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Manipulation of process parameters to achieve different ternary phase microparticle configurations

Wei Li Lee [a], Wan Ling Foo [a], Effendi Widjaja [b], Say Chye Joachim Loo [a,*]

[a] School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore
[b] Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833, Singapore

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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

* Corresponding author. Tel.: +65 6790 4603; fax: +65 6790 9081.
E-mail address: joachimloo@ntu.edu.sg (S.C.J. Loo).

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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