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Manipulation of process parameters to achieve different ternary phase microparticle configurations

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ABSTRACT

Ternary phase microparticles of poly(D,L-lactide-co-glycolide) (50:50), poly(L-lactide) and poly(caprolactone) were fabricated through a one-step solvent evaporation technique. The purpose of this study was to examine the effects of various process parameters on the final configuration (i.e. polymer distribution and dimensions) of these composite microparticles and, subsequently, propose their mechanism of formation. Particle morphologies and configurations were determined using scanning electron microscopy, polymer dissolution tests and Raman mapping. It was found that a starting polymer solution prepared below the cloud point and an increased oil to water ratio will facilitate polymer configurations close to thermodynamic equilibrium, which is dictated by the interfacial energies of the components. By varying the polymer mass ratio or adjusting the precipitation rate, through stirring speed and oil to water ratio, a wide range of microparticles with different core–shell dimensions and embedded particulate sizes can also be fabricated. At the same time, lowering the polymer solution concentration and increasing the stirring speed may result in smaller microparticles. Correlation of these process parameters with the final composite particle morphology was thus established. This understanding should allow the controlled fabrication of ternary phase composite microparticles through a single step solvent evaporation technique.

1. Introduction

Over the past few decades microparticles of biodegradable polymers, such as poly(lactic acid) (PLA) and poly(lactic acid-co-glycolic acid) (PLGA), have received much attention in the biomedical arena. This is because drug-loaded polymeric microparticles have the potential for controlled drug release [1,2] while at the same time protecting the drugs from degradation before they reach the release site. However, conventional single walled microparticles have several inherent limitations, such as an initial burst release and the inability to provide constant (zero order) drug release and/or a pulsatile release of therapeutic agents [1].

In an attempt to better control drug release kinetics, multiparticulate or microcapsule drug delivery devices [3–6] that compose a polymer shell surrounding one or many micron sized particulates have been used to circumvent some of these limitations. As such, different microparticles consisting of two immiscible polymers have been fabricated and reported [3–10]. Most notably, Pekarek et al. reported on the preparation of double walled microspheres consisting of a core of poly[1,3-bis-(p-carboxyphenoxy propane)-co-(sebacic anhydride)] (20:80) surrounded by an outer layer of poly(L-lactide) (PLLA) through a solvent evaporation technique [7–8]. As compared with other earlier attempts where prefabricated microspheres were further coated using a hot melt technique, pan coating or fluidized beds to produce double walled microparticles [10], the solvent evaporation technique is a one-step process that gave double walled microparticles with higher yields, uniform wall thickness and a controllable particle size within the range 20–1000 μm. Subsequently, Leach et al. [11] studied the effect of fabrication conditions such as weight ratio, polymer solution concentration, temperature and air flow on the formation efficiency of double walled microspheres.

Previous studies have shown that the degradation behavior of multi-layer polymer films differs from that of single layer polymer films, with the degradation of the top polymer layer accelerating hydrolysis of the underlying layers [12,13]. Thus it is postulated that a composite multi-layer or multi-phase microparticulate system may also possess unique hydrolytic degradation characteristics that differ from single walled particles. As such, these multi-layer or multi-phase microparticles may offer greater versatility in controlling the drug release kinetics and profile, by manipulating the particles' layer thicknesses, configurations or even size [5,6,14–24]. Matsumoto et al. [5,6] demonstrated that the outer, non-drug-holding poly(D,L-lactide) layer of multi-reservoir type microspheres suppressed the initial burst of cisplatin located in

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the PLGA core, thus achieving sustained release. Similarly, Shi et al. [15] also reported that a nearly complete and sustained release of hydrophilic bovine serum albumin and hydrophobic cyclosporin A can be achieved from poly(orthoester) (POE)–PLGA double walled microspheres.

Although the fabrication of double walled microparticles using a solvent evaporation method has been established [7–11,14–18], there has been no report on the fabrication of microparticles consisting of three different polymers – ternary phase microparticles. Introducing an additional polymer to form a multi-phase composite microparticle could, therefore, be an attractive and robust approach to delivering multiple drugs, through selective localization of each drug in the individual polymer phases. Such particulate systems may, at the same time, also provide sustained and controlled release of drugs. Alternatively, the additional polymer in ternary phase microparticles may also provide a means to release drugs in a sequential manner to achieve a pulsatile drug delivery profile.

The objective of this paper was, therefore, to report on the fabrication of ternary phase polymer composite microparticles composed of poly(D,L-lactide-co-glycolide) (50:50) (PLGA), poly (L-lactide) (PLLA) and poly(caprolactone) (PCL), through a one-step solvent evaporation technique. The effects of process parameters, such as polymer solution concentration, stirring speed, oil to water ratio and polymer mass ratio, on the final microparticle configuration were investigated. From these results the mechanism of formation of ternary polymer composite microparticles will be proposed. Using this knowledge different configurations of microparticles could be fabricated, thus providing a greater degree of freedom in designing microparticles that can provide the desired drug release profile suited to different applications.

2. Materials and methods

2.1. Materials

PLLA (intrinsic viscosity IV: 2.38, Bio Invigor), PLGA (50:50) (IV: 1.18, Bio Invigor), PCL (Aldrich) and poly(vinyl alcohol) (PVA) (molecular weight 30–70 kDa, Sigma–Aldrich) were used without further purification. The properties of the polymers used in this study are listed in Table 1. High performance liquid chromatography (HPLC) grade dichloromethane (DCM) and tetrahydrofuran (THF) (Tedia Co., Inc.) were used as solvents as received.

2.2. Polymer cloud point

Before any microparticle fabrication the cloud point of each polymer, i.e. the polymer solution concentration at which one polymer becomes immiscible with the other two polymers, was first determined. First, a total polymer mass of 0.3 g was weighed at a mass ratio of 3:2:1 (PLLA:PLGA:PCL). A 2% w/v homogeneous polymer solution, consisting of PLLA (0.15 g), PLGA (0.1 g) and PCL (0.05 g), was then prepared by dissolving the polymers in 15 ml DCM. The ternary polymer solution was then transferred to a 20 ml graduated cylinder and allowed to sit undisturbed in a fume hood at room temperature. When distinct phases became apparent in the solution the volume of the solution was recorded [25,26]. The formation of a distinctive yellowish liquid phase is indicative of the phase separation of PLGA.

At the same time, to determine the amount of DCM partitioned into each polymer phase, the volume fraction of DCM in each polymer phase was measured. The volume of DCM in each phase was calculated from the volume difference between the polymer liquid phase and the polymer that was added. Each polymer phase was extracted using a syringe and its volume was measured. To determine the polymer, i.e. PLGA, PLLA or PCL, in each phase the extracted polymer phase was analyzed by Fourier transform infrared spectroscopy (FTIR).

Table 1
Polymers used in this study.

Polymer	Intrinsic viscosity (dlg^{-1})	M_n ($gmol^{-1}$)*
PLLA	2.38	1.64×10^5
PLGA	1.18	5×10^4
PCL	–	10.7×10^4

* Number-average molecular weight as determined by SEC (size exclusion chromatography).

2.3. Fabrication of microparticles

PLLA/PLGA/PCL composite microparticles were prepared using an (oil/water) emulsion solvent evaporation technique [10]. Briefly, the three polymers were first dissolved in DCM. The resultant polymer solution was then added to an aqueous 0.5% w/v PVA solution and emulsified using an overhead stirrer (Calframo BDC1850-220) at room temperature (25 °C). The evaporation of DCM will give rise to phase separation of PLLA, PLGA and PCL, to yield ternary phase composite microparticles. Finally, the microparticles were filtered, rinsed with deionized water, lyophilized and stored in a desiccator for further characterization.

Microparticles with different configurations were prepared in the same manner by altering the starting ternary polymer solution concentrations, stirring speed, oil to water ratio and polymer mass ratio. Table 2 summarizes the process parameters that were altered in this study. A reference ternary phase microparticle (particle R) was first fabricated for subsequent comparison with other microparticles.

2.4. Characterization

2.4.1. Microparticle configuration and polymer distribution study

2.4.1.1. Scanning electron microscopy (SEM). The surface and internal morphologies of the microparticles were analyzed using scanning electron microscopy (SEM). The microscope employed was JEOL JEM-6360A, which was operated at a voltage of 5 keV. Before analysis the samples were first mounted onto metal stubs and cross-sectioned approximately at the center line with a razor blade. Samples were then coated with gold using a sputter coater model SPI-Module. For every sample batch that was fabricated 10 microparticles were randomly chosen to be viewed by SEM. Since particle configurations were found to be consistent within each batch, only one representative SEM micrograph will subsequently be shown.

2.4.1.2. Dissolution method. The dissolution method devised by Lee et al. [14], based on the solubility differences of the polymers in THF (i.e. PLGA and PCL are soluble in THF, PLLA is not), was used to determine the final configuration or polymer distribution within the ternary polymer microparticles. Briefly, the cross-sectioned composite particles were first immersed in THF, without agitation, for 2 days to dissolve the PLGA and PCL. The cross-sectioned particles were then collected for SEM analysis.

2.4.1.3. Raman mapping. To confirm the above results, Raman mapping was utilized to further verify the final particle configuration. Composite microparticles that had been pre-sectioned were placed under the microscope objective with laser power of up to ~20 mW. For particle R Raman point by point mapping measurements were then performed on an area of 300×200 μm with a step size of 5 μm in both the x and y directions using a Raman microscope (In-Via Reflex, Renishaw) equipped with a near infrared enhanced

Table 2
Parameters used to fabricate the PLLA/PLGA/PCL composite microparticles.

	Polymer solution concentration (% w/v)	Stirring speed (rpm)	Oil-to-water ratio	Polymer mass ratio		
				PLLA/	PLGA/	PCL
Ternary-phase reference microparticles (Particle R)	6	300	0.02	0.15 g	0.1 g	0.05 g
				3:	2:	1
Effect of starting polymer solution concentration	2	300	0.02	0.15 g	0.1 g	0.05 g
	4					
	10			3:	2:	1
Effect of stirring speed	6	150	0.02	0.15 g	0.1 g	0.05 g
		400		3:	2:	1
		500				
Effect of oil-to-water ratio	6	300	0.1	0.15 g	0.1 g	0.05 g
			0.03			
			0.0125	3:	2:	1
			0.0083			
Effect of polymer mass ratio				0.1 g	0.15 g	0.05 g
	6	300	0.02	2:	3:	1
				0.05 g	0.15 g	0.05 g
				1:	3:	1
				0.15 g	0.1 g	0.1 g
				3:	2:	2

deep depleted hermoelectrically Peltier cooled CCD array detector (576 × 384 pixels) and a high grade Leica microscope. The sample was irradiated with a 785 nm near infrared diode laser and a 50× objective lens was used to collect the backscattered light. Measurement scans were collected using a static 1800 groove per mm dispersive grating in a spectral window from 300 to 1900 cm^{-1} and the acquisition time for each spectrum was ~35 s. Spectral preprocessing, including removal of spikes due to cosmic rays, were then carried out first, before the Raman mapping data was further analyzed using the band target entropy minimization

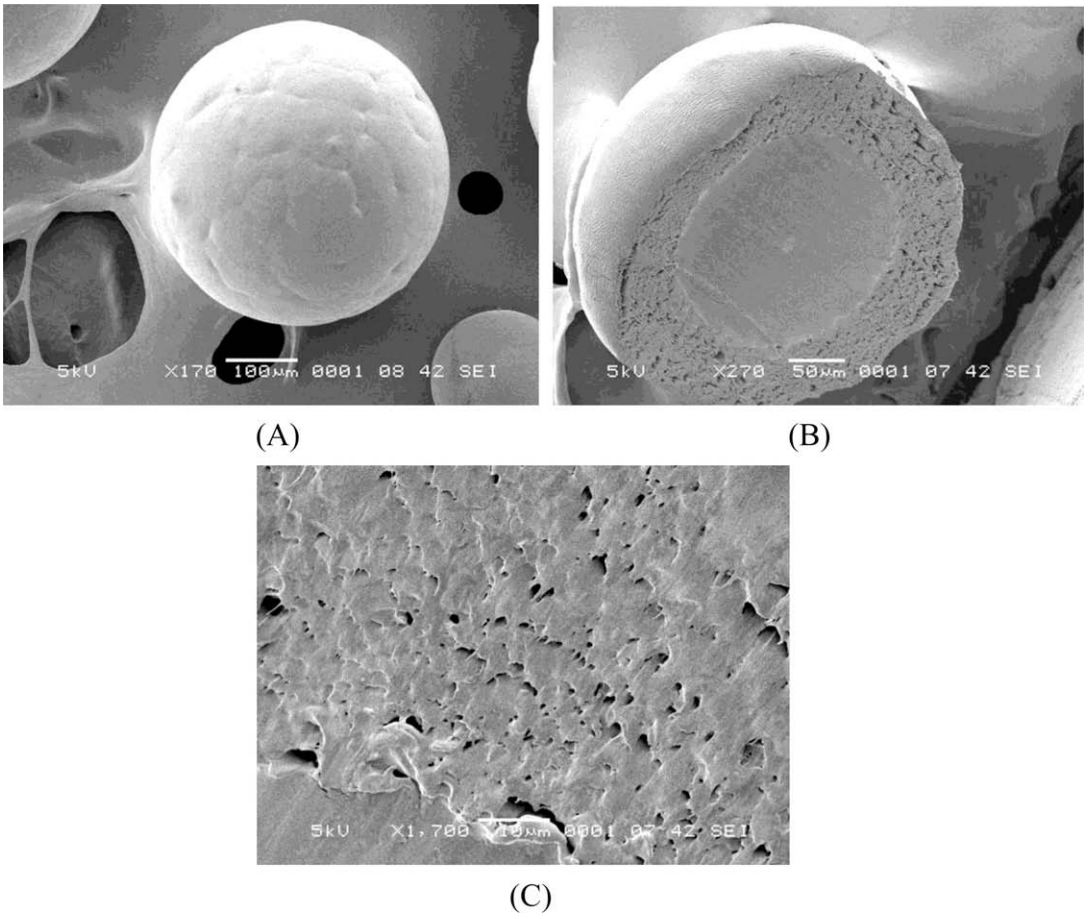

Fig. 1. SEM micrographs of PLLA/PLGA/PCL composite double-walled (DW) microparticles (particle R, reference particle). (A) External morphology. (B) Cross-sectional view of a microparticle. (C) Close-up view of the shell.

(BTEM) algorithm. The BTEM algorithm [27,28] was developed in order to reconstruct pure component spectral estimates. When all normalized pure component spectra of all underlying constituents have been reconstructed, the relative contributions of each measured point of these signals can be calculated by projecting them back onto the baseline-corrected and normalized data set. The spatial distribution of each underlying constituent (i.e. PLLA, PLGA and PCL) can then be generated.

2.4.2. Differential scanning calorimetry (DSC)

Thermal analysis of the microparticles was performed using differential scanning calorimetry (DSC) (DSC-Q10, TA Instruments). To avoid oxidative degradation, the sample and reference pans were purged with nitrogen at a constant flow rate of 48 ml min^{-1}. Approximately 5 mg of each sample was heated from −50 °C to 200 °C at a scan rate of 5 °C min^{-1}.

3. Results

3.1. Fabrication of ternary phase reference microparticles (particle R)

A reference set of ternary phase microparticles (particle R) was first fabricated before varying the process parameters as summarized in Table 2. Fig. 1 shows the SEM micrographs for particle R, of the exterior (Fig. 1A) and interior (Fig. 1B and C). From the micrographs the size range of the particles was ~300–400 μm. The microparticles were spherical with smooth exterior surfaces. Cross-sectioning the microparticles revealed a double walled structure (Fig 1B). In addition, a close-up view of the microparticle showed that the shell was less dense than the core (Fig 1C).

DSC thermograms of this composite microparticle showed two glass transition temperatures, due to PLLA and PLGA, and two melting temperatures, due to PLLA and PCL. This implies that the polymers were phase separated and formed an immiscible blend, resulting in the formation of ternary phase microparticles (Fig. 1). The thermal properties of the pure polymers and particle R are summarized in Table 3.

Fig. 2 shows SEM micrographs of cross-sectional views of particle R after dissolution in THF, showing a highly porous shell. DSC analysis of the shell showed that this polymer was PLLA (T_g = 76 °C, T_m = 178 °C) (data not shown). The microparticle configuration of particle R was further verified using Raman mapping. The Raman mapping results (Fig. 3) showed that the core–shell configuration was PLGA and PLLA, respectively, with PCL uniformly dispersed in the PLLA shell. In addition, the sizes of these PCL particulates were found to be 1–10 μm (Fig 2B). PLLA/PLGA microparticles without PCL were also fabricated for comparison, and SEM micrographs showed a dense PLLA shell due to the absence of

Table 3
Thermal properties of pure polymers and composite microparticles obtained from DSC thermograms.

Polymers	Glass transition temperature, T_g (°C)		Melting temperature, T_m (°C)	
PLGA	43		–	
PLLA	74		180	
PCL	–		60	
PLLA/PLGA/PCL	PLLA: 63	PLGA: 45	PLLA: 176	PCL: 52

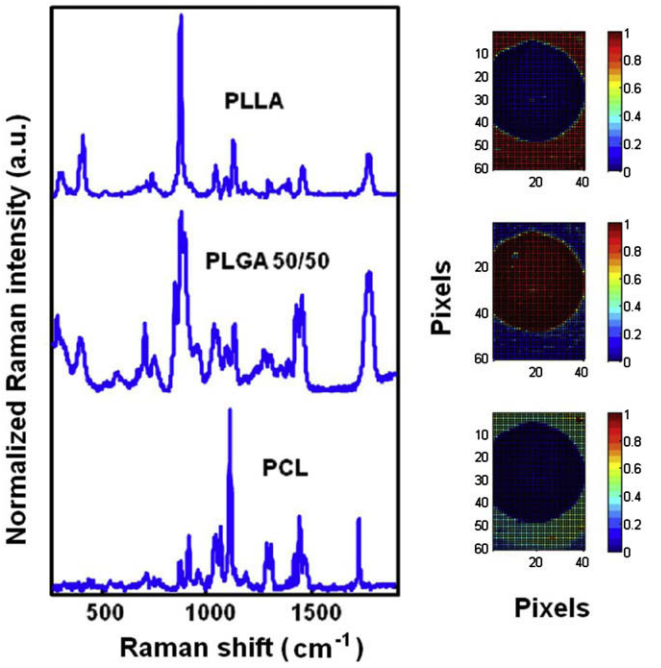

Fig. 3. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a PLLA/PLGA/PCL DW composite microparticle (particle R). The axes of score images are in pixels. These can be directly converted to distances by multiplying the number of pixels by 5 μm.

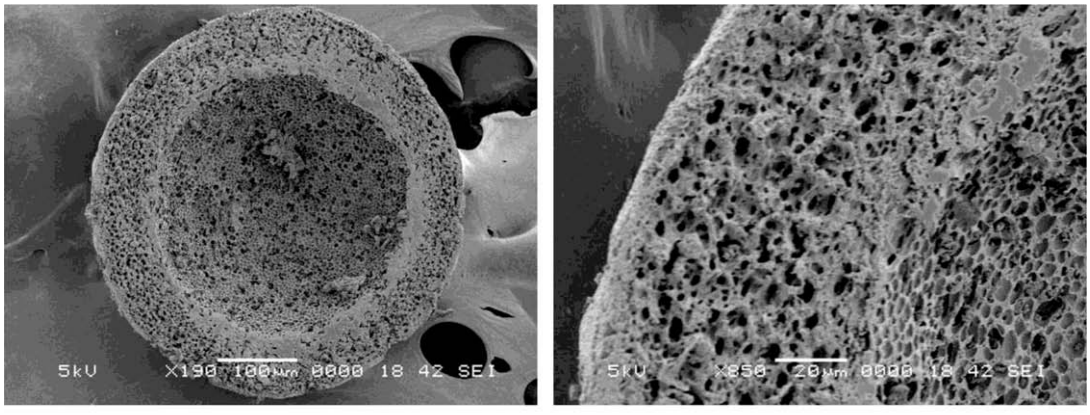

Fig. 2. SEM micrographs of PLLA/PLGA/PCL composite DW microparticles (particle R) after dissolution with THF. (A) Cross-sectional view of a whole microparticle. (B) Close-up view of porous shell.

PCL (Fig. 4A and B). Raman mapping confirmed that the dense shell was PLLA and the core PLGA (Fig. 4C). The existence of two glass transition temperatures (T_g of PLGA = 41 °C, T_g of PLLA = 67 °C) and one melting temperature (T_m of PLLA = 177 °C) again implied that PLLA and PLGA were phase separated (data not shown). In summary, the configuration of the PLLA/PLGA/PCL microparticles formed was therefore a PLGA core with a PLLA shell impregnated with PCL particulates.

3.2. Effects of process parameters on particle configuration

3.2.1. Effect of starting polymer solution concentration

Two cloud points were observed in this ternary polymer solution. The first phase separation occurred at 3.75% w/v, at which PLGA was phase separated from the PLLA/PCL solution, while the second cloud point was determined to be 7% w/v, where PCL and PLLA phase separated. When the initial polymer solution concentration was prepared at 2% w/v a double walled configuration was similarly observed, but the core was more porous than the shell (Fig. 5A). The porous and less dense core indicates that a greater amount of PCL was dispersed in the PLGA. When the concentration was increased to 4% w/v the configuration of microparticles (Fig. 5B) remained double walled, similarly to particle R (Fig. 1B). Further increasing the concentration increased the size of the microparticles. However, at 10% w/v (Fig. 5C) no double walled structure was obtained. Instead a coalescence of all the polymers was observed.

3.2.2. Effect of stirring speed

Changes in stirring speed did not affect the final configuration of the microparticles. However, the PCL particulates in the PLLA shell were largest in size at the lowest stirring speed of 150 rpm (Fig. 6A). This was confirmed by the THF dissolution test, which showed large pores (20–50 μm) in the PLLA shell (Fig. 6D). In addition, an increase in stirring speed reduced the overall size of the microparticles (Figs. 1B and 6A–C).

3.2.3. Effect of the oil to water ratio

The effect of the oil to water ratio on microparticle structure was studied and the resulting SEM micrographs are shown in Fig. 7. For the lowest volume of PVA solution (oil/water ratio = 0.1) the PLGA coalescence phase was not located at the center of the particle but was observed to migrate away from the center (Fig. 7A). At the same time, the PCL particulates in the PLLA shell were observed to be larger in size (10–25 μm) (Fig. 7A, B, E and F). DSC analysis of microparticles prepared at an oil/water ratio of 0.1 again clearly demonstrated only PLLA (T_g = 74 °C, T_m = 178 °C) as present in the microparticles after dissolution (data not shown). However, Figs. 1B, 7C and D show that increasing the volume of the continuous phase did not result in any further effect

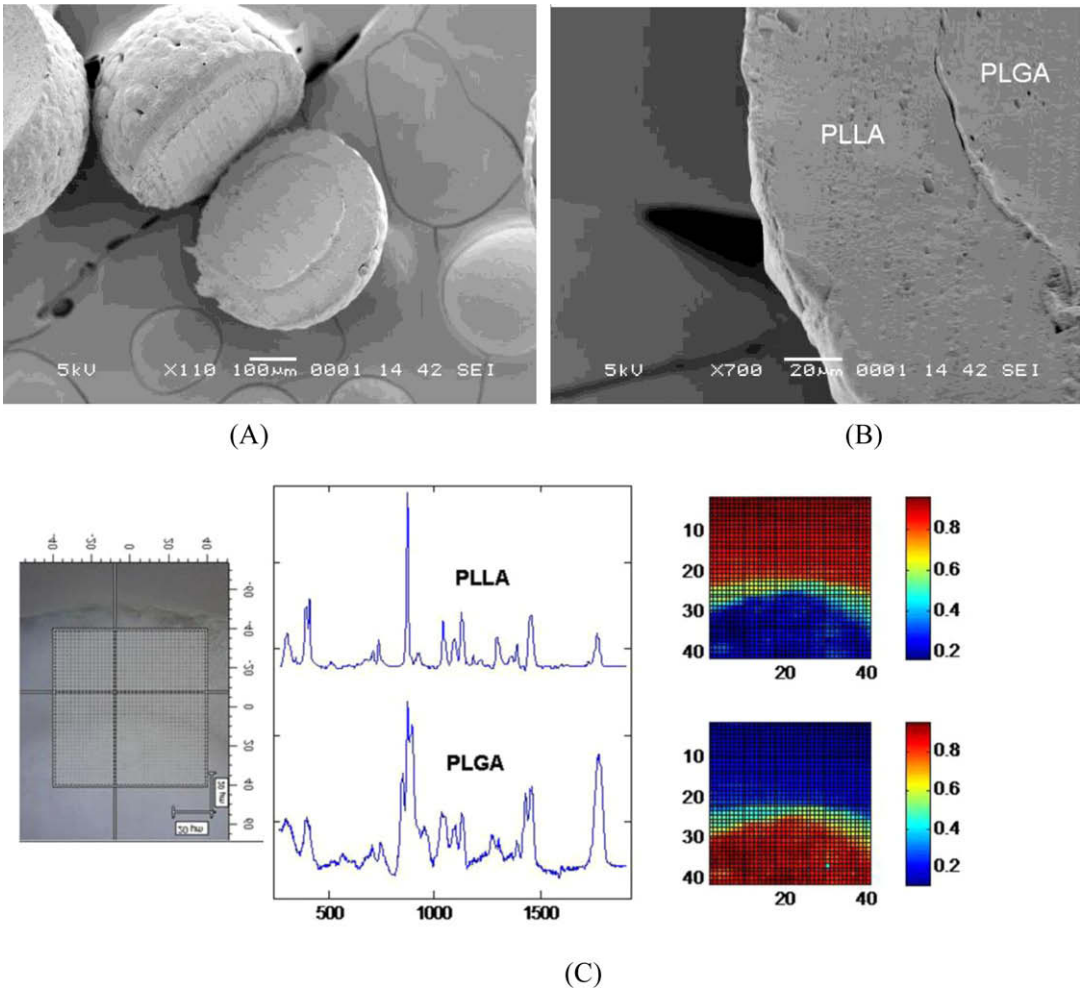

Fig. 4. PLLA/PLGA double walled microparticles. (A) SEM micrograph of cross-sectional view of a whole microparticle. (B) SEM micrograph of a close-up view of the shell. (C) Pure component Raman spectra estimates and their associated score images obtained via BTEM from a double walled microparticle. The axes of score images are in pixels. These can be directly converted to distances by multiplying the number of pixels by 2 μm.

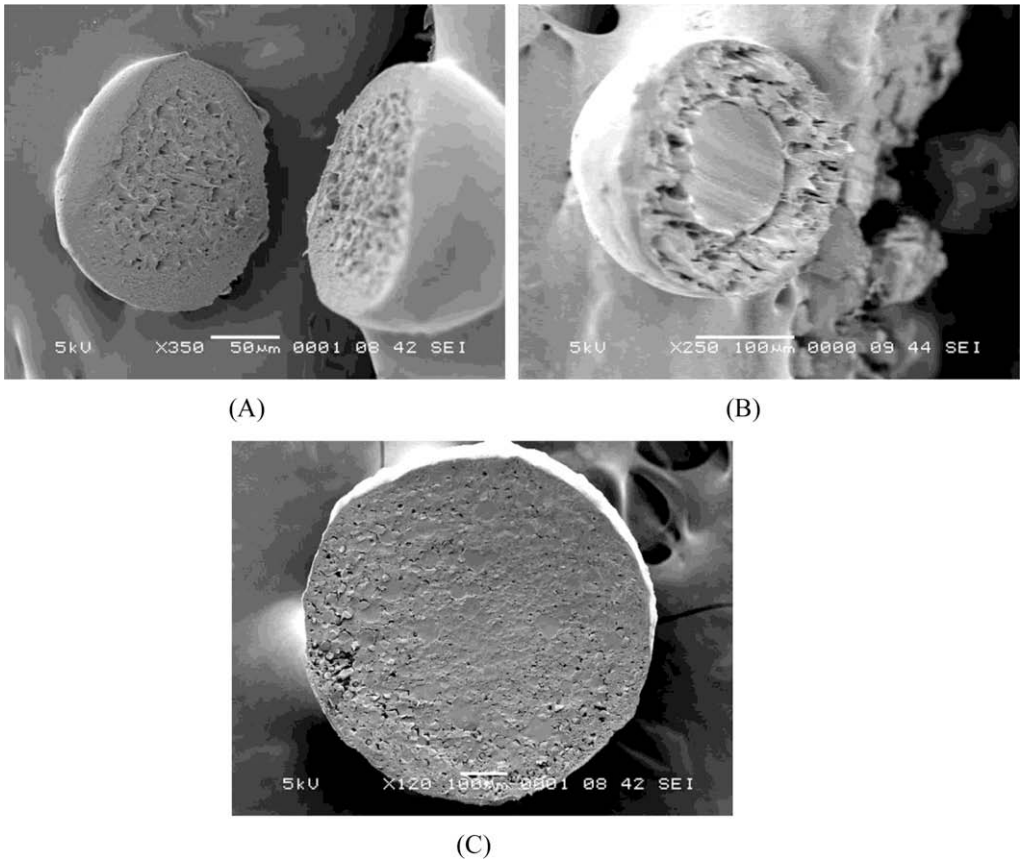

Fig. 5. SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared at various polymer solution concentrations. (A) 2% w/v; (B) 4% w/v; (C) 10% w/v.

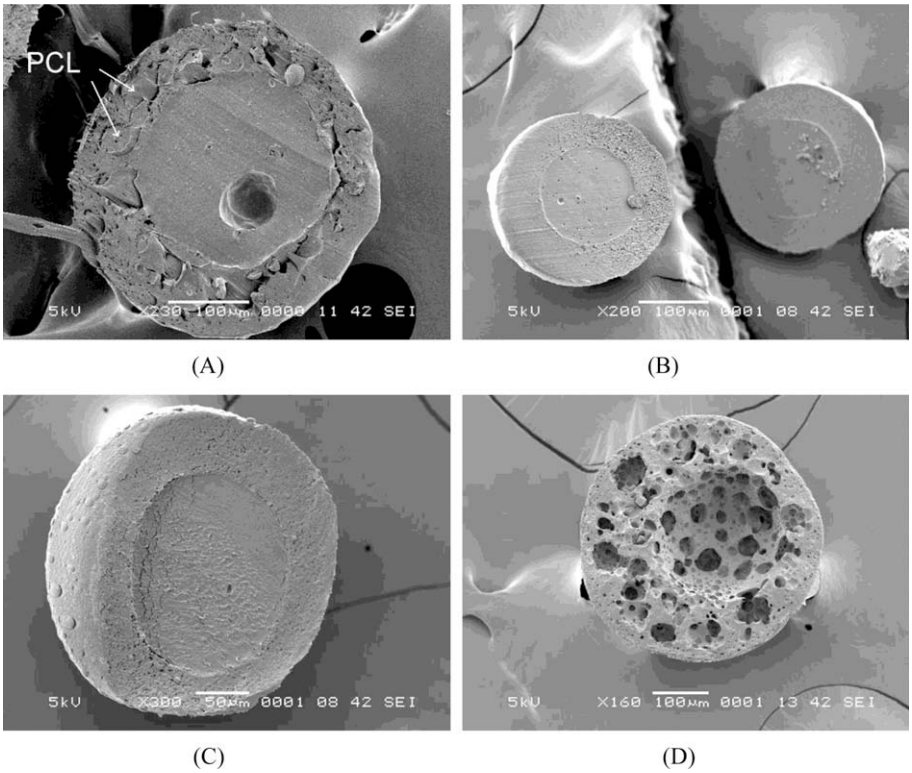

Fig. 6. SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared at various stirring speeds. (A) 150 rpm.; (B) 400 rpm.; (C) 500 rpm. (D) Cross-sectional view of a microparticle fabricated at a stirring speed of 150 rpm. after dissolution with THF.

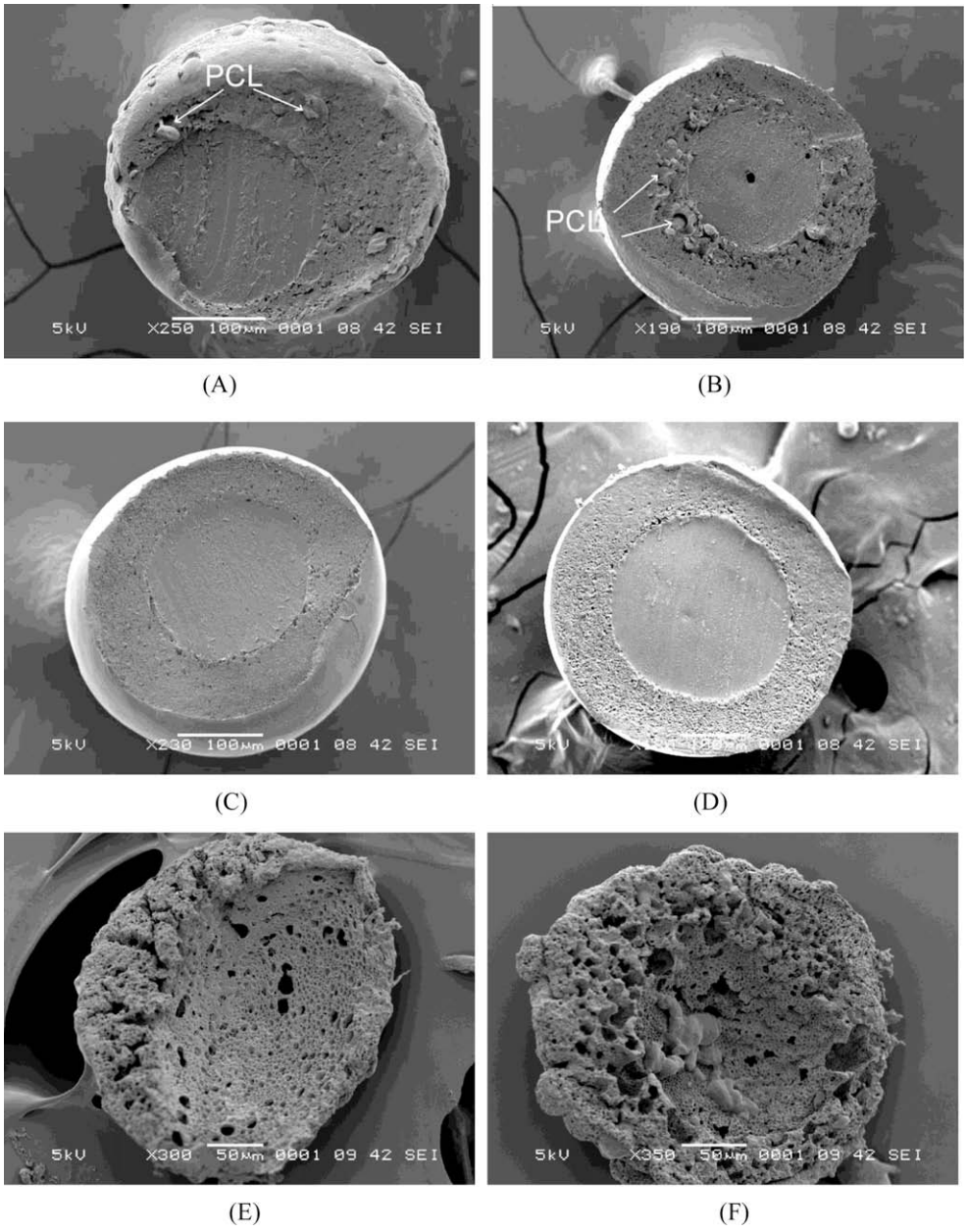

Fig. 7. SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared using various volumes of aqueous PVA solutions. (A) 50 ml (o/w: 0.1); (B) 150 ml (o/w: 0.03); (C) 400 ml (o/w: 0.0125); (D) 600 ml (o/w: 0.0083). (E and F) Cross-sectional views of a microparticle prepared at o/w ratios of 0.1 and 0.03, respectively, after dissolution with THF.

on the final double walled configuration of the microparticles. Raman mapping (Fig. 8) again confirmed the same configuration of microparticles prepared using a 400 ml PVA solution as that of particle R.

3.2.4. Effect of polymer mass ratio

Fig. 9 shows that changing the mass ratio of the polymers in the starting solution changed the size of the embedded PCL particulates and the core–shell dimensions. Increasing the mass of PLGA (PLLA:PLGA:PCL = 2:3:1) produced particles with larger core diameters and thinner shells (Fig. 9A). The inner core diameter increased dramatically when the PLGA mass was further increased to PLLA:PLGA:PCL = 1:3:1 (Fig. 9C).

Fig. 9E and F shows that an increase in the amount of PCL (PLLA:PLGA:PCL = 3:2:2) resulted in the formation of larger PCL particulates (~40 μm) in the PLLA shell. The SEM micrographs of double walled composite microparticles after dissolution with THF (Fig. 9B, D and F) show that the shell and core composition were PLLA and PLGA, respectively, and the shell was similarly impregnated with PCL particulates.

4. Discussion

4.1. Particle configuration

Various configurations of the composite microparticles were obtained by changing the starting polymer solution concentration. For reference particle R the initial polymer solution concentration was 6% w/v, which was above the cloud point of 3.75% w/v at which the PLGA phase separates from PCL/PLLA. When the polymer solution was initially poured into aqueous PVA solution the PLGA

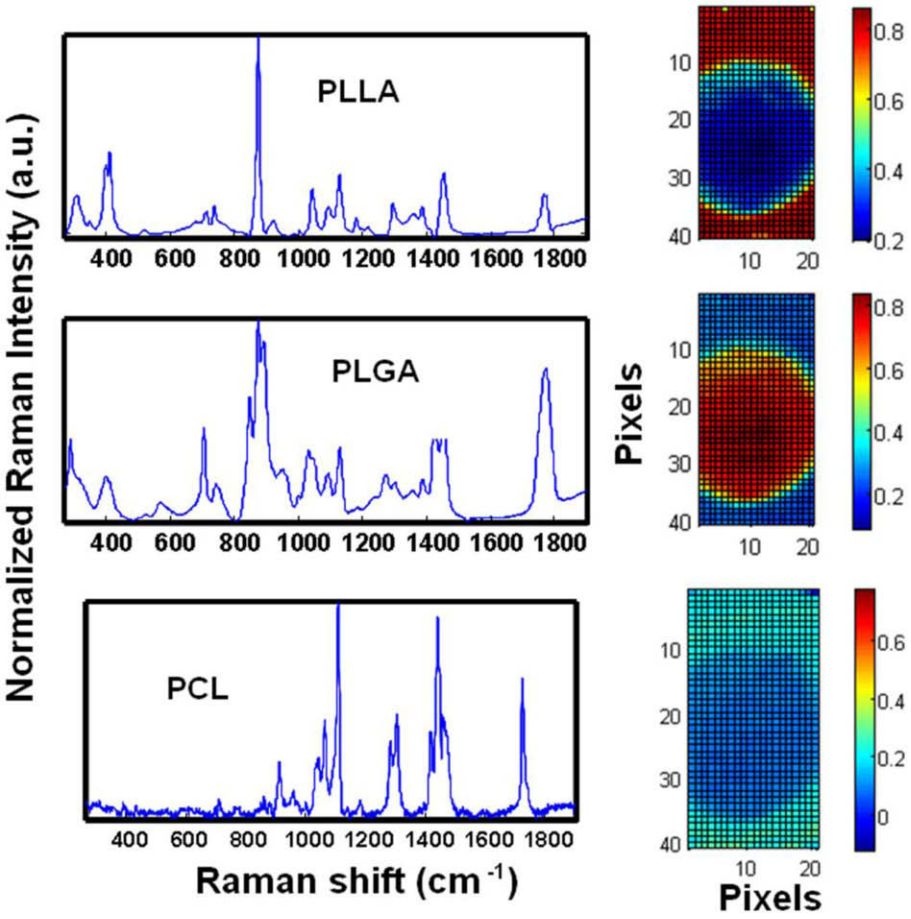

Fig. 8. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a PLLA/PLGA/PCL DW composite microparticle prepared using 400 ml PVA solution. The axes of score images are in pixels. These can be directly converted to distances by multiplying the number of pixels by 10 μm.

phase separated as small droplets containing DCM and polymer (known as the coacervate phase) within the emulsion droplets [25]. These droplets started to coalesce, becoming larger in size, while PLLA and PCL were still miscible within the emulsion droplets. After reaching the second cloud point at 7% w/v PCL and PLLA began to phase separate. During solvent evaporation a greater amount of DCM was partitioned into the PLGA coacervate droplets, due to a higher degree of interaction between DCM and PLGA. This was shown by a higher volume fraction of DCM in the PLGA phase (90.63%) as compared with the PLLA phase (82.71%) (results not shown). DCM-rich PLGA coacervate droplets were therefore more hydrophobic than the PLLA–DCM phase and tended to migrate towards the inner core, away from the aqueous solution. PLLA coacervate droplets, on the other hand, had a greater affinity for the surrounding aqueous phase. At the same time, PCL together with PLLA also migrated to the outer region of the emulsion droplet, due to poor intermixing of PCL with PLGA that had coalesced in the core and the limited mobility of PCL in the dispersing PLLA phase. Subsequently, the faster solidification of PLLA would then shorten the time allowed for PCL to further coalesce and migrate, leaving PCL to be dispersed and embedded as particulates in the PLLA shell. At the same time, the high molecular weights (high viscosities) of PLLA and PCL may also hinder the coalescence and migration of PCL. Further removal of solvent will finally cause PLLA to precipitate to form the outer shell, entrapping the PCL particulates.

For a starting homogeneous polymer solution of 2% w/v the polymers inside the emulsion droplets remained miscible until phase separation of PLGA at the first cloud point at 3.75% w/v. The high molecular weights (high viscosities) of PLLA and PCL would make the phase separation of PLGA from PCL/PLLA difficult. The surface tensions of PLGA, PLLA and PCL obtained by the Owens and Wendt approach [29] are 37.7, 36.1 and 37.5 mJ m^{-2}, respectively. Therefore, homogeneous dispersion of the PLGA coacervate droplets together with a lower interfacial energy between PLGA and PCL would enhance the interaction and intermixing of PLGA and PCL coacervate droplets when the solution reached the second cloud point. This would give rise to more PCL being dispersed in the PLGA core (Fig. 5A). Polymer solution concentrations of 4% w/v and 6% w/v, both of which fall between the first and second cloud points, produced microparticles with the same configuration, with PLGA in the core while PCL was embedded in the PLLA shell. Similar results were observed by Leach et al., who prepared microparticles at two different polymer concentrations below the cloud point which had the same double walled configuration [11]. For a polymer concentration of 10% w/v the high polymer concentration (increased viscosity) decreased the ability of the polymers to move and coalesce with their respective phases, thus resulting in a microparticle structure with no distinctive layers (Fig. 5C), a phenomenon again reported by Leach et al. [11] and Lee et al. [14].

During the solvent removal process DCM must first diffuse into the aqueous phase and then evaporate at the water/air interface [30]. The solubility of DCM in water is about 2 vol.% [31]. In this study 250 ml of aqueous PVA solution was therefore considered the critical volume, since 5 ml of DCM was used in dissolving the polymers. At a high oil to water ratio the water content is relatively

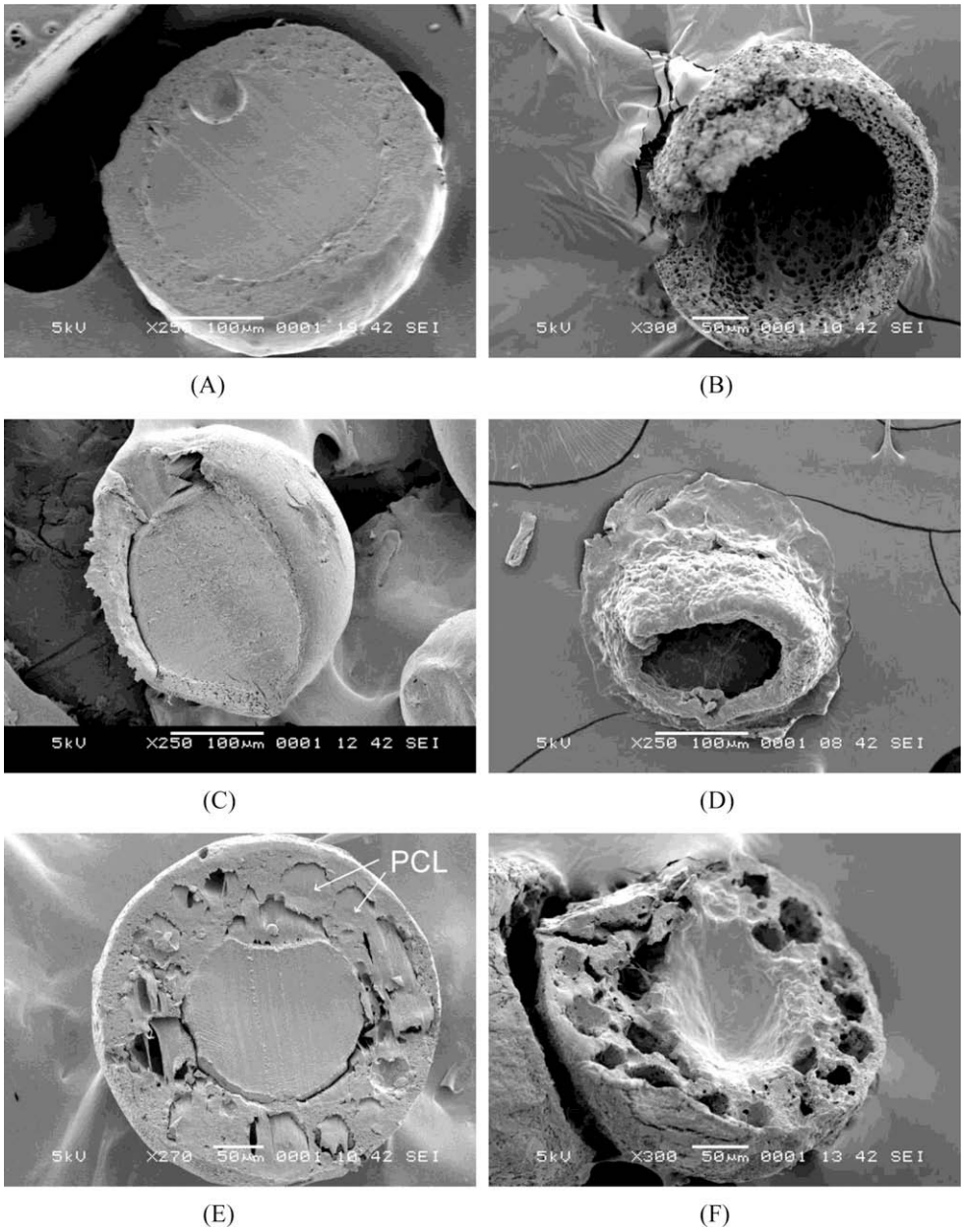

Fig. 9. SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared at various mass ratios of PLLA, PLGA and PCL. (A) 2:3:1 PLLA:PLGA:PCL; (C) 1:3:1 PLLA:PLGA:PCL; (E) 3:2:2 PLLA:PLGA:PCL; (B, D and F) Corresponding cross-sectional views after dissolution with THF.

low (50 ml). As such, the slow diffusion of DCM into the aqueous phase leads to slower precipitation of the polymers. At a later stage of solvent evaporation the small amount of remaining DCM no longer makes the PLGA coacervate droplets more hydrophobic than PLLA. The increased hydrophilicity of PLGA then causes it to migrate towards the external aqueous phase when the precipitation rate is slow enough (Fig. 7A). Beyond the critical volume of 250 ml the amount of aqueous solution did not seem to have any effect on the final configuration of the particles. It is possible that when the aqueous volume is too large DCM rapidly diffused into the water. Thus the solvent extraction rate was unaffected by the aqueous volume beyond a certain critical point.

In contrast to other reported work [5,6,11,16], we did not find any effect of polymer ratio that gave rise to a core–shell phase inversion. It is believed that a core–shell inversion only occurs when the polymer solution is prepared above the cloud point, a biphasic polymer solution is created and a well-controlled precipitation rate is achieved. When this binary polymer solution was initially poured into the aqueous PVA solution there was no preferential distribution of polymer when forming an interface with the surrounding aqueous environment or with the other polymers. The higher mass polymer usually formed the outer shell, which engulfed the polymer phase of lower mass. This occurred when the precipitation rate was fast enough to kinetically trap the non-equilibrium configuration in order for phase inversion to take place, as observed in a study by Matsumoto et al. [5,6]. We believe that this contradiction with our study may be reconciled by the fact that the considerably lower starting polymer concentration of 6% w/v could provide sufficient time and mobility for the polymers to reconfigure themselves according to thermodynamic equilibrium, which is dictated by changes in interfacial energies as the solvent evaporates [26].

As a result of kinetic factors governing the solidification of polymers, different polymer concentrations (below, above or be-

tween the two cloud points) and oil to water ratios produced microparticles of different configurations from a ternary polymer system.

4.2. Size of embedded particulates and dimensions of the core/shell of microparticles

The PCL particulate size can also be manipulated by specific process parameters. Larger PCL particulates dispersed in the PLLA shell were observed in microparticles produced at a lower stirring speed or volume of PVA solution. The decrease in polymer precipitation rate at lowering stirring speeds resulted in PCL coacervate droplets having more time to coalesce inside the PLLA matrix before the solidification of PLLA took place. Similarly, PCL coacervate droplets had more time to coalesce as DCM distribution into the aqueous phase was slower for lower amounts of PVA solution (higher oil to water ratios). Furthermore, higher amounts of PCL also facilitated coalescence of the PCL coacervate droplets during the solvent evaporation process, resulting in larger PCL particulates.

With a lower PLLA to PLGA ratio a PLGA core of larger diameter and a thinner PLLA shell were observed. It has been shown that similar cloud points were obtained for different polymer mass ratios in a binary polymer system [5,17]. Therefore, in this study the same initial polymer solution concentrations were used with different polymer mass ratios in the solvent evaporation process. Lee et al. [14] obtained similar results in their PLGA/PLLA binary polymer studies and proved that it is possible to fine tune the dimensions of the shell and core by changing the mass ratio of the different polymers.

4.3. Size of microparticles

Microparticle size is dependant upon the starting polymer solution concentration and stirring speed. Lowering the polymer solution concentration (from 6% to 4% w/v) may result in finer emulsion droplets [31], thus achieving smaller microparticle sizes while retaining the same internal morphology. In addition, a higher stirring speed may also produce finer emulsion droplets through strong shear forces [30,32], resulting in a smaller particle size while retaining the double walled structure.

5. Conclusions

Ternary phase PLLA/PLGA/PCL composite microparticles, with a PLGA core and PLLA shell impregnated with PCL particulates, were fabricated using a one-step solvent evaporation method. Various process parameters were found to give rise to different configurations of the resulting microparticles. A starting polymer solution prepared below the cloud point and an increase in the oil to water ratio facilitated configurations towards more thermodynamic equilibrium ones, dictated by the interfacial energies of the components. Changes in the shell polymer layer thickness and PCL particulate size can be achieved by manipulating the polymer mass ratio or by adjusting the precipitation rate through the stirring speed and oil to water ratio. It was also observed that lowering the polymer concentration and increasing the stirring speed achieved smaller particle sizes while retaining the double walled structure. A correlation between the final microparticle configuration and the process parameters was thus established. This relationship allows us to further understand and improve the fabrication of ternary phase composite microparticles through a one-step solvent evaporation technique.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 3, 4, and 8, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2009.10.028)

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