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Review

Are pharmaceutics really going supercritical?

Irene Pasquali, Ruggero Bettini*

Department of Pharmacy, University of Parma, 43100 Parma, Italy

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ABSTRACT

The present work focuses on some applications of supercritical fluids in the pharmaceutical field, provides a critical review of the most recent advances and aims to give a vision on the future of this technology. In particular, processes such as particle and crystal engineering, formation of cyclodextrin complexes, coating, foaming and tissue engineering, extrusion, production of liposomes, formulation of biotechnological compounds, sterilization and solvent removal are described and discussed.

Contents

* Corresponding author at: Department of Pharmacy, University of Parma, Viale G.P. Usberti 27/A, 43100 Parma, Italy. Tel.: +39 0521 905089; fax: +39 0521 905006.
E-mail address: bettini@unipr.it (R. Bettini).

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1. Introduction

The pharmaceutical industry has always represented a rapidly changing environment where the advances are driven by the discovery of new drugs and the development of new technologies.

Although much progress has been made in the past years, pharmaceutical companies are still facing a multitude of challenges: the statement "innovate or die" cannot be longer considered simply a provocation. Innovation in pharmaceutics is mainly represented by the discovery and development of new active pharmaceutical ingredients (APIs) which is a very long, risky and expensive process; it can take up to 16 years and its cost can exceed $3.1B (Waller et al., 2007). According to Paul (http://www.health-itworld.com) this is an issue that should be addressed by identifying new and better ways to improve efficacy and effectiveness of drug discovery and clinical trials. Besides, the development of sophisticated new drug delivery systems as well as the exploitation of innovative drug delivery routes (e.g. transdermal, pulmonary, nasal, etc.) may represent methods to improve the efficacy and/or safety of the APIs.

In this respect, regulatory and professional bodies have taken relevant initiatives: (i) EUFEPS *new safe medicine faster* considers how to rethink and accelerate drug development; (ii) ISPE *product quality lifecycle implementation* aims at providing practical global approaches to ICH guidance implementation; (iii) FDA *critical path initiative* was launched to stimulate and facilitate an effort to modernize the scientific process from discovery to medicinal products. This last initiative presents, among the others, one topic relevant to innovation in pharmaceutical manufacturing with respect to the production of biological (cell, tissue engineering, vaccine, etc.), devices, drugs, and nanotechnology.

The innovation in the pharmaceutical plants is, and will be more and more, a crucial issue for sustaining and enhancing the pharmaceutical productivity. The need of developing innovative technologies and processes was already stressed by an article published on the Wall Street Journal some years ago (Abboud and Hensley, 2003).

Medicines are highly regulated products and their quality is subjected to strict legal requirements. Quality is not created spontaneously or cannot longer simply be tested *a posteriori*, but it should be now designed into the product (Quality by Design, ICHQ8). In this regard, a quite recent initiative established by the FDA, the Process Analytical Technologies (PAT) aims at understanding and controlling the manufacturing process to improve both drug product quality and manufacturing efficiency. All the above-mentioned initiatives are linked to the new regulatory frame created by the ICHQ8 (Pharmaceutical development), ICHQ9 (Quality risk management) and ICHQ10 (Pharmaceutical quality system) guidelines (http://www.ich.org).

Therefore, the new paradigm for pharmaceutical industry implies the application of science-based evidences in drug development and production as well as a strong need to simplify, control and understand the process.

Moreover, pharma companies are more and more urged to develop production processes with very low environmental impact in particular for reducing the use of volatile organic compounds in medicine manufacturing (Hay and Khan, 2002) as well as the residues in the finished product (Ph. Eur. 6.0).

Since the early 1980s, technologies based on supercritical fluids (SCFs) have created great expectations in the pharmaceutical area owing to the great number of features that could be exploited, among which the environmental friendliness is probably the most popular (Ginty et al., 2005). A SCF can be used as solvent, antisolvent or processing aid in many pharmaceutical operations.

Compared with conventional unit operations, techniques based on SCFs can afford the peculiar features of the dense gases, such as high compressibility and diffusivity, very high evaporation rate and the possibility of fine tuning the solvent power through density modulation (temperature and/or pressure variation).

Other benefits of supercritical fluid technologies, strictly related to the above-mentioned new paradigm in pharmaceutics, are linked to the reduced complexity of the process which stems from a diminution of the number of steps as well as to the improved process understanding and control.

However, the way from bench to plant has not been so fast as it was expected and hoped at the begging.

Pharmaceutical manufacturing must meet stringent regulatory requirements (Good Manufacturing Practices, cGMP), and safety rules (Environmental, Health Safety, EHS). This last point implies particular attention in the case of the SCF-based operations that involve mechanical, chemical, and biological hazards (Clavier and Perrut, 2004). Mechanical hazards are related to the use of high pressure, while particular attention has to be paid when flammable and/or explosive fluids are handled. The corrosion is an issue that must be evaluated as well; supercritical water oxidation leads to extreme corrosion and special alloys are required. Furthermore, leakage of carbon dioxide (which is the most used SCF) in a closed room could lead to asphyxia in humans, so all possible CO_2 emissions must be collected into an over dimensioned vent line, ensuring a good dispersion of the gas in the outside atmosphere.

Apart from regulatory requirements another major issue that should be addressed when dealing with supercritical fluid technologies is represented by the cost of the equipments owing to the fact that the field is still relatively young and such technologies are not yet widespread.

The present work focuses on some applications of SCFs in the pharmaceutical field, providing a critical review of the most recent advances and it aims to give a vision on the future of this technology. In particular, processes such as particle and crystal engineering, formation of complexes with cyclodextrins (CDs), coating, foaming and tissue engineering, extrusion, production of liposomes and biotechnological compounds, sterilization and solvent removal are described and discussed. Particle and crystal engineering is only briefly discussed in this paper as it has already been reviewed elsewhere (Pasquali et al., 2006, 2008a).

2. Supercritical fluids

The first report which describing a potential process using a supercritical fluid as medium for particles production was published by Hannay and Hogarth: "We have then, the phenomenon of a solid with no measurable gaseous pressure, dissolving in a gas. When the solid is precipitated by suddenly reducing the pressure, it is crystalline, and may be brought down as snow in the gas, or on the glass as a frost, but it is always easily redissolved by the gas on increasing the pressure" (Hannay and Hogarth, 1879).

A fluid reaches the supercritical region when heated and pressurized above its critical pressure and temperature; the critical point represents the end of the vaporization curve in the PT phase diagram (Fig. 1). The supercritical *status* does not represent a specific aggregation state, but it corresponds to a region where the physico-chemical properties of the material are intermediate between those of the liquid and the gas. The macroscopic appearance of a fluid at the critical point is that of a homogeneous and opalescent system without apparent phase separation because, at this point, the density of the gas and liquid are identical.

Like a gas the SCF shows lower viscosity and higher diffusivity relative to the liquid. These properties facilitate mass transfer phenomena, such as matrix extraction or impregnation. Like a liquid, the SCF shows a density value high enough for exerting solvation

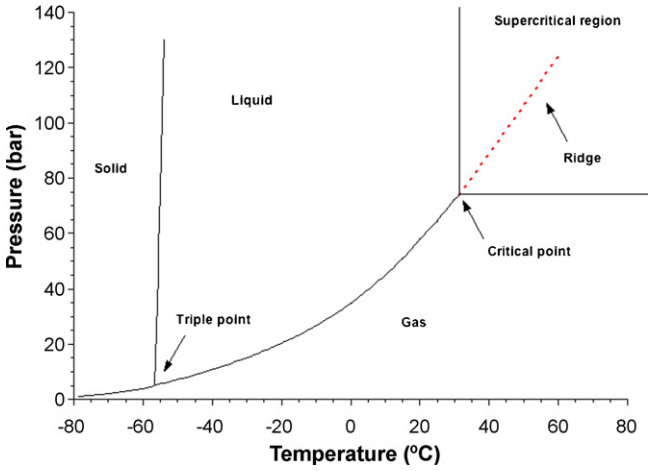

Fig. 1. CO_2 phase diagram.

effects. A SCF is dense but highly compressible, thus, any pressure change results in density alteration and, consequently, in solvent power variation (Brunner, 1994). In the vicinity of the critical point, the compressibility is high, and a small pressure change yields a great density modification.

At the microscopic level, the opalescence observed at the critical point can be interpreted by considering that the fluctuations of the local density occurs with a correlation length of the same order of magnitude than that of the visible light. The inhomogeneity is a fundamental concept that characterizes the supercritical status, and the density fluctuations are an indication of the nonuniform distribution of the molecules in the space occupied by the fluid. Molecules organization inside the supercritical region with areas of specific local densities both for the gas and liquid was reported (Nishikawa and Morita, 2000). This organization is dynamic and the density fluctuation allows explaining the increase of the fluid compressibility. In fact, in the CO_2 phase diagram it is possible to identify into the critical region a ridge that separates the liquid-like (upper) and gas-like (lower) regions (Fig. 1).

All gases become supercritical above their critical coordinates, although in some cases extremely high pressure and temperature may be required. Carbon dioxide (SC-CO_2) is the most widely used SCF for pharmaceutical applications because of its low critical temperature (31.2 °C) and pressure (7.4 MPa), inexpensiveness, non-flammability, recyclability and environmental benignity.

3. Processing using supercritical fluids

Supercritical fluids have been proposed for many different unit operations, nevertheless all processes are based on some techniques which can be divided in four groups:

- Operations where the SCF acts as a solvent (RESS, RESOLV);
- Operations where the SCF acts as an antisolvent (GAS, PCA, ASES, SEDS);
- Particles from a gas-saturated solutions (PGSS, DELOS, CPCSP);
- CO_2-assisted spray-drying (CAN-BD, SAA).

3.1. Operations where the SCF acts as a solvent

The RESS process (*Rapid Expansion of Supercritical Solution*) consists of the saturation of the supercritical medium with a solute followed by a rapidly depressurization (expansion) of the solution through a heated nozzle at supersonic speed (Krukonis, 1984). The rapid and uniform pressure drop, obtained by passing from supercritical to ambient conditions, leads to a dramatic and instantaneous decrease of solvent power (solute supersaturation) and as a consequence, to a rapid nucleation of the solute in form of very small particles with uniform size (Phillips and Stella, 1993). These particles are completely dry, solvent free, and they do not need further processing.

Factors influencing the properties of the precipitated particles are: solute solubility in SC-CO_2, pressure/temperature in the precipitation vessel, temperature, geometry and size of the nozzle, distance of the jet stream and angle of impact against the surface (Kayrak et al., 2003).

Advantages of RESS are the simple control of process parameters, the relatively easy implementation on lab-scale when a single nozzle is used, and the absence of organic solvents. On the other hand, important disadvantages have limited the development of this technique: the difficulty in scaling-up, the possible particles aggregation and nozzle blockage (York, 1999), the need of large amount of SCF, and, overall, the poor solubility of most pharmaceutical compounds in supercritical CO_2. In some cases, the SC-CO_2 solvent power can be enhanced with the use of a co-solvent which modifies the polarity of the dissolving phase and, as in the case of the short chain alcohols, features H-bonding (Kompella and Koushik, 2001).

RESOLV (*Rapid Expansion of a Supercritical Solution into a Liquid Solvent*) represents a variation of RESS. This technique was studied in order to minimize the particles aggregation during the jet expansion. Here, the supercritical solution is depressurized through an orifice into a collection chamber containing an aqueous solution at room temperature. Various water-soluble polymers or surfactants are added to the aqueous medium for stabilizing the obtained nanoparticle suspension (Meziani et al., 2005).

3.2. Operations where the SCF acts as antisolvent

These techniques were proposed to process molecules with poor solubility in SCFs (Gallagher et al., 1989). Here the pressurized CO_2 acts as an antisolvent for precipitating a solute from an organic solvent solution.

The solute is first dissolved in an organic solvent, then the pressurized CO_2 is put in contact with the solution. The principle is based on (i) the ability of the organic solvent to dissolve a large amount of gas; (ii) the mutual miscibility of the organic and SCF phases; and (iii) the low affinity of the SCF for the solute. The CO_2 diffuses into the organic solvent leading to the solvent evaporation into the gas phase; then, the volume expansion determines a density reduction, which lowers the solvent power of the organic solvent which leads to the precipitation of the solute.

Different processes based on the different mixing modes between the organic solution and the SCF were designed.

In the *Gaseous Antisolvent* (GAS) the precipitation vessel is loaded with the solution then, the SCF is introduced into a vessel until the final pressure is reached.

In the *Particles by Compressed Antisolvent* (PCA) and *Supercritical Antisolvent* (SAS), the CO_2 (supercritical for SAS, or subcritical for PCA) is first pumped inside the high-pressure vessel until the system reaches the fixed pressure and temperature, then, the organic solution is sprayed through a nozzle into the SCF bulk determining the formation of the particles that are collected on a filter at the bottom of the vessel.

The *Aerosol Solvent Extraction System* (ASES) is similar to the SAS technique except that, in this case, the solution and the antisolvent are simultaneously sprayed into the precipitation vessel.

Simultaneous spraying of the solution and the antisolvent occurs in the case of *Solution Enhanced Dispersion by Supercritical Fluids* (SEDS) as well. Compared with the other antisolvent

techniques SEDS is characterized by the use of a co-axial nozzle designed with a mixing chamber (Hanna and York, 1995) which allows the attainment of better mixing by enabling a turbulent flow. SEDS was also successfully used to process aqueous solution of peptides and proteins despite the limited solubility of water in SC-CO_2. SEDS overcomes this problem by using a co-axial three-component nozzle, in which the organic solvent, the SCF, and the aqueous solution, as separate streams, meet in the mixing chamber before being introduced in the precipitation vessel (Moshashaee et al., 2003).

Although the antisolvent techniques are very promising for particles engineering, the complete solvent removal still represents an issue: a final wash-out treatment with supercritical CO_2 through the precipitated particles may be required.

3.3. Particles from gas-saturated solutions (PGSS, DELOS, CPCSP)

Particles from gas-saturated solutions (PGSS), in its most interesting application for pharmaceutics, implies the dissolution of the compressed gas (super- or subcritical) into a melted material (usually a polymer) followed by the rapid depressurization of the gas-saturated solution through a nozzle that causes the formation of particles (Graser and Wickenhaeuser, 1982).

The interaction between CO_2 and the polymer reduces interchain polymer bonds giving rise to an enhanced polymer segmental mobility that in turn results in a reduction of the glass transition or melting point. The extent of the glass transition or melting point depression depends on the amount of CO_2 dissolved in the polymer (Kazarian, 2000; Pasquali et al., 2008b,c). In the rubbery or liquid state the polymer can be used as coating agent, to produce foams, or to incorporate drugs, affording a molecular dispersion which can be extruded or sprayed at lower pressure to obtain drug loaded microparticles. Obviously, polymer plasticization can be also induced thermally or by means of organic solvents, however major drawbacks of these approaches are the difficulty to process heat-sensitive materials and the possible presence of residual solvent in the final product. These are points of major concern especially in pharmaceutical, biotechnological and food applications.

This technique offers numerous advantages: the pressure used is lower than that necessary for RESS; the required amount of gas is relatively small; no organic solvents are required; it can work in continuous mode; the process affords a good yield.

On the other hand, the poor control of the particles size and particle size distribution represents an issue that has still to be addressed.

Nonetheless the balance between advantages and drawbacks should be considered positive, and this technique might play a prominent role in the future of drug delivery especially for the preparation of microparticulate systems.

Several PGSS plants are already running with capacities of some hundred kilograms per hour (Natex, Austria, Thar Technologies, USA, Uhde HPT) (http://www.natex.at, http://www.thartech.com, http://www.UhdeHPT.com).

Based on the principle of PGSS also DELOS and CPCSP processes have been developed.

In the *Depressurization of an Expanded Liquid Organic Solution* (DELOS) the CO_2 expands in an autoclave where an organic solution of the solute to be micronized is dispersed. Then the ternary mixture solute–solvent–compressed gas is depressurized by rapid reduction of the system pressure to atmospheric conditions in an expansion chamber. During the expansion the mixture cools down: the temperature drop is the driving force that causes the nucleation and precipitation of the drug (Ventosa et al., 2001, 2003). In this process the CO_2 does not act as an antisolvent, but as a co-solvent to nebulize and cool the organic solution. The process is not necessarily supercritical, in fact the operative pressure does not exceed the critical point of the CO_2/solvent mixture. The *Continuous Powder Coating Spraying Process* (CPCSP) was proposed by Weidner et al. (2001) for coating powders. In the CPCSP, the main components (binder and hardener) are melted in separated vessels to avoid a premature interaction with the polymer. The molten polymer is fed into a static mixer, and homogenized with compressed carbon dioxide. Then, the different components are intensively mixed and the formed solution expanded through a nozzle into a spray tower.

3.4. CO_2-assisted spray-drying (CAN-BD, SAA)

In these aerosolization techniques the supercritical CO_2 is used to assist the nebulization of the solution of the compound to be processed. The mechanism of this process is close to classical micronization by spray-drying. The substance is dissolved or suspended in water or ethanol or both, and the solution or suspension is intimately mixed with the SC-CO_2. The formed emulsion is rapidly decompressed through a suitable device.

In the case of CAN-BD (*Carbon dioxide Assisted Nebulization with a Bubble Dryer®*) the near-critical or supercritical CO_2 and the solution are pumped through a near zero volume to give rise to an emulsion which expands through a flow restrictor into a drying chamber at atmospheric pressure to generate aerosols of microbubbles and microdroplets that are dried by a flux of warm nitrogen (Sievers and Karst, 1997, 2000).

In the case of SAA (*Supercritical Fluid-Assisted Atomization*) (Reverchôn, 2002) the supercritical CO_2 and the solution are mixed into a vessel loaded with stainless steel perforated saddle which assures a large contact surface between liquid solution and the SCF; then the mixture is sprayed in a precipitator at atmospheric pressure under a flow of hot N_2.

The main difference between CAN-BD and SAA processes is represented by the mixing part of the equipment and, therefore, by the extent of solubilization of the SC-CO_2 in the liquid solution.

In summary many different techniques were developed based on the characteristics of the material to be treated and/or the final product. This underscores the versatility of the SCF-based techniques: a process limitation in one area often opens up new research and solutions in other directions. For instance, if the starting material is soluble in the SCF the RESS will be preferred while in case of the low solubility an antisolvent process will be used. When organic solvent has to be avoided, *i.e.* in the case of biological products, processes such as CAN-BD or SEDS with three co-axial nozzle should be selected. Finally, the characteristics of the desired product (micronized, coated, encapsulated, etc.) would drive the selection of the technology as well.

4. Particle and crystal engineering

Solid dosage forms are mostly used to administer drugs, and the selection of a solid with specific or even tailor-made characteristics (size, shape, crystal structure, morphology, drug release profile) is a major issue in modern drug delivery (Pasquali et al., 2006). Conventional processes for the production of particles (crushing/milling, air micronization, sublimation, re-crystallization from solvents, spray- and freeze-drying) often offer limited control over the properties of the produced powders.

The importance of solid-state, crystallographic purity as well as of the careful monitoring of drugs and excipients polymorphism is emphasized also by Regulatory Bodies (ICHQ6A, decision tree #3 and #4) (http://www.ich.org). As far as the crystal structure is concerned conventional manufacturing methods do not guarantee sufficient control on the powder characteristics; main

consequences are physico-chemical instability and poor product shelf-life.

SCF-based technologies represent a well-documented alternative for particle design and crystal engineering (Chow et al., 2007; Moribe et al., 2008; Pasquali et al., 2006, 2008a; Yasuji et al., 2008). The above-mentioned peculiar features of supercritical fluids enable to achieve crystallization conditions which may lead to very small particles with consistent particle size distribution, crystal structure and surface properties (Kordikowski et al., 2001; Rehman et al., 2003). A specific advantage that should be underlined is represented by the almost instantaneous pressure variation in supercritical processes which leads to uniform conditions in the whole solution. Classical crystallization processes exploit temperature variation where the rate-limiting step is related to the thermal conductivity of the medium, while in the SCF process the propagation of the pressure perturbation is correlated to the speed of sound.

Furthermore, traditional methods for the production of drug microparticles require numerous manufacturing steps that can be avoided, in case of SCF techniques, bringing to a simplification and better control of the process (Fig. 2).

The nozzle geometry is certainly a key point in the microparticles production. The design proposed by SEDS technology has represented a milestone on the way to more efficient, reproducible and performing equipments. The geometry of this nozzle allows working at high Reynolds (good mixing) and Weber (small droplet size) numbers by increasing the velocity of the fluid (Pasquali et al., 2008a). This leads to an improved mass transfer and nucleation rate affording the production of particles with small size and little agglomeration (Palakodaty et al., 1998).

A recent significant advancement in this field has been proposed by XSpray Microparticles (http://www.xspray.com). This company designed a new nozzle, RightSize Nozzle Head, based on a circular geometry enabling easier and consistent scaling-up.

5. Cyclodextrins inclusion complexes

Cyclodextrins are cyclic oligosaccharides able to fully or partially include in their hydrophobic internal cavity a guest molecule of appropriate size. This complexation allows to improve some physico-chemical properties such as solubility, dissolution rate, stability, as well as organoleptic characteristics. Conventional preparation methods of CDs inclusion complexes include kneading, co-precipitation, co-evaporation, co-grinding, freeze- and spray-drying (Brewster and Loftsson, 2007).

Studies on the feasibility of supercritical fluid processes to produce inclusion complexes between drugs and CDs in the solid-state are reported in literature.

A successful complexation (94% inclusion yield) between piroxicam and β-cyclodextrin (β-CD) was obtained by Van Hees et al. (1999). The inclusion experiments were performed by keeping a physical mixture of β-CD and piroxicam for 6 h in contact with CO_2 at 150 °C and 15 MPa without the use of organic solvents.

By using a similar method Moneghini et al. tried to include nimesulide into β-CD. The obtained results indicated that the drug was only partially included (Moneghini et al., 2004). However, the physico-chemical characterization of the obtained product pointed out the existence of interactions between drug and carrier that led to an increased *in vitro* drug dissolution rate.

Charaoenchaitrakool et al. (2002) prepared a complex of methyl-β-cyclodextrin (M-β-CD) and ibuprofen affording the M-β-CD solid/liquid transition as a consequence of the interaction with SC-CO_2. The same research group used an ASES process for producing micron-sized naproxen formulation incorporating hydroxylpropylated- and methylated-β-CD (Mammuccari et al., 2006).

A controlled particle deposition process using SC-CO_2 (CPD) was developed by Turk et al. (2007) to produce complexes of racemic ibuprofen and β-CD. In this method, ibuprofen and β-CD were filled into two separates cartridges as pure compound and in another cartridge, as a physical mixture: the cartridges were inserted in a high-pressure cell heated and pressurized at the desired values (40 °C and 25 or 30 MPa). After 15 h under stirring, the system was depressurized within 10 min. The maximum inclusion yield, in the case of separate cartridges was 88%, and 60.5% in the case of the physical mixture. Both the dissolution rate coefficient and the dissolved amount after 75 min of the CPD complexes were found to be significantly higher than those of unprocessed ibuprofen alone and ibuprofen/β-CD physical mixture.

A SEDS process (10 MPa, 40–80 °C) was carried out to produce crystalline channel-type and amorphous γ-CD particles and crystalline channel-type γ-CD complexes in a single-step process (Toropainen et al., 2007). The increase of process temperature changed the crystallinity of γ-CD. In particular, at 80 °C amorphous γ-CD was obtained, while the complexes crystallized a tetragonal channel-type form to hexagonal channel-type form. The dissolution behavior of budesonide/γ-CD complexes depended on their crystal structure: the tetragonal dissolved faster than hexagonal form.

This represents a nice example of γ-CD crystallinity change with subsequent variation of the dissolution rate of complexed budesonide stemming from the modification of the operative process conditions.

SAS process was used for the preparation of an inclusion complex between simvastatin (SV) and hydroxypropyl-β-cyclodextrin (HP-β-CD) (Jun et al., 2007). The activity of the SV/HP-β-CD complexes was tested *in vivo* in comparison with uncomplexed simvastatin. SV/HP-β-CD inclusion complex performed better than SV in reducing total cholesterol and triglyceride levels; this could be primarily attributed to the improved solubility and dissolution of the complex.

In another study, the feasibility of the ASES process for preparing solid-state inclusion complexes of itraconazole (ITR) with HP-β-CD was investigated (Lee et al., 2008). ITR/HP-β-CD complexes with a significantly higher dissolution rate (90% of ITR dissolved within 5–10 min) than the unprocessed ITR and its physical mixture were obtained.

6. Coating

Conventional coating processes are carried out by nebulizing an aqueous or organic solution of the coating material (sugar, polymer, wax) onto the solid dosage form.

The use of aqueous solutions eliminates many disadvantages associates with organic solvents; however, it increases the drying time due to the higher latent heat of vaporization of the water relative to organic solvents. Furthermore, the number of the material, in particular polymers, that can be dispersed or dissolved in water is limited. Last but not least, the coating of the small particles still represents an issue.

Solventless coating technologies may overcome some of the above-mentioned disadvantages, and reduce the overall cost by eliminating the slow and expensive process of solvent removal (Bose and Bogner, 2007).

A fluidized-bed coating process based on the RESS was described by Tsutsumi et al. (1995). The coating granulation process consists of three main steps: extraction, expansion and fluidization (Wang et al., 2001). In a typical experiment, CO_2 flows through the melted

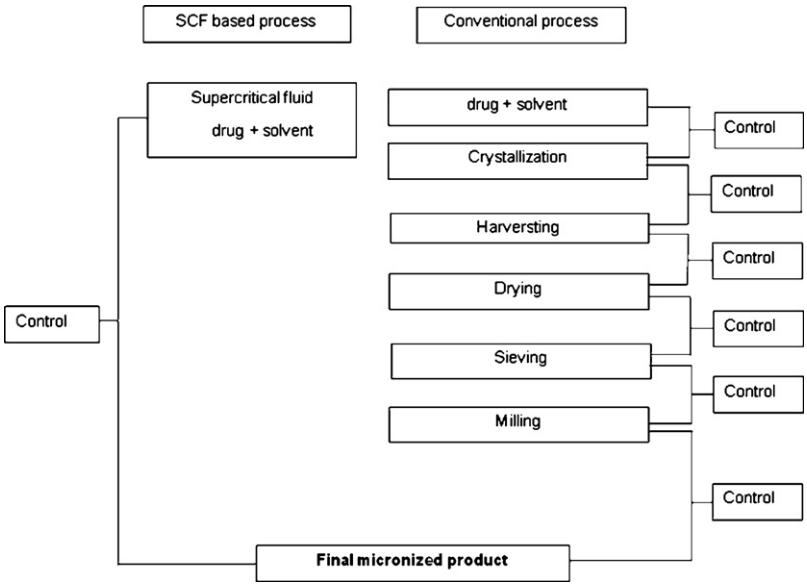

Fig. 2. Process simplification by SCF relative to conventional micron-sized crystalline powder production.

coating material in an extraction column, where the CO_2 becomes saturated with the solute. The bed is fluidized by adding pure CO_2 via a bypass line; thus the CO_2 acts as carrier fluid as well. The CO_2, saturated with the coating material, expands through a nozzle into the fluidized bed, which consists in a column installed inside an autoclave. The solid final product is separated from the gas by a cyclone and several filters (Kröber and Teipel, 2005). The column is equipped with two pressure sensors: one at the bottom and one on the top of the column. These allow the control of the pressure drop across the fluidized bed and calculation of the minimum fluidization velocity. Below this last the bed is packed, while above the bed expands and the particles move (Schreiber et al., 2002).

Process key parameters are temperature, pressure, solidification kinetics of the coating material, and fluidization gas velocity (Schreiber et al., 2002; Vogt et al., 2005).

The relatively low pressure and temperature as well as the absence of organic solvents enable the use of such process for encapsulating sensitive material like proteins (Rosenkranz et al., 2008). These authors tried to coat with paraffin irregularly shaped particles of bovine serum albumin (BSA) and insulin, by means of RESS process in a high-pressure fluidized bed running with SC-CO_2. The fluidization behavior of BSA was improved by adding free-flowing lactose. Lactose was mixed with BSA in different volume percentages and the minimum fluidization velocity of each mixture was measured at fixed pressure and temperature (8–12 MPa and 40–55 °C) by stepwise increasing the SC-CO_2 flow rate. It was noticed that the minimum fluidization velocity decreased by increasing pressure as well as by decreasing temperature; similar effects were found also by Vogt et al. (2005). The obtained coated particles were tested for dissolution behavior in comparison to the uncoated particles. Dissolution retardation of even more than 180 min, indicating improved encapsulation, was achieved for certain experiments.

Although this technique appears to be very promising, to date only few experiments have been done and too few data are available to express an opinion about its future application in pharmaceutics.

A supercritical fluid coating process by using a simple autoclave equipped with an rotating impeller was used to prepare BSA microparticles coated with two lipids (Ribeiro Dos Santos et al., 2002). Protein particles were coated with trimyristin (Dynasan® 114) and Gelucire® 50-02, two glyceride mixtures with a melting point of 45 and 50 °C, respectively. Dynasan® 114 afforded a discontinuous coating constituted by disordered micro-needles. This led to a drug release kinetics exhibiting a significantly high initial burst (35% in 5 min); then 70 and 85% of the protein was released in 30 min and 5 h, respectively. On the other hand, a prolonged protein release (over 24 h) was achieved with particles coated with Gelucire® 50-02, which produced a more homogeneous film coating. It was also shown that BSA did not undergo any degradation following the SC-CO_2 treatment.

Noteworthy, lipids are quite soluble in pressurized CO_2, while the most commonly used coating materials including many polymers show very low solubility in this supercritical fluid (Thies et al., 2000). This problem can be overcome by adding a co-solvent to the coating solution. Compared to conventional techniques the amount of organic solvent used here is greatly reduced.

Benoit et al. (1998) patented an antisolvent process to prepare microcapsules containing active ingredients with a polar polymer film (polysaccharide, cellulose derivates, acrylic or methacrylic polymers, polymers of vinyl esters, polyesters, polyamides, polyanhydrides, polyorthoesters, or polyphosphazenes). The procedure implied the suspension of the active ingredient in an ethanol solution of the polymer, followed by mixing with SC-CO_2 for the ethanol extraction.

Mishima et al. used a method called rapid expansion from supercritical solution with a non-solvent (RESS-N) for the microencapsulation of proteins (lipase and lysozyme) with polymers such as polyethylene glycols (PEGs), polymethylmethacrylate (PMMA), polylactic acid (PLA), polylactide-*co*-glycolide (PGLA) and PEG–PPG (polypropylenglycol)–PEG triblock copolymers (Mishima et al., 2000). The polymer, protein and ethanol were placed inside an autoclave, then the mixture was stirred, and the CO_2 pumped in at fixed temperature and pressure. Non-agglomerated proteins containing microparticles were obtained upon the expansion of the polymeric solution. The thickness of the polymer coating, the mean particle diameter as well as the particle size distribution, could be controlled by changing the polymer feed composition (Mishima et al., 2000).

Shekunov and co-workers developed a new method, called *particles from SCF extraction of an emulsion* (PSFEE), to encapsulate drug into microparticles (Chattopadhyay et al., 2003a,b). The polymer (PLGA, 5–20%, w/w) and a model drug like indomethacin,

ketoprofen, griseofulvin, or cholesterol acetate (10–20%, w/w) were dissolved in water-saturated ethyl acetate. This solution was then dispersed in an ethyl acetate saturated aqueous solution of polyvinyl alcohol (molecular weight 35,000–50,000) to form an emulsion. The emulsion was introduced into an autoclave from the top using a fine nozzle while the SC-CO_2 was introduced from the bottom. The operating pressure and temperature were chosen in such a way that the density of the CO_2 was lower than density of the emulsion. The SC-CO_2 interacted with the emulsion droplets and extracted the solvent causing the precipitation of the material dissolved in the droplets. The residual solvent content of the particles was consistently below 50 ppm, independently of the organic solvent used, and well below the regulatory-accepted levels (Chattopadhyay et al., 2005). Major feature of PSFEE is the combination of the flexibility of different emulsion systems (production of particles with well-defined size and structure, including coated, hollow or porous materials) with the possibility to develop a large-scale system.

The same authors exploited the PGSS method to produce composite particles for taste masking and controlled release. Polyethylene glycol, Eudragit RS and tripalmitin were used as excipients with model active ingredients such as acetaminophen, ketoprofen, and trypsinogen (Shekunov et al., 2003). A mixture of polymer and drug was plasticized with SC-CO_2; then, it was depressurized through a computer-controlled valve with a nozzle orifice. The size of the obtained micronized particles decreased by decreasing the nozzle diameter, increasing pressure differential and increasing the amount of CO_2 in the mixture.

Apart from particulate systems, SC-CO_2 can be used also to coat medical devices for controlled-release applications. A cardiac stent containing the drug RWJ-53380 was coated with PLGA by means of a SCF fluid deposition process (Metha and Corbo, 2001). This PLGA-coated stent released approximately 66 μg of the drug in 1 h, and 134 μg in nearly 18 h.

7. Foaming and tissue engineering

The plasticization of glassy polymers induced by SC-CO_2 plays an important role in the foaming process. A foam has a porous structure formed by non-connected (closed) cells with a diameter of about 10 μm or less and cell density larger than 10^9 cells/cm^3 (Baldwin et al., 1996).

Foaming of biodegradable polymers with SC-CO_2 has a great potential in numerous biomedical applications ranging from drug delivery to tissue engineering. Foamed materials often represent basic constituents for the preparation of sponge scaffolds used for tissue engineering. These scaffolds provide a temporary artificial support for cell adhesion and growing *in vivo*. Thus, they should have fundamental characteristics such as high porosity, appropriate pore size, biocompatibility, biodegradability and proper degradation rate. Besides, the polymeric scaffold may incorporate several growth factors or growth supplements. In this respect the attainment of the suitable drug delivery rate may represent a major challenge for formulators (Whitaker et al., 2001).

Among the technologies developed to fabricate these polymeric porous sponges, SCFs occupy a prominent position owing to their great capability to generate porous materials at relatively low temperature and without the need of organic solvents.

The production of foams and porous structures implies the saturation of the polymer with SC-CO_2 at constant temperature and pressure; then the system is brought to the supersaturated state either by reducing pressure or by increasing temperature, eventually leading to phase separation, nucleation and growth of pores.

A method to fabricate porous sponges of poly(D,L-lactic-*co*-glycolic acid) was described by Mooney et al. (1996). Solid discs of PGA, PLA and PLGA were saturated with CO_2 at 5.5 MPa for 72 h at room temperature, then, thermodynamic instability was created by rapidly decreasing the CO_2 pressure. Gas molecules cluster formed nuclei to minimize their free energy. Dissolved gas molecules diffused to these pore nuclei creating macropores. Sponges with porosity of up to 97% and uniform distribution of macropores (10–100 μm) were obtained. The porosity and the pore structure depended on the amount of gas dissolved in the polymer (which can be controlled by varying the gas pressure and temperature), on the rate and type of gas nucleation, and on the diffusion rate of the gas molecules through the polymer (Goel and Beckam, 1994; Park, 1995).

Tsivintzelis et al. carried out experimental and theoretical work on the preparation of microcellular polystyrene foams and porous structures of biodegradable poly(D,L-lactic acid) prepared, using SC-CO_2 as a blowing agent. These authors accurately described the effect of pressure, temperature, and depressurization rate on the obtained structure (Tsivintzelis et al., 2007). The size of the pores decreased and the pores population density increased with pressure increase, temperature decrease, and depressurization rate increase.

Several studies indicated that amorphous polymers absorb a larger amount of CO_2 with respect to the crystalline counterparts, therefore they are more suitable for porous foam formation. It was reported that crystalline poly(vinylidene fluoride) (PVDF) did not afford foam formation and the product was characterized by poor cell structure (Siripurapu et al., 2002). However, when PVDF was blended with PMMA, significantly improved morphologies were obtained due to foaming. The addition of PMMA to PVDF increased the solubility of the SC-CO_2 into the polymer blend leading to a greater density of the bubble nucleation sites and, consequently, to cell uniformity in the foam.

Other authors demonstrated that porous foams incorporating biologically active agents can be prepared by using a supercritical fluid based technique (Hile et al., 2000). PLGA foams incorporating basic fibroblast growth factor (bFGF) and BSA were generated both using the traditional solvent casting-salt leaching technique and a SC-CO_2-based technique.

Prior to the SC-CO_2 processing, an emulsion between an aqueous protein solution and a polymer solution in an organic (methylene chloride) solvent was prepared. The emulsion was treated with CO_2 at 35 °C and 80 bar for 24 h, then, the pressure was suddenly decreased (10–12 s). The release and activity of bFGF from the foams obtained with SC-CO_2 were determined and compared with those from the foams prepared by traditional solvent casting-salt leaching technique. Protein release rate was greater from structures made with CO_2 relative to those made by the salt leaching technique, even though a large initial burst was observed in the latter case. Residual methylene chloride levels were measured in the foams made with CO_2 and were found to be above the limits reported by the US Pharmacopeia, thus requiring further solvent removal prior to the *in vivo* use.

Other authors investigated the drug release of chlorhexidine diacetate from a porous polymeric system based on poly(ethylmethacrylate) (PEMA) and tetrahydrofurfurylmethacrylate (THFMA) obtained using a supercritical fluid-assisted impregnation and foaming technique (Gong et al., 2007). SC-CO_2 efficiently foamed the drug–polymer system and after fast depressurization afforded a highly porous structure which dramatically increased the release rate of chlorhexidine diacetate with respect to the conventional cured samples.

A fluorescently labeled protein (rhodamine-labeled antibody to immunoglobuline, 165 kDa) was successfully impregnated into

polymethylmethacrylate, swollen with CO_2 (Sproule et al., 2004). The experiments were carried out with a view cell that allowed *in situ* quantification of the polymer swelling by laser dilatometry. Foaming was studied by means of a X-ray computed tomography, while protein impregnation was verified by confocal microscopy.

Finally, Barry et al. produced bovine chondrocytes containing methacrylate scaffolds. Polymer foaming was achieved by placing PEMA/THFMA discs under CO_2 at 40 °C and 10 MPa for a time ranging from 1 to 48 h, then by rapidly depressurizing the system (Barry et al., 2004). The study demonstrated that the change in the structure of the substrate from flat disk to foam enhanced the cell phenotype retention, although the authors concluded that further modifications in the SC processing were required to obtain optimum porosity for cell migration and cartilage tissue formation.

8. Extrusion

The above-mentioned capability of SC-CO_2 to plasticize polymers at low temperature can be exploited in the extrusion process. Here, the SC-CO_2 can both change the rheological properties of the material, and behave as an expansion agent. The dissolution of a large amount of SC-CO_2 determines a polymer expansion and viscosity reduction. The viscosity reduction results in lower mechanical constraints and decreases the required operating temperature, thus allowing processing of thermolabile compounds.

Verreck and co-workers carried out extrusion of PVP-VA (polyvinylpyrrolidone-*co*-vinyl acetate), Eudragit and ethylcellulose, by means of a twin-screw extruder in which pressurized CO_2 was injected at a constant pressure rate (Verreck et al., 2006). The physico-chemical characteristics of the polymer before and after injection of CO_2 were evaluated. The specific surface area and the porosity of the polymers increased after treatment with carbon dioxide, eventually resulting in enhanced polymer dissolution in water. The expansion of the CO_2 at the nozzle determined a change of the morphology of the system toward a foam-like structure. The same observations were done in the case of itraconazole/polymer combinations as well (Verreck et al., 2007).

Another work illustrates the use of SC-CO_2-assisted extrusion for the preparation of a hot melt extruded monolithic polymer matrix for oral drug delivery (Lyons et al., 2007). The polymer used was polyethylene oxide (PEO) while the model drug was carvedilol. The SC-CO_2-assisted process gave rise to extruded material with faster dissolution compared to that obtained with a classical extrusion process.

In conclusion this represents an interesting example of process "contamination" where the introduction of a SCF technology into a conventional technique leads to a better performing material.

9. Liposomes preparation

The preparation of stable liposomes formulation on industrial scale is still a major issue in pharmaceutics mainly due to the need of the large amount of organic solvents and the high energy consume. In this respect, SCF-based technologies have attracted a great deal of interest during the past 10 years, as a green alternative to classical methods.

Pioneering work in this field was done by Frederiksen and coworkers, based on a modification of RESS process (Frederiksen et al., 1997, 1998). The apparatus was mainly composed by two parts: a high-pressure system in which phospholipids and cholesterol were dissolved into the supercritical phase and a low pressure system in which the supercritical phase (containing also a various amount of ethanol) was expanded and simultaneously mixed with a water phase containing dextran and fluorescein isothiocyanate to yield liposomes. Liposomes with a diameter of 200 nm were obtained. Noteworthy, this technique get the same encapsulation efficiency but required 15-fold less organic solvent, compared to the ethanol injection method of Batzri and Korn (1973).

Castor and Chu (Castor, 2005; Castor and Chu, 1998) presented a Supercritical Fluids™ CFN (SFS-CFN) apparatus for making liposomes containing hydrophobic or hydrophilic drugs featuring critical, subcritical or near-critical CO_2. Such fluids were used to solvate phospholipids, cholesterol and other raw materials. After a specific mixing time the resulting mixture was decompressed by means of an injection nozzle into a chamber that contained phosphate-buffered saline or an other biocompatible solution with the hydrophilic drugs (recombinant proteins, RNA, DNA). SCF depressurization and phase-conversion into a gas (with formation of bubbles) created a deposition of the phospholipids in the aqueous phase and, consequently, the formation of phospholipid bilayers encapsulating the hydrophilic drug. In the case of hydrophobic drugs, the phospholipids and the target compounds were solvated simultaneously in a SCF "cocktail" which was dispersed continuously into the aqueous environment. In a third variation of this technique, hydrophobic drugs and phospholipids were directly solvated in the SCF prior to injection into an aqueous solution.

The characteristics of the obtained liposomes depended on several process parameters: size and design of the nozzle (affecting the bubble sizes and therefore the size of liposomes formed); decompression rate which defines the characteristics of the deposited phospholipids as well as the intensity of the mixing (slow decompression produced smaller and more uniform liposomes); interfacial forces between the SCF and the aqueous phase; charge distribution of the liposomes and its interaction with the surrounding aqueous medium (pH, ionic strength and electrolyte composition); the nature of compounds being encapsulated.

It was found that liposomes formed with a 0.5 mm nozzle with lecithin at 28 MPa and 60 °C had an average diameter of 478 nm with unimodal distribution, while liposomes formed with 0.6 mm nozzle under identical condition had an average diameter of 326 nm. The incorporation efficiency varied between 1% for small unilamellar vesicles and 88% for some multilamellar vesicles (Castor and Chu, 1998). The stability of liposomes was also tested, by measuring the particle size distribution as a function of time. SC-CO_2 liposomes proved to be stable at 4 °C over a 6-month period.

This technique also afforded liposomes incorporating molecules such as paclitaxel, camptothecin, betulinic acid, bryostatin 1, vincristine and doxorubicin, with diameter between 100 and 300 nm. *In vitro* and *in vivo* results suggested that these formulations are more effective than the commercial available ones.

More recently, other authors (Otake et al., 2001) produced liposomes by using the method described by Castor and Chu. Phospholipids and ethanol were introduced in a view cell, along with the CO_2. The cell temperature was then raised up to 60 °C, namely a temperature higher than the phospholipid phase transition temperature, while the pressure was kept at 20 MPa. An aqueous dispersion of liposomes was obtained through the formation of an emulsion by introducing an aqueous glucose solution into the cell. Then, the pressure was reduced to release the CO_2, leading to a homogeneous liposomial dispersion. Transmission electron microscopy indicated that the vesicles obtained were large ellipsoidal unilamellar liposomes with a diameter from 0.1 to 1.2 μm, enabling a high entrapping efficiency (up to 20% for hydrophilic substances and 63% for hydrophobic compounds). The same method was used to prepare chitosan-coated cationic liposomes for DNA transfection (Otake et al., 2006). Pressurized CO_2 forms carbonic acid in contact with water thus it lowers the water

pH. This phenomenon was exploited for preparing chitosan-coated cationic liposomes of L-α-dipalmitoylphosphatidylcholine in one step without adding non-volatile acids. The entrapping efficiency was 17% with or without the addition of chitosan (those prepared by the Bangham method (Bangham et al., 1965) afforded a entrapping efficacy of 2%), while the liposomial dispersion was stable at room temperature in a sealed tube for over 30 days.

10. Biotechnological compounds processing

The dramatic development of biotechnologies has made available a new generation of drugs represented by peptides, nucleotides and DNA fragments. Formulation handling, stability as well as the selection of suitable administration routes for these drugs represent the great challenge of these new frontier (Conti et al., 2000).

Many researchers focused on SCFs technologies as an alternative to the classical methods for the production of therapeutic proteins delivery systems, because of the already mentioned mild operative temperature and absence of organic solvents, which may lead to protein denaturation (Okamoto and Danjo, 2008). In general, biotechnological compounds are high molecular weight, hydrophilic substances thus they show low solubility in SC-CO_2. Many literature reports refer to the use of SC-CO_2 as an antisolvent to precipitate proteins from a solution (Bouchard et al., 2007; Bustami et al., 2000; Moshashaee et al., 2000; Nesta et al., 2000; Okamoto et al., 2003; Palakodaty et al., 1998; Sarkari et al., 2003; Velaga and Carlfors, 2005; Winters et al., 1996).

The SAS technique was used to obtain microparticulate (1–5 μm) protein powders (insulin, trypsin, and lysozyme) from a dimethylsulfoxide (DMSO) solution (Winters et al., 1996). Analysis of the Raman spectra revealed minimal (lysozyme), intermediate (trypsin), and appreciable (insulin) changes in the secondary structure with respect to the starting material. The main modification, during supercritical fluid-induced precipitation, is the formation of β-sheet structure (from 29, 36, and 54% to 56, 47 and 62% for insulin, lysozyme, and trypsin, respectively) with concomitant decrease of α-helix (from 46, 34, and 16% to 12, 27, and 11%). Raman spectra showed that at high operating temperatures, the pressure led to more extensive β-sheet-mediated intermolecular interactions in the precipitates. Interestingly, Raman and FT-IR spectra indicated that proteins recovered their native conformation almost completely upon re-dissolution in water (recovery of biological activity ~100, 88–100, and 64–94%, for insulin, lysozyme and trypsin, respectively).

SEDS was used as well to produce micronized lysozyme particles from a DMSO solution (Moshashaee et al., 2000) which retained a biological activity ranging from 44 to 100% (standard deviation up ±12.3%). The pressure had the most significant influence on the biological activity, while temperature, solution concentration and flow rate had a minimal effect.

DMSO is one of the most commonly used solvents in the SCF antisolvent technique, owing to its good solvent ability for proteins and miscibility with SC-CO_2. However, it may disrupt the protein conformation (potential loss of secondary and tertiary protein structure) and it may remain as a residue in the final product (Huang et al., 1995; Jackson and Mantsch, 1991). Therefore, a precipitation from an aqueous protein solution should be in principle preferred, but the low solubility of water in SC-CO_2 (less than 0.5 mol% at 50 °C; Wiebe, 1941) makes almost impossible the use of this solvent in a conventional SAS technique.

To overcome this problem some authors (Moshashaee et al., 2003) modified the nozzle design in the SEDS process. As already stated, here the organic solvent, the SC-CO_2, and the aqueous solution are introduced, as separate streams, inside the precipitation vessel through a co-axial three-component nozzle. The presence of the organic solvent facilitated the evaporation of water into the SCF. In this modified version of the SEDS process, the protein was maintained in an aqueous environment, before the rapid water evaporation into the SC-CO_2, thus minimizing the contact between the protein and the organic solvent–SC-CO_2 mixture (Palakodaty et al., 1998).

The comparison of lysozyme Raman spectra collected before and after processing indicated that minor disturbance (negligible at 200 bar and 40 °C) of the secondary structure occurred in SEDS-processed samples (Moshashaee et al., 2003). The attainment of a single phase (water–ethanol and SC-CO_2) at the equilibrium represents a key factor for obtaining a high quality product. Furthermore, the biological activity seemed to be related to the ethanol/water ratio in the ternary system; in particular, the lower was this ratio, the higher the biological activity was. This finding was confirmed also by Bouchard et al. (2007).

ASES and PCA were used to obtain lysozyme, albumin, insulin, recombinant human deoxyribonuclease (rhDNase) (Bustami et al., 2000) and α-chymotrypsin (Sarkari et al., 2003), respectively.

More recently, other authors established a method for stabilizing proteins implying the spraying an aqueous protein solution into a mixture of SC-CO_2 and ethanol (Jovanovic et al., 2004). The effect of sucrose, and trehalose on lysozyme or myoglobin SCF formulations was studied in comparison with freeze-drying formulation (Jovanovic et al., 2006). In a further work, the same authors investigated the optimization of the protein-to sugar ratio and the SCF composition for ensuring the stability of the protein in the solid-state (Jovanovic et al., 2008b). Furthermore, they extrapolated process and formulation parameters to obtain a stable powder of human serum IgG (Jovanovic et al., 2008a). This work put into evidence the role of a buffer solution to avoid the protein denaturation, as already underscored by Sellers et al. (2001): the buffer counteracts the pH decrease steaming from the interaction between CO_2 and water.

CAN-BD features SC-CO_2 and warm nitrogen as dispersing and drying agents, respectively (Sievers et al., 2007). Vaccine formulations were stabilized using saccharides (trehalose, lactose) and polymers (PVP). The obtained powder had low water content, spherical shape and presented a mass median aerodynamic diameter (MMAD) suitable for inhalation (1.9 μm).

11. Sterilization

Microbial inactivation by pressurized CO_2 was studied by numerous investigators (Spilimbergo and Bertucco, 2003; Spilimbergo et al., 2002, 2003; White et al., 2006; Zhang et al., 2006). This technology is already applied in the food industry, and can be exploited also in the pharmaceutical field to sterilize polymeric material and fine particles (Dillow et al., 1991).

Different hypothesis about the mechanism of microbial inactivation by CO_2 were proposed: cytoplasmatic pH decrease (acidification); modification of cell membrane and extraction of cell wall lipids; cell rupture due to fast depressurization; inactivation of key enzymes for cell metabolism; extraction of intracellular substances (Spilimbergo and Bertucco, 2003).

The application of this technology to the pharmaceutical field was described by Dillow et al. (1991, 1999). Polylactide-*co*-glycolide and polylactic acid were treated with SC-CO_2 at pressure between 14 and 21 MPa and temperatures between 30 and 45 °C for a time ranging from 0.6 to 4 h. Agitation, pressure cycling, and presence of water were found to be the main parameters enhancing the steril-

Table 1
Ethyl acetate content of PNU-159548 raw (untreated) material and upon treatment with SC-CO_2 for 3 h at different temperature and pressure (Bettini et al., 2002)

	Ethyl acetate (ppm)
Untreated material	24,000
40 °C, 30 MPa	9,500
40 °C, 40 MPa	60
45 °C, 30 MPa	30
45 °C, 40 MPa	6

ization. Biomedical polymers were sterilized also by Wayne et al. (2005) by treating the material at 7–24 MPa, 25–60 °C, for a time ranging from 20 min to 12 h and by adding peroxides and/or carboxylic acids. This patent claims the inactivation of bacterial spores, which represents the major issue with this technology (Spilimbergo et al., 2002).

Quite recently, Rocco et al. patented a method for sterilizing glucocorticosteroids by SC-CO_2 implying the treatment at 125 °C and 80 bar for 25 min (Rocco, 2007).

12. Solvent removal

Residual solvent removal by SCF exploits the great diffusivity of the compressed gas as well as the easy evaporation of organic solvent into the supercritical phase. The efficiency of the process is a function of the solid/solvent and the solvent/SCF affinity (Herberger et al., 2003; Rodier et al., 2005; Ruchatz and Kleinebudde, 1997).

Thiering et al. (2001) underlined that said efficiency can be increased at high CO_2 densities (improved solvent miscibility), and at high temperatures (increased solvent vapor pressure).

Bettini et al. (2002) investigated the extraction of ethyl acetate from an alkylating anticancer drug by using SC-CO_2. The effects of temperature (40–45 °C), pressure (30–40 MPa) and CO_2 flux (100–500 mL min^{-1}) on the solvent removal were investigated. The treatment with SC-CO_2 for 3 h at 45 °C and 40 MPa reduced ethyl acetate contents from 2.4% to 6 ppm (Table 1).

Falk and Randolph investigated the influence of process parameters on the residual dichloromethane (DCM) level in gentamycin-loaded poly(L-lactide) microparticles obtained by a PCA method (Falk and Randolph, 1998). They found that the increase of the CO_2 flow rate and volume resulted in a lower level of residual DCM.

Kamihiri et al. studied the removal of seven organic solvents from antibiotics (penicillin G, and streptomycin sulfate) with SC-CO_2, and found that the solvent extraction depended on pressure, temperature and extraction time (Kamihiri et al., 1987). Furthermore, the addition of water as an entrainer resulted in a significant increase in the extraction rate. The authors attributed this phenomenon to a change in the interaction and affinity between the antibiotic and organic solvents.

13. Conclusions

From the data presented in this review we can now try to give an answer to the question posed in the title.

An exponential trend in the number of scientific publications and patents can clearly be evidenced. Obviously most of these publications reflect academic interest in understanding the fundamentals of SCF; the field is in fact particularly suitable for thorough theoretical examination.

Besides, a continuous effort toward the application can be noticed although the number of industrial successes is to date still very low.

Nevertheless, some indications allow forecasting a significant industrial development in the near future. One of them is for instance represented by the ongoing research carried out by Eiffel Technologies Ltd. in partnership with MAP Pharmaceuticals. These companies are developing three formulations for inhalation, containing micronized powders produced with a SC-CO_2-based process: two of them, for the treatment of asthma are in Phase II clinical studies, while the third, intended for migraine therapy is expected to enter into Phase III clinical studies in early 2008 (http://www.mappharma.com).

Pulmonary drug delivery would likely drive the industrial development of SCF in the near future in the pharmaceutical field.

SCF-based techniques will probably integrate rather than replace existing and well-established techniques such as solvent coating, granulation, spray-drying, etc.

In this respect, the "hybrid" extrusion technology featuring supercritical CO_2 may be considered an appropriately illustrative example.

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