Revolutionizing drug delivery through biodegradable multilayered particles

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Revolutionizing drug delivery through biodegradable multilayered particles

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Abstract

Modern drug discovery technologies are discovering more and more potent therapeutic agents with narrow therapeutic windows, thus necessitating the improvement of current particulate drug delivery systems. Conventional single-layered polymeric particles have limited control over drug release profiles, including burst release, the inability to provide zero-order, pulsatile, time-delayed release and controlled release of multiple drugs. In an attempt to better control drug release kinetics, the development of multilayered microparticles has been introduced. In this review, we give an overview of the fabrication and characterization techniques of multilayered polymeric microparticles. We also focus on the one-step solvent evaporation technique, and the key process parameters in this technique that affect the formation of microparticle configurations. In addition, the benefits and challenges of multilayered microparticulate system for drug delivery were discussed. This review intends to portray how distinctive structural attributes and degradation behaviours of multilayered microparticles can be exploited to fine-tune drug release profiles and kinetics.

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Introduction

Over the past decades, scientists in the pharmaceutical field have developed numerous innovative drugs to combat a long list of diseases. However, development of a new drug is only one aspect in disease treatment, as the recent shift in paradigm has been towards pharmaceutical reformulations. Administration of medication via a device that better control drug release kinetics is therefore gaining practical importance. Controlled release aids in improving therapeutic efficacy, reducing undesired side effect of drugs and allowing for less frequent administration, thereby improving patient compliance (Uhrich et al., 1999; Freiberg and Zhu, 2004).

Poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA), owing to their excellent biocompatibility and biodegradability, are the most frequently used polymers for the encapsulation of therapeutic agents and releasing it over a prolonged time (Peppas et al., 2000). These polymers can be fashioned into microparticles (Faisant et al., 2002; Mohamed and van der Walle, 2008), nanoparticles (Soppimath et al., 2001), films (Wang et al., 2006; Chia et al., 2008; Xie et al., 2008), scaffolds (Li et al., 2002; Thakur et al., 2008), pins (Heslinga et al., 2009), sutures (Stamboulis et al., 2002) and injectable depots (Wang et al., 2003; Wang et al., 2004; Raman and McHugh, 2005). In recent decades, the polymeric particles have garnered much interest in the field of drug delivery because of their versatility in tailoring the drug release profiles, facile administration routes and relatively simple fabrication technique (Brannon-Peppas, 1995; Berkland et al., 2004a; Freiberg and Zhu, 2004; Freitas et al., 2005).

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Considered for parenteral, oral, nasal or pulmonary administration, the polymeric particulate system has the potential to protect drugs from premature degradation, while at the same time providing controlled and sustained drug release (Freitas et al., 2005). Manipulation of the polymer chemistry, particle size, size distribution and/or morphology provides some flexibility in modulating drug release (Jain, 2000; Berkland et al., 2001; Berkland et al., 2002; Berkland et al., 2003; Klose et al., 2006; Mao et al., 2007). However, monolithic polymer particle-mediated drug delivery systems have several inherent problems, such as a high initial "burst" of the encapsulated agent (Jalil and Nixon, 1990; Huang and Brazel, 2001; Sheikh, 2007), limited control of drug release kinetics (Pekarek et al., 1994b, a; Sanchez et al., 1996; Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998; Krishnamachari et al., 2007; Naha et al., 2008), a lack of constant (zero-order) or pulsatile release of drugs and the inability to deliver multiple drugs from a single particle (Lee et al., 1997; Shi et al., 2003; Berkland et al., 2004a; Choi et al., 2010). The development of multilayered particulate system that comprises a polymer shell surrounding one or many micron-sized polymeric particulates would be an important step towards a robust approach to fine-tune the drug release rates, through selective localization of each drug in the individual polymer phases (Rahman and Mathiowitz, 2004; Matsumoto et al., 2005; Koppolu et al., 2010; Lee et al., 2011; Khung et al., 2012; Lee et al., 2012b), while other layers can serve as rate-limiting layers.

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This review aims to provide an overview of biodegradable multilayered particulate systems developed for controlling the drug release profiles. As such, this review is divided into two parts. The first part is to discuss several fabrication techniques of multilayered particles. The second part is to discuss the drug release properties of this "designer" multilayered particulate drug-delivery system with the alteration of particle morphology, layer configuration, drug-layer localization. This review does not focus on the release of encapsulated substance from

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polyelectrolyte-multilayer (layer-by-layer) particles, multilayered multiblock copolymer particles and multicompartmental capsules, which have been reviewed in detail elsewhere (Johnston et al., 2006; Tanaka et al., 2009; Becker et al., 2010; Delcea et al., 2010; Delcea et al., 2011; Such et al., 2011). The term ‘‘microparticle’’ as used below refers to a sphere typically from 1 to 500 μm in diameter. Alternative terms such as microspheres and microcapsules are also used in the literature.

Limitations of drug release from single-layered particles

Polymeric single–layered (neat) particles have been extensively investigated in the medical industries to deliver drugs. However, this monolithic particle-based drug delivery system often shows a high initial “burst” caused by the rapid release of the drug particles trapped on the surface. This burst release is detrimental, especially for the drug with narrow therapeutic window and high toxicity. In addition, low hydrophilic drug encapsulation efficiency is usually observed for the single-layered particles (Lee et al., 2002). Another major problem is that a single-layered particle is unable to achieve a zero-order drug release rate attributed to the change in diffusion distance of the drug in the polymer particles (Pekarek et al., 1994a). Additionally, the bulk degradation of commonly used polymers in the drug delivery, such as PLGA and PLA, does not enable constant release (Burkersroda et al., 2002; Loo et al., 2005; Loo et al., 2006). In general, the drug is first released through diffusion due to low mass loss of polymer, followed by erosion-controlled rapid release (Wang et al., 2006). Layer localization of the drug in the neat particles is also not achievable, giving rise to an inability to release drugs in a pulsatile or sequential manner from a single particle. In an attempt to better control drug release kinetics, multilayered particles have been employed to circumvent some of these limitations.

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Fabrication methods of multilayered microparticles

Overview of various fabrication methods

In the past years, several fabrication methods of double-layered microparticles have been devised. These methods included the coating technologies that use hot-melt technique (Mathiowitz and Langer, 1989), dip coating (Gopferich et al., 1994), fluidized beds(Watano et al., 2004), or spray drying (Lee et al., 1997; Wang and Wang, 2002); precision particle fabrication technology that employs a series of concentric nozzles to create a compound jet (Berkland et al., 2004a; Berkland et al., 2004b; Pollauf and Pack, 2006); and emulsion solvent evaporation method (Pekarek et al., 1994a). One of the processes for preparing double-walled microspheres is hot-melt technique. The core particles are first suspended in the melted coating polymer surrounded by a non-solvent and it is subsequently cooled for solidification. However, this hot-melt method is not preferable for the polymer with high melting point as well as thermo-labile drugs and polymers. On the other hand, dip and pan coating often result in uneven or inconsistent coverage coating and are only applicable to particles of sizes larger than 100 μm (Pekarek et al., 1994a). The non-uniformity in the coating layer would then cause a lot of problems in a delivery system because the drug release kinetic would be significantly dependent on the diffusion mechanism of drug through the outer layer. Although fluidization bed technique (or air suspension coating technique) generally produces uniform coating, this method is restricted to particles larger than 40μm in diameter due to the agglomeration problem of small particles (Watano et al., 2004). Double-walled microspheres can also be fabricated by spray drying the suspension of core microparticles in a non-solvent containing the dissolved coating polymer (Wang and Wang, 2002). The encapsulation of nanoparticles within microparticles (composite microparticles)

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also relies on the use of a non-solvent (Sheikh Hassan et al., 2009). The pre-synthesized nanoparticles are embedded in microparticles using the emulsion solvent diffusion evaporation technique. In these aforementioned fabrication techniques, the core is formed first and then each of the coating layers is added in the separate steps, thus further reducing the process yield. Therefore, it is imperative to develop a single-step fabrication technique to form the multilayered microparticles.

For the one-step fabrication of double-walled microspheres, Pollauf and Pack (2006) developed a precision particle fabrication that employs three coaxial nozzles to spray concentric jets of core and annular polymer solutions, which disrupt into uniform core-shell droplets through acoustic excitation. The polymer mass ratio, solution concentrations liquid flow rates and solvent extraction rate can be altered to vary the core-shell dimension and particle size. This technique has been used to prepare double-walled microspheres of uniform sizes. The disadvantage is that complex fabrication equipment was involved.

As compared to other fabrication techniques, the emulsion solvent evaporation technique is a one-step process that produces multilayered microparticles with higher yields, uniform layer thickness and a controllable particle size within a range of 20 µm -1000 µm (Pekarek et al., 1994a). Only simple and economical lab apparatus, such as an overhead stirrer etc., is required in this fabrication method, as compared to the fluidized bed method and precision particle fabrication technique where complex and expensive fabrication equipment are required. In addition, insoluble drug particles cannot be directly mixed into the coating layer and this becomes one of the concerns of dip coating and fluidization techniques. Through a one-step solvent evaporation method, drug particles can be dispersed in the polymer solution

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and do not necessarily have to be dissolved in the polymer solution. On another note, the solvent evaporation technique has been widely employed to fabricate the microparticles in a small scale for research purpose (Freitas et al., 2005). This article reviews the current state of the art in one-step solvent evaporation technique devised for preparing multilayered particles.

One-step preparation of multilayered microparticles by the emulsion solvent evaporation technique

The usual solvent evaporation method utilizes the formation of an oil-in-water emulsion with the presence of surfactant (Freitas et al., 2005). The polymer is dissolved in a volatile organic solvent, such as dichloromethane (DCM), ethyl acetate etc., and mixed with the substance to be encapsulated, before pouring into an aqueous continuous phase. The spherical emulsion droplets are created and subsequently hardened as the solvent evaporates, yielding solid microparticles. This solvent evaporation method has been used extensively to fabricate PLGA and PLLA neat microparticles. Ogawa et al. (1988) successfully used a water-in-oil-in-water solvent evaporation technique to encapsulate Leuprorelin or leuprolide acetate into single-layered PLGA microspheres and this is now marketed as Lupron Depot. Other popular commercial products produced by solvent evaporation are Enantone Depot/Trenantone/Enantone Gyn.

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In the modified solvent evaporation technique employed to prepare double-walled microspheres, which was first reported by Pekarek et al. (1994a), two polymer solutions were mixed before pouring into the aqueous non-solvent phase. During the solvent extraction and evaporation, the oil phase (containing polymer and solvent) becomes more concentrated and

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two immiscible polymers begin to undergo phase separation when the solution concentration is above the cloud point. Cloud point is the polymer solution concentration at which the polymers become immiscible with each other and begin to phase separate, while these polymers still remain dissolved in the solvent (Loxley and Vincent, 1998; Pollauf and Pack, 2006). Matsumoto et al. (1997) found that the cloud point depends on the solubility parameter of solvent. The solvent investigated were acetonitrile, methylene chloride and ethyl acetate. A higher solubility parameter resulted in higher cloud point. The independence of cloud point with the polymer mass ratio was observed in their work. In addition, the phase separation between two polymers with high molecular weights occurred more easily, leading to a lower cloud point.

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During polymer phase separation, if the polymer precipitation rate is slow enough, two polymers will configure themselves in their thermodynamically stable configuration as dictated by the interfacial tensions of two polymers and the external aqueous phase using the spreading coefficient theory. Spreading coefficient theory was developed by Harkins (1952) for a liquid to spread on a solid or liquid surface in terms of interfacial energies of the components. Torza and Mason (1970) subsequently extended Harkin's theory to a system in which two immiscible phases are dispersed in a third immiscible phase. Eq. (1) can be used to calculate the spreading coefficient for the determination of the ability of one polymer to engulf the other.

$$\lambda_{AB} = \gamma_{BC} - \gamma_{AC} - \gamma_{AB} \qquad \textbf{Eq. (1)}$$

γ_{AC} and γ_{BC} are the interfacial energies between the continuous phase (C) and polymer A or B, respectively, whereas γ_{AB} is the interfacial energy between polymer A and B. Qualitatively, one phase will spread onto the other when the spreading coefficient is positive. There are

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three possibilities to describe the configuration of the two immiscible polymers, which are complete engulfment leading to complete encapsulation, partial engulfment and no engulfment (complete separation of two polymers). **Table 1** shows the three possible combinations of the spreading coefficients. One should note that the spreading coefficient will change when the solvent is extracted since the change in polymer solution concentration will alter the effective interfacial energies. In addition, the process of forming internal configurations of particles is dynamic, because polymer phases within the emulsion droplets become less mobile during solvent extraction. When the solvent content decreases to a certain extent, the polymer phases within the emulsion droplets would not have enough mobility to configure themselves according to the thermodynamic equilibrium state, as dictated by the spreading coefficient. Therefore, the spreading coefficient theory would be only used as an indirect estimation of the final configurations of the particles after hardening process (Pollauf and Pack, 2006; Wang et al., 2009).

Due to the dynamic process of transition from liquid phase to solid phase during solvent extraction, kinetic factors also play a role in determining the final configuration of the particles. When the precipitation rate is considerably fast, the polymer phases may be kinetically entrapped in a non-equilibrium configuration as a transient intermediate within the microparticles. Therefore, there are two scenarios of forming the double-layered structure. In the first scenario where the complete engulfment is predicted by the spreading coefficient, the precipitation rate should be slow enough such that one polymer has sufficient time to spread onto the other. On the other hand, the second route involves the careful adjustment of the polymer precipitation rate so as to kinetically trap the polymers in the double-layered structure as a non-equilibrium configuration.

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Different microspheres comprising two immiscible polymers have been fabricated and reported. Most notably, Pekarek et al. (1994a) reported on the production of double-walled microspheres made of a core of poly(1,3-bis-(p-carboxyphenoxy propane)-co-(sebacic anhydride))20:80 (P(CPP:SA)20:80) and an outer layer of poly(L-lactide) (PLLA) through an emulsion solvent evaporation technique. Leach et al. (1999) further studied the effect of process parameters, such as the polymer mass ratios, starting polymer solution concentration, temperature and air flow on the formation efficiency of double-walled microspheres. It was found that these parameters determine the polymer precipitation rate and time allowed for the polymers to phase separate and configure themselves before the polymers precipitate. In general, lowering the polymer solution concentration, temperature and air flow result in an extended solvent extraction time, which in turn facilitates the formation of thermodynamically-driven configurations. It was also reported that varying the polymer mass ratio may lead to core-shell inversion; however, the process efficiency of getting double-walled structure was reduced. The configurations of the microspheres can thus be controlled by manipulating various process parameters.

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Double-walled microspheres comprising hydrophilic polymers can also be similarly produced through this one-step solvent evaporation method (Mathiowitz et al., 2003). Through the use of hydrophilic polymers, biological active materials and living cells can be encapsulated into the microspheres as organic solvent is not employed in the fabrication. Additionally, polymeric microcapsules with liquid cores were prepared by the controlled phase separation of polymer within the emulsion droplets (Sanchez et al., 1996; Loxley and Vincent, 1998). Liquid cores (non-solvent for the polymer) consisted of hexadecane or decane. Particle

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morphologies were strongly dependent on the type of non-solvent and the emulsifier, suggesting the importance of oil-water interfacial tension on final capsule configuration, as explained by the spreading coefficients. The thickness of microcapsule was found to be manipulated by the polymer concentration in the oil phase.

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Phase separation phenomenon of immiscible polymers determines the formation of double-layered structure. Rahman and Mathiowitz (2004) demonstrated the fabrication of double-walled microspheres with biodegradable PLLA and PLGA. Immiscibility was demonstrated in the case of polymers with certain molecular weight and chemical structure. It was also found that the polymer mass ratio has no effect on the cloud point. The same polymer solution concentration can thus be used with the system of different polymer mass ratios. In addition, the morphology of the composite microparticles was dependent on the polymer mass ratios (Yang et al., 2003). Double-walled structure with a poly(ortho ester) (POE) core and PLGA shell was observed when the POE content was 50, 60 or 70% in weight. A relatively uniform structure was observed for the microparticles with the POE content of 10, 20, 30 and 40%. With the POE content of 80 and 90%, no double-walled structure but with two distinct phases was observed as the low PLGA content was insufficient to encapsulate the POE phase. The formation of core-shell structure was reported to be driven by the affinity of hydrophilic PLGA and external aqueous medium during the fabrication process, forming PLGA shell and POE core. Besides the fabrication of single-reservoir type (double-walled) microspheres, a multi-reservoir type of microspheres composed of a PLGA shell surrounding many micro-sized poly(DL-lactide) (PDLLA) particulates had also been prepared by Matsumoto et al. (1997). This multi-reservoir type of structure was formed by mixing two polymer solutions above the cloud point and achieving a high polymer precipitation rate.

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Although the solvent evaporation method with the use of a single common solvent gives core-shell structure, each layer is impregnated with small particulates of the other polymer (Lee et al., 2002). This is due to insufficient time for the polymers to coalesce with their respective phases during solvent extraction; polymer solution becomes more viscous, inhibiting migration of polymer phases. In order to form a pure layer structure, Naraharisetti et al. (2005) devised a modified solvent evaporation to form the double-layered PLLA/PLGA microparticles based on the insolubility of PLLA in ethyl acetate (EA). PLLA and PLGA were dissolved separately in DCM and EA, respectively. PLLA-DCM and PLGA-EA were mixed and sonicated before adding into the aqueous phase. It was observed that the shell was composed of PLGA only, while the core was a mixture of PLGA and PLLA.

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The fabrication of microparticles comprising three different polymers – ternary-phase microparticles via a one-step solvent evaporation method was subsequently reported by Lee et al. (2010a). Double-layered ternary-phase microparticles, with a PLGA core and PLLA shell impregnated with poly(caprolactone) (PCL) particulates, were formed. 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 the polymers configuring themselves more towards thermodynamic equilibrium configurations dictated by the interfacial energies of the components. Change in the sizes of PCL particulates can be achieved by manipulating the polymer mass ratio or by adjusting the precipitation rate through stirring speed and oil-to-water ratio. Subsequently, the same group investigated how the intrinsic properties of a multiple-polymer system and different process parameters can affect the formation of multilayered microparticles, where

each different polymer coalesces with its respective phase and forms a layer within a multilayered structure (Lee et al., 2010c, 2012c). It was found that triple-layered and quadruple-layered microparticles composed of immiscible polymers can be produced when the polymer solution concentration was prepared above the cloud point. Multiple-polymer system with low viscosity and low cloud point would facilitate the coalescence of the respective polymer phases in forming multilayered structure. The polymer precipitation rate was carefully adjusted by optimizing process parameters so as to kinetically trap the multilayered structure in a non-equilibrium configuration. As such, polymer mass ratios determined the final layer configuration, where a polymer with higher mass (or volume) formed the outer layer. The mechanism involved in the formation of the multilayered microparticles is summarized in **Figure 1(a)**, and the SEM image of a quadruple-layered (multilayered) microparticle is shown in **Figure 1(b)**. Change in layer thickness and layer inversion could also be achieved by altering the polymer mass ratios. It was also demonstrated that triple-layered microparticles of sizes ranging from 20 µm to 400 µm could be achieved by changing the stirring speed and surfactant concentration. However, when the particle size was significantly reduced to approximately 2 µm, the process parameters were carefully manipulated to extend the phase separation time by increasing oil-to-water ratio and saturating the continuous aqueous phase with solvent. This was to provide sufficient time for the respective polymer phases to coalesce to form the core-shell structure. On the contrary, a more rapid solvent extraction would occur with smaller emulsion droplets due to the shorter diffusion distance, leading to a blended structure (Katou et al., 2008; Lee et al., 2012a).

Encapsulation of drugs into the multilayered microparticles

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Drug-loaded double-layered polymeric microspheres can be fabricated using an oil-in-water (singe-emulsion) or water-in-oil-in-water (w/o/w, double-emulsion) solvent evaporation technique. In the oil-in-water emulsion, the drug is dissolved together with the polymer in the oil phase (Mao et al., 2008). On the other hand, the introduction of another water phase to dissolve hydrophilic drugs in the w/o/w emulsion had been reported to give a more uniform distribution of hydrophilic drug within the microparticles (Mao et al., 2007; Wang, 2009).

There are several reports on the fabrication of double-layered polymeric microparticles as drug carriers. Matsumoto et al. (1997; 2005) demonstrated that the distribution of cisplatin in the multi-reservoir type microparticles composed of PLGA multi-cores and the outer non-drug-holding PDLLA layer. Distribution theory as described in Eq. (2), which states that the distribution of a drug compound is dependent on the solubility parameter of the polymer solutions and that of the drug, may explain the distribution of cisplatin and amino acids within biphasal polymeric solutions.

$$\log\frac{X_{PLGA}}{X_{PDLLA}} = V_{drugs}\frac{\left(\delta_{drug} - \delta_{PDLLA-DCM}\right)^2 - \left(\delta_{PLGA-DCM} - \delta_{drug}\right)^2}{2.3\,RT}$$

Eq. (2)

where X_{PLGA} and X_{PDLLA} are the concentrations of drug in the PLGA and PDLLA phase, respectively; δ_{drug}, $\delta_{PLGA-DCM}$ and $\delta_{PDLLA-DCM}$ are solubility parameters of drug, PLGA-DCM phase and PDLLA-DCM phase, respectively. V_{drug} is the molecular volume of the drug; R is the gas constant and T is the absolute temperature. Different solvent systems were shown to change the location where the drug was distributed due to the effect of different solubility parameters. In the case of positive value, drugs had a higher tendency to be localized in the PLGA matrix; when the value was negative, drugs had a tendency to be distributed in the

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PDLLA layer; where the value was zero, the drugs tended to be dispersed on the interface between PLGA and PDLLA matrix.

Although the distribution theory proposed by Matsumoto et al. (1997) demonstrated the overall aspects of drug distribution within the polymers, it has the limitation of being polymer solution concentration dependent. As with the spreading coefficient theory, the drug distribution cannot be directly predicted as the effective solubility parameters will change with time as the particles harden. To better understand the drug distribution, the distribution theory is therefore examined at the starting polymer solution concentrations and the system with no solvent present.

The distribution of drugs within double-layered polymeric microparticles is dependent on the relative hydrophobicity of the drugs and the polymers. Qualitatively, hydrophobic drugs are distributed within the more hydrophobic polymer phase, whereas hydrophilic drugs are localized in the more hydrophilic polymer. Lee et al. (2002) and Tan et al. (2005) gave the similar explanation for the drug distribution based on the drug-polymer affinity. Higher drug encapsulation efficiency was obtained for double-layered microparticles relative to that of the single-layered microparticles. The hydrophobic shell could prevent the hydrophilic drug from dispersing into the continuous aqueous phase during fabrication process (Lee et al., 2011). Another study by Lee et al. (2010c) demonstrated the same drug-layer localization for triple-layered microparticles. Strong affinity between hydrophobic long ethylene chains of poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA) and hydrophobic ibuprofen (IBU) drove the drug to be entrapped within the EVA core. Metoclopramide HCl (MCA) (hydrophilic), on the other hand, was predominantly well-dispersed in the relatively more

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3 hydrophilic PLGA shell. Therefore, different drugs, based on their hydrophilic-hydrophobic nature, can be localized in different polymeric layers, thus resulting in specific layer localization of drugs.

Encapsulation of protein and its layer localization within the double-walled microspheres have been studied by several research groups. Rahman and Mathiowitz (2004) used bovine serum albumin (BSA) label with fluorescein isothiocyanate (FITC-BSA) as a model protein to investigate its localization within the double-walled microspheres. Different polymer mass ratios resulted in different drug-layer localization. For polymer mass ratio PLLA:PLGA 1:1, the FITC-BSA was found to be localized in the outer PLLA layer. This suggests that the FITC-BSA has a higher affinity with the outer layer of PLLA due to the combination of the hydrophilicity of the external aqueous phase and its affinity for PLLA. When the PLLA:PLGA mass ratio was changed to 1:3, the protein was localized in the core. However, the exact drug localization mechanism was not discussed in detail. Same localization was also observed for the microspheres with high drug loading. The solvent evaporation method employed to produce double-layered microparticles results in a loss in bioactivity of hydrophilic protein as a result of the exposure of protein at the organic/aqueous interface. Kokai et al. (2010b) investigated the use of an anionic surfactant, docusate sodium salt (AOT) to reduce lysozyme denaturation during fabrication process. It was found that the addition of AOT in the PLGA solution phase caused the core-shell polymer inversion. This could possibly be due to the increase charge of the PLGA solution, causing PLGA to move outward and to form an interface with external aqueous phase.

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Through the localization of drugs in different layers, two or more particle populations can be readily combined into the multilayered microparticles as a single dosage form, creating synergistic and multiple strengths of a single microparticle. Shi et al. (2003) have shown the selective localization of hydrophilic BSA and hydrophobic cyclosporine A in the PLGA shell and the poly(ortho ester) core, respectively, within a double-walled microsphere. Also, it had been demonstrated that three different drugs were selectively localized in different layers in the quadruple-layered microparticles (Lee et al., 2012c). In another study, Lee et al. (2011) reported on the encapsulation of both hydrophilic metoclopramide HCl (MCA) and hydrophobic ibuprofen (IBU) into the double-layered ternary-phase microparticles. MCA and IBU were localized in the core and shell, respectively, as shown in **Figure 2**. The inclusion of hydrophobic and rubbery PCL particulates (as a third phase) within the shell of the ternary-phase microparticles resulted in a good dispersion of IBU. The exterior morphology of the particles was thus found to be dependent on a combination effect of the drug properties and polymer types.

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Characterization of multilayered microparticles

Determination of external and internal morphologies

The surface and cross-sectional morphologies of multilayered microparticles are characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and optical microscopy analyses. For the internal structure analysis using SEM, the samples are first mounted onto metal stubs and cross-sectioned approximately at the centreline of a microparticle with a razor blade (Lee et al., 2002). For the smaller-sized particles, such as 40 μm, the particles can be fractured by placing them on a metal stage submerged in liquid nitrogen and then chopping with a razor blade (Berkland et al., 2004b).

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Alternatively, the microparticles are embedded in frozen gelatin and subsequently cut on a cryostat into sections of 10 ± 40 µm in thickness (Leach et al., 1999). After sectioning, the crossed polarizer is used to study the internal configuration of the polymers, and to identify the two layers based on differences in crystalline structure and thus the efficiency of the formation of double-layered structure. For closer examination, the microparticles are embedded in LR white embedding medium, cut into thickness of 70-80 nm, stained and examined by TEM (Pekarek et al., 1994a).

Differential scanning calorimetric (DSC) is used to investigate the thermal properties of the multilayered microparticles (Pekarek et al., 1994b; Lee et al., 2010a). In general, the existence of the distinct glass transition and/or melting temperatures, each representative of the original component polymers, indicates the formation of the phase-separated and multi-phase structure. In other words, these temperatures in the DSC thermogram would be shifted or merged for the polymers that are miscible.

Polymer and drug distribution

In the polymer distribution study, a direct and simple method was devised to determine the composition of the shell and core based on the different solubilities of PLLA and PLGA in ethyl acetate (Lee et al., 2002). In addition, an IR study through Fourier transformed infra-red (FTIR) microscope can be employed in order to study the composition of each layer (Lee et al., 2002; Tan et al., 2005; Wang, 2009). Alternatively, an X-ray photoelectron spectroscope (XPS) can be utilized to determine the chemical composition of the microparticles' surfaces

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by comparing the carbon/oxygen ratio of the double-layered microparticles to that of the respective neat polymer (Yang et al., 2003).

For the identification of the drug distribution within the core-shell microparticles, confocal laser scanning microscope and fluorescence microscopy were used to observe the distribution of bovine serum albumin (BSA) within microparticles using BSA fluorescence (a fluorescently labeled protein) (Shi et al., 2003; Rahman and Mathiowitz, 2004). Observations using optical microscope would be carried out to identify the drug distribution within the double-walled microspheres by observing the distinct color crystalline drug filaments distribution (Lee et al., 2002; Tan et al., 2005).

Although these characterization analyses could provide information of double-layered microparticles with high magnification and high resolution, they lack more detailed information on the chemical compounds as well as their spatial distributions. Widjaja et al. (2003; 2010) have devised a novel characterization approach based on the combined use of Raman microscopy and band-target entropy minimization (BTEM) analysis to identify polymers, drugs, degradation products and impurities present within the microparticles and their distributions in two spatial dimensions. The pre-sectioned microspheres can be easily mounted under the microscope objective and hundreds or thousands of Raman spectra can be collected from a particular area of sample using Raman point by point mapping measurements. BTEM analysis has been employed to recover the underlying pure component spectra of major (i.e. polymers and drugs) and minor (i.e. solvent, contaminates) components from samples.

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Hydrolytic degradation of multilayered microparticles

Previous studies have shown that the degradation properties of multilayered polymer films are distinctive from that of single-layered polymer films; whereby the degradation of the top polymer layer would accelerate degradation of the underlying layers (Loo et al., 2008; Loo et al., 2010). Therefore, a composite multilayered microparticulate system would also possess unique hydrolytic degradation characteristics and morphological attributes that differ from single-layered particles. As such, these multilayered microparticles may offer greater versatility in controlling drug release kinetics and profile.

Leach and Mathiowitz (1998) studied the degradation behaviour of double-walled microspheres with a core of P(CPP:SA)20:80 and shell of PLLA. The fast degradation of the P(CPP:SA)20:80 inner core enhanced the degradation rate of the PLLA shell as these degradation products of P(CPP:SA)20:80 were trapped by the PLLA shell. As such, the PLLA shell of the double-layered microspheres degraded slightly faster in comparison to the single-layered PLLA microspheres. The degradation of these double-walled microspheres implanted subcutaneously and intramuscularly in rats was subsequently discussed (Pekarek et al., 1996; Leach et al., 1998). Degradation characteristics of samples implanted subcutaneously and intramuscularly were found to be identical. In correlation to in vitro studies, the in vivo degradation kinetics of the microspheres showed the same trends, with the microspheres degrading more rapidly in vivo.

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Similarly, the degradation rate of the POE core was enhanced due to the acidic microenvironment created by the degradation products of PLGA shell (**Figure 3a**) (Yang et al., 2003). The PLGA shell exhibited slightly slower degradation after the complete erosion of POE, as compared to the neat PLGA microparticles (**Figure 3b**). The degradation products of PLGA could diffuse more easily from the shell into the surrounding medium, whereas more degradation products accumulated within the neat PLGA microspheres, accelerating degradation. Therefore, one can see that the distinct degradation behaviour of double-walled microspheres arises from the unique structural composition and configuration.

In other studies, degradation characteristic of PLLA and PLGA microspheres in inversed core-shell configuration has been reported by Tan et al. (2005). The degradation of PLGA that formed the inner core was observed to be slower as compared with that of the PLGA shell. The outer layer acted as a barrier protecting the core polymer from undergoing rapid hydrolytic degradation. Irradiation using gamma-ray has been utilized to fine-tune the degradation of double-walled PLGA/PLLA microspheres. As reported by Lee et al. (2002), therapeutic dosage of 50 Gy was found to have a mid effect on the degradation of polymer. For industrial sterilization of 25 kGy, gamma irradiation decreased molecular weight, as evidenced by lower glass transition temperature and melting point.

Furthermore, there have been several research papers highlighting the degradation study of ternary-phase/triple-layered microparticle systems. The introduction of additional polymer altered the morphological structure of the microparticles. This change in morphology would in turn alter the degradation characteristics of particles. In the study conducted by Lee et al. (2011), a faster degradation (decrease in molecular weight) of the PLLA shell of ternary-

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phase microparticles was observed as compared to binary-phase microparticles. Shells of ternary-phase microparticles were less dense due to the presence of PCL particulates, which resulted in higher water uptake and thus faster hydrolytic degradation. Lee et al. (2010b) also reported on the in vitro hydrolytic degradation of triple-layered microparticles. The middle layer of PLLA degraded more rapidly than single-layered PLLA microparticles because the degradation products of PLGA shell migrated into PLLA, creating more hydrophilic and acidic microenvironment. Different polymer degradation kinetics were observed by altering the polymer mass ratios (i.e. layer thicknesses) of triple-layered microparticles as a result of distinct buffering effect and extent of autocatalysis (Lee et al., 2012d).

The degradation study of multilayered microparticles would then provide important insights into the drug release mechanisms from this particulate system.

Drug release from multilayered microparticles

In drug delivery system, conventional single-layered microparticles with high initial burst caused by drug trapped on the surface have their disadvantages for clinical usage. In order to eliminate initial burst release and better control drug release, the multilayered microparticles with drug encapsulated were thus introduced.

Controlled drug release from double-layered microparticles

Several papers reported on the development of double-walled or binary-phase polymer microspheres as drug carriers for sustained and controlled release. Matsumoto et al. (1997;

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2005) demonstrated that the multi-reservoir type microspheres retarded the initial burst of Cisplatin localized in the PLGA cores due to the presence of the outer non-drug-holding poly(D,L-lactide) (PDLLA) layer, resulting in a sustained release. In addition, Lee et al. (2002) demonstrated that the PLLA shell of double-walled microspheres suppressed the rapid release of highly water soluble etanidazole located in the PLGA core, thus achieving a low initial burst, followed by a lag release phase before being released in a linearly sustained manner for about 300 h. These double-walled microspheres would be combined with traditional neat particles which normally provide initial burst or other type of double-layered particles, giving rise to pulsatile or double-burst release. This release kinetic would be attractive for cancer therapy or vaccine delivery (Sanchez et al., 1996; Lee et al., 1997). It is suggested that the initial drug release was controlled by diffusion followed by the degradation-controlled release through a rate-limiting shell. The lag release phase can be further manipulated through the use of gamma irradiation, which causes significant decrease in molecular weight of polymer. It was shown that double-walled microspheres irradiated with 25 kGy released the drug 14 days earlier than the non-irradiated microspheres (**Figure 4**). Similarly, Naraharisetti et al. (2005) and Tan et al. (2005) reported on a sustained release of gentamicin and doxorubicin, respectively, from the PLGA core through a rate-limiting barrier of PLLA shell. In contrast, drug encapsulated within the shell was released rapidly for the double-walled microspheres with inverse core-shell configuration, which resemble those of single-layered microspheres (Tan et al., 2005). In another study by Wang (2009), further suppression of initial burst release can be obtained by increasing the thickness of non-drug-loaded outer layer. Change in polymer properties (e.g. lactide ratio and molecular weight) also provides a degree of freedom in altering drug release kinetics. For example, the degradation rate of shell polymer was accelerated by the degradation products of core

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polymer, which exhibited faster degradation due to its low lactide ratio and molecular weight, thus resulting in a more rapid release.

The importance of core-shell structure was demonstrated by Pollauf et al. (2005), who compared the drug release profiles of the core-shell microparticles with blended microspheres. Piroxicam (the model drug) release from the blended microspheres was much more rapid than that from the core-shell microspheres. The use of a polymer shell containing little or no piroxicam could delay drug release, whereas piroxicam was uniformly distributed in the blended microspheres. Furthermore, combining monodispersed core-shell microspheres of different shell thicknesses at a certain composition was found to provide a zero-order release. Berkland et al. (2004a) in another study demonstrated that increasing the PLLA shell thickness (by increasing PLLA content) shifted the release profile of piroxicam from a biphasic pattern observed for neat PLGA microspheres to linear release for the PLLA shell of ~10 μm, in monodispersed double-walled microspheres. However, one disadvantage was the decrease in encapsulation efficiency of drug possibly due to an increased amount of solvent, which caused the drug to move toward the droplet surface by mass action during precision particle fabrication process.

Protein / growth factor release from double-walled microspheres

Different strategies have been developed to minimize the loss in protein bioactivity and high burst release (Bilati et al., 2005; Schoubben et al., 2009; Yuan et al., 2009). Double-walled microspheres offer an alternative for sustained protein release. Shi et al. (2003) reported on a nearly complete release of proteins (i.e. hydrophilic bovine serum albumin (BSA) and

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hydrophobic cyclosporin A (CyA)) from POE/PLGA double-walled microspheres (**Figure 5**). The porous BSA-loaded PLGA shell prevented the formation of an acidic microenvironment which caused the destabilization of acid-labile biomacromolecules (BSA). Higher water uptake of double-walled microspheres increased the water solubilization of CyA, thus accelerating the release of CyA. The porous BSA-loaded PLGA shell also further enhanced the release of CyA. As such, it should be noted that the distinct morphology of double-layered microparticles and encapsulation of multiple drugs altered the drug release profiles. Rahman and Mathiowitz (2004) also studied the release profiles of BSA labeled with fluorescein isothiocynate (FITC-BSA) from double-walled PLLA/PLGA microspheres. They found that using a specific drug loading, almost zero-order release can be achieved with microspheres where the protein was loaded in the core. The advantages of the double-walled microspheres were also pronounced at higher drug loading (20%) where the release tended to be faster as a result of a higher concentration gradient between the microparticle matrix and the release medium. Interestingly, a thicker shell (by increasing shell polymer content) exhibited a faster initial release. This release result could be due to a higher content of semi-crystalline PLLA which accelerated the release of FITC-BSA. The release rate can therefore be fine-tuned by adjusting the polymer mass ratio, cystallinity of polymer and layer localization of drug. In the protein release study conducted by Kokai et al. (2010b), the inclusion of protein stabilizer (i.e. docusate sodium salt (AOT)) into the double-walled PLLA/PLGA microspheres caused lysozyme to be released more rapidly as AOT addition resulted in the core-shell inversion and localization of lysozyme in the PLGA shell. Within the first week of protein release, the bioactivity of lysozyme with the encapsulation of AOT was significantly reduced as a result of greater amount of protein exposed to organic/aqueous interface damage and an increased molar ratio of protein to AOT. Bioactivity of release lysozyme increased after one week and was similar to protein encapsulated alone. In a

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subsequent study, double-walled microspheres were loaded with neurotrophic factor and subsequently incorporated into porous PCL nerve guide (Kokai et al., 2010a). The nerve guides were implanted in a rat sciatic nerve gap to evaluate the efficacy of neurotrophic factor release from nerve guides for nerve regeneration. Double-walled microspheres preserved the bioactivity of neurotrophic factor and released it to the internal region of the nerve guide, promoting tissue formation and proliferation of Schwann cells. This study successfully demonstrated the applicability of double-walled microspheres through in vivo studies.

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Proteins are mostly hydrophilic and difficult to be encapsulated within hydrophobic polymers. To better control the protein release, protein can be loaded into the hydrophilic polymeric particles that create a more benign environment and then embedded into hydrophobic polymer, forming composite microparticles (Schoubben et al., 2009; Yuan et al., 2009). The hydrophilic polymer, such as alginate, dextran, protects protein from the direct contact with the organic solvent and vigorous agitation, thus maintaining structural integrity of protein. The hydrophobic polymer (e.g. PLGA or PLLA), on the other hand, is used to control the release. Yuan and Liu (2012) recently developed a core-shell microsphere system where the protein was loaded into dextran nanoparticles and then encapsulated within the microspheres composed of the PLLA shell and PLGA core. The protein-loaded nanoparticles were selectively distributed in the PLGA core due to their hydrophilic nature. By carefully adjusting surfactant types, nanoparticle loading, etc, these double-walled microspheres exhibited zero-order release and preserved protein bioactivity for 60 days.

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To effectively deliver growth factors to stem cells for tissue regeneration, PLGA-alginate core-shell microcapsules was developed through a coaxial electro-dropping method (Choi et al., 2010). The release results showed that two different biomolecules can be released together at different doses over time. The release profile of each biomolecule can be manipulated based on its localization in the core-shell domain. Early release of the biomolecule from the shell was observed, followed by another biomolecule from the core. This dual factor core-shell system provides an avenue to better regulate stem cell differentiation.

Site-specific delivery through multiparticulate systems

In addition, site-specific drug delivery to the small intestine or colon (pH ~7), through the oral route, can be achieved using multilayered particulate system with an enteric shell layer, while the drug is encapsulated within the inner core (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998; Krishnamachari et al., 2007; Naha et al., 2008). The pre-synthesized polymer cores were microencapsulated in Eudragit polymer through a solvent evaporation technique in an oil phase. As observed from in vitro release study, drug was released from the cores, once the enteric polymer had dissolved at pH above 7. This delivery system would be effective in treating colonic diseases locally such as Crohn's disease and ulcerative colitis. Although only single-drug loaded systems were demonstrated, one can envision that a multilayered particle coated with an enteric layer would be delivered to intestine or colon, while multiple drugs can be encapsulated in different layers for controlled release.

Tunable drug release profiles of ternary-phase / triple-layered microparticles

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While extensive studies of drug release from double-layered (binary-phase) microparticles have been conducted, a recent study by Lee et al. (2011) investigated how double-layered ternary-phase can achieve distinctive and unique drug release profiles when particles are transformed from being binary-phase to ternary-phase. The use of multi-phase/multilayered microparticles with introducing additional polymers was shown to facilitate the design of microparticles to alter the drug release profiles. The PCL particulates within the PLLA shell in ternary-phase microparticles resulted in a less dense and more rubbery shell matrix, which accelerated the drug diffusion from the core, as compared to a slower release from binary-phase microparticles. Drug release kinetics can be further manipulated by changing the polymer mass ratios. When the PCL content was increased, a more rubbery shell yielded a rapid zero-order release kinetic. For dual-drug release, the release of IBU located in the shell was found to influence the release kinetics of MCA in the core. Binary-phase PLLA(shell)/PLGA(core) microparticles yielded a huge (60%) burst release of IBU which caused the formation of surface pores and subsequently increased water uptake, accelerating PLLA degradation and thus MCA release. Ternary-phase microparticles, on the other hand, were shown to release two drugs in a sequential manner, with the release of IBU within the first 10 days, followed by the release of MCA after 10 days (**Figure 6**). A lag in the release phase of MCA was observed for ternary-phase microparticles, as IBU was well-dispersed in the shell containing the PCL particulates and consistently released. Ternary-phase microparticles, therefore, provide a more controlled release of multiple drugs in the investigated study.

The development of particulate systems using triple-layered microparticles, with an additional layer to double-layered microparticles, can be an attractive and robust approach in realizing a variety of drug delivery kinetics. This can be achieved through the manipulation

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of their layer compositions, configurations and thicknesses. Lee et al. (2012d) compared the ibuprofen release profiles of triple-layered microparticles with those of double-layered and single-layered microparticles. Degradation characteristics and particle structural attributes were tuned to alter the drug release properties of these microparticles. The triple-layered microparticles exhibited intermediate release kinetics due to faster PLLA mid-layer degradation arising from the migration of acidic PGA oligomers from the PLGA shell (**Figure 7**). With the layer inversion between PLLA and PLGA in the triple-layered microparticles, the more hydrophobic PLLA shell reduced the water uptake, thus slowing the degradation of polymers and achieving the time-delayed release. Through specific tailoring of layer thicknesses, a linearly sustained release could also be achieved.

Practical aspects of multilayered microparticles for drug delivery

Multilayered particles with controllable particle sizes, layer thicknesses, configurations and localization of drug can be fabricated through a simple, economical, and versatile one-step fabrication technique. The use of multilayered particulate system may pose a significant impact in functioning across a wide range of biomedical applications, where some layers can contain multiple drug substances (poly-pharmacies), bio-imaging dyes, while others can act as rate-limiting layers. Multilayered microparticles can be envisioned to provide pulstaile drug release kinetics, which is favourable for vaccination and local tumor therapy. It would also appear that delivery of radiosensitizers and/or anticancer drugs through the use of multilayered microparticles can be appropriate for cancer treatment over prolonged periods and with minimal systemic side effects. Also, the advantage of having multiple layers also allows for different drugs to be selectively localized in different layers for a time-controlled sequential release, which favours current chemotherapy regimen. In addition, drug delivery to

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the small intestine or colon, through the oral route, can be achieved using multilayered microparticles with an enteric shell layer, with other layers providing the means to achieve controlled release of single or multiple drugs. Multilayered particulate device can also function as a 3D scaffold and deliver bioactive molecules to cells for tissue engineering. This multilayered particulate system therefore allows for the tailoring of particle designs for a range of biomedical applications. Furthermore, the multilayered delivery system could achieve the controlled release of a wide range of substances, such as therapeutic agents, insecticides, fertilizers, indicators, etc. Polymer particles with different morphologies would thus be of great interest to a wide range of applications including pharmaceuticals, printing, perfumery, cosmetics, and agrochemicals.

Nevertheless, the considerably large size of the multilayered microparticles is a hindrance for intravenous injection. Intramuscular, subcutaneous injection or even surgical operations become a necessity for implantation. The optimized particle size is usually a compromise between two main factors. The decrease in particle size allows for better syringability and only requires smaller needle gauge, which translates into reduced patient discomfort. On the other hand, larger particles reduce the clearance from the injection site by macrophages. Therefore, 10 µm is generally considered to be an ideal lower size limit in order to avoid massive particle uptake by macrophages and is large enough to remain at the site of injection. Considering the above-mentioned determinants, an average particle size of about 30 µm (in diameter) therefore seems appropriate for depot applications, and the particle size can be controlled by manipulating certain fabrication parameters.

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Though the solvent evaporation technique is valuable for fabricating new formulations for controlled release purposes, routine validation of bulk pharmaceutical formulas would be time consuming to assess because it requires sectioning single microparticles. In addition, the fabricated microparticles must be subjected to an extensive washing process to remove the excess surfactant and solvent. Moreover, the organic solvent used to dissolve the hydrophobic polymers is a hazardous substance which presents toxicity problems and requires careful handling and disposal. Therefore, great effort should be made to improve the physicochemical and toxicological properties of the actual drug dosage formulations. Also, an acidic microenvironment in the degraded microparticles (e.g. polyesters) could lead to the destabilization and non-covalent aggregation of encapsulated acid-labile biomacromolecules.

In order to better utilize this controlled delivery system for practical therapeutic applications, it is inevitable that the in vivo performance of these multilayered microparticles should be rigorously investigated. However, to date, there is a dearth of studies that focus on the in vivo behaviour of these multilayered microparticles. With continued advances to these “designer” multilayered particulate drug-delivery systems, drug dosage forms can be tailored to provide specific release kinetic for individualized medicine by designing appropriate microparticle structures for a range of biomedical applications.

Conclusions

An assortment of “designer” multilayered microparticles with different particle parameters (e.g. particulate size, layer thickness, polymer configuration, homogeneity of drug dispersion, etc.) can be fabricated through the one-step solvent evaporation technique. Phase separation

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3 and precipitation rates of polymers can be manipulated by various process parameters to form
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5 different configurations of the resulting microparticles. Selective localization of drugs/
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7 proteins in different layers of the multilayered microparticle was also possible. The in vitro
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9 drug release profiles of multilayered microparticles have been reported. Multilayered
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11 particulate system provides unique and better controlled drug release properties, including
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13 reduced burst release, pulsatile release, time-delayed release, zero-order release and release of
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15 multiple drugs, as compared to neat microparticle system. Therefore, the use of multilayered
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17 microparticles can offer great versatility in controlling the drug release kinetics and profiles,
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19 by manipulating the particles' layer thicknesses, configurations, polymer types, layer-drug
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21 localization and degradation rates.
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46 ## Declaration of Interest
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Figure captions

Figure 1. (a) Scheme of the mechanism involved in the formation of the multi-layered microparticles, and (b) SEM image of a cross-sectioned quadruple-layered microparticle.

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Figure 2. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-IBU-loaded double-layered ternary-phase PLLA/PLGA/PCL microparticle.

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Figure 3. Changes in peak molecular weight of POE and PLGA in the neat microspheres and the double-walled POE/PLGA microspheres as a function of incubation time. (A) POE; (B) PLGA.

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Figure 4. Release profile of double-walled PLLA/PLGA microspheres with irradiation dose of 0 Gy (●), 50 Gy (▲) and >25 kGy (♦).

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Figure 5. Release profiles of (a) BSA and (b) CyA from the neat POE, PLGA and double-walled POE/PLGA microspheres.

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Figure 6. Sequential release of metoclopramide HCl and ibuprofen from MCA-IBU-loaded double-layered ternary-phase PLLA/PLGA/PCL microparticles.

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3 Figure 7. Release profiles of ibuprofen from the single-layered EVA, double-layered
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5 PLLA/EVA and triple-layered PLGA/PLLA/EVA microparticles.
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Table 1 Three possible configurations based on spreading coefficients

Complete engulfment	Partial engulfment	No engulfment
$\lambda_{AC} < 0$	$\lambda_{AC} < 0$	$\lambda_{AC} < 0$
$\lambda_{CB} < 0$	$\lambda_{CB} < 0$	$\lambda_{CB} > 0$
$\lambda_{BA} > 0$	$\lambda_{BA} < 0$	$\lambda_{BA} < 0$

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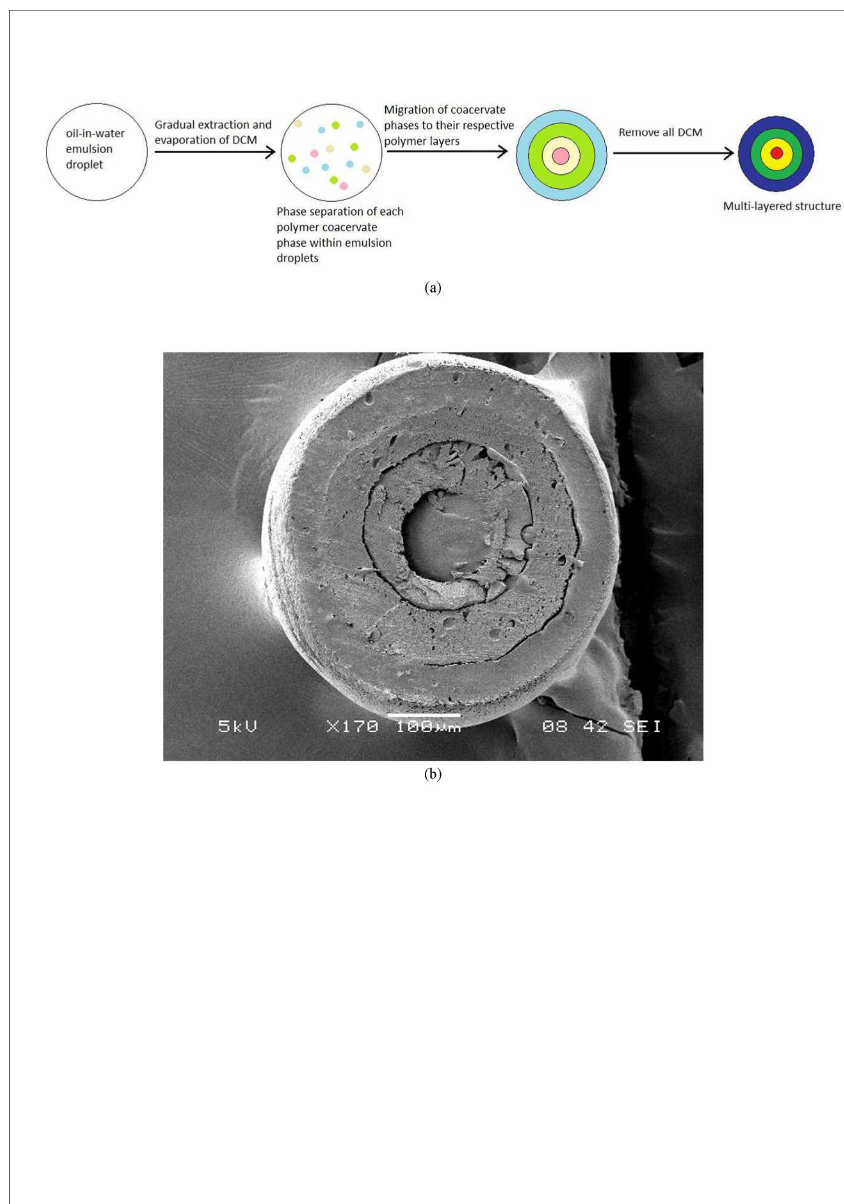

Figure 1. (a) Scheme of the mechanism involved in the formation of the multi-layered microparticles, and (b) SEM image of a cross-sectioned quadruple-layered microparticle.
(reprinted from Lee et al., 2012c with permission from Springer)

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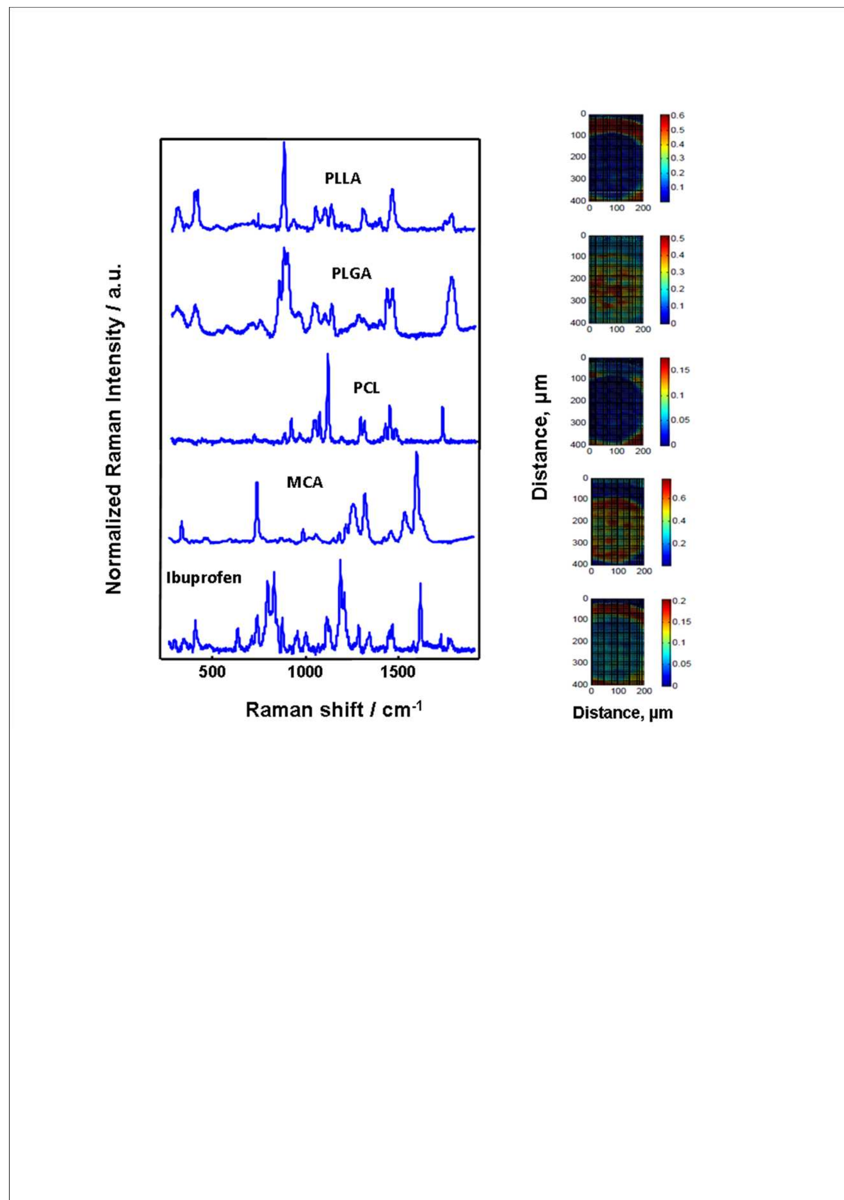

Figure 2. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-IBU-loaded double-layered ternary-phase PLLA/PLGA/PCL microparticle. (reprinted from Lee et al., 2011 with permission from Elsevier)

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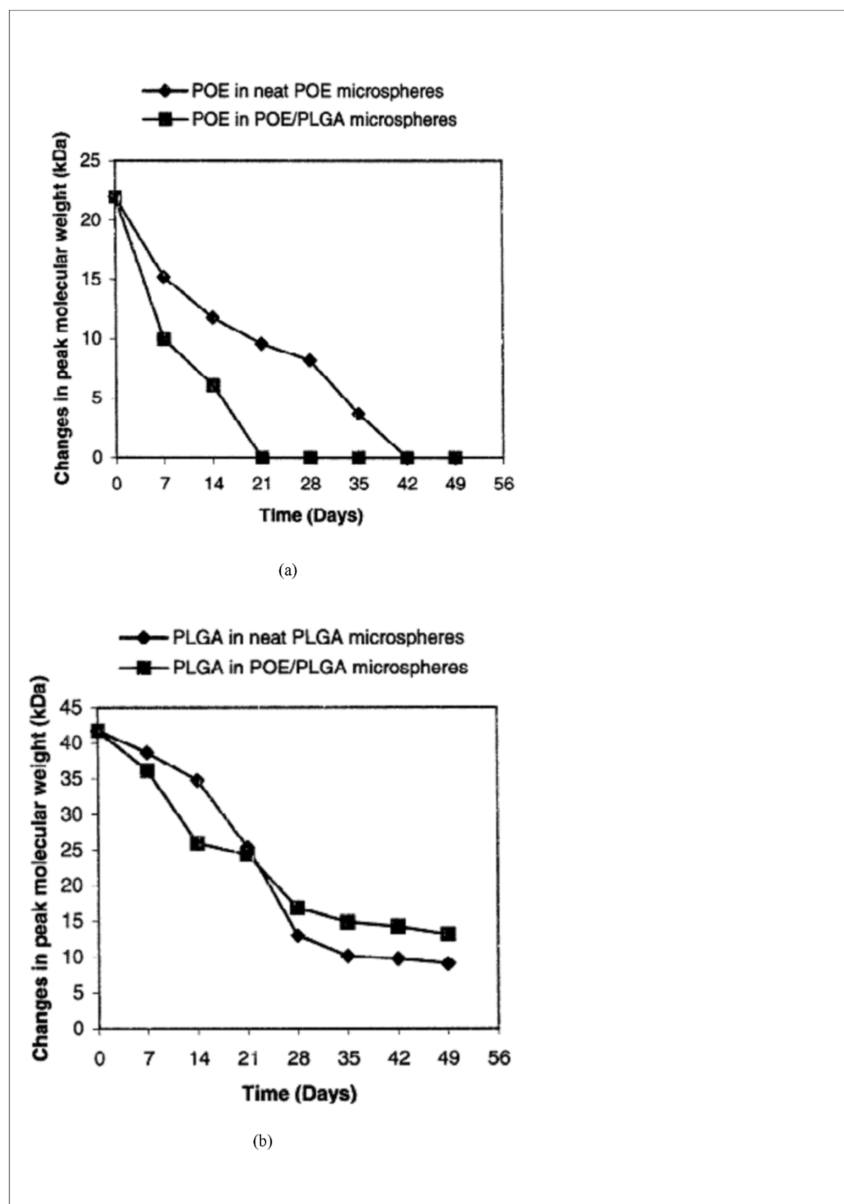

Figure 3. Changes in peak molecular weight of POE and PLGA in the neat microspheres and the double-walled POE/PLGA microspheres as a function of incubation time. (A) POE; (B) PLGA. (reprinted from Yang et al., 2003 with permission from Elsevier)

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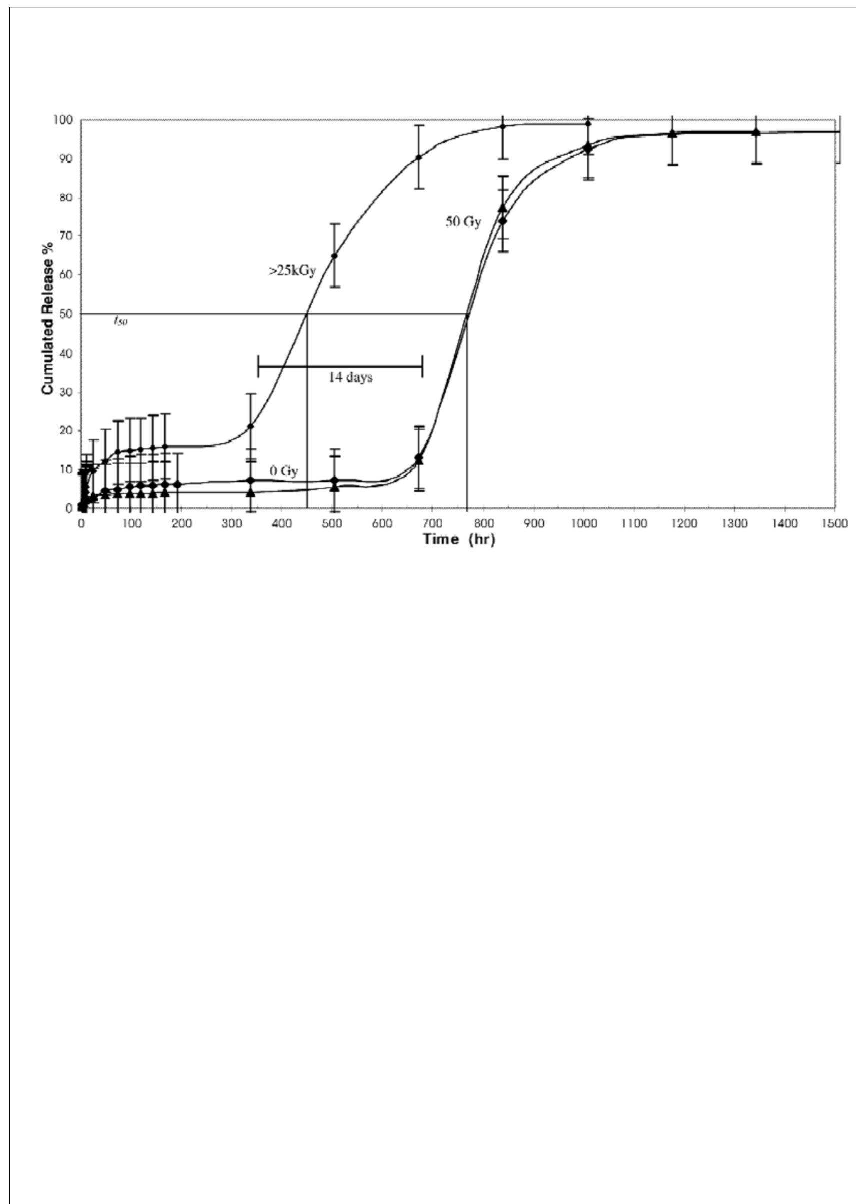

Figure 4. Release profile of double-walled PLLA/PLGA microspheres with irradiation dose of 0 Gy (•), 50 Gy (▲) and >25 kGy (♦).
(reprinted from Lee et al., 2002 with permission from Elsevier)

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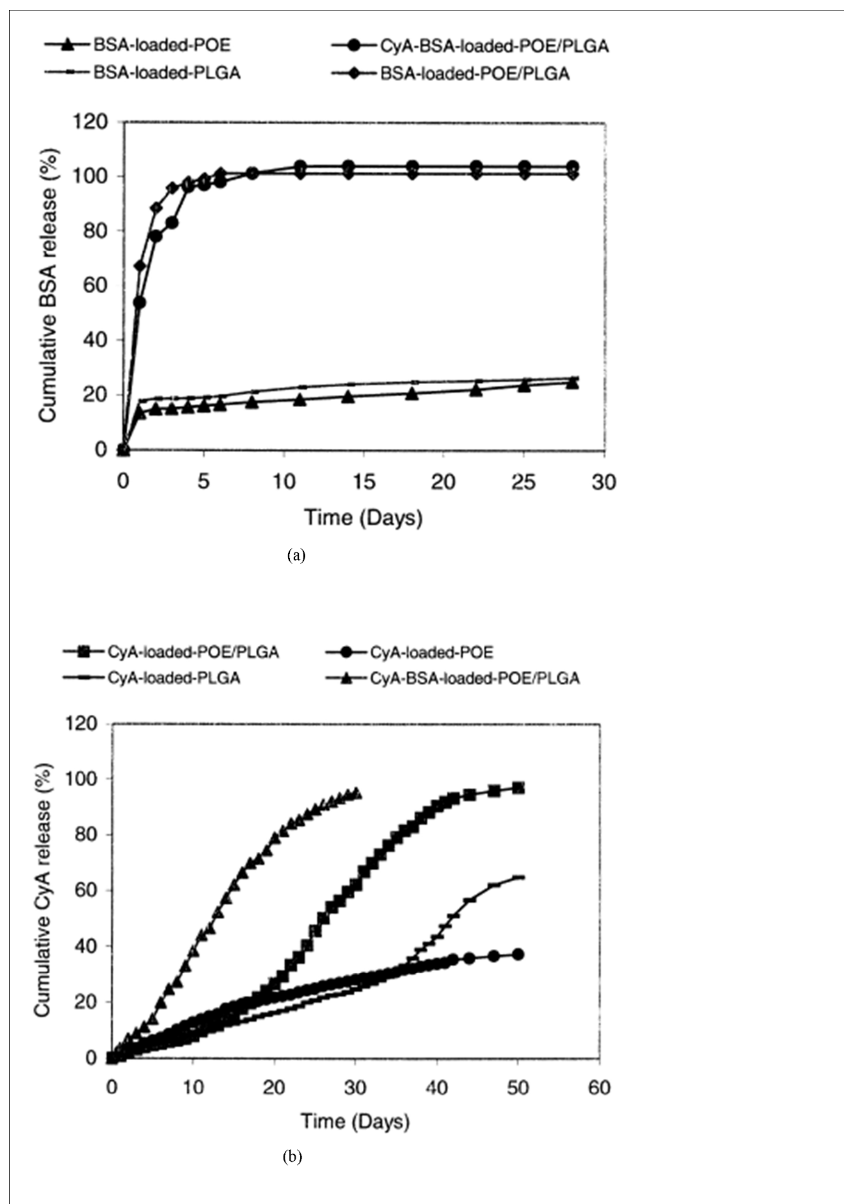

Figure 5. Release profiles of (a) BSA and (b) CyA from the neat POE, PLGA and double-walled POE/PLGA microspheres.
(reprinted from Shi et al., 2003 with permission from Elsevier)

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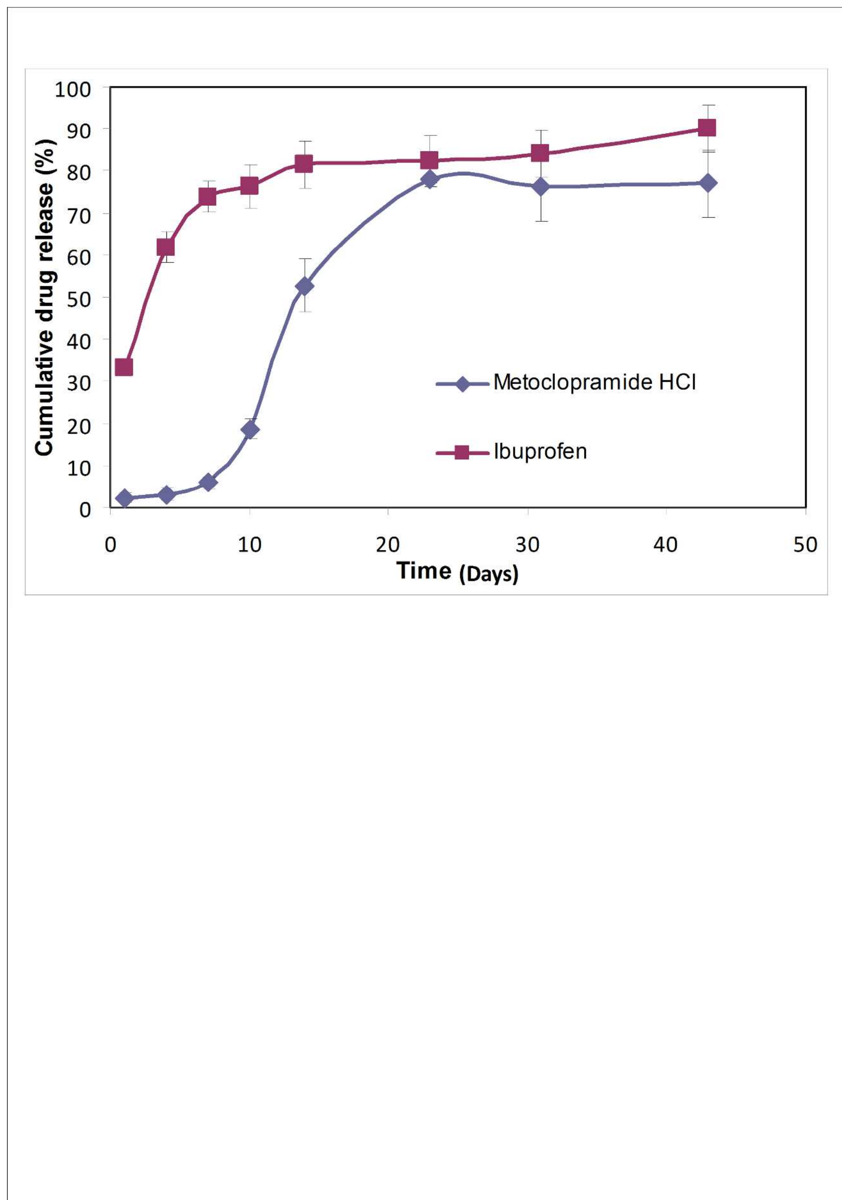

Figure 6. Sequential release of metoclopramide HCl and ibuprofen from MCA-IBU-loaded double-layered ternary-phase PLLA/PLGA/PCL microparticles.
(reprinted from Lee et al., 2011 with permission from Elsevier)

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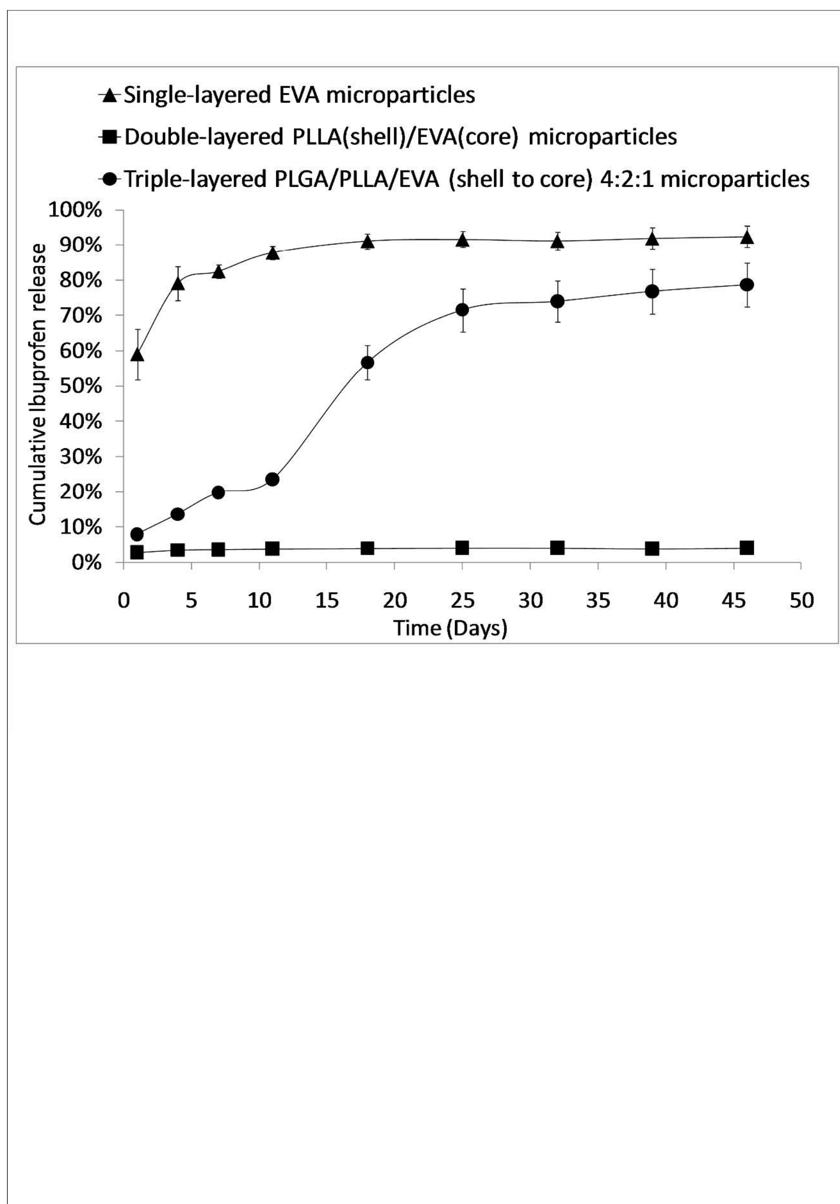

Figure 7. Release profiles of ibuprofen from the single-layered EVA, double-layered PLLA/EVA and triple-layered PLGA/PLLA/EVA microparticles.
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