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Preparation of Budesonide and Budesonide-PLA Microparticles Using Supercritical Fluid Precipitation Technology

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ABSTRACT The objective of this study was to prepare and characterize microparticles of budesonide alone and budesonide and polylactic acid (PLA) using supercritical fluid (SCF) technology. A precipitation with a compressed antisolvent (PCA) technique employing supercritical CO_2 and a nozzle with 100-µm internal diameter was used to prepare microparticles of budesonide and budesonide-PLA. The effect of various operating variables (temperature and pressure of CO_2 and flow rates of drug-polymer solution and/or CO_2) and formulation variables (0.25%, 0.5%, and 1% budesonide in methylene chloride) on the morphology and size distribution of the microparticles was determined using scanning electron microscopy. In addition, budesonide-PLA particles were characterized for their surface charge and drug-polymer interactions using a zeta meter and differential scanning calorimetry (DSC), respectively. Furthermore, in vitro budesonide release from budesonide-PLA microparticles was determined at 37°C. Using the PCA process, budesonide and budesonide-PLA microparticles with mean diameters of 1 to 2 µm were prepared. An increase in budesonide concentration (0.25%-1% wt/vol) resulted in budesonide microparticles that were fairly spherical and less agglomerated. In addition, the size of the microparticles increased with an increase in the drug-polymer solution flow rate (1.4-4.7 mL/min) or with a decrease in the CO_2 flow rate (50-10 mL/min). Budesonide-PLA microparticles had a drug loading of 7.94%, equivalent to ~80% encapsulation efficiency. Budesonide-PLA microparticles had a zeta potential of -37 ± 4 mV, and DSC studies indicated that SCF processing of budesonide-PLA microparticles resulted in the loss of budesonide crystallinity. Finally, in vitro drug release studies at 37°C indicated 50% budesonide release from the budesonide-PLA microparticles at the end of 28 days. Thus, the PCA process was successful in producing budesonide and budesonide-PLA microparticles. In addition, budesonide-PLA microparticles sustained budesonide release for 4 weeks.

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INTRODUCTION Budesonide, a potent corticosteroid with high glucocorticoid receptor affinity and prolonged tissue retention [1], inhibits inflammatory symptoms such as edema and vascular hyperpermeability [2, 3]. For the treatment of chronic inflammatory respiratory disorders such as asthma, nasal polyps, bronchiectasis, and chronic obstructive pulmonary disease, budesonide is currently marketed as a nasal spray (Rhinocort) and an orally administered dry powder inhaler (Pulmicort). In addition, budesonide is indicated in the treatment of Crohn's disease and inflammatory bowel disease, for which it is marketed as ileal release capsules (Entocort). Because the inflammatory disorders of the respiratory tract and the colon are chronic and the existing budesonide therapy for these disorders is available as immediate release formulations, there is a need to develop sustained release budesonide formulations that can prolong the drug release and hence, the anti-inflammatory action. In addition, for the first time, we observed that budesonide at low concentrations inhibits the expression of vascular endothelial growth factor and multidrug resistance-associated protein (MRP1), 2 key proteins that are overexpressed in various tumors [4, 5]. Thus, sustained release budesonide formulations may also be used for cancer therapy.

A common method for the controlled release of drugs is the use of microparticles of carrier-drug composites [6]. A typical carrier is a biodegradable polymer in which the drug is distributed uniformly. The drug is released from the carrier at a controlled rate due to drug diffusion and or degradation. In this study, we selected polylactic acid (PLA; molecular weight 100 000) as a model biodegradable polymer and utilized supercritical fluid (SCF) tech-

nology for preparing sustained release budesonide particles. Indeed, this technique has previously been used to produce polymer-drug microparticles [7-16] capable of controlling drug release. SCF technology is an environmentally benign technique that allows more precise control of particle morphology [17] while minimizing organic solvent use. It is not the purpose of this study to tailor particles for a particular disorder. However, it is the purpose of this study to demonstrate the feasibility of formulating polymeric budesonide particles.

Production of micron-sized drug and/or polymer particles using SCF technology utilizes the principle of drug precipitation with a compressed antisolvent (PCA) [17]. In this process, the solutes are first dissolved in a liquid solvent known to have partial or full miscibility with an SCF antisolvent (typically CO_2). The liquid solution is then sprayed through a capillary or a nozzle into flowing SCF. The liquid solvent then dissolves into the antisolvent, while the solutes do not. As a result, the solutes often crystallize into micron-sized particles. The particle size distribution and morphology has been shown to be affected by process variables such as flow rate, solute concentration, and antisolvent temperature and pressure [13-14, 18]. This process has been shown to produce particles with a low residual organic solvent content [19]. In addition, the process requires much lower operating temperatures, when compared to spray drying, thereby avoiding drug degradation [19]. There have been numerous studies using PCA to produce drug particles [19-20], superconductor particles [21], polymer particles [8, 14-15, 22-24], and many other particles.

To understand the drug and polymer behavior under the conditions of our study, we prepared plain drug and plain PLA particles prior to producing drug-carrier particles. The PCA apparatus used in this study employed a coaxial spray nozzle to deliver the drug solution and CO_2 to the precipitation chamber (PC). Solutions of drugs and/or polymers were sprayed through a capillary, with CO_2 flowing cocurrently in an annular region about the capillary. Coaxial nozzles have been used in the literature in an attempt to obtain better control over the product morphology [8, 13, 18, 23-24].

MATERIALS AND METHODS

Materials

The PLA used in this study (molecular weight 100 000, as reported by the supplier) was obtained from ICN Biomedicals (Costa Mesa, CA). Methylene chloride (99.9%) was obtained from Fisher Scientific (Pittsburgh, PA). The drug, budesonide (99.9%), was obtained from Sigma-Aldrich (St Louis, MO). All materials were used as received.

Apparatus

The precipitation experiments were performed using the experimental apparatus shown in **Figure 1**. CO_2 and the organic solution flow cocurrently into the top of the PC (Model 19TM40, Jerguson, Strongsville, OH). The volume of the PC is approximately 80 mL. The organic solution consisted of budesonide, PLA, or both budesonide and PLA dissolved in methylene chloride. The organic solution was delivered at a constant flow rate using a metering pump (Model B-100-S, Eldex Laboratories, Napa, CA). The organic solution flow rates (at a given metering pump setting) were determined during a separate calibration run. This was done by recording the change in the mass of the organic solution vial over a set spray time (usually 1 minute). A pulse dampener (Model 12-0625, Scientific Systems, San Jose, CA) was used to minimize the fluctuations in the upstream pressure. The organic solution entered the PC via a spray nozzle. The spray nozzle consisted of a 24-cm long piece of fused silica capillary tubing with 100-µm inner diameter (ID) (Part 1900331, Alltech, Deerfield, IL). The capillary tubing was connected to the stainless steel tubing using 2 adapters (Models EZRU21 and ZU1T, Valco, Ontario, Canada). The exposed capillary tubing between the adapters was protected from breakage by installing a metal collar that held the adapters together. The seals on the capillary tubing were achieved using graphite ferrules (RF-100/0.4-G, Alltech). The tip of the capillary tubing extended approximately 1.6 mm further into the PC than the stainless steel sheath. The CO_2 entered through the annular space between the capillary tubing (outer diameter = 0.4 mm) and the stainless steel sheath (ID = 1.5 mm) at the top of the PC. The CO_2 antisolvent was delivered to the PC at the desired operating temperature and pressure using a digitally controlled high-pressure syringe pump (Model 260 D, Isco, Lincoln, NE). For flow rates above 30 mL/min, an additional syringe pump was employed. The CO_2 flow rate was controlled using a back pressure regulator (Model BP66, Go, Spartanburg, SC) on the outlet line of the PC. The regulator was heated in a water bath to prevent freezing due to CO_2 expansion. The CO_2 flow rate was obtained from the display on the syringe pump controller. The temperature of the PC was controlled using 2, 250-W, strip heaters (Model OT-1202, Omega, Stamford, CT) attached to the sides of the vessel. The temperature inside the PC was measured using an RTD (resistance temperature detector) probe (Omega). The pressure was measured with a digital pressure gauge (Model PG-5000, Psitronix, Chicago, IL).

Particle Formation Procedure

The PC was filled with CO_2 and allowed to equilibrate at the desired operating temperature and pressure. Valve 3 was then opened to allow CO_2 to flow through the PC. When the CO_2 flow rate stabilized at the desired value (and the PC reached thermal equilibrium), the organic

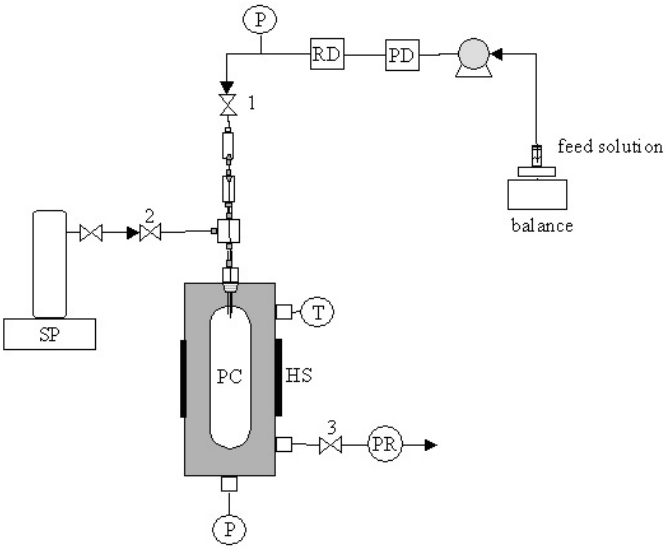

Figure 1. PCA apparatus. HS indicates heating strip; PC, precipitation chamber; PD, pulse dampener; PR, pressure regulator; RD, rupture disk; SP, syringe pump.

solution was sprayed into the vessel. Typical experiments involved 2, 20-plus-second, sprays at 1.4 mL/min (for larger flow rates, the spray time was adjusted to deliver an equivalent mass of solution). With each spray, the PC was filled with a dark cloud. After each spray, the PC was purged with at least 1 equivalent volume of pure CO_2 to remove any residual solvent (after which the contents of the PC were clear). After the final purge, the solvent line was disconnected and valve 1 was opened to allow the CO_2 to push out any residual solution from the capillary (to prevent plugging). The PC was then slowly depressurized (over at least 30 minutes)
through valve 2 while being maintained at the experimental temperature. The particles were then collected on a 0.5-µm sintered metal filter (Mott Corporation, Farmington, CT) placed inside the nut on the effluent port. The morphology and particle size distribution of the particles were determined using a Zeiss (Thornwood, NY) Model DSM940 scanning electron microscope (SEM). The particles were transferred from the filter to an SEM stub with double-sided carbon tape attached to it. The stub was then sputter-coated for 2 minutes with gold palladium. Each precipitation experiment was performed at least twice in order to validate the results. In general, the particle size varied by less than 10% between runs. Particle sizing was done visually from the SEM photographs by sizing more than 200 particles for each experiment.

Determination of the Drug Content of the Microspheres

The loading efficiency of budesonide in the polymeric microparticles was determined by extracting and quantifying the encapsulated budesonide using an high performance liquid chromatography (HPLC) assay. Budesonide-PLA microparticles (2 mg) were added to 2 mL of methylene chloride and subjected to shaking at room temperature for 16 hours, to ensure complete dissolution of the particles. The resulting solution was evaporated to dryness under nitrogen, and the dried residue was reconstituted with 200 µL of acetonitrile: aqueous buffer mixture (70:30). This reconstituted solution was vortexed for 1 minute and centrifuged at 12 000g for 5 minutes; the supernatants were injected onto the HPLC column.

Zeta Potential Measurements

The zeta potential of budesonide-PLA microparticles was measured using a zeta-plus instrument (Brookhaven Instruments Corporation, Huntsville, NY). A sample of microparticles weighing 1.5 mg was suspended in 5 mL of distilled water. The suspended particles were then sonicated for 60 seconds. The zeta potential was measured using palladium electrodes. The mean of readings from 3 different batches was taken as the zeta potential of the budesonide-PLA particles.

In Vitro Drug Release

The in vitro release of budesonide from PLA microparticles was carried out at 37°C. A 250-mL suspension of the budesonide-PLA microparticles (10 mg/mL) was taken into a floatable dialysis membrane obtained from Spectrum Laboratories (Rancho Dominguez, CA) (molecular weight cutoff: 50 000; sample volume of dialysis membrane: 500 µL), and the device was allowed to float in a centrifuge tube containing 50 mL of phosphate buffered saline (PBS; pH 7.4) containing 0.025% sodium azide. Sodium azide (0.025% wt/vol) was included to arrest bacterial growth in the release medium. The centrifuge tubes were placed in a shaking water bath incubator maintained at 37°C. Aliquots of 1 mL were removed from the 50-mL tube on various days up to 25 days, and the lost volume was replaced with fresh PBS. The budesonide released into the medium was analyzed using an HPLC assay as described below.

HPLC Assay

A previously reported reverse-phase HPLC method [25] was used for quantifying budesonide released from the PLA microparticles. A Waters HPLC system including a solvent delivery pump (Waters TM 616), a controller (Waters 600 S), an autoinjector (Waters 717 plus), and a photo diode array detector (PDA) (Waters 996, Millford, MA) was used in this study. The peak areas were integrated using Millennium software (Version 2.15.01). A 25-cm-long Discovery C-18 column (Supelco, Emeryville, CA) with a particle diameter of 5 µm and a pore size of 100 Å was used for drug elution. During the assay, budesonide was eluted isocratically at a mobile phase flow rate of 0.8 mL/min and monitored with a PDA detector operating at 250 nm. The mobile phase for the assay consisted of a methanol and aqueous buffer mixture (69:31 vol/vol). The buffer was prepared in water containing 0.1% acetic acid at pH 3. The run time for the

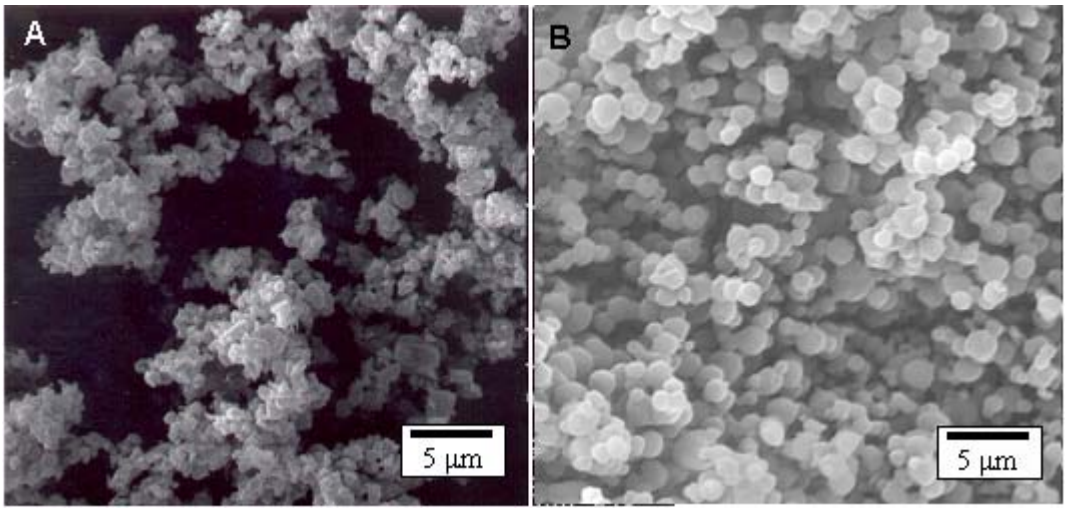

Figure 2. Comparison of SEM photographs for (A) unprocessed and (B) processed budesonide particles. The particles were processed using condition set 1 (Table 1); kV is 10, magnification is x3000.

Table 1. Summary of Results for Budesonide Particle Formation

Process Conditions							Particle Size Results*						
			CO_2		Flow Rate								
	Temperature	Pressure	Density	Concentration	$CHCl_3$	CO_2	Frequency (%)					$\overline{D}_N$	σ
Set no.	(°C)	(bar)	(g/cm³)	(wt%)	(mL/min)	(mL/min)	<1†	1-1.5	1.5-2	2-2.5	>2.5	µm	µm
1‡	40	85.8	0.38	1.00	1.4	30	3	65	27	5	0	1.45	0.34
2	35	78.9	0.38	1.00	1.4	30	—	—	—	—	—	—	—
3	45	92.7	0.38	1.00	1.4	30	0	10	43	33	14	1.83	0.38
4	40	92.7	0.55	1.00	1.4	30	—	—	—	—	—	—	—
5	40	139	0.76	1.00	1.4	30	—	—	—	—	—	—	—
6	40	85.8	0.38	0.25	1.4	30	7	80	13	0	0	1.21	0.20
7	40	85.8	0.38	0.50	1.4	30	5	46	46	3	0	1.38	0.30
8	40	85.8	0.38	1.00	2.7	30	0	15	49	29	6	1.82	0.38
9	40	85.8	0.38	1.00	4.7	30	0	21	40	28	11	2.02	0.68
10	40	85.8	0.38	1.00	1.4	10	0	27	38	29	6	1.85	0.41
11	40	85.8	0.38	1.00	1.4	50	9	61	15	15	0	1.40	0.43

*Dashes indicate that no particles were obtained.
†Numbers in this column are size ranges in µm.
‡Base case conditions.

assay was 20 minutes, and the retention time for budesonide was 12 minutes.

Thermal Analysis

The thermal analysis of pure budesonide, pure PLA, the physical mixture of budesonide-PLA, and SCF-derived budesonide-PLA microparticles was carried out using a differential scanning calorimeter (Shimadzu DSC-50 System, Shimadzu Scientific Instruments, Columbia, MD). Briefly, during each scan, 1 to 2 mg of the samples were placed in sealed aluminum sample pans and scanned at a rate of 5°C/min to determine the melting temperatures of the drug and the polymer.

RESULTS AND DISCUSSION

Budesonide Microparticles

An SEM photograph of unprocessed budesonide is shown in **Figure 2**. The particles were highly agglomerated with particle sizes ≤1 µm. The budesonide particles produced using the base case process conditions are also shown in **Figure 2**. The processed particles were larger and less agglomerated than the unprocessed material. The particles produced in this study look very similar to those produced by Steckel et al [19], who reported particle sizes ranging from 1 to 10 µm. They may have found larger particles because they used a much larger nozzle and drug solution flow rate. A summary of the

budesonide particle size results is given in **Table 1**. The average particle diameters ($_N$) ranged from 1.21 to 2.02 µm and the standard deviation of the particle diameters (σ) ranged from 0.20 to 0.68 µm.

Effect of CO_2 Temperature and Density

Experiments were performed to investigate the influence of CO_2 temperature and pressure in the PC, CO_2 and organic solution flow rates, and drug solution concentration on the particle size distribution and morphology. To investigate the influence of each process variable, experimental conditions were chosen such that comparison of results could be made in which only 1 process variable was manipulated (and the other variables were generally set to the base case conditions). The base case conditions were as follows: CO_2 temperature = 40°C, CO_2 pressure = 85.8 bar, CO_2 flow rate = 30 mL/min, drug solution flow rate = 1.4 mL/min, and drug concentration = 1 wt%. These conditions were a variation of those used by Steckel et al [19] to process several different steroid compounds (including 1 budesonide sample). Steckel et al [19] used much higher flow rates (drug flow rate = 63 mL/min and CO_2 flow rate ~250 mL/min) and a much larger nozzle diameter (300 µm), and their apparatus was different. However, they did not report the effect of varying the process variables.

To successfully produce microparticles, the conditions must be chosen such that the solvent is soluble in the SCF antisolvent. The solubility of solutes in SCF solvents can be correlated in terms of SCF density. The density of pure CO_2 is plotted in **Figure 3** as a function of temperature and pressure. In the literature there are limited data on the solubility of methylene chloride in CO_2 [26, 27]. However, in general, methylene chloride is miscible in supercritical CO_2 [26]. The average CO_2 density (33-43°C) at the methylene chloride/CO_2 critical points reported by Reaves et al [27] is also shown in **Figure 3**. Thus, as long as one operates sufficiently above this density, the methylene chloride is not likely to condense out in the precipitation chamber. The operating points used in the study (except for the point at 40°C and 139 bar) are also shown in **Figure 3**. To produce microparticles, not only must the solvent be miscible in the SCF, the solute must be insoluble in the resulting solvent/antisolvent mixture. Steckel et al [19] reported that budesonide was insoluble in supercritical CO_2 for the process conditions used in the majority of the experimental runs in this study (40°C and 85 bar).

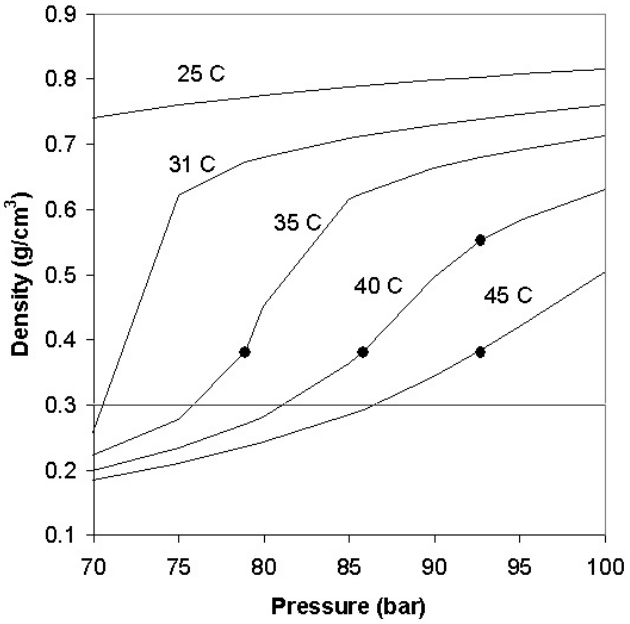

Figure 3. Density of CO_2 versus pressure along various isotherms. The curves represent pure CO_2 densities calculated using the equation of state given by Angus et al [28]. The dashed line represents the average CO_2 density (between 33 and 43°C) at the methylene chloride/CO_2 critical points reported by Reaves et al [27]. The closed circles represent the operating points that were investigated (same for the run at 40°C and 139 bar).

To optimize budesonide particle size, we investigated the effect of temperature and density on particle formation in supercritical CO_2. To investigate the effect of temperature, experiments were performed at 35, 40, and 45°C at a constant density of 0.38 g/cm^3. The experiments were run at a constant density (rather than at a constant pressure) since the properties of CO_2 (ie, viscosity, phase behavior, and interfacial tension with another fluid) correlate better with density. At 35°C (**Figure 4A**), the budesonide collected had a crystalline structure and no round particles were observed. At 40°C (**Figure 2B**) 1- to 2-µm round budesonide particles were formed. At 45°C (**Figure 4B**) the particles were slightly larger and less spherical. A possible explanation for this is that the particle growth rate increased with temperature. The melting of budesonide was not a factor since the melting point of budesonide is 245 to 255°C.

To investigate the effect of density, experiments were performed at 3 different densities—0.38, 0.55, and 0.76 g/cm^3—at a constant temperature of 40°C. At 0.38 g/cm^3 (**Figure 2B**), round 1- to 2-µm budesonide particles were collected. However, at 0.55 g/cm^3 (**Figure 4C**) the budesonide collected looked like nonuniform, nonspherical crystal-like structures. At 0.76 g/cm^3, the precipitation chamber did not get cloudy during the spraying of the drug solution and no budesonide was observed on the outlet filter. This suggests that budesonide is somewhat soluble in the CO_2 at this density, preventing reprecipitation during the purge and

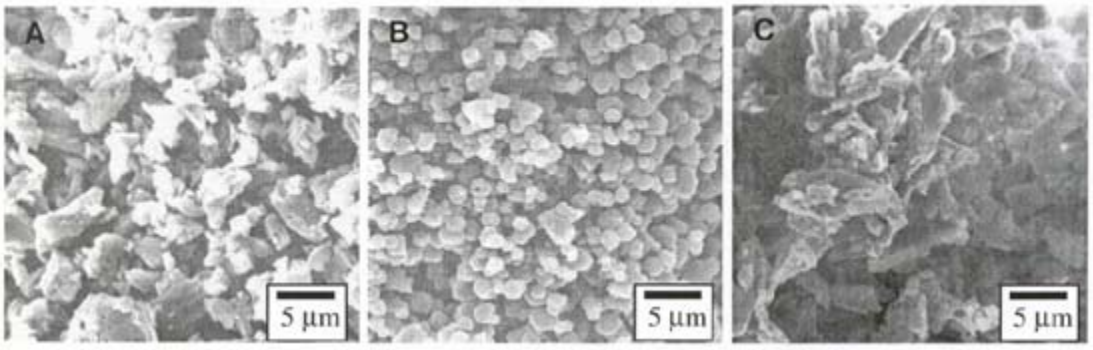

Figure 4. SEM photographs of budesonide particles prepared at different CO_2 temperatures and pressures: (A) 35°C, 78.9 bar (set 2); (B) 45°C, 92.7 bar (set 3); and (C) 40°C, 92.7 bar (set 4); kV is 10, magnification is x3000.

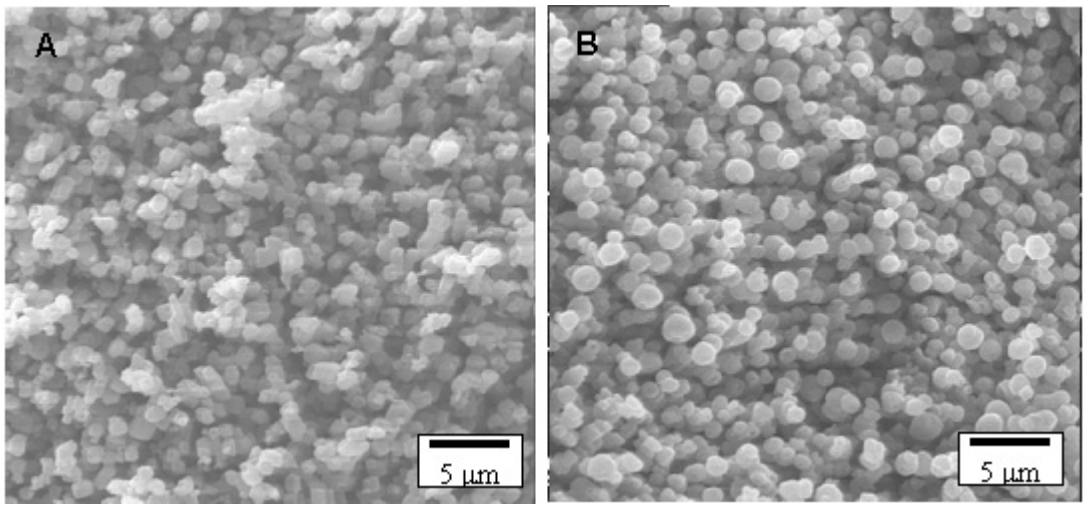

Figure 5. SEM photographs of budesonide particles prepared from different budesonide concentrations in methylene chloride: (A) 0.25 wt% (set 6), and (B) 0.50 wt% (set 7); kV is 10, magnification is x3000.

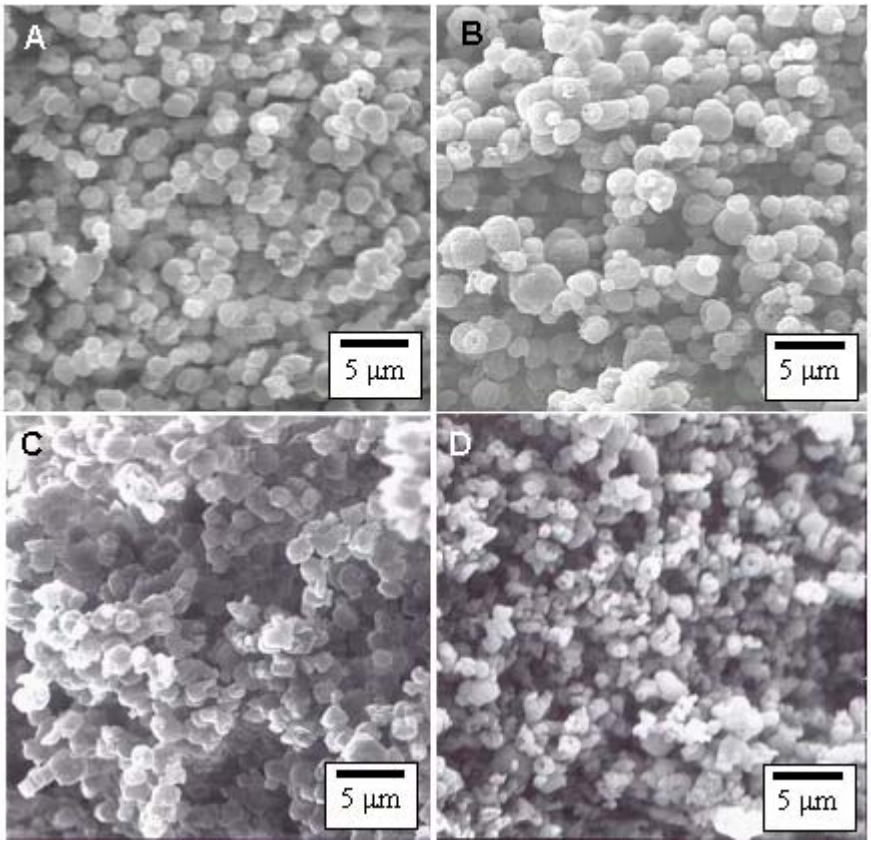

Figure 6. SEM photographs of budesonide particles prepared from different budesonide solution and carbon dioxide flow rates: (A) 2.7 mL/min, 30 mL/min (set 8); (B) 4.7 mL/min, 30 mL/min (set 9); (C) 1.4 mL/min, 10 mL/min (set 10); and (D) 1.4 mL/min, 50 mL/min (set 11); kV is 10, magnification is x3000.

facilitating removal of budesonide from the vessel during the purge.

Effect of Drug Concentration

Drug solution concentrations of 0.25, 0.50, and 1.00 wt% budesonide in methylene chloride were investigated. At 0.25 wt% budesonide, the particles were fairly agglomerated and mostly nonspherical (**Figure 5A**). At 0.5 and 1.0 wt% budesonide (**Figures 5B** and **2B**, respectively), the particles were less agglomerated. There was a negligible difference in the particle size and morphology for the runs at 0.5 and 1.0 wt% budesonide.

Effect of Flow Rates Through the Coaxial Nozzle

The drug solution flow rates that were investigated were 1.4, 2.7, and 4.7 mL/min (**Figures 2B**, **6A**, and **6B**, respectively). As the flow rate increased, the average particle sizes and polydispersites increased. This trend was also observed by Randolph et al [15] for PLA particle formation. According to Randolph et al [15], the methylene chloride concentration increased as the flow rate increased, which decreased the driving force for the mass transfer of methylene chloride into the CO_2. The decreased mass transfer rates decreased the supersaturation ratio, which then caused a decrease in the nucleation rate. A decrease in the nucleation rate would then lead to larger particles.

The CO_2 flow rates that were investigated were 10, 30, and 50 mL/min (ρ = 0.38 g/cm^3). The SEM results are shown in **Figures 6C**, **2B**, and **6D**, respectively. As the CO_2 flow rate increased, the average particle size decreased slightly, from 1.85 to 1.40 µm; however, this change is within the overall standard deviation range noted above.

PLA Microparticles

There have been several studies in the literature in which PLA particles have been produced using PCA [8, 14-16]. In some of these studies, and in this study, the CO_2 was flowing while the organic solution was being sprayed into the precipitation vessel. The PLA particles produced in this study (**Figure 7A**) appear very similar to those produced previously [15]. The particles had an average diameter of 1.27 µm, with a standard deviation of 0.42 µm. It was determined that 1 wt% PLA in methylene chloride was approximately the maximum concentration that would produce particles using the current nozzle dimensions. When the PLA concentration was increased to 2 wt%, PLA fibers were produced. In addition, increasing the solution flow rate at this concentration caused the upstream pressure to increase

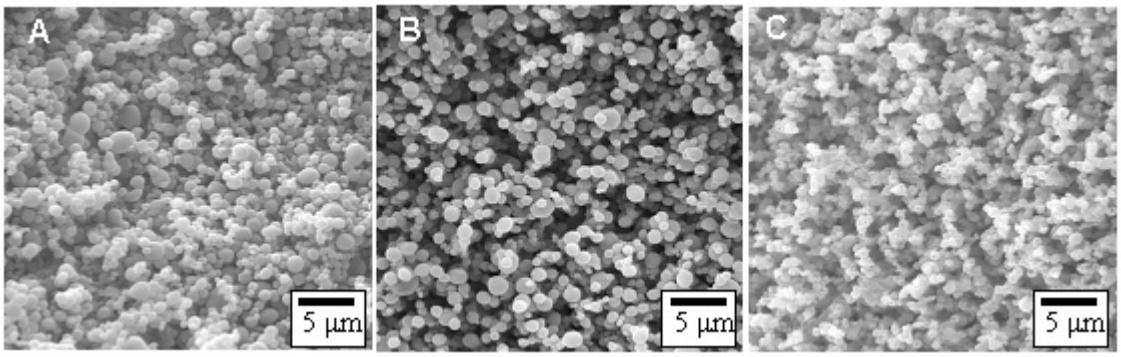

Figure 7. SEM photograph of budesonide-PLA particles: (A) 0 wt% budesonide, (B) 9.1 wt% budesonide, and (C) 33.3 wt% budesonide in PLA. Process conditions: temperature is 40°C, pressure is 85.8 bar, PLA concentration in methylene chloride is 1 wt%, CO_2 flow rate is 30 mL/min, and solution flow rate is 1.4 mL/min; kV is 10, magnification is x3000.

unacceptably (because of the higher viscosity of the polymer solution) without halting fiber formation.

Budesonide and PLA Microparticles

Coprecipitation experiments were performed using 2 different budesonide concentrations, 0.1% and 0.5%, in methylene chloride (**Figures 7B** and **7C**, respectively). The PLA concentration was fixed at 1 wt% in methylene chloride. At the lower budesonide loading (9.1 wt/wt% on a solvent-free basis), the particles produced were visually indistinguishable from pure PLA particles. In addition, the average particle diameter (1.26 µm) and the standard deviation (0.30 µm) determined from the SEM images were very close to the values for the pure PLA particles produced in this study. The average size of these particles determined using laser light scattering technique was 0.536 ± 0.037 µm (**Table 2**). On the other hand, at the higher budesonide loading (33 wt/wt%) on a solvent-free basis, there was microparticle aggregation. Particle sizing could not be done for the higher drug loading because of the high degree of agglomeration in the samples.

Table 2. Characterization of Budesonide-PLA Microparticles*

Parameters	
Size (µm)	0.536 ± 0.037
Zeta potential (mV)	–37 ± 4
Drug loading (%wt/wt)	7.94 ± 1.64
Encapsulation efficiency (%)	79.4 ± 7.72

* PLA indicates polylactic acid. All data are expressed as mean ± standard deviation for $n = 4$.

Pharmaceutical Characterization of Budesonide-PLA Particles

Particle Charge

Particle charge is an important parameter for drug delivery. To determine the charge on SCF-processed budesonide-PLA particles, we measured the zeta potential on these particles. The zeta potential, a measure of the surface charge of dispersed particulate systems, depends on the composition of the particles and the dispersion medium. The results of our studies indicated that the SCF-processed budesonide-PLA microparticles had a negative zeta potential (-37 ± 4 mV). The zeta potential plays a significant role in determining the stability of the dispersed systems, including microparticles [29].

Budesonide Encapsulation Efficiency

Budesonide loading in the SCF-derived PLA microparticles was performed using HPLC analysis. The results of our studies indicated that budesonide loading (%wt/wt) in the PLA microparticles was 7.94% ± 1.64%, which corresponds to an encapsulation efficiency of 79.4% ± 7.72% (**Table 2**). Compared to the encapsulation efficiency of budesonide in budesonide-PLA nanoparticles (65%) prepared using a solvent evaporation technique [30], the encapsulation efficiency of budesonide in SCF-processed PLA particles was higher. The most likely reason for this difference involves extensive drug partitioning between 2 immiscible phases (water and oil), which is often encountered in conventional solvent evaporation methods. This partitioning often results in reduced drug encapsulation efficiencies compared to the single-step SCF process. Interestingly, the high encapsulation efficiency in SCF-derived PLA microparticles suggests that supercritical CO_2 does not extract a detectable amount of budesonide under the conditions of microparticle formation. The possible encapsulation of the budesonide by PLA was tested using an in vitro release study.

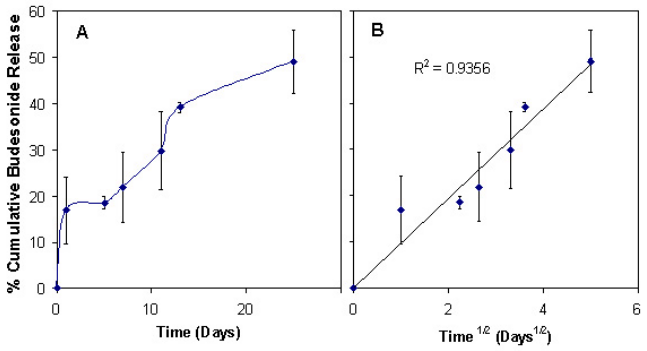

Figure 8. In vitro budesonide release from PCA-derived microparticles (initially containing 9.1 wt% budesonide). Budesonide was eluted into PBS at 37°C. Error bars represent the standard error based on three measurements. (A) Cumulative budesonide release vs time. (B) Cumulative budesonide release vs time$^{1/2}$.

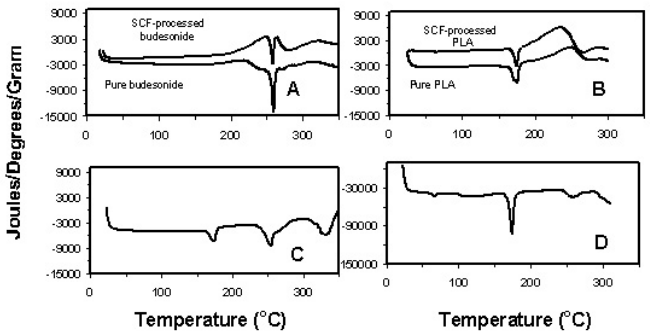

Figure 9. DSC curves: (A) pure budesonide and SCF-processed budesonide, (B) pure PLA and SCF-processed PLA, (C) physical mixture of budesonide and PLA, and (D) SCF-derived budesonide-PLA microparticles.

In Vitro Drug Release

To characterize the release profile of budesonide from the PLA microparticles, the rate of release of budesonide into PBS was measured in vitro at 37°C. Budesonide-PLA microparticles prepared using PCA technique contained 7.94% budesonide by weight. A sustained release of the drug was observed over 4 weeks (**Figure 8**). The release profile was linear with the square root of time, indicating that budesonide is likely entrapped within the PLA matrix. Unlike diffusion through the membrane of a reservoir system, where the rate of drug release is linear with time, matrix-controlled diffusion is linear with square root of time for spherical structures such as microspheres and nanospheres. Falk et al [11] obtained similar release profiles for PLA-encapsulated rifampicin, gentamicin, and naltrexone particles. The initial burst release was reasonably small (a 17% release after 1 day). The cumulative amount of budesonide release from these microparticles at the end of 4 weeks was 50% of initial drug loading.

Thermal Analysis

DSC is a useful technique for determining the changes in the thermal transitions of the drug and the polymer in pharmaceutical products. By performing DSC analysis of the drug product before and after the supercritical gas phase precipitation, one can determine the influence of this process on the thermal properties of the drug and the polymer. The peak size and shape of the DSC curves are useful in determining the crystallinity of the drug and the polymer. In recent studies, it has been reported that SCF processing alters the crystallinity of the drugs. For instance, SCF processing reduced the crystallinity of flunisolide, prednisolone, and triamcinolone acetonide [19]. Also, SCF processing of piroxicam and β-cyclodextrin inclusion complexes resulted in a complete loss of the crystalline endothermic peak of piroxicam [31]. A reduction in the crystalline nature of a substance in general improves the solubility and possibly release of the drug from the particles. Besides altering the crystallinity of small molecules, SCF processing also resulted in the decrease in the crystallinity of a high-molecular-weight biodegradable polymer such as PLA [32].

The DSC curves of budesonide and PLA before and after the PCA process are shown in **Figures 9A** and **9B**. The DSC curves for pure budesonide and PLA showed endotherms around 252 and 172°C, respectively. These endotherms are characteristic for the melting behavior of PLA and budesonide. However, upon SCF processing, the intensity of the melting peaks of budesonide and PLA were reduced, suggesting a reduction in the crystallinity of budesonide and PLA. The exotherm observed in **Figure 9A** for the SCF-processed budesonide could be a result of some molecular events that are leading to the recrystallization of budesonide following melting. The DSC curve for the physical mixture showed 2 peaks, an endotherm at 172°C corresponding to PLA, followed by the endothermic melting peak at 245 to 255°C, characteristic of crystalline budesonide (**Figure 9C**). However, the DSC curve for the SCF-processed budesonide-PLA microparticles indicated the endotherm corresponding to PLA at 172°C, but the corresponding endotherm for budesonide was considerably reduced in its intensity (**Figure 9D**). There may be several reasons for this change in budesonide peak: transformation of budesonide into an amorphous state, dissolution of budesonide in the PLA matrix, or loss of drug (budesonide could be extracted by CO_2) during the treatment. Drug extraction by vented CO_2 can be ruled out, because the drug loading in the particles is close to its theoretical value. As the SCF-processed budesonide-PLA microparticles indicated loss of drug crystallinity, the drug solubility, release, and membrane transport would likely be higher.

CONCLUSION The PCA method used in this study successfully produced budesonide and budesonide-PLA microparticles in the micron size range, with a narrow size distribution. The CO_2 temperature and pressure and the budesonide concentration chosen were critical to the successful production of the microparticles. Also, proc-

ess variables such as flow rates of drug solution and supercritical CO_2 influenced the budesonide microparticle morphology. The budesonide-PLA particles showed controlled release of budesonide over 4 weeks, which indicates that the budesonide was trapped within the PLA matrix. In addition, the budesonide encapsulation studies indicated 80% budesonide encapsulation efficiency, suggesting that much of the budesonide was utilized in the PLA matrix, unlike in conventional methods for the preparation of drug-containing polymer microparticles.

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