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Application of Supercritical Fluid Technology for Preparation of Drug Loaded Solid Lipid Nanoparticles

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Abstract

Small changes in pressure or temperature, close to the critical point, lead to large changes in solubility of supercritical carbon dioxide (CO_2). Environmentally friendly supercritical CO_2 is the most popular and inexpensive solvent which has been used for preparation of nanodrugs and nanocarriers in drug delivery system with supercritical fluid technology. Delivery of a drug is one of the most challenging research areas in pharmaceutical sciences. With a combination of drugs and innovative delivery systems such as lipid nanocarriers, drugs efficiency and safety have been improved significantly. There are various techniques available to produce drug loaded solid lipid nanoparticles. Among them, supercritical fluid technology has been identified as potentially effective and applicable approach which has attracted increasing attention during recent years. This technique has several advantages such as avoid the use of solvents, particles are obtained as a dry powder, instead of suspensions, mild pressure and temperature conditions can be applied. Nevertheless, little attention has been paid to formation of drug loaded solid lipid nanoparticles by supercritical fluid technology. In this paper, we present a brief introduction to solid lipid nanocarriers. Then a general overview of different processes of supercritical fluid technology has been provided and also case studies are presented to show the potential benefits of this approach in drug loaded solid lipid nanoparticle production.

Keywords: *Drug Solubility; Drug Delivery; Lipid Solubility; Supercritical Fluid Technology; Solid Lipid Nanoparticles.*

1. INRODUCTION

Drug delivery systems provide important tools to introduce drug at preselected target in therapeutic dose into the body. Many different types of drug delivery vehicles are currently apply in successful transportation of the drug. As a vehicle for controlled release of drug entities, nanodisperse systems such as liposomes, nanoemulsions, and nanoparticles are gaining more and more attention [1]. Nanoparticles have unique properties due to high surface to mass ratio compared to other particles. Therefore, they can bind, adsorb and carry other compounds such as drugs, probes and proteins. Solid lipid nanoparticles are one of the most important entities essentially required for successful transport of loaded drugs. Several conventional techniques have been utilized for drug loaded solid lipid nanoparticles production. These include high pressure homogenization (hot or cold), ultra-sonication, microemulsion technique, solvent emulsification/evaporation and etc.

The disadvantages of using these conventional techniques are energy intensive process, poor drug loading capacity, broad particle size distributions, biomolecule damage, large amounts of solvent used and associated disposal problems and solvent residues. To overcome most of these problems, supercritical fluid technology using carbon dioxide as a solvent is introduced to nanoparticle preparations without using organic solvents in any stage of the process. Despite the fact that supercritical fluid technology were introduced as an important and applicable technique for drug loaded solid lipid nanoparticles production in literatures, but few papers have been published in this area. Hence, this paper aims to bridge the gap between supercritical fluid technology and drug loaded solid lipid nanoparticles production.

In this paper, at first a brief introduction to solid lipid nanoparticles is presented. Also, different processes of supercritical fluid technology are shortly described. Then a comprehensive review of application of supercritical fluid technology in drug micronization and nanocomposite drug production is described. In addition, this paper tries to highlight key points for preparation of drug loaded solid lipid nanoparticles with different supercritical fluid processes.

2. SOLID LIPID NANOPARTICLES (SLN)

Poor drug solubility, poor absorption, drug distribution to non-targeted sites and high fluctuations in drug plasma levels are significant problems in traditional drug delivery systems. One of the approaches was introduced at the beginning of the 1990 to troubleshoot these problems were solid lipid nanoparticles (SLN) [2]. They are rapidly emerging as a new class of efficient and safer drug delivery system. SLNs represent a promising new approach to improve the oral bioavailability of the poorly soluble drugs [3].

Solid lipid nanoparticle is composed of spherical solid lipid particles ranging from 50 nm to 1μm, which are dispersed in water or in aqueous surfactant solution. They have many advantageous such as small size, large surface area, high drug loading, good biocompatibility and low toxicity. Potential disadvantages of SLNs are such as poor drug loading capacity, drug expulsion after polymeric transition during storage, relatively high water content of the dispersions (70-99.9%) and the low capacity to load water soluble drugs [4]. SLN get together the advantages of polymeric nanoparticles, emulsion and liposomes without some of their disadvantages [5].

SLNs are composed of biodegradable lipids like highly purified triglycerides, monoglycerides, fatty acids, complex glyceride mixtures or even waxes which are solid at physiological temperature. Polymorphism, crystallinity miscibility and physicochemical structure have to be considered in selection of lipids [6]. The lipid crystalline structure is a key factor to determine whether a drug would be expelled or incorporated into the lipid carrier system [7]. Incorporated drugs in lipid nanocarriers are located between fatty acid chains, between the lipid layers and also in crystal imperfections. Thus a highly ordered crystal lattice cannot accommodate large amounts of drug. Therefore the use of more complex lipids is more reasonable for higher drug loading [8]. Current methods to produce SLNs include high-pressure homogenization, ultrasonication, microemulsion technique, solvent emulsification/ evaporation, solvent emulsification-/diffusion and supercritical fluid technology [2].

High pressure homogenization is a reliable and powerful technique which has been widely used for SLNs production. In this technique, melted lipid containing drug is pushed with high pressure (100-2000 bars) through a narrow gap of few micron ranges. [3]. Shear stress and cavitation are the forces which cause the

disruption of particle down to the submicron range [9]. Basically, there are two approaches for production by high pressure homogenization, hot and cold homogenization techniques. For both techniques, the drug is dissolved or dispersed or solubilized in the melted lipid at approximately 5-10ºC above the melting point [9, 10]. Lower particle size of drug loaded solid lipid nanoparticles could be achieved by higher temperature [10].

Ultrasonication or high speed homogenization is another technique which can be used for SLN production. Two methods of sonication are commonly used, bath sonicator and probe tip sonicator. For large amount of diluted lipid dispersions, bath sonicator is applied. The probe tip sonicator is more suitable for dispersions, which require high energy in a small volume [2].

Microemulsion technique is based on the dilution of microemulsion [11]. Microemulsions are thermodynamically stable liquid mixtures which are composed of oil, water and surfactant and can be formed by spontaneous homogenization. Due to the dilution step, achievable lipid contents are significantly lower compared with the high pressure homogenization method [12].

In solvent evaporation method, the lipid is dissolved in an organic solvent and the solution is emulsified in an aqueous phase. After evaporation of the solvent, the lipid precipitates to form nanoparticles [12]. Advantage and disadvantage of different techniques of SLN preparation are summarized in Table 1 [13, 14].

Table 1. *Advantage and disadvantage of different techniques for SLN production.*

Technique	Advantage	Disadvantage
High pressure Homogenization	a. Low capital cost. b. Demonstrated at lab scale.	a. Energy intensive process. b. Demonstrated at lab scale c. Biomolecule damage. d. Polydisperse distributions. e. Unproven scalability
Ultrasonication	a. Reduced shear stress	a. Potential metal contamination. b. Physical instability like particle growth upon storage
Solvent Evaporation	a. Scalable. b. Mature technology. c. Continuous process. d. Commercially demonstrated	a. Extremely energy intensive process. b. Polydisperse distributions. c. Biomolecule damage.
Micro-emulsion	a. Low mechanical energy input. b. Theoretical stability	a. Extremely sensitive to change. b. Labor intensive formulation work. c. Low nanoparticle concentrations.

SLNs could be produced in different types such as homogeneous matrix, drug enriched shell and drug enriched core. SLN with homogeneous matrix is a solid solution of lipid and drug which is produced by cold homogenization. Drug enriched shell is achieved by hot homogenization when drug concentration in the melted lipid is low. Drug enriched core can form when drug concentration in the melted lipid is high and close to its saturation solubility [15, 16].

3. SUPERCRITICAL FLUID TECHNOLOGY

Supercritical fluid technology has been widely used for various applications such as extraction, reaction, chromatography and material processing. Formation of microparticles or nanoparticles with unique morphology is the most outstanding characteristic of particle formation from supercritical fluid technology. However the understanding of applying supercritical fluid technology to particle formation is still in their infancy [17]. Much research has been published about applications of supercritical fluid technology on the preparation of nano-materials [18-20].

Supercritical fluids (SCF) have unique properties such as high diffusivity, low viscosity, and high compressibility. These attractive solvents are increasingly replacing the organic solvents for many industrial processes. Supercritical CO_2 (SC-CO_2) is the most common SCF, because it is non-toxic, non-flammable, easy to obtain, and easily accessible critical condition such as T_c=304.25K and P_c=73.7 bar) [21]. Small changes in the temperature or pressure near the critical point result in significant changes in the solubilizing power of supercritical fluid and in turn density. Therefore, density depends on the applied temperature and pressure.

Different supercritical fluid processes are being developed to design particles with several purposes in drug delivery system. Formulation options such as dry powders, nanoparticle suspensions, microspheres or microcapsules and emulsion suspension could be designed with these processes [22]. These include the rapid expansion of supercritical solutions (RESS), the gas antisolvent process (GAS), supercritical antisolvent process (SAS) and its various modifications, and the particles from gas-saturated solution (PGSS) processes.

Supercritical fluid technology is an alternative method of preparing SLNs. This technique has several advantages such as avoid the use of solvents, particles are obtained as a dry powder, instead of suspensions, mild pressure and temperature conditions can be applied [10]. In addition, the density of a supercritical fluid modulates with pressure, thus easy separation and recovery of solvent and antisolvent can be performed by a simple downstream depressurization step with changes of pressure. These advantages make particle formation with supercritical fluids attractive for pharmaceutical applications [23]. Following this session, different processes of supercritical fluid technology which could be used for SLNs preparation, are described in details.

3.1. Rapid Expansion of Supercritical Fluid Technology

Rapid expansion of supercritical solutions (RESS) process is a powerful technique to produce ultrafine particles of uniform size. This process is based on large variations in the solvent power of the supercritical fluid with changes in pressure. This two-step process is illustrated in Fig.**1**. In this process, at first, supercritical fluid is saturated with a solid solute. When, supercritical fluid is expanded across a heated nozzle into a low pressure chamber, the density of the solution decreases dramatically and the solute precipitates uniformly due to high supersaturation. Then ultrafine and porous particles are produced and collected from the gaseous stream [20]. The morphology of the precipitated product, crystalline or amorphous, depends on the chemical structure of the material and on the RESS parameters such as temperature, pressure drop, impact distance of the jet against a surface and nozzle geometry [18]. The mass transfer in RESS process is severe and so high supersaturation is produced. High supersaturation leads to fast nucleation and hence it is difficult to control precipitation step [24].

Several strategies have been employed to micronize poor water soluble drugs to enhance their water solubility and in turn bioavailability.

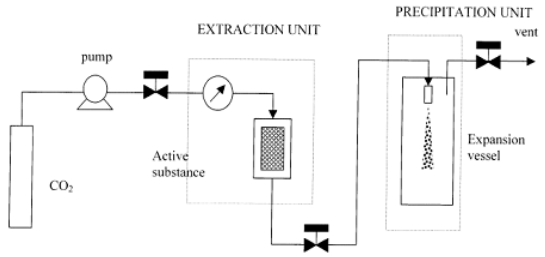

Figure 1. *Rapid expansion of supercritical fluid process [20]*

For this purpose, RESS is one of the most important techniques. This process has been reported in the literature for the micronization of pharmaceutical compounds such as carbamazepine, nabumetone, ibuprofen, griseofulvin, acetaminophen and erythromycin [25-30]. In recent years, much research has been performed on the production of sub-micron and nano-sized particles of pharmaceutical compounds but less attention has been paid to co-precipitation of two or more solutes in RESS process. As drug loaded SLNs are composed of two solutes, therefore co-precipitation of drug and lipid has to be investigated.

In order to recover large enough amounts of products, the solid active pharmaceutical ingredient must have a good solubility in the supercritical fluid [31]. Therefore, the solubility of solutes in supercritical CO_2 is probably the most important property that is needed to be known for the production of micronized solutes by RESS. This limitation is even more severe for co-precipitation application in which both the drug and lipid have to be soluble in SCF-CO_2 [32]. Hence, following this session solubility of common lipids and different drugs are presented.

Two most common saturated fatty acids which widely used in drug loaded SLN preparation are palmitic acid and stearic acid. The solubility of two fatty acids in supercritical carbon dioxide at 328 K and 318 K between 128 bar to 226 bar are shown in Table **2**. Solubilities of stearic acid and palmitic acid are high enough in SCF-CO_2 [33]. Hence, in preparation of SLN with RESS process, temperature and pressure of extraction unit can be considered a bit more than critical pressure and temperature of SCF-CO_2.

Table 2. *The solubilities of stearic acid (SA) and palmitic acid (PA) in SC- CO_2 [33]*

Temperature(K)	**Pressure(bar)**	**Solubility of SA mole fraction(*10^{-4})**	**Temperature(K)**	**Pressure(bar)**	**Solubility of PA mole fraction(*10^{-4})**
308	128.5	0.74	308	128.5	2.85
	148.1	0.89		148.1	3.14
	167.7	1.03		167.7	3.53
	197.1	1.19		197.1	4.01
	226.5	1.24		226.5	4.45
318	128.5	0.83	318	128.5	3.74
	148.1	1.48		148.1	5.44
	167.7	2.12		167.7	6.98
	197.1	2.87		197.1	8.65
	226.5	3.24		226.5	10.45

Akbari and co-workers have studied on micronization of stearic acid with RESS process. Results showed that the RESS processing of stearic acid leads to spherical particles in the range from 40 nm to 200 nm which are about 600 times smaller than the unprocessed powder as reflected by scanning electron microscopy (SEM) observations. Also, further increase in extraction pressure and extraction temperature causes the agglomeration of stearic acid nanoparticles, attributed to increase of stearic acid solubility in supercritical CO_2. Fourier transform infrared spectrophotometry (FTIR) and X-ray diffraction (XRD) analysis of the obtained product indicated the degree of crystallinity of stearic acid nanoparticles was reduced without any chemical structural change. Differential scanning calorimetry (DSC) analysis showed a 2.7◦K decrease in the melting temperature from that of bulk stearic acid [34].

Triglycerides such as tristearin, tripalmitin, trilaurin and trimyristin are widely used as lipid excipient in drug loaded SLNs [35]. The solubilities of triglycerides in supercritical carbon dioxide at 328 K and 325 K between 80 bars to 300 bars are shown in Table **3** and **4**. Therefore, due to high solubility of these lipids in supercritical CO_2, RESS can be a good candidate of supercritical fluid process to be used to produce drug loaded SLNs.

Solubility of drug is other important parameter which has to be considered in drug loaded SLN preparation with RESS process. In Table 5, solubilities of drugs in different temperatures and pressures are presented. Solubility data of different drugs show that lipids are more soluble in supercritical CO_2 than most of drugs. Of course, some drugs like aspirin, mefenamic, ibuprofen and cephexin have fair solubility. Solubility of these drugs is a bit more than lipids like stearic acid.

As a large number of studies have been performed on micronization of different drugs in supercritical CO_2 with RESS process and also in regard to high solubilities of lipid in SCF-CO_2, therefore it would be possible to dissolve drug and lipid simultaneously in supercritical CO_2 in extraction unit of RESS process.

Table 3. *The solubilities of tristearin (TS) and tripalmitin (TP) in supercritical fluid CO_2 [35]*

Temperature(K)	**Pressure(bar)**	**Solubility of TS mole fraction(*10^{-4})**	**Temperature(K)**	**Pressure(bar)**	**Solubility of TP mole fraction(*10^{-4})**
308	171	0.034	**308**	105	0.025
	234	0.039		127	0.046
318	150	0.023	**318**	9.5	0.052
	200	0.057		10.5	0.057
	268	0.069			
325	108	0.016	**325**	108	0.044
	127	0.022		127	0.152
	153	0.038		153	0.359
	185	0.072		185	0.633
	240	0.139		240	1.071

Table 4. *The solubilities of trimyristin (TM) and trilaurin (TL) in supercritical fluid CO_2 [35]*

Temperature(K)	Pressure(bar)	Solubility of TS mole fraction(*10^{-4})	Temperature(K)	Pressure(bar)	Solubility of TP mole fraction(*10^{-4})
308	83	0.109	**308**	83	1.401
	90	0.196		90	2.233
	105	0.429		105	4.178
	127	0.625		127	7.079
	171	1.357		171	14.949
	264	2.102			
318	95	0.255	**318**	95	1.179
	105	0.539		105	2.624
	125	1.063		125	5.221
	150	1.802		150	8.386
	200	3.664		200	18.696
	268	5.716			

Table 5. *The solubilities of different drugs in supercritical CO_2*

Drug	Temperature range	Pressure range	Solubility (mole fraction)	Ref
Carbamazepine	313(K)- 328 (K)	100bar-350 bar	$0.1*10^{-5}$-$3.8*10^{-5}$	36
Norfluxacin	318(K)-323(K)	100bar-300 bar	$1.4*10^{-6}$-$24.4*10^{-6}$	37
Ofluxacin	313(K)-323(K)	100bar-300 bar	$0.4*10^{-6}$-*10^{-6}1.3	37
Dexamethasone	308(K)-328(K)	150bar-350 bar	$1.3*10^{-6}$-$2.8*10^{-6}$	37
Piroxicam	312 (K)	100 bar- 190 bar	$1.3*10^{-5}$-$4.41*10^{-5}$	38
Mefenamic acid	308(K)-323 (K)	160 bar-400 bar	$8.31*10^{-5}$- $5.94*10^{-3}$	38
Fluirbiprofen	303(K)-323 (K)	80 bar-250 bar	$1.67*10^{-5}$-$1.97*10^{-4}$	39
Cyproheptadine	308(K)-338 (K)	160 bar-400 bar	$3.35*10^{-5}$-$3.09*10^{-3}$	40
Acetazolamide	313(K)-323 (K)	150 bar-200 bar	$0.57*10^{-5}$-$1.73*10^{-5}$	41
Beclomethasone	338(K)-358 (K)	200 bar-400 bar	$1.17*10^{-6}$-$7.07*10^{-6}$	42
Budesonide	338(K)-358 (K)	200 bar-400 bar	$1.04*10^{-6}$-$9.62*10^{-6}$	42
Aspirin	318(K)-328 (K)	100 bar- 200 bar	$2.18*10^{-3}$-$7.57*10^{-3}$	43
Medroxyprogesterone	308(K)-348 (K)	122 bar-304 bar	$0.19*10^{-4}$-$4.13*10^{-4}$	44
Cyproterone acetate	308(K)-348 (K)	122 bar-355 bar	$0.13*10^{-4}$-$2.61*10^{-4}$	44
Erythromycin	313(K)-333 (K)	100 bar- 300 bar	$3.8*10^{-4}$-$21*10^{-4}$	45
Cholesterol	318K	100 bar- 240 bar	$6.49*10^{-6}$-$94*10^{-6}$	46
Metronidazole	308(K)-348 (K)	122 bar-355 bar	$0.7*10^{-4}$-$45.5*10^{-4}$	47
Naproxen	308(K)-348 (K)	122 bar-355 bar	$0.1*10^{-4}$-$2.0*10^{-4}$	47
Tebuconazole	323(K)-338 (K)	100 bar- 300 bar	$0.35*10^{-5}$-$18.57*10^{-4}$	48
Imipramine	313(K)-323 (K)	300 bar- 500 bar	$6.4*10^{-6}$-$9.9*10^{-6}$	49
Artemisinin	310(K)-338 (K)	100 bar- 270 bar	$0.099*10^{-3}$-$2.65*10^{-3}$	50
Methimazole	308(K)-348 (K)	122 bar-355 bar	$0.15*10^{-4}$-$1.9*10^{-4}$	51
Atropine	308(K)-348 (K)	122 bar-355 bar	$0.6*10^{-4}$-$1.67*10^{-3}$	52
Diazpam	308(K)-348 (K)	122 bar-355 bar	$1.6*10^{-4}$-$1.11*10^{-3}$	52
Codeine	308(K)-348 (K)	122 bar-355 bar	$0.4*10^{-5}$-$0.94*10^{-4}$	52
Lovastatin	308(K)-348 (K)	122 bar-355 bar	$1.1*10^{-5}$-$1.14*10^{-4}$	53
Atrovastatin	308(K)-348 (K)	122 bar-355 bar	$1.12*10^{-6}$-$1.45*10^{-3}$	53
Simvastatin	308(K)-348 (K)	122 bar-355 bar	$2.0*10^{-6}$-$5.35*10^{-4}$	53
Fluvastatin	308(K)-348 (K)	122 bar-355 bar	$5.0*10^{-6}$-$6.0*10^{-4}$	53
Rosuvastatin	308(K)-348 (K)	122 bar-355 bar	$3.0*10^{-6}$-$2.44*10^{-4}$	53

Then with co-precipitation of drug and lipid in the expansion unit, fine and porous composite particles (drug loaded SLNs) can be produced. Following this session, there are some case studies about co-precipitation phenomena in RESS process.

Turk and co-workers have studied on formation of composite drug–polymer particles by co-precipitation during RESS process. In CO-RESS both, the drug (phytosterol) and the biodegradable polymer (poly-lactic acid) are dissolved in supercritical CO_2, followed by the rapid expansion of the ternary mixture. The experimental data of PLA and phytosterol solubility are in the range of $1.2–2.8\times10^{-4}$ g l-PLA/g CO_2 and 7.8-9.8 $\times10^{-4}$ g l-phytosterol / g CO_2 at 20 MPa. The simultaneous co-precipitation of the solutes occurs and composite particles were produced in the different morphologies and composition profiles. Agglomerated particles with small primary particles in the range of 50 nm were obtained. SEM pictures, DSC and XRD analysis of the composite product indicated that phytosterol might nucleate first and was surrounded by poly-lactic acid coating. In addition, an increase in pre-expansion temperature resulted in a lower amount of the polymer in the powder. Also, experiments showed that CO-RESS can be applied for preparation of submicron composite particles with different solutes [54].

Sane and co-worker have studied on formation of retinylpalmitate-loaded poly (l-lactide) nanoparticles using RESS into an aqueous solution (RESOLV) containing a stabilizing agent. The effect of rapid expansion processing conditions such as degree of saturation, pre-expansion temperature, and concentrations of poly(l-lactide) (PLLA) and retinylpalmitate (RP) on the particle size, form, and RP loading was systematically investigated. Solubility of PLLA is significantly less than RP in supercritical CO_2 and, therefore, PLLA is the major component precipitated during expansion step in RESS process just below the cloud point curve. Result in this study showed that spherical PLLA/RP nanoparticles with an average size range of 40–110 nm and RP loadings of 0.9–6.2 wt% were consistently produced by RESOLV. The entrapment capacity of RP in PLLA nanoparticles was determined by pre - expansion temperature and concentration of RP. It has been observed that increasing the pre-expansion temperature from 343K to 373 K and the concentration of RP from 0.05 to 0.15 wt% increased the encapsulated RP content at least twofold. Totally, the entrapment of RP in the PLLA nanoparticles increased with increasing pre-expansion temperature, degree of saturation, and RP concentration while decreased with increasing PLLA concentration [55].

Mishima and co-workers have studied on formation of polymer microparticles containing proteins such as lysozyme and lipase by RESS process. The polymers were poly (ethylene glycol), poly (methyl methacrylate), poly (L-lactic acid), poly (DL-lactide-co-glycolide) and poly (propylene glycol). A suspension of protein in CO_2 containing co-solvent and dissolved polymer was sprayed through a nozzle to atmospheric pressure. Results showed that the solubilities of these polymers in CO_2 increase significantly with low-molecular-weight alcohols as co-solvents. Also, the effect of various parameters, the pre-expansion temperature, pre-expansion pressure, feed composition, injection distance and polymer molecular weight were investigated on diameter particle size. Effect of pre-expansion temperature, pre-expansion pressure, polymer molecular weight, and injection distance were negligible on particle size distribution of microcapsule but it could be controlled by feed composition. Also, physical properties such as molecular weight and glass transition temperature of the polymer materials have not been varied through the RESS processing. Experiments showed that the produced globular

microparticles were reasonably mono-disperse in size [56].

Tom and co-workers have studied on precipitation of composite poly(l-lactic acid)-pyrene particles by RESS process. The maximum solubility of pyrene in supercritical CO_2 is of the same order of magnitude of LPLA in CO_2 – $CHCLF_2$ mixtures. In this research, fluorescence and transmission microscopy applied to observe pyrene in the co-precipitate because pyrene is fluorescent. Results showed that increasing pyrene concentration (0.0008 wt %-0.0013 wt %) led to increase in the fluorescence of microspheres. As pyrene concentration was more than 0.002 wt %, pyrene agglomerates separate from the microspheres were also observed. Therefore, the key result of this research is the limitation of pyrene concentration in supercritical CO_2. Also, these experiments showed the uniform incorporation of pyrene microparticles within polymer microspheres. Thus, results of this research confirmed the potential application of RESS technique to perform co-precipitation of composite particles with multiple substances [57].

Akbari and co-workers have studied on formation of ibuprofen loaded stearic acid nanoparticles by co-precipitation of rapid expansion of supercritical solutions. The maximum solubility of ibuprofen is of the same order of magnitude of stearic acid in supercritical CO_2. CO-RESS experiments were carried out with the physical mixture of ibuprofen and stearic acid with different mass ratio (1:1, 1:5, and 1:10) as solutes in different experimental conditions. Results showed that composite nanoparticles had a distinct spherical shape with smooth surface and RESS process could produce composite particles of SLNs with high drug loading capacity (35 wt % - 65 wt %). It was observed that with increase of lipid (to 10 times), drug loading only less than 2 times decrease. Hence, optimum drug incorporation efficiency can be obtained at low initial mass ratio of the physical mixture of ibuprofen and stearic acid. XRD patterns along with DSC show that ibuprofen was found in both amorphous and crystalline form within lipid matrix. FTIR study showed that molecular interactions that could alter the chemical structure of the ibuprofen did not occur. As melting temperature of lipids is low, thus pre-expansion temperature was less than melting temperature of lipid. Because liquid lipid droplet were formed and quickly solidified within the expansion unit, leads to form large particles and change morphology of particles [58].

Akbari and co-workers have studied on formation of drug-stearic acid composite nanoparticles by co-precipitation of rapid expansion of supercritical solutions. Carbamazepine and miconazole nitrate were used as drugs model. The maximum solubility of stearic acid is tenfold order of magnitude of carbamazepine in supe-rcritical CO_2. CO-RESS experiments were carried out with the physical mixture of carbamazepine and stearic acid with different mass ratio (1:1 and 1:5) as solutes. Results showed that RESS process could produce composite particles of SLNs with maximum drug loading capacity of 2.2 wt %. Also, FTIR characterization of co-precipitated miconazole- stearic acid nanoparticles by RESS process did not show any functional group of miconazole nitrate in the product. Therefore, stearic acid with high solubility in supercritical CO_2 inhibited the dissolution of miconazole nitrate in extraction unit [59].

As mentioned in previous section, major disadvantages of SLN production with traditional techniques is poor drug loading capacity. Main factors influence on loading capacity of a drug in lipid in traditional processes. They are: solubility of drug in melted lipid, miscibility of drug and melted lipid, chemical and physical structure of solid lipid and also polymorphic state of lipid material [13]. Loading capacity of different drugs loaded on solid lipid nanoparticles are listed in Table 6. As can be seen, loading capacity

of drugs which have been prepared with traditional techniques is low.

Table 6. *Loading capacity of different drugs loaded solid lipid nanoparticles*

Drug	**Lipid**	**Techniques of preparation**	**Drug Loading capacity (wt %)**	**Ref**
Tamoxifen	Phospholipon and Palm oil	High pressure Homogenization	4.5-18	60
Simvastatin	glycerylbehenateglyceryl	Hot melt emulsification	6	61
Andrographolide	palmitostearate	High pressure	3.49	62
Dexamethasone Acetate	glycerol tristearate, Soybean lecithin Monostearin	Homogenization	8.79	63
Curcumin	Trimyristin, and soy	High pressure	4.35	64
Atorvastatin	phosphatidylcholine		6.08	65
Triptolide	-	Homogenization	1.02	
		Emulsion-evaporation	18	66
Ibuprofen	polyoxyl 40 hydrogenated castor oil	Hot Homogenization Micro-emulsion	9.63	67
Norfloxacin	stearic acid	Hot homogenization and ultrasonic technique		68
	stearic acid			

For example as shown in Table **5**, drug loading of ibuprofen loaded stearic acid nanoparticles produced by homogenization is 18 % while RESS process could produce composite particles of ibuprofen loaded stearic acid with high drug loading capacity in the range of 35 wt % - 65wt % [58]. Therefore, drug loaded solid lipid nanoparticles with high loading capacity can be produced by RESS process.

Based on results of case studied which are shortly described in this section, there are important key points which have to be considered for drug loaded SLNs preparation with RESS process. These include:

- Select proper lipid and drug which have fair solubility in SCF-CO_2.
- If solubility of drug and lipid are the same order of magnitude, to obtain high drug loading capacity, the process should be optimized with initial mass ratio of physical mixture of lipid and drug. The best example for this system is stearic acid and ibuprofen.
- If solubility of lipid is more than drug, during co-precipitation process, drug with less solubility forms nuclei and lipid precipitate on drug nucleus and form shell. Also, feed composition of RESS process will be key parameter which has to be optimized for producing drug loaded SLN with suitable drug loading capacity. The best example for this system is stearic acid and carbamazepine.
- Temperatures of extraction unit and pre-expansion unit have to be less than melting temperature of lipids.
- In extraction unit during the solubilization step, lipid matrix may behave as a co-solvent.

- During co-precipitation step in expansion unit, nucleation and growth kinetics of nuclei are important phenomena which have to be considered.
- In RESS process, co-precipitation is extremely fast and it is very difficult to control the morphology and loading of the composites. To troubleshoot this problem, it is better to precipitate lipid over previously formed microparticles of drug.
- High shear mixer in the pre-expansion unit improves the control and homogeneity of the process.

3.2. Supercritical Antisolvent Solution (SAS)

Supercritical anti-solvent (SAS) process has been developed in order to micronize pharmaceutical compounds that have poor solubility in SCFs and cannot be processed by RESS technique [69]. In this process, liquid solution contains the solute to be micronized and the solute should be insoluble in the SCF. In addition, the liquid solvent should be completely miscible with SCF. The liquid solution in contact with the supercritical fluid induces super-saturation and precipitation of the solute (Fig.**2**). Due to the high solubility of organic solvents in SC-CO_2, solvent-free products are produced. The mixing between the supercritical anti-solvent and the liquid is faster than in conventional liquid anti-solvent processes, thus it leads to higher supersaturations and smaller particle size [70]. Several SAS operating parameters can influence particle size and particle size distribution. These parameters are: pressure, temperature, concentration in the liquid solution, carbon dioxide molar fraction and liquid jet characteristics [71]. Morphologies frequently observed in SAS processing include crystals with various habits and sizes, long rods, butterfly-like particles, snowballs, and starbursts. The type of morphology depends on the nucleation and growth kinetics of the material and on the degree of supersaturation of the liquid-rich phase [72]. The most frequently morphology for pharmaceutical applications is spherical nanoparticles with mean diameter between 30-300 nm range and microparticles in the 0.2-10 μm [71].

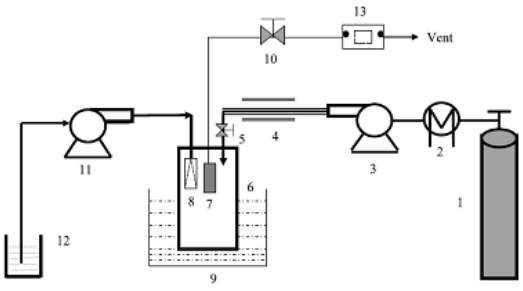

Figure 2. *SAS process(1) CO2 Cylinder; (2) refrigerator; (3) metering pump; (4) heating tape; (5) on-off valve; (6) precipitation chamber; (7) filter; (8) nozzle; (9) water bath; (10) metering valve; (11) HPLC pump; (12) suspension; (13) pressure gauge; (14) mass flow meter; (15) wet gas meter. [73]*

For solid lipid nanoparticles production, at first drug and lipid have to be dissolve in organic solvent like dichloromethane, dimethyl sulfoxide, acetone, methanol, ethanol and etc. Fortunately SAS process in terms of solvent choice is completely flexible [73]. Then, solution of the solutes of interest are bringing into contact with a supercritical fluid. The supercritical fluid saturates the liquid solvent which contains drug and lipid. The saturation of the liquid solvent by the supercritical fluid causes the co-precipitation of the solutes by an antisolvent effect.

The lipid material and drug must be soluble in a suitable organic solvent that is miscible with the supercritical fluid. Thus, there is a limitation in the choice of compounds and solvents, which usually causes failure in the SAS process [74]. If the drug and the lipid material are not soluble in the same solvent, it is possible to prepare two different solutions with each of the substances, and then subject

simultaneously to SAS precipitation [30]. Following this session, examples of co-precipitation by SAS process are described.

Wenfeng and co-workers have studied on co-precipitation of paclitaxel (PTX) and poly (L-lactic acid)(PLLA) by SAS process using different solvents. These are: dichloromethane (DCM), the mixtures of DCM/ethanol (EtOH) and DCM/dimethyl sulfoxide (DMSO). Fine particles with a narrow particle size distribution were obtained with different solvents. XRD results showed that the micronized PTX was dispersed into the PLLA polymer matrix in an amorphous form. Also, they have investigated the effects of process parameters on co-precipitation of PTX and PLLA by SAS process. The results indicated that particle morphology, particle size and PTX loading can be manipulated by adjusting these parameters [75].

Majerik and co-workers have aimed to improve the bioavailability of poor water soluble drug using SAS process. Oxeglitazar as drug was co-precipitated with various solubilizing excipients: polyoxyethylene–polyoxypropylene block copolymers (Poloxamer 188 and 407), polyethylene glycol (PEG 8000) and polyvinilpyrrolidone (PVP K17) with different solvents (ethanol, tetrahydro-furan, dichloromethane, chloroform, *N*-methyl-2-pyrrolidone, dimethylsulfoxide). Precipitated particles with different formulations were compared in terms of particle morphology, crystallinity, poly-morphic purity, residual solvent content, precipitation yield and dissolution kinetics. Results showed that SAS formulations of oxeglitazar–PEG 8000, Poloxamer 188 and 407 contained needle-shape drug crystals that were partly incorporated in polymeric spheres while experiments with PVP K17 resulted in semi- amorphous solid dispersions with high density and good flowability. In spite of the greater particle size, SAS formulations exhibited significantly greater dissolution rate compared to raw drug and physical mixtures [76].

Montes and co-workers have studied on co-precipitation of ethyl cellulose (EC) and amoxicillin (AMC) by SAS process using a mixture of DCM and DMSO as solvents. Sizes of composite particles were significantly smaller than those of the corresponding raw materials. Also results showed that an increase in the operating temperature led to a slight increase in the particle size of the precipitated particles but an agglomerate of particles occurred at higher temperatures. An increase in pressure led to a smaller particle size. X-ray photoelectron spectroscopy (XPS) analyses confirmed the presence of amoxicillin on the surface in some of the experiments. In all cases, the release of amoxicillin from the precipitates was slower than that from a solution of the pure drug [77].

Uzun and co-workers have used batch SAS process to co-precipitate cefuroxime axetil amorphous (CFA, antibiotic) and polyvinylpyrrolidone (PVP-K30) for preparing drug – polymer composite particles. Spherical particles having mean diameters of 1.88–3.97 μm were produced. Mean particle size of precipitated particles was not affected significantly with the change of process parameters. It was only affected by pressure change. It was observed that temperature and polymer /drug ratio affected the particle morphology. The drug release rate of co-precipitated CFA–PVP (1/1) particles was almost 10 times slower than the drug alone [78].

Montes and co-workers have studied on ampicillin (AMP) and ethyl cellulose (EC) composites production by SAS process. Successful precipitation of AMP and EC were carried out by this process and the particles precipitated were significantly smaller than the unprocessed compounds. Also, the particle morphologies of obtained products were quasi-spherical. As the temperature of the experiments was increased, the particle size of the

composites increased and increase in pressure did not have an appreciable effect on the particle size of composites. Also results showed that higher temperatures and pressures led to higher AMP loading percentages. XPS spectra show that the AMP is present not only in the core of the composites but also on the surface. The drug release profiles from precipitates in which ampicillin was present on the surface is faster than in cases where it was not present [79].

Durate and co-workers have studied on the preparation of acetazolamide composite microparticles by SAS process. Preparing composite via the SAS performed as a semi-continuous or a batch operation process. Eudragit (an anionic polymer based on methacrylic acid esters) were used as drug carrier. Results showed that particles produced by the semi-continuous mode were recovered as a powder made of spherical and elongated structures and in the batch mode, the precipitated composite powder showed large agglomerates, made of rods incorporated within a continuous film. Compared to the semi-continuous mode, the batch operation produced particles of larger mean diameter size and lower yield. Also, composites produced by semi-continuous technique have a drug release rate controlled by a diffusion mechanism, whereas for composites produced by the batch operation, the polymer swelling also contributes to the overall transport mechanism [80].

Based on results which are shortly described in this section, there are important points which have to be studied for drug loaded SLNs preparation with supercritical antisolvent process. These include:

- Select proper solvent which can solve lipid and drug simultaneously
- Solvent have to be miscible with the supercritical fluid
- High efficiency of loading is one of the key parameters in this process. It depends on a number of parameters, particularly on the initial concentrations of core and coating materials, and the affinity between these two materials (lipid and drug). If this affinity does not exist, segregated precipitation may occur. Surfactants can modify this affinity.
- If active substance is successfully embedded in an amorphous state into lipid matrix, the particle size of composite can be smaller compared to micronized pure active substance under the same processing conditions.
- Temperature of process has to be less than melting temperature of lipid.

3.3. Particle from Gas Saturated Solution/Suspension Method (PGSS)

The production of microparticles of materials of relatively low melting temperatures, such as polymers and lipids can be achieved by PGSS process. Also, this process can be used to entrap of active ingredients in polymer or lipid matrices to produce composite products. PGSS process takes advantages of the fact that the solubilities of compressed gases in liquids and solids like polymers are usually high [18]. As polymers are saturated with carbon dioxide, their melting temperatures decrease. The schematic illustration of the PGSS techniques is shown in Fig. **3**. In PGSS technique, SC-CO2 dissolves in a melted lipid or plasticized polymer at high pressure and gas solution saturates. The rapid expansion of solution through a nozzle results in precipitated particles. Strong cooling due to the Joule Thomson effect during expansion is the driving force for particle formation.

As melting temperature of lipids are low (Table **6**), therefore this process is a good candidate for SLN production. The PGSS technique requires neither drug particles nor lipid to dissolve in SCO2. No organic solvent is used in PGSS process in contrast to anti-solvent techniques. Also, this

method can be applied for a mixture of drug and lipid to produce drug loaded solid lipid nanoparticles.

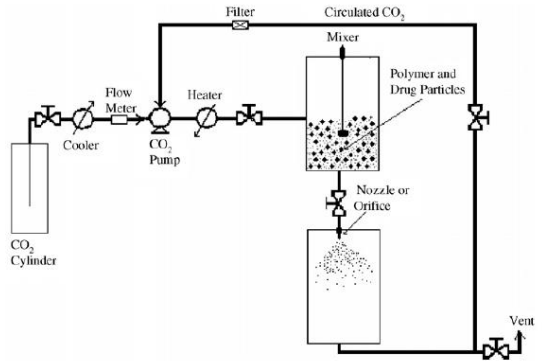

Figure 3. *PGSS equipment concept [24]*

One of the main advantageous of PGSS technique is the simplicity of this process, leading to low processing cost and wide range of products [69]. This process carried out in lower pressure and lower consumption of gas than RESS process. The crystallinity, particles size and particle size distribution can be influenced by the process parameters such as nozzle diameter, pre-expansion pressure and temperature, and flow rate of carbon dioxide. High saturation pressure induces high content of CO_2. It makes the nucleation process occur faster and leads to the formation of small particles. Also, the disruption of the lipid matrix with potential formation of shapeless particles may occur. This desirable effect may induce a burst release of the active compound [81]. Following this session, examples of co-precipitation by PGSS process are described.

Vezzu and co-workers have studied on production of lipid microparticles containing ribonuclease A (as proteins) functionalized with polyethylene glycol (PEG) by PGSS technique. Tristearin (TS), phosphatidylcholine (PC) and PEG were used as excipient to produce the lipid matrix. The effects of the operative variables (mixing and nozzle temperature, process pressure and organic solvent) on the microparticles size and yield were evaluated. Results showed that the temperature of the PGSS mixing chamber affected both the product yield and the particle size. As the temperature of the mixing chamber increased, the average particle size increased too. The pressure led to a reduction in particle size in range 130–140 bar. A reduction of the particle size was also achieved using DMSO as organic solvent. The ribonuclease A and PEGylated ribonuclease A lipid microparticles produced had an average size of about 5μm with a polydispersivity index of 1.7[82].

González and co-workers have studied on encapsulation efficiency of solid lipid hybrid particles prepared using the PGSS technique. The manufacture of particulate hybrid carriers containing a glyceryl monostearate, a hydrogenated castor oil and active pharmaceutical compound, ketoprofen, was investigated with the aim of producing controlled drug delivery systems based on solid lipid particles. Ketoprofen was partially solubilized in the supercritical CO_2 during lipid melting and mixing step. During expansion, the quick and sudden depressurization taking place through the nozzle and it led to ketoprofen supersaturation and crystals precipitation on the surface of the formed lipid particles. A lipidic matrix of GMS and HCO (50 wt%) was used to form composite powders loaded with active ingredients. Experiments were performed at 13MPa and 345K. Solid lipid particles were loaded with ketoprofen in percentages of 16.1wt%. Results showed that hydrophobic drugs, such as ketoprofen, were more efficiently encapsulated in the lipophilic lipidic matrix as a molecular dispersion [83].

Elvassore and co-workers have studied on lipid system micronization for pharmaceutical applications by PGSS technique. A thermodynamic study of gas solubility and melting temperature depression was carried out by a perturbed-hard-sphere-chain equation of state. Depending on the initial pressure and temperature, the expansion of the system

can lead to either solid or liquid or solid-liquid lipid particles. The pressure-temperature charts showed three regions above the P-T solid-liquid-fluid coexistence curve, from which solid, solid-liquid or liquid products can be obtained. Micro-particles of lipids and lipid mixtures were successfully produced by PGSS. The effect of operative temperature, pressure and type of nozzle on the resulting product was investigated. The particle size was reduced by increasing pressure and decreasing temperature. The mean dimension of particles was about 2 mm. It was observed that capsule-like precipitated lipid microparticles produced and it presented an internal cavity, high porosity on the internal shell and compact and homogeneous external surface [84].

Sousa and co-workers have studied on feasibility of using solid lipid particles as carrier for active substancesby PGSS technique. Glyceryl monostearate was applied as lipid and caffeine as active substance. Lipid loaded caffeine successfully prepared using the PGSS technique at 13MPa and 335K and exhibited a mean particle size of 5.49 µm. SEM image of composite particles showed extremely porous, but more similar to needle aggregates [85].

Based on results of researches which are shortly described in this section, there are important points which have to be studied for drug loaded SLNs preparation with PGSS process. These include:

- Select lipid with low melting temperature
- Select proper drug with high solubility in melted lipid
- Optimize process conditions (temperature, pressure, nozzle dimensions, …)

3.4. Gas Anti Solvent Method (GAS)

Solid compounds which are not soluble in supercritical fluids can be recrystallized by gas antisolvent process. In this process, dense gases are used as the antisolvent. Reduced temperature and pressure of dense gas should be between 0.9 and 1.2 [86]. GAS process is based on the supersaturation of a liquid solution by dissolution of supercritical fluid. In GAS process, at first, the solute is initially solubilized in an organic solvent. Solvent should be completely miscible with the supercritical fluid. A volumetric expansion of the liquid solution occurs upon gradual addition of supercritical fluid to the solution. It causes a reduction of the solvent power and thus dissolved solute of interest precipitate (Fig. **4**). The rate of supersaturation buildup in the solution can be controlled by the volumetric liquid expansion profile as a function of the process time, thus influencing the nucleation and growth rates. Different parameters such as operating temperature, the pressure profile, the type of solvent and antisolvent, and the stirring power input, influence on volumetric expansion profile [87]. Two different mass transport mechanisms govern particle precipitation. First, high solubility of pressurized CO_2 in liquid organic solvents leads to CO_2 penetration into the injected solution. Thus, the volume of the liquid solution increases. Second, due to the low solubility of organic solvents in pressurized CO_2 and due to a lower density of the bulk CO_2 compared with the liquid solution, the organic solvent evaporates slowly into the bulk CO_2, relative to the absorption rate [88]. This process performs at moderate pressure (5-8 MPa) and obtained ultrafine particles are normally in the range 1-10µm [20].

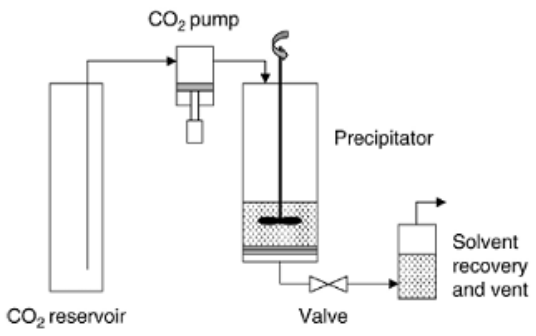

Figure 4. *GAS equipment concept [20]*

Elvassore and co-workers have studied on production of insulin-loaded poly (ethylene glycol)/ poly(l-lactide) (PEG/PLA) nanoparticles by gas antisolvent techniques. Spherical polymeric nanoparticles with a smooth surface were produced. Particles had low mean particle diameters and narrow distribution profiles (400-600 nm). Also, produced particles possessed plastic character and compact internal structure. Also, results indicated that the GAS process could be successfully used for the entrapment of therapeutic agents, such as proteins and peptides, with limited structural denaturation. The process led to produce high product yield, extensive organic solvent removal, and maintenance of >80% of the insulin hypoglycemic activity. The PEG played a key role in the simultaneous solute precipitation and influenced on the release behavior and the chemical, physical properties of the precipitated particles [89].

Sala and co-workers have studied on the polymorphic nature of microcrystalline solids of stearic acid prepared by gas antisolvent crystallization techniques. FT-IR spectra and PXRD patterns of the crystalline solids obtained by GAS process indicated that the resulting crystalline solid was composed of the C form with a small amount of the E form. The different polymorphic nature obtained in the GAS experiment is a consequence of the weak antisolvent character of CO2 over the system “stearic acid/ethyl acetate/CO2” [90].

Based on results of researches which are shortly described in this section, there are important points which have to be studied for drug loaded SLNs preparation with GAS process. These include:

- Select proper solvent which can solve lipid and drug simultaneously
- Solvent have to be miscible with the supercritical fluid
- Temperature of process has to be less than melting temperature of lipid
- To obtain high drug loading capacity, optimize the concentration of drug and lipid in solvent
- Optimize process conditions (temperature, pressure, nozzle dimensions, …) to obtain high volume expansion

4. CHARACTERIZATION OF LIPID-DRUG COMPOSITE

Particle size and particle size distribution, drug loading capacity, morphology of particles, crystallinity, chemical structure and melting behavior are important parameters which have to be evaluated for produced drug loaded SLN. Different techniques are used to characterize lipid - drug composites. Main include: high performance liquid chromatography (HPLC), microscopy, X-ray diffraction, differential scanning calorimetry and infrared spectroscopy.

Drug loading capacity is one of the most important parameters in SLN characterization. Type of lipid and method of SLN preparation strongly influence on drug loading capacity. For the determination of the drug loading, a known amount of drug loaded SLN is dissolved in organic solvent. Then the drug is analyzed by high performance liquid chromate-graphy (HPLC) or spectrophotometer [91].

Two different kinds of measurement are widely used for particle size and particle size distribution. These are: microscopic image analysis and light scattering techniques [31]. Microscopy such as scanning electron microscopy and atomic force microscopyare employed to examine the surface morphology and microstructure of the lipid materials and drugs while light scattering is used to measure the hydrodynamic diameter of a suspension of SLNs [92-93].

X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material. The presence of a new polymorph is deduced from additional

shoulders or shifts in the peak position in the X-ray powder patterns [94]. Lipid and drug may exhibit polymorphism, which is defined as the ability of a substance to exist in two or more crystalline phases [95-96].

DSC gives an insight into the melting and recrystallisation behavior of crystalline material like lipid nanoparticles. The break down of the crystal lattice by heating the sample yields inside information on, e.g. polymorphism, crystal ordering or glass transition processes [97]. If DSC curve of drug loaded SLN showed two endothermic peaks (lipid and drug), therefore both of them have already crystal structure but if DSC curve shows endothermic peak of lipid, drug would be in amorphous state.

The lipid crystalline structure was a key factor to determine whether a drug would be expelled or firmly incorporated into the lipid system. The lipid-forming highly crystalline state would lead to drug expulsion, but the lattice defects of the lipid structure could offer spaces to accommodate the drugs [29]. As the CI is significantly decreased, disorder in lipid crystal structure allows the creation of voids that accommodated larger amounts of bioactive molecules, minimized their expulsion, and could also modulate their release. The CI (crystallinity index) was calculated from the melting enthalpies using following equation [98, 99]:

$$CI(\%) = \frac{\Delta HLM}{\Delta HPL} * fm * 100 \qquad (1)$$

where enthalpy LM is the melting enthalpy of the lipid mixture, enthalpy PL is the melting enthalpy of the pure lipid and *fm* is a factor that consider the concentration of solid lipid in the matrix (0.90 for the mixtures containing 90 of solid lipid, respectively). The broadening of the heating peak and decline in the melting point and melting enthalpy in the thermogram of SLNs compared with pure components can be attributed to the colloidal dimension of the composite particles. The decrease of the heat of fusion was used to estimate the amount of drug in the drug loaded lipid nanoparticles. Therefore we assumed ideal mixture behavior and the following relationship between the heat of fusion of the pure substances h_i and the mixture h_M was used by using equation 2 [54]:

$$H_M = x_1 h_1 + (1 - x_1) h_2. \qquad (2)$$

XRD and DSC analysis can confirm if in a co-precipitation process, drug as active substance has been efficiently incorporated in carrier matrix (lipid) or not [31].

Fourier transform infrared (FTIR) spectroscopy is a label-free, non-destructive analytical technique that can be used extensively to study conformational information about the drug loaded solid lipid nanoparticles. FTIR analysis can confirm if in a co-precipitation process, drug and lipid exist in SLNs [100].

5. CONCLUSION

This paper provides an overview of production of drug loaded solid lipid nanoparticles as drug delivery system with various techniques of preparation. Supercritical fluid technology is a relatively novel technique for micronization of drugs. Different supercritical fluid processes are being developed to design particles with several purposes in drug delivery system. These include the rapid expansion of supercritical solutions (RESS), the gas antisolvent process (GAS), supercritical antisolvent process (SAS) and its various modifications, and the particles from gas-saturated solution (PGSS) processes. Supercritical fluid technology has been advised as an applicable approach for drug loaded SLN preparation in the literatures but few papers have been published in this area. This review tries to find main points to investigate the feasibility of applying supercritical fluid processes in drug loaded solid lipid nanoparticles preparation and also can fill the gap between them.

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