Notes

High Molecular Weight Poly(L-lactide) and Its Microsphere Synthesized in Supercritical Chlorodifluoromethane

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Biodegradable poly(L-lactide) (PLLA) microspheres have been utilized in clinical and agricultural applications of the controlled release of bioactive materials[1] and fertilizers.[2] Stringent requirements must, however, be fulfilled before a polymer can be used in biomedical applications. The purity of the polymer product is of concern because residual monomers, catalysts, and solvents may pose risks when such materials are in contact with biological fluids or tissues.[3] The organic solvents used in the microsphere fabrication process, typically methylene chloride and chloroform, are known to be difficult to remove completely, and residual solvent may cause harmful side effects, for example, carcinogenesis, neurotoxicity, or teratogenicity.[4] The U.S. Pharmacopoeia limits the maximum amount of solvent in pharmaceuticals.[5] In addition, when PLLA is synthesized by the ring-opening polymerization (ROP) of L-lactide (L-LA), it is almost inevitable that the as-polymerized product contains some quantity of residual monomer, which was found to enhance polymer hydrolytic degradation.[6]

The growing demand for microsphere preparations without residual solvent has catalyzed the development of new methods of polymer synthesis in supercritical carbon dioxide (scCO$_2$).[7] Continuous precipitation polymerizations of semicrystalline fluoropolymers in CO$_2$ have been studied by Desimone et al.[8] Unfortunately, many polymers except for specific fluoropolymers and polysiloxanes have limited solubility in scCO$_2$, which reduces possible homogeneous polymerizations to an extremely narrow range.[9] Thus far, most studies have focused on heterogeneous-phase polymerizations and on the development of new surfactants.[10] For example, the emulsion polymerization of DL-lactide and glycolide within scCO$_2$ with a fluorocarbon polymer surfactant as an emulsifying agent demonstrated low molecular weights (MWs) ranging from 2500 to 30 300 g/mol at various polymerization times.[11] Wood et al.[12] reported that cross-linked PMMA was produced by dispersion polymerization in subcritical 1,1,1,2-tetrafluoroethane. The most important feature of Wood's work is the relatively low reaction pressure (10−20 bar) as compared to polymerization in scCO$_2$ (170−350 bar) at similar temperatures. The polymers can be formed as uniform microspheres by addition of a stabilizer. In the case of emulsion and suspension processes, however, there can often be problems with residual surfactants and stabilizers, which are associated with the expense of adding these materials and later removing them. Recently, cloud point measurements by Lee et al. showed that PLLA is readily soluble in polar chlorodifluoromethane (R22), due to a specific interaction between the hydrogen atom in R22 and the polymer ester group.[13] Thus, the ROP of L-LA in supercritical chlorodifluoromethane (scR22) should allow high degrees of monomer conversion and high MWs in a homogeneous phase. An attractive feature of R22 is its lower ozone depletion than chlorofluoromethane,[14] and in fact it is used in medical application.

One of the methodologies of using supercritical fluids for the production of microspheres is the supercritical antisolvent (SAS) recrystallization process. This involves feeding a spray of liquid solution from a capillary nozzle as fine droplets into a vessel containing the supercritical fluid.[15] The liquid solution, including solute, then dissolves in the supercritical fluid on contact. High supersaturation of the solute can thus be achieved, and small particles of solute precipitated.

To the best of our knowledge, the polymerization of L-LA in scR22 has never been studied. Thus, here we describe how scR22 can be utilized as the solvent for the ring-opening solution polymerization of L-LA, followed by the in situ fabrication of PLLA microspheres, without any residual monomer and solvent, by using a continuous SAS process.

The experimental apparatus is schematized in Figure 1. Polymerization was conducted in a 52 mL stainless steel high-pressure cell equipped with a magnetic stirring bar and an electrically heating mantle. L-LA (3.0 g) and tin(II) octoate (Sn(Oct)$_2$) solution (0.43 mL of a 0.12 M solution in toluene; 5.2×10^{-5} mol of Sn(Oct)$_2$) were added to the cell, and the toluene was removed under vacuum. The reactor was heated to 50 °C and purged with nitrogen for 5 min. While being cooled to room temperature, it was evacuated for a few hours via a trap cooled by liquid nitrogen and then purged with nitrogen for an additional 10 min. The cell was then disconnected from the nitrogen line, evacuated, and connected to the R22 feed system. The cell was then filled with liquid R22 to ca. 30 bar at 50 °C using a high-pressure metering pump and then gradually heated to

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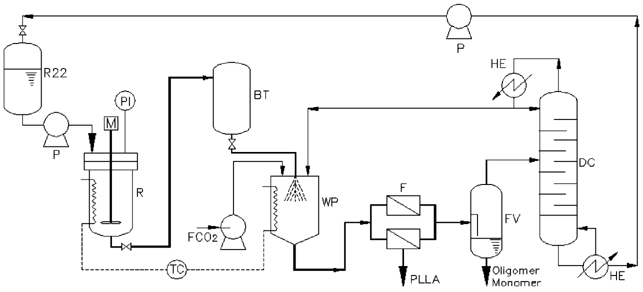


Figure 1. Scheme of the apparatus used for batch experiments: R22, chlorodifluoromethane; P, plunger pump; PI, pressure indicator; R, reactor; TC, temperature controller; FCO_2, CO_2 feed; BT, buffer tank; WP, windowed precipitation cell; F, filter; FV, flash vaporizer; DC, distillation column; HE, heat exchanger.

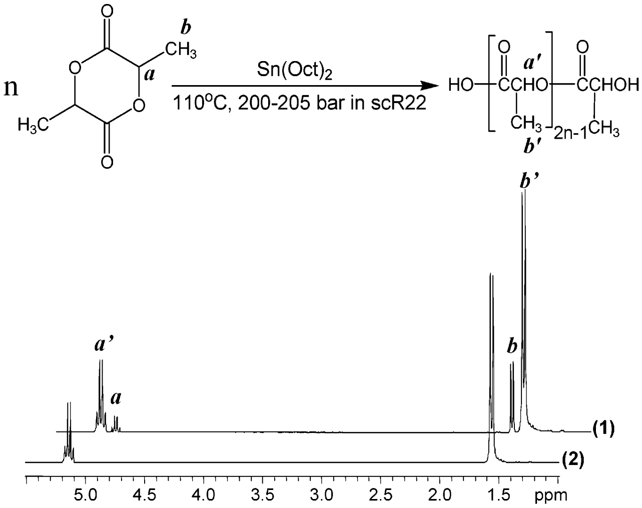
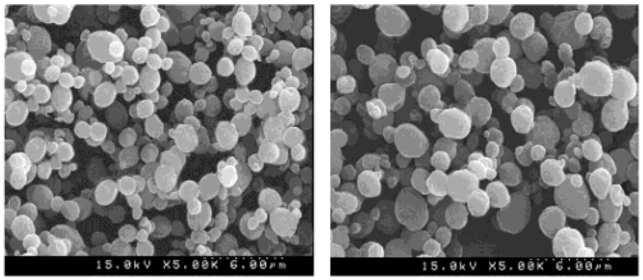


Figure 2. ^1H NMR spectra for PLLA synthesized (1) before and (2) after SAS processes.

110 °C to achieve a pressure of 200−205 bar. Polymerization was allowed to proceed for 24 h. During the polymerization, the polymer products and the reactants, including L-LA and initiator, were dissolved in scR22. Following the polymerization, the R22 solution, containing polymer and unreacted monomer, was atomized into the high-pressure precipitator vessel, which was swept with CO_2 antisolvent at 8 L/min at 40 °C under 100 bar, through a nozzle at 0.30 mL/min. Upon injection into the precipitator, PLLA particles were formed, and a mixture of CO_2 and R22, including unreacted monomers, flowed out of the precipitator. To wash out residual solvent and unreacted monomer from the recrystallized particles, the precipitator was filled with CO_2 for several minutes.[16]

Typical ^1H NMR spectra for the PLLA synthesized before and after SAS process are shown in Figure 2. ^1H NMR results suggested that most monomers were polymerized and that residual monomers were removed efficiently by the SAS process. Since both R22 and CO_2 are gases at normal pressure, it is expected that extremely little residual solvent remains in the PLLA microspheres. Nevertheless, we measured residual solvent by gas chromatography using the headspace method.[17] We found no solvents in the PLLA microspheres at the ppb level. Most of the particles were found to be spherical with a smooth surface (see Figure 3). The residual level of tin catalyst in PLLA was measured to be 0.37 and 0.34 wt % for before and after SAS processes, respectively. Thus, few of tin catalyst were washed out by SAS process. The mean particle sizes[18] were in the range 0.5−2.5 μm at a precipitator temperature of 35−40 °C. The conversion of monomers and M_n of the resulting polymers, obtained by polymerization initiated with $Sn(Oct)_2$, are given in Table 1. All of the monomer conversions are more than 70%, and the MWs of the PLLA were very high MWs (172 000 g/mol),[19] which are not achievable using supercritical fluids, such as $scCO_2$, as polymerization solvents. The polymerization of L-LA proceeds in a homogeneous state in scR22, whereas it proceeds heterogeneously in $scCO_2$. This difference is the result of the solubility of PLLA in $scCO_2$ or scR22, as indicated in the phase diagram of PLLA in supercritical fluids.[13] The growing molecules precipitated from $scCO_2$ had low MWs due to the poor solubility of PLLA in $scCO_2$. On the other hand, the specific interaction between the hydrogen in R22 and the ester group in PLLA made homogeneous polymerization possible, as occurs in solution polymerization, which resulted in the high MW PLLAs obtained.



(1) 35 °C (2) 40 °C

Figure 3. SEM photographs of PLLA microspheres prepared at 100 bar, 0.03 in. nozzle diameter, 0.3 mL/min solution flow rate, and 8 L/min CO_2 flow rate.

Table 1. Molar Ratios, M_n, and MWD for Polymerization of L-LA Initiated with $Sn(Oct)_2$ in Supercritical Chlorodifluoromethane at 110 °C under 200−205 bar[a]

entry	$[LA]_0/[Sn]_0$	conv (%)	$M_{n,GPC}/10^3$ (g/mol)[b]	MWD
1	406	71.2	172.6	1.64
2	295	73.0	171.3	1.69
3	197	73.3	172.1	1.70
4	100	73.5	170.9	1.75
5[c]	10000		3.5	1.40

[a] c_{R22} (R22 concentration) = 37.4% w/v, $[LA]_0$ is the initial L-LA concentration, 0.4 mol/L, and $[Sn]_0$ is the initial $Sn(Oct)_2$ concentration. [b] Determined by GPC measurements. [c] Polymerization in $scCO_2$; the literature value from ref 24.

A coordination insertion mechanism for L-LA polymerization with $Sn(Oct)_2$ has been postulated, involving covalent tin alkoxide bond formation and cleavage of the lactone acyl−oxygen bond.[20] Interestingly, the $Sn(Oct)_2$ concentration used in this study was about 10 times higher than that used in general bulk polymerization.[21] This feature may be attributed to competition between monomer and solvent for coordination to alkoxide.[22] When the $[LA]_0/[Sn]_0$ ratio was varied from 406 to 100, the $M_{n,GPC}$ ranged from 172 600 to 170 900 g/mol with increasing $Sn(Oct)_2$ concentration. Taking into account that $[LA]_0 = 0.4$ mol/L was used and that 72% of the L-LA was consumed to produce a PLLA of $M_n = 172\,600$ (equivalent to the number-average degree of polymerization $P_n = 1200$), the concentration of macromolecules present is 2.4×10^{-4} mol/L. As the resulting PLLA of $M_n = 172\,600$ was obtained without purposely adding alcohol, approximately 2.4×10^{-4} mol/L macromolecules was formed by the adventitious compound (e.g., any compound containing the hydroxyl group), converting

$Sn(Oct)_2$ into the actual initiator, tin alkoxide.[23] For comparison, the ROP of poly(glycolide-*co*-L-lactide) in $scCO_2$ from the literature is given in entry 5 (Table 1), where the growing polymer chain precipitated from $scCO_2$, resulting in a low MW, 3500 g/mol. The results obtained show that the synthesis of high-MW PLLA is more possible in scR22 than in $scCO_2$.

In conclusion, this process presents several advantages compared to the traditional polymerization in organic solvents or in $scCO_2$ for preparing PLLA microspheres. First, the synthesis of high-MW PLLA, followed by the preparation of PLLA microspheres, without residual monomer is possible. Second, the supercritical solvent used in this study is of low flammability and could be completely separated from the polymer by depressurization. Finally, these microspheres do not contain any surfactants or stabilizers.

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