Controlled drug release from multi-phase polymeric particles

Lee, Wei Li

2012

Lee, W. L. (2012). Controlled drug release from multi-phase polymeric particles. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/48050>

<https://doi.org/10.32657/10356/48050>

NANYANG
TECHNOLOGICAL
UNIVERSITY

CONTROLLED DRUG RELEASE FROM
MULTI-PHASE POLYMERIC PARTICLES

LEE WEI LI

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2012

CONTROLLED DRUG RELEASE FROM MULTI-PHASE POLYMERIC PARTICLES

LEE WEI LI

School of Materials Science and Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2012

Acknowledgements

The author would like to express his utmost gratitude to the following people for the author's accomplishments and completion of his PhD research and thesis:

My heartfelt thanks to my supervisor, Assistant Professor Loo Say Chye Joachim, who is also the main initiator behind this research project. His invaluable guidance and support that had been rendered throughout the duration of my PhD research is greatly appreciated. I also attribute all my publications to his patience in going through and correcting the manuscripts.

Special thanks to the collaborator of this research work, Dr. Effendi Widjaja, from Institute of Chemical and Engineering Sciences (ASTAR) and MERCK, for his help in performing the Raman mapping analysis. I am also thankful to Dr. Shi Wenxiong for his assistance with MATLAB programming software.

I would like to thank the students (Loei Cedric, Foo Wan Ling, Hong Meiju, Pattara-orn Yu, Lin Anli, Low Zheng Yang, Seh Yi Chuan, Lim Wei Yang Alvin, Loh Mei Ting Eugenia, Zhang Yingyan Joanne) for their assistance provided in this research work.

I would also like to express my thankfulness to laboratory technicians (Mr. Nelson Ng, Mr. Wilson Lim, Ms. Yuan Quan, Ms. Sandy Leong, Ms. Irene Heng, and Ms. Sharon Tan), friends and colleagues of Biomaterials Lab for their kindly support that they have given me without which I would not have been able to work comfortably throughout my candidature.

Table of Contents

Title page	i
Acknowledgements	ii
Table of Contents	iii
Summary	viii
Chapter 1 Introduction	1
1.1 Background	1
1.2 Problem statement	4
1.3 Objectives	6
1.4 Scope	7
Chapter 2 Literature Review	10
2.1 Materials background	10
2.1.1 Biocompatible polymers	10
2.1.1.1 Poly(D,L-lactic-co-glycolic acid) (PLGA)	10
2.1.1.2 Poly (L-lactic acid) (PLLA)	11
2.1.1.3 Polycaprolactone (PCL)	12
2.1.1.4 Poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA)	13
2.1.2 Drug candidates	13

2.1.2.1	Ibuprofen (IBU)	13
2.1.2.2	Metoclopramide monohydrochloride monohydrate (MCA)	14
2.2	Limitations of drug release from single-layered microparticles	15
2.3	Fabrication methods of double-layered microparticles	16
2.3.1	Comparisons of various fabrication methods	16
2.3.2	Emulsion solvent evaporation method	18
2.3.2.1	Fabrication of microparticles	18
2.3.2.2	Encapsulation of drugs into the microparticles	25
2.4	Characterization of double-layered microparticles	27
2.4.1	Determination of external and internal morphologies	27
2.4.2	Polymer and drug distribution	28
2.5	In vitro behavior of double-layered microparticles	30
2.5.1	Hydrolytic degradation	30
2.5.2	Drug release	31
2.6	Polymeric particles of various sizes	34
2.6.1	Single-layered particles	34
2.6.2	Double-layered particles	35
2.6.3	Effect of particle sizes on drug release	37
2.7	Modeling of drug release from polymeric microparticles	39
2.8	Summary	41
Chapter 3	Materials and Methods	43
3.1	Materials	43

3.2	Polymer cloud point	44
3.3	Contact angle measurement	45
3.4	Fabrication of microparticles	45
3.5	In vitro hydrolytic degradation	51
3.5.1	Water uptake	52
3.5.2	Mass loss	52
3.6	In vitro drug release	53
3.7	Characterization techniques	53
3.7.1	Scanning electron microscopy (SEM)	53
3.7.2	Particle size analysis	54
3.7.3	Dissolution method	54
3.7.4	Raman mapping	54
3.7.5	Differential Scanning Calorimetry (DSC)	55
3.7.6	Size-exclusion chromatography (SEC)	56
3.7.7	Thermogravimetric analysis (TGA)	57
3.7.8	UV/Vis spectrophotometer	57
3.7.9	Fourier transformed infrared spectroscopy (FTIR)	58
3.7.10	Confocal laser scanning microscopy (CLSM)	58
3.7.11	Focus ion beam (FIB)	58
3.7.12	Field emission scanning electron microscope (FESEM) fitted with a transmission electron detector (TED)	59
3.8	Statistical analysis	59

Chapter 4 Results and Discussion	60
4.1 Fabrication of multi-phase and multi-layered microparticles	60
4.1.1 Double-layered ternary-phase microparticles	60
4.1.1.1 Particle configuration	61
4.1.1.2 Size of embedded particulates and core-shell dimensions	72
4.1.1.3 Size of microparticles	77
4.1.1.4 Drug localization within the microparticles	77
4.1.1.5 Summary	82
4.1.2 Triple-layered and quadruple-layered microparticles	83
4.1.2.1 Fabrication parameters affecting the triple-layered configuration	83
4.1.2.2 Fabrication parameters affecting layer thickness and layer inversion	91
4.1.2.3 Drug localization in triple-layered microparticles	94
4.1.2.4 Fabrication of quadruple-layered microparticles	98
4.1.2.5 Summary	101
4.1.3 Double-layered sub-micron particles	102
4.2 Drug release from multi-phase and multi-layered microparticles	107
4.2.1 Double-layered ternary-phase microparticles	107
4.2.1.1 In vitro drug release	108
4.2.1.2 Hydrolytic degradation of microparticles	117
4.2.1.3 Summary	127
4.2.2 Triple-layered microparticles	129
4.2.2.1 Morphologies of the triple-layered microparticles	129
4.2.2.2 In vitro behaviors of triple-layered microparticles	131
4.2.2.3 Effect of layer thicknesses and configurations on ibuprofen release	140
4.2.2.4 Summary	149

4.2.3	Effect of the size of multi-layered microparticles on the drug release	151
4.2.3.1	Double-layered microparticles	151
4.2.3.2	Triple-layered microparticles	161
4.2.3.3	Summary	171
4.2.4	Implications of drug eluting multi-phase microparticles	171
4.3	Mathematical modeling of ibuprofen release from triple-layered microparticles	173
4.3.1	Theoretical model	174
4.3.2	Particles with different layer thicknesses	179
4.3.3	Particles with various sizes	185
4.3.4	Summary	190
Chapter 5	Conclusions	192
Chapter 6	Recommendations for Future Work	197
Chapter 7	References	202
Appendices		216

Summary

The study of the fabrication of multi-phase particles (i.e. double-layered ternary-phase microparticles, triple-layered and quadruple-layered microparticles) and their drug release profiles is reported in this thesis. The overall objective is to investigate the use of multi-phase/multi-layered microparticles with different morphologies and layer configurations as a means to control and fine-tune the drug release profiles. The results obtained show conclusive evidence that different microparticle configurations (i.e. polymer distribution and dimensions) can be achieved through the manipulation of process parameters in a one-step solvent evaporation technique. A starting polymer solution prepared below the cloud point, low stirring speed and an increased oil-to-water ratio facilitated the polymers to configure themselves towards thermodynamic equilibrium configurations. A considerably high polymer precipitation rate was needed to kinetically trap the triple-layered structure when the polymer solution was prepared above cloud point. Layer thickness and configuration were altered by changing the polymer mass ratios. Drugs can be localized within specific layers of the microparticles. A correlation of these process parameters to the final particle morphology was thus established. The ternary-phase and triple-layered microparticles were observed to provide unique and better controlled drug release as compared to single-layered and double-layered microparticles. This was due to their distinctive structural attributes and degradation behaviors of the multiple-polymer system. Drug release profiles can be further altered by changing the physicochemical properties of each polymer phase. A modeled relationship between the physicochemical processes and drug release was established for triple-layered microparticles of different layer thicknesses and particle sizes. It is concluded that the multi-phase/multi-layered microparticles can be used to better control the drug release profiles and kinetics.

Chapter 1 Introduction

1.1 Background

Paracelsus, the father of medicine is often cited as the person who coined the phrase, “The dose makes the poison”. In other words, it is the administered dose that set a drug from either having a therapeutic value or being a poison. However, what Paracelsus failed to mention is the mode of drug delivery and frequency of administered medication. In the past, scientists in pharmaceutical fields have been coming up with innovative drugs to combat the ever long list of diseases. However, development of a new drug is insufficient in treating diseases. Administration of medication via a device that better control drug release kinetics is gaining practical importance. Controlled release aids in improving therapeutic efficacy, reducing undesired side effect of the drug and allowing for less frequent administration, thereby increasing patient compliance [1, 2].

The biodegradable polyesters poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA), owing to their excellent biocompatibility, are the most frequently used biopolymers for the microencapsulation of therapeutic agents and releasing it over a prolonged time [3]. These polymers can be fashioned into microparticles [4, 5], nanoparticles [6], films [7-9], scaffolds [10, 11], pins [12], sutures [13] and injectable depots [14-16]. 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 [1, 17-19].

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 [19]. Manipulation of the polymer chemistry, particle size, size distribution and/or morphology provides some flexibility in modulating drug release [20-25]. Unfortunately, monolithic polymer particle-mediated drug delivery systems have several inherent problems, such as a high initial “burst” of the encapsulated agent [26-28], limited control of drug release kinetics [29-35], a lack of constant (zero-order) or pulsatile release of drugs and the inability to deliver multiple-drugs from a single particle [17, 36-38]. Owing to the limited and non-versatile control over drug release from the monolithic system, multiparticulate drug delivery system that composes a polymer shell surrounding one or many micron-sized particulates may provide improved control over drug release rates, through selective localization of each drug in the individual polymer phases [39-46].

The fabrication of multi-phase/multi-layered microparticles with introducing additional polymers would therefore be an important step towards an attractive and robust approach to provide a variety of drug delivery kinetics and profiles, where some layers contain drug substances; others are rate-limiting layers. Several processes have been developed to fabricate double-layered microparticles, including hot melt technique [47], pan- or dip-coating [48], fluidized beds [49], spray-drying [50], a precision particle fabrication technology [51-53], and emulsion solvent evaporation [30]. Unlike other fabrication

process where prefabricated microparticles were further coated, the emulsion solvent evaporation technique is a one-step process used to fabricate double-layered microparticles with higher yields, highly uniform coating, and a controllable particle size ranging from 20 to 1000 μm . The solvent evaporation technique is also more economical since expensive and complex fabrication equipment is not required. In addition, poorly soluble drugs can be directly loaded into the coating layer in the solvent evaporation technique, in comparison to the fluidized bed method. On another note, the solvent evaporation technique has been widely employed to fabricate the microparticles in a small scale for research purpose [54]. A fabrication technique that requires only single step and provides easy formation of ternary-phase and multi-layered (more than two layers) particles with layer localization of drugs would therefore facilitate the development of different ingenious designs of the particulate drug-delivery system.

Through the layer localization of drugs in different layers, two or more different drugs could be released at different doses with time [38]. Furthermore, two or more particle populations can be readily combined into the multiparticulate system as a dosage form, allowing for easy adjustment of pharmacokinetic profile and/or creation of synergistic and multiple strengths of a single microparticle. Previous studies have shown that the degradation behavior of multi-layered polymer films differ from that of single-layered polymer films; whereby the degradation of the top polymer layer would accelerate hydrolysis of the underlying layers [55]. Therefore, it is postulated that a composite multi-layer or multi-phase microparticulate system may also possess unique hydrolytic degradation characteristics and morphological attributes that differ from single-layered particles. As such, these multi-layered or multi-phase microparticles may offer greater

versatility in controlling drug release kinetics and profile, by manipulating the particles' layer thickness, configuration, size and the degradation rates [17, 56].

1.2 Problem statement

Although the fabrication of double-layered (binary-phase) microparticles using a single-step solvent evaporation method has been established, there has been no report on the fabrication of microparticles consisting of three different polymers – ternary-phase microparticles. In addition, the development of multi-layered (more than two layers) particles in a single-step still remains a major challenge. The process becomes even more intricate with each additional polymer. As such, composite microparticles prepared through the solvent evaporation method at present are only double-layered, although the third or more layers can be added in separate steps. These additional steps would result in quality-control problems and a decrease in yield [47, 57]. There is therefore the lack of extensive research work on one-step fabrication of multi-phase and multi-layered (more than two polymer phases) microparticles with specific localization of drugs in different layers. With regards to the papers that discussed the formation of double-layered microparticles [43, 58], very few have discussed comprehensively key process parameters affecting the formation mechanisms of the final microparticle configuration, and no papers has reported on the fabrication of double-layered sub-micron particles.

Modern drug discovery technologies are discovering more and more potent therapeutic agents which exhibit narrow therapeutic ranges, thus necessitating the improvement of current particulate drug delivery systems. The problem in polymer microparticle-based

drug delivery systems can thus be summarized as “the limited control of drug release profiles”. Multi-phase microparticles may offer improved control of the drug release rates. While studies of drug release from double-layered microparticles have been reported, no such studies have been conducted using double-layered ternary-phase microparticles. Therefore, it is of interest to understand how the release profiles and kinetics of double-layered microparticles are altered when particles are transformed from being binary-phase to ternary-phase by introducing an additional polymer to the particle. In addition, since there are currently no papers that explore the use of triple-layered microparticles to control the drug release, it is also of interest to know how triple-layered microparticles with different layer configurations (inversion of outer layers) and thicknesses can provide unique drug release properties, as compared to those of double-layered and neat microparticles. Particle size is an important parameter that could affect the degradation and drug release properties of the polymer matrix [59, 60]. However, the effect of the size of biodegradable multi-layered particles on the drug release rate has yet to be studied.

This thesis therefore aims to fill the gap by developing a fabrication technique for multi-phase and multi-layered particles that provides easy manipulation of particle morphology, layer configuration, drug-layer localization and particle size. Since there are also currently no papers that explore the use of multi-phase microparticles as a means to control the rate of drug release, it is therefore of interest to investigate the drug release properties of this “designer” particulate drug-delivery system.

1.3 Objectives

The focus of this thesis is to study the controlled drug release from multi-phase polymeric particles, and the main objective is to investigate the use of multi-phase and multi-layered microparticles with different layer configurations and particle sizes as a means to control and fine-tune the drug release profiles. In fulfilling the objective, the research work was divided into three parts.

The first part is to conduct an in-depth study on the fabrication of ternary-phase polymeric microparticles as well as multi-layered (from double-layered to quadruple-layered, and potentially multilayered) microparticles with controllable layer configurations and morphologies, through a one-step solvent evaporation technique. The effects of process parameters on the final microparticle configuration will be investigated. From these results, key process parameters affecting the formation of the multi-phase and multi-layered configurations are identified and the formation mechanisms are subsequently proposed. Drugs with different hydrophobicities will be used to understand how the specific localization of drugs in different layers of the microparticles can be realized through this one-step fabrication technique. Through this knowledge, different configurations and sizes of microparticles can be achieved, thus providing a greater degree of freedom in designing microparticles that would provide a variety of drug release profiles and kinetics.

The second part is to study the drug release properties of the double-layered ternary-phase and triple-layered microparticles with the alteration of layer thickness, configuration, particle size and drug type encapsulated in comparison to binary-phase and neat microparticles. The drug release results will be explained by investigating the rates of hydrolytic degradation and morphological differences of these microparticles.

The third part is to model and quantitatively describe the experimental drug release profiles of triple-layered microparticles with different layer thicknesses and particle sizes. At the end of thesis, it would then be determined if the use of multi-phase/multi-layered microparticles as drug delivery devices can provide a versatile and robust approach to control the drug release kinetics.

1.4 Scope

In this work, poly(D,L-lactide-co-glycolide) (50:50) (PLGA), poly(L-lactide) (PLLA), poly(caprolactone) (PCL) and poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA) will be used for the formation of multi-phase/multi-layered microparticles. These biocompatible polymers were selected for based on the mutual immiscibility. Ibuprofen (IBU), and metoclopramide monohydrochloride monohydrate (MCA) will be used as model drugs with different hydrophobicities to understand how the localization of drugs in different layers of the microparticles and controlled drug release can be realized.

Only the neat, double-layered, double-layered ternary-phase, triple-layered and quadruple-layered microparticles will be fabricated through a one-step emulsion solvent evaporation technique. The particle sizes range from sub-micron to $\sim 400\text{ }\mu\text{m}$. Effects of process parameters, such as initial polymer solution concentration, stirring speed, oil-to-water ratio and polymer mass ratio on the morphology of the particles will be investigated. These process parameters can be easily adjusted and manipulated in the solvent evaporation technique without any special equipment setup. Process parameters were chosen as it was hypothesized that the precipitation rate of polymer could be altered by polymer solution concentration, stirring speed and oil-to-water ratio. By varying the polymer mass ratios, a wide range of microparticles with different core-shell dimensions and embedded particulate sizes could be fabricated. A correlation of these process parameters to the final composite particle morphology will then be established. This understanding would allow for a controlled fabrication of multi-phase/multi-layered microparticles through a single-step solvent evaporation technique.

For the drug release studies conducted on the ternary-phase and triple-layered microparticles in comparison to binary-phase and neat microparticles, particle sizes of all microparticles investigated will be approximately $300\text{ }\mu\text{m}$. As it is difficult to study the internal morphology of small microparticles, larger microparticles will be involved in this study for further investigation. On the other hand, multi-layered particles of sizes ranging from sub-micron to $\sim 200\text{ }\mu\text{m}$ will be employed to study the effect of particle size on the rate of drug release.

For the in-vitro hydrolytic degradation, the microparticles will be incubated at 37°C with moderate shaking. The pH of the solution will be monitored over time to ensure that the pH was maintained at 7.4 throughout the study.

A mathematical and semi-empirical model will be developed to quantitatively describe the observed drug release profiles of triple-layered microparticles. Based on the dependency, this model would be applicable to other reservoir-membrane system.

Chapter 2 Literature Review

2.1 Materials background

2.1.1 Biocompatible polymers

2.1.1.1 Poly(D,L-lactic-co-glycolic acid) (PLGA)

PLGA (**Figure 2.1**) is a biocompatible (Food and Drug Administration, FDA, approved) and biodegradable copolymer of lactide and glycolide synthesized via random ring-opening co-polymerization [61]. Unlike the homopolymers such as polylactide and polyglycolide which exhibit low solubility, PLGA can be dissolved by a wide range of organic solvents, including dichloromethane (DCM), tetrahydrofuran (THF), acetone or ethyl acetate. PLGA undergoes bulk degradation through random hydrolytic scission of its ester linkages in the presence of water. The rate of water uptake of PLGA is much faster than the rate of hydrolysis of the polymer. The degradation rate of PLGA is dependent on several factors, such as the lactide and glycolide ratio, crystallinity and molecular weight [62]. PLGA 50:50 is fully amorphous as a result of disorderly and poor packing of constituent PLA and PGA. This high free volume encourages the water uptake, thus enhancing the hydrolytic scission of the ester linkage in PLGA 50:50. The degradation products with carboxylic end-groups would further enhance the hydrolysis, giving rise to an autocatalytic degradation. In vivo, lactic and glycolic acids are

metabolized and eliminated from the body as carbon dioxide and water via tricarboxylic acid cycle [61].

Figure 2.1 Chemical structure of PLGA

2.1.1.2 Poly (L-lactic acid) (PLLA)

PLLA (**Figure 2.2**) is the optically active L- form of stereoisomeric polylactide. PLLA is biodegradable and FDA approved. It undergoes degradation in water by hydrolysis of the ester groups, similar to PLGA. PLLA has semi-crystalline morphology and it is relatively more hydrophobic than PLGA due the presence of more methyl groups in PLLA, which hinders the water penetration [63]. Due to its chemical structure and hydrophobic nature, the complete degradation time is longer than PLGA. Henceforth, PLLA has a long degradation profile which makes it not very suitable as drug release matrices. In this study, PLLA was used together with PLGA to fabricate multi-phase and multi-layered microparticles to fine-tune the degradation rates of polymers.

Figure 2.2 Chemical structure of PLLA

2.1.1.3 Polycaprolactone (PCL)

PCL (**Figure 2.3**) is a biodegradable, biocompatible and semi-crystalline polymer with a low melting point of around 60°C and a low glass transition temperature of about –60°C [64]. Therefore, PCL is in rubbery state at room temperature (25°C). The degradation mechanism of PCL has been credited to a random chain-scission at the ester bonds through hydrolysis. Due to its low ester bonds to carbon ratio, the degradation of PCL is even slower than that of PLLA.

Figure 2.3 Chemical structure of PCL

2.1.1.4 Poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA)

EVA (**Figure 2.4**) is the biocompatible copolymer of ethylene and vinyl acetate. EVA, as a drug delivery device, is commonly used in biomedical applications. It is a very rubbery and elastic material that exhibits low glass transition temperature. The resultant rubbery nature attributes to an increment in permeability of EVA [65]. Although EVA does not undergo degradation, it is relatively inert and causes little or no adverse reactions to the tissues and human body [66]. EVA can be found in products such as endovascular prosthesis and drug-loaded polymeric coating on heart stents.

Figure 2.4 Chemical structure of EVA

2.1.2 Drug candidates

2.1.2.1 Ibuprofen (IBU)

Iso-butyl-propanoic-phenolic acid (molecular weight of 206.29 g/mol) or ibuprofen (**Figure 2.5**) is in the class of non-steroidal anti-inflammatory drug (NSAID) with

analgesic and antipyretic action. Half-life of ibuprofen is approximately 2 h. Ibuprofen has the log P (n-octanol/water) of 3.68 and possesses hydrophobic nature [67]. It can only dissolve very slightly in water (< 1 mg/mL). Subsequently, its hydrophobic nature results in good solubility in organic solvent. In this thesis, ibuprofen was used as a model hydrophobic drug compound and would be selectively encapsulated within the specific polymer phase of the multi-phase microparticles.

Figure 2.5 Chemical structure of ibuprofen

2.1.2.2 Metoclopramide monohydrochloride monohydrate (MCA)

Metoclopramide hydrochloride (molecular weight of 354.3 g/mol, **Figure 2.6**) is in a white drug crystal form and is highly soluble in water (650mg/mL). MCA has half-life of 5-6 h. It can be used to prevent postoperative nausea and vomiting associated with various gastrointestinal disorders; or treat patients with diabetic gastroparesis [68]. In addition, MCA may be used as a radiosensitizing agent in the treatment of non-small cell lung carcinoma and glioblastomas. The highly hydrophilic nature would drive MCA to be distributed in the relatively hydrophilic polymer phase of the multi-phase microparticles.

Figure 2.6 Chemical structure of MCA

2.2 Limitations of drug release from single-layered microparticles

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 [43]. In addition, low hydrophilic drug encapsulation efficiency is usually observed for the single-layered particles [39]. Another major problem is that a single-layered microparticle is unable to achieve a zero-order drug release rate. This is attributed to the change in diffusion distance of the drug in the polymer microparticles [30]. Additionally, the bulk degradation of commonly used polymers in the drug delivery, such as PLGA and PLLA, does not enable constant release [69-72]. In general, the drug is first released through diffusion due to low mass loss of polymer, followed by erosion-controlled rapid release [9]. Layer localization of the drug in the neat microparticles is also not achievable, giving rise to an inability of releasing drugs in a pulsatile or sequential manner from a single particle [38]. As such, limited

control of drug release from single-layered microparticles and achievement of optimal performance must still be overcome. In this thesis, multi-phase and multi-layered microparticles will be used to alleviate the drug release problems associated with single-layered microparticles.

2.3 Fabrication methods of double-layered microparticles

Although single-layered particles are able to load and deliver drugs, several problems such as high initial burst release, lack of time-delayed release and low drug encapsulation efficiency for hydrophilic drugs still exist. Fabrication of double-layered microparticles can facilitate the design of microparticles for controlled drug release systems, thus revolutionizing drug delivery.

2.3.1 Comparisons of various fabrication methods

In the past years, several fabrication methods of double-walled microspheres have been devised. These methods included the coating technologies that use hot-melt technique [47], dip coating [48], fluidized beds [49], or spray drying [37, 50]; precision particle fabrication technology that employs a series of concentric nozzles to create a compound jet [17, 51, 53]; and emulsion solvent evaporation method [30]. 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 [30]. 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 40 μm in diameter due to the agglomeration problem of small particles [49]. Double-walled microspheres can also be fabricated by spray drying the suspension of core microparticles in a non-solvent containing the dissolved coating polymer [50]. 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 multi-layered microparticles.

For the one-step fabrication of double-walled microspheres, D.W. Pack et al. 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 [51]. 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. The disadvantage is that complex fabrication equipment was involved.

As compared to other earlier attempts, the emulsion solvent evaporation technique is a one-step process that gives double-walled microspheres with higher yields, uniform layer thickness and a controllable particle size within a range of 20 μm –1000 μm [30]. 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 to 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 and do not necessarily have to be dissolved in the polymer solution.

Therefore, in this thesis, the economical, reliable and single-step emulsion solvent evaporation process was employed to fabricate multi-phase microparticles and tailor their structural architectures.

2.3.2 Emulsion solvent evaporation method

2.3.2.1 Fabrication of microparticles

The usual solvent evaporation method utilizes the formation of an oil-in-water emulsion with the presence of surfactant [19]. 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. 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 [73]. Other popular commercial products produced by solvent evaporation are Enantone Depot/Trenantone/Enantone Gyn.

In the modified solvent evaporation technique employed to prepare double-walled microspheres, which was first reported by Pekarek et al. [30], 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 two 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 [51, 74]. If the polymer precipitation rate is slow enough, two polymers will configure themselves in their most 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 Harkin for a liquid to spread on a solid or liquid surface in terms of interfacial energies of the components [75]. Torza and Mason extended Harkin's theory to a system in which two immiscible phases are suspended in a third immiscible phase [76]. **Eq. 2.1** can be used to calculate the spreading coefficient for the determination of the ability of one polymer to engulf the other.

$$\lambda_{AB} = \gamma_{BC} - \gamma_{AC} - \gamma_{AB} \quad \textbf{(Eq. 2.1)}$$

γ_{AC} and γ_{BC} are the interfacial energies between the continuous phase 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 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 2.1** shows the three possible combinations of the spreading coefficients.

Table 2.1 Three possible configurations based on spreading coefficients

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

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.

One should be noted that the spreading coefficient will change when the solvent is extracted since the change in polymer solution concentration will alter the effective interfacial energies. Therefore, the spreading coefficient theory would be only used as an indirect estimation of the final configurations of the particles after hardening process [51, 77]. Besides, there was no mention of the spreading coefficient when introducing more than two polymers. The addition of more polymers would then complicate the fabrication process. As such, there is a need to study and rationalize the formation mechanism of multi-phase and multi-layered microparticles, which this thesis aims to investigate.

Different microspheres comprising two immiscible polymers have been fabricated and reported. 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 (P(CPP:SA)20:80) surrounded by an outer layer of poly(L-lactide) (PLLA) through an emulsion solvent evaporation technique [30].

Leach et al. 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 [58]. It was found that these parameters determine the precipitation rate and time allowed for the polymers to phase separate and configure themselves before the polymers precipitate. The lower polymer solution concentration resulted in high efficiency of forming the double-walled structure. When

the polymer solution concentration was further increased, the process efficiency decreased. The higher solution concentration exhibiting increased viscosity decreased the ability of the polymers to move and phase separate, thus resulting in non-distinctive layered configuration. The low temperature slowed down the solvent extraction rate, thus allowing more time for phase separation. However, the low temperature would retard the phase separation by decreasing the polymer chain mobility. In addition, the cloud point might be altered when the temperature is lowered. The air flow would change the solvent evaporation rate. As such, the process time and polymer precipitation rate decreased and increased, respectively, with increasing air flow. 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.

Rahman et al. subsequently demonstrated the fabrication of double-walled microspheres with biodegradable PLLA and PLGA [43]. The correct conditions of immiscibility for phase separation of several polymers were investigated. 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. However, the mechanism of polymer distribution of PLLA and PLGA within the microspheres was not reported in the paper.

It had been reported that the morphology of the composite microparticles was dependant on the polymer mass ratio, as reported by Yang et al. [78]. 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 would not be great enough 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. However, Leach et al. had reported that the core-shell inversion could occur when changing the polymer mass ratios regardless of the polymer properties [58]. The contradiction of the finding should be reconciled, which will be investigated in this thesis.

Naraharisetti et al. devised a modified solvent evaporation to form the double-layered PLLA/PLGA microparticles based on the insolubility of PLLA in ethyl acetate (EA) [44]. PLLA and PLGA were dissolved separately in DCM and ethyl acetate, 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.

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. [42]. This multi-reservoir type of structure was formed by mixing two polymer solutions above

the concentration at which phase separation has been achieved. In their study, it was observed that the critical polymer solution concentration inducing phase separation 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 also 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. Preparing the starting polymer solution concentration above or below the cloud point would play an important role in forming the multi-layered microparticles, which will be demonstrated in this thesis.

The formation mechanism of multi-phase/multi-layered microparticles fabricated through the solvent evaporation method has yet to be investigated in great depth and detail. By now, there are only few papers reporting on the effect of process parameters on the formation of double-layered microparticles. There was however no mention of the effect of other process parameters, including stirring speed and oil-to water ratio, in their work. In addition, those studies conducted on particle fabrication were only restricted to the neat, double-layered and binary-phase microparticles. A comprehensive study on the fabrication of multi-layered/multi-phase microparticles comprising more than two polymers was therefore lacking in this field of research.

2.3.2.2 Encapsulation of drugs into the microparticles

Drug-loaded double-layered polymeric microspheres can be fabricated using the oil-in-water (single-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 [79]. 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 [25, 80].

There are several reports on the fabrication of double-layered polymeric microparticles as drug carriers for sustained and controlled release. Matsumoto et al. 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 [41, 42]. Distribution theory as described in **Eq. 2.2**, 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.

$$\log \frac{X_{PLGA}}{X_{PDLLA}} = V_{drug} \frac{(\delta_{drug} - \delta_{PDLLA-DCM})^2 - (\delta_{PLGA-DCM} - \delta_{drug})^2}{2.3 RT} \quad (\text{Eq. 2.2})$$

, 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 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. 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.

Qualitatively, the distribution of drugs within double-layered polymeric microparticles is dependent on the relative hydrophobicity of the drugs and the polymers. In general, hydrophobic drugs are distributed within the more hydrophobic polymer phase, whereas hydrophilic drugs are localized in the more hydrophilic polymer. Lee et al. [39] and Tan et al. [40] gave the similar explanation for the drug distribution based on the drug-polymer affinity. This led to the several reports of single hydrophilic drugs being loaded into the hydrophilic polymer core of double-walled microspheres, giving rise to a more controlled drug release [39, 40, 81].

Besides encapsulating single drug into the double-walled microspheres, Shi et al. have reported on the selective localization of hydrophilic bovine serum albumin and hydrophobic cyclosporine A in the PLGA shell and the poly(ortho ester) core, respectively [36]. However, up until now, this is the only one paper reporting on the encapsulation of multiple drugs into the double-layered microparticles fabricated by the solvent evaporation method. In this thesis, the study on dual-drug-loading within the multi-phase microparticles would then provide more insights into the multiple drug release kinetics from a single particle.

There is hardly any journal that relates the effect of an additional polymer to the dispersion manner of the drug. In this thesis, the effect of adding the hydrophobic PCL as a third phase into the double-layered microparticles on the dispersion manner of hydrophobic ibuprofen will be investigated, which make this study a novel contribution.

2.4 Characterization of double-layered microparticles

2.4.1 Determination of external and internal morphologies

Following fabrication, the surface and cross-sectional morphologies of double-layered 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 [39]. 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 [53]. Alternatively, the microparticles are embedded in frozen gelatin and subsequently cut on a cryostat into sections of $10 \pm 40 \mu\text{m}$ in thickness [58]. 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 [30].

Differential scanning calorimetric (DSC) is used to investigate the thermal properties of the double-layered microparticles [30]. In general, the existence of the two 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.

2.4.2 Polymer and drug distribution

In the polymer distribution study, a direct and simple method was devised by Lee et al. to determine the composition of the shell and core based on the different solubilities of PLLA and PLGA in ethyl acetate [39]. In addition, an IR study through Fourier transformed infra-red (FTIR) microscope can be employed in order to study the

composition of each layer [39, 40, 80]. Alternatively, an X-ray photoelectron spectroscope (XPS) can be utilized to determine the chemical composition of the microparticles' surfaces by comparing the carbon/oxygen ratio of the double-layered microparticles to that of the respective neat polymer [78].

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) [36, 43]. 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 [39, 40].

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. Therefore, more precise species identification of polymer, drugs and impurities present within the microparticles and their spatial distributions cannot be directly achieved using these techniques. A novel characterization approach based on the combined use of Raman microscopy and band-target entropy minimization (BTEM) analysis is adopted in this thesis.

2.5 In vitro behavior of double-layered microparticles

After the fabrication of double-layered microparticles, the next step would be to carry out the drug release and degradation study. Understanding the drug release properties and degradation behaviors will allow one to fine-tune and design the double-layered microparticle configuration to function across a wide range of applications.

2.5.1 Hydrolytic degradation

Leach et al. studied the degradation behavior of double-walled microspheres with a core of P(CPP:SA)20:80 and shell of PLLA [82, 83]. The experimental result showed that the inner core of P(CPP:SA)20:80 degraded first. These degradation products were trapped by the PLLA shell, and remained in the core for the period of investigation. The fast degradation of the P(CPP:SA)20:80 inner core enhanced the degradation rate of the PLLA shell. As such, PLLA outer shell of the double-layered microspheres degraded slightly faster in comparison to the single-layered PLLA microspheres. Similarly, Yang et al. reported that the degradation rate of the POE core was enhanced due to the acidic microenvironment created by the degradation products of PLGA [78].

On the other hand, degradation characteristic of PLLA and PLGA microspheres in inversed core-shell configuration has been studied by Tan et al. [40]. The degradation of polymer that formed the inner core was observed to be slower as compared with that of

the shell polymer. The outer layer acted as a barrier protecting the core polymer from rapid hydrolytic degradation. These previous findings showed advantages on controlled release of a drug located in the core as the release rate can be controlled through the degrading shell and core.

There have been several papers conducted on the degradation study of double-layered microparticles, while ternary-phase microparticle system has yet to be investigated. Based on the previous papers, it is hypothesized that the introduction of additional polymer would change the morphological structure of the microparticles. This morphological change would in turn alter the degradation characteristics, and thus providing distinct drug release kinetics.

2.5.2 Drug release

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 have higher drug encapsulation efficiency as well as better controlled drug release, the double-walled microspheres with drug encapsulated were employed.

There are several reports on the use of double-walled or binary-phase polymer microspheres as drug carriers for sustained and controlled release. Matsumoto et al. demonstrated that the outer non-drug-holding poly(D,L-lactide) layer of the multi-

reservoir type microspheres suppressed the initial burst of Cisplatin from that localized in the PLGA core, thus achieving sustained release [41, 42]. In addition, Lee et al. demonstrated that the PLLA shell of double-walled microspheres retarded the rapid release of highly water soluble etanidazole located in the PLGA core, thus achieving a low initial burst, followed by a lag phase before releasing in a linearly sustained manner [39]. Similarly, Tan et al. and Naraharisetti et al. reported on a sustained release of doxorubicin and gentamicin, respectively, through a rate-limiting barrier of PLLA shell [40, 44]. Interestingly, further suppression of initial burst release can be obtained by increasing the thickness of non-drug-loaded outer layer, which was reported by Wang [80]. The rapid linear release at the later stage was due to significant changes in the porosity and extensive degradation of the rate-limiting shell. On the other hand, 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, as reported by Tan et al. [40].

Furthermore, Shi et al. also reported that a nearly complete release of hydrophilic bovine serum albumin (BSA) and hydrophobic cyclosporin A (CyA) from POE/PLGA double-walled microspheres can be achieved [36]. The rapid and complete release of BSA was attributed to the easy diffusion of the acidic degradation products of PLGA and POE into the surrounding medium resulting from the porous BSA-loaded PLGA shell, thus preventing the formation of an acidic microenvironment which caused the destabilization of acid-labile biomacromolecules. For the release of CyA, the acidic degradation products of POE and PLGA and higher water uptake increased the water solubilization of CyA, thus accelerating the release of CyA. As such, it should be taken that the distinct

morphology and degradation behavior of double-layered microparticles can provide a better controlled drug release. In this thesis, the use of multi-phase/multi-layered microparticles with introducing additional polymers would facilitate the design of microparticles to control and alter the drug release profiles.

Rahman et al. also demonstrated fabrication of double-walled PLLA/PLGA microspheres which encapsulated BSA labeled with fluorescein isothiocyanate (FITC-BSA) by using solvent evaporation method [43]. The result showed that by varying the mass ratio of PLGA: PLLA from 1:1 to 3:1, the localization of FITC-BSA in double-walled microspheres changing from outer shell to inner core. However, the mechanism was not reported in the paper. They found that using a specific drug loading of 5 %, almost zero-order release can be achieved with a mass ratio of PLLA:PLGA 1:3.

It has been documented that several drug release studies conducted on double-layered microparticles have shown a lower initial burst release of drug distributed within the core as compared to the neat microparticles. However, these studies were mainly conducted for the double-layered microparticles with single drug loaded in the core. It is therefore of interest to understand how different drugs localized in different layers can affect the drug release. The release of drug located in the shell would affect the release kinetics of the drug in the core. In this thesis, the release kinetic of multiple drugs from multi-phase microparticles will be investigated. In addition, this thesis will study, for the first time, how double-layered ternary-phase and triple-layered microparticles can achieve

distinctive and unique drug release profiles when particles are transformed from being binary-phase to ternary-phase.

2.6 Polymeric particles of various sizes

2.6.1 Single-layered particles

Single-layered particles with different sizes had been fabricated by emulsion solvent evaporation method for the past few decades. The difference in sizes could have direct implications on the medical applications. To obtain the required sizes, many different process parameters, such as the initial polymer concentration, stirring speed, surfactant concentration, oil to water (o/w) ratio had been manipulated [84].

For instance, studies conducted had shown that an increase in the PLGA polymer concentration could lead to a larger particle size. This phenomenon was probably caused by an increase in the viscosity of the polymer solution. The high viscosity acts as a resistance against the shear forces during emulsification and increases the chance of agglomeration, forming larger particles.

Another study shows that increasing stirring speed decreases the particle size [85, 86]. This is because the shear stress increases with increasing stirring speed. The larger

tangential stress leads to a decrease in droplet size. Additionally, an increase in stirring speed also reduces the relative viscosity of the emulsion, leading to a decrease in particle size.

The amount of PVA concentration can also influence the size of particles [84]. The content of PVA surfactant was crucial in stabilizing the emulsion droplets and preventing their agglomeration. A higher amount of PVA concentration can protect smaller droplets from coalescence, thus forming smaller microparticles.

Altering the oil-to-water ratio was another way to manipulate the size of the particles. Studies had shown that an increase in the oil-to-water ratio can lead to a decrease in particle size. This can be attributed to the fact that a larger amount of organic solvent available for diffusion prevented the coalescence of droplets [84].

2.6.2 Double-layered particles

A solvent evaporation method is a one-step process that is easy and cheap to produce double-layered particles. For the past few years till now, efforts had been placed to manipulate different process parameters to obtain different double-layered particles of controllable sizes. However, the reported smallest sizes for double-layered microparticles fabricated by the solvent evaporation technique are only around 50 μm in diameter [40, 78]. At present, there has been no article reporting on the formation of double-layered

particles of sizes smaller than 50 μ m. One reason being that the smaller the particle sizes, the more challenging it is to fabricate and characterize them. Process parameters had to be further manipulated and better resolution devices are required to determine their internal morphologies.

Fabrication of double-layered particles of controlled size is more challenging than single-layered particles as the formation of double-layered particles is a dynamic process as the phase separation occurs. In the formation of single-layered particles, high-speed shearing or acoustic agitation, lowering of starting polymer concentration, and increasing PVA concentration would generate smaller emulsion droplets, and easily produced final particle sizes down to sub-micron or nano level. However, using high-speed shearing, lowering of starting polymer concentration and increasing PVA concentration on a binary polymer system to produce small emulsion droplets does not guarantee the formation of a double-layered structure. These smaller emulsion droplets generated would result in changes in the phase separation behavior due to the higher surface area-to-volume ratio [87]. Therefore, it would be of interest to study how particle size affects the internal morphology, which will be investigated in this thesis.

2.6.3 Effect of particle sizes on drug release

Polymer-based particulate drug delivery systems provide some versatility in the controlling of drug release rate. The versatility comes from the ability to manipulate polymer chemistry, such as polymer composition, monomer ratios, molecular weight and polymer type. One of the physical parameters of a particle that can deeply affect drug release is the particle size.

Two opposing drug release phenomena that can be observed are rapid drug release with smaller particle and vice versa. The first phenomenon can be attributed to higher diffusion rate of drug from the encapsulation location. Considering all other properties being constant, smaller microparticles generally release encapsulated compounds more quickly and over shorter period of time [5, 88]. The main reason for this phenomenon is the shorter diffusion length and higher concentration gradient of drug in the smaller microparticles. According to Fick's laws of diffusion, high diffusion rates are expected in smaller diffusion pathway length. Another reason that particle size can affect drug release is the surface area-to-volume ratio. The higher surface area as a result of reduced particle size allows the encapsulated drug to be released faster. Particles of appropriate size range would be used for certain applications. One such application is subcutaneous or intramuscular administration of drug delivery depots. Particles ranging from 5 μm to 150 μm are large enough to stay at the injection site while small enough to be administered through a small-gauge hypodermic needle [17]. Berkland et al. fabricated PLGA-based particles of various sizes through spray forming technique [17]. Subsequently, they were able to obtain zero-order drug release through mixing of different sizes microparticles. In

addition, Narayani et al also demonstrated that linear release profiles were possible by a means of combining different sizes of gelatin microspheres [89]. Therefore, manipulation of microparticle size would offer opportunity to control the release kinetics.

Particle size can also affect the degradation of degradable microparticles, and hence affecting the drug release rate. In large PLGA microparticles, autocatalytic effect can accelerate degradation rate, thus accelerating the drug release [90]. This was in contrary with the fact that the drug release is primarily diffusion-controlled. The longer diffusion length in large microparticles hindered the acidic degradation products to diffuse out into the bulk fluid. This accumulation of acidic degradation products results in an accelerated degradation [22, 91-94]. Therefore, unlike large microparticles, small microparticles would tend to degrade at a slower degradation rate. Klose et al. reported that the rapid degradation of larger microparticles allowed for similar drug release rates for a wide range of microparticles with various sizes [60]. The autocatalytic effect on the larger microparticles increased drug mobility, and compensated for the decrease in drug concentration gradients with increasing size of microparticles.

The research gap of the drug release from microparticles arises from the fact that there has been no paper reporting on the effect of sizes of multi-layered microparticles on degradation and drug release. So far, the studies conducted were only on single-layered particles. Hence, even after being able to fabricate multi-layered microparticles, there is an increasing field of interest to understand how their size difference affect their degradation behaviors and drug release profiles. The presence of two contradicting drug

release effects (i.e. diffusion and autocatalysis) from varying particle sizes prompts a further study of drug release mechanisms of multi-layered microparticles. Venturing into this unknown field would also help to answer many of the enquiries such as how big should the size of a multi-layered microparticle be when administering to a patient. This would then establish a solid platform for many subsequent applications to be.

2.7 Modeling of drug release from polymeric microparticles

Mathematical modeling of drug release provides insight into the underlying mass transport and chemical processes involved in the drug release mechanism. As such, the optimized design of a device for a certain drug release profile can be predicted from a systematic approach.

For the diffusion-controlled drug release from a microsphere, it is quantified by Fick's second law of diffusion (**Eq. 2.3**). D and C are the diffusivity and concentration of drug in the polymer, respectively.

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left[D r^2 \frac{\partial C}{\partial r} \right] \quad (\text{Eq. 2.3})$$

In reservoir system, it assumes that a drug reservoir is surrounded by the polymer shell with outer radius R and inner radius R_i , in which drug has to diffuse through a layer with

thickness of $R-R_i$. The drug distribution within the shell ($R_i \leq r \leq R$) was obtained by solving Fick's second law of diffusion, where a constant reservoir concentration ($C_r = C_i/K_r$) is kept at the surface $r = R_i$, where K_r is the partition coefficient of drug at the interface between the reservoir and shell matrix. On the other hand, drug concentration at the surface of the shell is assumed to be zero for no mass transfer resistance and the surrounding medium is in perfect sink condition [95]. By considering the time is long enough, the drug release rate equation can be simplified and described in **Eq. 2.4**. Here, D is the constant diffusion coefficient of the drug through the shell matrix.

$$\frac{dM}{dt} = 4\pi \frac{RR_i}{(R - R_i)} DC_i \quad (\text{Eq. 2.4})$$

One should be noted that **Eq. 2.4** is only applicable for the non-erodible reservoir-membrane drug delivery system. For the bulk-eroding systems (such as polyesters), the mobility of the polymer chains increases with decreasing molecular weight as a result of degradation, resulting in increased probabilities for the drug molecules to diffuse from one cavity to another [23, 59, 96, 97]. Therefore, the constant diffusion coefficient D_o , should be replaced with a time-dependent diffusion coefficient, D_t . According to Charlier et al., as the molecular weight follows a first-order exponential decay, D_t is expressed as $D_t = D_o \exp(kt)$, where k is the degradation rate constant and t is time [98]. On the other hand, since the drug content in system is finite, one must consider that the reservoir drug concentration becomes non-constant when all of dispersed drug is dissolved [99]. However, no studies have been conducted to address this issue for a drug-loaded sphere of reservoir-membrane system.

There are several papers reporting on the mathematical model, considering drug diffusion with non-constant diffusivities resulting from polymer degradation, for the drug release from PLGA-based microspheres [23, 59, 60, 100, 101]. The majority of the drug release models was based on the Higuchi model for non-erodible systems and then implemented the time-dependant diffusivity to take into account the matrix degradation. However, by now, a huge research gap can be seen from the fact that there has been no report conducted on the modeling of drug release from the biodegradable multi-layered microspheres. In this thesis, a detailed theoretical analysis of drug release will be conducted for the first time for a non-constant reservoir with a degradable shell matrix in the multi-layered microparticle system.

2.8 Summary

It has been reported that double-layered microparticles can be fabricated through a one-step solvent evaporation method and offer better control drug release kinetics compared to the neat particles. However, a review of journal papers has shown that there is a dearth of data on the fabrication of microparticles consisting of more than two different polymers – ternary-phase microparticles and triple-layered microparticles. There are numerous papers that discuss about the drug release properties of the double-layered microparticles, but none discussed about the drug release profiles of double-layered ternary-phase and triple-layered microparticles. The drug release study of multi-layered particles of various sizes has also yet to be done. Introducing an additional polymer into double-layered microparticles would provide a greater degree of freedom in designing

microparticles that would provide a variety of drug release profiles and kinetics. This would be an important achievement toward their development into a viable and versatile platform for drug delivery. Therefore, ternary-phase and triple-layered microparticles will be extensively discussed in this thesis, where a discussion on their formation mechanisms and in vitro drug release properties, supported by their degradation studies will be conducted in great depth and detail. A mathematical model for the drug release profiles of triple-layered microparticles will also be presented to better understand the underlying drug release mechanisms.

Chapter 3 Materials and Methods

3.1 Materials

Poly(L-lactic acid) (PLLA, intrinsic viscosity/IV: 2.38, Bio Invigor), poly(DL-lactic-co-glycolic acid, 50:50) (PLGA, IV: 1.18, Bio Invigor), PCL (molecular weight (MW): 80 kDa, Aldrich), poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA, molecular weight/MW 42 kDa, Aldrich), poly(styrene) (PS, monocarboxy terminated, MW 50kDa, Aldrich) and poly(vinyl alcohol) (PVA, MW 30-70 kDa, Sigma-Aldrich) were used without further purification. The drugs, ibuprofen (IBU) and metoclopramide monohydrochloride monohydrate (MCA), were purchased from Sigma-Aldrich. Solvents such as dichloromethane (DCM), chloroform, and tetrahydrofuran (THF) from Tedia Company Inc. were of High-Performance Liquid Chromatography (HPLC) grade. Phosphate-buffered saline (PBS) solution (pH 7.4) was purchased from OHME, Singapore. All drugs and solvents were used as received, unless otherwise noted. Laser grade Coumarin-6 and Rhodamine 6G fluorescent dyes were purchased from Aldrich and used as received.

3.2 Polymer cloud point

Before any microparticle fabrication, the cloud points of polymers, i.e. polymer solution concentration at which one polymer becomes immiscible with the other polymers, were first determined. The polymers were selected on the basis of mutual immiscibility. To determine the polymer cloud point, a homogenous polymer solution (2% (w/v)) was prepared in which polymers were dissolved in DCM. The polymer solution was then transferred to a graduated cylinder and allowed to sit undisturbed in a fume hood at room temperature. When cloudiness became apparent due to polymer phase separation, the volume of the solution was recorded [51, 74].

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 distinct 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 type of polymer in each phase, the polymer phase that was extracted was analyzed by Fourier transformed infrared spectroscopy (FTIR).

3.3 Contact angle measurement

Polymer films for each different polymer were prepared by dissolving them in DCM (10% (w/v)) and film casting it on a glass slide. After the films were casted, the solvent was allowed to evaporate and the films were dried in a vacuum oven for 3 days to ensure that the organic solvent has fully evaporated. Contact angle of each probe liquid (water, cyclohexane and glycerol) was measured against a film of each of the polymer using a FTA 200 goniometer system with First Ten Angstroms analysis software. Measurements were taken at room temperature (25 °C).

3.4 Fabrication of microparticles

Composite microparticles were prepared using an emulsion solvent evaporation method [30, 102]. All polymers were first dissolved together in DCM. After which, the resultant polymer solution was first ultra-sonicated for 1 min and was subsequently added to a PVA aqueous solution (0.5% (w/v), unless otherwise noted) and emulsified using an overhead stirrer (Calframo BDC1850-220) at room temperature (25°C). DCM and PVA were chosen as the solvent and surfactant, respectively, in this work because they are approved by the Food and Drugs Administration (FDA). Dichloromethane is less toxic than other common solvents such as chloroform. PVA is also known as non-toxic and widely used as an emulsion stabilizer in the solvent evaporation method [103]. The evaporation of DCM results in the phase separation of polymers, yielding multi-phase composite microparticles. Finally, the microparticles were centrifuged, rinsed with de-

ionized water, lyophilized, and stored in a desiccator for further characterization. Processing parameters, such as the starting polymer solution concentration, stirring speed, oil-to-water ratio, and polymer mass ratio, were altered to understand how these parameters affect the formation of internal configuration of microparticles. **Table 3.1** summarizes process parameters that were altered in the formation of ternary-phase PLLA/PLGA/PCL microparticles. A reference ternary-phase microparticle (particle R1) was first fabricated for subsequent comparison with other microparticles.

Table 3.1 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		
<i>Ternary-phase reference microparticles (Particle R1)</i>	6	300	0.02	0.15g	0.1g	0.05g
				3	: 2	: 1
<i>Effect of starting polymer solution concentration</i>	2	300	0.02	0.15g	0.1g	0.05g
	4			3	: 2	: 1
	8			3	: 2	: 1
<i>Effect of stirring speed</i>	6	150	0.02	0.15g	0.1g	0.05g
		400		3	: 2	: 1
		500		3	: 2	: 1
<i>Effect of oil-to-water ratio</i>	6	300	0.1	0.15g	0.1g	0.05g
			0.03	3	: 2	: 1
			0.0125	3	: 2	: 1
			0.0083	3	: 2	: 1
<i>Effect of polymer mass ratio</i>	6	300	0.02	0.1g	0.15g	0.05g
				2	: 3	: 1
				0.05g	0.15g	0.05g
				1	: 3	: 1
				0.15g	0.1g	0.1g
				3	: 2	: 2

For the formation of triple-layered PLGA/PLLA/EVA microparticles, a reference microparticle (particle R2) was first fabricated for comparison with sample microparticles (S) that were fabricated under different processing parameters. **Table 3.2** details the processing parameters investigated in this study.

Table 3.2 Parameters used to fabricate the PLGA/PLLA/EVA composite microparticles

Sample	Polymer solution concentration [% w/v]	Stirring speed [rpm]	Oil-to-water ratio	Polymer mass ratio		
				PLGA / PLLA / EVA		
R2	7.5	400	~0.013 (350mL PVA solution)	0.2g 4	0.1g 2	0.05g 1
S1	4	400	0.013	0.2g 4	0.1g 2	0.05g 1
S2	15	400	0.013	0.2g 4	0.1g 2	0.05g 1
S3	7.5	150	~0.013 (350mL PVA solution)	0.2g 4	0.1g 2	0.05g 1
S4	7.5	2000	~0.013 (350mL PVA solution of 2% (w/v))	0.2g 4	0.1g 2	0.05g 1
S5	7.5	400	~0.094 (50mL PVA solution)	0.2g 4	0.1g 2	0.05g 1
S6	7.5	400	0.013	0.5g 10	0.1g 2	0.05g 1
S7	7.5	400	~0.013 (350mL PVA solution)	0.1g 2	0.2g 4	0.05g 1

For the fabrication of quadruple-layered microparticles, the same method to form the particle R2 was used, but with the polymer mass ratios of PLGA:PLLA:PS:EVA 0.4g:0.2g:0.1g:0.04g or 10:5:2.5:1 and at the starting polymer solution concentration of 13% (w/v).

For the formation of double-layered microparticles of sub-micron size, **Table 3.3** summarizes the settings used. The polymer mass ratio and the initial polymer solution concentration were fixed at PLLA/PLGA 0.2g:0.1g and 6% (w/v), respectively.

Table 3.3 Parameters used to fabricate the PLLA/PLGA composite microparticles of various sizes

Sample	Stirring Speed (rpm)	PVA concentration in external aqueous phase (%w/v)	Oil-to-water ratio	Remarks
D1	350	0.5	0.02	-
D2	2000	6	0.02	-
D3	2000	6	0.1	1mL DCM added in PVA solution; Coumarin-6 & Rhodamine 6G added to polymer solution (1% w/w)

Drug-loaded double-layered ternary-phase PLLA/PLGA/PCL microparticles with a mass ratio of 3:2:1 were prepared using the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method. To achieve a more uniform distribution of hydrophilic drug (MCA), the aqueous drug solution was first prepared and emulsified with the polymer solution, rather than adding solid drug particles to the polymer solution. Briefly, the polymers (0.3 g, 6% w/v) and the hydrophobic drug (IBU) (20% w/w) were first dissolved in DCM (organic phase). Hydrophilic drug (20% w/w) was then dissolved in 0.1 mL deionized water (internal aqueous phase). Both solutions were mixed and ultra-sonicated for 30 sec using an ultrasonic processor (Sonic Vibra-Cell VC 130) to prepare the first water-in-oil emulsion. After which, other process parameters were the same as that used for particle R1.

Other ternary-phase PLLA/PLGA/PCL, binary-phase PLLA/PLGA and neat microparticles were similarly prepared whereby the drug type and polymer mass ratios were correspondingly altered. Deionized water was still used as the internal aqueous phase for the fabrication of the ternary-phase PLLA/PLGA/PCL and binary-phase PLLA/PLGA microparticles without any hydrophilic drug.

For the fabrication of ibuprofen-loaded triple-layered microparticles, ibuprofen (5 or 20% w/w) was first added to the polymer solution and the resulting solution was subsequently ultra-sonicated for 1 min to achieve complete homogenization. The method to obtain triple-layered PLGA/PLLA/EVA 4:2:1 microparticles was similarly employed to produce IBU-loaded triple-layered microparticles with different mass ratios (PLGA/PLLA/EVA

10:2:1, 10:5:1 and 2:4:1), double-layered PLGA/EVA 6:1 microparticles, double-layered PLLA/EVA 6:1 microparticles and neat microparticles. For the fabrication of MCA-loaded triple-layered microparticles, MCA (0.0175 g or 5% w/w) was first dissolved in water (0.1 mL, the internal aqueous phase). The polymer solution and the aqueous MCA solution were mixed and ultra-sonicated for 30 s to produce the first water-in-oil emulsion. This primary w/o emulsion was then poured into the PVA solution. The same parameters to obtain particle R2 were employed to produce MCA-loaded triple-layered microparticles.

Drug-loaded microparticles of different sizes were prepared in the similar manner as mentioned above, unless otherwise stated, by altering the polymer solution concentration, stirring speed, oil-to-water ratio, PVA concentration and theoretical drug loading correspondingly. To obtain the triple-layered microparticles with various size ranges, the poly-dispersed microparticles were sieved with various test sieves with pore size of 150 μ m, 106 μ m, 75 μ m, 53 μ m, 38 μ m (Cole-Parmer[®] U.S.A Standard Test Sieve) for 30 minutes.

3.5 In vitro hydrolytic degradation

Microparticles (50 mg) were weighed and placed in vials containing 30 mL of PBS (pH 7.4) (n = 3). Samples were incubated at 37 °C with moderate shaking. The pH of the solution was monitored over time to ensure that the pH was maintained at 7.4 throughout the study. Microparticles were removed from the vials at pre-designated times.

3.5.1 Water uptake

After rinsing with distilled water, the microparticles were weighed and then vacuum-dried to constant weight to determine the difference in weights. The water uptake was calculated at each time point according to the following equation (**Eq. 3.1**):

$$\text{Water Uptake} = 100\% \times \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \quad (\text{Eq. 3.1})$$

, where W_{wet} and W_{dry} are the weights of the wet and dry microparticles, respectively, measured at time t . Values obtained from three samples were averaged and reported.

3.5.2 Mass loss

Mass loss was calculated based on the difference between W_{dry} and the original weight (W_{initial}) of the sample. The values were normalized by dividing with their original weights and expressed in terms of percentages.

3.6 In vitro drug release

Drug-loaded microparticles (5mg) was accurately weighed in triplicate and placed in vials containing 5mL of PBS (pH 7.4) and incubated at 37 °C in a shaking incubator. At predetermined time intervals, 1 mL of medium from each vial was removed and analyzed using a Shimadzu UV-2501 UV-Vis spectrophotometer ($\lambda_{MCA} = 309$ nm, $\lambda_{IBU} = 220$ nm) before replacing the removed volume with fresh PBS solution. The percentage cumulative release of the drug was studied as a function of incubation time.

3.7 Characterization techniques

3.7.1 Scanning electron microscopy (SEM)

The surface and internal morphologies of the microparticles were analyzed using scanning electron microscopy (SEM) (JEOL JSM-6360A) at an operating voltage of 5 kV. Before analysis, the samples were first mounted onto metal stubs and cross-sectioned at their center with a razor blade. Samples were then coated with gold using an SPI-Module sputter coater. At least three independent batches for each particle type were fabricated, and ten microparticles from each independent production batch were randomly chosen for SEM analysis. Particle morphologies and configurations were found to be consistent within each independent batch for a particle group type. Hence, only one representative SEM micrograph is shown.

3.7.2 Particle size analysis

Particle size analysis was done using the SEMAfore or ImageJ software from the SEM images. The mean diameter of the microparticles was taken to be the number average of the diameters of the particles captured on the SEM image.

3.7.3 Dissolution method

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

3.7.4 Raman mapping

Raman mapping was utilized to verify the final particle configuration (i.e., polymer distribution) and drug distribution within the microparticles. Microparticles that had been pre-sectioned were placed under a microscope objective with a laser power of up to approximately 20 mW. Raman point-by-point mapping measurements on IBU-MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticles (as an example) were performed on an

area of 400×200 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 deep depleted thermoelectrically 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 20× 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 approximately 35 s. Spectral pre-processing, including the removal of spikes due to cosmic rays, was carried out before the Raman mapping data were further analyzed using the band target entropy minimization (BTEM) algorithm [104-107]. The BTEM algorithm was developed to reconstruct pure component spectral estimates. When all of the normalized pure component spectra of all underlying constituents had been reconstructed, the relative contributions of each measured point of these signals could be calculated by projecting them back onto the baseline-corrected and normalized data set. The color-coded scale represents the intensities of score image of each observed component, in which the summation of the intensities (color-coded scale) of all components at each particular pixel is equal to unity. These score images can be used to show the spatial distribution and the semi-quantitative content for all observed component in the microparticles.

3.7.5 Differential Scanning Calorimetry (DSC)

Thermal analysis of the microparticles was performed using a modulated differential scanning calorimetry (MDSCTM) (DSC 2920, TA Instruments, USA). To avoid oxidative

degradation, the sample and reference pans were purged with nitrogen at a constant flow rate of $48 \text{ mL}\cdot\text{min}^{-1}$. Approximately 5 mg of each sample was heated from -40°C to 200°C for the first heating ramp, cooled to -40°C , and finally reheated on the second ramp to 200°C , all at a rate of $5^\circ\text{C}\cdot\text{min}^{-1}$. The endothermic relaxation peak disappears on cooling the sample and reheating, thus the thermal properties of the degrading polymer in the equilibrium state were obtained. Thermograms were analyzed using TA universal analyzer software for the determination of glass transition temperatures (T_g) and melting temperatures (T_m).

3.7.6 Size-exclusion chromatography (SEC)

The polymer's molecular weight at each time was determined using size-exclusion chromatography (SEC) (Agilent 1100 Series LC System), which was performed at 30°C with chloroform as solvent, using a reflective index detector (RID), and the flow rate used was $1 \text{ mL}\cdot\text{min}^{-1}$. The molecular weights of the samples were obtained relative to a calibration curve constructed using polystyrene standards (165-5000 kDa). The polymers in the multi-phase and multi-layered microparticles were separated by the dissolution method, based on the solubility differences of the polymers in THF. Eight milligrams of microparticles was first immersed in 1 mL of THF to dissolve the polymers. PLLA remnants (not dissolvable in THF) and the polymer solution were later separated by centrifugation. The solvent in the polymer solution was evaporated slowly in air at room temperature for 48 h. The remaining solvent and PLLA were further dried in an oven at 40°C for one week. After which, the polymer mixture and PLLA were dissolved separately in 1 mL chloroform and analyzed using SEC.

3.7.7 Thermogravimetric analysis (TGA)

Encapsulation efficiency is defined as the ratio of actual to theoretical drug loading within the microparticles. For quantification of hydrophobic IBU, thermogravimetric analysis (TGA Q500 V6.5 Build 196) was used. Approximately 10 mg of the drug-loaded particles was placed on a platinum pan, and the sample was heated at $10\text{ }^{\circ}\text{C min}^{-1}$ from room temperature to $160\text{ }^{\circ}\text{C}$ under nitrogen at a flow rate of 60 mL min^{-1} , followed by isothermal heating at $160\text{ }^{\circ}\text{C}$ for 60 min before ramping to $500\text{ }^{\circ}\text{C}$. IBU is known to decompose at 160°C , which is lower than MCA ($\sim 280^{\circ}\text{C}$) and the polymers ($\sim 300^{\circ}\text{C}$). The mass loss percentage at 160°C determined in the TGA analysis was taken as the weight percentage (wt%) of IBU in the microparticles. All measurements were done in triplicate.

3.7.8 UV/Vis spectrophotometer

For quantification of hydrophilic MCA loading, approximately 10 mg of microparticles were first weighed and dissolved in 1 mL of DCM. MCA was then extracted using 10 mL of deionized water, into which the hydrophilic drug preferentially partitioned. The drug concentration was determined using a UV-Vis spectrophotometer (Shimadzu UV-2501) at 309 nm.

3.7.9 Fourier transformed infrared spectroscopy (FTIR)

The Perkin Elmer system 2000 FTIR with 16 scan per sample over the range of 4000-400 cm^{-1} was employed to obtain FTIR spectra. Polymer dissolved in DCM was first dripped onto potassium bromide (KBr) discs using dropper. The wetted discs were then dried in a vacuum oven for an hour before FTIR characterization.

3.7.10 Confocal laser scanning microscopy (CLSM)

Particles loaded with dyes were first placed onto a glass slide and subsequently covered with a cover slip. The attachment of cover slip was then secured with nail polish. Images were taken using a Zeiss LSM710 confocal laser scanning microscope with a Plan-Apochromat 63x/1.40 Oil objective and ZEN software (Carl Zeiss).

3.7.11 Focus ion beam (FIB)

Cross-sections of sub-micron particles were prepared and imaged using focused-ion beam (FIB) microscopy (FEI Nova Nanolab 600i). The particles were first mounted onto a lacey copper grid. A Ga^+ ion beam with 30 keV with a beam current of 26 pA was used for milling the target particles. The samples were then imaged by the secondary electrons formed during milling. Reducing the beam current to 9 pA helped to minimize specimen damage during ion beam scanning.

3.7.12 Field emission scanning electron microscope (FESEM) fitted with a transmission electron detector (TED)

The particles were first suspended in ethanol and few drops of suspension were subsequently deposited onto a continuous copper grid. After which, it was allowed to air dry. The particles were viewed under JEOL JSM-7600F FESEM fitted with a TED at an accelerating voltage of 30 kV.

3.8 Statistical analysis

Drug release, mass loss and water uptake data from different particles were evaluated by unpaired Student's t-test and the one-way ANOVA analysis followed by Tukey-test. Differences were considered statistically significant when $P \leq 0.05$.

Chapter 4 Results and Discussion

4.1 Fabrication of multi-phase and multi-layered microparticles

This section of the chapter reports on the investigation into the effects of process parameters, including initial polymer solution concentration, stirring speed, oil-to-water ratio and polymer mass ratio, on the final microparticle configurations. From these results, the formation mechanism of multi-polymer composite microparticles will be proposed. A correlation of the final microparticle configuration to process parameters is thus established. This relationship allows for a further understanding and improvement on the fabrication of multi-phase and multi-layered composite microparticles through a one-step solvent evaporation technique.

4.1.1 Double-layered ternary-phase microparticles

Though the fabrication of double-layered microparticles using a solvent evaporation method has been established, there has been no report on the fabrication of microparticles consisting of three different polymers – ternary-phase microparticles. The formation mechanism of ternary-polymer composite microparticles is proposed in this section. Through this knowledge, different configurations of ternary-phase microparticles can be fabricated, thus providing a greater degree of freedom in designing microparticles that can provide the desired drug release profile.

4.1.1.1 Particle configuration

A reference set of ternary-phase microparticles (particle R1) was first fabricated before varying process parameters as summarized in **Table 3.1**. **Figure 4.1** shows the SEM micrographs for particle R1, on the exterior (**Figure 4.1(a)**) and interior (**Figures 4.1(b-c)**). From the micrographs, the size range of the particles was approximately 300-400 μm in diameter. The microparticles were spherical with smooth exterior surfaces. Cross-sectioning of the microparticles revealed a double-layered structure (**Figure 4.1(b)**). In addition, a close-up view of the microparticle showed that the shell was less dense than the core (**Figure 4.1(c)**).

(a)

(b)

(c)

Figure 4.1 SEM micrographs of double-layered ternary-phase PLLA/PLGA/PCL microparticles (particle R1, reference particle). (a) External morphology, (b) Cross-sectional view of a microparticle, (c) Close-up view of the shell.

DSC thermogram of this composite microparticle showed two glass transition temperatures, from PLLA and PLGA, and two melting temperatures, from PLLA and PCL. This implies that the polymers were phase-separated and formed an immiscible blend, resulting in the formation of ternary-phase microparticles (**Figure 4.1**). The thermal properties of the pure polymers and particle R1 are summarized in **Table 4.1**.

Table 4.1 Thermal properties of pure polymers and composite microparticle obtained from DSC thermograms

Polymers	Glass transition temperature, T _g		Melting temperature, T _m (°C)	
	(°C)			
PLGA	43		-	
PLLA	74		180	
PCL	-		60	
PLLA/PLGA/PCL	PLLA: 63	PLGA: 45	PLLA: 176	PCL: 52

Figure 4.2 shows the SEM micrographs of cross-sectional views of particle R1 after dissolution in THF, where a highly porous shell was observed. DSC analysis of the shell showed that this polymer was PLLA ($T_g = 76\text{ }^{\circ}\text{C}$, $T_m = 178\text{ }^{\circ}\text{C}$). The microparticle configuration of particle R1 was further verified using Raman mapping. Raman mapping results (**Figure 4.3**) showed that the core-shell configuration was indeed 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 (**Figure 4.2(b)**). PLLA/PLGA microparticles without PCL were also fabricated for comparison, and the SEM micrographs showed a dense PLLA shell due to the absence of PCL (**Figures 4.4(a-b)**). Raman mapping confirmed that the dense shell was PLLA and the core was PLGA (**Figure 4.4(c)**). The existence of two glass transition temperatures (T_g of PLGA = $41\text{ }^{\circ}\text{C}$; T_g of PLLA = $67\text{ }^{\circ}\text{C}$) and one melting temperature (T_m of PLLA = $177\text{ }^{\circ}\text{C}$) again implied that PLLA and PLGA were phase-separated. In summary, the configuration of

PLLA/PLGA/PCL microparticles formed were therefore PLGA-core with a PLLA-shell impregnated with PCL particulates.

(a)

(b)

Figure 4.2 SEM micrographs of double-layered ternary-phase PLLA/PLGA/PCL microparticles (particle R1) after dissolution with THF. (a) Cross-sectional view of a whole microparticle, (b) Close-up view of porous shell.

Figure 4.3 Pure component Raman spectra estimates and their associated score images obtained via BTEM from a PLLA/PLGA/PCL microparticle (particle R1).

(a)

(b)

(c)

Figure 4.4 PLLA/PLGA double-layered microparticles. SEM micrographs of (a) cross-sectional view of a whole microparticle, (b) close-up view of the shell, (c) Pure component Raman spectra estimates and their associated score images obtained via BTEM.

For reference particle R1, the initial polymer solution concentration was 6% (w/v), which was above the cloud point of 3.75% (w/v) at which PLGA phase separates from PCL/PLLA. When the polymer solution was initially poured into the aqueous PVA solution, PLGA phase separated as small droplets containing DCM and polymer (known as the coacervate phase) within the emulsion droplets [74]. These droplets start 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 [108]. This was shown from a higher volume fraction of DCM in the PLGA phase (90.63%) as compared to the PLLA phase (82.71%). DCM-rich PLGA coacervate droplets were therefore more hydrophobic than the PLLA-DCM phase and had higher tendency 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 a poor intermixing of PCL with PLGA that had coalesced in the core and the limited mobility of PCL in a dispersing PLLA phase at high polymer solution concentration. 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 weight (high viscosity) of PLLA and PCL could also hinder the coalescence and migration of PCL. Further removal of solvent caused PLLA to precipitate and form the outer shell while entrapping the PCL particulates.

Various configurations of the composite microparticles were obtained by changing the starting polymer solution concentration. When the initial polymer solution concentration was prepared at 2% (w/v), a double-layered configuration was similarly observed but this time, the core was more porous than the shell (**Figure 4.5(a)**). The porous and less-dense core indicates that a greater amount of PCL was dispersed in the PLGA core. At starting homogeneous polymer solution of 2% (w/v), the polymers inside the emulsion droplets remained miscible until phase separation of PLGA ensued at the first cloud point at 3.75% (w/v). The high molecular weights (high viscosity) of PLLA and PCL would make the phase separation of PLGA from PCL/PLLA difficult. The surface tensions of PLGA, PLLA and PCL obtained from Owens and Wendt approach are 37.7 mJ/m², 36.1 mJ/m² and 37.5 mJ/m² respectively [109]. Therefore, the 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 reaches the second cloud point. This would result in more PCL being dispersed in the PLGA core (**Figure 4.5(a)**). On the other hand, when the starting polymer solution concentration was increased to 4% (w/v), the configuration of microparticles (**Figure 4.5(b)**) remained double-layered, similar to particle R1 (**Figure 4.1(b)**). Both of these concentrations (4 and 6% (w/v)) fall between the first and second cloud point, thus producing microparticles with the same configuration where PLGA remained in the core, while PCL was embedded in the PLLA shell. Similar results were also observed by Leach et al., where microparticles prepared at two different polymer concentrations, but below the cloud point, had the same double-walled configuration [58]. Further increasing the starting polymer solution concentration to 8% (w/v) (above the cloud point of all polymers) (**Figure 4.5(c)**), no double-layered structure was obtained. Instead a coalescence of all the polymers was observed. The high polymer concentration

(increased viscosity) decreased the ability of the polymers to move and coalesce with their respective phases; a phenomenon similarly reported by Leach et al. [58] and Lee et al. [39] .

(a)

(b)

(c)

Figure 4.5 SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared at various polymer solution concentrations. (a) 2% (w/v), (b) 4% (w/v), (c) 8% (w/v).

The effect of oil-to-water ratio on the microparticle structure was studied and the SEM micrographs are shown in **Figure 4.6**. For the lowest volume of PVA solution (oil/water ratio = 0.1), the PLGA coalescence phase was not located at the centre of the particle but was observed to migrate away from the centre (**Figure 4.6 (a)**). DSC analysis of microparticles prepared at oil/water ratio of 0.1 again clearly demonstrated only PLLA ($T_g = 74\text{ }^{\circ}\text{C}$, $T_m = 178\text{ }^{\circ}\text{C}$) present in the microparticles after dissolution. During the solvent removal process, DCM must first diffuse into the aqueous phase and then evaporate at the water/air interface [54]. The solubility of DCM in water is about 2% (v/v) [79]. In this study, 250 mL of aqueous PVA solution was therefore considered as a 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 low (50 mL). As such, the slow diffusion of DCM into the aqueous phase leads to a slower precipitation of the polymers. At a later stage of the solvent evaporation, the small amount of remaining DCM no longer made PLGA coacervate droplets more hydrophobic than PLLA. As such, PLGA expressed its increased hydrophilicity by migrating towards the external aqueous phase when the precipitation rate was slow enough (**Figure 4.6(a)**). However, **Figures 4.1(b), 4.6(c) and 4.6(d)** showed that further increasing the volume of the continuous phase did not result in any further effect on the final double-layered configuration of microparticles. Raman mapping (**Figure 4.7**) again confirmed the same configuration of microparticles prepared using 400 mL PVA solution as that of particle R1. Beyond the critical volume of 250 mL, the amount of aqueous solution did not seem to have any effect on the final configuration of particles. It is possible that when the aqueous volume was too large, DCM diffused rapidly into the water. Therefore, the solvent extraction rate was unaffected by the aqueous volume beyond a certain critical point.

Figure 4.6 SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared using various volumes of aqueous PVA solutions. (a) 50mL, (b) 150mL, (c) 400mL, (d) 600mL and (e,f) the cross-sectional views of a microparticles prepared at o/w of 0.1 and 0.03 respectively after dissolution with THF.

Figure 4.7 Pure component Raman spectra estimates and their associated score images obtained via BTEM from a PLLA/PLGA/PCL composite microparticle prepared using 400 mL PVA solution.

As a result of kinetic factors governing the solidification of polymers, different starting polymer concentrations (below, above or between the two cloud points) and oil-to-water ratios were observed to produce microparticles of different configurations from a ternary-polymer system.

4.1.1.2 Size of embedded particulates and core-shell dimensions

For the lower volume of PVA solution, the PCL particulates in the PLLA shell were observed to be larger in size (10-25 μm) (**Figures 4.6(a), (b), (e), (f)**). PCL coacervate droplets had more time to coalesce, as DCM distribution into the aqueous phase was slower for lower amount of PVA solution (higher oil-to-water ratio).

Changes in stirring speed did not affect the final configuration of the microparticles. However, at the lowest stirring speed of 150 rpm, PCL particulates in the PLLA shell were observed to be the largest in size (**Figure 4.8(a)**). This was also confirmed from the THF dissolution test, which showed large pores (20-50 μm) in the PLLA shell (**Figure 4.8(d)**). The decrease in polymer precipitation rate, at lowering stirring speeds, provided more time for PCL coacervate droplets to coalesce inside the PLLA matrix before the solidification of PLLA took place.

Figure 4.8 SEM micrographs of PLLA/PLGA/PCL composite microparticles prepared at various stirring speeds. (a) 150 rpm, (b) 400 rpm, (c) 500 rpm and (d) the cross-sectional view of a microparticle fabricated at stirring speed of 150 rpm after dissolution with THF.

Figure 4.9 shows that changing the mass ratio of the polymers in the starting solution changed the size of the embedded PCL particulates and core-shell dimensions. **Figures 4.9(e) and 4.9(f)** showed 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.

Higher amount of PCL facilitated the coalescence of the PCL coacervate droplets during the solvent evaporation process, resulting in larger PCL particulates. The PCL particulate size can therefore be manipulated by specific process parameters. Larger PCL particulates dispersed in PLLA shell was observed for the microparticles produced at a lower stirring speed, volume of PVA solution and higher amounts of PCL.

When the polymer mass ratios were varied, the SEM micrographs of double-layered ternary-phase microparticles after dissolution with THF (**Figures 4.9(b), (d) and (f)**) again showed that the shell and core composition were PLLA and PLGA, respectively, and the shell was similarly impregnated with PCL particulates. By increasing the mass of PLGA (PLLA: PLGA: PCL = 2: 3: 1), particles with larger core diameters and thinner shells were observed (**Figure 4.9(a)**). The core diameter increased dramatically when PLGA mass was further increased to PLLA: PLGA: PCL = 1: 3: 1 (**Figure 4.9(c)**). It had been shown that similar cloud points were obtained for different polymer mass ratios in the binary-polymer system [42, 43]. Therefore, in this study, the same initial polymer solution concentration was used with different polymer mass ratios in the solvent evaporation process. Lee et al. 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 accordingly by changing the mass ratio of different polymers [39].

Figure 4.9 SEM micrographs of PLLA/PLGA/PCL microparticles prepared at various mass ratios of the PLLA, PLGA and PCL (a) 2 : 3 : 1 (PLLA : PLGA :PCL) , (c) 1 : 3 : 1, (e) 3 : 2 : 2 and their corresponding cross-sectional views (b, d, f) after dissolution with THF.

Contrary to other reported work [40, 42, 58], we did not find any effect of polymer ratio that gave rise to a core-shell phase inversion. As such, it is believed that a core-shell inversion occurs only when the polymer solution is prepared above the cloud point in which 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 a polymer in forming an interface with surrounding aqueous environment or with other polymers. The higher mass polymer usually formed the outer shell that engulfed the polymer phase with lower mass. This occurred when the precipitation rate was fast enough to kinetically trap the non-equilibrium configuration in order for the phase inversion to take place, as observed from the previous studies [40, 42]. We believe that this contradiction with our study may be reconciled by the fact that the considerably low starting polymer concentration at 6% (w/v) (below the cloud point of all polymers) could provide sufficient time and mobility for the polymers to reconfigure themselves according to the thermodynamic equilibrium, which is dictated by the changes in interfacial energies as solvent is extracted [51].

4.1.1.3 Size of microparticles

Lowering the polymer solution concentration (from 6% (w/v) to 4% (w/v)) (**Figures 4.1(b) and 4.5(b)**) resulted in finer emulsion droplets, thus achieving smaller microparticle sizes, while retaining the same internal morphology [79]. In addition, an increase in stirring speed reduced the overall size of the microparticles (**Figures 4.1(b), 4.8(a-c)**). Higher stirring speed may also provide finer emulsion droplets through strong shear forces [19, 54], resulting in smaller particle sizes while retaining the double-layered structure. As such, microparticle size was found to be dependent upon starting polymer solution concentration and stirring speed.

4.1.1.4 Drug localization within the microparticles

A range of drug-loaded microparticles were fabricated to understand how drugs can be localized in different layers. **Figure 4.10(a)** shows the Raman mapping of IBU-MCA-loaded double-layered ternary-phase PLLA/PLGA/PCL (3:2:1) microparticles. PLGA and PLLA comprised the core and shell structures, respectively, with PCL uniformly dispersed as particulates within the PLLA shell. Hydrophilic MCA was encapsulated within the relatively more hydrophilic PLGA core, while hydrophobic IBU was localized in the relatively more hydrophobic shell, based on polymer-drug affinity [36, 39, 40, 110-112]. **Figure 4.10(b)** shows the Raman mapping of IBU-MCA-loaded binary-phase PLLA/PLGA microparticles. Similarly, MCA was localized in the PLGA core, while IBU was predominantly dispersed at the edge of the PLLA shell. It had been shown by

Miyajima et al. that the use of DCM did not affect the physico-chemical properties of various drugs [113].

Figure 4.10 Pure component Raman spectra estimates and their associated score images obtained via BTEM from (a) a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle, (b) a MCA-IBU-loaded PLLA/PLGA microparticle.

Figure 4.11 (Left column: binary-phase microparticles, Right column: ternary-phase microparticles; Row 1: Non-drug loaded, Row 2: MCA-loaded, Row 3: IBU-loaded, Row 4: MCA-IBU-loaded) shows SEM micrographs of microparticles prepared by the emulsion solvent evaporation method. The diameter of the IBU-MCA-loaded double-layered ternary-phase PLLA/PLGA/PCL (3:2:1) microparticles measured by SEM was $255.4 \pm 70.1 \mu\text{m}$. Comparable particle sizes were also observed for the other microparticle samples (i.e., binary-phase and neat). The non-drug loaded binary-phase and ternary-phase microparticles exhibited smooth and non-porous exterior surfaces. Cross-sectioning the microparticles revealed a porous PLGA core due to the repulsion of the internal aqueous droplets by the relatively more hydrophobic PLLA/PCL phase during the fabrication process. It was observed from the SEM micrographs that the presence of drugs does affect the morphological makeup of the microparticles. Microparticles containing MCA only were observed to have porous surfaces, possibly due to the hydration of MCA that drives the external water to diffuse into the nascent emulsion droplets by osmotic pressure. Binary-phase microparticles (with IBU) were observed to have uneven surfaces that are likely due to the drug particles that reside close to the surface [59], as was also evident from the Raman results (**Figure 4.10(b)**). Therefore, the presence of hydrophobic and rubbery PCL, as particulates, allows for better dispersion of hydrophobic IBU in the shell of the ternary-phase microparticles (**Figure 4.10(a)**), resulting in a smoother surface. The surface morphology of the microparticles is therefore dependent on a combination of effects, such as the drug and polymer types.

Figure 4.11 External and internal morphologies of the double-layered binary-phase PLLA/PLGA and ternary-phase PLLA/PLGA/PCL microparticles.

Table 4.2 shows the encapsulation efficiency of IBU and MCA in the various microparticles. A higher MCA encapsulation efficiency was achieved for double-layered microparticles relative to that of the single-layered microparticles; this difference highlights the advantages of double-layered microparticles. It is generally believed that the hydrophobic PLLA shell prevents the aqueous MCA droplets from dispersing into the external aqueous medium during fabrication [39, 40], thus entrapping more MCA within the core. There was, however, no significant difference in the drug loading efficiency between binary-phase and ternary-phase microparticles.

Table 4.2 Encapsulation efficiencies of drugs (%)

Particle configuration	Neat	Neat	Neat	Binary phase double-layered	Ternary phase double-layered		
Polymers	PLGA	PLLA	PCL	PLLA/PLGA	PLLA/PLGA/PCL		
Mass Ratios				2:1	3:2:1	6:2:1	3:2:2
MCA only	30.1 ± 2.4	-	-	52.9 ± 5.4	57.5 ± 4.1	55.4 ± 3.3	54.1 ± 4.2
IBU only	-	54.4 ± 4.7	58.4 ± 7.3	53.4 ± 3.3	54.1 ± 2.5	-	56.4 ± 3.5
MCA +	-	-	-	52.3 ± 4.2	51.7 ± 4.9	-	54.3 ± 3.8
IBU				63.2 ± 4.2	64.2 ± 3.2		65.5 ± 2.1

4.1.1.5 Summary

Double-layered ternary-phase PLLA/PLGA/PCL 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 the polymers configuring themselves more towards thermodynamic equilibrium configurations dictated by the interfacial energies of the components. The changes in polymer layer thickness and PCL particulate size can be achieved by manipulating the polymer mass ratio or by adjusting the precipitation rate through 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-layered structure. For the drug-loaded microparticles, MCA and IBU were localized within the PLGA core and the shell, respectively, based on the polymer-drug affinity. The presence of PCL within shell of the double-layered ternary-phase microparticles resulted in a good dispersal of IBU in the shell compared to the binary-phase microparticles, which may in turn provide a more controlled release.

4.1.2 Triple-layered and quadruple-layered microparticles

Ternary-phase microparticles composed of a core and a shell impregnated with the particulates of third polymer were fabricated using a one-step solvent evaporation method. Following which, it is of interest to investigate how the intrinsic properties of a multiple-polymer system and different process parameters can affect the formation of multi-layered microparticles, where each different polymer coalesces and forms a layer within a multi-layered structure. Key process parameters affecting the formation of the triple-layered and quadruple-layered, potentially multi-layered, configurations are identified and the formation mechanisms of these microparticles are subsequently proposed.

4.1.2.1 Fabrication parameters affecting the triple-layered configuration

A reference triple-layered microparticle of PLGA/PLLA/EVA (particle R2) was first fabricated under the conditions listed in **Table 3.2**. **Figures 4.12(a) and (b)** show the SEM micrographs for particle R2. The particles were spherical and exhibited a triple-layered morphology. Particle sizes measured from SEM were 275.9 ± 70.9 μm in diameter. Large particles were intentionally fabricated to facilitate Raman mapping to ascertain the effects of specific fabrication parameters on particle configuration. Layer thickness measurements of the mid-layer and outer shell were approximately 30 μm and 20 μm , respectively. Polymer layer configuration was verified using Raman mapping (**Figure 4.12(c)**), and the results showed that the outermost shell, middle layer, and core were PLGA, PLLA, and EVA abundant regions, respectively.

(a)

(b)

(c)

Figure 4.12 PLGA/PLLA/EVA triple-layered microparticles (particle R2). SEM micrographs of (a) external morphology, (b) cross-sectional view of a microparticle. (c) Pure component Raman spectra estimates and their associated score images obtained via BTEM.

(a) Polymer solution concentration

The effect of various fabrication parameters on particle configuration was subsequently investigated. One key parameter studied was initial polymer solution concentration. Preliminary studies indicated that the polymer cloud point at which the three polymers were phase-separated was observed at 5% (w/v). When the initial polymer solution concentration was prepared below the cloud point at 4% (w/v) (S1), no triple-layered configuration occurred; instead, two separate polymer cores bounded by a shell were observed (**Figure 4.13(a)**). After Tetrahydrofuran (THF) dissolution, only the PLLA shell remained (**Figure 4.13(b)**); the two soluble cores were therefore identified as PLGA and EVA. The diluted polymer solution prepared at 4% (w/v) resulted in a slower precipitation rate because the Dichloromethane (DCM)-laden system requires a longer time for the DCM to be extracted. Similar to the formation of double-layered ternary-phase microparticles, the DCM-rich PLGA coacervate droplets as a result of solvent partitioning had a tendency to migrate towards the inner core and away from the aqueous solution. The DCM-poor PLLA coacervate droplets, on the other hand, had a greater affinity for the surrounding aqueous phase, thus forming the outer shell. At the same time, the amphiphilic EVA polymer prefers to achieve a partially engulfed configuration (forming an interface with PLLA, PLGA, and the aqueous phase) to minimize the interfacial free energy and attain the optimum thermodynamic condition [114]. This leaves EVA to be kinetically entrapped as a transient intermediate within the microparticles S1. Thus, at a polymer solution concentration of 4% (w/v), multiple cores with no triple-layered configuration were observed. At a higher polymer concentration of 15% (w/v) (S2), the viscosity of the polymer solution increased. This decreased the ability

of the polymers to move and coalesce with their respective phases, thus resulting in a microparticle structure with no distinctive layers (**Figure 4.13(c)**), which concurred with the result observed from the PLLA/PLGA/PCL system [108]. To achieve distinctive triple-layered configuration (R2), it is therefore critical to use a ternary-polymer system with low viscosity and low cloud point at which all three polymers are immiscible. This would facilitate the coalescence of the respective polymer phases in forming a triple-layered microparticle configuration. On the other hand, higher cloud point of all polymers and the presence of more viscous PCL with high molecular weight would account for the non-triple-layered structure obtained for the previously discussed PLLA/PLGA/PCL polymer system.

(a)

(b)

(c)

Figure 4.13 SEM micrographs of cross-sectional view of PLGA/PLLA/EVA composite microparticles. (a) Particle S1, (b) particle S1 after dissolution with THF, (c) particle S2.

(b) Stirring speed

By reducing the stirring speed to 150 rpm (S3), two cores encapsulated within a shell were observed (**Figure 4.14(a)**). THF dissolution tests once again showed that the shell was PLLA, and the cores were PLGA and EVA (**Figure 4.14(b)**). Lowering the stirring speed to 150 rpm (S3) had a similar effect as lowering the polymer solution concentration as both would result in a decreased polymer precipitation rate [86], giving similar particle configurations as observed for S1 and S3. On the other hand, increasing stirring speed to 2000 rpm (S4) reduced the overall size of the triple-layered microparticles to $25.5 \pm 7.4 \mu\text{m}$, with a triple-layered microparticle of size approximately $15 \mu\text{m}$ shown in **Figure 4.14(c)**. Raman mapping results (**Figure 4.14(d)**) for S4 showed that the outermost shell, middle layer, and core were PLGA, PLLA, and EVA abundant regions, respectively. It had been reported that increasing stirring speed did not affect the final configuration of microparticles [39, 40, 108]. Therefore, it is believed that although increasing the stirring speed reduced the size of the nascent emulsion droplets which might lead to a slightly faster polymer precipitation rate, it did not have any significant effect on the triple-layered configuration of the microparticles [87]. Here, triple-layered microparticles of sizes ranging from 10 to $400 \mu\text{m}$ could therefore be achieved by changing stirring speeds.

Figure 4.14 SEM micrographs of cross-sectional view of PLGA/PLLA/EVA microparticles. (a) Particle S3, (b) particle S3 after dissolution with THF, (c) particle S4, (d) Pure component Raman spectra estimates and their associated score images obtained via BTEM.

(c) Oil-to-water ratio

The effect of oil-to-water ratio on microparticle morphology was also studied. When the oil-to-water ratio was increased, by reducing the volume of the aqueous poly(vinyl alcohol) (PVA) solution to 50 mL (S5), the EVA core was found to migrate to the outer shell and was partially engulfed in the microparticle, while PLGA and PLLA remained as the core and shell respectively (**Figures 4.15(a)** and **(b)**). This can be attributed to the slow diffusion of DCM into the aqueous phase, leading to a slower precipitation of the polymers, which allowed EVA to be partially engulfed (forming an interface with PLLA, PLGA, and the aqueous phase) in a thermodynamically stable configuration. The polymer precipitation rate therefore needs to be carefully adjusted for the formation of a triple-layered configuration. It has to be slow enough to allow sufficient time for the polymers to move into and coalesce with their respective phases, while at the same time, it has to be fast enough to reduce the time for the polymers to reconfigure themselves according to thermodynamic equilibrium, which is dictated by the changes in interfacial energies as the solvent is extracted [110].

Figure 4.15 SEM micrographs of PLGA/PLLA/EVA composite microparticles (particle S5). (a) Cross-sectional view of a microparticle, (b) A microparticle after dissolution with THF.

A triple-layered morphology (R2) was formed only when the polymer solution was prepared above the cloud point at which a tri-phasal polymer solution was first created and a well-controlled polymer precipitation rate was subsequently achieved. When this tri-phasal polymer solution was initially poured into the aqueous PVA solution, the three polymers immediately phase separated as small droplets containing DCM and polymer (the coacervate phase) within the emulsion droplets [74]. The time available for solvent partitioning between each polymer coacervate phase was reduced at a considerably higher precipitation rate [115, 116]. As a result, the surface tension of each of the polymer-DCM phases was about the same due to similar DCM content, with no preferential distribution of a particular polymer forming an interface with surrounding aqueous environment or with other polymers. Eventually, the final polymer layer configuration was therefore determined from the polymer mass ratios, where a higher mass (or volume) polymer subsequently formed the outer shell and engulfed the polymer phase with lower mass (or volume) [40].

4.1.2.2 Fabrication parameters affecting layer thickness and layer inversion

When the polymer mass ratio (PLGA: PLLA: EVA) was modified from 4: 2: 1 (R2) to 10: 2: 1 (S6), the PLGA shell thickness increased, resulting from a higher PLGA content (**Figure 4.16(a) and (c)**). The ratio of the outer diameter to the second inner diameter increased from ~1.23 (R2) to ~1.54 (S6). Similar effect was also observed for the double-layered ternary-phase microparticles.

On the other hand, when changing the mass ratio to 2: 4: 1 (S7) (**Figure 4.16(b)**), a change in the polymer layer configuration, or layer-inversion, was observed. Raman mapping results (**Figure 4.16(d)**) for S7 showed that the outermost shell, middle layer, and core were now PLLA, PLGA, and EVA abundant regions, respectively. This concurred with the report by Tan et al. who observed that the polymer with higher mass usually formed the outer shell, which engulfed the polymer phase with lower mass [40]. It is to be noted that the final polymer layer configuration was determined by the polymer mass ratios only when the precipitation rate was high enough to kinetically trap the non-equilibrium configuration. It is shown that, for the formation of double-layered ternary phase microparticles, the considerably lower starting polymer concentration of 6% (w/v) and higher oil-to-water ratio would have provided sufficient time for the polymers to reconfigure themselves accordingly to thermodynamic equilibrium, as determined by the changes in interfacial energies of the components during solvent extraction. Therefore, for this set of parameters, the thermodynamic factor prevailed over kinetics and no layer inversion was observed. On the other hand, for the formation of triple-layered microparticles, a considerably higher precipitation rate reduced the time for solvent partitioning between each polymer coacervate phase, thus allowing kinetic factors to dominate over the thermodynamic factor. As such, this caused higher mass polymer to engulf lower mass polymer, according to the polymer mass ratios.

(a)

(b)

(c)

(d)

Figure 4.16 SEM micrographs of cross-sectioned PLGA/PLLA/EVA triple-layered microparticles and their corresponding pure component Raman spectra estimates and their associated score images obtained via BTEM. (a, c) Particle S6, (b, d) particle S7.

4.1.2.3 Drug localization in triple-layered microparticles

Drugs were subsequently added to the polymer solution during fabrication. For comparison purposes, fabrication parameters were kept the same as that of particle R2 (**Table 3.2**). Drug localization in the microparticles was verified using Raman mapping. Ibuprofen was found to localize in the EVA core. The pure component Raman spectrum of Ibuprofen (**Figure 4.17(a)**) could not be separated from the EVA via BTEM analysis because Ibuprofen homogeneously dispersed within the EVA matrix and, as a result, there is no spatial variation between the two components. During the fabrication process, strong affinity between hydrophobic long ethylene chains of EVA and hydrophobic Ibuprofen drove the drug to be localized in the EVA core, and resulted in high encapsulation efficiency (88.0%) of Ibuprofen. Comparatively, the Raman mapping result (**Figure 4.17(b)**) for the ibuprofen-loaded triple-layered PLGA/PLLA/EVA 4:2:1 microparticles at higher actual loading of approximately 15% w/w showed that the pure-component Raman spectrum of ibuprofen crystal could be separated from that of EVA via BTEM analysis because the concentration of ibuprofen in EVA matrix was above its solubility limit. When the polymer mass ratio of PLGA/PLLA/EVA was changed from 4:2:1 to 2:4:1, a layer inversion between PLGA and PLLA was again observed (**Figure 4.17(c)**). PLLA and PLGA formed the outermost shell and the middle layer, respectively, where ibuprofen was still localized in the EVA core. Therefore, it is shown that the layer morphology can be manipulated by altering the respective polymer content, while the layer localization of drug remains unchanged.

On the other hand, BTEM analysis was successful in separating the Raman signal of

Metoclopramide HCl from the polymeric matrix. As shown in **Figure 4.17(d)**, Metoclopramide HCl was predominantly well-dispersed in the PLGA matrix. The Metoclopramide HCl aqueous droplets had a stronger affinity with a relatively more hydrophilic PLGA. It is clear that some peaks of Metoclopramide HCl in the estimates are shifted and the bands are broader. The BTEM spectral estimate of Metoclopramide HCl having broader bands is assigned as the amorphous form. However, the encapsulation efficiency of Metoclopramide HCl was lower (61.4%) than Ibuprofen. This was due to the fact that during the fabrication process, some Metoclopramide HCl diffused into the external aqueous phase from the PLGA shell as a result of its high solubility in aqueous medium and shorter diffusion distance. Similar drug localization results were observed by Shi et al., where hydrophilic bovine serum albumin and hydrophobic Cyclosporin A were loaded in the PLGA shell and poly(orthoester) (POE) core, respectively, due to the difference in their hydrophilicities [36]. Drug localization in a specific polymer is therefore driven by drug-polymer affinity. This drug-polymer affinity allows for different drugs, based on their hydrophilic-hydrophobic nature, to be localized in different polymers, thus resulting in specific layer localization of drugs.

(a)

(b)

(c)

(d)

Figure 4.17 The pure component Raman spectra estimates and their associated score images obtained via BTEM. (a,b,c) IBU-loaded, (d) MCA-loaded.

4.1.2.4 Fabrication of quadruple-layered microparticles

In order to illustrate the potential of the solvent evaporation technique for the fabrication of multi-layered microparticles (more than two layers), quadruple-layered microparticles were fabricated using the same approach as that of triple-layered microparticles. **Figures 4.18(a) and (b)** show the quadruple-layered PLGA/PLLA/PS/EVA (shell to core) microparticles. The process of the internal phase separation of polymers in the emulsion droplet is summarized in **Figure 4.18(c)**. Similar to the triple-layered microparticles, the starting polymer solution concentration of 13% (w/v) was prepared above the cloud point (12% w/v) of all polymers. The oil-to-water ratio which is lower than 0.02 had to be used based on the solubility of DCM in water, allowing for DCM to diffuse rapidly into the water. The increase in polymer precipitation rate was achieved by increasing the stirring speed. The quadruple-layered structure was therefore kinetically trapped as a transient intermediate at a considerably high precipitation rate, where a higher mass polymer formed the outer layer, engulfing the polymer phase with lower mass.

(a)

(b)

(c)

Figure 4.18 (a) SEM image of a cross-sectioned quadruple-layered microparticle, (b) its corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM, and (c) Scheme of the mechanism involved in the formation of the multi-layered microparticles.

It was found in this study that by manipulating specific process parameters, formation of double-layered to quadruple-layered, and potentially multi-layered, microparticles was achievable, based on the inherent phase separation of immiscible polymers in the emulsion droplets during solvent extraction and evaporation. Nevertheless, spreading coefficient, which can be theoretically predicted from the interfacial tensions of the polymers (in the molten state and at the starting polymer concentrations) and its outer aqueous phase, is able to indirectly estimate the configuration (i.e. complete engulfing, partial engulfing or no engulfing) of the two immiscible polymer phases in the emulsion droplet [30]. However, the spreading coefficient changes continually during solvent removal since the change in polymer solution concentration will alter the effective interfacial energies [51]. In addition, the process of forming the particle's internal structure is dynamic, as the mobility of the polymer phases in the system decreases steadily during solvent extraction. When the solvent content decreases to a certain level, the polymer phases within the emulsion droplets cannot move at all to reach the thermodynamic equilibrium configuration dictated by the spreading coefficient. Furthermore, partial encapsulation at which one polymer does not completely engulf the other polymer is often observed if the polymer precipitation rate is slow enough to facilitate the formation of thermodynamic equilibrium configuration [51, 110]. Therefore, it is preferred to adjust the solvent extraction rates (or polymer precipitation rate) by optimizing process parameters so as to kinetically trap the multi-layered structure in a non-equilibrium configuration. The polymers in the multi-layered microparticles are thus selected on the basis of mutual immiscibility only; on the other hand, the surface tension of each of the polymer is not a key criterion as a result of kinetics factors governing the phase separation and solidification of polymers.

4.1.2.5 Summary

Triple-layered and quadruple-layered microparticles comprising immiscible polymers were fabricated using a one-step solvent evaporation method. These multi-layered configurations formed only when the polymer solution was prepared above the cloud point. Careful adjustments of the polymer precipitation rate also facilitated the formation of multi-layered microparticles since fast precipitation rate decreased the ability of the polymers to move and coalesce with their respective phases; slow precipitation rate facilitated configurations towards more thermodynamic equilibrium ones. Manipulation of layer thickness and configuration could also be achieved by altering the polymer mass ratios. Drug localization in specific polymeric layers was observed to be dependent on the affinity of the drug to the polymer. This one-step solvent evaporation technique is therefore a simple, economical, reliable, and versatile method to fabricate drug-loaded multi-layered microparticles where the layer thickness and configuration can be designed as desired.

4.1.3 Double-layered sub-micron particles

The particles investigated in the previous sections were about 300 μm in diameter for the ease of imaging. However, it is certainly of interest to investigate whether the same phase segregation behavior is seen with 100x smaller particles. The solvent extraction rate would probably change due to a difference in surface area-to-volume ratio.

A reference set (D1) of large-sized double-layered microparticles was initially fabricated before proceeding in the variation of process parameters as summarized in **Table 3.3**. The SEM micrographs of the reference double-layered microparticles are shown in **Figure 4.4**. The microparticle size was $276 \pm 58.5 \mu\text{m}$.

As shown in **Figure 4.19**, the SEM images of particle D2 show the same spherical and smooth exterior surfaces but with a smaller particle size of $18.3 \pm 4.8 \mu\text{m}$ as compared to particle D1. The stirring speed and PVA concentration were increased to reduce the particle sizes. The increase in stirring speed would cause the reduction in relative viscosity due to the shear thinning effect, and breaks emulsion into smaller droplets, which explains the drastic decrease in particle size. An increase in the PVA concentration would help to stabilize smaller emulsion droplets, and would also increase the viscosity of the continuous phase, which amplifies the shear effect on the droplets upon stirring, thus leading to decreased particle sizes [19]. However, the cross-sectional view of particle D2 revealed no distinctive double-layered structure (**Figure 4.19(b)**), when the particle size was greatly reduced. This observation reinforces the fact that smaller nascent emulsion

droplet size resulted in a rapid diffusion of DCM into the aqueous phase due to higher surface area-to-volume ratio and shorter diffusion distance, leading to a faster precipitation of the polymers, as reported by Katou et al. [87]. For particle D2, it is to be noted that when the nascent emulsion droplets were significantly reduced to a certain size ($\sim 20\ \mu\text{m}$ in the investigated study), a much faster polymer precipitation rate was achieved. At this very high polymer precipitation rate, the polymers inside the emulsion droplets did not have enough time and mobility to migrate to their respective phases in order to form the core-shell structure. The existence of two glass transition temperatures (T_g of PLGA = $42\ ^\circ\text{C}$; T_g of PLLA = $66\ ^\circ\text{C}$) and one melting temperature (T_m of PLLA = $175\ ^\circ\text{C}$) again implied that PLLA and PLGA were phase-separated, since the starting polymer solution concentration was prepared above the cloud point of 4% (w/v).

Figure 4.19 SEM micrographs of PLLA/PLGA microparticles (D2). (a) Surface view of microparticles. (b) Cross-sectional view.

The formation of sub-micron particles ($2.3 \pm 1.3 \mu\text{m}$) (**Figure 4.20(a)**) brings the project to another dimension due to the fact that viewing of the cross-sectional morphology of sub-micron particles becomes difficult. The usual method of cross-sectioning the particles with a razor blade to be viewed under SEM could not be utilized for the sub-micron particles. To overcome this limitation, the use of confocal laser scanning microscopy (CLSM) was proposed. Particles (D3) loaded with rhodamine 6G and coumarin-6 dyes were fabricated. **Figure 4.20(b)** clearly shows the CLSM image of particle D3, with the green ring (coumarin-6) and the red core (rhodamine 6G), suggesting the core-shell structure. When phase separation occurred, coumarin-6 preferentially partitioned to the PLLA shell due to its relatively higher hydrophobicity, whereas the more hydrophilic rhodamine 6G partitioned into the PLGA core. The CLSM image thus represents the PLGA(core)-PLLA(shell) structure of the microparticle. Another characterization method, focused ion beam (FIB), was utilized to further ascertain the internal morphology of particles D3. FIB image (**Figure 4.20(c)**) again shows the double-layered structure. Furthermore, a structure with bright ring and dark core was observed under FESEM fitted with a transmission electron detector (TED) under dark field mode. This FESEM (with TED) image (**Figure 4.20(d)**) reveals the hollow core structure within the particles as a result of the complete erosion of PLGA core after 90 days in vitro, leaving behind the PLLA shell. As such, the CLSM, FIB and FESEM images do affirm the formation of double-layered sub-micron particles.

Figure 4.20 (a) SEM, (b) CLSM, (c) FIB and (d) FESEM (fitted with TED) images of double-layered PLLA/PLGA microparticle (D3).

An extended solvent extraction time to form the double-layered particles of sub-micron size was achieved by increasing the oil-to-water ratio and saturating the external aqueous phase with DCM. A decrease in the diffusion rate of the DCM from the emulsion droplets

to the external aqueous phase provided more time for the coacervate droplets to phase separate and coalesce with their respective phases. On top of that, the average particle size was decreased even further to the average size of 2.3 μm . This reduction in size was due to the decrease in precipitation rate when the volume of external aqueous phase was decreased. During the emulsification process, the dispersion volume of the emulsion droplets gradually decreases. This causes an increase in the viscosity of the dispersed droplets, affecting the droplet size equilibrium [84]. The slow precipitation rate used in the study, and thus the corresponding longer time for DCM to be extracted would reduce the probability of nascent emulsion droplets to coalescence and agglomerate during the early step of the emulsification, resulting in the decreased particle size.

The study of the formation of double-layered sub-micron particles is one of the novel contributions to scientific knowledge in this thesis. This study is of interest not only because of the dearth of journal papers on the double-layered sub-micron particles, but it will also allow better understanding of how polymer precipitation rate can be carefully adjusted to control the internal configuration of the particles.

4.2 Drug release from multi-phase and multi-layered microparticles

With regards to the published literatures dealing with the drug release kinetics of single-layered and double-layered microparticles, there is no any paper that compares the double-layered ternary-phase and triple-layered microparticles with those microparticles, which makes this study a novel contribution in this thesis. This chapter reports on the investigation into the influence of an additional polymer to form a ternary-phase structure on the drug release properties of microparticles, and establishes a relationship between different particle parameters and the drug release kinetics. Such particulate systems would thus demonstrate the potential versatility and unique drug delivery properties of multi-phase microparticles, through the manipulation of particle parameters, including layer thicknesses, structural configurations and sizes.

4.2.1 Double-layered ternary-phase microparticles

Double-layered ternary-phase microparticles composed of a poly(D,L-lactide-co-glycolide) (50:50) (PLGA) core and a poly(L-lactide) (PLLA) shell impregnated with poly(caprolactone) (PCL) particulates were loaded with ibuprofen (IBU) and metoclopramide HCl (MCA) through a one-step fabrication process. MCA and IBU were localized in the PLGA core and in the shell, respectively. The aim of this study was to study the drug release profiles of these double-layered ternary-phase microparticles in comparison to binary-phase PLLA(shell)/PLGA(core) microparticles and neat

microparticles. The external and internal morphologies of these microparticles were discussed in **Section 4.1.1.4**.

4.2.1.1 In vitro drug release

(a) Microparticles with MCA only

Figure 4.21(a) shows the release profiles of MCA from MCA-loaded microparticles. As expected, single-layered PLGA microparticles exhibited a fast release of MCA. The presence of a shell in the binary- and ternary-phase microparticles, however, retarded the rapid release of MCA from the PLGA core, with the ternary-phase microparticles having intermediate release kinetics. The PLLA shell, therefore, acted as a rate-limiting barrier that impedes rapid drug diffusion from the core. For ternary-phase microparticles, the PCL particulates with a very low glass transition temperature (about -60°C) resulted in a less dense and more rubbery shell [117]. Hence, at drug releasing condition of 37°C and surrounded by PBS medium, PCL chains are in a highly mobile and flexible rubbery state with sufficient free volume [96], which increases probabilities for the drug molecules to diffuse from one cavity into another through the shell; thus, a relatively faster release was observed for ternary-phase microparticles compared to binary-phase microparticles. Similar release results for binary-phase PLLA/PLGA particles have also been reported by others [39, 40, 80, 81].

(a)

(b)

Figure 4.21 Release profiles of MCA from (a) PLGA, PLLA/PLGA, and PLLA/PLGA/PCL microparticles, (b) ternary-phase PLLA/PLGA/PCL microparticles in the mass ratio of 3:2:1, 3:2:2 and 6:2:1.

Changing the particle parameters also had an effect on drug release kinetics. Based on **Figure 4.21 (b)**, it is clear that the dense and thicker shell of ternary-phase microparticles

(6:2:1) further suppressed the initial rapid release. When the PCL content was further increased (3:2:2) to form a more rubbery shell, rapid zero-order release kinetics were observed. Altering the properties of the shell by changing the polymer mass ratios can, therefore, result in different release kinetics and provide time-delayed drug release kinetics.

(b) Microparticles with IBU only

Figure 4.22(a) plots the release profiles of IBU from binary-phase and neat microparticles. It was observed that IBU release from the neat PCL microparticles proceeded relatively quickly in comparison to neat PLLA microparticles due to a highly flexible rubbery state of PCL chains. On the other hand, it was easier for IBU to be released into the PBS medium from the PLLA shell than neat PLLA microparticles because the diffusion distance for IBU was shorter for the former, resulting in a faster release from binary-phase microparticles. In the case of IBU-loaded multi-phase microparticles (**Figure 4.22(b)**), binary-phase microparticles exhibited a very high initial burst release at day 1; this burst was somewhat suppressed in the ternary-phase microparticles. This observation can be attributed to the morphology of the shell. Binary-phase microparticles were observed to have surface irregularities (**Figure 4.11**) due to drugs that reside close to the surface (**Figure 4.10(b)**), resulting in easy water ingress. This ingress of water accelerated the release of these surface drug particles and thus caused a huge burst release. Ternary-phase microparticles, on the other hand, exhibited a slightly smaller burst, as the hydrophobic IBU was well dispersed within the PLLA-PCL

shell (**Figure 4.10(a)**). Increasing the PCL content (3:2:2) further reduced the burst release of IBU due to the favorable dispersion of hydrophobic IBU in the PLLA-PCL shell.

(a)

(b)

Figure 4.22 Release profiles of IBU from (a) neat PLLA, neat PCL and PLLA/PLGA microparticles, (b) PLLA/PLGA, PLLA/PLGA/PCL 3:2:1 and PLLA/PLGA/PCL 3:2:2 microparticles.

(c) Microparticles with both MCA and IBU

Drug release profiles of microparticles with more than one drug were also compared. The cumulative release of IBU from MCA-IBU-loaded microparticles is plotted in **Figure 4.23(a)**. The release profile of IBU from dual-drug-loaded microparticles was similar to that of single-drug-loaded microparticles (IBU only). Binary-phase microparticles again showed a higher burst release of IBU when compared to ternary-phase microparticles. Similarly, a higher PCL content (3:2:2) reduced the initial release of IBU.

Binary-phase microparticles exhibited the fastest release of MCA (**Figure 4.23(b)**); ternary-phase microparticles significantly retarded the rapid release of MCA. However, the release of MCA was faster from ternary-phase 3:2:2 microparticles. The more rubbery shell matrix accelerated the diffusion of MCA.

(a)

(b)

Figure 4.23 Release profiles of (a) IBU and (b) MCA from MCA-IBU-loaded PLLA/PLGA, PLLA/PLGA/PCL3:2:1, and PLLA/PLGA/PCL 3:2:2 microparticles.

(d) Single-drug-loading vs. dual-drug-loading

It is of interest to understand how multiple drug loading can affect the release of a drug from these microparticles. Interestingly, from the release profiles of IBU and MCA (**Figures 4.24(b) and (c)**), it was evident that the drug release kinetics for both drugs (IBU or MCA only) were not affected by the presence of other drugs in ternary-phase microparticles. In addition, single- and dual-drug-loaded binary-phase microparticles have showed similar drug delivery profiles for IBU (**Figure 4.24(a)**). The only exception was for MCA release from binary-phase microparticles. As shown in **Figure 4.24(d)**, a lag in the release of MCA was observed for single-MCA-loaded binary-phase microparticles, while MCA-IBU-loaded binary-phase microparticles showed rapid release. This result was likely due to the huge (60%) initial burst release of IBU from MCA-IBU-loaded binary-phase microparticles that subsequently resulted in the rapid release of MCA. The initial suppression of IBU release from ternary-phase microparticles therefore prevented the rapid release of MCA that was observed in binary-phase microparticles, as the rapid release of IBU from the shell would have resulted in a more porous shell. Therefore, it was observed that MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 particles were able to release multiple drugs in a sequential manner, with the release of IBU within the first 10 days, followed by the release of MCA after 10 days.

(a)

(b)

(c)

(d)

Figure 4.24 Release profiles of IBU from (a, b) IBU-loaded and MCA-IBU-loaded microparticles. Release profiles of MCA from (c, d) MCA-loaded and MCA-IBU-loaded microparticles.

4.2.1.2 Hydrolytic degradation of microparticles

(a) Microparticles with MCA only

The release profile and kinetics of MCA-loaded microparticles can be explained by the hydrolytic degradation of these microparticles. As reported, ternary-phase microparticles were observed to have intermediate release kinetics, between those of neat (fastest) and binary-phase (slowest) microparticles. This result is in agreement with the faster degradation of the PLLA shell of ternary-phase microparticles and correspondingly higher water uptake (**Figure 4.25(a) and (b)**). The degradation of PLLA is much slower than PLGA 50:50. PLLA was thus considered as a rate-limiting phase for drug release from the core due to its high glass transition temperature, increased polymer crystallinity and hydrophobicity [118]. As such, only changes in molecular weight of PLLA over the incubation time were shown. Ternary-phase microparticle shells were less dense due to the PCL particulates, whereas the PLLA shells of binary-phase microparticles were denser. SEM micrographs (**Figures 4.26(a) and (c)**) show that the shells were relatively dense at day 4 and retarded the release of MCA. Subsequently, after 10 days, the increased formation of pores and the enhanced degradation of the shell, along with the extensive erosion of the PLGA core (**Figures 4.26(b) and (d)**), allowed for faster diffusion of MCA from the core. The degradation of PLGA is clearly evident from the Raman mapping, which shows the presence of poly(glycolic acid) (PGA), a product of PLGA hydrolysis, in the core after 4 days (**Figure 4.27(a)**); at this time, MCA was still present within the PLGA core. After 14 days of immersion (**Figure 4.27(b)**), the PLGA core had substantially degraded to form PGA, while some MCA was observed to have

diffused into the PLLA/PCL shell. Faster MCA release can therefore be attributed to polymer degradation, which is enhanced by the higher water uptake of the ternary-phase microparticles, arising from their porous PLLA/PCL shell. Raman mapping confirmed the absence of MCA after 30 days (**Figure 4.27(c)**).

(a)

(b)

Figure 4.25 Changes in (a) molecular weight of PLLA and (b) water uptake as a function of incubation time.

(a)

(b)

(c)

(d)

Figure 4.26 SEM images of the degrading MCA-loaded PLLA/PLGA microparticles after (a) 4 days (b) 14 days in vitro. SEM images of the degrading MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticles after (c) 4 days (d) 14 days in vitro.

(a)

(b)

(c)

Figure 4.27 Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticle (a) after 4 days, (b) after 14 days, and (c) after 30 days in vitro.

(b) Microparticles with IBU only

Unlike MCA-loaded microparticles, there was no distinctive difference in the SEC results for IBU-loaded microparticles (**Figure 4.28**). The results show a similar decreasing rate for the molecular weights of PLLA in binary- and ternary-phase microparticles. Therefore, there was no significant difference in the rate of hydrolytic degradation of PLLA for both ternary-phase and binary-phase microparticles.

Figure 4.28 Changes in molecular weight of PLLA as a function of incubation time.

(c) Microparticles with both MCA and IBU

The release kinetics of microparticles loaded with MCA and IBU can be explained using SEM micrographs, SEC results and Raman mapping results. The fast release of IBU from binary-phase microparticles resulted in larger pores in the shell and on the surface to allow for the faster release of MCA, as shown in **Figures 4.29(a) and (b)**. On the other hand, the shell matrix of ternary-phase microparticles after 4 days of immersion remained relatively unchanged (**Figures 4.29(c) and (d)**). The water uptake and the changes in the molecular weight of PLLA in binary-phase microparticles (**Figures 4.30(a) and (b)**) were also found to be more significant than those of ternary-phase microparticles, thus explaining the faster MCA release from binary-phase microparticles. This result could be due to the fact that the microparticles loaded with MCA had a higher osmotic driving force for water ingress through the porous shell of the binary-phase microparticles [119], thus accelerating hydrolytic degradation.

Figure 4.29 Internal and external morphologies of (a,b) the degrading MCA-IBU-loaded PLLA/PLGA microparticles and (c,d) the degrading MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles after 4 days in vitro.

(a)

(b)

Figure 4.30 Changes in (a) molecular weight of PLLA and (b) water uptake as a function of incubation time.

Raman mapping was again utilized to study the drug release mechanism of MCA-IBU-loaded ternary-phase PLLA/PLGA/PCL 3:2:1 microparticles. Initially, MCA was dispersed in the PLGA core, with IBU localized in the shell (**Figure 4.10(a)**). After 4 days of release (**Figure 4.31(a)**), the presence of PGA in the microparticles was observed. Some of the PLGA had degraded to form PGA. At this time, most of the MCA was still dispersed in the PLGA core, affirming the slow release of MCA. It was also observed that some IBU had diffused into the PLGA core during this time. After 11 days of release (**Figure 4.31(b)**), some MCA has dispersed into the PLLA/PCL shell and was subsequently released into the medium, resulting in an increased release rate (**Figure 4.23(b)**). During this time, no Raman signals of IBU were detected, which could be attributed to the very low IBU concentration in the particles, with about 80 % IBU having been released (**Figure 4.23(a)**).

(a)

(b)

Figure 4.31 Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle (a) after 4 days, and (b) after 11 days in vitro.

4.2.1.3 Summary

The drug release kinetics and profiles of binary- and ternary-phase microparticles are clearly distinct. The presence of PCL particulates within the PLLA shell (ternary-phase) played an important role in determining how hydrophilic drugs (present in the core) and hydrophobic drugs (present in the shell) were released. SEM, SEC and Raman mapping results show that two key factors are involved in differentiating the release kinetics of binary- and ternary-phase microparticles: morphological differences and the rate of hydrolytic degradation of the microparticles.

The release of hydrophobic IBU was largely determined by microparticle morphology, whereby the morphological differences between the binary- and ternary-phase microparticles accounted for the difference in the release kinetics of IBU. The hydrophobic IBU, which was loaded within the shell, was better dispersed in the ternary-phase microparticles than in the binary-phase microparticles. IBU was poorly dispersed in the PLLA shell, resulting in an uneven particle surface that contributed to the rapid release of IBU from binary-phase microparticles. The increase in the PCL content to PLGA/PLLA/PCL 3:2:2 showed a further retardation of the release of IBU from ternary-phase microparticles, confirming that shell properties were crucial in determining the release kinetics of hydrophobic drugs (in the shell). The single- (IBU) and dual- (IBU and MCA) drug-loaded microparticles showed similar drug delivery profiles of IBU, implying that the release of IBU from the shell was not affected by the presence of MCA in the core.

The release of MCA from single MCA-loaded microparticles was also influenced by the presence of PCL. Higher shell porosity, along with the embedding of rubbery PCL particulates in the PLLA shell, enhanced the degradation rate of the shell of the ternary-phase microparticles. Water uptake also increased, resulting in faster MCA release than that from binary-phase microparticles. This faster release resulted in an intermediate release rate of MCA from ternary-phase microparticles, between that of neat (too rapid) and binary-phase microparticles (longer lag phase).

The release of hydrophilic MCA from dual-drug-loaded microparticles (containing both MCA and IBU) was determined by two factors: first, the initial release kinetics of IBU and second, the degradation rate of PLLA. Rapid IBU release from binary-phase microparticles was found to result in faster MCA release. This result can be attributed to the formation of surface pores resulting from the fast IBU release, which subsequently increased water uptake and further accelerated PLLA degradation and, thus, MCA release. On the other hand, MCA-IBU-loaded ternary-phase microparticles exhibited a lag in the release phase of MCA, exhibiting a similar drug release rate as MCA-loaded ternary-phase microparticles. This implies that, unlike binary-phase microparticles, the presence of IBU in ternary-phase microparticles did not significantly affect the release of MCA, as IBU was well dispersed in the shell and gave rise to a more controlled release of MCA.

From this study, we determined that drug release profiles and kinetics can be altered by “designing” multi-phase microparticles that suit a particular drug delivery application.

4.2.2 Triple-layered microparticles

Although the double-layered microparticles have been widely investigated, there has been no paper conducted on the drug release from triple-layered microparticles. Besides overcoming the problems of conventional single-layered microparticles, the development of particulate systems using triple-layered microparticles, with an additional layer to double-layered microparticles, could be an attractive and robust approach in fine-tuning and realizing a variety of drug delivery kinetics and profiles. This would be achieved through the manipulation of their layer compositions, configurations and thicknesses, which demonstrates the novel contribution of this thesis.

4.2.2.1 Morphologies of the triple-layered microparticles

Figure 4.32 shows the SEM micrographs of the triple-layered microparticles prepared with emulsion solvent evaporation method. The microparticles had a smooth surface, uniform layer thickness and were spherical in shape. Particle sizes of ibuprofen-loaded triple-layered PLGA/PLLA/EVA 4:2:1 microparticles (**Figure 4.32(a)**) at lower theoretical loading of 5% w/w measured by SEM were $270.4 \pm 81.1 \mu\text{m}$. Average layer thickness measurements of the mid-layer and outer shell were approximately 35 μm and 21 μm , respectively. Comparable particle sizes were also observed for microparticles produced from other polymer pairs and amount of drug used. When the polymer mass ratio of PLGA/PLLA/EVA 4:2:1 was changed to 10:2:1 (**Figure 4.32(b)**), the outermost PLGA shell thickness increased to approximately 56 μm , while the thickness of mid-layer

was about 24 μm . On the other hand, microparticles with the mass ratio of PLGA/PLLA/EVA 10:5:1 (**Figure 4.32(c)**) had the thicker outer layers (PLGA, $\sim 33 \mu\text{m}$, and PLLA, $\sim 51 \mu\text{m}$) as compared to the mass ratio of 4:2:1. SEM micrograph in **Figure 4.32(d)** shows the triple-layered structure for the microparticles with the mass ratio of PLGA/PLLA/EVA 2:4:1, as also evident from the Raman mapping result **Figure 4.17(c)**.

Figure 4.32 SEM images of cross-sectional views of triple-layered PLLA/PLGA/EVA microparticles. (a) PLGA/PLLA/EVA 4:2:1, (b) PLGA/PLLA/EVA 10:2:1, (c) PLGA/PLLA/EVA 10:5:1, and (d) PLGA/PLLA/EVA 2:4:1

4.2.2.2 In vitro behaviors of triple-layered microparticles

The ibuprofen-loaded triple-layered PLGA/PLLA/EVA 4:2:1 microparticles were fabricated and compared with the single- and double-layered microparticles loaded with ibuprofen localized within EVA in the hydrolytic degradation and drug release studies. The actual drug content was $4 \pm 0.5\%$ w/w in all cases. Comparable particle sizes were also observed.

(a) Morphologies of the degrading microparticles

Figures 4.33(a) and (b) show the morphologies of degrading ibuprofen-loaded triple-layered PLGA/PLLA/EVA microparticles. The PLGA shell was observed to undergo significant degradation, of which pores were formed within this layer over time (**Figure 4.33(a)**). The morphology of PLLA, on the other hand, remained relatively unchanged. After 25 days, the PLGA shell was apparently almost absent, leaving behind double-layered PLLA/EVA microparticles (**Figure 4.33(b)**). It was also observed that the PLGA shell in double-layered PLGA(shell)/EVA(core) microparticles completely degraded after 25 days (**Figure 4.33(c)**). Double-layered PLLA(shell)/EVA(core) microparticles, on the other hand, remained dense and intact even after 46 days in vitro (**Figure 4.33(d)**).

Figure 4.33 SEM images of the degrading ibuprofen-loaded microparticles. Triple-layered PLGA/PLLA/EVA 4:2:1 microparticles after (a) 18 days, (b) 25 days in vitro. (c) Double-layered PLGA/EVA microparticles after 25 days in vitro. (d) Double-layered PLLA/EVA microparticles after 46 days in vitro.

(b) Water Uptake Study

Water uptakes of the various ibuprofen-loaded microparticles, as a function of incubation time, are plotted in **Figure 4.34**. The water uptake of the double-layered PLLA/EVA

microparticles was the lowest. The semi-crystalline morphology and relatively hydrophobic nature of PLLA prevents any appreciable water uptake. Comparatively, double-layered PLGA/EVA microparticles and triple-layered PLGA/PLLA/EVA microparticles exhibited increasing water uptakes with the progression of polymer degradation. It is known that PLGA 50:50 is in amorphous phase and possesses more free spaces for water diffusion into the polymer. Triple-layered PLGA/PLLA/EVA microparticles showed a lower water uptake as compared to the double-layered PLGA/EVA microparticles. This may be due to the presence of PLLA middle layer which reduced the amount of water absorption.

Figure 4.34 Water uptake of the degrading double-layered PLLA/EVA microparticles, double-layered PLGA/EVA microparticles and triple-layered PLGA/PLLA/EVA microparticles as a function of incubation time.

(c) Mass loss study

Figure 4.35 plots the mass losses of various ibuprofen-loaded microparticles. PLLA shell of the double-layered PLLA/EVA microparticles eroded very slowly, with the mass loss less than 10% in 46 days. Double-layered PLGA/EVA microparticles underwent rapid mass loss from day 11 and reached a plateau with approximately 80% mass loss, leaving behind EVA (**Figure 4.33(c)**). The mass loss of PLGA/PLLA/EVA triple-layered microparticles was also characterized by rapid mass loss after day 11, with no appreciable mass loss before that, which is coincided with the mass loss from double-layered PLGA/EVA microparticles. The rapid mass loss resulted from the formation of soluble oligomers and monomer products [9]. After day 25, these ibuprofen-loaded triple-layered microparticles exhibited unchanged mass loss of 55%, showing that PLGA has completely degraded and become soluble, as evident from **Figure 4.33(b)**. Mass loss of PLLA in triple-layered microparticles was considered relatively insignificant, due to its high molecular weight and less rapid degradation rate than PLGA, with little soluble oligomers/monomers leaching out from the polymer.

Figure 4.35 Mass loss of the degrading double-layered PLLA/EVA microparticles, double-layered PLGA/EVA microparticles and triple-layered PLGA/PLLA/EVA microparticles as a function of incubation time.

(d) Molecular Weight Analysis

Polymer degradation, through hydrolysis, was further substantiated through changes in their molecular weights. The molecular weight of EVA was found to be consistent throughout the degradation study. As such, only the molecular weights of PLGA and PLLA were plotted. **Figure 4.36** plots the changes in the molecular weights of double-layered PLGA/EVA, double-layered PLLA/EVA and triple-layered PLGA/PLLA/EVA microparticles loaded with ibuprofen. It was observed that the molecular weights of PLGA in both the triple-layered PLGA/PLLA/EVA and double-layered PLGA/EVA microparticles had similar degradation rates, implying that the presence of PLLA did not affect the degradation rates of PLGA. According to a degradation mechanism of PLGA proposed by Raghuvanshi et al. [120], the initial stage of degradation involves random chain scission, where the molecular weight of PLGA decreased significantly, but no

appreciable mass loss and no soluble degradation products leaching out were observed for the triple-layered microparticles up to 11 days (**Figure 4.35**). At day 25, with substantial mass loss of the PLGA shell (**Figure 4.35**), PLGA was no longer detectable on the SEC for both triple-layered and double-layered microparticles. On the other hand, PLLA was observed to undergo more rapid degradation in the triple-layered microparticles than in the double-layered PLLA/EVA microparticles. It was due to the migration of acidic PLGA oligomers into the PLLA layer. As the PLGA shell degraded, some soluble oligomers were leached out (resulting in mass loss), while the remaining oligomers diffused towards the PLLA middle layer. These migrating oligomers increased the hydrophilicity and acidity of the PLLA layer, thus accelerating its hydrolysis degradation. PLLA in double-layered PLLA/EVA microparticles had a slower degradation rate. This can be attributed to the fact that these microparticles exhibited low water uptake (**Figure 4.34**) and the absence of PLGA oligomers moving inward to the PLLA layer.

Figure 4.36 Changes in molecular weight of PLGA and PLLA in the double-layered PLLA/EVA microparticles, double-layered PLGA/EVA microparticles and triple-layered PLGA/PLLA/EVA microparticles as a function of incubation time.

(e) Differential Scanning Calorimetry (DSC)

Polymer degradation would be inferred from the changes to the thermal properties of the polymers. **Figure 4.37** plots the change in T_g of the double-layered and triple-layered microparticles with incubation time. T_g of PLGA decreased at a similar rate for both the double- and triple-layered microparticles. Subsequently, at day 25, the T_g of PLGA was absent from the DSC thermogram as the PLGA shell had completely degraded, as evident from the morphological and mass loss studies (**Figures. 4.33(b), (c) and 4.35**). No significant change in T_g of PLLA in double-layered PLLA/EVA microparticles was observed, while the T_g of PLLA from triple-layered microparticles decreased consistently with degradation time. The decrease in T_g therefore implied a significant decrease in molecular weight of PLLA in triple-layered microparticles, to a value below the molecular weight-threshold for constancy of T_g , as shown in **Figure 4.36**.

The distinct degradation characteristics of triple-layered microparticles could therefore present important insights into the possible drug release profiles from these novel microparticles.

Figure 4.37 Changes in glass transition temperature of PLGA and PLLA in the double-layered PLLA/EVA microparticles, double-layered PLGA/EVA microparticles and triple-layered PLGA/PLLA/EVA microparticles as a function of incubation time.

(f) In vitro drug release

The release profiles of ibuprofen from the various microparticles with ibuprofen localized in the EVA core are plotted in **Figure 4.38**. It was observed that each microparticle system exhibited a different release pattern of ibuprofen. The single-layered EVA microparticles yielded a very fast release due to its highly flexible rubbery state. Ibuprofen releases from neat PLGA and PLLA microparticles were relatively faster than that from the double-layered PLGA/EVA and PLLA/EVA microparticles, respectively. This could result from the fact that the additional diffusion pathways in the shell for the drug to be released from the core. On the other hand, in the case of double-layered and

triple-layered microparticles, the shell provided additional barriers to diffusion of drug encapsulated in the EVA core, thus limiting initial bursts and reducing rapid loss of drugs. The more resistant PLLA shell of the double-layered PLLA/EVA microparticles, as evident from its slow degradation rate (**Figure 4.36**), lower water uptake (**Figure 4.34**) and higher T_g (**Figure 4.37**), effectively retarded the diffusion of ibuprofen localized in the EVA core. On the other hand, the release of ibuprofen for double-layered PLGA/EVA microparticles was relatively faster. This was due to a more rapid degradation rate of the relatively hydrophilic PLGA shell (**Figure 4.36**). More interestingly, the release of ibuprofen from triple-layered microparticles was more consistent and observed to go through two stages: with an initial release of approximately 20% at day 11, followed by a more rapid release up to 32 days where it plateaus off. Although low mass loss was observed for the triple-layered microparticles before 11 days, the decreasing molecular weights of PLLA and PLGA as a result of degradation (**Figure 4.36**) and increasing water uptake (**Figure 4.34**) resulted in an increasing drug release dominated by diffusion in its first stage of degradation. Subsequently, as the PLGA degradation entered into the second stage of degradation process after 11 days, the rapid mass loss caused the formation of pores in PLGA layer (**Figures 4.33(a) and 4.35**), giving rise to a more rapid release of ibuprofen through the massively degrading PLLA middle layer (with significant decrease in its molecular weight) and more porous, eroding PLGA shell.

Figure 4.38 Release profiles of ibuprofen from the single-layered, double-layered PLGA/EVA, double-layered PLLA/EVA, and triple-layered PLGA/PLLA/EVA microparticles.

4.2.2.3 Effect of layer thicknesses and configurations on ibuprofen release

The effects of layer thicknesses and configurations on in vitro release of ibuprofen from the triple-layered microparticles at two different actual drug loadings of $4 \pm 0.5\%$ w/w and $15 \pm 1\%$ w/w) were investigated.

(a) Low drug loading ($4 \pm 0.5\%$ w/w)

From **Figure 4.39**, it was observed that increasing outer layer thicknesses resulted in a reduction in drug release rates. This could be attributed to a longer travelling distance for ibuprofen to diffuse through the thicker outer layers. Importantly, the reduced release in the first stage (before 11 days) and leveling of the second phase of ibuprofen release resulted in a shift towards more sustained and zero-order release for the triple-layered microparticles at the mass ratios of PLGA/PLLA/EVA 10:2:1. The change in ibuprofen release kinetics of triple-layered microparticles with different layer thicknesses can be attributed to the distinct degradation behaviors of PLLA layers. As observed from **Figure 4.40(a)**, the decrease in molecular weight of PLLA in triple-layered PLGA/PLLA/EVA 10:2:1 microparticles was slower than the triple-layered microparticles with mass ratios of 4:2:1 and 10:5:1. The higher content of fully amorphous PLGA would provide more open structure for basic ions (from the phosphate buffer) diffusing into the PLLA middle layer of triple-layered PLGA/PLLA/EVA 10:2:1 microparticles, neutralizing the generated acids. The hydrolytic degradation of the ester bonds in PLLA was therefore reduced, resulting in a slower decrease in molecular weight of PLLA. On the other hand, higher PLLA contents (thus thicker PLLA layer) for the triple-layered microparticles with mass ratios of PLGA/PLLA/EVA 4:2:1 and 10:5:1 would result in the occurrence of autocatalytic effects for the degradation of PLLA, where the concentration gradients and mass transport of acidic degradation products decrease with increasing diffusion pathway lengths. It is observed from **Figure 4.40(b)** that T_g of PLLA in triple-layered PLGA/PLLA/EVA 10:2:1 microparticles decreased to lesser extent, suggesting less free volume in polymer. At the same time, T_g of PLGA decreased at a similar rate for triple-layered microparticles with different polymer mass ratios. The less rapid decrease in

molecular weight and higher T_g of PLLA therefore contributed to more sustained and slower release of ibuprofen from triple-layered PLGA/PLLA/EVA 10:2:1 microparticles.

Figure 4.39 Release profiles of ibuprofen from the triple-layered microparticles with different polymer layer thicknesses.

(a)

(b)

Figure 4.40 (a) Changes in molecular weight of PLLA and (b) changes in glass transition temperature of PLGA and PLLA in the triple-layered PLGA/PLLA/EVA microparticles with different layer thicknesses as a function of incubation time.

Figure 4.41 plots the release kinetics of ibuprofen from the triple-layered microparticles with different configurations of outer two layers (inversion of PLLA and PLGA layers). It can be observed that triple-layered PLGA/PLLA/EVA 2:4:1 microparticles with PLLA outermost shell (while PLGA formed the middle layer) had a slower release kinetic than that from the triple-layered microparticles with PLGA outermost shell. This difference in ibuprofen release rates from triple-layered microparticles with layer inversion can be attributed to the different water uptakes of the microparticles. As seen in **Figure 4.42(a)**, the water uptake was lower when PLLA formed the outermost shell (PLGA/PLLA/EVA 2:4:1) because of the relatively hydrophobic PLLA acting as a barrier for water influx. With a lower water uptake, chain scission at the ester bonds in polymers is slowed down, leading to a less rapid decrease in molecular weights. **Figure 4.42(b)** plots the changes in molecular weight of PLLA and PLGA in triple-layered microparticles with reversed layer over time. The plot shows that with forming PLLA outermost shell, the decrease in molecular weight was slower up to day 18, attributed to its lower water uptake, resulting in a lag release phase of ibuprofen. In other words, the molecular weight of PLLA decreased more rapidly when PLGA was the external coat of PLLA. This can be explained by the acidic microenvironment created by the hydrolysis of the PLGA outermost layer upon immersion in PBS medium. After about 20 days, drastic decrease in the molecular weight of PLLA was observed for the triple-layered PLGA/PLLA/EVA 2:4:1 microparticles (**Figure 4.42(b)**), and the PLGA middle layer had significantly eroded leaving behind a gap between PLLA shell and EVA core (**Figure 4.43**). Both accelerated ibuprofen release.

Figure 4.41 Release profiles of ibuprofen from the triple-layered microparticles with layer inversion.

(a)

(b)

Figure 4.42 (a) Water uptake and (b) changes in molecular weight of triple-layered PLGA/PLLA/EVA microparticles with layer inversion as a function of incubation time.

Figure 4.43 SEM image of the degrading triple-layered PLGA/PLLA/EVA 2:4:1 microparticles after 25 days in vitro.

(b) High drug loading ($15 \pm 1\%$ w/w)

The release profiles comparing the $15 \pm 1\%$ w/w ibuprofen-loaded microparticles using the mass ratios of PLGA/PLLA/EVA 4:2:1, 10:2:1 and 2:4:1 are shown in **Figure 4.44**. The fastest release of ibuprofen due to diffusion took place with the 4:2:1 microparticles. Similar to the microparticles with lower drug loading, it was observed that the thicker outer layers of 10:2:1 microparticles caused a reduction in drug release rates. At day 4 (**Figure 4.45(a)**), some ibuprofen was observed to have diffused into the PLLA and PLGA layers, resulting in drug releasing into the PBS medium. The migration of acidic PLGA oligomers into the inner PLLA and EVA layers was clearly evident from Raman mapping ((**Figure 4.45(b)**)); during which the PLGA shell had extensively eroded, leaving behind double-layered PLLA/EVA microparticles after 25 days. At this time point, no Raman signals of IBU were detected, which could be attributed to very low IBU concentration in the particles arising from about 90 % IBU release. On the other hand, with the layer inversion between PLLA and PLGA, triple-layered PLGA/PLLA/EVA 2:4:1 microparticles with PLLA outermost shell had a slower release kinetic than that from the triple-layered microparticles with PLGA outermost shell, which was consistent with the microparticles with lower drug loading. The release profiles indicate that drug release from the triple-layered microparticles can still be manipulated by controlling the layer thickness and configuration when the actual drug loading is increased from $4 \pm 0.5\%$ w/w to $15 \pm 1\%$ w/w. Therefore, the advantages of the triple-layered microparticles were also pronounced at higher drug loading where the release tended to be faster as a result of a higher concentration gradient between the microparticle matrix and the release medium.

Figure 4.44 Release profiles of ibuprofen from the triple-layered microparticles at higher drug loading with different layer configurations.

(a)

(b)

Figure 4.45 Pure component Raman spectra estimates and their associated score images obtained via BTEM from a ibuprofen-loaded triple-layered microparticles (a) after 4 days, (b) after 25 days in vitro.

4.2.2.4 Summary

The triple-layered PLGA/PLLA/EVA microparticles with ibuprofen localized in the EVA core were fabricated using a one-step solvent evaporation method. The drug release properties of the triple-layered microparticles were compared with the single-layered and double-layered microparticles. The single-layered EVA microparticles exhibited a very fast release. The more resistant PLLA shell of the double-layered PLLA/EVA microparticles effectively reduced the drug release rate, while the release of ibuprofen for

double-layered PLGA/EVA microparticles was relatively faster due to a more rapid degradation of the PLGA shell; with the triple-layered microparticles having an intermediate release kinetic. The middle PLLA layer in the triple-layered microparticles was observed to degrade more rapidly compared to the PLLA shell in the double-layered microparticles. This was attributed to the migration of the PLGA oligomers to the PLLA layer, which accelerated hydrolysis of PLLA. Drug release profiles of triple-layered microparticles can be altered by changing the layer thicknesses. Using a $4 \pm 0.5\%$ w/w ibuprofen loading, a sustained and zero-order release was obtained with a PLGA:PLLA:EVA ratio of 10:2:1 due to the less rapid decrease in molecular weight and higher T_g of PLLA. For the layer inversion, triple-layered microparticles with PLLA shell further slowed down the release of ibuprofen because of the relatively hydrophobic PLLA acting as a barrier for water influx. Similar effects of particle parameters on drug release were observed at higher loading of $15 \pm 1\%$ w/w. This triple-layered microparticle system proved to offer the unique drug delivery properties, as compared to single- and double-layered microparticles, providing a robust approach to fine-tune and achieve the desired drug release kinetics.

4.2.3 Effect of the size of multi-layered microparticles on the drug release

Particle size is an important parameter in studying the drug release properties of microparticles. The particle size not only affects the diffusion distance of the drug but also the mass transport of acidic degradation products, and consequently, its rate of hydrolytic degradation. Although it is widely accepted that the particle size affects the degradation rates of polyesters, the significance of this effect on the drug release rate is uncertain. There is also a lack of data relating the sizes of multi-layered microparticles to their hydrolytic degradation and drug release rates; in which this thesis aims to contribute. This section studies the effects of particle sizes on the degradation and drug release behaviors of multi-layered microparticles, including double-layered and triple-layered microparticles. It would be determined if the changes in particle size would have a significant effect on the rate of drug release.

4.2.3.1 Double-layered microparticles

(a) MCA-loaded microparticles

MCA-loaded double-layered PLLA/PLGA microparticles of different sizes were fabricated at theoretical drug loading of 20% (w/w). As discussed previously, MCA was localized in the porous PLGA core of the double-layered microparticles. The porous PLGA core was formed by the repulsion of the internal aqueous phase by hydrophobic

PLLA phase during the fabrication process. The respective samples' particle sizes and their MCA encapsulation efficiencies are shown in **Table 4.3**. The encapsulation efficiency of drug decreased with decreasing particle sizes. This can be attributed to the fact that a small microparticle resulted in a short diffusion distance for MCA to leach out, contributing to the lower drug encapsulation efficiency. There was a noticeable decrease in encapsulation efficiency of MCA when the particle size decreased to approximately 20 μm in diameter (particle PS3). SEM images (**Figure 4.46**) of particles PS1, PS2 and PS3 revealed the presence of pores on the surface. Porous surface for PS3 were seemingly much pronounce than PS1 and PS2. This can be attributed that the thin polymeric wall would have a higher chance of collapse as the emulsion droplets shrink upon solvent removal [121], forming holes on particle surface and thus a much lower encapsulation efficiency of drug.

Table 4.3 Particle sizes (PS) and drug encapsulation efficiencies of different samples

Sample	Particle size in diameter (μm)	Encapsulation efficiency (%)	NaCl in the external aqueous phase
PS1	286.5 $\pm$ 63.2	53.4 $\pm$ 4.8	No
PS2	68.4 $\pm$ 24.7	45.8 $\pm$ 2.8	No
PS3	19.4 $\pm$ 10.1	5.3 $\pm$ 0.7	No
PS4	17.6 $\pm$ 6.5	25 $\pm$ 1.6	Yes
PS5	2.2 $\pm$ 0.9	0.3 $\pm$ 0.0	Yes

(a)

(b)

(c)

(d)

(e)

(f)

Figure 4.46 External and internal morphologies of the MCA-loaded double-layered microparticles. (a, b) Particle PS1, (c, d) particle PS2 and (e, f) particle PS3.

Particle PS4 was fabricated under the same conditions as PS3, but now with NaCl (1%w/v) added into the external aqueous phase. **Figures 4.47(a) and (b)** shows a dense surface morphology and double-layered structure for particle PS4. An increase in drug encapsulation efficiency from 5.3 to 25% (by 5-fold) was observed when comparing particle PS3 and PS4. The addition of salt, NaCl, was thus observed to increase the drug loading efficiency. It is generally believed that the addition of NaCl to the external aqueous phase (building up osmotic pressure) slows down the process in which the external aqueous phase flows into the emulsion droplets [122, 123]. This would then reduce the loss of drug to the external aqueous phase and form a dense surface, thus enhancing the drug loading efficiency. Subsequently, the MCA-loaded particles in even smaller size ($\sim 2\ \mu\text{m}$), particle PS5 (**Figure 4.47(c)**), were fabricated under the same conditions as particle D3 but with the addition of NaCl into the external aqueous phase rather than adding dyes in the polymer solution. As reported in **Section 4.1.3**, particle D3 was shown to yield double-layered structure (**Figure 4.20**), so as particle PS5. However, particle PS5 exhibited a very low encapsulation efficiency of MCA as a result of very small particle size.

(a)

(b)

(c)

Figure 4.47 External and internal morphologies of the MCA-loaded double-layered microparticles. (a, b) Particle PS4, (c) particle PS5.

(b) In vitro drug release

Figure 4.48(a) plots the MCA release profiles of the microparticles of sizes approximately 20 μm . There was a huge initial burst (90%) of MCA from the single-layered PLGA microparticles. Particle PS3 did not effectively reduce the initial burst release due to the highly porous surface morphology (**Figure 4.46(e)**). On the other hand, the dense surface of particle PS4, arising from the addition of NaCl in the external aqueous phase during fabrication process, effectively suppressed the high initial burst release. This again proves the need of a rate-limiting barrier (PLLA shell) to retard this initial burst release by forming double-layered microparticles.

(a)

(b)

Figure 4.48 MCA release profiles of (a) microparticles of sizes approximately 20 μm , and (b) double-layered microparticles of various sizes.

Figure 4.48(b) plots the release profiles of MCA from the double-layered microparticles of various sizes. As compared to particle PS1, particle PS2 showed a slower drug release rate while particle PS4 and PS5 exhibited higher initial burst release and faster drug release rates. Particles PS1 and PS2 were found to yield comparable drug loading efficiencies, as shown in **Table 4.3**. On the other hand, the ability of particle PS4 to suppress the initial burst release together with its higher encapsulation efficiency of drug provides a better option over particle PS3 for comparison with other particles.

As shown in the drug release profiles, particle PS2 was observed to exhibit slower drug release in comparison to particle PS1. This result can be explained by the significantly

decreased degradation rate of the rate-limiting PLLA shell, as evident from the SEC and DSC results (**Figure 4.49**). This decreased degradation rate of particle PS2 was possibly due to the fact that the shorter diffusion length with decreasing particle size would allow the acidic degradation products to diffuse out more easily into the bulk fluid. Consequently, polymer degradation of PLLA and PLGA could not be accelerated by these acidic oligomers (autocatalysis). In the DSC result (**Figure 4.49(b)**), PLGA of particle PS1 was not detectable at day 14, which is consistent with the extensive erosion of PLGA as shown in **Figure 4.50(b)**, showing more rapid PLGA degradation for particle PS1 as compared to particle PS2. **Figure 4.50** shows the morphological changes of double-layered microparticles over degradation time. Cross-sectional views of particle PS2 show that the MCA-loaded PLGA core and PLLA shell remained relatively intact at day 14, as compared to particle PS1, and thus retarded the release of MCA. The extensive erosion of the PLGA core in particle PS2 only occurred after 18 days, along with the increased formation of pores in PLLA shell, which accelerated the diffusion of MCA from the core.

(a)

(b)

Figure 4.49 Changes in (a) molecular weight of PLLA and (b) glass transition temperature of PLGA and PLLA as a function of incubation time.

(a)

(b)

(c)

(d)

(e)

Figure 4.50 SEM images of the degrading MCA-loaded double-layered microparticles. Particle PS1 after (a) 4 days and (b) 14 days in vitro. Particle PS2 after (c) 4 days, (d) 14 days and (e) 18 days in vitro.

As seen in the SEC results (**Figure 4.50(a)**), slower decreasing rates of PLLA molecular weight over incubation time with decreasing particle sizes were observed. However, the release of MCA from particles PS4 and PS5 was faster than that from the microparticles with larger sizes (particles PS1 and PS2), in which the smallest particle PS5 exhibited the fastest release rate. Moreover, particles PS4 and PS5 had relatively substantial burst release at day 1. This can be explained by another competing factor, where the huge initial burst release was due to the high surface area-to-volume ratio of small-sized particles which provided more accessible surface area for MCA to flow out into the releasing medium. Within this particle size range (around 2-20 μm), the effect of degradation rates was overcompensated by the effects of the increasing surface area-to-volume ratio with decreasing system dimension, thus resulting in increasing drug release rate with decreasing particle size.

4.2.3.2 Triple-layered microparticles

(a) In vitro drug release

Figure 4.51 shows the IBU release profiles of triple-layered PLGA/PLLA/EVA 8:2:1 microparticles in comparison to double-layered and single-layered microparticles with the size ranges of 38-10 μm , in which IBU was localized in EVA. The actual drug loading of all samples measured by TGA were approximately 3% (w/w). As expected, each microparticle system exhibited a different release rate of IBU, which is consistent with the results observed for the microparticles system with the sizes 350-250 μm . Single-

layered EVA microparticles released the entire encapsulated drug within a day. This fast release rate was largely due to the highly flexible rubbery state of EVA. The shell layer of the multi-layered microparticles provides an additional barrier to the diffusion of encapsulated drug from EVA core, thus reducing the burst release of IBU. Triple-layered microparticles had an intermediate drug release rate between double-layered PLGA/EVA 10:1 and PLLA/EVA 10:1 microparticles. This concurred with the drug release study conducted on the microparticles system with the sizes 350-250 μm which reported on the presence of the degrading PLLA layer as a rate-controlling barrier in triple-layered microparticles. As such, through the comparison with the previous study conducted on larger particles, it can be observed that the triple-layered microparticles still exhibited distinct and more sustained drug release kinetics when the particle size is reduced. It is thus of interest to study the effect of the size of triple-layered microparticles on drug release.

Figure 4.51 Release profiles of ibuprofen from the single-layered, double-layered and triple-layered microparticles with the size range of 38-10 μm .

Figure 4.52 Release profiles of ibuprofen from triple-layered microparticles of various sizes.

Figure 4.52 shows the cumulative IBU release versus time for five different particle size ranges. The mean sizes (in diameter) of the triple-layered microparticles, ranging from 20 to 137.3 μm , are given in **Table 4.4**. Clearly, it was observed that each microparticle system of different sizes exhibited a different release pattern of ibuprofen in which a trend of an increasing drug release rate with decreasing particle size was shown. Surprisingly, the phenomenon observed by Klose et al. [23, 60] who reported that autocatalytic effects compensated for the increased diffusion pathway lengths of drug, resulting in the similar release rates of particles with various sizes, was not seen in the present investigated system. To better understand the drug release mechanism, the degradation behavior of the polymer within the triple-layered microparticles was studied in detail.

Table 4.4 Mean diameter and actual drug loading of triple-layered microparticles of different particle size ranges

Size range (μm)	Mean diameter (μm)	Actual drug loading (%w/w)
150 – 106	137.3	2.976
106 – 75	79.4	3.314
75 – 53	56.2	2.923
53 – 38	41.6	3.865
38 – 10	20	3.026

(b) Polymer degradation

The decrease in molecular weight (ln MW) of PLLA in triple-layered microparticles of various sizes upon exposure to incubation medium is shown in **Figure 4.53**. A decrease in molecular weight indicates the hydrolytic degradation of polymers. PLLA was considered as a rate-limiting phase for drug release from the core due to its high glass transition temperature, semi-crystalline phase and hydrophobic characteristics. PLGA is known to be more susceptible to hydrolytic degradation than PLLA. As such, only changes in molecular weight of PLLA over the incubation time were shown. It was found that the PLLA degradation rate increased with increasing microparticle size. When PLGA shell degraded, the acidic PLGA degradation products that migrated into the PLLA layer

created an acidic micro-environment which accelerated the degradation of PLLA. However, this autocatalytic effect could be reduced through the rapid diffusion of acidic oligomers away from the surface of PLLA for the smaller microparticles due to shorter diffusion pathway lengths. In addition, due to concentration gradients, basic ions from the phosphate buffer could diffuse into the microparticles more easily with decreasing particle dimension, thus neutralizing the generated acids. The pseudo first-order equation describing an exponential decrease in the molecular weights of polymers with degradation time ($MW = MW_0 \exp(-k_{deg}t)$) was fitted into the experimental results [98, 124], where MW_0 is the initial polymer molecular weight before immersion in the release medium. **Table 4.5** shows the apparent degradation rate constant, k_{deg} , of PLLA decreased with decreasing system dimension, with a good agreement ($R^2 > 0.9$ in all cases). This again indicates that the occurrence of autocatalysis was significant in these investigated microparticles.

Figure 4.53 Plot of $\ln(\text{molecular weight})$ of PLLA with degradation time

Table 4.5 Effect of microparticle sizes on the degradation rate of PLLA

Size range (μm)	Initial molecular weight (kDa)	degradation rate constant, k_{deg} (day^{-1})
150 – 106	210	0.0418
106 – 75	202	0.0395
75 – 53	205	0.0352
53 – 38	207	0.0332
38 – 10	199	0.0237

Polymer degradation leads to a change of thermal properties of polymers. This change is reflected as a decrease of T_g in DSC analysis when the molecular weight significantly decreases to a value below the molecular weight-threshold for constancy of T_g , resulting in increased polymer chain mobility. As size range 38 – 10 μm and the rest showed two distinct T_g decreasing rate, only DSC results of triple-layered microparticles of size range 38 – 10 μm and 75 – 53 μm are plotted in **Figure 4.54**.

Figure 4.54 Change in glass transition temperature of PLLA and PLGA over incubation time

T_g of PLLA in the microparticles with size range 38 – 10 μm remained constant over 32 days of incubation unlike the decreasing trend displayed for 75 – 53 μm . The influence of particle size on degradation can again be attributed to the short intrinsic diffusion length in smaller particles (i.e. size range 38 – 10 μm). This resulted in rapid diffusion of remnant acidic oligomers away from the polymer matrix, and thus less pronounced autocatalytic effect of PLLA for the smaller microparticles.

Interestingly, the T_g of PLGA in microparticles in size range 38 – 10 μm decreased in a faster rate than the other samples, hence indicating a faster degradation rate of PLGA. This is in contrary to the general assumption that larger microparticles degrade faster than the smaller ones due to the autocatalytic effects. Morphological results shown by SEM images could be an explanation for this phenomenon. **Figure 4.55(a) and (b)** are the

cross-sectional SEM micrographs of triple-layered microparticles with size range 75-53 μm before immersion in PBS and at day 8 of degradation. The results show that the polymers in the triple layer configuration did not degrade homogeneously. Although pitting and pore formation can be observed on the external morphology for microparticles in size range 75-53 μm at day 8 in vitro, the triple-layered structure still remained its integrity. On the other hand, **Figure 4.55(c) and (d)** showed the morphological changes of degrading triple-layered microparticles of size range 38 – 10 μm . Similar to previous observation for size range 75-53 μm , polymers did not degrade homogeneously. However, the PLGA shell was observed to have disintegrated substantially for the microparticles of size range 38 – 10 μm at day 8. This extensive disintegration of PLGA in microparticles of size range 38 – 10 μm can also be observed from the mass loss result (**Figure 4.56**). Higher mass loss (20%) was measured for size range 38 – 10 μm as compared to the other size ranges with the mass losses less than 10% at day 8 in vitro. As such, it was found that the microparticles of sizes ranging 38 – 10 μm underwent more rapid degradation of PLGA shell than those microparticles in size range 75-53 μm , arising from the decrease of structural integrity of PLGA which would further promote the water uptake of the microparticles. This observation also indicates that the resultant disintegrated structure of PLGA shell would enhance the leaching of acidic degradation products, thus causing autocatalysis in PLLA layer to be less pronounced in the microparticles of size range 38 – 10 μm .

(a)

(b)

(c)

(d)

Figure 4.55 SEM micrographs of cross-sectional view of triple-layered microparticles with (a,b) size range 75-53 µm and (c,d) size range 38-10 µm before immersion in PBS and at day 8 of degradation.

Figure 4.56 Mass loss of triple-layered microparticles of various sizes over degradation time

Considering results from SEC molecular weight analysis (**Figure 4.53**) and DSC T_g analysis (**Figure 4.54**), autocatalytic effect does occur in larger particle, and this leads to faster degradation rate of PLLA layers. The middle PLLA layer was considered to function as a rate limiting barrier due to its high molecular weight, slow degradation rate and increased crystallinity. This would then be expected that the drug can be released faster from the larger particles. However, the experimental drug release results shown in **Figure 4.52** indicate that drug release rates decreased with increasing particle sizes. The explanation for the difference in experimental and expected drug release rates was discussed below. Although the autocatalytic effects were less pronounced in the smaller microparticles, it did not significantly suppress the degradation of PLLA as shown in the SEC results. As such, the restriction of IBU release through slowly degrading PLLA layer was overcompensated by the decreasing diffusion pathway lengths (increasing surface

area per unit mass), resulting in more rapid drug release from smaller-sized particles. This finding will be further substantiated by implementing a drug release model, as discussed in **Section 4.3**.

4.2.3.3 Summary

It was reported by several papers that the microparticle size is one of the factors that may affect the hydrolytic degradation behaviors, and thus drug release rates. Although it was found from this thesis that the increased microparticles size resulted in faster degradation of the rate-limiting PLLA layer due to autocatalytic effects, this accelerated degradation was found to be insignificant in enhancing the drug release rates. This proves that the drug release from the investigated multi-layered microparticles was primarily controlled by diffusion, where the drug release rates increased with decreasing particle sizes through a decreased diffusion pathway length. The effect of changes in degradation rates, arising from the changes in particle size, on drug release may thus be ignored when the degradation of polymers is not significantly suppressed in the small-sized multi-layered particles.

4.2.4 Implications of drug eluting multi-phase microparticles

Multi-phase particles with controllable particle sizes (micron and sub-micron sizes), layer thicknesses, configurations and localization of drug can be fabricated through a simple,

economical, and versatile one-step synthesis technique. The use of multiparticulate system is an important step towards a robust approach in providing a variety of drug delivery kinetics and profiles, where some layers can contain drug substances, bio-imaging dyes, while others can act as rate-limiting layers. For example, the shell in a multi-layered microparticle can act as a rate limiting barrier that reduces burst release of drugs. Multi-layered microparticles can also provide pulstaile drug release kinetics, which is favorable for vaccination and local tumor therapy. In addition, drug delivery to the small intestine or colon, through the oral route, can be achieved using multi-layered microparticles with an enteric shell layer, with other layers providing the means to achieve controlled and sustained release. The advantage of having multiple layers/phases also allows for different drugs to be selectively localized in different layers for a pre-determined time-controlled release at different time points. Through specific tailoring of layer thicknesses, zero-order release rates can also be achieved with these particles. This invention can therefore allow for the tailoring of particle designs for a range of biomedical applications. Furthermore, the investigated delivery system could achieve the controlled release of wide range of substances, such as therapeutic agents, insecticides, fertilizers, indicators, etc. Polymer particles with different morphologies would therefore be of great interest to a wide range of applications including pharmaceuticals, printing, perfumery, cosmetics, and agrochemicals.

Though the 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. 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.

4.3 Mathematical modeling of ibuprofen release from triple-layered microparticles

The triple-layered microparticles system investigated in this thesis can be envisioned and classified as a membrane-reservoir drug release system, in which ibuprofen was localized in the EVA core surrounded by non-drug holding outer layers of PLGA/PLLA. Since the initial drug content in the microparticles was kept below the drug solubility limit, dissolved drug that existed in the system was gradually depleted upon drug release. As such, one must consider that the reservoir drug concentration becomes non-constant. At present, no paper has been published yet to rigorously report on a mathematical model to illustrate an entire course of drug release from a non-constant reservoir with a degrading membrane. A temporal idiosyncrasy of initial lag release phase from a membrane-reservoir system will also be included in the model. This study therefore aimed to extend the previously reported analyses of membrane-reservoir dosages systems and to propose a mathematical model for biphasic drug release pattern of triple-layered microparticles. This would allow for better understanding the underlying chemical and physical processes involved in the drug release mechanisms.

4.3.1 Theoretical model

In the modeling analysis, drug release from the triple-layered PLGA/PLLA/EVA (shell to core) microparticles with ibuprofen localized in the EVA core follows a three-step sequence:

- (1) water penetration into the microparticles;
- (2) drug release to the surrounding release medium by diffusion process;
- (3) a membrane-reservoir system when drug diffusion through the outer layer is a rate-limiting step.

There are several assumptions which were considered in the modeling of drug release:

- (1) The surrounding water bath is perfectly stirred.
- (2) Sink conditions. At the particle surface, drug concentration is zero when mass transfer limitation does not exist and the volume of surrounding medium is large [125].
- (3) Drug dissolution in the core and water penetration are much more rapid than the drug diffusion through the outer layers.
- (4) No initial burst (no substantial amount of drug in the coating layer).
- (5) The diffusion of drug molecules is Fickian.
- (6) Diffusion coefficient and partition coefficient of drug are independent of its concentration.

(7) Polymers are completely immiscible and phase-separated.

As reported previously, before drug is released through a rate-limiting membrane in the membrane-reservoir system, a slow initial drug release is often observed when little or no drug is in the coating layer [99]. Lag time is a finite time interval required to establish a steady state of drug concentration. This suggests that, in the investigated system, the drug was first released by simple diffusion [39, 126] while the drug was subsequently released through a rate-limiting outer layer (membrane) that was undergoing hydrolytic degradation.

Therefore, the Fick's second law of diffusion under non-steady state condition for drug release from microsphere is utilized.

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left[D r^2 \frac{\partial C}{\partial r} \right] \quad (\text{Eq. 4.1})$$

D is the diffusion coefficient of drug, C is the drug concentration in the polymer matrix, t is time and r is the position in the radial position. In membrane-reservoir system, drug located in the reservoir (core) has to diffuse through a membrane layer with thickness of $R - R_i$, where R and R_i are the outer and inner radii, respectively [127]. The drug distribution within the shell ($R_i \leq r \leq R$) is obtained by solving Fick's second law of diffusion, where the reservoir concentration ($C_r = C_i / K$) is kept at the surface $r = R_i$. K is

the drug partition coefficient at the interface between the reservoir and shell matrix. By considering the time is long enough, the steady state of drug concentration across the membrane is achieved, and the solution to this diffusion problem is shown below (**Eq. 4.2**):

$$\frac{dM}{dt} = 4\pi \frac{RR_i}{(R - R_i)} DC_i \quad (\text{Eq. 4.2})$$

This mathematical model (**Eq. 4.2**) was combined with an equation considering the fact of polymer degradation. For the bulk-eroding systems (such as polyesters), the mobility of the polymer chains increases with decreasing molecular weight as a result of polymer degradation, increasing the probabilities for the drug molecules to move from one open volume to another [59]. Therefore, the diffusivity of the drug is not constant; instead, a time-dependent diffusion coefficient, D_t , was utilized. According to Charlier et al., D_t is expressed as $D_t = D_o \exp(k_{\text{deg}}t)$ due to an exponential decay of the molecular weight (pseudo-first-order degradation kinetics), where k_{deg} is the degradation rate constant, D_o is the diffusion coefficient of drug in the non-degraded polymer ($t=0$) and t is time [98]. As such, **Eq. 4.2** is modified to be as below (**Eq. 4.3**):

$$\frac{dM}{dt} = 4\pi \frac{RR_i}{(R - R_i)} D_o \exp(k_{\text{deg}}t) C_i \quad (\text{Eq. 4.3})$$

On the other hand, since the dissolved drug content (below the drug solubility limit in the polymer matrix) in the system is finite, it is to be noted that the reservoir drug concentration becomes non-constant upon drug release and it is governed by the drug release rates. C_r has to be determined as a function of time according to mass balance using the following equation (**Eq. 4.4**):

$$-V\left(\frac{dC_r}{dt}\right) = \frac{dM}{dt} \quad (\text{Eq. 4.4})$$

$$-V\left(\frac{dC_r}{dt}\right) = 4\pi \frac{RR_i}{(R - R_i)} D_o \exp(k_{\text{deg}} t) C_r K \quad (\text{Eq. 4.5})$$

The left hand side of **Eq. 4.5** is the decreased amount of drug located in the core and the term on the right hand side represents the amount of drug released into the surrounding medium. The variable concentration C_r can then be determined, as shown in **Eq. 4.6**:

$$C_r = C_o \exp \left\{ -\frac{K}{V} \left(4\pi \frac{RR_i}{(R - R_i)} D_o \right) \left(\frac{1}{k_{\text{deg}}} \right) [\exp(k_{\text{deg}} t) - \exp(k_{\text{deg}} t_a)] \right\} \quad (\text{Eq. 4.6})$$

D_o is the diffusion coefficient of drug in the non-degraded polymer ($t=0$), t_a is the end of simple diffusion-controlled release (stage 1) and at the same time, the commencement of

rate-limiting membrane-controlled release (stage 2), and C_o is the drug concentration in the core at time t_a .

The fraction of drug release from the triple-layered microparticles at the stage 2 only is $(M_t/M_\infty)_{\text{stage 2}} = V/M_\infty * (C_o - C_r) = V/(C_o V) * (C_o - C_r) = 1 - C_r/C_o$ and thus

$$\left\{ \frac{M_t}{M_\infty} \right\}_{\text{stage 2}} = 1 - \exp \left\{ -\frac{K}{V} \left(4\pi \frac{RR_i}{(R - R_i)} D_o \right) \left(\frac{1}{k_{\text{deg}}} \right) [\exp(k_{\text{deg}} t) - \exp(k_{\text{deg}} t_a)] \right\} \quad (\text{Eq. 4.7})$$

The complete analytical solution for the entire course of drug release is described below:

Eq 4.8 is used for the period of $t \leq t_a$, which describes the simple diffusion-controlled release at stage 1.

$$\frac{M_t}{M_\infty} = a\sqrt{t}, \text{ at } t \leq t_a \quad (\text{Eq. 4.8})$$

According to the analyses above, both modes of drug release are combined in the proposed model for the period of $t > t_a$. The cumulative fraction of drug release from triple-layered microparticles for the period of $t > t_a$ is described as the sum of drug release due to a simple diffusion-controlled release (first term on the right-hand side) plus the rate-limiting membrane-controlled release (second term on the right-hand side), as described in **Eq. 4.9**.

$$\frac{M_t}{M_\infty} = a\sqrt{t_a} + (1 - a\sqrt{t_a})[1 - \exp\{-\frac{K}{V}(4\pi\frac{RR_i}{(R - R_i)}D_o)(\frac{1}{k_{deg}})[\exp(k_{deg}t) - \exp(k_{deg}t_a)]\}],$$

at $t > t_a$

(Eq. 4.9)

MATLAB, a programming software, was then used to fit the proposed model, **Eqs. 4.8 and 4.9**, to the experimental data of drug release from the triple-layered microparticles with different layer thicknesses and various sizes. The correlation coefficients (R^2) between the experimental and theoretical results were compared to obtain a precise theoretical description for the drug release model.

4.3.2 Particles with different layer thicknesses

The diffusion coefficients of drug, D_o , in the non-degraded PLLA and PLGA at time=0 were determined by fitting $D = D_o \exp(k_{deg}t)$ and **Eq. 4.10** to sets of experimentally measured ibuprofen release rates of PLGA- and PLLA-based neat microparticles.

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{\pi^2} \exp(-\frac{n^2 \pi^2}{R^2} Dt) \quad (\text{Eq. 4.10})$$

Eq. 4.10 describes the dissolved drug system where initial drug loading is below the drug saturation limit in the polymer matrix; therefore the drug is dissolved uniformly in the polymer matrix [95]. The dissolved drug system can be confirmed by the absence of the melting point of the drug crystal in DSC thermogram. M_t/M_∞ is the cumulative fraction of drug release at time t , and R is the radius of the microparticles. The diffusion coefficients of ibuprofen, D_0 , in the non-degraded PLLA and PLGA were found to be $4.1829 \times 10^{-12} \text{ cm}^2/\text{s}$ and $6.59 \times 10^{-10} \text{ cm}^2/\text{s}$, respectively. **Figure 4.57** plots the ratio of diffusion coefficient of drug in PLGA to that in PLLA over the degradation time for the triple-layered PLGA/PLLA/EVA 4:2:1 microparticles. After 10 days in vitro, the diffusion coefficient of drug in PLGA is about 2000 times higher than that in PLLA. As reported previously in this thesis, rapid mass loss causing the formation of pores in PLGA shell was observed for the triple-layered PLGA/PLLA/EVA 4:2:1 microparticles after 10 days in vitro (**Figure 4.35**). The porosity of the PLGA shell would allow for the direct access to the release medium to the inner layers [128]. As such, the partitioning of drug from PLLA middle layer into PLGA shell and the release through the PLGA shell would be rapid. These results imply that the rate-limiting step would predominantly occur in PLLA due to its high glass transition temperature, long polymer chain lengths, semi-crystalline phase and slow degradation rate. The effect of PLGA shell on the drug release rates during stage 2 is thus omitted from the release models since only the rate-limiting step of PLLA layer determines the release rate and is reflected in the models.

Figure 4.57 Plot of ratios of diffusion coefficient of ibuprofen in PLGA to that of PLLA over the degradation time.

Figure 4.58 shows the plots of the model and experimental release profiles with good correlation coefficients ($R^2 \approx 0.99$). From **Figure 4.58**, biphasic drug release patterns were observed for all triple-layered microparticles with different thicknesses but with comparable particle sizes. As highlighted in the theoretical model, ibuprofen was first released through a simple diffusional process while the drug was subsequently released through a rate-limiting outer layer of PLLA that was undergoing hydrolytic degradation. The plot of the initial drug release data for cumulative release percentage versus square root of time shows a linear profile ($R^2 > 0.99$ at all cases) (**Figure 4.59**), suggesting the diffusion-controlled initial release of drug residing within the outer layers [39, 126, 129, 130], during which some ibuprofen could have diffused into the outer layer.

Figure 4.58 Model and experimental data matching of ibuprofen release from triple-layered PLGA/PLLA/EVA microparticles . (a) 4:2:1, (b) 10:2:1 and (c) 10:5:1.

Figure 4.59 Plot of cumulative release from triple-layered PLGA/PLLA/EVA microparticles versus square root of time

Table 4.6 lists the values of all known parameters and parameters determined by the model for the triple-layered microparticles with different layer thicknesses but with comparable particle sizes. The constant characteristic of diffusion of the triple-layered PLGA/PLGA/EVA 4:2:1 microparticles was found to be the highest, as compared to the other triple-layered microparticles in different layer thicknesses, indicating the fastest rate of diffusional release for the thinnest non-drug holding outer layers (the shortest diffusion length). On the other hand, drug diffusion of the microparticles with the mass ratios of PLGA/PLLA/EVA 10:2:1 seems to be slower than 10:5:1 microparticles, partly, because of the slower degradation of PLLA layer as shown by **Figure 4.40** and **Table 4.6**. The end of stage 1, at day 18.1, was observed for the triple-layered PLGA/PLLA/EVA 10:2:1 microparticles. This longer time required to establish a pseudo-steady state drug concentration gradient across the membrane with drug partitioning can be again attributed to the slower degradation of PLLA layer, which resulted in a denser and less permeable matrix structure. It was also observed that the partition coefficient of drug increased with increasing PLLA degradation rates. In the early days of degradation, PLLA is a relatively hydrophobic and highly glassy polymer. Therefore, very limited free volume was available for ibuprofen to partition into the PLLA layer. This drug partitioning has to be dependent on polymer degradation to cause chain scission and polymer relaxation to produce more “open” network. Therefore, the creation of more free volume as a result of the significantly decreased molecular weight (degradation) facilitates the drug partitioning and promotes further release.

Table 4.6 Models parameters of ibuprofen release from triple-layered PLGA/PLLA/EVA microparticles with different layer thicknesses

Parameters	Description	Unit	4:2:1	10:2:1	10:5:1
<i>Known parameters</i>					
R_{particle}	Radius of particle (PLGA+PLLA+EVA)	μm	135.2	134.7	141.5
R	Second inner radius (PLLA+EVA)	μm	114.4	79.2	108.6
R_i	Innermost radius (EVA)	μm	79.3	54.6	57.1
k_{deg}	Degradation rate constant of PLLA	day^{-1}	0.0357	0.0089	0.0330
D_o	Drug diffusion coefficient in PLLA phase	cm^2/s		4.1829×10^{-12}	
<i>Parameters determined by the model</i>					
a	Constant characteristic of diffusion	$\text{s}^{-0.5}$	0.07256	0.04148	0.04642
t_a	End of simple diffusion-controlled release (stage 1)	days	9.3	18.1	11
K	Partition coefficient of drug, [PLLA]/[EVA]	-	0.547	0.138	0.334
<i>Goodness of fit</i>					
R^2	Correlation factor	-	0.98457	0.99605	0.99157

4.3.3 Particles with various sizes

Same approach was utilized to model the drug release profiles of triple-layered PLGA/PLLA/EVA 8:2:1 microparticles and to determine the model parameters of ibuprofen release from triple-layered PLGA/PLLA/EVA 8:2:1 microparticles of various sizes, as listed in **Table 4.7**. As shown in **Figure 4.60**, good agreement between model and experimentally measured data for the microparticles with various size ranges was obtained ($R^2 > 0.99$). The constant characteristics of diffusion were observed to increase with decreasing particle size. This result can be explained by the fact that a shorter diffusion pathway and higher drug concentration gradient can be achieved with decreasing particle size, thus accelerating the diffusional release. Interestingly, it was found that the end of stage 1 for all the investigated size range was at around day 8. Although the decreasing polymer degradation rate with decreasing device dimension would hinder the drug diffusion through the membrane, the decreased particle size (decreased diffusion pathway length) was shown to compensate for this effect in the investigated system, as the degradation of polymers was not significantly suppressed in smaller particles. Similar to the previous result of the particles with different layer thicknesses, the partition coefficient of drug increased with increasing degradation rate of PLLA rate-limiting layer. Denser structure of slowly degrading PLLA would hinder the partitioning of drug from the core into the PLLA layer. Despite exhibiting lower partition coefficient of drug as a result of slower PLLA degradation, the drug release from the smaller-sized triple-layered microparticles still proceeded relatively faster at stage 2 due to a decreased diffusion pathway length, as shown in **Figure 4.61**, suggesting that the drug release was diffusion (geometry)-controlled rather than partition- and degradation-

controlled in the investigated system (size range 150-10 μ m in diameter). This result substantiates that the drug diffusion through the PLLA layer is too low due to the high T_g and glassy structure of PLLA, enabling the diffusion to dominate the drug release.

(a)

(b)

(c)

(d)

(e)

Figure 4.60 Model and experimental data matching of ibuprofen release from triple-layered PLGA/PLLA/EVA microparticles . (a) 38-10 μm , (b) 53-38 μm (c) 75-53 μm , (d) 106-75 μm , and (e) 150-106 μm .

Table 4.7 Parameters of ibuprofen release from triple-layered PLGA/PLLA/EVA 8:2:1 microparticles of various sizes

Parameters	Description	Unit	38- 10µm	53- 38µm	75- 53µm	106- 75µm	150- 106µm
<i>Known parameters</i>							
R _{particle}	Radius of particle (PLGA+PLLA+EVA)	µm	10	20.8	28.1	39.7	68.65
R	Second inner radius (PLLA+EVA)	µm	7.4	15.5	20.9	29.5	51.0
R _i	Innermost radius (EVA)	µm	5.1	10.7	14.4	20.3	35.2
k _{deg}	Degradation rate constant of PLLA	day ⁻¹	0.0237	0.0332	0.0352	0.0395	0.0418
D _o	Drug diffusion coefficient in PLLA phase	cm ² /s	4.1829x10 ⁻¹²				
<i>Parameters determined by the model</i>							
a	Constant characteristic of diffusion	s ^{-0.5}	0.26592	0.24188	0.24015	0.18603	0.12797
t _a	End of simple diffusion-controlled release (stage 1)	days	8	8	8	8.8	8
K	Partition coefficient of drug, [PLLA]/[EVA]	-	0.008	0.030	0.040	0.104	0.131
<i>Goodness of fit</i>							
R ²	Correlation factor	-	0.99165	0.99693	0.99231	0.99857	0.9951

Figure 4.61 Cumulative ibuprofen release from triple-layered microparticles of various sizes during stage 2.

4.3.4 Summary

Appropriate release models for the triple-layered microparticles with different layer thicknesses and particle sizes, where ibuprofen was localized in the core, have been proposed and which demonstrated good agreements with experimentally measured data. The triple-layered microparticles released its drug through initial simple diffusion and then followed by a release through a rate-limiting outer layer (membrane) that was undergoing hydrolytic degradation. The proposed model therefore consists of two terms: simple diffusion-controlled release and rate-limiting membrane-controlled release. Herein, the non-constant reservoir and degrading membrane are taken into account, in which the PLLA layer plays an important role in controlling the drug release due to its highly glassy and slowly degrading characteristics. The constant characteristic of diffusion was found

to increase with decreasing particle size and layer thickness. For the triple-layered microparticles with the comparable sizes, longer time for establishing a drug concentration gradient across the membrane with low drug partitioning was observed for the microparticles with the slower degradation of PLLA layer, which resulted in a denser and less permeable matrix structure. For the triple-layered microparticles with the various sizes, similarly, the partition coefficient of drug increased with increasing degradation rate of PLLA rate-limiting layer, although slower drug release was observed for larger-sized particles that underwent faster degradation (autocatalysis). This observation suggests that the release of ibuprofen was diffusion (geometry)-controlled rather than partition-controlled in the investigated system (size range 150-10 μ m in diameter). The applicability of this modeling analysis has been demonstrated to be effective over a range of layer thicknesses and particle sizes and to provide mechanistic insights into the drug release profiles.

Chapter 5 Conclusions

The fabrication of multi-phase particles (i.e. double-layered ternary-phase microparticles, triple-layered and quadruple-layered microparticles) and their drug release properties were reported in this thesis. The first objective was therefore to devise a one-step fabrication technique for the formation of ternary-phase polymeric microparticles as well as multi-layered (from double-layered to quadruple-layered, and potentially multilayered) microparticles. The results obtained show conclusive evidence that various process parameters were found to form different configurations of the resulting microparticles. A considerably low starting polymer solution (below the cloud point), low stirring speed and an increase in the oil-to-water ratio facilitated the configurations towards more thermodynamic equilibrium ones, dictated by the interfacial energies of the components.

For the double-layered ternary-phase PLLA/PLGA/PCL microparticles, 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. Higher amount of PCL resulted in larger PCL particulates dispersed in the PLLA shell. The decrease in polymer precipitation rate at lower stirring speed and lower PVA volume provided more time for PCL coacervate phases to coalesce before polymer solidification.

Triple-layered PLGA/PLLA/EVA and quadruple-layered PLGA/PLLA/PS/EVA microparticles composed of immiscible polymers can be formed only when the polymer

solution was prepared above the cloud point. The solvent extraction rate (or polymer precipitation rate) was carefully adjusted by optimizing process parameters so as to kinetically trap the multi-layered structure in a non-equilibrium configuration. The very fast precipitation rate decreased the ability of the polymers to move and coalesce with their respective phases; a slow precipitation rate facilitated a thermodynamically stable configurations. The time available for solvent partitioning between each polymer coacervate phase was reduced at a considerably high polymer precipitation rate. Therefore, the final layer configuration was determined by the polymer mass ratios, where a polymer with higher mass (or volume) formed the outer layer. Manipulation of layer thickness and configuration could also be achieved by altering the polymer mass ratios. Layer thickness was found to increase with increasing polymer content, and the higher mass polymer engulfed the polymer with lower mass.

It was also observed that lowering the polymer concentration and increasing the stirring speed as well as surfactant concentration reduced the particle sizes while retaining the multi-layered structure. However, it is to be noted that when the nascent emulsion droplets composed of PLLA and PLGA were significantly reduced to a certain size (~20 μm in the investigated system), the high surface area-to-volume ratio and short diffusion distance would lead to faster precipitation of the polymers. An extended solvent extraction time was therefore needed to form the double-layered sub-micron PLLA/PLGA particles by increasing the oil-to-water ratio and saturating the external aqueous phase with DCM.

An assortment of “designer” 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. The second objective was therefore to study the drug release profiles of double-layered ternary-phase and triple-layered microparticles. Here, two hypotheses were investigated. Firstly, it is hypothesized that ternary-phase and triple-layered microparticles will provide unique and better controlled drug release properties as compared to neat and binary-phase microparticles. Secondly, it is hypothesized that double-layered ternary-phase and triple-layered microparticles can offer great versatility in controlling the drug release kinetics through the manipulation of particle parameters, such as layer thicknesses, structural configurations and even particle sizes.

The drug release properties of the double-layered ternary-phase PLLA/PLGA/PCL microparticles were compared with those of binary-phase PLLA/PLGA and neat microparticles. It was found that MCA and IBU were localized in the PLGA core and in the shell, respectively. Drug localization in specific polymeric layers was observed to be dependent on the affinity of the drug to the polymer, resulting in specific layer localization of drugs. The MCA-loaded ternary-phase microparticles yielded an intermediate rate of release, between that of neat microparticles and binary-phase microparticles. Changing the polymer mass ratios of the ternary-phase microparticles also changed the release kinetics. In contrast, the presence of PCL resulted in the good dispersal of IBU in the ternary-phase microparticles, thus retarding the initial burst release of IBU and providing better controlled release. Similarly, the rate of IBU released can also be altered by changing polymer mass ratios. From this study, we determined that drug release profiles and kinetics can be altered by “designing” microparticles that suit a particular drug delivery application.

The drug release properties of the triple-layered PLGA/PLLA/EVA (shell to core) microparticles were compared with the single-layered and double-layered microparticles, with ibuprofen localized in the EVA core. The single-layered EVA microparticles exhibited a very fast release. Triple-layered microparticles exhibited an intermediate release kinetic and a more consistent (close to zero-order) release rate, in comparison to double-layered microparticles of PLLA/EVA and PLGA/EVA. In this system, it was found that the middle PLLA layer in the triple-layered microparticles degraded more rapidly than expected. Herein, degradation rate can be tailored to adjust drug release rate through the use of two polymers as the outer layers. Drug release profiles of triple-layered microparticles can be altered by changing the layer thicknesses and configurations. The more sustained and zero-order release from microparticles of PLGA:PLLA:EVA 10:2:1. Triple-layered microparticles that possess unique hydrolytic degradation characteristics and distinct structural attributes would therefore offer a promising strategy in achieving controlled drug release.

The accelerated degradation of the rate-limiting polymer (i.e. PLLA) due to autocatalytic effects was found to be insignificant in enhancing the drug release rates. It was observed that the drug release from the core of multi-layered microparticles increased with decreasing particle size. The suppressed release of ibuprofen through slowly degrading PLLA layer was overcompensated by the significant decrease in diffusion pathway length with decreasing particle size.

A relationship between the physicochemical processes and the drug release was established for triple-layered microparticles of different layer thicknesses and particle sizes, and the third objective was met. This relationship allows for better understanding

on how the underlying mass transport mechanisms control drug release from the investigated system. It is thereby concluded that the use of multi-phase/multi-layered microparticles can provide a versatile and robust means to control the drug release kinetics.

Chapter 6 Recommendations for Future Work

Based on existing knowledge of the effects of multi-phase/multi-layered microparticle configurations on drug release, it will help set directions for the future work that can be carried out. The following proposed work could be conducted in the near future to substantiate the research work that has been established in this thesis. These studies will give a greater and deeper understanding on the drug release profiles of multi-phase microparticles.

1. Investigation into drug release mechanism from MCA-loaded single-layered PLGA microparticles

PLGA microparticles containing MCA were observed to have porous surfaces (**Figure 6.1**), possibly due to the hydration of MCA that drives the external water to diffuse into the nascent emulsion droplets by osmotic pressure. This porous structure may result in the high initial burst release and subsequent linear release profile (**Figure 6.2**). More studies including mass loss, SEC and water uptake should be undertaken to understand the drug release mechanism.

(a)

(b)

Figure 6.1 (a) External and (b) internal morphologies of the neat PLGA microparticles.

Figure 6.2 MCA release profile of MCA-loaded single-layered PLGA microparticles

2. In vivo degradation and drug release studies of multi-phase microparticles

In vitro hydrolytic degradation and drug release studies were reported in this thesis. However, it would be of greater interest to study and understand the in vivo degradation rates and drug release behavior of these multi-phase microparticles comprising fully

biodegradable polymers. A study on the in vivo behavior of biocompatible multi-phase microparticles would give a more complete insight into how the use of multi-phase microparticles can provide a means to control the drug release profiles.

3. Drug release study of multi-phase microparticles comprising other biodegradable polymers

The study of drug release could also be extended to other biodegradable polymers, such as polyanhydrides, poly(ethylene glycol) and polyphosphoesters. The fabrication of the multi-phase microparticles composed of other different polymers would also be an interesting study. This would then substantiate the formation mechanisms proposed in this thesis. The degradation and drug release studies of these microparticles would subsequently be investigated, where different type of therapeutic agents (e.g. antibodies, peptides, proteins, etc) can also be loaded into these microparticles.

4. Fabrication of multi-phase/multi-layered nano-sized particles and its drug release study

Formation of double-layered particles were found capable of achieving the sub-micron range ($\sim 2\mu\text{m}$) using the solvent evaporation method in this thesis. Further fine-tuning of process parameters would be done to push the limit into the nano-sized region. This would potentially create a wider field of medical applications. Characterization of these nanoparticles may require the use of other equipments such as the transmission electron

microscope (TEM), where the particles are loaded with heavy metal to provide the better contrast. Subsequently, the degradation and drug release studies could be conducted.

5. Modeling drug release from the multi-layered microparticles loaded with dispersed drug

The entire release process of coated dosage form with dispersed drug in the core can be separated into two distinctive stages. The first stage is a zero-order release kinetic for a constant reservoir system, where the drug concentration is above its saturation limit in the core matrix. The second stage is typified by the drug release from a non-constant reservoir, which has been reported in this thesis. As such, a modeling study of an entire drug release process from a constant reservoir transforming into a non-constant reservoir would be presented. This would then extend the use of theoretical model derived from this thesis and give a better understanding on how drug loading affects the drug release kinetics.

6. Investigating the potential applications of the multi-phase microparticles

Due to the specific localization of drugs in different layers, the multi-phase and multi-layered microparticles investigated in this thesis would be used for delivering two or more than two drugs in succession or pulsatile manner. This new technology for microparticles

fabrication would have the potential for opening new doors in the field of vaccine delivery or other drug delivery-related applications.

Chapter 7 References

- [1] Freiberg S, Zhu X. Polymer microspheres for controlled drug release. *Int J Pharm* 2004;282:1-18.
- [2] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. *Chem Rev* 1999;99:3181-3198.
- [3] Peppas NA, Huang Y, Torres-Lugo M, Ward JH, Zhang J. Physicochemical foundations and structural design of hydrogels in medicine and biology. *Annu Rev Biomed Eng* 2000;2:9-29.
- [4] Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. *J Pharm Sci* 2008;97:71-87.
- [5] Faisant N, Siepmann J, Benoit JP. PLGA-based microparticles: elucidation of mechanisms and a new, simple mathematical model quantifying drug release. *Eur J Pharm Sci* 2002;15:355-366.
- [6] Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release* 2001;70:1-20.
- [7] Xie J, Tan JC, Wang CH. Biodegradable films developed by electrospray deposition for sustained drug delivery. *J Pharm Sci* 2008;97:3109-3122.
- [8] Chia NK, Venkatraman SS, Boey FYC, Cadart S, Loo JSC. Controlled degradation of multilayered poly (lactide co glycolide) films using electron beam irradiation. *J Biomed Mater Res* 2008;84:980-987.
- [9] Wang X, Venkatraman SS, Boey FYC, Loo JSC, Tan LP. Controlled release of sirolimus from a multilayered PLGA stent matrix. *Biomaterials* 2006;27:5588-5595.

-
- [10] Li WJ, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospun nanofibrous structure: a novel scaffold for tissue engineering. *J Biomed Mater Res* 2002;60:613-621.
- [11] Thakur RA, Florek CA, Kohn J, Michniak BB. Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing. *Int J Pharm* 2008;364:87-93.
- [12] Heslinga MJ, Mastria EM, Eniola-Adefeso O. Fabrication of biodegradable spheroidal microparticles for drug delivery applications. *J Control Release* 2009;138:235-242.
- [13] Stamboulis A, Hench LL, Boccaccini AR. Mechanical properties of biodegradable polymer sutures coated with bioactive glass. *J Mater Sci-Mater Med* 2002;13:843-848.
- [14] Raman C, McHugh AJ. A model for drug release from fast phase inverting injectable solutions. *J Control Release* 2005;102:145-157.
- [15] Wang L, Venkatraman S, Kleiner L. Drug release from injectable depots: two different in vitro mechanisms. *J Control Release* 2004;99:207-216.
- [16] Wang L, Kleiner L, Venkatraman S. Structure formation in injectable poly (lactide-co-glycolide) depots. *J Control Release* 2003;90:345-354.
- [17] Berkland C, Cox A, Kim K, Pack DW. Three-month, zero-order piroxicam release from monodispersed double-walled microspheres of controlled shell thickness. *J Biomed Mater Res* 2004;70A:576-584.
- [18] Brannon-Peppas L. Recent advances on the use of biodegradable micropraticles and nanoparticles in controlled drug-delivery. *Int J Pharm* 1995;116:1-9.
- [19] Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *J Control Release* 2005;102:313-332.
-

-
- [20] Berkland C, King M, Cox A, Kim K, Pack DW. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J Control Release* 2002;82:137-147.
- [21] Berkland C, Kim KK, Pack DW. Fabrication of PLG microspheres with precisely controlled and monodisperse size distributions. *J Control Release* 2001;73:59-74.
- [22] Berkland C, Kim K, Pack DW. PLG microsphere size controls drug release rate through several competing factors. *Pharm Res* 2003;20:1055-1062.
- [23] Klose D, Siepmann F, Elkharraz K, Krenzlin S, Siepmann J. How porosity and size affect the drug release mechanisms from PLGA-based microparticles. *Int J Pharm* 2006;314:198-206.
- [24] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide)(PLGA) devices. *Biomaterials* 2000;21:2475-2490.
- [25] Mao S, Xu J, Cai C, Germershaus O, Schaper A, Kissel T. Effect of WOW process parameters on morphology and burst release of FITC-dextran loaded PLGA microspheres. *Int J Pharm* 2007;334:137-148.
- [26] Jalil R, Nixon JR. Microencapsulation using poly (L-lactic acid) II: Preparative variables affecting microcapsule properties. *J Microencapsulation* 1990;7:25-39.
- [27] Jalil R, Nixon JR. Microencapsulation using poly (L-lactic acid) IV: Release properties of microcapsules containing phenobarbitone. *J Microencapsulation* 1990;7:53-66.
- [28] Sheikh H. Effect of the microencapsulation of nanoparticles on the reduction of burst release. *Int J Pharm* 2007;344:53-61.
- [29] Pekarek KJ, Jacob JS, Mathiowitz E. One-step preparation of double-walled microspheres. *Adv Mater* 1994;6:684-687.
-

-
- [30] Pekarek KJ, Jacob JS, Mathiowitz E. Double-walled polymer microspheres for controlled drug-release. *Nature* 1994;367:258-260.
- [31] Sanchez A, Gupta RK, Alonso MJ, Siber GR, Langer R. Pulsed controlled-release system for potential use in vaccine delivery. *J Pharm Sci* 1996;85:547-552.
- [32] Lorenzo-Lamosa ML, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Design of microencapsulated chitosan microspheres for colonic drug delivery. *J Control Release* 1998;52:109-118.
- [33] Rodriguez M, Vila-Jato JL, Torres D. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. *J Control Release* 1998;55:67-77.
- [34] Krishnamachari Y, Madan P, Lin S. Development of pH-and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. *Int J Pharm* 2007;338:238-247.
- [35] Naha PC, Kanchan V, Manna PK, Panda AK. Improved bioavailability of orally delivered insulin using Eudragit-L30D coated PLGA microparticles. *J Microencapsulation* 2008;25:248-256.
- [36] Shi M, Yang YY, Chaw CS, Goh SH, Moolchala SM, Ng S, et al. Double walled POE/PLGA microspheres: encapsulation of water-soluble and water-insoluble proteins and their release properties. *J Control Release* 2003;89:167-177.
- [37] Lee HK, Park JH, Kwon KC. Double-walled microparticles for single shot vaccine. *J Control Release* 1997;44:283-293.
- [38] Choi DH, Park CH, Kim IH, Chun HJ, Park K, Han DK. Fabrication of core-shell microcapsules using PLGA and alginate for dual growth factor delivery system. *J Control Release* 2010.
-

-
- [39] Lee TH, Wang JJ, Wang CH. Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies. *J Control Release* 2002;83:437-452.
- [40] Tan EC, Lin RY, Wang CH. Fabrication of double-walled microspheres for the sustained release of doxorubicin. *J Colloid Interface Sci* 2005;291:135-143.
- [41] Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H. Drug release characteristics of multi-reservoir type microspheres with poly(DL-lactide-co-glycolide) and poly(DL-lactide). *J Control Release* 2005;106:172-180.
- [42] Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H, Kobayashi M. The polymer-alloys method as a new preparation method of biodegradable microspheres: Principle and application to cisplatin-loaded microspheres. *J Control Release* 1997;48:19-27.
- [43] Rahman NA, Mathiowitz E. Localization of bovine serum albumin in double-walled microspheres. *J Control Release* 2004;94:163-175.
- [44] Naraharisetti PK, Ning Lew MD, Fu YC, Lee DJ, Wang CH. Gentamicin-loaded discs and microspheres and their modifications: characterization and in vitro release. *J Control Release* 2005;102:345-359.
- [45] Kokai LE, Ghaznavi AM, Marra KG. Incorporation of double-walled microspheres into polymer nerve guides for the sustained delivery of glial cell line-derived neurotrophic factor. *Biomaterials* 2010;31:2313-2322.
- [46] Kokai LE, Tan H, Jhunjunwala S, Little SR, Frank JW, Marra KG. Protein bioactivity and polymer orientation is affected by stabilizer incorporation for double-walled microspheres. *J Control Release* 2010;141:168-176.
- [47] Mathiowitz E, Langer RS. Preparation of multiwall polymeric microcapsules. US Patent No. 4861627, 1989.
-

-
- [48] Gopferich A, Alonso MJ, Langer R. Development and characterization of microencapsulated microspheres. *Pharm Res* 1994;11:1568-1574.
- [49] Watano S, Nakamura H, Hamada K, Wakamatsu Y, Tanabe Y, Dave RN, et al. Fine particle coating by a novel rotating fluidized bed coater. *Powder Technol* 2004;141:172-176.
- [50] Wang FJ, Wang CH. Sustained release of etanidazole from spray dried microspheres prepared by non-halogenated solvents. *J Control Release* 2002;81:263-280.
- [51] Pollauf EJ, Pack DW. Use of thermodynamic parameters for design of double-walled microsphere fabrication methods. *Biomaterials* 2006;27:2898-2906.
- [52] Pollauf EJ, Kim KK, Pack DW. Small-molecule release from poly (D, L-lactide)/poly (D, L-lactide-co-glycolide) composite microparticles. *J Pharm Sci* 2005;94:2013-2022.
- [53] Berkland C, Pollauf E, Pack DW, Kim K. Uniform double-walled polymer microspheres of controllable shell thickness. *J Control Release* 2004;96:101-111.
- [54] O'Donnell PB, McGinity JW. Preparation of microspheres by the solvent evaporation technique. *Adv Drug Delivery Rev* 1997;28:25-42.
- [55] Loo SCJ, Tan WLJ, Khoa SM, Chia NK, Venkatraman S, Boey F. Hydrolytic degradation characteristics of irradiated multi-layered PLGA films. *Int J Pharm* 2008;360:228-230.
- [56] Panyam J, Dali MA, Sahoo SK, Ma WX, Chakravarthi SS, Amidon GL, et al. Polymer degradation and in vitro release of a model protein from poly(D,L-lactide-co-glycolide) nano- and microparticles. *J Control Release* 2003;92:173-187.
- [57] Mathiowitz E, Jacob JS, Chickering Iii DE, Pekarek KJ. Preparation of multiwall polymeric microcapsules from hydrophilic polymers. US Patent No. 6511749, 2003.
-

-
- [58] Leach K, Noh K, Mathiowitz E. Effect of manufacturing conditions on the formation of double-walled polymer microspheres. *J Microencapsulation* 1999;16:153-167.
- [59] Siepmann J, Faisant N, Akiki J, Richard J, Benoit JP. Effect of the size of biodegradable microparticles on drug release: experiment and theory. *J Control Release* 2004;96:123-134.
- [60] Klose D, Siepmann F, Elkharraz K, Siepmann J. PLGA-based drug delivery systems: Importance of the type of drug and device geometry. *Int J Pharm* 2008;354:95-103.
- [61] Maurus PB, Kaeding CC. Bioabsorbable implant material review. *Operative Techniques in Sports Medicine* 2004;12:158-160.
- [62] Miller RA, Brady JM, Cutright DE. Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. *J Biomed Mater Res* 1977;11:711-719.
- [63] Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials* 2000;21:2335-2346.
- [64] Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A. Poly-[epsilon]-caprolactone microspheres and nanospheres: an overview. *Int J Pharm* 2004;278:1-23.
- [65] Shin SC, Byun SY. Controlled release of ethinylestradiol from ethylene-vinyl acetate membrane. *Int J Pharm* 1996;137:95-102.
- [66] Patrick CW, Mikos AG, McIntire LV. *Frontiers in tissue engineering*: Elsevier Science & Technology, 1998.
- [67] Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennern H, Hussain AS, et al. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol Pharm* 2004;1:85-96.
-

-
- [68] Yavagal DR, Karnad DR, Oak JL. Metoclopramide for preventing pneumonia in critically ill patients receiving enteral tube feeding: a randomized controlled trial. *Crit Care Med* 2000;28:1408.
- [69] Loo SCJ, Ooi CP, Boey YCF. Radiation effects on poly (lactide-co-glycolide)(PLGA) and poly (-lactide)(PLLA). *Polym Degrad Stab* 2004;83:259-265.
- [70] Loo SCJ, Tan HT, Ooi CP, Boey YCF. Hydrolytic degradation of electron beam irradiated high molecular weight and non-irradiated moderate molecular weight PLLA. *Acta Biomater* 2006;2:287-296.
- [71] Loo SCJ, Ooi CP, Boey FYC. Influence of electron-beam radiation on the hydrolytic degradation behaviour of poly (lactide-co-glycolide)(PLGA). *Biomaterials* 2005;26:3809-3817.
- [72] Burkersroda F, Schedl L, Gopferich A. Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials* 2002;23:4221-4231.
- [73] Ogawa Y, Yamamoto M, Takada S, Okada H, Shimamoto T. Controlled-release of leuprolide acetate from polylactic acid or copoly (lactic/glycolic) acid microcapsules: influence of molecular weight and copolymer ratio of polymer. *Chem Pharm Bull* 1988;36:1502-1507.
- [74] Loxley A, Vincent B. Preparation of poly(methylmethacrylate) microcapsules with liquid cores. *J Colloid Interface Sci* 1998;208:49-62.
- [75] Harkins WD. *The physical chemistry of surface films*: Reinhold, 1952.
- [76] Torza S, Mason SG. Three-phase interactions in shear and electrical fields. *J Colloid Interface Sci* 1970;33:67-83.
- [77] Wang Y, Guo BH, Wan X, Xu J, Wang X, Zhang YP. Janus-like polymer particles prepared via internal phase separation from emulsified polymer/oil droplets. *Polymer* 2009;50:3361-3369.
-

-
- [78] Yang YY, Shi M, Goh SH, Moochhala SM, Ng S, Heller J. POE/PLGA composite microspheres: formation and in vitro behavior of double walled microspheres. *J Control Release* 2003;88:201-213.
- [79] Mao SR, Shi Y, Li L, Xu J, Schaper A, Kissel T. Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. *Eur J Pharm Biopharm* 2008;68:214-223.
- [80] Wang Z. A water-in-oil-in-oil-in-water (W/O/O/W) method for producing drug-releasing, double-walled microspheres. *Int J Pharm* 2009;374:90-95.
- [81] Tan HX, Ye J. Surface morphology and in vitro release performance of double-walled PLLA/PLGA microspheres entrapping a highly water-soluble drug. *Appl Surf Sci* 2007;255:353-356.
- [82] Leach KJP, Mathiowitz E. Degradation of double-walled polymer microspheres of PLLA and P(CPP : SA)20 : 80. I. In vitro degradation. *Biomaterials* 1998;19:1973-1980.
- [83] Leach KJP, Takahashi S, Mathiowitz E. Degradation of double-walled polymer microspheres of PLLA and P(CPP : SA)20 : 80. II. In vivo degradation. *Biomaterials* 1998;19:1981-1988.
- [84] Mainardes RM, Evangelista RC. PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. *Int J Pharm* 2005;290:137-144.
- [85] Zhao H, Gagnon J, Hafeli UO. Process and formulation variables in the preparation of injectable and biodegradable magnetic microspheres. *Biomag Res Technol* 2007;5:2.
- [86] Meng FT, Ma GH, Qiu W, Su ZG. W/O/W double emulsion technique using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of microparticles. *J Control Release* 2003;91:407-416.
-

-
- [87] Katou H, Wandrey AJ, Gander B. Kinetics of solvent extraction/evaporation process for PLGA microparticle fabrication. *Int J Pharm* 2008;364:45-53.
- [88] Siepmann J, Faisant N, Benoit JP. A new mathematical model quantifying drug release from bioerodible microparticles using Monte Carlo simulations. *Pharm Res* 2002;19:1885-1893.
- [89] Narayani R, Panduranga Rao K. Gelatin microsphere cocktails of different sizes for the controlled release of anticancer drugs. *Int J Pharm* 1996;143:255-258.
- [90] Siepmann J, Elkharraz K, Siepmann F, Klose D. How autocatalysis accelerates drug release from PLGA-based microparticles: A quantitative treatment. *Biomacromolecules* 2005;6:2312-2319.
- [91] Dunne M, Corrigan OI, Ramtoola Z. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. *Biomaterials* 2000;21:1659-1668.
- [92] Shenderova A, Burke TG, Schwendeman SP. The acidic microclimate in poly (lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharm Res* 1999;16:241-248.
- [93] Fu K, Pack DW, Klibanov AM, Langer R. Visual evidence of acidic environment within degrading poly (lactic-co-glycolic acid)(PLGA) microspheres. *Pharm Res* 2000;17:100-106.
- [94] Brunner A, Mader K, Gopferich A. pH and osmotic pressure inside biodegradable microspheres during erosion. *Pharm Res* 1999;16:847.
- [95] Arifin DY, Lee LY, Wang CH. Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. *Adv Drug Delivery Rev* 2006;58:1274-1325.
-

-
- [96] Lao LL, Venkatraman SS, Peppas NA. Modeling of drug release from biodegradable polymer blends. *Eur J Pharm Biopharm* 2008;70:796-803.
- [97] Lao LL, Venkatraman SS, Peppas NA. A novel model and experimental analysis of hydrophilic and hydrophobic agent release from biodegradable polymers. *J Biomed Mater Res* 2009;90:1054-1065.
- [98] Charlier A, Leclerc B, Couarraze G. Release of mifepristone from biodegradable matrices: experimental and theoretical evaluations. *Int J Pharm* 2000;200:115-120.
- [99] Zhou Y, Chu JS, Li JX, Wu XY. Theoretical analysis of release kinetics of coated tablets containing constant and non-constant drug reservoirs. *Int J Pharm* 2010;385:98-103.
- [100] Faisant N, Akiki J, Siepmann F, Benoit JP, Siepmann J. Effects of the type of release medium on drug release from PLGA-based microparticles: Experiment and theory. *Int J Pharm* 2006;314:189-197.
- [101] Siepmann J, Gopferich A. Mathematical modeling of bioerodible, polymeric drug delivery systems. *Adv Drug Delivery Rev* 2001;48:229-247.
- [102] Loo SCJ, Lee WL. Multi-phase microparticles and method of manufacturing multi-phase microparticles. WO Patent WO/2010/140,987, 2010.
- [103] Chu KC, Jordan KJ, Battista JJ, Dyk JV, Rutt BK. Polyvinyl alcohol-Fricke hydrogel and cryogel: two new gel dosimetry systems with low Fe³⁺ diffusion. *Phys Med Biol* 2000;45:955.
- [104] Seah KHR, Garland M, Loo SCJ, Widjaja E. Use of Raman Microscopy and Multivariate Data Analysis to Observe the Biomimetic Growth of Carbonated Hydroxyapatite on Bioactive Glass. *Anal Chem* 2009;81:1442-1449.
-

-
- [105] Widjaja E, Li CZ, Chew W, Garland M. Band target entropy minimization. A general and robust algorithm for pure component spectral recovery. *Anal Chem* 2003;75:4499.
- [106] Widjaja E, Lee WL, Loo SCJ. Application of Raman microscopy to biodegradable double-walled microspheres. *Anal Chem* 2010;82:1277-1282.
- [107] Widjaja E, Garland M. Reverse engineering of multi-layer films. *Mater Today* 2011;14:114-117.
- [108] Lee WL, Foo WL, Widjaja E, Loo SCJ. Manipulation of process parameters to achieve different ternary-phase microparticle configurations. *Acta Biomater* 2010;6:1342-1352.
- [109] Krevelen DW, Hoftyzer PJ. Properties of polymers, their estimation and correlation with chemical structure: Elsevier Science & Technology, 1976.
- [110] Lee WL, Widjaja E, Loo SCJ. One-step fabrication of triple-layered polymeric microparticles with layer localization of drugs as a novel drug-delivery system. *Small* 2010;6:1003-1011.
- [111] Lee WL, Hong M, Widjaja E, Loo SCJ. Formation and degradation of biodegradable triple-layered microparticles. *Macromol Rapid Commun* 2010;31:1193-1200.
- [112] Lee WL, Loei C, Widjaja E, Loo SCJ. Altering the drug release profiles of double-layered ternary-phase microparticles. *J Control Release* 2011;151:229-238.
- [113] Miyajima M, Koshika A, Okada J, Kusai A, Ikeda M. The effects of drug physico-chemical properties on release from copoly (lactic/glycolic acid) matrix. *Int J Pharm* 1998;169:255-263.
- [114] Kim MK, Yuk SH, Jhon MS. Surface and Interfacial Energetic Analysis of Amphiphilic Copolymers. *Bull Korean Chem Soc* 1987;8:159.
-

-
- [115] Thomasin C, Nam-Tran H, Merkle HP, Gander B. Drug microencapsulation by PLA/PLGA coacervation in the light of thermodynamics. 1. Overview and theoretical considerations. *J Pharm Sci* 1998;87:259-268.
- [116] Thomasin C, Merkle HP, Gander B. Drug microencapsulation by PLA/PLGA coacervation in the light of thermodynamics. 2. Parameters determining microsphere formation. *J Pharm Sci* 1998;87:269-275.
- [117] Chen L, Yang J, Wang K, Chen F, Fu Q. Largely improved tensile extensibility of poly (L-lactic acid) by adding poly (caprolactone). *Polym Int* 2010;59:1154-1161.
- [118] Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater* 2003;5:1-16.
- [119] Frank A, Rath SK, Venkatraman SS. Controlled release from bioerodible polymers: effect of drug type and polymer composition. *J Control Release* 2005;102:333-344.
- [120] Raghuvanshi RS, Singh M, Talwar GP. Biodegradable delivery system for single step immunization with tetanus toxoid. *Int J Pharm* 1993;93:1-5.
- [121] Rosca ID, Watari F, Uo M. Microparticle formation and its mechanism in single and double emulsion solvent evaporation. *J Control Release* 2004;99:271-280.
- [122] Chen JL, Yeh MK, Chiang CH. The mechanism of surface-indented protein-loaded PLGA microparticle formation: the effects of salt (NaCl) on the solidification process. *J Microencapsulation* 2004;21:877-888.
- [123] Yeh MK, Chen JL, Chiang CH. Vibrio cholerae-loaded poly (DL lactide co-glycolide) microparticles. *J Microencapsulation* 2002;19:203-212.
- [124] Kenley RA, Lee MO, Mahoney TR, Sanders LM. Poly (lactide-co-glycolide) decomposition kinetics in vivo and in vitro. *Macromolecules* 1987;20:2398-2403.
- [125] Vergnaud JM. Controlled drug release of oral dosage forms: CRC, 1993.
-

- [126] Langer RS, Wise DL. Medical applications of controlled release: CRC Press, Boca Raton, Fla, 1984.
- [127] Crank J. The mathematics of diffusion: Oxford University Press, 1983.
- [128] Tiwari SK, Tzezana R, Zussman E, Venkatraman SS. Optimizing partition-controlled drug release from electrospun core-shell fibers. *Int J Pharm* 2010;392:209-217.
- [129] Baker RW. Controlled release of biologically active agents: John Wiley & Sons, 1987.
- [130] Baker RW, Lonsdale HK. Controlled release: mechanisms and rates: Plenum Press, New York, NY, 1974.

Appendices

List of papers published from the work:

Refereed Journal Articles

1. W.L. Lee, C. Loei, E. Widjaja, S.C.J. Loo, Altering the drug release profiles of double-layered ternary-phase microparticles, *J. Control. Release* 151 (2011) 229-238.
2. W.L. Lee, E. Widjaja, S.C.J. Loo, One-step fabrication of triple-layered polymeric microparticles with layer localization of drugs as a novel drug-delivery system, *Small* 6 (2010) 1003-1011.
3. W.L. Lee, P. Yu, M. Hong, E. Widjaja, S.C.J. Loo, Designing multi-layered particulate systems for tunable drug release profiles, *Acta Biomater.* (2012), *accepted (in press)*.
4. W.L. Lee, M. Hong, E. Widjaja, S.C.J. Loo, Formation and degradation of biodegradable triple-layered microparticles, *Macromol. Rapid Commun.* 31 (2010) 1193-1200.
5. W.L. Lee, W.L. Foo, E. Widjaja, S.C.J. Loo, Manipulation of process parameters to achieve different ternary-phase microparticle configurations, *Acta Biomater.* 6 (2010) 1342-1352.
6. W.L. Lee, E. Widjaja, S.C.J. Loo, Designing drug-loaded multi-layered polymeric microparticles, *J. Mater. Sci.: Mater. Med.* 23 (2012) 81-88.
7. E. Widjaja, W.L. Lee, S.C.J. Loo, Application of Raman microscopy to biodegradable double-walled microspheres, *Anal. Chem.* 82 (2010) 1277-1282.

Patents

1. S.C.J. Loo, W.L. Lee, Multi-phase microparticles and method of manufacturing multi-phase microparticles, **PCT/SG2010/000215** filed on 7 June 2010.