Biopolymer-solid lipid hybrid microparticles as a nutraceutical co-delivery carrier in the gastrointestinal tract

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2017

Ho, C. G. (2017). Biopolymer-solid lipid hybrid microparticles as a nutraceutical co-delivery carrier in the gastrointestinal tract. Master's thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/72883>

<https://doi.org/10.32657/10356/72883>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**BIOPOLYMER-SOLID LIPID HYBRID
MICROPARTICLES AS A NUTRACEUTICAL CO-
DELIVERY CARRIER IN GASTROINTESTINAL TRACT**

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SCHOOL OF MATERIALS SCIENCE & ENGINEERING

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A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Master of Engineering
2017

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

07 July 2017

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Date

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Abstract

Many obstacles are faced during oral delivery, for example, low bioavailability, multiple dosing, and side effects in the gastrointestinal tract. Two types of drug delivery systems are studied to address these problems of nutraceutical and drug delivery in the gastrointestinal tract. Firstly, a combination of water-in-oil-in-water (W/O/W) double emulsion and hot emulsification fabrication techniques are employed to design a novel alginate-solid lipid hybrid microparticles (SLM) to co-encapsulate two bioactive compounds, riboflavin and quercetin. Secondly, acarbose is reformulated into alginate-PLGA hybrid core-shell microparticles to investigate the release kinetics of acarbose in a simulated gastrointestinal tract for oral administration. In SLMs, riboflavin and quercetin are shown to localize in different carrier materials, with the encapsulation efficiency up to 50 % and 89 %, respectively. The release profiles show complete release within 24 hours except for Compritol 888 Ato. However, Compritol with the incorporation of liquid lipid can achieve the total release of 90%, which proves the potential for the Compritol formulation to be used in oral delivery. Now, the SLMs exhibit similar release kinetics for two bioactive compounds according to Higuchi's equation, and anomalous transport in power's law. Release profile and release kinetics suggest that SLM can successfully co-encapsulate and co-deliver two bioactive compounds despite their hydrophilicity. In the second part of the project, the uncoated alginate-PLGA hybrid microparticles exhibit sustained-release of acarbose up to 24 h, whereas commercial tablet Glucobay® exhibits fast release in less than 5 mins. Hybrid microparticles coated with polyelectrolytes can further retard the release in simulated gastric fluid from 70% to 35%. Both coated and uncoated formulations can be best-fitted into power's law. From the fitted data, the uncoated formulation exhibits anomalous transport, however, the release exponent change to Case II transport for coated formulation due to the effect of the additional coating layer. In a nutshell, both studies provide evidence that the novel hybrid SLM and modified polymeric system are promising oral delivery systems to suit different needs for nutraceutical and drug delivery in the gastrointestinal tract.

Acknowledgements

First and foremost, I would like to express my deepest appreciation to my supervisor, Professor Joachim Loo Say Chye for the opportunity given to join this group and start this meaningful project. Without his guidance and encouragement, this project will not be at this stage.

I would also like to thanks my fellow group members who had provided valuable advice, especially Dr. Baek Jongsuep and Kaarunya, for their experience shared during the work. Special thanks go to Betty Fan and Tan Bee Yi, with the constant encouragement and for the expertise help with the confocal microscopy and Raman mapping. Not forgetting also Wilson Poh, Lui Yuan Siang, Jerome Lim and Vidya for the assistance and support given.

Special thanks to the collaborators of the research work, Prof. Nuannoi, and Jidapa, from Suranaree University of Technology, Thailand, for their help in the pharmacokinetics study. I am also thankful to another group of collaborators from NTU LKC Medicine School, Prof. Yusuf, and Surabhi, for the assistance in animal work.

Last but not least, I would like to express gratitude to every staffs and group members that I have failed to mention.

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List of Abbreviations

Alg-PLGA	Alginate-PLGA
CLSM	Confocal Laser Scanning Microscopy
DCM	Dichloromethane
DSC	Differential Scanning Calorimetry
EE	Encapsulation Efficiency
GIT	Gastrointestinal Tract
HPMC	Hydroxypropylmethyl cellulose
MS	Mass spectrometers
NaAlg	Sodium Alginate
NLC	Nanostructured Lipid Carrier
NSAIDs	Nonsteroidal anti-inflammatory drugs
O/W	Oil-in-water (emulsion)
PBS	Phosphate Buffer Saline
PVA	Poly(vinyl) Alcohol
PLGA	Poly (lactide-co-glycolide)
PLLA	Poly (L-lactide)
Q3G	Quercetin-3-O-glucuronide
QT	Quercetin
RF	Riboflavin
RP- HPLC	Reversed phase high performance liquid chromatography
SEM	Scanning Electron Microscopy
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SLM	Solid Lipid Microparticles
SLN	Solid Lipid Nanoparticles
S/O/W	Solid-in-oil-in-water (emulsion)
W/O	Water-in-oil (emulsion)
W/O/W	Water-in-oil-in-water (emulsion)
XRD	X-ray Diffraction

Chapter 1

Introduction

This chapter describes the introduction of the thesis. Two projects are introduced separately for oral drug delivery systems, namely hybrid alginate-solid lipid microparticles (SLMs) and Acarbose reformulation in hybrid alginate-polymeric system. Both systems aim to address the problems and limitations faced in the delivery of compounds to the gastrointestinal tract (GIT), such as low stability, low absorption, and poor bioavailability. Besides this, co-encapsulation and co-delivery of two nutraceuticals are introduced in the novel hybrid SLMs, ought to achieve a synergistic effect of bioactive compounds, protect the unreleased compounds from degradation, and to improve the absorption and bioavailability in the GIT by delivery systems.

1.1 Background

Recently, drug delivery systems have emerged as “smart” systems for time-controlled delivery of the drug of interest to a targeted site at a predetermined dose. Many types of carrier have been devised to cater for the needs of different delivery routes such as parenteral, sub-mucosal, pulmonary, and parenteral. In spite of that, oral administration remains the most widely accepted among other delivery routes because of its non-invasive, ease-of-use, and better patient compliances.

The challenge of designing a drug delivery system for oral administration lies not only in the harsh gut milieu, but also the continuous secretion of mucus in the gastrointestinal tract (GIT) [1]. This has always been one of the many obstacles faced during oral delivery because degradation of substances occurs in the protease-rich and acidic environment of the GIT. In addition, many drugs and compounds possess low stability, poor solubility, low absorption and bioavailability in the GIT, which impedes the prospective progress of the drug delivery system during clinical trials. Thus, drug encapsulation in polymeric, solid lipid, and liposomal system have been studied extensively to address these problems. However, emulsion techniques often exacerbate the leaching of hydrophilic compound in to the aqueous continuous phase and leads to low encapsulation efficiency. In light of this problem, hydrogel such as alginate system will be adopted in this studies with slight modification, which can potentially improve the loading of hydrophilic compounds [2].

In this thesis, two separate systems, namely solid lipid microparticles and polymeric system will be introduced in part I and part II, respectively. These two systems are potentially important in the oral delivery of nutraceutical and drug to the gastrointestinal tract.

Part I: Solid lipid microparticles (SLM)

The past few years have witnessed an invasion of fast food chains all around the world. This, in tandem with our hightail lifestyle, has revolutionized the way we eat. Processed foods and fast foods are now considered as substitutes for a healthy meal because they entertain the need of our busy lifestyles. The prolonged consumption of unhealthy foods

can lead to a number of diseases such as obesity, cancer, and heart diseases which are now recognized as an epidemic [3]. In addition, respective treatments can be costly and difficult to follow. In light of this conundrum, complementary or alternative beneficial products for the prevention of these diseases are now seen as a necessity to improve one's health, which is why nutraceutical as nutritional supplement and functional foods are currently the new paradigm of a healthy diet.

“Nutraceutical” or nutrition supplement which promotes health and prevents diseases prevention has emerged in the past decades. The word “nutraceutical” is the combination of “nutrition” and “pharmaceutical”, referring to the food products that provide medical and health benefits [3]. Hippocrates, the father of Western medicine had said “Let thy food be thy medicine”, which emphasized the concept of using nutraceutical to treat disease. Nutraceutical is mostly the herbal extracts or the nutrients found in foods, they can be incorporated as functional food or used as a compound to be delivered directly. However, nutraceutical is usually used in conjunction or complementary with another drug rather than the primary treatment because of its low solubility, cell permeability, and low stability in the GIT [4].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drug that provides painkilling, fever reducing, and anti-inflammatory effects. However, the side effects associated with NSAIDs in oral administration is the instability of drug in acidic condition, gastric ulceration, and even gastric bleeding. Therefore, the common strategy to reduce this side effect is through enteric coating or encapsulation to achieve controlled release. Encapsulation has been used as a strategy to enhance the bioavailability and bioaccessibility of drugs or bioactive compounds that cannot be easily incorporated into food products. Biodegradable polymer especially polymeric system, has been studied extensively as a controlled-release system due to the advantages over conventional carrier such as tablet or gelatin capsule [5]. However, the shortcomings of the polymeric system are the involvement of organic solvents and the difficulty of scaling up for production. The concern about toxicity of polymeric system still persists if the organic solvent is not properly handled. Furthermore, the forming of polymeric particles is mainly driven by the evaporation rate of organic solvent, therefore it is difficult to scale up when optimization is required. Solid lipid particles have been recognized as a promising alternative drug delivery carriers to liposomes and

polymeric particulate system because it circumvents the prevalent limitations of those systems and at the same time possesses dominance over the rest [6]. In this study, solid lipid microparticles (SLM) without the use of organic solvent will be the choice of delivery system to encapsulate and deliver nutraceutical to the GIT, in order to improve the bioavailability, bioaccessibility, and bioactivity of nutraceutical.

Prevention is better than cure because nutraceutical literally prevents the diseases before the medical treatment using NSAIDs, for example, quercetin (a type of nutraceutical supplement) that possesses anti-inflammatory properties. Quercetin is not sensitive to low pH and is not known to have any side effect to the GIT. The only problem of quercetin in clinical application is its low absorption and bioavailability in the GIT. Therefore, microencapsulation as a drug delivery system with improved absorption of bioactive compounds in the GIT is highly essential. The strategy of using the particulate delivery system to address this problem is by releasing the bioactive compounds in a controlled manner without exceeding the therapeutic doses, as well as protecting the unreleased substance within the particles.

Quercetin has been shown to have a better effect and enhanced bioavailability in the presence of riboflavin [7, 8]. This gives rise to the idea of co-encapsulating these two bioactive compounds in the newly developed biopolymer-solid lipid hybrid microparticles to elicit the synergistic action in order to enhance the bioavailability and increase the intestinal absorption. Co-encapsulation strategies have been applied successfully in delivering two or more drugs in one carrier [9, 10], the same can be applied in co-encapsulating two nutraceutical bioactive compounds [11-13], which has shown to be possible. Synergistic effect of two nutrients in the co-encapsulation has become appealing because this maximizes the effect of each and exemplify the advantages of co-encapsulation. Furthermore, co-administration of lipids and emulsifiers has been proved to enhance the absorption of quercetin by oral administration [14].

Part II: Acarbose reformulation

The second part of this thesis describes the reformulation of a diabetic drug, acarbose into alginate-PLGA (Alg-PLGA) hybrid microparticles. Acarbose belongs to an oral α -glucosidase inhibitor, which is used as an oral antidiabetic agent in the management of type II, or non-insulin-dependent diabetes mellitus (NIDDM). Diabetes mellitus is a chronic disease that is caused by the impairment of carbohydrate, lipid, and protein metabolism. Acarbose literally inhibits the α -glucosidase enzymes required to digest carbohydrates in the small intestine, and thus reduces the digestion of complex carbohydrates. Acarbose only acts locally in small intestine due to its low absorption and bioavailability into human body.

Multiple dosing (three times a day) of oral antidiabetic agent is required every day before meals, because acarbose is excreted easily due to its high water solubility and short half-life of two hours. The commercial tablet Glucobay which contain acarbose has shown a remarkable effect in reducing postprandial glucose levels significantly by various clinical trials conducted in patients with NIDDM. However, approximate 20 % of the patients suffered from the gastrointestinal adverse effects such as flatulence, diarrhea, and abdominal pain in a randomized trial [15]. The adverse side effects were caused by the fast disintegration of Glucobay which releases Acarbose at a very high dosage in the stomach. These side effects are dose-dependent. The clinical strategy to minimize the side effects is by beginning with a low dose before gradually increasing it to the desired dosage [16]. Hence, a controlled and sustained-release formulation can obviate the need for multiple dosing and reduces the gastrointestinal side effects. With the sustained-release formulation, patients no longer need to take Glucobay together with the first bite of every meal. Single dose of acarbose in the new polymeric formulation can lasts for one whole day, patients can eat whenever they want because the acarbose is continuously presents in the small intestine.

1.2 Problem Statement

Part I: Solid lipid microparticles

NSAIDs drug has always faced the stability problem in oral administration. To address this problem, the replacement or synergistic effect of NSAIDs with bioactive compounds were studied extensively. However, many hydrophobic bioactive compounds, for instance, quercetin has low oral bioavailability which is less than 17% in rats [17], and only 1% in human [18]. The minimal absorption and low bioavailability in the GIT have limited the use of quercetin in clinical application despite its beneficial properties. Therefore, the challenge here is the need to design a controlled-release formulation for bioactive compounds which does not exceed the maximum absorption to prevent wastage, as well as to enclose the unreleased compounds within particles.

Part II: Acarbose reformulation

Acarbose has been reformulated into sustained-release formulations using hydroxypropyl methylcellulose (HPMC), guar gum, or Eudragit [19-21]. This sustained-release tablet or microsphere formulation could achieve a near zero order or Higuchi release pattern. However, none of these studies investigated the release kinetics of acarbose in a simulated gastrointestinal tract for oral administration. Besides that, hygroscopic drugs such as acarbose are not suitable to be compressed into a tablet due to the lack of drug protection to the humid environment. Therefore, alternative formulation was sought to protect the drug from degradation and oxidation. To date, no polymeric particulate system is used to reformulate acarbose due to the hydrophobic nature of polymer such as PLGA. Acarbose, being a hydrophilic and hygroscopic drug, is not easy to be encapsulated especially in the sustained-release formulation because hydrophilic drug tends to partition out during fabrication. Therefore, a recent invention of alginate-PLGA core-shell hybrid microparticles formulation was adapted to improve the encapsulation efficiency for the acarbose [22].

1.3 Hypothesis

Part I: Solid lipid microparticles

The hypotheses are food grade natural biopolymer-solid lipid hybrid microparticles will act as co-delivery depots for two bioactive compounds with different hydrophilicity. The hydrophilic riboflavin and hydrophobic quercetin will localize in alginate and solid lipid respectively, which in turn increase the encapsulation efficiency of each. Besides that, commercial solid lipid such as Precirol Ato 5 and Compritol 888 Ato can be better materials than synthetic polymer such as PLGA for oral drug delivery system [23], with satisfactory release profile in order to address the low bioavailability problem in the GIT. Besides that, riboflavin and quercetin will be more stable within solid lipid, and drug expulsion can be minimized by introducing liquid lipid [24].

Part II: Acarbose reformulation

The hypothesis is the reformulation of acarbose in polymeric microparticles will improve patient appliances and avoid multiple dosing. The coated polyelectrolytes will retard the release of drug in SGF to minimize the adverse side effect in GIT.

1.4 Objectives

Part I: Solid lipid microparticles

The main objective of the main project is to design food grade microparticles that are safe to consume and become an alternative to the polymeric microparticles with good controlled release of nutraceutical in the GIT. The objectives of this project are as follows:

1. Develop a new solid lipid formulation to co-encapsulate and deliver two model bioactive compounds (riboflavin and quercetin) simultaneously with different hydrophilicity.
2. Characterize and study the physicochemical properties of SLM.

3. Characterize and study the bioactive compounds loading capacity, release profile and release kinetics.

Part II: Acarbose reformulation

Acarbose is reformulated into a polymeric system for oral delivery. The MPs are then coated with polyelectrolytes to achieve a retardation of drug release in simulated gastric fluid. The objectives of Part II are as follows:

1. Reformulate acarbose into Alg-PLGA hybrid core-shell microparticles.
2. Coat the hybrid MPs with PEI and PAA polyelectrolytes.
3. Characterize and study the physicochemical properties of MPs.
4. Study the release behavior in simulated GIT fluids, comparing the effective retardation of drug release in simulated gastric fluid by coated polyelectrolytes.

1.5 Scope of work

This project seeks to develop a new solid lipid formulation to co-encapsulate and deliver two bioactive compounds which have the potential to act synergistically. This includes the fabrication techniques with the variation of the type of solid lipid and the incorporation of liquid lipid. Two model bioactive compounds, quercetin and riboflavin with different hydrophilicity were loaded in the SLM to examine the release behavior and release kinetics in different formulations.

The second part of the thesis covers the reformulation of a glucosidase inhibitor in Alg-PLGA hybrid microparticles. The MPs were coated with polyelectrolytes to achieve a retardation of drug release in SGF.

Chapter 2

Literature Review

This chapter describes the literature review for this thesis. Firstly, the materials used for the fabrication of microparticles, especially two types of solid lipids: Compritol 888 Ato and Precirol Ato 5. Besides that, the structure, composition, and the possible problems and limitations of the model bioactive compounds (quercetin and riboflavin) in oral delivery are introduced. Secondly, the fabrication method for the novel hybrid SLMs and the possible pros and cons of this system are also described. Last but not least, the various release kinetics models which usually used for fitting of the release profile are studied, in order to predict the possible release behavior of the model bioactive compounds from this novel system.

2.1 Materials background

Compritol 888 Ato (also named Glyceryl Dibehenate) is the product of esterification of glycerin and behenic acid (C_{22} fatty acid) without the use of any catalysts. Compritol 888 Ato is amphiphilic in nature due to the presence of partial acylglycerols, with the combination of monoglycerides (13-21% w/w), diglycerides (40-60% w/w) and triglycerides (28-32% w/w) of glycerol [25]. It has a hydrophilic-lipophilic balance (HLB) at around 2 and with a melting point at 70°C [24]. The chemical stability of Compritol is relatively high due to the peroxide value lower than $6 \text{ meqO}_2\text{kg}^{-1}$ [26].

Figure 1 Structure of (A) monobehenate, (B) dibehenate and (C) tribehenate [24]

Precirol Ato 5 has the chemical name of Glyceryl Palmitostearate. It is made up of monoglycerides (8-22% w/w), diglycerides (40-60% w/w) and triglycerides (25-35% w/w), with a melting point in the range of $53\text{--}57^{\circ}\text{C}$ and HLB value at 2 [25]. Precirol Ato 5 is a multifunctional excipient that is used as a lubricant, sustained release agent, and the unique properties of being used as a taste masking agent for the bitter oral drug. Precirol Ato 5 has a very similar composition with Compritol 888 Ato, the main difference is Precirol Ato 5 has shorter fatty acid chain length (C_{16-18}), which attribute less pronounced hydrophobic property, as compared to Compritol 888 Ato (C_{22}) [24].

Quercetin (3,3',4',5-7-pentahydroxyflavone) is a flavonoid that can be found in apple, onion or green tea. Quercetin is a polyphenol which is well-known as the most potent

antioxidants and highest antiradical properties among flavonoids [27]. The polyphenol structure of quercetin which contains numerous hydroxyl groups and double bonds contributes to the stabilization of scavenging free radicals by donating electrons, and this effect can reduce the oxidative stress in the human body, and thus helps to prevent degenerative disease [28]. The most significant impact of quercetin as a nutraceutical substance is the anti-inflammatory and anticancer effects. However, quercetin shows low aqueous solubility, poor systemic bioavailability, and bitter taste, which restricts the oral use of this compound [13].

The absorption mechanism of quercetin from GIT is still not fully understood, but it was believed that the quercetin in GIT undergoes cleavage by cytosolic β -glucosidase (CBG) within the enterocyte and deglycosylation by luminal hydrolysis by lactase phlorizin hydrolase (LPH) [29]. The metabolism of quercetin aglycone is then transformed into quercetin glucuronide conjugates form in the circulatory system. However, many types of quercetin metabolites have been discovered in plasma after oral administration of quercetin, as shown in Figure 2. Therefore, the detection and quantification of the major quercetin metabolites in plasma are definitely essential for example with the help of ultra-high performance liquid chromatography equipped with mass spectrometer (UPLC-MS), in order to have a big picture of the GIT metabolism and absorption of quercetin. Wittig *et al.* have used HPLC-MS in reaction monitoring mode to detect five quercetin glucuronides in human plasma [30]. Subsequently, Mullen *et al.* were able to identify 23 quercetin metabolites by HPLC with photodiode array (PDA) and MS detection [31]. A further study with the use of HPLC-MS with ion monitoring mode was able to identify three major metabolites that are quercetin-3-glucuronide (IV in Figure 2), quercetin-3'-sulphate (VI in Figure 2) and isorhamnetin-3-glucuronide (V in Figure 2) [32]. In addition, it was proved that quercetin-3-O-glucuronide (Q3G) was the most abundant metabolite in rat plasma [33]. This information definitely aids in the quantification of quercetin in the pharmacokinetic study for oral administration.

Figure 2 Structure of quercetin and its metabolites [34]

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a water-soluble vitamin (Vitamin B₂) that can be easily found in the milk or dairy products. Despite the fact that wide range of food sources contain riboflavin, riboflavin deficiency problem has become endemic in many countries due to a diet which lack of consumption of dairy products and meat, especially adolescents and elderly in many countries especially underdeveloped countries [35, 36]. Riboflavin is relatively stable to heat, but sensitive and can degrade under the light.

Figure 3 Structure of Riboflavin

Riboflavin is known as a hydrophilic photosensitizer, it can induce a change in the chemical substance through a hydrogen atom or electrons subtraction (type I pathway) or singlet oxygen generated from triplet oxygen (type II pathway) [8]. It is believed that the photosensitization of riboflavin by the additional hydroxyl groups can enhance the antioxidant property of phenolic compounds [8]. Therefore, this fact leads to the idea of encapsulating riboflavin together with quercetin, which can increase the antioxidant effect of quercetin, and deliver riboflavin concurrently in the same system.

2.2 Microparticles vs. nanoparticles

Basically, microparticles refers to the particles with the diameter range from 1 μ m to 1000 μ m [37], whereas nanoparticles include nano-sized to submicron sized particles. Little attention has been paid to solid lipid microparticles because nano- or submicron particles have higher applicability. However, for specific administration routes, for example, transdermal, intravenous and oral administration, micron size is more appropriate in delivering the drug to the target. Owing to the larger size of microparticles, the release can be more sustained with higher loading capacity [23], which means a lesser amount of microparticles with similar dosage need to be consumed. In addition, the toxicological issue faced by nanoparticles will not be a problem to microparticles [38]. Because of the above characteristics, microparticles seems to be more promising to be administered orally and deliver the nutraceutical into the GIT.

2.3 Fabrication methods of solid lipid microparticles

In order to obtain controlled release delivery system, the drugs or bioactive compounds must be trapped within the solid lipid rather than physically mixed. Several fabrication techniques have been devised since the emergence of solid lipid particulate system. These methods included solvent evaporation, melt dispersion, high-pressure homogenization, microchannel, spray drying, spray congealing and cryogenic micronisation [6]. Among these techniques, solvent evaporation emulsion and melt dispersion are most commonly used due to the absence of technically sophisticated equipment.

Solvent evaporation emulsion is the techniques widely used in polymer microparticles fabrication. The fabrication methodology is illustrated in Figure 4 Method 1. Solid lipid system can also be fabricated using solvent evaporation method due to its hydrophobic nature. Firstly, solid lipids are dissolved in the organic solvents, followed by the emulsification in a surfactant-containing aqueous phase, for examples poly (vinyl alcohol) (PVA) or Tween 80 solution. The oil-in-water (O/W) emulsion is stirred for few hours to ensure the complete evaporation of the solvent. The solvent evaporation techniques have been modified into various methods in order to form the desired morphology and structure, such as oil-in-oil-in-water (O/O/W), solid-in-oil-in-water (S/O/W), and water-in-oil-in-water (W/O/W) double emulsion. They are actually the similar methodology, only an extra step to form a primary emulsion is needed. For instance, $W_1/O/W_2$ emulsion, an aqueous phase W_1 is pre-mixed in organic solvent to form a primary emulsion, followed by adding this emulsion into a surfactant solution W_2 to form a secondary emulsion.

Figure 4 Schematic of W/O/W double emulsion. Solvent evaporation (Method 1) and hot homogenization (Method 2).

Melt emulsification, or known as hot homogenization is the most popular fabrication method in solid lipid system. This fabrication technique is very similar to solvent evaporation, but devoid of the organic solvent. The solid lipid is preheated at a temperature above the lipid melting temperature, and this molten lipid replaced DCM

as oil phase in the emulsion, as shown in Figure 4 Method 2. The advantage is the lack of the use of organic solvents, which can enhance the stability of particles and drugs. However, the drawback of melt emulsification is not suitable for heat-labile drugs or compounds.

2.4 Advantages & Limitation of solid lipid microparticles

Solid lipid such as Compritol® 888 Ato and Precirol® Ato 5 are widely used as the excipient in the tablet drug carrier and are known as “generally recognized as safe” (GRAS) substances in the pharmaceutical industry. Solid lipid nanoparticle (SLN) and Nanostructured lipid carrier (NLC) has gained the interest of many research groups because of the enhancement of the bioavailability of the poorly aqueous soluble drug, improvement of swallowability and taste masking properties [6, 24].

The concept of solid lipid particles is derived from the commonly used oil-in-water (O/W) emulsions, with the replacement of liquid lipid to solid lipid that exists as solid form at room temperature. In addition, solid lipid particles have lower production cost, ease of scaling up, avoid the use of organic solvent and increased bioavailability of sparingly water-soluble drug [23]. Moreover, solid lipid matrix can modify the release and protect the incorporated labile substances [23]. As aforementioned, the toxicity issue associated with the nanosized material will not be the concern to solid lipid microparticles.

Solid lipids are known to be hydrophobic in nature, which is very similar to liposome and polymeric particulate system, only poorly aqueous soluble drug with similar hydrophobicity or lipophilic drug can be encapsulated in this system easily. Although solid lipid particles usually exhibit high encapsulation efficiency of lipophilic substances, hydrophilic substances are not very well encapsulated in solid lipid particles [39]. Lee *et al.* have developed a multi-layer particulate drug delivery system with synthetic polymer, which successfully co-encapsulated two hydrophobic drugs Doxorubicin and Paclitaxel at separate layers [9]. This author further co-encapsulated hydrophilic drug Metoclopramide HCl and a hydrophobic drug Ibuprofen using the same system [10]. However, hydrophilic drug or good aqueous soluble drug tends to partition to the aqueous phase in this emulsion technique and resulting in a relatively

low encapsulation efficiency of hydrophilic Metoclopramide HCl. Therefore, Alan *et al.* have further developed a one-step double emulsion solvent evaporation with concurrent ionotropic gelation to fabricate a core-shell alginate-PLGA/PLLA structure, which improves the encapsulation efficiency of Metoclopramide HCl [22]. However, synthetic polymers are well-known as a long term controlled release agent which may not suit the oral administration because of its incomplete release within the duration in GIT. Baek *et al.* have designed polymeric floating microparticles for multiple drugs, the release profile seems promising for oral administration, which can achieve more than 80% release up to 24 hours [40]. The possible fast release within one day was attributed by the porosity caused by the fast solvent evaporation rate in reduced pressure.

Sustained release formulation for hydrophilic compounds for example peptide and protein has been studied extensively because these substances have many attractive properties. Although synthetic polymers such as PLGA and PLLA are biodegradable, biocompatible, and approved by Food & Drug Administration (FDA) to be used as a carrier for drug delivery system, the skepticism of the safety of the polymeric particles still exists due to the involvement of toxic organic solvent in the emulsion. The employment of toxic organic solvent may have deleterious effects to protein drugs [41]. Therefore, solid lipid system without the use of organic solvent during fabrication has become attractive for sensitive drugs. Nevertheless, the hydrophilic nature of proteins is expected to be poorly encapsulated within hydrophobic solid lipid matrix, extensive studies are still on-going to solve this problem.

Another drawback of solid lipid particles is the polymorphic transition (as shown in Figure 5) during storage. This is always observed when it comes to hot homogenization techniques, whereby the solid lipid changes from β to α form in the molten state, then the α form is retained after rapid cooling or crystallization step [24]. During storage, the crystal structure of solid lipid can slowly change from metastable state (α or β' polymorph) to a more stable state, which is the β form. After this phase transformation, there is a movement within the solid lipid matrix and the drugs have the chance to diffuse out from the carrier. This leads a relatively low loading capacity of the drug due to drug expulsion over a certain period of storage. Nanostructured lipid carrier (NLC) has been introduced by blending or replacing part of the solid lipid with liquid lipid (oil)

[42]. The liquid lipid has introduced the imperfection in the solid lipid lattice to accommodate drugs, which resulted in minimizing or avoiding drug expulsion during storage [43].

Figure 5 Polymorphic transition of solid lipid [24]

2.5 Fabrication of alginate-PLGA core-shell microparticle system

Synthetic polymers such as PLGA and PLLA are popular materials in fabricating polymeric microparticles and nanoparticles. Various fabrications for polymeric particulate system has been well established, includes emulsion solvent evaporation, solvent diffusion, nanoprecipitation and coacervation. However, these polymeric systems always lead to a low encapsulation efficiency of hydrophilic compound due to their relatively high hydrophobicity [44]. The hydrophilic compounds tend to partition to the external aqueous phase during fabrication due to poor affinity with hydrophobic polymer. Therefore, hydrogel system such as alginate, xanthan gum, and chitosan materials are usually a better choice to encapsulate hydrophilic compound for sustained-release formulation [2]. However, the sustained-release of hydrogel system for oral delivery is limited because hydrogel and swell and dissolve in the aqueous GIT environment.

To overcome the limitation of both polymeric and hydrogel system, a combination of both materials into a hybrid hydrogel-polymer particulate system has appeared. This composite can provide relatively high encapsulation efficiency of hydrophilic compound due to the affinity with hydrogel, yet provide a sustained release manner over longer period. The first generation of hybrid particulate system required the prefabrication of hydrogel and then dispersed the hydrogel into solid-in-oil-in water (S/O/W) double emulsion fabrication [45, 46]. After few modification, the hybrid

hydrogel-polymer particulate system has finally come to a single step double emulsion technique with concurrent internal gelling of hydrogel [22]. This technique reported a hydrophilic alginate hydrogel-core with hydrophobic PLGA shell. This system also proved a higher encapsulation efficiency of hydrophilic drugs. However, this system has not been used for oral delivery especially passing through the acidic gastric fluid. Thus, this thesis adopted this technique to study the GIT fate of this particulate system.

2.6 Drug release kinetics

In drug delivery field, one of the most challenging areas is the prediction of drug release from the particulate system as a function of time. Various mathematical dependent models have been developed to aid in explaining the drug release kinetics from the dissolution profiles. This offers a way to understand whether the drug release systems are diffusion-controlled, chemically-controlled or swelling-controlled [47]. There is no model that is universal to every system, a combination of different mathematical models can provide a more accurate insight of the mechanism of drug release and a better prediction from a different perspective. In this report, four mathematical models will be employed to study the drug release kinetics, namely zero-order, first-order, Higuchi equation and Korsmeyer-Peppas equation.

Eq. (1) describes the most simplified zero order drug release model. This model only applies to dosage forms that release the drug linearly with time, and the drug release rate is independent of concentration.

$$Q_t = Q_0 + K_0 t \quad (1)$$

where Q_t is the amount of drug released at time t , Q_0 is the initial amount of drug released at time=0, K_0 being the zero order release constant. The release kinetics can be plotted as the cumulative amount of drug released versus time [48]. This model is usually used to describe dosage forms such as transdermal system and matrix tablets with low soluble drugs.

Eq. (2) shows the first order model is dependent on concentration that is used to describe the absorption and elimination of drugs.

$$\log C = \log C_0 - Kt/2.303 \quad (2)$$

where C_0 is the initial drug concentration in the solution and K is the first order rate constant [49]. The *in vitro* release analysis can be plotted as log cumulative drug release percentage versus time. First order model is usually used to describe water-soluble drugs in porous matrices.

Higuchi equation was the first mathematical model proposed to describe drug release behavior from particulate system.

$$f_t = Q_t = A\sqrt{D(2C_0 - C_s)C_s t} \quad (3)$$

where Q_t represents the amount of drug release at time t , C_0 is the initial drug concentration, C_s is the drug diffused into the medium, and D is the diffusion coefficient of the drug [50].

Higuchi equation can be used in transdermal and some matrix systems with water-soluble drugs encapsulated. The data can be plotted as cumulative drug release percentage versus square root of time. Higuchi equation mainly describes the diffusion process of drug that follows the Fick's law which is dependent on square root time.

Korsmeyer-Peppas equation was developed after the Higuchi equation to define the water transport in the swellable glassy polymers [51]. This equation was initially developed for thin films that have a polymer relaxation and a diffusional component. For other geometries such as a sphere, different n -values was further derived as shown in Table 1, where k is the release rate constant showing the geometrical characteristics of the particles system, and n is the diffusional exponent of the transport mechanism.

$$\frac{M_t}{M_\infty} = kt^n \quad (4)$$

This power law only valid for the normalized drug release at any time lower than 60 %. The data can be plotted as log cumulative drug release percentage versus log time [52, 53]. The Fickian diffusion-controlled and relaxation-controlled are defined by n equal to 0.43 and 0.85, respectively. The Fickian diffusional release is characterized by the effect approximate to square root of time, whereas Case II shows the normalized water uptake at any time is not fully linearly proportional to time (near zero order release) caused by the relaxational swelling and water transport of the sample. The anomalous release is the intermediate between Fickian diffusional and Case II drug transport.

Table 1 Exponent n and drug release mechanism of Peppas equation from spherical polymeric system

Exponent, n	Drug release mechanism
0.43	Fickian diffusion
$0.43 < n < 0.85$	Anomalous transport
0.85	Case-II transport

Chapter 3

Hybrid Solid Lipid Microparticles (SLMs)

Chapter 3 demonstrates the thorough study of the SLMs. A combination of W/O/W double emulsion and the melt emulsification technique was employed to design this novel formulation. After which, the characterization of the physicochemical properties of the studies are conducted. Next, the release study of the quercetin and riboflavin is studied in a simulated gastric fluid and simulated intestinal fluid. Lastly, the release profile is fitted into the release kinetics models. This study shows that this novel SLMs formulation has the potential to co-encapsulate and co-deliver two bioactive compounds with different hydrophilicity, and allow the controlled release of nutraceuticals with a synergistic effect.

Materials and Methods

3.1 Materials

Glyceryl Dibehenate (Compritol 888 Ato) and Glyceryl Palmitostearate (Precirol Ato 5) were obtained from Masung & Co. Ltd, Korea. Alginic acid sodium salt from brown algae, Tween 80, and poly (vinyl alcohol) (PVA, molecular weight 30-70 kDa) were purchased from Sigma-Aldrich. The bioactive compound, riboflavin and quercetin were purchased from Sigma-Aldrich. Liquid lecithin was purchased from the local supermarket. Phosphate buffer saline (PBS) and methanol were obtained from Gibco and Tedia Company Inc, respectively. All chemicals and solvents were used as received unless otherwise noted. Nile red and calcein were used for confocal laser scanning microscopy (CLSM).

3.2 Particles Fabrication

Hot homogenization with slight modification was employed to fabricate biopolymer-solid lipid hybrid microparticles [22]. Briefly, 2 % (w/v) sodium alginate (NaAlg) was prepared by dissolving 10 mg of NaAlg in 0.5 ml of deionized water. Riboflavin (theoretical drug loading of 2 % w/w) were dissolved/dispersed in the internal aqueous phase. Tween 80 (1 % (w/v)) was added to prevent the partition of riboflavin and alginate into the external aqueous phase, as well as to increase the solubility of riboflavin in the internal water phase. Solid lipid (Precirol Ato 5 or Compritol 888 Ato, 1 g), 30mg of liquid lecithin and quercetin (theoretical drug loading of 2 % w/w) were mixed at 10 °C above the melting temperature. For dye-loaded SLM, nile red and calcein were separately mixed with molten solid lipid and alginate solution, respectively. Subsequently, NaAlg-riboflavin solution was added dropwise into molten solid lipid and emulsified for a few minutes under magnetic stirring to form primary water-in-oil (W/O) emulsion. The temperature of primary emulsion was held at the same temperature. This primary emulsion was then dispersed into 100 ml of 0.5 % (w/v) PVA solution, with a constant stirring of 400 rpm by overhead stirrer (Calframo BDC1850-220) at the same temperature. After 10 seconds, the emulsion was rapidly cooled down to room temperature by ice/water bath. Hardening of microparticles occurred immediately below the melting temperature. The microparticles were then

filtered, rinsed with deionized water and lyophilized. The lyophilized microparticles were kept in the desiccator for further characterization.

3.3 Quantification of quercetin and riboflavin

Quercetin was analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) with the ultraviolet detector, whereas riboflavin was detected using fluorescent detection by a plate reader (Infinite® M200, Tecan, Switzerland). The HPLC detection was achieved by Agilent 1100 Series with Ascentis® C18 column (250 mm x 4.6 mm, pore size: 5 µm), methanol and water (70:30 v/v) as mobile phase at UV detection wavelength 370 nm. Fluorescent detection of riboflavin was achieved at excitation wavelength 450 nm and emission wavelength at 527 nm. The column temperature was set at 35 °C and the concentrations were measured isocratic elution with a flow rate of 1 ml/min.

3.4 Particles Characterization

Scanning Electron Microscopy (SEM)

The cross-sectional morphology of the microparticles was observed using scanning electron microscopy (SEM, JEOL JSM-6360A, Tokyo Japan). The samples were mounted onto a metal stub and cut into half using a razor blade, followed by coating in a gold sputter coater (SPI-Module) prior to SEM observation. The particle size was analyzed using ImageJ software.

Confocal Laser Scanning Microscopy (CLSM)

Nile red and calcein were used to stain solid lipid and alginate respectively. Dyes were mixed with polymers before fabrication as mentioned previously. Dried microparticles loaded with dye were placed onto the glass slide and covered with a coverslip. CLSM images were captured using Zeiss LSM710 confocal laser scanning microscope with 10x dry objective lens and ZEN software (Carl Zeiss). For nile red, the excitation wavelength was centered at around 550 nm, with the emission wavelength of 630 nm.

For calcein, the excitation wavelength and emission wavelength were fixed at 495 nm and 516 nm, respectively.

Raman Mapping

DXR™ 2xi Raman Imaging Microscope (Thermo Scientific) was used to collect the Raman imaging data in order to observe the polymers and drugs distribution. Dried microparticles were cross-sectioned using razor blades and mounted into the sample chamber. Raman mapping was performed with a step size interval of 5 μm with a 532 nm laser. Spectrum range was collected from 50 to 3200 cm^{-1} .

Differential Scanning Calorimetry (DSC)

The thermal analysis of microparticles was performed by differential scanning calorimeter (TA Instrument-Q10). The samples were sealed in an aluminum pan and scanned between 0 °C to 100 °C with a heating rate of 5 °C/min. The melting point peaks revealed the crystallography difference of solid lipid microparticles.

X-Ray Diffraction (XRD)

Powder X-ray diffraction using a $\text{CuK}\alpha$ radiation (40 kV voltage, 20 mA) were used to determine the crystallographic change of solid lipid microparticles. Measurements were performed at 2θ angles scanning interval from 5 ° to 35 °, with step scan of 1 s/step.

3.5 Encapsulation Efficiency

Encapsulation efficiency is the percentage of actual drug loading divided by the theoretical drug loading. Five mg of microparticles were accurately weighed and dispersed in 10 ml of methanol, followed by sonication for at least 30 mins to ensure the breakage of microparticles to release quercetin and riboflavin completely. The supernatant was then collected, filtered through a 0.22 μm syringe filter and analyzed using the aforementioned HPLC method.

3.6 Stability of bioactive compound

Free quercetin, free riboflavin and the mixture of both compounds were dissolved in methanol and kept in 37 °C. Besides that, four SLM formulations were also kept in 4 °C and 37 °C separately. The bioactive compounds from SLM were extracted using methanol and determined using HPLC at predetermined time points. The stability of free bioactive compounds and the compounds within microparticles (n = 3) were studied up to three months.

3.7 *In Vitro* release study

Hybrid microparticles (5 mg, n = 3) were immersed in a vial containing 3 ml of dissolution medium (2 hours in simulated gastric fluid and up to 24 hours in simulated intestinal fluid) and placed at 37 °C with constant stirring. An aliquot of dissolution medium (1 ml) was collected at predetermined time points, and the same volume of medium was replaced with fresh dissolution medium to maintain sink condition. The concentration of quercetin and riboflavin were determined using HPLC and plate reader, respectively.

3.8 Results and Discussion

In a preliminary experiment, solid lipid was dissolved in dichloromethane (DCM) in order to employ double emulsion to fabricate core-shell structure microparticles [22]. However, in the primary emulsion, phase separation happened immediately and the mixture quickly separated into water and DCM layers due to poor wettability and low affinity between solid lipid and alginate (Figure 6, P2), whereas P1 is the PLGA-DCM and water emulsion which formed a stable primary emulsion. The orange is the color contributed by riboflavin for color visualization of the phase separation. Due to the repulsion between hydrophobic solid lipid and hydrophilic alginate, the partitioning of internal aqueous phase to the outer phase in W/O/W double emulsion led to a low encapsulation efficiency of hydrophilic compounds. This phenomenon was in accordance with the result reported previously [39]. The second problem was the difficulty in dissolving Compritol in DCM because Compritol had low solubility in DCM. Solid lipid must be fully dissolved in the oil phase in order to form a good

emulsion in solvent evaporation fabrication technique. Moreover, solid lipid microparticles employed by solvent evaporation techniques led to weak mechanical properties due to the porous structure caused by fast solvent evaporation rate [54]. These are the limitations faced by solid lipid system, and thus there are very few investigations on the solvent evaporation technique on solid lipid system for hydrophilic compounds [54, 55].

Figure 6 Stable primary emulsion (P1), Phase separation of DCM and water layers (P2)

In order to achieve a good retention of hydrophilic riboflavin and alginate, hot homogenization without the use of organic solvent was employed to ensure the internal phase was trapped within the solid lipid particles. The emulsifying time is the main parameter for alginate and riboflavin encapsulation because the partitioning of inner water phase can lead to the destruction of the integrity of microparticles. The emulsifying time was optimized to be 10 seconds in order to allow enough emulsion time to attain good spherical particles, as well as to reduce the loss of riboflavin to the outer aqueous phase. Besides that, alginate was chosen as the incorporated biopolymer because of its stability, biocompatibility, safe to consume, and especially its inertness, which does not react with other drugs and polymers. Whey protein isolate was also used

to investigate in the preliminary experiment, but whey protein isolate aggregated at high temperature in the presence of NaCl [56]. Therefore, careful selection of biopolymer is very important.

Table 2 Composition of SLM to achieve different release profile

Sample	Solid Lipid	Liquid Lipid	Particles Size (μm)	Encapsulation Efficiency (%)	
				Riboflavin	Quercetin
SL1	Precirol (1 g)	-	254.5 $\pm$ 96.5	34.65%	54.42%
SL2	Precirol (0.8 g)	Oleic Acid (0.2 g)	402.7 $\pm$ 147.7	49.94%	89.00%
SL3	Compritol (1 g)	-	374.6 $\pm$ 119.1	49.00%	80.11%
SL4	Compritol (0.8 g)	Oleic Acid (0.2 g)	504.8 $\pm$ 164.4	48.57%	80.93%

To achieve different drug release kinetics, several formulations of hybrid microparticles were fabricated (details summarized in Table 2). Two types of solid lipid with different composition and hydrophobicity were chosen to study the difference in drug entrapment and release kinetics. The idea of using liquid lipid such as oleic acid came from nanostructured lipid carrier (NLC), which was employed to solve the drug expulsion problem during phase transformation [26]. Besides this, the liquid lipid can also improve the encapsulation efficiency due to the higher solubility of the hydrophobic drug. While all the samples fabricated with the same techniques, they differed in the type of solid lipid and liquid lipid used. The same amount of quercetin and riboflavin were loaded for standardization. Lecithin was used as the surfactant due to the compatibility with lipids and it was proved to increase the absorption of quercetin in the GIT when administered together with lipid [14]. The fabrication rationale is illustrated in Figure 7.

3.8.1 Physico-chemical characterization of SLMs

SEM images of SLM are shown in Figure 8 where the cross-sectioned morphology on the left and surface morphology is at the right column. The SLM were very brittle, the particles broke immediately after a slight cut by the razor blade. The brittle fracture

could minimize the damage of the cross-sectioned surface. The surface morphology of SLM appeared to be spherical but with a certain roughness despite the type of solid lipid. Particles size of SLM ranged from 150 μ m to 650 μ m, Compritol had larger size than Precirol (data tabulated in Table 2) because Compritol has longer carbon chain and that probably increased the viscosity of molten solid lipid. SLM with the incorporation of liquid lipid (SL2 and SL4) were larger than the SLM without liquid lipid (SL1 and SL3). This was due to the higher viscosity of oleic acid than the molten solid lipid. The wide size distribution was attributed by the short emulsifying time that minimized the partition of riboflavin solution to the outer aqueous phase before the solidification of solid lipid. From the cross-sectional images, some of the big pores were noticed within the microparticles. The pores were formed when the riboflavin-alginate solution trapped within the particles during emulsifying, and these two compounds were expected to localize in the pores after water removal by lyophilization. However, both quercetin and riboflavin have rod-like shape and it was very difficult to distinguish the compounds based on SEM images.

Figure 7 Fabrication schematic of hybrid alginate-solid lipid microparticles, a combination of W/O/W double emulsion with hot homogenization.

In order to have a better understanding of the localization of alginate and two bioactive compounds, confocal microscopy and Raman mapping were conducted. From the CLSM images (Figure 9), solid lipid and alginate were stained with Nile red (red) and calcein (green) respectively. Quercetin and riboflavin are not visible under CLSM, therefore two dyes were used to replace these two bioactive compounds in order to

Figure 8 SEM images of SLMs loaded with riboflavin and quercetin. Left: cross-sectioned surface; Bottom row: surface. (a-d) SL1 to SL4. Scale bar shows 100 μm .

study and predict the localization of compounds within SLM. Nile red could represent the possible location of quercetin whereas calcein showed the localization of riboflavin

due to the similarity in hydrophilicity. From CLSM images, it is interesting to note that calcein actually distributed everywhere, together with a small cluster of calcein representing the alginate within the pores. This phenomenon could be explained by the very high drug loading of riboflavin (20 mg riboflavin compared with 10 mg alginate) and low solubility of riboflavin in water. The undissolved riboflavin might not stay with water during primary emulsification. Another hypothesis was the short emulsifying time might not allow enough time for a stable emulsion, and riboflavin was trapped in solid lipid matrix during the solidification. Besides that, solid lipid might possess amphiphilic behavior, which could attract riboflavin and calcein by affinity. CLSM, in this case, gave an idea but insufficient proof for the possible localization of alginate. Thus, Raman mapping was conducted as a complementary study to the CLSM.

Figure 9 CLSM of SLMs loaded with dyes. (a) Nile red, (b) Calcein, (c) overlay image of solid lipid and alginate, (d) overlay with bright field image.

Raman mapping (Figure 10) shows the localization of the bioactive compounds and polymers by the degree of intensity. The particle with big pores was selected for the ease of Raman mapping. Red shows the highest intensity and the presence of the compound at a particular location, whereas blue being the lowest intensity and the absence of the compound. For the carrier materials, alginate was shown to be localized within the pore whereas solid lipid was everywhere except in the pore, which proved the hypothesis was correct. As for the bioactive compounds, riboflavin distributed everywhere in Raman mapping, which was in concordance with the confocal microscopy result explained previously and therefore proved the reliability of the

confocal microscopy result. One thing to note is that the Raman intensity of riboflavin at the solid lipid matrix was higher than the intensity in the pore. However, the optical microscope image shown in the middle had darker orange in the pore than the matrix. That was probably because of the different scanning height for the pore and the cross-sectioned surface that caused the inverted result.

Figure 11 reports the DSC thermograms of SLM at different storage conditions. The melting points revealed the crystallography of the samples. Take SL1 as an example, the curve (i) is the Precirol raw material, the thermogram shows the endothermic peak at 57.1 °C, together with a shoulder peak at 62.1 °C. Fresh sample (curve ii) being the newly fabricated sample, shows two different melting temperatures at 52.2 and 57.4 °C. The difference in the shoulder peak indicated the change in the crystalline form due to the rapid cooling in fabrication. This phenomenon is in agreement with many studies reported that solid lipid microparticles transformed from β to α form in a molten state, and the metastable α form was trapped after the rapid cooling [24]. Curve (iii) to (v) reveal the crystallography form of samples under three different storage conditions after 3 months. At 4 °C, the endothermic peaks were almost the same as the peaks of the fresh sample. This suggests no phase transformation occurred at this temperature. Storage at 37 °C showed a complete transformation from α back to β form, which had a similar melting temperature with the untreated Precirol. The crystallography of SLM at 25 °C storage temperature was still in the middle of transformation. SL3 with Compritol without liquid lipid showed the similar trend as SL1.

Figure 10 Raman mapping of cross-sectioned SLMs.

Figure 11 (b) and (d) show SL2 and SL4 with the incorporation of oleic acid with a melting point at 10.6 °C. However, the oleic acid peak was rarely observed in curve (iii) to (iv). This was probably due to the low amount of liquid lipid and it was not comparable with a very high amount of solid lipid in SLM. Nevertheless, oleic acid did not affect the main peaks at the range between 50 to 75 °C. All samples exhibited similar crystallography before and after storage, suggesting all samples experienced a complete phase transformation from α to β form after 3 months at 37 °C.

Figure 11 DSC reveals phase transformation of SLMs under different storage conditions. (a-d) SL1 to SL4

XRD was used to support the DSC results. As depicted in Figure 12, the untreated Precirol showed two peaks at 19.3° and 22.8°, whereas the fresh SL1 showed the overlapped peaks at 21.2° and 22.1°. This suggests that the fresh SLMs having the

different form with the untreated Precirol, and it is in concordance with the DSC results shown previously. After a storage of three months, storage at 4 °C remained similar crystallography with the fresh sample, whereas the storage at 37 °C showed a complete change to the most stable β state. SL2 with the incorporation of oleic acid actually slowed down the phase transformation. Nevertheless, SL2 was still in the middle of transformation to the β form in the storage of 37 °C. The XRD results followed the trend of DSC results whereby the crystallography of Precirol fully transformed from α to β form in the storage of 37 °C after three months. For Compritol formulation, SL3 did not show obvious changes in 2θ , but SL4 showed crystallography change after storage. This suggests that Compritol experienced a partial phase transformation back to stable form probably due to high melting temperature and more time permitted for solid lipid to change back to stable form during solidification, but oleic acid might affect the melting point of solid lipid and hindered the phase transformation back to the stable state.

Figure 12 XRD shows the phase transformation from α to β phase after storage. (a-d) SL1 to SL4

3.8.2 Stability study

The main purpose of studying the phase transformation of SLMs is to investigate the drug expulsion that often occurs in solid lipid system [24]. Therefore, the amount of drug remaining in the SLMs was studied by comparing the encapsulation efficiency before and after phase transformation. Firstly, the stability test of two bioactive compounds was conducted at different conditions. Figure 13a shows the stability test of free quercetin and free riboflavin, whereas Figure 13b shows the mixture of both bioactive compounds in methanol over three weeks. In Figure 13a, free riboflavin was stable across 21 days but free quercetin started to degrade and showed a decrease in concentration after 7 days. This phenomenon showed that quercetin is not stable in methanol after 7 days. Whereas in Figure 13b, the mixture of quercetin and riboflavin showed similar trend whereby quercetin showed a drop in concentration and riboflavin was stable after 21 days of storage. No interaction between quercetin and riboflavin was observed due to the fact that riboflavin had a constant concentration throughout 21 days and the mixture showed the same trend with the free compound (Figure 13a).

Figure 13 Stability test: (a) Quercetin and riboflavin stored separately at 37°C, and (b) Quercetin and riboflavin mixture at 37°C

Figure 14 shows four formulations stored at 37°C and 4°C separately and it was observed that quercetin and riboflavin have constant encapsulation efficiency across 3 months, suggesting that these two bioactive compounds remained intact and well protected within SLM regardless of the storage temperature. Even though there is some fluctuation in term of encapsulation efficiency, at least no drastically decrease in encapsulation efficiency across 3 months was observed. The inconsistency could be caused by the HPLC condition and human error. During the rearranging of the SLM

matrix, drug expulsion often occurred when the drug was squeezed out from the solid lipid particles [24]. No compound expulsion was observed in our case probably due to the porous structure of the SLM which provided spaces for the bioactive compounds movement during phase transformation. Instead of partitioning out from the SLM, the bioactive compounds escaped into the pores. Thus, a porous SLM structure could be the next solution to the drug expulsion problem.

Figure 14 Stability test of bioactive compounds encapsulated in solid lipid system:

(A) Quercetin at 37°C; (B) Quercetin at 4°C; (C) Riboflavin at 37°C; (D) Riboflavin at 4°C.

3.8.3 Release profile and release kinetics study

The solubility of quercetin in water, SGF and SIF at room temperature are only 7.7 $\mu\text{g/ml}$, 5.4 $\mu\text{g/ml}$, and 28.87 $\mu\text{g/ml}$, respectively [57]. Thus, to achieve a sink condition in *in vitro* release study, 1 % (w/v) of Tween 20 and 1 % (w/v) of Tween 80 were used to increase the solubility of SGF and SIF, respectively. Normally the reported gastric emptying time is 3 to 5 hours, 2 h of release study in SGF is usually employed for nanoparticles, whereas 5 h prolonged release is for floating microparticles [40]. In this

study, SLMs floated on the SGF for the first few hours, therefore a prolonged gastric retention time (GRT) of 5 h was studied. Although the release profile shows result up to 24 hours, the main absorption period is only up to 10 or 12 hours, which is the period when the maximum absorption happens in colon and ileum [58]. Moreover, quercetin was known to have low bioavailability and bioaccessibility (~7 % in native form) [59], sustained release in gastrointestinal tract would definitely be advantageous.

Figure 15 shows the release profile of quercetin and riboflavin from four different formulations SL1 to SL4 after 3 months of storage. All of the samples achieved 100 % total release within 24 h except SL3 (Compritol without oleic acid). Quercetin and riboflavin exhibited relatively similar release rate for all formulations whereby a very consistent release rate was observed up to 12 h, regardless of the type of release medium. Compritol is always known to have good sustained release properties due to its complex structure [24], thus it was not surprising that lower than 40 % of total release was observed for both bioactive compounds in SL3. However, the incorporation of oleic acid into Compritol formulation (SL4) could compensate and accelerate the release rate. This made Compritol formulation useful in the oral delivery if complete release could be achieved within 24 h.

In addition, the release rate of quercetin and riboflavin was very similar regardless of the partition coefficient of the compounds ($\log p$ of riboflavin is -1.1, $\log p$ of quercetin is 2.16). This is a very interesting phenomenon because usually hydrophilic compound was expected to be released faster than a hydrophobic compound. The hydrophobic-hydrophobic interaction between quercetin and solid lipid should lead to a slower release rate. This could be explained by a large amount of riboflavin was trapped within the pore and riboflavin took time to diffuse out to the dissolution medium, thus a sustained release could be achieved. Another explanation was that the alginate within the pore contracted at low pH and a certain amount of riboflavin was trapped within alginate, thus slow release when exposed to SGF [60]. Nevertheless, the release profile showed the potential of these formulations to deliver both hydrophilic and hydrophobic compounds simultaneously at the similar rate.

Figure 15 *in vitro* release profile of quercetin and riboflavin after storage (a-d) SL1 to SL4.

To elucidate the drug release mechanism from different formulations, the release data were fitted to various models and summarized in Table 3 and Table 4. From the data, both quercetin and riboflavin could be well fitted into Higuchi and Korsmeyer-Peppas equation. Only riboflavin in the Compritol formulation (SL3 and SL4) had high correlation coefficient for zero-order, suggesting riboflavin released at a near constant rate which was independent of the concentration.

Firstly, all data could be fitted into Higuchi equation. Generally, formulations with Compritol fitted into the equation better than Precirol, showing a higher correlation coefficient. Higuchi equation mainly described the diffusion behavior of drugs followed the square root of time, which means Compritol followed diffusion-controlled more than Precirol. The rate constant k showed relatively close value for both compounds in SL1 and SL2, suggesting quercetin and riboflavin in Precirol formulation exhibited very similar release pattern. The similar Higuchi rate constant value is highly desirable for more than one bioactive compounds in a single system because similar rate constant k shows the similarity in the release pattern and release rate. High Higuchi

rate constant k was desired in this case in order to have a complete release within 24 h. For Compritol, the rate constant k value of quercetin and riboflavin in SL4 is considerably close. Unfortunately, the incomplete release of drug in SL3 led to a lower k value.

Table 3 Release kinetics of quercetin from SLMs.

Formulation	Correlation Coefficient (r^2)				Rate constant k ($\text{h}^{-1/2}$)	Release exponent n
	Zero- order	First- order	Higuchi equation	Korsmeyer- Peppas equation	Higuchi equation	Korsmeyer- Peppas equation
SL1	0.7000	0.5541	0.8730	0.9479	25.799	0.6632
SL2	0.6965	0.5474	0.8842	0.9584	25.141	0.6390
SL3	0.7842	0.6317	0.9078	0.8630	8.0967	0.5767
SL4	0.7955	0.6758	0.9195	0.8582	20.950	0.5128

Table 4 Release kinetics of riboflavin from SLMs.

Formulation	Correlation Coefficient (r^2)				Rate constant k ($\text{h}^{-1/2}$)	Release exponent n
	Zero- order	First- order	Higuchi equation	Korsmeyer- Peppas equation	Higuchi equation	Korsmeyer- Peppas equation
SL1	0.6238	0.4227	0.8389	0.9742	26.763	1.0766
SL2	0.6735	0.4937	0.8930	0.9848	25.004	0.7690
SL3	0.9732	0.6998	0.9713	0.9960	10.686	0.7120
SL4	0.8908	0.6424	0.9714	0.9962	19.702	0.7893

Secondly, both quercetin and riboflavin were best fitted into power law, with the highest correlation coefficient among all the models. Power law was further derived from Higuchi equation, to further distinguish the particulate system into diffusion-controlled and matrix erosion-controlled. For quercetin, the release exponent n fall between $0.43 < n < 0.85$, which indicated that the release mechanism involved anomalous

transport (Fickian diffusion together with matrix erosion). However, riboflavin in SL1 was a Case II transport ($n = 1.0766$), whereas other formulations followed anomalous transport. The case II transport of riboflavin in SL1 showed that the erosion of solid lipid was the predominant factor for the release rate. However, quercetin in the same formulation exhibited much slower release (anomalous transport), which showed the mathematical model was accurate when compared with the release profile result shown in Figure 15. This also further showed that SL2 with the incorporation of oleic acid could slow down the erosion of solid lipid and change the release kinetics towards a diffusion type, and hence the release exponent n in SL2 of both compounds was lower than SL1. This power law could further distinguish the compounds release pattern in Precirol to be the matrix erosion-controlled, while Compritol was closer to the diffusion-controlled.

The previous release profile and release kinetics data of both bioactive compounds showed that SL1 and SL2 having desirable release pattern. Therefore, these two formulations were used for the second comparison of release profile before and after storage to investigate the different profile after a phase transformation. It was hypothesized that the release profile can be altered due to the changes in polymorph. From Figure 16, it was obvious that the quercetin and riboflavin release profile were very different for fresh and stored SLM. Quercetin in freshly prepared SLM had slower release rate and incomplete total release after 24 h. Whereas for riboflavin, the release pattern was almost the same except the release rate. That was probably due to the compact structure of fresh SLMs, quercetin was trapped in the solid lipid matrix and thus the slow diffusion rate, but that did not affect the diffusion rate of riboflavin that stayed inside the pore. After storage, the compounds diffusion rate and mechanical strength of SLMs changed after polymorphic transformation. The solid lipid matrix was not as stiff as the fresh sample and that led to higher and faster diffusion rate.

Figure 16 Comparison of release profile for quercetin and riboflavin before and after storage. (a) Quercetin in SL1, (b) Riboflavin in SL1, (c) Quercetin in SL2 and (d) Riboflavin in SL2.

Chapter 4

Acarbose Reformulation in Polymeric System

This chapter demonstrates a reformulation of an oral antidiabetic agent in the hybrid polymeric system for oral delivery. A W/O/W double emulsion with internal gelling method is employed to encapsulate acarbose. Extra polyelectrolytes coating layers are coated onto the microparticles to manipulate the release profile of acarbose in the simulated gastric and intestinal fluid. The retardation of acarbose release in the stomach can definitely reduce the side effects suffered by the patients. Moreover, this system also proves the possibility in encapsulating hydrophilic compound into the polymeric system.

Materials and Methods

4.1 Materials

Poly (DL-lactide/glycolide) 50:50 (intrinsic viscosity 1dl/g) was obtained from Purac Biomaterials. Alginic acid sodium salt from brown algae, poly (vinyl alcohol) (PVA, molecular weight 30-70 kDa), Span® 80, Tween® 80, Calcium chloride (CaCl_2), formic acid, polyethylenimine (PEI, high molecular, water free), poly (acrylic acid) (PAA) and Acarbose $\geq 95\%$ were obtained from Sigma-Aldrich. Sodium chloride (NaCl) was purchased from J.T Baker Ltd. Phosphate buffer saline (PBS) and HPLC grade Acetonitrile (ACN) were obtained from Gibco and Tedia Company Inc, respectively. All chemicals and solvents were used as received unless otherwise noted.

4.2 Preparation of Alg-PLGA Microparticles

A slight modification of W/O/W double emulsion solvent evaporation with concurrent internal gelling method was employed [22]. Briefly, 2 % (w/v) sodium alginate (NaAlg) solution was prepared by dissolving 20 mg of NaAlg in 1ml of deionized water. NaCl (2 mg) was used as an osmolyte, 100 mg Acarbose and Tween 80 (1 % (w/v)) were added in the internal aqueous phase. Separately, PLGA (10 % (w/v)) was dissolved in 4 ml DCM, Span 80 (1 % (w/v)) was added into the oil phase as surfactant. Subsequently, NaAlg-Acarbose solution was added dropwise into PLGA/DCM solution and emulsified under magnetic stirring to form primary water-in-oil (W/O) emulsion. CaCl_2 (50 mM) was dissolved in 100 ml of PVA (0.5 % (w/v)) solution. The primary emulsion was then poured into the PVA solution, with a constant stirring at 300 rpm to form secondary water-in-oil-in-water (W/O/W) emulsion. The secondary emulsion was stirred for 3 hours to ensure DCM was completely evaporated. The microparticles were then filtered, rinsed with deionized water and lyophilized.

The coating onto Alginate-PLGA microparticles was done by polyelectrolyte multilayers coating technique [61]. The microparticles were dispersed into a PEI solution (50 wt %) with gentle stirring for 5 minutes. The PEI solution was then decanted, followed by the addition of PAA solution (50 wt %) as the second layer with the same method. After that, the microparticles were filtered, rinsed and air-dried.

Figure 17 Principle of double emulsion (W₁/O/W₂) emulsion with concurrent ionotropic gelation.

4.3 Particles Characterization

Scanning Electron Microscopy (SEM)

The cross-sectional morphology of the microparticles was captured using scanning electron microscopy (SEM, JEOL JSM-6360A, Tokyo Japan). The samples prior to analysis were mounted onto a metal stub and were cut into half using a razor blade. The stub was then coated in a gold sputter coater (SPI-Module) before analysis. The particle size was analyzed using ImageJ software.

Confocal Laser Scanning Microscopy (CLSM)

Coumarin 343 and calcein were used to stain PLGA and alginate respectively. Microparticles loaded with dye were placed onto the glass slide and covered with a cover slide. CLSM images were captured using Zeiss LSM710 confocal laser scanning microscope with 10x dry objective lens and ZEN software (Carl Zeiss). For Coumarin 343, the excitation wavelength was centered at around 440 nm, with the emission wavelength of 510 nm. For calcein, the excitation wavelength and emission wavelength were fixed at 495 nm and 516 nm, respectively.

Raman Mapping

DXR™ 2xi Raman Imaging Microscope (Thermo Scientific) was used to collect the Raman imaging data in order to observe the polymers and drugs distribution. Microparticles were cross-sectioned using razor blades and mounted into the sample chamber. Raman mapping was performed with a step size interval of 5 μm with a 532 nm laser. Spectrum range was collected from 100 to 3200 cm^{-1} .

4.4 Encapsulation Efficiency

Encapsulation efficiency is the percentage of actual drug loading divided by the theoretical drug loading. Microparticles (5 mg) were accurately weighed and dissolved in 1 ml of DCM, followed by adding 3 ml of SGF (n=3) with a constant stirring at 300 rpm. The hydrophilic acarbose is soluble in the water-based medium and the supernatant was analyzed using reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC-UV). The detection was achieved by Agilent 1100 Series with Ascentis® C18 column (250 mm x 4.6 mm, pore size: 5 μm), acetonitrile and water (80:20 v/v) with 0.1 % (v/v) formic acid as mobile phase at UV detection wavelength 210 nm. The column temperature was set at 35 °C and the concentrations were measured isocratic elution with a flow rate of 0.5 ml/min.

4.5 *in vitro* release study

Microparticles (50 mg) were immersed in a vial containing 3 ml of dissolution medium (2 hours in simulated gastric fluid (SGF) and subsequently transferred to simulated intestinal fluid (SIF)) and placed at 37 °C with constant stirring. An aliquot of dissolution medium (1 ml) was collected at predetermined time points, and the same volume of medium was replaced with fresh dissolution medium to maintain sink condition. The concentration of Acarbose was determined using the aforementioned HPLC method.

4.6 Kinetics modeling on drug release profile

The kinetics of drug release profile were analyzed with fitting to zero-order cumulative amount of drug release vs time), first order (log cumulative percentage of drug remaining vs time), Higuchi (cumulative percentage of drug released vs square root of time) and Korsmeyer-Peppas (log cumulative percentage drug released vs log time) equations.

4.7 Results and Discussions

Alginate particulate system does not provide sustained release and burst release is often observed, whereas PLGA monolithic polymeric system does not provide good entrapment of hydrophilic drug due to its hydrophobic nature. Furthermore, acarbose is hygroscopic and highly soluble in water (partition coefficient of -6.4), the entrapment of this hydrophilic drug can be very challenging. Therefore, it was hypothesized that high encapsulation efficiency and sustained-release could be achieved for the hydrophilic drug by combining alginate and PLGA into a single system, and this has proved by Alan *et al.* [22].

W/O/W double emulsion solvent evaporation with concurrent ionotropic gelation technique was employed to fabricate Alg-PLGA hybrid microparticles. The fabrication rationale is illustrated in Figure 17. The CaCl_2 was driven into the core by the salt concentration gradient and ionotropic gelation of alginate happened before the hardening of PLGA. Crosslinked alginate core plays a role in entrapping hydrophilic drug whereas PLGA shell acts as a rate-limiting layer. Therefore, alginate-PLGA hybrid microparticles have become a strong candidate for the acarbose reformulation.

4.7.1 Physico-chemical characterization of Alg-PLGA MPs

Figure 18 shows the SEM images of the fabricated microparticles. The blank microparticle (Figure 18a) was observed to be a core-shell structure, with an average size of $385.25 \pm 105.16 \mu\text{m}$. However, the acarbose loaded microparticles (Figure 18b) showed a change in the structure but with similar size ($357.38 \pm 77.39 \mu\text{m}$). With the presence of acarbose, the PLGA shell became thicker and porous, while the middle core

of alginate remained within the shell. Figure 18c, d, and e represent the surface morphology of blank, PEI coated and PAA coated microparticles, respectively. The surface morphology of PEI coating showed a smoother texture than blank microparticles, whereas PAA coating had a wrinkle and rough surface than PEI coating. This suggests a high probability of polyelectrolytes attachment onto the microparticles.

Figure 18 SEM images of cross-sectioned microparticles. (a) Blank and (b) acarbose loaded MPs. Surface morphology of MPs. (c) Blank, (d) with PEI coating, and (e) with PAA coating.

In order to have a better understanding of the distribution of polymers and drug, CLSM was employed since acarbose morphology was not distinguishable under SEI images. Calcein is a hydrophilic fluorescence dye which has high affinity with alginate, and hence hydrophilic acarbose was hypothesized to have similar distribution with calcein due to the hydrophilic affinity with alginate. CLSM images (Figure 19) confirmed that coumarin 343 (blue) and calcein (green) were well separated and distributed at shell and core, respectively.

Raman mapping was utilized to further confirm the localization of polymers and drug. As depicted in Figure 20a, Raman mapping showed the strong intensity of PLGA and alginate at shell and core, respectively. The distribution of acarbose was further distinguished by Raman mapping. Acarbose was shown by strong intensity of Raman

signal at the same location with alginate, which suggests acarbose was entrapped within the alginate hydrogel due to the strong affinity and high hydrophilicity.

Figure 19 CLSM of microparticles loaded with dyes. (a) PLGA, (b) alginate, (c) overlay image of PLGA and alginate, (d) overlay image with brightfield

4.7.2 Acarbose release profile and release kinetics

It has reported that the administration of acarbose is always associated with gastrointestinal adverse side effects such as flatulence, diarrhea, and abdominal pain [15]. Besides that, acarbose acts locally in the small intestine and inhibits the α -glucosidase enzymes. It would definitely be advantageous if the pH-triggered PAA coating can minimize the release of acarbose in the stomach in order to reduce the side effect suffered by patients, as well as a sustained release formulation to reduce the administration frequency. The typical gastric emptying time in the stomach, followed by the food digestion in the intestinal region was simulated for the drug release study. As such, the release profiles were conducted in SGF for 5 h, followed by SIF up to 24 h, as shown in Figure 21.

Figure 20 Raman mapping of cross-sectioned microparticles. a) Blank microparticles, b) Acarbose loaded microparticles.

Release studies of acarbose loaded hybrid microparticles with and without coating were conducted as a comparison, as well as the commercial acarbose tablet, GlucobayTM. From Figure 21, GlucobayTM showed a burst release in SGF within few minutes, which proved that the tablet is not a sustained-release formulation and acarbose released in the stomach at a very high dose. This result emphasized the necessity to reformulate acarbose to reduce the release rate in the stomach because the overloaded drug in the stomach is the main cause of the detrimental side effects to the diabetes patients. The sustained release of non-coated Alg-PLGA hybrid formulation showed a total release of 70 % in SGF and a complete release in SIF at 24 h. While PAA coated hybrid formulation, the release rate of acarbose in SGF was reduced to about 35 %, and a similar release profile with uncoated formulation was observed in SIF. The pH sensitive PAA coating provided an initial retardation of acarbose release.

Figure 21 Release profiles of Glucobay™ Tablet, Alg-PLGA hybrid microparticles with and without PAA coating.

The release profile of drug from PLGA-based microparticles is governed by the rate of polymer degradation together with the drug diffusion rate. The typical release pattern of the hydrophilic drug in PLGA microparticles follows the pseudo first-order degradation rate constant, which has a reported three-phasic release patterns [62]. At the early stage, the initial burst was observed, followed by a zero order release rate, and finally a final rapid drug release stage. PLGA-based microparticles were reported to have a sustained release of the drug up to 3 weeks or a month [22, 62]. However, the release rate in this study was hastened to only 24 h. This phenomenon could be explained by two reasons. The initial burst release was primarily controlled by the drug diffusion and the drug solubility. The partition coefficient of acarbose is about -6.4, and this led to a high release rate due to the fast diffusion rate of the hydrophilic drug into the dissolution medium. Secondly, the formulation in this study was modified into a core-shell structure with a thin PLGA shell, so the length of drug diffusion pathway was obviously reduced. Therefore, the release rate achieved a very steep zero-order release kinetics after the initial burst. However, the release profile in Figure 21 did not achieve a final rapid drug release at the last stage. A slowing down release rate was observed after transferring from SGF into SIF. This is because the degradation rate constant of polymer slightly decreased with the increasing pH of the release medium [62]. Thus, the changes in PLGA degradation kinetics explained the slow release of acarbose in neutral pH (SIF, pH = 6.8).

Table 5 Release kinetics of acarbose from Alg-PLGA with and without coating.

Formulation	Correlation Coefficient (r ²)				Rate constant k (h ^{-1/2})	Release exponent n
	Zero- order	First- order	Higuchi equation	Korsmeyer- Peppas equation	Higuchi equation	Korsmeyer- Peppas equation
Alg-PLGA MPs	0.6124	0.5039	0.8879	0.9868	22.56	0.5149
Coated Alg- PLGA MPs	0.7213	0.4537	0.9000	0.9424	18.667	1.7324

To elucidate the drug release mechanism from the hybrid microparticles, the release data were fitted to various models and summarized in Table 5. Both formulations could be well fitted into Higuchi equation, with a high correlation coefficient. The Higuchi rate constant k of non-coated MPs was higher than the coated MPs, which is in accordance with the release profile whereby the non-coated MPs showed a faster release than non-coated MPs.

In addition, both formulations could be best fitted into Korsmeyer-Peppas equation, with the correlation coefficient of 0.9868 and 0.9424 for non-coated and coated Alg-PLGA MPs, respectively. The release exponent n of non-coated Alg-PLGA ($n = 0.5149$) was best described by non-fickian diffusion mechanism, which is governed by erosion controlled and diffusion release of acarbose across the PLGA shell. Whereas for the coated Alg-PLGA ($n = 1.7324$), the release mechanism was characterized by super case II transport, which could be explained by the state transition or erosion of polymeric chains that swelled in the simulated fluids [63].

However, if the drug release data fitted separately into the Korsmeyer-Peppas model for SGF and SIF, only SGF contributed to the super case II transport ($n = 2.0527$), whereas SIF release data was very similar to the non-coated Alg-PLGA which described by non-fickian diffusion mechanism ($n = 0.6491$). In this case, the super case II transport in SGF was mainly affected by the retardation of release by the pH sensitive

PAA coating. This result is in concordance with the explanation of Conte *et al.* which described the multi-layer matrix tablets using power law [64]. The additional PEI and PAA layers limited the water influx and slowed down the core hydration, which in turn led to a slower release rate. Besides that, the PEI and PAA barriers reduced the surface area directly exposed to the dissolution medium and thus a slower erosion for PLGA shell. The much higher n value in SGF ($n = 2.0527$) was caused by the acceleration of release rate over time due to the high water solubility and high diffusion of acarbose regardless of the coating layers. As a result, the decreased release rate shifted the release kinetics towards a zero order release, which is independent of the drug concentration.

Chapter 5

Conclusion

This chapter concludes the thesis by showing the successful encapsulating nutraceuticals and drug into the SLMs and polymeric systems, respectively. These two systems has become the potential delivery systems for oral delivery to the gastrointestinal tract. It is hoped that this formulation can improve the stability of the encapsulated compounds, enhance the absorption, and improve the bioavailability in the GIT.

Food grade biopolymer-solid lipid hybrid microparticles was successfully developed using the combination of WOW emulsion and hot melt homogenization techniques, which has the potential to co-encapsulate and deliver two bioactive compounds with different hydrophilicity. Riboflavin and quercetin were successfully encapsulated into the SLM as model compounds which could be delivered to the GIT simultaneously.

From SEM images, all samples were spherical in shape with a rough surface, and the porous internal structure was observed. Confocal microscopy and Raman mapping showed the localization of quercetin and riboflavin in the solid lipid matrix and pores, respectively. DSC and XRD have shown that the SLM underwent phase transformation after three months' storage. The stability test of bioactive compounds in the formulation has been studied and no interactions were observed between quercetin and riboflavin. Besides that, no degradation of bioactive compounds happened within the microparticles up to three months.

Precirol and Compritol with the incorporation of oleic acid have shown a remarkable release profile whereby quercetin and riboflavin release pattern and release rate were similar, despite their hydrophilicity. The release profile could be well fitted into Higuchi equation and power law. These two equations also showed that quercetin and riboflavin release kinetics was very similar.

Whereas for alginate-PLGA microparticles, acarbose has been successfully encapsulated in this hybrid microparticles with improved encapsulation efficiency. Although acarbose altered the core-shell inner structure of the alginate-PLGA hybrid microparticles, acarbose was still proved to be localized within the PLGA shell by confocal microscopy and Raman mapping. The PAA coating has been shown to retard the burst release in SGF by one fold, which could reduce the adverse effect of acarbose acted to the stomach, as well as a sustained-release to reduce multiple dosing within 24 h.

In a nutshell, the hybrid biopolymer-solid lipid microparticles has proved to have the potential to co-encapsulate and co-deliver two bioactive compounds with different hydrophilicity, and allow the controlled release of nutraceuticals with a synergistic

effect. The alginate-PLGA hybrid microparticles has also proved to encapsulate and deliver acarbose in a controlled manner, which could largely improve patient compliance.

Chapter 6

Recommendations

Future works on the SLM and Alg-PLGA hybrid microparticles are proposed below, in order to gain a full picture of the properties of this particulate system. The recommendations focus on the microparticles design, drug loading and the release behavior of nutraceuticals. Besides that, future works in animal work such as pharmacokinetics study of nutraceuticals and postprandial glucose level test for acarbose are proposed.

6.1 Optimization of SLM formulation

Given that the SLMs are fabricated with the combination of hot homogenization and W/O/W double emulsion, the structure of the microparticles is largely determined by the mechanical shear forces and the emulsifying time before solidification. Due to the high repulsive force between hydrophilic compounds and solid lipid, the emulsifying time must be compromised to minimize the loss of hydrophilic compounds in W/O/W secondary emulsion. Therefore, a better fabrication technique can be explored to increase the encapsulation efficiency of hydrophilic compounds. For instances, the core-shell structure of polymeric system introduced by Alan *et al.* [22] is a good way to retain hydrophilic compound in the core. However, the high repulsive force by solid lipid will be the main obstacle.

In order to maintain a stable secondary emulsion, the repulsive force between solid lipid and hydrophilic compounds can be minimized by the surfactant. However, several types of surfactant have been tried in the preliminary study for example Span 80, Tween 80, and Polyglycerol polyricinoleate (PGPR). None of them could form a stable emulsion when solid lipid was dissolved in DCM for solvent evaporation technique. Therefore, another type of surfactant can be explored.

Furthermore, a combination of different types of solid lipid to achieve different release profile is also an interesting topic to study. Many types of solid lipid with a range of hydrophilicity can be investigated. Some less hydrophobic lipid such as stearic acid, or amphiphilic lipid may have good retention of the hydrophilic compound.

6.2 Animal works

Quercetin and riboflavin have been successfully encapsulated and the *in vitro* release profile was studied. Pharmacokinetic (PK) study is proposed as the next study, in order to evaluate how good is the enhancement of quercetin absorption in the presence of lipid, lecithin, and riboflavin. Normally quercetin has very low bioavailability in human, it is hoped that the bioavailability can be improved with the sustained-release by encapsulation.

Before the PK study, a Caco-2 cell permeability assay can be done first to have an insight of how well the bioactive compounds can penetrate through the Caco-2 cell monolayer to mimic human intestinal epithelial cells. The transport of the compounds from apical across basolateral compartment is monitored over 2 hours, and the permeability coefficient (P_{app}) can be calculated from the equation below [65]:

$$P_{app} = \left(\frac{\frac{dQ}{dt}}{C_0 \times A} \right)$$

where dQ/dt is the rate of compound permeation across the cells, A is the area of the monolayer, and C_0 is the initial concentration. From the permeability coefficient value, one can compare with reference compounds, atenolol and propranolol, which are known to be 50 % and 90 % of human absorption, respectively [65].

In addition, the antioxidant effect of quercetin and riboflavin can be tested by a DCFH-DA test. Basically, this test uses t-BOOH to stimulate cells to generate reactive oxygen species (ROS) and 2',7'-dichlorofluorescein-diacetate (DCFH-DA) is used to detect and quantify the intracellular produced H_2O_2 . Therefore, the antioxidant such as quercetin can reduce the ROS and the degree of ROS reduction can be quantified. These will be collaborating with a research group from Suranaree University of Technology, Thailand to do the pharmacokinetic, cell work, and antioxidant study after the *in vitro* study is done.

6.3 Further nutraceutical synergistic effect with drug

Other than quercetin, there are many hydrophobic bioactive compounds such as curcumin, piperine, and many polyphenols also faced low bioavailability problem in the clinical application. As mentioned previously, nutraceuticals are usually used as a prevention rather than a cure. Recently, researchers are looking to push nutraceuticals into the grey area whereby nutraceuticals are used as an immediate cure. One way of doing this is using nutraceutical in complementary with the drug. The synergistic effect between nutraceuticals and drugs have been studied extensively in the past few years. It is hoped that the synergistic effect can help to reduce the drug dosing to the human

body in order to minimize the side effects or the risk of poisoning. One example is the thought of using one of the bioactive compounds to act synergistically with painkiller, or NSAIDs such as ibuprofen, to reduce ibuprofen dosage and perhaps with additional good effects.

Chapter 7

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