

Supercritical Fluid Technology: A Review

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ABSTRACT

The challenges ever faced by pharmaceutical industry is mainly due to discovery of new drugs and development of new technologies. Supercritical fluid (SCF) technology is one such technique, which has become an important tool in the production of different particulate systems along with extraction and drying of protein and peptides during last couple of decade because of its specific properties such as flexibility in use, reduced environmental concern and its simplicity. In this review, we briefly describe the operating principles and parameters influencing each one of SCF processes along with their merits and perspectives. The application of SCF technology in pharmaceutical industry, including particle and crystal engineering, composite particles' preparation, coating of solid dosage form, liposome preparation, extraction and protein and peptide drying are discussed.

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Introduction

Although SCF technology is in use from late 19th century as a tool to understand the natural mineralization, the commercial exploitation of SCF technology has began in the 1970s. This was particularly motivated by environmental concern, capability of some SCFs for replacing toxic industrial solvent and finally, the SCF processes might be economical to liquid extraction and distillation methods [1, 2].

A fluid is said to be supercritical, when its pressure and temperature exceed their respective critical value (T_c - critical temperature and P_c - critical pressure). In the phase diagram (Fig. 1), the critical point located at the right upper end and the phase area beyond of this point is the SCF region [3]. Above the T_c , it is not possible to liquefy a gas by increasing the pressure. In other words, a SCF can behave as either a liquid or a gas, but is actually neither. The physicochemical properties of a SCF compared with those of liquid and gas were presented elsewhere in the literature [4].

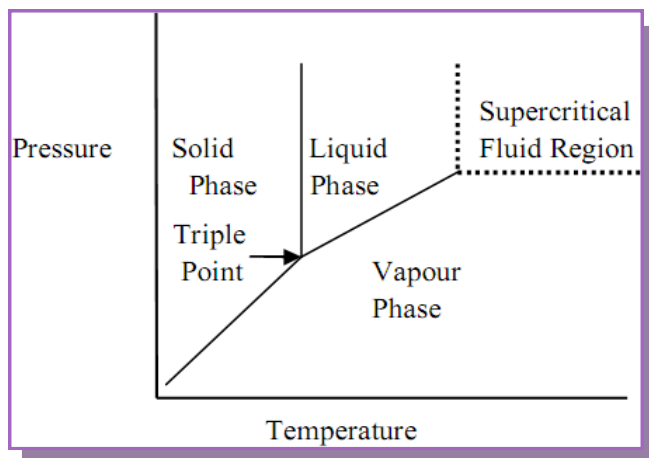


Fig. (1). Typical diagram of supercritical region

However, the SCF has a unique thermo-physical property. As the pressure is raised, the density of the gas increases without significant increase in viscosity while the ability of the fluid to dissolve compounds also increases. A gas may have little to no ability to dissolve a compound under ambient condition can completely dissolve the compound in supercritical range. Therefore, SCF provide a greater avenue as its solvation power is altered by careful control of changes in temperature and/or pressure [5].

All gases can form SCF above specific sets of P_c and T_c values, but in most of the cases, the transition to

the supercritical state occurs at high temperatures not compatible with pharmaceutical compounds (e.g. SC water) (Table 1). In addition, the P_c , T_c values increase with the molecular weight or intermolecular hydrogen bonding or polarity [6]. Among the number of gases tried as SCF, CO_2 is considered as the best option for SCF technique because of its low critical point (31.3 °C, 7.4 MPa), attractiveness for heat sensitive materials, it is inert, leaves no traces behind after the process, as well as being inexpensive, non-inflammable, having GRAS (generally regarded as safe) status and being easy to recycle or to dispose off. By incorporating a small amount of volatile cosolvent, often a polar or proteic compound such as acetone or ethanol, the solvation power of a particular SCF in less soluble solvent like water can be improved [7,8].

Table 1. Critical parameters of selected compounds [9]

Com- pounds	T_c (°C)	P_c (MPa)	D_c (g/ ml)	Solubility Parameter (cal/cm^3) ^{1/2}
H ₂ O	374	22	0.315	23.4
N ₂	-147	3.39	1.16	--
Xe	16.6	5.9	1.10	6.1
SF ₆	45.5	3.8	0.74	5.5
N ₂ O	36.5	4.1	0.45	7.2
C ₂ H ₄	9.1	5.1	0.22	--
CHF ₃	25.9	4.7	0.526	5.4
CO ₂	31.3	7.4	0.468	7.5

D_c density at critical conditions

In this review, we present various SCF processes with their advantages and limitations. In particular, different parameters influencing the above processes are discussed. Further, pharmaceutical applications of SCF processes including particle and crystal engineering, composite particles preparation, coating of solid dosage form, liposome preparation, extraction, protein and peptide drying and supercritical fluid chromatography (SFC) are presented through some selected examples.

Processes Using Supercritical Fluid

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SCF technology can be classified in to three broad categories depending on the way SCF-CO₂ is being used.

- ◆ SCF-CO₂ used as solvent for active substances and its excipients (RESS, PGSS, RESOLV, RESAS, DELOS)
- ◆ SCF-CO₂ used as antisolvent for the precipitation of active substances and their excipients in organic solvent (GAS, ASES, PCA, SAS, ASAIS, SEDS)
- ◆ SCF-CO₂ assisted spray drying or aerosolization based methods (CAN-BD, SAA).

SCF as a Solvent

Rapid Expansion of Supercritical Solutions (RESS)

RESS process is consisting of two steps; (a) dissolving the solid substance in a SCF and (b) formation of particles due to supersaturation. In the RESS process, at first SCF-CO₂ is pumped at desired pressure and temperature to extraction chamber containing solid substance(s) through heat exchanger as seen in the Fig. (2). The SCF percolates and dissolves the solid substance(s) in the extractor and then the resulted solution is depressurized through a heated nozzle or capillary at supersonic speed into a low pressure chamber. The supercritical solution is expanded adiabatically in the chamber, which leads to a rapid drop in temperature and pressure and spontaneous formation of droplets/particles. During the rapid expansion of the supercritical solution, the density and solvent power decrease significantly, resulting in super saturation of the solution and consequently precipitation of desire particles free of a residual solvent. This process is also called supercritical fluid nucleation (SFN) [10].

The parameters influencing RESS process are classified into pre-expansion and post-expansion condition. Pre-expansion condition includes equipment related parameters (temperature and pressure) and raw material related parameters like SCF, structure of solute (crystalline or amorphous, composite or pure) and cosolvent. The post-expansion condition depends on nozzle temperature, geometry, size, distance and angle of impact against the surface of the jet stream [11-15].

The advantages of RESS process are that it is simple, effective when single nozzle is used and it minimizes the use of organic solvent and reuses the SCF in continuous process. The main drawback is represented by poor solubility of most of the

pharmaceutical material (e.g. polymer) in SCF-CO₂, which, in turn require large amount of fluid, and therefore, RESS increases the cost of production. Difficulty of scaling up the process because of particle aggregation and nozzle blockage caused by cooling due to the rapid expansion of the supercritical solution and also poor control over particle size distribution [16].

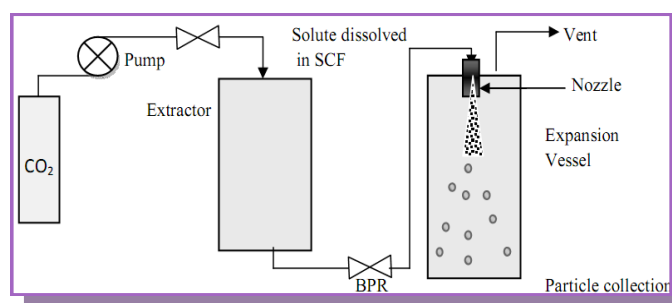


Fig. (2). RESS equipment concept

RESS with Solid Cosolvent (RESS-SC)

To overcome the low solubility of polar drug in SCF and the aggregation of particles in the expansion zone, the RESS process is modified to use a solid cosolvent (RESS-SC) [17]. The concept is based on the solubilization of solute and solid cosolvent in the SCF followed by expansion of the resulted solution through the nozzle in the expansion vessel. Finally, the solid cosolvent is removed by sublimation.

The solid cosolvent should have sufficient high vapour pressure for easy removal by sublimation, sufficient solubility in SCF, solid at nozzle exit point and non-reactive to SCF and the desired solute. One of such solvent is menthol which is used as solid cosolvent to phenytoin in the production of fine particles. The solubility of phenytoin in SCF is only 3 μmol/mol, but when used with menthol as solid cosolvent at 196 bar and 328 K, the solubility improved by 400 times. This improvement in solubility is attributed to the interaction between phenytoin and menthol [17]. Same pattern of solubility improvement was observed for drug salicylic acid or phenanthrene when benzoic acid is used as solid cosolvent [18].

Continuous RESS Process

This process combines the principles of RESS and SEDS process. In this method, like RESS process, SCF is introduced into a high pressure vessel upon

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preheating it to desired operating temperature. After maintaining the stable temperature and pressure in the high pressure vessel, polymer solution is delivered into the same chamber through co-axial nozzle. When the spraying of polymer solution is finished, the high pressure vessel is depressurized in ambient vessel and the product is collected. The ability of this technique to prepare microparticles continuously and constantly is the advantage over individual process [19]

Pre-Filtration RESS (PF-RESS) Process

Chiou *et al.* [20] developed this novel RESS technique in the intention to control the formation of fine particles and to obtain particles with narrow size distribution. The principle involved in the particle formation by PF-RESS process is to use a porous membrane with pore size less than 1 μm in order to pre-filter the SCF containing drug/polymer(s) in the extraction chamber. This can avoid the particle of large size to pass through the membrane and get into the precipitation unit. They successfully prepared microparticles of meloxicam of average size less than 2 μm with a narrow particle size distribution range between 0.5 μm and 5 μm .

Particle Formation from Gas Saturated Solutions (PGSS)

Many pharmaceutical materials are polar or high molecular weight substance, such as protein and peptide and due to which it is difficult to dissolve them in CO_2 , which has no polarity even in supercritical state. High amount of CO_2 is needed to compromise this low solubility, which in turn increases operational cost. In PGSS process, the polymer(s) are first melted or suspended in solvent at a given temperature in an autoclave and then solubilizing SCF-CO_2 in above melted or liquid suspended substance(s), leading to a so called gas saturated solution or suspension that is further depressurized through a nozzle with the formation of droplets or solid particles Fig. (3). Unlike to RESS technique, the principle governing PGSS process involves both the pressure and temperature- and solvent-induced phase separation.

Advantages of PGSS process are; (i) substance need not be soluble in SCF-CO_2 , (ii) simplicity of this process, leading to low processing cost and wide range of application, (iii) can be used with suspensions of active ingredient(s) in polymer(s) or other carrier

substance leading to composite particles, (iv) can be applied to process inorganic powders to pharmaceutical compounds, and (v) low solvent gas usage and pressure than RESS process as operational condition [21]. Care must be taken for thermolabile solute and moreover this technique compromises with microparticles. Insulin SLNs of size less than 500 nm were prepared by using DMSO as solvent and the lipid mixture of tristearin, phosphatidylcholine and dioctylsulfosuccinate [22].

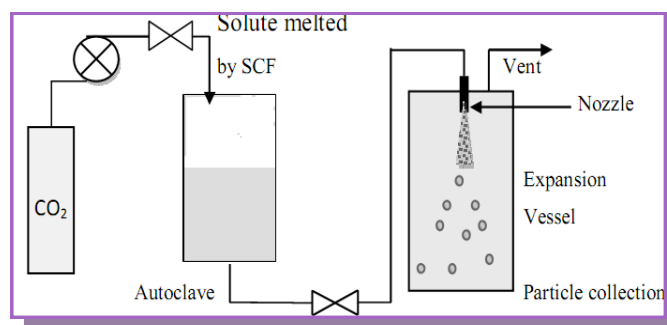


Fig. (3). PGSS equipment concept

Rapid Expansion of a Supercritical Solution into a Liquid Solvent (RESOLV)

RESOLV method consists of spraying of solution (drug in SCF-CO_2) into an aqueous medium from vessel maintain at given temperature and pressure. The rapid expansion of solution and followed by quenching leads to particle formation. Number of water soluble polymer (e.g. PVP) may be added to the aqueous medium to stabilize the particulate suspension. Finally, particles recovered from the suspension [23]. The advantage of this method is that the possibility of stopping the particle growth in the precipitator. RESOLV method is having the same limitation as RESS. The other problem is the recovery of particle from the aqueous solvent [24].

Rapid Expansion from Supercritical to Aqueous Solutions (RESAS)

This is a modification of RESS technique and is developed so that the stabilization of submicron particle in the aqueous phase became feasible. In this process, the supercritical solution (SCF with polymer and drug) is expanded through a nozzle in to an aqueous solution containing stabilizers. Usually, non-ionic surfactants such as lecithin, polysorbates and poloxamer are the choice for the stabilization because of their low toxicity. Parameters that are influencing the resultant particle

(Continued on page 17)

size are stabilizer type, concentration of stabilizer in aqueous phase, solid to surfactant ratio and finally the temperature of the stabilizer solution [25, 26].

Nanosizing of particles, high drug payload and long term stability are making this technique attractive than RESOLV method. But there are some demerits like it is not a suitable method for the drug which are unstable in aqueous solution and broad particle size distribution [26]

Depressurization of an Expanded Liquid Organic Solution (DELOS)

In DELOS process, the substances are first dissolved in suitable organic solvent and then it is mixed with SCF-CO₂ in a vessel of particular temperature and pressure. This mixture is depressurized through a nozzle into a vessel to form fine particle [27]. Here, the SCF-CO₂ is used as co-solvent to the organic solvent. The main advantage of this technique in comparison to PGSS is that the thermo-sensitive material can be handled to prepare fine particle without melting it.

SCF as an Antisolvent

The low solubility of pharmaceuticals in SCFs limited the large scale production of micro/nano sized particles by PGSS and RESS method. Using SCFs as antisolvent was thought off by many researchers to solve the above problem. Here, the solute is insoluble in an antisolvent, whereas the antisolvent should be completely miscible with liquid solvent. This is based on the principle that when a solution sufficiently expanded by a gas, the liquid phase is no longer a good solvent for the solute and particle formation by precipitation occurs. The SCFs as antisolvent includes GAS, SAS, ASES, PCA and SEDS processes.

Gaseous Anti Solvent (GAS)

GAS is a batch process where the precipitator is partially filled with the solution of solute of interest and then the supercritical antisolvent is pumped into the vessel, preferably from the bottom until the fixed pressure is reached as shown on Fig. (4). The particles precipitates as the gas concentration in the solution increases with pressure. After a holding time, the expanded solution is made to pass through a valve present above the precipitator to wash and clean the precipitated particles. A clear disadvantage of this technique is the lack of control on the particle formation,

which prevent the formation of mono dispersed particles.

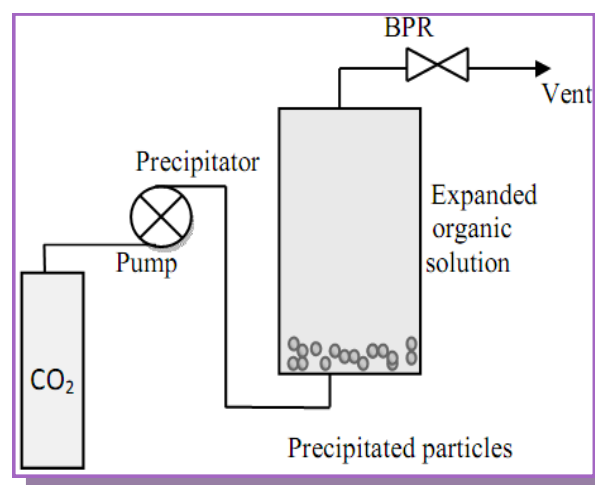


Fig. (4). GAS equipment concept

Aerosol Solvent Extraction System (ASES), Particles by Compressed Antisolvent (PCA), Supercritical Antisolvent (SAS)

The SCF is first pumped to the top of the high pressure vessel until the system reaches a constant temperature and pressure Fig. (5). Subsequently, active substance solution is sprayed as fine droplets into above SCF bulk phase through an atomization nozzle. The large volume expansion of drug solution in vessel, resulting dissolution of SCF into liquid droplets and, subsequently, in super saturation due to reduction in solvent power leading to nucleation and formation of small and mono disperse particles. Particles are collected on a filter at the bottom of the vessel. The SCF and organic solvent mixture flow down to a depressurized tank where suitable temperature and pressure condition allow gas-liquid separation. After the collection of sufficient quantity of particles, the spraying of liquid solution has to be stopped. Furthermore, to remove residual solvent, pure SCF continues to flow through the vessel [2]. The ASES can be modified with the addition of precipitation of compressed anti solvent (PCA) which was proved to be more efficient in the production of a great variety of organic and biopolymer based particles.

Main advantage of this technique over GAS is its suitability for continuous operation, which prerequisite for large scale mass production of particles. Complex

(Continued on page 18)

mass transfer process is one of the major limitations in SAS scale up. Complex mass transfer process is originated due to two issues. First one is the result of variety of jet dispersion patterns in the supercritical

Atomization of Supercritical Antisolvent Induced Suspension (ASAIS)

This is another modification to SAS technique. In ASAIS process, antisolvent induced precipitation occurs

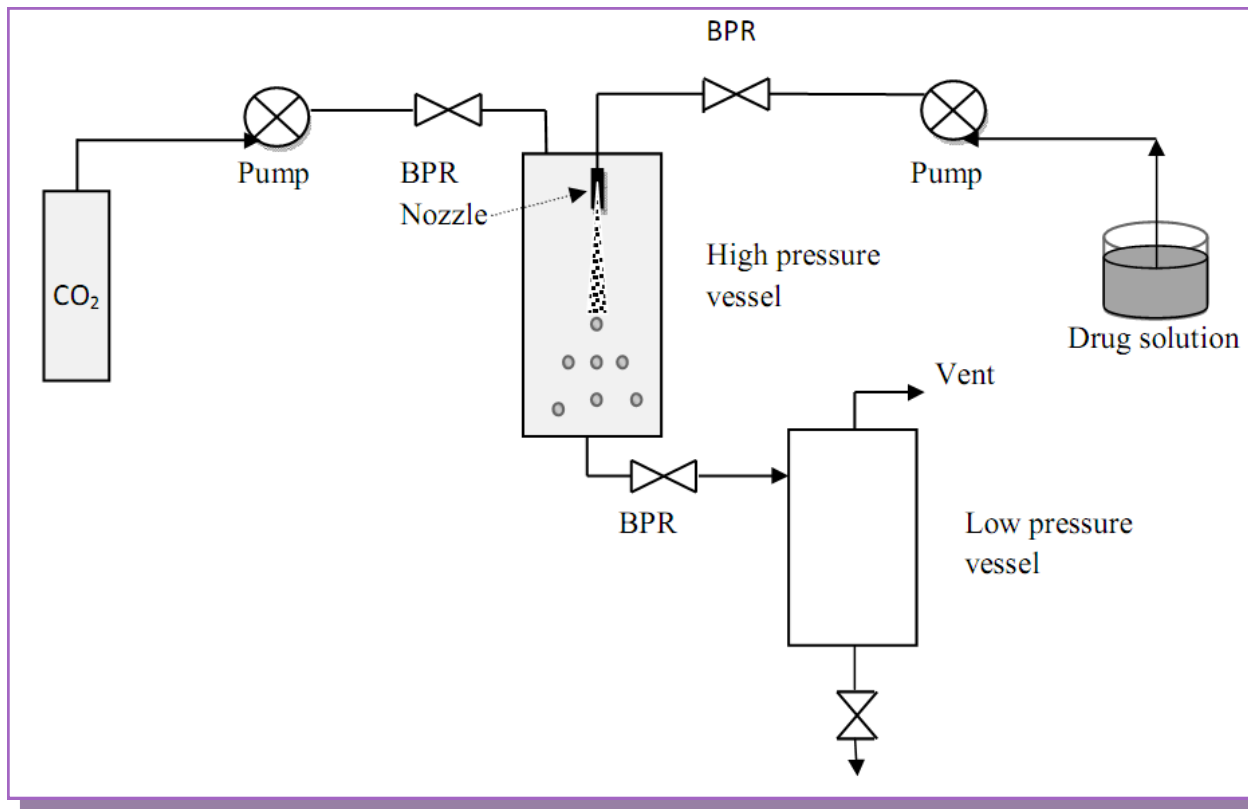


Fig. (5). ASE/SAS/PCA equipment concept

spray leading to formation of droplets of non uniform sizes. It is obvious that mass transfer within the droplets of smaller size is considerable faster than that of larger droplets owing to higher surface area of the former. Furthermore, there is more time for crystal growth in larger particles. Another problem related to mass transfer in SAS is due to residence time of particles under supercritical condition until the cycle ends. Particle may still grow, when present on the filter for separation, under above condition. To solve above problems in SAS, concentric tube antisolvent reactor (CTAR) technique was developed. In this process, particles are formed inside a small concentric tube instead of usual spraying of drug solution into SCF [28, 29]. Filter is used for the harvesting of particles, which is again more problematic owing to small concentric tube in CTAR.

in a small tube, where antisolvent mixed with the solution to generate a suspension. This suspension of particles is then sprayed into a precipitator at atmospheric condition for solvent separation, which eliminates the high volume and high pressure precipitator. In addition, very small to moderate antisolvent concentration is required. Contrary to both SAS and CTAR process, the particles recovery is performed by cyclone separator rather than using filter. Here, the first step (suspension formation) occurs in the small tube and next step in the precipitator and finally particle recovery in cyclone separator [30].

Solution Enhanced Dispersion by Supercritical Fluids (SEDS)

This is a modification of SAS process in which the SCF and drug solution are introduced simultaneously in to the precipitation vessel at particular temperature and pressure through the coaxial nozzle. The design of

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co-axial nozzle is such that to facilitate the dispersion of drug solution by SCF, thereby enhancing mass transfer and formation of fine particles Fig. (6). In addition, the high velocity of SCF allows intense mixing with drug solution. Here, the SCF serves both as an antisolvent and as a dispersion medium [31].

The particle formation/size by SEDS depends on the mass transfer of SCF into sprayed droplets and by the rate of solvent transfer into the SCF phase. In general, high mass transfer causes faster supersaturation and smaller particle size with less agglomeration [32]. Most often two way coaxial nozzle is used where both drug solution and SCF are introduced into precipitation chamber as separate stream. Basic operational principle of GAS/SAS/ASES/PCA/SEDS is described as follows. A ternary system is produced by the introduction of SCF in to chamber containing polymer and solvent homogenous binary system. Upon change in pressure, compositional quenching takes place leading phase separation and particle formation.

Baldyga *et al.* [33] used coaxial two-component nozzle with a mixing chamber in nozzle to prepare paracetamol particles. The intention was to partially mix alcoholic drug solution with SCF in the nozzle chamber before introduction into precipitation chamber. Mixing in the nozzle chamber creates high supersaturation, which enables to start nucleation and growth immediately after entering into precipitation chamber.

In order to obtain ultrafine particles with narrow size distribution, He *et al.* [34, 35] used SEDS with prefilming atomization (SEDS-PA) process. For the above purpose they have used twin-fluid atomizer. The principle involved in this process is to drive the liquid to be atomized along a surface as a film within the nozzle and consequently reaching at the atomizing edge. As a result, separation of liquid film takes place leading to the formation of fine droplets. The liquid to be atomized was driven along the coaxial annular passage and formed to a thin swirl liquid film by the spiral slots liquid distributor with an angle (45°) of inclination relative to the central axis of the atomizer. At the exit of the atomizer the atomizing dense gas stream impinges on the thin swirl film at 45° and followed by vigorous interaction between jet stream and annular liquid sheet resulting in the formation of fine droplets.

To overcome the limitation of water solubility in SCF, SEDS has been further modified in order to process water soluble compounds (e.g. protein and peptides). The above modification includes the use of three way coaxial nozzle to introduce aqueous drug solution, SCF and organic solvent (polar) in to particle formation chamber as separate stream. The organic solvent acts both as precipitating agent and a modifier, enabling the non-polar SCF to remove water [36]. The use of ultrasonic nozzle is a further modification of SEDS [37]. The formation of fine droplets is based on the induction of ultrasonic waves of frequency between 10-100 kHz, caused by the vibration of ultrasonic coaxial nozzle.

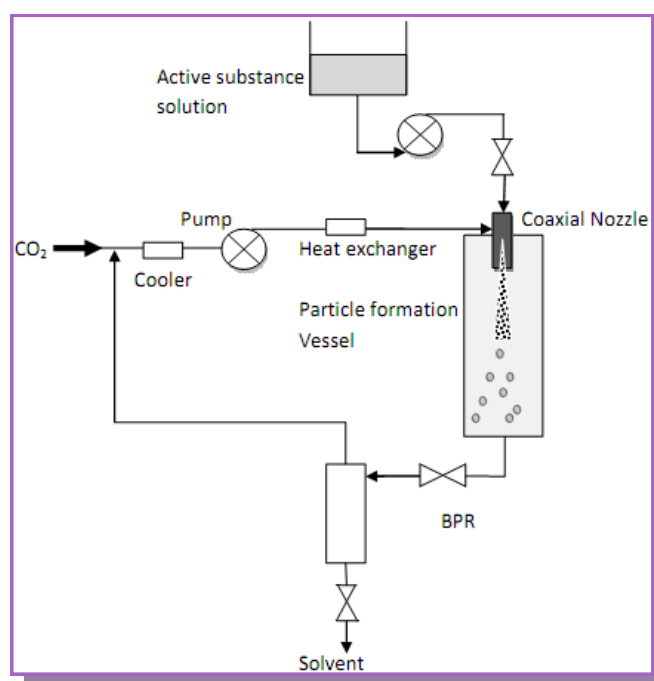


Fig. (6). SEDS equipment concept

Supercritical Fluid Extraction of Emulsions (SFEE)

This technique is based on counter-current extraction of emulsion by SCF. The process is as follows, first the o/w emulsion is introduced into the extraction chamber (at particular temperature and pressure) through the nozzle present at the top at a constant rate Fig. (7). Simultaneously, SCF from the side bottom of the extraction chamber is introduced. This counter-current flow leads to the expansion of organic phase of the emulsion, consequently, the precipitation of dissolved substances into composite nano-particles [38].

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The emulsion droplet diameter is the key size control parameter, besides fraction of lipid and drug in organic solvent. The advantage of this method over traditional methods like evaporation and liquid extraction are fast and complete removal of the solvent and formation of uniform particle size. In addition, whenever lipid is used as matrix material, the thermodynamic stability is established due to plasticizing effect of lipid as the depression of lipid melting point occur in the extraction column. Furthermore, lipid is purified due to extraction of impurities present along with lipid [38].

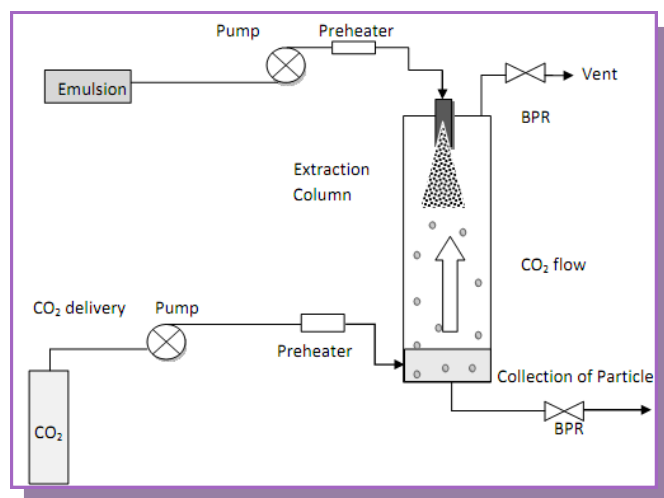


Fig. (7). SFEE equipment concept

SCF-CO₂ Assisted Spray Drying (Aerosolization-based) methods

These techniques used SF to assist or enhance the nebulization or aerosolization of the solution of the substance to be processed, which is then rapidly dried in a drying atmosphere to form fine particles. There are two methods based on this principle.

Carbon dioxide Assisted Nebulization with Bubble Dryer (CAN-BD)

This process focused on the nebulization of the liquid solution rather than using dense gas (SCF) to achieve precipitation by solubility reduction for the solute to be micro- or nano-sized. At first, the solute(s), preferably in between 1% to 10%, is dissolved or suspended in aqueous or organic solvent or their mixture and then mixed intimately with near critical or SC by pumping both fluid through a near zero volume tee as shown in Fig. (8), to generate emulsion. The resultant emulsion is rapidly expanded through a flow restrictor to near atmospheric pressure to form aerosol consisting of micro droplets and micro bubbles. The

aerosol is formed due to sudden dispersion of the liquid solution caused by rapid expansion of compressed gas. The drying chamber is filled with heated air or nitrogen gas to maintain the desired temperature for rapid drying of aerosol droplets or micro bubbles. Dry particles are collected on a filter placed at the outlet of the drying chamber [39].

Parameters influencing the particle formation are flow rate of solution (for lab scale 0.3-0.6 ml/min is sufficient), percentage of dissolved or suspended substance, inner diameter flow restrictor (50-175 μm and length \square 10 cm), temperature of the drying chamber, residence time of droplets or micro bubbles (as micro bubbles are dried faster than droplets) [39].

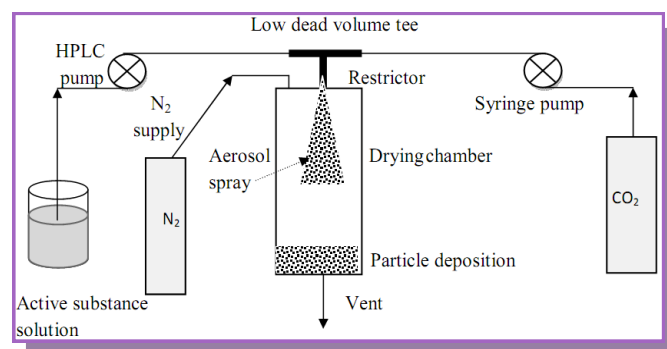


Fig.(8). CAN-BD equipment concept

Advantages of CAN-BD process are; (i) minimum decomposition of thermolabile drugs, (ii) preferred method for water soluble drug, (iii) organic solvent compatible with SCF can be substituted in part or totally for water, and (iv) very fine size of the produced particle (<3 μm diameter) [40]. There is need to heat tee and restrictor to a temperature in the range 50 to 100 $^{\circ}\text{C}$ in order to avoid restrictor obstruction during expansion.

Supercritical Fluid-Assisted Atomization (SAA)

SAA process is based on the solubilization of SCF in aqueous solution to be dried and subsequently atomization through a thin wall nozzle at atmospheric pressure. The difference between SAA and CAN-BD is the region where the mixing is achieved [41, 42].

Application of SCF Technology

Particle and Crystal Engineering

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Crystalline solids that are the same compound/ composition but have different crystalline forms are called polymorphs or modification [43, 44]. Pseudo polymorphs (solvates and hydrates) are molecular adducts that contain solvent molecule (water in case of hydrates) in the crystal lattice.

Traditional process such as crushing/milling, micronization, spray drying, freeze drying and crystallization used to produce particles. These processes offer limited control over the physicochemical properties of the produced particles including size, shape and crystalline purity due to numerous unintentional conversion of polymorphs, desolvation of solvates, and solvates formation occur during aforementioned processes [45]. Furthermore, these methods require more manufacturing steps to produce drug particles. Regulatory authority like International Conference on Harmonization (ICH), in the guideline Q6A, emphasizes the importance of solid state and crystallographic purity of the drug and excipients [46]. Similarly, FDA requires proof of crystalline form of drug along with the relationship between structure and stability of the crystal [47]. In above situations, SCF technology could be used as viable means of controlling crystal formation.

The influence of GAS technique on the polymorphism of a poorly water soluble drug, puerarin, was investigated by Li *et al.* [48]. It was known fact that puerarin commercially available in crystal form I. At optimum conditions, a more orderly and pure form of crystal of size 30.34 μm with needle-like shape was generated. In addition to this, at 60 mg/ml of solute concentration and methanol as solvent, two new crystal forms (form III and form IV) of puerarin were produced. Puerarin in form II has the smallest particle size and fastest dissolution rate compared to other forms. This was attributed to the metastable nature of form II, while form IV exists in stable form. Of all the process parameters, the type of solvent had tremendous influence on the external shape of crystal. For instance, puerarin crystal exhibited needle-like appearance with ethanol. When the solvent changed to methanol, the crystal appeared as long column and acetone produced long needles with brushes.

Different crystal forms of terbutalin sulphate were produced including stoichiometric monohydrate and amorphous material by SEDS technique [49]. One

crystalline and another semicrystalline form of terbutalin sulphate were obtained at the condition of higher temperature (50 °C), lower pressure (150 bar), and ethanol as solvent but in different volume of precipitation vessel. Particles in smaller (50 ml) volume of precipitation vessel were exposed to partially mix ethanol-rich phase resulting in lower supersaturation level, subsequently microparticles of 100% crystallinity were obtained. In contrast, the particles in the large (500 ml) precipitation vessel were growing in a well mixed CO₂-rich environment which is characterized by a high level of supersaturation in the beginning and followed by relatively low supersaturation particle formation process. The last stage of particle growth is considered as particle conditioning. A more stable and less energetic surface of terbutalin sulphate microparticles were obtained due to above phenomenon, which in turn improved powder flow and aerosolization performance.

Zhiyi *et al.* [50] prepared microparticles of water soluble drug, cefadroxil, from water-ethanol mix solvent by SAA process. They investigated the influence of operational parameters, including mixing vessel pressure and temperature, solution concentration, and solution feed rate on particle morphology (PM), particle diameter (PD) and particle diameter distribution (PDD). Among all the operational parameters, the influence of mixing vessel pressure was significant. The PD decreases, the PDD become narrower and the average particle diameter become small with the increase of the mixing vessel pressure in the range of 6-10 MPa, when other operational conditions were fixed at: the mixing vessel temperature 60 °C, the solution concentration 8 mg/ml and feed rate of 3 ml/min. Further increase in mixing vessel pressure resulted in smaller PD but wider PDD. The diameter of most of the particles obtained was below 1 μm in the above condition and having concave morphology. With the increase of solution concentration from 4-10 mg/ml, PD becomes larger, PDD becomes wider and PM becomes more irregular. Finally, it was concluded that the optimal operation condition for preparing microparticles of cefadroxil is: the pressure of 10 MPa and the temperature of 60 °C in the mixing vessel, the solution concentration of 4 mg/ml and the solution feed rate of 3 ml/min.

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Nijlen *et al.* [51] compare different micronization process, such as mechanical grinding, Jet milling and SCF technique (RESS process) on the basis of artemisinin particle size obtained. The lowest median diameter of particle of $4.1 \pm 0.2 \mu\text{m}$ is obtained by Jet milling. The mechanical grinding and micronization by RESS process produce median particle diameter of $27.4 \pm 4.6 \mu\text{m}$ and $10.6 \pm 0.5 \mu\text{m}$, respectively. The optimal condition for RESS technique was found to be 300 bar pressure and temperature of $80 \text{ }^\circ\text{C}$ in extraction column.

Hezave *et al.* [52] studied various parameters such as extraction temperature, extraction pressure, nozzle length, effective nozzle diameter and spraying distance on the particle size and morphology of diclofenac particles obtained by RESS method. They found that the increase in extraction temperature, nozzle length, effective nozzle diameter and spraying distance lead to increase in mean particle size of precipitated particles. But extraction pressure had an opposite effect on particle size. This was explained on the basis of solvation power of SCF. The solvating strength of SCF increases at higher extraction pressure, subsequently the concentration of diclofenac in SCF increases. Resulting higher drug concentration leads to higher supersaturation and results in smaller size of precipitated diclofenac particles. The mean particle size of diclofenac was in the range of 10.92 to $2.23 \mu\text{m}$ in all the experimental condition as comparison to the original particle of $38.12 \mu\text{m}$. Furthermore, the morphology of particles was changed from irregular to quasi-spherical and irregular.

Martin *et al.* [53] studied the effect of various parameters such as temperature of precipitation chamber, density, drug concentration and flow rate of SCF and drug solution on the particle size and morphology of microparticle of budesonide prepared by PCA method. They found that the particle growth rate increased by increasing the chamber temperature from 35 to $45 \text{ }^\circ\text{C}$. At $40 \text{ }^\circ\text{C}$, round budesonide particle size of $1\text{-}2 \mu\text{m}$ were obtained. At lower density (0.38 g/cm^3) the particles formed were fairly spherical. Increase in drug concentration and its flow rate increase the particle size, while particle size decreased by increasing SCF flow rate.

Charpentier *et al.* [54] investigated various parameters such as nozzle diameter, pre-expansion

pressure and pre-expansion temperature on the particle size and morphology of beclomethasone-17,21-diapropionate (BDP) microparticles prepared by RESS process. They found that both particle diameter and width of distribution increased as the nozzle diameter increases and the morphology of BDP particles changed from spheres to elongated crystal. Increase in pre-expansion pressure from 1,900 to 4,100 psi and pre-expansion temperature from 35°C to 50°C resulted in the increase in particle size. The former was attributed to the agglomeration of particles in the capillary as it was at lower temperature and latter result was explained by the fact that higher temperature solutions have a higher cloud-point pressure leading to earlier crossing of cloud point by the solution while passing through the nozzle and consequently, nucleation to start earlier which allow more time to grow and coalesce to form larger particles.

Kim *et al.* [55] used RESS process to prepare ultrafine lidocaine particles and studied the whole diameter of orifice nozzle and aspect ratio (length/diameter) of capillary tube along with extraction temperature and pressure. They observed fine particles with average particle diameter of 100-300 nm with spherical morphology. The average particles diameter increases with whole diameter of nozzle. This was explained by the fact that the increase in whole diameter causes a moderate concentration gradient of lidocaine around expansion device and consequently, premature precipitation resulting in larger particles. The increase in the aspect ratio above 200 creates concentration gradient followed by precipitation in capillary tube due to prolonged precipitation time.

Reverchon *et al.* [56] prepared cromolyn sodium microparticle by SAA process and analyzed the influence of precipitation temperature, drying temperature and concentration of drug on particle size and morphology. In all the trial, they found particle size in the range $1\text{-}5 \mu\text{m}$ with spherical shape. There was an insignificant increase in particle size with the temperature from 100 to $140 \text{ }^\circ\text{C}$. An increase in drug concentration from 25 mg/ml to 115 mg/ml caused the increase in particle size. This was explained by considering the fact that viscosity and surface tension increased at higher drug concentration resulting in the formation of larger

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primary droplets followed by the formation of secondary droplets. The degradation of product was observed at higher temperature.

Atila *et al.* [57] prepared digitoxin nanoparticle by RESS technique and investigated the effect of process parameters such as spray distance, flow rate and pre-expansion temperature on the particle size. The particle size of digitoxin particles decreases with the increase of spray distance and flow rate. The former case is the resultant of two competing phenomena. Firstly, as the spraying distance is short, residence time of droplets in expansion chamber decreases and leading to smaller particle size. In contrary, short spraying distance leads to coalescence of droplets due to decreasing angles between droplets. In the later case, the residence time of droplets inside the nozzle and in the expansion chamber decreased by increasing the flow rate. This decreases particle growth time and resulting in smaller particle size. However, particle size increases by increasing pre-expansion temperature. In all conditions, the particle size of digitoxin was decreased from 0.2-8 μm to 68-458 nm.

Keshavarz *et al.* [58] studied the effect of extraction temperature, extraction pressure and spray distance on the formation of raloxifen nanoparticles prepared by RESS process. They found that by increasing extraction pressure from 10 to 18 MPa and spray distance from 5 to 10 cm, the particle size decreased. However, by increasing extraction temperature from 40 to 60 $^{\circ}\text{C}$ particle size became smaller, but further increase in temperature to 80 $^{\circ}\text{C}$ decreases the particle size. The later part can be explained by the fact that higher temperature causes higher level of supersaturation due to increase in solubility at higher temperature. This higher degree of supersaturation increases the number of nuclei formation which in turn increases the probability of collision and followed by large particle formation. A particle size of 14.11 nm was obtained at optimum condition of 50 $^{\circ}\text{C}$ temperature, 17.7 MPa extraction pressure and spraying distance of 10 cm.

Varshosaz *et al.* [59] investigated the effect of process parameters, such as the extraction column temperature and nozzle temperature on the amorphous nanoparticles (without additives) of cefuroxime axetil prepared by RESS technique. They tried three level of

temperatures each for extraction column (60-90 $^{\circ}\text{C}$) and nozzle (50-70 $^{\circ}\text{C}$) and found that lowest particle size (158.57 nm) is obtained with nozzle temperature at 60 $^{\circ}\text{C}$ and column temperature at 90 $^{\circ}\text{C}$.

Montes *et al.* [60] precipitated amoxicillin nanoparticles by SAS process using N-methylpyrrolidone and CO_2 as solvent and antisolvent and investigated the effect of initial drug concentration, flow rate of drug solution, temperature, pressure and nozzle diameter on particle size and size distribution. They observed that increase in the initial drug concentration leads to larger particles sizes with a wider size distribution. This result is attributed to higher condensation rate from the higher drug concentration dominates the higher supersaturation from higher drug concentration. Higher flow rate leads to smaller particles sizes due to higher degree of mixing. Other factors did not have significant effect on particle size. Spherical nanoparticles with mean size diameter of 216-505 nm were obtained by this method.

Young *et al.* [61] prepared cyclosporine nanoparticles by RESAS technique and compared the effect of various stabilizes such as non-ionic surfactant (e.g. Tween 80, Pluronic F127, Myrtj 52) and phospholipid based surfactant on the particle size. Among non-ionic surfactant, Tween 80 produces smaller particle sizes ranges from 660 nm to 970 nm, whereas phospholipid based surfactant produces cyclosporine particles (200-300 nm) which was smaller than particles produced by Tween 80 at similar surfactant concentration and drug/surfactant ratio (as the drug/surfactant ratio exceeds 0.6 the particle size markedly decreases due to insufficient surface coverage by surfactant in aqueous solution). This result is attributed to the aggregation of large number of surfactant for phospholipid vesicle than that for micelle i.e. in a single vesicle the local concentration of surfactant that can coat a growing drug particle is higher than single micelle. Furthermore, the preferred curvature of the surfactant is more favorable for vesicle than micelle as the interface with water is less curved for vesicle as compared to micelle. In addition, vesicles are relatively stable, so the growth of the drug particles by collision/coagulation is minimized.

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Composite Particles

Microparticles: Microparticles are colloidal particles ranging in size from 1 μm to 1000 μm . They can be composed of either a homogeneous polymer-drug complex (microspheres) or a central drug core surrounded by a solid polymeric shell (microcapsules). Microspheres are defined as solid, spherical particles containing active substance either in solution or crystalline form. Microcapsules are spherical particles containing drug concentrated in the center core, which encloses particles of solids, liquids or gases within the polymeric embryonic membrane [62]. Polymer used to prepare microparticles can be either natural such as albumin, collagen, chitosan and alginate or synthetic [e.g. poly (lactic acid), poly (caprolactone), poly (methyl methacrylates), and poly (alkyl cyano-acrylates)].

Brion *et al.* [63] used PGSS technique to produce microparticle of solid dispersions containing a new chemical entity (YNS3107) and hydrophilic polymers such as PEG 400, PEG 4000 and Poloxamer 407. They found that the major parameters influencing the particle diameter are autoclave temperature and pressure. Smaller average particle diameter were obtained by increasing autoclave pressure as it increases the CO_2 content within the droplets and resulting in lower solid-lipid equilibrium condition time (SLEC) (more the SLEC, the less the number of collision between droplets). Similarly, temperature close to melting point of polymer matrix reduces the droplet coalescence by shortening SLEC time. Finally, they concluded that the optimal condition is temperature (62 $^\circ\text{C}$) and pressure (177 bar) to produce smallest microparticles of mean diameter $30.4 \pm 2.3 \mu\text{m}$.

Jordan *et al.* [64] prepared microsphere of human growth hormone by PGSS process (CriticalMix™) using PLGA and PLA in different ratios for subcutaneous injection. They observed that 90 % of the particles are below 100 μm and volume mean diameter (is the diameter at the 50 % point of the entire volume diameter) is 61 μm , which indicates these particles can be injectable. The encapsulation efficiency is in the range of 97.1% to 100 %, which is nearly double the value obtained by emulsification method.

Duarte *et al.* [65] compared the particle size of naproxen microparticles prepared by both solvent evaporation and SAS techniques. The polymers used

were methylcellulose and ethylcellulose and solvent system composed of dichloromethane and dimethylsulfoxide for SAS method. The microparticles obtained by SAS method were of significantly smaller in diameter (16.91 μm) and narrow size distribution than that of produced by solvent evaporation method. Furthermore, the particle size was further reduced to 4.71 μm , when ethylcellulose used alone.

Zhang *et al.* [66] prepared microparticles of morphine by SEDS technique and investigated the effect of flow rate of drug solution (aqueous), polymer solution [Poly-(L-lactic acid in dichloromethane)] and antisolvent (ethanol) on particle size and morphology. They observed that by increasing the flow rate of ethanol from 0.5 to 1.5 ml/min, irregular shape, uneven size and amorphous form became converted to ellipsoidal shape, uniform size and micron and submicron particles. But, the opposite effect was seen in case of flow rate polymer solution. The morphology of microparticles changed from irregular to axiolitic form with less agglomeration as the flow rate of dichloromethane was decreased from 1.5 to 0.5 ml/min. The microparticles were formed at the appropriate flow rates ratio of ethanol to dichloromethane. By increasing this ratio, the agglomeration can be prevented as the mass transfer was faster than the separation of fine droplets. Finally, they concluded that the obtained morphine microparticles possessed ellipsoidal shape and smooth surface with mean diameter of 2.45 μm at the optimum condition (the flow rates ratio of aqueous solution, ethanol and dichloromethane is 1/7.5/2.5).

Uzun *et al.* [67] used SAS process to prepare composite microparticles of cefuroxime axetil with polyvinylpyrrolidone, and obtained spherical particles of smaller size as comparison to larger and plate-like particles formed when drug or polymer was precipitated alone. This was explained by the fact that polymer inhibits particle growth of drug by blocking surface of particles and the consequence was larger surface area and smaller particle size. This was conformed by FT-IR study as there was an interaction between carboxylic groups of polyvinylpyrrolidone with amine group of cefuroxime axetil.

Patomchaivivat *et al.* [68] prepared microparticle of rifampicin and poly (L-lactide) by SAS

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technique and studied the effect of polymer:drug ratio on the morphology and size of particle. By fixing the conditions, such as 2 % of drug and polymer in methylene chloride, 172 bar of pressure, 40 °C of temperature and 0.82 of CO₂ molar fraction, they found few spherical particle at 5:5 polymer:drug ratio, but as the polymer: drug ratio increased to 7:3 or more, number of spherical particle increased along with lower particle size (the particle size was 3.68 μm at 9:1 polymer:drug ratio). At low polymer content, the solution was atomized into droplet having less amount of polymer which was not sufficient enough for hardening the droplet into spherical microparticle.

Both CO₂- and N₂-assisted atomization processes were used to develop ibuprofen/lipid (myristic acid and tripalmitin) composite microparticles. The average size of obtained particles was slightly larger than that of pure lipid particles in case of N₂-assisted process due to the difficulty of solidification using N₂. In CO₂-assisted process, the mean particle size was slightly smaller than that of pure myristic acid, but slightly larger than that of pure tripalmitin particles. The morphology of composite microparticles was similar to that of pure lipid particles [69].

Nanoparticles: Nanoparticles are carrier for drugs or other active molecules of nanometer size range (10 nm-1μm). Nanoparticles can be developed by using either non-biodegradable/biodegradable polymer (polymeric nanoparticles) or solid lipid (Solid Lipid Nanoparticles). Solid Lipid Nanoparticle (SLN) is a colloidal drug carrier system consisting of spherical solid lipid particles in the nanometer range, which are dispersed in aqueous or in water surfactant solution. The essential excipients used in SLNs are solid lipids (e.g. tristearin, stearic acid) as matrix and amphiphilic material (e.g. phospholids, bile salts and poloxamer) as surface stabilizer.

Chattopadhyaya *et al.* [38] prepared SLN suspension of model drug indomethacin and ketoprofen by SFEE method. Stable aqueous SLNs suspensions with mean volume diameter <50 nm were produced by this method, which was less than that of produced by high pressure homogenization and microemulsion technique. No crystalline drugs were detected in the formulations and also the degree of crystallinity was reduced in case of lipids (tripalmitin, tristearin and Gelucire 50/13). At stable condition, drug present in SLN above the

saturation level (10 % and 20 % for tripalmitin and Gelucire 50/13, respectively).

Coating

Traditional coating process involves the application of coating solution/suspension to the exterior of solid dosage form. This process associated with many disadvantages like solvent residue in the final dosage form, cost and environmental concern due to the use of organic solvent. To avoid above concern, organic solvent replaced with aqueous solvent. However, it increases drying time and a number of polymers are not soluble in aqueous solvent. The SCF technology is used to overcome the above shortcomings.

Santos *et al.* [70] developed coated microparticle of bovine serum albumin (BSA) using SCF coating process. The process was carried out in a simple autoclave equipped with a rotating impeller and the coating material selected are trimyristin (Dynasan® 114) and Gelucire 50/02 (mixture of glycerides and fatty acid esters) having melting point 45 °C and 50 °C, respectively. SCF condition used was temperature ranging between 35-45 °C and pressure about 200 bar. Gelucire® 50/02 produces consistent and homogeneous coating over BSA particles. This can be explained by the fact that Gelucire® 50/02 consists of mixture of glycerides and fatty acid which prevent the crystallization. On the other hand, Dynasan® 114 crystallized on BSA particles in the form of micro-needle resulting in discontinuity in coating layer. Due to this, Dynasan® 114 coated microparticles exhibit a high initial burst release of 35 % over in 5 minutes and 70 % release over 30 minutes. In case of Gelucire® 50/02 coated microparticles provide prolonged release of BSA over 24 hr.

Microparticles of red phosphorous (RP) coated with paraffin were developed by RESS technique with a newly designed nozzle. The characteristic feature of this model nozzle was the width of the aperture, which can be adjusted so that the supercritical solution expands rapidly through an exit with a controllable size. In addition, it can not easily be stopped (as seen in case of conventional nozzle) which makes the encapsulation process smooth. The obtained coated particle size of RP was 65 μm, when the raw RP particle of 45 μm size was

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taken. The surface of the coated microparticle shown to be perfectly smooth, indicating that the RP particles were completely and effectively encapsulated by the paraffin [71].

Mishima *et al.* [72] used RESS method with a non-solvent for the encapsulation of proteins (lipase and lysozyme). They have used number of polymers such as polymethylmethacrylate, polyethylene glycol (PEG), polylactic acid, poly lactide-co-glycolide and PEG-PPG (polypropylene glycol)-PEG triblock co-polymer and found non-agglomerated protein containing microparticles in the entire batch.

Extraction

Extraction by SCF involves the dissolution of the desired component from sample of plant and animal origin under the controlled condition of temperature and pressure followed by the separation of desired component from the SCF by a significant drop in solution pressure [73].

Conventional extraction processes require large amount of hazardous solvent (e.g. chloroform, methanol) and are generally cumbersome. Soxhlet extraction technique was developed in the intention to make extraction process continuous and was used for extraction of volatile oil and lipids. This process is associated with disadvantages such as requirement of highly pure solvents, use of hazardous and flammable liquid organic solvent, potential toxic emission during extraction, and finally, it is a time consuming process [74]. Supercritical fluid extraction (SFE) is routinely used for the extraction of lipids, essential oils from plant or animal material because of its environmentally friendly nature.

The major advantages of SFE over conventional solvent extraction process are: (i) the penetration power of SCF into porous solid materials is higher than liquid solvent due to its low viscosity and high diffusivity, (ii) a complete extraction is possible in SFE as a fresh fluid is continuously forced to flow through the samples, (iii) the solvation power of the SCF can be adjusted according to requirement by varying temperature and pressure, resulting a high selectivity, (iv) suitable for thermolabile material, (v) it can be associated with various compound detecting tool like gas chromatography and mass spectroscopy, which is useful in direct quantification in addition to extraction [75-78].

Essential oils extraction: Essential oils were traditionally extracted from seeds, roots, flowers, herbs and leaves using hydrodistillation. Thermal degradation, hydrolysis and solubility of some compounds in water may alter the flavour and sometimes fragrance of essential oils. SFE technique is used to avoid these problems. The optimum operating conditions for extraction of essential oils by SFE method are: pressure in the range of 90-100 bar and temperature ranges from 40-50 °C, since at these conditions all the essential oil compounds are highly soluble in SCF-CO₂ [79-82]. For instance, linalool, a terpene is completely miscible with SCF-CO₂ at temperature of 40 °C and pressure more than about 85 bar [82].

Seed oil extraction: Seed oils were generally extracted using hexane. The major problem associated with this method was difficulty in hexane removal after extraction and thermal degradation during the extraction, which necessitates the use of SFE technique [83-86].

Salgin *et al.* [87] employed SFE process for the extraction of jojoba oil and investigated the effect of process parameters such as pressure, temperature and particle size of jojoba seeds, flow rate of CO₂ on efficiency of extraction. They found the performance was increased (from 45.8 to 47.3 %) with the increase in temperature from 70 to 80° C and for pressure, the performance increased by increasing pressure from 200 to 600 bar. By increasing the CO₂ flow rate from 0.5 to 2 ml/min and reducing the particle size to half (i.e. 2.18 to 1.09 mm), the significant increase in performance was observed.

Preparation of Liposomes

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (e.g. phosphatidylcholine, cholesterol) [88]. Several methods are commonly used to prepare liposomes, like micro-emulsification, sonication, french pressure cell, membrane extrusion, organic solvent injection and reverse phase evaporation [89]. All the aforementioned methods are associated with problems, such as need of large amount of organic solvent, many steps involved and high energy consumption, which limit their wide

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application. SCF technique is used to solve above problems.

Frederiksen *et al.* [90] prepared liposomes by using modified RESS process. They have dissolved phospholipids and cholesterol in SCF under high pressure followed by depressurization in a low pressure chamber and simultaneously mixed with water containing dextran and fluorescein isothiocyanate to produce liposomes. In this method, the diameter of obtained liposomes was 200 nm and the organic solvent required was 15 times less than ethanol injection method [91]. Till date many scientist used SCF principle to prepare liposomes [92-94].

More recently the concept of pro-liposomes was developed in the view of the shortcomings such as oxidation, fusion, aggregation, and phospholipid hydrolysis resulting in physico-chemical instability associated with liposomes [95, 96]. Pro-liposomes can be defined as dry and free flowing particulate system with loaded drugs, which upon dispersion in water converted in to liposome suspension.

Feix *et al.* [97] prepared pro-liposomes of vitamin-D3 (VD) composed of hydrogenated phosphatidylcholine by SAS method and studied the effect of operational conditions such as temperature, pressure and composition on the loading of VD in pro-liposome. They found temperature of 45 °C, pressure of 8 MPa and mass ratio of 15 % between VD and lipid provide the optimum condition. The loading efficiency was found to be 12.89 %. In addition, they compared the encapsulation efficiency of pro-liposomes prepared by SAS method and ultrasonic dispersion method and found 100 % of encapsulation in case of SAS method upon dispersion in buffer which was more than that obtained by ultrasonic dispersion method.

Drying of proteins and peptides

The number of protein such as cyclosporine, insulin, protein hydrolysate are introduced to the market, which is the result of genetic engineering [98]. The important aspect about protein and peptide is the stabilization as they are unstable in liquid formulation because of chemical and physical degradation reaction, which necessitates to store in dry form [99]. Many traditional drying techniques, such as freeze drying, spray drying, vacuum drying and spray freezing drying, have been used for a long time to stabilize above

compounds. But all the above techniques are having drawbacks. For instance, freeze drying is an expensive process due to high energy and time consumption and not providing complete recovery of the intact protein due to process induced degradation (during freezing and drying phase). SCF process is an alternative method to above due to its mild process conditions, cost effectiveness and possible sterilizing properties [100].

Bouchard *et al.* [101] dried lysozyme particles by using SEDS technique and investigated the particle morphology and molecular integrity. They used ethanol along with SCF to increase solubilization power of CO₂ in aqueous solution. They observed three types of particles: (a) agglomerated nanoparticles (200-300 nm) with spherical shape, which agglomerated in to spheroid cluster (5-50 μm), (b) microparticles with ellipsoids to spheroid shape, and (c) microspheres. The agglomerated nanoparticles are formed at highest ethanol concentration because the mass transfer rate of ethanol in to the droplets exceeds the mass transfer of water to extractant phase. The microparticles were produced under competitive rate of antisolvent precipitation and water extraction. The microsphere formation can be explained by the fact that water extraction exceeds antisolvent effect of the ethanol.

Impregnation

Impregnation is generally used to incorporate active ingredients in the polymeric matrix. Traditionally, impregnation was carried out by two steps; (1) active ingredients is first dispersed or dissolved in a suitable solvent, and (2) the polymer is soaked in the so formed dispersion or solution [102]. The presence of residual solvent in the final substrate can lead to some toxic effects, which was the major concern associated with traditional method. In addition to its nontoxic nature, supercritical impregnation is having swelling and/ or plasticizing effect on the polymer. Impregnation of polymer can be achieved either by deposition or molecular dispersion phenomenon [103, 104]

Masmoodi *et al.* [105] carried out supercritical impregnation of Intraocular lenses (IOLs) made up of polymethylmethacrylate with cefuroxime sodium through batch process (a high pressure impregnation step followed by a depressurization step) and investigated

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various experimental conditions, such as pressure, temperature, impregnation duration and cosolvent on the impregnation yield. They observed that impregnation yield vary between 0.001-0.029 mg_{drug}/ mg_{IO_{LS}} and this is enhanced by using the cosolvent (ethanol). The mechanism of impregnation is deposition and *in-vitro* drug release show a burst release followed by slow release.

Supercritical Fluid Chromatography and its application

Supercritical fluid chromatography (SFC) uses CO₂ as mobile phase to dissolve compounds. Unfortunately, CO₂ is not a good solvent for polar compounds. But, this problem can be corrected by adding moderate amount of organic solvent, called as modifier [106]. The popularity of SFC lies on its advantage over HPLC such as (a) faster and more efficient separation of compounds due to lower viscosity and higher diffusivity of CO₂, (b) most of the pharmaceutical ingredients used for the synthesis purpose are as soluble or more soluble in mixture of CO₂ and organic modifier, (c) recovery of purified compounds from the collected fractions is easier and economical as solubility in CO₂ decreases rapidly with decrease in pressure, (d) moreover, mobile phase, CO₂, is cheaper, greener and safer as compared to organic solvent [106-108]. There are, generally, two types of SFC, namely packed column and preparative scale.

The application of SFC includes: (a) chiral (enantiomer) separation e.g., separation of chiral sulfoxide belonging to the family of substituted benzimidazoles by using Chiralpak AD and methanol as stationary phase and modifier, respectively [109]. Separation of 44 pairs of enantiomers (β -blockers, β -agonist, benzodiazepines, non-steroidal anti-inflammatory drugs, barbiturates, free and derivatized amino acids) were carried out by using common stationary phase (Chiralcel OD and AD, Chirobiotc V and T), CO₂ modified with 5-30 % of methanol [110]. Liu et al. [111] performed the enantiomeric separation of macrocyclic glycopeptide (teicoplanin) and some of its common derivatives, (b) Separation of achiral compounds such as the separation of mixture of estrogen metabolites [112] and separation of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine by SFC coupled with light scattering and mass

spectrometric detection [113], (c) analysis of peptides- among the peptides separated and analyzed by SFC are cyclosporin [114], actinomycin D and vancomycin [108], gramycidin A, B, and C [115], (d) Coupling of SFC with mass spectroscopy, which helps to obtained good peak shape and signal in chromatogram [116]. A large number of drugs and materials used for various SCF techniques are listed in Table. 2.

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Table 2. Active ingredients and materials used for different SCF techniques with their applications

Sl.no	SCF Technique	Active Ingredient	Material	Application	Ref.
1	RESS	Artemisinin		Micronization	51
		Diclofenac		Micronization	52
		Beclomethasone-17,21-dipropionate		Particle morphology	54
		Lidocaine		Particle Morphology	55
		Digitoxin		Micronization	57
		Raloxifen		Micronization	58
		Cefuroxime axetil		Nanonization	59
		Red phosphorous	Paraffin	Coating	71
		Lipase and lysozyme	Polymethylmethacrylate, PEG, polylactic acid (PLA), polylactide-co-glycolide (PLGA) and PEG-PPG (polypropylene glycol)-PEG	Coating	72
		Dextran and fluorescein isothiocyanate	Phospholipids and cholesterol	Liposomes	90
2	PGSS	YNS3107	PEG 400, PEG 4000 and Poloxamer 407	Micronize solid dispersion	63
		Human growth hormone	PLGA and PLA	Microparticles	64
3	RESAS	Cyclosporine	Phospholipid	Nanoparticles	61
4	GAS	Puerarin		Polymorphs	48
5	PCA	Budesonde	Polylactic acid	Microparticles	53
6	SAS	Amoxicillin		Micronization	60
		Naproxen	Methyl cellulose and ethyl cellulose	Microspheres	65
		Cefuroxime axitel	Polyvinylpyrrolidone-K30	Microparticles	67
		Rifampcin	Poly (L-lactide)	Microparticles	68
		Vitamin-D3	Phosphotidylcholine	Pro-liposome	97
7	ASAIS	Theophylline		Polymorphs	30
8	SEDS	Terbutalin sulphate		Polymorphs	49
		Morphine	PLA	Microparticles	66
		Lysozyme		Drying of proteins and peptides	101
9	SFEE	Indomethacin and ketoprofen	Tripalmitin, tristearin and Gelucire 50/13	Solid lipid nanoparticles	38
10	SFE	Jojoba oil		Extraction	87
11	CAN-BD	Ibuprofen	Myristic acid and tripalmitin	Solid lipid microparticles	69
12	SAA	Cefadroxil		Micronization	50
		Cromolyn sodium		Micronization	56

Conclusion:

A large number of SCF based processes were developed in recent years. There are still possibilities to improve the existing SCF processes by optimizing operational conditions (physical and chemical parameters). Furthermore, many new processes can be developed by understanding the properties of SCFs, nature of solute and their interaction. Of course, SCF based techniques are superior over existing and well established techniques such as milling/crushing for size reduction, Soxhlet extraction, spray coating, impregnation by soaking, etc. However, extensive research is required to make it feasible in industrial scale.

For instance, a commercial operation under the trade name of HIPLEX process is used for the processing of soybean at SafeSoy technologies in Ellsworth, Iowa. This CO₂-assisted process resulting in between 80-90% vegetable oil recovery for soybeans and over 90% recovery for canola oil [117]. Another extraction process in commercial scale using SCF technology is being carried out at Proderma Biotech (Indo Dutch Joint Venture company created between FeyeCon B.V of Holland and by Indian Promoters of Chemcaps for promoting the Super Critical Fluid technology in India) [118].

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