Expert Opinion

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Supercritical fluid technology for enhanced drug delivery

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The rapid advances in the development of formulation and delivery systems based on micron-sized and nanoscale drug particles will create significant benefits to the pharmaceutical industry. Complementary to traditional methods, supercritical fluid techniques have found many useful, and sometimes unique, applications in the production and processing of drug particles. In this article background information is provided on a variety of supercritical fluid techniques relevant to drug formulation and delivery, recent advances and novel applications are highlighted, and the successful development of a new supercritical fluid rapid expansion technique for producing exclusively nanoscale drug particles will be discussed. Challenges and opportunities for further development and future applications are also reviewed.

Keywords: aqueous suspension, drug, nanoparticle, polymer, RESOLV, stabilisation, supercritical fluids

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1. Introduction

Recently, there has been significant effort directed toward the development of drug formulation and delivery techniques and systems, especially for drugs of no or poor aqueous solubility. Well-established statistics suggest that approximately one-third of the drugs listed in the United States Pharmacopeia are insoluble or poorly soluble in water [1,2]. In fact, > 40% of new drug development fails because of the aqueous solubility problem and related negative effects on biopharmaceutical properties (such as inadequate control of drug particle size and polymorph formulation instability). The poor aqueous solubility presents significant problems to drug development in general, and to the preclinical screening of candidate drugs, particularly because of their often erratic and highly variable performance and serious bioavailability issues in clinical applications. A commonly employed approach has been to introduce the required aqueous solubility by chemically modifying the drug molecules. In addition to being difficult and costly, this approach is highly drug specific and not generally applicable; therefore, there is a growing interest in the development of more effective and versatile techniques for the formulation of water-insoluble drugs. Popular techniques include the micronisation of drug powders [3,4], the dispersion of micron- and submicron-sized particles [5-7,201], the selection of surfactants to enhance solubility [8-10,202], and the use of organic solvents or buffers [11-13]. These methods are limited by issues such as broad particle size distribution, excessive solvent use and disposal, thermal and chemical denaturation of products, trace residues, and inter-batch particle size variability. Other formulation methods have been successful for specific drug candidates, including the use of emulsions and microemulsions [14,15], solid dispersion [16], inclusion complexes with cyclodextrins [17], and drug-carrier polymers and liposomes [18-21]. A commonly encountered problem has been that some drugs of low aqueous solubility also have difficulty in dispersing into the oil cores in emulsions and microemulsions; in fact, it remains an ongoing challenge to find reliable and versatile solutions for the formulation-related problems with hydrophobic drugs.

The recent advances in nanoscience and nanotechnology have added a new dimension to the development of drug formulation techniques. Specifically, nanosizing drug particles has been identified as a potentially effective and broadly applicable approach for the handling of insoluble and poorly soluble drugs. It has been predicted that such technology may have a tremendous impact on drug discovery and testing, and also on improving the performance of drug products suffering from formulation-related issues [22-25]. In addition, the technology capable of size-selective preparation of nanoscale to micron-sized drug particles may have significant implications beyond the mitigation for water insolubility; for example, smaller-diameter drug particles correspond to a faster dissolution rate, thus potentially have higher activity (therapeutic effect related to bioavailability) and easier absorption. Among traditional techniques for particle size reduction are grinding, crushing, milling, recrystallising from solution with antisolvents, and freeze- and spray-drying. These processing techniques generally lead to micron-sized particles with a broad particle size distribution. A further reduction in size for nanoscale drug particles is considered to be extremely challenging [22]. Other related issues include possible thermal and/or chemical denaturation of the products in the processing, unwanted residues and inter-batch variability in particle sizes and properties [22].

Generally, there are two strategies in nanosizing drug particles; mechanical milling [26-32] and precipitation/condensation [14-16,33,34]. In the former, colloidal mills or jet mills are common, and more advanced pearl milling and high pressure homogenisation have also been used; for example, large micron-sized drug particles are dispersed and stabilised in aqueous solution, and then either milled or high-pressure homogenised to form suspended drug nanoparticles [35]. This strategy has been applied to a variety of drugs. A significant limitation has been the particle agglomeration. In the other strategy, the drug is first dissolved in a solvent to form a solution. The solution is then added to a non-solvent of the drug for precipitation into drug nanoparticles. In a variation of the method, macromolecules, such as surface modifiers and polymers, were added to the drug solution to aid the particle formation. The precipitation and condensation techniques have found numerous applications in the preparation of drug particles from solution, emulsion, microemulsion and hydrosols; however, the requirement of miscibility between the solvent and non-solvent of a particular drug represents a significant limitation of the techniques.

Several supercritical fluid processing techniques have been identified as promising in the desired particle size reduction. These techniques not only complement the traditional methods, but also offer unique advantages in some applications [36-43]; for example, there have been numerous investigations on the use of supercritical carbon dioxide as an ideal solvent system in the processing of pharmaceutical products [42-51]. It has also been demonstrated that supercritical fluid processing techniques can be applied to the preparation of particles from a variety of materials at moderate temperatures and with minimum solvent residues [50,51]. Recently, these techniques have been extended to the processing of nanoscale materials, including nanosized drugs and pharmaceuticals [52-57]. This article provides background information on the supercritical fluid processing techniques relevant to drug formulation and delivery, highlights the recent advances and novel applications, and discusses the successful development of a new supercritical fluid rapid expansion technique for producing exclusively nanoscale drug particles.

2. Supercritical fluid processing for particle formation

A supercritical fluid is a solvent that is above the critical temperature and pressure, where the fluid remains a single phase. Among the most important properties of a supercritical fluid are the low and tunable densities, which can be easily varied from gas- to liquid-like via a simple change in pressure at a constant temperature or vice versa, and the unusual solvation effects at densities near the critical density (often discussed in terms of solute–solvent and solute–solute clustering). Commonly used supercritical solvents include CO_2, ethylene, ethane, fluoroform and ammonia, although their flammable properties and toxicity with some of these may limit their uses for pharmaceutical purposes. Supercritical CO_2 is an ideal choice in the processing of pharmaceuticals. It has a near ambient critical temperature (~ 31°C), a relatively low critical pressure (73.8 bar), and it is non-toxic, non-flammable, abundant and inexpensive. As CO_2 is nonpolar, a polar modifier, such as ethanol, may be added as a cosolvent to improve the solubility of some drug molecules [58,59].

Supercritical fluid technology has shown great promise in addressing many of the challenges facing the pharmaceutical industry in drug delivery systems, including particle generation and processing techniques and issues such as controllable particle size and shape, cleanliness, environmental responsibility and scalability [42-57]. Several supercritical fluid methods have been successfully developed, leading to micron-sized particles of different shape, size and morphology [42-51]. These particle design and formation processes offer many drug formulation options such as dry powders, nanoparticle suspensions, microspheres or microcapsules as drug carriers, and drug-impregnated excipients [42-57]. Among the widely investigated and most relevant techniques are supercritical antisolvent (SAS) and rapid expansion of supercritical solutions (RESS) [43-51,60-62].

In SAS, the particle formation is carried out through precipitation in a compressed fluid under supercritical conditions (**Figure 1**). Obviously, the solute must be insoluble in the supercritical antisolvent. The method also requires that the antisolvent be miscible with the solution. As with any precipitation process, the antisolvent can be added to the solution (normal-addition precipitation) or the solution can be added to the antisolvent (reverse-addition precipitation). In the normal addition SAS, a solute is dissolved in a liquid

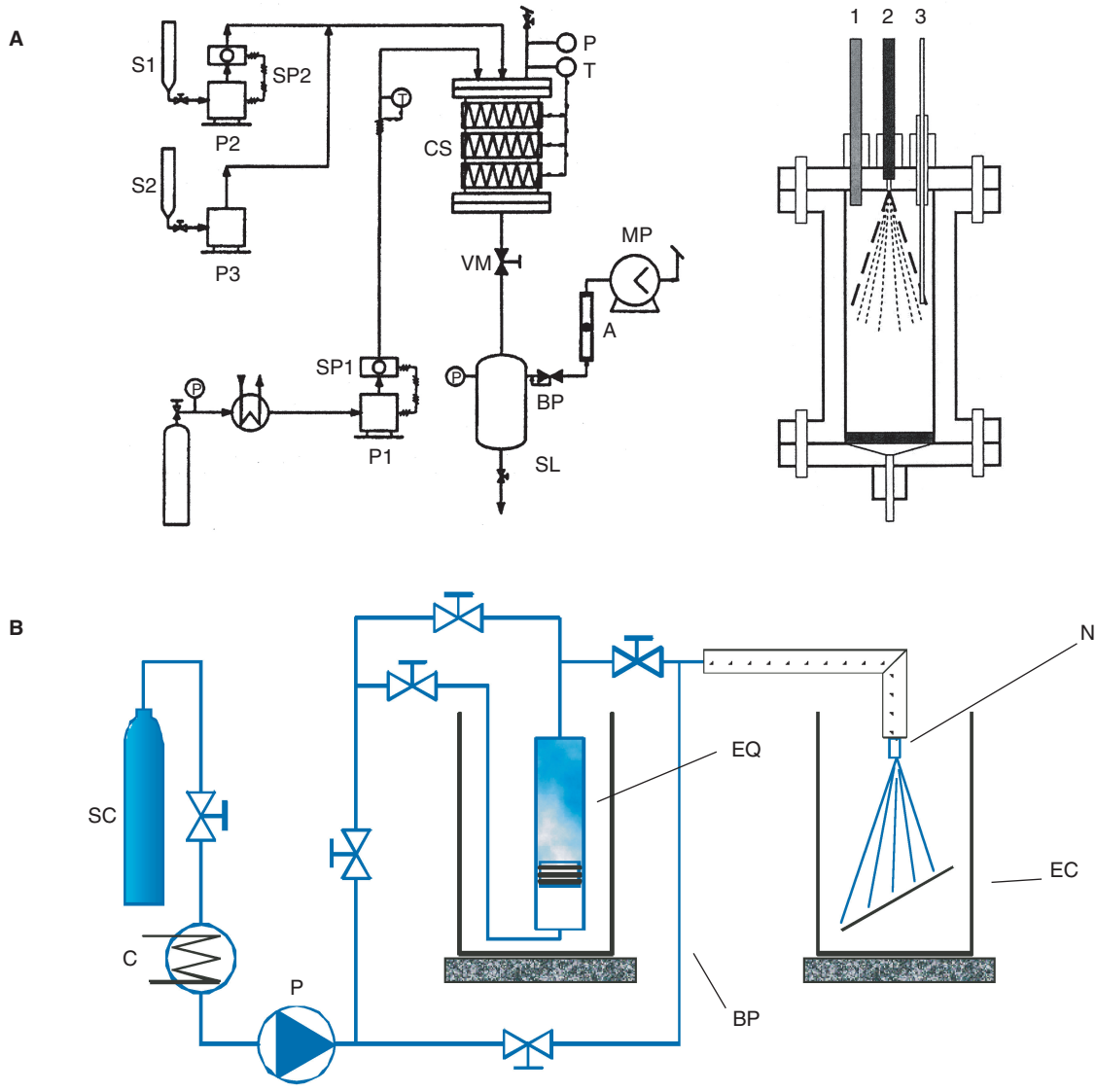

Figure 1. A. Left: A typical supercrital antisolvent apparatus; Right: Schematic representation of the precipitation chamber (1: Supercritical CO_2 inlet; 2: Liquid solution inlet; 3: Pressure and temperature measurements). B. A typical RESS apparatus. Reprinted with permission from WEBER M, THIES MC: Understanding the RESS process. In: *Supercritical Fluid Technology in Materials Science and Engineering: Synthesis, Properties, and Applications.* YP Sun (Ed.), Marcel Dekker, New York, NY, USA (2002):387-437 [83].
A. A: Calibrated rotameter; BP: Back-pressure regulator; CS: Precipitation vessel; MP: Wet test meter; P1/2/3: High pressure pump; S1/2: Liquid solution supplies; S: Liquid separator; SP1/2: Pressure dampeners; VM: Micrometering valve.
B. BP: Bypass line for solvent; C: Solvent cooler; E: Expansion chamber; EQ: Equilibrium cell; N: Nozzle; P: Solvent pump; RESS: Rapid expansion of supercritical solutions; S: Solvent cylinder.

solvent, and a supercritical antisolvent is added to the solution in a partially filled, closed container that is initially at ambient pressure [61-62]. The decrease in solubility of the solute with an increasing antisolvent fraction in the mixture results in the solute precipitation. The size and size distribution of the precipitated particles are dependent on the selection of the solution/antisolvent system, the solution concentration, the relative solution and antisolvent quantities, the rate of the antisolvent addition and the degree of mixing [61]. In the reverse-addition SAS, a liquid solution is sprayed through a nozzle into a supercritical antisolvent. The rapid diffusion between the solvent from the sprayed solution droplets and the bulk supercritical antisolvent results in the solute precipitation. The precipitate is then washed with the antisolvent and filtered to obtain the desired particles. The SAS method has been used for preparing a variety of micron and submicron particles and fine powders from inorganics, polymers, pigments, proteins and pharmaceuticals (acetaminophen, naproxen, salmeterol xinafoate etc.) [61-66]; for example, fine particles of trypsin, lysozyme and insulin proteins with diameters of 1 – 5 microns were produced by spraying the protein solution in dimethyl sulfoxide (DMSO) through

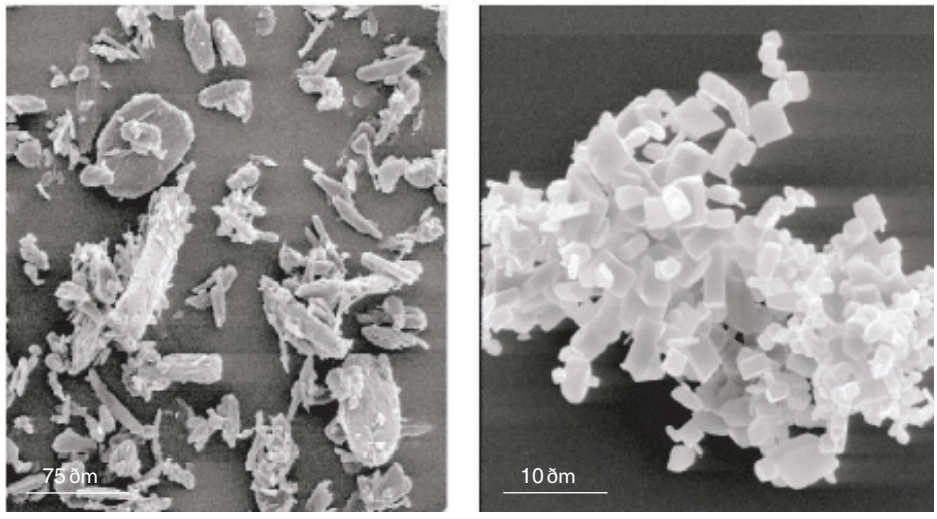

Figure 2. Scanning electron microscopy microphotographs of nicotinic acid. The image on the left shows the received sample, whereas the image on the right shows the sample prepared by using solution enhanced dispersion by supercritical fluids (SEDS). Reprinted from YORK P: Strategies for particle design using supercritical fluid technologies. *Pharm. Sci. Technol. Today.* (1999) **2**(11):430-440, copyright (1999), with permission from Elsevier.
SEDS: Solution enhanced by supercritical fluids.

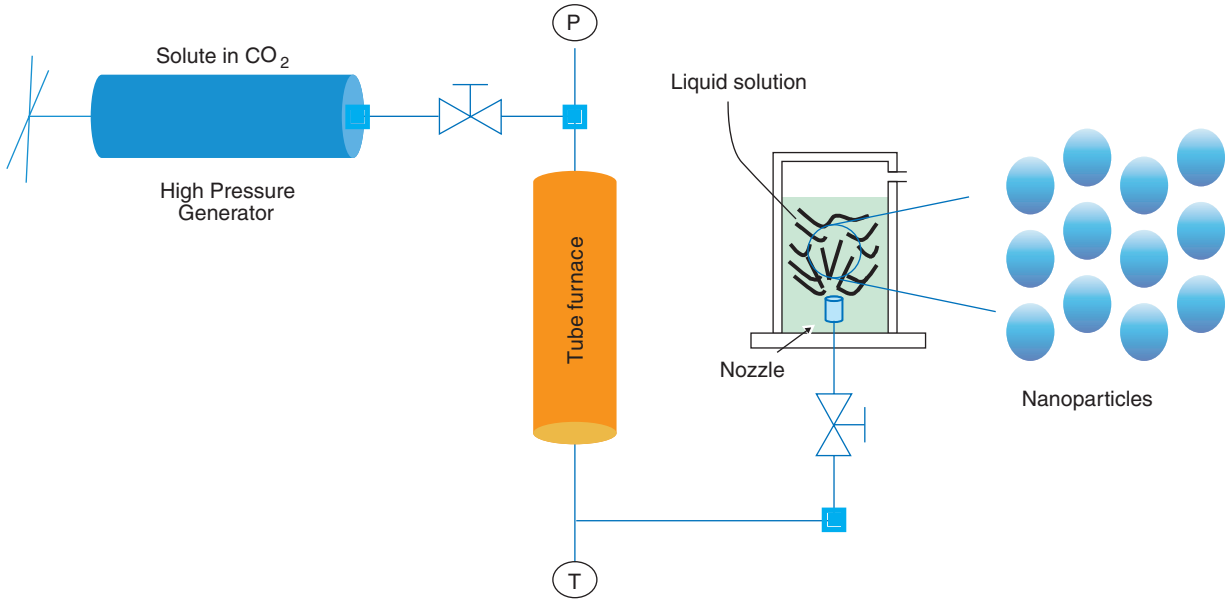

Figure 3. Experimental setup for the preparation of polymeric and drug nanoparticles via rapid expansion of a supercritical solution into a liquid solvent. Reprinted with permission from PATHAK P, MEZIANI MJ, DESAI T, SUN YP: Nanosizing drug particles in supercritical fluid processing. *J. Am. Chem. Soc.* (2004) **126**(35):10842-10843, copyright (2004) American Chemical Society.
P: Pressure; T: Temperature; RESOLV: Rapid expansion of a supercritical solution into a liquid solvent.

a small orifice into supercritical CO_2 [63]. The particle sizes could be varied via changing processing conditions, (e.g., larger particles could be obtained by decreasing the pressure or increasing the temperature of the supercritical antisolvent or by using a larger diameter expansion nozzle).

A major disadvantage of the SAS method is that the particle formation is followed by a lengthy drying period, which often leads to particle agglomeration and aggregation [37,38,66-68]. However, the problem may be minimised via the intensive mixing of the solution with the supercritical antisolvent, leading to more efficient mass transfer and smaller droplet size. One way to achieve the intensive mixing in a slightly modified SAS is by using an ultrasonic nozzle, where the increased mass transfer rate due to sonic waves in an energising gas stream leads to the formation of discrete submicron drug particles [45,203]. With sound waves rather than inertial and frictional

forces for droplet formation, large-diameter nozzles (instead of capillary or micro-orifice nozzles) could be used in the process for fine-particle production [203]. Another conceptually similar modification to the traditional SAS, or the solution enhanced dispersion by supercritical fluids (SEDS) technique [43,69,204], is to use the supercritical fluid as both an antisolvent and a 'spray enhancer', where a nozzle with two co-axial passages allows the simultaneous introduction of the drug solution and supercritical antisolvent into the particle formation vessel with controlled temperature and pressure. This modified SAS process has been used for the formulation of various drug particles, including nicotinic acid (**Figure 2**), paracetamol, salmeterol xinafoate, fluticasone propianate and water-soluble proteins [43]. The process has also allowed the manipulation of the particle size, shape and morphology via changing the process working conditions; for example, by processing in different regions of the supercritical phase with the same organic solvent, polymorphs of salmeterol xinafoate and fluticasone propianate were obtained from the SEDS process [204]. The same process was also applied to prepare the microfine particles of lysozyme from aqueous solution of the protein [69].

In addition to SEDS, other variations of SAS include precipitation with compressed antisolvent (PCA) and the aerosol supercritical extraction system (ASES) [43-48]. Since many compounds of interest have higher solubility in liquid solvents than in low-temperature supercritical fluids, SAS and other variations generally allow higher throughputs than the RESS method discussed below.

The RESS process has been widely studied as an effective technique for particle formation [37-48,70-84]. It differs from the SAS process in that, in RESS, the solute is dissolved in a supercritical fluid to form a solution and then the solution is rapidly expanded through a small nozzle or orifice into ambient air [75-77]. For solutes, poorly soluble or insoluble in the supercritical fluid, cosolvents may be added to increase their solubility in the preparation of supercritical solution for rapid expansion. A typical RESS apparatus for particle production is illustrated in **Figure 1**. The rapid reduction of pressure in the expansion is accompanied by a high degree of supersaturation in a very short period of time, resulting in the homogeneous nucleation to form particles of relatively narrow size distributions. The resulting material morphology depends on both the material structure and the RESS processing parameters, such as the nozzle geometry and the operating temperature and pressure (thus the fluid density) upstream and downstream from the nozzle [72,82]. In addition to the formation of particles from a variety of materials, including inorganics, ceramics, organics and polymers, the RESS technique has been applied to the processing of various drugs, such as salycilic acid, phenanthrene, aspirin, ibuprofen, lecithin, progesterone, nifedepin, testosterone and drug-polymer systems [37-48]. Although particles of a few microns are generally obtained as primary products, smaller particles 100 – 300 nm have been produced in RESS with the use of appropriate nozzles [46,70]. Smith and colleagues carried out mechanistic studies of the RESS process aimed, not only at the processing of various materials, but also the evaluation of the effects of processing conditions on the product morphology, size and size distribution [72,80-81]. The results from their experiments and others suggested that both nano- and micron-sized particles were present in the expansion jet, despite the fact that the end products were largely microparticles [41,83,84]. Theoretical calculations offered a reasonable explanation: agglomeration during the rapid expansion process is responsible for the growth of particles beyond the nanoscale and for the formation of the observed microparticles.

For nanoscale particles, Sun and colleagues made a simple but significant modification to the traditional RESS by using a liquid solvent or solution at the receiving end of the supercritical solution rapid expansion, otherwise known as the rapid expansion of a supercritical solution into a liquid solvent (RESOLV). This processing technique has been used to produce exclusively nanoscale particles from metals, semiconductors, and other materials [55-57,85-93]. A typical RESOLV apparatus for the production of nanoparticles is shown in **Figure 3**.

Mechanistically, the liquid at the receiving end of the rapid expansion in RESOLV probably suppresses the particle growth in the expansion jet, making it possible to obtain nanoscale particles only; thus, the nanoparticles produced in the expansion process could be prevented from aggregating by the presence of a polymeric or other protection agent in the receiving solution [55-57,85-93]. As recognised by Sun and colleagues, the RESOLV process for nanoparticle production has a unique feature that requires no nanoscale templating agents for the nanoparticle formation because the templating effect is provided by the supercritical fluid rapid expansion process, thus offering a clean way to directly couple the nanoparticles with biological species [91-93]. For example, Sun and colleagues have recently demonstrated that the semiconductor and metal nanoparticles coated directly with natural protein species could be prepared via RESOLV [91-93]. The understanding of the process is still limited, especially for the formation of polymeric and drug nanoparticles. Presented in **Figure 4** is the current state of knowledge on the complexity of particle formation in RESOLV [57]. The process steps and parameters primarily responsible for the particle sizes and morphology are still to be investigated, along with how the nanoparticulate system with a given set of properties is formed in the process. These issues can, to a reasonable extent, be addressed by empirical means, such as by manipulating the various process conditions and by using suitable additive molecules. The solute concentration, pre-expansion temperature and pressure, types of supercritical and receiving solvents and nozzle characteristics and so on, are among the variables and their effects to be examined.

Despite the lack of a clear mechanistic picture, the RESOLV process obviously offers some unique features that are valuable to the preparation of nanoscale systems highly relevant to the drug formulation and delivery. Exclusively nanoscale (< 100 nm) particles can be produced in RESOLV; the nanoparticles can be suspended in an aqueous medium,

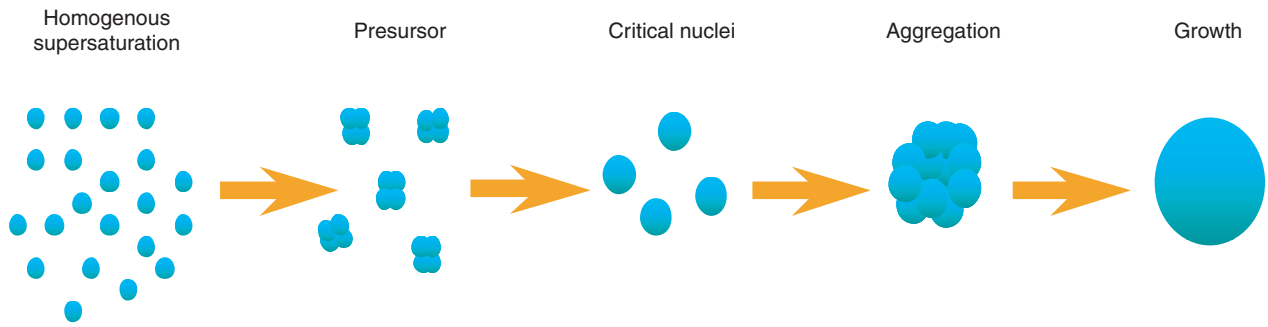

Figure 4. Stages for the formation of nanoparticles via rapid expansion of supercritical solution.

amenable to the use in delivery systems or the coupling with biological species. The process can be kept clean and free from unwanted impurities. Highlighted in Section 3 are examples, results and issues in the use of the RESOLV process for producing polymeric nanoparticles and nanosizing poorly water-soluble or -insoluble drugs.

3. Rapid expansion of a supercritical solution into a liquid solvent for polymeric nanoparticles

Polymeric nanoparticles present an effective and versatile platform for various drug delivery needs [44,47,77]. The traditional RESS process has been particularly popular in the processing of polymeric materials. According to the original report by Krukonis [70], the rapid expansion of a polypropylene solution in supercritical propylene resulted in the formation of fibre-like particles. Since then, particle formation in RESS from a large number of polymeric materials has been reported [80-82]; however, nanosizing the particles has apparently not been a focus of the community. Instead, much effort has been directed towards an understanding of the RESS process for different product morphology, such as particles versus fibres [71,80-82,94,95]. Several research groups also investigated the effect of RESS processing conditions, such as nozzle geometry and pre-expansion temperature and pressure, on the product size and morphology. A more recent emphasis has been on the processing of mixtures to obtain microcapsules or microspheres incorporated with an active ingredient [44,46, 96-98]; for example, Debenedetti reported the encapsulation of anticholestrol drug into polylactic acid, and more recently, Mishima and colleagues reported the formation of microspheres of flavones embedded in an excipient [97,98].

As discussed earlier, the traditional RESS process typically produces micron-sized polymeric particles, with only a few exceptions [40,79-84]. Mechanistic modeling of different solutes suggests that the particle growth, due to efficient condensation and coagulation in the expansion jet, is responsible for the observation of primarily microparticles [40,79-84]. Ginosar and colleagues [84] have shown through experiments that both nano- and micron-sized particles are present in the expansion jet and that the larger particles are found at a distance from the expansion nozzle, consistent with the predicted agglomeration mechanism. In this regard, the receiving liquid in the RESOLV process effectively 'captures' the originally formed nanoscale polymeric particles, overcoming some of the particle growth in the expansion jet to keep the particles exclusively nanoscale (< 100 nm).

The production of polymeric nanoparticles via RESOLV is best demonstrated by the highly CO_2-soluble polymer, polyheptadecafluorodecyl acrylate (PHDFDA) [55]. Experimentally, PHDFDA was dissolved in liquid CO_2 in a syringe pump, and the resulting solution was pressurised and heated to the desired pre-expansion pressure and temperature, and then rapidly expanded through a nozzle (capillary or orifice) into an ambient aqueous medium. With only water at the receiving end, the initially formed PHDFDA nanoparticles quickly agglomerated in the aqueous suspension to form large aggregates. It should be noted, however, that this agglomeration is fundamentally different from the agglomeration and coagulation in the expansion jet in RESS discussed earlier. This is due to the agglomeration occuring after the formation of initial nanoparticle suspension (or understood as being beyond the expansion jet) and, therefore, is on a longer time scale. As a result, the postformation particle agglomeration can be overcome relatively easily by protecting the initially formed suspension with the use of stabilisation agents, a common strategy for colloidal systems; for example, when an anionic surfactant sodium dodecyl sulfate (SDS) was presented even at a relatively low concentration (20 mM), the aqueous suspended PHDFDA nanoparticles could be protected to remain exclusively nanoscale (~ 40 nm in average diameter according to scanning electron microscopy [SEM] analysis; **Figure 5**). Similarly, water-soluble polymers can be effective stabilisation agents for the initially formed aqueous suspension of polymeric nanoparticles in RESOLV. In the presence of polyvinyl alcohol (PVA) in the aqueous receiving solution, as an example, the PHDFDA nanoparticles from RESOLV remained homogeneous without any significant precipitation. An SEM image of the PVA-protected PHDFDA nanoparticles is also shown in **Figure 5**. In fact, nanoparticles of a polymer protected by another polymer of very different solubility

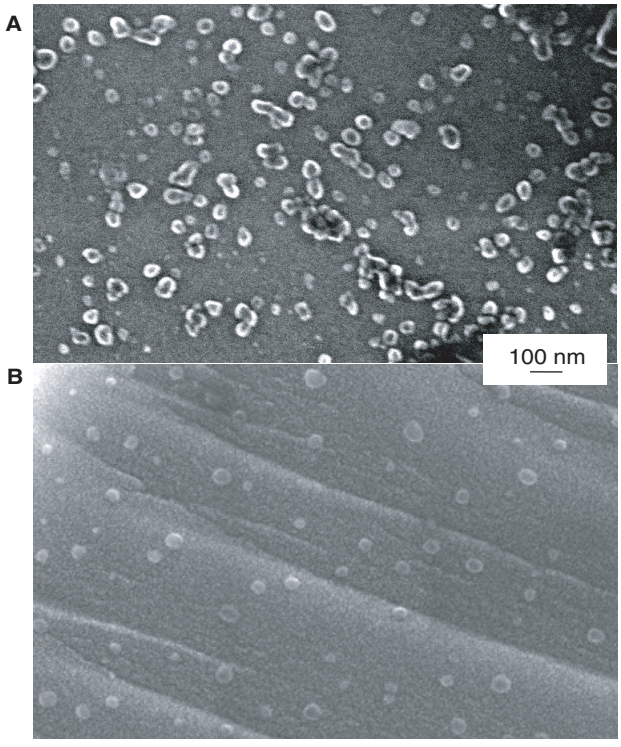

Figure 5. A. Scanning electron microscopy images of polyheptadecafluorodecyl acrylate nanoparticles obtained from rapid expansion of a supercritical solution into a liquid solvent with aqueous sodium dodecyl sulfate solution.
B. Aqueous polyvinyl alcohol solution at the receiving end of the rapid expansion. Reprinted with permission from MEZIANI MJ, PATHAK P, HUREZEANU R, THIES MC, ENICK RM, SUN YP: Supercritical-fluid processing technique for nanoscale polymer particles. *Angew. Chem. Int. Ed.* (2004) **43**(6):704-707.

characteristics represent an interesting nanocomposite configuration. The PVA-stabilised PHDFDA nanoparticles were embedded in a PVA polymer matrix as nanoscale templates, which could subsequently be extracted easily by using supercritical CO_2 to yield nanoporous PVA thin films (**Figure 6**) [57], which is also relevant to delivery applications.

Biocompatible and biodegradable polymers are important to drug formulation and delivery systems; in fact, there is a growing need for more effective and versatile techniques for the processing of such polymers. The RESOLV process has been applied to the preparation of nanoparticles from the biodegradable polymer, poly-l-lactic acid (PLA). The polymer has a low solubility in supercritical CO2 at moderate pressure and temperature, but is insoluble in water. Thus, PLA was dissolved in supercritical CO2 at a higher pressure (550 bar) in the syringe pump to reach the desired solution concentration (0.1 mg/ml). With the rapid expansion at 80°C pre-expansion temperature into an ambient aqueous medium, there was the obvious formation of PLA nanoparticles according to the monitoring by SEM analyses. In addition to the protection of PHDFDA nanoparticles, surfactants and water-soluble polymers were found to be effective in the stabilisation of the initially formed

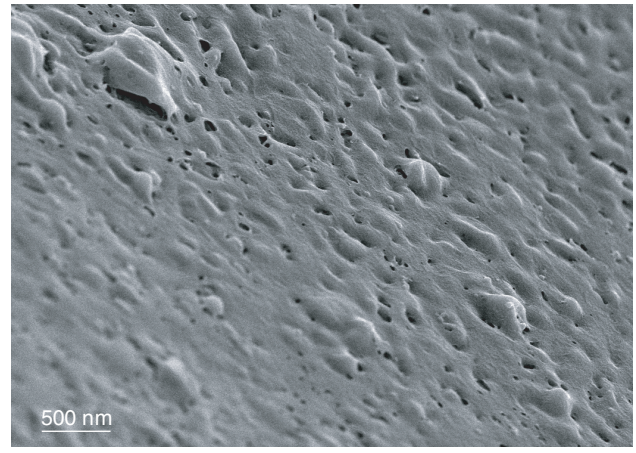

Figure 6. A scanning electron microscopy image of the polyvinyl alcohol film with nanoscale pores after the removal of embedded polyheptadecafluorodecyl acrylate nanoparticles. Reprinted with permission SUN YP, MEZIANI MJ, PATHAK P, QU L: Polymeric nanoparticles from rapid expansion of supercritical-fluid solution. *Chem. Eur. J.* (2005) **11**(5):1366-1373.

PLA nanoparticle suspension; for example, **Figure 7** shows an SEM image of the RESOLV-produced PLA nanoparticles under the surfactant SDS protection.

These examples demonstrate the great potential of RESOLV in the preparation of aqueous suspended polymeric nanoparticles. This is a unique method to produce exclusively nanoscale particles from polymers in a clean and versatile fashion; in fact, when the method was first reported, Dr Mark Lavine highlighted the work in the editor's choice section of *Science*, commenting that 'The RESOLV process could be used to capture and make free-standing polymer nanoparticles with dimensions below 100 nm for applications such as drug delivery' [99]. It is also important that these nanoparticles are produced as a suspension, stable when protected, which offers many subsequent formulation possibilities, such as encapsulating active ingredients, coupling with biological species and incorporating into desired delivery systems.

4. Rapid expansion of a supercritical solution into a liquid solvent for drug nanoparticles

In the formulation and delivery of drugs that are insoluble or poorly soluble in water, particle size reduction has emerged as a promising strategy. Particulate drug delivery systems are useful for administering drugs by various routes, including aqueous suspensions for oral, parenteral, or topical applications, and dry powders for inhalation and other uses [44,100]. The purpose of these delivery systems can be either immediate or sustained release of the therapeutic agent conjugated with a polymer excipient. As the particle size and size distribution of a drug

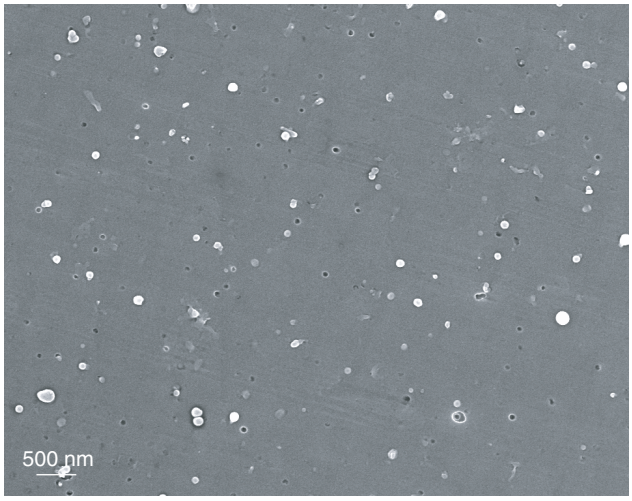

Figure 7. An Scanning electron microscopy image of sodium dodecyl sulfate-protected poly-L-lactic acid nanoparticles produced via rapid expansion of a supercritical solution into a liquid solvent.

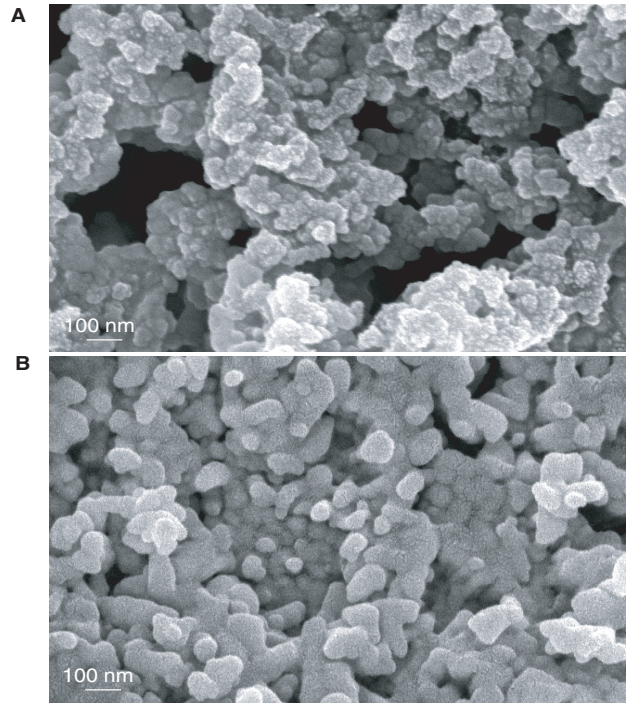

Figure 8. A. Scanning electron microscopy images of ibuprofen. B. Naproxen nanoparticle samples obtained from rapid expansion of a supercritical solution into a liquid solvent with the expansion into water only. Reprinted with permission from PATHAK P, MEZIANI MJ, DESAI T, SUN YP: Nanosizing drug particles in supercritical fluid processing. *J. Am. Chem. Soc.* (2004) **126**(35):10842-10843, copyright (2004) American Chemical Society.

may directly affect its pharmokinetics, it is important to tailor the drug particle size in a dosage to the route of administration. This is an area that has seen extensive research, especially concerning the use of traditional techniques [100,101]. However, it remains a challenge to prepare homogeneously distributed nanoscale drug particles and their stable suspensions in a versatile and controllable fashion; in fact, nanosizing drug particles has implications beyond the water solubility issue. Nanoscale particles are better suited, and sometimes the only option, for intravenous delivery [102-110]. Other widely acknowledged advantages of nanoparticles over microparticles include improved oral bioavailability, higher concentration in suspension, reduced toxicity, higher intracellular uptake, longer retention in tumour tissue, decreased mononuclear phagocytic system uptake, longer circulating capacity in the blood, and higher stability against enzymatic degradation (especially for protein, peptide and nucleic acid drugs) [111-117]. Nanoscale drug particles can also penetrate deep into tissues through fine capillaries, such as liver sinusoidal capillaries, which contain pores with an average diameter of 100 nm [118,119].

The RESOLV technique has demonstrated great potential in the production of exclusively nanoscale drug particles [56]. The initial success has been on the processing of water-insoluble drugs into aqueous suspensions of the drug nanoparticles. Two famous drugs used in the demonstration were ibuprofen and naproxen, which are somewhat soluble in supercritical CO_2 but practically insoluble in water [59,120]. The experimental procedure of RESOLV for the production of drug nanoparticles was similar to that for polymeric nanoparticles (**Figure 3**). For example, the solubility of ibuprofen in supercritical CO_2 under different temperature and pressure conditions was first evaluated in preparation for the RESOLV experiment. In RESOLV, ibuprofen was introduced into the syringe pump to form a solution in liquid CO_2, which was then heated to reach the pre-expansion temperature of 40°C and a pressure of 200 bar. Various expansion nozzles could be used but the expansion through a 50-micron orifice was common and adequate [121]. When the receiving end of the rapid expansion was pure water only, the formation of ibuprofen nanoparticles was followed by agglomeration in the suspension into larger aggregates. The SEM imaging provided the evidence that these aggregates were indeed composed of nanoparticles (**Figure 8**); however, the RESOLV processing of naproxen was similar [56], because the drug is slightly less soluble in supercritical CO_2, methanol in small quantity (2 weight %) was used as a cosolvent [56]. The same formation and agglomeration of naproxen nanoparticles were observed (**Figure 8**).

The drug particles produced in the RESOLV process are exclusively nanoscale, but without protection these suspended nanoscale particles can quickly form larger aggregates and precipitate, similar to many other colloidal systems. Thus, the colloidal stabilisation of the initially formed drug nanoparticles

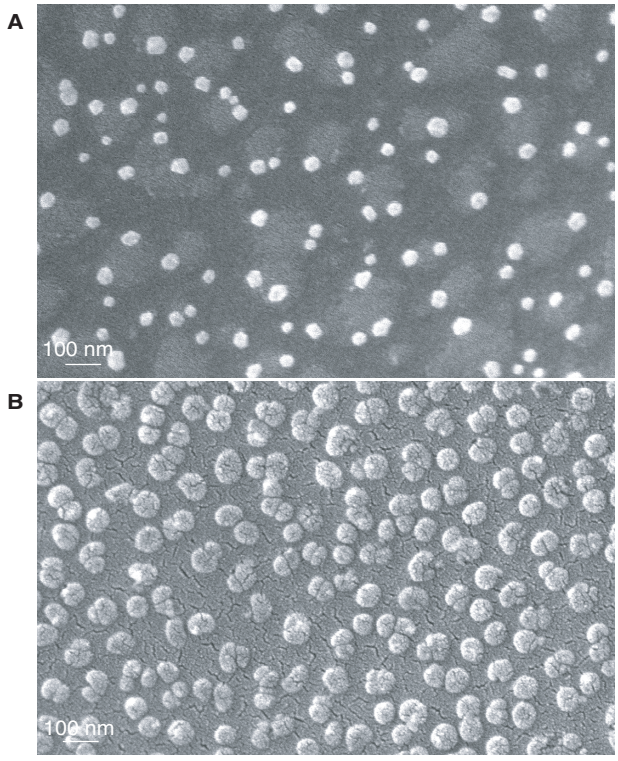

Figure 9. A. Scanning electron microscopy images of the Ibuprofen. B. Naproxen nanoparticle samples obtained from rapid expansion of a supercritical solution into a liquid solvent with aqueous poly-*N*-vinyl-2-pyrrolidone solution at the receiving end of the rapid expansion. Reprinted with permission from PATHAK P, MEZIANI MJ, DESAI T, SUN YP: Nanosizing drug particles in supercritical fluid processing. *J. Am. Chem. Soc.* (2004) **126**(35):10842-10843, copyright (2004) American Chemical Society.

becomes a critical issue. For both ibuprofen and naproxen nanoparticles, the water-soluble polymer poly-*N*-vinyl-2-pyrrolidone (PVP) was found to be an effective stabilisation agent. When PVP was present in the receiving aqueous solution in RESOLV, the suspensions of ibuprofen and naproxen nanoparticles remained stable without the formation of larger aggregates and precipitation [56]. This was confirmed by SEM analyses designed to reflect the dispersion and size distribution of the suspended nanoparticles; for example, as shown in **Figure 9**, the ibuprofen nanoparticles (40 nm average diameter) were relatively narrowly distributed (8.5 nm size distribution standard deviation), as were the naproxen nanoparticles [56]. The aqueous suspensions of the PVP-protected drug nanoparticles appeared homogeneous and remained stable for an extended period of time. It should be noted, however, that because PVP was in the receiving solution during the RESOLV process, it had some effect on the sizes of the initially produced drug particles. This was reflected by the dependence of the average nanoparticle size on the molecular weight of the polymeric stabilisation agent. The use of higher molecular weight PVP for naproxen clearly decreased the average nanoparticle size [56]. Although such a dependence offers additional opportunities to manipulate the size of the drug nanoparticles in RESOLV, it may become an unwanted interference in some cases. Alternatively, the stabilising agent may be added post-expansion instead of being present in the receiving solution pre-expansion. It should, in principle, be a feasible option to protect the initially formed drug nanoparticles by rapidly mixing in the stabilisation agent immediately following the rapid expansion; however, the required experimental setup for such an option will obviously be more complicated.

Many other water-soluble polymers, surfactants and food emulsifiers can also be used to protect the drug nanoparticles produced in RESOLV and stabilise the aqueous suspensions for formulation and other purposes; for example, SDS was found to be an excellent stabilising agent for ibuprofen nanoparticles [56].

Another example for the RESOLV processing of water-insoluble drugs was for amphotericin B, an antiparasitic drug of choice against many severe, life threatening fungal infections [122]. The significant limitation in the clinical administration of this drug is associated with its poor solubility. Other delivery strategies, such as intravenous injection and infusion routes, have also encountered major problems, including considerable fluctuations in blood level and serious side effects such as nephrotoxicity [23,123]. In alternative therapies, new delivery techniques, such as the use of liposomes, emulsions and lipids have been reasonably successful, as measured by the increase in aqueous solubility and reduction in toxic effects, without the loss of antimycotic properties of the drug [124,125]. However, the ability of these delivery vehicles to reach specific tissues is severely limited by issues including recognition and rapid clearance from the circulation, due primarily to the large sizes of the drug particles [22,23,31]. As a result, comparable efficacy can only be achieved at higher than conventional dosages, which is undesirable as the elimination of the drug from the body is known to be very slow [126,127]. The ratio between the dissolution rate and the clearance rate is crucial to the therapeutic applicability of the drug; therefore, an effective and versatile option for increasing the solubility and efficacy of the drug is to nanosize the drug particles [22,23,128].

The RESOLV technique was used to produce nanoscale amphotericin B particles. The experimental procedure was similar to that used in the processing of ibuprofen and naproxen. The drug was dissolved in CO_2 with DMSO as a cosolvent, and the supercritical solution was rapidly expanded into an aqueous solution containing a stabilisation agent; for example, the aqueous suspension of amphotericin B nanoparticles under the protection of a PVA polymer was stable. The result of SEM analysis suggested that the nanoparticles (~ 40 nm average diameter) were size-wise narrowly distributed and also well dispersed in the stabilised suspension.

In the nanosizing of drug particles, the physical parameters and properties of the nanoparticles could be influenced or

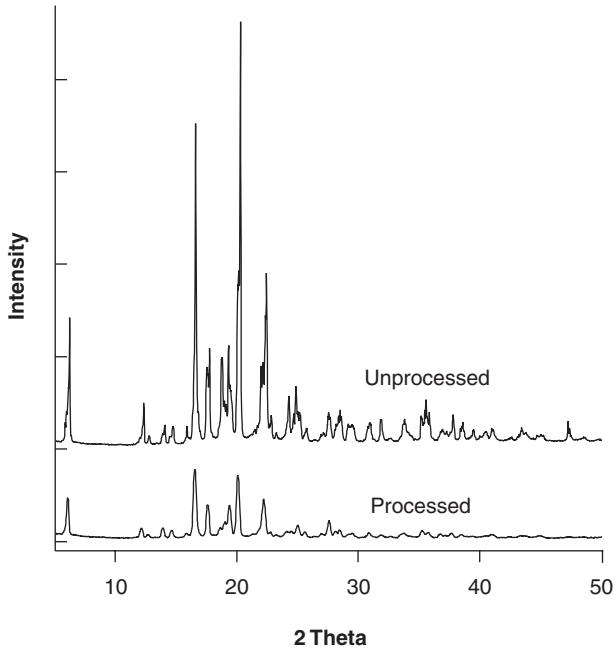

Figure 10. X-ray powder diffraction results of ibuprofen before and after rapid expansion of a supercritical solution into a liquid solvent processing.

manipulated by varying the RESOLV processing conditions; for example, in the production of ibuprofen nanoparticles a change in the pre-expansion temperature from 40 to 120°C increased the average particle size from 40 to 52 nm [56]. Obviously, the ability to control the drug particle size has important implications in drug delivery systems.

The nanosized drug particles from RESOLV were found to be somewhat different from the initial drug samples in their physical properties. According to differential scanning calorimetry (DSC) results, the melting point on their nanosizing, decreased by 2.5 and 5.6°C for ibuprofen and naproxen, respectively, indicating a significant decrease in their degree of crystallinity. The conclusion of lower crystallinity was supported by the X-ray powder diffraction results (**Figure 10**) [120]. In the drug formulation and delivery, it is widely acknowledged that a reduction in the crystallinity of drug particles increases their bioavailability [129,130].

In summary, the RESOLV technique offers a clean way to produce polymeric nanoparticles and to process drugs (especially those that are poorly soluble or insoluble in water) into nanoscale particles. It is a combination of two closely related processes; the formation of nanoparticles exclusively and the protection of the initially formed nanoparticles from agglomerating into large aggregates. The latter represents a different set of technical challenges from those found in the development of supercritical fluid technology; however, because of the unique experimental configuration, where the nanoparticles are formed in a suspension, many known colloidal stabilisation strategies can be applied. In addition, the flexibility of the experimental configuration of RESOLV may also enable the encapsulation of drugs into polymeric nanoparticles as carriers; for example, preliminary results have suggested that 'nanocomposites' of amphotericin B in PLA nanoparticles can be produced by using a supercritical solution of the drug–polymer mixture in RESOLV. There are many opportunities for further investigations.

5. Expert opinion

The supercritical fluid processing techniques offer alternative, or even unique, solutions to many problems in drug formulation and delivery, especially with respect to the production of micron-sized and nanoscale drug particles and related systems. Examples for the attractive and advantageous features of these techniques for particle formation include, amongst others, moderate operation conditions, low levels of residual solvents and products with targeted properties. As highlighted, SAS and RESS methods have attracted considerable fundamental and applied research effects in addition to applications at a primarily laboratory scale for a wide range of drugs and drug-embedded polymeric particles. The SAS method is often preferred in the processing of drugs and polymers with limited solubilities in supercritical CO_2, whereas the RESS method is more advantageous for being single step, a relatively low processing temperature and much reduced organic solvent usage. The applicability of the RESS method will likely be enhanced by a better understanding of the drug solubility in supercritical CO_2, the use of cosolvents and the investigation of other environmentally acceptable supercritical solvents.

The RESOLV method has already demonstrated great potential in the nanosizing of drug particles, especially those drugs with little or no solubility in water. The seemingly simple difference of RESOLV from RESS is particularly significant to the processing of drugs. The product from RESOLV is in the form of a suspension, which not only makes it possible to protect the drug particles from agglomeration, but also offers much needed flexibility in the further processing of drug particles for their coupling with biological species and for specific formulation and delivery systems. Another unique feature is that the RESOLV method produces exclusively nanoscale drug particles.

There are still enormous challenges in the supercritical fluid processing of drug particles and related systems for formulation and delivery applications. Issues and opportunities, such as the manipulation or even control of particle morphology, size, dispersity and crystallinity, remain to be explored and investigated. There is also the need to direct attention toward the modeling and mechanistic understanding of the particle formation in the supercritical fluid processes, in addition to the influence of processing parameters and conditions on the product properties. As most of the existing applications are only at small

laboratory scale, investigations on scale-up and other operational variables are required. Nevertheless, the authors are optimistic that supercritical fluid processing will play an important and valuable role in drug formulation for enhanced delivery applications.

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