8

The Encapsulation Art: Scale-up and Applications

M.A. Galán, C.A. Ruiz and E.M. Del Valle

8.1 Control Release Technology and Microencapsulation

Controlled release technologies are invaluable scientific tools for improving the performance and safety of chemicals. They involve materials such as barriers surrounding active materials to deliver the latter at the optimum time and rate needed[1,2].

The technical objective of this science is to find and use judiciously barriers, usually specially designed polymers (but may include adsorption inorganics or complexing with certain chemicals). Such formulations have also provided drug manufacturers, in particular, with a method for the measured, slow release of drugs as well as a business tactic for extending patent life and usefully differentiating their product. Methods include the following[1,2]:

- Designing the barrier surrounding the active chemical so as to change its permeability for the extraction and thus provide a tortuous path. Means include designing the barrier material to swell or slowly dissolve in the extracting fluid.
- Selection of an inorganic material which will adsorb the active material within its layered or porous structure, thus again providing a torturous path for the extracting fluid.
- Designing a chemical which will complex the active material and release it at a controlled rate under the right environmental conditions.
- Controlling the chemistry of the active material itself to release only under certain environmental conditions.

The application may require release in different ways: (a) constant release over time; (b) release rate diminishing with time; and (c) 'burst release', where all of the active

Chemical Engineering: Trends and Developments. Edited by Miguel A. Galán and Eva Martin del Valle
 ISBN 0-470-02498-4 (HB)

material is released suddenly at a particular time, such as after the drug has passed through the stomach into the intestines. Designing the barrier makes use of the dissolving rates or swelling rates of the barrier polymer. In turn, the dissolving or swelling rates and permeability may depend on the pH, moisture, and temperature of the environment, chemical properties of the encapsulating polymer, its size, shape, and thickness.

Microencapsulation is an important sub-category of controlled release technology. Active materials are encapsulated in micrometer-sized capsules of barrier polymers designed to control the rate of release of the active materials that are encapsulated. The term 'microencapsulation' is often confused with 'controlled release' but the latter is much more inclusive, as indicated above[1,3].

8.2 The Microcapsule

In its simplest form, a microcapsule consists of a small ball surrounded by a homogeneous coating. The material enclosed in the microcapsule is called[4]:

- *Core*, or *nucleus*, internal phase, encapsulated, active substance, etc.

The coating is also called:

- *Shell*, envelope, external phase, membrane.

Although this is the most common type, there are several other kinds of microcapsules, depending on the technology used in their production. Their size usually varies from 1 to 2000 μm.

Capsules smaller than 1 μm are called nano-capsules because their size is measured in nanometers. When the core and the coating are not really separated, the microcapsule, or nanocapsule, is called microparticle, or nanoparticle.

The architecture of microcapsules is generally divided into several arbitrary and overlapping classifications. One such design is known as matrix encapsulation. Where the matrix particle resembles that of a peanut cluster. The core material is buried to varying depths inside the wall material. The most common type of microcapsule is that of a spherical or reservoir design. It is this design that resembles a hen's egg. It is also possible to design microcapsules that have multiple cores which may be an agglomerate of several different types of microcapsules[1,3,5]. If the core material is an irregular material, such as occurs with a ground particle, then the wall will somewhat follow the contour of the irregular particle and one achieves an irregular microcapsule. The last well-known design for a microcapsule is that of a multiple wall. In this case, multiple walls are placed around a core to achieve multiple purposes related to the manufacture of the capsules, their subsequent storage and controlled release.

8.2.1 Properties of the Core and the Capsule

The core, which is the substance to microencapsulate, can have different characteristics. It may belong to various categories of chemical substances. It can be liquid or solid,

acid or basic, in powder or rough crystals. In addition to the requirement to induce microencapsulation of certain substances, the choice of the microencapsulation method and the coating material also depends on the characteristics of the active principle[6,7].

The choice of the coating material (capsule) often depends on the purpose, or the purposes, of microencapsulation. Not all membranes are able to confer specific properties onto the microencapsulated product. The choice of the right coating is often crucial in achieving the microencapsulation purpose. Some one hundred suitable substances have been described (and already used) to form a microcapsule film. The most commonly used substances are collected in Table 8.1.

Therefore, the first process consists of forming a wall around the core material. The second process involves keeping the core inside the wall material so that it does not release. Also, the wall material must prevent the entrance of undesirable materials that may harm the core. And finally, it is necessary to release the core material at the right time and at the right rate.

Microencapsulation is like the work of a clothing designer. He selects the pattern, cuts the cloth, and sews the garment in due consideration of the desires and age of his customer, plus the locale and climate where the garment is to be worn. By analogy, in microencapsulation, capsules are designed and prepared to meet all the requirements in due consideration of the properties of the core material, intended use of the product, and the environment of storage.

In a discussion of microencapsulation technology, particularly when one is talking about quantities and cost, it is necessary to understand that encapsulation is a volume process, independent of the density or value of the core material. Thus, microencapsulators frequently state that it is just as expensive on a volume basis to encapsulate diamond as graphite. Likewise, on a volume basis it is just as expensive to encapsulate paraffin wax as tungsten metal. Also, when experimenting with or acquiring microcapsules, it should be emphasized that it is necessary to use common, consistant terminology because of the preference for discussing microcapsules in terms of the core material, particularly when one is discussing the cost of production[7–9].

8.2.2 Microcapsules Uses

The uses of microcapsules since the initial coacervation work in the 1940s are many and varied. A good early review of these uses that also includes pharmaceuticals and

Table 8.1 *Materials used as coating material*

• *Agar*	• *Albumin*
• *Cellulose and its derivatives*	• *Gelatin*
• *Arabic gum*	• *Hydrogenated fats*
• *Glutens*	• *Glycerides*
• *Polyamides*	• *Acrylic polymers*
• *Polyesters*	• *Polyvinyl pyrrolidone*
• *Polyethylene glycols*	• *Polystyrene*
• *Starch*	• *Stearic acid*
• *Paraffins*	• *Waxes*
• *Polyvinyl, myristic, stearyl alcohols, etc.*	• *Others*

agricultural materials is provided by Gutcho[6]. The uses of microcapsules that are of interest here include the following[10]:

1. Reduce the reactivity of the core with regard to the outside environment, for example oxygen and water.
2. Decrease the evaporation or transfer rate of the core material with regard to the outside environment.
3. Promote the ease of handling of the core material:
   a. prevent lumping;
   b. position the core material more uniformly through a mix by giving it a size and outside surface matching the remainder of the materials in the mix;
   c. convert a liquid to a solid form; and
   d. promote the easy mixing of the core material.
4. Control the release of the core material so as to achieve the proper delay until the right stimulus.
5. Mask the taste of the core.
6. Dilute the core material when it is only used in very small amounts; but, achieve uniform dispersion in the host material.

8.2.3 Release Mechanisms

A variety of release mechanisms have been proposed for microcapsules; but, in fact, those that have actually been achieved and are of interest here are rather limited. These are as follows:

1. A compressive force in terms of a 2 point or a 12 point force breaks open the capsule by mechanical means.
2. The capsule is broken open in a shear mode such as that in a Waring blender or a Z-blade type mixer.
3. The wall is dissolved away from around the core such as when a liquid flavoring oil is used in a dry powdered beverage mix.
4. The wall melts away from the core releasing the core in an environment such as that occurring during baking.
5. The core diffuses through the wall at a slow rate due to the influence of an exterior fluid such as water or by an elevated temperature[1–4].

8.2.4 Release Rates

The release rates that are achievable from a single microcapsule are generally ‘0’ order, 1/2 order, or 1st order. ‘0’ order occurs when the core is a pure material and releases through the wall of a reservoir microcapsule as a pure material. The 1/2-order release generally occurs with matrix particles. 1st-order release occurs when the core material is actually a solution. As the solute material releases from the capsule the concentration of solute material in the solvent decreases and a 1st-order release is achieved. Please note that these types of release rates occur from a given single microcapsule. A mixture of microcapsules will include a distribution of capsules varying in size and wall thickness. The effect, therefore, is to produce a release rate different from ‘0’, ‘1/2’, or ‘1’ because of the ensemble of microcapsules. It is therefore very desirable to examine carefully on

an experimental basis the release rate from a collection of microcapsules and to recognize that the deviation from theory is due to the distribution in size and wall thickness[9,11].

8.2.5 Microcapsule Formation

The general technology for forming microcapsules is divided into two classifications known as physical methods and chemical methods. The physical methods are generally divided into the following[1–3,5,6]:

Spray coating

Pan coating: This is a mature, well-established technology initially patented by a pharmacist in the 19th century by the name of Upjohn. Generally, it requires large core particles and produces the coated tablets that we are familiar with.
Fluid bed coating: One version of this coating is known as Wurster coating and was developed in the 1950s and 1960s. The Wurster coater relies upon a bottom-positioned nozzle spraying the wall material up into a fluidized bed of core particles. Another version sprays the wall material down into the core particles.

Annular jet. This technology was developed by the Southwest Research Institute and has not been extensively used in the food industry. It relies upon two concentric jets. The inner jet contains the liquid core material. The outer jet contains the liquid wall material, generally molten, that solidifies upon exiting the jet. This dual fluid stream breaks into droplets much as water does upon exiting a spray nozzle.

Spinning disk. A new method was developed by Professor Robert E. Sparks at Washington University in St Louis. This method relies upon a spinning disk and the simultaneous motion of core material and wall material exiting from that disk in droplet form. The capsules and particles of wall material are collected below the disk. The capsules are separated from the wall particles (chaff) by a sizing operation.

Spray cooling. This is a method of spray cooling a molten matrix material containing minute droplets of the core materials. This method is practiced by the Sunkist Company.

Spray drying. Spray dryers can be used from small to very high productions depending on their design. They can reach evaporative capacities of up to 15 000 lb/h. Even though the cost of the equipment is expensive, the cost of maintenance is low due to the small number of moving parts and the use of resistant materials. The purity of the product will be maintained since the food particles do not have any contact with the surface of the equipment until they are dried, minimizing problems in sticking and corrosion. The simple operating system and the cleaning conditions for spray dryers contribute to the low labor cost. Another advantage of using the spray drying method is that a low bulk density of the product can be obtained.

Spray chilling. This is a process of spray chilling the wall around an atomized core. The resulting capsules move countercurrent to a flow of tempered air and are collected in a large container below the spray nozzle. It is practiced currently by the Durkee Company.

Co-extrusion processes. Liquid core and shell materials are pumped through concentric orifices, with the core material flowing in the central orifice, and the shell material flowing through the outer annulus. A compound drop forms that is composed of a droplet of core fluid encased in a layer of shell fluid.

The centrifugal nozzle technique was developed by SwRI. It requires that, initially, both the core and the wall are pumpable liquids (or thin slurries). Both fluids are fed into a special nozzle so that a coaxial stream is formed at the nozzle. This nozzle is spun rapidly, which stretches out the liquid ligaments and breaks off individual droplets. Surface tension pull the 'shell' material around the core droplet to form a complete covering. The wall material must be selected so that it will solidify before the particle is collected.

Use of supercritical fluids. Supercritical fluid (SCF) technology is now considered as a very innovative and promising way to design particles, especially for therapeutic drug formulation [12].

The advantages of SCF technology include use of mild conditions for pharmaceutical processing (which is advantageous for labile proteins and peptides), use of environmentally benign nontoxic materials (such as CO_2), minimization of organic solvent use, and production of particles with controllable morphology, narrow size distribution, and low static charge[12].

SCF technology is making in-roads in several pharmaceutical industrial operations including crystallization, particle size reduction, and preparation of drug delivery systems, coating, and product sterilization. It has also been shown to be a viable option in the formulation of particulate drug delivery systems, such as microparticles and nanoparticles, liposomes, and inclusion complexes, which control drug delivery and/or enhance the drug stability.

The number of methods for *chemical encapsulation*[1–5] is actually far less. They are necessary because they are very effective in encapsulating liquids and small core sizes. In particular, it is possible to encapsulate flavors and fragrances down to 10 μm in size.

1. Coacervation: This is a term borrowed from colloid chemistry to describe the basic process of capsule wall formation. The encapsulation process was discovered and developed by colloid chemist Barrett K. Green of the National Cash Register (NCR) Corporation in the 1940s and 1950s. Actually, coacervative encapsulation (or microencapsulation) is a three-part process: *particle or droplet formation*; *coacervative wall formation*; and *capsule isolation*. Each step involves a distinct technology in the area of physical chemistry. The first coacervative capsules were made using gelatin as a wall in an 'oil-in-water' system. Later developments produced 'water-in-oil' systems for highly polar and water-soluble cores.

   Simple coacervation involves the use of either a second more water-soluble polymer or an aqueous non-solvent for the gelatin. This produces the partial dehydration/desolvation of the gelatin molecules at a temperature above the gelling point. This results in the separation of a liquid gelatin-rich phase in association with an equilibrium liquid (gelatin-poor), which under optimum separation conditions can be almost completely devoid of gelatin.

   Complex coacervation was conceived in 1930s, B.K. Green, a young chemist just out of school, was intrigued by the dearth of information in the collaid field of liquids

dispersed in solids. It was the first process used to make microcapsules for carbonless copy paper. In complex coacervation, the substance to be encapsulated is first dispersed as tiny droplets in an aqueous solution of a polymer such as gelatin. For this emulsification process to be successful, the core material must be immiscible in the aqueous phase. Miscibility is assessed using physical chemistry and thermodynamics. The emulsification is usually achieved by mechanical agitation, and the size distribution of the droplets is governed by fluid dynamics.

2. Organic phase separation: Sometimes, this technique is considered as a reversed simple coacervation; a polymer phase separates and deposits on a 'core' that is suspended in an organic solvent rather than water.
3. Solvent evaporation: A polymer is dissolved in a volatile solvent. The active material is then suspended in this fluid. The mixture is added to carrier, and the solvent is evaporated, precipitating the polymer on the active and forming microspheres.
4. Interfacial polymerization: Includes a number of processes in which a wall is formed from monomers at the interface of a core and the suspension medium.

8.2.6 Use of Supercritical Fluids for Particle Engineering

In recent years, the crystal and particle engineering of pharmaceutical materials and drug delivery systems with SCF technology has gained momentum due to the limitations of conventional methods[13–19]. This technology offers the advantage of being a one-step process, and appears to be superior to other conventional incorporation methods such as emulsion evaporation methods[17–21].

Application of SCF is now the subject of increasing interest especially in the pharmaceutical industry and there are three aims[16–18]: increasing bioavailability of poorly soluble molecules; designing sustained-release formulations; and formulation of active agents for new types of drug delivery that are less invasive than parental delivery (oral, pulmonary, transdermal). The most complex challenge is related to therapeutic delivery, as it is extremely difficult to obtain a satisfactory therapeutic delivery effect due to biomolecule instability and very short half-life *in vivo*.

We will describe the general SCF techniques used for particle engineering, examples of drug delivery systems prepared with SCF processes, and factors influencing the characteristics of SCF products, and scale-up issues associated with SCF processes[22,23].

8.3 Supercritical Fluids

At the critical temperature (T_c) and pressure (P_c), a substance's liquid and vapor phases are indistinguishable. A substance whose temperature and pressure are simultaneously higher than at the critical point is referred to as a supercritical fluid (Figure 8.1). Of particular interest for SCF application are the ranges $1 < T/T_c < 1.1$ and $1 < P/P_c < 2$[24]. In this region, the SCF exists as a single phase with several advantageous properties of both liquids and gases. The physical and thermal properties of SCFs fall between those of the pure liquid and gas. SCFs offer liquid-like densities, gas-like viscosities, gas-like compressibility properties, and higher diffusivities than liquids. The properties of SCFs, such as polarity, viscosity, and diffusivity, can be altered several-fold by varying the operating temperature and/or pressure during the process. This flexibility enables

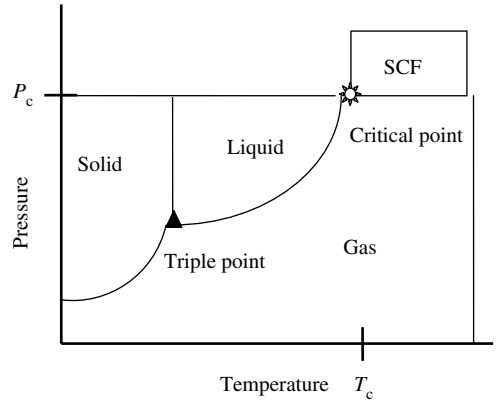

Figure 8.1 *Pressure–temperature phase diagram for a pure component*

the use of SCFs for various applications in the pharmaceutical industry, with the drug delivery system design being a more recent addition. Commonly used supercritical solvents include carbon dioxide, nitrous oxide, ethylene, propylene, propane, *n*-pentane, ethanol, ammonia, and water. Of these, CO_2 is a widely used SCF in the pharmaceutical processing due to its unique properties[21]:

- Behaves like a hydrocarbon solvent. An excellent solvent for aliphatic hydrocarbons with an estimated 20 carbons or less and for most aromatic hydrocarbons. Cosolvents, such as methanol and acetone, enhance the solubility of polar solutes in CO_2. Organic solvents, such as halocarbons, aldehydes, esters, ketones, and alcohols, are freely soluble in supercritical CO_2.
- Allows the processing of thermolabile compounds due to its low critical temperature.
- Does not react strongly (chemically) with many organic compounds.
- Can be used as solvent or antisolvent.
- Diffusion coefficients of organic solvents in supercritical CO_2 are typically one to two times higher than in conventional organic solvents.
- Easy to recycle at the end of the process.
- Nontoxic, noninflammable, and inexpensive.

8.4 Engineering Particle

Particle formation by supercritical methods is emerging as a viable platform technology for pharmaceuticals and drug delivery systems. Several requirements should be considered for an ideal particle-formation process:

- Operates with relatively small quantities of organic solvent(s).
- Molecular control of the precipitation process.
- Single-step, scalable process for solvent-free final product.
- Ability to control desired particle properties.
- Suitable for a wide range of chemical types of therapeutic agents and formulation excipients.

- Capability for preparing multi-component systems.
- Good manufacturing performance (GMP) compliant process.

SCF processing is recognized as achieving many of these objectives, particularly with recent developments in the scale of operation[25]. However, although move literature is appearing, fundamental mechanistic understanding of the SCF solvent and antisolvent processes is in its infancy[26–28]. Studies in progress that couple computational fluid dynamics with advanced laser-based 'real-time' particle imaging techniques under supercritical conditions, such as particle imaging velocimetry[29], will undoubtedly improve basic knowledge, process design, and define the boundaries and limitations of SCF particle-formation processes. Indeed, several recent reports have highlighted situations where specific particle design and crystal engineering targets have not been completely met[30,31]. Improved understanding of the complex interplay of the rapid physical, chemical, and mechanical processes taking place during particle formation by SCF techniques will help resolve such situations. Nevertheless, major benefits for SCF processing from the viewpoint of drug delivery have been demonstrated over recent years.

The different SCF particle-formation processes can be divided into six broad groups.

1. RESS: This acronym refers to 'rapid expansion of supercritical solutions'; this process consists of solvating the product in the fluid and rapidly depressurizing this solution through an adequate nozzle, causing an extremely rapid nucleation of the product into a highly dispersed material. Known for a long time, this process is attractive due to the absence of organic solvent use; unfortunately, its application is restricted to products that present a reasonable solubility in supercritical carbon dioxide (low polarity compounds).
2. Supercritical anti-solvent and related processes (GAS/SAS/ASES/SEDS): In these processes, the SCF is used as an antisolvent that causes precipitation of the substrate(s) dissolved initially in a liquid solvent. This general concept consists of decreasing the solvent power of a polar liquid solvent in which the substrate is dissolved, by saturating it with carbon dioxide in supercritical conditions, causing the substrate precipitation or recrystallization. Depending on the desired solid morphology, various methods of implementation are available:

   a) GAS or SAS, gas antisolvent or supercritical antisolvent, recrystallization: This process is used mostly for recrystallization of solids dissolved in a solvent with the aim of obtaining either small size particles or large crystals, depending on the growth rate controlled by the antisolvent pressure variation rate.
   b) ASES, aerosol solvent extraction system: This name is used when micro- or nanoparticles are expected; the process consists of pulverizing a solution of the substrate(s) in an organic solvent into a vessel swept by an SCF.
   c) SEDS, solution-enhanced dispersion by supercritical fluids: A specific implementation of ASES that consists of co-pulverizing the substrate(s) solution and a stream of supercritical carbon dioxide through appropriate nozzles.

3. PGSS: This acronym refers to 'particles from gas-saturated solutions (or suspensions)'. This process consists of dissolving an SCF into a liquid substrate, or a solution of the substrate(s) in a solvent, or a suspension of the substrate(s) in a solvent followed by a rapid depressurization of this mixture through a nozzle causing the formation of solid particles or liquid droplets according to the system.

4. DELOS: This acronym refers to 'depressurization of an expanded liquid organic solution', where an SCF is used for the straightforward production of micrometer-sized crystalline particles from organic solution. In this process, SCF acts as a co-solvent, being completely miscible, at a given pressure and temperature, with the organic solution to be crystallized. The role of the SCF is to produce, through its evaporation, a homogeneous sub-cooling of the solution with particle precipitation[32].
5. SAA, 'supercritical assisted atomization': This process is based on the solubilization of a fixed amount of supercritical carbon dioxide in the liquid solution; then the ternary mixture is sprayed through a nozzle, and as a consequence of atomization, solid particles are formed[33].
6. CAN-BD: Carbon dioxide assisted nebulization with a bubble dryer is a new-patented process that can generate a dense aerosol with small droplet and microbubble sizes that are dried to form particles less than 3-μm diameter[34].

Several reviews have considered these alternative varieties of SCF particle-formation processes[26,35–37]. However, a critical requirement to direct both process understanding and selection of required working conditions for targeted particle properties is the phase behavior of the different SCF methods. A recent review has addressed this topic[26], highlighting the importance of linking the underlying thermodynamics of phase equilibria operating under defined SCF processing conditions with changes in crystallization/precipitation mechanisms for products with desired properties.

8.4.1 Processes for Particle Design

Rapid expansion of supercritical solutions. RESS (Figure 8.2) consists of saturating an SCF with the substrate(s), then depressurizing this solution through a heated nozzle into a low-pressure chamber in order to cause an extremely rapid nucleation of the substrate(s) in the form of very small particles – or fibers, or films when the jet is directed against a surface – that are collected from the gaseous stream[36].

The pure carbon dioxide is pumped into the desired pressure and preheated to extraction temperature through a heat exchanger. The SCF is then percolated through the extraction

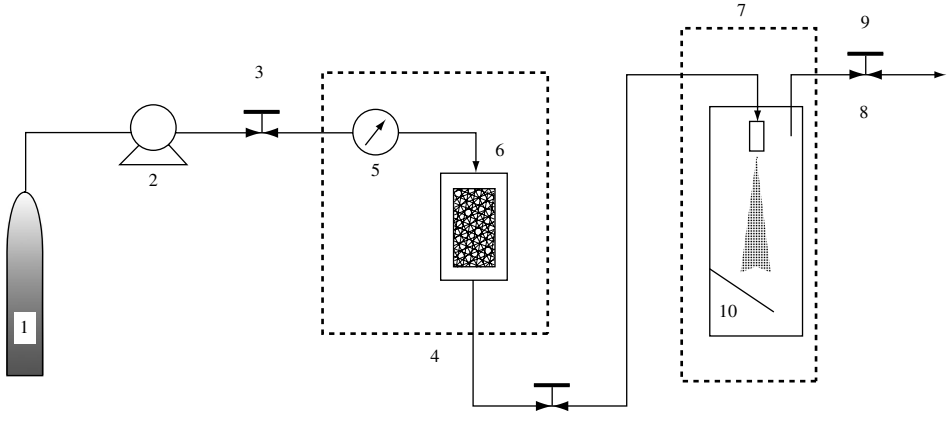

Figure 8.2 *RESS flow diagram: 1. CO_2, 2. pump, 3. valve, 4. extraction unit, 5. heat exchange, 6. solid material, 7. precipitation unit, 8. nozzle, 9. valve, 10. particle collection*

unit packed with one or more substrate(s), mixed in the same autoclave or set in different autoclaves in series. In the precipitation unit, the supercritical solution is expanded through a nozzle that must be reheated to avoid plugging by substrate(s) precipitation.

The morphology of the resulting solid material depends both on the material structure (crystalline or amorphous, composite or pure, etc.) and on the RESS parameters (temperature, pressure drop, distance of impact of the jet against the surface, dimensions of the atomization vessel, nozzle geometry, etc.)[38–51]. It is to be noticed that the initial investigations consisted of 'pure' substrate atomization in order to obtain very fine particles (typically of 0.5–20 μm diameter) with narrow diameter distribution; however, the most recent publications are related to mixture processing in order to obtain microcapsules or microspheres of an active ingredient inside a carrier.

This technology can be implemented in relatively simple equipment although particle collection from the gaseous stream is not easy. But the applications are limited as most attractive substrates are not soluble enough into the SCF to lead to profitable processes: a co-solvent may be used to improve this solubility, but it will be eliminated from the resulting powder, which is not simple and cheap[27].

Processing equipment requires a source of SCF, which passes through an extractor unit to a restricted orifice positioned in a particle collection-precipitation vessel held at a lower temperature and pressure (often ambient) than the extractor unit. There are several primary factors[52] found to influence the physical properties of particle size, shape, and surface topography of products. Those factors are

- dimensions of orifice (expansion device),
- time scale (typically 10^{-5} s),
- pressure/temperature conditions in precipitator,
- agglomeration phenomena during SCF solution expansion,
- phase process path followed during expansion.

For pharmaceutical organic materials studied for processing by RESS, SCF CO_2 is the preferred solvent. As SCF CO_2 is non-polar, those organics that are also non-polar can be expected to dissolve in SCF CO_2 and thus be suitable candidates for RESS processing. Examples include lovastatin[27], stigmasterol[51], salicylic acid, and theophylline[53]. Expansion of solutions to pressure conditions above ambient, and thereby at lower levels of supersaturation, can result in agglomeration of particles, whereas increased supersaturation during expansion leads to extremely rapid nucleation rates and micrometer- and sub-micrometer-sized particles. Several reports have considered using the RESS process for the direct formulation of drug:polymer systems by a coprecipitation strategy[54,55], with the objective of embedding drug molecules in a polymeric-core particle to provide a modified drug-diffusional flux. With evidence of phase separation for a lovastatin:poly(D, L-lactic acid) system[42,43], particles of a poly(L-lactic acid) coating on a core of naproxen have been prepared by careful control of processing conditions. Whilst most pharmaceutical compounds, produced by synthesis or natural compounds, exhibit solubilities below 0.01 wt.% under moderate processing conditions (below 60° C and 300 bar)[56], several low molecular weight hydrophobic compounds, including some steroids and biodegradable polymers, have been prepared in crystalline, micrometer-sized form with narrow distributions. However, predictive control of particle size and morphology remains a major challenge, along with processing and scale-up factors to eliminate particle aggregation and nozzle blockages caused by cooling effects on solution

expansion. This process can also be used with suspensions of active substrate(s) in a polymer or other carrier substance leading to composite microspheres.

RESS is a very attractive process as it is simple and relatively easy to implement at least at a small scale when a single nozzle can be used. However, extrapolation to a significant production size or use of a porous sintered disk through which pulverization occurs is extremely difficult to carry out. The reason is that in both cases, particle size distribution is not easy to control, and may be much wider than in the case of a single nozzle. Moreover, particle harvesting is complex, as it is in any process leading to very small particles[25].

However, the most important limitation of RESS development lies in the too low solubility of compounds in SCFs, which precludes production at acceptable costs, as, in most cases, use of a co-solvent to increase solubility in the fluid is not feasible[26].

Supercritical antisolvent and related processes (GAS/SAS/ASES/SEDS). Precipitation using SCFs as non-solvents or antisolvents utilizes a similar concept to the use of antisolvents in solvent-based crystallization processes.

In those processes, the SCF is used as antisolvent that causes precipitation of the substrate(s) dissolved initially in a liquid solvent. This general concept consists of decreasing the solvent power of a polar liquid solvent in which the substrate is dissolved, by saturating it with carbon dioxide in supercritical conditions, causing substrate precipitation or recrystallization[57]. Depending on the desired solid morphology, various ways of implementation are available.

GAS or SAS recrystallization: A batch of solution is expanded several-fold by mixing with a dense gas in a vessel (Figure 8.3). Owing to the dissolution of the compressed gas, the expanded solvent has a lower solvent strength than the pure solvent. The mixture becomes supersaturated and the solute precipitates in microparticles. This process has been called gas antisolvent or supercritical antisolvent recrystallization. As shown in

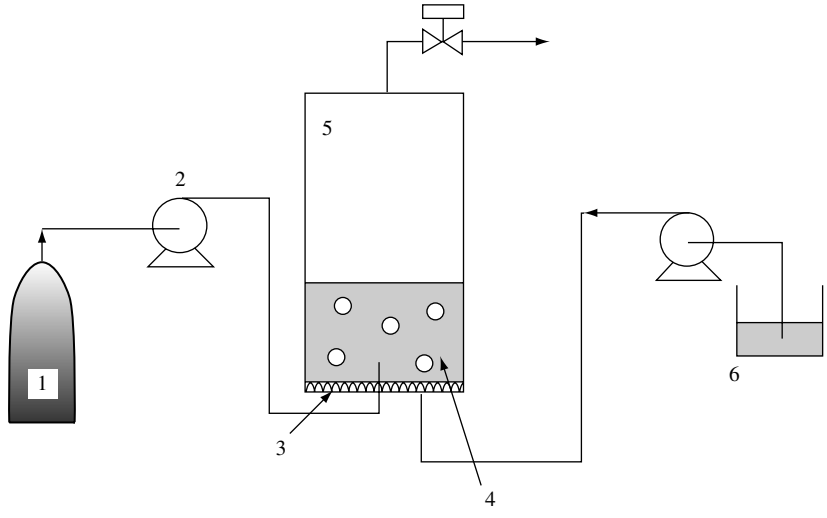

Figure 8.3 *GAS flow diagram: 1. CO_2, 2. pump, 3. particles, 4. expanded solution, 5. precipitator, 6. solution*

Figure 8.3 the precipitator is partially filled with the solution of the active substance. CO_2 is then pumped up to the desired pressure and introduced in the vessel, preferably from the bottom to achieve a better mixing of the solvent and antisolvent. After a holding time, the expanded solution is drained under isobaric conditions to wash and clean the precipitated particles.

With high solubilities of SCFs in organic solvent, a volume expansion occurs when the two fluids make contact, leading to a reduction in solvent density and parallel fall in solvent capacity. Such reductions cause increased levels of supersaturation, solute nucleation, and particle formation. This process, generally termed gas antisolvent recrystallization, thus crystallizes solutes that are insoluble in SCFs from liquid solutions, with the SCF, typically SCF CO_2, acting as an antisolvent for the solute. The GAS process was initially developed for crystallizing explosive materials[25].

Typically, the GAS process is performed as a batch process. Particles are formed in the liquid phase[37] and are then dried by passing pure SCF over product in the pressure vessel for extended periods. This situation, coupled with problems associated with heat generation during the addition of an SCF to solvent or solution[58], has resulted in modification of the process by several research groups[26] to improve both process and product control and to achieve a semi-continuous operation. In general, the developments involve spraying or aerosolizing the organic solvent drug solution into a bulk or flowing stream of SCF as the antisolvent. This is to maximize exposure of small amounts of solution to large quantities of SCF antisolvent to dissolve rapidly the solvent in the SCF, leading to dry particles and thereby reducing the drying stage in the GAS process.

ASES (aerosol solvent extraction system): This is the first modification of the gas antisolvent process and involves spraying the solution through an atomization nozzle as fine droplets into compressed carbon dioxide (Figure 8.4). The dissolution of the SCF into the liquid droplets is accompanied by a large volume expansion and, consequently,

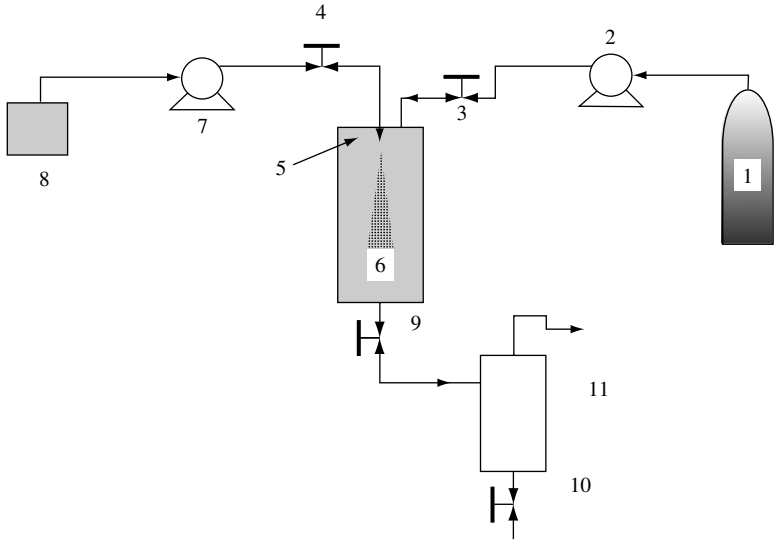

Figure 8.4 *ASES flow diagram: 1. CO_2, 2, 7. pump, 3, 4, 9, 10. valves, 5. nozzle, 6. high-pressure vessel, 8. active material+solvent, 11. low-pressure tank*

a reduction in the liquid solvent power, causing a sharp rise in the supersaturation within the liquid mixture, and the consequent formation of small and uniform particles[59]. The SCF is pumped to the top of the high-pressure vessel by a high-pressure pump. Once the system reaches steady state (temperature and pressure in precipitator), the active substance solution is introduced into the high-pressure vessel through a nozzle[60,61]. To produce small liquid droplets in the nozzle, the liquid solution is pumped at a pressure higher (typically ~20 bar) than the vessel operating pressure. Particles are collected on a filter at the bottom of the vessel. The fluid mixture (SCF plus solvent) exits the vessel and flows to a depressurization tank where the conditions (temperature and pressure) allow gas–liquid separation. After collection of a sufficient amount of particles, liquid solution pumping is stopped and pure SCF continues to flow through the vessel to remove residual solvent from the particles. This spray process has been called the aerosol solvent extraction system process[62].

SEDS (solution-enhanced dispersion by supercritical fluids): The second modification of the gas antisolvent process known as solution-enhanced dispersion by SCFs was developed by the Bradford University[61] in order to achieve smaller droplet size and intense mixing of SCF and solution for increased transfer rates. Indeed the SCF is used both for its chemical properties and as 'spray enhancer' by mechanical effect: a nozzle with two coaxial passages allows the introduction of the SCF and a solution of active substance(s) into the particle-formation vessel where pressure and temperature are controlled (Figure 8.5). The high velocity of the SCF allows breaking up the solution into very small droplets. Moreover, the conditions are set up so that the SCF can extract the solvent from the solution at the same time as it meets and disperses the solution. Similarly, a variant was recently disclosed by the University of Kansas[63], where the nozzle design leads to development of sonic waves leading to very tiny particles, around 1 μm.

Factors influencing particle properties when prepared by the SCF–GAS process[64,65] are

- solute solubility in organic solvent,
- solute insolubility in SCF,
- degree of expansion of organic solvent in SCF,
- organic solvent/SCF antisolvent ratio,
- rate of addition of SCF antisolvent,
- pressure of temperature conditions in precipitator,
- phase process path followed during particle nucleation.

In the SAS method, a solution of compound in an organic solvent is sprayed via a capillary-tube nozzle into a bulk of SCF[64,65], with pharmaceutical applications including polymers and proteins. A modification of the SAS process is the ASES, in which a drug or polymer solution is sprayed into a volume of SF for a period of time[66]. This step is followed by lengthy drying periods by flowing fresh SF over the particulate product. The precipitation with compressed antisolvent (PCA) process is similar in principle, with a liquid solution of a polymer delivered via a capillary tube into the antisolvent in a liquid (subcritical) or supercritical state[67]. Alternative polymeric particle topography and shapes have been reported, depending upon process paths followed in the phase diagram because of the polymer:SCF phase separation[68].

As with RESS, a range of pharmaceutical and polymer–drug systems have been successfully prepared, including micrometer-sized particles, albeit generally on a laboratory

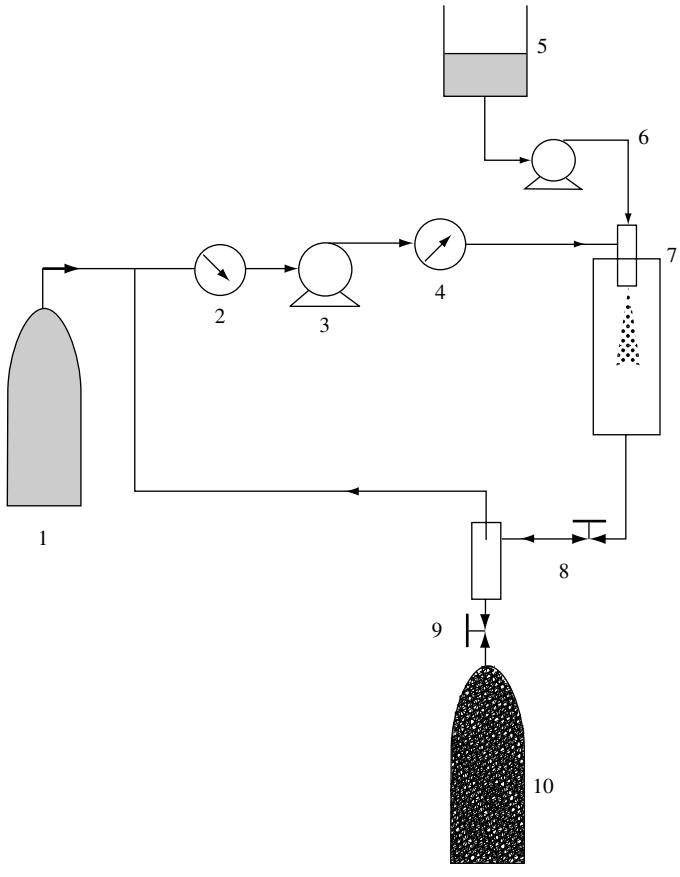

Figure 8.5 *SEDS flow diagram: 1. CO_2, 2. cooler, 3, 6. pumps, 4. heat exchange, 5. active substance solution, 7. particle formation vessel, 8, 9. valves, 10. solvent*

scale. Thus, the benefits of these SCF-antisolvent processes include a totally enclosed single-step process that requires reduced levels of organic solvent compared with conventional crystallization[63,64,69].

In the SAS and ASES techniques, the mass transfer of the SCF into the sprayed droplet determines the rate of particle formation, whereas particle agglomeration and aggregation phenomena are influenced by the rate of solvent mass transfer into the SCF from the droplet. The former mass transfer is dependent upon atomization efficiency and the latter on dispersing and mixing phenomena between the solution droplet and the SCF[37]. Thus, to minimize the particle agglomeration frequently observed and to reduce or eliminate drying times, increased mass-transfer rates are required. This has been successfully achieved in the SEDS process[67], which uses a coaxial nozzle design with a mixing chamber. This arrangement provides a means whereby the drug in the organic solvent solution interacts and mixes with the SCF antisolvent in the mixing chamber of the nozzle prior to dispersion, and flows into a particle-formation vessel via a restricted orifice. Thus, high mass-transfer rates are achieved with a high ratio of SCF to solvent, and the high velocities of the SCF facilitate break-up of the solution feed.

Over recent years, this process has been used for several drug and drug formulation applications, with evidence of successful process scale-up[25,37]. This process has been further developed to process water-soluble materials, including carbohydrates[37] and biologicals, with a three-component coaxial nozzle[28]. The metered and controlled delivery of an aqueous solution, ethanol, and SCF into the modified coaxial nozzle overcomes the problems associated with limited water solubility in $SFCO_2$, the most common SCF for pharmaceutical processing. These advances, particularly for scale-up and operation with aqueous solutions and SCF CO_2, strengthen the viability and industrial potential of SCF processing for pharmaceuticals.

There is no doubt that antisolvent processes have a bright future, especially for drug delivery systems, as they permit the monitoring of the properties and composition of the particles with great flexibility and for almost any kind of compounds. Nevertheless, scale-up is presently foreseen only for high-value specialty materials (pharmaceuticals, cosmetics, superconductors) with productions ranging from a few kilograms to a few hundred kilograms per day.

Regarding intellectual property, the situation may lead to some limitations of process applications until it will be cleared to ensure that no patent infringement is to be feared by potential users[26].

Particles from gas-saturated solutions/suspensions (PGSS). As the solubilities of compressed gases in liquids and solids like polymers are usually high, and much higher than the solubilities of such liquids and solids in the compressed gas phase, this process consists of solubilizing supercritical carbon dioxide in melted or liquid-suspended substance(s), leading to a so-called gas-saturated solution/suspension that is further expanded through a nozzle with formation of solid particles, or droplets[70,71] (Figure 8.6). Typically, this process allows the formation of particles from a great variety of substances that need not be soluble in supercritical carbon dioxide, especially with some polymers that absorb a large

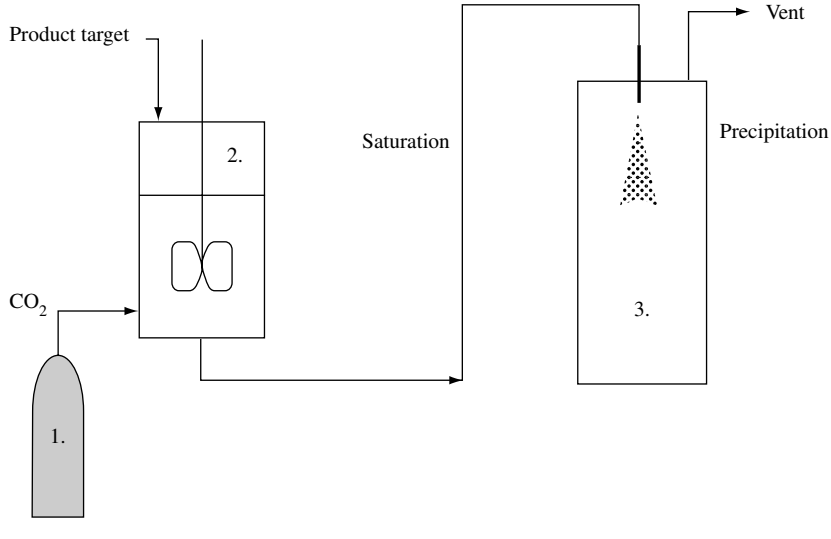

Figure 8.6 *PGSS flow diagram: 1. CO_2, 2. reactor, 3. precipitator*

concentration (10 – 40 wt.%) of CO_2 that either swells the polymer or melts it at a temperature far below (~10–50°C) its melting/glass transition temperature. A further variety of this process has been developed for controlling the porosity of polymer particles[68]. This procedure, called pressure-induced phase separation (PIPS), depends on a controlled expansion of a homogeneous solution of polymer and SCF in liquid or supercritical phase. By varying the polymer concentration and depressurizing via alternative phase transitions in the metastable or spinodal region of the phase diagram[70,71], the porosity of resulting polymeric particles can be increased with higher initial concentrations in the feed solution to the expansion orifice. As before, the process requires adequate solubility of the polymer and solute, if present, in the chosen SF for pharmaceutical vitality[72].

Particle design using the PGSS concept is already widely used at large scale, and is different from other process concepts presently under development yet. The simplicity of this concept, leading to low processing costs, and the very wide range of products that can be treated (liquid droplets or solid particles from solid material or liquid solutions or suspensions) open wide avenues for development of PGSS applications, not only for high-value materials but also perhaps for commodities, in spite of limitations related to the difficulty of monitoring particle size[72].

Recently, many patents were successively granted. Most are related to paint application (pulverization of suspensions to make coatings) and powder coating manufacture (combination of chemical reaction and pulverization of a suspension); more surprisingly, the basic PGSS process patent filed in 1995[73] for the formation of solid particles from polymer or solid substances has been successfully granted in Europe and recently in the US. Moreover, a patent for aerosol drug delivery[74] was also granted, describing several different processes and apparatus: the 'tee' process and equipment it is not clear that the pulverization it only caused by the mechanical effect of gas expansion well known for long, and a portable device for static nebulization using RESS or PGSS concepts.

Depressurization of an expanded liquid organic solution (DELOS). This new technology was developed by Ventosa *et al.*[32]. An SCF is used for the straightforward production of micrometer-sized crystalline particles from organic solution. In this process SCF acts as co-solvent being completely miscible, at a given pressure and temperature, with the organic solution to be crystallized. The role of the SCF is to produce, through its evaporation, a homogeneous sub-cooling of the solution with particle precipitation[32].

The driving force of a DELOS crystallization process is the fast, large, and extremely homogeneous temperature decrease experienced by a solution, which contains an SCF, when it is depressurized from a given working pressure to atmospheric pressure. In contrast to other already reported high-pressure crystallization techniques (RESS, GAS, PCA, PGSS), in a DELOS process the SCF behaves as co-solvent over the initial organic solution of the solute to be crystallized. Through a DELOS process it is possible to produce fine powders of a compound provided that a system 'compound/organic solvent/SCF' in a liquid one-phase state is found. In order to compare DELOS and GAS procedures, Ventosa *et al.*[32] crystallized 1,4-bis-(*n*-butylamino)-9,10-anthraquinone from 'acetone/CO_2' mixtures by both methods. The crystallization results obtained were analyzed upon the solubility behavior of 1,4-bis-(*n*-butylamino)-9,10-anthraquinone in 'acetone/CO_2' mixtures with different composition. They showed that in those ternary systems where the CO_2 behaves as co-solvent over a wide range of X_{CO_2}, like the case of 'colorant 1/acetone/CO_2'

system, this new process can be an alternative to the already reported GAS and PCA crystallization methods, where the CO_2 is used as antisolvent. In a DELOS process the extent of CO_2 vaporization at any point of the liquid solution is exactly the same; as a consequence the solution temperature decrease and the evolution of the supersaturation profile are extremely uniform over the entire system. Therefore, the design of the stirring system, which is usually a problem in many industrial processes performed in solution, is not a key point because the characteristics of the particles produced do not depend on the mixing efficiency.

Summarizing, the DELOS process is a promising new high-pressure crystallization technique, which can be a useful processing tool in the particle engineering of different compounds and materials of industrial interest.

Supercritical assisted atomization (SAA). This process is based on the solubilization of a fixed amount of supercritical carbon dioxide in the liquid solution; then the ternary mixture is sprayed through a nozzle, and as a consequence of atomization, solid particles are formed.

One of the prerequisites for a successful SAA precipitation is the complete miscibility of the liquid in the SCF CO_2, and the insolubility of the solute in it. For these reasons SAA is not applicable to the precipitation of water-soluble compounds due to the very low solubility of water in CO_2 at the operating conditions commonly used.

Reverchon and Della Porta[33] used SAA to produce tetracycline (TTC) and rifampicin (RF) microparticles with controlled particle size and particle size distributions in the range of aerosolizable drug delivery.

Water was used as a liquid solvent for TTC and methanol was used for RF; heated nitrogen was also delivered into the precipitator in order to evaporate the liquid droplets and generate the microparticles. SAA of these compounds was optimized with respect to the process parameters; then, the influence of the solute concentration in the liquid solution on particle size and particle size distribution was studied. The produced powders were characterized with respect to their morphologies and particle size: spherical particles with controlled particle size ranging between 0.5 and 3 μm were obtained for both drugs at optimized operating conditions[33].

Carbon dioxide assisted nebulization with a bubble dryer (CAN-BD®). CAN-BD®[34] can dry and micronize pharmaceuticals for drug delivery.

In this process, the drug, dissolved in water or an alcohol (or both), is mixed intimately with near-critical or supercritical CO_2 by pumping both fluids through a low volume to generate microbubbles and microdroplets, which are then decompressed into a low-temperature drying chamber, where the aerosol plume dries in seconds. CO_2 and the solution are mixed in the tee at room temperature and microbubbles and microdroplets formed are dried rapidly at lower temperatures (25−80° C) than are used in traditional spray drying processes. The residence time of the particles in the lab scale 750-mL glass drying chamber is less than 3 s. The primary advantage of this process is that there is less decomposition of thermally labile drugs. Secondly, no high-pressure vessels are needed in the CAN-BD process, except for the syringe pump, the 1/16 in. (outer diameter) stainless steel tubing, the low volume tee, and the flow restrictor, which allow fluid mixing at a moderate pressure (i.e. between 80 and 100 bar) and the expansion of the microbubbles and microdroplets to atmospheric pressure. Thirdly, these particles (hollow or solid) are generally formed in the optimum size range for pulmonary delivery

to alveoli (typically 99% are less than 3 μm in diameter). They have synthesized and measured the aerodynamic diameters of dried hollow and solid particles of various drugs and model compounds. Particles can be easily prepared and collected in a CAN-BD unit. Samples as small as 1 mL in volume can be dried for formulation studies, and scale-up of the CAN-BD process is in progress.

8.5 Factors Influencing Particle Properties

The characteristics of the particles produced using SCF technology are influenced by the properties of the solute (drug, polymer, and other excipients), type of SCF used, and process parameters (such as flow rate of solute and solvent phase, temperature and pressure of the SCF, pre-expansion temperature, nozzle geometry, and the use of coaxial nozzles)[12,14]. The influence of drug and polymer properties is discussed below.

Drug properties, such as solubility and partitioning of the drug into SCF, determine the properties of the particles formed. When SCF is intended as an antisolvent, if the drug is soluble in SCF under the operating conditions, it will then be extracted into SCF and will not precipitate out[75].

Similarly, during encapsulation of a drug in a polymer matrix, the properties of the drug influence the drug loading. Poly(Lactic acid) (PLA) microparticle formation using an antisolvent process with supercritical CO_2 indicated that an increase in liophilicity decreases the loading efficiency as well as release rate, possibly because lipophilic drugs can be entrained by supercritical CO_2 during SCF precipitation. Nucleation and growth rate influence the effective encapsulation and morphology of the particles. If the initial nucleation and growth rate of the drug are rapid and the polymer precipitation rate is relatively slow, then drug needles encapsulated in polymeric coat may be formed[16].

Polymer properties, such as polymer concentration, crystallinity, glass transition temperature, and polymer composition, are important factors that determine the morphology of the particles[12]. Increase in the polymer concentration may lead to formation of less spherical and fiber-like particles[20]. In an antisolvent process, the rate of diffusion of antisolvent gas is higher in a crystalline polymer compared to an amorphous polymer. This is because the crystalline polymer has a more ordered structure than the amorphous polymer. This leads to high mass-transfer rates in crystalline polymers, producing high supersaturation ratios and small particles of narrow size distribution. SCFs act as plasticizers for polymer by lowering their glass transition temperatures (T_g). Therefore, polymers with a low T_g tend to form particles that become sticky and aggregate. A change in polymer chain length, chain number, and the use of chain composition can alter polymer crystallinity, and, hence, the particle morphology.

8.6 Drug Delivery Applications of SCFs

Microparticles and nanoparticles. Drug and polymeric microparticles have been prepared using SCFs as solvents and antisolvents. Krukonis[15] first used RESS to prepare 5- to 100-μm particles of an array of solutes including lovastatin, polyhydroxy-acids, and mevinolin. In the past decade, simultaneous coprecipitation of two solutes, a drug and an excipient, gained interest. An RESS process employing CO_2 was used to produce

PLA particles of lovastatin and naproxen[17]. In these studies, supercritical CO_2 was passed through an extraction vessel containing a mixture of drug and polymer, and the CO_2 containing the drug and the polymer was then expanded through a capillary tube. A GAS process was used to produce clonidine-PLA microparticles[56]. 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 5–2000 nm[69]. 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[76]. 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 solvation 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[43].

Microporous foams. Using the SCF technique, Hile *et al.*[77] 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 bar and 35° C. The pressure was maintained for 24 h to saturate the polymer with CO_2 for the extraction of methylene chloride. Finally, the set-up was depressurized for 10–12 s, creating a microporous foam.

Liposomes. 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. Frederiksen *et al.*[78] developed a laboratory-scale method for preparation of small liposomes encapsulating a solution of FITC dextran (fluorescein isothiocyanatedextran), a water-soluble compound using supercritical carbon dioxide as a solvent for lipids[78]. 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 150–250 nm were obtained. Aphios Company's patent[79] (US Patent No. 5,776,486) on SuperFluidsTM CFL describes a method and apparatus useful for the nanoencapsulation of paclitaxel and campothecin in aqueous liposomal formulations called TaxosomesTM and CamposomesTM, respectively. These

formulations are claimed to be more effective against tumors in animals compared to commercial formulations.

Inclusion complexes. Solubility of poorly soluble drugs, such as piroxicam, can be enhanced by forming inclusion complexes with cyclodextrins. For many non-polar 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[80]. Cyclodextrins had previously been used for the entrapment of volatile aromatic compounds after supercritical extraction[81]. On the basis of this principle, Van Hees *et al.*[82] employed SCFs for producing piroxicam and β-cyclodextrin inclusion complexes. Inclusion complexes were obtained by exposing the physical mixture of piroxicam-β-cyclodextrin (1:2.5 mol:mol) to supercritical CO_2 and depressurizing this mixture within 15 s. Greater than 98.5% of inclusion was achieved after 6 h of contact with supercritical CO_2 at 15 MPa and 150° C.

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 polyethyleneglycol 4000 (PEG4000) increased the rate and extent of dissolution of carbamazepine[83]. In this method, a precipitation vessel was loaded with a 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.

Powders of macromolecules. Processing conditions with supercritical CO_2 are benign for processing macromolecules, such as peptides, proteins, and nucleic acids. Debenedetti[35] used an antisolvent method to form microparticles of insulin and catalase. Protein solutions in hydroethanolic mixture (20:80) were allowed to enter a chamber concurrently with supercritical CO_2. The SCF expanded and entrained the liquid solvent, precipitating sub-micrometer 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[84]. This led to the development of a method wherein the use of the organic solvents is completely eliminated to obtain fully active insulin particles of dimensions 1.5−500 μm. In this development, 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[85]. 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 non-polar 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 supercoiled state. Such macromolecule powders can possibly be used for inhalation therapies.[85,86].

Coating. SCFs can be used to coat the drug particles with single or multiple layers of polymers or lipids[24]. 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[82]. An active substance in the form of a solid particle or an inert porous solid particle containing an 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.

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[85]. The use of supercritical CO_2 for sterilizing PLGA microspheres (1, 7, and 20 μm) is described in US Patent No. 6,149,864[87]. The authors indicated that complete sterilization can be achieved with supercritical CO_2 in 30 min at 205 bar and 34° C.

Protein and biological materials. The considerable growth in biotechnology-derived therapeutic agents, including peptides, proteins, and plasmid DNA, has generated interest in non-oral routes of drug administration to bypass the damaging gastrointestinal effects for such materials[88]. The promise of using alternative delivery routes, via nasal, respiratory, transdermal via powder delivery and parenteral routes, is frequently constrained by requirements for stable, powdered products with specific particle size requirements. The complexity and sensitivity of these biologically sourced materials necessitate careful processing to ensure stability of product and provide appropriate physical characteristics[89]. The conventionally used and complex processes of freeze drying and spray drying are far from ideal. Taken together with the problems associated with downstream sieving or milling products prepared by these drying operations to achieve target particle sizes and size distributions, particle formation by SCF methods represents an attractive option. The application of SCF antisolvent methods has shown considerable promise in this field over recent years[38].

The low solubility of water in $SFCO_2$ has forced workers to use organic solvents including dimethylformamide (DMF) and dimethylsulfoxide (DMSO) as nonaqueous media for biological materials such as proteins[65,78,90]. Such solvents have limitations because proteins have low solubility and potential loss of secondary and tertiary protein structure in solution of these agents. Nevertheless, although extensive perturbation was evidenced in DMSO solutions and was partially present in the solid protein particles, micrometer-sized particles of insulin, lysozyme, and trypsin prepared by the SAS process essentially recovered biological activity on reconstitution[78,79].

The processing of labile biological materials from aqueous solutions is clearly preferred and modifications to the nozzle arrangement in the SEDS process have achieved this objective[12]. In this modification, the aqueous solution containing the biological material is only contacted momentarily with a potentially damaging organic solvent and SF in a three-component coaxial nozzle. This approach has been successfully applied to aqueous lysozyme solutions[91], with microfine product showing a spherical morphology with free flowing powder-handling properties.

8.7 Scale-up Issue

In recent years[52], a number of particle-formation techniques have shown considerable promise, only to falter on scale-up studies and in trying to achieve strict GMP requirements for the process.

At the same time, increased vigilance is being expressed by the regulatory agencies in facilities used for the preparation of drug substances in particulate form. This is occurring against a background of ambition by the pharmaceutical industry of global harmonization of material preparation and consistency of properties of powdered materials.

In many ways, SCF processing and controlled particle formation satisfies most of these demands directly by virtue of the inherent features of the process. As a single step, enclosed operation with mass balance, high yields of very consistent products can be achieved.

Materials of construction of equipment components are high-grade pharmaceutical-grade stainless steel; there are no moving parts and organic solvent requirements can often be reduced compared with crystallization processes[92].

To achieve commercial success, any method/technique developed should be scaled to produce quantities in batches for conducting further research or to market the product. From the perspective of scale-up, SCF technology offers several advantages. The processing equipment can be a single-stage, totally enclosed process that is free of moving parts and constructed from high-grade stainless steel, allowing easy maintenance and scale-up. It offers reduced solvent requirements and particle formation occurs in a light-, oxygen-, and possibly moisture-free atmosphere, minimizing these confounding factors during scale-up.

Some advances have been made in mechanistic understanding of SCF particle-formation processes and rigorous descriptions of mass transfer and nucleation processes are being developed[27]. The advances in the understanding of the mechanism of supercritical particle formation and SCF mass transfer are forming the basis for efficient scale-up of the laboratory-scale processes.

Such knowledge will form the basis for efficient scale-up of the laboratory-scale processes generally reported to date. The majority of studies deal with milligram quantities of product prepared by a batch process. For significant commercial viability, demonstration that the processes can be scaled to produce sufficient quantities of material for clinical trials and production batches is required[93].

While many investigators in the laboratory were only able to produce milligrams of the product, Thies and Muller[85] developed a scaled process of ASES capable of producing 200 g of biodegradable PLA microparticles in the size range $6-50\,\mu$m. On the other hand, industrial units, such as Bradford Particle Design, have resources for the production of up to 1 ton per year of GMP, cGMP compliant material. Scale-up studies with SEDS SCF processing underpinned by research into the physics, physical chemistry, and engineering of the process using a pilot plant to a cGMP small manufacturing plant have been straightforward[37]. Process conditions optimized at laboratory scale have been directly transferred to the larger scale equipment[52].

Engineering pharmaceutical particles by SCF SEDS processing, and the scalability of the process, provides much theoretical understanding of the process. As ever-increasing demand is made of particles by chemists, formulators, and regulators, for example in terms of chemistry of composition, size, and shape, as well as purity and low residual solvent, the SCF approach is likely to provide wide-ranging opportunities to meet such needs. With a proven ability to process delicate biological materials into stable and active particulates, the SEDS process also provides a much needed simplified and efficient alternative to both spray- and freeze-drying operations.

From a GMP perspective, several additional attractive features can be recognized for SCF particle-formation processes. For the antisolvent-based systems, the processing

equipment for the single-stage, totally enclosed process, which is free of moving parts, is constructed from high-grade stainless steel with 'clean in place' facilities available for larger-scale equipment. As well as having reduced solvent requirements compared with conventional crystallization, particle formation occurs in a light- and oxygen-free environment and, if required, moisture-free atmosphere. Although further engineering input is necessary to achieve true continuous collection and recovery of material at operating pressures, 'quasi-continuous' processing is already feasible with a switching device to parallel mounted particle-collection vessels[12].

Cost of manufacturing in pilot scale with SCF technology is comparable with (or may be better than) conventional techniques, such as single-stage spray drying, micronization, crystallization, and milling batch operations. Much has been achieved in a relatively short period since its introduction to pharmaceutical particle engineering, and the future looks attractive for SCF processing.

8.8 Conclusions

SCF technology can be used in the preparation of drug delivery systems and/or to improve the formulation properties of certain drug candidates. SCFs can be used to formulate drug carrier systems due to their unique solvent properties, which can be altered readily by slight changes in the operating temperature and pressure. In recent years, many pharmaceutical and drug delivery companies, some of which are listed in Table 8.2, have adopted SCF technology to obtain drug delivery solutions. The challenges being addressed with this technology include the formulation of poorly water-soluble compounds, obtaining particles of uniform size and shape, avoiding multistep processes, and reducing the excessive use of toxic organic solvents. SCF technology was successfully applied in the laboratory to the preparation of microparticles and nanoparticles or liposomes that encapsulate drug in a carrier, inclusion complexes, solid dispersions, microporous foams, and powders of macromolecules.

As requirements and specifications for 'smart' (those particles that deliver the drug in a controlled way) particles for drug delivery systems become more demanding, the traditional particle preparation and pretreatment procedures are often found to be unsuitable and inadequate. Key issues for emerging replacement technologies are that they provide opportunities for crystal engineering and particle design to be defined scientifically so that by manipulating the process the product can be fine-tuned, and that the process is readily scaled for manufacturing purposes according to the GMP principles and requirements. Recent research[25], development, and applications studies have shown that SF methods for pharmaceutical particle formation provide such a base technology. The SCF antisolvent principle and the SEDS process in particular provide wide scope for the diverse range of organic and biological materials used in single- and multicomponent particulate form in drug delivery systems. Products with targeted properties such as particle size or purity enhancement have been produced. In addition, several studies[40] have successfully addressed the important issue of scale-up and the inherent features of the SCF process enable GMP requirements to be readily accommodated. Indeed, many of the features recognized for an 'ideal' particle-formation process are substantially met by SCF technology.

Table 8.2 *Pharmaceutical and drug delivery companies using supercritical fluid technologies*

Company name	Market capital
Iomed	$9.69
Gentronics	$21.61
Flamel	$35.64
endorex	$37.70
Antares	$38.49
AP Pharma	$46.70
Elite	$57.77
Access	$58.53
AeroGen	$59.41
StemCells	$63.38
Sonus	$63.71
MexMed	$65.12
DepoMed	$65.70
MacroChem	$88.44
Boject	$99.22
Sheffield	$100.47
Generex	$103.39
Amarin	$114.19
Nastech	$120.91
Cygnus	$140.62
Aradigm	$142.36
Novavax	$215.27
Penwest	$283.86
Emisphere	$326.22
Cima	$356.47
Noven	$368.30
Atrix	$413.54
SkyePharma	$424.20
Durect	$449.79
Inhale	$724.85
Alkermes	$1684.80
Enzon	$2116.67
Andrx	$3445.73
Elan	$4772.94
Average	$503.40
Median	$108.79

However, whilst having many attractive features, further research and development of SCF processing for pharmaceuticals is required to consolidate current understanding and achieve, ultimately, predictive capability for particle design. With progress being made, attention should also continue to be directed to modeling the expansion and particle nucleation events in RESS processes, and the rapid cascade of the overlapping physical, mechanical, and chemical events occurring during SCF antisolvent particle-formation methods. Progress in these areas, coupled to engineering studies on plant design for continuous operation for SCF procedures, will undoubtedly strengthen rational approaches to particle design for drug delivery systems and facilitate confident installation

of manufacturing scale plant. Indeed, with continuing research in this expanding field, new possibilities are likely to be opened up, especially for biomolecules and bioreactions that are only possible by SF processing.

References

[1] Gutcho M.H. 1976. Microcapsules and Microencapsulation Techniques (Chemical Technology Review series). Noyes Data.

[2] Benita S. 1996. Microencapsulation: Methods and Industrial Applications (Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs). Marcel Dekker.

[3] Baxter G. 1974. In, Microencapsulation: Processes and Applications, Vandegaer J.E. (Ed.). Plenum Press, New York.

[4] Whateley T.L.. 1992. Microencapsulation of Drugs. Drug Targeting and Delivery, Vol. 1. T&F STM.

[5] Lim F. 1984. Biomedical Applications of Microencapsulation. CRC Press.

[6] Gutcho M.H. 1972. Capsule Technology and Microencapsulation. Noyes Data.

[7] Kuhtreiber W.M., Lanza R.P., Chick W.L. and Lanza R.P. 1999. Cell Encapsulation Technology and Therapeutics, 1st edition. Birkhauser, Boston.

[8] Benita S. 1997. Recent advances and industrial applications of microencapsulation. Biomedical Science and Technology, Proc. Int. Symp., 4th, Meeting Data, pp. 17–29.

[9] Brazel C.S. 1999. Microencapsulation: offering solutions for the food industry, Cereal Foods World, 44, 388–390.

[10] Heintz T., Krober H. and Teipel U. 2001. Microencapsulation of reactive materials, Schuettgut 7.

[11] Bencezdi D. and Blake A. 1999. Encapsulation and the controlled release of flavours, Leatherhead Food RA Ind. J., 2, 36–48.

[12] Kiran E., Debenedetti P.G. and Peters C.J. 2000. Supercritical Fluids Fundamentals and Applications, Chapter 7. Kluwer Academic Publishers.

[13] Kondo T. 2001. Microcapsules: their science and technology part III, industrial, medical and pharmaceutical applications, J. Oleo Sci., 50, 143–152.

[14] Kompella U.B. and Koushik K. 2001. Preparation of drug delivery systems using supercritical fluid technology, Crit. Rev. Ther. Drug Carrier Syst., 18(2), 173–199.

[15] Krukonis V.J. 1984. Supercritical fluid nucleation of difficult to comminute solids. Paper 104f presented at AIChE Meeting in San Francisco, California, November 1984.

[16] Tom J.W., Lim G.B., Debenedetti P.G. and Prod'homme R.K. 1993. Applications of supercritical fluids in controlled release of drugs. In, Supercritical Fluid Engineering Science. ACS Symposium Series 514, Brennecke J.F. and Kiran E. (Eds.). American Chemical Society, Washington, DC, p. 238.

[17] Kim J.H., Paxton T.E. and Tomasko D.L. 1996. Microencapsulation of naproxen using rapid expansion of supercritical solutions, Biotechnol. Prog., 12(5), 650–661.

[18] Bastian P., Bartkowski R., Kohler H. and Kissel T. 1998. Chemo-embolization of experimental liver metastases. Part I: distribution of biodegradable microspheres of different sizes in an animal model for the locoregional therapy, Eur. J. Pharm. Biopharm., 46(3), 243–254.

[19] Benoit J.P., Fainsanta N., Venier-Julienne M.C. and Meneibed P. 2000. Development of microspheres for neurological disorders: from basics to clinical applications, J. Control. Release, 65(1–2), 285–296.

[20] Taguchi T., Ogawa N., Bunke B. and Nilsson B. 1992. The use of degradable starch microspheres with intra arterial for the treatment of primary and secondary liver tumours – results of phase III clinical trial, Reg. Cancer Treat., 4, 161–165.

[21] Cohen S. and Bernstein H. (Eds.). 1996. Microparticulate Systems for the Delivery of Proteins and Vaccines (Knutson *et al*. chapter). Marcel Dekker, New York.
[22] Jallil R. and Nixon J.R. 1990. Microencapsulation using poly(L-lactic acid). III. Effect of polymer weight on the microcapsule properties, J. Microencapsul., 7(1), 41–52.
[23] Langer R. and Folkman J. 1976. Polymer for the sustained released of proteins and other macromolecules, Nature, 263, 797–800.
[24] Benoit J.P., Rolland H., Thies C. and Vande V.V. 2000. Method of coating particles and coated spherical particles. US Patent No. 6-087-003.
[25] York P. *et al.* 1998. In, Proc. Resp. Drug Delivery VI, Hilton Head, USA, pp. 169–175.
[26] Jung J. and Perrut M. 2001. Particle design using supercritical fluids: Literature and patent survey, J. Supercritical Fluids, 20, 179–219.
[27] Larson K.A. and King M.L. 1986. Evaluation of supercritical fluid extraction in the pharmaceutical industry, Biotechnol. Prog., 2, 73–82.
[28] Shekunov B.Yu. *et al.* 1999. Crystallization process in turbulent supercritical flows, J. Cryst. Growth, 198/199, 1345–1351.
[29] Shekunov B.Yu. *et al*. 1998. Pharm. Res., 15, S162.
[30] Ruchatz F., Kleinebudd P. and Müller B. *et al*. 1997. Residual solvents in biodegradable microparticles. Influence of process parameters on the residual solvent in microparticles produced by the aerosol solvent extraction system (ASES) process, J. Pharm. Sci., 86, 101–105.
[31] Phillips E.M. and Stella V.J. 1993. Rapid expansion from supercritical solutions: application to pharmaceutical processes, Int. J. Pharm., 94, 1–10.
[32] Ventosa N., Sala S. and Veciana J. 2003. DELOS process: a crystallization technique using compressed fluids, 1. Comparison to the GAS crystallization method, J. Supercritical Fluids, 26, 33–45.
[33] Reverchon E. and Della Porta G. 2003. Micronization of antibiotics by supercritical assisted atomization, J. Supercritical Fluids, 26, 243–252.
[34] Sievers R.E., Huang E.T.C., Villa J.A., Engling G. and Brauer P.R. 2003. Micronization of water-soluble pharmaceuticals and model compounds with a low-temperature bubble dryer, J. Supercritical Fluids, 26, 9–16.
[35] Debenedetti P.G. 1994. In, Supercritical Fluids: Fundamentals for Application, Vol. 273. NATO ASI Series E, 250–252.
[36] Reverchon E., Della Porta G., Taddeo R., Pallado P. and Stassi A. 1995. Solubility and micronization of griseofulvin in supercritical CHF_3, Ind. Eng. Chem. Res., 34, 4087–4091.
[37] McHugh M. and Krukonis V.J. 1994. Special Applications in Supercritical Fluid Extraction: Principles and Practice, 2nd edition. Butterworth-Heinemann, Boston.
[38] Weidner E. *et al*. 1996. In, High Pressure Chemical Engineering, Process Technology Proceedings, Vol. 12, Elsevier, 121–124.
[39] Jung J. and Perrut M. 2001. Particle design using supercritical fluids: literature and patent survey, J. Supercritical Fluids, 20, 179–219.
[40] Palakodaty S. and York P. 1999. Phase behavioral effects on particle formation processes using supercritical fluids, Pharm. Res., 34, 976–985.
[41] Smith R.D. and Wash R. 1986. US Patent No. 4-582-731, 15 April 1986 (Priority: 1 September 1983).
[42] Matson D.W., Fulton J.L., Petersen R.C. and Smith R.D. 1987. Rapid expansion of supercritical fluid solutions: solute formation of powders, thin films, and fibers, Ind. Eng. Chem. Res., 26, 2298–2306.
[43] Matson D.W., Petersen R.C. and Smith R.D. 1987. Production of powders and films from supercritical solutions, J. Mater. Sci., 22, 1919–1928.
[44] Petersen R.C., Matson D.W. and Smith R.D. 1987. The formation of polymer fibers from the rapid expansion of supercritical fluid solutions, Polym. Eng. Sci., 27, 1693–1697.

[45] Smith R.D. 1988. US Patent No. 4-734-451.
[46] Lele A.K. and Shine A.D. 1992. Morphology of polymers precipitated from a supercritical solvent, AIChE J., 38(5), 742–752.
[47] Phillips E.M. and Stella V.J. 1993. Rapid expansion from supercritical solutions: application to pharmaceutical processes, Int. J. Pharma., 94, 1–10.
[48] Reverchon E. and Taddeo R. 1993. Morphology of salicylic acid crystals precipitated by rapid expansion of a supercritical solution. In, I Fluidi Supercritici e Le Loro Applicazioni, Reverchon E. and Schiraldi A. (Eds.), 20–22 June 1993, Ravello, Italy, pp. 189–198.
[49] Berends E.M., Bruinsma O.S.L. and van Rosmalen G.M. 1994. Supercritical crystallization with the RESS process: experimental and theoretical results. In, Brunner G. and Perrut M. (Eds.), Proceedings of the 3rd International Symposium on Supercritical Fluids, Tome 3, 17–19 October 1994, Strasbourg, France, pp. 337–342, 4087–4091.
[50] Reverchon E. and Pallado P. 1996. Hydrodynamic modelling of the RESS process, J. Supercritical Fluids, 9, 216–221.
[51] Domingo C., Wubbolts F.E., Rodriguez-Clemente R. and van Rosmalen G.M. 1997. Rapid expansion of supercritical ternary systems: solute + cosolute $+CO_2$. The 4th International Symposium on Supercritical Fluids, 11–14 May 1997, Sendai, Japan, pp. 59–62.
[52] McHugh M. and Krukonis V. 1994. Supercritical Fluid Extraction Principles and Practice, 2nd edition, Butterworth-Heinemann.
[53] Nagahama K. and Liu G.T. 1997. Supercritical fluid crystallization of solid solution. The 4th International Symposium on Supercritical Fluids, 11–14 May 1997, Sendai, Japan, pp. 43–46.
[54] Godinas A., Henriksen B., Krukonis V., Mishra K.A., Pace G.W. and Vachon G.M. 1998. US Patent No. 0-089-852.
[55] Kim J.H. *et al.* 1996. Microencapsulation of naproxen using rapid expansion of supercritical solutions, Biotechnol. Prog., 12, 650–661.
[56] Mueller B.W. and Fisher W. 1989. Manufacture of sterile sustained release drug formulations using liquefied gases. W. Germany Patent No. 3-744-329.
[57] Brennecke J.F. and Eckert C.A. 1989. Phase equilibria for supercritical fluid process design, Am. Inst. Chem. Eng. J., 35, 1409–1427.
[58] Wubbolts F.E. *et al.* 1998. In, Proc. 5th Int. Symp. Supercrit. Fluids, Soc. Adv. Sup. Fluids, Nice, France.
[59] Mishima K., Matsuyama K., Uchiyama H. and Ide M. 1997. Microcoating of flavone and 3-hydroxyflavone with polymer using supercritical carbon dioxide. The 4th International Symposium on Supercritical Fluids, 11–14 May 1997, Sendai, Japan, pp. 267–270.
[60] McHugh M.A. and Guckes T.L. 1985. Separating polymer solutions with supercritical fluids, Macromolecules, 18, 674–681.
[61] Seckner A.J., McClellan A.K. and McHugh M.A. 1988. High pressure solution behavior of the polymer–toluene–ethane system, AIChE J., 34, 9–16.
[62] Robertson J., King M.B., Seville J.P.K., Merrifield D.R. and Buxton P.C. 1997. Recrystallisation of organic compounds using near critical carbon dioxide. The 4th International Symposium on Supercritical Fluids, 11–14 May 1997, Sendai, Japan, pp. 47–50.
[63] Liau I.S. and McHugh M.A. 1985. Supercritical Fluid Technology, Elsevier Science, Amsterdam.
[64] Tom J.E. 1993. Supercritical fluid engineering science: fundamentals and applications, ACS Symp. Ser., 514, 238–257.
[65] Yeo S.D. *et al.* 1993. Formation of microparticulate protein powders using a supercritical fluid antisolvent, Biotechnol. Bioeng., 45, 341–346.
[66] Bleich J. *et al.* 1993. Aerosol solvent extraction system – a new microparticle production technique, Int. J. Pharm., 97, 111–117.
[67] Dixon D.J., Johnston K.P. and Bodmeier R.A. 1993. Polymeric materials formed by precipitation with a compressed fluid antisolvent, AIChE J., 39, 127–139.

[68] Kiran E. and Zhuang W. 1997. Supercritical fluids: extraction and pollution prevention, ACS Symp. Ser., 670, 2–36.
[69] Hanna M. and York P. 1994. Patent WO 95/01221.
[70] Liau I.S. and McHugh M.A. 1985. Supercritical Fluid Technology. Elsevier Science, Amsterdam, p. 415.
[71] Weidner E., Steiner R. and Knez Z. 1996. In, Powder Generation from Polyethyleneglycols with Compressible Fluids. High Pressure Chemical Engineering, Rudolf von Rohr P. and Trepp C. (Eds.). Elsevier Science.
[72] Sievers R.E., Miles B.A., Sellers S.P., Milewski P.D., Kusek K.D. and Kluetz P.G. 1998. New process for manufacture of one-micron spherical drug particles by CO_2-assisted nebulization of aqueous solutions, Proceedings from Respiratory Drug Delivery IV Conference, Hilton Head, South Carolina, 3–8 May 1998, pp. 417–419. In, Supercritical Fluids, Chemistry and Materials, Poliakoff M., George M.W. and Howdle S.M. (Eds.). Nottingham, 10–13 April 1999.
[73] Sievers R.E. and Karst U. European Patent No. 0-677-332, 1995. US Patent No. 5-639-441, 1997.
[74] Weidner E., Knez Z. and Novak Z. 1995. European Patent No. EP 0-744-992, February 1995. Patent WO 95/21688, July 1995.
[75] Steckel H., Thies J. and Muller B.W. 1997. Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide, Int. J. Pharm., 152(1), 99–110.
[76] Pace G.W., Vachon M.G., Mishra A.K., Henrikson I.B. and Krukoniz V. 2001. Processes to generate submicron particles of water-insoluble compounds. US Patent No. 6-177-103.
[77] Hile D.D., Amirpour M.L., Akgerman A. and Pishko M.V. 2000. Active growth factory delivery from poly(D,L-lactide-co-glycolide) foams prepared in supercritical CO_2, J. Control. Release, 66(2–3), 177–185.
[78] Frederiksen L., Anton K., Hoogevest P.V., Keller H.R. and Leuenberger H. 1997. Preparation of liposomes encapsulating water-soluble compounds using supercritical carbon dioxide, J. Pharm. Sci., 86(8), 921–928.
[79] Castor T.P. and Chu L. 1998. Methods and apparatus for making liposomes containing hydrophobic drugs, US Patent No. 5-776-486.
[80] Lin S.Y. and Kao Y.H. 1989. Solid particulates of drug-b-cyclodextrin inclusion complexes directly prepared by a spray-drying technique, Int. J. Pharm., 56, 249–259.
[81] Kamihara H., Asai T., Yamagata M., Taniguchi M. and Kobayashi T. 1990. Formation of inclusion complexes between cyclodextrins and aromatic compounds under pressurized carbon dioxide, J. Ferment. Bioeng., 69, 350–353.
[82] Van Hees T., Piel G., Evrard B., Otte X., Thunus T. and Delattre L. 1999. Application of supercritical carbon dioxide for the preparation of a piroxicam-beta-cyclodextrin inclusion compound, Pharm. Res., 16(12), 1864–1870.
[83] Debenedetti P.G., Lim G.B. and Prud'Homme R.K. 2000. Preparation of protein microparticles by precipitation. US Patent No. 6-063-910.
[84] Tservistas M., Levy M.S., Lo-Yim M.Y.A., O'Kennedy R.D., York P., Humphery G.O. and Hoare M. 2000. The formation of plasmid DNA loaded pharmaceutical powders using supercritical fluid technology, Biotech. Bioeng., 72(1), 12–18.
[85] Thies J. and Muller B.W. 1998. Size controlled production of biodegradable microparticles with supercritical gases, Eur. J. Pharm. Biopharm., 45(1), 67–74.
[86] Kumagai H., Hata C. and Nakamura K. 1997. CO_2 sorption by microbial cells and sterilization by high-pressure CO_2, Biosci. Biotech. Biochem., 61(6), 931–935.
[87] Dillow A.K., Langer R.S., Foster N. and Hrkach J.S. 2000. Supercritical sterilization method, US Patent No. 6-149-864.
[88] Castor T.P. and Hong G.T. 2000. Methods for the size reduction of proteins, US Patent No. 6-051-694.

[89] Winters M.A. *et al*. 1996. Precipitation of proteins in supercritical carbon, J. Pharm. Sci., 85, 586–594.
[90] Schmitt W.J. 1995. Finely-divided powders by carrier solution injection into a near or supercritical fluid, AIChE J., 41, 2476–2486.
[91] Forbes R.T. 1998. Supercritical fluid processing of proteins. I: Lysozyme precipitation from organic solutions, In, Proceedings of the IChE World Congress on Particle Technology 3, Brighton, UK, pp. 180–184.
[92] Kamihara H., Asai T., Yamagata M., Taniguchi M. and Kobayashi T. 1990. Formation of inclusion complexes between cyclodextrins and aromatic compounds under pressurized carbon dioxide, J. Ferment. Bioeng., 69, 350–353.
[93] Steckel H. and Müller B.W. 1998. Metered-dose inhaler formulation of fluticasone-17-propionate micronized with supercritical carbon dioxide using the alternative propellant HFA-227, Int. J. Pharm., 173, 25–33.