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Supercritical Fluid Technology: An Overview of Pharmaceutical Applications

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Abstract: Supercritical fluid technologies (SCFT) represent a recent approach for obtaining pharmaceutical materials in pure physical form and the application of supercritical fluids is a superior alternative to conventional precipitation and extraction processes. Supercritical fluid technology (SCFT) offers exciting opportunities to produce and modify pharmaceutical substances and has the potential to revolutionize pharmaceutical processing using simple one-step process to produce micron-size products. Particle and delivery systems design are major developments of supercritical fluids applications. Solute supersaturation causing subsequent precipitation of small particles with a narrow size distribution has been achieved using SCFT. The materials such as nano- and micro- particles of high added value compounds: i.e. an active substance (drugs, but also magnetic substances for diagnostic applications) and a polymer for the preparation of delivery systems have proved better. Current pharmaceutical applications of SCFT include: drug extraction and analysis, drug particle and drug polymorph engineering, improve the solubility of a poorly soluble drug , purification and sterilization of medical components, recrystallize pharmaceuticals to nanosize, preparation of metal nanoparticles, chromatography, coating, convert highly brittle crystalline recipients to amorphous or non-crystalline forms, separate and analyze the drug enantiomers, micronization and preparation of drug delivery systems. Scientists are of the opinion that SCFT have the potential to meet challenges for the development processes of pharmaceutical products for 21st century.
KEYWORDS: Supercritical carbon dioxide; Drug delivery; Microparticles; anoparticles;· Micronization; Pharma ceutical technology.

1. Introduction

Supercritical fluid technology (SCFT) is a new method to produce fine drug particles and is valuable for product quality, batch consistency and the reduction of manufacturing barrier in many areas of pharmaceutical applications. The progress in SCFT is based on multidisciplinary approach and a considerable progress has been made over the last decade in the application of supercritical fluids (SCFs) to the processing of pharmaceuticals [1-6] and to the preparation of drug delivery systems [7-13]. The use of SCFs for the preparation and control of the specific physical form of pharmaceutical substances relevant to those fluids used for drug delivery systems have been described [14-17]. SCFs have been proposed as solvents, solutes, anti-solvents and reaction media and SCFT is very attractive for manufacturing innovative therapeutic particles, either of pure active compounds or mixtures of excipient and active compounds. Certainly, pharmaceutical companies can develop pharmaceutical products of much higher yields greater purity, and quality than possible by conventional chemical engineering unit operations using SCFTs.

The most commonly used SCFs for a variety of applications include supercritical fluid carbon dioxide ($SC\text{-}CO_2$), nitrous oxide, water, methanol, ethanol, ethane, propane, n-hexane and ammonia [18]. $SC\text{-}CO_2$ is an attractive solvent or anti-solvent as it is safe, inexpensive, readily available, and an ideal substitute for many hazardous and toxic solvents. $SC\text{-}CO_2$ exists when both the temperature and pressure equal or

exceed the critical point of 31°C and 73 atm and has both gas-like and liquid-like qualities, and it is this dual characteristic of SCFs that provides the ideal conditions for extracting compounds with a high degree of recovery in a short period of time. By controlling the level of pressure/temperature /modifier, SC-CO_2 can dissolve a broad range of compounds, both polar and non-polar. At present, carbon dioxide technology is one of the fastest growing new process technologies being adopted by the pharmaceutical industry. Supercritical water is a unique medium for safe destruction of dangerous waste by total oxidation due to its special physicochemical properties. For additional information's on the physicochemical properties and behavior of supercritical fluids, several well known texts can be consulted [4, 19].

2. Basic Techniques in SCF Technology:

Classification of SCFs based techniques can be proposed according to the role played by the SCFs in the process. Various SCF processes used in pharmaceutical processing [20, 21] include i) Rapid expansion of supercritical solutions (RESS), (ii) Supercritical antisolvent (SAS) precipitation technique (iii) Particles from Gas Saturated Solutions (PGSS), (iv) Gas antisolvent system (SAS), (v) Precipitation using compressed antisolvent (PCA) , (vi) Aerosol solvent extraction system (ASES), (vii) Solution enhanced dispersion by supercritical fluids (SEDS), (viii) Supercritical antisolvent system with enhanced mass transfer (SAS-EM), (ix) Impregnation or infusion of polymers with bioactive materials. SCFT, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.

3. Pharmaceutical Applications of SCFs:

Supercritical fluid extraction [22, 23] (SFE) and supercritical fluid chromatography (SFC) [24] are the most developed application of SCFT mainly for extraction and purification of active substances such as cosmetic and pharmaceutical products including phytopharmaceuticals / neutraceuticals. An effective method for isolating common imidazole drugs clotrimazole and ketoconazole from creams, tablets and cosmetic shampoos based on SFE was developed with extraction recoveries ranging from 90.8% to 97.2% [25]. The supercritical fluid impregnation proved to be feasible for the preparation of a new ophthalmic drug delivery system [26].

3.1. Particle generation and co-precipitation

The various stages of particle formation by supercritical fluid processing can be broadly classified into delivery, reaction, pre-expansion, expansion and collection. The importance of each of these processes in tailoring the particle morphology has been reported along with presenting various alternatives to perform these operations [17]. In pharmaceutical industry, fine particles (μm or nm) with uniform narrow size range are of particular interest. Various SCF processes [20, 21, 27-34] for particle formation include RESS, SAS, PGSS, SAS, PCA, ASES and SEDS.

RESS process was used to produce polymeric microparticles or microspheres loaded with pharmaceuticals for drug delivery applications [35, 36]. PGSS offers very promising perspectives of industrial development where supercritical carbon dioxide is used as a viscosity reducing agent [37]. The dissolution rate of griseofulvin produced by RESS was found to be about 2-fold higher than the common micronized material [38]. Researchers reported that the cyclosporine particles formed by RESS process were stabilized for drug concentrations as high as 6.2 and 37.5 mg/ml in 1.0 and 5% (w/w) Tween-80 solutions [39].

RESS process is a promising technique for the formation of submicron particles (≤100 nm) and that the improved dissolution behavior is influenced by particle size, surface area, and wettability of the processed powders as well as by the pH-value of the dissolution media [40]. The alternative strategies of using SCFT for crystal and particle engineering of pharmaceutical materials and drug delivery systems have shown great promise [41, 42]. The techniques for particle engineering biopharmaceuticals have also been reported [43].

The glucocorticoid was micronized by a new technique using supercritical carbon dioxide (aerosol solvent extraction system, ASES) resulting in very fine particles. The study proved the feasibility of reformulating fluticasone propionate with the alternative propellant HFA-227, thereby, indicating that the processing of steroids using SC-CO_2 proved to be a useful technique for the micronization and surface coating with a surfactant in one process step [44].

Co-precipitation of active pharmaceutical ingredients (API) with additives provided the ability to add functionality to API's at early stages of drug discovery and synthesis. Results of RESS aided co-precipitation studies involving drug–additive mixtures revealed habit modification, solubility enhancement, particle size reduction, eutectic formation, reduction in crystallinity, amorphous conversion, hydrate formation, polymorph conversion and selective extraction, thereby, suggesting altering the physicochemical and mechanical properties of API's using SCF co-precipitation [45].

3.2. Co-formulation

Co-formulation of drug and excipient is one of the emerging concept in Pharma industry. SCF based PCA process provided good approach for co-formulation and quality of co-formulation product [46]. Co-formulation particles of the non-steroidal anti-inflammatory drug ketoprofen and the amorphous

biodegradable polymer poly-lactic-co-glycolic acid ranging between 100 and 200 nm in size were reported by SC-CO_2 extraction of emulsions [47]. SCFT provided co-formulated samples with a rapid and enhanced dissolution profile for poorly water-soluble drugs [48]. The PCA process was used to maximize the drug loading in stable and fully amorphous solid dispersions of phenytoin in PVP [49].

3.3. Tissue engineering

SCFT is now emerging as an alternative to conventional materials' processing methods in the area of tissue engineering [50, 51]. SC-CO_2 processing may be used to form foamed scaffolds in which the escape of CO_2 from a plasticized polymer melt generates gas bubbles that shape the developing pores. Researchers described SCF processing methods of relevance to tissue engineering and controlled release strategies, with focus on the incorporation of bioactives such as protein growth factors [52].

Researchers have reported on poly(DL-lactic acid) (PDLLA) and poly(DL-lactic acid-coglycolic acid) (PLGA) with potential application as controlled release scaffolds for growth factor delivery. The pore size and structure of the PDLLA and PLGA porous scaffolds produced using SC-CO_2 can be altered by the processing conditions. A higher pressure and a longer soaking time allowed more CO_2 molecules to diffuse into the polymer matrix, leading to a higher nucleation density and hence the production of smaller pores. Higher temperatures produced foams with larger pores because increased diffusion rates facilitated pore growth. In addition, reducing the rate of depressurization allowed a longer period for pore growth and, therefore, larger pores were formed than with rapid depressurization. Further, the pore size of scaffolds also decreased with increasing glycolic acid content in the PLGA copolymers [53].

Polymers can be plasticized and even liquefied in the presence of SC-CO_2 at a low temperature, and lowering their viscosity allowed the efficient mixing of bioactive materials within the polymers without loss of activity. Using this technique, three-dimensional porous scaffolds have been fabricated by gas foaming for tissue engineering applications and microparticles have been prepared by PGSS for drug delivery purposes. Moreover, novel block copolymers can be synthesized by enzyme-catalyzed polymerization combined with controlled polymerizations in SC-CO_2 which have potential applications as drug carriers. Complex three-dimensional scaffolds for facial reconstruction could be fabricated using poly(DL-lactic acid) microparticles loaded with growth factors (e.g. a model protein RNase A) via the supercritical route [54].

Scientists tested a new supercritical fluid assisted technique for the formation of 3D scaffolds that consists of three sub-processes: the formation of a polymeric gel loaded with a solid porogen, the drying of the gel using SC-CO_2, the washing with water to eliminate the porogen. They obtained poly (l-lactic acid)) scaffolds with elevated porosity (>90%) and interconnectivity, with good mechanical properties (compressive modulus up to 81 kPa). Moreover, the coupling of fibrous nanostructure to micronic cells of controllable size was observed. In addition, scaffolds with predetermined shape and size were also produced in a short time (<30 h) and without an appreciable solvent residue (<5 ppm) [55].

3.4. Coating

An improved process for coating medical devices, particularly surgical devices such as stents with polymer or polymer and a pharmaceutical/therapeutic agent or drug using SC-CO_2 has been reported [56]. Scientists have reported a method for forming a continuous film on a substrate surface that involved depositing particles (mean particle size of less than 1 micron) onto a substrate surface and contacting the particle-deposited substrate surface with a supercritical fluid under conditions sufficient for forming a continuous film from the deposited particles. The method may be performed in a pressure vessel containing a compressible fluid. A particle-deposited substrate was provided in the pressure vessel and the compressible fluid was maintained at a supercritical or sub-critical state sufficient for forming a film from the deposited particles [57]. Pierre Fabre Medicament - active ingredient division - has recently enlarged its Drug Delivery Technology portfolio based on its knowledge in supercritical fluids. Cyclodextrin complexation in SC- CO_2 is a Pierre Fabre patented technology mainly used in the bioavailability enhancement of poorly soluble APIs [58]. Polymer fibers with a diameter of several ten to hundred nanometers have a very large surface area to volume ratio [59]. Hollow fibers by electrospinning in supercritical CO_2 are likely to provide a big potential in a wide range of applications [60].

3.5. Micronization and nanoization of Pharmaceuticals

Micronization is an important procedure used in the pharmaceutical industry to reduce the particle size of APIs. Challenges associated with the production of micro- and nano- particles for drug delivery can be addressed using SCFs [61]. Current SCF processes have demonstrated the ability to create nanoparticulate suspensions of particles 5 to 2,000 nm in diameter. RESS technique was employed for micronization of beclomethasone-17, 21-dipropionate, an anti-inflammatory inhaled corticosteroid commonly used to treat asthma. The results indicated that the RESS process conditions strongly influenced the particle size and morphology, thereby, suggesting that the RESS

technique is promising to produce particles suitable for pulmonary delivery [62].
The fine particles of antimalarial drug dihydro artemisinin were prepared by SCF process (RESS) using carbon dioxide as supercritical fluid [63]. A SC-CO_2 micronization technique based on gas-assisted melting atomization has been designed to prepare protein-loaded solid lipid submicron particles. The supercritical process was applied to homogeneous dispersions of insulin in lipid mixtures: (i) tristearin, Tween-80, phosphatidylcholine and 5 kDa PEG (1:0.1:0.9:1 and 1:0.1:0.9:2 weight ratio); and (ii) tristearin, dioctyl sulfosuccinate and phosphatidylcholine (1:1:0.5 weight ratio). Optimized process conditions yielded dry nonagglomerated powders with high product recovery (70%, w/w). Subcutaneous injection to diabetic mice of insulin extracted from the particles showed that the supercritical process did not impair the protein hypoglycemic activity [64]. Ipratropium bromide (IB) was micronized by means of a CO_2-based ASES in order to improve the particle shape and size characteristics for use in inhalation therapy [65].

Ibuprofen sodium has been successfully micronized using the SAS process to produce either crystalline rodlike particles with particle sizes of 1−5 μm, or amorphous spherical particles with particle sizes of about 500 nm [66]. Researchers have demonstrated micronization of an antibiotic compound, sulfamethoxazole, using SAS precipitation method. Further, the co-precipitation of sulfamethoxazole with a hydrophilic polymer hydroxypropyl cellulose in the continuous SAS process provided more enhanced dissolution rate [67]. Fine particles of acetaminophen were produced by Aerosol Solvent Extraction System (ASES) and the particle size distribution range also became narrow from 82 Mm to 4.9 Mm [68].
Micronisation using SCF technology resulted in a significant decrease in particle size as compared to untreated artemisinin and the dissolution rate of the micronised forms improved in comparison to the untreated form. The formation of solid dispersions with PVPK25 as a carrier improved the intestinal absorption characteristics of artemisinin [69].

The micronization of a non-steroidal anti-inflammatory drug, nabumetone, using RESS process showed that post-expansion temperature was the most significant factor, and a lower post-expansion temperature favored the production of smaller particles. Moreover, its dissolution rate was enhanced by 3.5 times after the RESS process [70]. ASES process was used for micronization of nonsteroidal anti-inflammatory drug $Cu_2(indomethacin)_4L_2$(Cu-Indo); (L = dimethylformamide [DMF]), which possessed very low solubility in SC-CO_2 using DMF as the solvent and CO_2 as the antisolvent. The particles obtained from the ASES process were changed from bipyramidal to spherical; with particle size less than 5 μm, as the concentration increased from 5 to 100 mg/g. A further increase in solute concentration to 200 mg/g resulted in large porous spheres, between 20 and 50 μ, when processing Cu-Indo by the ASES method. The dissolution rate of the micronized Cu-Indo was significantly higher than the commercial product [71].
Indomethacin-loaded poly(l-lactic acid)/poly(lactide-co-glycolide) (IDMC-PLLA/PLGA) microparticles were prepared using SEDS technique in an effort to obtain alternative IDMC formulation for drug delivery system.. *In vitro* release studies on the IDMC amorphously dispersed within the PLLA/PLGA matrix after the SEDS process revealed that the drug-loaded microparticles substantially enhanced the dissolution rate of IDMC compared to the free IDMC, and demonstrated a biphasic drug release profile. Furthermore, the results collectively suggested that IDMC-PLLA/PLGA microparticles prepared using SEDS would have potentials in anti-tumor applications as a controlled drug release dosage form without harmful organic solvent residue [72]. The micronization of some pharmaceutical compounds including sulfonamide drug and non-steroidal anti-inflammatory drug have been demonstrated, and the re-crystallized and micronized API showed significantly enhanced dissolution profiles [73].

The homogeneous shape theophylline (insoluble drug) microparticles (about 5 μm) produced by SEDS process could be used in inhalation therapy in clinic, thereby, suggesting its use in production with industrialization in the future because of the high yield (>70%) even in the lab scale [74]. Phenylbutazone was dissolved in acetone and SC-CO_2 was injected into the solution, thereby inducing supersaturation and particle formation using a supercritical fluid antisolvent process. The recrystallized particles showed cleaner surfaces and more ordered morphology compared to the particles obtained by other methods such as solvent evaporation [75]. Ethanol and SC-CO_2 were used as solvent and anti-solvent respectively, to precipitate arbutine (skin whitening agent in cosmetics and pharmaceuticals) as micro-particles using a supercritical anti-solvent under various conditions[76].
The PGSS process has been used to produce microparticles (30–250 μm) from the crystalline polymer poly(ethylene glycol) and poly(D,L-lactic acid) [77]. The PGSS process is particularly suitable for entrapment of delicate pharmaceuticals such as proteins. Furthermore, lysozyme, RNase, insulin and calcitonin have been successfully entrapped into the poly(D,L-lactic acid microparticles with minimal loss of structural integrity or functional activity by this technique [78]. Two different shapes of insulin microparticles were produced by SEDS process from

ethanol and dimethyl sulfoxide solution using supercritical carbon dioxide as an anti-solvent. Rod shapes of insulin particles were produced in the presence of ethanol and when dimethyl sulfoxide was used, spherical shapes of insulin particles were produced [79]. Using SCF technologies, SC-CO_2 can improve the solubility of poorly water-soluble drug substances using few or no organic solvents and with little or no heating [80-81]. The SAS process is a technology for the production of micro- and nanometer-sized particles for a wide variety of bio-medical pharmaceutical applications [82].

Although RESS process can produce pure and high-quality drug particles, but due to extremely low solubility of polar drugs in SC-CO_2, RESS has limited commercial applicability. To overcome this major limitation, a modified process rapid expansion of supercritical solution with solid cosolvent (RESS-SC) has been proposed and tested for phenytoin drug using menthol solid cosolvent. Phenytoin is soluble in pure SC-CO_2 up to only 3 μmol/mol but the solubility enhanced 400-fold, at 196 bar and 45 °C in the presence of menthol solid cosolvent. Further, RESS-SC process solid cosolvent helped in the formation of small nanoparticles [83]. Particles of lysozyme in the range of 0.1–5 μm were generated by high pressure CO_2 (at pressures between 8 MPa and 25 MPa) from aqueous ethanol solutions using an atomization process similar to the supercritical-assisted atomization technology [84].

Scientists have used SAS process for coating of fume silica with 50 nm diameter of nanoencapsulated acetaminophen. Both water insoluble and soluble substrates can be coated and encapsulated successfully in polymer by the SAS coating process [85]. Rapid expansion from supercritical to aqueous solution process was used to form stable suspensions of submicron particles of cyclosporine A, a water-insoluble drug. A solution of cyclosporine A in CO_2 was expanded into an aqueous solution containing phospholipid vesicles mixed with nonionic surfactants to provide stabilization against particle growth resulting from collisions in the expanding jet. Suspensions with high payloads (up to 54 mg/mL) were achieved with a mean diameter of 500 nm and particle size distribution ranging from 40 to 920 nm. This size range is several hundred nanometers smaller than that produced by same process for particles stabilized by Tween 80 alone [86].

Based on SCF technology, a recently developed process known as CO_2-assisted nebulization with a Bubble Dryer® (CAN-BD) has been demonstrated to have broad applicability to small-molecule as well as macromolecule substances, for example therapeutic proteins, anti-CD4 antibody (rheumatoid arthritis), α_1-antitrypsin (cystic fibrosis and emphysema), and trypsinogen (a model enzyme). Dry powders with enhanced apparent activity were formed in which stability and activity were maintained and which are fine enough to be inhaled and reach the deep lung [87].
Supercritical fluid extraction of emulsions (SFEE) is a novel process for the production of micro- and nanoparticles of bio-compatible and bio-degradable polymers such as poly-lactic-co-glycolic acid (PLGA) widely used as delivery devices for the administration of sensitive biopharmaceuticals such as proteins, peptides and genes. The potential of SFEE as an attractive and scalable process for the manufacturing of drug–PLGA composite particles for pharmaceutical applications has been exploited [88-89]. SCFs allow facile synthesis of nanocomposites, leading to some new nanomaterials with special structures that are very difficult to achieve by conventional methods [90].
Recent progress on nanoparticles processing using SCF technology has been reported [91-93]. The cefuroxime axetil nanoparticles produced as amorphous form by supercritical fluid technology have enhanced dissolution as compared to commercial cefuroxime axetil [94]. Inorganic nanoparticles have been synthesized in SCFs using precipitation-, microemulsion-, and spray-based approaches. Spray-based precipitation approaches have been the most effective for pharmaceutical nanoparticles, where the SCF is either the solvent or the nonsolvent, depending on the technology [95].

Recent advances in supercritical fluid extraction, precipitation, and solvent extraction have been employed to produce nanoparticle formulations for pulmonary delivery [96]. Scientists reported the application of a newly developed supercritical fluid processing technique, rapid expansion of a supercritical solution into a liquid solvent (RESOLV), to the nanosizing of potent antiparasitic drug Amphotericin B, and poly(l-lactic acid) and poly(methyl methacrylate) into nanoscale particles using a supercritical carbon dioxide-cosolvent system for their solubilization and processing. The process produced well-dispersed nanoscale particles suspended in an aqueous solution in each case, and the suspension was intrinsically stable or further stabilized in the presence of water-soluble polymers [97, 98]. The SCF technology based nanosized paclitaxel particles showed antineoplastic activity comparable to that of the commercial paclitaxel formulation [99].
Human growth hormone was successfully precipitated from aqueous solution using SEDS technology. The presence of sucrose in the protein solution promoted the precipitation of human growth hormone and improved dissolution [100]. Researchers have developed a novel apparatus and method for the submicronization of proteins based on the SCF process, ensuring the production of nanoscale protein particulates having

homogeneous physicochemical properties, thereby, enabling the development of transpulmonary and oral-administered formulations of protein drugs [101]. Scientists described the use of supercritical carbon dioxide as an antisolvent for the formation of nanoparticles that comprised biologically active therapeutics dispersed in a biodegradable polymer. The results showed that the release of a model compound, luciferin, from poly(lactic acid) (PLA) particles (approximately 250–350 nm in size) was observed for up to 40 days with up to 90% drug recovery, thereby, demonstrating that the process can be readily scaled to kilogram quantities [102].

Microparticles of budesonide alone, and budesonide and polylactic acid using SCF technology in SC-CO_2 were prepared and characterized. In addition, these microparticles sustained budesonide release for 4 weeks [103]. Using SCF technology, researchers demonstrated that the poly(DL-lactic acid) plasticized polymer and dry powder protein mixture can be sprayed to form solid polymer particles that encapsulate ribonuclease A and lysozyme without significant loss of enzymatic activity [104].

Different methodologies have been developed in order to incorporate protein within microspheres using supercritical fluids [105]. The use of supercritical fluid as an antisolvent caused precipitation of the substrates dissolved in a liquid solvent. Variations of this technique have led to the development of several different systems such as GAS or ASES) – also known as PCA, SAS and SEDS [106]. These specialized processes have been used to produce micron and submicron particles of proteins and peptides that are of defined morphology, size and internal structure [107]. In addition, if a polymer is added to the system as a carrier, this may lead to the formation of active protein-loaded micro/nanoparticles [108].

ASES technique has been used for the production of micronized steroids for lung delivery. In this direction, microparticles of budesonide, triamcinolone, acetonide, fluticasone-17-propionate, prendisolone, and flunisolide were all precipitated with average particle sizes of less than 5μm [109]. ASES systems were used to develop inhaled insulin [110].

The particle formation of lysozyme by supercritical fluid drying as influenced by varying flow rates of protein solution, supercritical carbon dioxide and ethanol, produced agglomerated nanoparticles, microspheres and irregular microparticles. Aagglomerated nanoparticles were produced under anti-solvent precipitation conditions, microspheres under water extraction conditions, and microparticles under competitive rates of both mechanisms [111]. GAS expansion of dimethylsulfoxide and *N*,*N*-dimethylformamide solutions with supercritical carbon dioxide produced biologically active powders of insulin with 90% of the particles smaller than 4 μm and 10% smaller than 1 μm [112].

SAS-EM provided a significantly improved method for the production of nano- and micro-particles with a narrow size distribution utilizing the properties of supercritical fluids and also the principles of virbrational atomization. Like the SAS technique, SAS-EM also uses a supercritical fluid as the antisolvent, but the dispersion jet was deflected by a vibrating surface that atomizes the jet into fine droplets. In addition, the vibrating surface also generated a vibrational flow field within the supercritical phase that enhanced mass transfer through increased mixing. Sizes of the particles obtained by this technique were easily controlled by changing the vibration intensity of the deflecting surface, which in turn is controlled by adjusting the power input to the vibration source. A major advantage of the SAS-EM technique is that it can be successfully used to obtain nanoparticles of materials that usually yield fibers or large crystals in SAS method. Microencapsulation via coprecipitation of two or more materials can also be achieved using the SAS-EM technique [113].

The SC-CO_2 methods for the preparation of prednisolone nanoparticles utilized the multicomponent system i.e. a ternary mixture of prednisolone, polyethylene glycol, and sodium dodecyl sulfate dissolved in methanol [114]. Recent developments in biodegradable particle formation using supercritical fluids have been reviewed with an emphasis on studies of micronizing and encapsulating poorly-soluble pharmaceuticals and gene [115]. In the area of polymer processing for pharmaceutical and medical applications, the formation of polymer–drug microparticles and microspheres, the production of simple or loaded membranes and the formation of temporary scaffolds were analyzed [116].

Poly (l-lactide) nanoparticles loaded with retinyl palmitate were successfully prepared by rapid expansion of a supercritical carbon dioxide (CO_2) solution into an aqueous receiving solution containing a stabilizing agent (RESOLV). Increasing the pre-expansion temperature from 70 to 100 °C and the concentrations of RP from 0.05 to 0.15 wt% increased the encapsulated RP content at least twofold [117]. Micronization of pharmaceuticals using the gas antisolvent technique was reported [118].

3.6. Co-crystals

The phase transformation of API to co-crystal depends on solution and co-crystal chemistry where nonstoichiometric concentrations of co-crystal reactants lead to thermodynamically favourable conditions for co-crystallisation. Pharmaceutical co-crystals can enhance specific properties of an API, like bioavailability and solid form stability. Using SCFT, co-crystal generation and particle formation occur in a

single-step. SCF being unique media, a possibility for the new molecular recognition events between two substances, results in polymorphic co-crystals.

Researchers reported the co-crystal formation tendencies in three different SCF techniques, focusing on distinct supercritical fluid properties - solvent, anti-solvent and atomization enhancer. Particulate indomethacin-saccharin co-crystals with different morphologies and sizes (nano-to-micron) were produced using supercritical fluid techniques, thereby, suggesting the potential of SCF technologies as screening methods for co-crystals with possibilities for particle engineering [119]. Process for preparing a co-crystal of an active substance and a co-crystal former, the process involving precipitating the active substance and the co-crystal former together from solution or suspension, in the presence of a supercritical or near-critical fluid, in particular using a GAS, SAS, SEDS or SAS-EM process has been reported [120]. RESS based co-crystallizations in inducing polymorph conversion and crystal disruption of chlorpropamide has been reported utilizing SC-CO_2 as the solvent. Co-crystallization studies revealed the formation of eutectic mixtures and solid solutions of chlorpropamide + urea which resulted in the crystal disruption of chlorpropamide and subsequent amorphous conversion at urea levels higher than 40% w/w. Unlike RESS, recrystallizations from liquid organic solvents lacked the ability to affect polymorphic conversions [121].

3.7. Inclusion complexes

Scientists reported the solubilization of the lipase from *Candida rugosa* in a fluorinated solvent, perfluoromethylcyclohexane (PFMC), in complex with a perfluoropolyether (PFPE) surfactant, KDP 4606 using hydrophobic ion pairing. The enzyme−surfactant complex formed a highly stable colloidal dispersion in both liquid and SC-CO_2 at high CO_2 densities (>0.92 and 0.847 g/mL, respectively), with 4% by volume PFMC as a cosolvent, yielding a fluid that was orange, optically translucent, and very nearly transparent. These studies indicated that nanoparticle aggregates of an enzyme−surfactant complex in CO_2, which are nearly optically transparent and stable to settling, are a promising new alternative to previous types of dispersions of proteins in CO_2 that either required water/CO_2 microemulsions or were composed of large particles unstable to settling [122].

Indomethacin exists entirely in an amorphous dispersion within the polymer matrix when hydroxypropylmethyl cellulose (HPMC)-indomethacin (4: 1, w/w) drug composites was prepared at 130 degrees C (17.2 MPa) via SC-CO_2 assisted impregnationatio. Hydrogen bonding was the primary interaction between HPMC and indomethacin involving the carboxylic acid carbonyl group of indomethacin and hydroxyl group of HPMC [123]. In addition to biocompatibility, clinical manageability and anti-bacterial effect of membrane lead to a successful treatment. Doxycycline grafted to the polymer surface enhanced guided tissue regeneration efficacy with antibacterial effect and increased hydrophilicity [124]. The shape of cyclodextrins assists the formation of cyclodextrins complexes with hydrophobic APIs. The resulting complex has a higher apparent solubility than the API alone. The processes with supercritical CO_2 enabled complexation of an insoluble API (or slightly soluble) with cyclodextrins. Formulplex® (improvement of bioavailability) is a novel patented formulation process in a supercritical medium complexation with cyclodextrin in a solid form. It permits enhancement of the apparent solubility of a poorly soluble drug [125].

Ketoprofen (KP) and a β-cyclodextrin (CD) with SC-CO_2 formed an inclusion complex. An increase in the parameters related to the process: pressure, temperature, maturation period, agitation and density of SC-CO_2 resulted in an increase in the association rate of KP with CD. The stoichiometry of complexation was found to be one molecule of KP with two molecules of CD. With control of both the operating conditions (pressure, temperature, maturation period, agitation and density of SC-CO_2) and the preparation of the mixing, high percentages of complexation was observed without the use of organic solvent [126]. SC-CO_2 was used to impregnate indomethacin (a non-steroidal anti-inflammatory drug) into chitosan thermosets for the preparation of controlled release formulations. The results suggested that the supercritical fluid impregnation process resulted in indomethacin being amorphously dispersed within the chitosan matrix. FTIR data suggesed that the aliphatic carbonyl group of indomethacin interacts with the NH_2 group of the chitosan backbone. *In vitro* dissolution studies (via UV–vis spectroscopy) revealed that the dissolution rate of indomethacin substantially increased after processing in SC-CO_2, particularly, under the experimental conditions 20.7 MPa and 70 °C [127]. The influence of temperature, residence time, water content and a ternary agent, l-lysine, was studied on preparation of piroxicam/β-cyclodextrin complexes by means of supercritical carbon dioxide. A complete inclusion was achieved for a piroxicam/β-cyclodextrin/l-lysine mixture by keeping a physical mixture of the three compounds (1:2:1.5 molar ratio) for 2 h in contact with CO_2 at 150 °C and 15 MPa [128]. Inclusion complex between ibuprofen and trimethyl-β-CD was successfully prepared using SC-CO_2 technique [129]. Inclusion of *RS*(±)-ibuprofen in β-cyclodextrin at the solid state was achieved using SC-CO_2. In addition, the dissolution rate of the inclusion complex formed was found to be significantly higher than that of untreated *RS*(±)-ibuprofen and its physical mixture with β-cyclodextrin [130]. Inclusion complexation of

itraconazole into β-cyclodextrin using SC-CO_2 significantly improved solubility of itraconazole in aqueous solutions. Higher inclusion yields were obtained in the SC- CO_2 method compared to physical mixing and co-precipitation methods. Further, both temperature and pressure had significant effects on itraconazole solubility in SC CO_2 and the inclusion yield of the complex prepared by SC CO_2 method [131]. The Supercritical Fluids Division of Pierre Fabre Medicament, which is France's second-largest independent pharmaceutical laboratory, has focused pharmaceutical operations (crystallisation, impregnation and complexation) at low temperatures, without the use of organic solvents or mechanical stress. Formulplex® is a patented supercritical CO_2 medium complexation process, which provided a means of obtaining inclusion complexes under mild conditions in order to increase the bioavailability of poorly soluble drugs [132]. Formulplex® has the advantage of being carried out under mild, solvent-free conditions. It also helps to reduce energy costs [133]. SEDS is a novel method to produce solid budesonide/γ-CD complex in a single-step process [134].Ibuprofen was successfully loaded into β-cyclodextrin granules using a supercritical fluid process for improved drug dissolution [135].

3.8. Solid Dispersions

Composite particle generation by SCF processes looks as a very promising solution to enhance the dissolution of poorly-soluble compounds and the most studies were conducted with hydrophilic polymers and cyclodextrins leading to size-controlled particles that rapidly release the active compound in the aqueous media [136].

A number of drying methods such as evaporation as a baseline, freeze drying, SCF, and a novel CO_2 sublimation are available. Based on drying time and production yields for all powders tested, SCF processing and CO_2 sublimation produce, by far, the most dispersible powder [137]. ASES process proved as a promising technique to reduce particle size and/or prepare amorphous solid dispersion of drugs in order to improve the solubility and bioavailability of poorly water-soluble drugs such as itraconazole [138]. Paclitaxel solid dispersion prepared by using the SCF process showed an improved solubility, thereby, being effectively used for the preparation of paclitaxel injection and oral preparation having a high bioavailability [139]. Dissolution studies of cefuroxime axetil solid dispersions with HPMC 2910/PVP K-30 prepared using (SEDS indicated that the dissolution rates were remarkably increased in solid dispersions compared with those in the physical mixture and drug alone. Thus, an amorphous or non-crystalline CA solid dispersion prepared using SEDS could be very useful for the formulation of solid dosage forms [140]. Micronized solid dispersions containing hydrophilic carriers and a new chemical entity, YNS3107 prepared by PGSS process enhanced the rate of dissolution of YNS3107 in the solid dispersion microparticles [141]. Precipitation with compressed antisolvent (PCA) provided an effective pharmaceutical formulation technology to improve the bioavailability of poorly water-soluble drug [142].

3.9. Powders of Macromolecules

Supercritical fluid is a useful medium to prepare dry tiny powders with advanced function. The powders prepared with supercritical fluid usually have a large surface area and a reduced crystallinity. This feature tends to increase the drug solubility, which is preferable especially to insoluble drugs for increasing their bioavailability. Supercritical fluid precipitation is also applicable to high molecular weight drugs. By selecting the running condition and/or additives, denaturation of proteins can be minimized. Sterilization of microorganisms and inactivation of viruses can be attained concomitantly with powder preparation in supercritical fluids [143]. Liposomal drug dry powder formulations (LDPF) have shown many promising features for pulmonary drug administration, such as selective localization of drug within the lung, controlled drug release, reduced local and systemic toxicities, propellant-free nature, patient compliance, high dose carrying capacity, stability and patent protection. LDPF can be formulated by supercritical fluid technologies. The prepared LDPF were evaluated *in vitro* and *in vivo* for lung deposition behavior and drug disposition in the lung using a suitable inhaler device and examples include: delivery of anticancer agents for lung cancer, corticosteroids for asthma, immunosuppressants for avoiding lung transplantation rejection, antifungal drugs for lung fungal infections, antibiotics for local pulmonary infections and cystic fibrosis and opioid analgesics for pain management using liposome technology [144]. The precipitation of proteins and genes with supercritical CO_2 is a promising way to produce protein and gene particles for inhalation [145]. A process based on W/O emulsion drying with supercritical CO_2 was reported to produce fine powders of bio-molecules such as inhalable protein directly from aqueous solutions [146]. A method for protein and polypeptide precipation by SCF microparticles and their stabilization processing and their protection and stabilization against denaturation has been reported [147].

3.10. Medium for Crystallization

The dissolution rate improvement in RESS for CO_2 soluble API and SAS for CO_2 insoluble API was found related to the specific surface area. Sulfamethizole crystallization was achieved using both the supercritical and liquid antisolvent processes

employing acetone and *N*,*N*-dimethyl formamide as solvents, and carbon dioxide and distilled water were used as antisolvents [148]. The potential for RESS-aided crystal doping in controlling the crystallinity levels in APIs as well as tailoring the polymorphism and particle morphology has been reported [149]. The recent developments in the area of particle engineering via crystallization for pulmonary drug delivery have been reported. Various excipient properties in combination with process variables influenced the morphology of the engineered particles. A wide range of pharmaceutical applications of large porous particles, particles with low surface energy, and particle aggregates has been reported [150]

3.11. As a Supercritical Bio-catalyst

Hydrophobic Ion Pairing was used to solubilize biomolecules in supercritical CO_2 and fluorous solvents, by pairing cationic sites on the surface of the biomolecule with fluorinated cationic surfactants forming a soluble neutral complex [151].

4. Supercritical fluids and polymorphism:

Crystallization under supercritical conditions has recently become one of the promising techniques to survey the polymorphism/crystal form landscape for new and improved materials. In the supercritical fluid experimental region, SCF technologies have demonstrated formation of new polymorphic forms which were not identified during routine screening [152]. Size and shape of the polymorph can be manipulated by controlling temperature and/or pressure during SCF processing in pharmaceutical area of polymorphism [153]. New polymorphs of flunisolide were produced using the SEDS [154] and two polymorphs of salmeterol xinafoate (SX-I and SX-II) produced by supercritical fluid crystallization displayed high polymorphic purity and distinctly different physical and surface properties [155].

The results on the effect of supersaturation and solvent on the polymorphic crystallization of stavudine showed that supersaturation predominantly control the occurrence of polymorphs. Crystallization of stavudine s from methanol or 2-propanol nucleates form I preferentially at a low supersaturation level, whereas form II can be obtained at a high supersaturation level. In case of 1-butanol as solvent, apart from forms I and II, another new metastable form IV crystallized out at a moderate supersaturation level. The relative stability of the above three polymorphs in decreasing order was found to be: I > IV > II [156]. The application of supercritical fluids in the control of process impurities such as chemical intermediates and residual solvent and in polymorphic control and chiral resolution has been reported [157].

5. SCF assisted impregnation:

The SCF impregnation proved effective for the preparation of a new ophthalmic drug delivery system which is a major advantage for the prevention of the inflammatory response after ophthalmic surgery [158]. Supercritical fluid assisted impregnation and foaming was engaged for preparation of porous chlorhexidine diacetate-poly (ethylmethacrylate) / tetrahydro furfuryl methacrylate (CX-PEM/THFM) drug release system, and results revealed that the drug release rate was almost as seven times faster in the SCF processed drug delivery system than conventional cured samples [159]. Supercritical carbon dioxide was used to impregnate into chitosan thermosets for the preparation of controlled release formulations and r results suggested that the supercritical fluid impregnation process resulted in indomethacin being amorphously dispersed within the chitosan matrix.. In vitro dissolution studies revealed that the dissolution rate of indomethacin substantially increased after processing in supercritical carbon dioxide, particularly, under the experimental conditions 20.7 MPa and 70 degrees C [160]. Supercritical fluid impregnation of dexamethasone in chitosan proved effective for the preparation of a drug delivery system for bone tissue engineering purposes [161]. Supercritical fluid assisted impregnation and foaming was employed for preparing porous chlorhexidine diacetate-poly(ethylmethacrylate) and tetrahydrofurfurylmethacrylate (CX-PEM/THFM) drug release system and the drug release rate was almost seven times faster in the SCF processed drug delivery system than conventional cured samples [162]

Conclusions:

Supercritical fluid technology have found unique applications in the production and processing of drug particles and is now considered to be an innovative and promising way to design and modify pharmaceutical substances. Carbon dioxide use as a supercritical solvent enabled the achievement of the supercritical region at moderate conditions of pressure and temperature, avoiding the degradation of thermolabile substances and providing simultaneously an inert medium suitable for processing easily oxidizable compounds. Microcrystals with nanoscale sub structures can now be designed and their functionality has contributed significantly to the stability and efficacy of the particulate dosage form. This innovative technology will help in meeting more strict requirements of regulatory authorities in terms of solid-state characterization, purity and environmental acceptability. SCF applications are being developed for both biopharmaceuticals and small molecules in which nano- and micro- particles with controlled morphology are being made. SCF processes offer some real opportunities for the formulation of proteins.

Supercritical fluid technology for the formation of composite micro/nano-spheres/capsules and impregnation of different polymers has been described.

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