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Preparation of drug delivery biodegradable PLGA nanocomposites and foams by supercritical CO_2 expanded ring opening polymerization and by rapid expansion from $CHClF_2$ supercritical solutions

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ABSTRACT

The synthesis of poly(lactic-*co*-glycolic acid) (PLGA) by the ring opening copolymerization of D,L-lactide and glycolide was performed at 110 °C to 130 °C using $Sn(Oct)_2$ as catalyst, 1,10-decanediol as initiator in a supercritical sc-CO_2 expanded medium at pressures of up to 3,500 psi. Due to the limited monomer solubility in sc-CO_2 at low temperatures (70 °C), only Mn = 2,500 is typically obtained. However, molecular weight increases with both temperature and sc-CO_2 pressure. Thus, Mn = 13,000 (PDI = 1.28) was obtained at 110 °C - 130 °C even in the absence of fluorinated surfactants. Biodegradable drug delivery nanocomposites based on dexamethasone and poly(lactic acid) (PLA) and poly(lactide-*co*-glycolide) (PLGA) were prepared by the rapid expansion of the corresponding supercritical $CHClF_2$ solutions (110 °C, 200-300 bar) in air (RESS) and in toluene (RESOLV). The RESS process leads to a broad particle size distribution (100-500 nm) while the RESOLV generates a narrower distribution centered around 100 nm and is accompanied by the formation of a few large particles, most likely due to aggregation.

INTRODUCTION

Currently, there is considerable interest in developing biodegradable nanoparticles as effective drug delivery devices. Organic biodegradable polymers play an important role in this area due to their ability to deliver a wide range of drugs to specific target areas of the body for sustained periods of time. The most widely used biocompatible/biodegradable drug delivery polymers are poly(lactic acid) (PLA), poly(glycolid acid) (PGA) and their copolymers, poly(lactide-*co*-glycolide) (PLGA). Polymer degradation and drug release rate can be manipulated by varying the copolymer composition. Conventional PLGA drug delivery systems are based on micron-sized polydisperse particles prepared by emulsion/solvent PLGA evaporation. There are relatively few reports on the preparation of PLGA nanoparticles.[1] However, they could offer more flexible formulation platforms vs. PLGA microspheres as they can be administered *via* a wider range of delivery routes, and can achieve both controlled and targeted delivery at the cellular level. Recently there is increased interest in synthesis and processing using inexpensive, non-flammable environment-friendly, "green" solvents such as supercritical sc-CO_2.[2] We have developed thermodynamically stable microemulsions in supercritical fluids (SCF)[3] as an attractive alternative to emulsions prepared in organic solvents. We are currently investigating the applicability of this methodology to the synthesis of biodegradable PLGA nanoparticles.[4].

Due to the limited monomer solubility in sc-CO_2 at low temperatures (70 °C), the synthesis of PLGA in sc-CO_2[5] typically proceeds with the formation of low molecular weight oligomers (Mn = 2,500).[5a] Addition of surfactants allowed for Mn of 13,000.[5b] The development of reliable, "green" procedures that do not require a templating effect for the production of narrow distribution polymer nanoparticles remains highly desirable. Recently, supercritical-fluid methodologies such as rapid expansion of supercritical solutions in air (RESS)[6] or in a solvent (RESOLV)[7] have emerged as promising avenues towards this goal. We are describing herein the synthesis of PLGA at temperatures of 110 °C to 130 °C and the use of the RESS and RESOLV techniques in the preparation of biodegradable polymer-drug nanocomposites.

EXPERIMENTAL

Materials. 1,4-dioxane-2,5-dione (glycolide, (G) 99.5+%) and dl-3,6-dimethyl-1,4-dioxane-2,5-dione (DL-Lactide, (L) 99.5+%, both from Purac America), Tin(II)-2-ethyl hexanoate ($Sn(Oct)_2$, 95%, Aldrich), 1,10-decanediol (Kodak), dexamethasone (Sigma, 98%) toluene (Fisher, 99.9%), THF (Fisher, 99.9 %), $CHClF_2$ (ABCR, 99%) and CO_2 (99.99%, Airgas) were used as received.

Equipment. Both the supercritical $CHClF_2$ mediated polymerization and nanoparticle preparation were conducted in a 54 cc high-pressure vessel, custom manufactured from 316 stainless steel and equipped with two sapphire windows (diameter = 1.25″, thickness = 0.5″), sealed on both sides with PEEK seals. Sc-CO_2 mediated polymerization for the foam were conducted in a 15 mL glass vial which was kept inside a high-pressure vessel. Bulk polymerizations were carried out in the same set-up in the absence of CO_2. After a specified period of time, the contents were depressurized through an expansion nozzle into a beaker and collected as fine powder.

Techniques. ^{1}H-NMR (500 MHz) spectra were recorded on a Bruker DRX-500 at 24 °C in $CDCl_3$. GPC analyses were performed at 34 °C on a Waters 150-C Plus gel permeation chromatograph equipped with a Polymer Laboratories PL-ELS 1000 evaporative light scattering (ELS) detector and with a Jordi Gel columns. THF was used as eluent at a flow rate of 1 mL/min. Number-average (M_n) and weight-average molecular weights (M_w) were determined from calibration plots constructed with polystyrene standards. The pore distribution of the foam was determined using a Quantachrome Poremaster Hg porosimeter. The concentration of dexamethasone in the drug-polymer composite was determined by HPLC. Samples for TEM imaging were prepared by placing a C-coated TEM copper grid inside the collection chamber. For RESOLV, a TEM grid was dipped in the liquid solvent after expansion of 30 minutes and was air dried. Conventional bright field TEM images were obtained from the samples in a Philips EM420T microscope operating at an accelerating voltage of 100kV.

Polymerizations Monomers (D,L-lactide, 2.5 gm, 17.3 mmol and glycolide, 0.5 g, 4.3 mmol), catalyst (tin octoate, 18 mg, 0.04 mmol) and initiator (1,10-decane diol, 3.7 mg, 0.02 mmol) were placed into the reactor. The vessel was sealed, evacuated and was heated to 90 °C. CO_2 or $CHClF_2$ were charged into the reactor at the desired pressure using a ISCO 260D syringe pump. The operating conditions were reached in approximately 1.5 hours. All starting materials melted at about 100 °C at 1500 psig (CO_2). The reaction was allowed to continue for a certain period of time. Subsequently, the vessel was cooled to room temperature and was depressurized. The contents were dissolved in $CHCl_3$ and were precipitated into MeOH. The solids were filtered and dried. Conversions were determined gravimetrically and by NMR.

Preparation of PLGA nanoparticles. In a typical experiment, 200 mg of PLGA was placed in the vessel which was charged with liquid $CHClF_2$, heated and pressurized at 110 ºC and 200 bar, respectively, thereby dissolving the PLGA. Upon equilibration, the SC solution was passed through an expansion nozzle (stainless steel capillary tubing) into a collecting chamber. The chamber was either filled with ambient air (RESS) or with liquid solvent (RESOLV).

Preparation of dexamethasone-PLA composites. 20 mg of dexamethasone and 180 mg of PLA was placed in the high pressure vessel which was then charged with $CHClF_2$. A single phase was reached at 110 ºC and 300 bars. The contents were depressurized through an expansion nozzle into a beaker and collected as fine powder.

RESULTS AND DISCUSSION

The experimental setup for the sc-CO_2 polymerizations is presented in Figure 1. The synthesis of PLGA in sc-CO_2 is presented in Scheme 1. Polymerizations were carried out at various temperatures, pressures, mole ratios, and for various reaction times in the presence and in the absence of sc-CO_2.

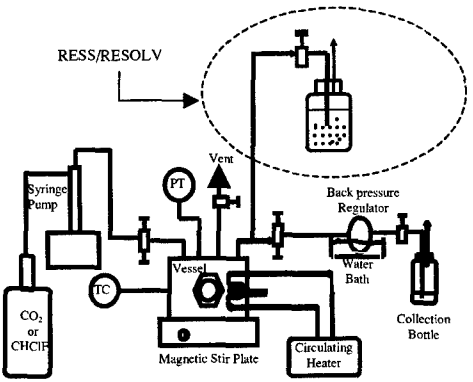
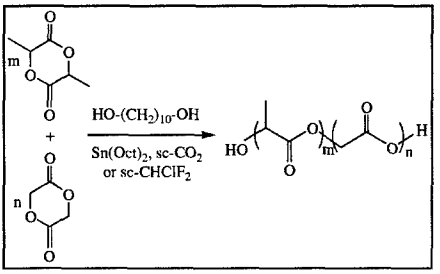


Figure 1. Experimental setup for PLGA synthesis and nanoparticle formation in SCF



Scheme 1: PLGA synthesis in SCF.

We first investigated the phase behavior of the monomers in sc-CO_2. The monomers had very low solubility in sc-CO_2. Under atmospheric pressure, the glycolide and DL-lactide monomers melt at about 87 °C and 128 °C respectively. However, under CO_2 pressure, melting/liquefaction of the lactide occurred at 90 °C at 4200 psi and at 110 °C at 1300 psi. Therefore sc CO_2 polymerizations were carried out at 110 °C - 130 °C. However, control experiments in the equipment described in Figure 1 in the absence of CO_2 were performed at 110 °C -150 °C and are presented in Table 1. The PLGA molecular weight increased with temperature and with time from 22,500 to 53,000. The conversion of the less reactive[8] lactide followed a similar trend. The polydispersities varied between 1.20 and 1.45 at 150 °C.

Table 1. Bulk copolymerization of d,l-lactide and glycolide without CO_2.

Exp	L/G/I/C[a]	Temp (°C)	Time (h)	L Conv (%)[b]	G Conv (%)[b]	L:G (PLGA)[b]	Mn[c]	PDI[c]
1	800/200/1/2	110	1.00	55	> 99	2.10:1	25,043	1.33
2	800/200/1/2	130	0.25	55	> 99	2.45:1	22,523	1.20
3	800/200/1/2	130	0.50	80	> 99	3.72:1	34,834	1.29
4	800/200/1/2	130	0.75	85	> 99	3.89:1	39,282	1.44
5	800/200/1/2	150	1.00	> 99	> 99	4.00:1	53,178	1.45

[a)]lactide/glycolide/1,10-decane diol/$Sn(Oct)_2$. [b)] by NMR, [c)] by GPC.

Table 2. Polymerization of d,l-lactide and glycolide in sc-CO_2.

Exp	L/G/I/C[a]	Temp (°C)	Press (psi)	Time (h)	L Conv (%)[b]	G Conv (%)[b]	L/G PLGA[b]	Mn[c]	PDI[c]
1	800/200/1/1	110	1,500	3	97	> 99	3.8/1	5,584	1.29
2	800/200/1/1	130	3,500	3	88	> 99	3.3/1	7,463	1.20
3	800/200/1/2	110	1,500	1	86	> 99	3.4/1	7,561	1.27
4	800/200/1/2	110	1,500	3	93	> 99	3.5/1	9,025	1.69
5	800/200/1/2	110	3,500	3	89	> 99	3.2/1	13,114	1.28
6	800/200/1/2	130	3,500	3	98	> 99	4.1/1	7,873	1.20

[a)]lactide/glycolide/1,10-decane diol/$Sn(Oct)_2$. [b)] by NMR, [c)] by GPC.

Selected examples of the PLGA synthesis in sc-CO_2 expanded media at 110-130 °C are presented in Table 2. While the molecular weights are lower than similar bulk conditions, they are larger than previously reported sc-CO_2 polymerizations at 70 °C.[5a] Thus, a 800/200/1/1 ratio of L/G/I/C already affords Mn = 5,500 at 110 °C (#1). An increase in temperature to 130 °C leads to a corresponding increase in Mn to 7,500 (#2). Doubling the amount of catalyst raises again Mn from 5,500 to 7500 (#3) and 9,000 (#4) at 1,500 psi after 1 and respectively 3 hours. A further increase in the CO_2 pressure leads to Mn as high as 13,000 and a relatively narrow molecular weight distribution (1.28). This is a large Mn for PLGA synthesis in CO_2 in the absence of a surfactant.[5a] A further increase in temperature to 130 °C while maintaining 3,500 psi increases just the conversion of the less reactive lactide while maintaining Mn at about 7,800. The increase in CO_2 pressure at a given temperature most likely increases the plasticization of the PLGA with CO_2, therefore depressing its Tg and allowing for better mass transfer processes.

While a 4/1 lactide to glycolide ratio was chosen based on the difference in their reactivity ratios,[8] at close to complete conversion, a similar composition was observed in the PLGA copolymer. Upon depressurization of the reactor, PLGA foams were typically obtained. An example of the foam is shown in Figure 2a while Figure 2b presents the foam morphology. The pore size distribution is presented in Figure 2c. For exp 4 in Table 2, the average pore volume was 3.9 cc/gram.

We have subsequently investigated the synthesis of PLA in sc-$CHCLF_2$. Due to the high solubility of PLLA in freon, relatively high molecular weight is readily accessible (Mn = 48,000, Mw/Mn = 1.16). NMR analysis of the precipitated product confirmed formation of PLA.

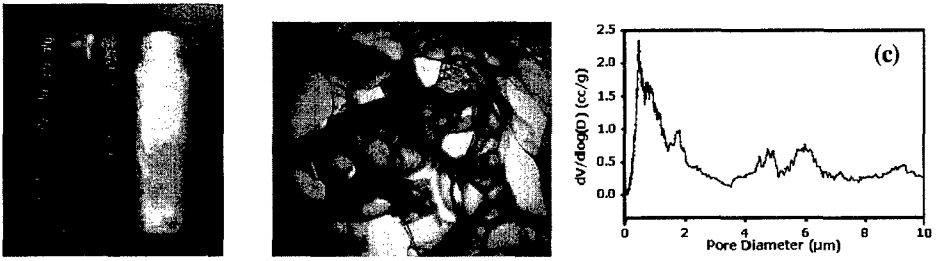


Figure 2. (a) PLGA foam after polymerization; (b) Morphology of PLGA foam 40X. (c) Pore size distribution of PLGA foam from Hg porosimetry.

PLGA and dexamethasone-PLA composite particles were produced using both RESS and RESOLV techniques. In both methods, the polymer was solubilized in sc-$CHClF_2$ and the sc solution was expanded through a nozzle in either air (RESS) or in a liquid solvent (RESOLV). During the expansion, the solvent power of supercritical fluid decreases dramatically and the solute precipitates. The high degree of supersaturation accompanying the rapid pressure reduction during the expansion results in homogenous nucleation and thereby the formation of well-dispersed particles containing 1 to 2 wt % dexamethasone.

Results from studies of different model solutes by the RESS process indicate that both nanometer- and micrometer-sized particles are present in the expansion jet. Figure 3 shows the TEM images of PLGA particles obtained from RESS at 110 ºC and 200 bars. It can be observed that the particles have a spherical morphology and the particle size distribution is relatively broad, ranging from 100-500 nm. At higher magnification, the particles with sizes smaller than 100 nm are not clearly seen probably due to weaker scattering. A modification to the traditional RESS process was performed by expanding the supercritical solution into a liquid solvent (RESOLV in toluene) to obtain smaller particles and narrower size distribution. In this case, the liquid solvent may suppress particle growth in the expansion jet. A TEM image of the resulting particles is given in Figure 3c. A narrower particle size distribution with sizes smaller than 100 nm can be achieved by the RESOLV procedure. However, due to possible aggregation a few isolated large particles (d = 1400 nm) were also observed.

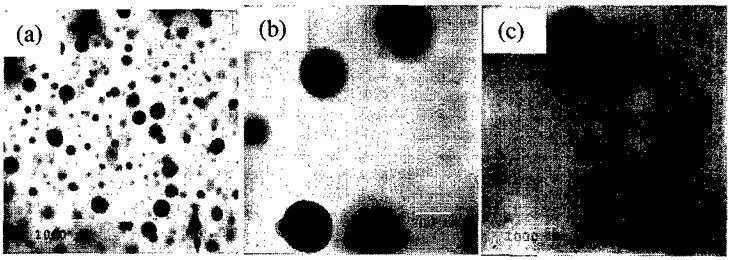


Figure 3. (a,b) TEM micrographs of PLGA particles obtained from the RESS process at two different magnifications. (c) TEM micrograph of PLGA particles obtained using RESOLV.

The use of a liquid at the receiving end of the rapid expansion process in RESOLV probably interferes or disrupts condensation and coagulation in the expansion jet, thus effectively quenching rapid particle-growth processes.

CONCLUSION

The synthesis of PLGA in sc-CO_2 expanded medium at 110°C to 130 °C using $Sn(Oct)_2$ as catalyst and 1,10-decanediol as initiator affords lower Mn as high as 13,000 (PDI = 1.28) obtained under supercritical conditions. PLGA and PLA–dexamethasone nanocomposites were prepared by the rapid expansion of the corresponding supercritical $CHClF_2$ solutions in air (RESS) and in toluene (RESOLV). The RESS process leads to a broad particle size distribution (100-500 nm) while the RESOLV generates a narrower size distribution centered around 100 nm. However this was accompanied by the formation of a few large particles, most likely due to aggregation. Optimization of these experiments is in progress.

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