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Review

Dense gas processing of polymeric controlled release formulations

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Abstract

Dense gas techniques, which utilize the properties of fluids in the vicinity of their critical points, are of increasing interest in the processing of pharmaceuticals. It is generally known that dense gases can be used for extractions, chromatographic separations and chemical synthesis due to their liquid-like solvation power and gas-like mass-transfer properties. The processes can be conducted at moderate operating conditions and are thus suitable for many heat-labile compounds such as proteins, biocompatible polymers and pharmaceuticals. Recent applications of dense gas techniques include micronization, crystallization of high-purity particles, sterilization and preparation of microencapsulated drug formulations. The following paper provides a brief overview of dense gases and various processing techniques to fabricate polymeric drug-loaded formulations for controlled release purposes.
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Keywords: Supercritical fluid; Dense gas; Controlled release; Microencapsulation; Particle formation

Contents

1. Introduction

A pure substance generally exists as either a solid, liquid or gas phase, which are clearly defined by phase boundaries, as shown in Fig. 1. Based on this pressure–temperature diagram for a pure substance, various phases can exist up to the critical point for all molecules. As pressure and temperature increase above the critical point, the liquid and gas phases become indistinguishable. Beyond the critical point, the substance exists as a supercritical fluid (SCF), which is a homogeneous medium. Therefore, it is possible to construct a path from point A to point B to induce a phase change from liquid to gas without passing through a distinct phase transition. A substance close to, but not necessarily above, its critical point is referred to as a dense gas (DG). The properties of DGs can vary widely and are generally

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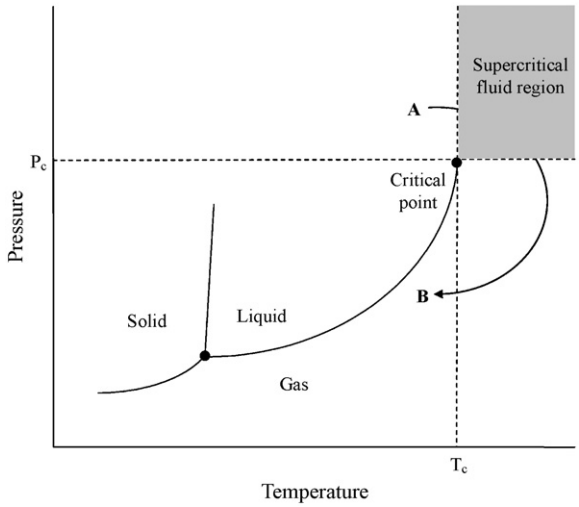

Fig. 1. Pressure–temperature diagram of a pure substance.

intermediate to those of gases and liquids. The density of a DG is generally closer to that of conventional liquids and several orders of magnitude higher than that of conventional gases and accounts for the solvating capabilities of the DGs.

The interest in DGs and their potential use for process improvements has significantly increased in the past decade. Due to the properties of these fluids, which can be tuned by small changes in pressure or temperature, DGs have potential as alternative media for classical separation processes and as reaction environments. Several conventional techniques, such as spray drying (Broadhead et al., 1992; Masters, 1979), emulsion–solvent extraction (Puisieux et al., 1994; Chasin and Langer, 1990; Bakan, 1994) and processes based on cavitation, attrition, high shear and impaction (i.e. high-pressure homogenization, microfluidization, media milling, air-jet milling and ball milling) (Byers and Peck, 1990; Rubinstein and Gould, 1987; Parrott, 1990; Aiache and Beyssac, 1994; Illig et al., 1996), have been utilized for particle processing. However, these processes may often incur undesired effects such as thermal and chemical degradation, inner-batch particle size variability and broad size distribution. Other disadvantages of some conventional particle processing techniques include the need for additional stages for the extraction of residual solvent and the extensive use of organic solvents, which may result in health and environmental concerns. Therefore, the limitation of conventional particle processing techniques has driven the focus of studies towards the potential replacement of traditional organic solvents with more environmentally friendly materials, such as DGs.

One of the applications of DG techniques in particle processing is the preparation of microencapsulated drug formulations. Microencapsulation can be defined as a process of coating or entrapping micron-sized material with another material without chemical interaction. Microencapsulation is often used to provide a protection for a drug from the reactive surroundings and to prevent drug degradation from light or exposure to oxygen. Furthermore, microencapsulation can be used to improve the formulation characteristics such as taste, stability and wettability. Microencapsulated formulations can also be used to extend the dosage time from a repeated to single administration and to provide controlled release of drugs in a desired part of the body. Therefore, microencapsulated drug formulations can be pharmaceutically beneficial since a drug is generally more effective for long-term treatment and exhibits fewer side effects when the drug concentration in the body is kept constant at some optimum level over the duration of therapy.

In this review, the concepts and parameters involved in the microencapsulation of pharmaceutical controlled release formulations and the utilization of different DG techniques for the preparation of microencapsulated drug formulations are discussed.

2. Microencapsulation in controlled release formulations

There have been significant developments in controlled release administration in the last three decades from primitive delayed-release dosage forms in the 1960s to highly sophisticated self-regulated delivery systems in the 1990s (Park, 1997). These advances have produced many clinically useful controlled release dosage forms and provided better shelf-life for many existing drugs. The market of pharmaceutical controlled release formulations is expected to rise exponentially from US$ 19 billion in 2000 to nearly US$ 42 billion by 2007 (Frost & Sullivan, 2001). The unveiling of the human genome and discovery of gene therapy for diseases are expected to surpass the development of drug delivery technologies to create novel pharmaceutical products (Frost & Sullivan, 2001). Consequently, research has been directed to the development of technologies for the production of controlled release formulations (Park and Mrsny, 2000).

Controlled release drug formulations can be described as formulations intended to provide temporal or spatial control of drug release in the body. Conventional drug administration, such as pills and tablet dose forms, may provide a single burst of drug concentration in the blood followed by a reducing drug concentration. In general, it is desirable to minimize the fluctuation of drug concentration in the blood and release the drug at a desired controlled rate. The therapeutic action of a drug is thus improved by controlled release behaviour by enhancing the durability and effectiveness of the drug in the body.

Controlled release formulations are commonly prepared with the use of microspheres or microparticles of drug–polymer composites where the active ingredient is distributed in the polymer matrix. In general, when biodegradable and bioerodible polymers are used in controlled release formulations, the polymers are degraded or eroded to non-toxic materials by body fluids over time. Thus, the removal of polymer material from the body after the drug release is unnecessary (Yolles et al., 1971; Frazza and Schmitt, 1970).

As illustrated in Fig. 2, controlled release systems can be categorized into two basic types, the matrix system and the core-and-shell system. In the matrix system, drug particles are distributed within the polymer matrix and the rate of drug release is determined either by the degradation of the polymer (erosion)

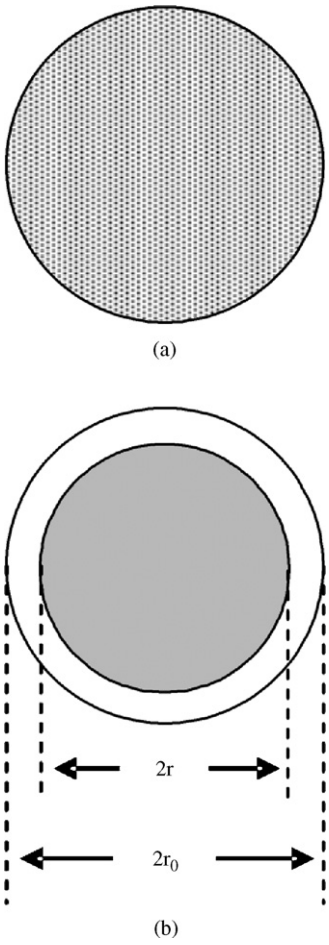

Fig. 2. Classification of controlled release system: (a) matrix system (b) core-and-shell system.

or the diffusion of the drug through the matrix. If the drug particles are distributed in the polymer matrix so that the diffusional release is slower than the erosion rate of the matrix, the release profile will be erosion dependent. If the drug release is diffusion dependent, the standard diffusion equations can be used to describe the release profile. In the core-and-shell system, the drug phase is contained within a rate-limiting polymeric phase. The release rate of the drug is then determined by the drug diffusion through the polymeric membrane surrounding.

The design of a controlled release system is a complicated process due to the interdependency of several factors, such as drug properties, route of administration and mechanism of release (Fig. 3) (Park and Mrsny, 2000). A drug may be released at zero-order rate from the ideal core-and-shell system providing the drug concentration remains constant and the polymeric wall erodes long after the drug has been released. The release rate is also affected by the changes of physical properties such as wall thickness and porosity. The coating thickness can be adjusted from nano- to micron-sized by changing the concentration of coating material from 3 to 30% of the total weight (Park, 1997). It is also possible for the polymeric membrane to rupture in the early stages, which causes dose dumping and rapid drug release into the circulation. The reservoir system is considered an ideal option if the polymer can be fabricated to degrade after the drug release. In general, the coating thickness and particle diameter increase with increasing drug concentration. In more advanced design, the application of several layers of polymer can be used for pulsed dosing at predetermined times (Park, 1997; Park and Mrsny, 2000).

Depending on the formulations, basic controlled release systems can also be modified into cylindrical (and planar) matrix devices or osmotic (and other membrane) systems. Among the above-mentioned controlled release systems, the core-and-shell controlled release system is the most common.

3. Polymers in controlled release formulations

Particle encapsulation has been demonstrated to be of particular interest in the control of drug release and it offers some advantages such as protection of bioactive agents from enzymatic degradation. The concept of incorporating polymers for controlled release was developed in the early 1970s when it was discovered that the then available forms of delivery using silicone rubber and polyethylene left foreign material in the body even after surgical removal of the device (Dunn and Ottenbrite, 1991). Only a few immunologically tolerable biodegradable natural polymers, including collagen and gelatin, were available and used for encapsulation at that time. Various polymers such as biodegradable, non-biodegradable, water-soluble and smart polymers have now been widely used in controlled release systems (Frazza and Schmitt, 1970; Dunn and Ottenbrite, 1991; Pitt et al., 1979; Bodmeier et al., 1989; Galaev and Mattiasson, 1999; Barker et al., 2000; Kikuchi and Okano, 2002; Ista and Lopez, 1998; Kim et al., 2003; Schild, 1992; Arvidsson et al., 2001; Donini et al., 2002).

- *Biodegradable polymers*, such as polyorthoesters, polyesters, polyamides and polyanhydrides, are synthetic or natural polymers that degrade and form non-toxic, or biocompatible, by-products in the body. Additional requirements for acceptable biodegradable polymers for controlled release systems include degradation to non-toxic metabolites, absence of impurities and ease of processing. Cyclazocine, which is made from poly(L-lactic acid), was the first synthetic biodegradable polymer used in parenteral administration with the implant method (Frazza and Schmitt, 1970). It was reported that the major drawback of the implant administration was the lengthy in vivo degradation time of several years. An absorbable compound of poly(glycolic acid) with a more acceptable period for complete in vivo degradation in 60 days was then developed by Frazza and Schmitt (Dunn and Ottenbrite, 1991). Following these early studies, many bioactive agents including anticancer agents, narcotic antagonists and steroids fabricated with various biodegradable polyesters have been generated into microparticles for a wide range of release behaviour (Pitt et al., 1979; Bodmeier et al., 1989).
- *Non-biodegradable polymers*, polymers which remain inert in the body for an indefinite time and are suitable for long term and extended drug delivery. Examples of non-biodegradable polymers are silicone, polyurethane and cellulose acetate.

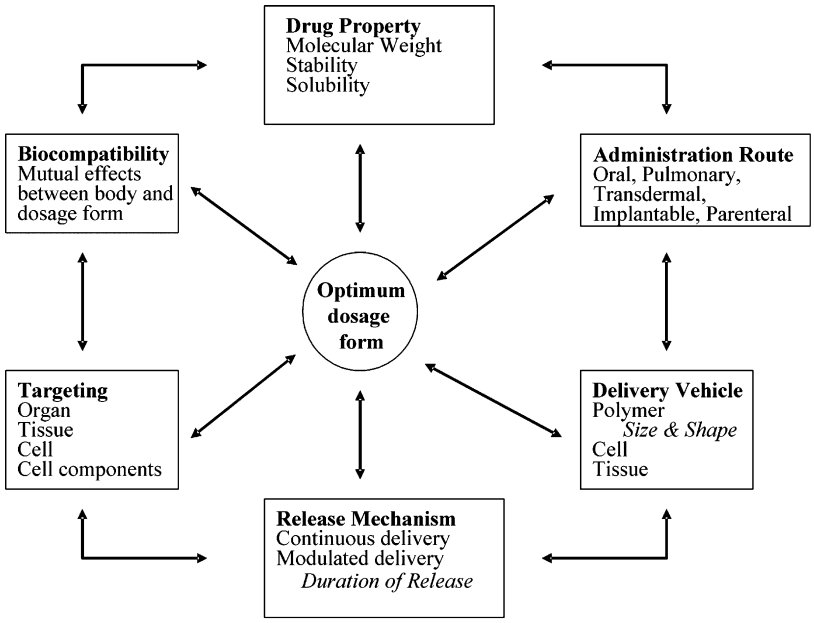

Fig. 3. Important interdependent factors in the design of controlled release systems (Park and Mrsny, 2000).

- *Water-soluble polymers*, such as polyethylene glycol, polyethyleneimine and polyacrylic acid, are polymers which can be solubilized in body fluids without any chemical degradation. Water-soluble polymers may undergo simple hydration, ionization or protonation and dissolve in the biological environment. Gelatin, starch and dextran are other examples of water-soluble polymers which are suitable for short-term (several hours to days) drug delivery due to their rapid dissolution in the biological fluids.
- *Smart polymers*, are synthetic polymers which are responsive to external stimuli. The most common smart polymers generally undergo conformational or phase changes in response to the variations in temperature and/or pH (Galaev and Mattiasson, 1999). Smart polymers have been developed and widely used in a range of applications such as microfluidic devices (Barker et al., 2000), pulsatile drug release systems (Kikuchi and Okano, 2002), bioadhesive mediators (Ista and Lopez, 1998) and nanoscale technologies (Kim et al., 2003). Other examples of smart polymers include poly(*N*-isopropylacrylamide) (Schild, 1992), poly(*N*-vinyl caprolactam) (Arvidsson et al., 2001), poly(acrylic acid) and poly(methacrylic acid) (Donini et al., 2002). Swelling and shrinking of the structure of smart polymers can be applied to pharmaceutical formulations to control the release of bioactive agents by subjecting the polymers to external stimuli such as pH or temperature. When a smart polymer is integrated as a coating agent or microcapsule wall, the conformational transition of a polymer structure can be utilized to allow drug release from the microcapsule (Galaev and Mattiasson, 1999).

4. Preparation of controlled release formulations

In recent years, many studies have been conducted to develop controlled release systems with optimum characteristics and performance. Manufacturing processes for the preparation of polymeric controlled release systems, which include conventional and novel processing techniques, have been evaluated over the years. In fact, conventional processes for the preparation of controlled release systems are still widely used by several pharmaceutical companies nowadays. However, due to the limitations of conventional processes (Leroux et al., 1996; Cohen et al., 2000), the alternative approach using DG techniques have become popular in the preparation of controlled release formulations with optimum characteristics and performance (Jung and Perrut, 2001; Reverchon, 2002; Reverchon et al., 1998; Subramaniam et al., 1997a; Chattopadhyay and Gupta, 2002; Yeo et al., 1993; Winters et al., 1996; Kim et al., 1996; Mishima et al., 2000; Tsutsumi et al., 1995; Wang et al., 2002; Falk and Randolph, 1998; Young et al., 1999; Elvassore et al., 2001a). Thus, the DG-processed products can be extended to pharmaceutical applications since they are solvent free and are of high-purity (Jung and Perrut, 2001; Reverchon, 2002; Reverchon et al., 1998; Subramaniam et al., 1997a).

The pharmaceutical industry currently experiences the challenge of drug formulations which involves active ingredients with low solubilities, high toxicities and low stabilities. The increasing interest in advanced controlled release formulations and the combination of their characteristics provide the increasing potential of DG technology in the pharmaceutical industry. The preparation of controlled release formulations using DG processing techniques offers several unique features such as the absence of moving parts in the system, the ability to perform the process in a single stage without any exposure of the products to the external environment, the use of high-grade stainless steel equipments and the inherent sterilizing effects (York, 1999). Furthermore, the DG technology may facilitate the development of significant improvements in the efficiency of both currently marketed drugs and new therapeutic entities.

In the formation of controlled release systems, DGs can be used as a solvent, solute, antisolvent and aerosolization aid depending on the desired characteristics of the products. The basic principles of DG particle processing techniques used in the production of drug-loaded polymeric systems are co-precipitation, particle coating and polymer impregnation. In the co-precipitation process, the drug, polymer and other ingredients are dissolved in a solvent to form a solution and then precipitated from the solution. However, the system may become complex in the presence of multiple solid phases upon phase separation. In the particle coating process, pure polymers are generally dissolved in a DG and sprayed onto the surface of a drug to form a thin polymeric coating layer. In the polymer impregnation process, on the other hand, the drug-saturated DG is passed through polymeric matrices and the drug is thus impregnated in the matrix.

Various DG processing techniques such as gas anti-solvent (GAS) (Chattopadhyay and Gupta, 2002; Yeo et al., 1993; Winters et al., 1996), rapid expansion of supercritical solution (RESS) (Kim et al., 1996; Mishima et al., 2000; Tsutsumi et al., 1995; Wang et al., 2002) and aerosol solvent extraction system (ASES) (Falk and Randolph, 1998; Young et al., 1999; Elvassore et al., 2001a) have been used to prepare controlled release systems of various drug formulations. Various drug/polymer systems prepared using different DG particle processing techniques are listed in Table 1 and the detail of each technique is discussed in the subsequent sections.

4.1. Gas anti-solvent (GAS)

The GAS process, which generally involves the use of DG and organic solvent as the anti-solvent and solvent respectively, can be used to recrystallize solid compounds that are insoluble in DGs. In the GAS process, the anti-solvent is gradually added into a high-pressure chamber containing a solute-laden solution. The solution expands and the consequent reduction in solvating power of the solvent results in supersaturation of the solution and subsequent solute precipitation (Fig. 4a). The expanded solvent is then purged out of the system and the precipitate is flushed with pure DG or SCF to remove the residual solvent. The ability of the DG to expand organic solvents and precipitate dissolved solutes from organic solutions was first investigated by Gallagher et al. (1989). Since then, the GAS process has been utilized for various applications including crystallization of drugs and polymeric microspheres (Ghaderi et al., 2000), explosives (Gallagher et al., 1992), purification of organic acids (Shisikura et al., 1994) as well as fractionation and purification of polymeric absorbents (McHugh and Krukonis, 1994).

Table 1
Drug/polymer formulations processed by various DG techniques

Process	Drug	Polymer	Solute	Solvent	Anti-solvent
GAS	Insulin (Elvassore et al., 2001a)	L-PLA, PEG	–	DMSO, DCM	CO_2
ASES	Hydrocortisone (Ghaderi et al., 2000)	D,L-PLGA	–	DCM, acetone, hexane	N_2, CO_2
	Diuron (Taki et al., 2001)	L-PLA	–	DCM	CO_2
	Insulin (Elvassore et al., 2001a)	L-PLA	–	DMSO, DCM	CO_2
	Lysozyme, *p*-HBA (Sze Tu et al., 2002)	L-PLA	–	Methanol, DCM	CO_2
	Cholesterol (Vega-Gonzalez and Subra, 2003)	PMMA, PCL	–	DCM	CO_2
	Theophylline (Grassi et al., 2003)	HPMC	–	DCM, ethanol	CO_2
	Hyoscine butylbromide (Bleich et al., 1994)	L-PLA	–	Methanol, DCM	CO_2
	Rifampin, natrexol (Falk and Randolph, 1998)	L-PLA	–	DCM	CO_2
	Albumin, estriol (Engwicht et al., 1999)	L-PLA	–	Methanol, DCM	CO_2
RESS	Lipase, lysozyme (Mishima et al., 2000)	D,L-PLGA, PMMA, L-PLA	–	CO_2/ethanol	–
	Acetylsalicylic acid, flavone (Matsuyama et al., 2003)	PEG, PMMA, ethyl cellulose	–	CO_2/ethanol	–
	Albumin (Dos Santos et al., 2003)	Lipids	–	CO_2	–
	Distyrylpyrazine (Pestov et al., 2003)	Polyacrylate	–	Chlorodifluoromethane	–
	Lovastatin (Tom et al., 1993)	D,L-PLA, poly(hydroxy acid)	–	CO_2	–
	Naproxen (Kim et al., 1996)	L-PLA	–	CO_2	–
PGSS	Ribonuclease A, β-D-galactosidase (Howdle et al., 2001)	D,L-PLA, PLGA, PCL	CO_2	–	–
Impregnation	Naproxen (Duarte et al., 2006)	Ethyl cellulose, methyl cellulose	–	DCM, DMSO, acetone	CO_2
	Nimesulide, piroxicam (Alessi et al., 2003)	PVP, PDMS	–	–	CO_2
	Indomethacin (Liu et al., 2005)	D,L-PLA, PLGA, PEG	–	Acetone	CO_2
Miscellaneous	Indomethacin, ketoprofen (Chattopadhyay and Huff, 2005)	Eudragit® RS, D,L-PLGA	–	Water, PVA, ethyl acetate	CO_2
	BSA (Ribeiro Dos Santos et al., 2002)	Dynasan® 114 and Gelucire® 50-02	–	CO_2	–

Note: L-PLA, poly(L-lactide acid); PEG, poly(ethylene glycol); D,L-PLGA, poly(D,L-lactic glycolic acid); PMMA, poly(methyl methacrylic acid); PCL, poly(caprolactone), HPMC, hydroxypropylmethylcellulose; *p*-HBA, *para*-hydroxybenzoic acid; BSA, bovine serum albumin; PVP, poly (vinyl pyrollidone); PDMS, poly(dimethylsiloxanes); DMSO, dimethylsulfoxide; DCM, dichloromethane; PVA, poly(vinyl accohol); CO_2, carbon dioxide; N_2, nitrogen.

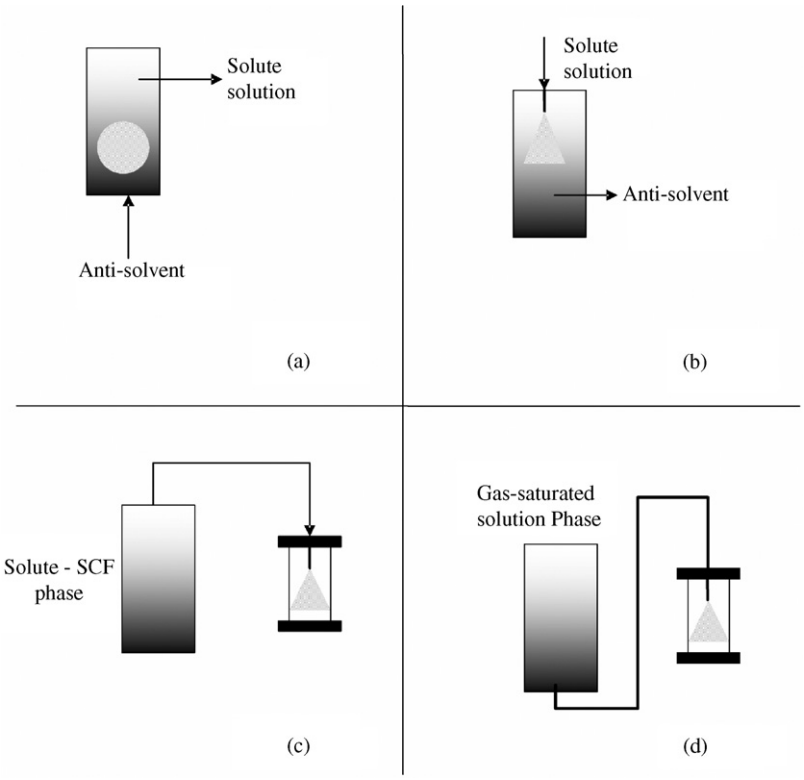

Fig. 4. Schematic diagram of particle processing by various SCF techniques. (a) GAS, (b) ASES, (c) RESS and (d) PGSS.

In principle, drug-loaded polymeric formulations for controlled release purposes can be fabricated using the GAS process in two different modes: (1) coating of pre-formed particles; (2) co-precipitation of the active ingredient and polymer. In both cases, drug-polymeric controlled release systems (i.e. matrix or core-and-shell system) can be formed upon recrystallization of the polymer during expansion.

The GAS technique is based on the anti-solvent effect of compressed gas and can be used to prepare hydrophobic polymeric materials containing hydrophilic and thermo-labile drugs, such as insulin, due to the mild operating temperature in the process (Ghaderi et al., 2000). Elvassore and co-workers studied the production of insulin-loaded poly(lactic acid) (PLA) microparticles using the GAS technique. In this study, insulin and PLA were dissolved in dichloromethane (DCM) and dimethylsulfoxide (DMSO), to which the DG CO_2 was added (Elvassore et al., 2001b). The thermodynamic aspects of the system were also studied by measuring the liquid solvent expansions and the precipitation pressures. It was reported that the precipitation of insulin and PLA was mainly caused by the presence of DMSO in the DCM/DMSO mixture and its corresponding thermodynamic properties (Ghaderi et al., 2000).

4.2. Aerosol solvent extraction system (ASES)

The ASES technique was first developed by Müller and Wassmus (1989) as an alternative technique for the formation of polymeric drug delivery systems and it has been extended to the encapsulation of various pharmaceutical compounds. The ASES technique is a continuous crystallization process that utilizes DGs as the anti-solvents to induce solute precipitation from a solution (Fig. 4b). The ASES process can be generated in different modes: (1) suspension of drug particles in polymer solution; (2) solution containing the drug and polymer; (3) the drug and polymer are separately dissolved in two different solvents and both solutions are simultaneously contacted with the DG anti-solvents. The solute-laden solution is either co-currently or counter-currently introduced through a nozzle into the DG anti-solvent. The solute-laden solution is dispersed as fine droplets by the spraying device, which facilitates the intimate mixing with the anti-solvent and the generation of very fine particles with narrow particle size distribution. The optimization of the operating parameters, such as nozzle geometry and orientation, operating pressure and temperature and solute concentration, offers more flexibility in the ASES process compared to the other precipitation techniques.

Insulin-loaded poly(ethylene glycol) (PEG)/PLA nanoparticles with different molecular weight (MW) of PEG and PEG/PLA ratio were produced by Elvassore et al. (2001b). The preparation of nanoparticles involved the addition of DCM solution containing PLA into the DMSO solution containing insulin or insulin/PEG. The resulting solution was then sprayed co-currently using a high-performance liquid chromatography (HPLC) pump through a nozzle into the high-pressure vessel containing CO_2 anti-solvent. Nanospheres with average particle diameter of 400–600 nm and narrow particle distribution were produced. Drug loading of more than 90% was obtained although only 80% of the biological activity of insulin was retained after ASES processing. In vitro dissolution studies showed that the resulting microencapsulated insulin/PEG/PLA

exhibited a slow but constant insulin release for more than 60 days.

Another co-precipitation process of herbicide agent, diuron, and L-PLA using the ASES technique was studied by Taki et al. (2001). The diuron and L-PLA were dissolved in DCM and the resulting solution was sprayed into a CO_2 anti-solvent media through a capillary nozzle. It was reported that high concentrations (~0.9 wt%) of diuron resulted in an early precipitation of diuron as long needles while a solution containing 0.1 wt% of diuron produced microspheres. The diuron (0.1 wt%) and L-PLA (1 wt%) compounds in DCM solution were precipitated as core-and-shell system microspheres with average particle diameter of 3 μm. The energy dispersive X-ray studies showed that diuron was entrapped inside the spherical particle of L-PLA with high drug loading characteristic. Moreover, the presence of chlorine arising from DCM in the microencapsulated particles was undetected by the energy dispersive X-ray analysis.

Wang and co-workers studied the use of hydrocortisone and PLGA as a model microencapsulated system and investigated the process parameters affecting the product characteristics. Hydrocortisone particles were suspended in poly(lactic glycolic acid) (PLGA)-DCM solution and sprayed into the high-pressure CO_2. Higher encapsulation efficiency was achieved with lower drug to polymer ratio (Wang et al., 2004). The polymer concentration affected the agglomeration behavior of coated particles. A higher polymer concentration leads to higher particle agglomeration and difficulty to achieve uniform polymer coating. Lower polymer concentration (i.e. in the order of 4.0 mg/ml) minimized the agglomeration of coated particles. Apart from the polymer concentration in the solution, particle agglomeration was also attributed to the operating pressure and temperature. If the glass transition temperature (T_g) of a polymer is depressed, the coated particles tend to agglomerate due to the higher pressure. When the operating temperature is above the T_g, the polymer coating on the particles surface appears to be sintered and leads to severe agglomeration. Operating temperatures below the T_g reduced particle agglomeration (Wang et al., 2004).

The ASES system can be modified by the incorporation of an ultrasonic nozzle, as has been developed by Subramaniam and co-workers. The ultrasonic nozzle is particularly effective in minimizing particle aggregation, especially when a solution containing suspended particles is used (Subramaniam et al., 1997b, 1998). The ultrasonic waves, having a frequency between 10 and 100 kHz, can be generated by a specially designed ultrasonic coaxial spray nozzle. In the ultrasonic coaxial nozzle, the solute-laden solution is contacted with the energized antisolvent. A spray of very fine droplets, which increases the total interface area within the solution and the anti-solvent, can be obtained. Furthermore, the ultrasonic nozzle favors the formation of smaller particles compared to traditional expanding devices (Subramaniam et al., 1997b). The efficiency of the ultrasonic nozzle has been reported by Subramaniam and co-workers. The characteristics of the anti-inflammatory drug, hydrocortisone, precipitated from DMSO using CO_2 as the antisolvent, were dramatically affected by spraying the solution through an ultrasonic nozzle. Needle-shaped particles with a length of 1 mm were generated using a traditional nozzle whilst 600-nm spherical particles were generated when ultrasonic nozzle was used (Subramaniam et al., 1998).

One of the benefits of the ASES process over conventional processes is the elimination of a separate stage for solvent removal, as the separation stage is easily carried out by flushing the precipitates with a fresh anti-solvent. Falk and Randolph (1998) investigated the effect of process parameters, such as the anti-solvent flowrate and post-precipitation anti-solvent flowrate and volume, on the residual DCM in gentamycin-loaded PLA particles. The residual solvent in the final products was minimized by increasing the anti-solvent flowrate during precipitation as well as post-precipitation anti-solvent flush volume. Conversely, higher residual solvent was obtained when the anti-solvent flowrate during the washing stage was increased.

4.3. Rapid expansion of supercritical solutions (RESS)

The production of microencapsulated formulations containing polymer and active ingredient can be achieved by the RESS process, providing both materials are soluble in a SCF. In the RESS process, the solutes are dissolved in SC media to form a solute-laden SC solution. The SC solution is then subjected to rapid expansion across a micron-sized capillary nozzle (Fig. 4c). The density and solvation power of the SCF dramatically decrease due to the rapid expansion resulting in a high degree of solute supersaturation and subsequent particle precipitation. The main characteristic of the RESS process is the combination of the high supersaturation ratio and rapid propagating mechanical perturbation (Tom et al., 1993). The rapid mechanical perturbation creates uniform conditions within the nucleating media, resulting in particle formation with narrow particle size distribution. The product characteristics are affected by various operating parameters such as solute concentration, pre-expansion temperature and pressure, as well as nozzle geometry.

Naproxen-loaded L-PLA microparticles have been produced using the RESS process by Kim et al. (1996). The extraction pressure and pre-expansion temperature used were varied at 170–200 bar and 90–115 °C respectively. The particle size of naproxen-loaded L-PLA microparticles was 2–5 μm with particle agglomeration up to 90 μm. The RESS process may be considered less economical as most polymers exhibit poor solubility in SC media at temperatures and pressures below 80 °C and 200 bar respectively. The exposure to a very high temperature may also be unsuitable for thermo-sensitive pharmaceuticals.

The production of protein-loaded microparticles by RESS-based enhanced mixing and spraying has been studied by Whitaker et al. (2005). It has been demonstrated that plasticized D,L-PLA and proteins, such as ribonuclease A, lysozyme, insulin and calcitonin, can be sprayed to form solid polymer particles that encapsulate the proteins (Whitaker et al., 2005). In this study, a helical impeller was installed inside the vessel to enhance the mixing prior to spraying to the precipitation chamber. The process relies on the ability of SC CO_2 to plasticize amorphous polymer, to depress the glass transition temperature of the polymer and to form a molten polymer phase containing large amounts of dissolved CO_2. The mixing-enhanced RESS process was able to produce microencapsulated particles rang-

ing in size from 10 to 300 μm. Furthermore, the proteins were successfully encapsulated in the D,L-PLA matrix while retaining the biological activity of the proteins after the fabrication of the microparticles.

A modification of the RESS process with non-solvent (RESS-N) containing solutes has been reported recently by Matsuyama et al. (2003). In general, the RESS-N utilizes a suspension of solute in CO_2 containing a co-solvent and dissolved polymer, which is sprayed through a nozzle to atmospheric pressure. In their study, the RESS-N process was applied to the formation of polymeric microcapsules (i.e. PEG, PLA) containing drugs such as *p*-acetamidophenol, acetylsalicylic acid, 1,3-dimethylxanthine, flavone and 3-hydroxyflavone. The drug suspension in CO_2 containing co-solvent (i.e. ethanol, methanol, 1-propanol, acetone and toluene) and dissolved polymer was sprayed to the atmospheric pressure. The primary particle diameter of the microencapsulated drugs processed by the RESS-N technique was in the range of 7–55 μm. Matsuyama and co-workers suggest that the RESS-N process is advantageous since: (1) the low solubility of polymer in pure CO_2 is significantly increased by the addition of low MW alcohols as co-solvents; (2) the adhesion within microparticles is minimized since remaining co-solvents in the mixture are non-solvents for the product and can be removed by the evaporation of CO_2; (3) the coating thickness can be controlled by adjusting the drug to polymer feed ratio.

The RESS process can be beneficial for the manufacturing of protein-loaded polymeric systems since it is a solvent-free technique and is able to generate materials with various density and surface roughness by varying in the kinetics of SCF evaporation (Whitaker et al., 2005). Moreover, the RESS process may be the most convenient SCF particle processing technique that simultaneously offers particle size reduction and impregnation without the concerns of residual organic solvent. However, the application of the RESS process is limited due to the difficulty of attaining reasonable solubilities of both materials in a single SCF media. There are also some difficulties in controlling the degree of incorporation of the drug into the polymer matrices in a complex situation where the RESS process is utilized for the production of drug-loaded polymeric particles. As a consequence, careful manipulation of operating parameters during the rapid particle formation stage may be necessary to avoid the separate formation of drug and polymer materials (Yeo and Kiran, 2005; Matsuyama et al., 2003).

4.4. Particles from gas-saturated solutions (PGSS)

In the PGSS technique, which was first developed in 1994 by Weidner et al. (1996), the SCF is dissolved in a solute matrix resulting in viscosity reduction and melting point depression of the solute. A gas-saturated solution is thus formed as the gas concentration in the molten solute increases with increasing pressure. Similar to the RESS technique, the gas-saturated solution is then expanded through a capillary nozzle to induce the particle precipitation (Fig. 4d). The molten solute precipitates out of the gas-saturated solution as fine particles due to the high level of supersaturation. The product characteristics may be modified by adjusting the process parameters, such as solute concentration, pre-expansion temperature and pressure, expansion temperature and pressure, and nozzle geometry.

The PGSS technique has been demonstrated as one of the SCF-based microencapsulation processes to produce thermally sensitive polymer composites (i.e. D,L-PLA and PLGA) containing bioactive materials (i.e. ribonuclease A, catalase and β-D-galactosidase) (Howdle et al., 2001). Howdle and co-workers demonstrated that the biological activity of the enzyme was preserved and the release of ribonuclease A from the PLA composites over a 70 day period showed an initial burst release of less than 10% of the dose over the first 2 days followed by 'close to' zero-order release kinetics. Moreover, it was reported that the pore structure of the polymer could be controlled by the depressurization rate.

The PGSS process offers a mechanism for controlling both macro- and micro-porosity via a single-step process. Therefore, the PGSS process, which utilizes the pressure-adjustable solvation power of the DGs, can be used to control the characteristics of microencapsulated drug formulations.

4.5. Impregnation

One of the distinguishing properties of DGs is the ability to diffuse into the amorphous or glassy region of polymers and swell the matrices. Supercritical fluids are able to increase the porosity of the structures, allowing solutes to be infused (i.e. impregnated) into polymer matrices. The impregnation process is influenced by the solute solubility in the DG media and its partition coefficient (Kikic and Vecchione, 2003). Dense gas-based impregnation techniques can be applied to the fabrication of drug/polymer systems for long-term drug-delivery and improvement of therapeutic dosing and stability (Jung et al., 2002; Busby et al., 2002). Dense gases can not only interact with polymers at temperatures higher than the melting point, but also with polymers in the glassy state. The DG dissolution, polymer matrix swelling and glass transition temperature of the polymer should all be considered in determining the DG–polymer interaction (Kikic and Vecchione, 2003). The conventional processes to generate drug impregnation into polymeric matrices involve the solubilization of the solute in a solvent, the solute partitioning between the solvent and polymeric substrate and solvent removal steps. The use of DGs as the solvent to entrap the drug can eliminate the product purification stage, since the DGs are converted into gas phase through the depressurization stage and are easily separated from the extract without altering the product properties.

Duarte et al. (2006) studied the preparation of controlled release microspheres using SCF technology for the delivery of the non-steroidal anti-inflammatory drug, naproxen. In this study, ethyl cellulose/methyl cellulose microspheres were prepared from DCM/DMSO using the ASES process. The cellulose polymer was then placed inside an impregnation cell where naproxen-saturated SC CO_2 was passed through for a predetermined period of time and at a very slow flowrate to induce the impregnation. The drug impregnation efficiency was reported to be 3.1% and the in vitro dissolution release studies showed that

the resulting naproxen-impregnated cellulose exhibited a very slow release profile and only 40% of naproxen was released after 8–10 h.

The SCF impregnation method was also carried out by Alessi et al. (2003) to study nimesulide- and piroxicam-impregnated poly(vinyl pyrolidone) (PVP) and poly(dimethylsiloxanes) (PDMS) particles. The maximum impregnation efficiency of nimesulide in PVP was reported to be 10%. However, the impregnation efficiency of nimesulide increased to 13% when PDMS (20 wt%) was added to PVP. It has been demonstrated that PDMS acts as a good co-solvent and thus enhances the solubility of nimesulide in SC CO_2 and forms better retaining agents when mixed with the PVP (Alessi et al., 2003).

Another SCF-based microencapsulation technique was utilized to impregnate indomethacin into biodegradable polymers, such as D,L-PLA, D,L-PLGA and PLA/PEG (Liu et al., 2005). The biodegradable nanoparticles were prepared from polymer solutions by anti-solvent-induced precipitation in the presence of surfactants. It was reported that indomethacin was incorporated into biodegradable nanoparticles with no change of particle size and morphology. Liu and co-workers claimed that the highest drug loading was obtained with D,L-PLA homopolymer while lower drug loading was obtained with D,L-PLGA copolymers due to the increasing fraction of glycolic acid. Furthermore, the drug loading in D,L-PLA microspheres increased markedly as the impregnation temperature was increased from 35 to 45 °C and the drug release was also delayed over 10 h (Liu et al., 2005).

Drug impregnation into polymer matrices from SC solution has several advantages, such as high drug penetrability into polymer matrices, controllable solvation power and impregnation rate and prevention of particle agglomeration due to the minimum usage of organic solvents (Kikic and Vecchione, 2003).

4.6. Miscellaneous processes

There are other DG-based processes which have been studied in the preparation of microencapsulated drug formulations including extraction of emulsions using DG media (Chattopadhyay and Huff, 2005) and particle formation based on the DG depressurization technique (Ribeiro Dos Santos et al., 2002).

The method of SCF-based extraction of emulsions (SFEE) has been developed recently by Chattopadhyay and Huff (2005). In their study, a crude emulsion was prepared by dissolving a known amount of indomethacin/Eudragit® RS into water-saturated ethyl acetate (EA) to form an organic solution. The resulting organic solution was then added into a known amount of EA-saturated aqueous poly(vinyl alcohol) (PVA) solution. The emulsion was injected through a capillary nozzle, which was used to enhance the atomization of emulsion into micro-droplets in the SC CO_2 media. After the contact with SC CO_2, an aqueous suspension was formed at the bottom of the vessel whilst the effluent SC CO_2 was purged from the top of the vessel. It was reported that the average diameter of microparticles was 1 μm and the residual solvent in the emulsion was less than 50 ppm. The main control parameter over product morphology was the emulsion droplet size. Precipitation of particles with different sizes can be accomplished by varying the emulsion formulations and optimization of the solvent–surfactant system (Chattopadhyay and Huff, 2005). The novel SCF-based emulsion extraction is beneficial compared to the traditional emulsion-based manufacturing techniques, which often bring various disadvantages such as the efficacy reduction of the pharmaceutical product due to the residual solvent impurities and increased toxicity (Shine, 1994).

Another microencapsulation technique using a novel DG process has been studied by Ribeiro Dos Santos et al. (2002). The microencapsulation process involves different steps to induce coating formation: (1) solubilization of the coating material in SCF media; (2) pressure reduction to induce the formation of coating layer; (3) final depressurization to atmospheric condition. In their study, the coating material (Dynasan® 114 or Gelucire® 50-02) and bovine serum albumin (BSA) were placed in a vessel equipped with an impeller. The system was then pressurized with CO_2 over a predetermined period until the desired SC conditions were reached. After the equilibration and solubilization of coating material, the vessel was cooled to induce pressure reduction. The pressure reduction changed the phase of CO_2 from SC to liquid, thus the coating material was precipitated on the insoluble BSA particles, which have the particle size of 50 μm, dispersed on the CO_2 media. The system was then depressurized to atmospheric to collect the coated particles. The particle diameter and BSA loading of the microencapsulated products were in the range of 50–500 μm and 13–67% respectively. In vitro dissolution studies showed that the lipid coating caused the initial burst release of BSA to decrease from 95% in 5 min to 62% in 15 min. The study also demonstrates that solid protein particles can be coated efficiently without any alteration of the structural integrity. It is thus advantageous compared to other SCF-based processes since the active materials remain in solid state and the heat exposure and water adsorption in the interface are avoided to retain the biological activity of proteins (Ribeiro Dos Santos et al., 2002).

5. Summary

Controlled release formulations are invariably more expensive than conventional formulations thus they can only be justified when they offer one or more therapeutic advantages. Apart from the improvement of pharmacological responses, the other advantages of controlled release systems include the maintenance of therapeutic drug levels and minimization of drug level fluctuation, total amount of drug used, patient compliance and reduction of side effects. On the other hand, there are some limitations of the controlled release formulations such as increased potential for first-phase metabolism, possible reduction in systemic availability and possibility of dose dumping in the burst release stage. The dose dumping characteristic is one of the crucial factors for potent drugs with narrow therapeutic index. Fortunately, the GMP and highly sophisticated techniques in the preparation of controlled release formulation have been able to reduce the probability of dose-dumping occurrence and minimize the other limitations (Welling, 1997).

The conventional techniques in the preparation of controlled release formulations, which include emulsification, spray drying, coacervation, in situ polymerization and solvent evaporation (Leroux et al., 1996; Cohen et al., 2000), are often associated with some drawbacks such as high residual solvent, increased toxicity, efficacy reduction and increased side effects (Shine, 1994). The alternative approaches using DG techniques have been widely studied in the preparation of controlled release formulations due to their optimum characteristics, performance and ability to minimize the drawbacks in comparison with the traditional processes. The unique properties of DGs make them attractive due to the ease of solvent removal, less processing steps involved and low handling and disposal expenses. The DG processing techniques such as GAS, ASES, RESS, PGSS and impregnation, have been widely studied in the preparation of various pharmaceutical controlled release formulations. Furthermore, DG processes have been demonstrated to produce polymeric controlled release formulations with characteristics of acceptable drug loading and ability to sustain the drug release over the desired period of time.

The major limitation of DG technology for the manufacturing on the commercial scale is the lack of fundamental data for accurately describing multicomponent phase behaviour involved in the process. The practical implementation of DG processes for particle production requires the predictability of product characteristics. Therefore, it is foreseeable that the utilization of DGs in commercial applications will be more commonplace with the improved understanding of multi-phase systems in DG environments (Foster et al., 2003).

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