Multi-drug-loaded gastric-floating microcapsules

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MULTI-DRUG-LOADED

GASTRIC-FLOATING MICROCAPSULES

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2014

**MULTI-DRUG-LOADED
GASTRIC-FLOATING MICROCAPSULES**

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Abbreviations

BCS	Biopharmaceutics Classification System
°C	Degree Celsius
Da	Dalton
DCM	Dichloromethane
FDDS	Floating Drug Delivery Systems
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscope
g	gram
GI	Gastrointestinal
GRT	Gastric Residence Time
HIV	Human Immunodeficiency Virus
IMMC	Inter-digestive Migration Myoelectric Cycle
IV	Intrinsic Viscosity
Log P	Partition Coefficient
min	minute
ml	millilitre
MSGF	Modified Simulated Gastric Fluid
MSIF	Modified Simulated Intestinal Fluid
NSAID	non-steroidal anti-inflammatory drug
O/W	Oil-in-Water emulsion
PBS	Phosphate Buffer Solution
PCL	polycaprolactone
PEG	polyethylene glycol
PLA	poly(lactic acid)
PLLA	poly(L-lactide)
PVA	poly(vinyl alcohol)
rpm	rotations per minute
SEM	Scanning Electron Microscope
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
THF	Tetrahydrofuran
UV-vis	Ultraviolet-visible Spectroscopy
v or V	Volume
V%	Volume percentage

w or W	Weight
w%	Weight percentage
W/O/W	Water-in-Oil-in-Water
μm	micrometer

Abstract

Recent advances in floating drug delivery systems (FDDS) have been developed to increase drug bioavailability and reduce patients' dosing frequency and pill burden, by means of an effective sustained release. Nevertheless, with most hydrophobic drugs unable to achieve complete release within the gastrointestinal (GI) residence period (10 hours for FDDS), an understanding of how to promote drug release in such systems is essential. This study explores modified gastro-retentive polymeric floating microcapsule fabricating processes that simultaneously release metformin hydrochloride, a hydrophilic drug, and fenofibrate, a hydrophobic drug, in a controlled manner, seeking to promote the release rate of the hydrophobic drug. A comparable study using a solvent evaporation method to create multi-drug gastric floating microcapsules achieved 86% metformin hydrochloride release and only 20% fenofibrate release within 10 hours. The first process developed in this study using a spray-coating technique could achieve 87% to 100% metformin hydrochloride release within 10 hours and 100% fenofibrate release between 1 to 10 hours by parameter variation. The second process created bi-layer gastric floating microspheres in a single-step process achieving 100% fenofibrate release within 10 hours. The study also explored a fabrication technique where spray-coated gastric floating microcapsules were capable of producing loading dose and maintenance dose.

Chapter 1 Introduction

1.1 Background

In the development of new medicines, delivery is an integral feature of every new drug, where in the present era no drug product enters the market without its own built-in delivery program. Among drug delivery systems, oral delivery encompasses of 60% of the pharmaceutical market [1]. As increasingly new biotechnological therapeutics growing along with new forms of drugs, peptides and proteins offering to treat different diseases, the challenge arises for pharmaceutical scientist on how to effectively and safely administer these drugs via a patient-friendly oral route. At present, oral delivery of drugs is undisputedly the most preferable route of drug delivery due to patient compliance, ease of administration, pain avoidance and flexibility in formulation [2].

Pioneering developments in manufacturing processes, excipients and coating technologies have propelled drug carrier design up the popularity list of oral drug delivery options for the research and development scientist. With the rising number of hydrophobic drugs, highly potent active pharmaceutical ingredients, and health supplements manufactured within drug company pipelines, the potential applications of drug carriers have grown substantially in recent years.

Most oral drug delivery systems are designed into 2 systems: immediate release and modified release systems. The immediate release drug release systems are created to disintegrate rapidly, and provide instant drug release. This lead to a fast increase and decrease in the drug plasma levels, and hence producing sharp fluctuations causing a reduction in drug effectiveness, and in some situations, an increased incidence of side effects [3, 4]. As a result, drug delivery systems of such nature have to be administered

several times per day in order to compensate the decrease in drug plasma concentration from metabolism and excretion [5].

To improve the pharmacokinetic profiles of active pharmaceutical ingredients and patient compliance, as well as reducing side effects, modified release systems were developed. These systems are developed for delayed release, extended release, programmed release and site-specific release, which offer the opportunity to provide constant drug plasma levels over an extended period of time following administration [6]. However, drug absorption in the gastrointestinal (GI) tract may be very short and highly variable in certain circumstances [7]. It is also reported that 40% of drug entities that are recently discovered are indicated to be hydrophobic and have poor aqueous solubility, therefore limiting drug concentration in systemic circulation for the desired pharmacological response [8]. These water-insoluble drugs would cause slow drug absorption leading to inadequate and low bioavailability [9]. Therefore, these conditions may require a rational drug design to lengthen gastrointestinal residence time and increase drug dissolution by structural modification, formulation, and site-specific delivery.

One of the successful methods to achieve a gastric retentive system is the utilization of increased buoyancy to achieve retention. Drug delivery systems, in recent years, have made scientific and technological advances in developing such systems to overcome these physiological adversities, i.e. short gastric residence times (GRT) and unsatisfactory drug absorption [10]. Floating drug delivery systems (FDDS) facilitates the retention of drugs in the stomach, prolonging overall gastrointestinal transit time and improve the oral bioavailability of the drugs providing site-specific absorption from the stomach or upper part of the small intestine[11]. FDDS could incorporate sustained release of drug compounds where the advantages prove much superior to conventional drug delivery

systems where drug level fluctuations leading to sub-therapeutic or toxic concentration could be avoided, thus minimizing the risk of failure of the medical treatment and undesirable side effects [12].

Amongst the broad range of drug delivery systems, the principle of buoyancy offers a simple and practical approach to achieve increased gastric residence time for continuous drug release before it reaches the absorption window, thus ensuring optimal bioavailability [13]. One such example reported by Tang et al. is that addition of vegetable oil into the matrix of an alginate drug carrier could increase the hydrophobicity of the material, thus, increasing the floatability and prolonging the GRT [14]. Furthermore, recent literatures have also designed FDDS available for multiple drug-loading using polyesters in favour of its versatility and biocompatibility. Hence, at present such FDDS are of much interest in the field of oral drug delivery.

Polyesters are a category of polymer which contains an ester functional group in the polymer chain. Polyesters such as poly(lactic acid) (PLLA), and polycaprolactone (PCL), are frequently used for encapsulation of active pharmaceutical agents due to its biocompatibility and biodegradability [15]. These polymers have been used for prolonged drug release and have been made into films [16], nanoparticles [17], microparticles [18], pins used for implants [19], sutures [20], and scaffolds [21]. Recently, polymeric particles have drawn much interest in the field of drug delivery due to its relatively easy fabrication method, versatility in manipulating drug release profiles, and simple administration routes.

With a drug delivery system that comprises a polymer shell made of polyesters, the shell will act as a floating device, storage for drugs, a controlling agent for the release of drugs, and yet still be safe for consumption. By manipulating this shell's size and structure, and

the combined use of vegetable oil and different polymers, an FDDS for controlled release of drugs of different hydrophobicities could be produced. Such a floating gastro-retentive delivery method can potentially improve bioavailability, reduce total drug dosage and minimize drug wastage [11, 22].

1.2 Problem Statement

FDDS have increasingly gain prominence due to its retentive abilities in the gastric system as extensive researches continue to be conducted within the oral drug delivery field. However, researches in FDDS have rarely been conducted beyond single drug formulation. Therefore, even with its gastric retentive capability, this delivery system will meet a limitation in patients with multiple diseases requiring different drug formulations. Furthermore, the lack of multiple drug formulation study also signifies that drugs with different pharmacokinetics profile would have to be prepared in different batches, with each batch formulated exclusively for a single type of drug. As such, patients would still be required to consume multiple capsules or tablets to overcome diseases, and increased cost and time is required to prepare multiple batches of different drug types.

Even while FDDS continue to gain increased implementation for drug delivery, loading drugs of different hydrophobicities into a single dosage form would be difficult. As such, loading both hydrophilic and hydrophobic drugs into a single drug delivery unit and monitoring the controlled release of the drugs is an area worth studying. Moreover, hydrophobic drug absorption persists to be a challenge within the field of oral drug delivery as there has been no study to address slow drug delivery leading to poor drug

absorption in FDDS. Lee et al. have reported the encapsulation of drug-loaded microparticles to create a multi-drug floating capsule, however drug release from fenofibrate, a hydrophobic drug, was slow and could not achieve effective release rates [12]. By developing a system capable of greater drug release of hydrophobic drugs, drug delivery along the GI tract would be more consistent and well-absorbed, producing less drug wastages and better bioavailability. Also, multiple drug-loaded systems run the risk of having drug-drug interaction resulting in a shorter shelf life [23]. As such, most FDDS limits the production of a single-type drug, resulting into patients consuming multiple tablets.

Reports have been made to suggest adding vegetable oil into the alginate matrix of the drug carrier where results have shown to increase the floatability and prolonging the GRT of oral drug delivery systems. However, such studies in polyesters such as PLGA, PLA, or PCL are still in infancy and a study of the effect of vegetable oil has on floatability in these polyesters is important. Hence, this thesis will seek to develop a fabrication technique for floating polymeric microcapsule containing drugs of different hydrophobicities, and also designing a drug delivery device capable of prolonged buoyancy and improved drug release of hydrophobic drugs in simulated gastric fluid.

1.3 Objectives

The aim of this project is to develop multi-drug floating microcapsules suited for drug release in simulated upper gastrointestinal tract. The main objective is to design polymeric microcapsules, containing both hydrophilic and hydrophobic drug compounds within each capsules, and to investigate the release enhancement of hydrophobic drugs for oral pharmaceutical formulation. In fulfilling the main objective, the research work is divided into three parts.

The first part of the study is on the optimization of the polymeric microparticles for improved drug release that is encapsulated within the polymeric microcapsules. By using a different polymer for the microparticles, the study aims to increase drug release rates for hydrophobic drugs. The study will compare the drug release rate from existing literature, the newly proposed polymer for the hydrophobic drug-loaded microparticles encapsulated within the microcapsules, and the free hydrophobic drug encapsulated within the microcapsules.

The second part of the study aims to design and create two new types of microcapsules to increase hydrophobic drug release rates. The first design utilizes a spray-coating technique to portray a versatile drug release system where release rates could be altered based on the parameters used in the spray-coating. The second design creates a one-step bi-layer microcapsules drug delivery system to increase hydrophobic drug release rates fabricated through a solvent evaporation method.

The final part of the study is to employ a spray-coating technique to produce microcapsules capable of providing loading dose and maintenance dose to patients. The

aim of the study is to create a double release system where microcapsules are able to induce a burst release for the loading dose and a sustained release for the maintenance dose for hydrophobic drugs to reach therapeutic levels rapidly and maintain drug plasma levels at an effective level.

1.4 Scope

The scope of this research includes two different fabrication techniques to create 3 different studies. The studies involve the solitary or combinational application of a solvent evaporation technique and a spray-coating technique.

The polymers used in this research are poly(L-lactide) (PLLA), poly(caprolactone) (PCL), and poly(ethylene glycol) (PEG) which are selected for the formation of microparticles and microcapsules, and the coating layers on microcapsules. Drugs included in this research consist of metformin hydrochloride (hydrophilic), fenofibrate (hydrophobic), and piroxicam which are used to monitor drug encapsulation drug release rates from the microcapsules.

The scope of this research will only utilize olive oil to manipulate buoyancy for the gastric floating microcapsules and will focus on the design and morphology of the microstructure, capsule floatability and in vitro drug releases.

Chapter 2 Literature Review

2.1 Gastro-retentive Drug Delivery

Among drug delivery systems, gastro-retentive drug delivery has an important function. The bioavailability for dosage forms which have absorption window in the stomach and small intestine is generally limited due to short residence time thus drug release is often short and incomplete [24]. By retention of oral dosage forms in the upper GI tract would allow prolong contact time of drug with the GI mucosa, leading to higher bioavailability, and hence therapeutic efficacy, reduced time intervals for drug administration, potentially reduced dose size and thus improved patient compliance [25]. Graff et al. reported that mean gastrointestinal time in young and middle-aged subjects to be approximately 5 hours, i.e. 30 mins to 3 hours in the stomach, 90 mins to 9 hours in the small intestine [26].

2.1.1 Factors Affecting Gastric Retention

2.1.1.1 The Gastric Emptying Process

The stomach is anatomically divided into four parts: cardia, fundus, corpus (body) and pylorus. The cardia receives the contents from the esophagus and transports it into the fundus (upper curvature of the stomach) and the corpus (main central region). These 2 sections, called the proximal stomach, serve as a reservoir for ingested contents which are then transported into the distal region, pylorus (lower curvature of the stomach), where it is the major site for mixing and squeezing the contents to facilitate gastric emptying [27].

The digestive work of the stomach is the formation of chime, a thick semifluid mass of partially digested food and digestive secretions, and its transfer to the duodenum [28].

The gastric emptying process follows an inter-digestive series of electrical events, known as the inter-digestive migration myoelectric cycle (IMMC), which occurs in the stomach and small intestine every 2 to 3 hours [25]. Occurrence of these events depends mainly of the state of the stomach: fasted-state or fed state. Onset of IMMC in the fed state is delayed as feeding causes a lag time resulting in a slower gastric emptying rate. The duration for gastric emptying can vary between 30 minutes to 3 hours depending on size, shape, density, caloric content and frequency of intake of the consumption [29-35].

2.1.1.2 The Gastric pH Level

The gastric pH level is an important criterion when selecting a drug substance, excipients, or drug carrier for designing gastro-retentive delivery systems, which may significantly affect the release of the orally administered drug. The gastric pH is influenced by many factors including age, diet, drugs, illnesses, pathological conditions, presence of gases or fatty acids, and other fermentation products [36-39]. The median value of gastric pH reported in fasted state is 1.7, while in the fed state the median gastric pH rose to 6.7 before returning to the fasted state value in less than 2 hours [40].

2.1.2 Gastric Retention Approaches

Delivery systems developed for gastric retention should be able to withstand gastric forces caused by peristaltic movement, i.e. the constant churning and grinding by smooth muscles in the stomach [41]. Gastro-retentive system should also be capable of resisting the gastric emptying process and aim to release the required dosage before being eliminated from the stomach. Some of the most common approaches used to increase the gastric residence time of pharmaceutical dosage forms include the usage of pharmacological agents which slow gastric motility [42, 43], bio-adhesive systems [44-

46], expandable or shape modifying systems [47-50], swelling systems [51-54] and high density controlled systems which are either, high density systems [55-58] or floating systems [12, 59-62].

Bio-adhesive systems involve the use of muco-adhesive polymer which sticks onto the mucous layer secreted by the goblet cells of the stomach forming a gel-like continuous, thin blanket over the mucosal epithelial surface [63]. However, the effectiveness of such a system would weaken due to the constant renewal of mucus along the gastric lining and presence of different soluble material in the gastric juice [64].

Expandable, shape modifying and swelling systems relies on size enlargement to a size larger than the pyloric sphincter, a ring of smooth muscle fibers around the lower opening of the stomach leading into the duodenum, in order to be retained in the stomach for a longer time. Swelling systems are normally produced from the osmotic absorption of water [41, 65]. However, these systems would require rapid and sufficient swelling before the gastric emptying takes place [66].

High density drug delivery systems will sediment at the bottom of the stomach and be retained in the folds of the stomach body below the pyloric region, yet, it is technically difficult to manufacture them with a large amount of drug (more than 50% w/w) and achieve the required density for gastric retention [67].

2.1.3 Floating Systems

A number of buoyant preparations including hollow capsules, granules, microspheres powders, and tablets, have been developed to increase gastrointestinal residence time.

Desai et al. reported a single-unit floating system that was capable of increasing total gastrointestinal residence time up to 10 hours in vivo in a fed-state [68]. However, single-unit floating systems such as hydrodynamically balanced systems and floating tablets are unreliable and irreproducible for prolonged gastric residence time due to the all or nothing emptying process [69]. On the other hand, multiple unit dosage forms, such as hollow microsphere, powder, and capsules, are more suitable since they could reduce the inter- and intra-subject variability in absorption and reduce the probability of dose dumping [70].

Hollow microspheres were developed by Kawashima et al. [69, 71] consisting of Eudragit S as an enteric polymer, and the drug loaded in the outer polymeric shell by an emulsion solvent diffusion method. The drug release profiles from the microspheres exhibited enteric behavior, and by changing the ratio of polymer to drug in the microspheres drug release rates could be altered. However, many drugs were not released from this type of microparticles at the pH of gastric fluids [72]. The system was therefore modified, by the addition of nonvolatile oil to the dispersed phase [73] or using Eudragit S/RL [74]. In a separate experiment, Lee et al. successfully encapsulated a multi-drug loaded floating microcapsule consisting of free metformin hydrochloride, metoprolol-loaded polylactide-co-glycolide (PLGA) particles and fenofibrate-loaded polycaprolactone (PCL) particles into microcapsules. However, fenofibrate release from capsules was low and far from achieving complete release in the GI tract [12]. While many multi-unit floating systems have been developed, to date there has been no study to address slow drug delivery leading to poor drug release in FDDS.

2.1.4 Fabricating Floating Microcapsules for Loading Dose and Maintenance Dose

Patients suffering from arthritis, sciatica, or cardiac arrest, normally require a loading dose of anti-inflammatory or pain relief drug to achieve rapid therapeutic dosage levels and a maintenance dose to sustain effective therapeutic conditions without reaching toxic levels [75]. Furthermore, in producing a microcapsule capable of sustained-release, it removes the hassle of frequent administering of maintenance dose, thus improving patient compliance. Joseph et al. conducted a study of piroxicam in rabbits and have reported that a combination of loading dose and maintenance dose will result in enhanced drug bioavailability. Furthermore, the study suggested that by having the combined dose, elderly patients could overcome drug variability in steady state levels due to erratic elimination of the drug [76].

2.2 Materials Background

The floating drug delivery system developed in this project is made up of a polymeric capsule loaded with free drug compound i) within the cavity of the polymeric capsule, ii) within the wall of the polymeric capsule, iii) within the surface coating on the polymeric capsule. The capsule is made up of PCL and poly-L-lactide (PLLA), while the surface coating is made up of PCL and/or polyethylene glycol (PEG). The drugs used are metformin hydrochloride, fenofibrate and piroxicam.

2.2.1 Polymers

2.2.1.1 Poly-L-lactide

PLLA is an FDA-approved biodegradable polymer, resulting from polymerization of L,L-lactide in which the monomers are linked by ester bonds. The polymer undergoes hydrolytic degradation of the ester bonds and degrades slowly in humid conditions above its glass transition temperature of 60 to 65°C [77]. PLLA can be fabricated using ring opening polymerization of lactide or a direct condensation of individual monomers. Polylactide has two stereoisomers - poly(D-lactide) and poly(L-lactide) - where PLLA is of higher crystallinity [78]. The presence of the methyl groups in its monomers provides steric hindrance towards penetrating water molecules, thus enabling it to have a hydrophobic nature [79]. PLLA is chosen as a viable option for the shell of the capsule due to its crystallinity and hydrophobicity, leading to a longer degradation time to be used for sustaining the release of easily water-soluble hydrophilic and amphiphilic drugs.

Figure 1: Formula structure of PLLA

2.2.1.2 Polycaprolactone

PCL is a biodegradable, semi-crystalline polyester having a low melting point of 60°C and a low glass transition temperature of – 60°C which causes it to be rubbery at room temperature [80]. PCL is typically prepared via ring opening polymerization of cyclic ester of ϵ -caprolactone using stannous octoate as the catalyst [81]. PCL undergoes chain scission of the ester bonds through hydrolysis and degrades slowly owing to its low ester bonds to carbon ratios resulting in a degradation rate that is even lower than PLLA [82]. However, its rubbery state above its glass transition temperature allows the polymer to be implemented in the microcapsule shell to facilitate and increase the rate of release of hydrophobic drugs.

Figure 2: Formula structure of PCL

2.2.1.3 Polyethylene glycol

PEG is an FDA-approved biodegradable polyether resulting from the polymerization of ethylene oxide. PEG has a wide range of physical properties due to its availability in different molecular weights and chain length. Depending on these properties, PEG could be in the form of liquid or low-melting point solids. PEG are amphiphilic and soluble in water as well as in many organic solvents (i.e. ethanol, acetone, and chloroform). Low molecular weight PEG, approximately 1kDa, are viscous and colorless liquids, while higher molecular weight PEG are waxy, white solids with melting points proportional to their molecular weights to an upper limit of about 67 °C [83]. Lower-molecular-weight PEGs are usually purer oligomers, often referred to as monodisperse, uniform or discrete. Very high purity PEG has recently been shown to be crystalline [84]. PEG could be removed from the body unaltered with clearance rates inversely proportional to polymer molecular weight [85]. This enables PEG with a wide range of applications in the biomedical industry where PEG have been used as an excipient, film coatings, lubricants, tablet binders and tissue engineering scaffolds [86, 87].

Figure 3: Formula structure of PEG

2.2.2 Drug Selection

2.2.2.1 Fenofibrate

Fenofibrate is a drug of the fibrate group used to help reduce cholesterol and triglycerides (fatty acids) in the blood. High levels of these types of fat in the blood are associated with

an increased risk of atherosclerosis (clogged arteries). Fenofibrate is used to treat high cholesterol and high triglyceride levels – patients at risk of coronary heart diseases [88]. According to biopharmaceutics classification system (BCS), fenofibrate is a class II drug (low solubility–high permeability) which has a very high log P of 5.24, making it practically insoluble in water (aqueous solubility < 0.5 mg/l), and has a half-life of 20 hours [89]. Due to its hydrophobic nature of a poorly soluble drug, fenofibrate dissolves only in organic solvents such as dichloromethane, ethyl acetate and hexane. The bioavailability of fenofibrate is approximately 69% at stomach, 73% at proximal small bowel, 66% at distal small bowel and only 22% at the colon [90].

Figure 4: Formula structure of fenofibrate

2.2.2.2 Metformin Hydrochloride

Metformin hydrochloride is an oral anti-hyperglycemic agent, a first-line drug of choice used in treatment of diabetes mellitus type 2, formerly known as non-insulin-dependent diabetes mellitus [91]. According to BCS, metformin hydrochloride is a class III drug (high solubility-low permeability) which has a log P of –1.43 with water solubility of 138 mg/ml, making it highly hydrophilic, and also having a half-life of approximately 6.2 hours [92]. The absolute oral bioavailability of metformin hydrochloride is about 40 to 60% with a complete gastrointestinal absorption within 6 hours after consumption [93].

Figure 5: Formula structure of metformin hydrochloride

2.2.2.2 Piroxicam

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class used to relieve postoperative pain, tenderness, swelling and stiffness caused by rheumatoid and osteoarthritis (arthritis caused by a breakdown of the lining of the joints), primary dysmenorrhea (menstrual cramps); and act as a painkiller, especially where there is an inflammatory component [94-96]. According to BCS, piroxicam is a class II drug (low solubility–high permeability) which has a high log P of 3.6 with water solubility of 23 mg/l, making it highly hydrophobic, and also having a half-life of approximately 37.5 hours [94, 97]. Solubility of piroxicam is also known to increase when pH of the solvent reaches above its pKa values. Ahmed et al. reported an increase in solubility of piroxicam from 0.0281 ± 0.001546 %w/w in SGF to 0.0281 ± 0.001546 %w/w in SIF [98].

Oral bioavailability studies have indicated that piroxicam experiences high permeability in the GI tract and undergoes complete GI absorption [76, 99]. However, piroxicam can induce damage to the walls of the final part of the digestive system, i.e. cecum, colon, rectum [100]. Therefore, drug delivery systems have to be designed to release drugs in the upper GI tract.

Figure 6: Formula structure of piroxicam

2.3 Fabrication Method

The floating microcapsules in this research study involve three fabrication parts: drug-loaded microparticles, floating microcapsules and drug-loaded coating layer. Microparticles are first fabricated with drugs and subsequently encapsulated into microcapsules. Both microparticles and microcapsules are prepared by a solvent evaporation method. Depending on the design of the experiment, some of these microcapsules are further spray coated with drug-loaded layer(s) (Figure 7).

Figure 7: Schematic design of a spray-coated microcapsule with microparticles encapsulated within

2.3.1 Microparticles

The formation of microparticles in this thesis utilizes an oil-in-water emulsion (O/W) and a solvent evaporation method. The emulsion consists of a mixture of two immiscible liquids, an oil phase and an aqueous phase. The oil phase is made up of polymer and drugs that will dissolve in a volatile solvent, such as dichloromethane, to facilitate evaporation. The water is made up of distilled water and a surfactant which will stabilize

the oil phase. The emulsion is stirred until all the oil solvent evaporates, allowing the polymer to solidify forming particles with drug encapsulated within [101]. Oil-in-water emulsion (O/W) is generally the simplest form of emulsion solvent evaporation technique. Residual solvents in microparticles preparations are associated with toxicological risks and may be retained in the microparticles as a residual organic volatile impurity. Bitz and Doelker used a rotary evaporator to remove the dichloromethane from the microparticles and researchers found that the level of residual dichloromethane was below the recommended 500 ppm for all the samples tested [102, 103].

2.3.2 Floating Microcapsules

The concept of floatation of polymeric microcapsule utilized in the study generally revolves around three factors: 1) forming an internal hollow cavity, 2) making the shell more hydrophobic, and 3) lowering the density of the shell. The approach used to obtain hollow cavities within a capsule is by using a water-in-oil-in-water (W1/O/W2) double emulsion solvent evaporation method. The internal aqueous phase is made up of water (W1), emulsified in an oil phase (O) made up of polymer in a volatile solvent for easy evaporation, which is subsequently emulsified in a continuous phase of water and surfactant (W2) where the surfactant is used for stabilizing the O/W emulsion. Murata et. al increased floatability of alginate beads by adding vegetable oil (olive oil) into the beads [104]. The increased hydrophobicity from the presence of olive oil within the hydrophilic alginate further promotes buoyancy of the beads. Yoo et al. first form a low density thin polymeric shell hollow microsphere by using vegetable oil, allowing the microspheres to float [105]. Lee et.al. made a similar study in polyesters to investigate the effect of olive oil in the formation of hollow microspheres where between 0.01 ml and 0.1 ml of olive oil per 300 mg of capsule polymer is the most ideal range for fabricating hollow-structured floating microspheres [12].

2.3.3 Drug-loaded Coating Layer

A novel way to strategize improved drug delivery of hydrophobic drugs is to localize them onto the surface of the microcapsules instead of encapsulating them within the capsules. Coating layers have been commonly used in studies such as to provide extended drug release, increase density of the drug carriers in high density gastric retention systems, coating of bioadhesives, or serve as a protection to activate site-specific delivery in oral drug delivery systems [106-109]. Ichikawa et al. developed a floating minicapsule containing granules that consisted of a core containing drug. The granules were coated with double layers with the inner coat a foamable layer and the outer layer an expansive polymer film [110]. While, there have been many studies reporting of a coating layer on oral drug delivery systems, none have reported the use of drug-loaded polymeric coating on these systems.

2.3.3 Emulsion Solvent Evaporation Method

Emulsion solvent evaporation is widely used to form microspheres. An emulsion requires a mixture of two immiscible liquids. Emulsion solvent evaporation process used for creating microspheres usually begins by dissolving or dispersing drugs in a volatile organic solvent, e.g. dichloromethane, for rapid evaporation, together with the drug-carrier forming material (polymer). This organic phase is then emulsified with another continuous phase, i.e. an aqueous medium, which is immiscible with the organic phase. Addition of surfactants is normally required to stabilize dispersed phase due to stirring motion. This stirring motion causes the organic solvent to be extracted from the dispersed phase and hence, solidifying these dispersed phase droplets into microspheres. Depending on the solvent involved, the microspheres dried and collected after oven drying or freeze drying [111].

Emulsion solvent evaporation technique is one of the most regular methods used as it is simple and cost efficient, and does not require a coacervating agent for phase separation or high temperature for solidification. However, problems with drug encapsulation and rate of solvent evaporation, are common problems encountered. Therefore it is essential to control fabrication parameters accordingly such as polymer solution concentration, stirring speed and oil-to-water ratio to tackle such problems. In addition, these parameters are also useful when tweaking size, morphology and the yield of microspheres [112-114].

2.4 Characterization of Sample

2.4.1 Morphology

The most fundamental approach to characterize sample morphology is by using imaging microscopy analysis obtain sample's surface topography and composition. A scanning electron microscope (SEM) is the most common instrument used for imaging microscopy analysis. Samples are coated with gold to be electrically conductive and to prevent accumulation of electrostatic charge, which disrupts the electron imaging. Electron imaging requiring nano-scale resolution would require a field emission scanning electron microscope (FESEM).

Analysis of the internal morphology of microspheres is done by slicing and cross-sectioning at the centerline of the sample with a razor blade [23, 115]. Samples which are soft or are easily flattened may be placed in liquid nitrogen for hardening to ease the slicing process. Alternatively, to aid in the slicing, microparticles can be placed on the sample holder, and then submerged in liquid nitrogen [116]. This will lower the temperature of the sample below the glass transition temperature, making it brittle and easy to slice.

2.4.2 Drug Loading and Distribution

For every drug delivery system, amount of active drug loaded is required to be determined to ensure dosage lie within the therapeutic window, i.e. a safe and effective range of drug dosage to treat diseases without reaching the toxic limit. Jain et al. demonstrated a drug extraction method of determining actual drug loading in Eudragit S microspheres where it was dissolved and filtrated through a membrane filter, and having the drug concentration determined spectrophotometrically by ultraviolet-visible

spectroscopy (UV-Vis) [22]. Another study conducted by Lee et al. determined the weight of drugs by using thermogravimetric analysis (TGA). By increasing the temperature, various components of the microsphere were decomposed and the weight percentage of each resulting mass change was measured. TGA could be used as an effective measurement in the event that the decomposition temperature of drug and the polymer are not similar.

Detection of drug loading for drugs, i.e. metformin hydrochloride, fenofibrate and piroxicam used in this research study would require several preparation steps. One of the simplest methods for such quantitative analysis is by using a UV-Vis spectrophotometer. Since, the three drugs used in this experiment contain molecules containing π -electrons or non-bonding electrons, they can absorb the energy in the form of ultraviolet or visible light from the spectrophotometer to detect drug concentration. The drug has to be separated from the polymer matrix using a suitable solvent (e.g. tetrahydrofuran), and subsequently placed into the medium where the drug solution would be analysed (e.g. simulated gastric fluid), and detected at a certain wavelength (e.g. 299nm for fenofibrate). A series of diluted concentration of the drug in the medium has to be measured to construct a calibration curve and serve for drug concentration measurement.

Swamy et al. prepared metformin hydrochloride to be tested for concentration level by UV-Vis through placing microspheres containing the drug into phosphate buffer solution (PBS) at 37°C for 48 hours, and then centrifuged it at 10,000 rpm to detect the drug concentration in PBS [117]. UV-Vis absorbance was determined using a spectrophotometer at the wavelength 230 nm. Ren et al. determined the drug loading concentration of fenofibrate in microparticles by dissolving a known amount of fenofibrate in ethanol and monitoring UV-Vis absorbance value at 286 nm [118]. Joseph et al. measured

drug entrapment in polycarbonate microspheres by dissolving them in dichloromethane and placing the solution in hydrochloric acid. The mixture is stirred for 6 hours and set to evaporate dichloromethane at 60°C. The mixture was filtered and the filtrate was measured at around 354 nm using the UV-Vis spectrophotometer [76].

Distribution of drugs within microspheres could be detected by several methods. One of the methods effectively used to provide visual indication of the distribution is through Raman microscopy. Effedi et al. performed Raman mapping measurements on double walled microspheres assisted with band-target entropy minimization to determine spatial distribution within the walls of the microspheres. The results showed that by combining Raman microscopy and band-target entropy minimization analysis, Raman mapping can serve as a sensitive characterization tool as it can give very specific information on the chemical species present and its spatial distribution [119].

2.5 In Vitro Analysis of Gastric Floating Microcapsules

To evaluate the performance of gastric floating microcapsules, studies should be conducted in vitro to monitor the features of the delivery system. With these in vitro studies, buoyancy and the rate of drug release of the microcapsules can be determined. Usually, oral drug delivery studies suited for release in the GI tract is conducted in simulated gastric fluid and simulated intestinal fluid to provide a closer representation of in vivo conditions. The simulated gastric fluid is fixed at about pH 1.2 comprising of HCl solution (0.1 mol/L) and Tween 20 (0.02 % w/v), while simulated intestinal fluid is fixed at about pH 6.8 using phosphate buffer solution (PBS). Samples are measured with known values and placed into the medium at 37°C and agitated between 50 and 250 rpm for 12 to 24 hours, with the medium extracted for measurement at selected time points [12, 14, 23, 120]. Through buoyancy and drug release tests, the floating microcapsule could be optimized to suit different drugs, conditions and to tackle different diseases.

2.5.1 Buoyancy Test

Several methods have been used for in vitro buoyancy testing. One such method is the percentage buoyancy test reported by Najmuddin et al. where samples were stirred for 12 hours, with the buoyant microspheres pipetted out and the sunk microspheres separated by filtration. Both sets of microspheres type were dried at 40°C overnight. The weight of each sample batch was then measured and the buoyancy of each respective sample was determined by the weight ratio of the floating microspheres to the sum of floating and sunk microspheres [121]. This percentage buoyancy can be calculated using the following equation:

$$\% \text{ Buoyancy} = \frac{W_f}{(W_f + W_s)} \times 100 \%$$

Where, W_f and W_s is the weight of floating and sunk microspheres respectively.

Another percentage buoyancy test conducted by Tang et al. determined buoyancy by counting the number of microparticles [14]. This percentage buoyancy can be calculated using the following equation:

$$\% \text{ Buoyancy} = \frac{N_f}{(N_f + N_s)} \times 100 \%$$

Where, N_f and N_s are the number of floating and sunk microspheres respectively.

This method introduced by Tang et al, is only applicable to microspheres that are large enough to be counted individually by eyesight.

2.6 In Vitro Drug Release

There have been many drawbacks with the usage of conventional oral drug delivery systems where there is a lack of controlled release, rapid GI tract transit period, lack of multi-drug release feature, and the inability to provide site-specific drug release. The experiments in this thesis aim to provide new designs on microcapsules that are capable of tackling such problems. Hence, it is important to evaluate available literature on current oral drug delivery systems that are feasible to be implemented.

2.6.1 Material Aspect

In the pursuit of controlled drug release, a multitude of experiments have involved using biocompatible polymers as a drug carrier for oral drug delivery. Hydrophilic drugs used in microspheres have been known to provide rapid initial drug release while hydrophobic drugs have normally shown retarded drug release results [122, 123]. Therefore, material selections in drug delivery experiments are important criteria based on the hydrophobicity of the drug used.

Reza et al. conducted a study to investigate the relationship between hydrophilic and hydrophobic types of polymers and their content level on the release profile of drug from matrix systems. It was reported that higher polymeric content in the matrix decreased the release rate of drug because of increased tortuosity and decreased porosity, while at lower polymeric level, the rate and extent of drug release was elevated. The report included a comparative study where carnauba wax had the slowest rate of drug release, Hydroxypropyl methylcellulose had the fastest drug release and Kollidon SR had an intermediate release profile. Release rate was also found to be the function of physico-

chemical nature of drug molecule. Theophylline and diltiazem HCl, being more hydrophilic, released faster than diclofenac sodium in all matrix systems [124].

2.6.2 Fabrication Parameters

Secondary factors such as amount of drug loading, microsphere size, precipitation kinetics and drug distribution could also affect drug release rate. One such example was reported by Bodmeier et al, where different amount of drug loading of quinidine was stored in polylactide microsphere and were monitored for their rate of release. In the study, it was reported that the microspheres with lower drug loading would result in a longer period of sustained release. It was reasoned that in highly loaded microspheres, the drug will diffuse into the release medium, leaving empty pores on interconnected channels. Subsequently, when drug within these pores have been dissolved, the drug release continued from regions with less accessibility [125]. In another study, Berklund et al. reported that piroxicam-loaded microspheres showed that decreasing microsphere size, would lead to a more uniform drug distribution, resulting in a prolonged drug release [126].

2.6.3 Design

Design of drug carrier is also another important criterion for drug release studies. Solid-single layer microspheres have illustrated rapid drug release especially with hydrophilic drugs [125] while some hydrophobic drugs in single-layered microspheres were still able to retard its release because the solubility of hydrophobic drugs in the release medium is not high. Khung et al. demonstrated this in single-layered particles, where ibuprofen showed an initial burst release of 95% compared to the initial release of 20% of the drug in encapsulated microparticles in hollow core microcapsules [127].

Hollow core microcapsules have been utilized as a drug delivery system due to the air contained within the empty cavity which allows the drug carrier to float in the stomach. Sato et al. investigated loading different hydrophilic drugs in the shell matrix of floating hollow microballoons of different polymer types (Eudragit, ethylcellulose, hydroxypropylmethylcellulose, and hydroxypropylmethylcellulosephthalate) [23]. However, by localizing the drugs within the shell matrix in the microballoons, drugs that are contained within the macropores of the matrix would be dissolved in the release medium, allowing the medium to penetrate into the microballoons easily, resulting in reduction of buoyancy. This behaviour was further highlighted when there was a burst release of Riboflavin, causing rapid decrease of buoyancy. Soppimath et al. further demonstrated that controlling the release of drugs could be done by altering the polymer type for the shell of the hollow microspheres [128].

The foundation of the drug delivery system in this project was largely based on Lee et al.'s work in microencapsulation of drug-loaded microparticles [12]. Model drugs of metformin hydrochloride, metoprolol tartrate and fenofibrate were loaded in polymers to create microparticles, where they were further encapsulated in PLGA, PLLA and hybrid PCL-PLLA microcapsules. A comparison between the different polymer-types used to fabricate different microcapsules showed drastic differences in the drug release of in those drug delivery systems. The PLGA microcapsules demonstrated burst release (95% in the first hour) for metformin hydrochloride, a hydrophilic drug, which causes a problem for sustained release for the purpose of generating higher bioavailability. On the other hand, PLLA and hybrid PCL-PLLA microcapsules suppressed release rates for fenofibrate (5% and 20% respectively in 10 hours), a hydrophobic drug, to a degree which causes insufficient drug release. Therefore, new designs to the drug delivery

system have to be implemented to control the release rates of both hydrophilic and hydrophobic drugs effectively in a sustained manner.

Yadav et al. reported a melt granulation technique which could be used to improve the solubility and dissolution characteristics of hydrophobic drugs. It used a process by which fenofibrate powder was agglomerated by meltable polymers and surfactants to create granules. The study reported the use of polyethylene glycol (PEG) and a surfactant, poloxomers-407. The drug release result showed a marked increase in the solubility and in vitro dissolution profiles of the melt granules compared to the free drug [129]. By applying this technique to the microparticles used in this thesis, we may observe a substantial enhancement in the drug release rate for the hydrophobic drug.

Roland et al. produced a controlled release delivery system within the intestines, where the calcium alginate microparticles were dip-coated with cellulose acetate phthalate as calcium alginate were susceptible to acidic dissolution medium and would disintegrate when the microparticles transit through the gastric system [107]. These coating layers called enteric coating, avoid drug delivery in the upper GIT and target drug delivery into the large intestinal regions, giving rise to new systems where drug delivery could be time and pH dependent [130-132]. A similar design could be explored by using a pH dependent polymer to be loaded with hydrophobic drugs that dissolves rapidly when it comes in contact with gastric fluid in order to promote the release rate for fenofibrate used in this thesis.

There have been many studies reporting the drug release of single-layered particles and hollow microcapsules, however, no study has been done to address slow drug delivery leading to poor drug absorption in FDDS, thus a need to explore a different ways to create

a drug delivery system suitable for sustained drug release for both hydrophilic and hydrophobic drugs in the GI tract.

Chapter 3 Materials and Method

3.1 Materials

Poly (L-lactide) (PLLA) (IV: 2.4, Purac), polycaprolactone (PCL) (molecular weight: 10 kDa, Sigma-Aldrich), poly ethylene glycol (PEG) (molecular weight: 10 kDa, Sigma-Aldrich), poly(vinyl alcohol) (PVA) (molecular weight: 30-70 kDa, Sigma-Aldrich) were used without further alteration. Poloxamer 127 (Pluronic F-68) were procured from Sigma-Aldrich. Drugs such as metformin hydrochloride, piroxicam and fenofibrate were purchased from Sigma-Aldrich. Solvents such as dichloromethane (DCM), ethyl acetate, and n-hexane were from Tedia Co., Inc., Sigma Aldrich and Fluka respectively. Phosphate-buffered saline (PBS) solution (pH 7.4) was purchased from OHME Singapore, was also used as the simulated intestinal fluid (SIF). All drugs and solvents were used as received, unless otherwise noted. The simulated gastric fluid (SGF) at about pH 1.2 composed of HCl solution (0.1 mol/L) and Tween 20 (0.02 % w/v), both from Sigma-Aldrich. The SGF was further modified by adding cremophor EL (4 % w/v) from Sigma-Aldrich (Modified SGF). The SIF was also further modified by adding cremophor EL (4 % w/v) and Tween 80 (0.5 % w/v).

3.2 Fabrication

3.2.1 Multi-drug Loaded Gastric Floating Microcapsules

3.2.1.1 Altered Fenofibrate by Melt Granulation Technique

A mixture of 0.2 g of fenofibrate with 0.2 g of surfactant, poloxamer-127 was grinded using a mortar and pestle for 10 min. The mixture was then placed in hot petri dish and heated at around 60°C in a temperature controlled water bath so as to melt the surfactant in which the drug was dispersed for 1 min. The formed molted mass is then cooled to room temperature and at the end of this granulation process, the granules were allowed to solidify at room temperature by spreading them in thin layers on microscope glass plates. The cooled granules were collected and stored in sealed bags and stored in the dessicator.

3.2.1.2 Drug-loaded Microparticles

Fenofibrate-loaded microparticles were fabricated using oil-in-water (O/W) emulsion. 0.2 g PCL and 0.07 g of fenofibrate were added to 2.85 ml of DCM. The polymer/drug solution was poured into 50 ml of PVA solution (4 % w/v), and the mixture was subjected to overhead stirring at 2000 rpm for 4 min, followed by quickly transferring to the rotary evaporator at ambient temperature (25°C) for solvent evaporation. The particles were collected by centrifugation, washed with de-ionized water, and then freeze dried. Altered fenofibrate-loaded microparticles used 0.18 g of melt fenofibrate granules instead of the free drug.

3.2.1.2 Encapsulation of Drug-loaded Microparticles and Free Metformin

Hydrochloride in Microcapsules

The pre-fabricated fenofibrate-loaded microparticles were first ground using a mortar and pestle then suspended in 5 ml of deionized water containing polyvinyl alcohol (PVA) (4 %

w/v). The suspension was placed under vortex until homogenous, followed by centrifugation and removal of supernatant. The wet microparticles were ground again and subsequently suspended again into 1 ml of PVA solution (0.1 % w/v) containing 0.1 g of metformin hydrochloride. The particles/water suspension was made homogenous and then added drop-wise into a PLLA-PCL solution (PLLA-PCL 75wt%:25wt% 0.3g PLLA, 0.09g PCL dissolved in 5 ml of DCM) and followed by 0.01 ml olive oil under magnetic stirring at 700 rpm. The mixture was emulsified in a 50 ml PVA aqueous solution (0.25 % w/v) containing 1 ml DCM under overhead stirring speed of 250rpm for 4 min. The emulsion droplet was poured into 150 ml PVA solution (0.25 % w/v) and transferred to a rotary evaporator at ambient temperature (25°C) for the solidification of microcapsules. The microcapsules fabricated were rinsed with deionized water 3 times and freeze-dried.

3.2.2 Spray-coated Gastric Floating Microcapsules

Encapsulation of free metformin hydrochloride in microcapsules was similarly prepared without fenofibrate-loaded particles as described above.

3.2.2.1 Spray-coating of Drug-loaded Layer on Microcapsules

The microcapsules (0.1g) were spray-coated using a MediCoat DES 1000 Benchtop Stent Coating System (Sono-TekCorp., Milton, NY) under a clean hood environment and then coated with the configuration as stated in table 1. Power for the frequency generator was set at 1.4 W and air shroud level was set at 2 psi.

Table 1: Configuration for fenofibrate spray-coating

Sample	1st Coating Layer		2nd Coating Layer		3rd Coating Layer	
	Solution	Flow Rate (ml/min)	Solution	Flow Rate (ml/min)	Solution	Flow Rate (ml/min)
B1	PCL: 0.1 g Feno: 0.014 g DCM: 1 ml	0.5	-	-	-	-
B2	DCM: 1 ml	0.5	PCL: 0.1 g Feno: 0.014 g DCM: 1 ml	1	-	-
B3	DCM: 1 ml	0.5	PCL: 0.1 g Feno: 0.014 g DCM: 1 ml	0.2	PCL: 0.1 g DCM: 1 ml	0.2

3.2.3 Bi-Layer Gastric Floating Microcapsules

1 ml of PVA solution (0.1 % w/v) containing 0.1 g of metformin hydrochloride was made homogenous by vortex. The solution was added drop-wise into a PLLA-PCL solution (PLLA-PCL 75wt%:25wt% 0.3 g PLLA, 0.09 g PCL dissolved in 3 ml of DCM) and followed by 0.01 ml olive oil under magnetic stirring at 700 rpm for 1 minute. The mixture was poured into another polymer solution (refer to Table 2 for concentration variations) added with 0.0196 g fenofibrate and magnetic stirred at 700 rpm for 1 minute. The mixture was emulsified in a 50 ml PVA aqueous solution (0.25 % w/v) containing 1 ml DCM under overhead stirring speed of 220 rpm for 4 min. The emulsion droplet was poured into 150 ml PVA solution (0.25 % w/v) and transferred to a rotary evaporator fixed at ambient temperature (25°C) for the solidification of microcapsules. The microcapsules fabricated were rinsed with deionized water 3 times and freeze-dried.

Table 2: Concentration variation for second polymer solution

Polymer Solution		
C1	C2	C3
PCL: 0.2 g Ethyl Acetate: 5 ml	PCL: 0.4 g Ethyl Acetate: 6 ml	PCL: 0.4 g Ethyl Acetate: 6 ml Olive Oil: 0.01 ml

3.2.4 Gastric Floating Microcapsules for Loading Dose and Maintenance Dose

1 ml PVA aqueous solution (0.25 % w/v) was added drop-wise into a PLLA-PCL solution (PLLA-PCL 75wt%:25wt% 0.3g PLLA, 0.09g PCL dissolved in 5 ml of DCM) and followed by 0.01 ml olive oil under magnetic stirring at 700 rpm. The mixture was emulsified in a 50 ml PVA aqueous solution (0.25 % w/v) containing 1 ml DCM under overhead stirring speed of 250rpm for 4 min. The emulsion droplet was poured into 150 ml PVA solution (0.25 % w/v) and transferred to a rotary evaporator at ambient temperature (25°C) for the solidification of microcapsules. The hollow microcapsules fabricated were rinsed with deionized water 3 times and freeze-dried.

The microcapsules (0.1 g) were then spray-coated using a MediCoat DES 1000 Benchtop Stent Coating System (Sono-TekCorp., Milton, NY) under a clean hood environment and then coated with the configuration as stated in table 3. Power for the frequency generator was set at 1.4 W and air shroud level was set at 2 psi.

Table 3: Configuration for Piroxicam spray-coating

Sample	1st Coating Layer		2nd Coating Layer		3rd Coating Layer	
	Solution	Flow Rate (ml/min)	Solution	Flow Rate (ml/min)	Solution	Flow Rate (ml/min)
D1	DCM: 1 ml	0.5	PEG: 0.1 g Pirox: 0.014 g DCM: 1 ml	0.2	-	-
D2	DCM: 1 ml	0.5	PCL: 0.1 g Pirox: 0.082 g DCM: 1 ml	0.2	-	-
D3	DCM: 1 ml	0.5	PCL: 0.1 g Pirox: 0.082 g DCM: 1 ml	0.2	PEG: 0.1 g Pirox: 0.021 g DCM: 1 ml	0.2

3.3 Characterization

3.3.1 Morphological Analysis

The surface and internal structure of the microcapsule were analyzed using scanning electron microscopy (SEM) (JEOL JSM-6360A) at an operating voltage of 5kV. Prior to analysis, the samples were first mounted on a metal stub and cross-sectioned at their centre with a metal blade. Samples were coated with gold using an SPI-Module sputter coater before analysis.

3.3.2 Determination of Actual Drug Loading

3.3.2.1 Metformin Hydrochloride

10 mg of microcapsule (n=2) was dissolved in 1 ml DCM by sonication. The drugs were extracted by adding 10 ml PBS where metformin hydrochloride is completely partitioned into PBS. Drug content in PBS was analyzed using a UV-Vis spectrophotometer (Shimadzu UV-250) at wavelength 233 nm.

3.3.2.2 Fenofibrate

10 mg of microcapsule (n=2) was dissolved in 1 ml DCM, followed by the addition of 5 ml n-hexane to precipitate polymers and metformin hydrochloride, leaving only fenofibrate dissolved within the DCM/hexane mixture. The suspension was centrifuged and the supernatant was extracted and left to dry in a bottle. Then the dried content was re-dissolved in modified SGF. Drug content in modified SGF was analyzed using a UV-Vis spectrophotometer (Shimadzu UV-250) at wavelength 299 nm.

3.3.2.3 Piroxicam

10 mg of microcapsule (n=2) was dissolved in 1 ml DCM, followed by the addition of 10 ml n-hexane to precipitate polymers and metformin hydrochloride, leaving only piroxicam dissolved within the DCM/hexane mixture. The suspension was centrifuged and the supernatant was extracted and left to dry in a bottle. Then the dried content was re-dissolved in modified SGF. Drug content in modified SGF was analyzed using a UV-Vis spectrophotometer (Shimadzu UV-250) at wavelength 335 nm.

3.3.3 Presence of Double Layer in Microcapsule

The method used to confirm the presence of a double layer in the capsule shell is based on the solubility differences of polymers in THF. PLLA is not soluble in THF, while PCL is soluble in THF. The microcapsules were immersed under agitation overnight in THF to dissolve PCL polymers. The samples were then collected for SEM imaging.

3.4 In vitro Buoyancy Test

The microcapsules in duplicate sets were dispersed in 20 mL test bottles filled with 15 mL of SGF. The bottles were immersed in a 37°C water bath and agitated by magnetic stirring at 250 rpm. After 24 hours, the number of microcapsules still floating was visually determined. The microcapsule buoyancy can be calculated with the following equation:

$$\% \text{ Buoyancy} = \frac{N_{float}}{(N_{total})} \times 100 \%$$

Where, N_{float} and N_{total} are the number of floating and total microcapsules respectively.

3.5 In vitro Drug Release

3.5.1 Drug Release in SGF

Duplicate of 10 mg of microcapsules were placed in 20 ml vials containing 10 ml SGF. Another duplicate set was prepared containing modified SGF. Both sets were maintained at 37°C in a water bath and stirred at 250 rpm. At different intervals within 24 hours, 1 ml of the mediums from each vial was drawn and fresh medium was added back. The collected SGF were analyzed using UV-Vis spectrometer (UV-2501, Shimadzu) at 233 nm for the release of metformin while the collected modified SGF were analyzed at 299 nm for the release of fenofibrate.

3.5.2 Drug Release in SGF and SIF

Duplicate of 10 mg of microcapsules were placed in 20 ml vials containing 10 ml of drug release medium simulating the gastric condition. Both vials were maintained at 37°C in a water bath and stirred at 250 rpm. At different intervals within 24 hours, 1 ml of the mediums from each vial was drawn and fresh medium was added back. At the time point where $t = 5$ hours, the vials containing SGF were replaced by another medium simulating the intestinal condition. The collected sets of medium were analyzed using UV-Vis spectrometer (UV-2501, Shimadzu) at their respective wavelengths for the release of the drugs as shown in Table 4.

Table 4: Type of medium used and UV-Vis wavelength

Drug	Medium used before $t = 5$ hours	Medium used after $t = 5$ hours	UV-Vis Wavelength
Metformin Hydrochloride	SGF	SIF	233 nm
Fenofibrate	Modified SGF	Modified SIF	299 nm
Piroxicam	Modified SGF	Modified SIF	Modified SGF: 335 nm Modified SIF: 361 nm

Chapter 4 Results and Discussion

4.1 Fabrication of Multi-drug Loaded Gastric Floating Microcapsules

Fabrication of multi-drug loaded gastric floating microcapsules was based on the work developed by Lee et al. where the microcapsules provided controlled and sustained release of multiple cardiovascular drugs to improve the pharmacokinetic profiles of multiple active pharmaceutical ingredients [12]. The microcapsules were reproduced and selected as a control group for this study.

Drug-loaded micro-particles (Figure 8a) were encapsulated with free metformin hydrochloride within microcapsules to provide controlled and sustained release. The fabrication method (as described in chapter 3.2.1) creates a single hollow cavity to contain the micro-particles. The microcapsule size had an approximate mean size of 600 μm with a smooth surface having no visible particles or pores on the surface of the shell (Figure 8b). Wall thickness is approximately 50 μm and the size of hollow cavity had an approximate mean size of 500 μm .

Figure 8: (a) Fenofibrate-loaded microparticles (b) View of a single microcapsules (c) Interior view of sliced microcapsules (d) View of metformin hydrochloride and fenofibrate-loaded particle within a microcapsule

In order for the micro-particles to be encapsulated within the microcapsules, a particle suspension mixed with PVA as a surfactant is used. By using PVA, the micro-particles are adequately dispersed to form a stable suspension suited for encapsulation. Micro-particles that are fabricated are approximately 5 to 20 μm. It has been determined that particle sizes of this range will provide a more stable particle suspension, allowing the particles to be encapsulated within the microcapsules (Figure 8c).

Buoyancy of the floating microcapsule is attributed by two factors: 1) Addition of olive oil, and 2) Formation of hollow cavity. The presence of olive oil within the polymer shell lowers the overall density of the capsules and increases the hydrophobicity of the polymer shell. The formation of the hollow cavity traps air molecules within the microcapsules

thus lowering the overall density and promoting buoyancy. Approximately 90% of the capsules remained floating after 24 hours (Figure 9).

Figure 9: In vitro buoyancy result of sample A1

The formation of the hollow cavity requires a combination of two factors: 1) Formation of the $W_1/O/W_2$ emulsion, and 2) Precipitation rate of capsule shell in the continuous phase. When the double emulsion was achieved in the continuous phase, i.e. PVA solution, the capsules needed to be hardened quickly otherwise the double emulsion would not hold and the internal water would release into the continuous phase. Hence, a rotary evaporator is essential to speed up the rate of precipitation.

The microcapsules, A1, had drug loading 10.91 ± 0.16 weight percentage (w%) and 1.89 ± 0.03 w% for metformin hydrochloride and fenofibrate respectively. Both drugs showed sustained release in SGF for 24 hours (Figure 10). Within this timeframe, metformin hydrochloride was completely released while only 40% of fenofibrate were released due to its hydrophobicity of the drug. The shell was made up of 75% PLLA and 25% PCL. A

dissolution test performed on the microcapsules revealed that PCL was embedded within the PLLA matrix. PCL was added to form a PLLA-PCL composite as PCL chains were in a highly mobile and flexible rubbery state with sufficient free volume at body temperatures, allowing higher probabilities of drug molecules to diffuse through the shell, which allows higher amount of fenofibrate to be released into the medium. The semi-crystalline PLLA provided the necessary retardation to limit water molecules from entering the microcapsule, thus the release of metformin hydrochloride, a highly hydrophilic drug, was impeded and able to provide a sustained release. However, assuming that floating systems could be retained in the GI tract for 10 hours as reported by Desai et al. [68], the fenofibrate in A1 was not be efficiently released (20% cumulative release in 10 hours) and drug absorption within the GI tract would be low, leading to low bioavailability and more drug wastage.

Figure 10: Cumulative drug release profile of sample A1 in SGF

Since in vivo study could not be performed on the floating microcapsule, another in vitro drug release test was conducted to simulate drug release condition along the GI tract as demonstrated in a study by Joseph et al. [76]. The experiment follows a procedure of releasing drugs from a floating device in the simulated gastric fluid for the first 5 hours, followed by releasing drugs in the simulated intestinal fluid for the remaining duration until 24 hours. The PLLA-PCL shell microcapsule loaded with both drugs, sample A1 was used for this release study. The release profile in SGF-SIF (Figure 11) was very similar to the results obtained when in vitro study was performed in SGF only (Figure 10). Approximately 93% of metformin HCl was released at 10 hours, while about 24% of fenofibrate was released within the same period. Therefore, if the microcapsule was still intact after leaving stomach, drugs could still be released sustainably in the small intestine where both drugs have been reported to be able to continue efficiently absorbing the drugs [90, 93].

Figure 11: Cumulative drug release profile of sample A1 in SGF-SIF

4.2 Approaches to Increase Drug Release Rate of Hydrophobic Drugs

4.2.1 Melt granules used in Microparticles

Yadav et al. reportedly used a melt granulation technique to improve the solubility and dissolution characteristics of fenofibrate [129]. By applying this technique, we would expect to observe an enhancement in the drug release rate for the hydrophobic drug. A test was conducted by fabricating the altered fenofibrate-loaded PCL microcapsules using the melt granules instead of free fenofibrate drug (Refer to Figure 12).

Figure 12: (a) Image of fenofibrate melt granules (b) Image of pure fenofibrate (c) Image of fenofibrate melt granules loaded in PCL microparticles (d) Image of fenofibrate-loaded PCL microparticles

However, the experiment using these microcapsules encapsulated with altered microparticles within, sample A2, showed a very modest increase in the drug release rate, an increase from 20% to 28% cumulative release in 10 hours (Figure 13). This increase is

due to the surfactant, poloxamer-127, being added with fenofibrate to form melt granules which served to increase the dissolution characteristic of fenofibrate. The results suggest that a different approach should be taken to enhance the release rate of fenofibrate.

Figure 13: Comparison of fenofibrate drug release rates to show the effect of melt granules used

4.2.2 Alteration of Polymer Used in Microparticles

An approach to change the polymer used in fabricating the fenofibrate-loaded microparticles where a pH-sensitive polymer soluble at pH values lower than 3 was intended to be used to promote the release. When in contact with the gastric fluid, the pH-sensitive would immediately dissolve and release the fenofibrate within it. Therefore, a test was first conducted using free fenofibrate to be encapsulated within the cavity of the hollow floating microcapsules to gauge the potential of the release rate (Figure 14).

Figure 14: Images of microcapsules encapsulated with free fenofibrate

However, a test encapsulating free fenofibrate powder, sample A3, showed no significant increment (an increase from 20% to 23% cumulative release in 10 hours), suggesting that the alteration of polymer used to fabricate microparticles would have limited effect (Figure 15). Although polymer selection of microparticles was explored to increase fenofibrate release rates, this same alteration concept on the microcapsule polymer could not be applied as it would cause a burst release instead of a sustained release, especially since a highly hydrophilic drug, metformin hydrochloride is also encapsulated.

Figure 15: Comparison of fenofibrate drug release rates to show the effect of free fenofibrate drug used instead of microparticles

4.2.3 Embedding of Drug Within the Wall of the Microcapsules

Another approach to increase hydrophobic drug release was to load the drug within the walls of the microcapsules. The initial intention was to localise the drug in the walls to promote more contact with the drug release medium as it was suspected that the air molecules present in the cavity of the microcapsules may prevent the fluid from reaching the drug thus contributing to lower drug release (Figure 16).

Figure 16: Images of microcapsules where fenofibrate is scattered within the shell matrix

However, subsequent drug release result from the amended fabrication, sample A4, showed a significant decrease in drug release rate of 20% to 7% cumulative release in 10 hours (Figure 17). It is believed that the semicrystalline PLLA that is used to fabricate the microcapsules traps the fenofibrate drug within its matrix and retards its diffusion out into the drug release medium. Therefore, an approach to design fenofibrate release outside the microcapsules have to be taken.

Figure 17: Comparison of fenofibrate drug release rates to show the effect of encapsulating fenofibrate within the shell matrix

4.2.4 Spray-coating of Drug-loaded Layer on Microcapsules

In order to produce a microcapsule for sustained release in both metformin hydrochloride and fenofibrate, and also to increase the drug release rate of fenofibrate, a fenofibrate-loaded PCL coating later was spray-coated on the microcapsule. The MediCoat coating system was used as a spray-coater which allows the polymer and drug solution to be spray-coated directly onto the surface of the capsule. Preliminary findings show that the microcapsules, B1, experienced a complete release within the first hour. Furthermore, when the coated solution of PCL, fenofibrate and DCM was coated directly on the microcapsules during the coating process, this porous coating layer tends to peel off under stirring during the in vitro drug release (Figure 18). Given the proposed drug release mechanism for having a sustained release, this peel-off problem will not be able to provide prolonged release within the gastric region since the peeled-off coating layer will sink towards the pylorus (lower region of the stomach).

Figure 18: (a) View of microcapsule, B1, before in vitro test (b) View of microcapsule, B1, after in vitro test

To overcome the coating layer from peeling off, 0.5 ml of DCM is spray-coated onto the surface of the microcapsules first before spray-coating the polymer and drug solution. Furthermore, the air shroud for spray-coating was reduced from 4 psi to 2 psi to prevent

excessive downward pressure of the coating solution and the power for the frequency generator was increased from 1 W to 1.4 W to allow finer spray droplets to produce a more continuous coating layer to prevent the coating layer from breaking under agitation. The function of the air shroud (nitrogen gas) is to direct the coating solution downwards onto the capsules. While increasing the air shroud pressure may suggest that the coating solution will be directed more towards the capsules, this strong downward pressure will induce increased force on the coating layer that was continuously being coated on the surface of the capsule. Since this coating layer is still in semi-liquid state (not dried yet), an air shroud pressure that is too high will cause this coating layer to be peeled off by the strong pressure of the nitrogen gas. On the contrary, if the air shroud pressure is set too low, it will cause the coating solution to miss the targeted area. Therefore, a balanced air shroud pressure is necessary to form a continuous layer. SEM images showed that by altering these parameters, the coating layer in sample B2 remained intact after 24 hours of agitation under drug release (Figure 19).

Figure 19: (a) View of microcapsule, B2, before in vitro test (b) View of microcapsule, B2, after in vitro test

2 batches of samples, B2 and B3, were fabricated to show the potential effect of the coating technique. The microcapsules, B2, had drug loading of 4.80 ± 0.07 w% and 0.86

± 0.05 w%, while B3 had drug loading of 2.14 ± 0.11 w% and 1.26 ± 0.05 w% for metformin hydrochloride and fenofibrate respectively. B2 had a coating thickness of $5 \mu\text{m}$ while B3 had a coating thickness of $10 \mu\text{m}$. The drug release profile in B2 (Figure 20) indicates a more rapid release where metformin hydrochloride was completely released in SGF within 10 hours while fenofibrate experienced a complete release in the first hour. On the other hand, B3 produced a more sustained release where metformin hydrochloride had 88% cumulative release after 10 hours while fenofibrate experienced a 97% cumulative release after 10 hours (Figure 21). Both release profile observed a marked increase from the unaltered floating microcapsules in sample A1 which fenofibrate only had a 20% cumulative release after 10 hours.

Figure 20: Fenofibrate and Metformin Hydrochloride release profile in SGF for sample B2

Figure 21: Fenofibrate and Metformin Hydrochloride release profile in SGF for sample B3

The sustained release was achieved due to 2 factors. First, the alteration in flow rate during spray-coating where the B3 had a reduced flow rate compared to B2. Since the spray-coating were conducted in a row-by-row sweeping mode, the reduced flow rate will allow DCM to evaporate easier resulting in the coated layer to solidify more before the next round of spray coating. This allowance provided a denser, less porous and more continuous coating layer in B3, giving a smoother and more consistent morphology (Figure 22), thus retarding the drug release. Second, B3 had an additional PCL layer spray coated on the fenofibrate-loaded PCL layer. The combination of both is believed to slow down the release rate of both metformin hydrochloride and fenofibrate, producing a sustained release profile. The introduction of this spray-coating method would provide high flexibility in achieving different types of drug release profiles, as well as the possibilities of having multi-layer coatings. Approximately 90% of sample B2 capsules and 95% of sample B3 remained floating after 24 hours (Figure 23).

Figure 22: (a) Side view of coating of sliced microcapsule, B2 (b) Side view of coating of sliced microcapsule, B3
 (c) External view of coating of microcapsule, B2 (c) External view of coating of microcapsule, B3

Figure 23: In vitro buoyancy result of sample B2 and B3

An in vitro drug release test to simulate in vivo conditions was conducted to simulate drug release condition along the GI tract. Only the gastric floating microcapsule sample B3 was used for this release study as the fenofibrate drug in B2 had a burst release within the first hour would not require further study in intestinal condition. The release profile in SGF-SIF (Figure 24) was very similar to the results obtained when in vitro study was performed in SGF only (Figure 21). Approximately 93% of metformin HCl was released at 10 hours, while about 95% of fenofibrate was released within the same period. Therefore, by comparing the results under the same conditions used in this in vitro test, fenofibrate drug release in B3 microcapsules (Figure 24) showed a significant improvement from A1, the unaltered microcapsules (Figure 11), which had a 24% cumulative release in 10 hours. Metformin hydrochloride drug release remained unchanged at 93% cumulative release in 10 hours.

Figure 24: In vitro drug release profile of sample B3 in SGF-SIF

4.2.5 Bi-Layer Gastric Floating Microcapsules

Although spray-coating of drug-loaded layer on microcapsules could potentially produce a flexible and multi-drug release system, it has certain limits to amount of drug being loaded into the coated layer as the spraying tip of the coating machine may not be able to effectively eject and spray the solution when the polymer or drug reaches above a certain concentration. Therefore, a one-step bi-layer microcapsule system is created to overcome high polymer or drug concentration used in the solution as well as to simplify process for convenience.

In this fabrication, a similar double emulsion solvent evaporation method is used with an extra solution containing PCL, fenofibrate and ethyl acetate added into the mixture. As a preliminary study, no drug is loaded within the hollow cavity while fenofibrate will be localized in the outer layer. This double layer structure (Figure 25) is first formed when the PCL-PLLA-DCM mixture is added into the PCL-Fenofibrate-Ethyl Acetate solution. Since PCL is soluble in both solvents while PLLA is soluble only in DCM, upon mixing, the PLLA will precipitate first thus forming an inner layer enveloped within the PCL-Fenofibrate-Ethyl Acetate droplet. After rotary evaporation, the ethyl acetate will be removed along with any residue DCM, therefore forming the outer layer of the microcapsules.

Figure 25: Illustration of bi-Layer gastric Floating microcapsules

In fabrication of the outer wall of the microcapsules, it is essential to note that the coverage of the capsule is dependent on the amount of polymer as well as the volume of ethyl acetate used. As shown in Figure 26, when the polymer concentration and solvent volume increased from formulation C1 to C2, a more continuous outer layer was produced.

Figure 26: (a) View of microcapsule, C1, with an incomplete coverage on the microcapsule (b) View of microcapsule, C2, with improved coverage

The presence of the double layer structure is characterized by a dissolution method as described in chapter 3.3.3. The bilayer microcapsule consists of 2 layers, the inner layer (PCL-PLLA), and outer layer (PCL). Evidence of this bi-layer can be seen in Figure 26a and 26a from the SEM images. After the dissolution test where THF is used to dissolve PCL, only 1 layer is expected to remain, i.e. inner layer. This can be seen in the SEM images in Figure 27b and 27c. This layer that remains is PLLA, since only PLLA does not dissolve in THF while PCL does. Furthermore, the dissolution study also revealed a single porous PLLA layer remaining, indicating that the increased porosity was formed due to the absence of PCL after the dissolution process (Figure 27b and 27d).

Figure 27: (a) View of bi-layer wall before dissolution test (b) Absence of double layer after dissolution test (c) View of sliced microcapsule after dissolution test (d) Porous surface due to PCL within the inner layer being dissolved after dissolution test

The microcapsules, C3, had an approximate mean size of 450 μm with inner wall thickness approximately 30 μm and outer wall thickness approximately 20 μm . The size of hollow cavity had an approximate mean size of 350 μm . C3 had metformin hydrochloride drug loading of 5.72 ± 0.03 w% fenofibrate drug loading of 5.75 ± 0.19 w%. The drug release profile achieved was a sustained release lasting approximately 10 hours. It is also worth noting that this system, C3, had a marked improved rate of release of fenofibrate, approximately 100% release in 10 hours (Figure 28), as compared to our unaltered microcapsules study, A1, 20% release in 10 hours (Figure 10). Metformin hydrochloride, on the other hand had a slight decrease from 86% cumulative release in 10 hours, shown in sample A1, to 82% cumulative release in 10 hours, shown in sample C3.

Figure 28: Drug release profile of sample C3 in SGF

During in vitro buoyancy testing, only 40% of microcapsules, C2, remained floating after 24 hours. To promote the buoyancy of the microcapsules, sample C3 had an additional

0.01 ml of olive oil added into the ethyl acetate solution. With this additional olive oil in ethyl acetate, buoyancy of the microcapsules increased to 90% of microcapsules after 24 hours during in vitro testing.

Figure 29: In vitro buoyancy result of sample C2 and C3

The in vitro drug release test to simulate drug release condition along the GI tract revealed that the release profile in SGF-SIF (Figure 30) was very similar to the results obtained when in vitro study was performed in SGF only (Figure 28). Approximately 91% of metformin HCl was released at 10 hours, while about 97% of fenofibrate was released within the same period. The results reaffirmed that metformin hydrochloride and fenofibrate release is not significantly affected by the change in medium from SGF to SIF as shown in sample A1 and B3.

By comparing the results under the same conditions used in this in vitro test, fenofibrate drug release in C3 microcapsules (Figure 24) showed a significant improvement from A1, the unaltered microcapsules (Figure 11), which only had a 24% cumulative release in 10

hours. Metformin hydrochloride drug release had a slight decrease from sample A1 by 2% when the medium was measured at 10 hours.

Figure 30: In vitro drug release profile of sample C3 in SGF-SIF

4.2.6 Gastric Floating Microcapsules for Loading Dose and Maintenance Dose

To produce a microcapsule capable of providing a loading dose and maintenance dose, a combination of two different drug release kinetics is required, i.e. a burst release for the loading dose and a sustained release for the maintenance dose. The loading dose will serve to reach therapeutic levels rapidly while the maintenance dose will keep drug dosage at effective levels without reaching the toxicity limit. Joseph et al. postulated that by having a loading dose and a maintenance dose, it would help patients overcome drug variability in steady state levels due to erratic elimination of drug, especially seen in the elderly [76]. The study concluded that combined dose (piroxicam loading dose 26% and maintenance dose 74% of total dosage) increased elimination half-life by six times and increase bioavailability of piroxicam in patients.

In order to design a delivery system to combine these release kinetics for the gastric floating microcapsules, the MediCoat coating system was used as a spray-coater which allows the polymer and drug solution to be spray-coated directly onto the surface of the capsule and to create coating layers with individual release kinetics. The proposed design required a drug-loaded coating layer which enables burst release and another drug-loaded coating layer providing a controlled release.

Two sets of coated microcapsules, D1 and D2, were first fabricated to study the individual release kinetics. D1 was coated with a piroxicam-loaded PEG layer to provide a loading dose for rapid release where PEG would dissolve in SGF upon contact, while D2 was coated with a piroxicam-loaded PCL layer to provide a maintenance dose for sustained release. Figure 31 shows the presence of coating layer on the microcapsules. The microcapsules, D1, had an approximate mean size of 750 μm with coating thickness approximately 40 μm . The size of hollow cavity had an approximate mean size of 500 μm .

On the other hand, D2, had an approximate mean size of 800 μm with coating thickness approximately 80 μm . The size of hollow cavity had an approximate mean size of 500 μm .

Figure 31: (a) Side view of coating layer of sample D1 (b) External view of microcapsules sample D1 (c) Side view of coating layer of sample D2 (d) External view of microcapsules sample D2

The microcapsules, D1, had piroxicam drug loading of 2.45 ± 0.04 w%, while D2 had piroxicam drug loading of 12.77 ± 0.07 w%. Initial findings show that the microcapsules, D1, which had a piroxicam-loaded PEG coating layer, experienced a complete release within the first hour in SGF (Figure 31). A one-hour test for sample D1 was conducted to ensure that the microcapsules experience a burst release to achieve rapid therapeutic levels. Results obtained confirmed an immediate release and piroxicam reached 96% cumulative release in SGF within the first 5 minutes (Figure 32). SEM images taken after the drug release in sample D1 showed a smooth external surface and the absence of the

coating layer, indicating that the drug-loaded PEG coating had dissolved in SGF (Figure 34).

Figure 32: Piroxicam release profile in SGF for sample D1

Figure 33: One-hour test of piroxicam in SGF for sample D1

Figure 34: (a) Side view of sliced microcapsule, D1, before drug release in SGF (b) External View of microcapsule, D1, before drug release in SGF (c) Side view of sliced microcapsule, D1, after drug release in SGF (d) External view of microcapsule, D1, after drug release in SGF

The drug release profile in D2, which had a piroxicam-loaded PCL coating layer (Figure 35) indicates a more sustained release where piroxicam 83% of piroxicam was released in SGF within 10 hour. A one-hour test for sample D2 was conducted to understand the drug release kinetics of piroxicam in SGF within the first hour. Results showed a sustained release of piroxicam where a cumulative release of 43% were reached in the first hour and only about 16% of piroxicam was released in the first 5 minutes (Figure 36). SEM images of sample D2 taken after the drug release in SGF showed a rough external surface and the presence of coating layer indicating that the PCL coating still remained and was not dissolved after the in vitro test (Figure 37).

Figure 35: Piroxicam release profile in SGF for sample D2

Figure 36: One-hour test of piroxicam in SGF for sample D2

Figure 37: (a) Side view of sliced microcapsule, D2, before drug release in SGF (b) External View of microcapsule, D2, before drug release in SGF (c) Side view of sliced microcapsule, D2, after drug release in SGF (d) External view of microcapsule, D2, after drug release in SGF

By analyzing the drug release results in D1 and D2, it is expected that the combination of both loading dose and maintenance dose would provide a rapid burst release and a sustained release to ensure dosage maintaining at effective levels. Hence, sample D3 was fabricated such that the first layer of piroxicam-loaded PCL coating was sprayed onto the floating microcapsules first before the next layer of piroxicam-loaded PEG coating was sprayed. By combining both dosage on the microcapsules instead of separated batches of microcapsules, microcapsules will be more efficiently utilized and patients will be required to consume less microcapsules per intake. Figure 38 shows the SEM images of the coated samples, D3.

Figure 38: (a) Side view of a sliced microcapsule, D3. (b) External view of a microcapsules, D3 (c) Close-up view on the coating layer of microcapsule, D3 (d) View of several coated microcapsules, D3

The microcapsules, D3, were fabricated according to the loading and maintenance dosage as reported by Joseph et al., i.e. piroxicam loading dose 26% and maintenance dose 74% of total dosage [76]. Sample D3 had a total piroxicam drug loading of 14.98 ± 0.24 w%. In vitro drug release in SGF showed that the microcapsules, D3, had a 56% cumulative release in the first hour followed by a sustained release reaching 91% cumulative release within 10 hours (Figure 39). The one-hour test for sample D3 that was conducted reported that piroxicam reached 29% cumulative release in SGF within the first 5 minutes (Figure 40), an 13% increase from the release study of sample D2 within the same timeframe. This release study of sample D3 showed the potential result of a loading dose may have on the pharmacokinetic of the drug. By utilizing this delivery system, rapid therapeutic drug level could be attained while dosage level could be further sustained within an

effective range. In vitro buoyancy testing showed that 95% of microcapsules, D3, remained floating after 24 hours (Figure 41).

Figure 39: Piroxicam release profile in SGF for sample D3

Figure 40: One-hour test of piroxicam in SGF for sample D3

Figure 41: In vitro buoyancy result of sample D3

The in vitro drug release test to simulate drug release condition along the GI tract revealed that the release profile of D3 in SGF-SIF (Figure 42) showed promising results. Approximately 80% of piroxicam was released at 5 hours in the gastric environment, and the remaining drug load was completely diffused out into the intestinal environment within the following 2 hours. This release profile indicated a more inclined release rate when SIF was introduced to the microcapsules. This is in line with reported literature that dissolution rate in SIF is higher than in SGF [98]. As a result, complete release is obtained after 7 hours. Since gastric floating capsules have been reported to transit to large intestines after 10 hours, the shortened timeframe for complete release would prove to benefit patients as piroxicam has been reported to cause irritation and damage to large intestines [100]. An in vivo test, however, is recommended to be conducted to further substantiate this viewpoint for these microcapsules.

Figure 42: In vitro drug release profile of sample D3 in SGF-SIF

Chapter 5 Conclusion

In conclusion, the key difference to overcome slow hydrophobic drug release from floating microcapsules and retaining its function to provide a multi-drug sustained release, containing both hydrophilic and hydrophobic entities, lies in the localizing of drugs within certain region of the PCL-PLLA microcapsules, i.e. on the external surface of the capsules. Design aspects, polymer characteristics and selection criteria, as demonstrated, would play a critical role in manipulating or limiting rate release when paired with drugs of different solubility. The two modified fabrication techniques, spray-coating technique and bi-layer microcapsules, seek to localize hydrophilic drug within the microcapsules while embedding hydrophobic drug within the outer layer of the microcapsules, each with its own advantages over flexibility in drug placement, ease of process and the amount of drug loading. In both processes, the highly hydrophobic drug, fenofibrate, achieved significantly higher rate of release while providing sustained release. The spray-coating technique was further implemented for the design of gastric floating microcapsules capable of producing loading dose and maintenance dose, where a piroxicam-loaded coating layer effected a burst release and sustained release to influence drug release kinetics. By the addition of 0.01 ml of olive oil to the delivery systems designed in this thesis, floating from the multi-unit drug delivery system is achieved.

Chapter 6 Future Work

The following proposed work will be conducted in the near future. These studies will further showcase the strength of microencapsulation of drug-loaded particles.

6.1 Encapsulate More Than Two Drugs in the Microcapsule

Diseases such as HIV, tuberculosis, and lupus all require multiple drug combination therapies. The fabrication technique and microcapsule structure developed in this study can encapsulate countless number of drugs with close to zero drug-drug interaction as long as the drugs can be encapsulated and protected within its polymeric micro-particles or layers.

6.2 pH-sensitive Enteric Coating on Multi-drug Microcapsules

Drugs that are destroyed by gastric juices or causing gastric irritation are sometimes administered in a dosage form with a coating that prevents dissolution in the acidic gastric contents. During oral administration of drugs like minocycline, it is absorbed from the stomach and causing stomach upset. This could be avoided by delaying drug release from microcapsules to release in the small intestine instead of the stomach by enteric coating, allowing the antibiotics to be absorbed in the small intestine. A pH-sensitive polymer could be coated to release when the microcapsules reach the small intestine, where pH levels is approximately 6.5.

6.3 In Vivo Buoyancy and Drug Release Studies

While in vitro buoyancy and drug release studies were reported in this report, it would be of great interest to study the buoyancy and drug release behavior of these floating microcapsules in vivo. An in vivo study will provide a clearer insight into how the

floatability, drug content and drug release can be further adjusted to enhance bioavailability without reaching toxic levels.

Chapter 7 References

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Chapter 8 Glossary

Sample A1

Microparticles:

0.2 g PCL, 0.07 g fenofibrate, 2.85 ml DCM

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Sample B1

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating Layer:

1st layer – 0.1g PCL, 0.014g fenofibrate, 1ml DCM (Flow rate: 0.5ml/min)

Sample B2

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating Layer:

1st layer – 1ml DCM (Flow rate: 0.5ml/min)

2nd layer – 0.1g PCL, 0.014g fenofibrate, 1ml DCM (Flow rate: 1ml/min)

Sample B3

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating Layer:

1st layer – 1ml DCM (Flow rate: 0.5ml/min)

2nd layer – 0.1g PCL, 0.014g fenofibrate, 1ml DCM (Flow rate: 0.2ml/min)

3rd layer – 0.1g PCL, 1ml DCM (Flow rate: 0.2ml/min)

Sample C1

Inner layer:

0.3g PLLA, 0.09g PCL, 3ml DCM

Outer layer:

0.2g PCL, Ethyl Acetate 5ml, 0.0196g fenofibrate

Sample C2

Inner layer:

0.3g PLLA, 0.09g PCL, 3ml DCM

Outer layer:

0.4g PCL, Ethyl Acetate 6ml, 0.0196g fenofibrate

Sample C3

Inner layer:

0.3g PLLA, 0.09g PCL, 3ml DCM

Outer layer:

0.4g PCL, Ethyl Acetate 6ml, 0.0196g fenofibrate, 0.01ml olive oil

Sample D1

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating layer:

1st layer – 1 ml DCM (Flow rate: 0.5ml/min)

2nd layer – 0.1g PEG, 0.014g Pirox, 1ml DCM (Flow rate: 0.2ml/min)

Sample D2

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating layer:

1st layer – 1 ml DCM (Flow rate: 0.5ml/min)

2nd layer – 0.1g PEG, 0.082g Pirox, 1ml DCM (Flow rate: 0.2ml/min)

Sample D3

Microcapsule:

0.3g PLLA, 0.09g PCL, 5 ml of DCM

Coating layer:

1st layer – 1 ml DCM (Flow rate: 0.5ml/min)

2nd layer – 0.1g PEG, 0.082g Pirox, 1ml DCM (Flow rate: 0.2ml/min)

3rd layer - 0.1g PEG, 0.021g Pirox, 1ml DCM (Flow rate: 0.2ml/min)