Protein Particle Formation for Pulmonary Delivery

A THESIS

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Abstract

Recently, there has been an increased focus on inhalation therapies in drug delivery research. It has been proven that many medications and vaccines can be inhaled in particle form, which has certain advantages. Respiratory diseases such as asthma and emphysema can be more directly treated by administering medications to the lungs. Even for non-respiratory conditions, the large internal surface area of the lungs provides a very effective means for entering the bloodstream.

Particle processing of pharmaceuticals is commonly done in batches with toxic organic solvents; a large portion of drug processing costs come from multiple solvent separation steps. Another problem with batch processes is that it is difficult to achieve consistent particle size and distribution. To overcome these issues, many innovative particle engineering methods have been developed using supercritical fluids as the solvents or anti-solvents. One such process, ASES (Aerosol Solvent Extraction System), has proven to yield particles of the ideal size to administer by inhalation (1-5 microns) and uniform distribution necessary for reliable dosage. Also, a supercritical fluid such as CO_2, which is gaseous at room temperature, completely separates upon returning to ambient conditions. This process has great potential to increase yield, increase throughput, and decrease processing costs. This purpose of this study is to find trends for development of mathematical models and to show potential for realistically scaling up for industrial production.

Key processing variables include drug solution flow rate, antisolvent flow rate, temperature, and pressure. Preliminary experiments explored the effects of these variables on particle morphology using Bovine Serum Albumin (BSA) as a less-expensive model system. It was found that increasing the system pressure decreases the size of the primary particles, but increases agglomeration due to frequency of particle collisions. Increasing the system temperature also decreases the particle size, which indicates the need for a balance between achieving high density and high viscosity in the antisolvent. For this system, solution and antisolvent flow rates appear to have the most pronounced effect on the resulting particles. This would indicate that turbulence and other mass transfer effects are the most important. Furthering studies with BSA on a larger scale will help to understand the effects of scale on the important processing variables.

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1. Introduction

1.1 Motivation

a. Pulmonary Administration

For thousands of years, inhalation has been used as a means for treating lung conditions. As recently as the 1990s, nebulizer and aerosol technologies have made substantial progress for systemic therapeutic application[1]. This can be largely attributed to the allure of developing new, non-invasive techniques for administering medications and vaccines. The large internal surface area of the lungs allows for effective uptake into the bloodstream that rivals the speed of injection; this makes pulmonary administration the most promising of the currently examined non-invasive delivery modes, including nasal, oral, and dermal[2]. In order to be inhaled, the drug must be in particulate form. The nominal particle size is 1-5 microns, which is a narrow range for effective absorption through the interior of the lung. Particles that are smaller tend to be exhaled before reaching the lung, and larger particles tend to become lodged in the natural mucous linings of the respiratory system[3]. As is evident, a narrow particle size distribution is vital for accurate dosaging.

b. Commercialization

Conventional particle production techniques are unsuitable for such fine powdered pharmaceuticals. Mechanical methods such as grinding and milling have large size variability, and can potentially denature the drug. Chemical techniques, such as recrystallization, require excessive solvent use[4]; along with solvent/evaporation techniques, the final product requires expensive separation steps, or possibly several days of drying[5]. Spray drying may require temperatures that thermally denature biological materials. In order to industrially produce pharmaceutical powders for inhalation, processes with gentler operating conditions, reduced solvent use, and more reliable particle distribution are required.

1.2 Supercritical Fluids (SCF) Processes

a. SCFs

A supercritical fluid is a continuous fluid phase that exists above a substance's critical temperature and pressure. At the critical point, illustrated in Figure 1-1, the gaseous phase and liquid phase are no longer distinguishable, and the fluid exhibits intermediate properties.

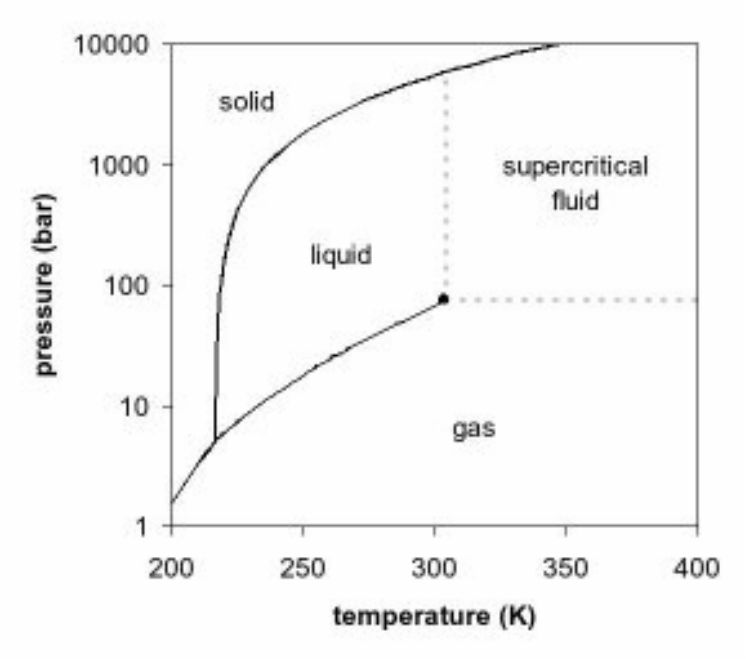


Figure 1-1. Carbon Dioxide Phase Diagram[6]

A SCF has low gas-like viscosity and high liquid-like density; this enables high diffusion rates and solvent properties, respectively. The density of a SCF also fluctuates significantly with temperature and pressure, as shown in Figure 1-2, which allows for varying the solvation power of the fluid[7]. These characteristics have been utilized in the development of many supercritical fluid particle formation processes. These processes circumvent many of the issues related to conventional particle formation methods.

A particularly common SCF for processing pharmaceuticals is carbon dioxide (SC-CO_2). The critical temperature is 31.1C, and for biological molecules, such as protein medications, low processing temperature is favorable for stability.

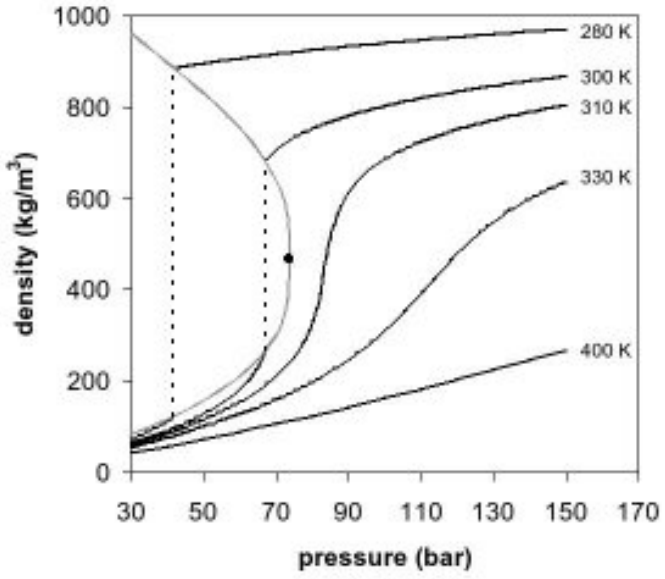


Figure 1-2. Carbon Dioxide Density Diagram[8]

In general, carbon dioxide is also non-toxic and does not require separation steps like an organic solvent; many pharmaceuticals are insoluble in SC-CO_2, so the CO_2 completely separates when the system is returned to ambient conditions. Typically protein medications are readily soluble in water, so aqueous solutions are used. The drawback is that CO_2 and water are relatively immiscible, so an organic solvent or co-antisolvent must be used. A few of the popular SCFs processes for making pharmaceutical particles, specifically with SC-CO_2, are subsequently described.

b. Rapid Expansion of Supercritical Solutions (RESS)

The RESS process is suitable for substances that are soluble in SC-CO_2; in this way, it is similar to a conventional recrystallization technique, only with the advantage of utilizing

temperature and pressure as a means to achieve supersaturation. Also, since SC-CO_2 is used as a solvent, no organic solvents are required. A schematic of the process is shown in Figure 1-3.

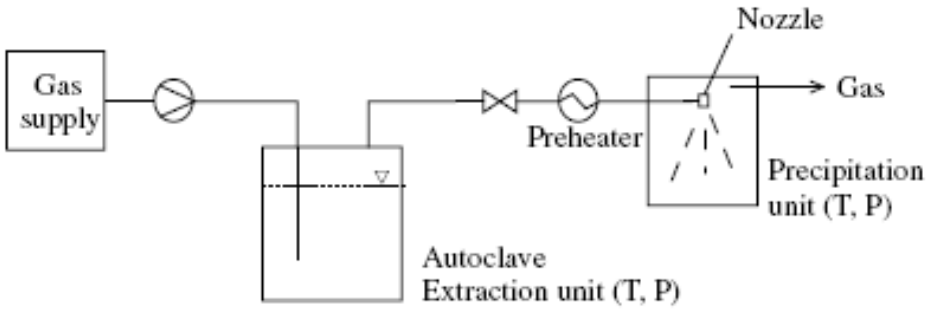


Figure 1-3. RESS Process Schematic[7]

The drug of interest is solubilized in SC-CO_2 in an autoclave; the temperature and pressure are chosen to have the maximum solubility without the formation of a liquid phase. This saturated solution is decompressed through a nozzle, such that the SC-CO_2 expands very rapidly, decreasing in density and solvent ability. Different types of nozzles can be used to change the morphology of the resulting particles[7]. This process has been studied extensively and successfully, and it is the most physically understood process of the ones mentioned here. Many mathematical models have been proposed for different physically realizable situations, such as one-dimensional solvent expansion and free jet expansion[9]. The disadvantages of this process include the large volumes of gas required due to the fact that even drugs soluble in SC-CO_2 are not soluble in large amounts. Also, the rapid depressurization of the solution through the nozzle poses a risk of freezing, as well as particle accumulation. The preheater used before the nozzle to reduce this freezing risk also has the potential of heating the solution to the crystallization density, which also is of concern.

c. Gas Antisolvent (GAS) Recrystallization

In the GAS process, SC-CO_2 is used as an antisolvent; as previously mentioned, most pharmaceutical compounds are insoluble in CO_2, so there are more powders that can be processed with this method. The drug is first dissolved in an organic solvent that is miscible with SC-CO_2 at the process temperature and pressure in a precipitation vessel. SC-CO_2 is then pumped into the bottom of the vessel, which dissolves into the solution. Because of the insolubility of the drug in SC-CO_2, particles precipitate from the expanding solution. This process has high throughput. A schematic of the GAS process is shown in Figure 1-4.

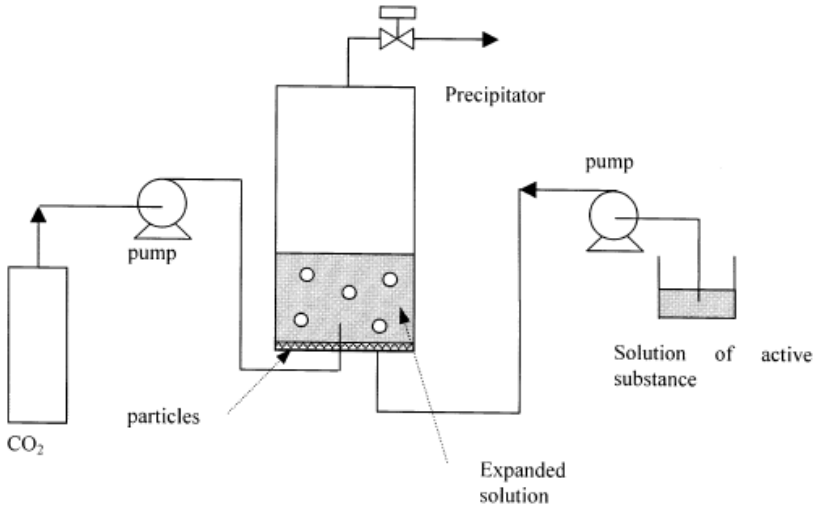


Figure 1-4. GAS Process Schematic[10]

One disadvantage of this process is that it operates as a batch. It also uses a significant amount of organic solvent, which inevitably requires stripping of residual solvent from the product[7].

d. Aerosol Solvent Extraction System (ASES)

The ASES process also utilizes the insolubility of most pharmaceuticals in SC-CO_2. It is similar to the GAS process in that the drug is dissolved in a solvent partially miscible with SC-CO_2, such that particles precipitate upon mixing. The difference is that the ASES process is a semi-continuous spray process. By using a nozzle to create an aerosol of the drug solution

before contacting the SC-CO_2 antisolvent, supersaturation occurs more rapidly and uniformly, producing smaller particles. The drug solution is pumped in though the inner diameter of a coaxial nozzle, while the antisolvent is pumped in through the outer diameter; the two meet at the tip of the nozzle, inside the pressurized vessel. The antisolvent expands the drug solution and particles precipitate. A filter at the bottom of the vessel catches the particles; for this reason, particle washing and collection is a batch process. A schematic of the ASES process is shown in Figure 1-5.

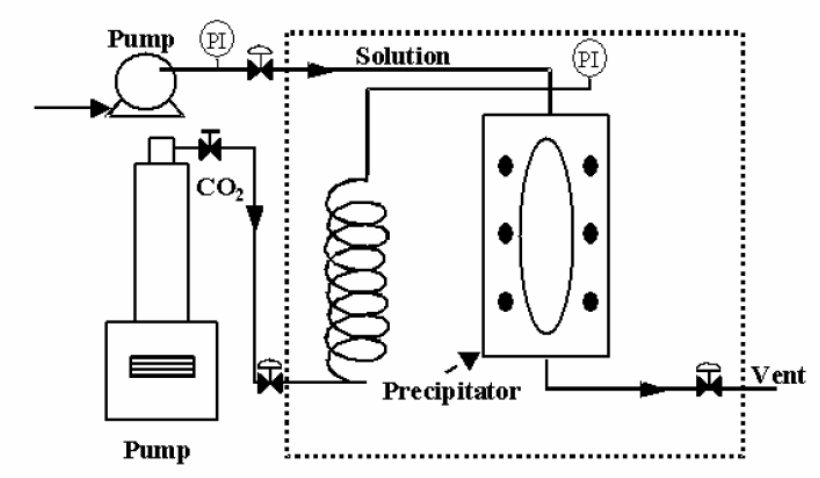


Figure 1-5. ASES Process Schematic[11]

The main disadvantage of this process is that the mechanism for particle formation is poorly understood. Also, when using aqueous drug solutions (which are common for proteins and other medications) a solvent or co-antisolvent must be used to overcome the relative immiscibility of water and CO_2.

1.3 Research Objectives

The goal of this research is to explore the effects of temperature, pressure, and solution and antisolvent flow rate on Bovine Serum Albumin (BSA) particles created using the ASES process. These investigations aid in the development of relationships between these processing

variables and the resulting particle size and morphology. Using the ASES process on an industrial scale is reliant on the development of theoretical models to predict and explain the process; another goal of this project is to apply knowledge gained and apply it to larger scale experiments. This ultimately lends to discussing heuristics for industrial scale-up.

2. Literature and Theoretical Review

2.1 ASES Literature Review

Since its development in 1989[12], the ASES process has been used extensively for particle production and microencapsulation. From explosives to pharmaceuticals, the superior properties of supercritical fluids have allowed for smaller, more uniform particles. Supercritical fluids have diffusivities that can be up to two orders of magnitude higher then traditional liquid solvents[13], while having similar densities. In combination with the aerosolization and rapid mass transfer provided by the coaxial nozzle, supersaturation and phase separation occur on the order of 10^{-5} seconds; particle diameters can be reached that are below the minimum possible with traditional liquid recrystallization or milling[13]. Additionally, the ASES method can produce particles with a larger percentage of fine particle mass (FPM, <5 μm). Steckel et al. compared budesonide particles made using the ASES process to ones made with the conventional jet-milling process; they were able to obtain 47.9% fine particle mass using the ASES process, as compared to 29.0% using jet-milling[14].

Fine particle mass is especially important for pulmonary delivery. Particles that are <5 μm in diameter are more able to penetrate into the smaller airways, which are often the target of local treatment in the lungs[15]. However, particles <0.5 μm are exhaled, so a narrow size distribution is required for efficient delivery[16]. Kim, et al. investigated the effects of flow rates and solvent type on the particle size and size distribution of poly(L-lactic acid) (PLLA) nano- and microparticles. Spherical particles were produced using 1-4 dioxane (5-10 μm), tetrahydrfuran (THF) (0.5-1.0 μm), and dichloromethane (DCM) (0.2-0.8 μm)[17]. A very comprehensive review of many of the polymers and pharmaceutical compounds micronised using the ASES process was written by Jennifer Jung and Michel Perrut[10]. As an example,

amoxicillin was precipitated from N-methylpyrollidone (NMP) by Reverchon with a particle size range of 0.2-0.8 μm[18]. As is evident, the ASES process has successfully produced particles in the size range of effective pulmonary delivery with narrow size distributions.

One drawback to the ASES process is agglomeration. With the high frequency of collisions in the precipitation chamber during the ASES process and the turbulence, this is not surprising. Particles of insulin, albumin, lysozyme, and myoglobin have all shown to form aggregates of primary particles[19]. A study by Bustami, et al. for micronizing the first recombinant protein approved for human therapy by inhalation, recombinant human deoxyribonuclease (rhDNase), had up to 88.7% aggregates[20]. Particle agglomeration may not be entirely undesirable, however. New inhaler designs, such as the Turbuhaler™, actually utilize agglomerated particles, which are more stable during storage[15]. When the inhaler is used, the shear forces from the air stream within the device break up the aggregates and disperse the individual particles. A method for reliably producing such agglomerates would still be required.

Aside from the morphological advantages over typical pharmaceutical processing, there are significant processing and health advantages of a SCF process such as ASES. Pharmaceutical processes for either recrystallizing drugs from solutions or isolating them from solid matrices primarily use large amounts of organic solvents[4]. Both waste streams containing organic solvents and trace amounts left in the final product are health concerns. Not only does the ASES process use small amounts of cosolvents, but low residual content can easily be achieved in addition to easy separation of the organic solvent from the effluent stream[21]. Ruchatz et al. were able to reduce residual methylene chloride content in polylactic acid particles to 100 ppm using a SCF washing step that proved to significantly decrease the amount of time required compared to conventional drying techniques[22]. Five different steroids, including the

common inhalant medication fluticasone, were successfully micronised using DCM as the solvent; the residual solvent content was <250 ppm and as low as 18 ppm[23]. Using large amounts of organic solvents is also a concern because some proteins will denature with exposure to dimethylsulfoxide (DMSO), dimethylformamide (DMF), and others[24]. Some cosolvents are less harmful to protein structure than others, such as ethanol[25]; in this study, lysozyme and trypsin were solubilized in a solution of water and ethanol. The lysozyme particles retained 95% activity, while less than 40% of the trypsin bioactivity was maintained. In a study by Winters et al., the stability and storage of proteins processed with ASES was investigated. Insulin, trypsin, and lysozyme were processed using DMSO as the solvent, which resulted in minimal to intermediate alteration in secondary structure. However, this did not affect the stability during storage as compared to conventionally lyophilized particles, and they were reconstituted successfully to native structure in aqueous solution[26]. It is evident that a specific protein requires individual consideration in choosing a cosolvent or coantisolvent.

Based on the results of a number of studies of the effects of processing variables on the resulting particle size and morphology, many aspects of the ASES process have different effects depending on the material being processed. In the case of hydrocortisone acetate precipitated from DMF, lower temperatures resulted in smaller particles and pressure had little effect on the properties of the product[27]. The previously mentioned study involving the micronisation of budesonide from DCM showed little variation in particle size or morphology when varying the CO_2 and solution flow rates over a limited range[14]. There was, however, a substantial effect on the aerodynamic properties of the particles obtained. The also previously mentioned investigation of the precipitation of rhDNase from aqueous solution showed that varying the processing temperature over 20-45ºC resulted in little change in particle size. However, there

was a substantial reduction in biological activity at temperatures above 35ºC[20]. Other systems have shown substantial morphological and size effects when varying temperature and pressure. Thies and Muller found that increased pressure, which translates into increased antisolvent density from 0.25 to 0.69 g/mL, dramatically decreased particle size of PLLA precipitated from DCM from 50 to 6 μm[28]. However, Randolph, et al. found that for the same system, increasing the density from 0.75 to 0.96 g/mL increased the particle size from 0.61 to 1.4 μm (referred to again later)[29]. These discrepancies allude to the fact that the complicated mechanisms involved in the ASES process are not well understood, and appear to vary with processing conditions and processing material. With this in mind, the two general theories applied to the ASES process will be explained subsequently.

2.2 Theoretical Models

a. Hydrodynamic Theory

The mechanism of particle formation in hydrodynamic theory focuses primarily on the aerosolization of the drug solution provided for by the coaxial nozzle. Discrete solution droplets are formed as the jet leaves the nozzle tip, from which particles nucleate on contact with the antisolvent. In this mechanism, the droplet size has the most influence on the resulting particle size. The size of the droplets formed is dependent on the relative external pressure forces due to the antisolvent and the surface tension holding the droplets in tact, as described by the Weber number[5]:

$$N_{We} = \frac{\rho_A U^2 D}{\sigma} \qquad 2.1$$

where ρ_A is the antisolvent density, U is the relative velocity of the solution and antisolvent, D is the droplet diameter, and σ is the interfacial surface tension. The greater the Weber number, the smaller the solution droplets (and resulting particles) will be. Since the surface tension is

relatively low and the density relatively high for SCFs, the Weber number is significantly large. Since the density of a SCF increases dramatically with increased pressure, this should result in smaller particles.

Within hydrodynamic theory, there are two different proposed mechanisms for the actual particle nucleation process. First, there is the one droplet-one particle theory, which as the name states, one particle precipitates from each droplet. Therefore, the particle size is directly dependent on the droplet size; pressure should have a dramatic effect on particle size. A visual representation of this theory is shown in Figure 2-1.

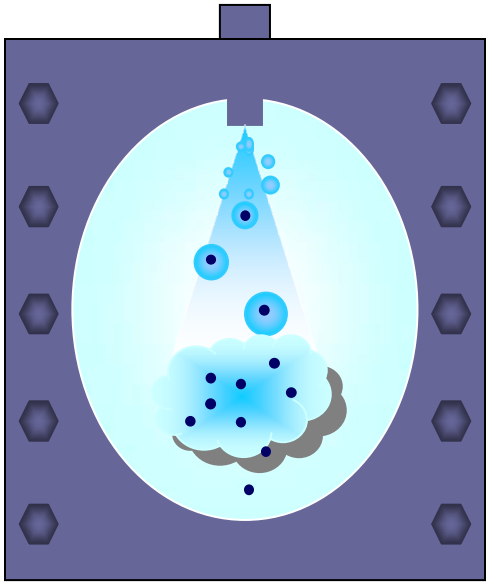


Figure 2-1. One Droplet-One Particle Theory

On the other hand, there is also the idea that multiple particles precipitate from each droplet. Multiple nucleation sites form within each particle as the antisolvent more rapidly penetrates the solution droplets. Therefore, increasing the mass transfer between the two phases would cause multiple smaller particles to form within each droplet; not only do small droplets result in small particles, but so do turbulent conditions, high diffusivity, etc. A visual representation is shown in Figure 2-2.

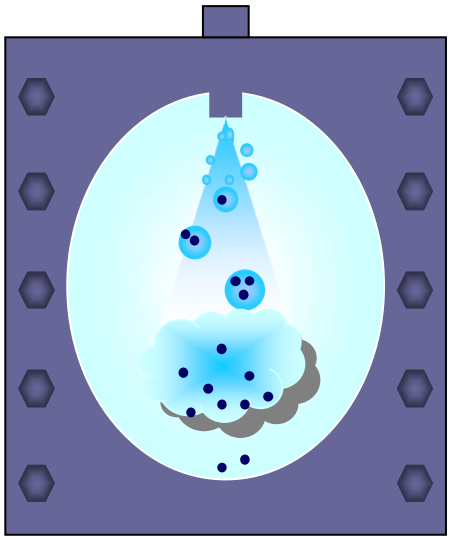


Figure 2-2. Multiple Particle Theory

b. Crystallization Theory

The mechanism of crystallization theory focuses primarily on turbulent mixing of the solution and antisolvent. In this case, the interfacial surface tension is assumed to be so small that the Weber number becomes infinitely large and discrete solution droplets do not form. Particle nucleation is then a result of supersaturation within the gaseous plume formed by the nozzle and is dependent on the mixture kinetics and mass transfer.

Figure 2-3. Crystallization Theory

In this manner, it is similar to a traditional solvent recrystallization process, only the rates of mixing in the ASES process are much higher due to the high density and low viscosity. Therefore, nucleation is much more uniform and produces much smaller particles. A visual representation is shown in Figure 2-3.

c. Proposed Models

The mechanism of particle formation in the ASES process is really a complicated mix of hydrodynamic, thermodynamic, and mass transfer effects. As stated by Foster et al., both of the previously described mechanisms are supported in the literature and the exact mechanism appears to be system dependent[11]. A review by Reverchon describes a few of these in detail[13]. As one example, Chang and Rudolph neglect the solute-solvent and solute-antisolvent interactions and treat the process as a binary system. The particle formation is explained based on expansion behavior predicted by the Peng-Robinson equation of state. Good results were obtained only in some cases, one being the precipitation of acetaminophen from toluene[30]. Rantakyla et al. made a model starting with experimental results obtained for PLLA in DCM. The model considered diffusion of the antisolvent into the droplet, the evaporation of the solvent from the droplet, and the solid particle formation. The solid particle formation was modeled with an equation developed by Tom and Debenedetti that described particle size distribution with time[31]. This model assumed that one particle formed from each droplet. A more recent model developed after the Reverchon review by Lengsfeld et al. included the Peng-Robinson binary phase equilibria, classic jet breakup theory, and transient surface tension[32]. This model can only be applied to dilute solution systems where the solute does not interfere with the binary dynamics. Importantly, the model determined that the particle nucleation in dilute systems results from mixing, and not from discrete droplet formation. This

model has limited applicability, especially considering that other experiments have shown that smaller particles form from more concentrated solutions[18].

3. Experimental

3.1 Experimental Setup

a. Apparatus

A schematic of the setup can be seen in Figure 3-1. The system can be used with any combination of drug solution, antisolvent, flow rates (often a molar ratio of 1:50 solution to antisolvent[33]), pressure, and temperature. The drug solution (A) is pumped by a High Performance Liquid Chromatography (HPLC) pump (B). A syringe pump (C) full of pure carbon dioxide is used to initially pressurize system as well as for flushing the vessel after spraying the drug. Another syringe pump (D) is used if the carbon dioxide used as the antisolvent has a modifier. The solvent and antisolvent flow through the coaxial nozzle (E) before reaching the precipitation vessel (F). The particles are caught in a filter (G) with a 0.5 micron mesh. The outflow is sent through a metering valve (H) used to maintain the antisolvent flow rate to a dewar (I) filled with ice to condense any solvent and un-precipitated material for recovery. The entire process is run inside a constant temperature water bath (J).

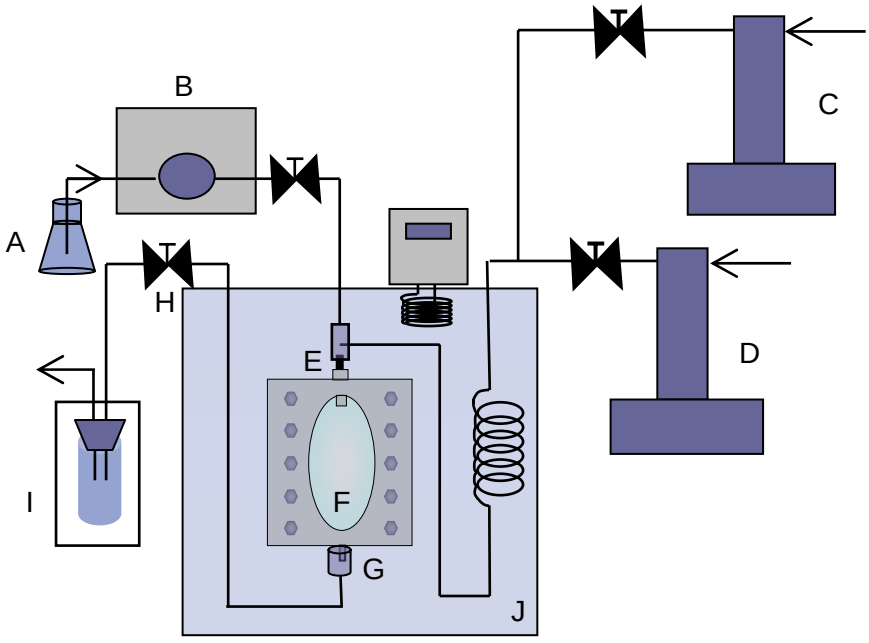


Figure 3-1. Experimental Apparatus

b. Coaxial Nozzle

The defining characteristic of the ASES process is the use of a coaxial nozzle. The inner nozzle is Polyethertetherketone (PEEK) tubing with a diameter of 50 microns and has the drug solution flowing through it. This feature, in combination with the large pressure drop between the pumping source and the precipitation vessel, creates an aerosol spray. The antisolvent then flows through the outer annular space of the nozzle between the PEEK tubing and the outer 1/16" stainless steel tubing. A side view and a cross-sectional view of the coaxial nozzle can be seen in Figure 3-2. The spraying further aids the mixing of the antisolvent and drug solution upon contact in the precipitation vessel. Super-saturation occurs due to the drug being insoluble in the antisolvent, and particles precipitate.

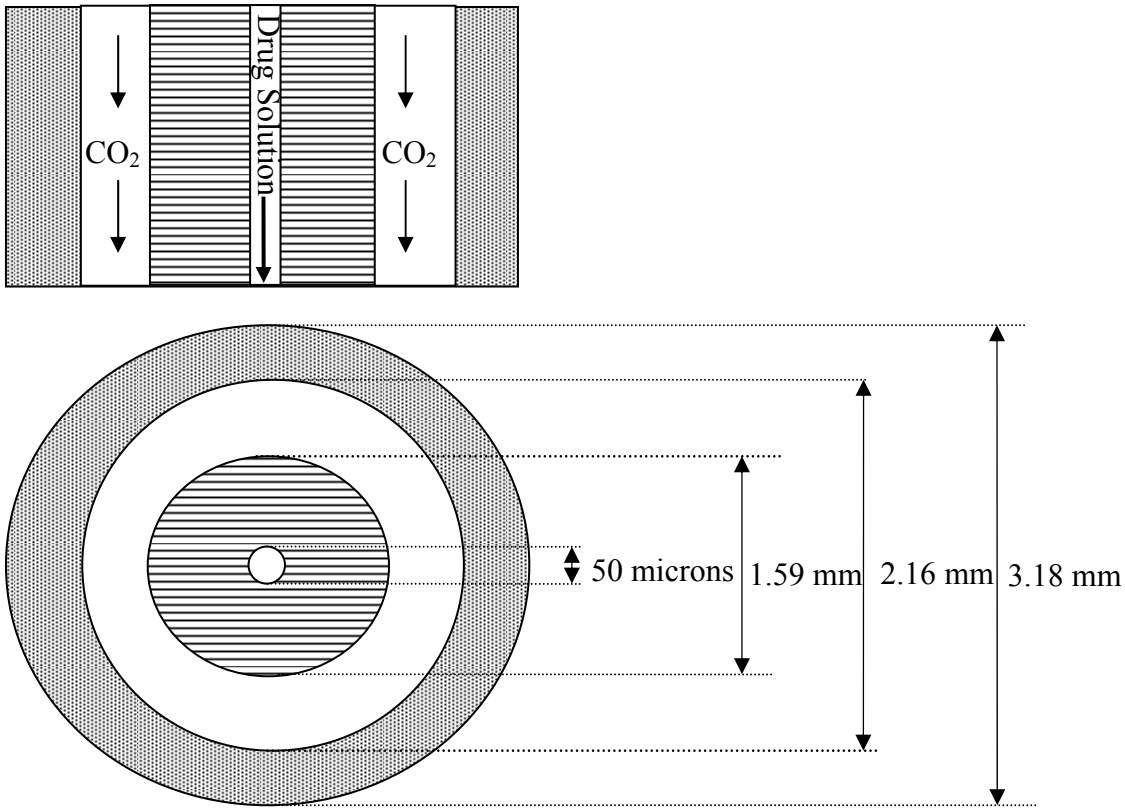


Figure 3-2. Coaxial Nozzle

Mawson et al investigated the use of a coaxial nozzle as opposed to a standard nozzle. Using the standard nozzle, solution droplets were evident throughout the vessel due to inadequate mixing with the antisolvent. Although the standard nozzle achieved better aerosolization due to the larger relative velocity between the solution and antisolvent, the poor mixing resulted from insufficient turbulence outside of the jet. Due to this, the particles dried more slowly and would become highly agglomerated. Using a coaxial nozzle increases mass transfer between the two phases and causes better particle nucleation[34].

3.2 Procedure

a. Antisolvent Preparation

Initially, pure SC-CO_2 was used as the antisolvent, which would reinforce the non-toxic, benign nature of the process. As was mentioned earlier, pure CO2 is relatively immiscible with water, which was the case with the aqueous protein solution. Inside the pressure cell, condensation formed on the glass as the SC-CO_2 antisolvent was unable to penetrate the drug solution spray. A photograph of this behavior during an experiment is shown in Figure 3-3.

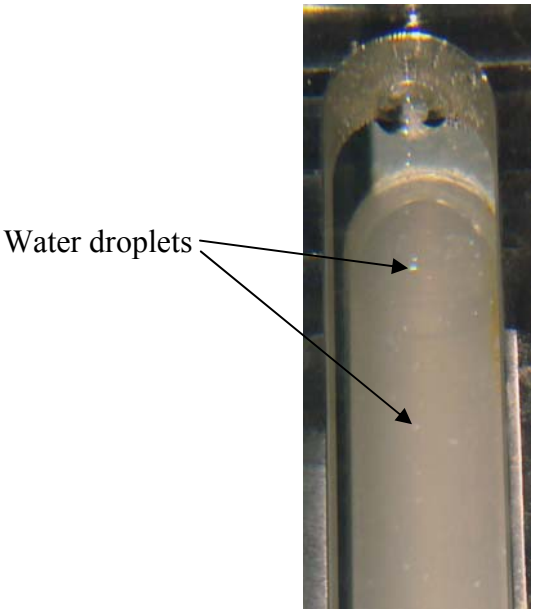


Figure 3-3. Immiscible Solution/Antisolvent

It was then evident that the system would require an organic solvent to increase the miscibility of the drug solution and the antisolvent. To do this, ethanol was added as a co-antisolvent[19]. To prepare the modified antisolvent, the ethanol was added in an amount equivalent to 20 mol% and the pump was cooled to add liquid CO_2. This mixture was cooled and heated several times to assure complete mixing within the syringe pump. Using a modified antisolvent required consideration of the ternary behavior of the water-CO_2-ethanol system to avoid phase splitting in the pressure vessel. Additionally, the pressure vessel was initially pressurized using pure CO_2 before adding the modified antisolvent to prevent phase splitting.

b. Drug Solution Preparation

The protein of interest in these experiments was Bovine Serum Albumin (BSA), which is often used as an inexpensive model protein in experimentation. As previously mentioned, the drug solution contained water as the solvent; BSA concentrations of 15-80 mg/mL were used. Even after employing the use of the modified antisolvent, the drug solution and antisolvent appeared to not be miscible enough for supersaturation. Increasing the amount of ethanol in the antisolvent made them too miscible; that is, condensation formed similarly to when pure SC-CO_2 was used. As a solution, the amount of ethanol was decreased in the antisolvent and a small amount of ethanol was added to the drug solution. However, when preparing the ternary drug solutions, gels would form. Phase diagrams such as that shown in Figure 3-4 demonstrate the behavior of these solutions. Region 1 would form a solution, while the others were gels or other forms of insoluble solids. The low concentrations to make a solution were undesirable, so aqueous solutions were again used. The solutions for the results comparison were 50 mg/mL BSA in HPLC grade water.

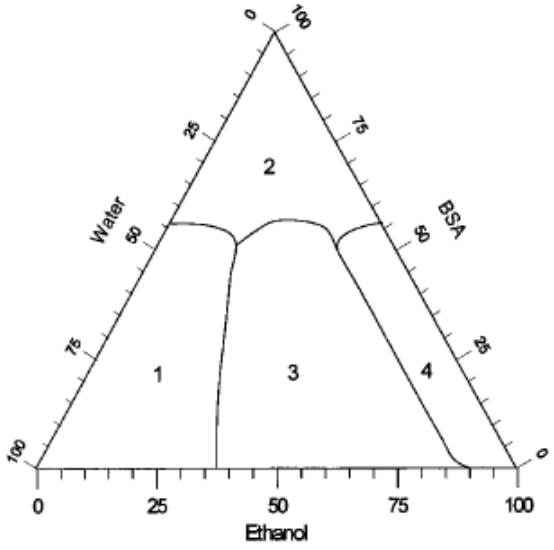


Figure 3-4. Ternary Phase Diagram (BSA-Ethanol-Water)[35]

c. Conducting the Experiment

The antisolvent is first prepared in a syringe pump to be cooled overnight and mixed in the hours preceding the experiment. A few hours before, the bath temperature is set, the drug solution is prepared and placed in the refrigerator, the vessel is pressurized with pure CO_2, and the antisolvent is heated to begin the mixing process. The antisolvent is heated by circulating the water from the bath through the pump jacket, cooled with ice water, and then heated again.

To begin an experiment, the antisolvent syringe pump is run at constant pressure, using the metering valve to set the initial flow rate. Once the initial antisolvent flow rate is set, the HPLC pump is used to increase the pressure of the drug solution to about 2000 psi greater than the system pressure before opening the valve to the nozzle. The drug solution is then sprayed in for approximately one minute to prevent the filter from clogging with particles. When particles are precipitating, a white fog appears inside the vessel, as seen in Figure 3-5.

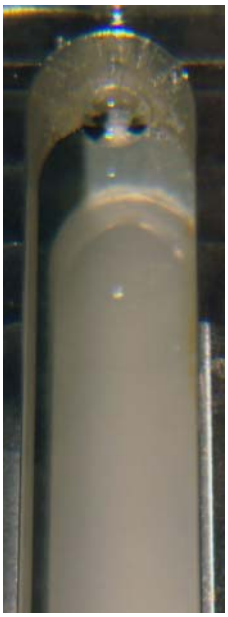
a.  b. 

Figure 3-5. Particle Precipitation (a. Pressurized Vessel b. Particle Fog)

If this is not evident, or there are visible droplets of solution falling from the nozzle, the flow rates are readjusted. If condensation forms, the solution valve is closed so that pure CO_2 can flush out the excess water and ethanol before attempting to resume spraying. After particles form, more antisolvent is flushed through, followed by the washing step.

d. Particle Recovery and Analysis

Following every run, the nozzle is back-flushed to remove any remaining liquid, and approximately 200 mL of pure carbon dioxide is used to wash any residual ethanol and water in the entire system. The system is then de-pressurized, and the precipitation chamber removed from the water bath to recover particles. Figure 3-6 shows an example of the filter, which fits snugly inside the large threaded fitting in the bottom of the vessel, removed from the vessel and covered in particles. The powder is collected in glass vials after preparing samples for Scanning Electron Microscopy (SEM) and stored in the freezer.

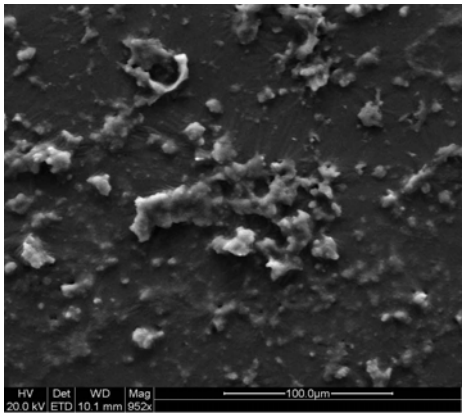
Figure 3-6. Filter with Particles

4. Results and Discussion

Due to the small amounts of powder recovered during successful runs and the often heavily agglomerated state of the particles, analysis was very qualitative. Ideally, powders consisting of spherical particles would be produced for size distribution analysis. In this case, SEM images were used as a basis for comparing and understanding the basic effects of pressure, temperature, and flow rate over small ranges.

4.1 Pressure Effects

All experiments were conducted well above the critical pressure of CO_2 (73.8 bar, 1070 psi). It was found that precipitation occurred for the system using the previously specified drug solution and antisolvent composition between 2000-2500 psi. Images of particles made at the upper and lower limits of this pressure range and 45ºC can be seen in Figure 4-1.

a. 

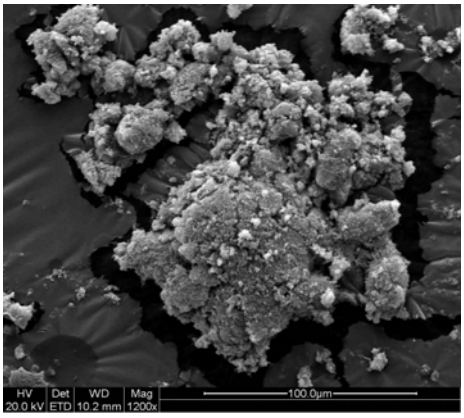
b. 

Figure 4-1. Pressure Effects (a. 2000 psi b. 2500 psi)

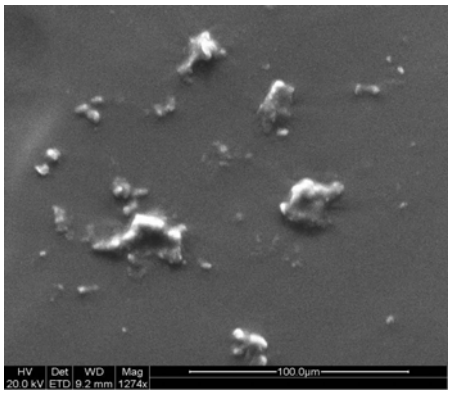
It is evident that the primary particle size is much larger at 2000 psi than 2500 psi. This is attributed to the fact that the increased system pressure increases the supercritical antisolvent density (see Figure 1-2); the antisolvent is more able to mix with and expand the drug solution. Obtaining smaller particles from larger pressures is consistent with both of the previously discussed theories; hydrodynamic theory would explain this as a result of greater jet-break up

from the increase in external pressure forces relative to the surface tension between the drug solution droplets and the antisolvent. The cause of the agglomeration is a little less clear. It could simply be an effect of the increased surface energy of the smaller particles, some effect related to the complicated solution behavior of BSA in the presence of water and ethanol, or it could be related to the drug solution concentration. Many studies find that increasing the drug solution concentration (up to a certain threshold) increases the rate of particle nucleation, and subsequently decreases agglomeration[19,36]. This possibility is not explored further experimentally here, but seems to be more consistent with crystallization theory. The decreased particle size with increased pressure is also consistent with this theory; only in that case, the smaller primary particles are a result of the increased mass transfer between the solution and antisolvent due to the increased antisolvent density. The increased supersaturation results in more sites of nucleation and therefore smaller resulting particles. In general, it appears as though both the hydrodynamic theory and the crystallization theory dominate at different conditions in the same system. This observation is supported by the work of others[5, 8].

4.2 Temperature Effects

The system temperature does not have much flexibility in the ASES process. The temperature must be above the critical temperature of CO_2 (31.1ºC), but also must remain within a range that will not accelerate protein denaturation. Taking this into consideration, all experiments were conducted in the range of 35-45ºC. Images of particles obtained at the upper and lower limits of this range and a pressure of 2000 psi are shown in Figure 4-2. As is evident, the primary particle size decreases as temperature increases. This is likely due to a combination of effects; the reduction in antisolvent viscosity (and increase in diffusivity) increases the mass transfer between the solution and antisolvent, and altering the mixture thermodynamics via

reduced solubility of the drug in the solution/antisolvent mixture and increased nucleation rates. From this and the previous effects of increased antisolvent density, the mass transfer (and therefore overall ability of the antisolvent to expand and supersaturate the drug solution) seems to be a balance of high density and high diffusivity. This implies that there is an optimum temperature and pressure that, when exceeded, will cease to reduce the resulting particle size.

a. 

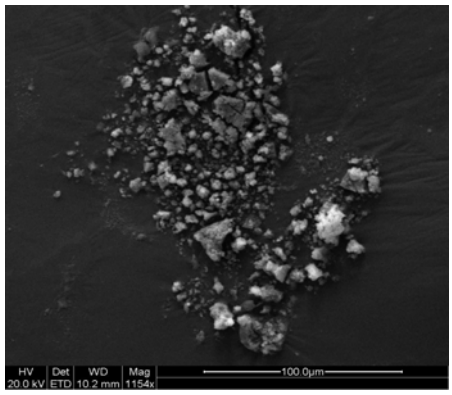
b. 

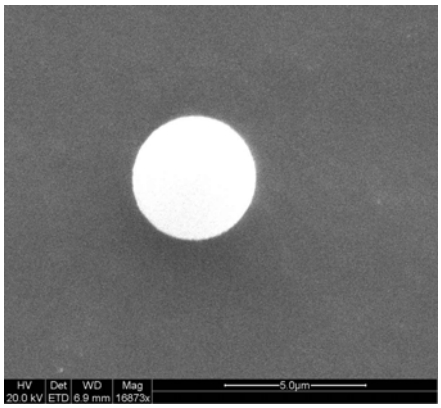
Figure 4-2. Temperature Effects (a. 35ºC b. 45ºC)

This is reflected in work by Randolph et al. that studied the effects of increasing the CO_2 antisolvent density[29]; increasing the density from 0.65 to 0.76 g/mL increased the resulting particle size. This implies that the reduction in diffusivity has a more dominant effect than the increase in antisolvent density.

4.3 Flow Rate Effects

Both the ratio of solution to antisolvent flow rate, as well as the overall magnitude of the flow rates, has an effect on the particle formation process. For the solution and antisolvent compositions specified in these experiments, ratios of antisolvent:solution ranging from 40:1 to 30:1 are effective. In general, it is observed that increasing the overall flow rates while maintaining the same ratio does not necessarily result in particle formation. This non-linearity is a factor when considering scale-up, and will be discussed further later. Figure 4-3 shows images

obtained from two portions of the same powder sample collected from a single experiment using a solution flow rate of 1.0 mL/min and an antisolvent flow rate of 30 mL/min. The experiments for investigating flow rate effects are at 40ºC and 2000 psi.

a. 

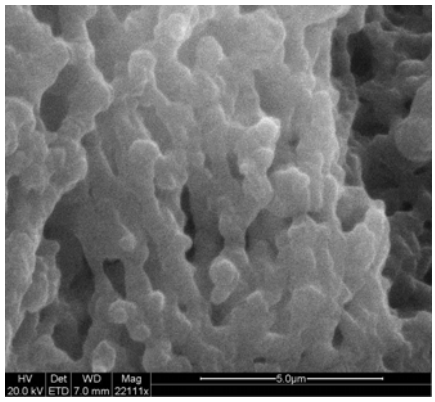
b. 

Figure 4-3. Particle Morphologies Observed (a. spherical b. fibrous)

Figure 4-3a is an example of a spherical particle in the desired size range. Figure 4-3b has the appearance of intertwined chains subject to high shearing effects, similar to that observed by Shekunov, et al. when using ASES to produce polymer microparticles. This is attributed to denatured protein chains in the solution; denatured protein chains lose their folded conformation and can become linearized, similar to polymer chains. This is not only an issue because of the loss of biological function, but also because this type of particle morphology could lead to filter clogging; the issue of denaturation will be addressed further when discussing scale-up.

According to theory, decreasing the flow rates should lead to larger particles; in hydrodynamic theory, this is because of lesser jet break up producing larger solution droplets, and due to decreased turbulence and mass transfer in crystallization theory. As was previously mentioned, the flow rate ratio can not be maintained as the overall magnitude of the flow rates change, so the combination of 0.5 mL/min drug solution and 20 mL/min antisolvent is used in the comparison. Figure 4-4 is an SEM image of the resulting powder.

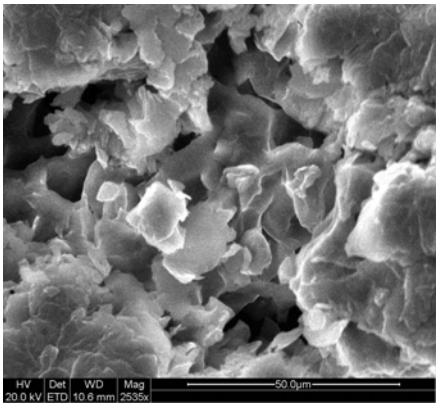


Figure 4-4. Amorphous Particles

Decreasing the overall magnitude of the flow rates results in precipitation, but the supersaturation is insufficient for obtaining discrete, spherical particles. This result is more consistent with the crystallization theory, where insufficient mass transfer results in slow nucleation so crystals form instead of particles.

4.4 Scale-up

As was previously mentioned, there is little known about the exact mechanism of supersaturation, nucleation, and crystal growth in the ASES process. The proposed models only pertain to specific conditions for a few systems; many systems, such as the one described here, show characteristics that can be explained by both models. Without a more comprehensive mathematical model, scale-up is very difficult. A few of the more important issues concerning industrial scale-up and how they relate to the previously described experiments will be discussed.

a. Considerations

The results described for this system indicate the sensitivity of the ASES process to many processing variables. This invariably indicates that the process itself is very complex and can not be easily scaled proportionally. The more obvious ways to increase the scale of this process would be to increase the flow rates, nozzle diameter, vessel size, or a combination

of these. To increase the flow rate and process more material, the nozzle diameter must be increased to achieve the same pressure drop. In doing this, the mixing dynamics change as the mass transfer must occur over a larger area; the dynamics of the system happen on such short time scales, that small changes can have a significant effect on the precipitation kinetics. The spray dynamics may also change, because as the scale gets larger, it will be more difficult to achieve aerosolization. Developing correlations that take all of these effects into account would be ideal, but that has yet to be accomplished. A more feasible short-term option would be to conduct experiments on larger scale equipment to find empirical relationships; this will only apply to a particular system, but it is an option.

Another consideration is that the aqueous system described here contacts CO_2, which can lower the pH. At such high pressures, the pH can become as low as 3.0[37]. When dealing with proteins, pH changes can cause denaturation. Aside from losing biological activity, precipitation may be induced by surface charge changes[21].

In pharmaceutical processes, residual solvent content is always a consideration for product purity. As stated previously, using SC-CO_2 in the ASES process with aqueous protein solutions requires the addition of a solvent; in this case, the antisolvent is modified with ethanol. Although the lab scale system is washed with a large volume of pure CO_2, there are instances where the particles redissolved upon depressurization because of residual solvent content. So, not only for product purity, but for product recovery, an efficient washing step is absolutely required. Effects such as cake resistance, filter pore size, miscibility of the solvent with CO_2, the affinity of the solvent for the particles, and the amount of solvent present need to be considered. Many studies have been done to investigate the amount of time required for effective washing[22, 38], which is typically much shorter than the times required for conventional

drying techniques. Ideally, the vessel should be designed to achieve a plug flow when washing instead of mixing; this would make a large narrow vessel more attractive. Practically, this puts a limitation on height for stability, as well as the dynamics of particle/antisolvent interactions as they come into contact for longer times.

Particle recovery is an issue with the ASES process. On the laboratory scale, the particle formation process is continuous, but the particle washing and recovery are done as a batch. To collect particles, the entire system is depressurized and the vessel must be taken apart. This would not be feasible on the industrial scale, because it is both labor and time intensive. Additionally, if particle recovery were made continuous in some way, there is still the issue of filter blockage, which would require system depressurization. One positive consideration concerning particle recovery is that for many pharmaceutical processes, production capacity can be on the order of only a few kilograms per year.

b. Design Options

To maintain the same spray dynamics when scaling-up, correlations are needed for increasing nozzle diameter, flow rates, solution concentration, etc. It is very likely that all of these variables are independent of each other with scale. However, a more plausible method is to determine the most influential processing variables and the effect of scale. As suggested by Thiering, et al, a process should be designed to achieve efficient mixing, such that the mixture kinetics is the limiting factor[21]. In doing so, variables such as temperature and pressure that affect the diffusivity and miscibility would be controlling. The simpler option for maintaining the same spray and mixing dynamics would be to use multiple identical nozzles in one large vessel. In the event that wall effects and other controlling factors are altered by using a large vessel, multiple identical units could be used in parallel.

To overcome the issues related to particle collection, multiple, independently pressurized particle collection units could be used. That way, particle production in the vessel could be continuous, and it would not need to be depressurized in order to collect particles. This method is favorable, because if there are issues with clogged filters, the process will not need to be taken offline. Another way to circumvent filter issues is to use other methods for particle collection. Traditional particle filtering methods such as cyclones could be used; issues with agglomeration would need to be investigated to use this type of separation. In the case of the innate electrostatic character of proteins, an electrostatic precipitator could be used to pull the particles out of the effluent stream with electric fields. The concern here would be possible reversible alteration in protein structure. Theiring et al. also suggested the possible use of a cyclone with charged walls to combine the features of the two separation techniques[21].

5. Conclusions and Recommendations

The Aerosol Solvent Extraction System (ASES) is a supercritical fluids process that is a viable option for the production of small particles for pulmonary drug delivery. Using supercritical carbon dioxide (SC-CO_2) as the antisolvent allows for low processing temperatures; this is especially important for biological molecules such as proteins. Particles can be produced in the optimal range for lung deposition with narrow size distribution and high bioavailability.

To date, the ASES process is not well understood. The work presented here supports theories applied in the literature to the ASES process, which shows promise for developing models. Increasing the system pressure decreases the size of the primary particles, but increases agglomeration due to frequency of particle collisions. Increasing the system temperature also decreases the particle size, which indicates the need for a balance between achieving high density and high viscosity in the antisolvent. For this system, solution and antisolvent flow rates appear to have the most pronounced effect on the resulting particles. This would indicate that turbulence and other mass transfer effects are the most important for this system of bovine serum albumin (BSA) and aqueous solution with ethanol-modified carbon dioxide antisolvent.

For this process to be feasible on an industrial scale, mathematical models and correlations for predicting the effects of processing variables on particle size and morphology are necessary. Applying the knowledge gained in this study to larger scale laboratory equipment will help to better understand the effects of scale on mixture thermodynamics, hydrodynamics, and mass transfer. It is also important to extend the studies to other model proteins to find general trends having to do with protein size, shape, and surface charge. The wide applicability, as well as the superior particle processing capabilities of the ASES process indicate that further work is necessary.

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