Powdered Lipid Nano and Microparticles: Production and Applications

Amandine Berton, Géraldine Piel and Brigitte Evrard*

Laboratory of Pharmaceutical Technology, University of Liège, Belgium

Received: July 8, 2011; Accepted: August 2, 2011; Revised: July 27, 2011

Abstract: This review details articles and recent patents in an emerging topic called powdered form of nano- and microparticles. Solid lipid particles were developed in the early 1990s and since, they have been considered as promising drug delivery systems, especially in providing a sustained release profile of the encapsulated drug. This kind of drug delivery system has several advantages, due to its physiological composition. It is generally well tolerated by the human body and are relatively stable during storage in comparison with other carriers like liposomes. The description of these powdered lipid particles, their different production processes and their applications are the focus of the article.

Keywords: Lipidic excipients, manufacturing process, microparticles, nanoparticles, pharmaceutical technology, supercritical fluids.

INTRODUCTION

It has become more and more evident that, in order to control, localize or improve drug delivery, the development of suitable delivery systems is necessary. Several drug carrier systems can be used, depending on the administration route, the drug properties and the intended drug release profile. The most often studied carriers in the controlled release of active pharmaceutical ingredients are:

- liposomes
- polymeric nano and micro particles
- cyclodextrines
- solid lipid particles

Solid lipid particles (SLP) were developed in the early 1990s and since, they have been considered as promising drug delivery systems, especially in providing a sustained release profile to the incorporated active substance. Solid lipid nanoparticles (SLN) are in the submicron size range (50 to 1000nm) while solid lipid microparticles (SLM) have a size above 1 μm. They are both composed of lipidic excipients that are in solid state at room temperature. Commonly used lipidic excipients for SLP manufacturing are fatty alcohols, fatty acids, fatty acid esters of glycerol, waxes, cholesterol.

SLP seem to provide an alternative drug carrier system to liposomes and polymeric nanoparticles. SLP combine several of those systems advantages while avoiding some of their disadvantages.

Due to their physiological composition they are well tolerated by the human body. SLP are physicochemically stable and can be produced relatively easily on a large industrial scale. In addition, raw materials and production costs are rather low. Their most important limitation is due to the fact that the active compound, which is incorporated into SLP, must be lipophilic enough to ensure high entrapment efficiency (EE). Moreover, these particles permit drug targeting and have the possibility to control the release of the drug. Nano and microparticles have been studied for several applications. The peroroal route is the most often cited. It includes SLM dispersion, tablets or capsules. They could also be parenterally administred apart from the intravenous route, owing to particle size of microparticles. They are potentially useful as a depot formulation when prolonged action is required. SLM can also be considered as a promising drug carrier system for pulmonary administration [1-7].

The most usual techniques for the formulation are : the solvent evaporation method, the hot melt dispersion technique, the spray drying and the spray congealing [4, 8-16]. These methods are multi step processes, or use organic solvent and work at high temperature. To avoid these drawbacks, a novel supercritical fluids technology called PGSS® (Particles from Gas Saturated Solutions) is being increasingly studied [17].

The objectives of this review are the description of solid lipid particles, production methods and their recent applications, preferably in dry powder form.

SOLID LIPID PARTICLE PREPARATION TECHNIQUES

Materials

A summary of frequent used lipidic materials in SLP formulation is presented in Table **1**. Following the preparation techniques, surfactants may also be incorporated in the formulation (e.g. Polysorbates, Poloxamer, phosphatidylcholine)

Patents Review

Table **2** presents some of the most relevant patents related to SLP published the last five years.

*Address correspondence to this author at the Laboratory of Pharmaceutical Technology, University of Liège, Tour 4, étage 2, Avenue de l'hôpital 1, 4000 Liège, Belgium; Tel: +32 4 3664306; Fax: +32 4 3664302; E-mail: B.Evrard@ulg.ac.be

1872-2113/11 $100.00+.00

Table 1. Summary of Frequent used Materials use in SLP Production

Category	Literature
Fatty alcohol	
Cetyl alcohol	[33]
Stearyl alcohol	[40, 43, 114]
Fatty acid	
Stearic acid (C18 fatty acid)	[33, 43, 115]
Fatty acid esters of polyglycerol	
Tetraglycerol pentastearate (TGPS)	[43]
Tetraglycerol monostearate (TGMS)	[43]
Fatty acid esters of glycerol	
Glyceryl monostearate	[10, 31, 34, 43, 116-118]
Glyceryl monobehenate	[31]
Glyceryl behenate	[21, 34, 75, 102, 119-122]
Glyceryl palmitostearate	[21, 123-126]
Glyceryl ditristearate	[21, 33]
Glyceryl tripalmitate	[14, 21, 81, 82, 127]
Glyceryl tristearate	[21, 128]
Hydrogenated fatty acid ester	
Hydrogenated hardened castor oil	[129]
Polar wax	
Complex mixture containing, e.g. esters of acids and hydroxyacids	[33, 41, 43, 123, 124, 130]
Others	
Saturated polyglycolized glycerides	[42, 123, 124, 131]
Beeswax	[76, 123, 124, 132]
Paraffin wax	[31]
Cholesterol	[22, 31]
Phospholipids	[22, 133, 134]
Microcrystalline wax	[40]

Preparation Techniques

Several techniques can be used to produce solid lipid nano - or microparticles: spray-drying, spray congealing, cryogenic micronization, electrospray and supercritical fluid process. For instance, Rodriguez patented an apparatus and a method for preparing lipidic solid forms with controlled release of the active ingredient [18]. The solid forms obtained can be used in the pharmaceutical field for oral administration in the form of powders with controlled release, or as intermediates for obtaining further forms such as capsules, tablets, suspensions and the like, or they can be used in cosmetics, fragrances, preservants and for alimentary purposes as well as in the veterinary field or, when releasing vegetal hormones, pesticides, or fertilizers, in the agroindustrial field. This chapter is dedicated to the description of production techniques of Solid lipid particles.

Spray Drying Method

Spray drying is a one-step process which converts a liquid feed to a dried particulate form [7, 19-25]. The feed can be a solution, a coarse or fine suspension, or a colloidal dispersion (e.g. emulsions, liposomes, etc.), which is first atomized to a spray form that is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the solvent to form dried solid particles. The dried particles are then separated from the gas by means of a cyclone, an electrostatic precipitator or a bag filter Fig. (**1**).

The three important steps of the spray drying process are atomization, drying and separation. The design may be "open cycle", where the drying gas (usually air) is not recirculated and is vented into the atmosphere. When spray drying is made from an organic solvent, a "closed cycle" layout is more suitable than an open one, since the risk of inflammability and explosion is higher in the latter when organic feeds are heated in the presence of oxygen [26].

Atomization is the process in which a liquid is sprayed into as a collection of droplets. The usual methods are centrifugal, pneumatic, ultrasonic and electrostatic atomization. Atomization method is particularly usefull for thermolabile materials and compounds with a low melting point or glass transition temperature. Indeed, the drying time varies from 100 milliseconds to seconds [27]. Once the liquid is atomized, the droplets are dried in solid particles by intimate contact with the heated gas in the drying chamber.

The important drying parameters are:

- Inlet and outlet temperatures,
- Feeding rate,
- Drying gas medium,
- Gas humidity,
- Gas flow rate
- Residence time.

These parameters affect the final size, shape, density, crystallinity and residual solvent content of the particles. The humidity level of the gas medium must be relatively low to avoid the formation of agglomerates in powders, especially for hygroscopic materials [26].

Compared to other SLP production methods, spray drying produces particles which are characterized by smaller and more homogeneous particle-size distribution. In the case of pulmonary administration, these particles result in a higher respirable fraction than mechanically micronized drugs [28]. However, the particles produced are not always spherical and may have convoluted surfaces, asperities and cavities. The shape is influenced by the drying rate, the surface tension and the viscosity of the liquid [27].

The main advantages of the spray drying technique are the ability to manipulate and control a variety of parameters such as solvent composition, solute concentration, solution and gas feed rate, temperature and relative humidity, droplet size, etc. Optimization of particle characteristics can be performed in terms of size, size distribution, shape, morphology and density, in addition to macroscopic powder properties like bulk density, flowability and dispersibility [26].

Table 2. Patents Reviewing.

Particles Type	Publication Year	Short descriptions	Ref.
SLP	2006	Amighi patented new compositions of (active) solid lipidic particles (SLP), e.g. for inhalation, and their use as carriers or as fillers in pharmaceutical compositions	[135]
SLP	2006	Marchaud has developed an anhydrous particle consisting of a carrier on which a mixture consisting of at least one principle and one self-emulsionable lipid composition is applied. The said composition contains caprylocapric macrogolgyceride	[136]
SLN	2006	Lee claimed a lipid particle comprising an amphiphile coated complex of a hydrophobic bioactive agent and an inverted hexagonal phase-forming lipid	[137]
SLM	2007	Winter developed a method to produce a dry powder comprising solid particles of the hydrophilic excipient, which may be a water-soluble saccharide such as mannitol or trehalose. At least part of these particles individually comprise one or more incorporated colloidal particles, such as one or more liposomes. An active ingredient is incorporated within, or associated with, the colloidal particles. The size of the solid particles selected is usually substantially larger than that of the colloidal particles	[138]
SLN	2007	Charcosset used a new method using a membrane reactor, which allows the preparation of solid lipid particles, especially nanoparticles	[139]
SLN	2008	Shekunov has developed an apparatus and a method of producing solid composite lipid/drug nanoparticles for controlled drug delivery that offers several advantages over conventional processing techniques, including the consistent production of solid composite lipid/drug particles having an average diameter below 100 nm, high drug loading and low temperature processing	[140]
SLP	2008	Battaglia patented a method for the preparation of solid lipid micro and nanoparticles comprising the phase of mixing an acid solution with a micellar aqueous solution comprising at least one water-soluble fatty acid salt in the presence of a non- ionic and biocompatible polymeric stabilising agent. The micro and nanoparticles can furthermore incorporate an active ingredient, in particular, a thermosensitive active ingredient	[141]
SLP	2009	Vanderbist claimed powder compositions for delivery by dry powder inhalers, said powder compositions comprising coated particles of active material. In particular, it relates to powder compositions containing high amounts of active material while still showing enhanced flow properties. It further relates to a method and to a suspension for manufacturing said powder composition	[142]
SLP	2009	Eskandar has developed novel pharmaceutical compositions and dry powder formulations that contain one or more drug substances encapsulated within lipid vesicles. The present invention also concerns processes for the manufacture of such compositions and formulations and methods of treating diseases or disorders with such compositions and formulations	[143]
SLM	2010	The Richard patent relates to growth hormone (GH) formulations with sustained-release properties, in particular human growth hormones (hGH) and methods for their preparation. The growth hormone formulations can be manufactured without denaturing the protein and can conveniently be administrated to the person in need by using a conventional syringe via a needle with a small diameter. The first step in the manufacture of microcapsules is preferably performed by using a spray-drying technique and the second step is performed by using a spray-congealing technique	[45]
SLN	2010	Keck patented compositions containing a combination of solid lipid particles (nanoparticles and/or microparticles) and metallic particles, whose composition can optionally- additionally contain oil droplets	[89]
SLM	2010	Fatmi has developed methods for enhancing the release and/or absorption of active agents that are not very water-soluble as described herein. The method involves dissolving, melting, or suspending anactive agent that is not very water-soluble in one or more molten fatty acids, conjugated fatty acids, (semi-) solid surfactants of high HLB value, and/or hydrophilic polymers	[56]
SLM	2010	Vassal used a novel pharmaceutical technology form for administration of Temozolomide in the form of solid lipid particles, especially a new form suitable for pediatric use and pharmaceutical compositions comprising such lipid particles loaded with Temozolomide	[144]
SLN	2010	Bondi claimed nanoparticulate lipid vectors containing riluzole, and the preparation and characterisation thereof. The systems obtained have a different biodistribution from the free drug *in vivo*, and can be used to prepare pharmaceutical formulations	[145]

(Table 2) Contd....

Particles Type	Publication Year	Short Descriptions	Ref.
SLP	2010	Baker patented targeted lipid particles, targeted lipids and their use as delivery agents	[146]
SLP	2010	Galuska has developed the production of lipid particles that are at least substantially spherical. Such particles can be used, e.g. as a shortening component in food products	[147]
SLN	2011	Brito patented dry powder compositions of an RNA active in RNA Interference, and their uses for medicaments and for delivery as therapeutics for influenza. The dry powder compositions of this invention may be used for aerosolized delivery to the lungs	[148]

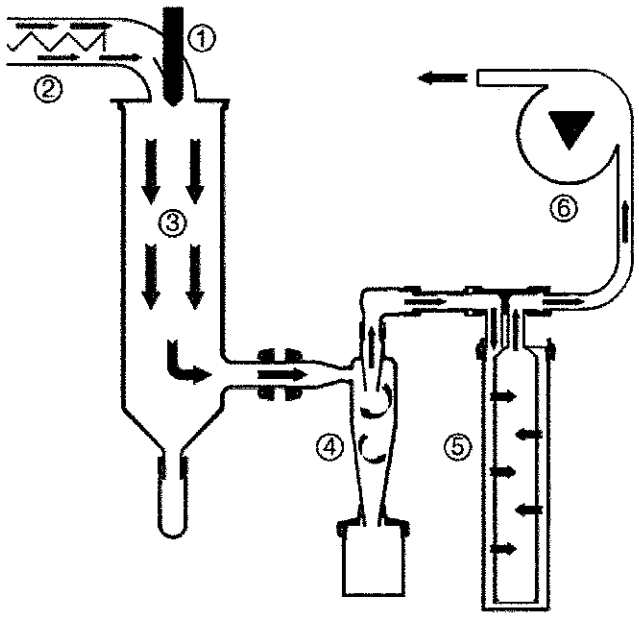

Fig. (1). Scheme of a Mini Spray Dryer [113]. 1. Two-fluid nozzle, operated by compressed air to disperse the solution into fine droplets; 2. Electric heating of the drying medium; 3. Spray cylinder for drying the droplets into solid particles; 4. Separation of the particles in the cyclone; 5. Outlet filter to remove fine particles; 6. Aspirator for generating the flow.

However, the spray drying process may induce degradation of some macromolecules as a result of a number of factors such as thermal stress during droplet drying, high shear stress in the nozzle and peptide/ protein adsorption at the greatly expanded liquid/air interface of the spray solution. Sebti *et al.* have developed a spray-drying technique to produce SLM. These SLM are composed of biocompatible phospholipids and cholesterol, and can be used as a carrier or filler to deliver drugs directly to the lungs via a dry powder inhaler [7].

Friesen patented a process which allows delivery of a spray solution comprising an active agent and a matrix material in an organic solvent to a spray-drying apparatus. The atomization of the solution into droplets within the spray-drying apparatus leads to the removal of at least one portion of the organic solvent from the droplets, forming and collecting several particles [25].

Poortinga developed a method for spray drying a high-viscosity fluid product using a spraying device. The method comprises the projection of the fluid product out of an outflow opening of the spraying device which leads to the development of droplets of the fluid product. The spraying device is designed to produce a pressure drop in the fluid product across the outflow opening, which is larger than 15 bars and, at least, partially dries the droplets in a drying medium, such as air, so that they become particles [24].

The Society *Anro spray solutions* patented a spray-drying device. The invention also relates to a system for spray-drying. A supply of the product to be spray-dried is connected via a line to an injection pipe to which a spray-drying device in line with the invention is connected. The spray-drying device and the injection pipe end are situated in a spray-drying tower [29].

Bot patented novel Spray-Dried Lipid Microparticles (SDLM) that are comprised of a lipid, a ligand and an agent [30]. The ligand is specific to a cell surface receptor, thereby enabling the targeting of the agent to cell bearing receptor specific to the targeting ligand.

Cryogenic Micronization

Lipid matrices, obtained either by melt dispersion (the drug is mixing in a molten lipid) or solvent stripping (the drug and lipid are dissolved into a solvent mixture under stirring, e.g. benzyl alcohol, ethanol [31]), are stored at -80°C and then micronised in a customised apparatus supplying liquid nitrogen during the process [32]. Finally, the obtained powders are sieved in an automatic sieving apparatus. This technique can be used for the production of particles of 5 to 5000μm in diameter according to the chosen sieves [31].

Del Curto patented lipid microparticles consisting of lipids and amphiphilic components, which allow to promote the incorporation of peptides and/or proteins,. They also describe a process to produce these microparticles. A cryogenic micronization manufacturing process for their preparation is also disclosed [32]. In the cryogenic micronization step, cooling is performed by insufflating liquid nitrogen before the drug loaded lipid matrix can be micronized.

Spray Congealing (also Called Spray Chilling)

Lipids are heated to a temperature above melting point. The drug is then dissolved into the melted lipid. The hot mixture is atomized through a pneumatic nozzle into a vessel which is stored in a carbon-dioxide ice bath. The particles obtained are then vacuum dried at room temperature for several hours [21, 23, 33-39].

In this technique, some variations can be performed. The melted mixture is atomized by ultrasound energy into small droplets that fall freely and solidify by cooling at room temperature [40-42].

Another variant of the spray congealing method is to use a rotating disc [43]. With this technique, the melted mixture is dropped onto a high-speed rotating disc. The rotation induces the molten mixture to spread between the disc periphery and the cooled surface from which microparticles are collected.

The technique can also be adapted depending on the type of particles to produce. Fine particle products with a mean particle size of 50-150 microns are often congealed in a co-current spray congealing chamber with high-speed rotary atomization. Particles with a mean particle size of 150-500 microns that melt and solidify slowly are congealed in a spray cooling chamber with a fountain nozzle. Finally, particles with a mean particle size of 500 to 2000 microns (also called prills) are congealed using a low-speed rotary atomizer with a specially designed prilling wheel.

Often additional cooling is needed to ease full congealing. Recent developments in spray cooling include the use of congealing chambers with integrated bag filters for congealing and separating the product in one unit. This design is very compact and thus space-saving [37].

Spray congealing was successfully employed for preparing SLM loaded with drugs such as clarithromycin, theophyllin, diclofenac, verapamil and indomethacin, but the preparation of protein lipid microparticles by spray congealing has been rarely reported [37].

Only a few spray congealing patents that produce solid lipid particles seem to have been published [44, 45]. Richard's patent relates to growth hormone (GH) formulations that have sustained-release properties, in particular human growth hormones (hGH) and methods for their preparation. The growth hormone formulations can be manufactured without denaturing the protein and can conveniently be administered to the person in need of it by using a conventional syringe via a needle with a small diameter. The first step in the manufacture of microcapsules is performed preferably by using a spray-drying technique and the second step is performed by using a spray-congealing technique [44, 45]. However, this process is generally patented for other kinds of particle [46]. Petersen patented a process and an apparatus for spray drying or spray cooling, particularly for spray drying liquids containing dissolved and/or suspended solid materials or for spray cooling congealable liquids [47].

Electrospray

The electrostatic atomizer comprises a nozzle connected to a high-voltage power supply and is supplied with a liquid to be atomized [16, 48, 49] Fig. (2). In a general process, a polymer solution is contained in a syringe, with a metal capillary connected to a high-voltage power supply as one electrode. A metal foil collector is placed opposite the capillary as a counter electrode. Depending on the properties

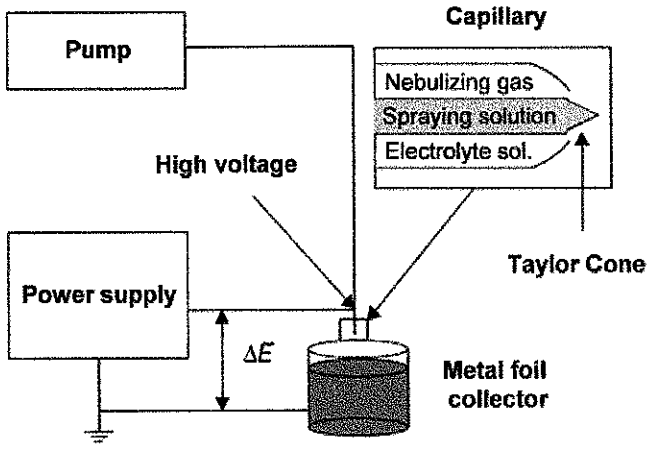

Fig. (2). Illustration of a system used for nanoparticle preparation by means of electrospray [48].

of the liquid, the flow rate and the voltage applied can be modulated. The cone-jet mode is the method used to prepare nanoparticles in which liquid emerging at the nozzle forms hemispherical drops because of the surface tension (electrospray). By increasing the electrical field, the hemispherical drops can be changed to a conical shape, which breaks up into highly charged droplets. By selecting suitable conditions, droplets can be produced with a close size distribution and nano or micrometer size range. Solid particles can be formed by evaporating solvent from the droplets produced traveling through the electrical field [16]. The electrospraying method might be employed to prepare monodisperse lipid-based nano- and microparticles in powder form in a single-step for drug delivery [16]. Bussano *et al.* have developed new insulin-loaded lipid microparticles using the electrospray procedure [50]. The authors showed that the electospray method did not affect the secondary structure of insulin.

Zhang patented a method for preparing lipid particles which produced discrete droplets of vesicle-forming lipids in a solvent, where the droplets have a diameter and a volume suitable for *in vivo* administration [51]. The lipid particles are administered preferably either parenterally or intratumorally. In one embodiment, the droplets are generated by a system selected from the group consisting of a nebulizer, an atomizer, a venturi mist generator, a focused acoustic ejector, and an electrospray device.

Supercritical Fluid Technology

Supercritical fluid (SCF) is obtained above the critical pressure and temperature. Above this fluid's critical point, the solubility of a substance can be modulated by a relatively small change in pressure. Rapid depressurization of the mixture creates a high degree of supersaturation. Due to its low critical point at 31°C and 74 bars, and its low cost and non-toxicity, carbon dioxide is the most widely-used solvent in many SCF processes [52-56]. Its critical temperature makes supercritical CO_2 suitable for processing heat labile solutes at conditions close to room temperature [57]. The three main uses of SCF processes are:

Rapid Expansion of Supercritical Solutions (RESS)

In the RESS, the supercritical fluid is saturated with the substrate(s). This solution is then depressurized through a heated nozzle into a low pressure chamber in order to cause an extremely rapid nucleation of the substrate(s) in the form of very small particles [55].

Fulton and Deverman has patented a new method of formulation. They use a process which consists of forming a supercritical fluid solution of at least one supercritical fluid solvent and at least one solute. A depressurization of the solution of supercritical fluid through a nozzle under conditions sufficient to form solid particles of the solute that are substantially free of the supercritical fluid solvent, and electrostatically depositing the solid solute particles onto the substrate. The solute particles may have a mean particle size of less than 1 micron [58].

Supercritical Anti-Solvent and Related Processes (GAS/SAS/ASES/SEDS)

In this process, the supercritical fluid is used as an anti-solvent that causes precipitation of the substrate(s) dissolved initially in an organic solvent. The solute is recrystallized from the solution in one of the three ways [55, 59].

This process has been called gas anti-solvent (GAS) or supercritical anti-solvent (SAS) recrystallization Fig. (**3**).

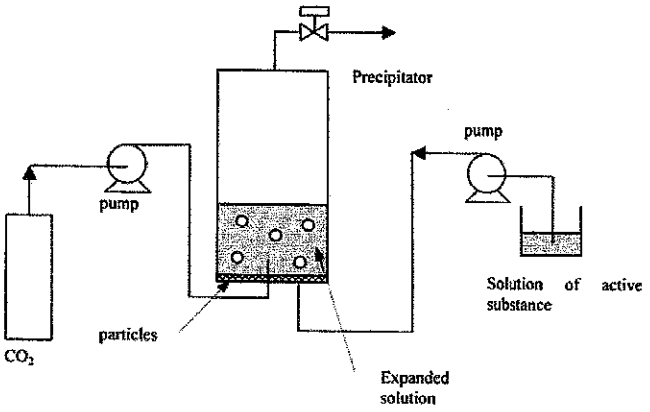

Fig. (3). GAS/SAS equipment concept [55].

The principle of this method is based on the ability of the organic solvent to dissolve a large amount of gas.. The gas diffuses into the organic solvent leading to the evaporation of this solvent into the gas phase. The volume expansion lead to a decrease of the density which is responsible of the lower solvent power and thus for the precipitation of the substrate. into microparticles. The second method is the aerosol solvent extraction system (ASES) process Fig. (**4**).

It involves spraying the solution through an atomization nozzle as fine droplets into compressed carbon dioxide. The dissolution of the supercritical fluid into the liquid droplets is followed by a large volume expansion and, consequently, a reduction in the liquid solvent power, causing a sharp rise in the supersaturation within the liquid mixture, and the consequent formation of small and uniform particles. The third method, solution enhanced dispersion by supercritical fluids

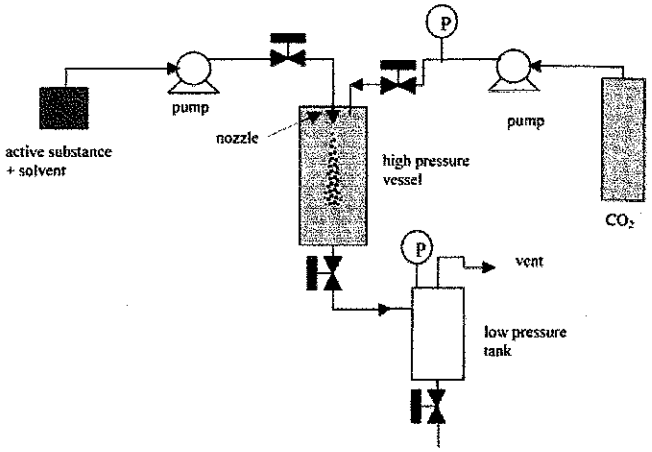

Fig. (4). ASES equipment concept [55].

(SEDS) was developed by Bradford University in order to achieve smaller droplet size and intense mixing of supercritical fluid and solution for increased transfer rates Fig. (**5**) [60].

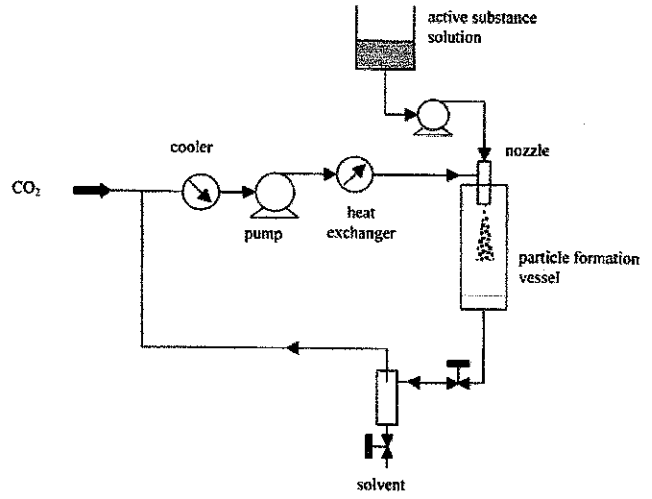

Fig. (5). SEDS equipment concept [55].

Actually, the supercritical fluid is used both for its chemical properties and as a 'spray enhancer' by mechanical effect: a nozzle with two coaxial passages allows the introduction of the supercritical fluid and a solution of active substance(s) into the particle formation vessel where pressure and temperature are controlled. The high velocity of the supercritical fluid allows the break up of the solution into very small droplets. Moreover, the conditions are set up so that the supercritical fluid can extract the solvent from the solution at the same time as it meets and disperses the solution.

These three processes, that can produce micro- and nanoparticles, are used for processing solids which are not able for dissolving into supercritical fluids, so RESS is impossible, or sensitive to high shear stress such as peptides or proteins. Moreover, addition of a carrier (often a polymer or a lipid) to the active solution can lead to the formation of active substance loaded micro- and nanospheres.

Bradford University has developed a method for formulating particles [60] which comprises the co-introduction of a supercritical fluid and a vehicle containing at least one substance in solution or suspension, into a particle formation vessel. The temperature and pressure are controlled, so that dispersion and extraction of the vehicle occur substantially, simultaneously with the action of the supercritical fluid. The invention also provides a particulate product of such a method; apparatus for use in carrying out the method; and a nozzle for use in the apparatus for co-introducing fluids into a particle formation vessel.

Ru patented a preparation method for the supercritical anti-solvent of water-soluble nano glycyrrhizic acid powder [61]. The formulation produced shows a smooth surface, a good water solubility, a high yield and no residual solvent. The cost of the production technology is low and the industrialization is easy.

Krukonis has developed a method for recrystallizing solid materials from systems comprised of a solute, which is the eventual material recrystallized, a liquid which is a suitable solvent for the solute, and a gaseous component which is soluble within the solvent and whose presence therein causes the solvent to approach or attain a supersaturated state, thereby precipitating (recrystallizing) the solute material [59].

Particles from Gas-Saturated Solutions/Suspensions (PGSS)

Actually, the solubility of compressed gases in liquids and solids (e.g. polymers) is usually high and usually higher than the solubility of such liquids and solids in the compressed gas phase. So, the process consists in solubilizing supercritical fluid in melted or liquid-suspended substance(s), leading to a so-called gas-saturated solution/ suspension that is further expanded through a nozzle with the formation of solid particles, or droplets Fig. (**6**). This process allows the production of particles from a variety of substances that do not need to be soluble in supercritical carbon dioxide, especially with some polymers that absorb a large concentration (10-40 wt.%) of CO_2 that either swells the polymer or melts it at a temperature much below (10-50°C) its melting/glass transition temperature. This process can also be used with suspensions of active substrate(s) in a polymer or another carrier substance leading to composite microspheres [17, 55, 62-72].

Weidner patented a process for preparing particles and/or powders [63]. A compressible fluid is introduced under pressure in the substance or substance mixture to dissolve it. The resultant solution is then expanded so that particles are formed during the expansion process.

Fine drug particles produced by a SCF process are less electrostatic than those produced by mechanical means. These particles show better flowability characteristics and a more dispersible following discharge from a dry powder inhaler. Moreover, SCF manufactures respirable drug particles that are intrinsically more uniform in terms of crystallinity, morphology, particle size distribution and shape than those produced via jet milling [73].

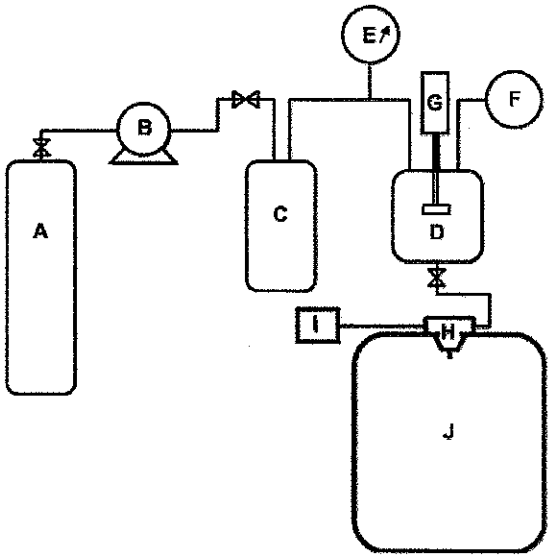

Fig. (6). PGSS equipment concept [17]. Scheme of PGSS™ equipment: A. CO2 storage tank, B. pump, C. heat exchanger, D. mixing vessel, E. manometer, F. back pressure regulator, G. rotative stirring agitator, H. nozzle, I. air supply and J. expansion chamber.

POWDERED SOLID LIPID PARTICLE ADMINISTRATION ROUTES

Solid lipid particles are considered to be a promising drug carriers system. However, SLP have been rather unexploited so far. To date, only a few complete studies on solid lipid nano- and microparticles have been published. Therefore, little data is currently available on SLP *in vivo* administration, drug release and biocompatibility.

Nevertheless, as assumed for SLN [74], it could be hypothesized that SLM are well tolerated by the human body because they are made of physiological compounds. On the other hand, the surfactant toxicity has to be considered, though not more than other drug carrier systems.

This chapter presents an overview of powdered solid lipid particles administration routes and main applications, and corresponding *in vivo* drug release and biocompatibility studies carried out so far.

The *in vivo* fate of the SLP will depend mainly on the following points:

A. Administration route

B. Interactions of the SLP with the biological surroundings including:

b1. Distribution processes (adsorption of biological material on the particle surface and desorption of SLP components into the biological surroundings)

b2. Enzymatic processes (e.g. lipid degradation by lipases and esterases).

SLP are composed of physiological or physiologically related lipids or waxes. Therefore, pathways for transportation and metabolism are present in the body which may contribute to a large extent to the *in vivo* fate of the carrier. Probably the most important enzymes of SLP degradation are lipases, which are present in various organs and tissues.

Peroral Administration

The peroral route is the most often cited for SLP administration [33, 34, 40, 43, 75-77]. It includes aqueous SLM dispersion, SLM tablets, pellets or capsules. However, data on *in vivo* drug release and biocompatibility studies is often missing. Demirel has perorally administered SLM suspensions to rabbits; such suspensions were composed of glyceryl behenate and caprylocaproyl macrogol-8-glycerides as a lipidic matrix, polysorbate 80 as a surfactant and an antiparkinsonian agent as the active substance. The bioavailibility of piribedil-SLM was found to be higher than pure piribedil [75].

Considering that SLP lipidic matrices are composed of lipidic excipients and that most surfactants have already been used perorally, the authors do not seem to have ever cast any doubt on SLP biocompatibility after oral administration.

The environment of the stomach favors particle aggregation due to the acidity and high ionic strength. It can be expected that food will have an important impact on SLP performance. However, no data has been published on this issue to our knowledge. Penkler has described an increasing bioavailability and prolonged plasma level after peroral administration of lipid nanodispersions containing cyclosporine to animals [78].

SLN can protect encapsulated peptides from degradation upon contact with gastrointestinal fluids. Zhang has studied and characterized lectin-modified solid lipid nanoparticles (SLN) containing insulin and evaluated the potential of the lectin-modified colloidal carriers for oral administration of peptide and protein drugs [79]. Their results on oral administration to rats demonstrated that SLN promoted the oral absorption of insulin.

Wang *et al.* have developed a new formulation in which Poly (lactic-co-glycolic acid) (PLGA) was used as a co-emulsifier in the preparation of insulin-loaded solid lipid nanoparticles with hydrogenated castor oil as lipid matrix and lecithin as surfactant by double-emulsion technique. They have demonstrated in an *in vivo* study that PLGA could enhance the entrapment of insulin in the solid lipid nanoparticles, and more importantly, prolong the time of hypoglycaemic activity of the insulin-loaded SLN [80].

Parenteral Administration

SLP are also parenterally administered apart from the intravenous route for microparticles, owing to particle micronic size. Some studies have been carried out on *in vivo* SLM drug release and SLM biocompatibility.

Reithmeier [81, 82] has studied the biocompatibility of SLM composed of a microcrystalline triglycerides as lipidic matrix and polyvinyl alcohol as a surfactant by implanting SLM subcutaneously in mice. Polymeric microparticles composed of poly(lactic-co-glycolic), a well-known approved polymer often used for parenteral applications, were also implanted and used as a reference. The study showed only a slight inflammation reaction in the implantation area, but that was the case for both SLM and polymeric microparticles. It has been concluded that studied SLM showed comparable biocompatibility to polymeric microparticles that have been approved for use in parenteral administration.

Del Curto [31] has produced solid lipid microparticles composed of glyceryl monobehenate and containing gonadotropin release hormone by co-melting process. After subcutaneous injection in rats, studied SLM proved to give the incorporated active substance a sustained release profile. Antide-Solid lipid microparticles are then potentially useful as a depot formulation when prolonged action is required.

Yang has studied the pharmacokinetic and body distribution of captothecin after i.v. administration in mice. SLN induced a higher AUC/dose and mean residence time especially in the brain, heart and reticuloendothelial cells containing organs other than drug solution [77].

Transdermal Administration

SLP can also be administrated by transdermal application [83-85]. SLN have been found to modulate drug release in the skin and to improve drug delivery to particular skin layers *in vitro* [86]. Liu has produced sodium diclofenac solid lipid nanoparticles by a modified emulsion/solvent evaporation method for transdermal delivery [87]. The *in vitro* release of SLN showed a two-step release pattern: one initial burst release followed by a second slow-release phase. In the *in vitro* cutaneous permeation studies, the value of flux obtained for the sodium diclofenac solution was higher than that of the SLN suspension. SLN had also been shown to improve the dermal localization of the active compound. El-Kamel has produced and characterized a formulation of testosterone (TS) solid lipid microparticles and it seems to be applied as a transdermal delivery system [88]. Keck has developed compositions which can be used as dermal formulations to help the skin restore to normal conditions in the case of e.g. irritated skin, or to support the medical therapy of skin with atopic dermatitis symptoms, atopic dermatitis, psoriasis or related diseases (e.g. accompanied by a distorted barrier function of the skin and a microbial load) [89].

Solid lipid nanoparticles have gained widespread interest in recent years as drug carriers for dermatological purposes, aiming for a better permeation or prolonged action on the skin or in specific skin layers [90-93]. Compared to liposomes, SLN show several advantages such as excellent tolerability, good physical stability, protection of labile drugs, a high drug payload, controlled drug release, low cost and ease of production [2, 86]. For cosmetic application, SLN exhibit an occlusive effect, which improves skin hydration and protection against sun exposure [94-99]. As these carriers could be applied to damaged skin, possible cytotoxicity of these preparations should be evaluated.

Pulmonary Administration

Powdered SLM are considered as a promising drug carrier system for pulmonary administration even if they have been rather unexploited so far [100, 101]. However, a preliminary *in vivo* tolerance study has been carried out on rats with SLM composed of glyceryl behenate as a lipidic matrix and poloxamer 188 (Lutrol® F68) as a surfactant. SLM dispersions in PBS were administered intratracheally. Results did not show significant differences between placebo groups

and SLM-treated rats. It has been concluded that the studied SLM seem to be well tolerated by the lower airways, but tolerance must still be assessed after repeated administration [102]. Jaspart has studied the use of SLM to obtain a sustained release profile to a model drug, salbutamol acetonide (SA). SA was synthesized from salbutamol in order to increase the lipophilicity of this molecule and thereby increase its incorporation efficiency into SLM. SA-loaded SLM were then produced by a hot emulsion technique followed by high-shear homogenization. ASA *in vitro* release study from SLM showed that the release rate increased with SA loading but remained in every case lower than the dissolution rate of pure SA [13]. Sanna has investigated the effects of processing conditions on the characteristics of solid lipid microparticles with a potential application as carriers for pulmonary administration [102]. The results suggested that a single intratracheal administration of the SLM does not induce a significant inflammatory airway response in rats and that the SLM might be a potential carrier for encapsulated drugs via the pulmonary route. Weers has developed a particulate composition suitable for drug delivery, preferably via inhalation. The particulate compositions of the present invention exhibit an increased gel-to-liquid crystal transition temperature resulting in improved dispersibility and storage stability [103]. Dellamary patented powdered pharmaceutical compositions for drug delivery that exhibit improved stability and dispersability over the shelf life of the composition. More particularly, this invention relates to a highly stable metal ion-lipid microparticle for drug delivery. The powdered formulations described in the Dellamary's invention can be applied to inhalation therapies, powders for reconstitution, dry powders and suspensions due to their unique powder stability [104]. Morton patented particles and methods to produce particles. In particular, the invention relates to methods of making composite active particles comprising a pharmaceutically active material for pulmonary inhalation, the method comprising a jet milling process [105].

GENE THERAPY

Gene therapy represents a new paradigm in the prevention and treatment of many diseases, including genetic disorders, such as cystic fibrosis, haemophilia and many somatic diseases, such as tumours, neurodegenerative diseases and viral infections, such as AIDS. Solid lipid nanoparticles have been increasingly studied as a potential anticancer drug delivery carrier as a result of their physical stability, protection of labile drugs from degradation, easy preparation, and low toxicity [2]. The use of biocompatible compounds for preparing SLN avoids the toxicity problems caused by polymeric nanoparticles [106-108]. SLN chemical composition can be modulated, that is, the particle surface charge, thus allowing oppositely charged molecules to bind by means of electrostatic interactions, is another important feature of these systems [109]. However, in most cases, although the surface charge can be easily increased, in the absence of endosomolytic agents such as chloroquine, gene transfer efficiency mediated by SLN-derived gene vectors (even when the lipid composition is optimised) remains lower than that observed with standard transfection agents [110].

Bondi` and co-workers evaluated the suitability of new cationic SLN based on glyceryl behenate as matrix lipid, dimethyldioctadecylammonium bromide (DDAB) as a charge carrier and Pluronic F68 as a surfactant, as non-viral transfection agents for gene delivery on the human hepatoma (HuH-6) cell line [111, 112]. Plasmid DNA encoding the b-galactosidase gene (plasmid pCMV-b-gal) under the control of the human cytomegalovirus (CMV) was used in this study as the reporter gene, and the expression gene coding for b-galactosidase was estimated by measuring the corresponding activated substrate. The authors showed that cationic SLN–DNA complexes could promote transfection of liver cancer cells. Cationic SLN were able to form stable complexes with DNA and to protect DNA against DNase I digestion. *In vitro* studies on human liver cancer cells demonstrated a very low degree of toxicity of both SLN and SLN–DNA complexes. Further, SLN-DNA complexes were able to promote transfection of liver cancer cells. These data suggest that the cationic SLN may be potentially useful for gene therapy.

CURRENT & FUTURE DEVELOPMENTS

Solid lipid nano - and microparticles can reasonably be considered as promising drug carrier systems even if up to now they have been studied little. Actually, SLP show several advantages, such as a physiological composition which limits the toxicity, the relative low cost prices of SLM raw materials and production processes and finally, the possibility of producing SLP on a large industrial scale. However, some drawbacks must also be evaluated. Actually, the drug to be incorporated into SLP must be lipophilic enough to obtain high entrapment efficiency. The entrapment efficiency is also influenced by several other parameters such as the preparation method, the physical state of drug and lipid excipient nature. Moreover, it is necessary to make a compromise in desired characteristics to optimize formulation parameters and production techniques.

The physicochemical characteristics of SLP are key factors for the selection of the product process. Spray drying and supercritical fluid methods offer more flexibility and the possibility of morphology control in addition to size control, but they may sometimes yield amorphous material or undesired polymorphs.

Most research articles present a complete characterization of SLP, including size and shape determination, solid state analysis and drug loading capacity studies although such characterization requires several analytical methods and is generally slow and difficult to process.

The *in vitro* drug release studies that have been carried out tend to prove the ability of SLP to administrate the incorporated substances. Therefore solid lipid nano- and microparticles can be considered as a promising drug carrier system, which can be used by different administration routes (i.e. peroral, parenteral, topical and pulmonary routes).

However most of the promising drug release results obtained by *in vitro* experimentation must be confirmed by *vivo* studies. In the same way, SLP biocompatibility aspects and *in vivo* fate also have to be evaluated even if the physiological used materials tend to suggest that SLP are well tolerated by the human body.

ACKNOWLEDGEMENTS

The authors would like to thank the Walloon Region (Belgium) for its financial support to this research work.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Mehnert W, Mader K. Solid lipid nanoparticles, Production, characterization and applications. Adv Drug Deliv Rev 2001; 47:165-96.

[2] Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of art. Eur J Pharmaceut Biopharmaceut 2000; 50: 161-177.

[3] Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery: II. Drug incorporation and physicochemical characterization. J Microencapsul 1999; 16(2): 205-13.

[4] Jaspart S, Piel G, Delattre L, Evrard B. Solid lipid microparticles: formulation, preparation, characterisation, drug release and applications. Expert Opin Drug Deliv 2005; 2 (1):75-87.

[5] Ravi KMNV. Nano and microparticles as controlled drug delivery devices. J Pharm Pharmaceut Sci 2000; 3(2): 234-58.

[6] Dalpiaz A, Cacciari B, Mezzena M, Strada M, Scalia S. Solid lipid microparticles for the stability enhancement of a dopamine prodrug. J Pharm Sci 2010; 99(11): 4730-7.

[7] Sebti T, Amighi K. Preparation and *in vitro* evaluation of lipidic carriers and fillers for inhalation. Eur J Pharm Biopharm 2006; 63(1): 51-8.

[8] Battaglia L, Gallarate M, Cavalli R, Trotta M. Solid lipid nanoparticles produced through a coacervation method. J Microencapsul 2010; 27(1): 78-85.

[9] Bondi ML, Craparo EF. Solid lipid nanoparticles for applications in gene therapy: A review of the state of the art. Expert Opin Drug Deliv 2010; 7(1): 7-18.

[10] Garcia-Gonzalez CA, Sampaio da Sousa AR, Argemi A, Lopez Periago A, Saurina J, Duarte CM, *et al.* Production of hybrid lipid-based particles loaded with inorganic nanoparticles and active compounds for prolonged topical release. Int J Pharm 2009; 382(1-2): 296-304.

[11] Hitzman CJ, Elmquist WF, Wiedmann TS. Development of a respirable, sustained release microcarrier for 5-fluorouracil II: *In vitro* and *in vivo* optimization of lipid coated nanoparticles. J Pharm Sci 2006; 95(5): 1127-43.

[12] Hitzman CJ, Elmquist WF, Wattenberg LW, Wiedmann TS. Development of a respirable, sustained release microcarrier for 5-fluorouracil I: *In vitro* assessment of liposomes, microspheres, and lipid coated nanoparticles. J Pharm Sci 2006; 95(5): 1114-26.

[13] Jaspart S, Bertholet P, Piel G, Dogne JM, Delattre L, Evrard B. Solid lipid microparticles as a sustained release system for pulmonary drug delivery. Eur J Pharm Biopharm 2007; 65: 47-56.

[14] Mehnert W, Mader K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001; 47(2-3): 165-96.

[15] Passerini N, Qi S, Albertini B, Grassi M, Rodriguez L, Craig DQ. Solid lipid microparticles produced by spray congealing: Influence of the atomizer on microparticle characteristics and mathematical modeling of the drug release. J Pharm Sci 2010; 99(2): 916-31.

[16] Trotta M, Cavalli R, Trotta C, Bussano R, Costa L. Electrospray technique for solid lipid-based particle production. Drug Dev Ind Pharm 2010; 36(4): 431-8.

[17] Brion M, Jaspart S, Perrone L, Piel G, Evrard B. The supercritical micronization of solid dispersions by Particles from Gas Saturated Solutions using experimental design. J Supercrit Fluids 2009; 51(1): 50-6.

[18] Rodriguez, L.C., Cavallari, C., Motta, G. Apparatus and method for preparing solid forms with controlled release of the active ingredient. WO9603979 (**1996**).

[19] Pilcer G, Sebti T, Amighi K. Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation. Pharmaceut Res 2006; 23(5): 931-40.

[20] Depreter F, Amighi K. Formulation and *in vitro* evaluation of highly dispersive insulin dry powder formulations for lung administration. Eur J Pharm Biopharm 2010; 76(3): 454-63.

[21] Eldem T, Speiser P, Hincal A. Optimization of spray-dried and congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. Pharmaceut Res 1991; 8(1): 47-54.

[22] Brasseur S, Amighi K, Moes AJ. Evaluation of solid lipid microparticles for controlled delivery to the pulmonary tract. In: Spa, Belgium 2000; 11.

[23] Killeen MJ. Spray drying and spray congealing of pharmaceuticals. Encyclopedia Pharmaceut Technol Edited by Swarbrick J, Boylan JC 2000: 207-21.

[24] Poortinga, A.R.J. Method and apparatus for spray drying and powder produced using said method. US2011052786 (**2011**).

[25] Friesen, D.D., Baumann, J.M., Dubose, D.B., Millard, D.L. Spray-drying process. WO2010111132 (**2010**).

[26] Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm 2010; 392(1-2): 1-19.

[27] Johnson KA. Preparation of peptide and protein powders for inhalation. Adv Drug Deliv Rev 1997; 26(1): 3-15.

[28] Steckel H, Brandes HG. A novel spray-drying technique to produce low density particles for pulmonary delivery. Int J Pharmaceuti 2004; 278(1): 187-95.

[29] Van Krimpen, H.R. Spray-drying device WO2011028105 (**2011**).

[30] Bot, A., Dellamary, L., Smith, D., Woods, C.M. Engineered spray-dried lipid-based microparticles for cellular targeting. WO0164254 (**2001**).

[31] Del Curto MD, Chicco D, D'Antonio M, Ciolli V, Dannan H, D'Urso S, *et al.* Lipid microparticles as sustained release system for a GnRH antagonist (Antide). J Control Rel 2003; 89: 297-310.

[32] Del Curto, D., Esposito, P. Lipid microparticles by cryogenic micronization. US2004091522 (**2004**).

[33] Savolainen M, Herder J, Khoo C, Lovqvist K, Dahlqvist C, Glad H, Juppo AM. Evaluation of polar lipid-hydrophilic polymer microparticles. Int J Pharmaceut 2003; 262(1-2): 47-62.

[34] Savolainen M, Khoo C, Glad H, Dahlqvist C, Juppo AJ. Evaluation of controlled-release polar lipid microparticles. Int J Pharmaceut 2002; 244: 151-61.

[35] Albertini B, Mezzena M, Passerini N, Rodriguez L, Scalia S. Evaluation of spray congealing as technique for the preparation of highly loaded solid lipid microparticles containing the sunscreen agent, avobenzone. J Pharm Sci 2009; 98(8): 2759-69.

[36] Albertini B, Passerini N, Pattarino F, Rodriguez L. New spray congealing atomizer for the microencapsulation of highly concentrated solid and liquid substances. Eur J Pharm Biopharm 2008; 69(1): 348-357.

[37] Maschke A, Becker C, Eyrich D, Kiermaier J, Blunk T, Gopferich A. Development of a spray congealing process for the preparation of insulin-loaded lipid microparticles and characterization thereof. Eur J Pharm Biopharm 2007; 65(2): 175-87.

[38] Passerini N, Albertini B, Perissutti B, Rodriguez L. Evaluation of melt granulation and ultrasonic spray congealing as techniques to enhance the dissolution of praziquantel. Int J Pharm 2006; 318(1-2): 92-102.

[39] Passerini N, Gavini E, Albertini B, Rassu G, Di Sabatino M, Sanna V, *et al.* Evaluation of solid lipid microparticles produced by spray congealing for topical application of econazole nitrate. J Pharm Pharmacol 2009; 61(5): 559-67.

[40] Passerini N, Perissuti B, Albertini B, Voinovich D, Moneghini M, Rodriguez L. Controlled release of verapamil hydrochloride from waxy microparticles prepared by spray congealing. J Control Release 2003; 88: 263-275.

[41] Rodriguez L, Passerini N, Cavallari C, Cini M, Sancin P, Fini A. Description and preliminary evaluation of a new ultrasonic atomizer for spray-congealing processes. Int J Pharmaceut 1999; 183: 133-43.

[42] Passerini N, Perissutti B, Moneghini M, Voinovich D, Albertini B, Cavallari C, *et al.* Characterization of carbamazepine-Gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasounds. J Pharm Sci 2002; 91(3): 699-707.

[43] Akiyama Y, Yoshioka M, Horibe H, Hirai S, Kitamori N, Toguchi H. Novel oral controlled-release microspheres using polyglycerol esters of fatty acids. J Control Rel 1993; 26(1): 1-10.

[44] Richard, J., Baldascini, H.G., Agostinetto, R., De Angelis, L., Deschamps, F. Solid lipid microcapsules containing growth hormone in the inner solid core WO2009080164 (**2009**).

[45] Richard, J., Fais, F., Baldascini, H. Solid lipid microcapsules containing hGH. WO2010017965 (**2010**).

[46] Faure, A.V.J.F.M., Mertens, R.J.M., Kiekens, F.R.I. Process for the preparation of a solid dosage form, in particular a tablet, for pharmaceutical use and process for the preparation of a precursor for a solid dosage form, in particular a tablet. US2011082214 (**2011**).

[47] Petersen, T.S. Process and apparatus for spray drying or spray cooling. US6151798 (**2000**).

[48] Peltonen L, Valo H, Kolakovic R, Laaksonen T, Hirvonen J. Electrospraying, spray drying and related techniques for production and formulation of drug nanoparticles. Exp Opin Drug Deliv 2010; 7(6): 705-719.

[49] Rohner TC, Lion N, Girault HH. Electrochemical and theoretical aspects of electrospray ionisation. Phys Chem Chemical Physics 2004; 6(12): 3056-68.

[50] Drechsler MD, Obermeier I, Doring Y, Lackner KJ, Modrow S, von Landenberg P. Generation of multifunctional murine monoclonal antibodies specifically directed to the VP1unique region protein of human parvovirus B19. Immunobiology 2008; 213(6): 511-7.

[51] Zhang, Y. Preparation of lipid particles. KR20060123170 (**2006**).

[52] Amidi M, Pellikaan HC, de Boer AH, Crommelin DJA, Hennink WE, Jiskoot W. Preparation and physicochemical characterization of supercritically dried insulin-loaded microparticles for pulmonary delivery. Eur J Pharm Biopharm 2007; In Press, Corrected Proof.

[53] Bahrami M, Ranjbarian S. Production of micro- and nano-composite particles by supercritical carbon dioxide. J Supercrit Fluids 2007; 40(2): 263-83.

[54] Knez Z, Weidner E. Particles formation and particle design using supercritical fluids. Curr Opin Solid State Mat Sci 2003; 7(4-5): 353-61.

[55] Jung J, Perrut M. Particle design using supercritical fluids: Literature and patent survey. J Supercritical Fluids 2001; 20(3):179-219.

[56] Fatmi, A., Kim, T.K.E. Methods for enhancing the release and absorption of water insoluble active agents. WO2010075065 (**2010**).

[57] Dunbar C. Dry powder formulations for inhalation. Drug Deliv Syst Sci 2002; 2(3): 78-80.

[58] Fulton, J.L.D. Electrostatic deposition of particles generated from rapid expansion of supercritical fluid solutions. WO03101624 (**2002**).

[59] Krukonis, P.M., Coffey, M.P. Gas anti-solvent recrystallization process. US5360478 (**1991**).

[60] Hanna, M.Y.P. Method and apparatus for the formation of particles. WO9501221 (**1995**).

[61] Ru, J.Q.L., Wei, W., Baoyou, Z., Lin, Z., Xiaonan, Z., Xiuhua, Z., Baishi, Z., Chunlin, Z., Yuangang, Z. Preparation method for supercritical anti-solvent of water-soluble nano glycyrrhizic acid powder. CN101756905 (**2010**).

[62] Nizet D, Jaspart S, Brion M, Piel G, Delattre L, Evrard B. Supercritical fluids technology to produce solid lipid microparticles. In: 13th Forum of Pharmaceutical Sciences, Spa, Belgium 2007.

[63] Weidner E. Process for the production of particles or powders. Brevet 1997.

[64] Weidner E, Knez Z, Novak Z. PGSS (Particles from gas saturated solutions)- a new process for powder generation. In: Strasbourg, France 1994; 229-35.

[65] Weidner E, Petermann M, Knez Z. Multifunctional composites by high-pressure spray processes. Curr Opin Solid State Mat Sci 2003; 7(4-5): 385-90.

[66] Choi JA, Jung II, Lim GB, Ryu JH. Preparation of drug-loaded polymeric particles using a PGSS process and their characterization. J Biosci Bioengin 2009; 108: S26.

[67] Elvassore N, Flaibani M, Vezzu K, Bertucco A, Caliceti P, Semenzato A, Salmaso S. Lipid system micronization for pharmaceutical applications by PGSS techniques. In: 2003; *Proc 6th Int Symp Supercrit Fluids, Versailles*, France, 2003.

[68] Fages J, Lochard H, Letourneau JJ, Sauceau M, Rodier E. Particle generation for pharmaceutical applications using supercritical fluid technology. Powder Technol 2004; 141(3): 219-26.

[69] Garcia-Gonzalez CA, Argemi A, de Sousa ARS, Duarte CMM, Saurina J, Domingo C. Encapsulation efficiency of solid lipid hybrid particles prepared using the PGSS (R) technique and loaded with different polarity active agents. J Supercrit Fluids 2010; 54(3): 342-7.

[70] Mandzuka Z, Knez Z. Influence of temperature and pressure during PGSS (TM) micronization and storage time on degree of crystallinity and crystal forms of monostearate and tristearate. J Supercrit Fluids 2008; 45(1): 102-111.

[71] Martin A, Weidner E. PGSS-drying: Mechanisms and modeling. J Supercrit Fluids 2010; 55(1): 271-81.

[72] Salmaso S, Elvassore N, Bertucco A, Caliceti P. Production of solid lipid submicron particles for protein delivery using a novel supercritical gas-assisted melting atomization process. J Pharm Sci 2009; 98(2): 640-50.

[73] Malcolmson RJ, Embleton JK. Dry powder formulations for pulmonary delivery. Pharmaceut Sci Technol Today 1998; 1(9): 394-98.

[74] Mehnert W, Mader K. Solid lipid nanoparticles - Production, characterization and applications. Adv Drug Deliv Rev 2001; 47(2-3): 165-96.

[75] Demirel M, Yazan Y, Muller RH, Kilic F, Bozan B. Formulation and *in vitro-in vivo* evaluation of piribedil solid lipid micro- and nanoparticles. J Microencapsul 2001; 18(3): 359-71.

[76] Giannola LI, Di Stefano V, De Caro V. White beeswax microspheres: A comparative *in vitro* evaluation of cumulative release of the anticancer agents fluorouracil and ftorafur. Pharmazie 1993; 48(2):123-26.

[77] Yang S, Zhu J, Lu Y, Liang B, Yang C. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm Res 1999; 16(5): 751-7.

[78] Penkler LJ, Van Rooyen PH, Wessels PL. Conformational analysis of mu-selective [D-Ala2,MePhe4]enkephalins. Int J Peptide Protein Res 1993; 41(3): 261-74.

[79] Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm 2006; 327(1-2):153-9.

[80] Wang SL, Xie SY, Zhu LY, Wang FH, Zhou WZ. Effects of poly (lactic-co-glycolic acid) as a co-emulsifier on the preparation and hypoglycaemic activity of insulin-loaded solid lipid nanoparticles. IET Nanobiotechnol 2009; 3(4):103-8.

[81] Reithmeier H, Herrmann J, Gopferich A. Lipid microparticles as parenteral controlled release device for peptides. J Control Release 2001; 73: 339-50.

[82] Reithmeier H, Herrmann J, Gopferich A. Development and characterization of lipid microparticles as a drug carrier for somatostatin. Int J Pharmaceut 2001; 218: 133-43.

[83] Liu W, Hu M, Xue C, Xu H, Yang X. Investigation of the carbopol gel of solid lipid nanoparticles for the transdermal iontophoretic delivery of triamcinolone acetonide acetate. Int J Pharmaceut 2008; 364(1): 135-41.

[84] Priano L, Esposti D, Esposti R, Castagna G, De Medici C, Fraschini F, *et al.* Solid lipid nanoparticles incorporating melatonin as new model for sustained oral and transdermal delivery systems. J Nanosci Nanotechnol 2007; 7(10): 3596-601.

[85] El-Kamel AH, Al-Fagih IM, Alsarra IA. Effect of sonophoresis and chemical enhancers on testosterone transdermal delivery from solid lipid microparticles: An *in vitro* study. Curr Drug Deliv 2008; 5(1): 20-6.

[86] Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev 2001; 47(2-3): 165-96.

[87] Liu DF, Ge YF, Tang Y, Yuan YB, Zhang Q, Li R, Xu QW. Solid lipid nanoparticles for transdermal delivery of diclofenac sodium: Preparation, characterization and *in vitro* studies. J Microencapsulation 2010; 27(8): 726-34.

[88] El-Kamel AH, Al-Fagih IM, Alsarra IA. Testosterone solid lipid microparticles for transdermal drug delivery. Formulation and physicochemical characterization. J Microencapsulation 2007; 24(5): 457-75.

[89] Keck, C. Compositions containing lipid micro- or nanoparticles for the enhancement of the dermal action of solid particles. WO2010051918 (**2010**).

[90] Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002; 54: S131-S155.

[91] Borgia SL, Regehly M, Sivaramakrishnan R, Mehnert W, Korting HC, Danker K, *et al.* Lipid nanoparticles for skin penetration enhancement-correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. J Control Release 2005; 110(1): 151-63.

[92] Weyenberg W, Filev P, Van den Plas D, Vandervoort J, De Smet K, Sollie P, *et al.* Cytotoxicity of submicron emulsions and solid lipid nanoparticles for dermal application. Int J Pharmaceut 2007; 337(1-2): 291-8.

[93] Santos MC, Mehnert W, Schaller M, Korting HC, Gysler A, Haberland A, *et al.* Drug targeting by solid lipid nanoparticles for dermal use. J Drug Target 2002; 10(6): 489-95.

[94] Wissing SA, Muller RH. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity - *in vivo* study. Eur J Pharmaceut Biopharmaceut 2003; 56(1): 67-72.

[95] Wissing SA, Muller RH. Cosmetic applications for solid lipid nanoparticles (SLN). Int J Pharmaceut 2003; 254(1): 65-8.

[96] Wissing SA, Muller RH. The development of an improved carrier system for sunscreen formulations based on crystalline lipid nanoparticles. Int J Pharmaceut 2002; 242(1-2): 373-5.

[97] Wissing SA, Muller RH. Solid lipid nanoparticles as carrier for sunscreens: *In vitro* release and *in vivo* skin penetration. J Control Release 2002; 81(3): 225-33.

[98] Uner M, Wissing SA, Yener G, Muller RH. Skin moisturizing effect and skin penetration of ascorbyl palmitate entrapped in solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) incorporated into hydrogel. Pharmazie 2005; 60(10): 751-55.

[99] Uner M, Wissing SA, Yener G, Muller RH. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for application of ascorbyl palmitate. Pharmazie 2005; 60(8): 577-82.

[100] Yazan Y, Demirel M, Buyukkoroglu B, Selen KB. Solid lipid microparticle versus inclusion complex of piribedil. In: 2004/05//; Sevilla, Spain.

[101] Jaspart S, Kirschvink N, Chiap P, Delattre L, Crommen J, Gustin P, *et al.* Solid lipid microparticles for administration by inhalation: optimisation of manufacturing parameters and preliminary tolerance study. Blankenberge, Belgium 2004.

[102] Sanna V, Kirschvink N, Gustin P, Gavini E, Roland I, Delattre L, *et al.* Preparation and *in vivo* toxicity study of solid lipid microparticles as carrier for pulmonary administration. AAPS Pharm Sci Tech 2004; 5(2): e27.

[103] Weers, J.G., Tarara, T.E., Dellamary, L.A., Riess, J.G., Schutt, E.G. Phospholipid-based powders for drug delivery. WO0185136 (**2001**).

[104] Dellamary, L.A., Riess, J., Schutt, E.G., Weers, J.G., Tarara, T.E. Stable metal ion-lipid powdered pharmaceutical compositions for drug delivery and methods of use. WO0185137 (**2001**).

[105] Morton, D.S.J. Dry powder composition comprising co-jet milled particles for pulmonary inhalation. EP2286797 (**2011**).

[106] Choi HK, Kang KW, Chun MK, Kim O, Subedi RK, Ahn SG, *et al.* Doxorubicin-loaded solid lipid nanoparticles to overcome multidrug resistance in cancer therapy. Nanomed-Nanotechnol 2010; 6(2): 210-13.

[107] Bondi ML, Craparo EF. Solid lipid nanoparticles for applications in gene therapy: A review of the state of the art. Exp Opin Drug Deliv 2010; 7(1): 7-18.

[108] Brioschi AM, Calderoni S, Zara GP, Priano L, Gasco MR, Mauro A. Solid lipid nanoparticles for brain tumors therapy: state of the art and novel challenges. Nanoneuroscience Nanoneuropharmacol 2009; 180: 193-223.

[109] Tabatt K, Kneuer C, Sameti M, Olbrich C, Muller RH, Lehr CM, *et al.* Transfection with different colloidal systems: Comparison of solid lipid nanoparticles and liposomes. J Control Release 2004; 97(2): 321-32.

[110] Olbrich C, Bakowsky U, Lehr CM, Muller RH, Kneuer C. Cationic solid-lipid nanoparticles can efficiently bind and transfect plasmid DNA. J Control Release 2001; 77(3): 345-55.

[111] Bondi ML, Azzolina A, Craparo EF, Lampiasi N, Capuano G, Giammona G, *et al.* Novel cationic solid-lipid nanoparticles as non-viral vectors for gene delivery. J Drug Target 2007; 15(4): 295-301.

[112] Bondi ML, Craparo EF, Giammona G, Cervello M, Azzolina A, Diana P, *et al.* Nanostructured lipid carriers-containing anticancer compounds: Preparation, characterization, and cytotoxicity studies. Drug Deliv 2007; 14(2): 61-7.

[113] Pilcer G. New highly effective dry powder tobramycin formulations for inhalation in the treatment of cystic fibrosis. Univ Libre de Bruxelles, Laboratoire de Pharmacie Galénique et de Biopharmacie institut de. Pharmacie 2008.

[114] Souto EB, Wissing SA, Barbosa CM, Muller RH. Comparative study between the viscoelastic behaviors of different lipid nanoparticle formulations. J Cosmetic Sci 2004; 55(5): 463-71.

[115] Cavallari C, Rodriguez L, Albertini B, Passerini N, Rosetti F, Fini A. Thermal and fractal analysis of diclofenac/Gelucire 50/13 microparticles obtained by ultrasound-assisted atomization. J Pharm Sci 2005; 94(5): 1124-34.

[116] Pilaniya U, Pilaniya K, Chandrawanshi HK, Gupta N, Rajput MS. Formulation and evaluation of verapamil hydrochloride loaded solid lipid microparticles. Pharmazie 2011; 66(1): 24-30.

[117] Hao J, Fang X, Zhou Y, Wang J, Guo F, Li F, Peng X. Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design. Int J Nanomed 2011; 6: 683-92.

[118] Nayak AP, Tiyaboonchai W, Patankar S, Madhusudhan B, Souto EB. Curcuminoids-loaded lipid nanoparticles: novel approach towards malaria treatment. Colloids Surf B Biointerfaces 2010; 81(1): 263-73.

[119] Rodriguez L, Passerini N, Cavallari C, Cini M, Sancin P, Fini A. Description and preliminary evaluation of a new ultrasonic atomizer for spray-congealing processes. Int J Pharm 1999; 183(2): 133-43.

[120] Frederiksen HK, Kristensen HG, Pedersen M. Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin-incompatibility of the lipid and the pyrethroid and biological properties of the formulations. J Control Release 2003; 86: 243-52.

[121] Dalpiaz A, Mezzena M, Scatturin A, Scalia S. Solid lipid microparticles for the stability enhancement of the polar drug N6-cyclopentyladenosine. Int J Pharm 2008; 355(1-2): 81-6.

[122] Aji Alex MR, Chacko AJ, Jose S, Souto EB. Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. Eur J Pharm Sci 2011; 42(1-2): 11-18.

[123] Bodmeier R, Wang J, Bhagwatwar H. Process and formulation variables in the preparation of wax microparticles by a melt dispersion technique. I. Oil-in-water technique for water-insoluble drugs. J Microencapsulation 1992; 9(1): 89-98.

[124] Bodmeier R, Wang J, Bhagwatwar H. Process and formulation variables in the preparation of wax microparticles by a melt dispersion technique. II. W/O/W multiple emulsion technique for water-soluble drugs. J Microencapsulation 1992; 9(1): 99-107.

[125] Yang R, Gao RC, Cai CF, Xu H, Li F, He HB, Tang X. Preparation of gel-core-solid lipid nanoparticle: a novel way to improve the encapsulation of protein and peptide. Chem Pharm Bull 2010; 58(9): 1195-202.

[126] Reitz C, Strachan C, Kleinebudde P. Solid lipid extrudates as sustained-release matrices: the effect of surface structure on drug release properties. Eur J Pharmaceut Sci: Offic J Eur Federat Pharmaceutical Sci 2008; 35(4): 335-43.

[127] Erni C, Suard C, Freitas S, Dreher D, Merkle HP, Walter E. Evaluation of cationic sold lipid microparticles as synthetic carriers for the targeted delivery of macromolecules to phagocytic antigen-presenting cells. Biomaterials 2004; 23: 4667-76.

[128] Masters DB, Domb AJ. Liposphere local anesthetic timed-release for perineural site application. Pharmaceut Res 1998; 15(7): 1038-45.

[129] Rodriguez L, Albertini B, Passerini N, Cavallari C, Giovannelli L. Hot air coating technique as a novel method to produce microparticles. Drug Dev Ind Pharm 2004; 30(9): 913-23.

[130] Benita S, Zouai O, Benoit JP. 5- Fluorouracil: Carnauba wax microspheres for chemoembolization: An *in vitro* evaluation. J Pharm Sci 1986; 75(9): 847-51.

[131] Demirel M, Yazan Y, Muller RH, Kilic F, Bozan B. Formulation and *in vitro-in vivo* evaluation of piribedil solid lipid micro - and nanoparticles. J Microencapsulation 2001; 18(3): 359-71.

[132] Yener G, Incegul T, Yener N. Importance of using solid lipid microspheres as carriers for UV filters on the example octyl methoxy cinnamate. Int J Pharmaceut 2003; 258: 203-7.

[133] Fang JY, Fang CL, Liu CH, Su YH. Lipid nanoparticles as vehicles for topical psoralen delivery: Solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur J Pharm Biopharm 2008; 70(2): 633-40.

[134] Puri A, Loomis K, Smith B, Lee JH, Yavlovich A, Heldman E, *et al.* Lipid-based nanoparticles as pharmaceutical drug carriers: From concepts to clinic. Crit Rev Therapeut Drug Carrier Syst 2009; 26(6): 523-80.

[135] Amighi, K., Sebti, T. Solid lipid particules as pharmaceutically acceptable fillers or carriers for inhalation. WO2006066367 (**2006**).
[136] Marchaud, D.B. Anhydrous solid particles containing a lipid composition and pharmaceutical composition containing said particles. WO2006021709 (**2006**).
[137] Lee, J.K. Lipid particles comprising bioactive agents, methods of preparing and uses thereof. WO2006068890 (**2006**).
[138] Rachel JD, Lack EB, Larson B. Incidence of complications and early recurrence in 29 patients after facial rejuvenation with barbed suture lifting. Dermatol Surg 2010; 36(3): 348-54.
[139] Charcosset, C.F. Novel method for preparing solid lipidic particles using a membrane reactor. WO2007000531 (**2007**).
[140] Shekunov, B.Y.C., Huff, R.W. Method for producing solid-lipid composite drug particles. US2008286365 (**2008**).
[141] Battaglia, L.S.T., Cavalli, R. Method for the preparation of solid lipid micro and nanoparticles. WO2008149215 (**2008**).
[142] Vanderbist, F.B., Amighi, K., Pilcer, G. Improved pharmaceutical dry powder compositions for inhalation. WO2009050217 (**2009**).
[143] Eskandar, F. Liposomal dispersion and dry powder formulations comprising oligonucleotides having improved downstream prossessing properties. WO2009027337 (**2009**).
[144] Vassal, G.P., Ioulalen, K., Brun, B.C., Lassu, N. Galenic form under the form of solid lipid particles, useful in pediatrics, comprise temozolomide in lipid matrix, where the matrix comprises triglycerides with saturated fatty acids, and a mixture of fatty acids with specific fatty acids. FR2935270 (**2010**).
[145] Bondi, M.L.G., Craparo, E.F., Drago, F. Nanostructured lipid carriers containing riluzole and phamaceutical formulations containing said particles. US2010247619 (**2010**).
[146] Baker, T.S.W., Catterall, C.F., Eaton, M.A., Norman, T.J., Parker, D. Targeted lipid particles. US7718157 (**2010**).
[147] Galuska, P.E., Rasmussen, L.M. Processes for producing lipid particles. US2010233344 (**2010**).
[148] Brito, L.C., Ge, Q., Treco, D. Dry powder compositions for rna influenza therapeutics. US2011077284 (**2011**).