CRYSTALLIZATION AND PLASTICIZATION OF POLY(L-LACTIDE) (PLLA) WITH SUPERCRITICAL CO$_2$

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This work investigates the structure and solid-state transitions occurred in PLLA polymers in supercritical carbon dioxide (scCO$_2$), with the aim to understand formation of microparticles for drug delivery applications. It is shown that PLLA exposed to CO$_2$ form consistently crystalline structures for both low- and high-molecular weight PLLA. However these structures were susceptible to pressure and temperature changes despite the supposed rigidity of the crystalline lamellar regions. The tendency of PLLA particles to form agglomerates is explained by a combination of a kinetic mechanism during nucleation and a slower process of structural changes occurred after this nucleation stage.

1. INTRODUCTION

The incorporation of a drug in a polymer for sustained release has numerous advantages for drug delivery. Recently much research has centred on the use of biodegradable microspheres. Several techniques have been suggested for the preparation of these materials, including solvent evaporation, coacervation and spray-drying. Supercritical antisolvent crystallization offers novel means of preparation of biodegradable microparticles. However, supercritical fluids, including most commonly used carbon dioxide, are excellent plasticizers of many polymers, in particular those with a significant amorphous phase, causing swelling and agglomeration in particles. Amorphous polymers are often preferable for drug formulation, allowing for more uniform release and more efficient drug loading than highly crystalline materials. Thus a compromise has to be found by optimising processing conditions to prevent agglomeration in CO$_2$ whilst retaining sufficient drug-release properties of the semi-crystalline polymers. Over the recent years it has become aparent that of the biodegradable polyesters only PLLA can be processed as its semi-crystalline nature prevents plasticization in CO$_2$. Recent work has attempted to address this issue. For example, Ghaderi et al. [2] used mixtures of CO$_2$ and N$_2$ to reduce plasticization of polymers by CO$_2$ whist retaining its ability to extract the organic solvent. Breitenbach *et al* [3] have processed co-polymers containing L-lactide blocks, thus retaining some crystallinity, some other authors [4] found that at least 25% w/w crystallinity was required in PLLA to stabilise the amorphous fraction and prevent agglomeration. Polymer structure and plasticization effect by CO$_2$ are considered in this work. The aim is to de-couple hydrostatic, plasticising and the thermal effects of CO$_2$, and investigate the mechanism of crystallization and particle agglomeration.

2. MATERIALS AND METHODS

PLLA was purchased from Boehringer Ingelheim and used as received. The reported molecular weight for the oligomer was 2,050 and the polymeric form was reported to have an intrinsic

viscosity of 1.0 dL/G in chloroform at 25°C which allowed the mean viscosity molecular weight of PLLA to be calculated as 30,000 using Mark-Houwink Equation for PLLA [5].

The experimental arrangement of the SEDS™ process [6] was used. The key component is the two-channelled coaxial nozzle fitted onto the closure of a 50 ml capacity stainless steel tubular vessel. Liquid carbon dioxide is pumped at constant flow rates of up to 18.0 ml/min at pressure between 80 and 200 bar by a high pressure reciprocating pump (Jasco, UK). The state of the CO_2 is changed from liquid to supercritical by passing the liquid CO_2 through a heat exchanger in the air oven, which was maintained at a temperature above the critical temperature of CO_2, between 40°C and 70°C. A solution of PLLA in dichloromethane at a concentration between 1 and 8 %w/v was delivered to the nozzle using an HPLC liquid solution pump (Jasco, UK).

Particle size and morphology were examined by scanning electron microscopy (Hitachi, S-520, Japan). The particle size distribution was determined using an AeroSizer® (Amherst Process Instruments, Hadley, MA) fitted with the dry-powder AeroDisperser device. The instrument was calibrated using a 10μm standard (Sigma, Poole, UK). The density of PLLA required for this analysis was determined by helium pyconometry. AeroSizer® parameters used were: shear force at medium, feed rate at medium, deagglomeration was at high and the pin vibration on.

The role of CO_2 in structural changes (lamellar formation) was investigated using monolithic samples of amorphous PLLA that were prepared by quench-cooling from the melt to 0°C. Monoliths were used to overcome problems related to background scattering by small particles. Only high molecular weight PLLA was used. Samples were exposed to either CO_2 or N_2 at temperatures ranging from 25°C to 120°C and 80 bar to 200 bar. Nitrogen was used as a hydrostatic control, preliminary experimentation showed that amorphous PLLA did not crystallize in the presence N_2 below its glass transition temperature, *i.e.* did not plastisize PLLA. The structural studies involved investigation of the long- and short-range crystallinity using wide- and small-angle X-ray scattering (WAXS and SAXS correspondingly), combined with thermodynamic analysis by differential scanning calorimetry (DSC). The SAXS measurements were carried out using the method described in work [1], *ex situ* for the majority of samples. A single sample was studied *in situ* in the diamond cell to determine the plasticization pressure of CO_2. Additionally, a comparative study was completed on PLLA microspheres, for both oligomeric and polymeric materials, obtained using spray-drying process followed by plasticization with CO_2.

3. RESULTS AND DISCUSSION

3.1 PARTICLE SIZE AND MORPHOLOGY

Precipitation with scCO₂ of oligomeric PLLA produced plate-like microparticles, under all operating conditions, with irregular shape and sub-micron dimension along the plate edge. Particle size was determined for all samples of oligomeric PLLA in the range between 2 and 6 μm in terms of the mean geometric diameter. The volume geometric diameter was significantly greater, between 5 and 20 μm, indicating particle agglomeration. The measured particle size increased with the solution concentration. The processing pressure had only a minor impact on oligomeric PLLA mean particle size at low temperatures. A reduction in processing temperature however resulted in a smaller mean particle diameter which was attributed to a lesser agglomeration at lower temperatures.

Polymeric PLLA gave agglomerated spherical particles or fibres if the solution concentration exceeded 2 %w/v. The primary particles were seen to be between 0.5 and 2 μm under all operating conditions, however the particle size data had shown extensive and strong particle agglomeration up to 200 μm volume diameter, which prevented any consistent analysis of particle size distribution. It should be noted that particle size data presented in the literature [2,7] for PLLA processed using $scCO_2$ involved sonication prior to sizing.

The particle morphology of CO_2 - processed PLLA is influenced by both the polymer molecular weight and by particle agglomeration. On exposure to supercritical CO_2, amorphous oligomeric PLLA microspheres produced by spray-drying fused, whilst polymeric PLLA microspheres agglomerated. Thus a transition from amorphous to semi-crystalline structure during or after precipitation can result in strong particle agglomerates. The agglomeration is also a function of polymer concentration, in the extreme case leading to formation of fibres. This is common in processes that involve the atomisation of polymer solutions and indicative of atomisation failure in viscous, high concentration polymer solutions. As dichloromethane and $scCO_2$ are miscible at the pressures and temperatures studied in this work, the micro-mixing process with $scCO_2$ is also affected by the solution viscosity. High polymer concentrations or poor CO_2 flow rates would lead to poor mixing and result in fibre formation or strong particle agglomeration. In addition, it is well known that the nucleation rate within the spinodal region, for melts and concentrated solutions alike, can be significantly reduced by higher viscosity and slower molecular diffusivity in this region. This effect may also lead to larger or more agglomerated particles. Clearly, all these phenomena occur in the solvent-reach phase during the particle nucleation process and proceed even before the semi-crystalline structure of the polymers is formed.

3.2 POLYMER STRUCTURE

Polymer crystals typically exist as lamellae and as such display periodicity of the order between 100 - 300 Å. Peaks present in SAXS profiles result from differences in electron density between crystalline and amorphous lamellar phases, whereas peaks present in WAXS reflect long-range ordering of the polymer crystal structure of periodicity somewhat between 1 - 10 Å.

Amorphous oligomeric and polymeric PLLA give a broad halo between 8° and 25° 2θ, which is characteristic for this amorphous material (Fig.1A). All CO_2-processed oligomeric and polymeric PLLA gave diffraction peaks. These peaks are located at 16.6° and 19.0° 2θ, which is characteristic for the PLLA unit cell [8]. Both samples produced by plasticization of the amorphous materials and by antisolvent precipitation in CO_2 gave the same long-range structure (Fig.1 B and C). Crystallinity calculated from WAXS data was about 50% for both the oligomeric and polymeric PLLA, this value was also confirmed by independent DSC analysis (not reported here). The presence of crystalline domains in SEDS - processed PLLA is not unexpected, as this technique has been shown to induce nucleation in small molecules. Spray-dried material exposed to supercritical CO_2 also contains crystalline domains. This certainly a result of plasticization by supercritical CO_2, which suppresses the glass transition temperature, improves polymer mobility and thus promoting crystallization.
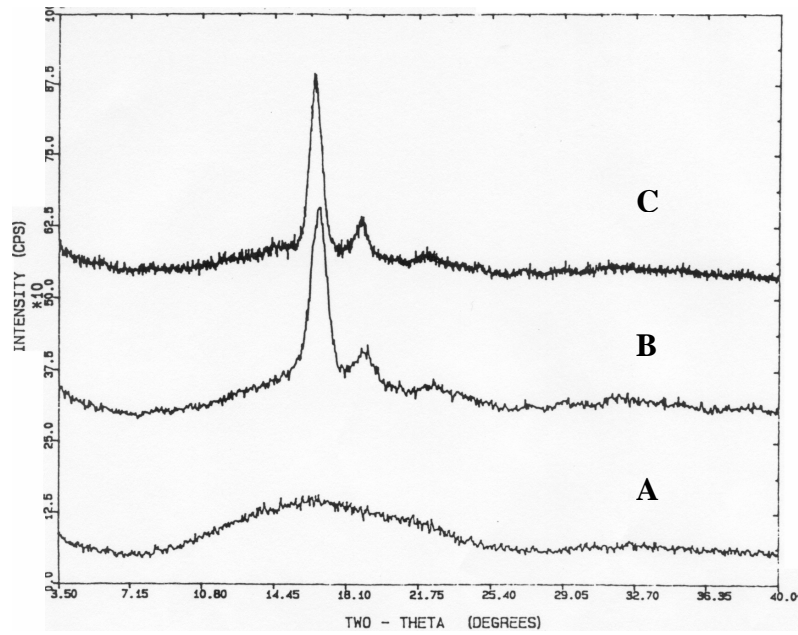
Figure 1: *WAXS profiles of polymeric PLLA: (A) spray-dried, (B) spray-dried PLLA exposed to CO_2 and (C) produced by SEDSÔ process.*
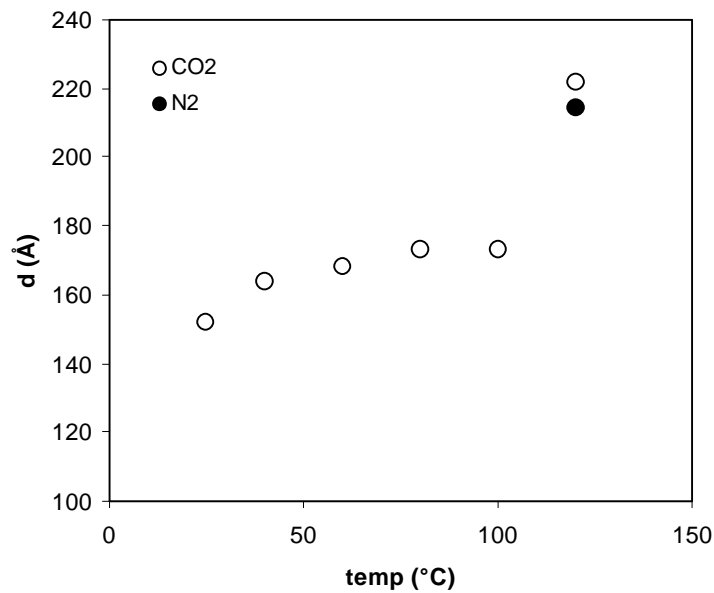

Figure 2: *The effect of CO_2 and N_2 temperature on the lamellar thickness of PLLA monoliths at 80 bar.*

SAXS data is more sensitive to structural changes between the amorphous and crystalline regions of the polymer and therefore gave more detail on the structural changes by plasticization in CO_2. *In situ* analysis of quenched polymer samples using SAXS technique showed that at ambient temperature about 20°C, annealing in CO_2 led to lamellar formation at pressure as low as 20 bar, this low pressure indicating excellent plastisizing properties of CO_2 even in the gaseous state.

Plasticization of PLLA films used for *in situ* study occurred on the time-scale of several minutes, which probably reflected both the diffusion time of CO_2 in the polymer matrix and relatively slow formation of lamellae in the viscous media of plastisized PLLA. From Fig. 2 it is apparent that annealing PLLA with nitrogen at 80 bar only resulted in detectable lamellar formation at 120°C. Annealing with CO_2 at 120°C also resulted in lamellar formation at a similar d-spacing of approximately 220Å. At lower temperatures only annealing in CO_2 led to lamellar formation.
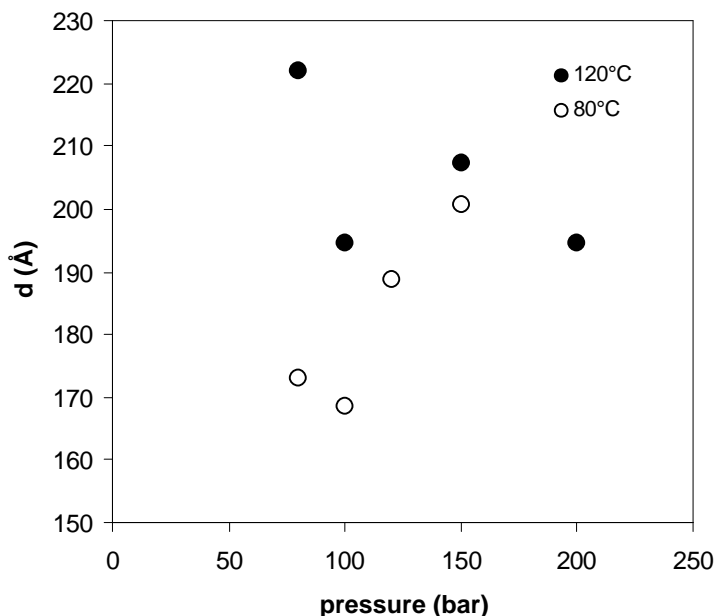

Figure 3: The effect of the processing pressure at 80°C and 120°C on the lamellar thickness of PLLA monoliths obtained at 80 bar.

Thus it can be concluded that CO_2 is involved in crystallization of PLLA at lower temperatures and the short-range structures produced are different, than those produced by melting at high temperature. The same can be said about the pressure effect as shown in Fig.3. At 80°C, lamellar thickness increased with rising pressure, from 160Å to 210Å concurrently with increase in CO_2 density. The lamellar thickness of PLLA annealed in N_2 at 120°C was similar to PLLA annealed at these conditions. It is apparent that CO_2 induces lamellar crystallization at low temperatures. The glass transition can be considered in terms of free volume changes. As CO_2 plastisize PLLA, it increases free volume and thus effectively decreases the glass transition temperature. This increases molecular mobility and results in crystallization. As seen from the comparative study with nitrogen, the hydrostatic increase in pressure had no affect on lamellar thickness, the suppression of the glass transition was dominated by the CO_2 density effect. The crystallization process however is limited by the degree of plasticization and may not produce structures which are the most thermodynamically stable for the polymer. Therefore the particles formed may undergo a slower "ripening" process resulting in a structure closer to that obtained in melt. It is likely that a certain degree of mobility or freedom remain within the plastisized semi-crystalline PLLA structure after the nucleation process. For example, increase of the lamellar thickness in response to the CO_2 density changes can be considered as a recrystallization process leading to formation of larger lamellae. This process is relatively slow and may particularly be important for sub-micron polymer particles which have a large specific surface area, where the molecular mobility is further increased and there is a possibility of close contact between particles.

CONCLUSIONS

Plasticization of many polymers by CO_2 results in the polymers existing in a rubbery state which prevents formation of particles during the antisolvent precipitation process. Semi-crystalline PLLA can be processed because the semi-crystalline domains stabilize the amorphous domains. However, as shown in this work, oligomeric PLLA and, to the larger extend, polymeric PLLA can still form strong agglomerates which prevent dispersion of these particles in a dry-powder device. It has been found that this agglomeration depends on the nucleation process, solution viscosity and the quality of the solvent used in the feed solution. Thus the processing conditions have to be optimised for each molecular weight of the polymer to avoid "kinetic" agglomeration as opposite to agglomeration due to plasticization effect. In addition, the SAXS data suggested that the lamellar structure of PLLA depends on the pressure and temperature during plasticization with CO_2 and may change with time. Lamellae formation in the plastisized PLLA is a relatively slow process compared to the particle nucleation. Therefore it is suggested that phase changes occurring on the particle surfaces after the nucleation step may also contribute to the particle agglomeration.

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