A Review on Micronization Techniques

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Abstract: Drug powders containing micron-size drug particles are used in several pharmaceutical dosage forms. Many drugs, especially newly developed substances, are poorly water soluble, which limits their oral bioavailability. The dissolution rate can be enhanced by using micronized drugs. Small drug particles are also required in administration forms, which require the drug in micron-size size due to geometric reasons in the organ to be targeted (e.g., drugs for pulmonary use). The common technique for the preparation of micron-size drugs is the mechanical commination (e.g., by crushing, grinding, and milling) of previously formed larger particles. In spite of the widespread use of this technique, the milling process does not represent the ideal way for the production of small particles because drug substance properties and surface properties are altered in a mainly uncontrolled manner. Thus, techniques that prepare the drug directly in the required particle size are of interest. Because physicochemical drug powder properties are decisive for the manufacturing of a dosage form and for therapeutic success, the characterization of the particle surface and powder properties plays an important role. This article summarizes common and novel techniques for the production of a drug in small particle size. The properties of the resulting products that are obtained by different techniques are characterized and compared.

Micronized powders are of interest in many industrial fields; pharmaceuticals, catalysts, pigments, and biopolymers, for example, are some categories of products that can be used as micro-sized particles. Traditional techniques used to produce micronic powders are based on high-temperature reactions that require high energies, on jet milling that is characterized by low efficiencies and mechanical stress, and on liquid solvents precipitation that has a poor control on particle size and can pollute the product. Generally, the control of the powder size and the span of its distribution are still very approximate.

In the last few years, several supercritical fluids-based techniques have been proposed for the production of micronic and nanometric particles. These processes try to take advantage of some specific properties of gases at supercritical conditions such as enhanced solubilization power and its modulation, large diffusivities, solventless or organic solvent reduced operation, and the connected possibility of controlling powder size and distribution. Techniques like the rapid expansion of supercritical solutions (RESS), supercritical antisolvent precipitation (SAS), particle generation from gas-saturated solutions (PGSS), and new atomization processes have been critically reviewed in this work.

1. Introduction

The demand for pharmaceutical materials including finely ground active substances and excipients is growing. Injectable drugs and dry powder inhalants require particle size distribution in the range of D_{97} of 2-20 microns, with a steep distribution curve and minimum of fine and over sized particles. This can be accomplished by either wet or dry processes. Conventional dry size reduction of pharmaceutical powders was accomplished by impact size reduction. Equipment commonly used falls into the category of either mechanical impact mills or fluid energy impact mills. Examples of mechanical impact mills are hammer and screen mills, pin mills, and air-classifying mills. Spiral jet mills and fluid –energy mills are examples of fluid-energy impact mills.

1.1. What is micronization?

Micronization is a term used to describe size reduction where the resulting particle size is less than 10 microns. Micronization size reduction involves acceleration of particles so that grinding occurs by particle-to-particle impact or impact against a solid surface.

Fluid-energy mills are used for micronization because of the high impact velocities possible as results of particle acceleration in a fast gas stream. Particle velocities in jet mill are in the range of 300-500 meters per second, compared to 50-150 meters per second in a mechanical impact mill. In fact, the generic term has been used to describe

various type of spiral jet miils or "pancake mills". There are many and varied reasons that manufacturers choose to grind pharmaceutical powders. Among these are increased surface areas, improved bioavailability, and increased activity. Dry powder inhalants and injectable compounds benefit from finer and more defined particle size distributions. Reproducible steep particle size distribution , those with a minimum of fine particles and strict control of oversized particles, combined with improved methods to measure particle size distributions has a change in micronizing techniques.

The spiral jet mill is gradually yielding to the next generation of higher-technology fluidized-bed jet mill combines high-energy micronization with an integral forced vortex air classifier. This combination allows greater control of the maximum product particle size and usually a reduction in generated fines. In pharmaceutical products, the particle size of drugs and components may affect processing and bioavailability. An increasing number of compounds that are investigated in industrial drug discovery have low aqueous solubility. For class II compounds (according to the biopharmaceutical classification), dissolution rate is the limiting factor for bioavailability. According to the Noyes-Whitney equation, particle size reduction, resulting in increased surface area, is a very promising approach to enhance dissolution rate and, thus, the bioavailability of poorly water-soluble compounds.

2. fluid-energy impact milling & micronization techniques

2.1. Spiral jet mill micronization

The spiral jet mill is suitable for the fine and ultra fine size reduction of materials up to a Moh's hardness of 3 that display brittle crystalline grinding characteristics. They are typically used in applications where a ultrafine portion is required. The spiral jet mill is simple in design, consisting of a flat cylindrical grinding chamber with several nozzles arranged tangentially in the peripheral wall, a pneumatic feed injector, and a feed funnel. Operation is just as simple. The feed is accelerated into the grinding chamber through the feed injector. The material inside the grinding chamber is subjected to two opposing force :the free vortex created by centrifugal force(mass force)imparted on the particle by the nozzles, and the drag force created by the airflow as it spirals toward the center of the mill. The larger particles are affected to a greater degree by the mass force, circulating around the periphery of the mill and colliding with other particles. As the particles become finer, the drag force exerts greater effects, drawing the particles with the airstream to the central outlet of the mill. Feed particle size is critical, restricted by the size of the feed injector, for mills of 200-300, the feed size can be a maximum of 1.5mm, the smaller –size mills, the feed size is correspondingly finer. There are several factors, both operational and physical, which affect the fineness of the end product, such as feed rate, nozzle pressure, nozzle angle, air flow rate, feed particle size, chamber diameter and width, and product outlet diameter. All of these variables can be adjusted during operation, but it is more likely that once operation has begun, only feed rate will be varied to meet the required particle size distribution. The size range of spiral jet mills employed in size reduction of pharmaceutical poders includes units from 50mm to 500mm, but most are in the 100mm and 200mm size range.Table 1 shows some typical mill sizes with their relative fineness and throught ranges.

Grinding chamber diameter (mm)	50	100	200	315
Powder fineness (d_{97} in microns)	3–80	4–90	5–100	6–110
Throughput at d_{97} = 10 microns (kg/hr)	0.6–1	2.5–4	10–15	20–35
Throughput at d_{97} = 20 microns (kg/hr)	1–2	4–8	20–30	45–70
Air volume (m³/hour)	15	50	190	460

Table 1

There are several manufacturer of spiral jet mills which meet the general design and performance criteria outlined above. There may be slight differences in design, manufacturing methods, and product collection requirements. There are two types of spiral jet mill designs, with single or dual product collection points. A jet mill system with a single product collection point is easier to clean and sterilize, is more compact, and does not split product into two fractions. It is also easier to design in 10 BAR PSR constructions. Spiral jet mills are effective tools for micronization, especially in the pharmaceutical industry, but they have several limitations. First, as mentioned above, is the limitation in feed size due to the method of product injection. Oversized feed particle can cause blockage in the feed hopper and result in variations in particle size distribution caused by fluctuating feed rates. This can be controlled by presizing the feed, using a properly designed feed system, and applying vibration to dislodge buildup in the feed chute. There is also possibility of buildup and scaling in the mill due to the impact which occurs on the mill walls, this is especially problem with sticky substances such as steroids. But perhaps the most serious drawback is the lack of control of particle size distribution, especially top size limitations. These limitations led to the development of fluidized-bed jet mill.[1]

2.2. Fluidised-bed jet mill micronization

The fluidized-bed jet mill is suitable for the fine and ultrafine size reduction of any material up to a Moh's hardness of 10 that can be fluidized by the expanded compressed gas in the grinding chamber. They are typically used in applications where a fine to ultrafine micronization is required, and they are not limited for feed size, heat sensitivity of material, or abrasive characteristics. They are characterized by decreased energy consumption, reduced wear and buildup grinding chamber, steeper particle size distribution, and low noise emission. The fluidized-bed jet mill actually consists of two distinct segments and thus processes. The lower grinding section comprises the actual grinding chamber with several nozzles arranged radially in the chamber wall and gravity feed inlet.
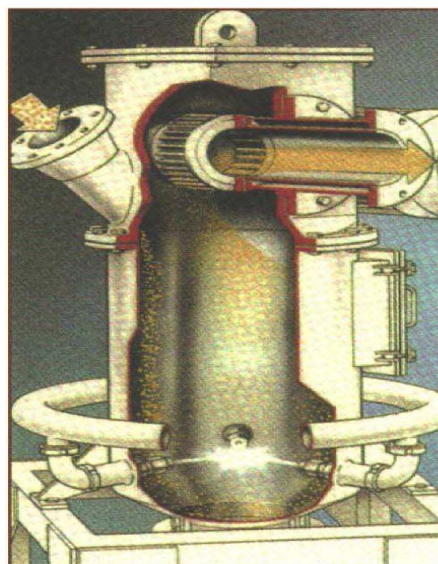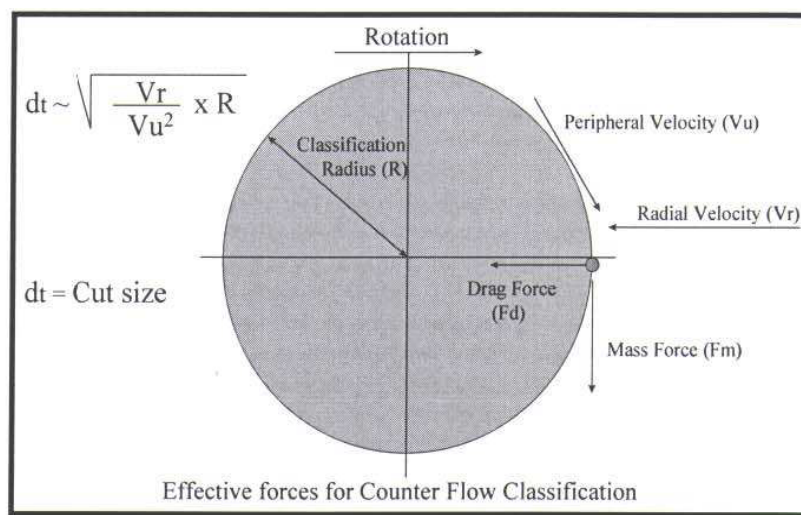

Figure1: **Fluidized-Bed jet mill**

The upper classifier section is a centrifugal forced vortex air classifier which is responsible for particle-size control. The two processes together to give the fluidized-bed jet mill its characteristics steep particle-size distribution and sharp top size control. Operation of the fluidized-bed jet mill is quit simple. Feed material is introduced to the grinding chamber through a large cavity feed inlet. During normal operation, there is fluidized bed of material inside the grinding chamber .Material is entrained by the high velocity gas streams created by the nozzles, and size reduction occurs as a result of particle-to-particle collision in the gas stream and at the local point of the nozzles. The expanded gas conveys ground particles upward towards the centrifugal air classifier. The classifier allows material of a given fineness to exit the mill while rejecting oversized particles back into the grinding chamber for additional size reduction. Equilibrium is established with an internal recirculation: the introduction of fresh feed material and constant discharge of ground material from the mill. The key to maintaining a consistent particle-size distribution is the integral air classifier. Air classification is defined as the separation of the bulk material according to the settling velocity in a gas. As in the spiral jet mill, the same two opposing force –mass force and drag force-are acting on the particles. Mass force is the force exerted on the particle by acceleration due to gravity, inertia, or centrifugal force. Drag force is the force exerted on a particle by the surrounding medium as affected by its aerodynamic properties. In the centrifugal air classifier the mass force is exerted by the peripheral velocity of the classifier wheel. The drag force is exerted on the particle by the carrying fluid, which in the case of a jet mill is the expanded grinding gas. The particle size at which the mass force and drag force act equally on the particle is defined as the cut point. As in the spiral jet mil, the mass force exerts a greater influence on the particles which are coarser than the cut size, and they are returned to the grinding zone of the jet mill. The drag force acts upon the particles which are finer than the cut size, and carried through the classifier wheel and recovered as product.

$$dt \sim \sqrt{\frac{Vr}{Vu^2}} \times R$$

dt = Cut size

Effective forces for Counter Flow Classification

OPERATING PRINCIPLE OF A CLASSIFIER

Figure:2

Most of the limitations inherent in the spiral jet mill do not exist in the fluidized-bed jet mill. There is no real limitation on feed size as the gravity feed inlet varies in size from two to six inches. The problem of the material buildup and scaling in the mill is also

virtually nonexistent. Material does not circulate or impact against the mill walls; in fact, the vertical velocity of air and product in the chamber is only about 1.5 meters per second. The most improvement in the fluidized-bed jet mill process is the ability to control the particle-distribution of the product. The upper particle size of the product is strictly controlled by adjustment of the integral air classifier .By increasing the rotational velocity of the classifier wheel, a greater mass force is exerted on the particles, and smaller particles will be rejected and returned to the grinding zone. The end result is a finer particle size distribution. Conversely, decreasing the classifier speed will allow larger particles to pass through the classifier wheel, the end result being a coarser particle size distribution. Airflow also has an effect. A higher airflow through the classifier wheel result in an increased drag force and a coarser particle-size distribution. With this degree of control, a fluidized-bed jet mill is able to produce an infinitely adjustable particle-size distribution. Table 2 illustrates the effect of adjustment of various operating parameters on the particle-size distribution (PSD).

Table 2.	Adjustment of Particle Size in an Alpine® AFG Fluidized-Bed Jet Mill			
Nozzle Diameter	Grinding Pressure	Total Air Volume	Classifier RPM	Particle Size
Constant	Constant	Constant	Higher	Finer
Constant	Constant	Constant	Lower	Coarser
Constant	Higher	Higher	Constant	Coarser
Constant	Lower	Lower	Constant	Finer
Smaller	Constant	Lower	Constant	Finer
Larger	Constant	Higher	Constant	Larger

Because of the integrated classifier, spatter grain particle are virtually eliminated from the finished product. Control of the upper particle size also reduces the possibility of overgrinding the product in order to ensure a top size. The first graph, comparing the resulting particle-size distribution from a fluidized-bed jet mill to that from a spiral jet mill clearly shows a more precise cut point and a reduction in the ultrafine fraction.
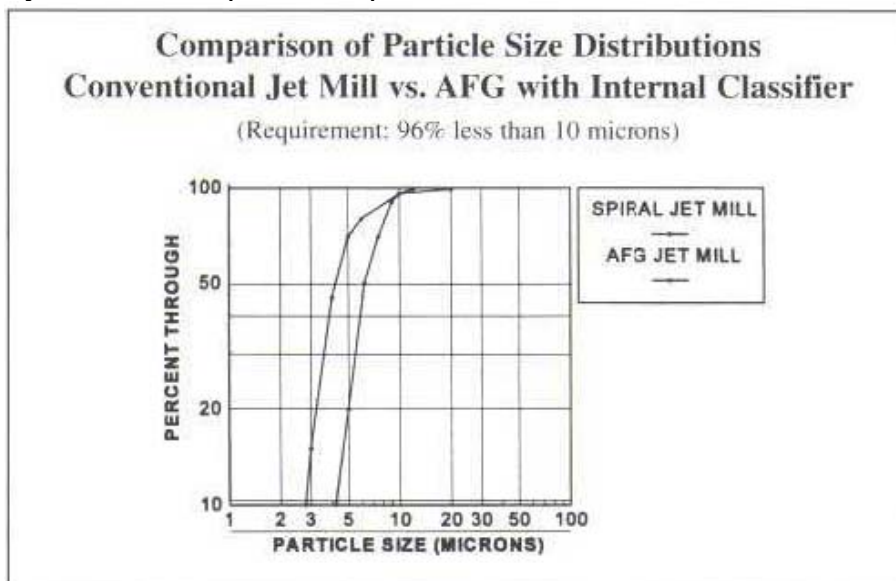

Figure:3

The following graph indicates the improvement in specific energy which can be expected when processing in a fluidized-bed jet mill.
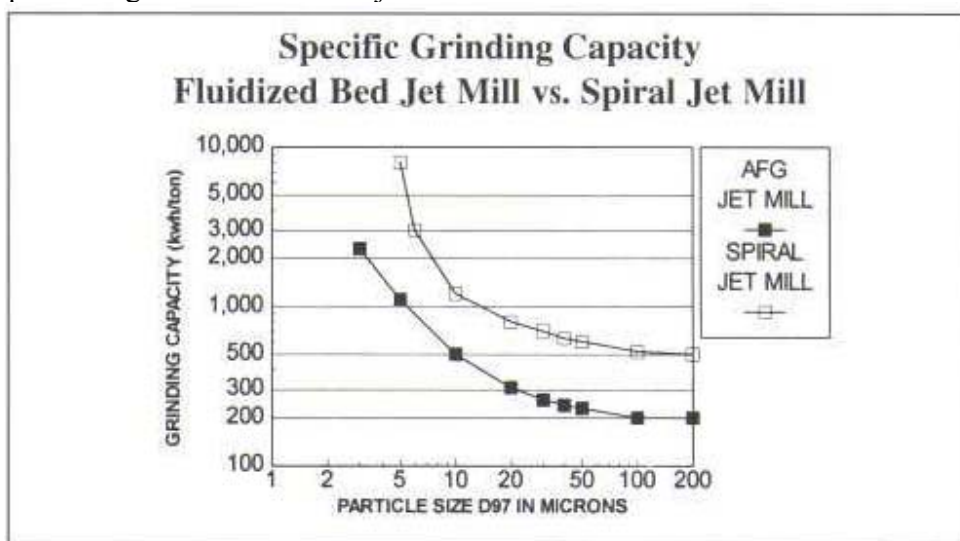

Figure:4

Fluidized-bed jet mills are available in sizes ranging from 100mm to 1250mm grinding-chamber diameter. For most pharmaceutical applications, through, the most common sizes are 100mm to 400mm.Table 3 shows some typical mill sizes with their fineness and throughput ranges.[1]

Grinding chamber diameter (mm)	100	140	200	400
Powder fineness (d_{97} in microns)	2–40	3–60	4–100	5–120
Grind Air volume (m³/hr) Minimum Maximum	50 70	110 150	200 300	800 1200
Throughput at d_{97} = 10 microns (kg/hr)	5–8	10–15	20–30	80–120
Throughput at d_{97} = 20 microns (kg/hr)	10–15	20–30	40–60	160–240

Table 3

3. Supercritical Fluids Micronization techniques:

3.1 INTRODUCTION:

During the last few years, the possibility of application of supercritical fluids has received an increasing attention from the scientific community with the aim to develop new technologies for the production of materials or to substitute the traditional technologies based on the use of organic solvents. Solid material properties at micro- and nano-size level are connected to their chemical composition as well as to their particle size. The production of solid materials with specific properties is very important for many industries, for example, catalysts, coatings, electronics, ceramics, superconductors, dyestuff, pigments, and pharmaceuticals. In these fields, research is very active in improving the properties of these materials by proposing new processes and technologies that can be "green technologies", as well. Indeed, organic solvents are process fluids currently used in these industries. They produce pollution not only of the process products, but also of the processed material and of the environment with relevant social and industrial costs. The supercritical fluids, instead, can produce new and improved products with new and advanced processes. Moreover, they have the advantage that they do not pollute the extracts, residues, and, in many cases, the environment. Supercritical fluids are characterized by a continuous adjustable solvent power/selectivity obtained by varying pressure and temperature. Therefore, the same supercritical solvent can be used in different sections of the plant to obtain different extraction/separation performances. At last, the diffusivity of supercritical fluids is similar to that of gases (they are gases, indeed); thus, it is about two orders of magnitude larger than that of liquid solvents. As a consequence, process times can be greatly reduced, and no wastes are produced.

Since the size and size distribution and sometimes even the morphology of particles produced in different industries are usually not appropriate for the subsequent use of those materials, particle design has been gaining increasing importance in manufacturing advanced ceramic materials, dyes, explosives, catalysts, coating materials, microsensors, polymers, pharmaceuticals, and many other chemicals. For example, when dealing with aerosol delivery of pharmaceuticals in the lungs by inhalers, a narrow size distribution of fine particles is necessary in order to maximize the efficiency of the drug, and hence to minimize the required dosage. This results in the decrease of side effects of the drug while maintaining the same therapeutic result. Another important reason for applying particle design is the increasing number of newly developed drugs that are poorly soluble in both aqueous and organic media. An alternative and promising approach is the production of micro- or nanoparticle pharmaceuticals with improved solubilities. This was explained extensively by Muller et al. Several conventional methods are currently in use for producing fine powders, including jet and ball milling, spray drying, and recrystallization using solvent evaporation or liquid anti-solvent. But all these techniques have in common the disadvantage of poor control of the size distribution of the particles, i.e., a wide range of particle sizes is usually produced. In addition, each method has its own specific disadvantages. For example, spray drying usually requires high operating temperatures, which may cause thermal degradation of sensitive materials such as the majority of food ingredients and pharmaceuticals. Solvent evaporation and liquid antisolvent recrystallization face solvent and anti-solvent residual problems. In the past two decades, many researchers have tried to solve these shortcomings of conventional techniques Of particle design by investigating the potential of SCF. In 2001, Jung and Perrut performed an extensive review on the different techniques available for particle

design using SCF. This study aims to continue that work, highlighting the most recent developments in the gas anti-solvent and other SCF techniques for particle design.

3.2 Rapid Expansion of Supercritical Solution (RESS)

Krukonis was the first scientist who ever tried to apply SCF, which had previously been used for extraction operations, for recrystallizing solid materials with the intention of producing fine particles with narrow size distributions. The most attractive features of SCF in this respect include enhanced solubility power compared to regular gases, sensitivity to small changes in either temperatures or pressures and fairly mild operating conditions. The technique proposed by Krukonis was named Rapid expansion of supercritical solutions (RESS). In the RESS process, the supercritical fluid acts as the solvent. The solid material to be micronized is first solubilized in the supercritical fluid using an extractor. Therefore, a supercritical solution is discharged from the extractor. If this solution is expanded via a nozzle and sprayed into an expansion vessel, precipitation of particles will occur, as the conditions are no longer supercritical. Fine particles with narrow size distribution can be produced at relatively low temperatures using the RESS process. However, a major limitation of this process is that the solubilities of many materials, especially pharmaceuticals, are usually very low in SCF. This makes RESS not attractive for industrial-scale productions of such low-soluble materials. But as a general rule, if the solute has a significant solubility in the SCF, the RESS process will be the first choice for particle design because of its simplicity. The rapid expansion of supercritical solutions (RESS) consists of saturating a supercritical fluid with a solid substrate, then this solution is depressurized through a heated nozzle into a low-pressure chamber in order to cause an extremely rapid nucleation of the substrate in the form of very small particles or fibers or films that are collected from the gaseous stream.

Most of the newly developed active pharmaceutical ingredients are poorly soluble or insoluble in aqueous media. Particle size reduction of such pharmaceuticals is one of the clue to improve the dissolution, absorption and subsequent bioavailability. Grinding and spray drying are the major techniques for the size reduction, however, heat- or mechanical stress-induced degradation of the material and residual organic solvent often limit the application, respectively. Furthermore, the resultant particle size distribution is usually broad and does not reach to the few micron or sub-micron level. Supercritical fluids have been used for particle size reduction in chemical, cosmetic and pharmaceutical industries. Rapid expansion of supercritical solutions (RESS), gas antisolvent (GAS), aerosol solvent extraction system (ASES) and solution enhanced dispersion of solids (SEDS) are known as the preparation methods of drug fine particles and among them, RESS method is the only way to prepare the powder without using organic solvents. In the RESS process, the solute is dissolved in a supercritical solvent and the supercritical solution is rapidly expanded through a nozzle. Rapid phase change from supercritical fluid to the gas state induces the high supersaturation of the solute and results in the formation of very small particles. As a supercritical fluid, carbon dioxide (CO_2) has commonly been used because of its mild critical temperature (304.2 K) and pressure (7.39MPa). Supercritical CO_2 is advantageous to the environment due to the non-toxic and easily recycled properties and to the application for heat-sensitive pharmaceuticals. The RESS method has been applied for polymer coating, microencapsulation and micronization. For the purpose of micronization, some active

pharmaceutical ingredients, such as salicylic acid, griseofulvin, ibuprofen, have been used. The mean particle size of the drugs obtained by the RESS technique drastically reduced to micron order. In some cases, the particle size reached to sub-micron, however, it was very difficult to keep the size because of the crystal growth and agglomeration simultaneously occurred with the fine particle formation. A unique approach utilizing RESS is preparation of polymorphs. Deoxycholic acid sand carbamazepine polymorphs would be examples which have been reported. A meta-stable form of deoxycholic acid was obtained by storing a sample in a vessel filled with CO2 at 12MPa, 60 °C.
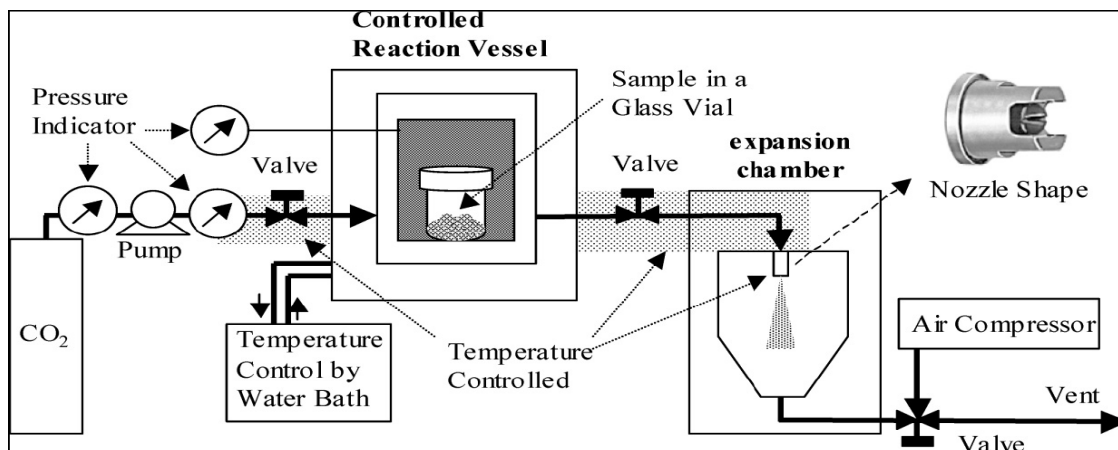

Figure 5: Rapid Expansion of Supercritical Solution (RESS) appratus

Apparatus and Settings: Supercritical fluid operating system based on the rapid expansion of supercritical solutions (RESS) method (SC sprayer®, Nikkiso, Co. Ltd., Japan) was schematically shown in Fig. 5. The setup consists of an extraction unit and a precipitation unit. The solvent CO2 was introduced to a temperature-controlled reaction vessel (internal volume: 90 ml) in the extraction unit by a pump NP-AX-403 (Nihon Seimitsu Kagaku Co., Ltd., Japan) up to a desired upper limit pressure (max. 29MPa). Because CO2 density varies depending on the temperature, lower limit of the pressure was adjusted to the values at which single-sprayed amounts of supercritical CO2 became a fixed amount (0.19 mol). After the extraction, definite amounts of sample/supercritical CO2 mixtures passed through a high-pressure stainless steel tubing and were expanded from a spray nozzle, which was composed of a tungsten carbide orifice UniJet flat spray tip (Spraying Systems Co., Japan), in the precipitation unit. Spraying period is less than 0.5 s. Temperature of the tubing and nozzle was usually the same as that of the extraction unit. At the precipitation unit, rapid phase change of sprayed supercritical CO2 into the gas state induced the high supersaturation of the solute and resulted in the formation of very small particles. The expansion chamber volume was 12 liter and the spraying distance from the nozzle to a 0.8 mm glass fiber prefilter (Millipore, MA, U.S.A.) placed on the bottom flange was 30 cm. The expanded gas was vented by using a compressor (Hitachi 0.75LP-7S ·T, Japan) and the vacuum flow rate was adjusted by an ejector cock to collect precipitated particles on the filter. The process parameter variables were optimized to obtain drug fine particles.

The use of supercritical fluids (SCF) is becoming increasingly important in pharmaceutical powder preparation processes, and in particular, the use of supercritical carbon dioxide. Carbon dioxide is commonly used as a supercritical fluid because it is non-toxic, non-flammable, and cheap. It has a low critical temperature and pressure (Tc = 31.1 ◦C and Pc = 73.8 bar) that allow for low temperature processing. In addition, carbon dioxide is a naturally occurring chemical that can be recycled from the atmosphere. From a pharmaceutical point of view, supercritical carbon dioxide has several advantages, including being solvent-free, and being able to be used in a single-stage process and at moderate processing temperatures. All of these are of advantage in protecting the environment, in industrial production, and in manufacturing heat-sensitive drugs. The rapid expansion of a supercritical solution technique, in which a solute drug is dissolved in supercritical carbon dioxide under high pressure, and then the resulting liquid is injected as a spray into an atmospheric chamber, is used to precipitate drugs after rapid decompression. The result is a decrease in particle size, enhanced drug solubility and dissolution rate for low solubility drugs, and drug bioavailability.
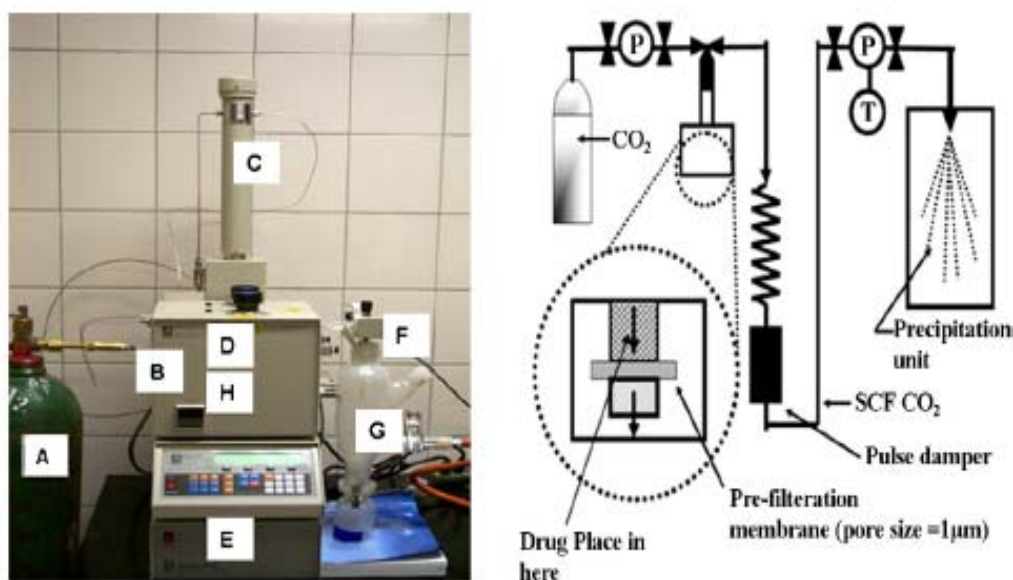

Figure 6:A picture and a schematic diagram of the carbon dioxide RESS apparatus used. Key: A=CO2 gas container; B= inlet valve; C= reducing union; D= extraction Chamber; E = pump; F = spray nozzle; G= collection chamber; and H= cartridge filtration membrane.

3.3 Particles from Gas-Saturated Solutions (PGSS)

The process of particles from gas-saturated solutions (PGSS) is another SCF process for particle design. In the PGSS process, a SCF or compressed gas is dissolved into a solution of a solute in a solvent or into a melted material. Then, a rapid depressurization of the mixture occurs through a nozzle, causing the formation of solid particles, or liquid droplets depending on the mixture and the conditions. Based on the principles of the PGSS process, a few other processes have been developed as well (CAN-BD/SAA/DELOS/CPCSP).[10]

3.4 CAN-BD: (CO2-ASSISTED NEBULIZATION WITH A BUBBLE DRYER®)

Supercritical or near-critical fluid processes for generating microparticles have enjoyed considerable attention in the past decade or so, with good success for substances soluble in supercritical fluids or organic solvents. In this review, we survey their application to the production of protein particles. A recently developed process known as CO2-assisted nebulization with a Bubble Dryer® (CAN-BD) has been demonstrated to have broad applicability to small-molecule as well as macromolecule substances (including therapeutic proteins). The principles of CAN-BD are discussed as well as the stabilization, micronization and drying of a wide variety of materials.[48] CAN-BD is a process patented by Sievers et al[48-53]. This invention covers two versions of the process, static and dynamic. The static version involves the pre-mixing of scCO2 and a solution containing a solute of interest at a pressure higher than the critical pressure of CO2. After equilibrium is established or approached, the mixture in a high pressure chamber is allowed to expand to atmospheric pressure through a flow restrictor (or a capillary tube) by expansion into a drying chamber.

The dynamic version involves continuous intimate mixing of a solution containing a solute of interest and scCO2 or near-critical CO2. In one version of this process, the two fluid streams become intimately mixed in a low dead volume tee and are then expanded through a flow restrictor to atmospheric pressure, where the plume of microbubbles and microdroplets are rapidly dried. This dynamic version of CAN-BD has been consistently, repeatedly and broadly successful in preparing protein particles that are usually stable, active and in the size range suitable for pulmonary delivery. This success has been achieved because the aqueous solution or suspension containing a protein or vaccine virus can be formulated to contain the appropriate stabilizers.

Recently, the CAN-BD process has been used to produce dry powders of live-attenuated measles vaccine virus (Edmonston- Zagreb) with good mechanical yield and with retention of viral activity as measured by a plaque forming unit assay that is comparable to commercial lyophilization[48,54-60]. CAN-BD has also been used to dry siRNA nucleotides[56.] Depending on formulation and laboratory processing conditions, typical lab scale yields range between 50% and 90%. In traditional spray drying, yield usually increases with scale, and the same may be realized for CAN-BD, in which droplet drying and particle collection is similar to traditional spray drying.

Principles of CAN-BD:

Unlike the anti-solvent processes, CAN-BD does not employ dense gases to achieve precipitation by solubility reduction of the solute(s) to be micronized. Rather they are used to enhance or facilitate the nebulization or aerosolization of a liquid solution, which is then rapidly dried to form particles by solvent removal. Organic or aqueous solutions are both readily processed by CAN-BD, although neither solvent type needs to be present for the processing of the other. CAN-BD is broadly applicable to the processing of aqueous protein solutions and therefore lends itself readily to studies undertaken to create dry solid formulations optimized for protein storage stability and retention of biological activity, and to develop such protein particles with morphology and size suitable for

pulmonary administration. They are here briefly described again. A liquid solution (organic or aqueous), typically containing 1% to 10% total dissolved solids, is brought into intimate contact with supercritical or near-critical CO_2 (usually at 1,200 to 1,500 psi and 20°C to 35°C, although a wide variety of conditions can be used) in a low dead volume tee. The resulting emulsion or solution mixture is rapidly expanded to near atmospheric pressure through a capillary flow restrictor, which is usually fused silica, stainless steel or PEEK with an inner of 50 to 175 µm and a length of 10 cm. upon expansion, the emulsion or solution forms a dense aerosol consisting of microdroplets and microbubbles. The aerosol is formed primarily due to the sudden physical dispersion of the liquid solution caused by the rapid expansion of compressed CO_2. Further break up of the microdroplets occurs due to the sudden release of any CO_2 that became dissolved in the liquid solution during intimate contact in the tee. At 1,000 to 2,000 psi, the solubility of CO_2 in water is about 2 to 2.5 mole % . The dense aerosol is delivered into a drying chamber (maintained at or near atmospheric pressure), into which preheated air or nitrogen gas is also delivered so as to maintain the chamber at a desired average drying temperature (typically 25°C to 65°C when processing aqueous protein solutions). Drying of an aerosol droplet is very fast. Adler and Lee[87] calculated that the total drying time in a Buchi spray-dryer (Tinlet=150°C, Toutlet=95°C) was less than 2 ms for a 8.6 µm droplet containing 10% (w/w) trehalose. In CAN-BD, the average residence time of a droplet/dry particle in the drying chamber has been estimated from chamber volume and flow rate calculations to be a few seconds .It should be noted that the droplet drying time will be shorter than the residence time. Microbubbles should dry even faster than microdroplets with the same diameter. In drying some substances by CAN-BD, hollow dry particles are formed. Dry particles are collected on a filter membrane, with pore sizes between 0.2 and 0.45 µm, located at the outlet of the drying chamber. CAN-BD can be operated as either a batch, semi-continuous, or continuous process. Typical flow rates on a lab-scale are 0.3 to 0.6 ml/min of liquid solution and 1 to 3 ml/min of dense CO_2. We have successfully scaled up CAN-BD to process up to 20 ml/min of liquid solution, and have more recently used flow rates as high as 30 ml/min, which is commercial production scale for high value pharmaceutical products. expansion, the emulsion or solution forms a dense aerosol consisting of microdroplets and microbubbles. The aerosol is formed primarily due to the sudden physical dispersion of the liquid solution caused by the rapid expansion of compressed CO_2. Further break up of the microdroplets occurs due to the sudden release of any CO_2 that became dissolved in the liquid solution during intimate contact in the tee. At 1,000 to 2,000 psi, the solubility of CO_2 in water is about 2 to 2.5 mole % . The dense aerosol is delivered into a drying chamber (maintained at or near atmospheric pressure), into which preheated air or nitrogen gas is also delivered so as to maintain the chamber at a desired average drying temperature (typically 25°C to 65°C when processing aqueous protein solutions). Drying of an aerosol droplet is very fast. Adler and Lee calculated that the total drying time in a Buchi spray-dryer (Tinlet=150°C, Toutlet=95°C) was less than 2 ms for a 8.6 µm droplet containing 10% (w/w) trehalose. In CAN-BD, the average residence time of a droplet/dry particle in the drying chamber has been estimated from chamber volume and flow rate calculations to be a few seconds .It should be noted that the droplet drying time will be shorter than the residence time. Microbubbles should dry even faster than microdroplets with the same diameter. In drying some substances by CAN-BD, hollow dry particles are formed. Dry particles are

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Organic solvents that are compatible with liquid carbon dioxide can be substituted in part or totally for water. Examples that the authors have used include ethanol, methanol, acetone, ethyl acetate and various mixtures of solvents, surfactants, buffers, stabilizers and other excipients. The solvent choice depends on the solubility and stability of the pharmaceutical to be micronized, and on the desired morphology and mean size of the particles.
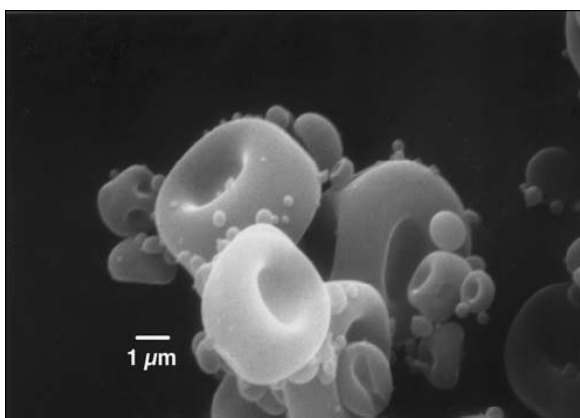

Figure 11: SEM image of particles of anti-CD4 antibody produced by CAN-BD at 50°C (Run A). SEM images obtained as described elsewhere.[79]
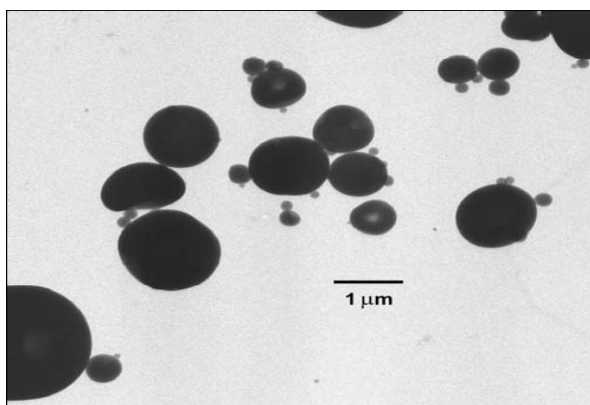

Figure12: SEM image of particles of anti-CD4 antibody produced by CAN-BD at 50°C (Run A). Particles were physically adhered to glow-discharged, carbon-coated, Form var coated copper grids by gently touching the activated side of the grid to the powder and then were visualized using a Philips CM 10 microscope operated at an accelerating voltage of 80 kV.
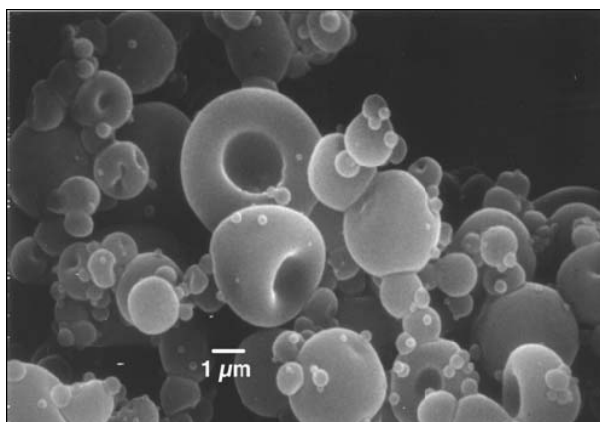
Figure13: SEM image of particles of anti-CD4 antibody produced by CAN-BD at about CAN-BD at about 30°C (Run B).

3.4 Supercritical Assisted Atomization: (SAS)

SAA is based on the solubilization of supercritical carbon dioxide in a liquid solution containing the drug; the ternary mixture is then sprayed through a nozzle, and microparticles are formed as a consequence of the enhanced atomization. SAA process parameters studied were precipitator temperature, nozzle diameter, and drug concentration in the liquid solution. Their influence was evaluated on morphology and size of precipitated particles. Spherical particles with mean particle size ranging from 1 to 3 μm of the new anti-asthma drug were produced by SAA. The mass median aerodynamic diameter (MMAD) of the SAA micronized particles and of the conventional jet-milled drug was used to compare the results obtainable using the 2 techniques. Particularly, MMADs from 1.6 to 4.0 μm were obtained by SAA at the optimum operating conditions and by varying the concentration of the solution injected. MMAD of 6.0 μm was calculated for the jet-milled drug. SAA samples also exhibited narrower particle size distribution (PSD). A good control of particle size and distribution together with no drug degradation was obtained by SAA process.

Supercritical Fluids (SFs) can take advantage of some specific properties of gases at supercritical conditions: a continuous adjustable solvent power/selectivity obtained varying pressure and temperature; diffusivities of 2 orders of magnitude larger than those of liquids are also obtainable. As a consequence, SFs can show very fast mass transfer and performances that cannot be obtained by conventional solvents. Mild operating conditions and solventless or organic solvent reduced operation are other advantages. Among all the possible SFs, carbon dioxide (CO_2) is largely used. It performs as a lipophilic solvent; it is nontoxic, nonflammable, and cheap; and its critical parameters are readily accessible on the industrial scale ($Tc = 37.1$°C; $pc = 73.8$ bar). For example, critical temperature is very near to the room temperature allowing the treatment of thermolabile compounds. Therefore, SFs have been proposed to develop improved and flexible micronization processes.

SAS process is based on drug solubilization in a liquid solvent; the solution is then sprayed in a high-pressure vessel containing the SF. The liquid solvent and the SF form a

solution, and the drug precipitates as microparticles. In PGSS, the drug is melted and saturated by SF, and then atomized obtaining microparticles. SAA process is based on the solubilization of a given percentage of supercritical CO2 in a liquid solution in which was previously dissolved the solute to be micronized. The solution is obtained in a high pressure vessel loaded with stainless steel perforated saddles that assures a large contact surface between liquid solution and SC-CO2. Then, the solution is atomized through a nozzle, and drug microparticles are obtained after the droplets are evaporated using warm nitrogen[94]. SAA process was successfully tested on various drugs such as erytromycin[95], rifampicin[96], tetracycline[96], terbutaline[97], and griseofulvin[98], and micronics particles with controlled size distributions were produced using water, methanol, or acetone, as liquid solvents. SAA process scale-up is now in progress. SAA process performance is particularly good in the particle size range appropriate for inhalable powders. Pharmaceutical companies therefore have shown interest in the preparation by SAA of new chemical entities, which are not easily prepared by traditional micronization techniques.

SAA Apparatus

SAA apparatus consisted of 2 high-pressure pumps (model 305, Gilson, Middleton, WI) that deliver the liquid solution and CO2 to a heated bath (Forlab model TR12, Carlo Erba, Milan, Italy) and then to the saturator. The saturator was a high pressure vessel (intense volume [IV] 50 cm3) loaded with stainless steel perforated saddles (specific surface area of ~10 m2/m3), which assures a large contact surface between liquid solution and CO2, allowing the dissolution of the gaseous stream in the liquid solution. Residence times in the saturator can vary from several seconds to minutes at the commonly adopted process conditions. The solution obtained in the saturator was sprayed through a 100-μm injector into the precipitator. Nitrogen was taken from a cylinder, heated in an electric heat exchanger (model CBEN 24G6, Watlow, St Louis, MO), and sent to the precipitator to assist liquid droplet evaporation. The precipitator was a stainless steel vessel (IV 3 dm3) operating at atmospheric pressure. The saturator and the precipitator were electrically heated using thin band heaters. A stainless steel frit at the bottom of the precipitator allowed the powder collection and the gaseous stream flow out. A condenser located after the precipitator was used to recover the liquid solvent.

Manometers, temperature controllers, and thermocouples complete the apparatus. The SAA layout is schematically reported in Figure 23. All the SAA experiments were performed in 2 replicates.
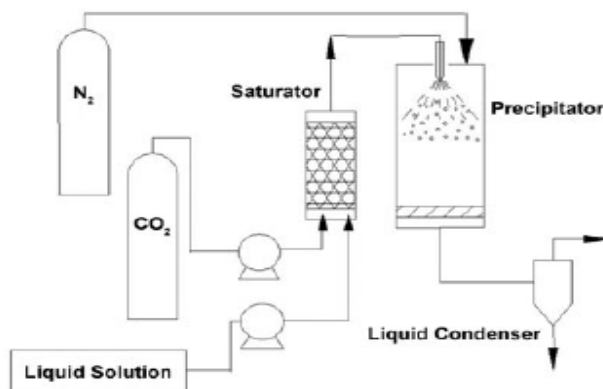

Figure 23. Schematic representation of the SAA apparatus.

Pharmaceutical aerosol delivery is undergoing dramatic changes in both inhaler device and formulation aspects. Particularly, there is a rapid move from traditional propellant driven metered-dose inhalers (MDIs) to the dry powder inhalers (DPIs). Powder drug inhalation does not depend on the use of chlorofluorocarbons as propellants, is less expensive, and does not require coordination between inhalation and device actuation. Recent improvements in the design, ease of use, and multidose capability, make DPIs attractive alternatives to pressurized MDIs (pMDIs) for aerosol therapy in ambulatory patients, especially for the therapeutic treatment of asthma and other bronchial diseases. Dispersed dry powders also generally have greater chemical stability than liquids used in atomizers.

Dry powders for aerosol delivery require a particle size distribution ranging between 1 and 5 μm to avoid impaction and/or sedimentation in the upper airways and with an optimum Mass Median Aerodynamic Diameter (MMAD) of 3 μm.Therefore, particle engineering using appropriate processes and excipients is required to produce particles of optimal size, morphology, and surface properties that would enhance aerosol efficiency. As a consequence, the preparation of drug microparticles with a controlled particle size distribution has become an important step for the development of aerosol delivery formulations.

Conventional methods used are jet-milling, spray-drying, and recrystallization. Several problems are associated with these processes. Some drugs are unstable under conventional jet-milling conditions; the particles would be jagged due to the jet-milling process and could hold electrostatic charges that impair their dispersion in an aerosol device. Thermally sensitive product can be degraded during the drying or can be contaminated by the solvent used during the crystallization process. Moreover, these methods would not provide an efficient control of the particle size; a broad particle size distribution is normally obtained.

SAA processes:

Reverchon tested the SAA process for producing some different kinds of compounds: superconductor and catalyst precursors, ceramics, and pharmaceutical compounds using some different solvents: water, methanol, and acetone. He micronized yttrium acetate, aluminium sulfate, zirconyl nitrate hydrate, sodium chloride, dexmethasone, carbamazepine, ampicillin, and triclabenzadol. Reverchon analysed the influence of the concentration of the liquid solution, kind of solvent and nozzle diameter on the particle size and particle size distribution of the SAA-produced powders. The process parameter that mainly controlled the particle size in the SAA process was the concentration of the liquid solution. Particle sizes ranged from 0.1 to 3 μm. Reverchon and Della Porta used the SAA process for producing nano- and microsized powders of some water-soluble and/or alcohol-soluble pharmaceutics. The same group applied the SAA process to micronize terbutaline from an aqueous solution. Reverchon and Della Porta used methanol as a solvent for producing rifampicin and water for producing tetracycline. Results show that the SAA process is a promising technique for producing controlled-size drug particles.

A Novel Technology for Microparticles Preparation of an Asthma-controlling Drug:

The aim of this work is to illustrate the results obtained micronizing by SAA the new chemical entity, HMR1031. This new drug was synthesized by Aventis Pharma and revealed a strong action as an asthma controlling drug during the preliminary in vitro and

in vivo tests; however, owing to its low decomposition temperature (~100°C), a partial drug degradation could be observed when micronization was attempted by conventional jet-milling and spray-drying. Moreover, particle size distributions outside the requested ranges were obtained by conventional milling.

The influence of some SAAprocess parameters onHMR1031 particles was also studied to evaluate the possibility of particle size tailoring. Micronized powders were characterized with respect to morphology, particle size, and particle size distribution. Drug degradation, solvent residue, and drug solid state were also monitored after SAA processing.[94]

Influence of the Drug Concentration and of the Nozzle Diameter on PSDs:

Systematic experiments were performed varying HMR1031 concentration in the methanol solution operating at the above reported optimized conditions. Particularly, HMR1031 concentration was varied from 50 to 150 mg/mL to explore the effect of this process parameter on the size and distribution of the precipitated powders. The morphology of HMR1031 particles obtained in all these runs was always spherical and noncoalescing. Some examples of the particles produced are shown in the SEM images reported in Figure 24 in which the experiments were performed at solute concentrations of 50 and 100 mg/mL, respectively. These SEM images were obtained with the same enlargement (20 K), therefore it is possible to make a qualitative evaluation of the broadening of particle size when the HMR1031 concentration in methanol is increased. PSDs of the SAA micronized drug were measured by laser diffraction and are reported in Figure 25, in terms of volumetric distributions in a cumulative form. The volume based particle size distributions enhance the contribution of the larger particles since the volume and not the diameter is the reported parameter. These distributions are the most relevant when a pharmaceutical compound is described, since the weight of the drug with a given particle size is the key parameter with respect to its therapeutic performance. The PSDs in Figure 3 indicate that HMR1031 microparticles produced at 50 mg/mL exhibit a D50 of 1.4 (± 0.1) μm and a D90 of 3.2 (± 0.2) μm; particles produced at 100 mg/mL have a D50 of 2.6 (± 0.1) μm and a D90 of 4.9 (± 0.2) μm, whereas microparticles produced at 150 mg/mL show a D50 of 3.6 (± 0.2) μm and a D90 of 5.4 (± 0.3) μm.

The PSD of a commercial jet-milled sample is also reported in Figure 3, for comparison purposes; an example of its morphology is illustrated in the SEM image reported in Figure 4. The size distribution of the commercial HMR1031 is wider with respect to the SAA micronized drug and nearly 50% of the milled powder is outside of the aerosol size range, as discussed in the Introduction. In particular, a D50 of 5.6 (± 0.2) μm and a D90 of 12.4 (± 0.4) μm was measured.

The PSDs were evaluated by laser diffraction method and were always in good agreement with the indications given by SEM images of the SAA micronized and jet-milled particles. However, SEM observations revealed that SAA micronized particles are spherical (see Figure 24) and can be hollow, as demonstrated in a previous work, whereas the jet-milled particles showed an irregular shape (see Figure 26)
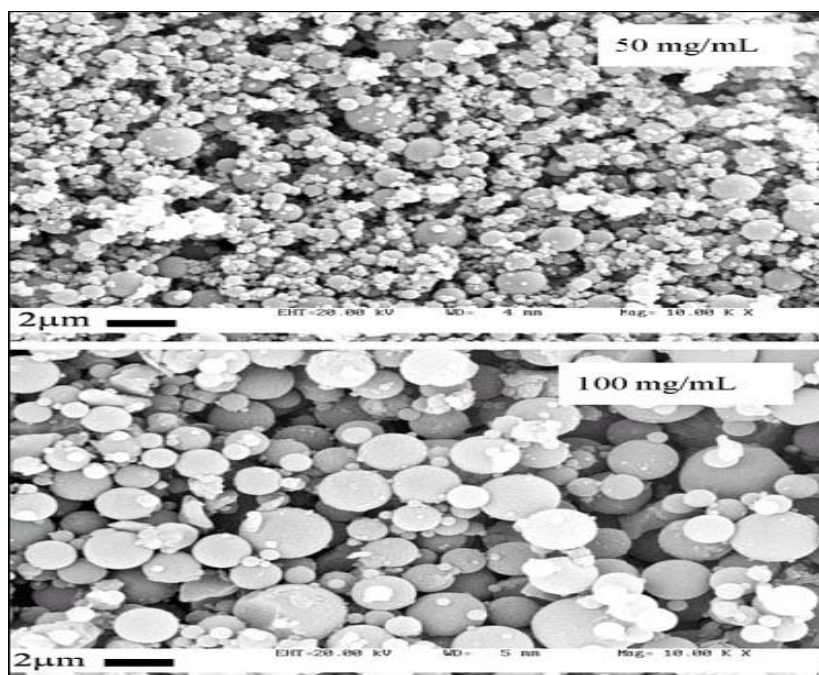
Figure 24. SEM images of HMR1031 precipitated by SAA from methanol operating at 80 bar, 80°C in the saturator and at a temperature of 50°C in the precipitator. The concentration of HMR1031 in the solution was 50 and 100 mg/mL, respectively. Both images are reported with a magnification of 10 K.
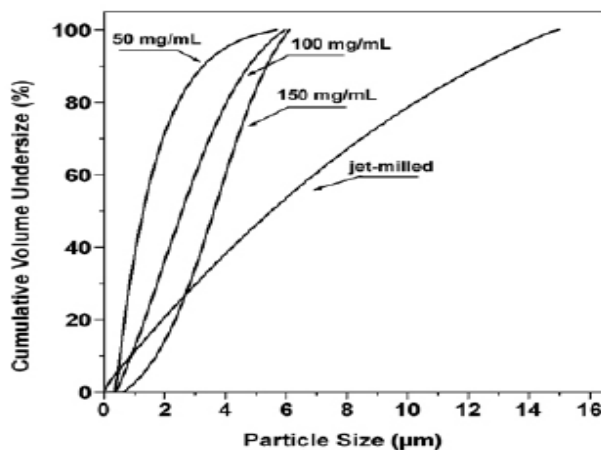

Figure 25. PSD curves of HMR1031 produced by SAA from methanol operating at 80 bar, 80°C in the saturator and at a temperature of 50°C in the precipitator. HMR1031 concentrations of 50, 100, and 150 mg/mL are reported. The PSD of the jet milled drug is also reported for comparison purposes.
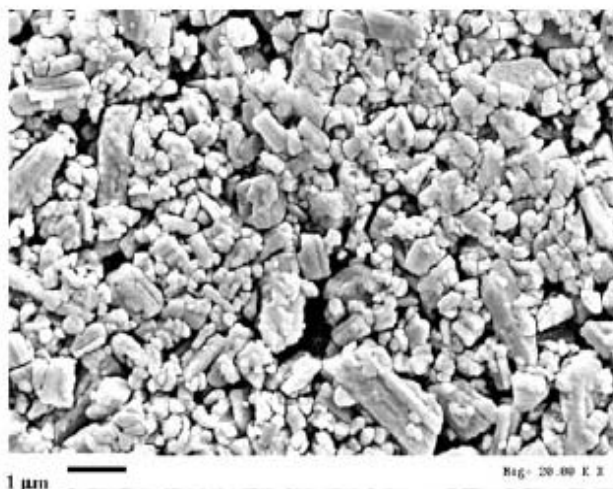
Figure 26. SEM image of jet-milled HMR1031.Magnification 20 K.[8]

SAA was successfully applied to a new chemical entity (HMR1031) that was problematic to process using traditional micronization techniques. The study of influence of drug concentration in the liquid solution on SAA performance revealed also the possibility of particle size tailoring depending on the requested target.

3.5 DELOS :(depressurization of an expanded liquid organic solution)
Recently, Ventosa et al[102-103]. proposed as a process the depressurization of an expanded liquid organic solution (DELOS). In the DELOS process, a compressed gas in an autoclave expands the liquid solution consisting of the solute to be micronized and a conventional solvent. At this stage, the compressed gas acts as a cosolvent, not as an anti-solvent. Therefore, no crystallization should occur at this stage, although the appearance of an undesired gas anti-solvent process is possible. The cosolvency effect of an anti-solvent has experimentally been shown in detail by Shariati and Peters for the system CO_2 + 1-propanol +salicylic acid. If this expanded solution of the ternary mixture of solute–solvent–compressed gas is depressurized by rapid reduction of the system pressure to atmospheric pressure in an expansion chamber, evaporation of the solution takes place resulting in a sharp decrease in solution temperature. As a consequence, a pronounced and homogeneous increase of supersaturation over all the solution takes place, which causes the precipitation of the solute as fine particles with narrow size distribution.

DELOS processes
Ventosa et al[102,103] tested the DELOS process for producing a colorant (1,4-bis-(n butylamino)-9,10- anthraquinone) powder from acetone solution using supercritical CO2. They could produce submicron and micron-sized particles. They also compared this process with the GAS process. The yield of the DELOS process was much higher than the GAS process.

3.6 CPCSP :(continuous powder coating spraying process)
The continuous powder coating spraying process (CPCSP) was proposed by Weidner et[105] al. as an alternative technique for the manufacture of powder coatings based on the PGSS process. This process is applicable to new coating materials which are low melting and fast-reacting components. In the CPCSP process, the main components (binder and

hardner) are melted in separate vessels in order to avoid a premature reaction of the polymers. The polymer melts are fed to a static mixer. In the static mixer, the melts are homogenized with compressed CO2 under a pressure up to 220 bar. The residence time is very short in the static mixer and also, due to the dissolved carbon dioxide, the melting point of the mixture decreases. Therefore, the temperature in the static mixer is set very low and reaction can be avoided. The solution formed in the mixer is expanded afterwards via a nozzle into a spray tower. Due to the senormous increase of the volume of the expanding CO2, the melt is atomized into fine droplets. Simultaneously, the droplets freeze and a fine powder coating is formed because of the temperature decrease in the spray tower caused by the expanding gas. With a blower, the gas is removed from the spray tower and by means of a cyclone and a filter the fine particles are separated from the gas. Weidner et al[105]. showed that it is possible to produce powder coatings with an average particle size of less than 40 μm, while manufacturing the coating powders using a conventional process results in particles larger than 40 μm.

CPCSP processes:

Weidner et al[105] showed the feasibility of the CPCSP process for producing powder coating particles by applying this process to micronizing low-melting polyester (melting temperature range: 80-90ºc). They studied the effect of temperature in the static mixer on the morphology and size distribution of the particles. They also showed the effect of pressure in the static mixer on the size distribution of particles.

3.11 Drug Delivery Applications of SCFs: -

(1) *Micro particles and Nanoparticles: -*

Drug and polymeric micro particles have been prepared using SCFs as solvents and antisolvents. Krukonis first used RESS to prepare 5- to 100-μm particles of an array of solutes including lovastatin, polyhydroxy-acids, and mevinolin. RESS process employing CO_2 was used to produce poly (lactic acid) (PLA) particles of lovastatin and naproxen. A GAS process was used to produce clonidine-PLA microparticles. In this process, PLA and clonidine were dissolved in methylene chloride, and the mixture was expanded by supercritical carbon dioxide to precipitate polymeric drug particles. SCF technology is now claimed to be useful in producing particles in the range of 5 to 2,000 nm. This patent covers a process that rapidly expands a solution of the compound and phospholipid surface modifiers in a liquefied-gas into an aqueous medium, which may contain the phospholipid. Expanding into an aqueous medium prevents particle agglomeration and particle growth, thereby producing particles of a narrow size distribution . However, if the final product is a dry powder, this process requires an additional step to remove the aqueous phase. Intimate mixture under pressure of the polymer material with a core material before or after SCF salvation of the polymer, followed by an abrupt release of pressure, leads to an efficient solidification of the polymeric material around the core material. This technique was used to microencapsulate infectious Bursal Disease virus vaccine in a polycaprolactone (PCL) or a poly (lactic-co-glycolic acid) (PLGA) matrix.

2) Micro porous Foam: -

Using SCF technique, Hile et al prepared porous PLGA foams capable of releasing an angiogenic agent, basic fibroblast growth factor (bFGF), for tissue engineering applications. These foams sustained the release of the growth factor. In this technique, a homogenous water-in-oil emulsion consisting of an aqueous protein phase and an organic polymer solution was prepared first. This emulsion was filled in a longitudinally

sectioned and easily separable stainless steel mold. The mold was then placed into a pressure cell and pressurized with CO_2 at 80 bars and 35°C. The pressure was maintained for 24 hours to saturate the polymer with CO_2 for the extraction of methylene chloride. Finally, the set-up was depressurized for 10 to12 seconds, creating micro porous foam.

3) Liposome: -

Liposomes are useful drug carriers in delivering conventional as well as macromolecular therapeutic agents. Conventional methods suffer from scale-up issues, especially for hydrophilic compounds. In addition, conventional methods require a high amount of toxic organic solvents. These problems can be overcome by using SCF processing. Fredereksen et al developed a laboratory scale method for preparation of small liposomes encapsulating a solution of FITC-dextran, a water-soluble compound using supercritical carbon dioxide as a solvent for lipids. In this method, phospholipid and cholesterol were dissolved in supercritical carbon dioxide in a high-pressure unit, and this phase was expanded with an aqueous solution containing FITC in a low-pressure unit. This method used 15 times less organic solvent to get the same encapsulation efficiency as conventional techniques. The length and inner diameter of the encapsulation capillary influenced the encapsulation volume, the encapsulation efficiency, and the average size of the liposomes. Using the SCF process, liposomes, designated as critical fluid liposomes (CFL), encapsulating hydrophobic drugs, such as taxoids, camptothecins, doxorubicin, vincristine, and cisplatin, were prepared. Also; stable paclitaxel liposomes with a size of 150 to 250 nm were obtained. Aphios Company's patent (US Patent No. 5,776,486) on SuperFluidsTM CFL describes a method and apparatus useful for the nanoencapsulation of paclitaxel and campothecin in aqueous liposome formulations called TaxosomesTM and CamposomesTM, respectively. These formulations are claimed to be more effective against tumors in animals compared to commercial formulations.

4) Inclusion complexes: -

Inclusion complexes with cyclodextrins. For many nonpolar drugs, previously established inclusion complex preparation methods involved the use of organic solvents that were associated with high residual solvent concentration in the inclusion complexes. Earlier, cyclodextrins were used for the entrapment of volatile aromatic compounds after supercritical extraction. Based on this principle, Van Hees et al employed supercritical fluids for producing piroxicam and ß-cyclodextrin inclusion complexes. Inclusion complexes were obtained by exposing the physical mixture of piroxicam-ß-cyclodextrin (1:2.5 mol-mol) to supercritical CO_2 and depressurizing this mixture within 15 seconds. Greater than 98.5% of inclusion was achieved after 6 hours of contact with supercritical CO_2 at 15 MPa and 150°C.

5) Solid Dispersions: -

SCF techniques can be applied to the preparation of solvent-free solid dispersion dosage forms to enhance the solubility of poorly soluble compounds. Traditional methods suffer from the use of mechanical forces and excess organic solvents. A solid dispersion of carbamazepine in polyethylene glycol 4000 (PEG4000) increased the rate and extent of dissolution of carbamazepine. In this method, a precipitation vessel was loaded with solution of carbamazepine and PEG4000 in acetone, which was expanded with supercritical CO_2 from the bottom of the vessel to obtain solvent-free particles.

6) *Powders of Macromolecules: -*

Processing conditions with supercritical CO2 are benign for processing macromolecules, such as peptides, proteins, and nucleic acids. Debenedetti0 et al used an antisolvent method to form microparticles of insulin and catalase. Protein solutions in hydroethanolic mixture (20:80) were allowed to enter a chamber cocurrently with supercritical CO_2. The SCF expanded and entrained the liquid solvent, precipitating sub micron protein particles. Because proteins and peptides are very polar in nature, techniques such as RESS cannot be used often. Also, widely used supercritical antisolvent processing methods expose proteins to potentially denaturing environments, including organic and supercritical nonaqueous solvents, high pressure, and shearing forces, which can unfold proteins, such as insulin, lysozyme, and trypsin, to various degrees. This led to the development of a method, wherein the use of the organic solvents is completely eliminated to sobtain fully active insulin particles of dimensions, 1.5-500 µm. In this invention, insulin was allowed to equilibrate with supercritical CO_2 for a predetermined time, and the contents were decompressed rapidly through a nozzle to obtain insulin powder. Plasmid DNA particles can also be prepared using SCFs. An aqueous buffer (pH 8) solution of 6.9 KB plasmid DNA and mannitol was dispersed in supercritical CO_2 and a polar organic solvent using a three-channel coaxial nozzle. The organic solvent acts as a precipitating agent and as a modifier, enabling nonpolar CO_2 to remove the water. The high dispersion in the jet at the nozzle outlet facilitated rapid formation of dry particles of small size. Upon reconstitution in water, this plasmid DNA recovered 80% of its original super coiled state. Such macromolecule powders can possibly be used for inhalation therapies.

7) *Coating:* -

SCFs can be used to coat the drug particles with a single or multiple layers of polymers or lipids. A novel SCF coating process that does not use organic solvents has been developed to coat solid particles (from 20 nm to 100 µm) with coating materials, such as lipids, biodegradable polyester, or polyanhydride polymers. An active substance in the form of a solid particle or an inert porous solid particle containing active substance can be coated using this approach. The coating is performed using a solution of a coating material in SCF, which is used at temperature and pressure conditions that do not solubilize the particles being coated.

8) *Product Sterilization: -*

In addition to drug delivery system preparation, SCF technology can also be used for other purposes, such as product sterilization. It has been suggested that high-pressure CO_2 exhibits microbicidal activity by penetrating into the microbes, thereby lowering their internal pH to a lethal level. The use of supercritical CO_2 for sterilizing PLGA microspheres (1, 7, and 20 µm) is described in US Patent No. 6,149,864. The authors indicated that complete sterilization can be achieved with supercritical CO_2 in 30 minutes at 205 bar and 34°C.

9) *Particulate Dosage Forms: -*

Some gases at certain pressures cause swelling of polymers likpolypropylene, polyethylene, and ethylene-vinyl acetate co-polymer and ethylene ethyl acrylate copolymer or drug carriers, and allow migration of active material in polymer matrix to give diffusion-controlled drug delivery systems. This specific behavior can be exploited for various purposes replacing the traditional techniques like Spray-drying, solvent evaporation and freeze-drying. This approach can be utilized as a solvent-free approach

to develop novel, controlled-release dosage forms and deposit thermo labile materials such as peptide drugs into the polymers.

In Pharmaceutical Industries: -

1) Medium for Crystallization: -

To generate high purity polymorphs, even with some morphological viz. high degree of Enantiomeric enrichment. SF technology appears to be a potential modality. Moreover, size and shape of the polymorph can be manipulated by controlling temperature and/or pressure during processing while degree of crystallization can be improved by manipulating the rate of crystallization & high degree of crystallinity. Better candidate in metered dose inhaler compared with conventionally crystallized and micronized drug.

2) Solubilization of pharmaceuticals: -

RESS technology has been used. Most of pharmaceutical compounds below 60 c and 300 bars showed a considerable higher solubility. In many a process of solubilization of polar or non-volatile compounds a limited solubility in SC CO_2 is fails to form a homogenous solution under practical conditions. To aid the solubilization in such cases the CO_2-philic solubilizers are being developed which rather the SC CO_2 insoluble substances and make them solubilize in SC CO_2.

3) Extraction and Purification: -

Supercritical fluid extraction technique could be utilized to separate impurities mainly organic complexes from the pharmaceuticals. Methods developed by Zoel are now widely used in industry as in caffeine production & Isolation of Taxol from the bark of the Taxus brevifolia in which SC CO_2 is used. Purification via SCF technology gives a better alternative to all conventional purification methods as it is almost automated, quick, high yielding, SCF methods are also reported for the extraction of bryostatins, natural products, production of fat free products.

4) Medium for Polymerization and Polymer Processing: -

Supercritical fluids mainly SC CO_2 is rapidly becoming an alternative solvent for polymerization. Solubility plays a very important role in the synthesis of polymers. Mainly two processes used 1) Step growth: SC CO_2 has been reported very yielding in the production of polycarbonates, polymides, polyesters, polypyrrols, polyphenoxides and silica gels. 2) Chain growth: free radical polymerization of styrenics, armlets and methacrylates, cationic polymerization of isobutylene. Supercritical CO_2 in polymerization is increased plasticization because of CO_2. The highly plasticized state of polymers is also results in increased polymerization rates by the enhanced diffusion of monomer into the polymer .

5) As a Supercritical Bio-catalyst: -

Randolph et al primarily found the enzyme alkaline phosphates active in a batch reaction system yhat employed SC CO_2 as solvent. In the comparison SC CO_2 as the adverse effect of pressure was less profound in case of compressed propane and ethane. Nakamura et al studied the acidolysis of trioline with stearic acid in SC CO_2 by using Lipase as a bio-catalyst .

6) Micronization of Pharmaceuticals: -

The RESS process has been shown to be capable of forming micron-sized particals. Krukonics, first extensively studied RESS in micronization of a wide variety of materials, including pharmaceuticals, biologicals, and polymers . He produced uniform submicron powder of estradiol. Loth and Hemgesberg studied the micronization of phenacetin by

RESS and compared with jet-milled phenacetin. The main limitation of RESS is the inability to process those materials which are insoluble or very less soluble in the SCF.So for this materials the SAS process has been successfully used to produced micron sized particles like insuline, bovine liver catalase, lysozyme, trypsin, methylprednisolone and hydrocortisone acetate. Insuline were in two crystalline forms; spheroidal (smaller than 1 micron) and needle (5 micron). ASES process has been studied for the preparation of a range of steroids for pulmonary delivery.

The special properties of SCFs bring certain advantages to chemical separation technique. Several applications have been fully developed and commercialized which include food and flavouring, pharmaceutical industry, inviormental protection for volatile and lipid soluble compounds, extraction of high value oils, extraction of natural aromas, recovery of aromas form fruits, meat and fish, isolaltion of lipid soluble compounds .

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