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SCHOOL OF CHEMICAL AND BIO ENGINEERIG

Production, Characterization and Optimization of Pharmaceutical grade Modified Potato starch for Tablet formation

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A Thesis Submitted to The School of Chemicals and Bio Engineering, Addis Ababa Institute of Technology in Partial Fulfillment of the Requirements for The Degree of Master of Science in Chemical Engineering (Process Engineering Stream)**.**

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School of Chemical and Bio Engineering

This is to certify that the thesis prepared by Mikias Delelegn, entitled: **"Production, Characterization and Optimization of Pharmaceutical grade Modified Potato starch for Tablet formation**" and submitted in partial fulfillment of the requirements for the degree of Masters of Science in Chemical Engineering (Process Engineering) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

Advisor	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date
Chair person	Signature	Date

DECLARATION

I certify that this research work titled: "**Production, Characterization and Optimization of pharmaceutical grade modified potato starch for tablet formation***"* has not been submitted in any form for another Degree, Diploma or an award at any University or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. The work was under the guidance of Dr. Hundsea Dessalegn instructor in Addis Ababa university, School of Chemical and Bio Engineering. Where material has been used from other sources it has been properly acknowledged / referred.

ABSTRACT

Pharmaceutical technologies restrict in all ingredient of the drugs but native starches are structurally weak and functionally so its needs to add values for application. The Objective is synthesis, characterize, and optimize pharmaceutical-grade potato starch from local potato and identify the physicochemical property of native, cross-linked and acetylated for formation of tablet by direct compression. Modified starch production was carried out by cross-linked and acetylated methods using modified agents of Sodium-hexa-meta phosphate and acetic-anhydride respectively. Full factorial methodology was applied to investigate and find optimum modified starch synthesis parameters. Reaction temperature (45-75) ℃, modified agent concentration (100 -300) % and Time (30-90 min) have been selected as the major study parameters. Peak viscosity and degree of cross-linked for Cross-linked potato starch and acetyl content and degree of acetylation for Acetyl potato starch have been examined as experimental response. A total of 27experiments were conducted, besides the proximate and compositional analysis of the raw-material. The important synthesis parameters, which affect the responses, were investigated during the analysis of variance (ANOVA). The regression analysis showed good fit of the experimental data to the second-order polynomial Quadratic model. A temperature of 45 ℃, modified agent concentration of 300 and reaction time of 30 min was found to be the optimum conditions. FTIR result confirm the presence of conjugated phosphate and acetyl groups at a band intensity of (1243 and 1748) cm^{-1} which implies that the starch was modified. Finally, the tablet containing APS need more lubricant for simplification of compression process and less compression force due to less density than Cross-linked potato starch.

Key words: Acetylation, acetyl starch, Cross-linked potato starch, Characterization and optimization.

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ACRONYMS

AAIT	Addis Ababa Institute of Technology
AAU	Addis Ababa University
ANOVA	Analysis of variance
AOAC	Association of official Analytical Chemistry
APS	Acetyl potato starch
AS	Acetyl starch
CLPS	Cross-linked potato starch
CLS	Cross-linked starch
CMS	Carbo-xymethyl starch
CSA	Central statistical Agency
DCLPS	Degree of Cross-linked potato starch
DAPS	Degree of Acetyl potato starch
DMS	Dual Modification starch
FDA	Food and Drug Administration
FTIR	Fourier transforms infrared spectra
HCl	Hydrochloric Acid
IPEC	International Pharmaceutical Excipients Council
NaOH	Sodium hydroxide
SHMP	Sodium-hexa-meta phosphate
Y	Yield of starch

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Starch is a source of carbohydrate available in many plants bowls of cereal, roots, tubers, seeds, and fruits that have semi-crystalline biopolymer properties. The origin of starch cause varies in shape, size, structure, and chemical composition that can affect the starch granules. Of starch. [1]. There are many sources of Starches in the world. They are isolated from different parts of the plant such as cereals (corn and wheat), tubers (potato), and roots (cassava, taro, sweet potato, arrowroot) [2]. Nowadays the number of researches increase to cope up with accelerating global needs so that the sources also increase such as ginger, cocoyam, sorghum, plantain, rye, barley, yam, enset, and colocasia have also been researched [3].

The Starch molecular structure, physical, chemical, biochemical properties, functionality, and uses are studied on different intensive research over many decades occupying a vast body of published literature reporting its preparative and analytical methods, [4]. The application area of starch is diversified as result the production techniques become diverse in native starches (NSs) and numerous starch modifications [5]. Most native starches are limited in their direct application because they are unstable concerning changes in temperature, pH, and shear forces. Native starches show a strong tendency for decomposition and retro-gradation [6]. The various starch modification techniques have been categorized into; physical, chemical, enzymatic, and genetic modification to improve the physical-chemical properties of the starch molecules [7].

The global market for industrial starches is growing, and the current demand is being met by a few number of crops, the most important of which are potato, maize, wheat, and tapioca. Starch commands more diverse markets due to the availability of newer processing processes and the present desire for biodegradable and renewable resources. [8]. Approximately, 60 million tons of starch is extracted annually worldwide from various cereal, tuber, and root crops, of which roughly 60% is used in foods and 40% in pharmaceuticals and for non-edible purposes such as textile, pulp, and breweries [4].

Freshly prepared starch paste has long been used as a binder in the production of tablet and capsule granules. The high demand for starch places great strain on the few known sources. In today's world, functional properties are rarely seen in naive starch. Modification of native starch alters the structural and morphological aspects of the starch, affecting compressibility qualities such as tableting, shape, crystalline character, swelling power, and moisture content. As a result, different starches may be able to aid improve tablet properties.[9].

Excipients are becoming more widely recognized as critical components of both traditional and innovative drug products, serving specialized tasks in the formulation of ideally stable, elegant, safe, and active therapeutic products [10]. Due to their suitable physicochemical properties, as well as their relative low cost and inertness, starches in both their native and modified forms are widely used as versatile excipients in various solid dosage forms, particularly as diluents, dis-integrant, and binding agents in tablet formulations [11].[12].

In this study, native potato starch was modified by cross-linked and acetylation methods by modified agent's sodium-hexa-meta phosphate and acetic anhydride respectively. Modified starch with the optimum conditions that consider the temperature, residence time, and concentration of modified agent was investigated and optimized using full factorial. Therefore, in this work, the preparation and characterization were systematically examined by analysis of surface morphology studies (SEM) and chemical functional groups (FTIR) of modified starch were described.

1.2. Statement of the Problem

Ethiopia ranks the second-largest country in sub-Saharan Africa in terms of population, the population has been rapidly growing and the projection shows that it steadily increase from 83.7 million in 2012 to 133.5 million in 2032 and will reach 171.8 million in 2050, by which it will be double in 83 years. The rate of population growth increased to 3.1 percent per year[13]. According to Alemayehu, 2014 in the next 10 years, the population number increase dramatically. In the report of WHO Ethiopia is 185 in measuring overall health system performance from 191 countries so one of the uncomfortable countries to live in because of the shortage of basic necessary ingredients to the people's life [14]. The health sector is one of the major sectors for any society which contains health centers, skilled employers, types of equipment, and drugs.

The drug is an important part of the medical field. The pharmaceutical drug contains active and excipients site. The active site is the ingredients, which directly treat the disease, and excipients are an ingredient to increase the effectiveness of the drug such as starch, protein, vitamin, fat, and another ingredient [10].

Starch is a carbohydrate polymer derived from the organs, such as roots, tubers, seeds, and fruits of various botanical sources. Its uses are based mainly on its adhesive`, thickening, gelling, swelling, and film-forming properties which the essential for the pharmacy industry. Starch extracted from various sources such as potato, yam, corn, beans, peas, unripe pawpaw, and banana. In Ethiopia number of pharmacy industry increase but there is no large-scale production of pharmaceutical-grade starch and modified starch-processing industry so all raw material imported from a different country such as India, and China. In This industry the excipient part of the drug a large portion and cost. One of the major excipients of drugs is starch so export starch cause the country to lose a large amount of foreign currency, decreasing the quality and productivity of the pharmaceutical industries, decrease the accessibility and the drug reach to the customer at compatible cost [15].

Dagim, Bubu, Belete, Gudene, Challa, Menagesha, Alemaya, and other potato tuber varieties have been discovered in Ethiopia. Belete was the main potato cultivar with the highest starch content. Belete potato variety was employed as a raw material in these studies to extract starch, which is widely used in food, chemicals, pharmaceuticals, and other industries. In the pharmaceutical industry, native and modified starches are used as pharmaceutical excipients. The white, soft, smooth dryness of native starch, as well as its ability to gel and impart viscosity, are all important characteristics that make it appealing for use as a medicinal excipient. [10]. In various studies, Ethiopian potato starch was used as a binding and lubricant in tablet manufacture. Because the active ingredients in many tablets are tiny, these research aimed to address the effect of starch as a filler. In tablet formulation, the filling procedure was crucial for achieving a constant size and boosting the tablet's effectiveness. Potatoes may be grown readily across more than 70% of the country.

Apart from these effects, recent studies on modified starch production have focused on the pharmaceutical industry because of the many properties of starch used in this industry, such as the use of modified starch production as a binder, disintegrate, lubricants, anti-adherents and diluents or fillers, and other properties. Furthermore, the majority of past studies have concentrated on the

process of finding new ideas. Physically, chemically, and enzymatically are the three basic strategies for modifying starch. Physicochemical analysis of potato starch, as well as a comparison and optimization research of the starch as a dis-integrant in tablet formulations, were published by Anteneh et al. CLASs showed enhanced powder flowability and tablet compressibility from anchote in another study published by Desalegn. In this work, potato starch was cross-linked and acetylated chemically, and the features, effects of the factors, and optimization conditions were compared. As a result, this study provides a significant opportunity for enterprises to determine the benefits and drawbacks of such chemical alterations. In general, this research suggested that native and modified potato starch be synthesized, characterized, and optimized in the pharmaceutical business. Finally, research on the efficacy of tablets as fillers and the properties of tablets containing modified potato starch.

1.3. Objectives

1.3.1. General Objective

The general objective of this study is to synthesize, characterize, and optimize pharmaceutical-grade potato starch from Belete potato variety for tablet formation.

1.3.2. Specific Objectives

The specific objectives are to:

- Determine, Compare and contrast physicochemical characteristics of cross-linked and acetylated starch such as solubility, density, starch hydration, crystallization, pH
- Investigate the effects of extraction process variables (temperature, concentration, and extraction time) on the degree of modification and determine the optimal operating condition
- Compare and contrast filling capacity and determine the weight, thickness, hardness, and friability of a tablet between acetylated and cross-linked potato starch in tablet formation by direct compression.

1.4. Significance of the study

The study has great significance in terms of assuring the production of an alternative form of top platform chemical which has a versatile application in different pharmaceutical industries. The

outcome of the study will have a significant advantage. The major outcome of this study is to compare and contrast the physicochemical properties of native and modified starch, identify the optimum condition of the process variable, identify which modification method is more productive then construct a conclusion, which type of modification of starch and conditions, are more suitable for drug formulation. And also help to observe the easy accessibility, productivity, and economical consequence

1.5. The Scope of the Project

The project gives special emphasis to develop pharmaceutical-grade potato starch production processes and their modification of the starch. The scope of work includes a preliminary assessment on laboratory scale, optimization and characteristics of modification of starch, raw material & equipment supplies availability, the concentration of chemicals, identify the optimum condition production of the starch, comparing physicochemical properties of native, cross-linked and acetylated potato starch, measure the effectiveness of the tablet formed by filling cross-linked and acetyl potato starch. Finally, construct a conclusion and recommendation on the preferable type of modification and condition of process variables.

CHAPTER TWO

2. Literature Review

This chapter contains available information related to starch modification and the effect of starch on tablet formation. Both theoretical and empirical literature is going to be reviewed throughout this chapter. In the theoretical aspect, sufficient and relevant theories on the topic under investigation are reviewed. Again, relevant and sufficient empirical works also will be investigated. Most materials contain starch raw materials, the importance of modification for tablet formation, and the Basic property of modified starch. Particularly, studies associated with the effect [2] of modification of starch on tablet formation, compare and contrast cross-linked, acetylation, and native starch.

2.1. Starch Raw Material

Starches can be used in a variety of places around the world. The raw material used in different studies differs depending on the goal, accessibility, and starch quality. They're found in a variety of plant bits, including cereals (corn and wheat), tubers (potato), and roots (cassava, taro, sweet potato, and arrowroot) [2]. Ginger, cocoyam, sorghum, plantain, rye, barley, yam, enset, and colocasia have all been researched to keep up with the increasingly global needs [3]. Particularly in the pharmacy industry to manufacturing drugs to fill the excipient part. In Ethiopia, corn, potato, and Enset are the major available raw material used for starch production.

2.2. Ethiopian Potato

The Lamiaceae family includes the Ethiopian potato, which is a tuber-bearing member of the Lamiaceae family such as Dagim, Bubu, Belete, Gudene, Challa, Mara, Shenkolla, Gabissa, Gera, Jalene, Gorebella, and other potato cultivars are grown in Ethiopia. The native starch extract from the Belete potato variety, which has the highest starch content and total starch yield, was used in these studies [17]. For use in pharmaceutical technology, unprocessed native starches are structurally weak and functionally limited. As a result, starch is chemically or physically changed to make it suitable for industrial application [86].

Potatoes are farmed in four main areas in Ethiopia: the central, eastern, northwestern, and southern regions. Ethiopia is one of Africa's top potato producers, with 70 percent of its fertile terrain suited

for potato production above 1500 meters [17]. According to the CSA, potato production capacity will reach 20.1t ha-1 in 2020, an increase of 3.6 percent every year. When compared to local, which is susceptible to illnesses that reduce production and has the largest tuber size, yield per plant, and early maturity to reach, the Belete potato variety, planted at altitudes of 1600–2800 meters, and improved variety Belete, have a positive benefit or yield difference when compared to local, which is sensitive to illnesses that reduce production and has the largest tuber size, yield per plant, and early maturity to reach. During the belg (short rain season—February to May) and meher (long rain season—June to October) seasons [18].

2.3. Starch for Tablet Formation and Its Property

Many types of research and modification are created to scale up the quality and the effectiveness of starch. Particular in the pharmacy industry to enhance the quality of drugs. Specific starch consumption in drug production varies from technology to technology. The raw material used for such an industry become increase from time to time. According to Phili F. Builders, Starch-based excipients have been shown to offer numerous advantages in drug production in terms of lower cost, safety, and product quality. It has also been evaluated and used as a drug carrier in controlled drug delivery systems [10].On other hand, it's is a versatile, cheap, and readily available material obtained from renewable sources that have found wide application in tableting as a binder, dis-integrant, diluents, and lubricant [18]. According to yalfal 2018, in Ethiopia, Production of starch for pharmaceutical industry almost insignificant imported from Asian and European countries. The production of starch from potato is advantageous due to easy accessibility and high yield starch.

2.4. Chemical Composition of Raw Material

The chemical composition of the potato starch is the basic element to understand the nature and the factor affecting the property of the potato starch. There are many variables to know the chemical composition such as; Amylose Content, amylopectin, Ash value, Protein content, Fat content, and Moisture content.

2.4.1. Estimation of Amylose Content and Amylopectin

Starch is a biodegradable polysaccharide consisting of two essentially unit's linear amylose and the branched amylopectin. Starch also one of such polysaccharides[19]. It is also known as a semi-crystalline material. These crystalline regions are predominantly made up of amylopectin polymers

of which the outer branches are hydrogen-bonded to each other to form crystallites that unravel during gelatinization. The amorphous regions of granules are mainly composed of amylose and amylopectin branch points [20]. Amylose and amylopectin make up 98 to 99% of the dry weight of native granules, with the remainder comprising small amounts of lipids, minerals, and phosphorus in the form of phosphates esterified to glucose hydroxyls [4]. The equation used to measure amylose and amylopectin content;

Equation 2.1 and 2.2. measuring Amylose and Amylopectin

$$\text{Amylose content (\%)} = 3.06 \text{ x absorbance x } 20 \quad (2.1)$$

$$\text{Amylopectin content (\%)} = 100 - \text{amylose content (\%)} \quad (2.2)$$

2.4.2. Ash value

The weight of the ash was then determined and the percentage ash value calculated using the relation below[21].

Equation 2.3. measuring percentage ash value

$$\text{Percentage ash value} = \frac{WA * 100}{WS} \quad (2.3)$$

Where, WA and WS are the weight of ash formed and the initial weight of starch powder, respectively.

2.4.3. Protein content

The protein contains sufficient amounts of all the essential amino acids required by the body for the growth and maintenance of lean muscle tissue. The interaction between protein and starch is mainly electrostatic, between the anionic groups of the starch and the positively charged groups of the protein. The protein-starch interactions in bulk solutions and at interfaces have an important influence on the stability properties of food dispersions. A binary mixture of protein and starch in an aqueous solution can exhibit one of the three different equilibrium situations: these are miscibility, thermodynamic incompatibility, and complexation [22].

2.4.4. Fat content

The importance of starch-lipid interaction is particularly increased in cereal starches [23]. Polar lipids have long been known to affect the behavior of starch pastes and are thought to interact with linear amylose chains to inhibit swelling and hydration. The effect of the lipid on starch gelatinization is related to hydrocarbon chain length. While short-chain polar lipids may accelerate the rate of gelatinization, medium and long-chain compounds inhibit the swelling of granules and uptake of water[24]. When granular starches are pasted in water in the presence of various surfactants, it would be expected that these adjuncts will affect the swelling of the granules and the concurrent solubility of the starch substance. If the surfactant complexes strongly with the linear fraction, then it should restrict granule swelling and solubility of those starches which contain a linear fraction [25].

The presence of higher lipid content can affect some physicochemical properties of starches significantly such as delaying/decreasing starch granules swelling, reducing the solubility, reducing the rate of gelatinization, and reduction of water uptake especially if the lipid is a medium and long-chain compound through interaction with linear amylose chains. It has also the potential to affect the size and shape of the starch granules at higher temperatures [26]: [3]: [5].

2.4.5. Moisture Content

The moisture content of bulk solid is the most important factor controlling the flow properties of granular materials. The surface moisture leads to the appearance of cohesive forces between particles of solids and adhesive forces between particles and the walls of the conveying duct. Both retard the flow of solids and under certain conditions may stop the flow entirely [27].

2.5. Starch Modification

Starch modifications were usually done to enhance or repress the inherent property of these native starches or to impact new properties to meet the requirements for specific applications. The modifications alter the properties of starch, including solution viscosity, association behavior, and shelf-life stability in final products and improve paste clarity and sheen, paste, and gel texture, film formation, and adhesion in the other hand decrease retro-gradation, gelling tendencies of pastes and gel syneresis [27]: [28].

Another purpose of starch modification is to stabilize starch granules during processing and make starch suitable for many foods, pharmaceutical, and industrial applications[29] : [28]. The process of starch modification involves the de-structuration of the semi-crystalline starch granules and the effective dispersion of the component polymers. In this way, the reactive sites (hydroxyl groups) of the amylopectin polymers become accessible to electrophilic reactants [30].

The functionality of starch depends on the molecular size, degree of crystallinity, amylose content, and viscosity properties, by and large, it could be achieved through modifications of the native starch [31]. Native starch irrespective of its source is undesirable for many applications, because of its inability to withstand processing conditions such as extreme temperature, diverse pH, high shear rate, freeze-thaw variation, water insolubility, tendency to easily retrograde and undergo syneresis and therefore form unstable pastes and gels [29]: [28]. Starch is modified chemically, enzymatically, and/or physically to enhance its positive attributes and/or to minimize its defect[32]. The starch modification involves the alteration of the chemical composition and physicochemical characteristics of the NS to improve its functional characteristics. The starch modification industry is constantly evolving. Modifications of starch include physical, chemical, and enzymatic methods.

2.5.1. Physical Methods

The physical modification involves physical treatment of starch granules thermally or by other physical means to impart certain needed properties or correct one or more shortcomings associated with native starch [33]. This modification is simple, cheap, and safe compared to chemically and enzymatically modify.

The physical modifications involve the treatment of NS granules under different temperature/moisture combinations, pressure, shear, and irradiation. It also includes mechanical attrition to change the physical size of starch granules [28]. Physical modification of starch is mainly applied to change the granular structure and convert native starch into cold-water-soluble starch or small crystallite starch. This set of techniques are generally given more preference as these do not involve any chemical treatment that can be harmful for human use [34]. Currently, physical methods are common modification in different food, pharmaceutical and other. These includes [26]: [35];

1. Pre-gelatinization
2. Radiation
3. Sonication
4. Heat-moisture treatment
5. Heat-moisture treatment,
6. Osmotic-pressure treatment
7. Thermal inhibition
8. Gelatinization and retro-gradation

2.5.2. Chemical Modification

The chemical modification involves the alteration of functional groups of the starch molecule, resulting in markedly altered Physicochemical properties [36]. The properties of starch derivatives obtained depend on the kind of starch bases used and their basic properties [37]. Its involves the introduction of functional groups on the starch molecule without affecting the morphology or size distribution of the granules. It generates significant changes in starch behavior, gelatinization capacity, retro gradation, and paste properties. Such modification helps in the stabilization of inter-molecular bonds at different positions and locations [26].

The chemical and functional properties achieved by modified starches depend, inter alia, on the starch source, reaction conditions (reactant concentration, pH, temperature, reaction time, and the presence of catalyst), type of substituent, degree of substitution (DS), complication with salts, covalent cross-linking and the distribution of the substituents in the starch molecule[28]. Chemical modification of starch changes the functionality of the starch. The chemistry involved in the modification of starch is quite straightforward and involves primarily reactions associated with the hydroxyl groups of the starch polymer [38].

Chemical modification of starches may yield starches with desirable functional properties that could be valuable in the food and pharmaceutical industries. The derived starches may have better properties as tablet excipients especially in direct compression manufacture of pharmaceutical tablets [39]. It is generally achieved through derivatization, such as acetylation, Carboxymethylation, cat ionization, oxidation, acid hydrolysis, substitution, and cross-linking. These techniques are however limited due to issues concerning consumers' safety and the environment. There is an evolving new trend called dual modification, which involves the

combination of physical, chemical agents, e.g., micro wave assisted acetylation or HHP-assisted phosphorylation [28].

Cross-linked Starch

Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds that act as a bridge between the starch molecules which alters not only the physical properties but also the thermal transition characteristics of starch [40]. Cross-linked starches (CLS) constitute a major class of modified starches. It reinforces the already present hydrogen bonds in the granules with new covalent bonds [41]. Cross-linking treatment is intended to add intra- and inter-molecular bonds at random locations in the starch granule that stabilize and strengthen the granule. Restricted water uptake could also be achieved by this method, due to the increased density of cross-links in the starch structure [37]:[42].

Phosphorylation is one of the most common methods used to modify starch and produce mono-starch and di-starch phosphate. The phosphate is bounded to starch molecules that cause changes in the functional properties of starch. Usually, sodium tri-meta-phosphate (STMP), sodium tripolyphosphate (STPP), Phosphoryl chloride (POCl3), epichlorohydrin (ECH), and sodium hexametaphosphate (SHMP) are common chemical agents used for phosphorylation and cross-linking and can serve as main cross-linking agents[43]. So, the type of cross-linking agent greatly determines the change in functional properties of the treated starches. The reactivity and concentration of reagents have also been reported to influence the degree of substitution of CLS [42].

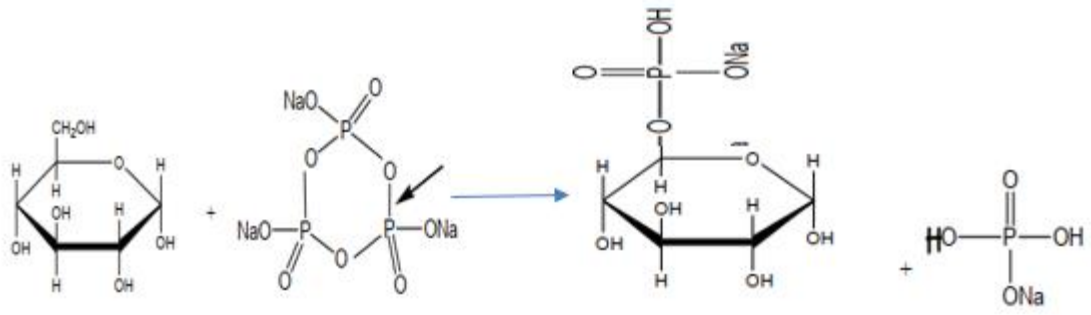

Figure 2.1**:** A scheme for chemical reactions involved during cross-linked of potato starch with SHMP.

Cross-linked starch needs more energy is required to separate the starch molecules, thus altering gelatinization. It also affects the granular swelling and produces starch that can tolerate high temperature, shear, and acidic conditions. When Starch has higher peak viscosity it shows a low level of degree of cross-linked, reduced breakdown viscosity than that of unmodified starch and may maintain granule integrity to keep the swollen granules intact. This helps to prevent loss of viscosity and provide resistance to mechanical shear. However, increasing the level of crosslinking will eventually reduce granule swelling and decrease the starch viscosity. Exceptions to this also occur. When cross-linked starch is previously gelatinized, for instance, increasing the level of crosslinking may increase granular swelling [9]. Such types of starch are more resistant to acid, heat, and shearing than native starch, and are therefore suitable for applications such as in canned food and pharmacy to increase the shelf life of the product [44].

Acetylation

According to "Lisie" Starch acetylation is a chemical modification by which part of the hydroxyl groups of glucose monomers is converted into acetyl group, altering the molecular structure of the starch. starch source, reactant concentration, catalyst type, concentration, reaction time, and suspension pH are the major factors that affect starch acetylation[45]. It's performed to improve the physical, chemical, and functional properties of starch.

In the acetylation process, the hydroxyl groups of the glucose monomers are replacing by acetyl groups (CH3COO-) therefore the acetylation is the esterification of hydroxyl groups in the an-hydro-glucose unit of the starch molecule. The starch acetate has applications that are regulated by its characteristics, such as the degree of substitution and the percentage of acetyl groups[46]. Acetylation using acetic anhydride occurs predominantly in the amorphous region of the granules. The insertion of acetyl groups promotes the reduction in the interactions between the outer chains of amylopectin and amylose chains, conferring new features to the polymer. Acetic anhydride is commonly used as an acetylating agent, and the reaction is activated in the presence of an alkaline catalyst [47].

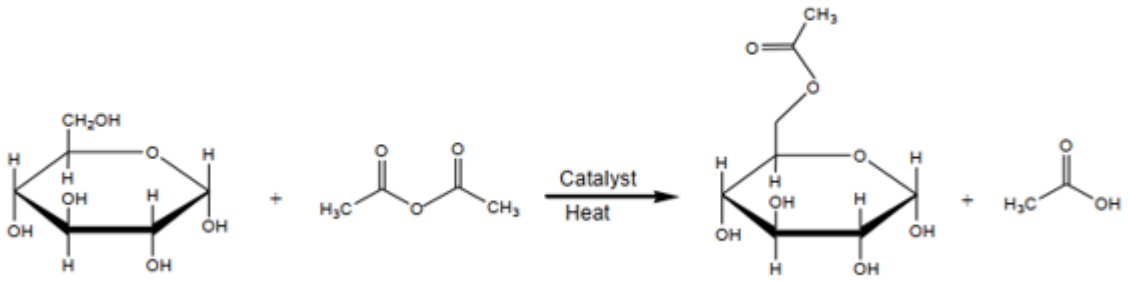

Figure 2.2: A scheme for chemical reactions involved during acetylation of potato starch with acetic anhydride.

Starch acetates of low DS are commonly used in the food industry for quality consistency, and as texture and stability enhancers. The Food and Drug Administration (FDA) maximum DS of acetylated starches for food application is 0.1. Starch acetate of high DS exhibits a high degree of hydrophobicity and thermo-plasticity and is soluble in organic solvents like chloroform and acetone, and is mostly used in nonfood applications. At 0.0275 DS, cornstarch exhibit lower paste gelling, this is practically lost at 0.05 DS. Most commercial starch acetates have < 0.05 DS. The introduction of the bulkier acetyl group compares with the hydroxyl group causes steric hindrance to the alignment of the linear chains. This allows for easy water percolation between chains thus increasing the granule swelling power and solubility resulting in lower gelatinization temperature [48]. A mechanism for the effect of acetylation was proposed: introduction of the acetyl group reduces the bond strength between starch molecules and thus makes the starch granule swell and solubilize more, giving improved freeze-thaw stability[44].

2.5.3. Enzymatic Modification

Enzymatic modification of starch has been used to increase the linear starch content, thus increasing complexing capability. It investigated the effect of pH and debranching on complex formation of waxy rice and fatty acids (FA) with different chain lengths and saturation and suggested that FA complexed to a greater extent with long linear starch chains as measured by a decrease in iodine absorbance of the complexes [49]. The enzymatic hydrolysis of granular starch also causes significant changes in the functional properties of starches such as gelatinization

temperatures, gel formation, and paste viscosity, which is very important to define the industrial uses of starches[50].

Enzymes are highly specific and they usually act under milder reaction conditions than traditional chemicals. Furthermore, they are readily biodegradable and usually lead to reduced or no toxicity when they reach the environment after use in industrial production. These properties allow manufacturers to produce the same or sometimes even better quality products with less raw material, chemical, water, and/ or energy consumption and with less problematic waste generation than traditional processes [51].

2.5.4. **Dual Modification**

Dual modifications include chemical modifications and different types of modifications combined. It has been used in industry to optimize modified starch functionality. This new approach involves the combination of chemical and physical agents (e.g., acetylation assisted by microwave, phosphorylation assisted by high pressures). Specifically, dual chemical modifications involve two processes of chemical modification (e.g., acetylation/oxidation and cross-linking/acetylation). Starches modified by two chemical methods, such as emulsifiers, agglutinants, and thickeners, are commonly used in the food industry and are included as adsorbents of heavy metals in the non-food industry [26].

2.6. Basic Physicochemical Property of Modified Starch

Modified starch has many properties that can affect the quality and effectiveness, particularly for pharmaceutical purposes. These are;

2.6.1. Densities and Related Properties

To manufacture a good tablet, the powder blend has to flow uniformly and form firm compaction. Good flow ability ensures uniformity in die fill and thus uniformity in tablet weight. It also facilitates the blending of fine powders encountered in direct compression blends [52]. The absolute and relative densities of a wide range of pharmaceutical solids and probed some of the influences of chemical structure, processing history, and dosage-form type on these properties. Monitoring the relative density of solid pharmaceutical materials can be useful during the design,

optimization, and scale-up of manufacturing processes for solid pharmaceutical dosage forms and may help achieve robust drug-product manufacturing processes [53].

The packing and cohesive properties of Bulk and tapped densities are important characteristics that affect powder storage, powder flow, die filling during compression [54]. There are various methods available to measure the powder flow. The compendia methods include measurement of the angle of repose bulk density, tapped density, Carr's compressibility index, or Hausner ratio [55]. Powders with high values of compressibility index are considered as materials with poor compressibility, indicating also relatively high inter-particle interactions [56].

Bulk Density

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the inter-particulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per milliliter (g/ml) although the international unit is kilogram per cubic meter because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm3). The bulking properties of a powder are dependent upon the preparation, treatment, and storage of the sample. The particles can be packed to have a range of bulk densities and the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made [57].

Eq. 2.3. Bulk density was determined as a mean of three measurements.

$$\text{Bulk density } (\rho b) = \frac{M}{Vb} \tag{2.3}$$

Where m is the weight of the powder and V_b is bulk volume

Tapped Density

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until a little

further volume or mass change is observed. The tapping is achieved by raising the cylinder and allowing it to drop under a specified distance [57].

Equation 2.4. Tapped density measurement

$$\text{Tapped density } (\rho t) = \frac{m}{Vt} \tag{2.4}$$

Where m is the weight of the powder and V_t is the tapped volume

Carr's Index

Carr's index also known as Carr's Compressibility Index is an indication of the compressibility of a powder. Compressibility is a measure of the relative volume change of a fluid or solid as a response to a pressure change or stress. It measures the relative significance of inter-particle interactions [58]. It is also an indirect method of assessing the flow of a powder. The maximum acceptable value for Carr's index is 15 %. The lower Carr's index of the material, the better the compressibility and flow ability. [18].

Equation 2.5. Carr's Index measurement

$$\text{Carr's Index (CI)} = \frac{(\rho t - \rho b) * 100}{\rho t} \tag{2.5}$$

Hausner Ratio

Hausner Ratio measures the friction condition and a number that is correlated to the flow ability in a morning powder mass. It is a characteristic of a powder in addition to the angle of repose and flow time [59]. It is used to show that the flow ability of a powder [60]. A Hausner ratio greater than 1.25 is considered to be an indication of poor flow ability and less than 1.25 is considered to be an indication of free-flowing [61].

It was used to measure and predict the propensity of a given powder sample to be compressed and which are understood to reflect the importance of inter-particulate interaction. These interactions are significant when tapped and bulk density will be huge difference magnitude. The lower the Hausner ratio, the lesser the tendency for densification to occur. As the values of these indices increase, the flow of the powder decreases [56].

Equation 2.6. Hausner ratio measurement

$$\text{Hausner ratio (HR)} = \frac{\rho t}{\rho b} \tag{2.6}$$

2.6.2. PH Determination

Control of acidity and/or alkalinity provides a useful method of ensuring identity, stability, and freedom from contamination. Water-soluble substances, which are naturally acidic or alkaline usually, have limits of acidity or alkalinity, which is expressed as a PH, rang for the solution of specified concentration. It measures the hydrogen ion (H^+) activity in a solution[62].

2.6.3. Analyses of Fourier Transform Infrared (FTIR) spectra

FTIR spectroscopy is a quick and simple technique for identifying compounds. The IR spectrum of a given compound is unique and characteristic. This is because the IR spectrum distinguishes between the different kinds of bonds in a molecule [64]. FT-IR spectra of the cross-linked and acetyl modified starch sample were acquired by Fourier transformed infrared spectrophotometer in transmittance mode to check structural changes due to modification.

2.6.4. Determination of Starch Hydration Capacity

The hydration of starch represents the water absorbed by the particle or the particle surface [65]. The concept of free and bound water within starch is of great practical significance. The status of water has an influence on the structural and textural properties of the food as well as its microbial stability [66].

Equation 2.7: The hydration capacity was determined using the equation below:

$$\text{Hydration capacity} = \frac{Ws}{Wd} \tag{2.7}$$

Where Ws and Wd are the weights of the sediment and dry sample, respectively.

2.6.5. Degree of Substitution (DS) or Degree of Acetylation Determination

The properties of starch acetates are a function of the acetyl content, type of starch, non-starch components, and method of pretreatment. And the measurement of the acetyl content is a prime method for the characterization of SAs [46]. Acetylated starches have improved properties over

their native counterparts and have been used for their stability and resistance to retro-gradation, acetyl groups upon acetylation reduce the bond strength between starch molecules and thereby increases the swelling power and solubility of the starch granule, decreases the coagulation of the starch and provides improved freeze-thaw stability. The extent of Physico-chemical property changes in the acetylated starch compared to the native starch is proportional to the degree of acetylation or degree of C=O substitution incorporated into the starch molecules [67]. Starch acetylation depends upon certain factors, such as starch source, reactant concentration, catalyst type, concentration, reaction time, and suspension pH [45].

2.6.6. Swelling Power and Solubility Determination

Swelling power and solubility provide evidence of the magnitude of the interaction between starch chains within the amorphous and crystalline domains (Okunlola& Akingbala, 2013). The extent of this interaction is influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation [69]. The swelling power indicates indirectly the water holding capacity of various types of native starch and its modified starches[70]. In the pharmacy industry to be potentially useful as a pharmaceutical excipient, the ability of starch granules to swell has important implications. Swelling power is not only a measure of the hydration capacity of the sample but is also indicative of the associative forces in the granules[71].

The percent solubility (%S) and SP were determined according to Eq.2.8 and 2.9, respectively.

$$\%S = \left(\frac{Wa}{Wb}\right) * 100 \tag{2.8}$$

$$\%SP = \frac{100}{Wc} * Wb * (100 - s) \tag{2.9}$$

Where; Wa is the weight (g) of soluble material in the supernatant, Wb is Weight (g) of precipitate, and; Wc is Weight (g) of a starch sample.

2.6.7. Peak Viscosity Determination

Peak viscosity was strongly influenced by variations in pH and was to some extent dependent on ion concentration. In hard tap water, the peak viscosity was slightly decreased by the anionic emulsifiers (SSL > CSL > DATE), due to the presence of Ca++ and Mg++ ions, while DGMS

gave a higher peak viscosity than in distilled water. the maximum viscosity obtained during the heating program (peak viscosity) is influenced by the addition of other materials such as sugars, salts, acids, proteins, fats, or surface-active agents of the starch[72].

2.6.8. Morphological Studies of Samples Under Scanning Electron Microscopy

Particle morphology is an important property in the characterization and identification of, especially powdered pharmaceutical excipients. It can also be used to predict certain functional properties that relate particularly to flow-ability and compatibility of the powder and disintegrating characteristics of tablets [73]. It was studied using Scanning Electron Microscopy (SEM).

2.7. Application Area of Starch

Starch has also versatile applications in the food, pharmaceutical, textile, plastic, and paper industries. Starch serves in food industries as a thickener, gelling aid, texturizer, emulsifier, appearance modifier, bulking agent, coating, adhesive, and lipid substitute [71]. It has also been used for centuries as a binder in making paper and this continues to be an important use. This paper will see the application area of starch in the pharmaceutical industry.

2.7.1. Applications in Pharmaceuticals

The International Pharmaceutical Excipients Council (IPEC) defines excipients as ―Substances, other than the API in finished dosage form, which has been appropriately evaluated for safety and are included in a drug delivery system to either aid the processing or to aid manufacture, protect, support, enhance stability, bioavailability or patient acceptability, assist in product identification, or enhance any other attributes of the overall safety and effectiveness of the drug delivery system during storage or use‖ [76].

Excipients are used to convert pharmacologically active compounds into pharmaceutical dosage forms suitable for administration to patients [77] and increasingly being known as important components of conventional and novel drug products, providing specific functions in helping the formulation of optimally stable, elegant, safe, and active drug products [78]: [79].

They are also necessary for ensuring the manufacturing process is thriving and the quality of the formulation can be guaranteed. The appropriate selection of excipients in the formulation is critical

in the development of a successful product [77]. Filler-binders or diluents, disintegrating agents, and lubricants are the major types of excipients that are used for the production of tablets, which are present in almost all tablet formulations [80]. The role of excipients in determining the quality of a formulation and in many cases the bioavailability of a drug from tablets has received considerable attention in recent years [81].

2.7.2. Classification of Pharmaceutical Excipients

Different types and categories of excipients are used in pharmaceutical dosage formulations, whether in the case of liquid, solid or semisolid preparations. This thesis, paper is all about the excipients used in the solid dosage forms, especially about the excipients that are commonly used within the formulations of a directly compressible tablet. Therefore, we will categorize the excipients that are largely used as powder excipients. Direct compression formulations can be developed with minimal numbers of excipients. In a conventional direct compressible tablet, the excipients used in the formula may be categorized as follows [10]:

1. Diluents or fillers
2. Binders
3. Dis-integrant
4. Glidants
5. Lubricants
6. Anti-adherents

Starches are the most commonly used pharmaceutical excipient in the manufacture of tablets and its native and modified forms are widely employed as versatile excipients in various solid dosage forms, especially as diluents, disintegrate, and binding agents in tablet formulations[11].

During recent years, starch has been taken as a new potential biomaterial for pharmaceutical applications because of the unique physicochemical and functional characteristics as well as its relatively low cost and inertness [12]. It also finds applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, water retention agent, adhesive, forming artificial red cells, forming bone tissue, film-forming polymer, used as sustained release polymer for drug, as anti-adherent and lubricant, filler, super dis-integrant and matrix former in capsules and tablet formulations during its manufacture; or protect, support or enhance stability, bioavailability, or patient acceptability; assist in product identification; or enhance any other attribute of the overall safety and effectiveness of the drug during storage or use [83].

CHAPTER THREE

3. MATERIAL AND METHODS

3.1. Raw Materials

The basic Raw material that was used for this project was potato. The material, potato, was collected from Holleta agricultural field, Ethiopia, and transported to AAIT, School of Chemical and Bio-Engineering Laboratory.

3.2. Chemical

All reagents will obtain from the school of bio and Chemical Engineering Chemical Store and Laboratory and from the market. sodium metabisulphite, sodium hydroxide, sodium Carbonate, SHMP, distilled water, acetic anhydride, Hydrochloric acid, and iodine solution.

3.3. Equipment Employed

Eyes goggle, hand glove, knife, cutter, muslin cloth, blender machine, hydrometer, refract meter, autoclave, Pyrex, viscometer, analytical balance, beaker for handling the sample, tightly sealed containers, water bath, UV/Visible Spectrophotometer, Buchner funnel, centrifuge, stirrer, oven, Spoon and Crucible.

3.4. Methods Employed

3.4.1. Isolation of Starch from Potato Tuber (Native Starch Extraction)

The procedure to extract, isolate, and purify the native potato starch was described by [12]. First, the fresh potato tuber was washed to remove the soil and unwanted materials that cover the surfaces. Then, the cleaned tubers were immersed in water, peeled, and cut up into small pieces then, milling small pieces of potato to increase the surface area and soaked with distilled water with the ratio of (1kg of potato:2liter distilled water) containing 0.075% w/v of sodium metabisulphite for 4hour.

The starch slurry materials were then passed through fine muslin to remove cell debris, the translucent suspension was collected and allowed to settle, and the supernatant decanted. Repeat the soaking and decantation process 3 times until the result of native starch become insignificant.

The sediment starch was washed several times with distilled water until the wash water was clear and free of suspended impurities. Finally, the starch was dried at room temperature, milled to a fine powder in a mortar and pestle, sieved through 224 µm mesh size, and kept in tightly sealed containers [12].

3.4.2. Determination of Percent Yield of Native Starch

Percentage yields of dry basis extracted native potato starch were determined according to the method described by the Association of Official Analytical Chemists with slight modification, [AOAC, 2000].

Equation 3.1; yield of native starch

$$\%\ \text{yield of native starch} = \frac{weigth\ \ of\ dry\ native\ starch * 100}{weigth\ of\ the\ sample} \qquad (3.1)$$

3.5. Modified starches Preparation

3.5.1. Preparation of Cross-linked Potato Starch (CLPS)

Cross-linking of potato starch was performed using the method described by Woo &Seib, [85]. As shown in Table 3.1. the native starch (50g, dry basis) disperse into 50ml distilled water mix for 30 minutes using a mixer. Then, prepare the solution which containing sodium carbonate (0.8g) in 20ml and SHMP at three different concentrations 100, 200, 300 (%) dissolved in 25ml amount of distilled water individually and add into the solution containing native starch then adjust the PH of the solution until become 11 by the solution which containing sodium hydroxide (1.2%, w/w) in distilled water then stirring until the solution completely homogenous by a magnetic stirrer. The starch suspensions were maintained at (45, 60, 75)^{0}C while stirring and held at these temperatures for (30, 60, 90) min contact time. Adjust the pH at 6.5 by 1.5 N hydrochloric acid of the suspension, after cooling to room temperature, to terminate the reaction.

The CLS slurries were then recovered by centrifuging at 3,000 rpm for 15 min and the Starch solution washed 3 times in distilled water (4*100ml) and keep in the open air for 10 minutes until the starch cake decant (the slurring and filtering steps were repeated twice) filtered through by decantation process after the cake become clean filter by a Buchner funnel and dried in a hot air

oven at 50 ^{0}C for 24 h. The materials were then powdered using mortar and pestle, passed through a 224 μm mesh sieve, and packed in an air-tight container

Table 3.1: Reaction conditions for the synthesis of CLPS

CLPS Batches	SHMP (%)	NaOH (%w/w)	Na_2CO_3 (g)	Reaction time (min)	Reaction temperature(^{o}c)
CLPS-A	100	1.2	0.8	30	45
					60
					75
				60	45
					60
					75
				90	45
					60
					75
CLPS-B	200	1.2	0.8	30	45
					60
					75
				60	45
					60
					75
				90	45
					60
					75
CLPS-C	300	1.2	0.8	30	45
					60
					75

				60	45
					60
					75
				90	45
					60
					75

Yield percentage of cross-linked starch

Percentage yields of dry basis cross-linked potato starch were determined according to the method described by the Association of Official Analytical Chemists with slight modification, [AOAC, 2000].

Equation 3.2: yield of cross-linked starch

$$\text{Yield (\%) of cross} - \text{linked starch} = \frac{\text{weight of cross} - \text{linked starch} \ * 100}{\text{Weight of native starch}} \quad (3.2)$$

Degree of cross-linking (DCL) Determination

The Degree of cross-linking affects many properties of the starch such as swelling power, viscosity, solubility, and paste in order to identify which degree of cross-linking is suitable for the property of the starch to produce excipient of the drug.

Procedure; The DCL of the modified starches was determined from the viscosity values, according to the method of[41]. The DCL was calculated by using the Equation 3.3.3below;

$$\text{DCL} = \frac{(\text{A} - \text{B})}{\text{A}} * 100 \quad (3.3)$$

Where A is the peak viscosity of the control sample (NS) and B is the peak viscosity of CLPS.

3.5.2. Acetylated Potato Starch (APS) Preparation

Acetylation of potato starch (K) with acetic anhydride (AA) was performed as follows[86]: As shown in table 3.2 native starch (10 g, dry basis) was dispersed in distilled water (113 ml) and stirred for 30 minutes at 25°C. Acetic anhydride was added dropwise100%, 200%, and 400% (based on the dry weight of starch) and prepare the solution of NaOH in 0.45 molarities concentration and add the NaOH solution into the starch solution until the pH becomes within the range of 8.0-8.4while maintaining constant stirring by a magnetic stirrer. The reaction vessel was sealed and reaction mixtures were heated to (45, 60, 75) ^{O}C, and held at this temperature for (0.5, 1, 1.5) hour while stirring. The pH of the starch suspension was then adjusted to 4.5 with 0.5 N HCl then, Starch solution washed 3 times in distilled water (200ml) and keep in the open air for 10 minutes until the starch cake decant (the slurring and filtering steps were repeated twice)filtered through by decantation process after the cake become clean filter by a Buchner funnel, and then oven-dried at 50 ˚C for 48 h. finally grounded the modified starch until the starch passed through 224 micrometers and packed an airtight container. Yield (%) of modified starch was calculated on a starch dry weight basis.

Table 3.2: Reaction compositions for potato starch acetates (APS)

APS	$\left(\frac{\text{AA} * 100\%}{\text{native starch}}\right)$	NaOH (%, w/w)	Reaction time (h)	Reaction temperature (^{o}C)
APS-A	100	1.8	0.5	45
				60
				75
			1	45
				60
				75
			1.5	45
				60
				75
	200	1.8	0.5	45

APS-B				60
				75
			1	45
				60
				75
			1.5	45
				60
				75
APS-C	300	1.8	0.5	45
				60
				75
			1	45
				60
				75
			1.5	45
				60
				75

Percentage yield of acetyl starch

Percentage yields of dry acetyl potato starch were determined according to the method described by the Association of Official Analytical Chemists with slight modification, [AOAC, 2000].

Equation 4.4 Yield of acetyl starch

$$\text{Yield (\%) of acetyl starch} = \frac{\text{weight of acetyl starch } * 100}{\text{Weight of native starch}} \qquad (3.4)$$

Degree of Substitution (DS) or Degree of Acetylation Determination

The method is described by colossi was employed to determine the DS [46]. First, 1 g sample of APS was placed in a 250 ml flask, and 50 ml of distilled water was added. The flask was agitated, heated to 90 ^{O}C over a water bath, held at this temperature for 30 min, and cooled; 40 ml of 0.5 N potassium hydroxide was added while swirling. The flask was stoppered and then allowed to stand

for 72 h with occasional swirling. The excess alkali was then back titrated with standard 0.5 N hydrochloric acid using phenolphthalein as an indicator. A Native starch (NS) was treated the same way, substituting the SA. The DS was determined using eq.3.5 bellow. Results were expressed as a mean of triplicate determinations.

$$\% \text{ A} = \frac{[v2 - v1] * N * 43 * 100}{(m * 1000)} \quad (3.5)$$

Where:

A - acetyl content;

v_1- the volume of 0.5 N HCl in mL used for titration of 1 g native starch(ml);

v_2- the volume of 0.5 N HCl in mL used for titration of 1 g sample(blank, ml);

N- the normality of HCl solution;

m -the weight of the sample;

43 - the molecular weight of the acetyl group;

$$\text{Degree of substitution} = \frac{162 * \% A}{(43 * 100) - (42 * \%A)} \quad (3.6)$$

Where;

A - acetyl content

43 - the molecular weight of the acetyl group;

162- the molecular weight of the hydro-glucose unit

3.6. Determination of Chemical Composition of Native and Modified Potato Starch

3.6.1. Raw Materials

The material (sample) that was used in the determination of chemical composition was native and dual modified starch. The sample was collected from AAiT, School of Chemical and Bio-Engineering Laboratory

3.6.2. Chemicals Used:

Orto-phosphoric acid, boiling chips, diethyl ether, sulphuric acid, hydrogen peroxide, ammonium, ethanol, sodium hydroxide, distilled water, and iodine are the chemical used to determine the chemical composition.

3.6.3. Equipment Used:

Volumetric flask, spectrophotometer, nickel crucible, oven, distiller, aluminum cup, desiccator, Petri dishes are equipment used to determine the chemical composition of the native and dual modified potato starch

3.6.4. Chemical Composition

The chemical composition of the potato starch is the basic element to understand the nature and factor affecting the property of the potato starch. There are many variables to know the chemical composition such as; amylose content, amylopectin, ash value, protein content, fat content, and moisture content

Estimation of Amylose Content and Amylopectin

Amylose content and amylopectin were determined by using the method of Juliano, 1971. Briefly, 0.10g of the sample was weighed into a 100 cm^3 volumetric flask, and 1cm^3 of 99 % ethanol and 9 cm^3 of 1 M sodium hydroxide solution was added carefully. The contents were mixed thoroughly and the sample solution was heated for 10 minutes in boiling water to gelatinize the starch. After cooling, the solution was made up to the mark with distilled water and shaken thoroughly. Five (5) $cm^3$of the starch solution in a 100cm^3 volumetric flasks was treated with 1.0 cm3 of 1M acetic acid and 2.0 cm^3 of iodine solution. The solution was diluted to the mark with distilled water and the absorbance was read using a spectrophotometer at 620 nm. The absorbance of the blank solution prepared accordingly was subtracted from that of the sample and an amylose and amylopectin content was calculated using equations (3.7) and (3.8) respectively:

$$\text{Amylose content (\%)} = 3.06 \times \text{absorbance} \times 20 \quad (3.7)$$

$$\text{Amylopectin content (\%)} = 100 - \text{amylose content (\%)} \quad (3.8)$$

Ash value

Ash value of the starch was obtained by using the method described by Analytical Methods [21]. A 2 g weight of each starch powder sample was poured into a nickel crucible, which was initially heated at 105^{o}C to a constant weight and allowed to cool. The crucible with its content was then gently heated until it was completely charred. Subsequently, the heat was increased gradually until most of the carbon vaporized.

The sample was finally heated strongly until the residue is free from carbon (i.e. almost white). The crucible with its content was allowed to cool and weighed. The heating and cooling step was then repeated until the weight of the residue (ash) was constant. The weight of the ash was then determined and the percentage ash value calculated using the relation below:

$$\text{Percentage ash value} = \frac{WA * 100}{WS} \tag{3.9}$$

Where, WA and WS are the weight of ash formed and the initial weight of starch powder, respectively.

Moisture

Moisture content will be determined according to AOAC [21], using the official method 925.09. Empty dishes and lids (made of porcelain) will be dried using an air drying oven for 1 hour at 100 ^{o}C, transferred to the desiccator (with granular silica gel), cooled for 30 minutes, and weighed (M1). The prepared samples will be mixed thoroughly and about 5.000g of fresh samples will transferred to the dried and weighed dishes (M2). The dishes and their contents will be placed in the drying oven and dried for 3 hrs at 105^{0}C, and then the dishes and their contents will be cooled in a desiccator to room temperature and reweighed (M3). Finally, moisture content was calculated by using the following

$$Moisture\ (\%) = \frac{(M2 - M3)}{(M2 - M1)} \times 100 \tag{3.10}$$

M1=mass of the dried dish, M2=mass of the dried dish and the sample before drying, and M3=mass of the dish and the sample after drying.

Crude Protein

Crude protein (N×6.25) will be determined by the Kjeldahl method [21]. All nitrogen will be converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated ortho-phosphoric acid-containing copper sulfate and potassium sulfate as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Digestion: about 0.50 g of fresh sample s will be taken in a Tecator tube, 6ml of an acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) will be added, mixed, thoroughly, and 3.5ml of 30% hydrogen peroxide will be added systematically. As soon as the violent reaction had ceased, the tubes will be shaken for a few minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.500 g of copper sulfate with 100 g of potassium sulfate) will be added into each tube and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 3700C, the tubes will be lowered into the digester. The digestion will be then continued until a clear solution was obtained, about 1 hr. The tubes in the rack will be transferred into the fume hood for cooling, a 15ml of deionized water will be added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution will be placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution will be transferred into the sample compartment of the distiller. The tubes will be rinsed with two portions of about 5 ml deionized water and the rinses will be added to the solution. A 25ml of 40% sodium hydroxide solution will be added into the compartment and washed down with a small amount of water, stoppered and the steam switched on. A 100ml solution of the sample will be distilled, and then the receiver will be lowered so that the tip of the condenser 30 is above the surface of the distillate. The distillation will be continued until a total volume of 150 ml is collected. The tip will be rinsed with a few milliliters of water before the receiver will be removed.

$$N=\frac{[(T-B)\times N_{HCl}\times 14]}{W}\times 100 \qquad (3.11)$$

$$P = F * N \qquad (3.12)$$

Where: -

1. T: volume in ml of the standard hydrochloric acid solution used in the titration for the test,
2. B: - Volume in ml of the standard hydrochloric acid solution used in the titration for the blank,
3. 14.00 is the molecular weight of nitrogen,
4. N: - is Nitrogen (%), F:- is conversion factor (6.25),
5. N_{HCl}: is the normality of HCl used (often 0.1N),
6. W: - is sample weight on a dry matter basis and P: - is a crude protein (%)

Crude Fat

Crude fat will be determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55 °C) in a soxhlet extractor [21]. The ether will be evaporated from the extraction flask. The amount of fat is quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as a percentage. The extraction flasks will be cleaned, dried in a drying oven at 70^0C for 1 hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed (W1). The bottom of the extraction thimble will be covered with about a 2cm layer of fat-free cotton. About 2.00 gram of fresh samples will be added into the extraction thimbles, and then covered with about 2cm layer of fat-free cotton. The thimbles with the sample content will be placed into a soxhlet extraction chamber. The cooling water will be switched on, and 50 ml of diethyl ether will be added to the extraction flask through the condenser. The extraction will be conducted for about 3 hrs. The extraction flasks with their content will be removed from the extraction chamber and placed in the drying oven at 70^0C for about 1hr, cooled to room temperature in the desiccators for about 30 minutes, and re-weighed (W2).

$$W = W2 - W1 \tag{3.13}$$

$$Fat(\frac{g}{100g}) = \frac{[W \times 100]}{Wo} \tag{3.14}$$

Where: W = weight of fat

W2 = weight of extraction flask after extraction (wt. of flask and fat);

W1 = weight of extraction flask before extraction (wt. of flask);

Wo = weight of fresh Sample.

3.7. Physicochemical characterization of starch

3.7.1. Densities and Related properties

Bulk Density

Determine the Bulk density of potato starches were carefully pouring 10 g powder into a graduated glass-measuring cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density. The bulk density (g/ml) was calculated by using Eq. 6.1. Bulk density was determined as a mean of three measurements.

$$\text{Bulk density } (\rho b) = \frac{M}{Vb} \tag{3.15}$$

Where m is the weight of the powder and V_b is bulk volume

Tapped Density

For tapped density, 10 g of powder was tapped in a graduated measuring cylinder 500 times using a tapped densitometer (ERWEKA, Germany) to attain a constant volume reading from the cylinder and the tapped density was calculated from the weight and tapped volume of the powder by using Eq. 6.2. Tapped density (g/ml) was determined as a mean of three measurements.

$$\text{Tapped density } (\rho t) = \frac{m}{Vt} \tag{3.16}$$

Where m is the weight of the powder and V_t is the tapped volume.

Carr's Index

It's calculated from the difference between the tapped and bulk density and divided by the tapped density.

$$\text{Carr's Index (CI)} = \frac{(\rho t - \rho b) * 100}{\rho t} \tag{3.17}$$

Hausner Ratio

Hausner ratio was obtained from the ratio of tapped density to bulk density of the starches.

$$\text{Hausner ratio (HR)} = \frac{\rho t}{\rho b} \tag{3.18}$$

True Density

True density determination, fluid displacement method was utilized using xylene as immersion fluid. 2 g starch samples were placed in a volumetric flask (25 ml) which was then filled with xylene and weighed following the determination of the weight of the empty volumetric flask and the volumetric flask filled with xylene. Xylene was added in such a way that it washes down and overlays the sample. After 10 min, the sediment starch was stirred with a small glass-stirring rod to release entrapped air. True density (g/ml) was calculated using equation 6.5. It was measured as a mean of three measurements.

$$\text{True density} = \frac{w1 * sg}{[w1 + w2 - w3]} \tag{3.19}$$

Where

W1= Weight (g) of a starch sample,

W2= Weight (g) of a volumetric flask filled with xylene,

W3= Weight (g) of volumetric flask plus sample plus xylene left after displaced by the sample, and

Sg= Specific gravity of xylene (g/ml) (0.87).

3.7.2. pH Determination

The pH of Potato starch was determined using the method described by Nwachukwu, [87]. It was done by shaking a 1% w/v dispersion of the starch in water for 5 min and the pH was determined using a digital pH meter.

3.7.3. Analyses of Fourier Transform Infrared (FTIR) Spectra

The sample was first ground in a mortar to reduce the average particle size. About 5-10 mg of the samples were equilibrated at 50 ^{O}C for 24 h and finely ground in a mortar to produce a uniform

particle size. The samples were diluted with an oily mulling agent in a mortar and pestle. FTIR of cross-linked and acetyl starches measure on spectrum 65 FT-IR (Perkin Elmer). Samples were recorded at a resolution of 4 cm-1 and wave numbers ranged between 4000 and 400 cm-1 with the number of 4 scans using potassium bromide (KBr) pellets. Then transfer the data into a graph by using origin lab software [88].

3.7.4. Determination of Starch Hydration Capacity

The hydration capacity of the starch was determined by using the method of Jubril [56]. A 1g weight of starch was placed in a plastic centrifuge tube, 10 ml distilled water was added and then closed. The contents were shaken for 2 min then allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10 min in a bench centrifuge. The supernatant decanted and the weight of the wet starch was recorded. The hydration capacity was determined using the equation below:

$$\text{Hydration capacity} = \frac{Ws}{Wd} \tag{3.20}$$

Where Ws and Wd are the weights of the sediment and dry sample, respectively.

3.7.5. Swelling Power and Solubility Determination

The swelling power (SP) and solubility of starches were determined using the method described elsewhere [71]. Starch samples (0.5 g each) were dispersed in distilled water (10 ml) in pre-weighed centrifuge tubes. Aqueous dispersions of starch samples were heated at reasonable intervals between 20 °C and 85 °C for 30 min, with shaking every 5 min and then left to cool to room temperature. Each sample was then centrifuged at 3000 rpm for 15 min and analyzed for the weight of sediment per gram of starch dry weight basis. The supernatant liquid 0obtained at each point was dried in a hot air oven for 2 h at 130 ^{0}C and weighed after cooling in a desiccator. All determinations were done in triplicate. The percent solubility (%S) and SP were determined according to Eq.6.7 and 6.8, respectively.

$$\%S = \left(\frac{Wa}{Wc}\right) * 100 \tag{3.20}$$

$$\%SP = \frac{100}{Wc * (100 - s)} * Wb \tag{3.21}$$

Where;

Wa is the weight (g) of soluble material in the supernatant,

Wb is Weight (g) of precipitate and;

Wc is the Weight (g) of a starch sample.

3.7.6. Peak Viscosity Determination

The peak viscosities of the cross-linked Potato starches and NPS samples were determined according to the method described by Jayakody *et al*, 2007 with a rotational viscometer using spindle number 4 at shearing stress of 200 rpm. Starch suspensions at 10% (w/v) concentration were prepared and shaken for 3min. The suspension was then heated from 50^{o}C to 90^{o}C in a water bath and meanwhile, their peak viscosities were recorded at 50, 70, and 90^{o}C from the digital display on the viscometer. After maintaining the samples at 90^{o}C for 3min, the peak viscosities of the same were read as they were cooled from 90 back to 50^{o}C. The maximum peak viscosities in the entire heating-cooling cycles were taken and used in the estimation of the DCL in the CLS sample.

3.7.7. Morphological Studies of Samples Under Scanning Electron Microscopy

The nature of the morphology of the granule, size of native and modified starch were viewed at various magnifications by SEM brand is field emission electron microscope with Model is inspect f50 to provide clear pictures of the sample. Modified starch samples had large granules as revealed at 4400 x magnification. Oval, round and flattened shapes with a smooth surface are exhibited different morphologies of the sample. Physical interactions of the molecules cause agglomeration, which was the immediate reason for observed Clusters of various sizes. The following are the procedure of SEM analysis result product at Addis Ababa Science and Technology University (AASTU) (Addis Ababa, Ethiopia):

1. Clean the sample holder specimens using acetone or isopropanol alcohol
2. Cut carbon graphite tips into small pieces and stick them on specimens
3. Fasten or stick the fine powder sample on specimens

4. Then open the scanning electron microscope by venting the vacuum.

5. Insert the samples into SEM using tweezers.

6. Pump the vacuum system to create under vacuum

7. Wait some minute till the minimum vacuum pressure reaches $1*10^{-3}$ pa

8. Annotation or rename the sample name

9. Click the beam on

10. Start to do surface morphology analysis by adjusting the breathiness, stigmatism, contrast, focusing, magnification, working distance, working voltage, and spot size.

11. Save the desired image what the researcher needs

3.8. Preparation and Evaluation of Tablets

3.8.1. Preparation of Tablets

Filler capacity of native, CLPS, and APS as per the method described by Mitrevej et al, 1996. As shown in Table 3.3. Each modified starch blended with the active site of a tablet (paracetamol) in a Turbula® mixer for 10 min followed by addition of lubricant (magnesium stearate) further blended for 5 min. The blend was then compressed into tablets with 500 mg size at a fixed compression force on an eccentric tablet machine that was fitted with 10 mm diameter flat-faced punches.

Table 3.3: Paracetamol tablet formulations by using CLPS and APS as a filler (F1-Formulation)

Ingredients (mg/tab)	F1	F2	F3	F4
Paracetamol(%)	53	53	53	53
CLPS(%)	45			
APS(%)		45		
Mg Stearate (%)	2	2	2	2
Total(mg)	500	500	500	500

3.8.2. Evaluation of the Formed Tablets

Weight and Thickness

Randomly selected 10 tablets and weighed individually on an analytical balance and then the average weight and standard deviations were calculated. Tablet thickness was measured using a sliding caliper scale.

Crushing Strength

Randomly selected ten tablets were taken from each type of modified starch and determined the crushing strength of the tablets by using a hardness tester. In this, each tablet was placed between two anvils, and the force that just caused the tablet to break was recorded as the crushing strength. The average crushing strength was taken.

Tensile Strength

The radial tensile strength was calculated using the data obtained from crushing strength, diameter, and thickness of the tablet using equation

$$\sigma = \frac{2F}{\pi DT} \tag{3.22}$$

Where σ x is the tensile strength, F is the force required to break the tablet, D is the diameter of the tablet, and T is the tablet thickness.

Friability

Twenty tablets of known weight from each batch were placed in the plastic chamber of a friability tester and were subjected to combined effects of abrasion and shock by running the apparatus at a speed of 20 rpm for 5 min. Afterward, the tablets were dusted and weighed, and the percent loss in weight was calculated as percent friability.

3.9. Experimental Design Data Analysis and Modeling

Design of Experiment

Factorial was adopted in the design of experimental combinations. The main advantage of factorial is to increase the number of experimental runs needed to provide sufficient information for statistically acceptable results. The analysis of variance (ANOVA) was used as one of the primary tools for statistical data analysis using design Expert software 11.

A three-variable (three levels of each) General factorial experimental design was employed. The parameters (Concentration of modified agent, time, and temperature) and their levels were chosen based on the literature and preliminary experiments. The optimum conditions were obtained from the Model graphs. These factors were; temperature (45, 60 & 75)^{0}C, STMP concentration (100, 200, 300) % and time (30, 60, 90) minute to cross-link and acetic anhydride concentration (100, 200, 300)%, temperature (45, 60, 75)^{0}C to acetylation and retention time of (30, 60, 90) minute. Finally, measure the Responses were peak viscosity and degree of substation for cross-linked and acetyl content and degree of substation for acetylation.

3.10. Process Description and Flow Diagram for Modified Pharmaceutical Grade Starch

3.10.1. Process Description

The production of pharmaceutical-grade modified starch from potato as it is explained in the process flow diagram in the figure above involves the transportation of potato to the storage and potato was a wash to clean, remove unwanted part of the potato, milling the potato and soak into distilled water with 0.075% w/v of sodium-metabisulphite for 4 hours. Then extract native starch by fine muslin and repeat soaking and extraction process 3 times. Until the amount of extracted native starch is insignificant. The dried sample at room temperature was milled into fine powder in a mortar and pestle.

Modified the starch to increase the effectiveness of the drug. For starch modified by cross-linking mix 50ml distilled water with 50g of native starch, then adjust pH at 11 by adding NaOH solution and put the solution at water bath, which the temperature set at 55^{o}C for 15minute. Then prepare 3 different solutions which contain STMP (100, 200, 300) % w/w. lastly, add STMP solution into native starch solution with sodium carbonate (8%)w/w, adjust the PH at 6.5 by adding 1.5N of

HCL solution centrifuge by 3000rpm for 15min, decant the modified starch, dried the modified starch, grounded the modified starch at 750 micrometers and Packed in an airtight container

Second, modified the starch by acetylation, modification by acetylation started by mixing 50g native starch with 113ml of distilled water then prepare a solution of 0.45M of NaOH to adjust the PH at 8 and heat at 50^{o}C for 10 minutes. Prepare the solution which contains modification agent Acetic anhydride in (100, 200, 300)w/w %. Add acetic anhydride solution into starch solution and mix by magnetic stirrer for (30, 60, 90) minutes. Adjust the PH of the solution at 4.5 by HCl. Finally dried the modified starch grounded the modified starch at 750micrometer and Packed it in an airtight container.

3.10.2. Processes Flow Diagram

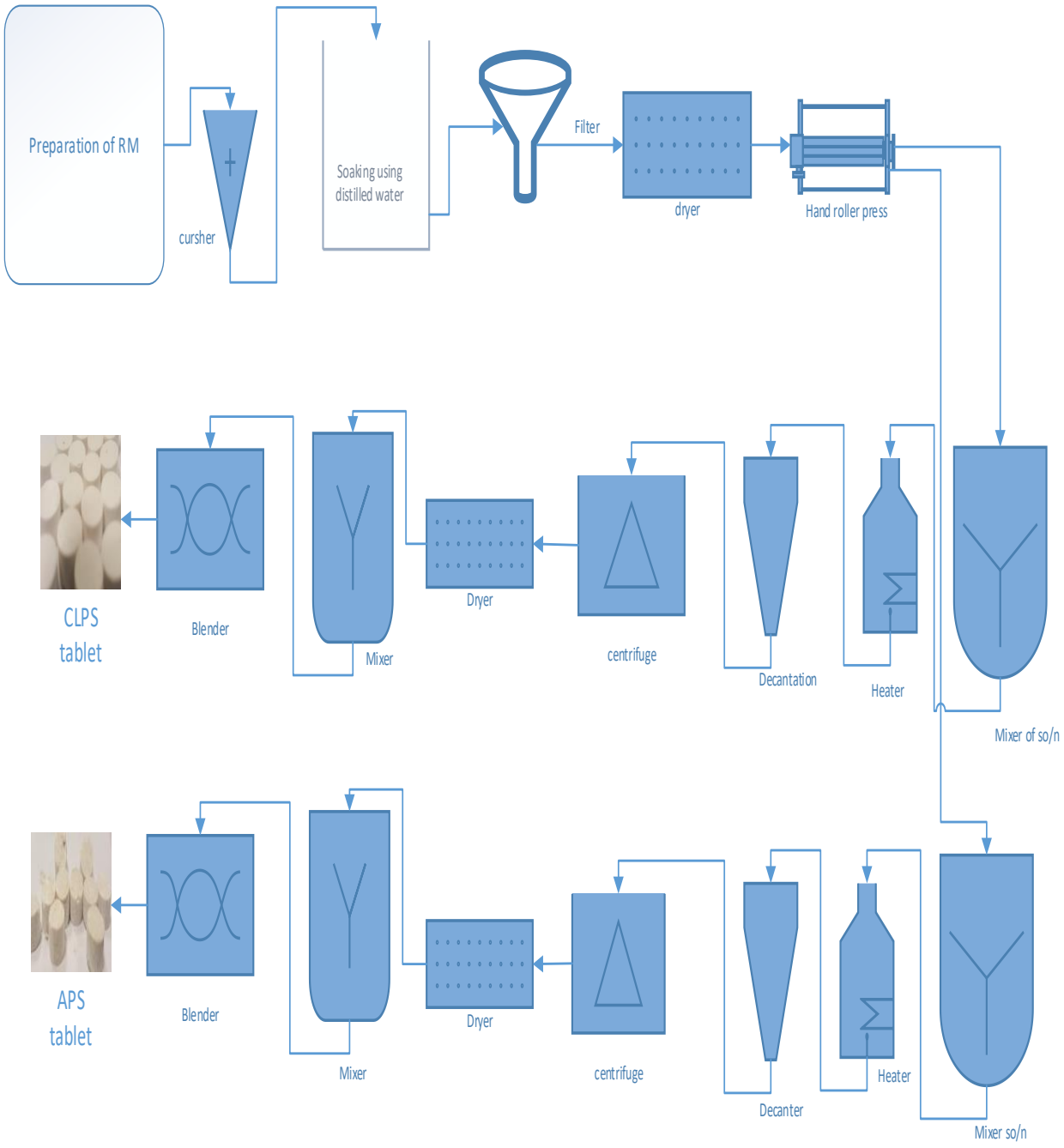

Figure 3.1: Process flow diagram of CLPS and APS containing tablet formulation.

CHAPTER FOUR

4. Results and Discussions

4.1. Chemical Composition

The potato starch contained 10.39% moisture, 0.25% crude ash, 0.30% crude protein, 0.15% crude fat, and 89.50% total starch. The results of very low contents of protein and fat indicated that the obtained potato starch was very pure and that the residual protein had been thoroughly removed during isolation. The isolated potato starch contained 30.52% amylose and 69.48% amylopectin. The yield of the cross-linked and acetylated average result was in the range of (87.5 – 94.30) % and (82.36 – 92.75) % respectively.

The comparison of the composition of native potato starch with other starch such as maize and Enset According to Gebre-Mariam *et al*., 1996 the table below. In this paper, the amylose content was 30.52. On the other hand, the moisture, Ash, Fat, and protein content of potato by Gebre-Mariam were higher than these papers.

Table 4.1. chemical composition of Enset, potato, and corn (Gebre-Mariam et al., 1996).

Starch components	Enset	Potato	Corn	Potato (this lab result)
Moisture(%)	14	13.5	13.0	10.39
Ash(%)	0.16	0.20	0.10	0.25
Fat(%)	0.25	0.05	0.50	0.15
Protein(%)	0.35	0.20	0.40	0.30
Amylose(%)	29.0	29.3	29.1	30.52

According to Assefa *et al*., 2016, the proximate composition of the starch on a dry weight basis was found to be 0.14% ash, 0.21% lipid, 0.43% protein, and 99.2% starch. The amylose content

was 30.6%. Its true density and moisture content values were 1.47 g/mL and 11.2%, respectively. In this paper, the proximate composition of the starch on a dry weight basis was found to be 0.25% ash, 0.15% lipid, 0.30% protein, and 89.50% starch. The amylose content was 30.52%. Its true density and moisture content values were 1.46 g/mL and 10.39%, respectively. these two papers almost have similar content of chemical composition [16].

4.2. Densities and Related Properties

As shown in Table 4.2. The bulk and tapped densities increase when the starch in both modifications but in some amount acetylated starch higher that cross-linked so cross-linked starch difficult to compress in tablet formation as a result of large volume. In carr's index and Hausner ratio native starch is higher than modified one and an insignificant difference between native and modified starch in true density. On other hand, the gap between bulk and tapped density decrease when native starch-modified in both modifications.

Table 4.2. Density and related property

Potato starch (PS)	Density and related property				
	Bulk Density	Tapped Density	Carr's Index	Hausner ratio	True Density
Native	0.56	0.70	20	1.25	1.46
Cross-linked	0.68	0.75	9.33	1.102	1.48
Acetylated	0.70	0.78	10.26	1.11	1.49

$$\text{True density} = \frac{w1 * sg}{[w1 + w2 - w3]} \quad (4.1)$$

Where

W1= Weight (g) of a starch sample,

W2= Weight (g) of a volumetric flask filled with xylene,

W3= Weight (g) of volumetric flask plus sample plus xylene left after displaced by the sample, and

Sg= Specific gravity of xylene (g/ml) (0.87).

4.3. Basic Characteristics of Native and Modified Starch

4.3.1. pH Determination

The PH value of modified starch is different APS is acidic property which is 4.65 and CLPS is near to neutral which is 6.80.

4.3.2. Analysis of Fourier Transforms Infrared Spectroscopy (FTIR)

The graph of FTIR help to determine the starch whether modified or not by observing the IR absorption peak of the sample. In figure 4.1. The characteristic IR absorption peaks CLPS are observed at 3599 cm-1 due to –O–H stretching vibrations, and the intense absorption peak that occurred at 2927 cm-1 was presumably originated from the stretching vibrations of the –C-H group. Another characteristic absorption band due to –C=C stretching vibrations in the medium appearance of the aromatic ring was presented at 1658 cm-1. The characteristic absorption bands occurred due to the –C–H stretching was shown at 1243 originated from the stretching vibrations of –P=O-group, and The characteristic IR absorption peaks ACPS are observed at3424 cm-1 due to –O–H stretching vibrations, and the intense absorption peak occurred at 3003 cm-1 due to stretching weak –O–H. Another characteristic absorption band due to –C=O stretching vibrations in the medium appearance of the aromatic ring was presented at 1748 cm-1. 1411 and 648 were another absorption band of FTIR result in ACPS grouped in –S=O and –C-H stretching respectively. For this study, FTIR data tell as native starch-modified or not, in CLPS of FTIR data 1243 stretching vibration tell as there was cross-linking of the native starch with phosphorus content compound so the starch hydroxyl functional group replace by SHMP functional group and in APS of FTIR data 1748 stretching vibration which shows that native starch hydroxyl group replace by –C=O functional group of acetic anhydride this implies that starch was acetylated.

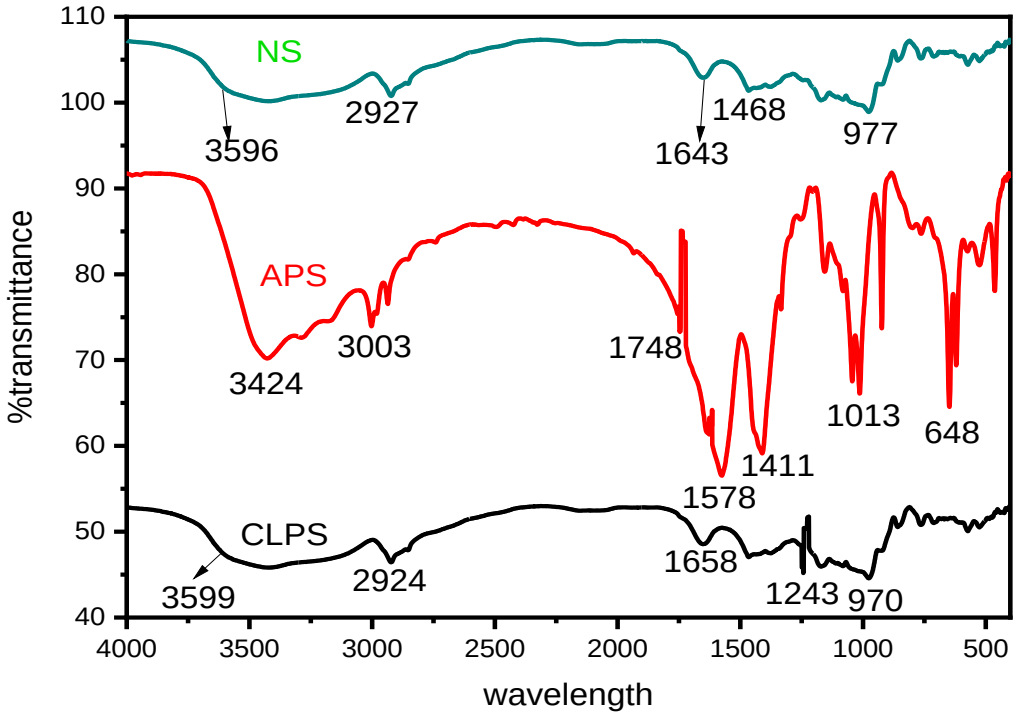

Figure 4.1: FTIR of native and modified starch

4.3.3. Determination of Starch Sydration Capacity

Table 4.3: Hydration capacity

Starch	Sediment(g)	Dry sample(g)	Hydration capacity
Native starch 1	1.35	1	1.35
Native starch 2	2.16	1	2.16
Native starch 3	1.56	1	1.56
Acetylated starch 1	2.54	1	2.54
Acetylated starch 2	3.44	1	3.44
Acetylated starch 3	2.157	1	2.157
Cross-linked starch 1	1.29	1	1.29
Cross-linked starch 2	4.5466	1	4.5466
Cross-linked starch 3	1.4092	1	1.4092

The average hydration capacity of NS, APS, and CLPS was 1.69, 2.71, and 2.415 by using table 4.2. The starch hydration capacity become increases when the starch-modified. From this study, the hydration of NS was lower than both modifications. When compare the hydration capacity of CLPS and APS, APS had more potential to hold the water.

4.3.4. Swelling Power and Solubility Determination

The swelling power of starch has been reported to depend on the water holding capacity of starch molecules by hydrogen bonding[89]. Hydrogen bonds stabilize the structure of the double helices in crystallites. When starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules are linked by hydrogen bonding to the exposed OH groups of amylose and amylopectin. This causes an increase in granule swelling and solubility[90].

4.3.4.1. Swelling Power and Solubility of Cross-linked and Acetylated Starch

Table 4.4: Swelling power and solubility of cross-linked potato starch

	Temperature effect		Time effect		Concentration effect	
	Lowest value	highest value	Lowest value	highest value	Lowest value	highest value
Solubility	26	50	10	22	40	60
Swelling power	2.76	11.56	1.97	3	0.66	1.5

Table 4.5: Swelling power and solubility of acetylated potato starch

	Temperature effect		Time effect		Concentration effect	
	Lowest value	highest value	Lowest value	highest value	Lowest value	highest value
Solubility	34	62	14	30	44	62
Swelling power	2.91	18.21	1.55	3.31	4.10	19.73

Temperature Effect on Swelling Power and Solubility

The solubility and swelling power of cross-linked and acetylated potato starch at temperatures ranging between 45 °C and 75 °C are depicted in Table 4.4 and 4.5. As expected, the swelling power and solubility of the starches increased with temperature. Solubility of cross-linked and acetylated potato starch was found to increase considerably from 26 - 50 and 34 -62 as well as

swelling power also increasing from 2.76 -11.56 and 2.91 - 18.21. From these results both solubility and swelling increasing in acetylated and cross-linked starch when the temperature increase. Acetylated starch was more solubility and swelling power value than cross-linked starch.

Time Effect on Swelling Power and Solubility

The solubility and swelling power of cross-linked and acetylated potato starch at a time ranging between 30 minutes and 90 minutes are depicted in Table 4.4 and 4.5. As expected, the swelling power and solubility of the starches increased with time. Solubility of cross-linked and acetylated potato starch was found to increase considerably from 10 - 22and 14 - 30 as well as swelling power also increasing from 1.97 - 3 and 1.55 – 3.31. From these result both solubility and swelling increasing in acetylated and cross-linked starch when the time increase. Acetylated starch was more solubility and swelling power value than cross-linked starch.

Concentration Effect on Swelling Power and Solubility

The solubility and swelling power of cross-linked and acetylated potato starch at a concentration ranging between 100 and 300 are depicted in Table 4.4 and 4.5. As expected, the swelling power and solubility of the starches increased with concentration. Solubility of cross-linked and acetylated potato starch was found to increase considerably from 40 - 60 and 44 - 62 as well as swelling power also increasing from 0.66 -1.5 and 4.10 – 19.73. From these results both solubility and swelling increasing in acetylated and cross-linked starch when the concentration increase. Acetylated starch was more solubility and swelling power value than cross-linked starch.

4.3.5. Morphological Studies of Samples

Figure 4.2: depicts scanning electron micrographs of cross-linked potato starch was composed of single entities of small and large grains with rounded shape. When acetylated potato starch was very small and the space between grains insignificant showing no signs of fissures even under high magnification.

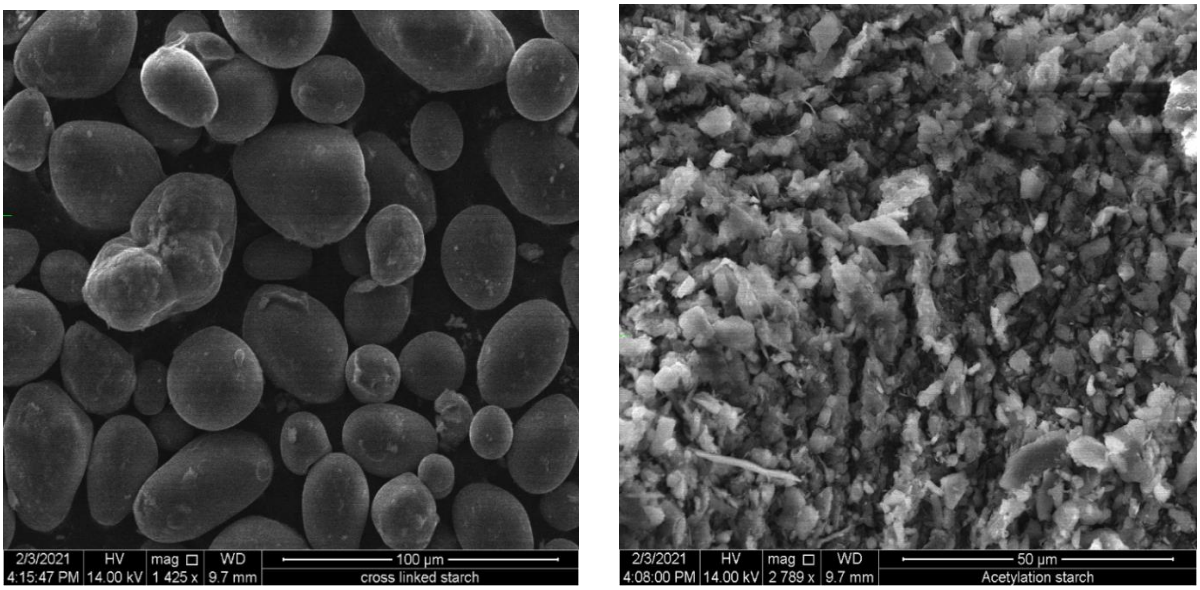

Figure 4.2: SEM of CLPS and APS

4.4. Formulation of Tablet

The tablet is formed by a direct compressor, which contains the active ingredient and modified starch with lubrication. From this study tablet that contains APS needed more lubricant as a result of the side of the tablet stack on the compressor. When the tablet containing CLPS was relatively, smooth the side of the tablet. On the other-hand APS moisture content higher than CLPS one sit's difficult to compressor until all moisture out from the surface.

When compare the density of APS denser than CLPS so tablet that contains APS is easy to form a more compact tablet as a result of the volume of APS is lower than CLPS. To get the more compact tablet, the density should be large with a small volume.

4.4.1. Basic Characteristics of Tablet

Weight, Tensile Strength, Crushing Strength, and Thickness

As shown in Table 4.6 and 4.7, the tablets contain the cross-linked starch weight, tensile strength, and crushing strength were higher than acetylated starch as a result of the density of cross-linked starch was better than acetylated one so when density inversely related to weight. On the other hand, thickness is constant for both acetylated and cross-linked starch and diameter directly related to crushing strength. According to laboratory results, tensile strength had direct dependence on crushing strength.

Table 4.6: Characteristics of the tablet of cross-linked starch

Run	Weight(g)	Thickness(mm)	Diameter (mm)	Crushing strength(N)	Tensile strength (10^6)
1	0.46	5	9.95	72	0.9218
2	0.465	5	9.94	70	0.8971
3	0.49	5	10.08	71	0.8973
4	0.485	5	9.93	77	0.9878
5	0.4725	5	9.92	80	1.027
6	0.47	5	9.93	71	0.9108
7	0.47	5	9.96	79	1.01
8	0.485	5	9.98	73	0.9318
9	0.49	5	10.12	75	0.944
10	0.48	5	10.07	78	0.9867

Table 4.7: Characteristics of the tablet of acetylated starch

Run	Weight(g)	Thickness(mm)	Diameter (mm)	Crushing strength(N)	Tensile strength
1	0.45	5	9.80	63	0.8189
2	0.465	5	9.83	62	0.8034
3	0.455	5	9.80	71	0.9229
4	0.475	5	9.88	60	0.7736
5	0.4625	5	9.86	70	0.9044
6	0.48	5	9.93	64	0.8210
7	0.4725	5	9.89	72	0.9274
8	0.485	5	9.91	67	0.8612
9	0.46	5	9.85	65	0.8406
10	0.4825	5	9.90	68	0.8750

Friability

Tablets made from ACPSs exhibited lower friability than those tablets made from CLPS. The loss in total weight of the tablets made from ACPS due to friability was 0.46 but those tablets made from CLASs lies in the range of 0.73 in the formulations. Their friability values were less than 1%, which ensures that the formulated tablets were mechanically stable. However, the friability of native starch was 1.39. Both CLPS and APS were friability less than 1% but NS had corresponding friability values greater than 1%, and they did not meet the pharmaceutical requirements (USP30/NF25, 2007).

4.5. Experimental Design Analysis of Potato Starch Modification

General factorial of Design-Expert 11.0.0 software was used for statistical analysis and to determine the optimal conditions of the starch modification process. The relationship between the factors and the performance measures is expressed by multiple regression equations, which can be used to estimate the expected values of the performance level for any factor levels.

Table 4.8: Build information summary

A. Build Information model summary

File Version	11.1.2.0		
Study Type	Factorial		
Design Type	Full Factorial	Runs	27
Design Model	2FI	Blocks	No Blocks
Center points	0		

B. Build Information of factors

Factor	Name	Units	Type	Low Actual	High Actual	mean	Std. Dev.
A	Concentration	%	Numeric	100	300	200	81.650
B	Temperature	Degree centigrade	Numeric	45	75	60	24.495
C	Time	Minute	Numeric	30	90	60	12.247

C_1. Build Information of Responses

Response	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev	Ratio	model
Peak viscosity	Cp	27	polynomial	1031	1681	1340.667	153.508	1.63	Linear
Degree of cross-linked	%	27	Polynimial	26.814	72.7753	63.043	8.138	2.714	Mean

Based on the full factorial result and using the relationships in Table 4.8, a total of 27 runs were performed which measures process stability and inherent variability. Peak viscosity and degree of cross-linked were used as the response and basis to evaluate the effects of various process parameters that are studied under the production process. Peak viscosity is defined as the maximum viscosity of modified starch recorded by vary the temperature (50, 70, 90)°C, and the Degree of cross-linked is defined as the ratio of the difference peak viscosity of modified and native starch to peak viscosity of native starch sample used. The responses from the various experimental runs with the design matrix of the full factorial are shown in table 4.7

Table 4.9: Experimental Design and result (modification of starch by cross-linked)

Run	Factor A Conc.(%)	Factor B Time (Min)	Factor C Temperature(^{0}C)	Response 1 Peak viscosity (Cp)	Response 2 DCLPS
1	100	30	60	1256	66.8339
2	200	30	75	1256	66.8339
3	300	90	75	1564	58.7008
4	200	90	75	1653	56.3507
5	100	90	60	1501	60.3644
6	300	30	45	1031	72.7753
7	100	90	75	1681	55.6113
8	200	30	60	1207	68.1278
9	200	60	60	1356	64.1933
10	200	60	75	1366	63.9292
11	100	30	45	1138	69.9498
12	300	90	45	1383	63.4803
13	300	60	45	1271	66.4378
14	100	90	45	1420	62.5033
15	200	60	45	1290	65.9361
16	200	90	60	1481	60.8925
17	200	30	45	1054	72.1679
18	100	60	75	1380	63.5595
19	300	30	75	1246	67.098

20	300	30	60	1181	26.8144
21	300	90	60	1463	61.3678
22	300	60	75	1357	64.1669
23	300	60	60	1346	64.4574
24	200	90	45	1386	63.4011
25	100	60	45	1303	65.5928
26	100	30	75	1271	66.4378
27	100	60	60	1357	64.1669

$C_{2.}$ Build Information of Responses

Response	Units	**Observations**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**	**Ratio**	Model
Acetyl content	%	27	Polynomial	5.1375	36.7875	22.86	9.26	7.16	2FI
Degree of acetylation		27	Polynomial	0.20377	2.16324	1.17	0.5873	10.62	Quadratic

Based on the full factorial result and using the relationships in Table 4.9, a total of 27 runs were performed which measures process stability and inherent variability. Acetyl content and degree of acetylation were used as the response and basis to evaluate the effects of various process parameters that are studied under the production process. Acetyl content is defined as the total content acetyl in modified potato starch express by present and degree of cross-linked is defined as the measurement of the degree of replacement of hydroxyl group of native starch by acetyl functional group. The responses from the various experimental runs with the design matrix of the full factorial are shown in table 4.8

Table 4.10: Experimental Design and result (modification of starch by acetylation method)

Run	Factor 1 Concentration %	Factor 2 temperature Degree centigrade	Factor 3 Time Minute	Response 1 Acetyl Content %	Response 2 Degree of Acetylation
1	200	75	90	13.031	0.562534
2	200	45	90	29.5625	1.56591
3	300	60	90	25.2025	1.25954
4	200	45	30	36.25	2.11431
5	300	75	30	22.575	1.09108
6	100	60	30	22.0375	1.05798
7	300	60	30	26.775	1.36596
8	100	45	60	25.73	1.29476
9	200	75	30	16.255	0.727979
10	200	60	90	17.8175	0.812699
11	200	60	60	21.5	1.02532
12	100	75	60	8.8	0.362711
13	100	75	30	10.4725	0.439502
14	200	45	60	34.15	1.93052
15	100	60	60	16.7625	0.755157
16	300	45	90	32	1.75372
17	300	45	30	36.7875	2.16324
18	300	45	60	35.275	2.02755
19	100	45	30	34.15	1.93052
20	200	75	60	15.6975	0.69849
21	100	45	60	31.015	1.67628
22	100	75	90	5.1375	0.203778
23	100	60	90	10.95	0.461941
24	300	60	30	30.4575	1.63339
25	300	75	60	17.308	0.784732

26	300	75	90	15.204	0.672701
27	200	60	30	26.2575	1.33046

According to Hyemi Heo, the DS was calculated by the phosphorus content of CLPS with an increase in the concentration of STMP/STPP, the DSS for the CLPS samples increased from 5.561 to 36.136. In this paper, the DS was calculated by the phosphorus content of CLPS With an increase in the concentration of SHMP, the DSS for the CLPS samples increased from 56.3507% to 72.7753%, and DS of starch by Acetylation method With an increase in the concentration of Acetic anhydride, the DSS for the APS samples increased from 0.203778 to 2.16324 but DS decrease with increasing temperature and time [91].

4.6. Model Equation

A model equation is a mathematical correlation that expresses the relation between the factors and the response. The model equation was developed to show the correlation between the hydrolysis parameters and the yield of cellulose nanocrystals (CNCs). Design expert v11 software quadratic model was found to be adequate for the prediction of the given yield as shown by the following equation Eq.4.2 and Eq. 4.3

Final Equation in Terms of Coded Factors of Acetyl content and Degree of acetylation

Acetyl Content = 22.86 + 4.25*A – 3.37 * B -9.47* C + 0.62* A*B + 1.46 * A*C + 0.33 *B *C

Degree of acetylation = 1.11 + 0.25 * A – 0.22 * B -0.61 * C + 0.022 * A * B + 0.042 * A * C + 0.065 * B *C – 0.033 * A^2 - 9.855E-003 * B^2 + 0.14 * C^2

Final Equation in Terms of Actual Factors of Acetyl content and Degree of acetylation

Acetyl Content = 75.75680 – 0.028230* concentration – 0.19756 * Time – 0.86986 * Temperature + 2.06361E-004 * concentration *Time + 9.72750E-004 * concentration * Temperature + 7.34259E-004 * Time * Temperature

Degree of acetylation = 6.52942 + 1.76785E-003 * concentration – 0.015988 * Time – 0.13147 * temperature + 7.38261E-006 * concentration * Time + 2.77540E-005 * concentration *

temperature + 1.43358E-004 * time * Temperature – 3.34390E-006 *concentration -1.09504E-005* Time +6.40845E-004 *Temperature

Final Equation in Terms of Coded Factors of peak viscosity and Degree of cross-linked

Peak viscosity = 1340.67 - 25.83*A + 160.67*B + 83.22*C

Degree of cross-linked = 63.04

Final Equation in Terms of Actual Factors of peak viscosity and Degree of cross-linked

Peak viscosity = 738.11111 – 0.25833*concentration + 5.35556*time + 5.54815* Temperature

Degree of cross-linked = 63.04263

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Where: A is the acid concentration (wt.%), B is the Temperature (℃), C is the time(min) of acetylated starch**.**

4.7. Analysis of variance (ANOVA) for Starch modification

The statistical significance and the goodness of fit of the developed quadratic model, as well as the effect of individual variables and their interactions, were analyzed by analysis of variance (ANOVA). The results of the analysis of variance are shown in table 4.10.

ANOVA for Selected Factorial Model for Cross-linked Starch

The F value and P-value of the obtained factorial model presented in table 4.8were found to be 132.03 and <0.0001 respectively. This implies that the factorial model is significant. As a result that, the model can sufficiently predict the Peak viscosity and Degree of cross-linked based on the investigated factors affecting the production process. The p-value of the main three effects modified agent concentration, Time, and Temperature i.e. A, B and C respectively were significant based on their estimated p values (<0.05) which indicated that the model was suitable for use in this experiment and suggesting that all parameters influence the Peak viscosity and Degree of cross-linked. The interactive effects of BC are significant ($p<0.05$).

Table 4.11: ANOVA for a selected factorial model for cross-linked starch

Response 1: Peak Viscosity

Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Model	6.013E + 005	3	62885.20	132.03	<0.0001	Significant
A-Concentration	12012.50	1	12012.50	7.91	0.0099	
B-Time	4.646E + 005	1	4.646E + 005	306.05	<0.0001	
C-Temperature	1.247E + 005	1	1.247E + 005	82.11	<0.0001	
Residual	34918.61	23	1518.20			
Cor Total	6.362E+05	26				

ANOVA for Selected Factorial Model for Acetylated Starch

In acetylated potato starch, the F value and P-value of the obtained factorial model presented in table 4.9had a small difference. For Acetyl content were found to be122.88 and <0.0001 and 136.10 and <0.0001 for Degree of acetylation respectively. These imply that the factorial model is significant. As a result, that, the model can sufficiently predict the Acetyl content and Degree of acetylation based on the investigated factors affecting the production process. The p-value of the main three effects modified agent concentration, Time, and Temperature i.e. A, B and C respectively were significant based on their estimated p values (<0.05) which indicated that the model was suitable for use in this experiment and suggesting that all parameters influence the Peak viscosity and Degree of cross-linked. The interactive effects of AB are significant (p<0.05).

Table 4.12: ANOVA for a selected factorial model for acetylated starch

Response 1: Acetyl Content

Source	Sum of square	Df	Mean square	F-value	P-value	
Model	2200.22	10	220.02	122.88	<0.0001	
A-concentration	329.22	2	164.61	91.94	<0.0001	significant
B-Temperature	1624.43	2	812.21	453.63	<0.0001	
C-Time	204.48	2	102.24	57.10	0.0042	
AB	42.10	4	10.52	5.88		

Residual	28.65	16	1.79			
Cor Total	2228.87	26				

Response 2: Degree of acetylation

Source	Sum of Squares	Df	Mean Square	F-value	P-value	
Model	8.75	6	1.46	136.10	<0.0001	significant
A-Concentration	1.17	2	0.5833	54.41	<0.0001	
B-Temperature	6.74	2	3.37	314.43	<0.0001	
C-Time	0.8460	2	0.4230	39.46	<0.0001	
Residual	0.2144	20	0.0107			
Cor Total	8.97	26				

4.8. Diagnostics plot of factorial Model

Normality of the Data

The normality of the data was done using a normal probability plot. These graphs show that the deviation of the variance from a straight line which implies the normal distribution whether close to a straight line or not helps to understand the experiment is done correctly or not.

Normality of the Data of Cross-linked Starch

The normal probability plot of the residual for both Peak viscosity and Degree of Cross-linked is shown in Figure 4.3: which reveals that the residuals for peak viscosity are falling reasonably close to the straight line with little scattering than the Degree of cross-linked. Both responses are close to a straight line, which implies that distributed normally showing no deviation of the variance. Therefore, the normality assumption was satisfied as the residual plot approximated along a straight line.

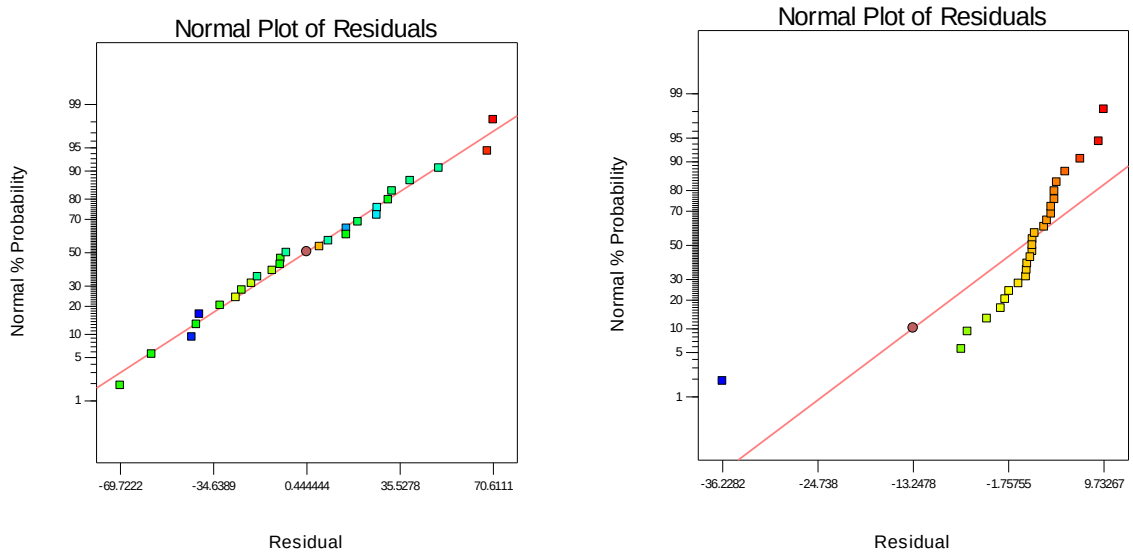

Figure 4.3: Normality of the Data of Cross-linked Starch

Normality of the Data of Acetylated Starch

The normal probability plot of the residuals for both Acetyl content and Degree of acetylation are shown in Figure 4.4: which reveals that the residuals for acetyl contents are falling reasonably close to the straight line with little scattering than the Degree of acetylation. Both responses are close to a straight line, which implies that distributed normally showing no deviation of the variance. Therefore, the normality assumption was satisfied as the residual plot approximated along a straight line.

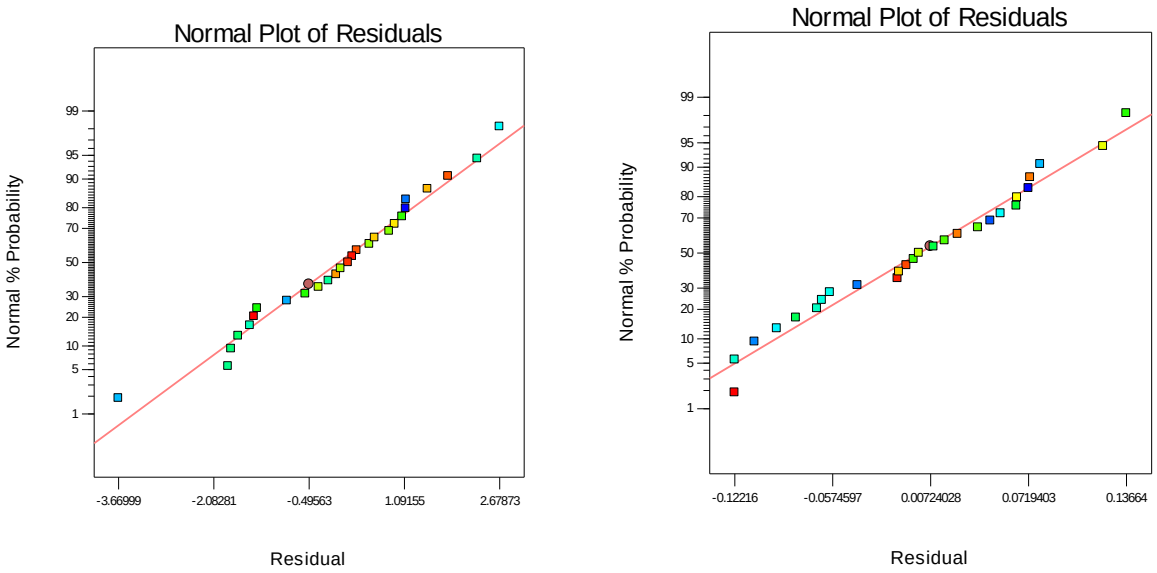

Figure 4.4: Normality of the Data of Acetylated Starch

4.9. Residuals Vs Run

Residuals Vs Run of Cross-linked Starch

Figure 4.5: presents a plot of residuals versus experimental run order. It checks for lurking factors that may have influenced the response during the experimental run. The plot should show an arbitrary random scatter. From the plot, it has been observed that all the data points lie within the limits.

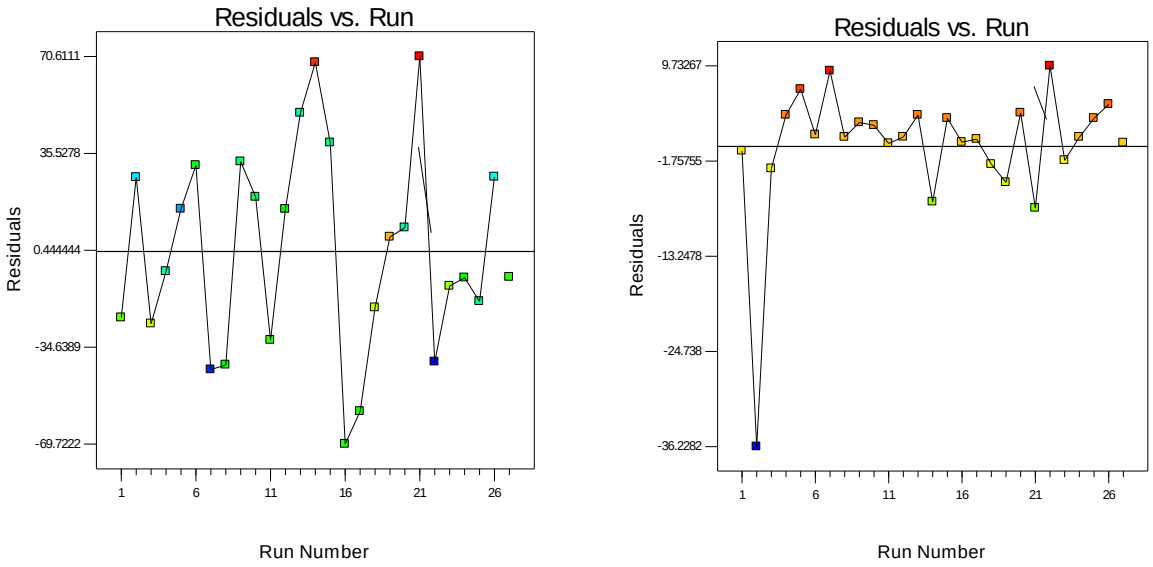

Figure 4.5: Residuals Vs Run of cross-linked Starch

Residuals Vs Run Acetylated Starch

Figure: 4.6: presents a plot of residuals versus experimental run order. It checks for lurking factors that may have influenced the response during the experimental run. The plot should show an arbitrary random scatter. From the plot, it has been observed that all the data points lie within the limits.

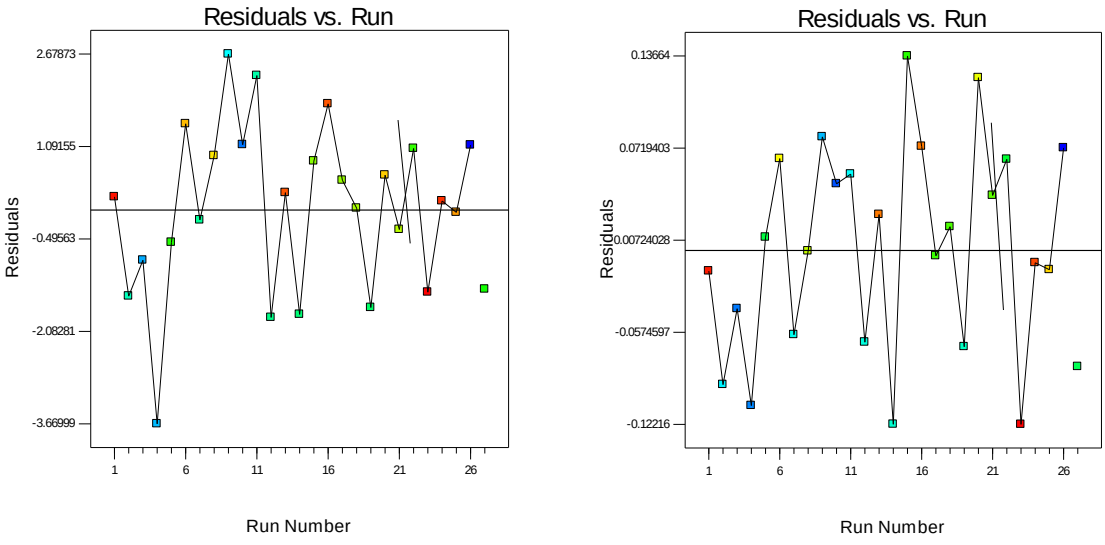

Figure 4.6: Residuals Vs Run Acetylated starch

4.10. Experimental variables Effects on the Response of Starch Modification

In both acetylation and cross-linked modification concentration, time, the temperature is the measure factors, which affect the degree of modification of the starch. Individual effects using a design expert software graph were used to investigate the cause-effect individual and interaction of three process variables: temperature (45- 75°C), time (30-90 min), and concentration (100 - 300 wt.%).

4.10.1. Variable Effect on Peak Viscosity and Degree of Cross-linked Potato Starch

Concentration Effect on Peak Viscosity and Degree of Cross-linked

The effect of modified agent concentration on peak viscosity and Degree of cross-linked are different when peak viscosity was inversely related but directly proportional to the Degree of cross-linked in Figure 4.7: when concentration increases from 100 to 300 at constant concentration and time peak viscosity decrease from 1138 to 1031 and Degree of cross-linked increasing from 69.9498 to 72. This is due to the increasing amount of functional group of a modified agent so that contact between the modified agent and native starch also increases.

Temperature Effect on Peak Viscosity and Degree of Cross-linked

The effect of temperature on peak viscosity and Degree of cross-linked also one major factor, which affects significantly. Temperature is directly related to peak viscosity and inversely related to the Degree of cross-linked. In Figure 4.8: when temperature increases at constant concentration and time from 45 to 75 peak viscosity increase from 1138 to 1271 and the Degree of cross-linked decreasing from 69.9498 to 66.4378. This is due to increasing temperature the potential to replace the hydroxyl group by modified agent decrease.

Time Effect on Peak Viscosity and Degree of Cross-linked

The effect of temperature on peak viscosity and Degree of cross-linked also another major factor, which affects the modification process. Timeless effect modification than temperature and its direct relation with peak viscosity and inversely related to Degree of cross-linked.

In Figure 4.9: when temperature increases at constant concentration and temperature from 30 to 90 peak viscosity increase from 1138 to 1420 and the Degree of cross-linked decreasing from 69.9498 to 62.5033. This is due to the increasing time the potential to replacing the hydroxyl group by modified agent decrease.

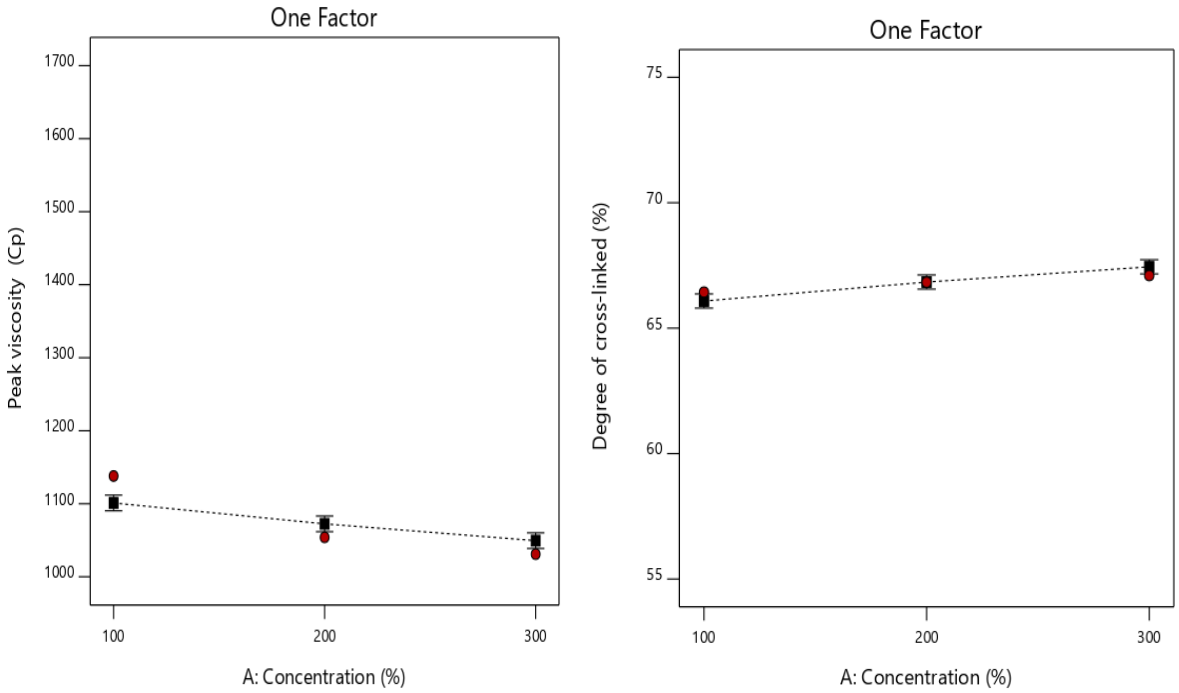

Figure 4.7: Concentration Effect on peak viscosity and Degree of cross-linked

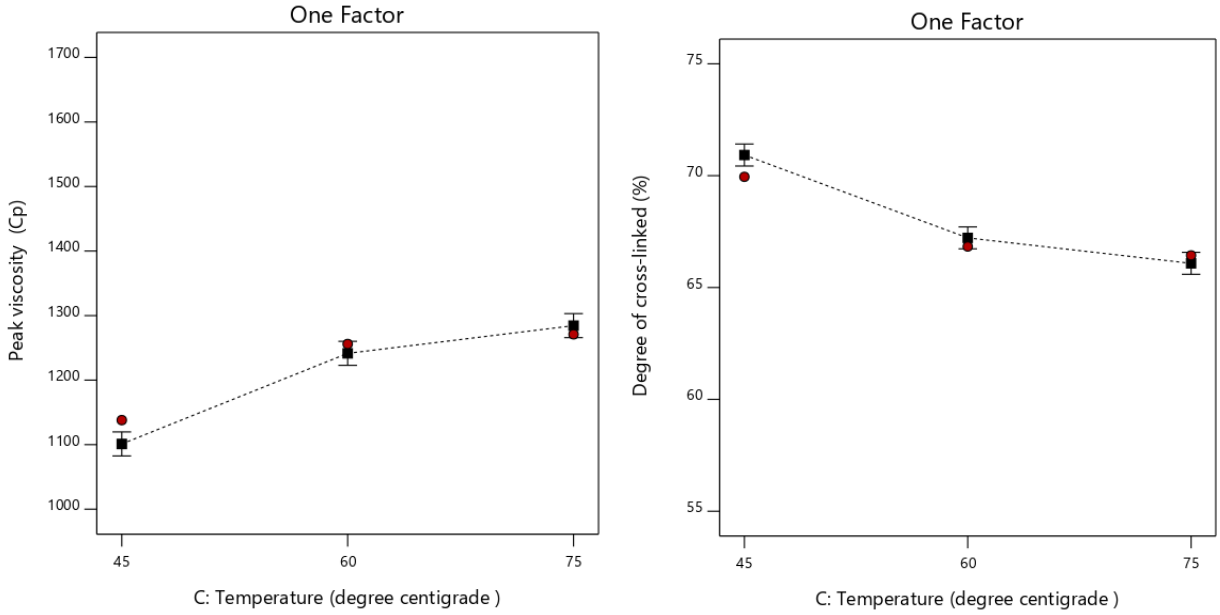

Figure 4.8: Temperature Effect on peak viscosity and Degree of cross-linked

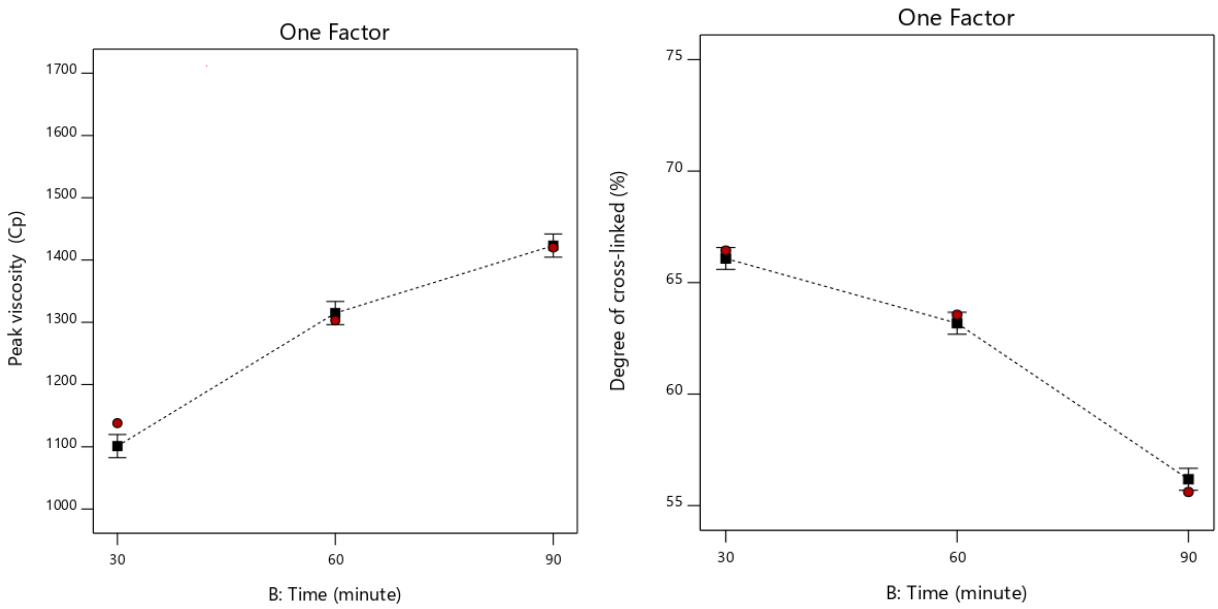

Figure 4.9: Time Effect on peak viscosity and Degree of cross-linked

4.11. Variable Effect on Acetyl content and Degree of Acetylation of Acetylated Potato Starch

Concentration Effect on Acetyl content and Degree of Acetylation

The effect of modified agent concentration on Acetyl content and Degree of acetylation are similar as a result of acetyl content and degree of acetylation are directly proportional to each other in Figure 4.10: when concentration increase from 100 to 300 at constant temperature and time Acetyl content increase from 34.15% to 36.7875 and Degree of acetylation increasing from 1.93052 to 2.16324. This is due to the increasing amount of Acetyl group of the modified agent so that contact between the Acetyl group and native starch also increase.

Temperature Effect on Acetyl content and Degree of Acetylation

The effect of temperature on Acetyl content and Degree of acetylation are similar as a result of acetyl content and degree of acetylation are directly proportional to each other in Figure 4.11: when temperature increase from 45 to 75 at constant concentration and time Acetyl content increase from 34.15% to 10.4725% and Degree of acetylation increasing from 1.93052 to

0.439502. This is due to increasing temperature Acetyl functional group's capability to replace the hydroxyl functional group of native starch become decrease.

Time Effect on Acetyl content and Degree of Acetylation

The effect of temperature on Acetyl content and Degree of acetylation are similar as a result of acetyl content and degree of acetylation are directly proportional to each other in Figure 4.12: when temperature increase from 30 to 90 at constant concentration and temperature Acetyl content increase from 34.15% to 25.73 and Degree of acetylation increasing from 1.93052 to 1.29473. This is due to increasing time Acetyl functional group capability to replace the hydroxyl functional group of native starch become decrease similar effect as a temperature.

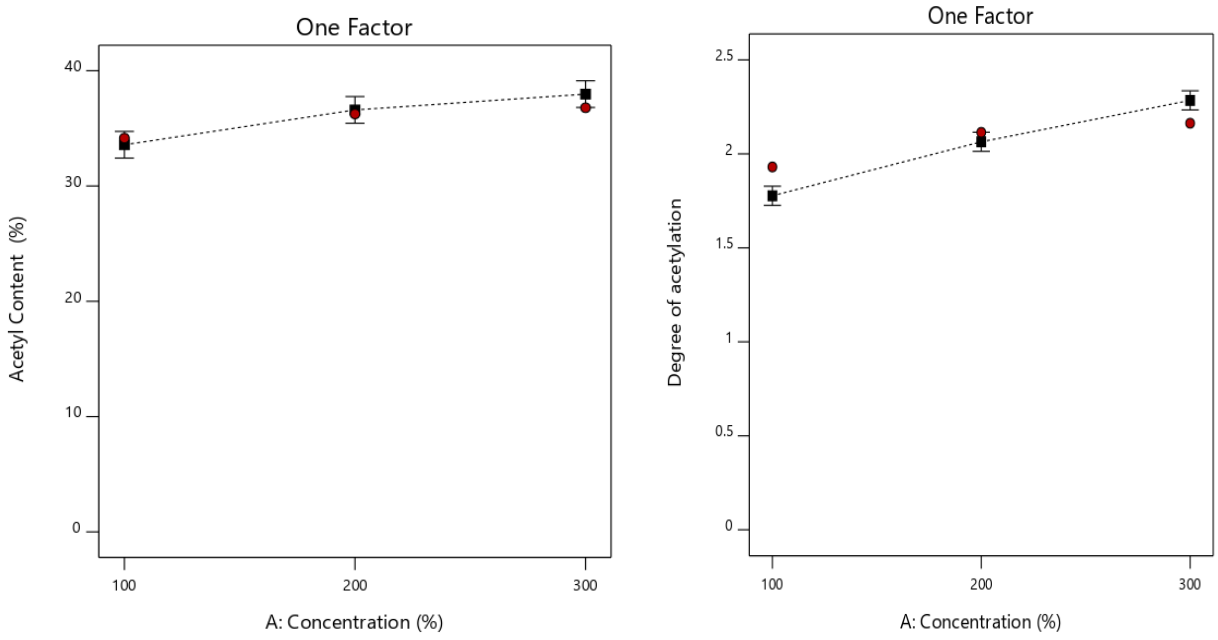

Figure 4.10: Concentration Effect on Acetyl content and Degree of acetylation

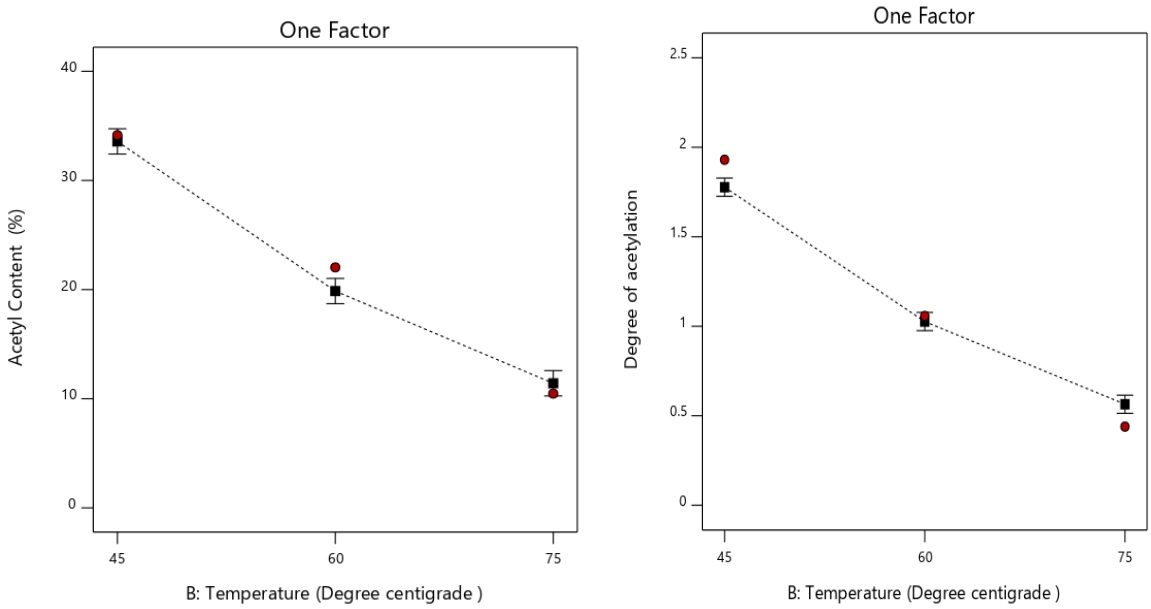

Figure 4.11: Temperature Effect on Acetyl content and Degree of acetylation.

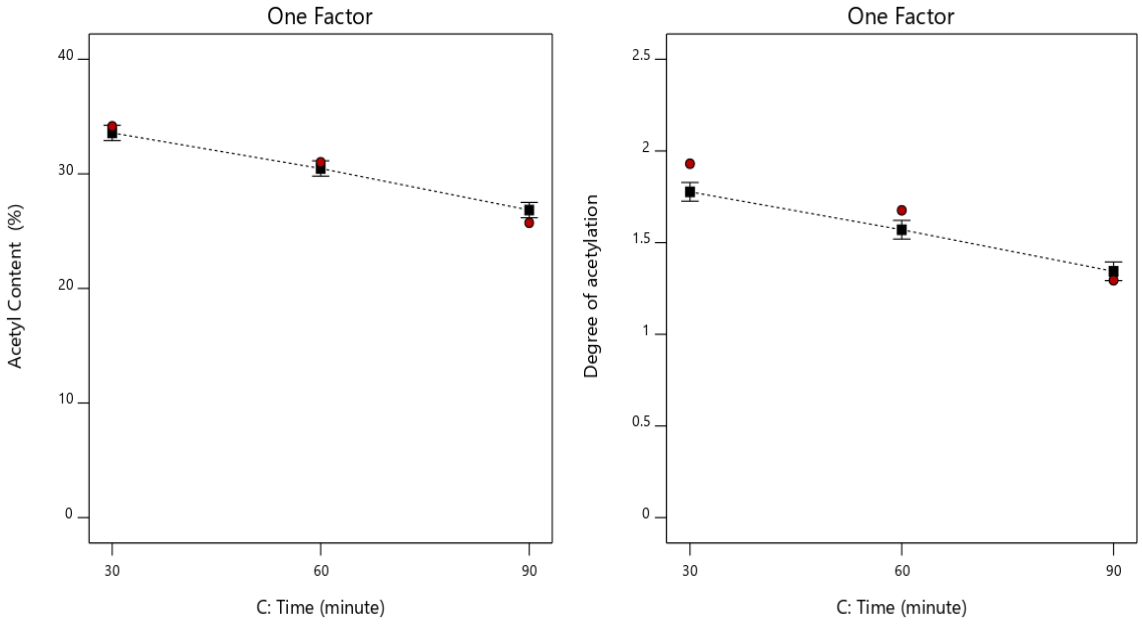

Figure 4.12: Time Effect on Acetyl content and degree of acetylation

4.12. Interaction effect of Temperature and Time on Modified Starch Response

4.12.1. Temperature and Time Effect on Peak viscosity and Degree of Cross-linked.

Interaction effects represent the combined effects of factors on the response. When an interaction effect was present, the impact of 1 factor depends on the extent of the other factor. The ability to estimate and test interaction effects between the factors involved is one of the advantages of DOE. Response surfaces were plotted using Design-Expert version 11.0.0 software to study the effects of parameters and their interactions on yield.

Figure 4.13: shows the interaction effect of Temperature and Time on the peak viscosity and Degree of cross-linked. As the temperature and time vary either increasing or decreasing, both respond. As shown in the Figures at minimum temperatures and minimum time the peak viscosity is low compared to peak viscosity at the maximum time and maximum temperatures. For instance, at a constant concentration condition at a temperature of 45℃ and time of 30minute, the corresponding peak viscosity is 1138Cp and the temperature and time rise to 75℃, and 90 minutes the peak viscosity become 1681Cp.On other hand at a constant concentration condition at a temperature of 45℃ and time of 30minute the Degree of cross-linked 69.9498 decreases to 55.6113 when the temperature and time at maximum value 75℃ and 90 minutes, Therefore, the interaction between temperature and time was significantly affecting both response and temperature and time are directly proportional each other.

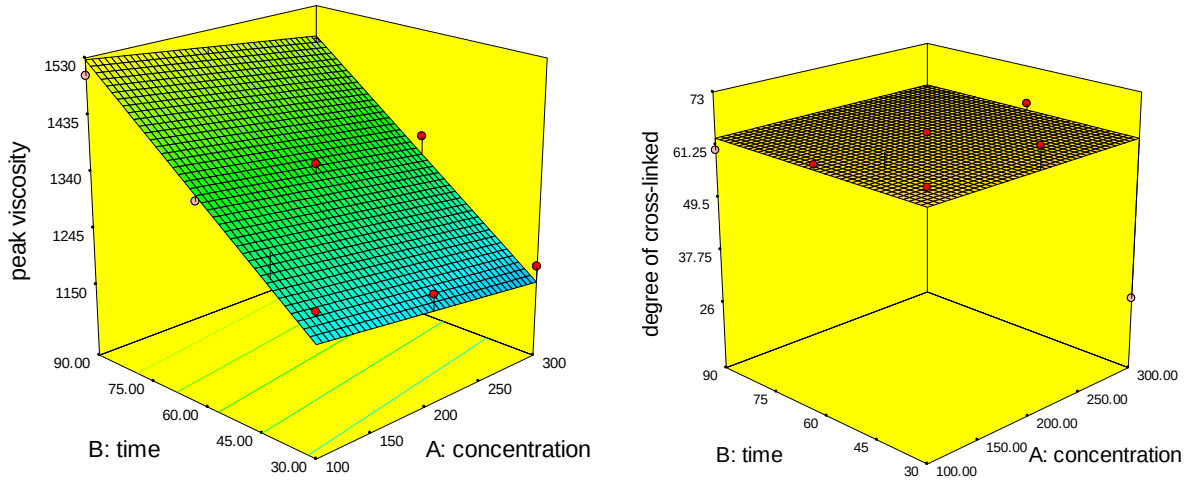

Figure 4.13: A (peak viscosity against time versus concentration) 3D Plot of peak viscosity and degree of acetylation against reaction time

4.12.2. Temperature and Concentration Effect on Acetyl content and Degree of Acetylation

Figure: 4.14: show the interaction effect of Temperature and concentration on the Acetyl and degree of acetylation. As the concentration and concentration vary either increasing or decreasing, both responses. As shown in the Figures at minimum temperature high value of both acetyl content and degree of acetylation but low concentration low value both acetyl content and degree of acetylation compared to both acetyl content and Degree of acetylation at maximum concentration and maximum temperatures. For instance, at a constant concentration condition at a temperature of 45℃ and concentration of 100, the corresponding acetyl content is 34.15% and the temperature and concentration rise to 75℃ and 300% the acetyl content become 22.575%, in other hands at a constant concentration condition at a temperature of 45℃ and concentration of 100% the Degree of acteylation1 was 1.93052 decrease to 1.09108 when the temperature and concentration at maximum value 75℃ and 300%Therefore, the interaction between temperature and concentration was significantly affecting both response and temperature and concentration are inversely proportional each other.

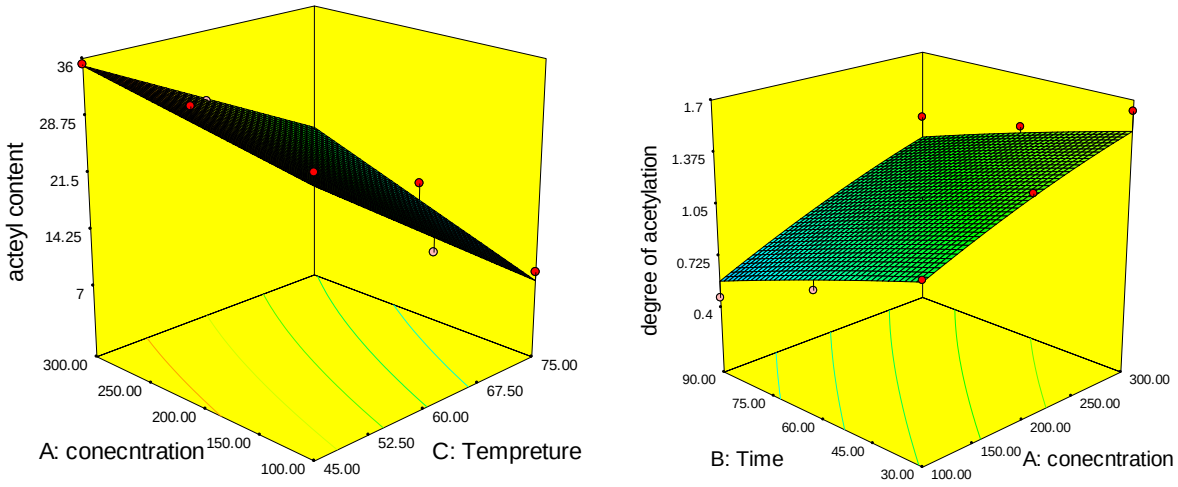

Figure 4.14: B (Acetyl content against temperature versus concentration) 3D Plot of Acetyl content and degree of acetylation against reaction time versus concentration

4.13. Optimization of Modified Potato Starch

Optimization of process variables had many goals such as maximize the degree of substitution of both modified starches, acetyl content of ACPS, and minimizing peak viscosity of CLPS. Economic benefit or increasing furfural yield by minimizing process cost. Determination of the optimum conditions for starch modification was obtained by Design Expert 11.0.0 using numerical optimization. Full factorial was used to maximize the desirability by finding out different combinations of independent process variables. As it has been discussed in the previous sections a prolonged concentration of modified agent will cause a maximizing degree of cross-linked and minimizing peak viscosity on the other hand increment both acetyl content and degree of acetylation, while an increase in temperature and time causes decrement of a degree of cross-linked and maximizing peak viscosity in other hand decrements both acetyl content and degree of acetylation. Therefore, optimizing process variables aimed to determine optimum conditions within the range of the values of the process variables by considering the constraints associated with variations of these process parameters.

Table 4.13: Optimization of cross-linked potato starch

Name	Goal	Lower limit	Upper limit
Concentration(%)	In range	100	300
Temperature(℃)	In range	45	75
Time(min)	In range	30	90
Peak viscosity(Cp)	maximize	1031	1681
Degree of cross-linked	maximize	26.8144	72.7753

Table 4.14: Optimization of acetylated potato starch

Name	Goal	Lower limit	Upper limit
Concentration(%)	In range	100	300
Temperature(℃)	In range	45	75
Time(min)	In range	30	90
Acetyl content(%)	Maximize	5.1375	36.7875
Degree of acetylation	Maximize	0.2038	2.1632

The values obtained for optimum production of modified potato starch were: For Both modifications method of maximum value of degree of substitution occur at concentration was 300%, temperature of 45℃ and reaction time of 30 min.

CHAPTER FIVE

5. Conclusion and Recommendation

5.1. Conclusion

The objective of this thesis was to assess the modification of potato starch by the cross-linked and acetylation method used as an excipient for tablet formation. The modification quality of modified starch measure by measuring peak viscosity and degree of cross-linked for CLPS and acetyl content and degree of acetylation for APS. Therefore, it has been concluded there is an inverse relationship between peak viscosity and degree of cross-linked for CLPS and a direct relationship between Acetyl content and degree of acetylation for APS.

From the compositional analysis carried out on particular native potato starch, it was observed that native potato starch (89.50%), crude protein (0.3%), crude fat (0.15%), ash content (0.25%), moisture content (10.39%), amylose content (30.52%) and amylopectin (69.48). when the starch-modified the density become increase. From this research we can conclude that density of APS > CLPS > NS.

In this study, the modification of potato starch by cross-linked and acetylation methods was investigated and optimized using the full factorial method. The study has shown that the experimental method can be used as an excellent tool to identify the interaction effect of the individual modification starch parameters. decreasing temperature and residence time while the increasing concentration of modified agents and decreasing peak viscosity and increases the degree of cross-linked for CLPS and increasing both acetyl content and degree of acetylation for APS. however, a temperature of 45 ℃, the concentration of modified agent of 300% and reaction time of 30 min was found as optimum values.

The FT-IR analysis of the product showed that the presence of characteristic absorption band due to –C=O stretching vibrations in the medium appearance of the aromatic ring was presented at 1748 cm-1conjugated carbonyl which shows the presence of acetyl functional group help to determine the starch was acetylated. Another hand the presence of characteristic absorption bands occurred due to the –C–H stretching was shown at 1243 originated from the stretching vibrations

of –P=O-group show the presence of phosphate-containing functional group help to determine the starch was cross-linked.

Tablet formed which containing CLPS had higher tensile strength, crushing strength and friability than APS.so CLPS advantageous as a result of did not crush in packaging and transportation of the tablet but APS also advantageous than CLPS because of friability is lower which help to increase the potential easily absorbed by the patient body. The higher value of viscosity of CLPS containing more smooth surface than APS so APS need more lubricant. According to density, APS was denser so it's more easily compressed but CLPS was less dense so it's difficult to compress.

5.2. Recommendation

There is a large production capacity of potatoes worldwide. Ethiopia has also an abundant amount of potato plants that grown both in nature and in nurture. However, this large cultivation of the potato, the plant is above 70% of Ethiopian farmers. On the other hand, starch modification is the interest of many research areas particularly in the pharmaceutical industry in that can fully or partially use as an excipient part of tablet formulation. Therefore, most of the current researcher's native starch modified to upgrade the quality and effectiveness of starch. Considering the results obtained in this research, the following recommendations are suggested for future works;

1. In these study, native potato starch modified by the cross-linked and acetylated method. But it is recommended that future studies should use another method of dual modification to investigate the effectiveness of modification by combining two or more methods of modification that could be a rise in the quality and quantity of degree of substitution functional group the starch native.

2. It is recommended that other researchers should be done further characterization of modified starch that was not used in this work such as dual modification and enzymatic modification.

3. Detailed economic feasibility studies in the production process are recommended since it is critical for the rationale of commercialization.

4. Further study and proposing suitable methods for obtaining modified starch should be investigated for the different application for the pharmaceutical industry to formulate tablet as a binder, Dis-integrant, Lubricants, and Anti-adherents

Finally, in this study, it has been discovered that a higher degree of substitution of the functional group of the native starch was achieved at a higher concentration of modified agent and lower temperature and time. Therefore, it has been recommended to study the maximum concentration of modified agent and lower temperature and time that higher degree of substitution. This would be helpful to produce modified starch which minimized the limitation of starch and upgrade the effectiveness of the tablet.

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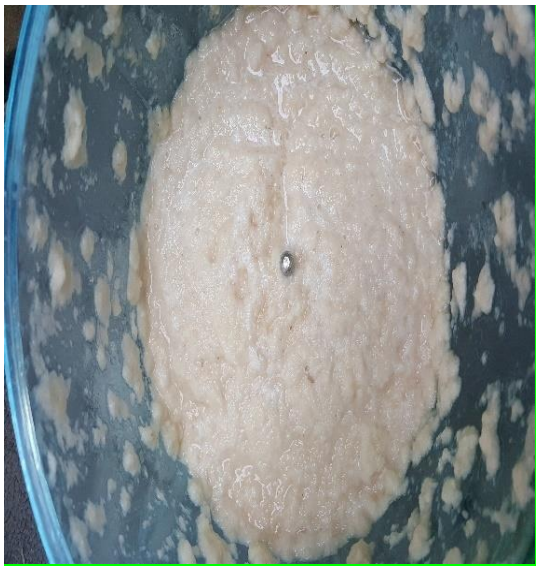
APPENDIXES

Appendix A. Lab work image

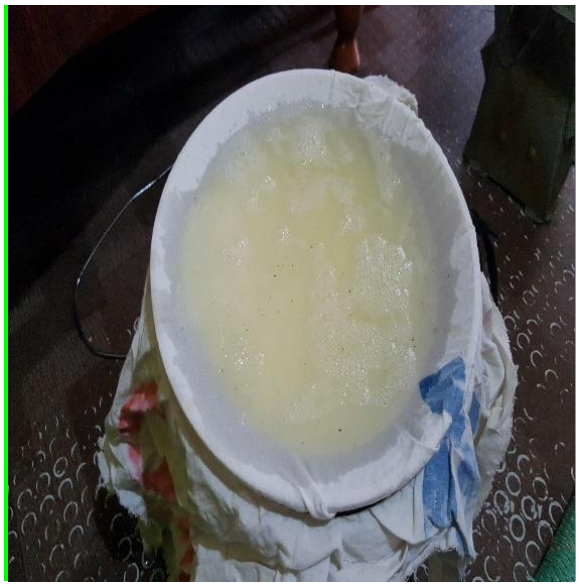
1. Raw material preparation and extraction of native starch

Preparation of potato (peel a potato).



Milling the raw material

soaking the potato in distillates water.



separation starch from slurry

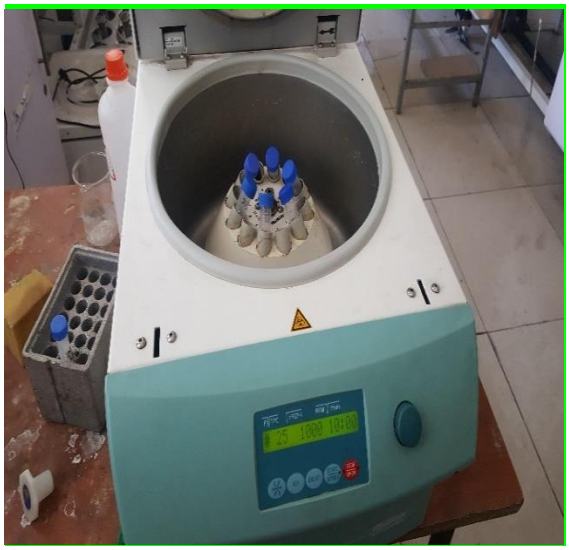
native starch

2. **Modification by starch by cross-linked and acetylation**

A B

C

D



E

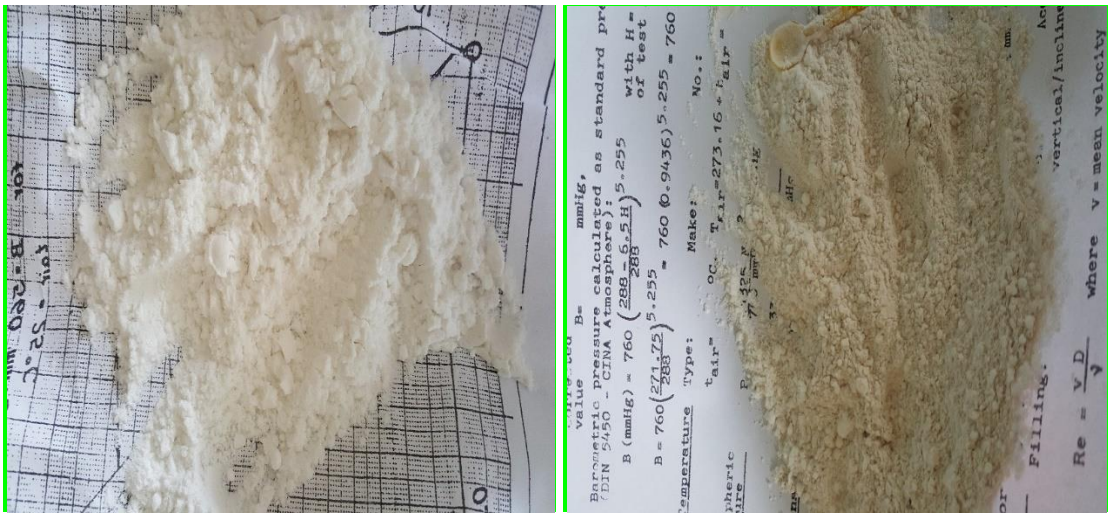
F

G H

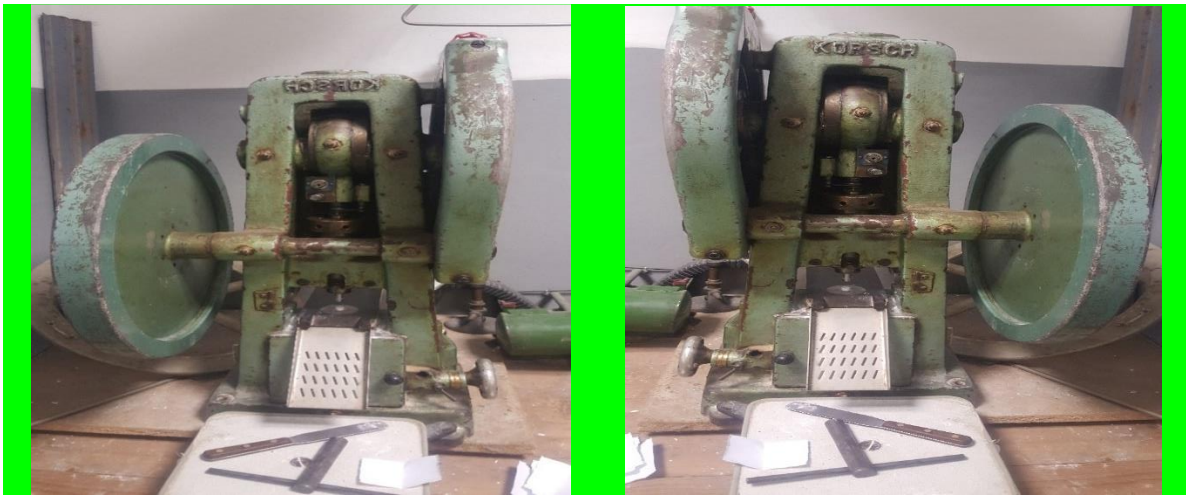
I J

Fig 2. modification of starch A) Mixing the starch with modified agent in distilled water B) PH adjustment C) Heating in water bath D) Decantation E) Centrifuge F) drying G)Shaking by shaker H) milling starch by mortal and pestle I) Titration to determine degree of acetylated J) Measuring peak viscosity to determine degree of cross-linked.

3. Preparation of tablet by using modified starch blended with the active site of tablet (paracetamol) in a Tubular mixer.



A



B

C


DIAMET. 9.35mm
HARDNESS 48.00N
CALEVA
DIAMET. 9.90mm
HARDNESS 60.00N
CALEVA

D

E

Fig 3 A) Mixing the starch with the active site, experiment, and lubricant B) Compression to form the tablet C) Formed tablet as sample D) Crushing strength E) Friability of CLPS and APS tablet.

Appendix B. Calculation of chemical composition of raw material

Estimation of amylose content and amylopectin

Amylose content (%) = 3.06 * absorbance * 20

= 3.06 * 0.498 * 20

= 30.52

Amylopectin content (%) = 100–amylose content (%)

= 100 – 30.52

= 69.48

Ash value

TW = 22.183g

WC = 22.178g

Ws = 2g

(Wa) = Total weight - Weight of crucible

= 22.183-22.178 =0.005

% Ash content = $\frac{Wa*100}{Wb}$ = $\frac{0.005*100}{2}$ = 0.25

Where: -

1. Wt =Total weight
2. Wc = Weight of crucible
3. Ws = Sample weight
4. Wa = Weight of ash
5. %AS = % Ash content

Crude protein

$$N=\frac{[(T-B)\times N_{HCl}\times 14]}{W}\times 100$$

$$=\frac{[(0.03239-0.030)\times 0.1\times 14]}{5}*100$$

$$= 0.0478$$

$$P = F * N$$

$$= 6.25*0.0478$$

$$= 0.3$$

Where: -

1. T: volume in ml of the standard hydrochloric acid solution used in the titration for the test=32.39ml,
2. B:- Volume in ml of the standard hydrochloric acid solution used in the titration for the blank=30ml,
3. 14.00 is the molecular weight of nitrogen,
4. N:- is Nitrogen (%), F:- is conversion factor (6.25),
5. N_{HCl}:-is the normality of HCl used = 0.1N,
6. W:- is sample weight on dry matter basis = 5g and P:- is a crude protein (%).

Crude fat

$$W = W2 - W1$$

$$= 100.0075 - 100$$

$$= 0.0075$$

$$\%Fat(\frac{g}{100g} = \frac{[W \times 100]}{Wo}$$

$$=\frac{[0.0075\times 100]}{5}$$

$$= 0.15$$

Where

W = weight of fat;

W2 = weight of extraction flask after extraction (wt. of flask and fat);

=100.0075g

W1 = weight of extraction flask before extraction (wt. of flask);

= 100g

Wo = weight of fresh Sample

= 5g

1. Moisture content

$$Moisture\ (\%) = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

$$= \frac{(48.91-48.7022)}{(48.91-46.91)} \times 100$$

= 10.39%

Where

1. M1=mass of the dried dish, = 46.91g
2. M2=mass of the dried dish and the sample before drying= M1+2g= 48.91g
3. M3=mass of the dish and the sample after drying. = M1+ 1.7922 = 48.7022

Characterization of tablet

Friability

Fcl = $\frac{WIcr-WFcr}{Wcr}$*100

=$\frac{4.7675-1.2872}{4.7675}$*100

=0.73%

Fac = $\frac{WIac-WFac}{Wiac}$*100

= $\frac{4.6875-2.6962}{4.6875}$*100

= 0.46%

Where

1. Fcl = friability of cross-linked starch containing tablet
2. Fac = Friability of Acetylated starch containing tablet
3. WIcr = initial weight of cross-linked starch

4. WFcr = final weight of cross-linked starch

5. WIac = initial weight of acetylated starch

6. WFac = final weight of Acetylated starch

7. T = time

8. Rpm = revolution per minute

Tensile strength (average)

$\sigma = \frac{2F}{\pi DT}$

$= \frac{2*74.6*1000000}{3.14*9.988*5}$ $= \frac{2*66.2*1000000}{3.14*9.865*5}$

$= 9.515*10^{-7}$ $=8.55*10^{-7}$

where:

1. σ is the tensile strength(cross-linked starch and Acetylated starch),

2. F is the force required to break the tablet(the average force CLPS and APS is 75 and 69),

3. D is the diameter of the tablet (lmm), and T is the tablet thickness(0.5mm).