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Synthesis and Characterization of Multifunctional Nanostructures Derived from Native Sago Starch For Potential Biomedical Applications

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

This study reports the potential application of hydroxypropyl starch and starch acetate nanoparticles as a controlled release nanocarrier for piperine. Hydroxypropyl starch and starch acetate were synthesized by modifying sago starch with hydroxypropylation and acetylation reaction. Hydroxypropyl starch nanoparticles with mean particle sizes of 110 nm were obtained by controlled precipitation through drop-wise addition of dissolved hydroxypropyl starch solution into excess absolute ethanol. Meanwhile, starch acetate with mean particle sizes of 140 nm was also successfully obtained by the same manner. Piperine was loaded onto hydroxypropyl starch nanoparticles, starch acetate nanoparticles, and native starch nanoparticles *via* the in-situ nanoprecipitation process. Hydroxypropyl starch nanoparticles and starch acetate nanoparticles achieved higher piperine loading capacity as compared to native starch nanoparticles with the maximum loading capacity of 0.46, 0.50, and 0.33 $mg.mg^{-1}$, respectively. Hydroxypropyl starch nanoparticles was able to retain piperine in the simulated stomach pH (1.2) where it was released in a slow and sustained manner within 24 hours, while piperine was release from starch acetate nanoparticles over a period of 28 hours in the simulated blood pH (7.4). On the other hand, the release rates of piperine from native starch nanoparticles were faster, whereby 96% of piperine was released within 16 hours at all pH tested in the same manner.

Keywords: Controlled release, hydroxypropyl starch nanoparticles, starch acetate nanoparticles, piperine

Sintesis dan Pencirian Nanostruktur Multifungsi yang Diperoleh daripada Kanji Sagu Asli untuk Aplikasi Bioperubatan yang Berpotensi

ABSTRAK

Kajian ini melaporkan potensi penggunaan nanopartikel kanji hidroksipropil dan kanji asetat sebagai nanopembawa pembebasan terkawal untuk piperina. Kanji hidroksipropil dan kanji asid disintesis oleh kanji sagu yang diubahsuai dengan reaksi hidroksipropilasi dan asetilasi. Nanopartikel kanji hidroksipropil dengan purata saiz zarah 110 nm diperolehi melalui pemendakan terkawal larutan hidroksipropil ke dalam etanol dalam jumlah yang banyak. Sementara itu, asetik kanji dengan purata saiz zarah 140 nm juga berjaya diperoleh dengan cara yang sama. Piperina telah dimuatkan ke nanopartikel kanji hidroksipropil, nanopartikel kanji asetik, dan nanopartikel kanji asli melalui proses nanopemendakan. Nanopartikel kanji hidroksipropil dan nanopartikel kanji asetat mencapai kapasiti muatan piperina yang lebih tinggi berbanding nanopartikel kanji asli dengan kapasiti muatan maksimum masing-masing 0.46, 0.50, dan 0.33 mg.mg^{-1}. Nanopartikel kanji hidroksipropil dapat mengekalkan piperina dalam simulasi pH perut (1.2) di mana ia dikeluarkan secara perlahan dan konsisten dalam masa 24 jam, manakala piperina dibebaskan daripada nanopartikel kanji asetat selama tempoh 28 jam dalam simulasi pH darah (7.4). Sebaliknya, kadar pelepasan piperina daripada nanopartikel kanji asli adalah lebih cepat di mana 96% piperina dibebaskan dalam masa 16 jam di semua pH yang diuji dalam kondisi yang sama.

Kata kunci: *Pembebasan terkawal, nanopartikel kanji hidroksipropil, nanopartikel kanji asetat, piperina*

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LIST OF ABBREVIATIONS

Ac	Acetyl content
Ace	Acetylated
AGU	Anhydroglucose units
C	Carbon
cm	Centimetre
C_3H_6O	Propylene oxide
$C_9H_6O_4$	Ninhydrin reagent
COOH	Carboxylic group
DS	Degree of Substitution
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Radiation
g	Gram
$g.g^{-1}$	Gram per Gram
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
KOH	Potassium hydroxide
mins	Minutes

$mg.mg^{-1}$	Miligram per Miligram
$mg.L^{-1}$	Miligram per Litre
$mg.mL^{-1}$	Miligram per Mililitre
mL	Mililitre
MS	Molar substitution
N	Normality
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NPs	Nanoparticles
OH	Hydroxyl group
OSA	Octenyl succinic anhydride
PBS	Phosphate Buffer Solution
rpm	Rotation per minute
SEM	Scanning Electron Microscope
STMP	Sodium trimetaphosphate
TEM	Transmission Electron Microscopy

UPW	Ultrapure water
$v.v^{-1}$	Volume per Volume
$v.w^{-1}$	Volume per Weight
W_d	Weight of dried starch nanoparticles
W_s	Weight of swollen nanoparticles
XRD	X-ray Difractogram
^oC	Degree celcius
%	Percentage
μm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background

The sago palm is a species of the genus *Metroxylon* belonging to the Palmae family. Sago starch is obtained from *Metroxylon sagu* and grown well in Southeast Asian countries as a tropical crop. It is relatively cheap and widely available in the South Asian region. A mature sago palm tree produces about 60×10^6 tonnes of sago starch annually in south East Asia (Uthumporn, Wahidah, & Karim, 2014). Starch is a typical biodegradable natural polysaccharide and it is a renewable substance produced by a lot of plants that contains chlorophyll such as corn, potato, wheat and barley as a source of stored energy (Qin, Liu, Jiang, Xiong, & Sun, 2016), in which it acts as a reserve food supply for the period of growth, dormancy, and germination (Bismark, Zhifeng, & Benjamin, 2016).

Starch, the second most abundant carbohydrate available from plant kingdom as a natural raw material. It is white in color and insoluble in cold water due to the polymerized structure, and it has hydrogen bonding between adjacent chains (Ye et al., 2017). Starch granules are mainly composed of amylose and amylopectin. The granules may vary in shape, size, structure and chemical composition depending on the starch source (Din, Xiong, & Fei, 2015). Due to its well-defined properties such as renewable, biodegradable, biocompatible and low cost, the biopolymer has a large potential as a flexible renewable resource to be applied in both food and non-food industry (Masina et al., 2017; Ye et al., 2017).

Starch in its native form is unsuitable for various applications due to its physical and chemical properties such as poor mechanical properties, insolubility in cold water, and high

viscosity. Various modifications such as blending, chemical modification, and physical modification have been performed on the starch to improve its properties so that it can be utilized in food and biomedical industries. Chemical modification has been the most effective method to alter the properties of the starch and improve its overall properties. Starch molecules possess large numbers of hydroxyl groups, providing the active site for modification *via* chemical reactions. Many kinds of research have been carried out on starch modifications in which various functional groups such as carboxyl, acetyl, and hydroxypropyl were being introduced to the starch structure (Chen et al., 2015; Shen, Xu, Kong, & Yang, 2015). Generally, chemical modification of starch is accomplished through derivatization such as esterification, etherification, oxidation and cross-linking (Chen et al., 2015; Din et al., 2015). The functional and chemical properties of the modified starches are affected by various factors such as starch source, reaction conditions, type and distribution of substituting agent along with the molecules as well as the degree of substitution (Din et al., 2015).

For the last two decades, nanotechnology has forth come as one of the most innovative technologies focusing on characterization, fabrication, and manipulation of the structure of matter at dimensions of roughly 10 to 100 nm (Ye et al., 2017). Since then, nanotechnology is one of the most active research areas in chemistry, physics, and medicine. The unique properties of nanoparticles which are significantly different from their bulk materials have attracted much attention. Nanotechnology is able to decrease the particle size of raw materials and also improves their functional properties (Qin et al., 2016).

Starch nanoparticles have drawn much attention due to their potential for mass production, low cost, and non-hazardous properties. Based on previous works reported,

starch nanoparticles are one of the most suitable candidates to be used in foods, cosmetics as well as pharmaceuticals area (Ye et al., 2017). Besides that, studies of starch nanoparticles in drug delivery system showed that these starch nanoparticles have a large potential as a nanocarrier in oral drug administration (Raghvendra et al., 2017; Qin et al., 2016; Din et al., 2015). This is due to their rather significant small size enables the penetration of cellular barriers that have been limiting other drug carriers (Raju, Benton, Pavitra, & Su, 2015). Previous research by Najafi et al. (2016) showed that nanoparticles with particle size less than 300 nm have higher encapsulation efficiency and the encapsulation efficiency of acetylated corn starch nanoparticles increases from 67.7 to 89.1% when the degree of substitution increases from 0.33 to 2.66. The high encapsulation efficiency indicates high affinity of the model drug (ciproflaxin) molecule for the acetylated corn starch nanoparticles matrix (Heydar, Najafi, Baghaie, & Ashori, 2016).

For the past hundred years, black pepper has been used as a spice for cooking and traditional medicine due to its well-known medicinal properties. Based on ayurvedic system of medicine, pepper fruit has anthelmintic, antiasthmatic, alternative and has been used to treat pain, piles, insomnia, and epilepsy. One of phenolic compound in black pepper that has attracted much attention is piperine. Piperine is a naturally occurring alkaloid which imparts pungency and flavour to pepper. Pharmacological research has recently shown that piperine has antidepressant, antipyretic, anti-inflammatory, antithyroid, hepaprotective, immunomodulatory and antitumor properties. Recent study suggests that blood cholesterol, triglycerides, and glucose could be reduced by application of piperine (Stojanovi et al., 2019; Gorgani, Mohammadi, Najafpour, & Nikzad, 2016). Also, some studies revealed that piperine is able to hinder the development of breast cancer and show anti-cancer activity.

Piperine can enhance the bioavailability of some therapeutic compounds such as sulfadiazine, streptomycin, and rifamicin by acting on hepatic enzymatic breakdown (Thenmozhi & Je, 2017; Gorgani et al., 2016; Wu et al., 2014).

1.2 Problem Statement

Piperine has many advantages, but it has not been widely used in biomedical field and formulations of therapeutic products due to its poor solubility in water which causes limited dissolution rate. The low solubility in water and poor dissolution of piperine is the primary limiting aspect in the absorption process for piperine that lead to low availability of orally administered drugs (Thenmozhi & Je, 2017; Wu et al., 2014). Starch has to be modified before it can be used to encapsulate piperine because in its native form, starch has poor mechanical properties, insoluble in cold water, and have high viscosity. Chemical modification will alter the properties of the starch and improve its overall properties (Chen et al., 2015; Shen, Xu, Kong, & Yang, 2015). To overcome the poor aqueous solubility of drugs candidate like piperine, many formulation strategies have been studied such as micronization, nanocrystallization, solid dispersion, micelle solubilization, as well as encapsulation in nanoparticles. By encapsulating piperine in nanoparticles, it will undergo self-dispersion process, where the piperine is encapsulated or dissolved in carrier excipients or vehicles in molecular state by solubilization or self-assembly. This method is able to maximize the dispersion of piperine and lead to more stable dispersion system, which have been widely applied to formulation of poorly water-soluble drugs candidate. When exposed to aqueous media, the hydrophilic polymeric nanocarrier will dissolves and piperine releases as fine particles (Zhang, 2018). Improving the solubility of piperine is important to avoid

dose-escalation, toxicity, and an increase in production cost during the development of drug process.

1.3 Objectives

The objectives of this study are:

1. To synthesize and characterize hydroxypropyl and acetylated starch nanoparticles.
2. To evaluate the potential of hydroxypropyl and acetylated starch nanoparticles as controlled release nanocarriers for piperine.

1.4 Scope of studies

In this study, sago starch was chemically modified by hydroxypropylation and acetylation process, respectively. Hydroxypropyl group was substituted on the sago starch in the presence of an alkaline catalyst, while acetyl group was substituted with the help of iodine as a catalyst. Chapter 1 describes the general introduction and justifications of this study. Chapter 2 discusses the literature review related to this study such as overview of starch, starch modifications and starch nanoparticles as controlled release nanocarriers. Chapter 3 explains the procedure involved in the preparation of hydroxypropyl starch nanoparticles from native sago starch which involved hydroxypropylation and nanoprecipitation process. This chapter also discusses the application of hydroxypropyl starch nanoparticles as controlled release nanocarrier for piperine. Chapter 4 presents the work on the preparation of acetylated starch nanoparticles for controlled release nanocarrier of piperine. Chapter 5 provides the conclusions of this study and recommendations for future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

Starch, the abundant natural polysaccharide obtained from plants is cheap, renewable and biodegradable. This biopolymer is the main carbohydrate in plants and it is a bulk energy storage molecule in plants. World produce around 58 million tonnes of starch extracted mainly from maize, wheat, and potatoes. Starch is a heterogeneous polymer of α-D-glucose units, and the anhydroglucose units (AGUs) are mainly linked by α-1,4 linear and α-1,6 branched bonds (Heydar et al., 2016; Yu et al., 2016).

There are two different structural forms of insoluble semi-crystalline granules in starch namely amylose and amylopectin. Amylose is a long linear polymer that contains up to 6000 AGUs of α-1,4 linkages. It has a tendency to retrograde and produce strong films as well as tough gels. Meanwhile, amylopectin is highly branched and is derived from α-1,4 linkages and α-1,6 linkages of glucose units. Amylopectin is more stable and when dispersed in water, it may produce weak films and soft gels. Amylose and amylopectin may form entanglements and the presence of the small number of proteins and lipids in starch may affect the physicochemical properties of starch to different extent depending on the botanical source of the starch. As shown in Figure 2.1, hydroxyl (OH) groups in starch structure are located at C-2, C-3 and C-6 making starch hydrophilic in nature (Bismark et al., 2016; Din et al., 2015).

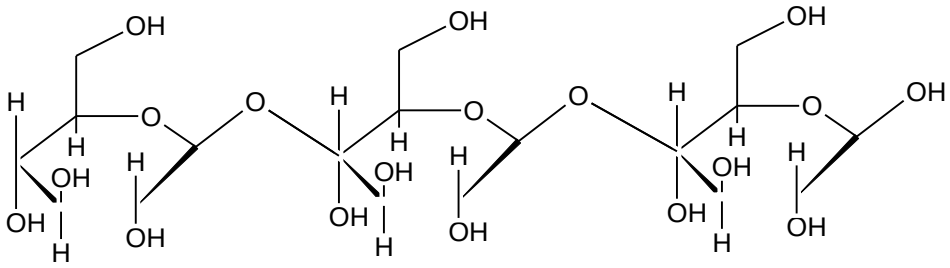


Figure 2.1: Molecular structure of starch

Starch-based products are usually safe for human consumption and it is widely used in food, textiles, cosmetics, plastics, adhesives, paper, and pharmaceutical industries. In its native state, starch has industrial applications due to its insolubility in cold water, instability of its pastes and gels, low shear stress resistance and thermal decomposition, coupled to high retrogradation and syneresis (Sukhija, Singh, & Riar, 2016; Chen et al., 2015; Lisie et al., 2015). Native starch has many hydroxyls and cyclic structures that cause starch material to be brittle (Bismark et al., 2016). In addition to being a major food item, starch is mainly used in various industies as coatings and sizing in paper, textiles and carpets, as binder and adhesives, as absorbents and also as bone replacement implants, bone cements, drug delivery systems, and tissue engineering scaffolds (Masina et al., 2017).

Starch exhibits limited applications in the native state due to poor processability and solubility in water and low shear stress resistance and thermal decomposition. Furthermore, native starch has a high degree of retrogradation and can easily undergo syneresis in addition to the gelling tendency of the pastes. Therefore, starch is modified by various modification methods aimed to correct one or some of the abovementioned limitations which will improve the versatility, meet the demanding technological needs, and applicability in various industries (Din et al., 2015; Castro, Evangelista, Carbinatto, Do, & Cury, 2013; Neelam, Vijay, & Lalit, 2012).

Generally, starch modification methods have been classified into four categories which are the physical, chemical, enzymatic, and genetical modification. These modifications aimed to improve the physicochemical properties and produce novel derivatives of starch with useful structural attributes (Neelam et al., 2012). A lot of new functionality and value-added attributes on starch could be produced by modifications which make it an ongoing process with the huge potential market (Din et al., 2015).

2.2 Physical modifications of starch

Physical modification of starch is usually applied to alter the granular structure and change the properties of native starch into cold-water-soluble starch or small-crystallite starch. These types of modifications are preferred since it does not involve any chemical treatment that could be harmful to human. Some of the physical methods for starch modifications are heat moisture treatment, annealing, retrogradation, freezing, ultrahigh-pressure treatment, glow discharge plasma treatment, osmotic-pressure treatment, thermal inhibition, and gelatinization. In previous study, high moisture treatment method was used to modify pulses starch and the result showed decrease in amylose leaching, swelling ratio, and peak viscosity, and increase in thermal stability, gelatinization temperatures and susceptibility towards acid hydrolysis (Bemiller & Huber, 2015; Neelam et al., 2012).

Another study showed that annealing modification on lentil, smooth pea, and wrinkled pea starch had resulted in a decrease in granular swelling and amylose leaching while the gelatinization temperatures, thermal stability, and susceptibility towards α-amylase increase. The retrogradation process involves linking the starch chains into ordered crystalline structures. The research found that the resulted retrograded starch demonstrated

resistant to digestibility by amylase enzymes, therefore it can be an alternative nutrient aid for diabetic patients. It was found that the properties of the modified starch are also affected by the source of the starch, amylose content and density of the paste (Din et al., 2015).

2.3 Chemical modifications of starch

On the other hand, chemical modification is the introduction of new functionality in starch. Starch molecules possess large numbers of hydroxyl groups that provide active sites for chemical modifications. Various functional groups such as carboxyl, acetyl, hydroxypropyl, amine and amide can be introduced into the starch structure to give specific improved desirable functional properties to the starch such as higher solubility, less tendency to retrograde, and less moisture absorbency (Yu et al., 2016; Chen et al., 2015; Castro et al., 2013). Usually, the amount of chemical needed to achieve the desired functional properties of the starch vary depending on the reagent type required for the substitution. The changes in the starch structure and regions after the addition of newly substituted groups can be detected by nuclear magnetic resonance (NMR) or scanning electron microscope (SEM) (Senanayake, Gunaratne, Ranaweera, & Bamunuarachchi, 2014).

Tay, Pang, and Chin (2012) had synthesized the water-soluble cross-linked starch-maleate monoester gel particles from native sago starch (*Metroxylon sagu*) using a facile and green approach. The sago starch was reacted with maleic anhydride in an aqueous medium and then precipitated in absolute ethanol to synthesize the starch-maleate monoester gel. During UV irradiation in the presence of cerium (IV) ammonium nitrate, starch-maleate gel transformed into cross-linked gel particles of mean diameter 445 ± 115 nm. FTIR spectroscopy was used to confirm the substitution of maleic anhydride onto starch, while

back-titration method was carried out to determine the degree of substitution and it was within the range of 0.03-0.21. The water absorbency and hydrophilicity of the starch-maleate samples of degree of substitution (DS) > 0.08 were higher than the starch-maleate samples of DS < 0.03. Since the starch-maleate is non-toxic, biocompatible and cheap, starch-maleate gel particles had a large potential to be utilized in biomedical applications or as drug delivery carriers (Tay, Pang, & Chin, 2012).

Starch nanoparticles were prepared through self-assembly of short-chain amylose debranched from cooked taro starch. Then, the starch nanoparticles were chemically modified by octenyl succinic anhydride (OSA) to improve their hydrophobicity. The particle size via OSA starch nanoparticles was found to increase as compared to native starch nanoparticles. OSA starch nanoparticles had a new absorption peak at 1727 cm^{-1} based on FTIR spectra. This indicates the characteristic peak of carbonyl, indicating the formation of the ester bond. The dispersibility of the modified starch nanoparticles in the mixture of water with nonpolar solvent increased with increasing of the degree of substitution (DS) (Jiang, Dai, Qin, Xiong, & Sun, 2016).

Sodium hypochlorite (NaOCl) and sodium trimetaphosphate (STMP) was used to chemically modify the elephant foot yam starch to prepare oxidized, cross-linked, oxidized cross-linked, and cross-linked oxidized starch. To indicate the modification of starch, the pasting, morphological, thermal, FTIR and XRD analysis were analyzed. The result showed significant improvement in water binding capacity and color value of the modified starch. In native and modified starch, the positive effect was observed on the swelling power and solubility but in cross-linked starch, the swelling power decrease and the amylose content increase. Based on the X-ray diffractogram, the degree of crystallinity of oxidized and dual

modified starch increases. Also, the morphology of the native, cross-linked, and cross-linked oxidized starch was smooth, whereas surface fissures were observed in oxidized and oxidized cross-linked starch (Sukhija et al., 2016).

2.3.1 Hydroxypropylation (Etherification)

Substitution of the hydroxypropyl groups with the starch chains could disrupt the inter- and intramolecular hydrogen bonds thus enhance the free movement of the starch chains in amorphous regions in the granule. The weakened internal bond structure in the starches granules enhances the functional characteristics of the starch such as freeze-thaw stability, reduced gelatinization temperature, high levels of peak viscosity and starch paste clarity due to the derivatized hydroxypropyl groups (Senanayake et al., 2014; Neelam et al., 2012).

The internal hydrogen bond strength and quantity in starch could be reduced by the bulky hydroxypropyl group. This substantially reduces starch ability to recrystallize, which in turn reduces the retrogradation property of the starch, and therefore yield starch derivatives that are stable at high temperatures. Hydroxypropyl starch have improved swellability and viscosity, making these starch derivatives applicable as excipients in formulations that needed cold storage stability, long-term freeze-thaw, and longer general shelf-life (Masina et al., 2017).

Numbers of researches have been done to explore the potential use of hydroxypropylated starch as a copolymer or an activated complex for copolymerization or grafting. For example, the use of the reducing ends of the hydroxypropyl starch derivative in the development of nanoparticles for sustained release of insulin application. The resulted

nanoparticles have high levels of amylase resistance, aided in targeting the particles for colon-specific drug delivery (Masina et al., 2017).

The changes in the physicochemical properties of starches obtained from different cultivars of sweet potatoes commonly consumed in Sri Lanka when they are being chemically modified by hydroxypropylation were analyzed. Due to the modification, significant changes of ($P < 0.05$) in the crude digestibility level, thermal properties, and the water separation (syneresis) of starch gels (7.0%) during cold and frozen storage were observed. The gel stability, water-solubility, digestibility and storage stability of the native starches in the cold storage was increased to a significant level by hydroxypropylation. Besides that, compared to native starch, hydroxypropylated starch has lower gelatinization temperature and retrogradation enthalpies. After two weeks, hydroxypropylated starch gels stored under cold storage did not show a syneresis, besides high stability of the starch gels in frozen storage within the four-week cycle (Senanayake et al., 2014).

Propylene oxide was also used to chemically modify the rice starch. The molar substitution of the resulted hydroxypropylated rice starch was ranging from 0.022 to 0.033. Hydroxypropylated starch has lower gelatinization properties compared to native rice starch, which was affected by the amount of propylene oxide used during the reaction. The native rice starch and hydroxypropylated rice starch with various concentrations of propylene oxide were used to prepare films. Based on the results, the hydroxypropylated rice starch film showed higher water vapor permeability, film solubility and transparency as compared to the native starch film (Woggum, Sirivongpaisal, & Wittaya, 2015).

2.3.2 Acetylation

Acetylation is one type of chemical modification that can be obtained by the esterification reaction between native starch and acetic anhydride, vinyl acetate or acetic acid. During acetylation, part of hydroxyl groups on anhydroglucose units of starch is substituted with acetyl groups, forming esters. The reactant concentration, pH, presence of catalyst and reaction time affect the number of acetyl groups incorporated into the starch molecule. Starch acetates can be classified based on the degree of substitution (DS). Usually, acetylated starch with low DS is used in the food industry, while starch with high DS has various applications such as hot melt adhesives, coating, biodegradable packaging materials, and drug delivery. A recent study also suggested the use of acetylated starch as the biodegradable materials for food packaging and various pharmaceutical applications (Masina et al., 2017; Bartz et al., 2015).

Acetyl group is much bulkier compared to the hydroxyl group, therefore, it sterically hinders the structural organization of starch chains in acetylated starch. Water percolation between chains is facilitated because repulsion is present in between the starch molecules. So, the swelling power and solubility of starch increased and energy required for gelatinization is less, resulting in a lower gelatinization temperature and enthalpy. In addition, starch chains are less able to form hydrogen bonds and re-associate due to steric hindrance, therefore, acetylated starch is less prone to retrogradation (Raj & Prabha, 2016; Ackar et al., 2015).

Bartz et al. (2015) had synthesized acetylated starch by reacting barnyardgrass starch with acetic anhydride and iodine as a catalyst. They reported some of the physicochemical,

functional, and morphological properties of the modified starch. The result showed an increase in the degree of substitution when the concentrations of iodine increase. In the morphological analysis, a low level of surface corrosion was observed and the overall relative crystallinity decrease while the shape of the starch granules remained unchanged. As a result of the acetylation reaction, the pasting temperature, enthalpy, other gelatinization temperatures decrease but the viscosity of the starch gel increase.

Much research on application acetylated starch in drug delivery has been done previously. Acetylated starch has demonstrated sustained drug release in some polymeric systems due to an increase in swelling and decreased enzyme sensitivity during gastrointestinal digestion. Studies done on the drug release profile of acetylated potato starch films and microparticles showed much slower release rate when compared to native starch. Reduced swelling in acetylated starch may increase retention time for oral starch-based delivery systems, thus prolong the retention time of the system in the stomach. This is highly applicable for drugs having low absorption and low pH stability since it constitutes a more controlled drug delivery system (Masina et al., 2017).

2.4 Starch nanoparticles

Nanotechnology is the use and manipulation of matter at a tiny scale. Nanoparticles are the wide class of ultrafine materials, included particulate substances, whose size is ranging from 1 to 100 nm. At this size, molecules function differently and give many new and interesting applications (Kumar, Jajodia, Kumar, & Gautam, 2017; Ranjit & Baquee, 2013). However, in nanotechnology, the size of nanoparticles is not strictly bound only up to 100 nm. Sometimes nanoparticles can be larger in size but still function effectively (Raju

et al., 2015). Studies found that material size is able to influence the physicochemical properties of a substance. Smaller size provides nanoparticles qualities that larger materials do not possess (Khan, Saeed, & Khan, 2017; Raju et al., 2015).

Some researchers also utilized starch nanoparticles with sizes ranging from 300 to 400 nm as a filler in composites. They found that the incorporation of starch nanoparticles have improved both the biodegradability and the mechanical properties of the composites. Other than that, several studies have proven the potential applications of starch nanoparticles in other industries like foods, cosmetics, and pharmaceuticals (Kim, Park, & Lim, 2014).

2.5 Preparation of starch nanoparticles

There are various methods that have been used to prepare starch nanoparticles, including acid hydrolysis, extrusion, high-pressure homogenization and emulsification and nanoprecipitation (Qin et al., 2016). However, these methods possess several disadvantages like requiring a long reaction time to produce nanoparticles, broad particle size distribution, the large volume of residue and a large number of emulsifiers needed (Campardelli, Della Porta, & Reverchon, 2012).

2.5.1 Nanoprecipitation

Nanoprecipitation method is a fast, easy, less energy consuming and reproducible method to fabricate synthetic and natural polymer nanoparticles (Campardelli et al., 2012). Typically, nanoprecipitation allows the preparation of very fine particles with better control over their particle properties such as size and morphology, plus it is suitable for producing nanoparticles from a range of different food ingredients (Qin et al., 2016) and biomaterials

such as starch since it does not employ toxic chemicals (Juna, Hayden, Damm, Kappe, & Huber, 2014).

Simple nanoprecipitation method was used to synthesize starch nanoparticles of particle size range between 300 nm and 400 nm from native sago starch. Controlled precipitation through drop-wise addition dissolved native starch solution to excess absolute ethanol formed starch nanoparticles. The size and shape of starch nanoparticles were modulated varying the synthesis parameters including the use of appropriate surfactant. During precipitation, starch nanoparticles were obtained with a mean diameter of approximately 150 nm in the presence of surfactants (Chin, Pang, & Tay, 2011).

Various native starches (waxy corn, normal corn, high amylose corn, potato, tapioca, sweet potato, and pea starch) were used to prepare starch nanoparticles by nanoprecipitation method. The mean particle sizes of starch nanoparticles obtained were mainly ranging from 30 to 75 nm. The researchers found that the smaller the native starch granules were, the smaller the respective starch nanoparticles obtained. All starch nanoparticles yielded have a V-type crystalline structure. In addition, high amylose content in native starch produces starch nanoparticles with higher crystallinity. The gelatinization enthalpy of the starch nanoparticles was lower than the corresponding native starch except for high amylose corn starch (Qin et al., 2016).

In addition, Juna et al. (2014) studied the influence of alkali concentration, temperature, the concentration of urea and KSCN on the shapes and sizes of waxy corn starch precipitates that were isolated via nanoprecipitation. The waxy corn starch was heated in 1M NaOH with varying temperatures employing a microwave sector to study its apparent

molecular characteristics and they found that the sizes of starch nanoparticles decreased from 600 to 238 nm with increasing temperature (Juna et al., 2014).

2.5.2 Acid hydrolysis

The preparation of starch nanoparticles through acid hydrolysis method usually involves a two-step hydrolysis reaction. Fast hydrolysis occurs during the first step, where the amorphous regions of the starch granules are attacked. In the second step, during the slow hydrolysis, the erosion of the crystalline regions occurs. Usually, starch nanostructures resulted from this method have high crystallinity and a platelet-like shape. This method generally involves diluting the starch in hydrochloric acid or sulfuric acid, maintained under constant stirring and controlled the temperature for more than 5 days (Silva et al., 2018; Kim, June, Kim, & Lim, 2013).

Research were carried out on starch nanoparticles prepared from waxy maize starch by acid hydrolysis and ultrasonication method, where the aqueous sulfuric acid solution was used to hydrolyze the waxy maize starch for 2 to 6 days. The results showed that at 40 ^{o}C reaction temperature, the starch exhibited higher crystallinity while at 4 ^{o}C, the starch hydrolyzed showed similar crystallinity to those of native starch. The starch hydrolyzed precipitates were redispersed in water and treated by ultrasonication. High yield (78%) and crystallinity of starch nanoparticles were observed when treated with 6 days hydrolysis at 4 ^{o}C followed by ultrasonic treatment. The starch nanoparticles obtained were globular in shape with particle size ranging from 50 to 90 nm (Kim et al., 2013).

2.5.3 High-pressure homogenization

High-pressure homogenization is one of the methods to produce starch nanoparticles by physical treatments with no addition of a chemical reagent. This method is simple, effective, and environmentally friendly besides it can reduce the processing time to produce starch nanoparticles. Moreover, by using this method, the yield of nanoparticles could be increased and various purification steps such as in acid hydrolysis could be avoided. The high-pressure homogenization method is usually used in food, pharmaceutical, chemical, and biotechnology industries and the size of nanoparticles obtained by this method may vary by 10 nm. During high-pressure homogenization, a partial or complete destruction of the crystalline structure could occur and only starch solution with low concentration could be used for homogenization (Silva et al., 2018).

Normal maize starch was treated by a combination of heat-moisture treatment and homogenization method to produce nanoparticles. The method yielded more than 80% nanoparticles with particle sizes of less than 50 nm. FTIR analysis showed that 60 minutes homogenization has caused obvious damage in the long-range crystalline structure of heat-moisture treatment starch, but the short-range chain associations remain intact (Park, Kim, Cho, Lee, & Kim, 2016).

2.5.4 Ultrasonication

Ultrasonication involves the use of ultrasound equipment to sonicate the starch solution under controlled temperature for a fixed duration. During ultrasonication, energy will be transferred into starch particles by cavitation. Shear forces resulted from high velocities microjets will break the covalent bonds of the starch molecules and therefore, the

particle size is reduced. The amylopectin, which is the crystalline structure of the starch is affected by ultrasonication, producing nanoparticles with low crystallinity. Nanoparticles formed by ultrasonication may vary in size ranging from 30 nm to 200 nm depending on various factors such as ultrasonication power and frequency, duration, and treatment temperature, as well as the concentration of the starch solution and source of the starch (Silva et al., 2018).

Starch nanoparticles were prepared by ultrasonic-assisted oxidation of waxy corn starch. The starch nanoparticles were prepared by three different methods, which is one-time oxidation followed by ultrasonication, twice oxidation and twice ultrasonication, and (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO)-mediated oxidation with ultrasonication. The results showed that the starch nanoparticles form with particle size ranging between 20 and 60 nm. The crystallinity of the starch nanoparticles decreased from 36.32% to 11.35%, 1.64%, and 1.72% compared to waxy corn starch. The starch nanoparticles were also found to have higher carboxyl and carbonyl content (Sun, Fan, & Xiong, 2014).

Table 2.1: Summary of disadvantages of various preparation method to prepare nanostructures

Preparation method	**Disadvantages**
Nanoprecipitation	Require large volume of non-solvent
Acid hydrolysis	Long reaction time, relatively low yield, high process temperature and formation of undesirable products and platelet-like shape nanoparticles
High pressure homogenization	Prerequisite of micronized drug particles and suspension formation using high-speed mixers before treated it to homogenization
Ultrasonication	Require high implementation cost

2.6 Starch nanoparticles as a nanocarrier

Polymer nanoparticles are receiving much attention in chemistry, pharmaceutics, and biomaterial science industry due to its potential application in biomedical and controlled release. The applications of starch nanoparticles as drug nanocarriers are widely known. Several studies have been carried out previously in this area. The physical and chemical properties of starch nanoparticles play an important role in navigating the biological barriers and therefore determining the overall success of the therapy. Starch has been applied in drug delivery and biocatalysts fields due to its ability to improve drug solubility and stability, reduce drug toxicity and side effects, and excellent biocompatibility and storage stability (Heydar et al., 2016).

For the past few decades, the encapsulation method has been widely applied in pharmaceuticals agriculture, food, cosmetics, and textile industries. Recently, modern technologies on advanced drug deliveries have been able to replace the conventional dosage forms that were lacked in flexibility and less sophisticated due to some issues such as bioavailability, stability, taste, and odor (Janeth et al., 2017; Ackar et al., 2015). Encapsulation has attracted much attention as a potential solution to overcome these challenges. Biopharmaceutical classification system stated that 40% of the currently commercialized drug molecules have poor solubility and 90% of drug molecules in drug development pipeline are also poorly soluble. Encapsulation plays a crucial role to prevent the degradation of active ingredients and to obtain controlled or targeted drug release systems. The use of biodegradable polymers in drug encapsulation is the best approach to prepare an ideal drug delivery system with safe, biocompatible, comfortable, easily administered and inert properties. Therefore, the encapsulation method could be used to

prolong drug release, protect the sensitive drug molecule from degradation, prevent drug incompatibility, control the drug's dosage, reduce toxicity, and stabilize the drug molecules (Janeth et al., 2017).

Native sago starch nanoparticles were investigated for its potential as a nanocarrier for curcumin. By using the *in situ* nanoprecipitation method and water-in-oil microemulsion system, curcumin was loaded onto starch nanoparticles. The results showed that the solubility of curcumin loaded starch nanoparticles in aqueous solution is better compared to free curcumin. Various formulation parameters such as types of the reaction medium, types of surfactant, surfactant concentration, loading time, and initial concentration of curcumin could affect the particle size and loading efficiency of curcumin loaded nanoparticles. Under optimum conditions, curcumin loaded starch nanoparticles with a mean particle size of 87 nm were successfully synthesized and achieved up to 78% of loading efficiency. Curcumin showed sustained release behaviour, where it was released from starch nanoparticles in a sustained manner under physiological pH within 10 days (Chin, Mohd Yazid, & Pang, 2014).

Acetylated starch nanoparticles with 312 nm size was successfully derived from corn starch, and it was studied for its potential as a matrix for the delivery of ciproflaxin. Acetylated corn starch nanoparticles were prepared by using nanoprecipitation method, by the dropwise addition of water to acetone solution of acetylated corn starch under stirring. The encapsulation efficiency of ciproflaxin was evaluated. The results show that the encapsulation efficiency of ciproflaxin increase as the degree of substitution increase. The lowest encapsulation efficiency obtained was 48.5% and the highest was 89.1% (Heydar et al., 2016).

A novel type of reduction-sensitive starch nanoparticles was prepared by using a reversed-phase microemulsion method. N,N-bisacryloycystamine with the disulphide linkages was used as a crosslinker, which was specifically cleaved by dithiothreitol. The starch nanoparticles were then tested for their potential application as drug delivery carriers. The starch nanoparticles were found to be able to load a model drug 5-aminosalicylic acid (5-ASA) efficiently and they also studied the in vitro drug release behaviours. The results showed that in the presence of dithiotherol, the disulphide crosslinked starch nanoparticles exhibited accelerated release behaviour. Therefore, N,N-bisacryloylcystamine showed promising potential as a biomaterial carrier for the application of drug controlled release due to its biocompatibility and biodegradability and also rapid drug release in response to dithiotherol (Yang, Huang, Gao, Liu, & Zhang, 2014).

2.7 Piperine

Herbs and spices and spices have been used in food and medicinal preparations for many years before. This is due to their delightful flavour and health benefits in addition to their beneficial pharmacological properties. Black pepper is one of the most consumed spices around the world, and it is rightly known as the King of Spices. In the past, black pepper has been used for many purposes and so as in the future. The pungency and flavour give value to the pepper and it is due to the presence of a naturally occurring alkaloid named as piperine, and also volatile essential oil. Piperine is the major constituents in pepper, and it is responsible for the pungency of piperine while the volatile oil imparts the aroma to it (Lee, Kim, Back, & Han, 2018; Gorgani et al., 2016; Wu et al., 2014).

Plants from the Piperaceae family contain varies amount of piperine, and some reported that black pepper has higher piperine content which is up to 9%. The chemical structure of piperine is identified as piperohylpiperidine with the chemical formula of $C_{17}H_{19}NO_3$. The IUPAC name is 1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl) piperidine. Piperine is a very weak base when undergoing acid or alkali hydrolysis, it decomposes to piperidine (Figure 2.2), volatile basic piperine (Lee et al., 2018; Gorgani et al., 2016; Pentak, 2015).

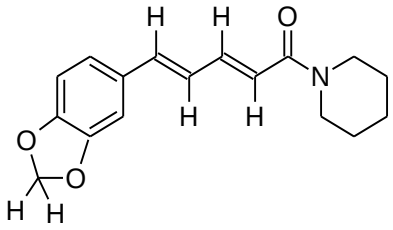


Figure 2.2: Molecular structure of piperidine

The interest in medicinal plants and natural products has been increasing and the development of plant-based pharmaceutical attracted much attention for better healthcare solutions. Piperine has large potential to be applied in pharmaceutical industry since it possesses many therapeutic properties such as anticancer, antioxidant, antimicrobial, antiasthmatic, anti-inflammatory, immunomodulatory, analgesic, and bio-enhancing activity (Budama-kilinc, 2019; Gorgani et al., 2016). Nevertheless, piperine has not been fully utilized for in pharmaceutical applications due to its poor solubility in water which leads to low availability (Thenmozhi & Je, 2017).

Piperine was studied as a potential drug molecule due to its anti-inflammatory and analgesic properties. To increase its bioavailability for topical applications, poly(lactic-co-glycolic acid) (PLGA) was used to encapsulate piperine. Piperine PLGA nanoparticles were

characterized by using spectroscopic and imaging methods. The high encapsulation efficiency of piperine was observed, which was up to 99% and the loading capacity is 3%. In vitro drug release study was also performed on the piperine PLGA nanoparticles using phosphate buffer solution, pH 7.2, where after 24 hours, 43.32% of piperine was released from the nanoparticles. The release study was completed within 240 hours, and 96.61% of the piperine was released (Budama-kilinc, 2019).

Piperine is also known for its bio-enhancing properties. When mixed with drugs or other active molecules, piperine promotes and augment their bioavailability without causing any synergistic effect with the drug (Mhaske, Sreedharan, & Mahadik, 2018). Piperine was used as a natural enhancer to increase bioavailability of curcumin in a study that aims to develop a transdermal curcumin delivery system. In this study, the amount of curcumin was fixed while the amount of piperine is varied. The result from an in vitro skin permeation study showed that piperine has successfully increased the permeation of curcumin. The permeation rate of curcumin increased up to 1.89 times when 7.41% of piperine was used compared to non-piperine contained membrane (Jantarat, Sirathanarun, Boonmee, & Meechoosin, 2018).

CHAPTER 3

HYDROXYPROPYL STARCH NANOPARTICLES AS CONTROLLED RELEASE NANOCARRIERS FOR PIPERINE

3.1 Introduction

Piperine is a naturally occurring alkaloid and it is the major bio-active component of pepper, which gives pungency and biting taste to it. Several studies have demonstrated that piperine possesses various beneficial health and therapeutic properties and most recently, piperine also showed chemopreventive and antioxidant activities (Gorgani et al., 2016). Besides, it also has anticarcinogenic, stimulatory, anti-inflammatory, antimicrobial and antiulcer activities (Baspinar, Üstündas, Bayraktar, & Sezgin, 2018; Gorgani et al., 2016). However, the pharmaceutical activities of piperine are limited due to its low water solubility and its toxic effect on the central nervous and reproductive system when being used in high concentration (Ezawa, Inoue, Tunvichien, Suzuki, & Kanamoto, 2016; Gorgani et al., 2016). These limitations have prompted many researchers attempted to encapsulate piperine onto various nanoparticles in order to enhance its water solubility, bioavailability and efficacy. There are several studies in which piperine was loaded onto nanoparticles. For example, piperine and curcumin was loaded onto zein-chitosan nanoparticles and 89% piperine encapsulation efficiency was achieved (Baspinar et al., 2018). Piperine also showed high encapsulation efficiency (90.5%) when being encapsulated onto nanosize liposomes (Pentak, 2015).

Studies have shown that starch nanoparticles is a promising nanocarriers for various drugs and nutraceutical products due to its advantages, such as improving drug solubility and stability, decreasing drug toxicity and high drug loading capacity. In view of this, some

researchers have attempted to load curcumin onto starch nanoparticles via in-situ nanoprecipitation method. Maximum loading efficiency of 78% was achieved and the curcumin was released in a sustained way within 10 days from the nanoparticles with mean particles size of 87 nm (Chin et al., 2014). A model drug 5-aminosalicylic acid was also loaded onto crosslinked starch nanoparticles which is 40 nm in size and the maximum drug loading capacity achieved was 0.302 $g.g^{-1}$ (Yang et al., 2014).

However, starch are naturally unsuitable for most of the technological and biomedical applications in its native state due to its high retrogradation and syneresis besides having poor processability and low water solubility. Therefore, all kind of modifications including blending and chemical modifications such as hydroxypropylation, oxidation and crosslinking were carried out to overcome the abovementioned limitations and improve its properties. Starch molecules possess large numbers of hydroxyl groups which could serve as the active sites for modification *via* various chemical reactions. Chemical modification is the most efficient tool to customize the overall performance of native starch (Chen et al., 2015).

Hydroxypropyl starch can be prepared by the etherification reaction of native starch with propylene oxide in the presence of strong alkaline catalyst. The hydroxyl groups are substituted with hydroxypropyl groups through the nucleophilic substitution reaction mechanism. Studies showed that hydroxypropylation reduced the retrogradation property of starch to recrystallize and it is more stable at high temperatures. The hydroxypropyl group has also been proven to have positive swelling effect on polymers in matrices with neutral pH, therefore providing a controlled and sustained, drug release profile (Masina et al., 2017).

There have been studies that have reported the synthesis of modified starch nanostructures (Table 3.1), however, up to our knowledge, this is the first time hydroxypropyl starch nanoparticles was prepared and loaded with piperine. In this work, we report the synthesis of hydroxypropyl starch by chemical modifications of the native sago starch using hydroxypropylation methods. Subsequently, piperine loaded hydroxypropyl starch nanoparticles were prepared by *in-situ* nanoprecipitation method. Formulation parameters that affected the loading capacity and release profiles of piperine were evaluated.

Table 3.1: Summary of synthesis of modified starch nanostructures

Starch source	**Modification**	**Morphology**	**Application**	**References**
Corn starch	Acetylation	Nanoparticles	Drug delivery	Heydar et al., (2016)
Maize starch	Crosslinking and esterification	Nanocrystals		Ren et al., (2016)
Corn starch	Oxidation	Nanofibers		Wang, (2015)
Soluble starch	Crosslinking	Nanoparticles	Drug delivery	Yang et al., (2014)

3.2 Materials and Methods

3.2.1 Materials

Native sago starch powder was obtained from a local grocery store in Kuching. Hydrochloric acid (HCl), sulphuric acid (H_2SO_4), sodium hydroxide (NaOH) and potassium hydroxide (KOH) were purchased from Merck. Propylene oxide was procured from Acros Organic (USA). Sodium thiosulfate were purchased from R&M Chemicals, Essex, UK. Piperine was acquired from Sigma Aldrich. Absolute ethanol was obtained from HmbG Chemicals (Hamburg, Germany). All chemicals were used without further purification. Ultrapure water (~18.2 MΩ•cm, 25 ^oC) was obtained from the Water Purifying System (ELGA, Ultra Genetic).

3.2.2 Synthesis of hydroxypropyl sago starch nanoparticles

Hydroxypropylation of sago starch was performed according to the reported method with slight modifications (Heydar et al., 2016; Ren et al., 2016; Yang et al., 2014). Anhydrous sodium sulfate (Na_2SO_4) (20%, $w.v^{-1}$) was added to the starch slurry (20%, $w.v^{-1}$). The starch slurry was stirred for 30 minutes and the pH was adjusted to 10.5 with the addition of NaOH (5%, $w.v^{-1}$). Then, propylene oxide (5%, 10%, 15% and 20%, $v.v^{-1}$) was added as an etherifying agent. The resulting suspension was mixed thoroughly and the reaction was maintained at 40 ^oC for 24 hours. The reaction was then terminated by adjusting the pH to 5.5 using HCl (10%, $v.v^{-1}$). The resulting hydroxypropyl starch solution was added drop-wise into absolute ethanol in the ratio of 1:20 under constant stirring (900 rpm). Hydroxypropyl starch nanoparticles were precipitated and were centrifuged at 950 rpm, washed 3 times with absolute ethanol and dried at 60 ^oC for 24 hours in the oven.

3.2.3 Molar Substitution (MS)

The hydroxypropyl group in the modified starch was determined according to the reported method (Hazarika & Sit, 2016; Wang, 2015). A sample of hydroxypropyl starch (0.05 g) was weighed into a 100 mL volumetric flask and 25 mL of sulphuric acid (H_2SO_4) (0.5 M) was added. A sample of native starch was prepared in the same manner. The flasks were placed and heated in boiling water until the solution became clear. The samples were allowed to cool before the contents were diluted to 100 mL with UPW. 1 mL of the solution was pipetted into 25 mL volumetric flask. The flasks were immersed in an ice bath before 8 mL of concentrated H_2SO_4 solution were added drop wise into each flasks. The solution was mixed well and the flasks were placed in a boiling water bath for 3 minutes. The flasks were then transferred to an ice bath until the solution was chilled. Approximately 0.6 mL of ninhydrin reagent ($C_9H_6O_4$) was added, and the flasks were immediately shaken well and leave at room temperature for 100 minutes. The volume of the solution in each flask was adjusted to 25 mL by adding concentrated H_2SO_4 and mixed well. After 5 minutes, a portion of the solution was transferred to a cuvette and the absorbance was measured at 590 nm, using starch blank as a reference. A calibration curve was prepared with an aliquot (1 mL) of standard solution, containing 10, 20, 30, 40 and 50 $mg.mL^{-1}$ of propylene glycol. The hydroxypropyl group was calculated using equation (3.1):

$$\text{Hydroxypropyl groups (\%)} = \frac{\text{C x 0.7763 x 10 x F}}{\text{W}} \qquad (3.1)$$

where C is the amount of propylene glycol in the sample solution determined from the calibration curve ($mg.mL^{-1}$), F is the dilution factor and W is weight of the sample (mg). The molar substitution (MS) of the modified starch was calculated using equation (3.2):

$$\mathrm{MS} = \frac{162\ \mathrm{W}}{100 - (\mathrm{M} - 1)W} \tag{3.2}$$

where W is the equivalent hydroxypropyl group in 100 g of starch and M is the molecular weight of C_3H_6O.

3.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of native starch, hydroxypropyl starch and hydroxypropyl starch nanoparticles were obtained using a Fourier Transform Infrared Spectrophotometer (FTIR) (SHIMADZU Model FTIR-8201PC). Dried starch powder samples was made into thin pellets with potassium bromide (KBr) and then scanned within the wavenumber ranges of 600 cm^{-1} and 4000 cm^{-1}.

3.2.5 Scanning Electron Microscopy (SEM)

The morphology of the samples were investigated using a scanning electron microscope (SEM) (JEOL Model JSM6390LA) at various magnifications. The samples were dropped on stainless steel plates, dried at room temperature, and coated with a layer of platinum using JEOL/JFC-1600 Auto Fine Coater.

3.2.6 Transmission Electron Microscopy (TEM)

An appropriate amount of nanoparticles was dispersed in absolute ethanol and sonicated before they were dropped onto the fomvar-coated copper grids. TEM micrographs of the nanoparticles were obtained *via* a transmission electron microscopy (TEM) (JEOL Model 1230).

3.2.7 Swelling ratio

The swelling property of hydroxypropyl starch nanoparticles was studied by immersing dried hydroxypropyl starch (1g) in PBS solution of various pH values (1.2, 7.4 and 8.6) at 37 ^{o}C. At every 4 hours time intervals (1-72 hours), samples were taken out from the PBS solution, dried with filter papers and weighed. The swelling ratio of hydroxypropyl starch nanoparticles was calculated using equation (3.3):

$$Swelling\ ratio\ (g/g)\ =\ \frac{Ws-Wd}{Wd} \tag{3.3}$$

where W_s is the weight of swollen hydroxypropyl starch nanoparticles and W_d is the weight of dried starch nanoparticles.

3.2.8 Loading of piperine onto hydroxypropyl starch nanoparticles

Piperine of various concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg.L^{-1}) were dissolved in 20 mL of absolute ethanol as a precipitating medium, then 1 mL of hydroxypropyl starch solution was added dropwise into the resulting solution and magnetically stirred for 30 minutes. Starch nanoparticles formed were separated from the reaction medium by centrifugation, and the concentration of piperine remained in the supernatant was quantified spectrophotometrically at a wavelength of 343 nm with a UV-vis spectrophotometer (Jasco V-630). The molar concentration of piperine was calculated from the absorbance value, based on a calibration curve of piperine in ethanol solution. The loading capacity of piperine for starch nanoparticles was calculated using equation (3.4):

$$Loading\ capacity\ (mg/mg)\ =\ \frac{[piperine]tot\ -\ [piperine]free}{weight\ of\ nanoparticles} \tag{3.4}$$

where $[piperine]_{tot}$ is the initial concentration of piperine used and $[piperine]_{free}$ is the concentration of piperine remained in the supernatant.

3.2.9 Piperine release studies

A predetermined amount of piperine loaded hydroxypropyl starch nanoparticles were dispersed in 20 mL of phosphate buffer saline (PBS) solution (pH 1.2, 7.4 and 8.6) at 37 ^{o}C. At predefined time intervals (4 hours), PBS was withdrawn from each of the release media and immediately replaced with the same volume of PBS solution. The molar concentrations of piperine in the supernatant were determined from the absorbance values measured at 655 nm against the calibration curve of piperine in PBS at pH 1.2, 7.4 and 8.6. The calibration standard was prepared by dissolving various known concentration of piperine (0.1, 0.2, 0.3, 0.4 and 0.5 mg.L^{-1}) in PBS solution (pH 1.2, 7.4 and 8.6). The absorbance value were then measured by using UV- vis spectrophotometer. A graph of absorbance versus concentration was constructed and a best fit straight line was drawn through the data points. The percentage of piperine released was measured according to equation (3.5):

$$Piperine\ released\ (\%) = \frac{[piperine]rel}{[piperine]load}\ x\ 100\% \qquad (3.5)$$

where $[piperine]_{rel}$ is the concentration of piperine released at time and $[piperine]_{load}$ is the concentration of piperine being loaded onto the hydroxypropyl starch nanoparticles.

3.3 Results and Discussions

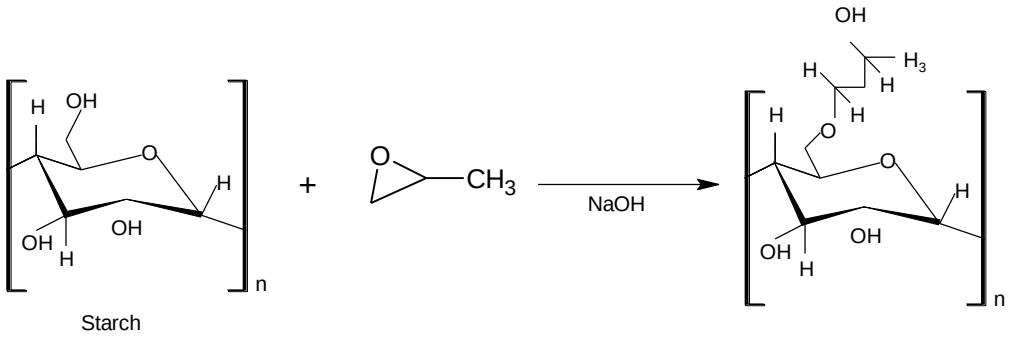
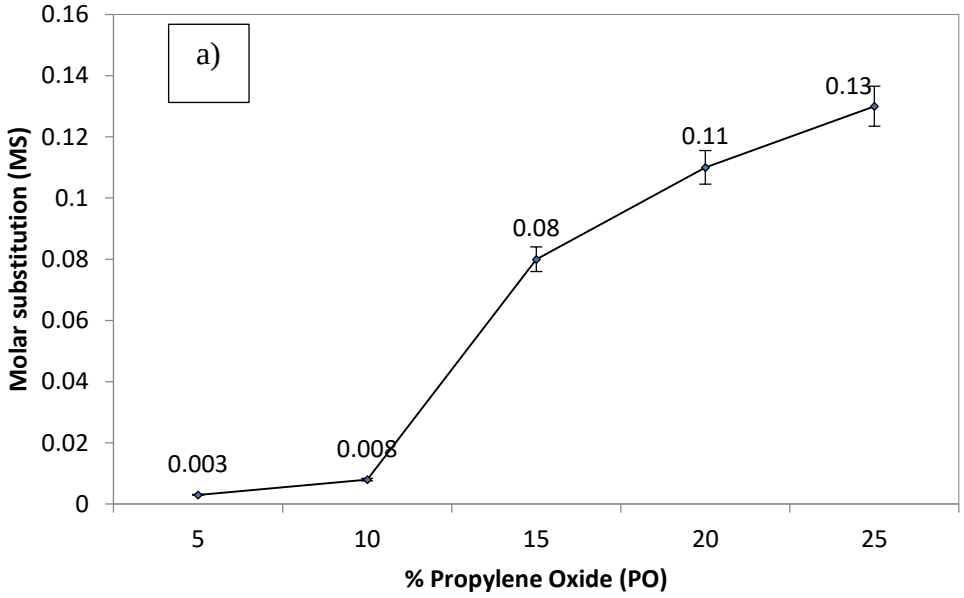


Figure 3.1: Reaction scheme of hydroxypropylation of native sago starch to form hydroxypropyl starch

Starch possesses a considerable amount of hydroxyl group that has anticipated to react with hydroxypropyl groups via etherification. As a result, some hydroxyl groups of the anhydroglucose unit of starch were replaced by hydroxypropyl groups. The reaction mechanism of reaction between native sago starch and propylene oxide is shown in Figure 3.1.

3.3.1 Molar Substitution (MS) of hydroxypropyl starch



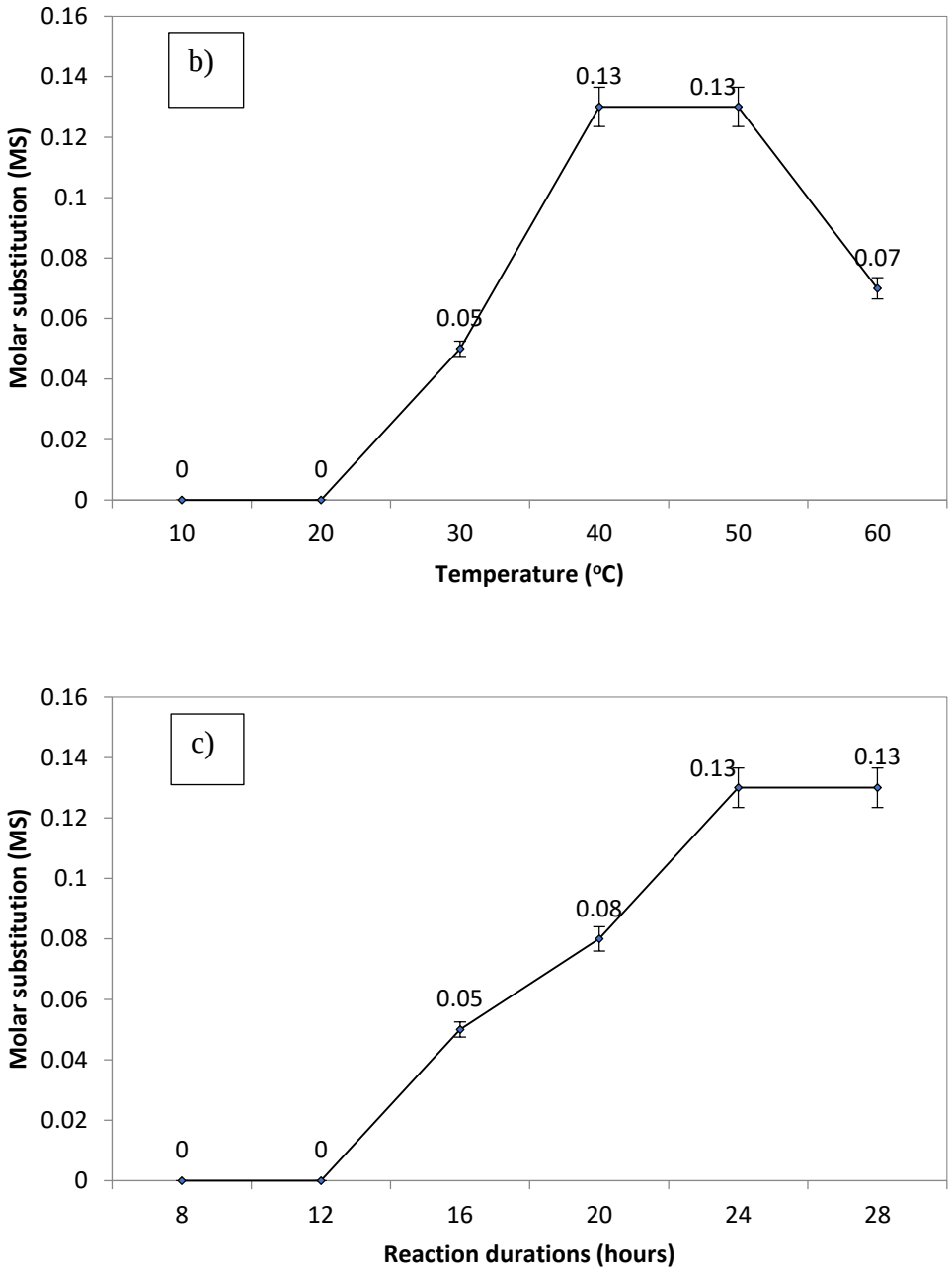


Figure 3.2: Effect of synthesis conditions on the molar substitution (MS) of hydroxypropyl starch (a) propylene oxide concentration (40 ^{o}C, 24 hours), (b) reaction temperatures (25% PO, 24 hours) and (c) reaction durations (25% PO, 40 ^{o}C), where n=3

The effects of etherification conditions (% propylene oxide, temperature and reaction duration) on the MS of hydroxypropyl starch was investigated to study the efficiency of hydroxypropylation. As shown in Figure 3.2 (a), it was found that the minimum and

maximum MS was obtained at 0.003 and 0.13 when 5 and 25% of propylene oxide was applied, respectively. Increasing the concentration of propylene oxide give rise to higher alkalinity condition which could promote swelling by the ionizing starch hydroxyl group, which in turn yielded higher substitution (Wang, 2015)**.** At 30% propylene oxide, the starch slurry gelatinized at room temperature and was not recoverable, therefore the reaction was not pursued. The effect of reaction temperatures on MS were investigated by heating the samples at various reaction temperatures.

Figure 3.2 (b) shows that hydroxypropylation did not occur at temperature less than 30 ^{o}C. Increasing reaction temperature of hydroxypropylation to around 40 to 50 ^{o}C showed a significant increase in MS, where MS 0.13 was obtained. However, when the temperature is further increased to 60 ^{o}C, the MS was reduced to 0.07 which could be due to degradation of the starch. The effect of reaction durations on MS of hydroxypropyl starch is shown in Figure 3.2 (c). There were no reaction occurred at reaction time less than 16 hours. After 16 hours reaction, hydroxypropyl starch with 0.05 MS was obtained. The maximum MS of 0.13 was obtained at 24 hours reaction time and when the reaction was pursued until 28 hours, the MS remained constant.

3.3.2 FTIR analysis

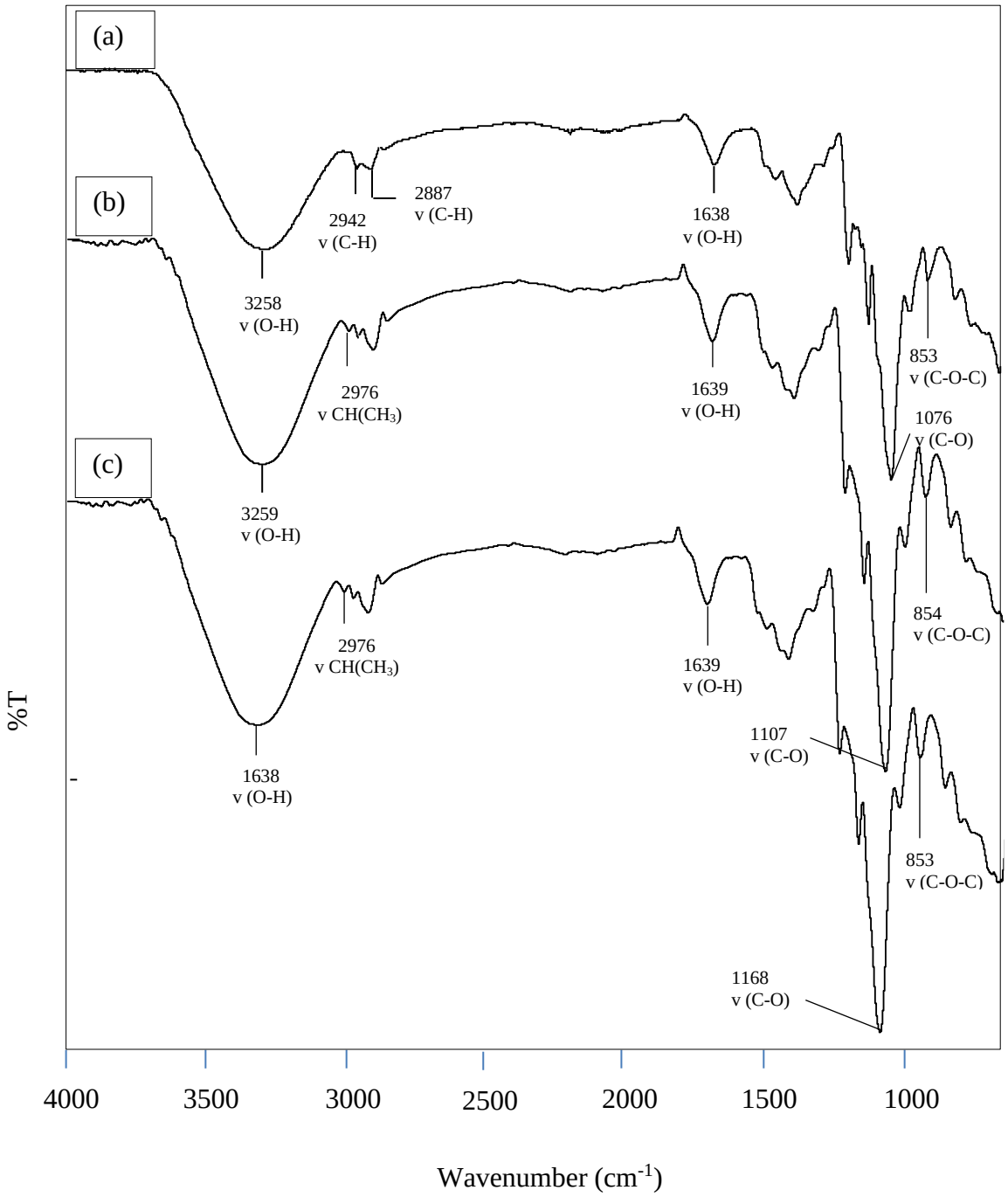


Figure 3.3: FTIR spectra of (a) native starch, (b) hydroxypropyl starch, and (c) hydroxypropyl starch nanoparticles

Figure 3.3 shows the FTIR spectra of native starch, hydroxypropylated starch and hydroxypropyl starch nanoparticles analysed by FTIR. Figure 3.3 (a) showed the spectrum of native sago starch with absorption bands at 2942 cm^{-1} and 2887 cm^{-1} indicating the presence of CH band stretching. The band at 1638 cm^{-1} was attributed to the OH group (O-H from moisture absorbency in starch) and the band at 3258 cm^{-1} was due to the OH groups of starch molecules (Hazarika & Sit, 2016) The peak at 1076-1168 cm^{-1} are mainly assigned to C-O stretch of C-O-H in starch (Chin et al., 2011). After hydroxypropylation, a new characteristic absorption band was observed at 2976 cm^{-1} which corresponded to the asymmetric CH(CH_3) stretching as shown in Figure 3.3 (b). The appearance of the peak at 2976 cm^{-1} was evidenced of hydroxypropylation of starch (Sukhija et al., 2016; Woggum et al., 2015). Besides that, the absorption peak at 853-856 cm^{-1} attributed to C-O-C glycosidic bonds of anhydroglucose unit (AGU) of starch molecules was not degraded after hydroxypropylation (Chin et al., 2011). The FTIR spectrum of hydroxypropyl starch nanoparticles (Figure 3.3 (c)) was similar to that of hydroxypropyl starch (Figure 3.3 (b)). This showed that nanoprecipitation process using ethanol as the precipitating medium did not break the bonds of the newly substituted groups.

3.3.3 Morphological study

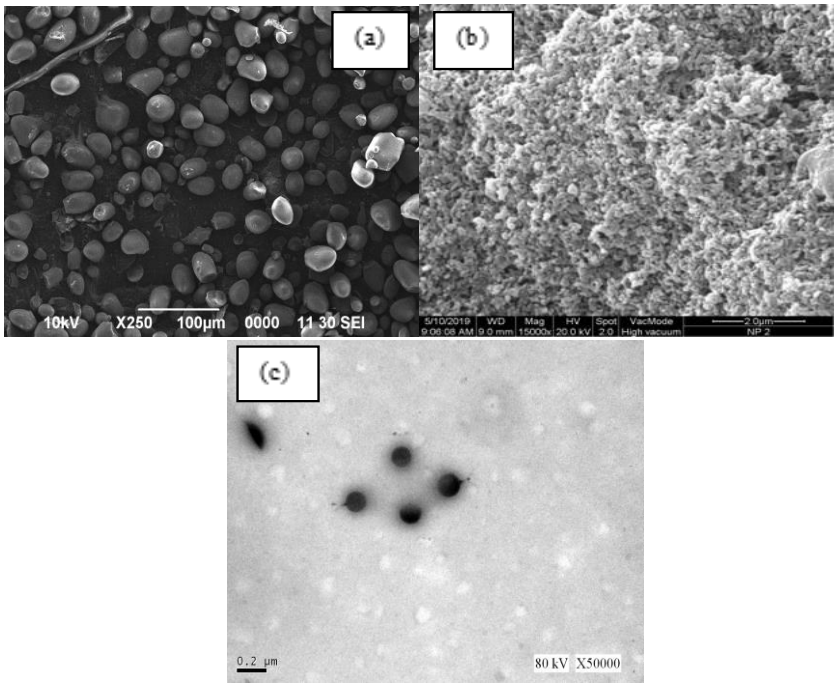


Figure 3.4: (a) SEM images of native sago starch, (b) FESEM images of hydroxypropyl starch nanoparticles and (c) TEM images of hydroxypropyl starch nanoparticles

As shown in Figure 3.4 (a), the morphology of native sago starch granules was observed to be smooth and oval in shape with particles sizes around 20 to 45 µm. After nanoprecipitation, the size and shape of the nanoparticles are completely different from the native starch. Hydroxypropyl starch nanoparticles are mainly spherical in shape with a mean particle sizes of 110 nm as shown in the SEM and TEM micrographs, respectively (Figure 3.4 (b) and Figure 3.4 (c)). During nanoprecipitation process, ethanol act as non-solvent to precipitate hydroxypropyl starch solution (solvent). Solvent interfacial deposition occurs at the interface of the solvent and non-solvent when the starch solution diffused into the dispersive medium, forming starch nanoparticles aggregation. Rapid desolvation of the starch renders the precipitation of nanoparticles (Aminian, Nafchi, Bolandi, & Alias, 2013).

3.3.4 Piperine Loading Capacity

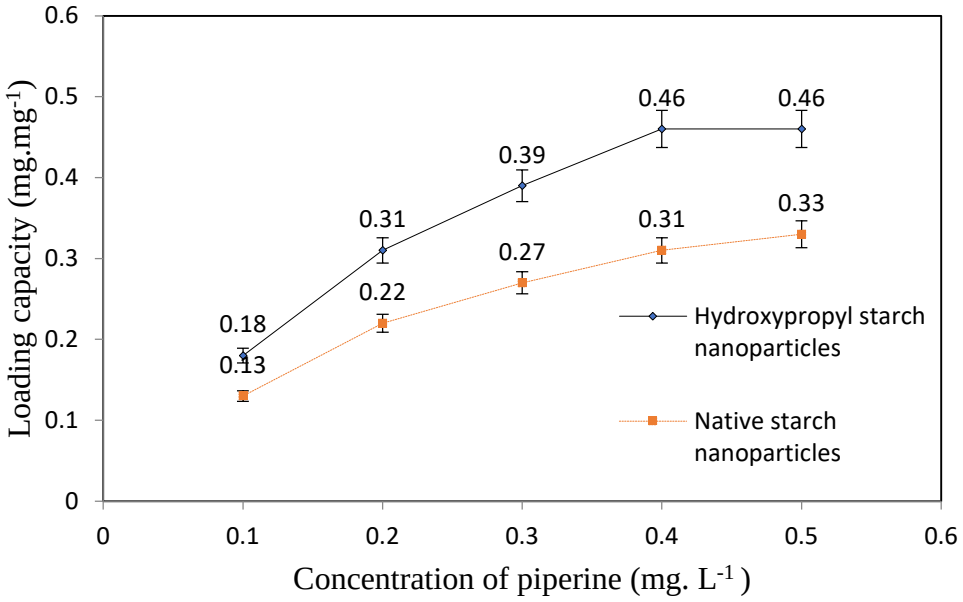


Figure 3.5: Loading capacity of piperine onto native starch nanoparticles and hydroxypropyl starch nanoparticles, where n=3

For comparison, the loading capacity of native starch nanoparticles and hydroxypropyl starch nanoparticles was investigated at different concentrations of piperine (0.1, 0.2, 0.3, 0.4 and 0.5 $mg.L^{-1}$) as shown in Figure 3.5. The loading capacity of piperine onto native starch nanoparticles increased almost linearly from 0.13 to 0.33 $mg.mg^{-1}$ as the concentration of piperine increased from 0.1 to 0.5 $mg.L^{-1}$. Whereas, for hydroxypropyl starch nanoparticles, the loading capacity increased from 0.18 to 0.46 $mg.mg^{-1}$ when the concentration of piperine increased from 0.1 to 0.4 $mg.L^{-1}$. When the concentration of piperine was further increased to 0.5 $mg.L^{-1}$, the loading capacity remained constant. This may be due to the saturation of the adsorption sites of hydroxypropyl starch nanoparticles (Aminian et al., 2013). Overall, the hydroxypropyl starch nanoparticles was observed to have higher loading capacity compared to native starch nanoparticles.

Due to higher drug loading capacity demonstrated by *in-situ* nanoprecipitation technique compared to conventional methods, it was used in this research to load the piperine onto the nanoparticles (Chin et al., 2014). Through etherification, the hydrophobicity of propylene oxide and the hydrophilicity of starch backbone is retained (Pec & Venskutonis, 2016). Adsorption of piperine onto hydroxyl propyl starch could occur through hydrophobic interactions. Piperine is hydrophobic thus are attracted by the hydrophobic surfaces of hydroxypropyl starch nanoparticles. During nanoprecipitation, the hydrophobic cores of hydroxypropyl starch nanoparticles were surrounded by hydrophilic outer shells, so the inner core serve as nano-carrier for piperine, thus explained the observed higher loading capacity (Janeth et al., 2017; Pec & Venskutonis, 2016).

3.3.5 Piperine release studies

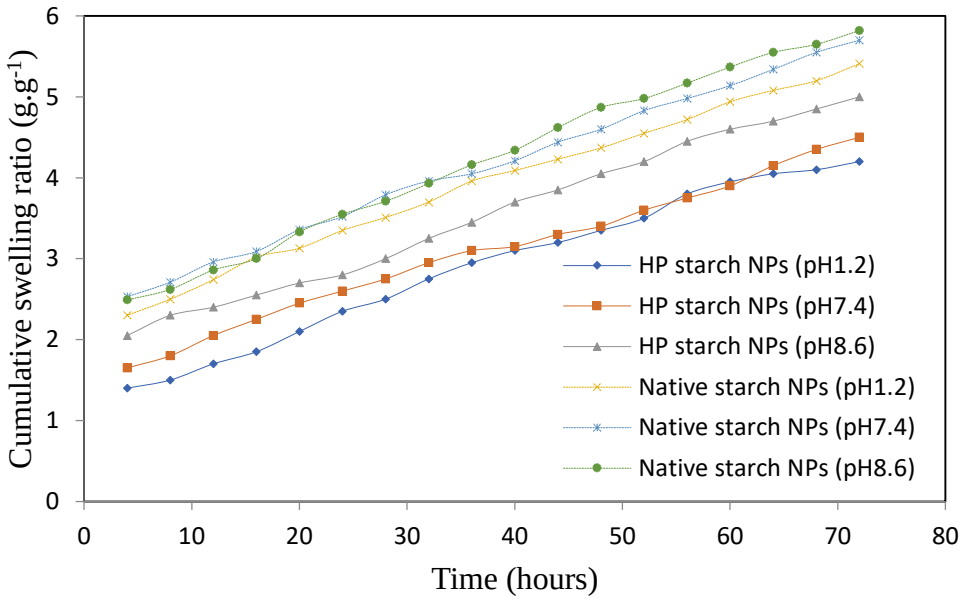


Figure 3.6: Swelling ratios of native starch nanoparticles and hydroxypropyl starch nanoparticles in various pH of physiological media (1.2, 7.4 and 8.6) as a function of time

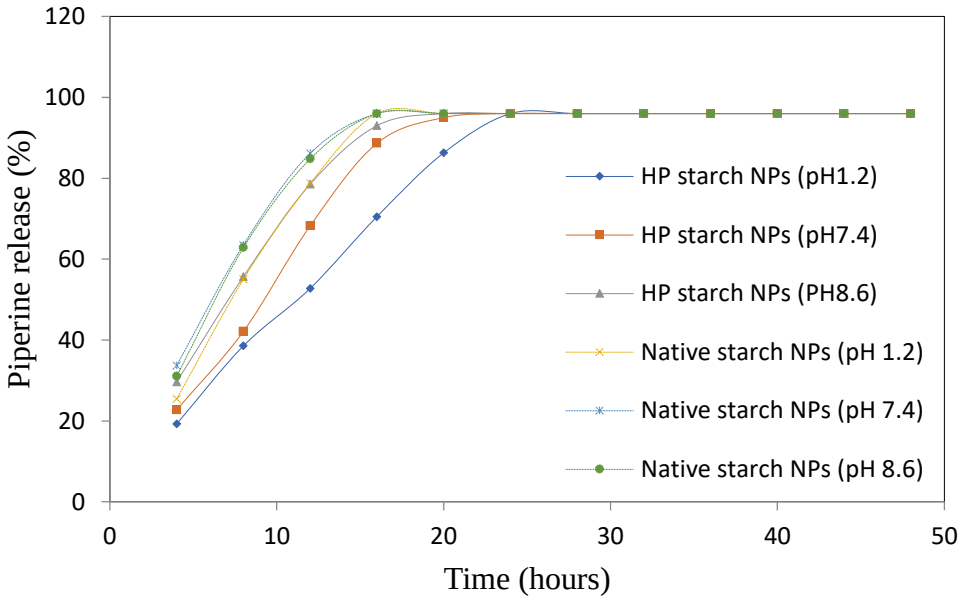


Figure 3.7: Percentage of piperine released from native starch nanoparticles and hydroxypropyl starch nanoparticles in media of various pH

The swelling behaviour of the native starch nanoparticles and hydroxypropyl starch nanoparticles and the release profiles of piperine from native starch nanoparticles and hydroxypropyl starch nanoparticles were investigated at various physiological pH values (1.2, 7.4 and 8.6) and the results were shown in Figure 3.6 and 3.7, respectively. The piperine release profiles were observed to be dependent on the swelling behaviour of the starch nanoparticles. For native starch nanoparticles, piperine was completely released after 16 hours. This could be due to the higher swelling ratio (3.93, 4.25 and 4.22 $g.g^{-1}$) at pH 1.2, 7.4 and 8.6, respectively. On the other hand, at pH 1.2, piperine was completely released from hydroxypropyl starch nanoparticles in a slow and constant manner within 24 hours. This observation could be due to the smaller swelling ratio (2.9 $g.g^{-1}$) of hydroxypropyl starch nanoparticles in the acidic medium (Parent, Baradari, Champion, Damia, & Viana-trecant, 2017). At pH 7.4 and 8.6 piperine was observed to slowly release from hydroxypropyl starch nanoparticles over the period of 16 and 12 hours, respectively. The

hydrophobic nature of hydroxypropyl functional group has resulted in relatively smaller swelling ratio of starch nanoparticles, which in turn, has caused the piperine to release in slower rates (Masina et al., 2017; Parent et al., 2017).

3.4 Summary

Hydroxypropyl starch were successfully synthesized from native sago starch by hydroxypropylation reaction and nanoprecipitation method was used to synthesize nanoparticles from the hydroxypropyl starch. This study showed that hydroxypropyl starch nanoparticles have higher loading capacity (0.33 $mg^{-1}.mg^{-1}$) of piperine, lower swelling ratio (2.9 $g^{-1}.g^{-1}$) and slower piperine release rates (24 hours) as compared to native starch nanoparticles. The results of this study clearly demonstrated that hydroxypropyl starch nanoparticles is a promising controlled release nanocarrier for piperine.

CHAPTER 4

STARCH ACETATE NANOPARTICLES AS CONTROLLED RELEASE NANOCARRIERS FOR PIPERINE

4.1 Introduction

Previous studies showed that piperine possesses various therapeutic properties such as antidepressant, antipyretic, anti-inflammatory, antithyroid, hepaprotective, immunomodulatory and antitumor. However, piperine also has low water solubility besides poor dissolution rate which leads to low availability, making it unsuitable for pharmaceutical applications (Gorgani et al., 2016). Nanoparticles have been widely used as nanocarriers for various drug formulations because of its ability to improve drug solubility and stability, reduce drug toxicity, in addition to having high drug loading capacity. Some researchers also suggested that encapsulating poorly water-soluble drugs candidates like piperine in nanoparticles could improve their solubility in aqueous solutions (Zhang, 2018).

In acetylation of starch, some anhydroglucose units of starch are being replaced by acetyl group to form modified molecular structure of starch. Starch acetylation, therefore, promotes reduction in the interaction between outer chains of amylopectin and amylose, thereby provides new features to the starch**.** Depending on their degree of substitution, starch acetate possesses various properties such as hydrophobicity and various non-food applications such as tablet binders, hot melt adhesives, coating, biodegradable packaging materials as well as pharmaceutical applications. Starch derivatives obtained by acetylation are also widely used in the paper, food, textile and biomedical applications due to their enhanced film-forming, stabilizing and thickening properties as compared with native starch (Heydar et al., 2016; Hong, Zeng, Brennan, Brennan, & Han, 2016; Raj & Prabha, 2016).

In this study, the preparation of starch acetate nanoparticles derived from native sago starch as controlled released nanocarriers for piperine was reported. Piperine possesses many therapeutic properties that have a high potential for pharmaceutical applications, but it has poor solubility and low availability. Hence, starch acetate nanoparticles were prepared in an attempt to improve the solubility and increase the availability of the piperine. The piperine loading capacity and piperine release profile of starch acetate nanoparticles was compared with that of native starch nanoparticles.

4.2 Materials and Methods

4.2.1 Materials

Native sago starch powder was obtained from a local grocery store in Kuching. Hydrochloric acid (HCl), sulphuric acid (H_2SO_4), acetic anhydride and potassium hydroxide (KOH) were purchased from Merck. Iodine and sodium thiosulfate were purchased from R&M Chemicals, Essex, UK. Piperine was acquired from Sigma Aldrich. Absolute ethanol was obtained from HmbG Chemicals (Hamburg, Germany). All chemicals were used without further purification. Ultrapure water (~18.2 MΩ•cm, 25 ^{o}C) was obtained from the Water Purifying System (ELGA, Ultra Genetic).

4.2.2 Synthesis of starch acetate

Acetylated sago starch was synthesized based on the method reported by Bartz et al. (2015) with slight modifications. A sample of native sago starch (1 g) was placed in a round bottom flask equipped with a reflux condenser, thermometer, and stirring system. Then, 4 ml of acetic anhydride was added. The resulting suspension was stirred continuously for 5 minutes at room temperature before iodine (0.3 g) which was previously dissolved in 1 ml

of acetic anhydride was added. The reaction temperature was varied (60 °C, 70 °C, 80 °C, 90 °C and 100 °C) for different reaction duration (10, 15, 20, 25, and 30 minutes). Saturated sodium thiosulfate solution (2 mL) was then added to stop the reaction. Samples of starch acetate obtained were washed with absolute ethanol (20 mL) to remove unreacted acetic anhydride and then dried at 60 °C for 24 hours.

4.2.3 Degree of substitution (DS)

The percentage of acetyl groups was determined using the titration method (Bartz et al., 2015). Approximately 1 g of each sample was added to a conical flask with 50 mL of ethanol (75%). These samples were heated in a water bath at 50 °C for 30 minutes and then 40 ml KOH (0.5 N) was added after cooling. The mixtures were continuously stirred at 200 rpm for 72 hours. Excess alkaline was titrated with 0.5 N HCl using phenolphthalein as an indicator. The average values for the titration of the native starch sample were used as blanks. The percentage of acetyl content (AC) was calculated according to equation (4.1):

$$\text{AC (\%)} = \frac{[(\text{mLblank} - \text{mLsample}) x\ MHCl\ x\ 0.043\ x\ 100]}{\text{Sample weight (g)}} \qquad (4.1)$$

DS is defined as the average number of sites per glucose unit that receives a substituent group based on equation (4.2):

$$\text{DS} = \frac{162 \text{ x AC}}{[4300 - (42 \text{ x AC})]} \qquad (4.2)$$

4.2.4 Synthesis of starch acetate nanoparticles

Acetylated starch nanoparticles were prepared from acetylated starch using the nanoprecipitation method. The resulted starch acetate solution was added drop-wise into absolute ethanol in the ratio of 1:20 under constant stirring (900 rpm). Starch acetate nanoparticles precipitated were then centrifuged, washed with absolute ethanol (to remove excess acetic anhydride) and dried at 60 ^{o}C for 24 hours (Chin et al., 2011).

4.2.5 Characterization of starch acetate nanoparticles

FTIR spectra of native starch and starch acetate nanoparticles were obtained using a Fourier Transform Infrared Spectrophotometer (FTIR) (SHIMADZU Model FTIR-8201PC). Dried starch powder samples were made into thin pellets with potassium bromide (KBr) and then scanned within the wavenumber ranges of 600 cm^{-1} and 4000 cm^{-1}. The morphology of starch acetate nanoparticles was investigated using a scanning electron microscope (SEM) (JEOL Model JSM6390LA) at various magnifications. Small amounts of the samples were dropped on the SEM plates, dried, and coated using JEOL/JFC-1600 Auto Fine Coater with a thin layer of platinum. TEM micrographs of the nanoparticles were obtained *via* transmission electron microscopy (TEM) (JEOL Model 1230). An appropriate amount of nanoparticles was dispersed in absolute ethanol and sonicated before they were dropped onto the fomvar-coated copper grids.

4.2.6 Swelling ratio studies

The swelling property of starch acetate nanoparticles was studied by immersing dried starch acetate (1g) in PBS solution of various pH values (1.2, 7.4 and 8.6) at 37 ^{o}C. At every 4 hours time intervals (1-72 hours), samples were taken out from the PBS solution, dried

with filter papers and weighed. The swelling ratio of starch acetate nanoparticles was calculated using equation (4.3):

$$Swelling\ ratio\ (g/g)\ =\ \frac{Ws-Wd}{Wd} \qquad (4.3)$$

where W_s is the weight of swollen starch acetate nanoparticles and W_d is the weight of dried starch acetate nanoparticles.

4.2.7 Loading of piperine onto starch acetate nanoparticles

Piperine of various concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 $mg.L^{-1}$) was dissolved in 20 mL of absolute ethanol as the precipitating medium, then 1 mL of acetylated starch solution was added dropwise into the resulting solution and magnetically stirred for 30 minutes. Starch acetate nanoparticles formed were separated from the reaction medium by centrifugation, and the concentration of piperine remained in the supernatant was quantified spectrophotometrically at a wavelength of 340 nm with a UV-vis spectrophotometer (Jasco V-630). The molar concentration of piperine was calculated from the absorbance value, based on a calibration curve of piperine in ethanol solution. The loading capacity of piperine for starch nanoparticles was calculated using equation (4.4):

$$Loading\ capacity\ (mg/mg)\ =\ \frac{[piperine]tot - [piperine]free}{weight\ of\ nanoparticles} \qquad (4.4)$$

where $[piperine]_{tot}$ is the initial concentration of piperine used and $[piperine]_{free}$ is the concentration of piperine remained in the supernatant.

4.2.8 Piperine release analysis

A predetermined amount of piperine loaded starch acetate nanoparticles were dispersed in 20 mL of phosphate buffer saline (PBS) solution (pH 1.2, 7.4 and 8.6) at 37 ^{o}C. At predefined time intervals (4 hours), PBS was withdrawn from each of the release media and immediately replaced with the same volume of PBS solution. The molar concentrations of piperine in the supernatant were determined from the absorbance values measured at 655 nm against the calibration curve of piperine in PBS at pH 1.2, 7.4 and 8.6. The percentage of piperine released was measured according to equation (4.5):

$$Piperine\ released\ (\%)\ =\ \frac{[piperine]rel}{[piperine]load}\ x\ 100\% \qquad (4.5)$$

where $[piperine]_{rel}$ is the concentration of piperine released at the time (t) and $[piperine]_{load}$ is the concentration of piperine being loaded onto the starch acetate nanoparticles.

4.3 Results and Discussions

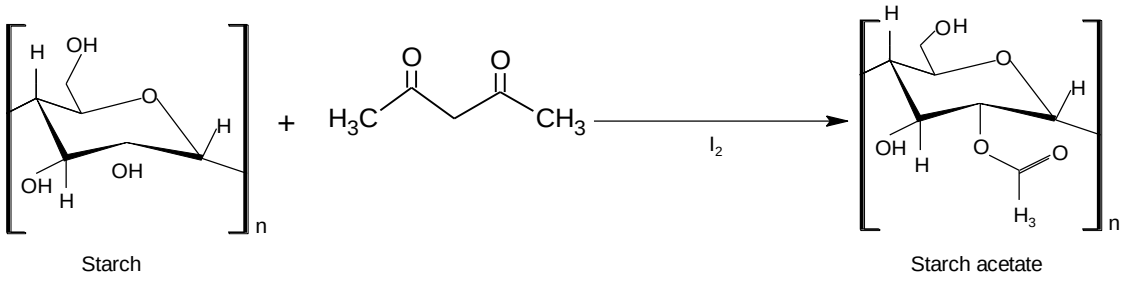


Figure 4.1: Schematic representation of the acetylation reaction of native sago starch with acetic anhydride to form starch acetate molecules

In acetylation, the hydroxyl groups in anhydroglucose unit of starch were replaced with acetyl groups. Figure 4.1 shows a schematic representation of the acetylation reaction of native sago starch with acetic anhydride to form starch acetate molecules.

4.3.1 Degree of substitution (DS)

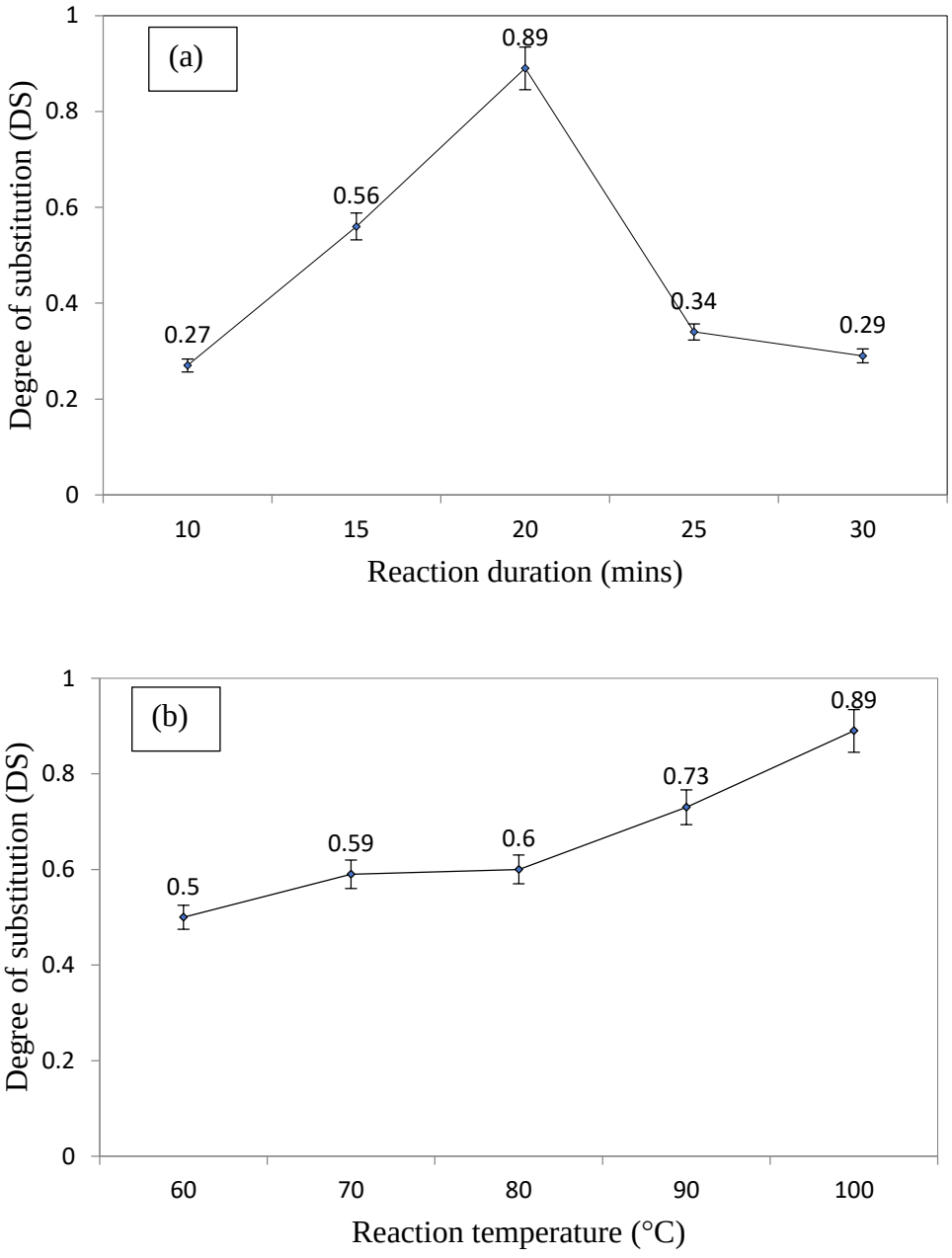


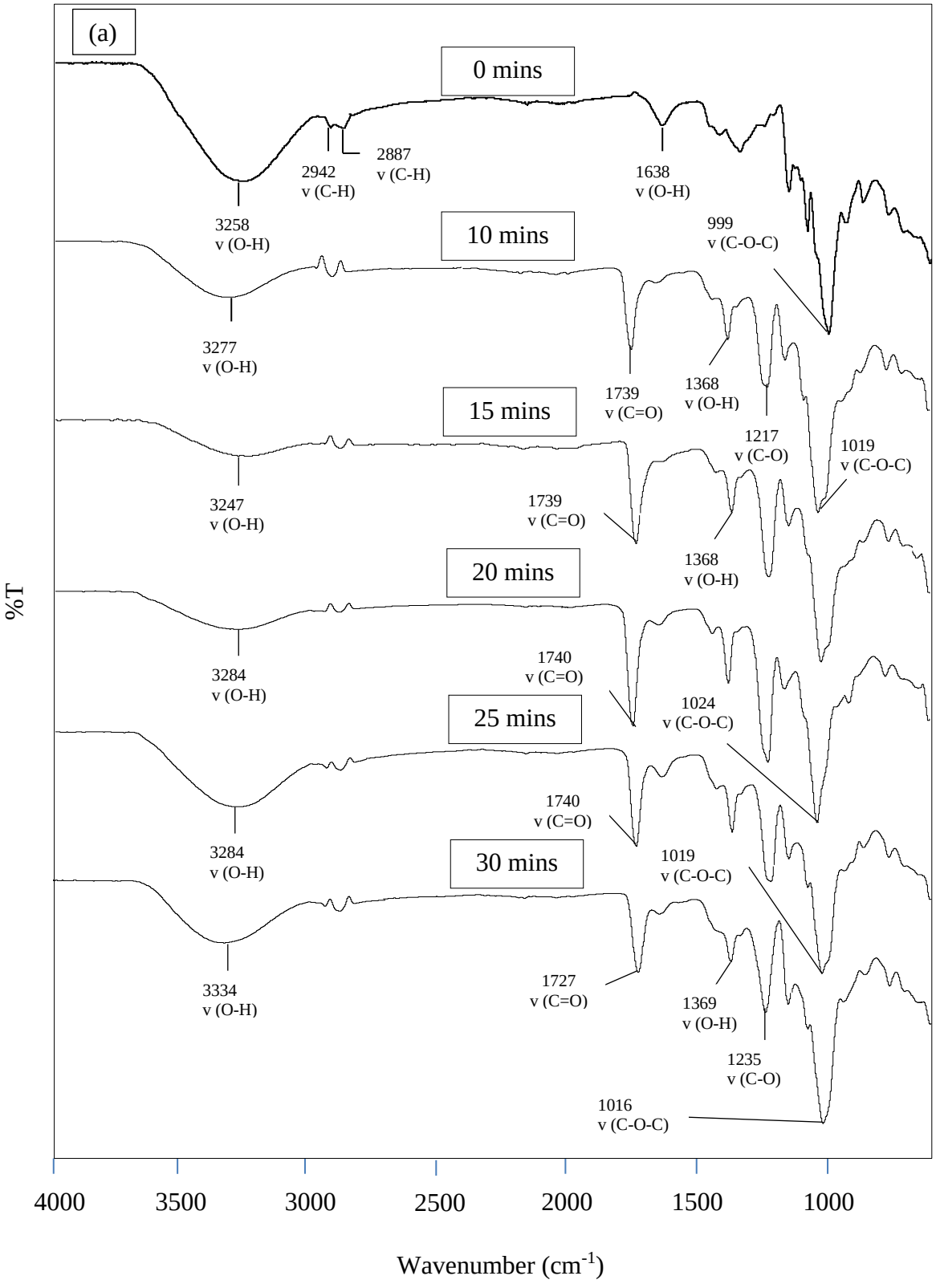
Figure 4.2: Effect of synthesis conditions on the degree of substitution (DS) of starch acetate (a) reaction durations at 100°C reaction temperatures, and (b) reaction temperatures at 20 minutes reaction time, where n=3

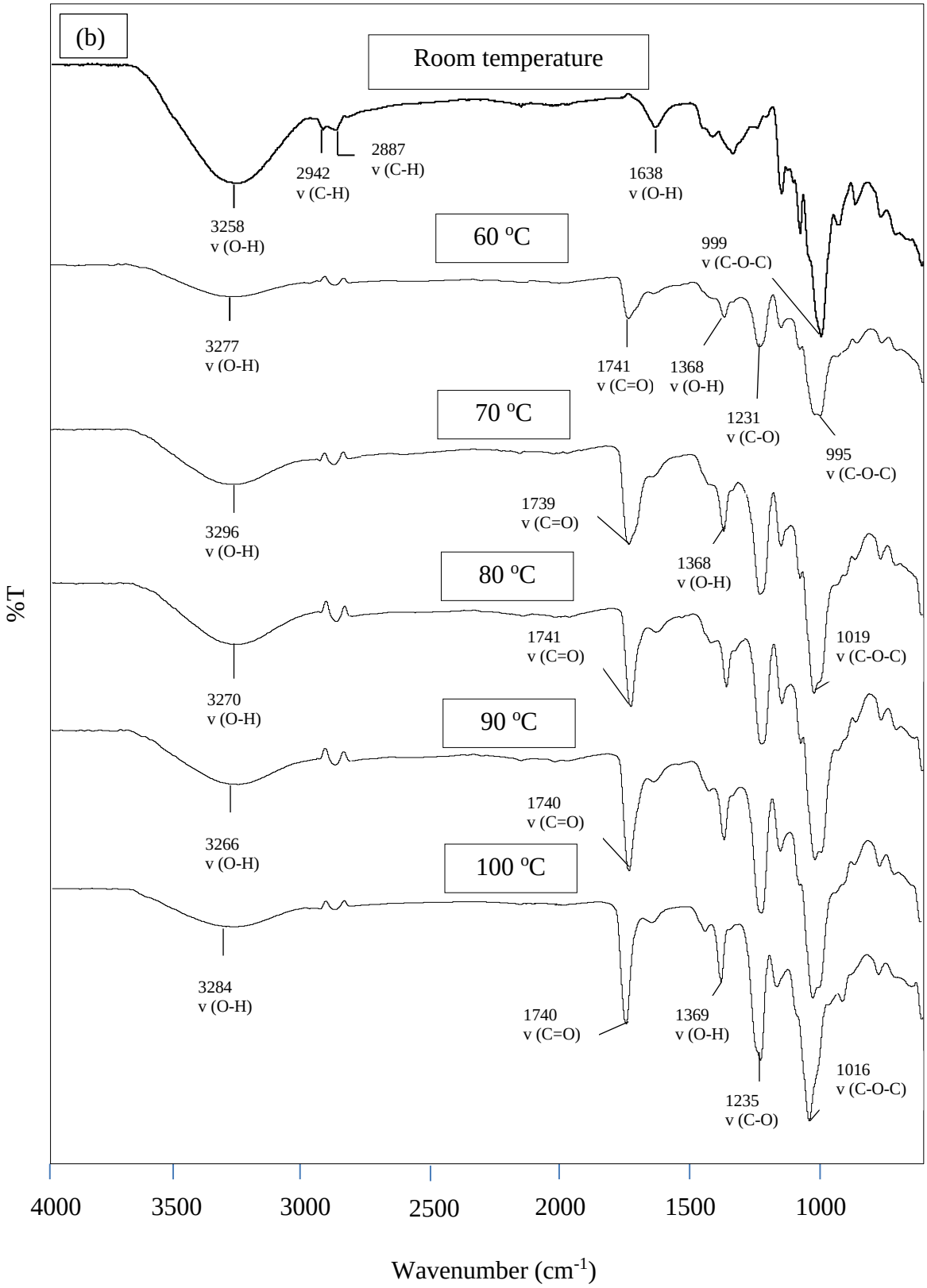
The DS was calculated as the average number of hydroxyl groups substituted per anhydroglucose unit. Treatment of starch with acetic anhydride for 10 to 30 minutes resulted

in a starch acetate with medium DS (0.1-1.0). As shown in Figure 4.2 (a), the DS was observed to increase with longer reaction duration from 10 to 20 minutes at 100 ^{o}C but decrease when the reaction was continued for 25 to 30 minutes, due to prolonged reaction that caused the acids to undergo hydrolysis, which breaks the ester bond (Bartz et al., 2015).

The effect of reaction temperatures on acetylation reaction was investigated by heating the samples for 20 minutes at various temperatures. It was found that acetylation reaction may occur at 60 ^{o}C as shown in Figure 4.2 (b), where starch acetate with 0.50 DS was obtained. It was observed that as the reaction temperature increased, the DS also increased. The highest DS of starch acetate was 0.89 which was obtained at 100 ^{o}C. This may be due to higher temperatures, the diffusion of the acetic anhydride and swelling of starch granules increase and disrupt the crystalline region of the starch molecules, leading to higher acceptability of amorphous regions of starch to acetic anhydride substitutions (Heydar et al., 2016). However, the reaction temperature of more than 100 ^{o}C was not pursued in this research since degradation of starch was observed at higher reaction temperature (> 100 ^{o}C).

4.3.2 FTIR analysis




(b)
Room temperature
3258
v (O-H)
2942
v (C-H)
2887
v (C-H)
1638
v (O-H)
60 ºC
3277
v (O-H)
1741
v (C=O)
1368
v (O-H)
999
v (C-O-C)
1231
v (C-O)
995
v (C-O-C)
70 ºC
3296
v (O-H)
1739
v (C=O)
1368
v (O-H)
80 ºC
3270
v (O-H)
1741
v (C=O)
1019
v (C-O-C)
90 ºC
3266
v (O-H)
1740
v (C=O)
100 ºC
3284
v (O-H)
1740
v (C=O)
1369
v (O-H)
1235
v (C-O)
1016
v (C-O-C)
%T
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

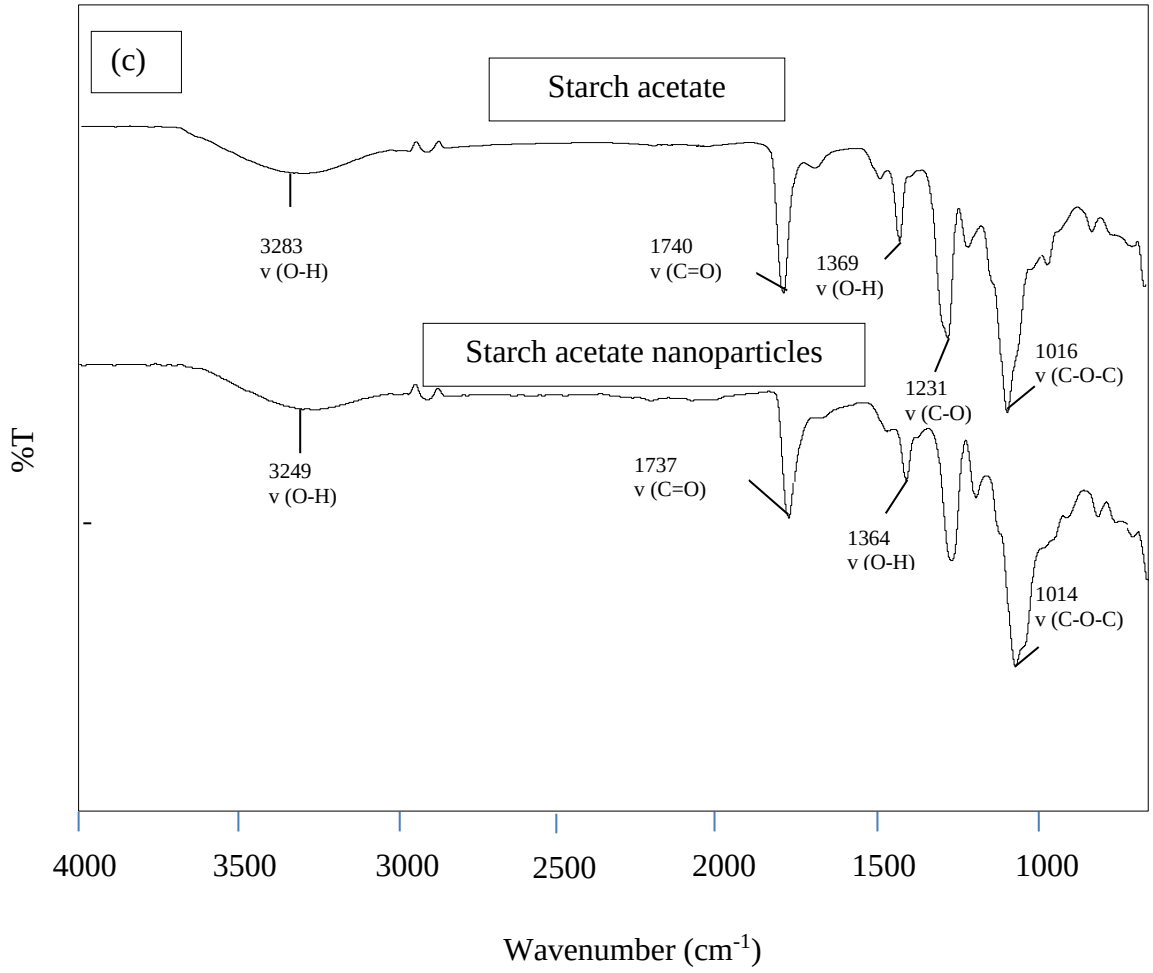


Figure 4.3: FTIR spectra of (a) starch acetate at various reaction durations (100°C reaction temperatures), (b) starch acetate at various reaction temperatures (20 minutes reaction time), and (c) starch acetate nanoparticles

FTIR spectra of starch acetate prepared at various reaction durations were shown in Figure 4.3 (a). As shown in Figure 4.3 (a), acetylation can occur within 10 to 30 minutes reaction. Acetylation of starch is confirmed by the appearance of new absorption peaks in the region 1738 t0 1727 cm^{-1} which corresponded to the ester group due to the stretching of the carbonyl bond (C=O). The other two major ester bands also appeared at the region 1388 cm^{-1} and 1217 cm^{-1} which are related to the stretching of the CH and C(=O)-O from acetyl group, respectively (Bartz et al., 2015; Gabardo, Ana, Travalini, & Colman, 2015;

Senanayake et al., 2014). All these peaks were absent in the FTIR spectra of native starch. The intensity of the carbonyl group was observed to gradually increase with reaction duration from 10 to 20 minutes and decreased as the reaction was continued to 30 minutes. The higher intensity of the absorption peak of the carbonyl group demonstrated the higher DS of acetyl group into the starch chain.

Meanwhile, Figure 4.3 (b) showed the FTIR spectra of starch acetate at various reaction temperatures. The characteristic peak of the carbonyl group increased with reaction temperatures from 60 to 100 ^{o}C. This concurs with the finding of the highest DS was achieved at 100 ^{o}C. The FTIR spectra of starch acetate nanoparticles as shown in Figure 4.3 (c) were similar to the spectra of starch acetate. This shows that the ester bonds were not broken during the formation of nanoparticles in nanoprecipitation process.

4.3.3 Morphological study

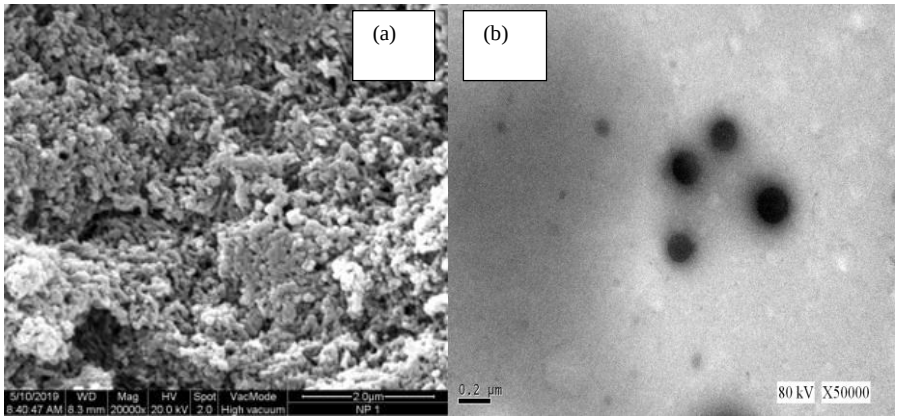


Figure 4.4: (a) FESEM images of starch acetate nanoparticles, and (b) TEM images of starch acetate nanoparticles

Figure 4.4 shows the FESEM and TEM images of starch acetate nanoparticles. As can be seen in Figure 4.4 (a), the starch acetate nanoparticles are mostly spherical in shape, with mean particle sizes of 140 ± 20 nm. The starch granules that were broken due to the

acetylation reaction had transformed into smaller nanoparticles with spherical shape during the nanoprecipitation process (Heydar et al., 2016).

4.3.4 Piperine loading capacity

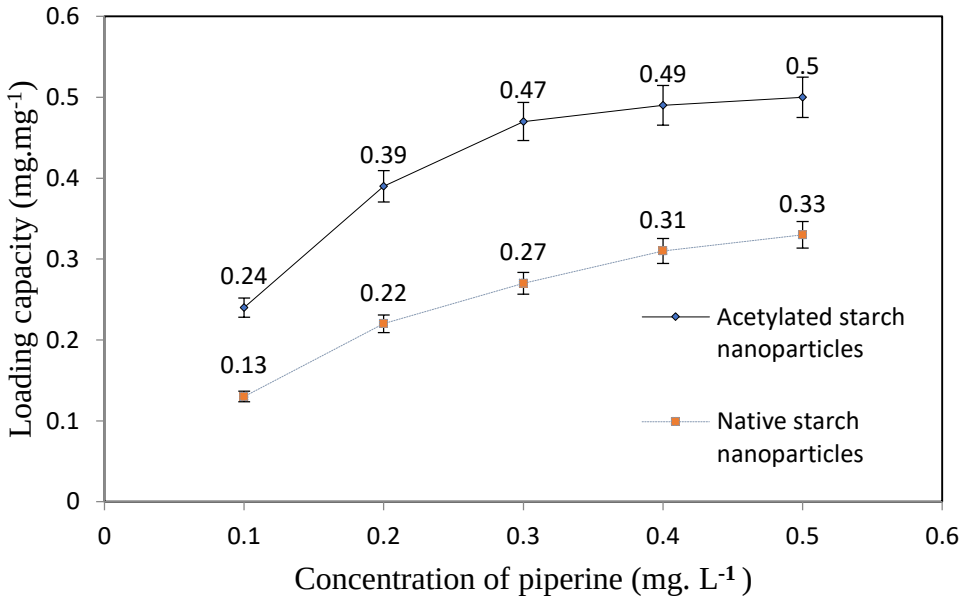


Figure 4.5: Loading capacity of piperine onto native starch nanoparticles and starch acetate nanoparticles, where n=3

The loading capacity of piperine onto the starch acetate nanoparticles was compared with the native starch nanoparticles at various piperine concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg.L^{-1}) (Figure 4.5). The loading capacity increased almost linearly from 0.13 to 0.33 mg.mg^{-1} when the concentration of piperine was increased from 0.1 to 0.5 mg.L^{-1} for native starch nanoparticles. Meanwhile, for starch acetate nanoparticles, the loading capacity increased significantly from 0.24 to 0.47 mg.mg^{-1} when the concentration of piperine increased from 0.1 to 0.3 mg.L^{-1}. Then, at piperine concentration 0.4 to 0.5 mg.L^{-1}, the loading capacity is almost constant, which are 0.49 and 0.5 mg.mg^{-1}, respectively. The adsorption sites of the starch acetate nanoparticles may have been saturated. The piperine loading capacity of the starch acetate nanoparticles was almost similar to that of

hydroxypropyl starch nanoparticles. The highest loading capacity achieved by starch acetate nanoparticles and hydroxypropyl starch nanoparticles was 0.5 and 0.46 $mg.mg^{-1}$, respectively.

4.3.5 Piperine release studies

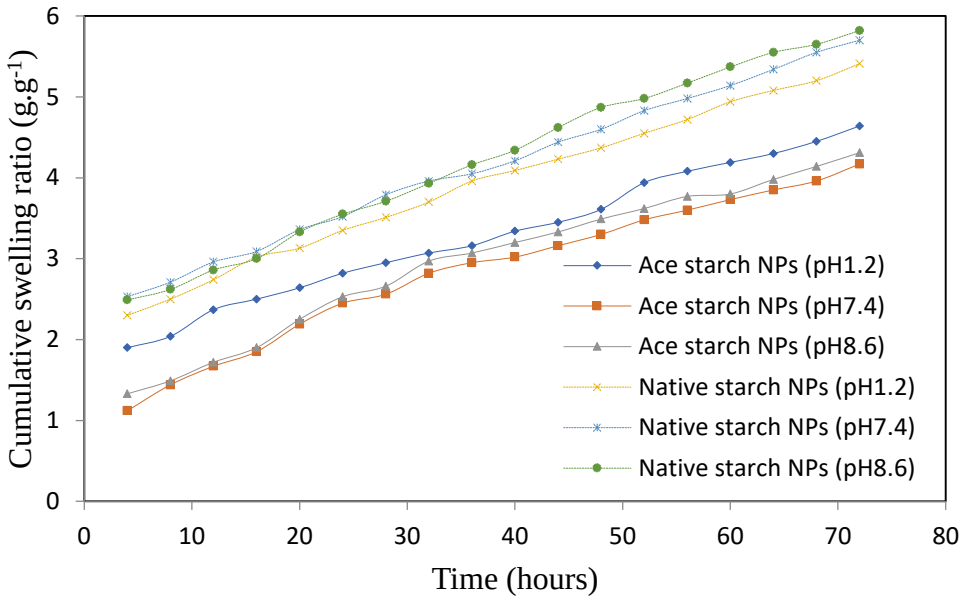


Figure 4.6: Swelling ratios of native starch nanoparticles and starch acetate nanoparticles in various pH of physiological media (1.2, 7.4 and 8.6) as a function of time

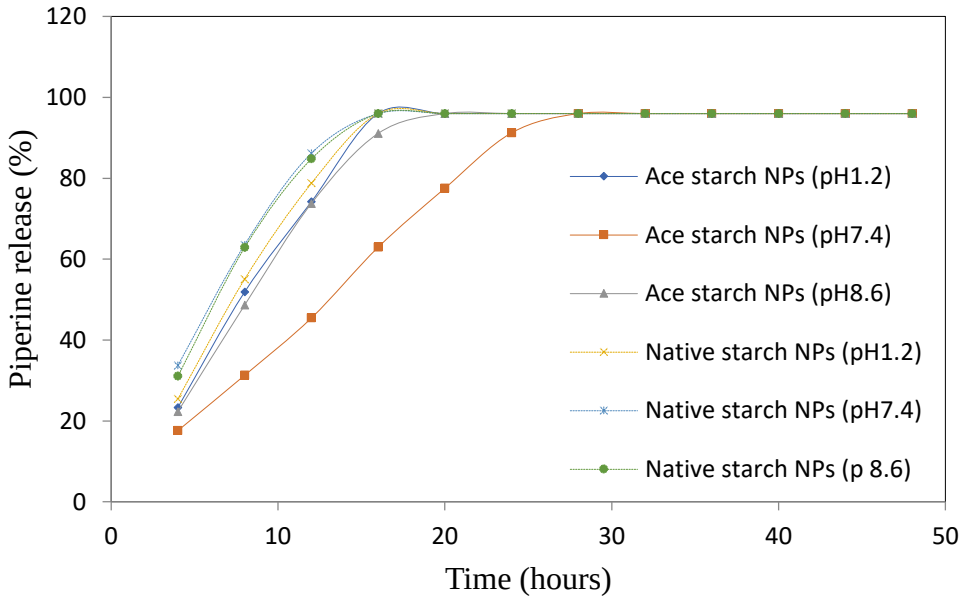


Figure 4.7: Percentage of piperine released from native starch nanoparticles and starch acetate nanoparticles in media of various pH

Figure 4.6 shows the swelling ratio of native starch nanoparticles and starch acetate nanoparticles while Figure 4.7 shows the piperine release profiles from native starch nanoparticles and starch acetate nanoparticles at pH 1.2, 7.4, and 8.6. Starch acetate nanoparticles exhibited lower swelling ratio at all pH tested compared to native starch nanoparticles. At pH 7.4, piperine was released from the starch acetate nanoparticles in a slow and sustained manner within 28 hours. This may be due to the smaller swelling ratio (2.85 $g.g^{-1}$) of starch acetate nanoparticles at a slightly basic medium. At pH 1.2 and 8.6, the swelling ratios of the starch acetate were 3.10 and 2.97 $g.g^{-1}$ respectively. Acetylation enhanced the properties of the native starch by reducing the swelling ratio, thus increased the retention time for piperine in starch acetate nanoparticles, and increased the residence time of the piperine in the system (Masina et al., 2017).

4.4 Summary

Native sago starch has been successfully modified by acetylation reaction to form starch acetate which was then converted into nanoparticles *via* the nanoprecipitation method. The starch acetate nanoparticles were evaluated as the controlled release nanocarriers for piperine. The results demonstrated that starch acetate nanoparticles have higher piperine loading capacity, lower swelling ratio, and slower piperine release rates as compared to native starch nanoparticles. The highest piperine loading capacity of starch acetate nanoparticles was 0.5 $mg.mg^{-1}$ and piperine was released from starch acetate nanoparticles within 30 hours in a slow and sustained manner. Therefore, this study suggested that starch acetate is a potential candidate for the controlled release of piperine.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Concluding remarks

Native sago starch has been successfully modified by chemical modification through both hydroxypropylation and acetylation reactions. For hydroxypropylation, the molar substitution (MS) of the hydroxypropyl starch has been modulated by various reaction parameters such as reaction temperatures, reaction durations, and the concentrations of propylene oxide based on three replicates (n=3). FTIR analysis of hydroxypropylated starch showed the present of a new characteristic absorption band at 2976 cm^{-1} which corresponded to the asymmetric $CH(CH_3)$ stretching. Subsequently, hydroxypropyl starch nanoparticles have been successfully synthesized *via* the *in-situ* nanoprecipitation process. Hydroxypropyl starch nanoparticles with a mean particle size of 110 ± 20 nm were synthesized.

Starch acetate was successfully prepared by acetylation reaction of native sago starch with acetic anhydride in the presence of an iodine catalyst. The degree of substitution (DS) of the starch acetate varied between 0.27 and 0.89. FTIR analysis confirmed the acetylation by the appearance of new characteristic peaks at the region of 1745 cm^{-1} , 1371 cm^{-1}, and 1240 cm^{-1} corresponded to the ester group due to the stretching of the carbonyl bond (C=O), stretching of the CH, and C(=O)-O from acetyl group, respectively. Starch acetate nanoparticles have been prepared by the nanoprecipitation method, forming nanoparticles with mean particle size of 140 ± 20 nm.

The potential application of hydroxypropyl starch and starch acetate nanoparticles as controlled release nanocarriers for piperine were evaluated at different physiological pH

(1.2, 7.4 and 8.6) by using UV-visible spectrophotometer and the results were compared with native starch nanoparticles. Hydroxypropyl starch nanoparticles exhibited higher piperine loading capacity as compared to the native starch nanoparticles with the maximum loading capacity of 0.46 ± 0.05 and 0.33 ± 0.05 $mg.mg^{-1}$, respectively. Meanwhile, the highest loading capacity achieved by starch acetate nanoparticles was just slightly higher than hydroxypropyl starch nanoparticles which were 0.50 ± 0.05 $mg.mg^{-1}$. Piperine was released from hydroxypropyl starch nanoparticles in a slow and sustained manner at pH 1.2 over the period of 24 hours, while piperine was released from starch acetate nanoparticles within 28 hours at pH 7.4. This may be due to the stronger interaction of hydrogen bonds between ester group (COOH) in starch acetate that lead to smaller swelling ratio at pH 7.4 (2.85 $g.g^{-1}$), compared to the swelling ratio of hydroxypropyl starch at pH 1.2 (2.9 $g.g^{-1}$). Whereas piperine was released from native starch nanoparticles within 16 hours at all pH tested. Thus, hydroxypropyl starch nanoparticles and starch acetate nanoparticles can be considered as a potential candidate for controlled release of piperine.

5.2 Recommendations

The present research demonstrated the potential application of hydroxypropyl starch nanoparticles and acetylated starch nanoparticles as the promising controlled release nanocarriers for piperine. However, further study to improve the properties and performance of the modified starch nanoparticles would be necessary. This is important in order to develop a better nanocarrier that is safe for human consumption, effective, and cost-effective before it can be commercialized. The ability of hydroxypropyl and acetylated starch nanoparticles to improve bioavailability of piperine should also be further explored. Many other parameters could be studied that may enhance the performance of the starch

nanoparticles like particle size. Besides that, more research should be carried out on other types of starch modifications for controlled release for piperine, to increase its availability.

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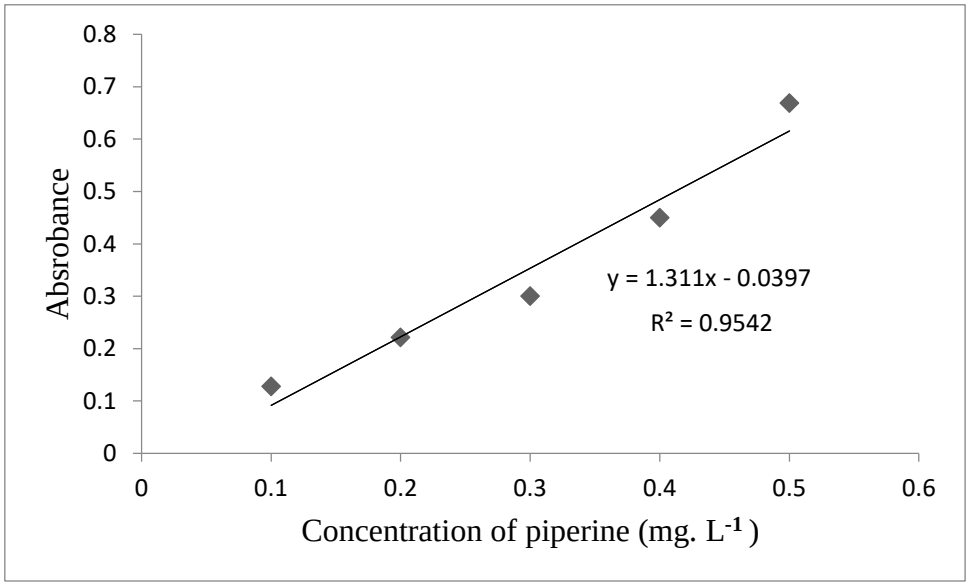
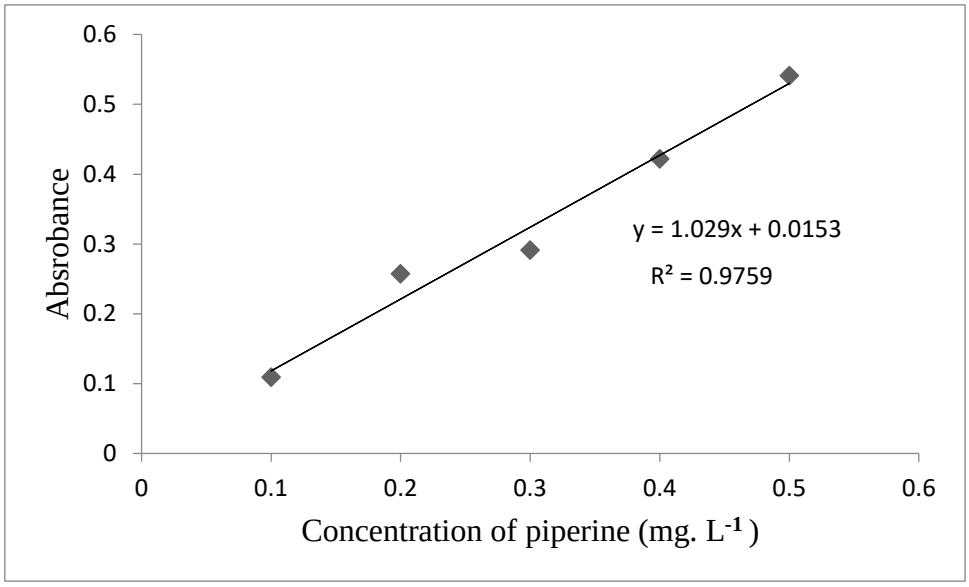
APPENDICES

Appendix A

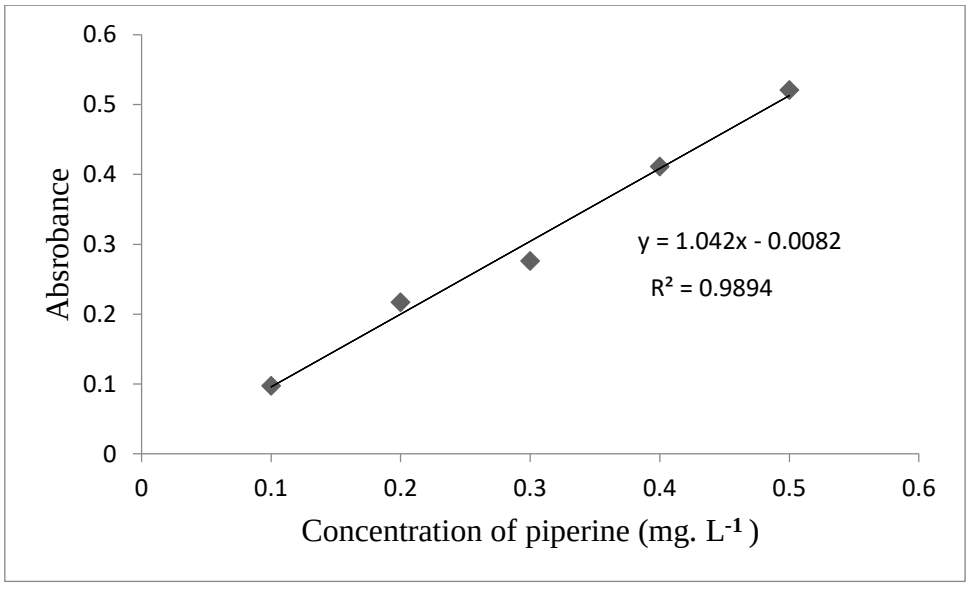
Calibration of piperine



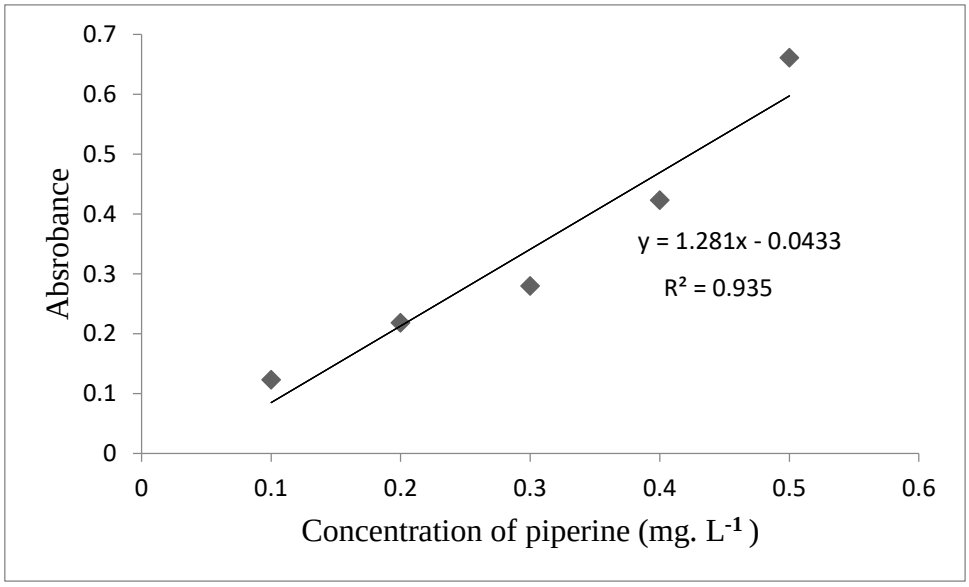
Appendix A1: Calibration curve of piperine in absolute ethanol. The linear regression equation of calibration curve of piperine in absolute ethanol is y = 1.311x with $R^2 = 0.9542$



Appendix A2: Calibration curve of piperine in pH1.2 phosphate buffer solution (PBS). The linear regression equation of calibration curve of piperine in absolute ethanol is y = 1.029x with R^2 = 0.9759



Appendix A3: Calibration curve of piperine in pH7.4 phosphate buffer solution (PBS). The linear regression equation of calibration curve of piperine in absolute ethanol is y = 1.042x with R^2 = 0.9894



Appendix A4: Calibration curve of piperine in pH8.6 phosphate buffer solution (PBS). The linear regression equation of calibration curve of piperine in absolute ethanol is y = 1.281x with R^2 = 0.9350