipitation Process. Biotechnol. Prog. **1993**, *9*, 429–435.
23. Mawson, S.; Kanakia, S.; Johnston, K.P. Coaxial Nozzle for Control of Particle Morphology in Precipitation with a Compressed Fluid Antisolvent. J. Appl. Polym. Sci. **1997**, *64*, 2105–2118.
24. Nesta, D.P.; Elliott, J.S.; Warr, J.P. Supercritical Fluid Precipitation of Recombinant Human Immunoglobulin from Aqueous Solutions. Biotechnol. Bioeng. **1999**, *67*, 457–464.
25. Winters, M.A.; Knutson, B.L.; Debenedetti, P.G.; Sparks, H.G.; Przybycien, T.U.; Stevenson, C.L.; Prestrelski, S.J. Precipitation of Proteins in Supercritical Carbon Dioxide. J. Pharm. Sci. **1996**, *85*, 586–594.
26. Hanna, M.; York, P. Method and Apparatus for the Formation of Particles., Patent No. PCT/GB95/01523, 1995.
27. Sloan, R.; Hollowood, M.E.; Ashraf, W.; York, P.; Humphreys, G.O. Supercritical Fluid Processing: Preparation of Stable Protein Particles. In *Proceedings of the Fifth Meeting on Supercritical Fluids. Materials and Natural Products Processing*; Nice, France, 1998; Vol. 1, 301–306.
28. Young, T.J.; Johnston, K.P.; Mishima, K.; Tanaka, H. Encapsulation of Lysozyme in a Biodegradable Polymer by Precipitation with a Vapor-over-Liquid Antisolvent. J. Pharm. Sci. **1999**, *88*, 640–650.
29. Tom, J.W.; Debenedetti, P.G. Particle Formation with Supercritical Fluids—A Review. J. Aerosol. Sci. **1991**, *22*, 555–584.
30. Philips, E.M.; Stella, V.J. Rapid Expansion from Supercritical Solutions: Application to Pharmaceutical Processes. Int. J. Pharm. **1993**, *94*, 1–10.
31. Knutson, B.L.; Debenedetti, P.G.; Tom, J.W. *Microparticulate Systems for the Delivery of Proteins and Vaccines*; Marcel Dekker, Inc.: New York, **1996**, *77*, 89–125.
32. Matson, D.W.; Peterson, R.C.; Smith, R.D. Production of Fine Powders by the Rapid Expansion of Supercritical Fluid Solutions. Adv. Ceramics **1987**, *21*, 109.
33. Kim, J.H.; Paxton, T.E.; Tomasko, D.L. Microencapsulation of Naproxen Using Rapid Expansion of Supercritical Solutions. Biotechnol. Prog. **1996**, *12*, 650–661.
34. Knez, Z.; Skerget, M.; Sencar-Bozic, P.; Rizner, A. Solubility of Nifedipine and Nitrendipine in Supercritical CO_2. J. Chem. Eng. Data **1995**, *40*, 216–220.
35. Ting, S.S.T.; MacNaughton, S.J.; Tomasko, D.L.; Foster, N.R. Solubility of Naproxen in Supercritical Carbon Dioxide With and Without Cosolvents. Ind. Eng. Chem. Res. **1993**, *32*, 1471–1481.
36. Young, T.J.; Mawson, S.; Johnston, K.P.; Henriksen, I.B.; Pace, G.W.; Mishra, A.K. Rapid Expansion from Supercritical to Aqueous Solution to Produce Submicron Suspensions of Water-Insoluble Drugs. Biotechnol. Prog. **2000**, *16*, 402–407.
37. Weidner, E.; Steiner, R.; Knez, Z. Powder Generation from Polyethylene Glycols with Compressible Fluids. In *Process Technology Proceedings 12, High Pressure Chemical Engineering*; Rohr, R.R.V., Trepp, C., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; 223–228.
38. Srcic, S.; Sencar-Bozic, P.; Knez, Z.; Kerc, J. Improvement of Nifedipine Dissolution Characteristics Using Supercritical CO_2. In *Proceedings of the 16th Pharmaceutical Technology Conference and Exhibition*; Athens, Greece, 1997; 59–69.
39. Florey, K.; Brewer, G.A. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1987.
40. Kiran, E.; Zhuang, Z. Miscibility and Phase Separation of Polymers in Near- and Supercritical Fluids. ACS Symp. Ser. **1997**, *670*, 2–36.
41. Mumenthaler, M.; Leuenberger, H. Atmospheric Spray-Freeze Drying: A Suitable Alternative in Freeze-Drying Technology. Int. J. Pharm. **1991**, *72*, 97–110.
42. Oyler, J.R. Liquid Substances Freeze-Drying Systems and Methods. U.S. Patent 5208998, 1993.
43. Briggs, A.R.; Maxwell, T.J. Process for Preparing Powder Blends. U.S. Patent 3721725, 1973.
44. Briggs, A.R.; Maxwell, T.J. Lyophilized Biological Products. U.S. Patent 3928566, 1975.
45. Briggs, A.R.; Maxwell, T.J. Method of Preparation of Lyophilized Biological Products. U.S. Patent 3932943, 1976.
46. Adams, T.H.; Beck, J.P.; Menson, R.C. Method and Apparatus for Making Novel Particulate Compositions. U.S. Patent 4211015, 1980.
47. Adams, T.H.; Beck, J.P.; Menson, R.C. Novel Particulate Compositions. U.S. Patent 4323478, 1982.
48. *The Montreal Protocol on Substances that Deplete the Ozone Layer*; 1987.

49. Williams, R.O.; Rogers, T.L.; Liu, J. Study of Solubility of Steroids in Hydrofluoroalkane Propellants. Drug Dev. Ind. Pharm. **1999**, *25*, 1227–1234.
50. Dunn, D.B.; Masavage, G.J.; Sauer, H.A. Method of Freezing Solution Droplets and the Like Using Immiscible Refrigerants of Differing Densities. U.S. Patent 3653222, 1972.
51. Buxton, I.R.; Peach, J.M. Process and Apparatus for Freezing a Liquid Medium. U.S. Patent 4470202, 1984.
52. Sauer, H.A. Method and Apparatus for Freeze-Freeze Drying. U.S. Patent 3484946, 1969.
53. Lilakos, L. Method and Apparatus for Cryogenic Crystallization of Fats. U.S. Patent 4952224, 1990.
54. Hebert, P.F.; Healy, M.S. Production Scale Method of Forming Microparticles. U.S. Patent 5922253, 1999.
55. Gombotz, W.R.; Healy, H.S.; Brown, L.R. Very Low Temperature Casting of Controlled Release Microspheres. U.S. Patent 5019400, 1991.
56. Gombotz, W.R.; Healy, M.S.; Brown, L.R.; Auer, H.E. Process for Producing Small Particles of Biologically Active Molecules. Patent WO90/13285, 1990.
57. Gusman, M.I.; Johnson, S.M. Cryochemical Method of Preparing Ultrafine Particles of High-Purity Superconducting Oxides. U.S. Patent 4975415, 1990.