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NOVEL PARTICLE ENGINEERING TECHNIQUES IN DRUG DELIVERY: REVIEW OF COFORMULATIONS USING SUPERCRITICAL FLUIDS AND LIQUEFIED GASES

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This paper aimed to survey the numerous publications and patents issued in the fields of supercritical fluid assisted and cryogenic formulation techniques with special regard to coformulations of active substrate – excipient composites. These methods open new possibilities in formulation of drug carrier composites with targeted physical properties including particle size, morphology, crystallinity, polymorphism and residual solvent content Both methods were proved to be viable alternatives to conventional formulation techniques.

Introduction

Lately, coformulations of active pharmaceutical ingredients (API) with biodegradable polymers draw more and more attention. Composites of drug-excipinet systems are widely used in controlled delivery systems and formulation of water insoluble APIs.

In controlled drug delivery the release of the API may be constant or cyclic over a long period, or it may be triggered by the environment (pH, enzymes, temperature, ion strength, chemical species, etc.). The main goal of controlled drug delivery is to achieve more effective therapies with fewer administrations and avoid both under- and overdosing. Depending on the route of administration (oral, transdermal, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular) drug formulation techniques must meet additional requirements concerning the physical properties of dry powder or emulsion. One of these properties is the particle size. For instant, only nanoparticles can be injected intravenously while particles in the range of 1-5 μm are suitable for pulmonary delivery, particles ranging from 5 to 100 μm are subcutaneously injectable. Conventional techniques for the production of drug-loaded microparticles including emulsion and double-emulsion solvent extraction, spray and freeze drying, liquid antisolvent and solvent evaporation are generally associated with high residual solvent contents, low encapsulation efficiencies and thermal degradations. Apart from spray-drying all of these techniques are multi-stage processes. In certain cases dry powder must undergo a micronisation step – usually by milling – to generate the required particle size and size distribution. Micronization techniques like air jet milling have several disadvantages (cohesive, electrostatically charged product with occasional crystallographic defects). Researches of recent years pointed out that supercritical fluid processes offer alternative single-step methods to prepare micronized dry powder of APIs with controllable physical properties including particle size, morphology, crystallinity, residual solvent content, etc [1].

Coprecipitations of drug-excipient systems are also widely used in formulation of poorly water soluble APIs [2]. The most important methods of drug solubilization are (1) formulation of solid dispersion (or solid solution), (2) preparation of surface modified nano and microparticles and (3) complexation of API in water soluble cyclodextrin derivates.

Solid dispersions of drug-excipient systems are composites containing nanosized particles of crystalline or amorphous API homogenously dispersed in the carrier particles. The solid dispersion that contains API dispersed on molecular level is referred to as solid solution. Formulations containing amorphous forms are generally more soluble than their crystalline counterparts but for the same reason they are less stable and may tend to crystallize during the product shelf life. These materials are often sensitive to water sorption, mechanical and thermal stresses. Surface modification techniques aim to hide the hydrophobic nature of the surface of drug-particles either by forming an adsorbed layer of surfactants or by coating with hydrophilic polymers. This approach requires smaller amount of excipient compared to solid dispersion [3]. The third method is forming inclusion complexes of APIs and water soluble cyclodextrin (CD) derivates. In addition to conventional techniques – hot melt extrusion, spray-drying and solvent evaporation – amorphous composites can be obtained by cryogenic formulation techniques discussed in the second chapter.

Coformulations using supercritical fluids

Since the first experiences of Hannay et al. in 1879 [4], application of dense gases in crystallization processes has made a long way [1,5]. This chapter reviews the different techniques published and patented so far in the field of particle design with supercritical fluids with special regard to coprecipitation of active substrate-carrier matrix composites. Beside the most important techniques such as the Rapid Expansion from Supercritical Solution (RESS), Particles from Gas-Saturated Solution (PGSS), Gas Antisolvent (GAS), Supercritical Antisolvent (SAS), Solution Enhanced Dispersion by Supercritical Fluids (SEDS), Precipitation with a Compressed fluid Antisolvent (PCA) and the Aerosol Solvent Extraction System (ASES), some other less known methods were as well discussed like Rapid Expansion from Supercritical Solution with a Non-solvent (RESS-N), Rapid Expansion from Supercritical to Aqueous Solution (RESAS), Rapid Expansion of Liquefied Gas Solution (RELGS), Rapid Expansion of Liquefied-Gas Solution and Homogenization (RELGS-H) and Polymer Liquefaction Using Supercritical Solvating (PLUSS) (see *Fig. 1*).

These methods use supercritical fluids (SCF) either as solvent (RESS) or antisolvent (GAS, SAS, ASES, SEDS) and/or dispersing fluid (GAS, SEDS, PGSS). As applied SCFs (usually CO_2) are gases at ambient temperature and pressure, they are easy to separate from both organic cosolvent and solid product, therefor supercritical technology is considered as clean, recyclable and "green" technology. In addition, carbon dioxide – the most commonly used fluid – is chemically inert, non-toxic, non-flammable and inexpensive. Having mild critical temperature (31,1 °C) and a critical pressure of 73,8 bar, CO_2 is suitable to treat heat-sensitive materials – even explosives – without any thermal degradation with relatively low energy costs.

Rapid Expansion of Supercritical Solution (RESS)

RESS consists in depressurising the solution of the solid substrate solvated in supercritical fluid through a heated nozzle (10-50 μm, i.d.), causing an extremely rapid nucleation of the substrat in question [4]. This process is attractive due to the absence of organic solvent, but efficiency and/or feasibility depends on the solubility of the treated substrate in SCF at the operating temperature and pressure.

To overcome the difficulties associated with $scCO_2$-insoluble polar compounds and high molecular weight polymers, Mishima et al. have invented a new method called Rapid Expansion from Supercritical Solution with a Non-solvent (RESS-N). This process involves a second solvent which solubilizes the solid substrates in supercritical conditions but doesn't dissolve them at atmospheric pressure. This cosolvent is homogenized in the pure SCF before the extraxtion unit, the modified SCF is saturated in the solid substrates and expanded through a nozzle to atmospheric pressure [6]. This method was afterwards used for encapsulation of proteins (lysozyme, lipase) as well as flavone and 3-hdroxyflavone in biodegradable polymers like: polyethylene glycol (PEG), poly(methyl methacrilate) (PMMA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA); Poloxamer and Eudragit E100 [7-9]. The concentrations of cosolvents must be at least 27 % (w/w) to achieve enough high solubility of polymer in $scCO_2$/cosolvent system and the active principle must be insoluble in the chosen cosolvent.

In 1994, Frederiksen et al. patented a method suitable to prepare liposomes containing at least one phospholipid, an excipient and a water soluble API [10,11]. Phospholipids and excipients were dissolved in an appropriate polar solvent and homogenized in $scCO_2$, this solution was afterwards expanded to atmospheric pressure and simultaneously dispersed in the aqueous solution of API. Residual organic solvent can be removed by evaporation, dialysis or gel filtration. Five years later, Ma Shuxian patented another process for preparation of liposomes by (1) dissolving a phospholipid, an excipient and a lipophilic API in compressed or supercritical CO_2 (2) releasing the CO_2 until the density of CO_2 is lower than that of lipophilic API (3) quickly depressurizing the vessel (4) loading a dispersing liquid and stirring in order to form API-coated liposomes [12].

To obtain sub-micron particles of water-insoluble solids Henriksen et al. submerged the nozzle of RESS process in an aqueous solution containing surfactants, typically phospholipids [13] . In another embodiment of the invention API is precipitated by Compressed Gas Antisolvent method and stabilized in the aqueous solution. Stabilized suspensions of cyclosporine, indomethacin and tetracaine HI nanoparticles were prepared. Young et al. detailed the precipitation of cyclosporine sprayed in a solution of Tween-80 polymer. The mean particle size was between 400 and 700 nm and the solubility of cyclosporine increased significantly [14]. RESAS (Rapid Expansion from Supercritical to Aqueous Solution) is a viable method to enhance the solubility of actives principles that are insoluble in water but soluble in $scCO_2$.

Godinas et al. invented a process wherein an active principle together with a surface modifier is dissolved in a dense gas which is afterwards expanded in an aqueous solution containing a second – optionally, identical to the first – surface modifier and other additives [15]. This technique was patented by the name of Rapid Expansion of Liquefied Gas Solution (RELGS) or Rapid Expansion of Liquefied Gas Solution and Homogenization (RELGS-H). Suspensions of fenofibrate and cyclosporine were prepared with mean particle sizes of 200 and 23 nm, respectively. The surface modifiers were Lipoid-80 and Tween-80 dissolved in $scCO_2$ in the case of fenofibrate and Tween-80 in fluid phase with egg phospholipid and mannitol in aqueous phase for cyclosporine. Obviously, the latter three inventions – RESAS, RELGS and RELGS-H – are applicable only in the case of water-insoluble compounds.

Also, in 1999, Bausch et al. patented a method wherein a biologically active substrate is dissolved in a compressed gas, liquid or SCF containing a surface modifier, and is either expanded to lower pressure or dispersed in an anti-solvent phase [16].

Table 1. Summary of drug-carrier systems precipitated by RESS and related methods.

Substrate	Excipient	Observation	References
Lovastatin	PLA	Needles of substrate in polymer microspheres	[17]
Pyrene	PLA	Solid dispersion	[18]
$ZnPc(SO_3H)_4$[a] FITC-dextran[b]	POPC[c] Cholesetrol	Liposomes, 200 nm	[10,11]
Naproxen	PLA	Microcapsules	[19]
Cyclosporine	Tween 80 Phospholipid	Suspension, 28 – 80,9 nm	[10]
Flavone 3-Hydroxyflavone	PEG	Mean particle size: 10 μm	[7]
Fenofibrate	Lipoid E80 Tween 80	Suspension, 0,2 μm	[15]
Cyclosporine	Tween 80	Mean particle size: 23 nm	
Tetrahydrolipstatin	Brij 96	Mean particle size: 1,4 – 2,1 μm	[16]
Saquinavir	Aerosol OT Brij 96	Mean particle size: 0,8 – 3,8 μm Mean particle size: 1,4 μm	
Cyclosporine	Tween 80	Suspension, 0,5-0,7 μm	[14]
3-Hydroxyflavone	Eudragit E100	Microcapsules	[8]
Lipase Lysosyme	PEG PLA PLGA PMMA Poloxamer	Microcapsules, 6 - 62 μm	[9]
Lidocaine	PEG 8000	Solid dispersion	[20]

[a] Zinc-Phthalocyanine Tetrasulfonate; [b] Fluorescein Isothiocyanate-dextran; [c] 1-palmitoyl-2-oleoylphosphatidylcholine;

Particles from Gas-Saturated Solution (PGSS)

PGSS consists in dissolving a compressed gas – supercritical fluid – in a molten solid or in a solution or suspension of solid substance followed by a rapid expansion to lower – atmospheric – pressure [21]. The very first application of the PGSS concept related mostly to paint and polymer industry particularly to powder coating [22]. Since the solubilities of compressed gases in liquids and solids like polymers are usually much higher then those of such liquids and solids in the compressed gas this method is proved to be more advantageous over RESS. Active ingredients can be micronized from aqueous solution as well according to the patent of Sievers et al. [23]. Since CO_2 is one of the most soluble gases in water, depressurization of the ternary mixture – solid-water-CO_2 – produce extremely high expansion. Water can be evaporated from the aerosol in high temperature furnace to obtain dry powder. In 1998 Shine et al. patented a method called Polymer Liquefaction Using Supercritical Solvating (PLUSS) whereby PGSS method is applicable below the melting point of the processed material [24]. As polymers in high pressure CO_2 atmosphere swell or melt at lower temperature, active substrates can be dispersed and encapsulated without any thermal degradation. Shakeseff and coworkers from the University of Nottingham have successfully applied $scCO_2$ as dispersing and plasticizing fluid to prepare composites of biologically active materials and polymers [25-28].

Kerc et al. described the micronization of three pure drugs (nifedipine, felodipine and fenofibrate) as well as their composites with PEG 4000 polymer by PGSS process [29]. The addition of polymer to the solid substance avoids agglomeration, enhances the solubility of treated drug in water and decreases considerably the process temperature [30].

Table 2 Summary of drug-carrier systems precipitated by PGSS and related methods.

Substrate	Excipient	Observation	References
IBDV vaccine[a]	Polycaprolactone	Agglomeration of fine particles	[24]
Nifedipine	PEG 4000	Non-regular porous particles	[30]
Nifedipine Felodipine Fenofibrate	PEG 4000	Mean particle size: 16 - 30 μm	[29]
Avidin	PLA	Solid solution	[28]
Avidin	PLA-PEG-biotin	Protein-loaded particles	[26]
rhBMP-2[b]	PLA	Porous scaffolds	[25]
Ribonuclease A Lysozyme	PLA	Protein-loaded particles, 10 - 300 μm	[27]
Theophylline	HPO[c]	Microcapsules, 2,5 - 3 μm	[31]

[a] Infectious Bursal Disease Virus; [b] Growth Factor recombinant human bonemorphogenetic protein-2; [c] Hydrogenated palm oil;

Gas Antisolvent (GAS)

When a gas is absorbed in a solution this latter gradually expands and losses its solvent strength. This sudden drop in solvent strength leads to the precipitation of dissolved substrates from the supersaturated solution. GAS process claims a precipitator, which is partially filled with the solution of API (and excipient). The supercritical fluid is preferably introduced at the bottom to achieve a better mixing. Unlike the other antisolvent processes described later, in that case the liquid phase is the continuous one and the antisolvent constitutes the dispersed phase. When precipitation is complete particles are washed in pure SCF.

Among the related patents the one of Krukonis et al. represents the most what we call GAS process [32]. Authors described crystallization of two difficult to handle explosives (RDX and NQ), an inorganic salt (cobalt chloride) and a pharmaceutical precursor (saligenin). Two years latter Pallado et al. patented a GAS application whereby microcapsules of APIs and biocompatible polysaccharides can be obtained [33].

Recently, Moneghini and Kikic achieved a remarkable enhancement of carbamazepine

dissolution rate using PEG 4000 polymer in GAS technology [34]. Drug and PEG 4000 particles were still distinguishable at D/P = 1:1,5. At D/P = 1:11 they precipitated together, but crystalline drug remained detectable.

Corrigan et al. compared three formulation methods including solvent evaporation, spray drying and GAS recrystallization [3]. Hydrocortisone and Polyvinylpyrrolidone (PVP) were coprecipitated in different D/P ratios. It was found that spray drying was the most effective process for eliminating hydrocortisone crystallinity, followed by GAS and solvent evaporation. However, the highest dissolution rate was observed in the case of solvent evaporation, followed by spray drying. The dissolution rates of particles from GAS recrystallisation were almost the same as those of physical mixture of drug and polymer.

Bertucco et al. described the encapsulation of potassium chloride and phenylpropanolamine in various polymers, including hydroxypropyl methylcellulose phthalate (HP-55), Eudragit E and ethylcellulose (EC) [35]. Authors used a stirred precipitator in batch mode. Polymer concentration was found to have strong influence on particle morphology: at low concentration only partial encapsulation was observed, at high polymer concentration particles were aggregated. In a previous study Bertucco et al. prepared controlled release particles of two steroids and a protein embedded in HYAFF-11 (hyaluronic acid benzylic ester) polymer [36]. Although, the authors called their process Supercritical Anti-Solvent, it corresponds much more to GAS, in our nomenclature. They obtained particles with an average diameter of around 4 μm. However, yields were rather poor for steroids due to their non-negligible solubilities in $scCO_2$.

Table 3 Summary of drug-carrier systems precipitated by GAS and related methods.

Substrate	Excipient	Observation	References
Insulin Calcitonin GMSCF[d]	HYAFF-7[a] HYAFF-11[b] HYAFF-11-p75[c]	Mean particle size: 0,1 - 1 μm	[33]
Steroids	HYAFF-11		[36]
KCl Phenylpropanol-amine	HP-55 Eudragit E100 EC	Mean particle size: 500 nm	[35]
Carbamazepine	PEG 4000	Heterogeneous precipitate	[34]
Hydrocortisone	PVP	Particles with crystalline substrate	[3]
Carbamazepine Theophylline	PEG 4000 HPMC	Solid dispersion	[37]
Carbamazepine	PEG 8000 Gelucire 44/14 Vitamin E TPGS [e]	Solid dispersion	[38]
Carbamazepine	PVP K30 Gelucire 44/14 Vitamin E TPGS	Solid dispersion	[39]

[a] Ethyl Ester of Hyaluronic Acid; [b] Benzyl Ester of Hyaluronic Acid; [c] Partial Benzyl Ester of Hyaluronic Acid; [d] Granulocyte Macrophage Colony Stimulating Factor; [e] D-α-tocopheryl polyethylene glycol 1000 succinate

Supercritical Antisolvent (SAS) Aerosol Solvent Extraction System (ASES) Precipitation with a Compressed fluid Antisolvent (PCA)

SAS (ASES, PCA) involves a capillary through which the solution of API is dispersed in a continuous SCF flow. The nomenclature is not consistent at this point; certain authors use the name of SAS as a synonym of GAS. In this work SAS (ASES, PCA) signifies the process wherein the solution is dispersed in the supercritical fluid or compressed gas by spraying through a capillary nozzle in a co-current gas anti-solvent flow. The main advantage of SAS over GAS is the faster expansion in steady state conditions, which results in higher nucleation rate, and hence smaller mean particle size and narrower size distribution. In addition, physical properties of processed powder

can be easily optimized by controlling the operating parameters like SCF flow rate, solution flow rate, nozzle diameter, pressure and temperature.

Fisher and Müller patented a coprecipitation process wherein a solution containing an active substrate, an excipient or both is dispersed in a SCF flow optionally containing the excipient, active substrate or both [40]. Authors used both single and multi-nozzle injection device and disclosed a number of ways in which the fluids can be contacted with one another. Sterilizing capacity of the invented method was proved by treating eight microorganisms – including bacterias, fungis and yeasts – mixed with PLA. Samples were totally sterile due to the treatment at 140 bar 50 °C. In another example, clonidin-HCL was coprecipitated with PLA from dichloromethane (DCM).

In 1998 William J. Schmitt patented a similar process. His method consists in dissolving a solid in an appropriate organic solvent and injecting the solution in supercritical fluid anti-solvent [41]. Triamcinolone acetonide particles were precipitated from terahydrofuran (THF) both in static $scCO_2$ and continuous flow with particle diameters ranging from 10 to 30 mμ and from 5 to 10 mμ, respectively.

Also, in 1998, Manning et al. patented a method, which extends SAS over hydrophilic pharmaceutical substrates by using an amphifilic additive [42]. This additive – preferably a surfactant – forms a hydrophobic ion pair (HIP) complex and solubilizes the active substrate. The resulting solution can already be subjected to any supercritical anti-solvent method.

Falk et al. prepared PLA micro-spheres with embedded APIs: gentamycin, naltrexone and rifampin by PCA process [43]. The solution containing both the API and the polymer was dispersed through an ultrasonic spray nozzle instead of a capillary tube. Gentamycin and naltrexone were solubilised in DCM by hydrophobic ion-pairing method; the chosen surfactant was sodium bis-2-ethylexyl-sulfosuccinate. Particles ranged from 0,2 to 1,0 μm in size, drug loading was higher for ion-paired pharmaceuticals due to their lower solubilities in $scCO_2$/DCM. In a subsequent study the authors focused on influence of process parameters on residual solvent level in PLA coated gentamycin particles [44]. Residual DCM content was found to decrease, when $scCO_2$ flow rate was higher during the precipitation.

Taki et al. used SAS method to prepare controlled release system for the herbicide, diuron [45]. Authors studied the effect of pressure, flow rate and composition on the solid dispersion of diuron in PLA. It was confirmed that the morphology of coprecipitated particles depends heavily on the concentrations of drug and polymer. Spherical particles of PLA entrapping diuron with mean particle size between 1 and 5 μm were successfully precipitated when the concentrations of diuron and PLA were below 0,1 and 3 % (wt.), respectively.

Sze Tu et al. studied the effect of the main process parameters, like: pressure, temperature, spraying velocity, solution concentration and solvent strength on ASES-precipitated PLA particles loaded with *para*-hydroxybenzoic acid (*p*-HBA) and lysosyme [46,47]. Authors used a multiple nozzle (three-passages) with a capillary tube of 180 μm i.d. in the middle. Initially, API and polymer were delivered in the same solution through the inner capillary tube. In a second stage, solution of active substrate was delivered in the inner nozzle and the polymer in the intermediate passage. The encapsulation efficiency – using the multiple nozzle – of *p*-HBA and lysosyme was 8,2 % and 12,4 %, respectively. Average drug loading was between 3 and 8 % (wt.) while theoretical drug loading was typically between 60 and 84 % (wt.).

Solution Enhanced Dispersion by Supercritical Fluids (SEDS)

SEDS was described in sequential patents associated with the name of the University of Bradford and recently Bradford Particle Design PLC [63-68]. This method claims a coaxial nozzle to co-introduce the SCF and the solution of API in the precipitation vessel. Due to the high velocity of SCF – driven preferably in the inner passage – a jet is forming at the outlet of the nozzle wherein the SCF brakes up the solution into small droplets and extracts the solvent. The high dispersion that characterises the jet leads to almost instantaneous precipitation of micron and sub-micron sized uniform particles. The main advantage of SEDS over other supercritical fluid based techniques is the fact that SCF plays both the role of dispersing fluid and antisolvent which allows better control over mean size and size-distribution of the product.

Table 4 Summary of drug-carrier systems precipitated by SAS, ASES, PCA and related methods.

Substrate	Excipient	Observation	References
Clonidin-HCL	PLA	Agglomerated spheres, 10 – 100 μm	[40]
Hyoscine butyl-bromide	PLA	Agglomerated particles, < 20 μm	[48]
Hyoscine butylbromide, Indomethacin, Piroxicam, Thymopentin	PLA	Microcapsules	[49]
Thymopentine	PLGA Lecithin	Microcapsules	[50]
Naproxen	PLA	Mean particle size: 5 μm	[51]
Steroids[a]	PC[b]	Mean particle size: 2 - 9 μm	[52]
Gentamycin, Naltrexone, Rifampin	AOT[c] PLA	Spherical particles, 0,2 - 1 μm	[42,43]
Tetracosactide	PLA	Microcapsules, 5,9 μm	[53,54]
p-HBA	PLA PLGA	Fibrous network Microcapsules	[46]
α-Chymotrypsin	AOT PLA	Spherical particles, 2 - 3 μm	[42]
Insulin	SDS	Spherical and irregular particles 1 - 5 μm	
Ribonuclease	PEG SDS	Fiber-like and spherical particles 0,5 - 1 μm	
Cytochrome C	SDS	Collapsed spherical particles, 5 μm	
Pentamidine	SDS	Spherical particles, 0,1 – 1 μm	
Streptomicin	AOT	Spherical particles, 0,4 – 1 μm	
Albumin Estriol	PLGA	Agglomerated spherical particles, 10 – 130 μm	[55]
Chimotrypsin-AOT Insulin-lauric acid conjugate Insulin Lysozyme	PLA	Mean particle size: 1 – 5 μm	[56]
Diuron[d]	PLA	Microcapsules, 1 - 5 μm	[45]
Insulin	PEG PLA	Drug-loaded spheres, 0,4 – 0,6 μm	[57]
Insulin	PLA	Drug-loaded spheres, 0,5 – 2 μm	[58]
p-HBA Lysosyme	PLA	Agglomerated irregular particles	[47]
Budesonide	PLA	Spherical particles, 1 – 2 μm	[59]
rhDNase[e] Lysosyme	Lactose	Amorphous agglomerated spheres	[60]
Copper indomethacin	PVP	Solid dispersion, 0,05 – 4 μm	[61]
Insulin	PEG PLA	Agglomerated spherical particles, 360 – 720 nm	[62]

[a] beclomethasone-17,21-dipropionate, betamethasone-17-valerate, budesonide, dexamethasone-21-acetate, flunisolide, fluticasone-17-propionate, prednisolone and triamcinolone acetonide; [b] Phosphatidylcholine; [c] bis-(2-ethylhexyl) sodium sulfosuccinate; [d] Herbicide; [e] Recombinant human deoxyribonuclease;

In their first SEDS patent York et al. reported the coprecipitation of salmeterol xinafoate with hydroxypropylcellulose from acetone using both two- and three-passage nozzles [63]. In both cases peaks of salmeterol xinafoate were weaker in X-ray Diffraction patterns due to the amorphous fraction of the incorporated drug.

Since water is hardly miscible with $scCO_2$, hydrophilic compounds like sugars can not be processed directly. For this reason York et al. have completed their previous patent by describing a

process in which sugars are precipitated from aqueous solution by mixing it with a cosolvent (ethanol or methanol) which make water miscible with $scCO_2$ in a restricted concentration range [64].

As amorphous phase drugs are generally considered to be meta-stable, their stability over the storage period at ambient temperature is a crucial point. To demonstrate the viability of SEDS York et al. has devoted a whole patent to coprecipitating drug-carrier systems [67]. The drugs were chosen to cover a broad range of polarities including the highly apolar ketoprofen and in ascending order of polarity, indomethacin, carbamazepine, paracetamol, theophylline and ascorbic acid. The coformulation of these drugs with hydrophilic hydroxypropylmethylcellulose (HPMC), PVP and hydrophobic EC polymers revealed that the more they are alike in polar and hydrogen bonding characteristics the higher concentration of amorphous phase can be achieved. The amorphous phase of indomethacin coformulated with all three excipient was the most dominant in PVP (60 %) followed by HPMC (35 %) and EC (25 %). Furthermore, York et al. have coprecipitated two cyclo-oxygenase-2 (COX-2) inhibitors with hydroxypropylcellulose (HPC) and Poloxamer 237 as well as an anti-diabetic drug with 75/25 DL-lactide-*co*-caprolactone. The reduction of crystallinity level was linear in the whole concentration range for Poloxamer 237 and above 20 % for HPC. All these drug-carrier systems proved to be stable for at least three months stored between 0 and 25 °C.

Juppo et al. studied the formation of solid dispersion of the model drug 2,6-dimethyl-8-(2-ethyl-6-methylbenzylamino)-3-hydroxymethylimidazo-[1,2-a]pyridine mesylate and two excipients: Eudragit E and mannitol [69]. Although mannitol gave no true one-phase dispersion (solid solution) it improves the solubility of the model drug. Eudragit E is known to inhibit crystallisation but due to its low glass transition temperature it gives no well-defined particles in SEDS and can dissolve in $scCO_2$/solvent mixture reducing significantly the yield of this method. Indeed, Eudragit E was found to form amorphous dispersion, but with extremely low yield (< 20%).

Ghaderi et al. used a combination of supercritical N_2 and CO_2 to incorporate hydrocortisone in PLGA matrix. Microparticles with hydrocortisone were more irregular in shape, but differed slightly in size from pure PLGA particles [70].

Table 5 Summary of drug-carrier systems precipitated by SEDS and related methods.

Substrate	Excipient	Observation	References
Salmeterol Xinafoate	HPC	Cristalline drug enbedded in polymer matrix	[63]
Hydrocortisone	PLGA	Microcapsules, 9 - 13 μm	[70]
Ascorbi acid	EC HPMC	Aggragated particles, 0,5 μm	[67]
Carbamazepine	EC HPMC	Aggragated acicular particles, 0,5 μm	
Indomethacin	HPMC	Amorphous aggragates and fibers, 0,05 - 1 μm	
	EC	Amorphous particles, 100 μm	
	PVP	Amorphous particles, 10 - 250 μm	
Ketoprofen	HPMC	Amorphous aggragates, 0,1 – 0,3 μm	
	EC	Aggregates	
Paracetamol	HPMC	Amorphous spheres, 3 – 200 μm	
	EC	Fine powder	
Theophyline	HPMC	Amorphous aggragates, 0,1 – 50 μm	
	EC	Amorphous aggragates, 1 – 70 μm	
Model drug[a]	HPC Poloxamer 237	Clusters and agglomerates of spheres, 2 – 32 μm	
Model drug[b]	HPC	Clusters and irregular agglomerates, 4 – 47 μm	
Plasmid-DNA	Mannitol	DNA-loaded particles	[71]
Chlorpheniramine maleate	Eudragit RL	Drug crystals incorporated in swelled polymer	[72]
Model drug[c]	Mannitol Eudragit E	Mixture of drug particles and polymer fibers, 1 - 20 μm Solid solution	[69]

[a] ((Z)-3-[1-(4-chlorophenyl)-1-(4-methansulfonyl)methylene]-dihydrofuran-2-one);
[b] ((Z)-3-[1-(4-bromophenyl)-1-(4-methansulfonyl)methylene]-dihydrofuran-2-one);
[c] 2,6-dimethyl-8-(2-ethyl-6-methylbenzylamino)-3-hydroxymethylimidazo-[1,2-a]pyridine mesylate;

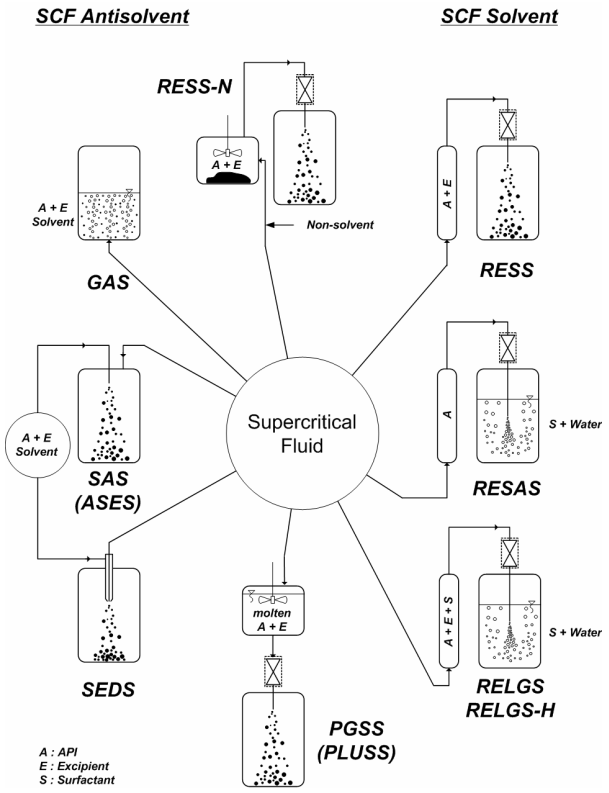

Fig. 1 Schematic diagram of supercritical particle engineering technologies.

Particle design using liquefied gases

Cryogenic particle design is present in particle engineering for some 30 years [73]. During this period several invention were published and patented. All of these methods take advantage of instantaneous freezing of a solution or suspension dispersed in cryogenic fluid. Inventions can be classified by the type of injection device: type of nozzle (capillary, rotary, pneumatic, ultrasonic), location of nozzle (above or under the liquid level); and the composition of cryogenic liquid (Hydrofluoroalkanes, N_2(l), Ar(l), O_2(l), organic solvents). First we considered the techniques where the orifice is located above the liquid surface, afterwards those of immersed nozzle.

Briggs and Maxwell invented the first process of spray freezing onto cryogenic fluid. Their first patent dates back to 1973 wherein the authors described a process of blending a solid biological product with solid sugar [74]. The chosen API together with the carrier sugar (mannitol, maltose, lactose, inositol or dextran) were dissolved in water and atomized above the surface of a boiling agitated fluorocarbon refrigerant. To enhance the dispersion of the aqueous solution authors placed a sonication probe in the stirred refrigerant. Solid particles were collected with a sieve and lyphilized. Freon 12 (dichlorodifluoromethane) was found suitable for this purpose because its boiling point (T_b= -30 °C) is sufficiently low to cause instantaneous freezing, but not enough low to form an extensive "vapor barrier" around the droplets which would hinder fast freezing. Several APIs were blended by this method including proteins, pharmaceuticals and enzymes (luciferase, hexokinase, glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), pyruvate kinase; luciferin, bovine albumin, morpholinopropane sulfonic acid (MOPS), 2,6-dichlorophenol indophenol (DIP), nicotinamide-adeninine-dinucleotide (NAD) and its reduced derivative (NADH).

In the following two patents, the authors completed the above list of APIs with blood serum, red blood cells, bacitracin, polymyxin B, tetracycline, chlorpromazine, maltase enzyme, testosterone, Vitamin C, cholesterol and gelatin [75,76]. Processed materials were characterized by high biological activity, homogeneity and stability.

In 1980 Adams et al. patented a method similar to the one of Briggs and Maxwell with the slight difference that they used needle (capillary) nozzles to disperse the solution or suspension onto the surface of stirred halocarbon refrigerant [77,78] (see *Fig. 2*). Blood plasma particles processed in Freon 12 ranged from 0.84 to 1.68 mm in diameter.

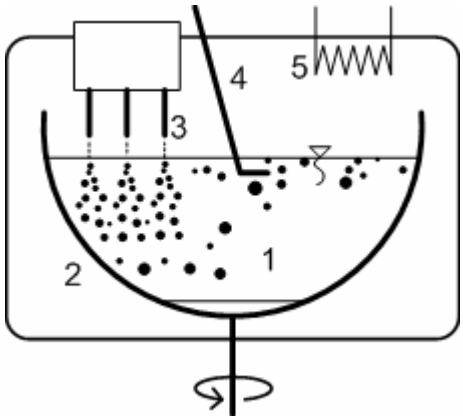

Fig. 2 Schematic diagram of the apparatus invented by Adams et al.
1. Refrigerant; 2. Rotated vessel; 3. Nozzles; 4. Wire screen; 5. Condenser

Hebert et al. prepared microparticles of controlled release device by spraying the solution containing an API and a biodegradable polymer into cold nitrogen gas [79] (see *Fig. 3*). Particles

were frozen partially in the gaseous phase and collected in the liquid phase at the bottom of the vessel where they solidified completely. In a second vessel liquid nitrogen was evaporated and residual organic solvent is removed by extraction.

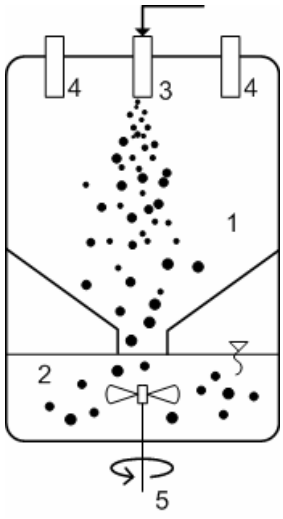

Fig. 3 Schematic diagram of the apparatus invented by Hebert et al.
1. Freezing vessel; 2. Extraction vessel; 3. Nozzle; 4.Liquified gas inlet; 5. Mixing means

Gombotz et al. patented a similar process to prepare microparticles of biodegradable polymers wherein the solution of API is atomized directly into liquid non-solvent or in liquified gas containing frozen non-solvent at a temperature below the melting point of the solution [80]. The solvent in the microspheres then thaws and is slowly extracted by the non-solvent. However, it can be difficult to find a good solvent, which extracts exclusively the organic solvent, and residual organic traces are generally hard to remove by extraction. Previously, Gombotz et al. published another process, which consists in atomizing the solution or suspension of API into a liquefied gas and lyophilizing the frozen particles [81]. Their method aimed to prepare microspheres of APIs including zinc insulin, catalase, heparin, hemoglobin, dextran, superoxide dismutase (SOD), horse radish peroxidase (HRP), bovin serum albumin (BSA), glycine and testosterone. Particles ranged from 10 to 90 μm in diameter and kept 70 – 95 % of their initial biological activity. To achieve a mean diameter smaller than 10 μm – which is desirable in the case of injectable polymeric microspheres of controlled drug delivery system – the lyophilized product was suspended in a non-solvent and exposed to ultrasonic energy. Owing to the porous structure and the great specific surface area that characterize the lyophilized product, particles were easy to disintegrate, leading to a mean diameter between 0,1 and 10 μm.

Lyophilization is a widespread process in pharmaceutical and food industry but rather expensive and time-consuming. Mumenthaler and Oyler used recirculated dry gas instead of vacuum to remove residual solvent from particles previously sprayed into cryogenic air [82,83] (see *Fig. 4*). During spray-freezing cold gas is supplied on the top of the vessel around the spray nozzle. When spray freezing is over, frozen particles are fluidized by passing the gas through the bed. Solvent vapors are continuously condensed in a heat exchanger. The temperature of dry gas must be carefully controlled to supply the heat of sublimation without melting the frozen droplets.

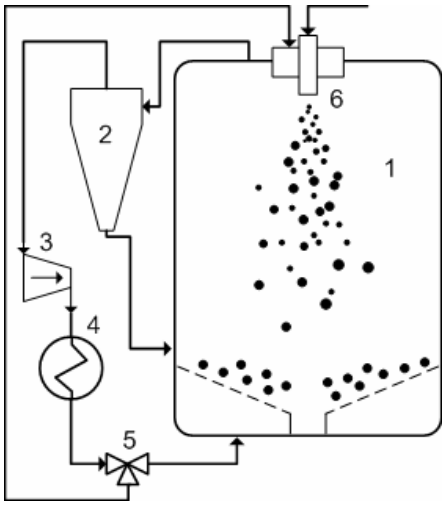

Fig. 4 Schematic diagram of the apparatus invented by Oyler.
1. Freezing and drying vessel; 2.Cyclone; 3. Turbine; 4.Heat exchanger; 5.Three-way valve; 6. Nozzle

More intense atomization can be achieved by submerging the nozzle into the cryogenic substance. Due to the liquid-liquid collision, atomization beneath the surface of cryogen results in smaller droplets which freeze much faster.

In 1969, Harold A Sauer patented the first method using submerged atomization device [84] (see *Fig. 5*). Solution was injected in liquid refrigerant through a heated nozzle at the bottom of the vessel. At the end of the atomization process, frozen droplets floating on the surface were collected in a spherical screen and dried in cold air or nitrogen gas. Residual moisture was removed by successively reducing the pressure in the chamber. The method developed by Dunn involves two immiscible halocarbon refrigerants. The boiling point of the denser refrigerant must be slightly above the melting point of the solvent while that of the lighter one is lower. Solution is dispersed through a heated nozzle in the denser phase from

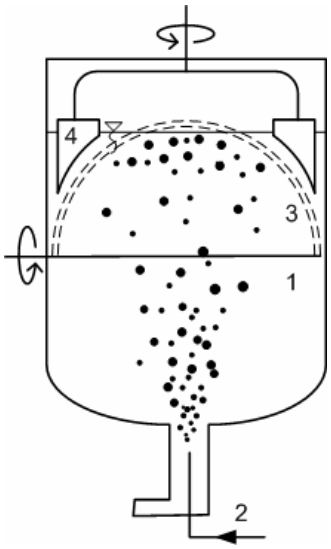

Fig. 5 Schematic diagram of the apparatus invented by Harold A Sauer.
1. Freezing and drying vessel; 2. Nozzle; 3. Screen hemisphere; 4 .Mixer paddle;

which rising solution droplets step in the lighter refrigerant and solidify [85] (see *Fig. 6*). The frozen particles floating on the surface of the upper refrigerant are collected and lyophilized. Authors described the precipitation of Aluminum sulfate in various Freon–based cryogenic systems.

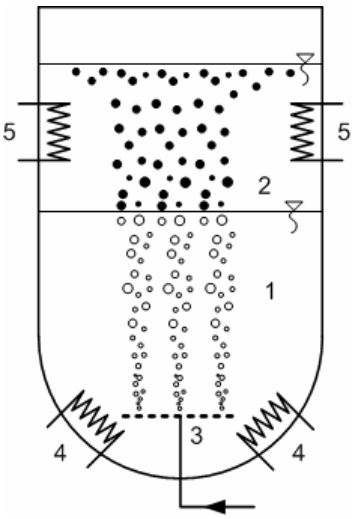

Fig. 6 Schematic diagram of the apparatus invented by Dunn et al.
1. Denser refrigerant (injection zone) ; 2. Lighter refrigerant (Freezing zone); 3. Atomization device; 4 Heating coil; 5. Cooling coil

Recently, Williams et al. invented a method called Spray-freezing into Liquid (SFL) which, due to an insulating nozzle, allows injection into extremely cold liquids – i.e. liquefied gases – without any nozzle blockage [86] (see *Fig. 7*). The authors recommended nozzles made of molded tip (polyetheretherketone, polyether block amide or polyurethane elastomers).

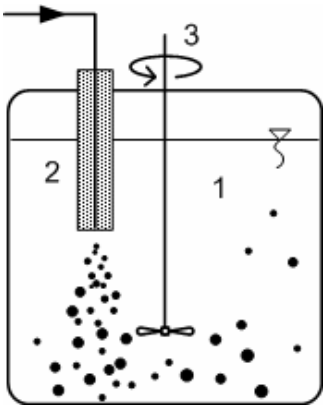

Fig. 7 Schematic diagram of the apparatus invented by Williams et al.
1. Liquified gas; 2. Insulating nozzle; 3. Propeller stirrer

Physical properties of the lyophilized solid particles were found advantageous in many respects foremost in the case of poorly water soluble APIs:

- Mean particle size: 0,68 – 16 μm
- Amorphous solid dispersion
- Great specific surface area: 3-117 m^2/g
- Porous structure
- Enhanced rate of dissolution
- Improved wettability
- Low residual solvent content.

Table 6 Summary of drug-carrier systems precipitated by cryogenic formulation techniques.

Drug	Excipient	Solvent	Observation	References
NAD, NADH, Luciferase, Luciferin, Hexokinase, G-6-PDH, LDH, Pyruvate kinase, Bovine albumin, MOPS, DIP	Mannitol, Maltose, Lactose, Inositol, PEG	Water (KCl, $NaHCO_3$)	Embedded substrates with high biological activities	[74]
Blood serum	Citric acid	Water	Embedded substrates with high biological activities	[75,76]
Maltase enzyme	Inositol, Mannitol			
Testosteron	Sodium monoglutamate			
Vitamin C	Inositol			
Cholesterol	SDS	Water/Ethanol		
SOD, HRP, Mitomycin C, Etoposide, Hemoglobin	PLA PLGA	DCM	Microspheres MPS [b] = 30 - 50	[80]
Carbamazepine	SDS	Water/THF	Solid solution MPS = 5,06 - 7,11 SSA [c] = 12,81 - 44,44	[86,87,90]
Danazol	PVA (22000) Poloxamer 407 PVP K15			
Insulin	Tyloxapol Lactose Trehalose	Water		
Danazol	HP-β-CD	Water/THF	Solid solution MPS = 7; SSA = 113,5	[86,88]
Danazol	PVA (22000) Poloxamer 407 PVP K15	Water/THF Water/Ethyl acetate[a] Water/DCM[a]	Solid solution MPS = 6,52 - 16,75 SSA = 8,9 - 83,06	[86,89]
Carbamazepine	Poloxamer 407 PVP K15	Water/THF Acetonitrile	Solid solution MPS = 0,68 - 7,06 SSA = 3,88 - 13,3	[86,91]
Salmon calcitonin, Tyloxapol	Lactose	Water	Solid solution MPS = 5,06 - 10,49 SSA = 11,04 - 19,16	[86]
Danazol	Poloxamer 407	THF		
Triamcinolone acetonide	Poloxamer 407 PVP K15			
Danazol	PVP K15	Acetonitrile DCM	Solid solution SSA = 28,50 -117,50	[92]

[a] Emulsion.; [b] Mean particle size (μm); [c] Specific surface area (m^2/g)

Conclusion

This paper aimed to survey the numerous publications and patents issued to date in the fields of supercritical fluid assisted and cryogenic particle design with special regard to coprecipitation of active substrate-excipient composites. All of these techniques have their own advantages and drawbacks. To choose the right method and the optimal working conditions preliminary experiments have to be carried out for each API and/or excipient. Lipophilic molecules are usually soluble in SCFs thus they can be processed by RESS technique. However, applicability of RESS is not restricted to lipophilic APIs, low yield associated with polar APIs and polymers can be increased by adding cosolvents to the SCF (RESS-N). Owing to the rapid expansion nucleation is more important than crystal growth

hence the sub-micron particles. To prevent particle agglomeration – one of the main drawbacks of RESS – supercritical solution can be expanded into an aqueous solution containing a stabilizer (RESAS, RELGS-H). Additional stabilizers can minimize particle aggregation and reduce the mean particle size by orders of magnitude [14]. Unlike the RESS, PGSS is applicable whether the API-excipient is soluble in SCF or not. This tecnique was developed in paint and polymer industry to prepare coated micro-particles. As most polymers swell and melt at lower temperature when placed in high pressure CO_2, thermolabil APIs (peptides, enzymes, viruses) can be as well encapsulated without considerable decrease of their biological activity (PLUSS). In contrast to PGSS, supercritical antisolvent techniques (GAS, SAS, SEDS, ASES, and PCA) require a solvent wherein the API and excipient are dissolved and which is miscible with the SCF at working conditions. The fact that these techniques use SCFs as antisolvents make them available for a wide range of APIs and excipients and allow milder working conditions compared to RESS. Furthermore they provide additional operating variables i.e. flow rates, concentrations, nozzle diameter and solvent, some of which proved to be critical in terms of particle size, morphology, polymorphism and crystallinity [93]. Aqueous solutions can be processed as well with cosolvents (ethanol or methanol) mixed in a three-passage nozzle [64]. SCF assisted precipitation techniques are successful candidates for drug-excipient coformulation for controlled release dosage forms. However amorphous solid solutions are great challenge for SCF assisted particle design, as it's not always possible to reduce product crystallinity to zero [67,69]. Pharmaceutical formulations using cryogenic fluids were originally developed to blend thermolabil biological substances (peptides, enzymes, cells) preserving their biological activities [74-76]. In recent years, Williams and co workers pointed out that formulation processes using liquefied gases can be successfully applied to enhance the solubility of poorly water soluble or insoluble APIs. Apart from applied solvent (organic solvent, aqueous solutions or emulsion) coformulations contained fine microparticles of amorphous solid solution with great specific surface area, enhanced solubility and low residual solvent content.

In future, SCF assisted and cryogenic particle design technologies can be alternatives to conventional formulation techniques due to their ability to produce micro-particles with targeted physical properties including particle size, morphology, crystallinity, polymorphism and residual solvent content.

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