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# Supercritical $CO_2$: A Clean and Low Temperature Approach to Blending $P_{DL}LA$ and PEG

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**The unique combination of the gas like viscosity and liquid like density of supercritical $CO_2$ ($scCO_2$) is exploited to blend poly(D,L-lactic acid) ($P_{DL}LA$) and poly(ethylene glycol) (PEG) at near ambient temperatures. This novel process lowers the polymer blend viscosity and also permits incorporation of thermally and solvent labile protein based drugs. A series of blends are prepared with agitation in $scCO_2$. Differential scanning calorimetry (DSC) data shows that miscible blends can be produced at moderate temperatures. A surprising region of miscibility is revealed between 8 and 25%w/w PEG. The properties of this miscible region are probed with high pressure parallel plate rheological studies, showing that the viscosity in $scCO_2$ is directly related to the miscibility. Using the particles from gas saturated solutions (PGSS) method, microparticles of these $P_{DL}LA$/PEG blends are produced using $scCO_2$ and it is determined that the yields obtained are proportional to the miscibility of the polymers. Thus $scCO_2$ provides a unique route to low temperature, solvent free processing that accesses a window of miscibility that has not previously been observed. Finally, DSC analyses of these sprayed microparticles confirm the presence of the same high miscibility region observed in the bulk samples prepared under supercritical conditions.**

## 1. Introduction

Biodegradable poly(lactic acid) (PLA) is commonly used for a wide range of biomedical applications, from drug delivery through to tissue engineering.[1–2] Unfortunately, it has a high viscosity[3] and therefore requires the use of either high temperatures[4] or environmentally damaging, volatile organic solvents[5] to facilitate processing. In general, the processing temperatures required for extrusion or compounding are close to the degradation temperature of PLA.[6] Additionally, in many applications, a key requirement is the encapsulation of a drug which may be both thermally labile and solvent sensitive, and so could be degraded significantly by the processing. One solution to overcome this problem is to reduce the processing temperatures by blending PLA with a lower viscosity material such as poly(ethylene glycol) (PEG). For drug delivery applications, the incorporation of PEG is also desirable to increase the hydrophilicity of the polymer. The blending of two miscible polymers results in a combination of their individual properties.[7] For example, a reduction in the viscosity of ultra-high molecular weight polyethylene (UHMWPE) has previously been observed following melt blending with PEG.[8] This reduction was attributed to the PEG molecules entering into the amorphous regions of the UHMWPE and acting as a lubricant to improve chain mobility.

A great deal of research has been reported on the formation of PLA/PEG blends. The tensile strength of PLA is reduced with the addition of PEG,[9] whilst the flexibility,[10] elongation point[11] and hydrophilicity[10] all increase. The majority of these studies have focussed on the semi-crystalline L-PLA form, because its crystalline nature makes processing more viable than for the amorphous DL-PLA form.[12] Miscible blends containing PLA are generally produced by solvent casting[10,13] or melt extrusion[9,11,14] requiring the use of solvents or high temperatures (ca. 180 °C) which may not be environmentally acceptable or appropriate in the presence of delicate drugs, in particular protein based therapeutics.

There are also some inconsistencies in the reported miscibility range of PLA and PEG. The majority of authors report the polymers to be miscible from 0 to 30%w/w PEG[9,11,14] when melt blended at 180–190 °C or solvent blended with dichloromethane but some quote this limit as 20%w/w.[10,15] One explanation is that the miscibility limit of the blends alters with PEG molecular weight; researchers have observed this limit to reduce from 30%w/w, for a molecular weight of 400, to 15%w/w, for a molecular weight of 10 000.[13] These miscible blends are also unstable at atmospheric conditions with phase separation[9,14] occurring over 20 days as a result of the crystallisation of the PEG domains within the miscible blend.[9,16] Phase

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separation is only observed in miscible blends with a glass transition temperature ($T_g$) below room temperature as the polymer chains have sufficient mobility to allow recrystallisation of the PEG molecules. This recrystallisation reduces the size of the amorphous regions leading to an increase in $T_g$. Once sufficient phase separation has occurred, the $T_g$ will rise above ambient temperature and the structure is then effectively locked in as the chains no longer have sufficient mobility to diffuse together over short to medium timescales.

In order to blend the two polymers, PLA must be liquefied and as previously discussed high temperatures and/or solvents are conventionally required. However, $scCO_2$ can be used to avoid these issues since the gas like properties of $scCO_2$ allow it to diffuse into the amorphous regions of polymers enhancing chain mobility.[17] This lowers the $T_g$ of amorphous polymers, plasticising them and allowing processing at much lower temperatures.[18–20] For example, it has been reported that the $T_g$ of poly (ethylene terephthalate) can be lowered by as much as 40 °C in the presence of $scCO_2$.[17]

The aim of the current study was to investigate the $scCO_2$ assisted blending of $P_{DL}LA$ and PEG in order to develop a more hydrophilic formulation and one that can be processed at near ambient temperatures. The miscibility of the two polymers was analysed by differential scanning calorimetry and the effect of this blending on the viscosity was determined by high pressure rheological techniques. This knowledge was then applied to the CriticalMix PGSS (polymers from gas saturated solutions) spraying process to optimise the production of polymer microparticles for drug delivery applications.[1]

## 2. Results and Discussion

### 2.1. Plasticisation Studies

The reduction of the $T_g$ and melting temperature ($T_m$) of polymers by $scCO_2$ enables processing at significantly lower temperatures than could be achieved at atmospheric pressure.[21,22] It is therefore hypothesised that $P_{DL}LA$ and PEG could form a miscible blend at relatively low temperatures in the presence of $CO_2$ thereby removing the need for higher temperature processing or the use of organic solvents.

A variable volume view cell was initially used for simple observations to determine the pressure and temperatures required to liquefy the physical mixtures of the polymers. The samples of PEG and $P_{DL}LA$ initially appeared as white flakes and a white powder, respectively, but in the presence of $scCO_2$ above specific conditions, both samples liquefied (**Figure 1**).

Under atmospheric conditions PEG has a melting point of 61.1 °C but in the presence of $scCO_2$ at approximately 175 bar this decreases to below 55 °C. This reduction has been previously attributed to the low viscosity and high diffusivity of $CO_2$ enabling excellent penetration into the free volume of PEG through Lewis acid-base interactions with the ether groups.[23] The inter/intra molecular interactions are then disrupted, mobilising the individual chains and consequently reducing the $T_m$. The viscosity of the liquefied polymer was qualitatively assessed just by observing the movement of a magnetic stir bar in the sample. Initially, the stir bar was fixed within the solid polymer powder. However, upon addition of $scCO_2$, the PEG was transformed to a liquefied state and the increasingly free rotation of the magnetic stir bar demonstrated a lowering of the viscosity.

The $T_g$ of $P_{DL}LA$ under atmospheric conditions is 52.8 °C but this can be depressed below 34 °C in the presence of $scCO_2$. This marked reduction illustrates the ability of $scCO_2$ to act as a plasticiser and lower the $T_g$ of amorphous polymers.[17] In contrast to the PEG case, the viscosity of the plasticised $P_{DL}LA$ was found to be high and almost no movement of the magnetic stir bar could be discerned. Increasing pressure and temperature had minimal effect. These observations clearly show the differences between amorphous and crystalline polymers as the low $T_g$ of PEG (<–90 °C) is responsible for the lower observed viscosity (**Table 1**).[24]

In general the blending of polymers should result in a combination of their original properties,[7] and it is hypothesised that the addition of the low viscosity PEG should decrease the

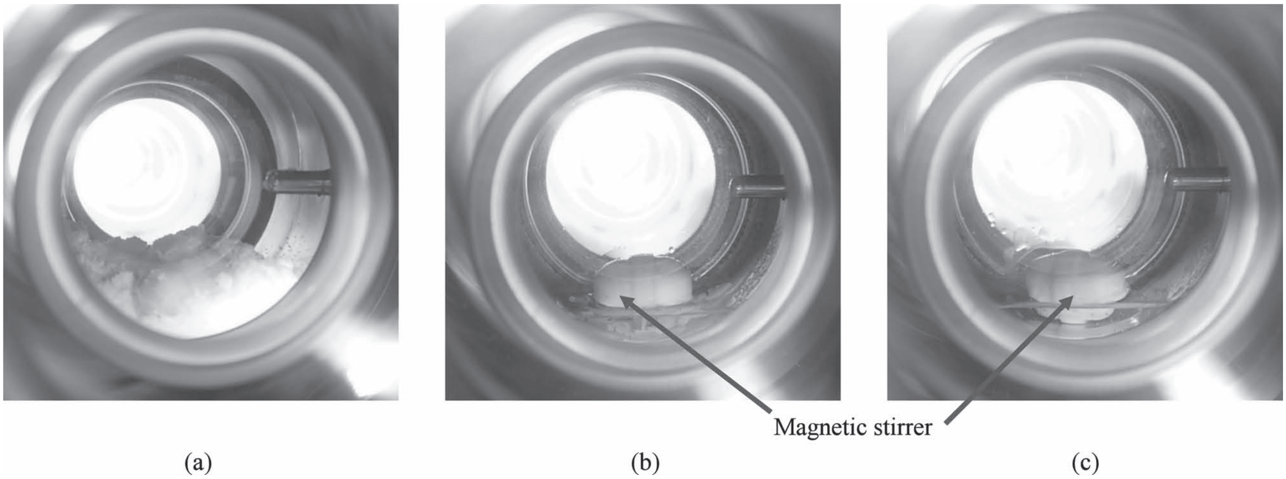

**Figure 1.** Transitions observed for $P_{DL}LA$:PEG 90:10 during the view cell experiment: a) initial mixture in the form of a white powder ($P_{DL}LA$) and white flakes (PEG), b) plasticisation of the $P_{DL}LA$ component to produce a viscous colourless liquid containing the suspended PEG flakes and c) melting of PEG and blending with $P_{DL}LA$ to give a low viscosity colourless liquid at 45 °C and 144 bar.

**Table 1.** Plasticisation conditions and observations of stir bar mobility for $P_{DL}LA$/PEG blends. The majority of the blends did not fully plasticise until 55 °C and 175 bar as a result of the high melting point of PEG. However, the blends containing low PEG contents were fully liquefied at 45 °C and approximately 165 bar, 16 °C below the atmospheric melting point of PEG. The addition of PEG to $P_{DL}LA$ clearly allowed movement of the stir bar qualitatively indicating a reduced viscosity.

| Composition of blend |  | Plasticisation/melting Conditions |  | Movement of the magnetic stir bar |
|---|---|---|---|---|
| [%w/w] |  | [°C] | [bar] |  |
| $P_{DL}LA$ | PEG |  |  |  |
| 100 | 0 | 34 | 76 | No |
| 90 | 10 | 45 | 144 | Yes |
| 80 | 20 | 44 | 165 | Yes |
| 70 | 30 | 55 | 174 | Yes |
| 60 | 40 | 57 | 163 | Yes |
| 50 | 50 | 54 | 162 | Yes |
| 40 | 60 | 53 | 160 | Yes |
| 30 | 70 | 53 | 167 | Yes |
| 20 | 80 | 53 | 154 | Yes |
| 10 | 90 | 53 | 153 | Yes |
| 0 | 100 | 55 | 168 | Yes |

viscosity of liquefied $P_{DL}LA$. In order to assess this theory qualitatively, the view cell experiments were repeated for a range of $P_{DL}LA$:PEG ratios.

A series of observations (Table 1) showed that each of the blends was completely liquefied at 55 °C and 165 bar. Blending can only occur once the polymers are in their liquefied state, and this can only happen upon the complete melting of PEG, despite the fact that the $P_{DL}LA$ component plasticises at a much lower temperature. Some of the blends exhibited a lower liquification temperature (45 °C), likely caused by liquefied $P_{DL}LA$ molecules penetrating into and disrupting the crystalline lattice of PEG.[25] Once liquefied, each blend allowed rapid movement of the magnetic stir bar, confirming that PEG is indeed effective in lowering the viscosity of $P_{DL}LA$, even at concentrations as low as 10%w/w. As PEG is able to enter into the free volume of $P_{DL}LA$, it separates the polymer chains and therefore increases their mobility. Although not previously reported for $P_{DL}LA$/PEG blends, PEG has been shown previously to be effective at reducing the viscosity of high molecular weight polymers, for example UHMWPE.[8]

### 2.2. Blending Studies

After obtaining the temperatures and pressures required to liquefy $P_{DL}LA$ and PEG, a high pressure autoclave[26] was employed to blend samples of $P_{DL}LA$/PEG (ca. 1 g) by mixing them in the presence of $scCO_2$. These samples were subsequently evaluated by DSC and NMR at atmospheric pressure to determine the miscibility and actual $P_{DL}LA$/PEG composition of the blends, respectively. Polymers miscible on a domain scale of 30 nm or less display only a single $T_g$, whereas immiscible blends show no deviation from the $T_g$ values of the pure components.[27] If the polymers are only partially miscible, two $T_g$'s will be displayed but their values will converge towards a central point according to the degree of miscibility.[28] An issue surrounding the analysis of the $P_{DL}LA$/PEG blends was that the $T_g$ of PEG is below -90 °C and so its detection is beyond the capabilities of the DSC instrument used. Thus the actual degree of miscibility could not be fully investigated but a comparison of the $T_g$ values of the $P_{DL}LA$ component of the blends could be made.

Researchers have previously reported $P_{DL}LA$ and PEG to be miscible at low PEG contents when blended thermally at 180–190 °C[11,29] or with solvents.[10,15] However, the $scCO_2$ blending resulted in high miscibility at low PEG contents (8–25%w/w) as shown by the significant ($p < 0.001$) reduction in the $T_g$ of $P_{DL}LA$ (**Figure 2**). Our data show that in the presence of $scCO_2$, blending of $P_{DL}LA$ and PEG can occur at moderate temperatures thus providing an alternative to the high temperatures and solvents required in conventional processes.[9–11,13,14,30]

Detailed investigation of the $T_g$ of the blends produced (Figure 2) shows a very surprising minimum in the observed $T_g$ of $P_{DL}LA$ on the addition of between 8 and 25%w/w PEG with the lowest value occurring at ca. 15%w/w PEG. This clearly demonstrates that the highest miscibility is achieved around these percentage compositions. Beyond this, the $T_g$ rises again indicating that effective blending is no longer achieved and the polymers begin to phase separate. Above 70%w/w PEG the $T_g$ of $P_{DL}LA$ could no longer be observed. This is because the $T_g$ of $P_{DL}LA$ and melting point of PEG occur at similar temperatures, so at high PEG contents the melting point transition swamps the $T_g$ of $P_{DL}LA$.[11] At the miscibility maximum (15%w/w PEG) the $T_g$ has been reduced by over 30 °C. NMR spectra of each individual blend were collected in order to accurately determine the composition of the blends and to accurately define the miscibility region.

In order for two polymers to form a miscible blend the mixing process must obey the Gibbs free energy law. This law states that for a spontaneous process to be feasible, the change in the Gibbs free energy must be negative.[31]

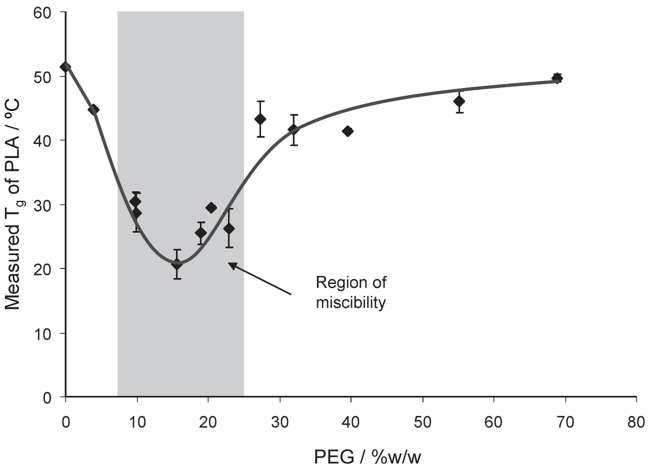

**Figure 2.** DSC data for the $T_g$ of $P_{DL}LA$ providing a miscibility analysis of a range of $P_{DL}LA$/PEG blends. An area of miscibility, represented by the shaded region, is observed between 5 and 25%w/w PEG. $p<0.001$ LSD 3.12 °C.

Thus for any form of blending to proceed, enthalpy must reduce whilst entropy rises. Since there is a high degree of chain entanglement within amorphous polymers, the entropy is initially very large. Therefore, whilst mixing always gives an increase in entropy the magnitude of the change is small causing its contribution to the Gibbs free energy to be negligible. As a result it is the enthalpy of mixing and experimental temperature that will primarily govern miscibility. Unless strong intermolecular forces are present between the two polymers, most blends have an endothermic enthalpy of mixing preventing intimate blending as the T$\Delta$S term is insufficient in magnitude to overcome it, meaning $\Delta$G will be positive. The composition of the blend can also impact on the enthalpy and hence the miscibility of polymers.[32] This results in regions of miscibility such as that observed for the $P_{DL}$LA/PEG blends (Figure 2). Although not previously reported for $P_{DL}$LA/PEG blends, a similar trend in the miscibility has been observed with PLA/poly(caprolactone blends).[33] However, the occurrence of phase separation above 15%w/w PEG has been previously reported for solvent blended crystalline $P_L$LA and PEG.[13]

### 2.3. Rheology of $P_{DL}$LA/PEG Blends

One consequence of this high miscibility region should be that the viscosity of the blend is significantly reduced, especially in the presence of $scCO_2$ and the viscosity should also follow the observed miscibility trend. Thus, high pressure rheological experiments were employed to probe the effect of blending PEG with $P_{DL}$LA in the presence of $scCO_2$.

The previous view cell experiments (Table 1) showed qualitatively that the addition of PEG to $P_{DL}$LA lowers the viscosity. Using a high pressure rheometer, quantitative data can be obtained to assess the effect of $scCO_2$ upon the pure components ($P_{DL}$LA, PEG) and their blends. The high pressure rheometer is limited to a pressure of 100 bar, and although this is lower than the 140 bar used in our initial studies, it is well known that once above the critical point, $CO_2$ pressure appears to have very little effect of the viscosity of polymers.[34]

At low shear rates, $P_{DL}$LA and the blends demonstrated shear thinning behaviour up to a shear rate of ca. 50 $s^{-1}$ (**Figure 3**a).[35] This behaviour is observed as the entangled chains within amorphous polymers are unravelled and aligned by the rotating motion.[36] Above a shear rate of 50 $s^{-1}$, the polymers and the blends display Newtonian behaviour and their viscosities become independent of shear rate.[35] Since the parallel plates are contained inside the high pressure vessel there is an absence of centrifugal effects on the rheological measurements at these high rotational speeds. In the presence of $scCO_2$, $P_{DL}$LA has a much higher viscosity than PEG at a shear rate of 344 $s^{-1}$ (215 Pa.s compared to 33 Pa.s). A significant ($p < 0.001$) reduction in the viscosity of $P_{DL}$LA is observed on the addition of PEG, and this is proportional to the amount of PEG within the blend. We hypothesise that the PEG molecules enter into the free volume between the $P_{DL}$LA chains, causing the chains to separate increasing their mobility and lowering viscosity. Similar behaviour has been previously reported for PEG/UHMWPE miscible blends.[8]

From the initial rheological studies it was shown that PEG can reduce the viscosity of $P_{DL}$LA. We hypothesised that viscosity would decrease with increasing blend miscibility, because at high miscibilities the PEG molecules should be completely dispersed within the $P_{DL}$LA and therefore might have a more pronounced effect. Thus a range of $P_{DL}$LA/PEG blends with concentrations in the miscibility region (i.e., 0, 6, 10, 15, 20, 24 and 30%w/w PEG) were analysed. The same blends were also previously investigated by DSC (Figure 2). A temperature of 40 °C was chosen to mimic the experimental conditions used in the PGSS process[1] where drug delivery devices can be fabricated at near ambient temperatures; a key advantage of the use of $scCO_2$.[1,37]

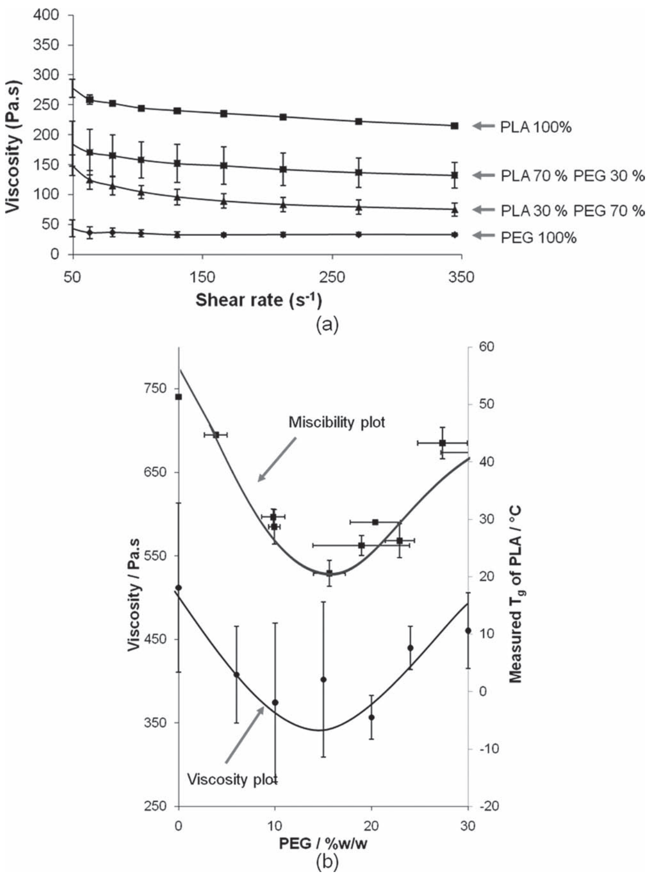

**Figure 3.** Rheological analysis of PLA/PEG blends in $scCO_2$. a) The addition of PEG causes a large reduction in the viscosity of PLA and is determined by the concentration of PEG within the sample. $p < 0.001$ LSD 23.5 Pa.s; b) Comparison of the viscosity of the PLA/PEG blends at 344 $s^{-1}$ (left axis) with the miscibility of the polymers (right axis). A general trend of decreasing viscosity with increasing miscibility is observed. $p = 0.189$, LSD 123 Pa.s.

The results revealed that even at low PEG concentrations the viscosity of $P_{DL}$LA at 344 $s^{-1}$ is reduced (Figure 3b). Whilst there is no significant ($p = 0.189$) difference between the viscosities of the blends in this high miscibility region, there is clearly a trend towards lower viscosities.

### 2.4. Use in Application

The PGSS process (CriticalMix) exploits the unique properties of $scCO_2$ to enable plasticisation and spraying of polymeric materials.[1,24,38,39] We have utilised this capability to encapsulate drugs and to create polymer microparticles in a process that can be operated at near physiological temperatures, thus preventing degradation of delicate protein based drugs such as human growth hormone.[1,37]

We have shown previously that PEG can be easily sprayed into small, spherical particles using a PGSS particle rig.[24,40] However, processing of $P_{DL}LA$ is more challenging, often producing irregular shapes and long fibres.[38] One suggestion for this observed disparity is the difference in the viscosities of the plasticised polymers. PEG, being crystalline, becomes a low viscosity liquid in the presence of $scCO_2$.[24] However, the amorphous nature of $P_{DL}LA$ ensures high viscosity[24,34] and makes it more challenging to atomise through the nozzle.[38]

We hypothesised that lowering the $P_{DL}LA$ viscosity by the addition of PEG (Figure 3) would enhance processability during the PGSS process. Moreover, since PEG is a common excipient used for introducing hydrophilicity to polymer particles, it was clear that there are sound formulation advantages in creating such microparticulate miscible blends. Particles were produced from polymer compositions within, or close to, the previously determined miscible region (Figure 2) using the PGSS rig. The microparticle samples were analysed using a range of analytical techniques to determine the effect of adding PEG and the miscibility of the polymers on the properties of the particles. A polymer mixture containing 70%w/w PEG was also sprayed to analyse the effects of very high PEG concentrations.

The mixing and spraying of the $P_{DL}LA$/PEG blends produced a fine white powder for all compositions and in each case the yield (mass of material weighed into the process vs. mass of material recovered) was determined as a good indicator of the ease of spraying and particle formation (**Figure 4**). At the very high PEG contents (towards 70%w/w) the yield is high because the high PEG content results in a significant ($p < 0.001$) reduction in the viscosity of the blend (Figure 3) and this clearly leads to more facile spraying. These yield data are consistent with the relationship between viscosity and spraying previously reported for PEG.[40] What is particularly striking, are the data obtained at lower PEG ratios, which show a sharp increase and a clear maximum yield as the PEG concentration reaches 15%w/w and then decreases again towards 30%w/w (Figure 4). Although the points in the plot are individual points, for a repeated manufacture of a formulation using the same process the standard deviation was found to be 5%. This error has been incorporated into the plot (Figure 4) to give an indication of the error which would be expected in this case.

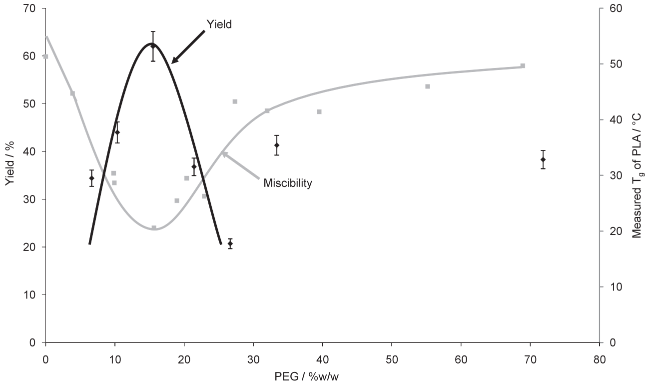

**Figure 4.** Yield of particles produced by PGSS for a range of PEG:$P_{DL}LA$ mixtures. Note that the yield rapidly increases up to a PEG content 15%w/w where it decreases again. At higher PEG contents, the viscosities are significantly lower and yields rise again accordingly. The standard deviation of a repeated manufacture of a formulation was 5%. This error has been added to this plot to give an indication of the expected error. Comparison with the data obtained from DSC studies of various blends showing that the blend miscibility trend correlates well with the yield data for collection of particles from the PGSS spraying.

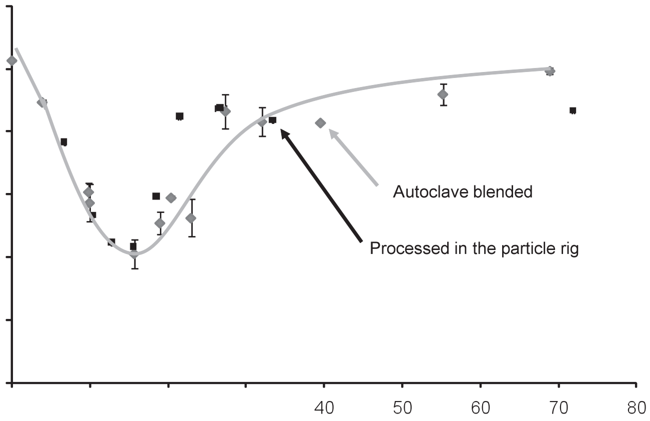

**Figure 5.** Comparison of the $T_g$ values of the particles obtained by PGSS spraying with blends prepared by simple $scCO_2$ mixing within an autoclave. The $T_g$ of the processed particles are very similar to those of the blends. Note: each of the data points has been plotted with error bars however some are too small to be observed.

Moreover, below 30%w/w PEG these yield data match precisely with the miscibility regions (Figure 2) that were established by measurement of the $T_g$ of the prepared blends and superimposing the two sets of data shows this clear correlation (Figure 4).

DSC analyses were performed on the microparticles obtained from these spraying experiments (i.e., each point in Figure 4) in order to determine the miscibility of the polymers following the PGSS processing (**Figure 5**). These data again show clearly the same increase in miscibility up to 15%w/w PEG as demonstrated by the $T_g$ data measured for $P_{DL}LA$ in the particles obtained from the spraying process. Superimposition of DSC data from the PGSS spraying experiments with those obtained from simple blending in $scCO_2$ (Figure 2) demonstrate excellent correlation and indicate that the very rapid and violent pressure release (effervescent atomisation) of the spraying process does not induce any significant phase separation in the polymeric microparticles (compare traces in Figure 5). Moreover, it is clear that model polymer blends obtained through autoclave mixing provide a valid route to estimating the microparticle compositions and optimal spray parameters for a range of formulations. These results also prove that, in the presence of $scCO_2$, $P_{DL}LA$ and PEG can be blended together at temperatures as low as 40 °C. Thus from a formulation perspective $scCO_2$ offers an ideal route to both introducing hydrophilic character and an opportunity to process delicate protein therapeutics at near ambient temperatures.

### 2.4.1. Particle Size Analysis

Particle size is also a key criterion that will determine the efficacy of any formulation, particularly with respect to control of the drug release profile and injectability. The microparticle products were further investigated to determine the effect of PEG content and polymer miscibility upon particle size and morphology. SEM images of the particles (**Figure 6**) show an increase in particle size on the addition of PEG up to 24%w/w. Each of the batches contains irregular shapes, but the tendency is towards a more spherical form as the concentration of PEG increases. The exterior surface of the particles also becomes increasingly smoother as the PEG content is increased. As the viscosities of the blends in the miscibility region are similar this has little impact on the size of the particles formed. Since the particles are produced via effervescent atomisation through the nozzle there is a very rapid release of most of the $CO_2$ dissolved in the polymer and at the surface in particular the polymer will harden quickly. However, some residual $CO_2$ will be trapped inside, particularly within the amorphous regions and as this tries to diffuse from the polymer, bubbles will be formed.[41] These bubbles will grow and coalesce, weakening the structure of the particle, until finally the polymer particle will explode forming smaller fragments. This process will also be affected as the ratio of PEG to PLA changes. The solubility of $CO_2$ in PEG is lower than in $P_{DL}LA$ because its crystalline nature makes it more difficult for the $CO_2$ molecules to penetrate into the free volume between the chains.[17,42] Thus, increasing the PEG content within the blends decreases the amount of dissolved $CO_2$ and hence reduces the level of entrapped $CO_2$ in each microparticle. Thus, one can hypothesise that fewer such explosions will occur leading to larger, smoother and more spherical particles at high PEG contents up to 24%w/w PEG. Above this range, atomisation effects will dominate and one would expect smaller particles as a result of simply spraying a much less vis-

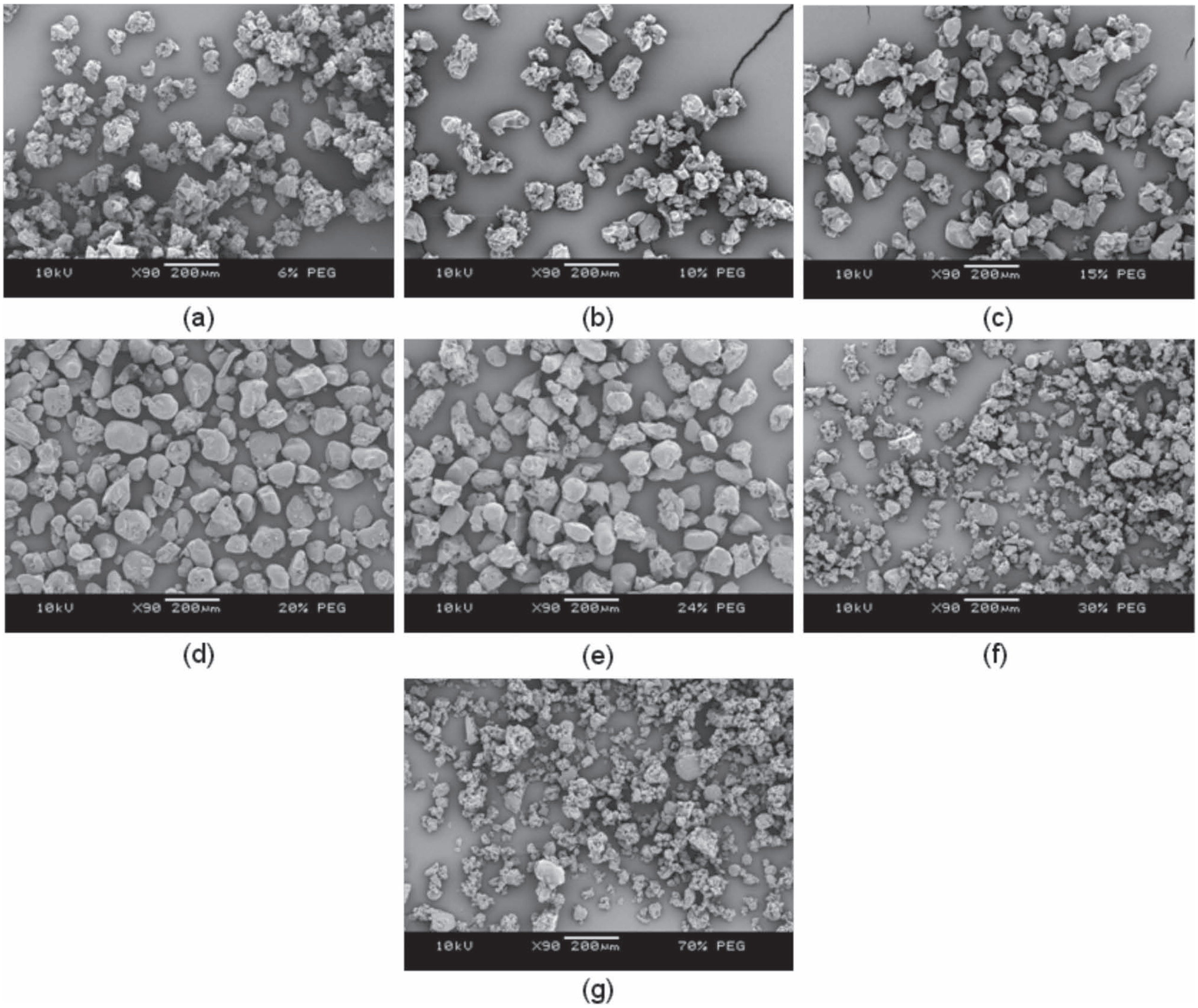

**Figure 6.** SEM images of the sieved $P_{DL}LA$:PEG particles, containing a) 6, b) 10, c) 15, d) 20, e) 24, f) 30 and g) 70%w/w PEG. As the PEG content is increased to 24%w/w the particles become larger, more spherical and less porous. Above this concentration, the particles decrease in size again. The SEM images also show that the porosity decreases as more PEG is added to the formulations demonstrating that at higher PEG concentrations, less $CO_2$ is retained to develop into pores.

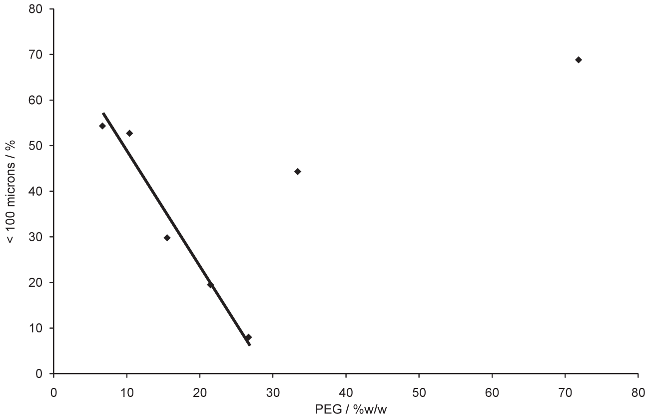

**Figure 7.** Demonstration of the yield of particles sized under 100 µm achieved via sieving of the sprayed microparticulate samples. Again, these data follow the trends outlined earlier related to viscosity and PEG loading.

cous liquid as demonstrated by the viscosity of the 70%w/w PEG blend being lower than that at 30%w/w (Figure 3)[40] and the lowering of particle size observed in the SEM images (Figure 6).

The batches were then sieved to quantify the percentage of particles smaller than 100 µm (**Figure 7**). These data clearly complement the SEM images showing that the yield of particles of less than 100 µm decreases linearly as the PEG concentration increases up to 30%w/w. Above this there is a clear tipping point and particle size then decreases again at higher PEG loadings and hence lower viscosities which makes atomisation easier and more rapid, therefore forming smaller particles.

## 3. Conclusions

Our data demonstrate that $P_{DL}LA$ and PEG can be blended using $scCO_2$ at temperatures as low as 40 °C. This avoids the high temperatures and solvents required with conventional methods, thereby opening up the opportunity to incorporate thermally and solvent labile protein based therapeutics. DSC analyses have proven that $P_{DL}LA$ and PEG are miscible between a PEG content of 5 and 25%w/w with the greatest miscibility occurring at 15% w/w PEG. Rheological studies have provided a quantitative method for analysing the viscosity of the blends. These results demonstrate that in the presence of $scCO_2$, $P_{DL}LA$ is much more viscous than PEG and that the combination of the two polymers can significantly reduce the viscosity of $P_{DL}LA$. No significant difference in the viscosity of the blends was detected as the PEG content was varied in the miscible region but, a reduction in the viscosity was observed at high miscibilities. $P_{DL}LA$/PEG microparticles were successfully generated using the CriticalMix process.[1] The yield was inversely related to the miscibility of the polymers showing that the miscibility does have a direct effect on the processability of the blends. Sieving of the particles showed the size to increase linearly upon the addition of PEG, to a content of 24%w/w, but then decrease again as further PEG is added. DSC studies following spraying have shown that the particles retain their miscibility during processing. This illustrates that $P_{DL}LA$ and PEG can be blended at temperatures as low as 40 °C in the presence of $scCO_2$.

## 4. Experimental Section

*Materials*: $P_{DL}LA$ R202H with an inherent viscosity of 0.23 dl/g ($M_w$ 11 000 a.m.u measured against PS standard in THF) was purchased from Boehringer Ingelheim and PEG 6000 ($M_w$ 6000) was supplied by Fluka. Both polymers were used as received. Pharmaceutical grade carbon dioxide (99.5% $CO_2$) and zero grade $N_2$ were supplied by BOC Special Gases.

*Plasticisation Studies:* The temperatures and $CO_2$ pressures required to either melt or plasticise $P_{DL}LA$, PEG and the $P_{DL}LA$/PEG blends were determined using a hydraulic variable volume view cell.[43] $P_{DL}LA$ was physically mixed with PEG in compositions of 0 to 100%w/w in 10%w/w increments. In a typical blending experiment, this polymer mixture (0.87 g) was loaded into the view cell at room temperature. The cell was sealed and charged with $CO_2$ (20 g) and any changes to the polymers noted. The view cell was then heated to 35 °C and the piston moved forward to generate a pressure of 170 bar. The polymer was allowed to soak in the $scCO_2$ for 1 hour before raising the pressure to 340 bar. The view cell was then heated to 45 °C whilst retracting the piston in order to maintain a pressure of 170 bar. The sample was then left to equilibrate again for 1 hour before raising the pressure to 340 bar by moving the piston forwards. This procedure was repeated at both 55 and 65 °C. At each pressure and temperature interval the blend was observed visually and any changes noted. A magnetic stir bar was also incorporated into the cell to qualitatively observe the viscosity of the liquefied materials.

*Blending of $P_{DL}LA$ and PEG*: Blends of $P_{DL}LA$ and PEG in various compositions were produced using a high pressure autoclave. In a typical experiment, the polymer mixture (0.5 g) was added to a 10 mL autoclave equipped with a magnetically coupled overhead stirrer.[26] The autoclave was charged with $scCO_2$ to 55 bar and heated to 60 °C. On reaching the correct temperature, the pressure was increased to 140 bar and the mixture stirred for 1 hour. After this time, the autoclave was allowed to cool to room temperature before being vented. The blends were stored in a freezer to prevent phase separation and analysed by differential scanning calorimetry (DSC) and NMR spectroscopy 1 to 3 days after production to ensure that no $CO_2$ remained.

*Differential Scanning Calorimetry (DSC)*: A TA-Q2000 DSC (TA instruments), calibrated with an indium standard, was used to analyse the $P_{DL}LA$/PEG blends and polymer microparticles. In a standard analysis, the sample (2–5 mg) was weighed into a Tzero DSC pan (TA instruments). This pan was capped with a Tzero DSC lid (TA instruments) and sealed with a Tzero press (TA instruments) using a Black Tzero lower die and a flat die (TA instruments). In a typical run, the temperature was increased from –90 to 100 °C at a rate of 10 °C/min. As no endothermic peak was present on the glass transition the result was read from the inflection point. The miscibility of the two polymers was determined by the shift in the $T_g$ of $P_{DL}LA$. As heat can increase the miscibility of blends resulting in further convergence of their $T_g$'s, the results were determined from the first cycle to ensure that any shifts were created by $CO_2$ processing only.

*Nuclear Magnetic Resonance (NMR)*: Accurate compositions of the $P_{DL}LA$/PEG blends were determined using $^1H$ NMR on a Bruker DPX-300 spectrometer operating at 300.14 MHz. Typically, 16–32 scans were co-added to improve the signal to noise ratio. The polymers were dissolved in $CDCl_3$ prior to analysis.

The peaks created by the CH (4.80 ppm) in $P_{DL}LA$ and the two $OCH_2$ (3.60 ppm) groups in PEG were used to calculate the actual weight composition of the samples (Equation 1). Each blend was sampled three times in order to produce an average.

$$Composition_A = \frac{(Integral_A/Proton_A) \times M_{wA}}{[(Intregal_A/Proton_A) \times M_{wA}] + [(Integral_B/Proton_B) \times M_{wB}]} \times 100\% \quad (1)$$

*Rheology of $P_{DL}LA$/PEG Blends*: High pressure rheological studies were performed using a Physica MCR301 rheometer (Anton Paar, Hertford, UK), with a high pressure parallel plate geometry of 20 mm in diameter and with a 1 mm gap (PP20/Pr), on the $P_{DL}LA$/PEG blends to determine the impact of PEG concentration on the viscosity of $P_{DL}LA$. In a typical experiment, the two polymer powders were physical mixed together to give the required composition. This mixture (0.5 g) was added to the cup of the high pressure rheometer which was then sealed and heated to 40 °C. $scCO_2$ was charged into the vessel, using a high pressure syringe pump, to generate a pressure of 100 bar, and the polymer mixture was allowed to soak for 1 hour. The experiments were performed at 100 bar instead of the 140 bar previously used as this was the maximum pressure rating of the rheometer. During this time, the syringe pump remained connected in order to compensate for any minor leaks within the system. After this hour, rotational tests were executed to measure the viscosity as the shear rate was varied on a log scale from 10 to 1000 $s^{-1}$. Each polymer mixture was analysed three times in order to obtain an average.[34]

*Production of $P_{DL}LA$/PEG Microparticles*: Microparticles of $P_{DL}LA$/PEG blends containing 6, 10, 15, 20, 24, 30 and 70%w/w PEG were produced using the particles from gas saturated solutions (PGSS) equipment following the procedure described previously.[1] $P_{DL}LA$ and PEG were placed in a high pressure vessel. This vessel was heated >32 °C and pressurised, with $CO_2$ to >75 bar. The liquefied polymers were then stirred for 1 hour, intimately mixing them together. This mixture was spayed through a nozzle, causing the formation of particles which were then collected by a cyclone.

*Scanning Electron Microscopy (SEM)*: High resolution, high magnification images of the microparticles produced using the CriticalMix process were obtained using a JSM-6060LV scanning electron microscope. The samples were attached onto aluminium stubs with adhesive carbon discs. Before analysis, the samples were sputter coated with gold for 3 minutes, under vacuum, using a SCD 030 sputter coater (Blazers) with a current of 0.1 A.

*Sieving*: In order to get an appreciation of their size and distribution, the polymer microparticles resulting from the CriticalMix process were sieved through a 100 µm sieve, using a Retch sieve shaker, at an amplitude of 1.5 mm for 30 min.

*Statistical Analysis*: Statistical analyses were performed where possible, using Microsoft Excel. Analyses were calculated using ANOVA and the Least Significant Difference (LSD) at the 5% confidence level. Any differences in results were considered significant if the p-value was less than 0.05.

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