



Faculty of Resource Science and Technology

**SYNTHESIS AND CHARACTERIZATION OF HYDROPHOBIC
STARCH NANOPARTICLES**

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**SYNTHESIS AND CHARACTERIZATION OF HYDROPHOBIC STARCH
NANOPARTICLES**

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This project is submitted in partial fulfilment of
the requirement for the degree of Bachelor of Science with Honours
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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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(Caryn Tan Sze Yee)

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LIST OF ABBREVIATIONS

Chemicals

DMSO: Dimethyl sulfoxide

DMAP: 2-(dimethyl-amino) pyridine

DMF: N, N-Dimethylformamide

DS: Degree of Substitution

SP: Swelling power

AGU: Anhydroglucose Unit

NaOH: Sodium hydroxide

HCl: Hydrochloric acid

KBr: Potassium Bromide

Rpm: Rate per minute

NU: NaOH/Urea

Symbols

⁰C: degree Celsius

wt %: weight percentage

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Synthesis and Characterization of Hydrophobic Starch Nanoparticles

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ABSTRACT

Sago starch is derived from one of the oldest tropical crops; sago palm (*Metroxylon sagu*) and have been widely exploited by men. As they are easily found at low cost with biodegradability properties, sago starch has been of great emphasized to act as a drug carrier. In this study, sago starch was subjected to chemical modification using esterification process to exhibit hydrophobicity using different parameters such as concentration, temperature and duration of reaction. Sago starch dissolved in 50mL of DMF with a ratio of 1:1.5 to oleic acid at the temperature of 100 °C with a reaction time of 2 hours in the presence of DMAP catalyst yield the highest degree of substitution at 0.12. The existence of a carbonyl peak at 1724 cm^{-1} in the FTIR spectrum confirmed the successful modification by means of introducing the fatty acid unto the native sago starch. The obtained starch oleate was dissolved in 0.8 wt% NU solution and added drop wise into an absolute ethanol solution to yield hydrophobic starch nanoparticles. The morphology of the hydrophobic starch nanoparticles were studied using SEM.

Key words: Native sago starch; Esterification; Starch-oleate

ABSTRAK

*Kanji sagu diperolehi daripada salah satu tumbuhan tropical yang tertua; pokok sagu (*Metroxylon sagu*) dan telah lama dieksploitasi oleh manusia. Oleh sebab kanji sagu senang didapati pada kos yang rendah dan adalah biodegradasi, mereka berpotensi untuk dijadikan sebagai pengangkut dadah. Dalam kajian ini, kanji sagu telah diubahsuai menggunakan tindakbalas kimia menggunakan proses pengesteran untuk memberi ciri-ciri hidrofobik dengan parameter yang berlainan seperti tahap kepekatan, suhu, dan tempoh masa tindak. Kanji sagu dilarutkan dalam 50 mL pelarut organik DMF dengan nisbah 1:1.5 oleh kanji sagu pada asid oleik, pada suhu 100 °C dengan tempoh masa tindak balas selama 2 jam bersama dengan pemangkin DMAP memberi darjah penggantian yang paling tinggi pada 0.12. Kemunculan keamatan serapan kumpulan karbonil dalam spektra FTIR pada 1724 cm^{-1} mengesahkan bahawa proses pengesteran telah berjaya dilakukan dengan pengenalan asid lemak ke dalam kanji sagu. Kanji-oleik yang berjaya dihasilkan dilarutkan dalam larutan 0.8 wt% NU dan ditambahkan setitik demi setitik ke dalam larutan ethanol untuk menyediakan kanji nanopartikel. Morfologi ester sagu dinilai menggunakan SEM.*

Kata kunci: Kanji sagu asli; Pengesteran; Kanji-oleik

1.0 Introduction

In the recent advancement of medical line, nanoparticles have been of interest for drug transport as their rather significant small size enables the penetration of cellular barriers that have been limiting other drug carriers. Starch, a polysaccharide found abundantly at low cost is being studied as the starting materials to fabricate nanoparticles due to cost efficiency and easy availability. However, a major setback is that starch is predominantly hydrophilic in nature while most drugs appear to be hydrophobic. Hence, modifications have to be done onto the native starch to obtain the desired characteristics.

There are many studies carried out on starches that have been chemically modified by many ways of chemical reaction onto the hydroxyl groups in the starch molecules such as substituted starches prepared by acylation of starch with fatty acid chlorides in organic solvents such as pyridine or dimethylacetamide (Kapusniak & Siemion, 2007; Wang *et al.*, 2008). Starch esters are modified starches synthesized with a variety of reactant such as acid anhydrides octenyl succinic anhydride (OSA), fatty acid chlorides and dodecenyl succinic anhydrides (DDSA) (Borredon *et al.*, 1999; Fowler *et al.*, 2002; Tukomane & Varavinit, 2008; Wang *et al.*, 2007).

In a related study, Veen *et al.* (2005) studied on the release mechanism of hydrophilic anhydrous caffeine, a chosen freely soluble model drug from hydrophobic matrix composed of modified potato starch - starch acetate. Abraham and Simi (2007) discussed on the synthesis of modified hydrophobic starch using fatty acids by incorporating potassium persulphate as a catalyst in DMSO solution. Horchani *et al.* (2009) synthesized a high degree of substitution of starch oleate via enzymatic esterification process using an

immobilized *Staphylococcus aureus* lipase. Another study also has shown the success in synthesizing starch maleate monoester gels (Tay *et al.*, 2012). Thus, this work aimed to prepare a hydrophobic starch ester through esterification reaction between native sago starch and oleic acid meanwhile, exploring the different synthesis parameters as well as to fabricate and characterize the hydrophobic starch nanoparticles. The method employed in the preparation of hydrophobic starch-oleic monoester gels was adapted from Chin *et al.* (2014) with modifications. The esterification reaction could occur through the substitution of carboxylic group of the oleic acid onto the free hydroxyl groups of the native sago starch molecules. Starch oleate monoesters gel were synthesized using the precipitation method by cross-linking native sago starch and oleic acid in N,N-Dimethylformamide (DMF) medium followed by precipitation in absolute ethanol.

Once the sample contains ester linkage, it will be then be used to synthesize hydrophobic starch nanoparticles. There are a few methods that could be used to produce starch nanoparticles such as emulsification (Jain *et al.*, 2008; Shi *et al.*, 2011), acid hydrolysis (Angellier *et al.*, 2004), and dialysis (Liu *et al.*, 2007; Namazi *et al.*, 2011). However, these methods possess several disadvantages such as requiring a long reaction time to produce nanoparticles, broad particle size distribution, leaving a large volume of residues and the need of a large amount of emulsifiers (Feng *et al.*, 2014). Hence, the fabrication of starch nanoparticles will be done using the simple nanoprecipitation method adapted from Chin *et al.* (2011) with modifications.

2.0 Literature Review

2.1 Starch

Starch, a biopolymer made of amylose and amylopectin are biocompatible, biodegradable, non-toxic polymer, low cost, abundantly found with bio-adhesion properties and has a huge importance in industrial application (Pang *et al.*, 2011). Natural starch are composed of two components, mainly amylose - a planar polysaccharides linked by 1,4-glycosidic bonds and amylopectin - a highly branched polymer linked by 1,6-glycosidic chains. With a decrease in the reserve of fossil fuels and an increase in concern of the environment, bio-based and biodegradable products made from starch has been gaining lots of attention as an alternative for a lower cost and safer choice (Angles & Dufresne, 2001). Mansou *et al.* (2010) stated that in biological application, starch polymers has a promising potential as a controlled release systems due to their similarity with the extracellular matrix in the human body that could be exploited for any systems such as the stimuli-responsiveness. The flexibility of starch polymers could be enhanced through the addition of plasticizers such as glycerol or water (Malafaya *et al.*, 2007; Bonacucina *et al.*, 2006). Besides, with the presence of cross-linking, it leads to advance in drug delivery systems by counterbalance for weak points of plasticized starch which has rapid response to moisture and shows low tensile strength.

However, the use of starch has been challenged by certain limitations namely incompatibility with hydrophobic polymers. Excellent hydrophilic property in unmodified starch has caused its limitations in various applications especially in drug delivery as many drugs are hydrophobic in nature though there are exception such as ophthalmologic drugs namely timolol and flurbiprofen (Coucke *et al.*, 2009). In other studies, researchers has

successfully synthesis starch-maleate monoesters from native sago starch and these monoesters has good potential in drug transport as they could entrapped most hydrophobic drugs. Hence, one of the alternatives to compensate this hydrophilic setback is to graft hydrophobic side chains such as oleic acid, unto the back-bone of these hydrophilic starches.

Oleic acid is an omega-9, monounsaturated fatty acid found naturally in products made from plant and animal resource. It is deemed one of the healthiest source of fat in the diet especially olive oil as its major component is the triglyceride esters of oleic acid. Other than that, there are a number of meat and plants products rich in oleic acid namely peanut, butter, sesame oil, avocados, eggs, chicken and more.

Other positive effects of oleic acid on the body are allowing free flow of blood without blocking the arteries, thus reducing the levels of low density lipoprotein (LDL) while increasing the levels of high density lipoprotein (HDL), rich in antioxidants, boost memory power, relieves asthma symptoms, obstructs progression of Adrenoleukodystrophy (ADL) which is a fatal disease affecting the adrenal glands and brain and many more.

2.2. Modification of starch

Generally, physical modification and chemical modification could be performed unto native starch to obtain the desired characteristics. Usual physical modifications include particle size reduction, pre-gelatinization and moisture adjustments (Light, 1989).

Starches are native biopolymers that are composed of various ratios of two glucose polymers which are the linear amylose and branched amylopectin. In every glucose unit of amylose and amylopectin molecules, there are three free hydroxyl groups (OH) that can be

substituted upon chemical modification, thus the average degree of substitution (DS) ranges from 0 up to 3.0. All three free hydroxyl groups of each anhydroglucose unit have different reactivity whereby the primary OH on C₆ is more reactive than the secondary OH on C₂ and C₃ due to steric hindrance (Kumar *et al.*, 2014). These available hydroxyl groups are capable in forming hydrogen bonds which undergo further chemical reactions such as oxidation and reductions, substitutions, and esterification to be substituted by other functional groups such as -CHO, -COOH that leads to the alteration of the chemical reactivity and the rheological properties of starch (Tomasik & Schilling, 2004). Hence, chemical modification aims to induce certain changes on these hydroxyl groups to obtain desired characteristics to expand its applications especially in drug delivery. Methods involving chemical modifications include acid hydrolysis, cross linking, oxidation and substitution reactions and are usually conducted in aqueous slurry or in extrusion conditions (semi-dry conditions) (Gotlieb & Capelle, 2005).

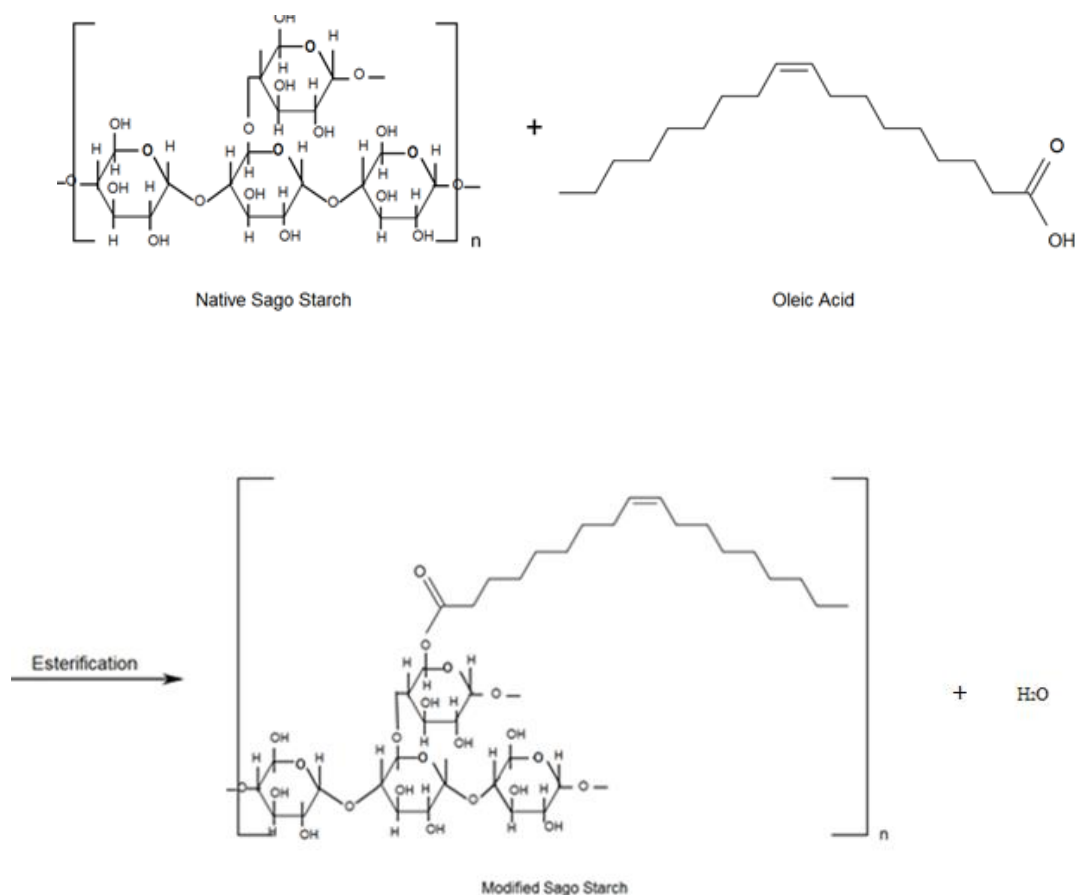


Figure 1: Scheme for reaction of native sago starch with oleic acid.

The introduction of an ester group into a polysaccharide establishes a crucial achievement as it resulted in modifying the hydrophilicity of the native starch and acquires new thermal and mechanical properties (Aburto *et al.*, 2000). The synthesis of fatty acid starch esters could be achieved through a variety of reacting materials. According to Yang and Montgomery (2008), reacting native starch with carboxylic acids with carbon numbers in between C4-C16, using fatty acid vinyl esters (Junistia *et al.*, 2008), or fatty acid chlorides (Aburto *et al.*, 1999) as reactants could synthesis starch esters. Sodium dihydrogen phosphate, potassium carbonate, sodium acetate and potassium methoxide could be used as catalysts while the reaction medium could be made of organic solvents such as DMSO and pyridine.

2.3 Hydrophobic starch nanoparticles

Nanoparticles are discrete particles in sizes ranging in between 10-1000 nm. Due to their extremely small size and large surface area, they are capable in penetrating most barriers and membranes. Nanoparticles synthesised from starch have prominently been noticed due to its unique properties, low cost, abundance, and biodegradability. Hence, it has been widely used in various applications including insecticides (Frederiksen *et al.*, 2002), tissue engineering scaffolds (Gomes *et al.*, 2002), as well as for drug delivery carriers (Mahkam, 2010).

Modified starch have been of much interest lately as chemical reactions with the available hydroxyl groups on amylose and amylopectin could be used to produce water-resistant materials and are capable in forming self-assembled nanoparticles for biomedical uses. On top of that, hydrophobic starch nanoparticles are one of the up-coming drug carriers as they possess prominent characteristics namely controlled solubility and biocompatible in vivo. Other application that hydrophobic starch nanoparticles have been noticed for due to their enhanced properties is that they could be used as emulsifiers or thickeners (Namazi *et al.*, 2011). Hermawan *et al.* (2015) used starch as the raw materials to be used as thermoplastics material by esterifying native starch with several fatty acids esters such as fatty acid methyl esters (FAME). Chemical modifications were performed to improve their hydrophobicity, thermal properties and mechanical strength. Kshirsagar and Singhal (2007) has reported the modification of native corn starch with oleic acid to form hydrophobic starch could be used as an alternative for gum Arabic for the wall material in microencapsulation for sensitive food constituents such as oils.

3.0 Methodology

3.1 Materials and Methods

Native sago starch (*Metroxylon sagu*) powder was purchased from local sundry shop at Kuching (Sarawak, Malaysia). 2-(dimethyl-amino) pyridine (DMAP), Oleic acid, sodium hydroxide, hydrochloric acid, N,N-Dimethylformamide (DMF) was purchased from J.T. Baker (Phillipsburg, USA), absolute ethanol, Ultrapure water (18.2M Ω ·cm) obtained from Water Purifying System (Model: ELGA, Ultra Genetic) was used throughout the experiments. Native sago starch powder and all chemicals were used without further purification.

3.2 Preparation of hydrophobic starch-oleic monoester gels

In the preparation of hydrophobic starch-oleic monoester gels, the approach is adapted from Chin *et al.* (2014) with modifications. Starch-oleate monoesters gel was synthesized using the precipitation method by cross-linking native sago starch and oleic acid in N,N-Dimethylformamide (DMF) medium followed by precipitation in absolute ethanol.

Table 1: Parameters involved in the synthesis of modified starch

Ratio of Starch to Oleic Acid (wt%)	Temperature (°C)	Reaction Time (Minutes)
1:1.5	30	60
1:1.5	30	120
1:1.5	30	180
1:1.5	50	60
1:1.5	50	120
1:1.5	50	180
1:1.5	100	60
1:1.5	100	120
1:1.5	100	180
1:3	30	60
1:3	30	120
1:3	30	180
1:3	50	60
1:3	50	120
1:3	50	180
1:3	100	60
1:3	100	120
1:3	100	180
1:5	30	60
1:5	30	120
1:5	30	180
1:5	50	60
1:5	50	120
1:5	50	180
1:5	100	60
1:5	100	120
1:5	100	180

3.2.1 Effect of ratio of starch to oleic acid

Starch-oleic monoester gels prepared by esterification of starch with oleic acid in DMF medium. 50 mL of N,N-dimethylformamide (DMF) were used to dissolve about 0.01 mole native sago starch powder. The solution was then stirred for 20 minutes to allow the powder to dissolve. Then, a known amount of oleic acid (at a ratio of 1:1.5, 1:3, and 1:5) and 30 μ L of 2-(dimethyl-amino) pyridine (DMAP) acting as catalyst were added into the mixture. Using magnetic stirrer, the mixture was left to stir at a constant stirring rate of 900 rpm at 30 °C, 50 °C and 100 °C for 1 hour, 2 hours and 3 hours followed by cooling of the mixture at room temperature. The mixture underwent drop wise precipitation and the precipitate was washed adequately with absolute ethanol.

Table 2: Ratio of starch to oleic acid

Starch (g)	Oleic Acid (mL)
1 (1.62)	1.5 (4.83)
1 (1.62)	3 (9.46)
1 (1.62)	5 (15.76)

Another set of experiment at a higher native starch powder to oleic acid ratio were conducted to study the differences between it and those of lower native starch powder to oleic acid ratio. A ratio of 1.5:1 of native starch powder to oleic acid was dissolved in 50 mL of DMF and was allowed to stir for 20 minutes. 30 μ L of 2-(dimethyl-amino) pyridine (DMAP) as the catalyst and the predetermined amount of oleic acid were then added and left to stir at a constant rate of 900 rpm at 30 °C for 1, 2, and 3 hours. It was then added drop wise into absolute ethanol, centrifuged, washed and dried.

3.2.2 Effect of catalyst

Another set of experiment was conducted with the absence of catalyst to study the importance of it in the fabrication of the modified sago starch. A ratio of starch to oleic acid at 1:1.5 was dissolved in 50 mL of DMF, and then stirred to allow the dissolution of the native starch power. It was then left to stir continuously at 900 rpm for an hour at 30 °C without the presence of 30 μ L of 2-(dimethyl-amino) pyridine (DMAP) catalyst at a constant stirring rate of 900 rpm. It was then added drop wise into absolute ethanol, centrifuged, washed and dried.

A different set of experiment was conducted using 1.0 g of native starch powder was dissolved in 30 mL N,N-dimethylformamide (DMF). The mixture was stirred for 20 minutes. 3 mL of oleic acid and 40 μ L of DMAP was added and refluxed at 100 °C. The mixture was stirred continuously at 900 rpm for 3 hours. It was then added drop wise into absolute ethanol, centrifuged, washed and dried.

3.3 Synthesis of hydrophobic starch nanoparticles

In this work, starch nanoparticles were synthesized using the simple one-step nanoprecipitation method by optimizing both the precipitating condition and the rate of precipitation in order to achieve controlled size particles. In this synthesis, the method was adapted from Chin *et al.* (2011) with modification. The modified sago starch were dissolve in a solvent mixture system composed of NaOH/urea (NU) (0.8:1 wt%). 1 wt% of modified sago starch solution was prepared by dissolving the modified sago starch precipitate in the NU solvent at ambient conditions. Then, while stirring it continuously with a magnetic stirrer at a constant stirring rate of 600 rpm, an aliquot of modified sago starch solution (1 mL) was added drop wise into a fixed quantity of absolute ethanol of 20 mL. This was to obtain a stable suspension of starch nanoparticles. The mixture obtained was then centrifuged. The regenerated starch nanoparticles was obtained by removing the supernatant. The regenerated modified starch nanoparticles was rinsed adequately with absolute ethanol to remove any remaining NaOH and urea. Figure 2 depicts the overall methodology involved.

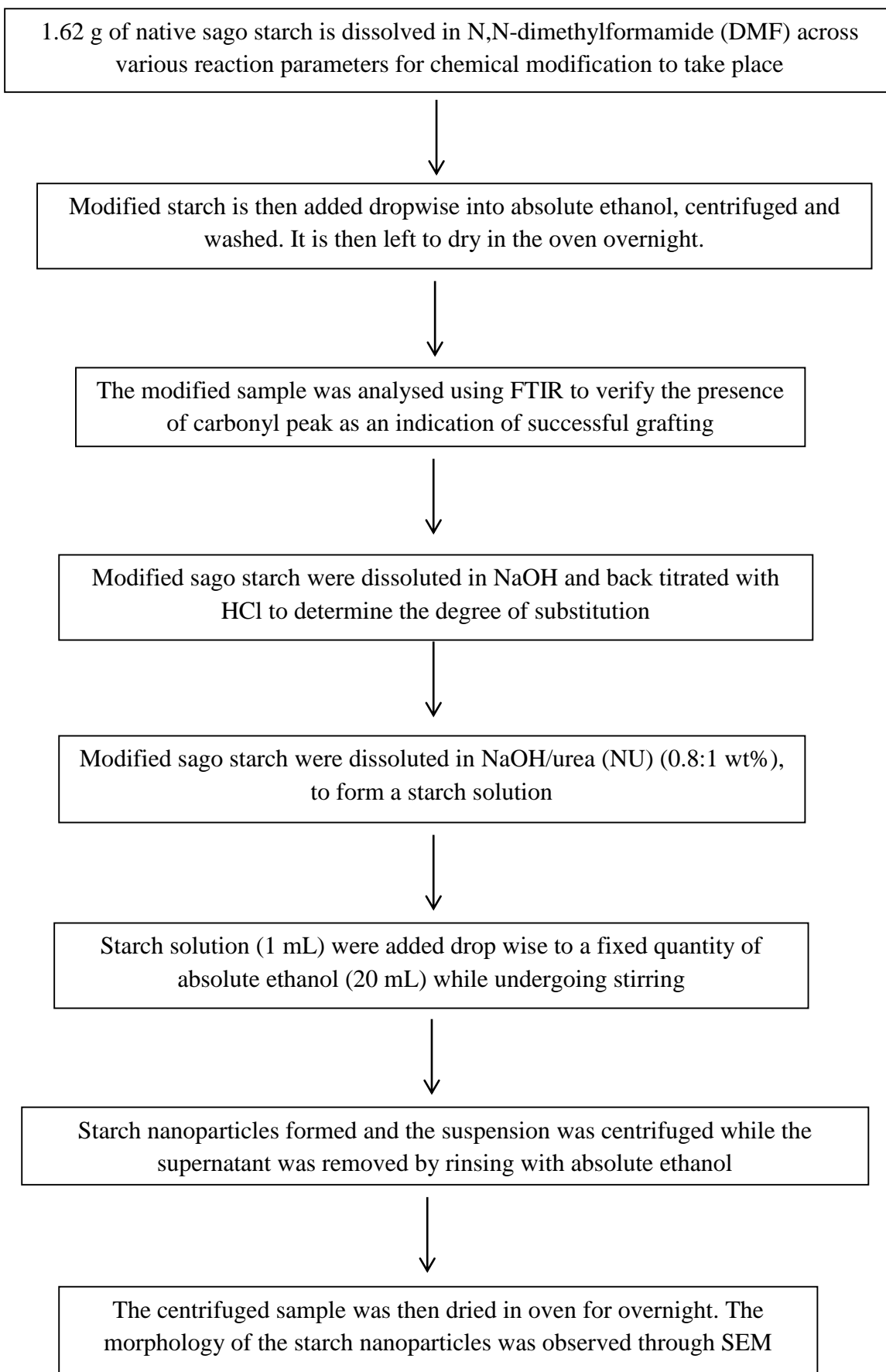


Figure 2: Flow chart of methodology

3.4 Characterization of hydrophobic starch monoester gel and nanoparticles

FT-IR Analysis

As a confirmatory test that modified hydrophobic starch-oleic monoester has been formed, fourier transformed infrared (FT-IR) analysis was carried out. Spectra of native sago starch and starch oleate were obtained from KBr/sample pellets, using a Perkin Elmer System Spectrophotometer while the spectrum of pure oleic acid was obtained using Thermo Scientific Nicolet iS10 FT-IR Spectrometer, both within the wavenumber range of 4000–400 cm^{-1} .

Degree of Substitution

Anhydroglucose unit (AGU) is the fundamental chemical building blocks of polysaccharides such as cellulose and starch while the degree of substitution is an indication of the average number of hydroxyl groups on each AGU. Theoretically, each AGU is approximately of 162.15 g/mole and starch holds the greatest acquirable value at 3.0.

According to Hermawan *et al.* (2015), degree of substitution (DS) increases with longer fatty acid chains and sago starch is far more reactive than potato starch. To determine the DS value, methods were obtained from Ogawa *et al.* (1999) with modifications. Back titration was used and the DS was calculated according to equations (1) and (2).

20 mL of 0.05 M NaOH was used to dissolve a known amount of starch-oleate with stirring at ambient temperature for 30 min. Using, phenolphthalein as an indicator, a