ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

PREPARATION AND EVALUATION OF CROSS-LINKED ANCHOTE (*COCCINIA ABYSSINICA)* STARCH FOR SUSTAINED RELEASE TABLET FORMULATION

DESALEGN ABEJE (B. PHARM)

MAY, 2018

PREPARATION AND EVALUATION OF CROSS-LINKED ANCHOTE (*COCCINIA ABYSSINICA)* STARCH FOR SUSTAINED RELEASE TABLET FORMULATION

A thesis submitted to the Department of Pharmaceutics and Social Pharmacy, College of Health Sciences, of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics

Desalegn Abeje (B. Pharm)

Under the supervision of Prof. Tsige Gebre-Mariam and Dr. Anteneh Belete, Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University

May, 2018

Addis Ababa University

College of Health Sciences, School of Pharmacy

Department of Pharmaceutics and Social Pharmacy

This is to certify that the thesis prepared by Desalegn Abeje, entitled: "Preparation and Evaluation of Cross-linked *Coccinia abyssinica* (Anchote) Starch for Sustained Release Tablet Formulation" submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved and signed by the examining committee:

Name	Signature	Date
Prof. Tsige Gebre-Mariam (Advisor)	_____	_____
Dr. Anteneh Belete (Advisor)	_____	_____
Dr. Efrem Nigussu (External Examiner)	_____	_____
Dr. Nisha M. Joseph (Internal Examiner)	_____	_____

_____

Head, Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University

ABSTRACT

Starch is a natural polysaccharide, inexpensive, renewable, biodegradable polymer and is one of the most abundant organic compounds in nature. Modification of starch is carried out to enhance the positive attributes and to eliminate the shortcomings of the native starches. Starch modification offers numerous possibilities to generate novel starches which includes new functional and value added properties as demanded by the industry. Chemical modification via cross-linking is possible due to the ubiquitous hydroxyl groups in starches that have been exploited for over a century, principally in the preparation of starch esters and ethers, in order to tune the structure of starches for specific applications. It is apparent that synthetic and semi-synthetic polymers used for sustained release preparations have got biodegradability and biocompatibility problems. In addition, cross-linked anchote starch (CLAS) has not yet explored in order to use as an alternative excipient for sustained release in the preparations of oral drug delivery system. Thus the aim of the present study was to extract starch from anchote *(Coccinia abyssinica),* to cross-link, characterize and explore its potential as a sustained release pharmaceutical excipient.

Anchote native starch (NS) was cross-linked with different concentration of sodium hexametaphosphate at 55^0C for 8h. Three types of CLASs with varying degrees of cross-linking (DCL) were prepared and labeled as CLAS-5, CLAS-10, and CLAS-15. Powder mixtures of ten ibuprofen (IBP) formulations were compressed by direct compression technique. Among these 9 of them were prepared by varying the type of cross-linked starches and the amount incorporated in each formulation. In addition formulation with NS (FNS) was prepared.

Swelling power (SP) and solubility of the CLASs decreased as concentration of SHMP increased and the water uptake decreased. Powder flowability of the CLASs was improved as compared with the NS. Tablet properties were also improved with CLASs than NS. *In-vitro* dissolution testing were performed in 0.1N HCl for the first 2h and in phosphate buffer (pH 7.2) for another 10h for all the formulations (F1-F9) since these formulations passed relevant tests like disintegration time, friability, and hardness. The release of IBP from the matrix tablets prepared with different drug to polymer ratios was investigated in an effort to develop sustained release tablet formulations. Formulation F1 and F2 was prepared from CLAS-5 as matrix forming polymer with the lowest DCL among the modified starches, and prepared by incorporating 20%

and 25% of the polymer respectively released greater than 90% of the drug content in nearly 6h. But formulation F3 was prepared from the same matrix forming polymer and yet prepared with 30% of the polymer released greater than 90% of the drug content in nearly 8h. Formulations F4, F5, and F6 were prepared from CLAS-10 as a matrix forming polymer and had medium DCL and prepared with 20%, 25%, and 30% of polymer, respectively released greater than 90% of the drug in nearly 10h due to the complete disintegration of the matrix tablets within this period of time. However, formulations F7, F8 and F9 were prepared from the other polymer CLAS-15 having the highest DCL and prepared with 20%, 25%, and 30% of polymer, respectively released greater than 90% of the drug slowly at the end of 12h. The *in-vitro* dissolution data were subjected to the various drug release kinetic models and the data best fitted the Higuchi's model. The mechanisms of drug release were anomalous or non-Fickian diffusion category due to the fact that the diffusional release exponent, n ranges between 0.6902 and 0.7699 thus all underwent both diffusion and polymer relaxation. FT-IR spectra indicated no interaction between ibuprofen and the drug release sustaining agent. In conclusion, CLASs can serve as sustained release excipient in matrix tablets.

Key words**:** *Coccinia abyssinica* starch, cross-linking, sodium hexametaphosphate, sustained release, ibuprofen, matrix tablet

ACKNOWLEDGEMENTS

I thank the Almighty GOD, the source of wisdom and strength for whose grace, mercy and protection this work comes to completion.

My first and foremost gratitude goes to my supervisors Prof. Tsige Gebre-Mariam and Dr. Anteneh Belete for their immense contributions, motivation, suggestions, their time and constructive criticism. I am indebted to them for their constant help with my research work. I really appreciate both of them for the support and assistance they provided me with.

I wish to acknowledge the Ethiopian Pharmaceutical Share Company (EPHARM) for kindly providing me ibuprofen powder, anhydrous lactose, sodium lauryl sulphate and colloidal silicon dioxide free of charge and for providing me access to their FT-IR facility. I am also grateful to the School of Chemical and Bio-Engineering, Addis Ababa Institute of Technology, Addis Ababa University for allowing me to use rotary viscometer.

I would also like to express my deep sense of gratefulness and thanks to all the staff of Department of Pharmaceutics and Social Pharmacy for their valuable guidance, personal assistance and motivation to carry out this work.

I am profoundly indebted to Ato Fekade for his help in bringing this research work to this stage. I am also highly thankful to my classmates for their supportive collaboration during the course and research work.

I would like to acknowledge Addis Ababa University for sponsoring my graduate study.

Last of course not the least; my special thanks go to my family and friends who supported me with words of inspiration throughout my entire study.

Desalegn Abeje,

May, 2018

TABLE OF CONTENTS

PAGE

ACRONYMS

ANOVA	Analysis of variance
BCS	Biopharmaceutics classification system
CLAS-5	Cross-linked anchote starch with 5% SHMP at 55^0C for 8h
CLAS-10	Cross-linked anchote starch with 10% SHMP at 55^0C for 8h
CLAS-15	Cross-linked anchote starch with 15% SHMP at 55^0C for 8h
CLS	Cross-linked starches
CSD	Colloidal silicon dioxide
DCL	Degree of cross-linking
DP	Degree of polymerization
DSC	Differential scanning calorimetry
ECH	Epichlorohydrin
FT-IR	Fourier transform-infrared
IBP	Ibuprofen
NS	Native Starch
NSAIDs	Non-steroidal anti-inflammatory drugs
$POCl_3$	Phosphoryl chloride
RH	Relative humidity
SEM	Scanning electron microscopy
SHMP	Sodium hexametaphosphate
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate

LIST OF FIGURES

PAGE

LIST OF TABLES

PAGE

1. INTRODUCTION

1.1. Starch

There is a strong and growing interest in the use of biopolymers in oral drug delivery systems (O'Brien and Wang, 2009a). Natural polymers, such as starches, have a range of potential applications in markets currently dominated by petroleum-based materials. Most importantly, their properties can be tailored to meet specific demands. Petroleum-based polymers create solid waste disposal problems, and thus attention has been focused on the application of starch as a biodegradable thermoplastic material (Manoi and Rizvi, 2010). Starch is one of the most commonly used excipients in the manufacturing of tablets as filler, disintegrant, or binder. Its availability and low cost have allowed it to be integrated into a wide variety of pharmaceutical formulations (O'Brien and Wang, 2009b).

Starch is a naturally occurring, biodegradable, cheap, renewable, and abundantly available polysaccharide molecule (Ashogbon and Akintayo, 2014). Starch is widely available and have been very useful in tablet production due to their inertness, cheapness and a lot of effort has also been expended on the development of new starches from local sources as pharmaceutical excipients (Onyishi *et al.*, 2013). Starch is one of the most important excipients used in the pharmaceutical industry. The International Joint Conference on Excipients rated starch among the top ten pharmaceutical ingredients (Stasiak *et al.*, 2014). Though, its intrinsic physicochemical properties make it less efficient as a multifunctional pharmaceutical excipient, native starch (NS) continue to function as a versatile polymer in the pharmaceutical sector. NS and its modification products continue to emerge with the spate of attention and research into this material. Some essential attributes that make NS attractive for use as a pharmaceutical excipient includes: their white, soft, smooth dryness as well as gelling, and viscosity imparting properties. Also, when they are modified, new attributes are impacted which expand their functions and applications, making them more efficient in both conventional and novel drug delivery systems (Builders and Arhewoh, 2016).

The properties of starch, which vary considerably between samples from different plants and between varieties within species, depend on the molecular composition and the structure of the components. These properties are of considerable importance for the diverse applications of

starch. The recent substantial increase in knowledge of the molecular structure of starch components and the architecture of starch granules has contributed significantly to an enhanced understanding of the structural base of starch functionality (Tester *et al.,* 2004; Vamadevan and Bertoft, 2015).

NSs are insoluble in water below their gelatinization temperature. This is a very important property, which enables an easy extraction of the starch granules from their plant source in aqueous systems (Swinkels, 2007). Starch is arguably one of the most actively investigated biopolymers in the world. It is a raw material of various botanical origins and it is the most important carbohydrate reserve in plants: it is used by the food, paper, chemical, pharmaceutical and textile industries, among many others. This biopolymer is the main source of carbohydrates in the human diet due to its abundance in nature, where it is present in the seeds, roots and stems of different plants (Andrade *et al.*, 2014).

Starch occurs as discrete particles, called granules. Granule size and shape of starch are reported to be primarily affected by the germplasm from which the starch is isolated. The other factors affecting starch granule morphology include climatic conditions and agronomic practices (Wani *et al.*, 2012). The structural design of polymer carriers intended for use in biochemical and biopharmaceutical delivery applications is based on several critical requirements including biocompatibility, biodegradability, bioresorbability, non-toxicity, stability on storage and appropriate size of the drug (Bajpai and Bhanu, 2007). More recently, starch and its derivatives have received greater attention for different pharmaceutical applications. Starch-based capsules, film coatings, microspheres, and subcutaneous implants have been studied, and tablet is the most common pharmaceutical form explored (Onofre *et al.*, 2009).

1.1.1. Amylose and amylopectin

Starch consists of amylose (Fig 1.1 (A)) and amylopectin branch units (Fig. 1.1 (B)). The NS structures contain starch–lipid complexes that contribute to the supramolecular complexity within starch granules (Shanks and Gunaratne, 2011). The strong self-association of amylose molecules might reduce their accessibility to reagents, while the less ordered amylopectin molecules are more receptive to modification, leading to higher reaction efficiency (Onofre *et al.*, 2009). Amylose has a high tendency to retrograde and produce tough gels and strong films.

In contrast, amylopectin, when dispersed in water, is more stable and produces soft gels and weak films. It is possible for entanglements to occur between amylose and amylopectin, along with the presence of minor components (proteins, phospholipids, and lipids), water, and very small amounts of phosphorus, magnesium, and calcium compounds and also have important impacts on the physicochemical properties of the starches from different botanical origin (Ashogbon and Akintayo, 2014; Lewicka *et al.*, 2015).

The location of amylose in a starch granule is still in dispute. Various possible locations have been listed: amorphous lamellae, amorphous growth ring, or interspersed or co-crystallized with amylopectin molecules. Amylose is actually helical. The interior of the helix contains hydrogen atoms and is therefore hydrophilic. The amylose content of starch has been reported to vary with the botanical source of the starch and is affected by the climatic and soil conditions during grain development. Typical levels of amylose in starches are 15% to 25%. Amylose content appears to be the major factor controlling almost all physicochemical properties of starch such as turbidity, syneresis, freeze–thaw stability, pasting, gelatinization, and retrogradation properties (Wani *et al.*, 2012). Amylose is considered primarily responsible for retrogradation processes, and this is the most significant property of this fraction. Amylopectin is much less prone to retrogradation than amylose. The association of dissolved amylopectin molecules is strongly inhibited by their highly branched structure. Therefore, amylopectin tends to be soluble, forming solutions that do not gel under normal conditions. The presence of the branched amylopectin fraction has a moderating influence on the retrogradation of the linear amylose fractions, slowing down its precipitation and diminishing its gel tendencies (Swinkels, 2007).

Amylopectin molecules are arranged radially within the starch granule with the ends of the chains pointing towards the surface. The clusters of double-helical chains in adjacent amylopectin molecules can align themselves to form semi-crystalline arrays within the granule. Compared to amylopectin, amylose is a smaller molecule with longer chains and a limited number of branch linkages (Denyer *et al.*, 2001). Starch granules are mainly found in seeds, roots and tubers, as well as in stems, leaves, fruits and even pollens. The granules occur in all shapes and sizes (spheres, ellipsoids, polygon, platelets, and irregular tubules) depending on the botanical source (Whistler, 2009; Ashogbon and Akintayo, 2014). Generally, granule size refers to the average diameter of the starch granule. Granule size can be determined by various

techniques like microscopy (light microscopy, scanning electron microscopy (SEM)), sieving, electrical resistance, laser light scattering, and field flow fractionation (Wani *et al.*, 2012). The starch granules are organized into more or less crystalline regions and amorphous regions. The amorphous regions are those where chain folding or multiple branching occur, preventing the formation of ordered polymer structures. In tuber and root starches, solely the amylopectin molecules constitute the crystalline structure. The amylose in these starches is present in the amorphous state and can be readily leached out preferentially from the granule (Swinkels, 2007). The presence or absence of crystalline order is often a basic factor underlying starch properties (Ispas-szabo, 2000).

NS granule is heterogeneous both chemically (e.g., amylose and amylopectin) and physically (e.g., crystalline and non-crystalline regions). Each plant species has a unique starch granular size ranging between 1-100μm (Hoover, 2001). The molecular composition and physical aspects of starch structure are examined in relation to starch properties and utility. The structures and molecular properties of amylose and amylopectin are further considered for their effects on properties of starch granules and pastes, and whether or not they act independently or in concert with one another. In addition, starches differ widely in size, shape and composition. Regardless of their absolute size, amylopectins are very large molecules that can be appreciated for their properties (Zobel, 1988).

The two starch components have different properties and are not suitable for the same applications (Stawski, 2008). Their proportion and physical organization inside the granule are responsible for the physicochemical and functional properties of starch as well as its susceptibility to enzyme attack which is particular to the different sources (Nunez-Santiago *et al.*, 2004). Hydroxyl group at carbon C6 is primary alcohol while at C2 and C3 the carbons are secondary alcohols. It is the presence of the three hydroxyl groups in glucose that makes it susceptible to substitution reactions and enables the number of possible modifications of starch (Lewicka *et al.*, 2015). Crystal form is considered to be an important contributing factor in determining overall granule properties (Whistler, 2009). Ability of polysaccharides to form a network structure (gel), even at low concentrations has been well known. This property to form a three-dimensional-network structure (gelation) offers an effective means of increasing the chemical stability and mechanical properties of the polymer. Starch-based systems are quite

promising in several biomedical applications, including drug delivery carriers (Mundargi *et al.*, 2008).

(A)

(B)

Figure 1.1: Structure of (A) linear amylose and (B) branched amylopectin

1.1.2. Sources of starch

Starch can be obtained from a variety of sources. In addition to the difference in amylose/ amylopectin ratio, starches from different sources also vary in their structural characteristics and consequently their physicochemical properties (Onofre *et al.*, 2009). A large number of starch resources are found in the tropic and subtropics regions which are being used as food while their properties remain to be determined (Alam and Hasnain, 2009).

Starch is found in high proportions in cereals and seeds (like corn, maize, wheat, rice, sorghum, barley, or peas) and in tubers or roots (like potato or cassava) of plants. Most of the starch produced worldwide is derived from corn, but other types of starch such as cassava, sweet potato, potato, and wheat starches are also produced in large amounts (Dupuis *et al.*, 2014). The source of a starch can be identified from its microscopic appearance. Starch occurs in practically

every type of tissue of green plants: leaves, roots, tubers, seeds and fruits. Storage of starch takes place in the underground organs of various plants, for example potato, sweet potato, tapioca, arrowroot and canna. The seeds of many plants contain starch as a reserve nutrient, for example grasses, rice, wheat, maize, sorghum, barley and oat. Starch constitutes the major portion of the carbohydrates of legume seeds (peas, beans, lentils). Starch occurs also as a component of many fruits, for example unripe apples, bananas and green tomatoes. The roots and tubers differ from the cereals in that they have considerable higher moisture content, but lower lipid content (Swinkels, 2007). The anchote starch *(Coccinia abyssinica)* is one of those starch-rich sources. Knowledge of the functional properties of anchote starch may also extend its use as a food source (Alam and Hasnain, 2009).

1.1.3. *Coccinia abyssinica* plant

Anchote tubers possess two variations in their tissue colour, red and white. Cultivation of anchote is particularly widespread in the western and southwestern regions of Ethiopia, at varying elevations of 1300–2800m with an annual rainfall of 762–1016mm. Anchote is propagated exclusively from seeds and harvested in 4 months. Anchote is the only plant in the *Cucurbitaceae* family which is known to produce edible starchy tubers. Anchote *(C. abyssinica)* is an indigenous tuber crop of the Ethiopian Highlands. In Ethiopia, anchote is associated with traditions of the Oromo tribe. Various anchote dishes are prepared for ceremonies and festivals in Oromia Region (Parmar *et al.*, 2017). Anchote is subsistence crop widely grown to fill food security during hunger months. Unlike many other crops, anchote can be grown with minimal inputs and it is able to produce reasonably well under unfavorable conditions such as low soil fertility, acidic soils or drought and under intercropping with cereals (Beruk, *et al.*, 2015).

A **B**

Figure 1.2: *Coccinia abyssinica* "Anchote" plant (A) and its tubers (B) (Photo taken by Desalegn A.)

1.1.4. Anchote tubers and their starches

Apart from cereals and pulses, Ethiopian agro ecosystems are highly suitable for the production of high quality roots and tubers (Fig. 1.2). Among the starchy root and tuber crops from Ethiopia, *C. abyssinica* commonly known by its vernacular name anchote is one of these root and tuber crops. It is popular in the western Oromia Region of the country. Apart from food, the crop is also used in traditional medicine. In Ethiopia, anchote is associated with traditions of the Oromo tribe. Various anchote dishes are prepared for ceremonies and festivals in Oromia Region The proximate analysis suggests that anchote tubers have marginally higher protein content than other common root and tuber crops in the region (such as cassava and sweet potato). It was reported that red anchote contained 1.12% ash, 3.58% protein, 0.26% fat and 95.04% starch but white anchote contained 1.1% ash, 2.77% protein, 0.41% fat and 95.72% starch (Parmar *et al.*, 2017). Anchote is specifically widely cultivated and used in Jimma, Illu-Abba Bora and Wallaga areas of the Oromia Regional State. Anchote is a fruit bearing plant. However, it is the only tuber bearing crop in the genus Coccinia. The carbohydrate content of anchote contributed the major part to its energy since it contained large quantities of starch (Habtamu and Kelbessa, 1997). In Ethiopia root and tuber crops play significant roles for food and nutritional security for farmers.

Apart from their regular uses as foods, these crops also have cultural and medicinal uses (Mekbib and Deressa, 2016).

1.2. Starch modification

The industrial utilization of NSs is limited because of inherent imperfect nature, such as water insolubility and their tendency to easily retrograde and undergo syneresis and therefore form unstable pastes and gels. Starch modification does not only decrease retrogradation, gelling tendencies of pastes and gel syneresis but also improves paste clarity and sheen, paste, and gel texture, film formation and adhesion. The aim of starch modification is to stabilize starch granules during processing and make starch suitable for many food and industrial applications (Guerra-Dellavalle *et al.*, 2009; Ashogbon and Akintayo, 2014).

Starches are inherently unsuitable for most applications and, therefore, must be modified chemically and/or physically to enhance their positive attributes and/or to minimize their defects (Whistler, 2009). Starch modification involves the alteration of the physico-chemical 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. Physical methods involve the use of heat and moisture, and chemical modifications introduce functional groups into the starch molecule using derivatization reactions (e.g., etherification, esterification, crosslinking), or involve breakdown reactions (e.g., hydrolysis and oxidation) (Singh *et al.*, 2007; Alcazar-Alay and Meireles, 2015).

1.2.1. Physical modification

Physical modification is simple, cheap, and safe. This kind of modification of starch can improve water solubility and reduce particle size. The methods 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 (Ashogbon and Akintayo, 2014). Pregelatinization, annealing and heat-moisture treatment are physical methods of modifying starch properties. While pregelatinization causes granules disruption, annealing and heat-moisture treatments acquire modified properties without rupturing the granules. These physical treatments can change certain starch properties using simple and environmentally safe

processes (Olasupo *et al.,* 2011). Physical properties of heat moisture treated starch depend on the starch origin and the treatment conditions used (Insha *et al.,* 2016).

1.2.2. Chemical Modification

Chemical modification is a classical way to effectively improve the functionalities of starch (Liu *et al.*, 2014). The properties of starch derivatives obtained depend on the kind of starch bases used and their basic properties (Odeku and Picker-Freyer, 2009). Self-association (induced by changes in pH, ionic strength or physical and thermal means), complexation with salts and covalent cross linking are some of the widely adopted strategies to modify starch (Mundargi *et al.,* 2008). Chemically modified starches retain their macromolecular nature whatever the chemical modification, while presenting a wide range of physiochemical properties. Chemical modification includes a series of reactions causing a change in the chemical structure of some of the glycosyl units starch macromolecules. They relate to the primary and secondary alcohol functions of glycosyl units (oxidation, esterification, and etherification), the glycosidic bond and pseudo aldehyde function (hydrogenation) (Lefnaoui and Moulai-Mostefa, 2015). Chemical reactions generally occur randomly with the primary hydroxyls, the secondary hydroxyls, the aldehydic reducing end groups and the glycol groups (Wang *et al.,* 2017). This kind of modification of starch affects its digestion in the small intestine, and the degree to which depends on starch source, type and degree of modification, extent of starch gelatinization, and the source of enzyme used (Shukri and Shi, 2015).

The introduction of functional groups into the starch molecule in chemical modification resulting in markedly altered physico-chemical properties. The rate and efficiency of the chemical modification process depends on the reagent type, botanical origin of the starch and on the size and structure of its granules. Channels that open to the granule exterior provide a much larger surface area accessible by chemical reagents, and provide easier access by the reagents to the granule interior. However, the reagent may diffuse through the external surface to granule matrix in the absence of channels (Singh *et al.,* 2007). 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 (Odeku and Picker-Freyer, 2009).

Chemically modified starches have also been shown to be promising in the pharmaceutical industry as sustained release matrices and starches cross-linked by various agents such as epichlorohydrin have retarded drug release from solid dosage forms at various levels (O'Brien *et al.,* 2009).

1.2.2.1. Acetylation

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 acetylation depends upon certain factors, such as starch source, reactant concentration, catalyst type, concentration, reaction time and suspension pH (Lisie *et al.,* 2015). The chemically modified starch by acetylation has been widely studied. The acetylation may be performed to improve the physical, chemical and functional properties of starch. In the acetylation process, the hydroxyl groups of the glucose monomers are converted to the groups CH_3COO- therefore the acetylation is an esterification of hydroxyl groups in the anhydroglucose unit of the starch molecule. The starch acetate has applications which are regulated by their characteristics, such as the degree of acetylation or the degree of substitution, and the percentage of acetyl groups (Colussi *et al.,* 2015). 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 (Bartz *et al.,* 2015).

1.2.2.2. Hydroxypropylation

Modification of NS by hydroxypropylation imparts some useful physicochemical properties, which increases its range of application in different food as well as non-food applications. Hydroxypropylation renders a hydrophilic character when introduced into the starch granule and it is known to weaken or to strain the internal bond structure that holds the granule together. This reduction in bond structure is reflected in starch pasting temperature (Pal *et al.,* 2002). Hydroxypropylated starch derivative formed by reaction of starch with propylene oxide. This modification improves the shelf-life, freeze-thaw stability, cold-storage stability, clarity and texture properties of starch paste. The mechanism for the base-catalyzed reaction of propylene

oxide with starch is considered to be substitutive nucleophilic bimolecular type. The higher the level of hydroxypropyl substitution, the lower the pasting temperature until the product becomes swollen. Hydroxypropyl groups also prevent retrogradation resulting in more fluid paste with improved clarity. This modification can render the desired textural properties in the product (Miyazaki and Van, 2006). The hydroxypropylation groups weaken or disrupt the internal bond structure that holds the starch granules together and influence physicochemical properties of products depending on the starch source, reaction conditions, the type of substituent groups employed, and the extent of substitution. The hydroxypropylation starches have been prepared from different sources, including potato and their physicochemical properties have been intensively studied as a function of the degree of molecular substitution. The effect of hydroxypropylation on rheological properties of starches was also examined (Pec and Venskutonis, 2016).

1.2.2.3. Carboxymethylation

Carboxymethylation of polysaccharide (Chemical modification method) is a vital and versatile transformation since it provides access to water soluble polymers and intermediate with valuable functional attributes and most widely used food colloids. Carboxymethyl starch is a starch derivative in which the –OH groups of the starch molecule, are partially substituted by ether group (-O-CH_2 COOH). It exhibits varying degree of viscosity depending on its degree of substitution (Nagar, 2013). Carboxymethyl starch is a polymer with great importance in pharmacy, medicine, cosmetics, food industry, environmental protection and many other industrial applications. The addition of bulky hydrophilic groups to polysaccharide chains resulted in reduced starch tendency to retrogradation (recrystallization), and cold water solubility. Moreover, it made the polymer less prone to damages caused by heat and microbial attack. Solubility of Carboxymethylated starch in cold water increases along with degree of substitution value increase, and the properties such as: water absorption, adhesiveness and film forming characteristics are improved simultaneously. Similarly, paste and film clarity as well as paste and gel storage stability are significantly improved. Carboxymethylated starch derivatives exhibited lower gelatinization temperature, specific changes in rheological properties and pH stability (Spychaj *et al.*, 2013). Chemical modifications overcome of NS functional problems. When the hydroxyl groups of starch are for instance (partly) substituted with sodium

monochloroacetate to give carboxymethyl starch, the starch becomes cold-water soluble. This modification procedure has a positive effect on the applicability in the mentioned areas. As such, this makes carboxymethylation a very successful modification reaction for various starches (Yao *et al.*, 2004)

1.2.2.4. Cross-Linking

Cross-linking is a key technique for modifying the properties of starch. Starch can be phosphorylated by reaction with various inorganic phosphate salts as well as specially developed organic reagents. STMP is used to cross-link starches by means of a covalent phosphate diester bond (Kulicke *et al.*, 1990; Thakur *et al.*, 2015). It is generally performed by treating starches (semi-dry or slurry) with multifunctional reagents capable of forming either ether or ester linkages between hydroxyl (–OH) groups on starch molecules. The effect of cross-linking on the properties of starch depends on the botanical source of starch concentration and type of cross-linking agents as well as reaction conditions used (Manoi and Rizvi, 2010; Wongsagonsup *et al.*, 2014).

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 (Jyothi *et al.*, 2006). 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 (Odeku and Picker-Freyer, 2009; Mirmoghtadaie *et al.*, 2009). Phosphorylation is one of the most common methods used to modify starch and produce monostarch and distarch phosphate. The phosphate is bounded to starch molecules that cause changes in functional properties of starch. Usually, sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), Phosphoryl chloride ($POCl_3$), epichlorohydrin (ECH) and sodium hexametaphosphate (SHMP) are common chemical agents used for phosphorylation and cross-linking and can serves as main cross-linking agents (Heebthong *et al.*, 2016). 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 (Singh *et al.*, 2007).

Cross-linking is performed to restrict swelling of the starch granule under cooking conditions or to prevent gelatinization of starch. For controlling the modification reactions and hence, to control and optimize the production and use of modified starches, it is necessary to be able to correlate the extent and location of cross-links along the starch backbone with the functionality obtained (Zhao *et al.*, 2015). It introduces intermolecular bridges between starch chains markedly reinforcing H-bonds holding the granule. It mainly takes place in amylopectin; therefore the reinforcement of amylopectin may lead to stronger and closer packing within the crystalline regions (Atichokudomchai and Varavinit, 2003; Wongsagonsup *et al.*, 2014).

The type of reagent used and cross-linking conditions determine the ratio of mono and di-type bonds (esters with phosphorous based agents) due to cross-linking reaction mechanism and available starch hydroxyls (Hirsch and Kokini, 2002). Cross-linking of starch molecules to give a distarch monophosphate is favored by alkalinity above pH 10 and by the presence of a neutral sodium salt. Otherwise, monostarch monophosphate esters are formed. Alkalinity increment of the cross-linking medium increases starch anion concentration; while increasing sodium ion concentration increases the uptake of alkali by starch as well as the ionic strength of the reaction medium (Woo and Seib, 1997). Cross-linking resulted in an ordered structure of the starch pastes, thus resulting in higher degree of retrogradation (Mirmoghtadaie *et al.*, 2009).

The type of cross-linking agent determines the changes in functional properties of a treated starch, because the molecular structures of the cross-linked systems produced by different cross-linking agents are different. Therefore, based on the reagent used for cross-linking, the final product is generally classified into three types: the first type is a mono-starch phosphate which is produced by esterification of starch with ortho-phosphoric acid, sodium or potassium ortho-phosphate, or STPP. The second classification is a di-starch phosphate which is produced when a NS is reacted with STMP or $POCl_3$. Final type of CLS is a phosphated di-starch phosphate which resulted from combined treatments of mono-starch phosphate and di-starch phosphate (Ashogbon and Akintayo, 2014). The solubility of the CLS decreases with an enhancement in the degree of cross-linking (DCL) when measured as a function of temperature. The decreased solubility can be explained by the fact that the DCL results in the compactness of granular structure in the starch molecules which ultimately cause less disintegration of starch granules during the process of gelatinization. Starch paste from CLS is highly viscous, compacted and less

likely to breakdown during severe agitation, cooking and exposure to low pH conditions (Zia-ud-Din *et al.,* 2017).

1.3. Modified starches in sustained release formulations

Polymers are often used in sustained release formulations to provide diverse functionality to the formulation in which they are employed (Onofre *et al.,* 2009). CLS have been developed as excipients for the formulation of controlled-release solid dosage forms for the oral delivery of drugs (Lenaerts *et al.,* 1998). Cross-linking is an effective way to render microparticles water-insoluble, and the release profile of encapsulated or entrapped materials could be controlled by altering the cross-linking degree. Hence CLS microparticles seem to be promising delivery vehicles for the controlled release (Li *et al.,* 2009).

Extended release may be achieved based on modifying drug dissolution by controlling the access of biologic fluid to the drug. This control can be achieved through barrier coating, micro encapsulation, complex formation, ion exchange resins, or by embedding the drug in a slowly eroding or hydrophilic matrix system. In delayed release dosage forms, enteric coating is generally used to protect drugs from the gastric acidic environment (Souza *et al.,* 2013). Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems (Irisappan *et al.,* 2014).

Polymers and release retarding materials used as matrix formers in matrix tablets play a vital role in controlling the drug release from the tablets. Though a variety of polymeric materials are available to serve as release retarding matrix materials, there is a continued need to develop new, safe and effective release retarding matrix materials for matrix tablets for controlled release. Modified starches have been used for various pharmaceutical purposes such as fillers, superdisintegrants and matrix formers in capsules and tablet formulations. One of the important modifications of starch is acetylated starch. Starch acetate is reported to have excellent bond forming ability and suitable for coating and controlled release applications (Chowdary and Radha, 2011).

1.4. Hydrophilic matrix tablets

Hydrophilic matrix can be utilized as a means to control the drug release rate. The preparation of a tablet with the matrix involves the direct compression of the blends of drug, release retardant and other additives, in which the drug is uniformly distributed throughout the matrix core of the release retardant. Upon immersion, drug release is controlled by a gel diffusion barrier that is formed and tablet erosion. The formulation of the drugs in gelatinous capsules or more frequently, in tablets, using hydrophilic polymers with high gelling capacities as base excipients is of particular interest in the field of controlled release (Velasco *et al.,* 1999). Drugs can be transported out of a matrix via movement through the free volume of the cavities of the matrix, jumping between the cavities due to the wriggling or movement of the matrix polymer chains. The proportion and structure of amylose and amylopectin in starches from different botanical sources strongly influenced the level of modification required to produce a satisfactory sustained release matrix. Hydrogels are hydrophilic polymers commonly used as sustained release agents in pharmaceutical formulations because of their ability to form a gel network upon swelling, which entraps the drug and acts as a barrier to its release to the surrounding medium (Onofre *et al.,* 2009).

The convenience and easiness to manufacture, resulting in low price of the dosage form, has contributed much to the popularity of hydrophilic matrix systems as a sustained release drug delivery system. Hydrophillic polymer matrix systems are widely used in oral controlled drug delivery systems because of their flexibility to obtain a desirable drug release profile, cost effectiveness, and broad regulatory acceptance. The release of drugs from hydrophilic matrix systems is controlled by one or more mechanisms that may be transport of solvent into the device, swelling of the matrix, diffusion of the solute through the swollen matrix, erosion of the swollen matrix, and so on (Shoaib *et al.*, 2006; Shah *et al.*, 2009). Polymer matrix systems have the advantages of prolonging drug release and reducing adverse effects in patients (Ghosh and Barik, 2010). Since the polymer is the most critical component of a controlled release tablet, which by virtue of its unique physiochemical properties is able to control the rate release, it has gained importance in the pharmaceutical application both as drug encapsulant and drug carrier (Teixeira, 2009).

Hydrophilic polymers absorb high amounts of water and its increased use in the drug delivery systems formulations is based on its ability to form a gel network in the swollen state which entraps the drug and acts as a barrier to its release to the medium. Its swelling ability is a fundamental property that should influence the drug release rates, by controlling both the diffusion rate of the penetrant into the matrix and the drug dissolution and diffusion throughout the gel layer of the swollen matrix (Carbinatto *et al.*, 2014). The key element to drug release from swellable polymers is the use of polymers that will undergo transition from the glassy to the rubbery state which is characterized by a gel-like layer, on hydration by water. This transition should occur fairly rapidly so that the drug has to pass through the viscous gel layer to be released. The rate at which the drug is released from the swellable hydrophilic matrices is determined by numerous processes such as hydration of the polymer that leads to swelling, diffusion of the drug through the hydrated polymer, drug dissolution and polymer erosion. Many of these processes occur simultaneously to release the drug (Nerurkar *et al.*, 2005; O'Brien and Wang, 2009b).

The physicochemical properties of the hydrogel network as well as the selection of drug-loading method will determine the mechanism(s) by which the loaded drug is released from the cross-linked matrix. Due to the usually high permeabilities of hydrogel networks and the advantages of *in-situ* fabrication, most research efforts are focused on understanding diffusion- controlled release of encapsulated drugs from three-dimensional hydrogel matrices (Lin and Metters, 2006). The cross-linked network enables the entrapment of drugs in the hydrogel domains. Since these hydrogels are not water-soluble, they do not dissolve, and erosion in the manner of linear polymers does not occur. Rather, when the hydrogel is fully hydrated, osmotic pressure from within works to break up the structure, essentially by slough off discrete pieces of the hydrogel. These hydrogels remain intact, and the drug continues to diffuse through the gel layer at a uniform rate (Khan and Jiabi, 1998). Delivery of drug from hydrophilic matrices is known to be affected by many factors such as the polymer swelling and erosion behavior, the drug dissolution characteristics, the drug/polymer ratio, the granulation technique and the tablet shape (Ahammed *et al.*, 2010).

1.5. The present study

Starches obtained from various roots and tubers have revealed suitable potential as excipients in tablet formulations in order to encourage future research endeavors. A lot of efforts have been made to develop locally produced starches as pharmaceutical excipients. Starch can generally be recognized as a common excipient in pharmaceutical industries and it is used as a disintegrant, binder, or filler (diluents). Since Ethiopia has a variety of plant species, those plant species can be taken as a source of starch for various functions. These consists of *Enset ventricosum* (Gebre-Mariam and Schmidt, 1996), *Dioscorea abyssinica* (Gebre-Mariam and Schmidt, 1998), Godare (Adane *et al*., 2006), Anchote (Nigussie *et al*., 2006), *Dioscorea bulbifera* (Mohammed *et al*., 2007), Cassava (Paulos and Gebre-Mariam, 2009), etc. Physicochemical properties of starches from these plants and their exploitation for tablet excipient have been investigated.

The present study was undertaken to determine certain functional properties of anchote (*C. abyssinica)* starch and to evaluate the effects of some modification treatments on these functional properties. The purpose of this research was to study the modification of anchote starch with cross-linking, and to study the effect on the sustained drug release properties of the starch. Hence this study was aimed to prepare hydrophillic matrix tablets of Ibuprofen (IBP) as a model drug, and cross-linked anchote starches as hydrophilic matrix to sustain drug release. In the present study some of the physicochemical properties of the underutilized anchote starches will be explored. SHMP also described as sodium polymetaphosphate is a white crystalline odorless powder used as a cross-linking agent. CLS have long been used as food additives because of their safety and low cost. CLS is capable of forming hydrophilic matrices upon contact with aqueous medium. Drug molecules can be entrapped or embedded into the matrices\ so that it will facilitate the drug release mechanisms. A few years ago it was discovered that they also possess unique features that suggest their use as an excipient for the manufacture of controlled release solid oral dosage forms of drugs (Lenaerts *et al.*, 1998).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used classes of drugs worldwide (Jan *et al.*, 2006; Li *et al.*, 2016). Ibuprofen (IBP), Fig.1.3, is one of well-known and first-line NSAIDs used in the treatment of rheumatic diseases to reduce the symptoms of chronic inflammation and pain. IBP is classified as BCS Class II drugs. It has poor water solubility and is

a good candidate for formulation into a modified release system because of its short half-life (Devrim and Canefe, 2006; Guerra-Ponce *et al.*, 2016).

Figure 1.3: Structure of ibuprofen

IBP,2-(4-Isobutyl phenyl) propanoic acid is a potent NSAID (Odeku *et al.*, 2014). It shows a plasma half-life of 1.8-2.0 h; as a result it has to be administered 3 to 6 times a day, making this drug a suitable candidate for a controlled/sustained release formulation (Potthast *et al.*, 2005; Swain *et al.*, 2016). Thus to improve patient compliance and bioavailability, to minimize total drug quantity accumulation on chronic use and reduce fluctuation in drug level sustained release of IBP is desirable (Shoaib *et al.*, 2006). It is well known that patient compliance is better when the drug dosing is only once or twice daily. Therefore, the aim of this work was to investigate potential of cross-linked anchote starch (CLAS) as sustained release carrier tablet formulation of IBP.

1.6. Objectives

1.6.1. General objective

- To evaluate sustained release properties of cross-linked local anchote (*C. abyssinica)* starch

1.6.2. Specific objectives

- To produce cross-linked starch from native starch of anchote *(C. abyssinica)* tubers, and determine the degree of cross-linking of the modified starches; and
- To evaluate the tableting properties and analyze the sustained release properties of the cross-linked starches

2. EXPERIMENTAL

2.1. Materials

Fresh tubers of anchote *(C. abyssinica)* were obtained from local farmers in the Kellam, West Wollega, Western Ethiopia. Sodium metabisulphite and sodium carbonate (Guangzhou Jinhaunda Chemical Reagent Co. Ltd., China), sodium hexametaphosphate (Uni-chem Chemical reagent Ltd., China), hydrochloric acid and magnesium stearate (BDH Chemicals Ltd., England), sodium hydroxide (Sigma-Aldrich, Sweden), potassium dihydrogen phosphate (Codex, Carlo Erba, Italy). Ibuprofen (IOL Chemicals and Pharmaceutical Co. Ltd, Barnala, India), lactose anhydrous (LAH) (Davisco food international, Inc-USA), sodium lauryl sulphate (JRS Pharma GmbH and Co. KG, Germany) and colloidal silicon dioxide (Aerosil®) (Evonica Specialty Chemicals, China) were kindly donated by the Ethiopian Pharmaceutical Share Company (EPHARM) and used as received.

2.2. Methods

2.2.1. Isolation of starch from *C. abyssinica* (Anchote) tuber

The starch was isolated and purified following the method described by Gebre-Mariam and Schmidt, (1996). Fresh *C. abyssinica* (anchote) tubers were cleaned and peeled. The tubers were then chopped and suspended in large quantities of distilled water containing 0.075% (w/v) of sodium metabisulphite. The material was allowed to settle overnight, and the supernatant was decanted. The sedimented starch was repeatedly treated with sodium metabisulphite solution until the supernatant was clear. The material was then passed through fine muslin to remove cell debris and the translucent suspension was collected and allowed to settle. The sedimented starch was then washed several times with distilled water by filtering and re-suspending until the wash water was clear and free of suspended impurities. The resulting starch was finally dried at room temperature, milled to fine powder in a mortar and pestle, sieved using 224μm mesh sieve and stored in airtight plastic container under dry conditions for further use.

2.2.2. Preparation of modified starches

2.2.2.1. Preparation of cross-linked *C. abyssinica* (Anchote) starch

Cross-linking of *C. abyssinica* starch was performed using the method described by Woo & Seib, (1997). NS (100 g, dry basis) was suspended in 200 ml of distilled water containing sodium hydroxide (1.2%, w/w); the slurry was adjusted to pH 11 with the alkaline solution and heated to 55 ^{0}C. After 15 min of heating, sodium carbonate (6%, w/w) and SHMP at three different concentrations 5, 10 & 15 (%, w/w) dissolved in minimal amount of distilled water were added to the medium while stirring. The starch suspensions were maintained at 55 ^{0}C while stirring and held at this temperature for 8 h (Table 2.1). The pH of the suspension was then adjusted to 6.5 with 1N hydrochloric acid, 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 later washed several times with distilled water, and dried in hot air oven (Kottermann® 2711, Germany) at 40 ^{0}C for 24 h. The materials were then powdered using mortar and pestle, passed through a 224 μm mesh sieve, and packed in air-tight container.

Table 2.1: Reaction conditions for the synthesis of CLASs

CLAS Batches	SHMP (%, w/w)	NaOH (%, w/w)	Na_2CO_3 (%, w/w)	Reaction time (h)	Reaction temperature (^{0}C)
CLAS-5	5	1.2	6	8	55
CLAS-10	10	1.2	6	8	55
CLAS-15	15	1.2	6	8	55

CLAS- Cross-linked anchote starch

2.2.3. Physicochemical characterization of modified starches

2.2.3.1. Determination of peak viscosity

The peak viscosities of the cross-linked anchote starches and NS samples were determined according to the method described by (Jayakody *et al.,* (2007) with a rotational viscometer (elcometer®, 2300RV1-L, IP20, Spain) using spindle number 4 at a shearing stress of 200 rpm. Starch suspensions at 10% (w/v) concentration were prepared and shaken for 3min. The

suspensions were then heated from 50^0C to 90^0C in a water bath and meanwhile, their peak viscosities were recorded at 50, 70 and 90^0C from the digital display on the viscometer. After maintaining the samples at 90^0C for 3min, the peak viscosities of the same were read as they were cooled from 90 back to 50^0C. The maximum peak viscosities in the entire heating-cooling cycles were taken and used in the estimation of the DCL in the CLS samples.

2.2.3.2. Determination of the degree of cross-linking (DCL)

The DCL of the modified starches was determined from the viscosity values, according to the method of (Jyothi *et al.,* 2006). The DCL was calculated by using Equation 2.1:

$$\text{DCL} = \frac{(\text{A}-\text{B})}{\text{A}} * 100 \quad \text{Eq. 2.1}$$

where A is the peak viscosity of the NS and B is the peak viscosity of CLAS. Results were expressed as a mean of triplicate determinations.

2.2.3.3. Determination of moisture content

The moisture content was determined as per the method described by Gebre-Mariam and Schmidt, (1996) and Carmona-Garcia *et al.*, (2009). 2g of starch was weighed into weighed, dried Petri-dish and heated at 120 ^{0}C for 4 h in a hot air oven (Kottermann® 2711, Germany) to a constant weight, and the final weight was noted. The moisture content was calculated using Equation 2.2, and the results are the mean of triplicate determinations:

$$\text{Moisture Content} = \frac{(\text{Wi}-\text{Wf})}{\text{Wi}} * 100 \quad \text{Eq. 2.2}$$

where Wi and Wf are starch weights before and after drying respectively.

2.2.3.4. Determination of solubility and swelling power

Solubility and swelling power were determined following the methods reported elsewhere (Wattanachant *et al.,* 2002). 0.5 g of starch samples (Wi) were dispersed in10 ml of distilled water in a pre-weighed centrifuge tubes. The slurries were heated in a thermostatically controlled water bath at 25, 37, 45, 55, 65, 75, 85 and 95^0C for 30 min with shaking every 2 min to keep the starch granules suspended. The heated slurries were then cooled to room temperature and

centrifuged at 3000 rpm for 15 min to separate gel (W_r) and supernatant. The supernatant (Ws) was decanted carefully and poured into Petri-dish for subsequent analysis of solubility pattern. The solid part was dried in an oven for 2h at 130^0C. Solubility and swelling power were determined using Equation 2.3 and 2.4 as follows:

$$\text{Solubility (\%)} = \frac{\text{Dry weight of solubilized starch (Ws)}}{\text{Dry weight of starch}} * 100 \quad \text{.....Eq. 2.3}$$

$$\text{Swelling Power} = \frac{\text{Wet weight of swollen starch sediments (Wr)}}{\text{Dry weight of starch (Wi)} * (100 - \text{\% Solubility})} \quad \text{.....Eq. 2.4}$$

2.2.3.5. Determination of moisture sorption pattern

Moisture sorption properties of native and CLS were determined according to the method described by Gebre-Mariam and Schmidt, (1996). Saturated solutions of different salts were prepared to provide different percentage relative humidity (RH). Pyrex desiccators containing distilled water and saturated salt solutions were prepared to provide different RH chambers and kept at room temperature. 2 g of NS and CLS samples were pre-dried in an oven for 4h at 120^0C and spread over petri dishes and transferred to particular RH chambers, and allowed to equilibrate for four weeks. The moisture content was determined based on the weight difference of the starches before and after equilibration.

2.2.4. Characterization of powder properties of the native and modified starches

Determination of Bulk (ρ_{Bulk}) and tapped densities (ρ_{Tapped})

Bulk densities of native and CLASs were determined by carefully pouring 60g starch powder into a 250ml graduated glass measuring cylinder. The cylinder was then lightly tapped. The volume was then read directly from the cylinder and used to calculate the bulk density. The ρ_{Tapped} was measured by applying 500 taps to 60 g of native and cross-linked anchote starch samples in a 250ml glass graduated cylinder using a mechanical tapper (ERWEKA®, Germany). The ρ_{Bulk} and ρ_{Tapped} (g/ml) were calculated by using Equation 2.5 and 2.6 respectively. Bulk and tapped densities were determined as a mean of triplicate measurements.

$$\rho\text{Bulk} = \frac{m}{Vb} \quad \text{..... Eq. 2.5}$$

$$\rho\text{Tapped} = \frac{m}{Vt} \quad \text{Eq. 2.6}$$

where m is the weight of the starch powder, Vb is bulk volume and Vt is the tapped volume.

Determination of Carr's index (CI) and Hausner's ratio (HR)

The compressibility index (CI) and HR were computed from the determined bulk and tapped densities of the powder using Equation 2.7and 2.8, respectively:

$$\text{CI}\ (\%) = \frac{(\rho\text{Tapped} - \rho\text{Bulk})}{\rho\text{Tapped}} * 100 \quad \text{Eq. 2.7}$$

$$\text{HR} = \frac{\rho\text{Tapped}}{\rho\text{Bulk}} \quad \text{Eq. 2.8}$$

Angle of repose and flow rate determination

Sixty grams of native and cross-linked anchote starch samples were poured into a plugged glass funnel with the tip, 10 cm above the flat surface of the bench. The starch powders were then allowed to flow freely through the orifice of the funnel to form a heap whose height and diameter were determined. The times taken for the starch powders to flow the orifice were noted. The angle of repose and flow rates were computed using Equation 2.9 and 2.10 respectively:

$$\text{Angle of repose}\ (\Theta) = \tan^{-1}(h/r) \quad \text{Eq. 2.9}$$

where, h = height of the powder and r = radius of circular heap

$$\text{Flow rate} = \frac{\text{weight of starch powder (g)}}{\text{time of flow (s)}} \quad \text{Eq. 2.10}$$

2.2.5. Fourier transform infrared spectroscopy (FT-IR) studies

FT-IR spectra of the modified starch sample, the model drug and physical mixtures of the drug and CLAS-15 were acquired by Fourier transformed infrared spectrophotometer (FT-IR- 8400S, SHIMADZU®, Japan) in transmittance mode in order to check structural changes due to the cross-linking reactions and to investigate any chemical interactions between the drug (IBP) and polymer matrix. The samples were first ground in a mortar to reduce the average particle size.

About 5-10 mg of the finely ground samples were mixed with an oily mulling agent (Nujol) in a mortar and pestle. The sample mixture was then placed onto the face of a potassium bromide (KBr) plate and the second window was placed on top of the first salt plates to form a thin film of the mull by compression between two plates. The sandwiched plates were placed in the infrared spectrophotometer and the spectra were obtained. FT-IR spectra of CLS of anchote, IBP and mixtures of IBP and CLS sample were scanned at a spectral resolution of 4 cm^{-1} with wave number range between 4000 and 400cm^{-1} and average of 20 scans using IR Solution software. The background spectrum was carried out before running each sample.

2.2.6. Tablet preparation and evaluation

2.2.6.1. Tablet preparation

IBP matrix tablets were prepared by direct compression method. Tablets each containing 200 mg IBP was prepared as per formula given in Table 2.2. All the powders except magnesium stearate were mixed in a Turbula mixer (Willy A. Bachofen AG, Turbula T2F, Basel, Switzerland) for 10 min. Finally, 0.5% w/w magnesium stearate was added and mixed for further 3 min. Different formulations were directly compressed into tablets on a single punch tablet compression machine (ERWEKA® GmbH, KORSCH, EKO, 7891, Western Germany), using 10 mm flat surface beveled punches, at a fixed compression pressure using microcrystalline cellulose powder at a hardness range of 70-90 N. The tablets were kept for 24 h at room temperature in air-tight glass containers before their properties were evaluated.

Table 2.2: Composition of 200 mg IBP controlled-release matrix tablets using CLAS blended mixtures.

Ingredients (mg/tab)	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
IBP	200	200	200	200	200	200	200	200	200
CLAS-5	80	100	120	_	_	_	_	_	_
CLAS-10	_	_	_	80	100	120	_	_	_
CLAS-15	_	_	_	_	_	_	80	100	120
LAH	114	94	74	114	94	74	114	94	74
SLS	2	2	2	2	2	2	2	2	2
CSD (Aerosil®)	2	2	2	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2	2	2	2
Total tablet weight	400	400	400	400	400	400	400	400	400

NB: LAH - Lactose anhydrous, SLS - Sodium lauryl sulphate, and CSD - Colloidal silicon dioxide (Aerosil®)

2.2.6.2. Tablet evaluation

2.2.6.2.1. Crushing strength

Crushing strength of the tablets was determined with Tablet hardness tester (Schleuniger, 2E/205, Zurich, Switzerland). Ten tablets were taken randomly from each formulation and the crushing strengths of the tablets were measured individually, the mean and standard deviation were calculated.

2.2.6.2.2. Thickness

The thickness of 10 matrix tablets were determined using sliding vernier caliper (Nippon Sokutei, Japan) and the results were expressed as mean values with standard deviations.

2.2.6.2.3. Tensile strength

The radial tensile strength (σ) was calculated using Equation 2.11:

$$\sigma = \frac{2F}{\pi DT} \quad \text{Eq. 2.11}$$

where, F is the force required breaking the tablets, D is the diameter (10 mm) of the tablets, and T is the thickness of the tablets.

2.2.6.2.4. Weight Variation

Tablets were evaluated for uniformity of weight. Twenty tablets of each formulation were weighed together and individually using an electronic balance (Mettler Toledo, PR 203, Switzerland) according to USP30/NF25, 2007. Average weight was calculated. The percentage of weight variation is calculated by using Equation 2.12. Each tablet weight was then compared with average weight to make certain whether it is within acceptable limits or not.

$$\%\ \text{of weight variation} = \frac{\text{Individu al weight} - \text{Average weight}}{\text{Average weight}} * 100 \quad \text{Eq. 2.12}$$

2.2.6.2.5. Friability

Friability of tablets was determined with a friability tester (ERWEKA® GmbH, Heusenstamm, TAR 20, Germany) according to USP30/NF25, 2007. Preweighed 10 tablets were needed for drum rotation at 25 rpm for 4 min. The tablet samples were then removed and dedusted and reweighed. The percentage weight loss of tablet was calculated by reweighing the tablets. The percentage friability was then calculated by using Equation 2.13:

$$\% F = \frac{W(\text{initial}) - W(\text{final})}{W(\text{initial})} * 100 \quad \text{.....Eq. 2.13}$$

2.2.6.2.6. Disintegration time

The disintegration time was determined according to the disintegration test for uncoated tablets of the United States Pharmacopoeia (USP 30/NF25, <701>, 2007). Six tablets were tested at a time using a disintegration apparatus (ERWEKA® GmbH, Heusenstamm, Germany) filled with distilled water maintained at 37± 2 ^{0}C as the immersion fluid. The tablets were considered completely disintegrated when all particles passed through the wire mesh.

2.2.6.2.7. Standard Curve of Ibuprofen in 0.1N HCl and in phosphate buffer solution

Aliquots of stock solution (100µg/ml) of IBP in 0.1N HCl (pH 1.2) in phosphate buffer at a (pH 7.2) were pipetted into a series of 100ml volumetric flask and diluted to volume to provide different concentrations. The UV absorbance readings were measured at λmax of 221 nm using UV-Visible spectrophotometer (SOLAR CM 2203, Minsk, Belarus). The calibration curve was constructed by plotting absorbance against concentration. The linearity curve ranged between 5-23 µg/ml in 0.1N HCl (pH 1.2) and in phosphate buffer at a pH 7.2.

2.2.6.2.8. *In-vitro* dissolution study

In-vitro dissolution study was carried out by using USP Type II dissolution apparatus (Paddle method) (ERWEKA® DT600, Heusenstamm GmbH, Germany) (USP30-NF25, <711>, 2007), and the dissolution test was performed on six tablets in pH 1.2 (900 ml) for the first 2 h and then in phosphate buffer (pH 7.2) from (2-12) h. The dissolution medium was kept in thermostatically

controlled water bath, maintained at 37 ± 0.5^0C and the stirring speed of the paddle was set at 50 rpm. At predetermined time intervals i.e., at 0, 15 and 30min, and then 1, 2, 3, 4, 6, 8, 10 and 12 h; 5 ml aliquots were withdrawn and compensated with equal volume of the dissolution medium kept at the same temperature in order to maintain the volume constant. Samples were filtered through Whatman filter paper and analyzed spectrophotometrically at 221 nm for the drug release after appropriate dilution. From the absorbance values, concentration was determined using the standard calibration curve of IBP. Results were plotted as cumulative percentage of IBP released versus time. All the experiments were performed in triplicate (mean ± SD, n=3).

2.2.6.2.9. Analysis of drug release kinetics

To obtain indications on the mechanism for the release rate kinetics, the results of *in-vitro* release profiles obtained for the formulations were fitted into the five models. Kinetic models were used to evaluate the *in-vitro* drug release: Cumulative percent drug released versus time (Zero-order kinetic model Equation 2.14); Log cumulative percent drug remaining versus time (First-order kinetic model Equation 2.15); Cumulative percent drug released versus square root of time (Higuchi's model Equation 2.16); Log cumulative percent drug released versus log time (Korsmeyer-Peppas Equation 2.17) and Hixson-Crowell Method Equation (2.18).

Zero Order Kinetics

$Q = Q_0 - K_0 t$ …… Eq. 2.14

where, Q=is the amount of drug remaining at time t; Q_0 = the quantity of drug present initially in the dosage form; and K_0 = zero order release constant

First Order Kinetics

$\ln Q = \ln Q_0 - K_1 t$……Eq. 2.15

where, Q = is the amount of drug remaining at time t; Q_0 = is the quantity of drug present initially in the dosage; and K_1 = first order release rate constant

Higuchi Model

$M_t/M_0 = K_H t^{1/2}$…… Eq. 2.16

where, Mt = amount of drug released at time t, M_0 = amount of total drug in the tablet and K_H = the Higuchi constant.

Korsmeyer-Peppas Release Model

$$M_t/M_0 = Kt^n \quad \text{Eq. 2.17}$$

Where, Mt = the amount of drug released at time t, M_0 = the amount of total drug in tablets, M_t/M_0 = the fractional drug release at time t, t = release time, K = a constant incorporating the structural and geometric characteristics of the matrix tablets and n = a diffusional exponent, indicative of the mechanism drug release.

Hixson-Crowell Method

$$Q_t^{1/3} = Q_0^{1/3} - K_{HC}t \quad \text{Eq. 2.18}$$

Where, Qt = the amount of drug remaining in time t, Q_0 = the initial amount of the drug in tablet and K_{HC} = the rate constant for Hixson-Crowell rate equation.

2.2.7. Data analysis

Statistical analysis of data was carried out using analysis of variance (ANOVA) and model dependent method on Origin® 7.0 statistical software (Origin Lab Corporation, Northampton, MA 01060, USA). At 95% confidence interval, ($p < 0.05$) were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Preliminary studies

Different reports indicate that chemical reactions like cross-linking of starch is affected by many factors, such as starch source, cross-linking reagent concentration and composition, the extent of substitution, pH, reaction time and temperature (Ashogbon and Akintayo, 2014). From literature search and various preliminary studies, sequences of cross-linking experiments were carried out at temperatures (45, 50, and 55 ^{0}C) in order to determine cross-linking conditions. The aim of literature survey and preliminary studies were to obtain suitable reaction conditions for the synthesis of cross-linked *C. abyssinica* starch with substantial increase in DCL for extended release application in tablets manufactured by direct compression method. It was found out that considerable percentage of DCL was obtained with the reaction condition of starches cross-linked at a temperature of 55 ^{0}C which were stirred for 8 h. Therefore, CLS at 55 ^{0}C with varying concentration of SHMP were selected for further studies, i.e., reaction condition (Table 2.1).

3.1. Characteristics of modified starches

3.1.1. Rheological properties

The viscosities of NS and the CLASs decreased on heating from 50 to 90^{0}C as shown in (Table 3.1). A possible explanation for this might be the deformation of the swollen anchote starch granules which ultimately led to a reduction in peak viscosities at higher temperatures. These results were in agreement with those of (Jing-ming & Sen-lin, 1990) for corn starch. The peak viscosities of the native and CLAS suspensions were found to be significantly different ($P < 0.05$). Examination of the rheological properties of starches is an important step in the characterization and understanding of their functional properties. When starch is cooked in excess of water, the granules swell and at the same time part of the components solubilize giving rise to a suspension of swollen particles dispersed in a macromolecular continuous phase. It is possible that when starch granules hydrate freely they are more fragile and prone to shear disruption, while those that swell partially are more resistant to rupture (Nunez-Santiago *et al.*, 2004). Using CLS with a proper cross-linking level could provide granule swelling without disruption and consequently control the rheological properties of starch (Wongsagonsup *et al.*,

2014). Strengthening bonding between starch chains by cross-linking will increase the resistance of the granule to swelling leading to lower peak viscosity (Liu *et al.,* 1999). This might be a possible explanation to the decrease in peak viscosities of the CLAS with increase in concentration of cross-linking agent as can be seen in Table 3.1. The order of peak viscosities of the NS and CLASs is CLAS-15 < CLAS-10 < CLAS-5 < NS. Singh *et al.,* (2007) reported that chemical modification leads to a considerable change in the rheological properties of starches. The increase in viscosity during the cooling period is indicative of not only the normal inverse relationship between the viscosity and temperature of suspensions but also of the tendency for various constituents present i.e., swollen granules, fragments of swollen granules, and colloidally dispersed and dissolved starch molecules to associate.

Table 3.1: Peak viscosities of NS and CLAS during the heating-cooling cycle of a 10 % w/v suspension at selected temperatures (Mean ± SD, n=3)

Sample	Peak viscosity in cP of increasing temperature in ^{0}C			Peak viscosity in cP of decreasing temperature in ^{0}C		
	50	70	90	90	70	50
NS	3347.78 (35.29)	2844.32 (27.14)	2227.35 (9.55)	2349.68 (44.11)	2914.09 (85.83)	3625.84 (24.83)
CLAS-5	1492.59 (25.43)	1420.9 (57.15)	1211.01 (21.62)	1246.32 (24.10)	1522.33 (98.68)	1626.46 (19.13)
CLAS-10	1240.15(39.79)	1142.96 (39.23)	1084.4 (95.74)	1173.99 (86.86)	1307.25 (84.04)	1391.6 (39.99)
CLAS-15	846.27 (10.76)	768.86 (33.03)	687.32 (76.78)	728.67 (14.25)	839.56 (37.19)	864.76 (47.00)

Figures in the parenthesis represent ±SD, n=3, ^{0}C- degree centigrade, cP- centipoises

3.1.2. Degree of cross-linking

Different types of CLAS with varying DCL were obtained by changing the starch to SHMP ratio. As shown in Table 3.2, the DCL of the starches increased in the following order CLAS-5 < CLAS-10 < CLAS-15 indicating that the DCL is directly proportional to the number of covalent bonds which are produced during reaction with a crosslinking agent. With increasing concentration of cross-linking agent, the peak viscosity decreases. The lower peak viscosity of the more highly cross-linked samples can be attributed to the higher density of cross-links, and is consistent with the decreasing swelling behavior with increasing cross-linking agent concentration. The DCL increased with the increasing of the concentration of the crosslinking agents, SHMP (Table 3.2).

Table 3.2: Peak viscosities and DCL of CLAS (mean ± SD, n=3).

CLS Samples	Peak Viscosity (cP)	Degree of Cross-linking (%)
CLAS-5	1626.46 (19.13)	55.14 (0.77)
CLAS-10	1391.6 (39.99)	61.62 (0.86)
CLAS-15	864.76 (47.00)	76.15 (1.24)

Figures in the parenthesis represent ± SD, n = 3

The peak viscosity of NS suspension was 3625.84 cP as can be seen in Table 3.1. Substantial reduction in peak viscosity in cross-linked starch compared with that of NS was due to phosphate intermolecular linkage in the starch molecules. Cross-linking decreases amorphous chain mobility and strengthens the starch molecular structure. Higher DCL suppresses swelling and lowers peak viscosity. Wongsagonsup *et al.,* (2014) reported that with increasing cross-linking level, the granular structure of the cross-linked tapioca starch was much stronger; probably due to decrease in the swelling power of the cross-linked tapioca starch with increasing reagent concentration leading to more cross-links, causing constraints on the swelling behavior. Hirsch & Kokini, (2002) and Heebthong *et al.,* (2016) also reported that the maximum peak viscosities were shown in lowest DCL due to the intermolecular bridge in starch molecule. Gels made from CLS samples showed greater strength. Lower peak viscosity with increasing DCL was observed.

3.1.3. Solubility and Swelling power (SP)

When starch is heated in excess water, the crystalline structure is disrupted and water molecules become linked by H-bonding to the exposed –OH groups of amylose and amylopectin. This causes an increase in granule swelling and solubility. SP and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. 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 (Hoover, 2001). Figures 3.1 and 3.2 depict the SP of native and CLAS. CLAS had reduced SP and solubility as compared to the native counterparts. SP generally decreases with increase in the concentration of the cross-linking agent, SHMP, due to limited hydration of the hydroxyl groups of the gel as a result of the increased number of interchain bridges introduced by the increased DCL. Heebthong, *et al., (*2016) reported that higher DCL lowered water solubility index since phosphate linkage prevented the breakage and leaching solid of starch granule that resulted in lower solubility. This result suggested that cross-linking strengthened the starch samples.

Introduction of phosphate cross-links into the starch restricted the mobility of the molecular structure, leading to a reduction in water solubility index of starch. Mirmoghtadaie, *et al.,* (2009) and Hirsch and Kokini, (2002) demonstrated that CLS exhibit lower solubility than their native equivalents, and solubility decreases further with an increase in the concentration of cross-linking agent, which may be attributed to an increase in rigidity compactness. Cross-linking reinforces the structure of starch granules and limits water absorption by restricting the mobility of starch chains in the amorphous region. The ranking of SP is: CLAS-15 < CLAS-10 < CLAS-5 < NS. Detduangchan, *et al.,* (2014) reported that increasing the cross-linking agent yielded the promotion of the cross-link density of starch, resulting in greater compactness and resistance to dissolution. Cross-linking delayed the gelatinization process by restricting swelling and reducing the hydration of starch.

As a result, the solubility of CLS decreased as compared to that of NS. Solubility and SP of all the starch samples were low at lower temperatures. This is due to the presence of widespread and strongly bonded structures which bring about better degree of associative forces in the granules

making the CLAS resistant to solubilization and swelling. The solubility and SP of all starch samples (native and cross-linked) were low at low temperatures but increased at higher temperatures. All CLS samples showed significantly lower SP ($P < 0.05$) beyond 75 ^{0}C compared with native counterparts. The reason might be at higher temperature NS gelatinized with relative ease but with CLS since they are connected with the covalent bond due to cross-linking they would not gelatinize. Wongsagonsup *et al.,* (2014) stated that the solubility of CLS is mainly associated with their swelling power behavior. The higher the SP, the higher the solubility is. At low levels of cross-linking (CLAS-5), the penetration of water molecules into the granules was enhanced which would facilitate leaching of starch molecules into the aqueous phase, resulting in increased solubility. In contrast, at higher cross-linking levels (CLAS-15), the SP decreased due to the excessive cross-linking that hinder the leaching of starch molecules from the starch granules and thus decreased solubility. The effect of cross-linking on swelling is reported in several studies (Chatakanonda, *et al.,* 2000, Singh *et al.,* 2007, Mirmoghtadaie, *et al.,* 2009, Detduangchan, *et al.,* 2014, Heebthong, *et al.,* 2016).

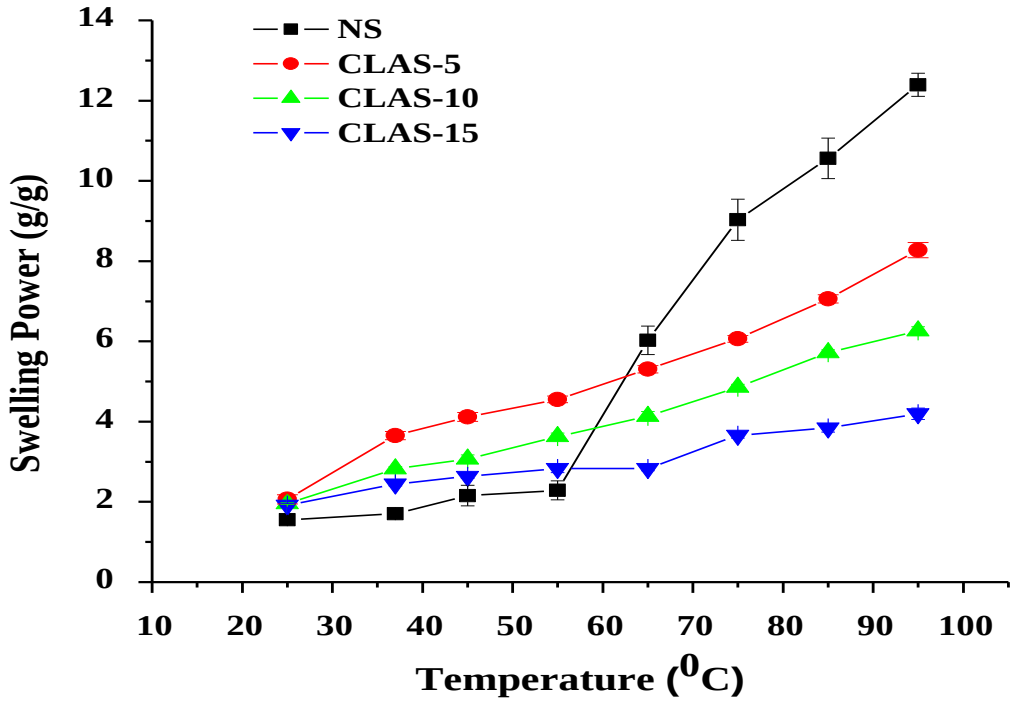

Figure 3.1: Swelling pattern of native and CLASs at various temperatures

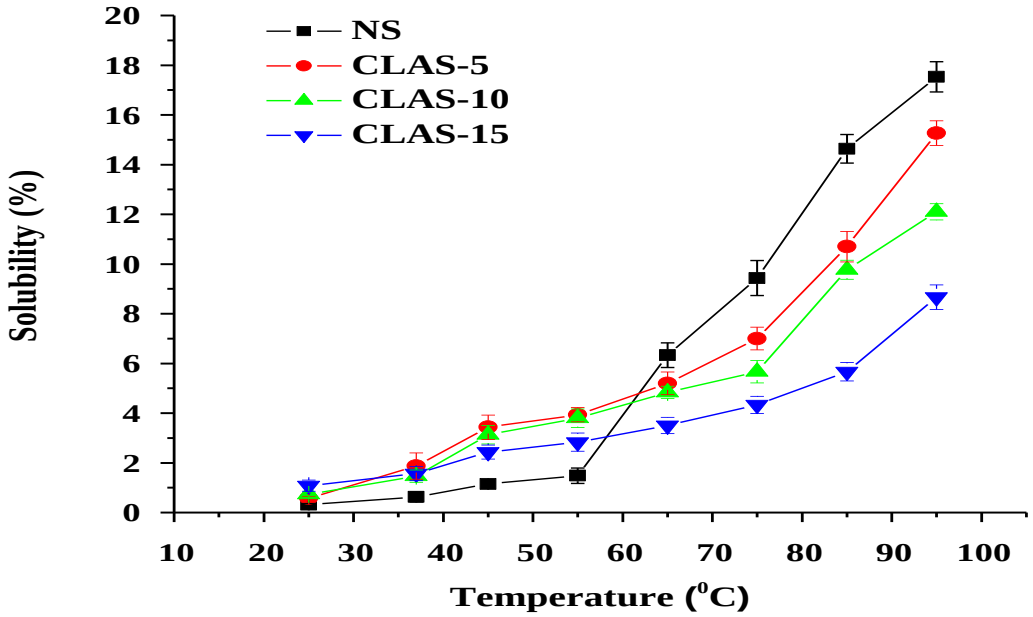

Figure 3.2: Solubility of native and CLASs at various temperatures

3.1.4. Moisture sorption pattern

Moisture is known to modify the flow and mechanical properties of many powders including starches. Therefore, knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction is critical (Gebre-Mariam and Schmidt, 1998). Starch based excipients are widely used in pharmaceutical industry, either as filler/binders and disintegrants or as programmed release excipients in oral dosage forms. During storage, starches being hygroscopic material sorb significant amount of moisture from the atmosphere over a wide range of relative humidities and temperatures. It is well known that this water significantly affects several physicochemical characteristics (Walia *et al.,* 2002; and Steendam, 2005). The knowledge and understanding of sorption isotherms is highly important in drug formulation and used for the predictions of quality, stability and shelf-life of the pharmaceutical products.

Moisture sorption behaviors of native and CLASs are shown in Fig. 3.3. The water uptake of the CLAS is lower than the native counterpart. Carmona-Garcia *et al.,* (2009) described that the reaction between the -OH groups of glucose units of starch and the bi- or poly-functional chemical reagent used in this chemical modification, SHMP, decreases the possibility of reaction

between -OH of starch chains and the water molecules and consequently the sorption of water to this polymer. As the concentration of cross-linking agent increased, water sorption capacities decreases. Cross-linking reinforces the structure of starch granules and limits water absorption by restricting the mobility of starch chains in the amorphous region as described by (Mirmoghtadaie *et al.*, 2009). Low level of DCL increases water sorption. (Heebthong *et al.*, 2016) reported at low level of cross-linking, the repulsion of negative charge of phosphate in starch chain could enhance hydration. On the other hand, at high DCL, the bonding between adjacent molecules restrict the swelling and hydration of starch granules. Cross-linking reinforces the structure of starch granules and limits water absorption and solubility of starch, thereby restricting the mobility of the starch chain in the amorphous region.

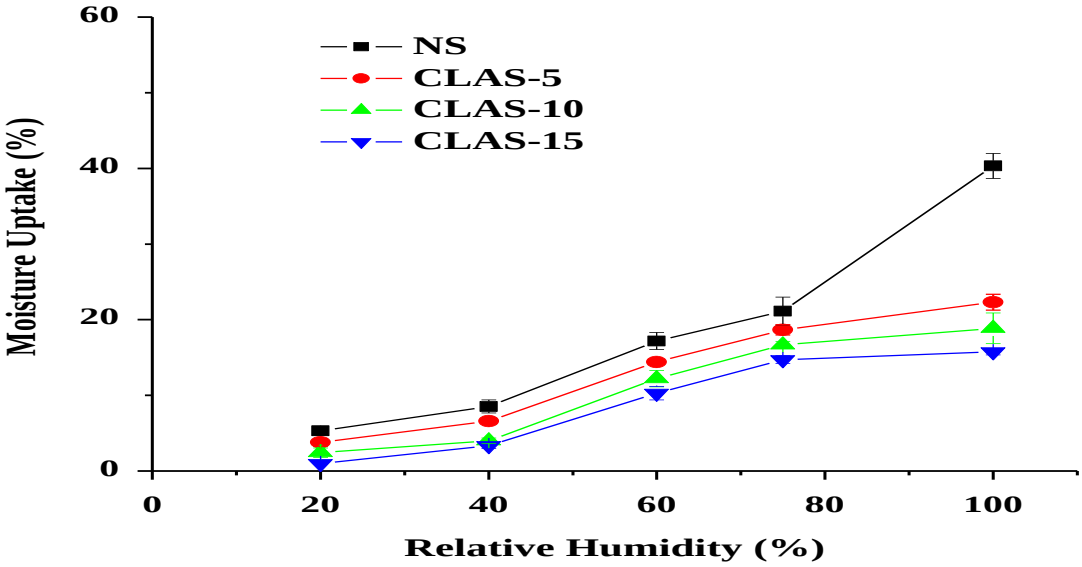

Figure 3.3: Moisture sorption patterns of native and CLASs after storage for 4 weeks.

3.1.5. Fourier transform infrared (FT-IR) spectra

The compatibility of drug and the polymer, CLAS-15 was studied with FT-IR. NS cross-linked with 15% SHMP was selected for FT-IR analysis. The FT-IR spectrum of pure IBP, CLAS-15, and physical mixture of IBP and CLAS-15 are shown in Fig. 3.4, Fig. 3.5 and Fig. 3.6, respectively. As can be seen from Fig. 3.4, the characteristic IR absorption peaks are observed at 3336.62 cm^{-1} due to –O–H stretching vibrations, and the intense absorption peak occurred at

1716.53 cm^{-1} was presumably originated from the stretching vibrations of –C=O group. Another characteristic absorption band due to –C=C stretching vibrations of the aromatic ring was presented at 1506.30 cm^{-1}. The characteristic absorption bands occurred due to the –C–H stretching was shown at 2954.74cm^{-1}, and 2852.52 cm^{-1} and the absorption band at 1417.58 cm^{-1} was due to –C–O–H bending vibrations of carboxyl moiety. Additional characteristic absorption peak presented at 1456.16 cm^{-1} is due to –C–H bending vibrations. The characteristic absorption peak at around 1315 up to 1475 cm^{-1} is attributed to –O–H bending (deformation) vibration. This finding was in close agreement with the data found in Dragan *et al.*, (2015). A characteristic IR spectra of the CLAS showed the absorption bands at 1271.00cm^{-1} and 1020.27cm^{-1} ascribed to P=0 and P–O–C stretching vibrations respectively were shown in Fig. 3.5, and in the physical mixture of IBP and CLAS-15. Detduangchan *et al.*, (2014) reported that in STMP cross-linked rice starch the peak at 1012.79 and 1261.38cm^{-1} corresponds to phosphate ester stretching vibrations of (P–O–C) and (P=O), respectively. Similar findings by (Jyothi *et al.*, 2006) reported that the IR spectra of the modified starches showed the typical peaks for the starch backbone. In addition, in the IR spectra of the CLAS-15, the characteristic absorption bands of starch at 1151.42 cm^{-1}, represented is due to C–C stretching vibrations. As a result, the FT-IR studies indicated that all the characteristic absorption peaks for IBP appeared in the physical mixture of IBP and CLAS-15 as well, and thus it is essentially be indicative and/or suggests that there was no incompatibility or chemical interaction of the drug with the polymer, CLAS-15. Consequently, the polymer could be used safely to formulate the matrix tablet.

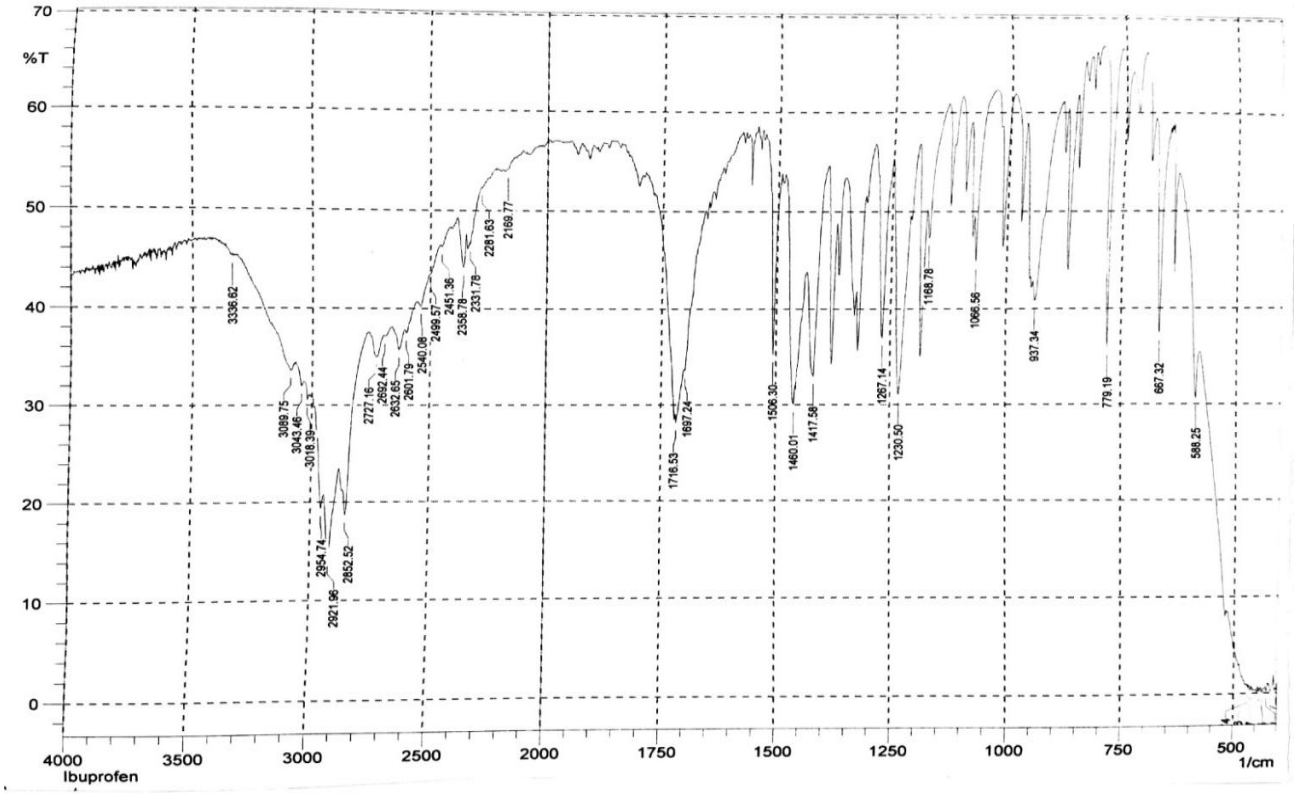

Figure 3.4: The FTIR spectrum of pure ibuprofen (API)

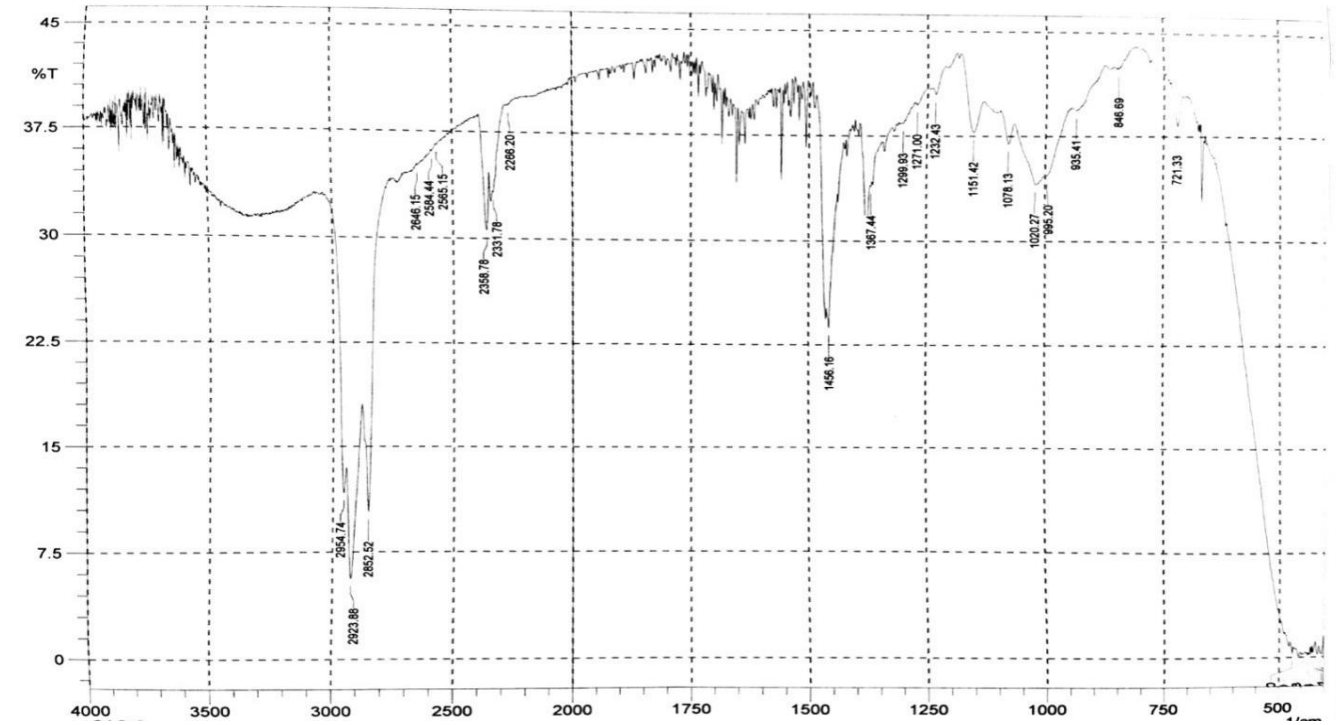

Figure 3.5: The FT-IR spectrum of cross-linked anchote starch (CLAS-15)

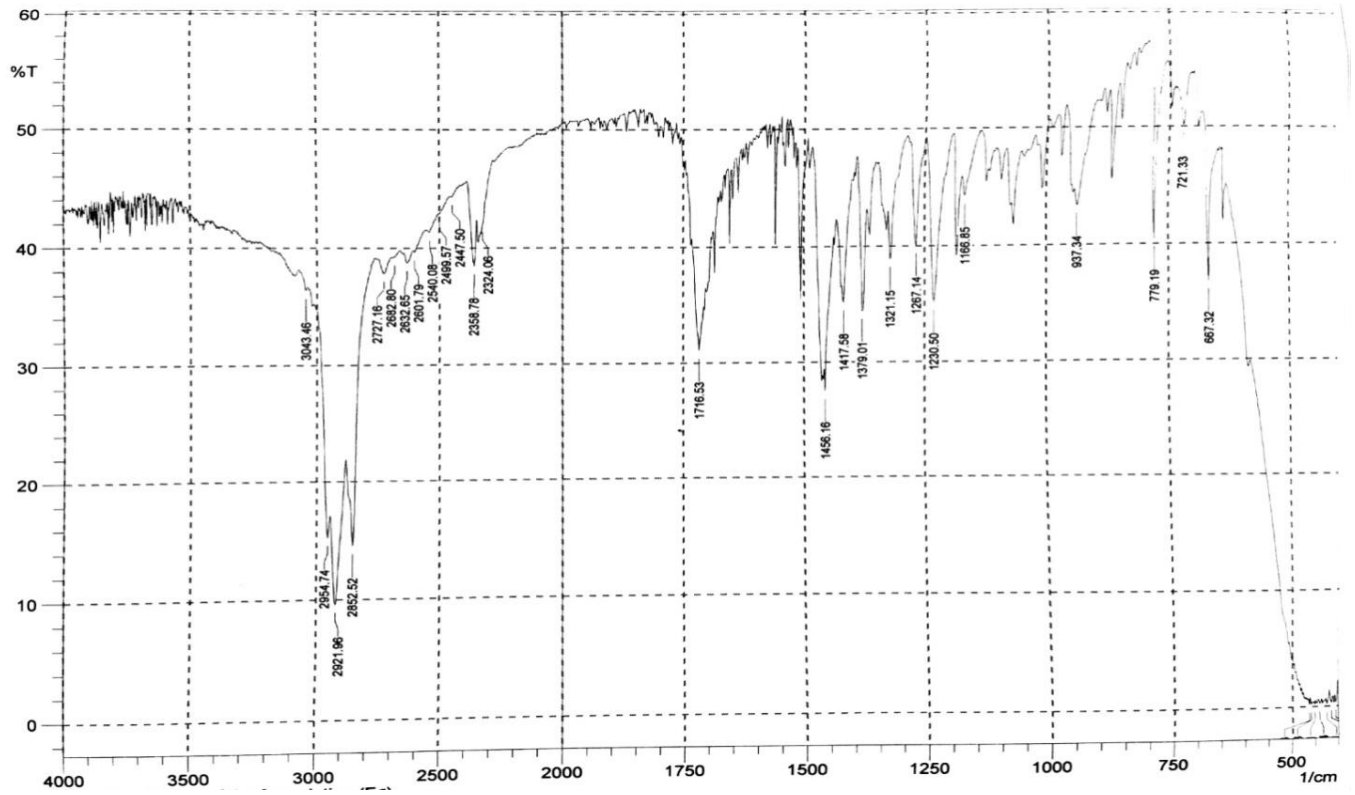

Figure 3.6: The FT-IR spectrum of physical mixture of ibuprofen and the CLAS-15

3.1.6. Powder properties

Flow property of pharmaceutical powders can be a critical attribute in the manufacture of pharmaceutical dosage forms, assisting or governing bulk movement of powders or granules during transfer, blending, granulation, and tableting (Tay*et al.,* 2017). Optimum flowability of powders is crucial in the manufacturing process of solid single dose preparations (Schussele and Bauer-Brandl, 2003). The bulk density and tapped density for the NS, CLAS-5, CLAS-10 and CLAS-15 values are shown in Table 3.3.

The bulk and tapped densities for the entire native and CLASs were found to be in the range between 0.48 ± 0.012g/ml to 0.69 ± 0.042g/ml and 0.67 ± 0.064g/ml to 0.79 ± 0.075g/ml, respectively. The results of angle of repose for CLAS-15 was 32.3^0 but that for CLAS-5 and CLAS-10 were 36.5^0 and 35.1^0, respectively which indicate that the powders show fair to good flow properties. This was additionally supported by their compressibility index (CI) values. CI for CLAS-5, CLAS-10 and CLAS-15 were 19.6, 14.1 and 10.2, respectively indicating fair to good flow properties of powders (USP30-NF25, 2007). The HR and CI are the indicators of the

flowability and consolidation properties of the powder mixtures. CI is a simple test to evaluate bulk and tapped densities of the powders and the rate at which they are packed down. HR indicates the flow properties of powder. The CI and HR enable the powder flows at minimum bulk density and consolidate to maximum density inside the die, prior to compression.

The moisture content of NS was found to be 9.58% ± 0.2103. The moisture content of NS and CLASs decreased in the following order: NS > CLAS-5 > CLAS-10 > CLAS-15 the values were 9.58%, 8.32%, 7.28%, and 5.93% respectively. One can clearly see that cross-linking decreases the moisture content of the NS significantly ($P < 0.05$). This finding was in close agreement with the data reported by Detduangchan, *et al.,* (2014).

Table 3.3: Powder properties of the native and CLASs (mean ± SD, n=3).

Powder properties	NS	CLAS-5	CLAS-10	CLAS-15
Bulk density (gm/ml)	0.48 (0.012)	0.54 (0.027)	0.61 (0.023)	0.69 (0.042)
Tapped density (gm/ml)	0.67 (0.064)	0.69 (0.032)	0.75 (0.092)	0.79 (0.075)
Carr's index (%)	27.9 (3.974)	19.6 (1.706)	14.1 (1.315)	10.2 (2.625)
Hausner's ratio	1.37 (0.097)	1.24 (0.033)	1.22 (0.115)	1.16 (0.183)
Angle of repose (^{0})	-	36.5 (1.323)	35.1 (1.852)	32.3 (3.751)
Flow Rate (g/sec)	-	2.97 (0.529)	2.47 (0.730)	2.18 (0.209)
Moisture content (%)	9.58 (0.210)	8.32 (0.69)	7.28 (0.802)	5.93 (1.383)

Figures in the parenthesis represent ± SD, n=3

- Starch powder didn't flow through the funnel

3.2. Evaluation of tablets

Various properties of IBP tablets are shown in Table 3.4.

Table 3.4: Crushing strength, weight, thickness, tensile strength, friability and disintegration time of tablets of native, CLASs

Formulation Codes	Crushing strength (N) ± SD	Weight (mg) ± SD	Thickness (mm) ± SD	Tensile strength (Kg/cm^2) ± SD	Friability (%) ± SD	Disintegration time (min)
FNS	73.0 (5.0)	398.3 (1.5)	3.97 (0.02)	11.7 (2.33)	1.7 (0.11)	6
F1	90.0 (3.0)	400.7 (3.06)	3.95 (0.01)	14.5 (1.85)	0.89 (0.06)	>120
F2	92 (6.8)	395.7 (4.04)	3.96 (0.03)	14.8 (2.3)	0.78 (0.08)	>120
F3	98.6 (4.6)	402.7 (4.93)	4.0 (0.02)	15.7 (0.8)	0.62 (0.09)	>120
F4	130.6 (4.04)	403.3 (9.3)	3.98 (0.02)	20.9 (2.34)	0.41 (0.15)	>120
F5	137.0 (6.24)	394.7 (6.2)	3.98 (0.03)	21.9 (1.51)	0.24 (0.08)	>120
F6	145.0 (7.55)	404.3 (7.8)	3.96 (0.02)	23.3 (1.83)	0.36 (0.09)	>120
F7	148.6 (8.02)	408.3 (6.6)	3.97 (0.03)	23.8 (2.93)	0.29 (0.3)	>120
F8	155.3 (6.03)	404.0 (4.6)	3.98 (0.02)	24.9 (1.22)	0.21 (0.13)	>120
F9	165.6 (2.52)	401.6 (3.2)	3.98 (0.02)	26.5 (2.16)	0.09 (0.04)	>120

Figures in the parenthesis represent ±SD, FNS-Formulation of NS

3.2.1. Weight Uniformity

As shown in Table 3.4, the average weight of 20 tablets was 394.7±6.2 to 408.3±6.6g. As per the USP30/NF25, (2007) pharmacopeial limit for the percentage deviation for tablets of more than 324 mg is ± 5%. It was also revealed that none of the formulation showed a deviation of more than ± 5% for any of the tablets tested. The average percentage deviation of all tablet formulations was found to be within the above stated limit.

3.2.2. Crushing strength

Tablet strength is an important quality factor that is tested during tablet production. Tensile strength and breaking force increase exponentially with increasing relative density for typical pharmaceutical powders (Razavi *et al.,* 2015). Preparation of the tablets from the CLS was likely to have stronger packing. When applying a compression force to the starch granules, the crystalline regions for the more crystalline starches could be forced closer together (Atichokudomchai and Varavinit, 2003). The mechanical properties of the tablets are also important because the tablets must be strong enough to withstand post-compaction operations such as handling, coating, packaging, storage, transport, etc. (Shang *et al.,* 2013).

As shown in Table 3.4, the hardness of the tablets of all batches ranged from 73.0 ± 5.0 to 165.6 ± 2.52 N and the thickness of tablets ranged from 3.95 ± 0.01 to 4.00 ± 0.02 mm. It was also revealed that tablets prepared from high levels of CLASs had a higher crushing strength than those tablets prepared from low levels of the modified starches. This can be exemplified by, the formulations like F7, F8, and F9 which showed a comparatively higher hardness value. The low hardness value observed with formulation FNS, F1, F2, and F3 perhaps due to the presence of low levels of CLASs in which the tablets were made of. This results were in close agreement with the previously published report by Nikolic *et al.,* (2015). They confirmed that high proportions of both drug and matrix polymer can significantly affect mechanical properties that are considered as an important quality attribute of the hydrophilic matrix tablets.

3.2.3. Tensile strength

Tablet tensile strength is an essential parameter to consider and is therefore standardly tested during the manufacturing process as reported by Juban *et al.,* (2017). As shown in Table 3.4, the

tensile strength of the tablets of all batches ranged from 11.7±2.33 to 26.5±2.16 Kg/cm^2. The tensile strengths of tablets prepared from using CLAS were higher than tablets prepared from the native counterpart. Their differences were statistically significant ($P<0.05$). The increase in tensile strength is attributed to the increase in compactibility of the CLASs. Compaction behaviour and properties of tablet (porosity and tensile strength) dramatically affected when the polymer passes the glass transition and becomes rubbery. Compression of a rubbery polymer results in extensive relaxation during the decompression stage, making it practically impossible to prepare strong compacts. Therefore, in practice, glassy polymers are applied as matrix-forming tablet excipients. Starches, for example, are rigid at low moisture content, whereas they become more ductile at higher moisture content. Moisture facilitates both elastic and plastic deformation of modified starches (Steendam, 2005).

3.2.4. Friability

As shown in Table 3.4, tablets made from CLASs exhibited lower friability than those tablets made from NS. The loss in total weight of the tablets made from NS due to friability was 1.7±0.09 (% ± SD) but those tablets made from CLASs lies in the range of 0.09±0.04 to 0.89±0.07 (% ±SD) in all the formulations (F1 to F9). Their friability values were less than 1% which ensures that the formulated tablets were mechanically stable. However, formulations FNS had corresponding friability values greater than 1%, and they did not meet the pharmacopoeial requirements (USP30/NF25, 2007).

3.2.5. Disintegration time

Tablets made from higher concentration of cross-linking agent took longer time to disintegrate as compared to those tablets which were made from lower concentration of cross-linking agent. Generally the disintegration times are related to hardness. When the hardness increased, the disintegration time increased. As shown in Table 3.4, tablets made from NS exhibited short disintegration time than those tablets prepared with all types of CLASs. The disintegration time of tablets with NS was found to be 6 min whereas the corresponding values for formulations F1-F9 showed a disintegration time of more than 2h. Tablets prepared by using NS disintegrated within very few minutes whereas tablets prepared by using a CLAS with a higher DCL since they have contained high concentrations of the cross-linking agent, SHMP. Thus, such tablets

remained intact for a longer period of time since their hardness values were higher and the rigid network of the cross-linking. As the DCL increased, the mechanical strength of the CLS increased and disintegration did not occur within 2h. Hence, the drug release from these CLS matrix tablets becomes extended for longer period of time. Wongsagonsup *et al.*, (2014) reported that cross-linking depressed the disintegration of starch granules. Cross-links prevent the starch granules from fully swelling and ultimately disintegrating.

3.2.6. Calibration curves

Figure 3.7 shows the standard calibration curve of ibuprofen in 0.1N HCl. The absorbance of the solution as a function of its concentration was plotted and a calibration curve with a linear regression equation of Y = 0.0349X + 0.0132 (where Y is the absorbance and X is the concentration in µg/ml) and a correlation coefficient (R^2 = 0.9996) was obtained.

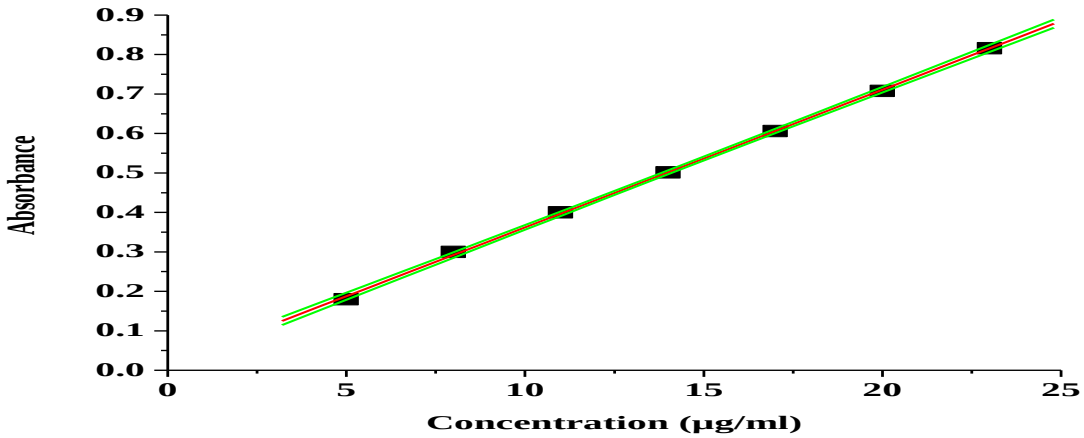

Figure 3.7: Standard calibration curve of ibuprofen at λmax of 221nm in 0.1N HCl (pH 1.2) with 95% confidence bands for the mean.

Figure 3.8 shows the standard calibration curve of ibuprofen in phosphate buffer solution. The absorbance of the solution as a function of its concentration was plotted and a calibration curve with a linear regression equation of Y = 0.0352X + 0.0074 (where Y is the absorbance and X is the concentration in µg/ml) and a correlation coefficient (R^2 = 0.9998) was obtained.

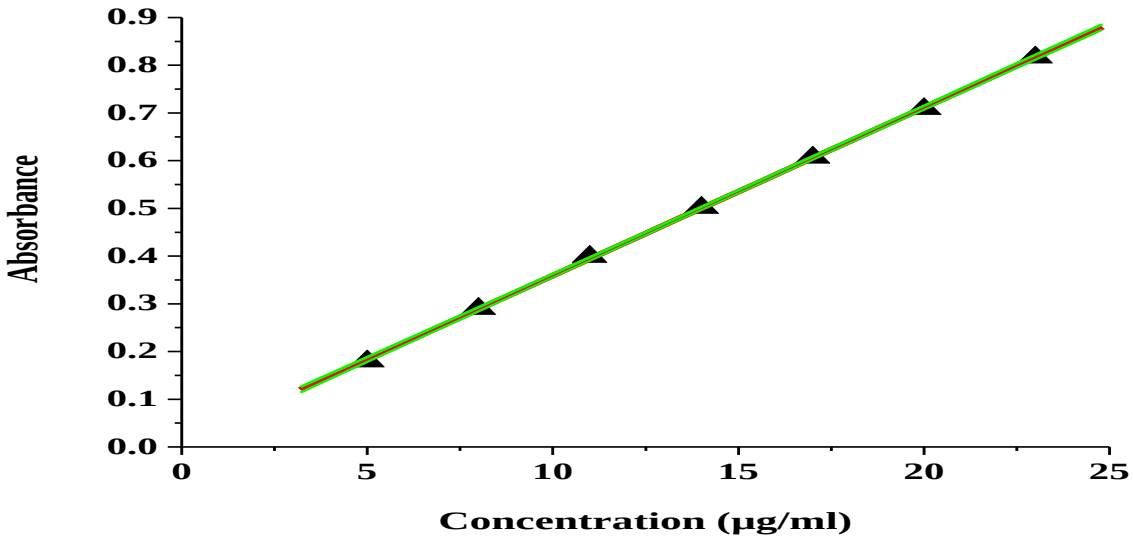

Figure 3.8: Standard calibration curve of ibuprofen at λmax of 221nm in phosphate buffer solution (pH 7.2) with 95% confidence bands for the mean.

3.2.7. *In-vitro* dissolution tests

In-vitro dissolution testing is an important tool used for development of dosage forms in the pharmaceutical industry. It is very widely used in formulation development, in monitoring the manufacturing process and as a quality control test. It can also be used to predict the *in-vivo* performance of certain products (Anand *et al.*, 2011).

The dissolution profiles of IBP from its different prepared tablets formulae (F1- F9) are depicted in Fig. 3.9. As shown in Fig. 3.9, increase in the polymer concentration resulted in the decrease of drug release which essentially sustained the drug effect for the intended purpose. CLASs reduced the drug release due to a reduction in the penetration of solvent molecule into the system. The rate of release was controlled by the permeability of matrix structure. This increased release retardant effect of highest amount of CLASs might be due to gel barrier formation which eventually impedes the penetration of dissolution medium to the matrix. It might also be due to matrix slow erosion. It was evident that increasing polymer concentration in the matrix essentially brings enhanced viscosity of the gel, which ultimately reduced the drug release.

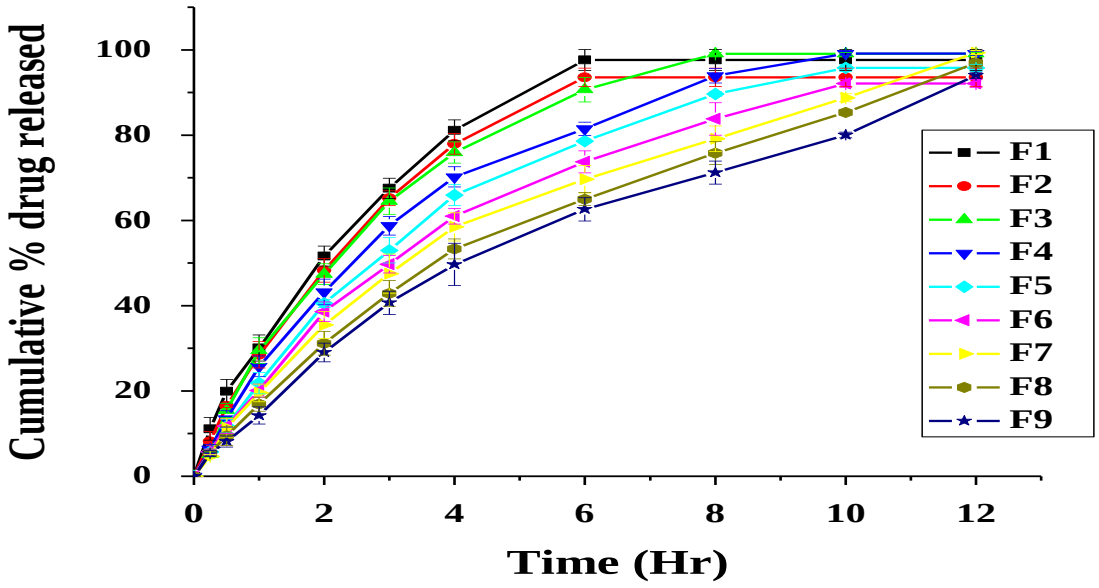

Figure 3.9: *In-vitro* release profiles of nine IBP matrix tablets formulations from CLASs tablets containing 200 mg IBP.

The *in-vitro* drug release showed that the dependence on the amount of the matrix forming polymer and the DCL. For the initial 2 h i.e., when the drug was in 1.2 pH buffer the drug release was found to be low in all the cases, indicating that the matrices did not release appreciable amount of the drug in the acidic medium which resist disintegration, however when transferred into intestinal media (pH 7.2), there was slow dissolution of the polymer resulting in the slow release of the drug and it can also be attributed to the fact that the swelling of matrices in the acidic medium was low. However the basic pH of the medium was able to penetrate and dissolve the matrix to release the drug by diffusion and polymer chain relaxation. Detduangchan *et al.*, (2014) reported that the dissolution gradually decreased with increased concentrations of the cross-linking agents. This may be explained by the fact that increasing the cross-linking agent resulting in greater compactness and resistance to dissolution. As the concentration and amount of polymer increased, larger amounts of drug got bonded in the polymer matrix as a result the rate of drug release from the matrices decreased. In Figure 3.9, it is also shown that Formulations F1 and F2 which were prepared from CLAS-5 and incorporated with 20%, and 25% of the polymer, respectively and since greater than 90% of the drug release was attained in about 6 h, there exist complete drug release in the stated period of time. Formulation F3 which was

prepared with 30% of the same polymer released greater than 90% of the drug in 8 h since this formulation was prepared from the lowest percentage of the cross-linking agent and were having the lowest DCL (55.14%) among the CLS in this study. Formulations F4, F5, and F6 which were prepared from CLAS-10 as a matrix forming polymer had medium DCL (61.62%) and incorporated with 20%, 25%, and 30% of the polymer respectively released greater than 90% of the drug in nearly 10h since the matrix tablets completely disintegrated within this period of time. However, formulations F7, F8 and F9 which were prepared from CLAS-15 had the highest DCL (76.15%) and incorporated with 20%, 25%, and 30% of CLAS-15, respectively released greater than 90% of the drug slowly at the end of 12h. Matrix tablets made from the highest concentration of cross linking agent CLAS-15, i.e., formulations F7, F8, and F9 swelled slowly that drug releases from them were lower.

Thus, a stable formulation was achieved by incorporating high level of CLASs, so that sustained release profile was maintained for an extended periods of time, 12h. Velasco *et al.,* (1999) reported that an increase in polymer concentration causes an increase in the viscosity of the gel. Therefore a reduction of the drug release was exhibited.

3.2.8. Drug Release Kinetics

In order to determine the mechanism and kinetics of drug release, the results of the *in-vitro* drug release study were fitted into various kinetic equations, namely, zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas model. The results of the *in-vitro* drug release studies are presented in Table 3.5 below.

Table 3.5: Mathematical Modeling and drug release kinetics of the various formulations with their corresponding R^2, K and n values

Models		F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order	R^2	0.9600	0.9548	0.8045	0.8516	0.8776	0.9003	0.9335	0.9548	0.9594
	K_0	-30.968	-30.206	-30.988	-30.811	-31.089	-30.228	-30.803	-30.427	-29.655
First order	R^2	0.8101	0.7793	0.5709	0.6345	0.6574	0.6789	0.6830	0.7351	0.7487
	K_1	0.3442	-0.3776	-0.1796	-0.1797	-0.1905	-0.1899	-0.1993	-0.2032	-0.2092
Higuchi	R^2	0.9970	0.9969	0.9346	0.9609	0.9732	0.9833	0.9954	0.9978	0.9950
	K_H	0.4576	0.4531	0.3352	0.3285	0.3286	0.3170	0.3192	0.3122	0.3031
Hixson-Crowell	R^2	0.8739	0.8532	0.6681	0.7224	0.7474	0.6707	0.7898	0.8291	0.8384
	K_{HC}	-0.6250	-0.6569	-0.3163	-0.3181	-0.3307	-0.3682	-0.3384	-0.3426	-0.3460
Korsmeyer-Peppas	R^2	0.9961	0.9914	0.9266	0.9615	0.9708	0.9774	0.9762	0.9918	0.9912
	K_{KP}	0.3059	0.2665	0.2286	0.2223	0.2000	0.1937	0.1778	0.1673	0.1535
	n	0.6902	0.7699	0.7250	0.7014	0.7338	0.7224	0.7552	0.7480	0.7631

Mulhbacher *et al.,* (2004) demonstrated that drug release can be targeted by varying the amount and type of co-excipients used in the formulation. The physicochemical properties of drug and polymer govern the release of drug from formulations which could also modify their release kinetics. The drug release from hydrophilic matrices is controlled by the following mechanisms: the polymer swelling and drug solubility, the drug diffusion, and the matrix erosion. O'Brien *et al.,* (2009) demonstrated that the kinetics of drug release from the swellable matrices is proposed to be governed by the structural features of the hydrogel of which the gel layer formed around the glassy unhydrated core of the tablet. Onofre *et al.,* (2009) also reported that drug release from a matrix is affected by many factors, such as diffusivity of the drug from the matrix to the solvent, porosity and tortuosity of the matrix, and swelling ability and erosion susceptibility of the matrix.

As the drug retarding polymer content increased the release significantly decreased, ($P < 0.05$), suggesting that the matrix with higher polymer content became more compact and rigid. As shown in Fig. 3.9, at 12 h release period formulations F7, F8, and F9 which were incorporated with 20%, 25%, and 30% (w/w) of the polymer released about 99.34%, 96.92%, and 94.06% of the drug with 12 h, respectively. The drug release was found to be inversely related to the amount and concentration of CLASs incorporated in the formulation. Higher amount of polymer was incorporated (30% of total tablet weight) in F9, and hence, due to the stronger gel layer formation, slower drug release was exhibited. From this it was evident that the extended drug release was clearly related to percentage of polymer incorporated in each formulation. One can appreciate that there is dependence between release time and the DCL in which the formulations were prepared. IBP tablets prepared from CLAS-5 released the drug relatively faster than tablets prepared from CLAS-10 and CLAS-15. The slower release for IBP tablets prepared from CLAS-10 and CLAS-15 might be due to slower water penetration with higher concentration of CLAS.

The model that best fitted the release data was determined by the highest r^2. As one can see from Table 3.5, the highest r^2 values for all the formulations were shown among the various release kinetic models of the drug. Among the various drug release kinetic models applied, the plot of the fraction of IBP released against square root of time yield the best linearity confirming that the release data followed the Higuchi model. Costa and Lobo, (2001); and Ghosh and Barik, (2010) reported that the swelling of the polymer would be expected to alter drug concentration gradient

in the gel layer. This is governed by Fick's law, the square root time dependent or Higuchi model. The n value was used to characterize different release mechanisms (Shoaib *et al.*, 2006). The data acquired from *in-vitro* release study were close-fitting to the Korsmeyer-Peppas model in order to determine the "n". Therefore, it was estimated by linear regression of log Mt/M_0 Vs log t of different formulations were shown in Table 3.5, obtained values of n ranges between 0.6900 and 0.7699 for IBP release for all the formulated matrix tablets which indicated that drug release mechanisms involving a combination of both diffusion and polymer chain relaxation. The range of values of the release exponent n, and the related transport mechanism for spherical sample, cylindrical sample, and thin film geometry as per Korsmeyer-Peppas model were shown in Table 3.6.

Table 3.6: Release exponent and mechanism of diffusional release from swellable release systems

Release exponent (n)			
Spherical sample	Cylindrical sample	Thin polymer film	Drug release mechanism
0.43	0.45	0.5	Fickian diffusion
0.43 < n < 0.85	0.45 < n < 0.89	0.5 < n < 1	Anomalous (non-Fickian) diffusion
0.85	0.89	1	Case-II transport (relaxation)
> 0.85	> 0.89	> 1	Super Case-II transport

Therefore, it was evident that the release of IBP from the formulated matrix tablets was controlled by the swelling of the polymer followed by drug diffusion through the swelled polymer and slow erosion of the tablet. The diffusional exponent i.e., n, is an indication of the mechanism of drug release and takes various values depending on the geometry of the release device (Ritger and Peppas, 1987).

4. CONCLUSION

Chemical method of modification via cross-linking was carried out to modify the anchote starch. CLASs were prepared by reacting different concentrations of SHMP and resulted in modified starches with varying DCL.

CLASs exhibited improved powder flowability and tablet compressibility. Solubility, SP properties and water uptake of the CLASs decreased with increasing concentrations of SHMP. The physicochemical property of the CLASs varies as compared with the NS. The CLASs had shown increased tablet hardness, disintegration time and decreased friability due to the fact that the tighter bonds hold firmly. CLASs had lower solubility and SP properties than the NS. The peak viscosities of the CLASs exhibited lower than the native counterpart and the DCL of the CLASs with higher concentrations of cross-linking agent have shown higher as compared to CLASs incorporated with relatively low concentrations of the cross-linking agent. Since the gel layer formed serves as a barrier increases with time, the drug release mechanism was following a combination of both diffusion and chain relaxation pattern.

FT-IR studies showed that there were no significant interactions between the CLAS-15 and the model drug, ibuprofen. According to the concentration and amount of the CLASs incorporated, the prepared tablets sustained the release of drug for 6, 8, 10, and 12 h. Increasing the amount of the CLASs resulted in a reduction in the drug release and considerable sustained action were achieved. The mechanisms of drug release from all IBP matrix tablets formulations exhibited anomalous or non-Fickian diffusion type. In general, CLAS has the potential as a pharmaceutical excipient in sustained release preparations.

5. RECOMMENDATIONS FOR FURTHER WORK

The followings are suggested for further investigations:

- Evaluate the application of CLASs in food and non-food industries,
- Investigate the *in-vivo* performance of CLASs in animal models and human volunteers, and
- Conduct accelerated and long term stability studies.

6. REFERENCES

Adane, M., Endale, A., Bultosa, G., Gamal, M. and Gebre-Mariam, T. (2006). Isolation and physico-chemical characterization of Godare (*Colocasia esculenta*) starch from Ethiopia, *Ethiop. Pharm. J* **24**: 13-22.

Ahammed, T., Hasan, M. and Islam, S. (2010). Effect of Granulation Technique and Drug-Polymer Ratio on Release Kinetics of Gliclazide from Methocel K4M Matrix Tablet. *Bangladesh Pharm J* **13**: 8–12.

Alam, F. and Hasnain, A. (2009). Studies on Swelling and Solubility of Modified Starch from Taro *(Colocasia esculenta)*: Effect of pH and Temperature. *Agric. conspec. sci* **74**: 45–50.

Alcazar-Alay, S.C. and Meireles, M.A.A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. *J. Food Sci. Technol* **35**: 215–236.

Anand, O.,Yu, L.X., Conner, D.P. and Davit, B.M. (2011). Dissolution Testing for Generic Drugs: An FDA Perspective. *The AAPS J* **13**: 328–335.

Andrade, M.M.P., Oliveira, C.S., Colman, T.A.D., Costa, F.J.O. and Schnitzler, E. (2014). Effects of heat-moisture treatment on organic cassava starch: Thermal, rheological and structural study. *J. Therm. Anal. Calorim* **115**: 2115–2122.

Ashogbon, A.O. and Akintayo, E.T. (2014). Recent trend in the physical and chemical modification of starches from different botanical sources: A review. *Starch/Stärke* **66**: 41–57.

Atichokudomchai, N. and Varavinit, S. (2003). Characterization and utilization of acid-modified cross-linked Tapioca starch in pharmaceutical tablets. *Carbohydr Polym* **53**: 263–270.

Bajpai, A. K. and Bhanu, S. (2007). Dynamics of controlled release of heparin from swellable

crosslinked starch microspheres. *J. Mater. Sci. Mater. Med* **18**: 1613–1621.

Bartz, J., Jorge, T., Marcos, A., Rosa, Z., Manoel, A., Alvaro, R., and Guerra, D. (2015).

Acetylation of barnyardgrass starch with acetic anhydride under iodine catalysis. *Food Chem*. **178**: 236-242.

Bemiller, J. and Whistler, R. (2009). Starch: Chemistry and Technology. 3rd Edn., Elsevier Inc. New York, pp. 96-120.

Beruk, B.D., Tadesse, F.T. and Dereje, H. (2015). Physical and Proximate Characterization of Anchote *(Coccinia abyssinica)* Accessions Grown under Hawassa and Wondo Genet Conditions, Southern Ethiopia. *Food Sci Qual Manag* **42**: 62–75.

Builders, P.F. and Arhewoh, M.I. (2016). Pharmaceutical applications of native starch in conventional drug delivery. *Starch/Stärke* **68**: 864–873.

Carbinatto, F.M., Castro, A.D., Evangelista, R.C. and Cury, B.S.F. (2014). Insights into the swelling process and drug release mechanisms from cross-linked pectin/high amylose starch matrices. *Asian J Pharm Sci* **9**: 27–34.

Carmona-Garcia, R., Mirna M. Sanchez-Rivera, M.M., Mendez-Montealvo, G., Garza-Montoya, B. and Bello-Perez, L.A. (2009). Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch *(Musa paradisiaca).Carbohydr Polym* **76**: 117–122.

Chatakanonda, P., Varavinit, S. and Chinachoti, P. (2000). Effect of Crosslinking on Thermal and Microscopic Transitions of Rice Starch. *LWT - Food Sci Technol* **33**: 276–284.

Chowdary, K. P. R. and Radha, G.V. (2011). Synthesis , Characterization and Evaluation of Starch Acetate as Rate Controlling Matrix Former for Controlled Release of Glipizide. *Asian J Chem* **23**: 502–504.

Colussi, R., Shanise, L., Vania, Z., Bartz, J., Jorge, T., Marcos, A., Rosa, Z., Manoel, A., Alvaro, R., and Guerra, D. (2015). LWT - Food Science and Technology Acetylation of rice

starch in an aqueous medium for use in food. *LWT - Food Sci. Technol* **62**: 1076–1082.

Costa, P. and Lobo, J. M. S. (2001). Modeling and comparison of dissolution profile. *Eur. J Pharm Sci* **13**: 123–133.

Cury, B. S. F., Castro, A.D., Klein, S.I., and Evangelista, R.C. (2009). Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 2: Physical parameters, cross-linking degrees and drug delivery relationships. *Int. J Pharm* **371**: 8–15.

Denyer, K., Johnson, P., Zeeman, S., and Smith, A.M.(2001). The control of amylose synthesis. *J. Plant Physiol* **158**: 479–487.

Detduangchan, N., Sridach, W. and Wittaya, T., Yai, H. (2014). Enhancement of the properties of biodegradable rice starch films by using chemical crosslinking agents. *Int Food Res J* **21**: 1189–1199.

Devrim, B. and Canefe, K. (2006). Preparation and evaluation of modified release ibuprofen microspheres with acrylic polymers (Eudragit®) by quasiemulsion solvent diffusion method: Effect of variables. *Acta Pol Pharm* **63**: 521–534.

Dragan, F.,Kacso, I., Dreve, S., Martin, F., Borodi,G., Bratu, I. and Earar, K. (2015). Compatibility Study of Ibuprofen with Some Excipients Employed for Solid Dosage Forms Compatibility Study of Ibuprofen with Some Excipients Employed for Solid Dosage Forms. *Rev. Chim* **66**: 191–195.

Dupuis, J.H., Liu, Q. and Yada, R.Y. (2014). Methodologies for Increasing the Resistant Starch Content of Food Starches:A Review.*Comp Rev Food Sci Food Safety* **13**: 1219–1234.

Gebre-Mariam, T. and Schmidt, P. C. (1996). Isolation and Physico-chemical Properties of Enset Starch. *Starch/Stärke* **48**: 208–214.

Gebre-Mariam, T. and Schmidt, P. C. (1998).Some Physico-chemical Properties of Dioscorea Starch from Ethiopia. *Starch/Stärke* **50**: 241–246.

Ghosh, S. and Barik, B. B. (2010). Formulation and in vitro evaluation of once daily sustained release formulation of aceclofenac. *Trop J Pharm Res* **9**: 265–273.

Guerra-Dellavalle, D., Sánchez-Rivera, M. M.; Zamudio-Flores, P. B.; Méndez-Montealvo, G., Bello- Pérez, L.A. (2009). Effect of chemical modification type on physicochemical and rheological characteristics of banana starch. *Rev Mex Ing Quím* **8**: 197–203.

Guerra-Ponce, W. L., Gracia-Vásquez, S.L., Patricia González-Barranco, P., Camacho-Mora, I.A., Yolanda Araceli Gracia-Vásquez Y.A., Orozco-Beltrán E., and Felton L.A. (2016). *In-vitro* evaluation of sustained released matrix tablets containing ibuprofen: A model poorly water-soluble drug. *Braz. J. Pharm Sci* **52**: 751–760.

Habtamu, F. and Kelbessa, U. (1997). Nutritional and antinutritional characteristics of Anchote *(Coccinia abyssinica). Ethiop. J. Health Dev* **11**: 163–168.

Heebthong, K. and Ruttarattanamongkol, K. (2016). Effects of Degree of Cross-Linking on Physical Properties , Pasting and Freeze-Thaw Stability of Cassava Starch Modified By Reactive Extrusion Process (Rex). Proceedings of The IRES 30th International Conference, Tokyo, Japan, 18th February 2016, ISBN: 978-93-85973-35-2, pp 49–54.

Hirsch, J. B. and Kokini, J. L. (2002). Understanding the mechanism of cross-linking agents ($POCl_3$, STMP, and ECH) through swelling behavior and pasting properties of cross-linked waxy maize starches. *Cereal Chem* **79**: 102–107.

Hoover, R. (2001). Compositions, molecular structure, and physiochemical properties of tuber and root starches:A review. *Carbohydr Polym* **45**: 253–267.

Insha, S., Singh, S. and Saxena, D. C. (2016). Food Hydrocolloids Effect of heat-moisture and acid treatment on physicochemical , pasting , thermal and morphological properties of Horse Chestnut *(Aesculus indica)* starch. *Food Hydrocoll* **57**: 103–113.

Irisappan, S. C. and P,S.K. (2014). Formulation and evaluation of floating microspheres of Cefdinir. *J Pharm Res* **8**: 212–216.

Ispas-szabo, P., Ravenelle, F., Hassan, I., and Preda, M. (2000). Structure–properties relationship

in cross-linked high-amylose starch for use in controlled drug release. *Carbohydr Res* **323**: 163–175.

Jayakody, L., Hoover, R., Liu, Q., and Donner, E.(2007). Studies on tuber starches. II. Molecular structure, composition and physicochemical properties of yam *(Dioscorea sp.)* starches grown in Sri Lanka. *Carbohydr Polym* **69**: 148–163.

Jing-ming, L. and Sen-lin, Z. (1990). Scanning Electron Microscope Study on Gelatinization of Starch Granules in Excess Water. *Starch/Stärke* **42**: 96–98.

Juban, A. Briancon, S. and Stephanie, T.H. (2017). Experimental study of tensile strength of pharmaceutical tablets: effect of the diluent nature and compression pressure. *Powders and Grains* **140**: 1–4.

Jyothi, A.N., Moorthy, S.N. and Rajasekharan, K.N. (2006). Effect of cross-linking with epichlorohydrin on the properties of cassava (Manihot esculenta Crantz) starch. *Starch/Stärke* **58**: 292–299.

Khan, G.M. and Jiabi, Z. (1998). Formulation and in vitro Evaluation of ibuprofen - carbopol 974P-NF controlled release matrix tablets III: influence of co-excipients on release rate of the drug. *J. Control. Release* **54**: 185–190.

Khan, G.M. and Zhu, J. (1999). Studies on drug release kinetics from ibuprofen – carbomer hydrophilic matrix tablets: influence of co-excipients on release rate of the drug. *J. Control. Release* **57**: 197–203.

Koo, S.H., Lee, K.Y. and Lee, H.G. (2010). Effect of cross-linking on the physicochemical and physiological properties of corn starch. *Food Hydrocoll* **24**: 619–625.

Kulicke, W.M., Aggour, Y. A. and Elsabee, M. Z. (1990). Preparation, Characterisation, and Rheological Behaviour of Starch-Sodium Trimetaphosphate Hydrogels. *Starch /Stärke* **42**: 134–141.

Kuu, W.Y., Chilamkurti, R. and Chen, C. (1998). Effect of relative humidity and temperature on moisture sorption and stability of sodium bicarbonate powder. *Int J Pharm* **166**: 167–

175.

Lefnaoui, S. and Moulai-Mostefa, N. (2015). Synthesis and evaluation of the structural and physicochemical properties of carboxymethyl pregelatinized starch as a pharmaceutical excipient. *Saudi Pharm J* **23**: 698–711.

Lenaerts, V., Moussa, I., Dumoulin, Y., Mebsout, F., Chouinard, F., Szabo, P., Mateescu, M.A., Cartilier, L., and Marchessault, R. (1998). Cross-linked high amylose starch for controlled release of drugs: Recent advances. *J. Control. Release* **53**: 225–234.

Lewicka, K., Siemion, P. and Kurcok, P. (2015). Chemical modifications of starch: Microwave effect. *Int J Polym Sci* **2015**: 1–10.

Li, B.Z., Wang, L.J., Li, D., Chiu, Y.L., Zhang, Z.J., Shi, J., Chena, X.D. and Mao, Z.H. (2009). Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. *J Food Eng* **92**: 255–260.

Lin, C.C. and Metters, A.T. (2006). Hydrogels in controlled release formulations: Network design and mathematical modeling. *Adv Drug Deliv Rev* **58**: 1379–1408.

Lisie, S., Mello, E., Rosana, C., Vânia, Z., Bartz, J., Marjana, R., Neftali, L., Villarreal, C., Alvaro, R., Guerra, D., and Rosa, Z. (2015). Structure, morphology and functionality of acetylated and oxidised barley starches. *Food Chem* **168**: 247–256.

Liu, H., Ramsden, L. and Corke, H. (1999). Physical Properties of Cross-linked and Acetylated Normal and Waxy Rice Starch. *Starch/Stärke* **51**: 249–252.

Liu, J., Wang, B., Lin, L., Zhang, J., Liu, W., Xie, J. and Ding, Y. (2014). Functional, physicochemical properties and structure of cross-linked oxidized maize starch. *Food Hydrocoll* **36**: 45–52.

Lorlowhakarn, K. and Naivikul, O. (2006). Modification of rice flour by Heat Moisture Treatment (HMT) to produce rice noodles. *Kasetsart J. Nat. Sci* **40**: 135–143.

Manoi, K. and Rizvi, S.S.H. (2010). Physicochemical characteristics of phosphorylated cross-

linked starch produced by reactive supercritical fluid extrusion. *Carbohydr Polym* **81**: 687–694.

Mekbib, Y. and Deressa, T. (2016). Exploration and collection of root and tuber crops in East Wollega and Ilu Ababora zones: Rescuing declining genetic resources. *Indian J Tradit Know* **15**: 86–92.

Mirmoghtadaie, L., Kadivar, M. and Shahedi, M. (2009). Effects of cross-linking and acetylation on oat starch properties. *Food Chem* **116**: 709–713.

Miyazaki, M. and Van, P. (2006). Recent advances in application of modified starches for breadmaking. *Trends in Food Sci Technol* **17**: 591–599.

Mohammed, K., Endale, A., and Gebre-Mariam, T. (2007). Isolation, Acetylation and Physicochemical Characterization of Kottee Harree *(Dioscorea bulbifera)* Starch. MSc thesis, School of Graduate Study, Addis Ababa University.

Mulhbacher, J., Ispas-Szabo, P. and Mateescu, M. A. (2004). Cross-linked high amylose starch derivatives for drug release: II. Swelling properties and mechanistic study. *Int. J. Pharm* **278**: 231–238.

Mundargi, R.C., Shelke, N.B., Rokhade, A.P., Patil, S.A., and Aminabhavi, T.M. (2008). Formulation and *in-vitro* evaluation of novel starch-based tableted microspheres for controlled release of ampicillin. *Carbohydr Polym* **71**: 42–53.

Nagar, V.V. (2013). Studies on preparation and functional properties of carboxymethyl starch from sorghum. *Intl Food Res J* **20**: 2205–2210.

Nerurkar, J., Jun, H.W., Price, J.C., and Park, M.O. (2005). Controlled-release matrix tablets of ibuprofen using cellulose ethers and carrageenans: Effect of formulation factors on dissolution rates. *Eur JPharm Biopharm* **61**: 56–68.

Nigussie, T., Endale, A., and Gebre-Mariam, T. (2006). Isolation, Characterization and Evaluation of Binding and Disintegrant Effects of Anchote Starch in Paracetamol Tablet Formulation. MSc thesis, School of Graduate Study, Addis Ababa University.

Nikolic, N.D., Medarevic, D.P., Duris, J.D., and Vasiljevic, D.D. (2015). Comparison of drug release and mechanical properties of tramadol-hydrochloride matrix tablets prepared with selected hydrophilic polymers. *Chem. Ind. Chem. Eng Q* **21**: 369–378.

Nunez-Santiago, M.C., Bello-Pérez, L.A. and Tecante, A. (2004). Swelling-solubility characteristics, granule size distribution and rheological behavior of banana *(Musa paradisiaca)* starch. *Carbohydr Polym* **56**: 65–75.

O'Brien, S. and Wang, Y. J. (2009a). Effects of shear and pH on starch phosphates prepared by reactive extrusion as a sustained release agent. *Carbohydr Polym* **77**: 464–471.

O'Brien, S. and Wang, Y. J. (2009b). Starch phosphates prepared by reactive extrusion as a sustained release agent Stephen. *Carbohydr Polym* **76**: 557–566.

Odeku, O. A., Okunlola, A. and Lamprecht, A. (2014). Formulation and in vitro evaluation of natural gum-based microbeads for delivery of ibuprofen. *Trop J Pharm Res* **13**: 1577–1583.

Odeku, O. A. and Picker-Freyer, K. M. (2009). Evaluation of the material and tablet formation properties of modified forms of Dioscorea starches. *DrugDev Ind Pharm* **35**: 1389–1406.

Olasupo, O.A., Augustine, O.A., Adenike, O.A. and Adekunle, A.I. (2011). Pasting Properties of Heat-Moisture Treated Starches of White and Yellow Yam (Dioscorae species) Cultivars. *Nature and Sci* **9**: 29–33.

Onofre, F., Wang, Y.J. and Mauromoustakos, A. (2009). Effects of structure and modification on sustained release properties of starches. *Carbohydr Polym* **76**: 541–547.

Onyishi, I.V., Chime, S.A. and Ugwu, J.C. (2013). Evaluation of binder and disintegrant properties of starch derived from Xanthosoma sagittifolium in metronidazole tablets *Afr. J. Biotechnol* **12**: 3064–3070.

Pal, J., Singhal, R. S. and Kulkarni, P.R. (2002). Physicochemical properties of hydroxypropyl derivative from corn and amaranth starch. *Carbohydr Polym* **48**: 1–5.

Parmar, A., Gebre, B.A., Legesse, A., Demelash, W., Fladung, K. and Hensel, O.(2017). Nutritional Comparison of White and Red *Coccinia Abyssinica* (Lam.). Accessions: An Under-Utilised Edible Tuber of the Ethiopian Highlands. *Foods* **6**: 2-8.

Paulos, G., Endale, A., and Gebre-Mariam T. (2009). Isolation, Acetylation and physicochemical characterization of cassava starch obtained from Ethiopia, *Ethiop. Pharm. J* **27**: 42-54.

Pec, L. and Venskutonis, P.R. (2016). Preparation and properties of propylene oxide and octenylsuccinic anhydride modified potato starches. *J Food Sci Technol* **53**: 4187–4196.

Razavi, S.M., Gonzalez, M. and Cuitino, A.M. (2015). General and mechanistic optimal relationships for tensile strength of doubly convex tablets under diametrical compression. *Int. J. Pharm* **484**: 29–37.

Ritger, P.L. and Peppas, N.A. (1987). A Simple Equation for Description of Solute Release. *J. Control. Release* **5**: 37–42.

Schüssele, A. and Bauer-Brandl, A. (2003). Note on the measurement of flowability according to the European Pharmacopoeia. *Int. J. Pharm* **257**: 301–304.

Shah, S.N.H., Asghar, S., Choudhry, M.A., Akash, M.S.H., Rehman, N. and Baksh, S. (2009). Formulation and evaluation of natural gum-based sustained release matrix tablets of flurbiprofen using response surface methodology. *Drug Dev. Ind. Pharm* **35**: 1470–8.

Shang, C.,Sinka, I.C., Jayaraman, B. and Pan, J.(2013). Break force and tensile strength relationships for curved faced tablets subject to diametrical compression. *Int. J. Pharm* **442**: 57–64.

Shanks, R.A. and Gunaratne, W. K. (2011). Gelatinization and retrogradation of thermoplastic starch characterized using modulated temperature differential scanning calorimetry. *J. Therm. Anal. Calorim* **106**: 93–99.

Shoaib, M.H., Tazeen, J., Merchant, H.A. and Yousuf, R.I. (2006). Evaluation of Drug Release

Kinetics From Ibuprofen Matrix Tablets Using Hpmc. *Pak. J. Pharm. Sci* **19**: 119–124.

Shukri, R. and Shi, Y.C. (2015). Physiochemical properties of highly cross-linked maize starches and their enzymatic digestibilities by three analytical methods. *J Cereal Sci* **63**: 72-80.

Singh, J., Kaur, L. and McCarthy, O.J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications-A review. *Food Hydrocoll* **21**: 1–22.

Souza, D.F., Goebel, K. and Andreazza, I.F. (2013). Development of enteric coated sustained release minitablets containing mesalamine. *Braz J Pharm Sci* **49**: 529–536.

Spychaj, T., Wilpiszewska, K. and Zdanowicz, M. (2013). Medium and high substituted carboxymethyl starch : Synthesis, characterization and application. *Starch/Staerke* **65**: 22-33.

Stasiak, M., Molenda, M., Horabik, J., Mueller, P. and Opalinski, I. (2014). Mechanical properties of potato starch modified by moisture content and addition of lubricant. *Int Agrophys* **28**: 501–509.

Stawski, D. (2008). New determination method of amylose content in potato starch. *Food Chem* **110**: 777–781.

Steendam, R. (2005). Amylodextrin and poly(DL-lactide) oral controlled release matrix tablets. Concepts for understanding their release mechanisms: Concepts for understanding their release mechanisms. Available at: https://www.rug.nl/.../amylodextrin-and-polydllactide-oral-controlled-r... (Accessed 01 June 2017).

Swain, R. P., Kumari, T. R. and Panda, S. (2016). Formulation development and evaluation of sustained release ibuprofen tablets with acrylic polymers (Eudragit) and HPMC.*Int J Pharm Pharm Sci* **8**: 131–135.

Swinkels, J.J.M. (2007). Industrial starch chemistry: Sources of starches. Available

at:www.agrobynature.com/IndustrialStarchChemistry.pdf (Accessed: 16 August 2016).

Tay, J.Y.S., Liew, C.V. and Heng, P.W.S. (2017). Powder Flow Testing: Judicious Choice of Test Methods. *AAPS Pharm Sci Tech* **18**: 1843–1854.

Teixeira, A.Z.A. (2009). Hydroxypropylcellulose controlled release tablet matrix prepared by wet granulation: Effect of powder properties and polymer composition. *Braz. arch. biol technol* **52**: 157–162.

Tester, R. F., Karkalas, J. and Qi, X. (2004). Starch - Composition, fine structure and architecture. *J* Cereal Sci **39**: 151–165.

Thakur, M. K. Kapelko-Zeberska, M., Zieba, T. and Singh, A.V. (2015). Surface modification of biopolymers: an overview, 1st edn., John Wiley & Sons, Inc., New York, pp. 335–369.

USP30-NF25 (2007) United State Pharmacopoeia 30-National Formulary 25. The United States Pharmacopoeial Convention.

Vamadevan, V. and Bertoft, E. (2015). Structure-function relationships of starch components *Starch/Staerke* **67**: 55–68.

Velasco, M.V., Ford, J.L., Rowe, P. and Rajabi-Siahboomi, A.R. (1999). Influence of drug:hydroxypropylmethylcellulose ratio, drug and polymer particle size and compression force on the release of diclofenac sodium from HPMC tablets. *J. Control. Release* **57**: 75–85.

Walia, P.S., Shogren, R.L. and Lawton, J.W. (2002). Mechanical properties of thermoplastic starch/poly(hydroxy ester ether) blends: Effect of moisture during and after processing. *J. Appl. Polym. Sci* **84**: 121–131.

Wang, L., Liu, X. and Wang, J. (2017). Structural properties of chemically modified Chinese yam starches and their films. *Int. J. Food Prop* **20**: 1239–1250.

Wani, A.A, Singh, P., Shah, M.A., Schweiggert-Weisz, U., Gul, K. and Wani, I.A.(2012). Rice Starch Diversity: Effects on Structural, Morphological, Thermal, and Physicochemical Properties A Review. *Comp Rev Food Sci Food Safety* **11**: 417–436.

Wattanachant, S., Muhammad, S.K.S., Hashim, D.M. and Rahman, R.A. (2002). Suitability of sago starch as a base for dual-modification. *J. Sci. Technol* **24**: 431-438.

Wongsagonsup, R., Pujchakarna, T., Jitrakbumrunga, S., Chaiwatb, W., Fuongfuchatc, A., Varavinitd, S., Dangtipe,S., and Suphantharikad, M.(2014). Effect of cross-linking on physicochemical properties of tapioca starch and its application in soup product. *Carbohydr Polym* **101**: 656–665.

Woo, K. and Seib, P.A. (1997). Cross-linking of wheat starch and hydroxypropylated wheat starch in alkaline slurrywith sodium trimetaphosphate.*Carbohydr Polym* **33**: 263-271.

Yao, J., Chen, W., Robbert, M., Manurung, K., Ganzeveld, and H.J.H. (2004). Exploratory studies on the carboxymethylation of cassava starch in water-miscible organic media Exploratory Studies on the Carboxymethylation of Cassava Starch in Water-miscible Organic Media. *Starch/Stärke* **56**: 100–107.

Yosef, Y. and Tileye, F. (2013). Micropropagation of anchote [*Coccinia abyssinica* (Lam.) Cog]: High calcium content tuber crop of Ethiopia. *Afr. J. Agric. Res* **8**: 5915–5922.

Zhao, J., Chend, Z., Jinb, Z., Waardc, P., Buwaldad,P., Gruppena, H. and Schols, H.A. (2015). Level and position of substituents in cross-linked and hydroxypropylated sweet potato starches using nuclear magnetic resonance spectroscopy. *Carbohydr Polym* **131**: 424–431.

Zia-ud-Din, Xiong, H. and Fei, P. (2017). Physical and chemical modification of starches: A review. *Crit Rev Food Sci* **57**: 2691–2705.

Zobel, H.F. (1988). Molecules to Granules: A Comprehensive Starch Review. *Starch/Stärke* **40**: 44–50.

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any University.

Name: Desalegn Abeje Minda

Signature _____

This thesis has been submitted for examination with my approval as a University advisor.

Name: Prof. Tsige Gebre-Mariam

Signature _____

Dr. Anteneh Belete

Signature _____

Place and date of submission: Addis Ababa, Ethiopia, May, 2018