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Preparation and Evaluation of Microwave-Assisted Acetylation of *Plectranthus edulis* (Ethiopian Potato) Starch as a Sustained Release Excipient in Tablet Formulations

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July, 2019

Addis Ababa

Preparation and Evaluation of Microwave-Assisted Acetylation of *Plectranthus edulis* (Ethiopian Potato) Starch as a Sustained Release Excipient in Tablet Formulations

A Thesis Submitted to Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Science, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmaceutics

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SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Ayantu Mosisa, entitled "Preparation and Evaluation of Microwave-Assisted Acetylation of *Plectranthus edulis* (Ethiopian Potato) Starch as a Sustained Release Excipient in Tablet Formulations" and 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.

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ACRONYMS

CI:	Carr's Index
DS:	Degree of Substitution
DSC:	Differential Scanning Calorimetry
FTIR:	Fourier Transform Infrared Spectroscopy
HR:	Hausner Ratio
MCC:	Microcrystalline Cellulose
NPES:	Native *Plectranthus edulis* Starch
NS:	Native Starch
EC:	Ethyl Cellulose
OSA:	Octenyl Succinic Anhydride
RH:	Relative Humidity
RE:	Reaction Efficiency
S:	Solubility
SA:	Starch Acetate
SP:	Swelling Power
SR:	Sustained Release
STMP:	Sodium Trimetaphosphate
XRD:	X-ray Diffraction

ABSTRACT

Starch has been the subject of intensive research over many decades due to the fact that native starches are diverse, biodegradable, applications are enormous and modifications to starch are numerous. In this study, *Plectranthrus edulis* (*P.edulis*) starch has been chemically modified under microwave heating with the aim of determining its potential for sustained release application. It was chemically modified by microwave-assisted acetylation using acetic anhydride as acetylating and sodium hydroxide as a catalyst. The reaction conditions were optimized for maximizing the degree of substitution (DS) and the DS selected were 1.77 and 0.44.

The prepared starch acetate (SA) were evaluated and compared with the native *P. edulis* starch and standard excipients in terms of various physicochemical characteristics including the chemical structural changes, powder properties, swelling power (SP) and solubility, moisture sorption pattern, compactibility and drug release properties. The Fourier transform infrared (FTIR) spectra of the modified starches verified the structural change in the starch molecules resulting from acetylation. The x-ray diffraction (XRD) and differential scanning calorimetry (DSC) studies shows acetylation reduced the formation of intermolecular hydrogen bonds and thereby resulted in the slight destruction of the ordered crystalline structure. The results of the powder properties (Carr's index and Hausner ratio, angle of repose and Kawakita analysis) indicated that acetylation improved the flow property of *P. edulis* starch. Moreover, microwave-assisted acetylation appreciably increased starch SP at a lower DS 0.44 than higher DS 1.77. Modification also increased solubility of native *P.edulis* starch. The water uptake of the *P.edulis* SA powders slightly increased with relative humidity (RH) at lower values but increased significantly at about 100% RH.

The effect of DS on the compactibility and drug release retardant effect were also evaluated by using anhydrous theophylline as model drug. The plain tablets of SA DS 1.77 shows higher tensile strength than the SA DS 0.44 tablets and it also shows significant tablet properties than native *P.edulis* starch tablet which reflected the better compactibility of the former modified starch. Plain tablets of SA DS 0.44 and microcrystalline cellulose (MCC) (Avicel PH 101) were completely disintegrated within few min while those made of SA with DS 1.77 and ethyl cellulose (EC), did not disintegrate at all during the measurement time of 2 h. The tensile strength (19.81 Kg/cm^2) of tablets containing 20% of theophylline and 79.5% *P. edulis* SA DS 1.77 with crushing strength of

150.2 N was significantly higher than tablets with crushing strength of 51.1 N because the tablets with crushing strength of 51.1 N has the highest porosity when compared to tablet with crushing strength of 150.2 N. The tablets of SA DS 1.77 has the highest tensile strength and the lowest porosity than tablets of SA DS 0.44 at the same percentage composition of theophylline and *P. edulis* SA which indicate the effect of DS.

The *in vitro* release rate was found to be significantly related to the DS and crushing strength, hence more sustained release (SR) of the drug was observed with the matrix incorporated with SA of higher DS and highest crushing strength. The drug release rate of theophylline changed from rapid release to sustained release as the DS increased from 0.44 to 1.77 and as crushing strength increased from 51.1 N to 150.2 N. The drug release rate also increased as the SA DS 1.77 percentage decreased from 79.5 to 59.5% (w/w). Further the drug release data were fitted to five kinetic models. Among the various drug release kinetic models applied, the best linearity was found with Higuchi's plot ($R^2 = 0.9988$) indicating the release of drug from the matrix as a square root of time dependent process based on Fickian diffusion and based on Korsemayer–Peppas model the release mechanism observed was anomalous diffusion (diffusion coupled with swelling). Therefore, it was concluded that *P. edulis* SA could have a potential for use as a sustained release excipient.

Key Words: Degree of Substitution, Microwave-assisted Acetylation, *P. edulis* Starch, Starch Acetate, Sustained Release

1. INTRODUCTION

Starch, a polysaccharide composed exclusively of D-glucose, is one of the most abundant organic compounds found on earth. Starch can be isolated from leaves, stems, tubers, seeds, and roots of higher plants where it serves as an energy reserve. Chemically, starch is a carbohydrate polymer consisting of anhydroglucose units linked by two different glucosidic bonds. It consists of two glucose molecules: amylose (15–30%), a linear polymer in which the glucose units are joined end-to-end by α [1→ 4] glucosidic linkage (Fig 1.1 A) and amylopectin (85–70%), a branched chain polymer consisting of much shorter chains of α [1→ 4] D-glucose residues connected by α [1→ 6] D-glucosidic linkages (Fig 1.1 B) (Moorthy 1996).

Amylose is linear or slightly branched, has a degree of polymerization up to 6000, and a molecular mass of 10^5 to 10^6 g/mol. The chains can easily form single or double helices. Amylopectin on the other hand has a molecular mass of 10^7 to 10^9 g/mol. It is highly branched and has an average degree of polymerization of 2 million, making it one of the largest molecules in nature. Chain lengths of 20 to 25 glucose units between branch points are typical. About 70% of the mass of starch granule is regarded as amorphous and about 30% as crystalline (Tester *et al*. 2004; Schwartz and Whistler 2009).

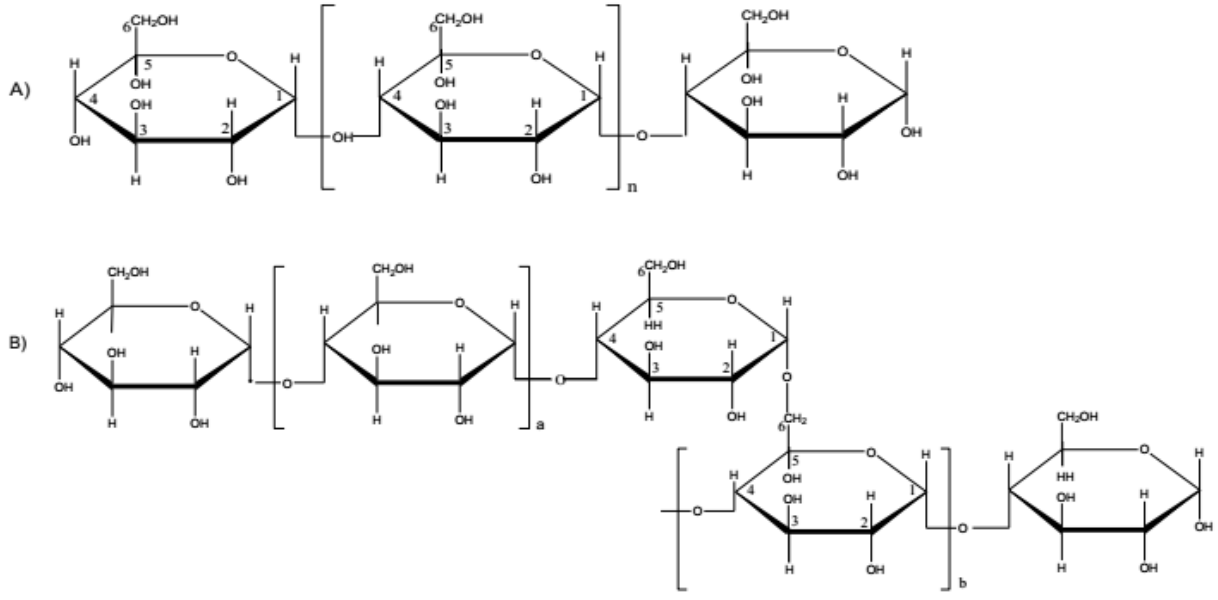


Figure 1.1: Structure of linear amylose (A) and branched amylopectin (B)

The world-wide market for industrial starches is expanding and the demand is met by limited range of crops, the most important of which are potato, maize, wheat and tapioca. Availability of novel processing techniques and current demand for biodegradable and renewable resources undoubtedly make starch to command more versatile markets (Schwartz and Whistler 2009).

1.1. Pharmaceutical applications of starch

Excipients are increasingly being recognized as important components of conventional and novel drug products, providing specific functions in aiding the formulation of optimally elegant, stable, safe, and active drug products. Starch in its native and modified forms has been widely used as pharmaceutical excipient. Starch-based excipients have been shown to offer numerous advantages in drug production in terms of safety and product quality. Starch is used as an excipient primarily in oral solid dosage formulations where it is utilized as a binder, diluent, and disintegrant (Bos *et al*. 1987). A survey of the literature shows the usefulness of starches from various botanical sources as pharmaceutical excipients. Its use is based on its adhesive, thickening, gelling, swelling and film-forming properties as well as its readily availability, low cost (Gebre-Mariam and Schmidt 1996).

Native starches (NS) should be modified by physical and chemical methods, as well as by enzymatic hydrolysis, in order to overcome their limitations and expand the application of starch as an excipient (Hong *et al.* 2014).The properties of starch are affected by its botanical source, but they all share common features: they are hygroscopic, insoluble in cold water, swellable up to 5–10% at 37 ºC and soluble in hot water at temperatures above the gelatinization temperature (Builders and Arhewoh 2016).

Major starch sources are cereals (40 to 90%), roots (30 to 70%), tubers (65 to 85%), legumes (25 to 50%) and some immature fruits like banana or mango, which contain approximately 70% of starch by dry weight. Tubers are considered to be the third important staple food crops after cereals and legumes. They are grown and consumed throughout the world in hot and humid regions. These contain 70–80% water, 16–24% starch and less quantities about <4% protein (Santana and Meireles 2014).

1.2. *Plectranthrus edulis* (Ethiopian potato)

Starch granules are synthesized in a broad array of plant tissues and within many plant species (Tester *et al.* 2004). The chemical composition and the physical characteristics are essentially typical to the biological origin of the starch, i.e. they are unique to each type of starch. Therefore, what has been discovered about the structural features of one type of starch does not necessarily apply to other types of starch (Schwartz and Whistler 2009). Ethiopia harbors many plants, out of which traditional starchy tubers and roots have the potential to be used as sources of starch, and replace commercial starches in various industrial applications.

Plectranthus edulis (Fig 1.2) is an indigenous annual tuber crop grown widely in the central, southern, western, northwestern and southwestern parts of Ethiopia (Gemeda 2000 and Mulugeta 2008). It is a dicotyledonous plant and belongs to the family Lamiaceae/Labiatae. In different growing areas of Ethiopia, different vernacular names are used for *P. edulis.* Among these are '*Dinicha Oromo*' in Oromia, '*Wolaita Dinich*' around Wolaita '*Agew Dinich'* in the northwest and '*Gurage Dinich'* around Gurage zone (Garedew *et al*. 2013). For generations, farmers in different parts of the country have been cultivating *P. edulis* primarily for its edible tuber. The leaves are also eaten by humans as green vegetable in some regions (Garedew *et al*. 2013).

The physicochemical properties of *P. edulis* have been characterized and the mean particle size and amylose content of the starch were found to be 36.20 ± 20.9 µm and 30.6%, respectively. Starch yield on dry weight basis was found to be 80.4% ± 0.45. The NS exhibits X-ray diffraction pattern that is typical of B-type (Assefa *et al*. 2015).

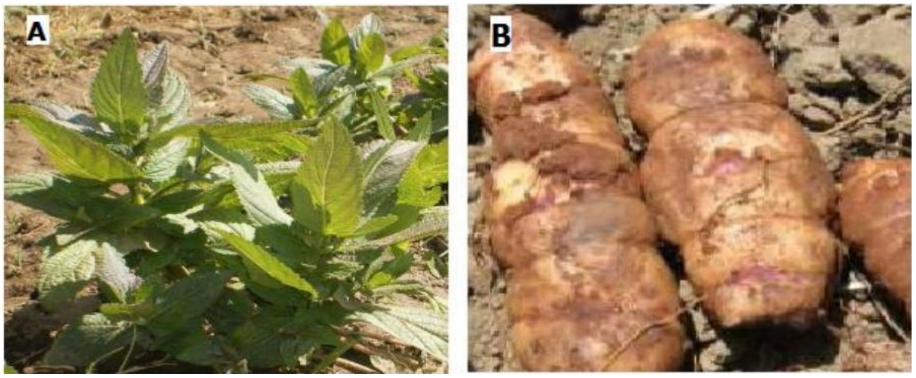


Figure 1.2: *P. edulis* plant (A) and its tuber (B) (Mekbib *et al*., 2007)

Due to its high yielding potential (Mulugeta *et al*. 2007), excellent caloric and nutrient source, wide adaptability, drought and insect pests resistance, and multifunctional usage, farmers and governmental and non-governmental organizations have recently shown a renewed interest in *P. edulis* (Mulugeta 2008).

1.3. Modification of starch

Modification of starch is carried out to overcome shortcomings of NSs and increase the usefulness of starch for industrial applications. Starch modifications, which are aimed at correcting one or some of the shortcomings associated with NS, involve the alteration of the physical and chemical characteristics of the NS to improve its functional characteristics (Singh *et al*. 2007). Modification of starch is an ongoing process as there are many possibilities. There is a huge market for the many new functional and beneficial properties resulting from these modifications (Kaur *et al*. 2012).

NSs, as natural and secure excipients, have been used as classic tablet disintegrant, fillers and binders. However, they suffer from low compactibility and elastic compression behaviors. Meanwhile, orally administered drugs comprising NSs are subject to being eroded by α-amylase in the gastrointestinal tract and thus fail to extend the drug release (Prado *et al*. 2009). Therefore, natural starches should be modified in order to overcome these limitations and expand the application of starch as an excipient. There are many different methods of modifying starch biopolymers. Generally, there are three broad classifications of starch modifications: chemical, physical and dual modification.

1.3.1. Physical modification

Physical modification is simple, cheap, and safe because it requires no chemicals or biological agents. Physical modification of starch can be applied alone or with chemical reactions to change the granular structure and convert NS into cold water-soluble starch or into small crystallite starch. Thermal treatment, sonication, radiation, and heat-moisture treatment are the most commonly employed physical modification techniques (Bhat and Karim 2009; Chung *et al*. 2009; Wadchararat *et al*. 2006 and Zuo *et al*. 2009).

Microwave or dielectric heating is an accepted new physical modification technique to run chemical conversion on lab scale as an alternative heating principle. It offers an advantage over

conventional techniques by reducing both the required amount of solvent and the reaction time, and the radiation can easily reach particles inside (Sanchez-Rivera *et al*. 2010). Therefore, to meet the demanding technological needs of today, the properties of starch from various sources are modified by a variety of modification methods.

The microwave heating process, the high temperatures attained and the ability to work under high pressure conditions for relatively short times make reactions faster than under conventional thermal conditions, and limit the occurrence of slower side reactions. Thus, greater yields are usually obtained (Sekhon 2010). The ability to reduce reaction times from days and hours to minutes and seconds has motivated various research areas.

1.3.2. Chemical modification

Chemical modification involves the introduction of functional groups into the starch molecules, resulting in markedly altered physicochemical properties. Such modification of native granular starches profoundly changes the proximate compositions, gelatinization, retrogradation, and pasting characteristics. Chemical modification is intended to facilitate intra- and inter-molecular bonds at random locations in the starch granule for their stabilization. The chemical and functional properties achieved by modified starches depend on starch source, reaction conditions (reactant concentration, pH, reaction time, and the present of catalyst), type of substituents, degree of substitution (DS), and the distribution of the substituents in the starch molecule (Wang and Wang 2002). Modification is generally achieved through derivatization such as etherification, esterification and cross-linking, oxidation, cationization and grafting of starch (Kaur *et al.* 2012).

Acetylation is one such method that has received significant attention in recent years. Acetic anhydride is commonly used as a versatile acetylating agent of organic substrates, essentially involving the substitution of free hydroxyl groups. During acetylation, three free hydroxyl groups on C2, C3 and C6 of the anhydroglucose unit of the starch molecule can be replaced with acetyl groups in a kinetically controlled reaction; therefore, the theoretic maximum DS is three. In fact, these three free –O–H groups have different reactivities. The primary –OH on C6 is more reactive and is acetylated more readily than the secondary ones on C2 and C3 due to steric hindrance (Xu *et al*. 2004).

1.3.3. Dual modification

Starch modification using a combination of chemical and physical or chemical and enzymatically methods have grown rapidly. Dual modification of starches has been demonstrated to introduce desirable properties to starch for specific applications. Cross-linking and substitution of starch are two such methods commonly utilized in industrial applications (Singh *et al*. 2007). Microwave and ultrasound irradiation was used for the esterification of carboxymethyl cold-water-soluble potato starch with octenylsuccinic anhydride. They were positively able to shorten the esterification time from a few hours to a few minutes. The derivatives displayed excellent emulsifying and surfactant performance properties (Cizova *et al*. 2008). Recently, alternative methods such as extrusion cooking and microwave techniques have been used for the chemical modification of starch. Such methods are especially useful in dry reactions, where the reactant and reagents are in the solid state.

1.4. Starch acetate (SA)

One of the important chemically modified starches is starch acetate (SA). SA with high DS (>1.0) has many good properties and applications such as hydrophobicity and its use as thermoplastic cellulose acetate substitutes (Xu *et al*. 2004). SA is modified starch produced by mixing the NS with acetylating agents in the presence of a catalyst (Fig 1.3) (Pohja *et al*. 2004 and Raatikainen *et a*l. 2002). SA possesses a number of beneficial properties compared to unmodified starch. Its flowing properties are suitable for direct compression, it exhibits both plastic deformation and fragmentation under pressure, it has excellent binding properties and, it produces tablets with sustained drug release properties (Korhonen *et al*. 2000; Korhonen *et al*. 2002; Pohja *et al*. 2004; Raatikainen *et al*. 2002 and Tarvainen *et al*. 2002).

In particular, the preparation of hydrophobic starch esters is promising to enhance the starch properties for controlled drug release application by increasing hydrophobicity, minimizing both swelling and enzymatic degradation. This has been emphasized in literature for SA as a matrix former (Korhonen *et al*. 2000; Mäki *et al.* 2007; Pohja *et al*. 2004; Singh and Nath 2012, Singh and Nath 2013; Van Veen *et al.* 2005), membrane former (Nutan *et al*. 2007; Tarvainen *et al*. 2004) and in microencapsulation (Tuovinen *et al.* 2004).

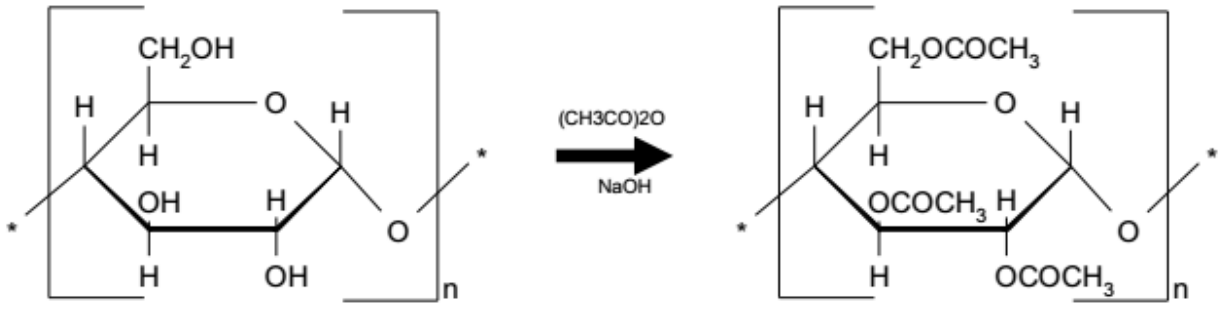


Figure 1.3: Acetylating NS monomer into fully acetylated (DS 3.0) SA monomer

Chowdary and Radha synthesized SAs with a DS of 1.48 - 1.50 by acetylation of potato starch with acetic anhydride. Matrix tablets of glipizide prepared by employing SA as matrix former in different proportions gave slow and controlled release for more than 24 h (Chowdary and Radha 2011).

The mechanism of drug release from the SA matrix tablets appears to be diffusional in most cases. The most significant variables that affect the release of drugs from the SA matrix have been reported to be particle and powder properties of SA, tablet properties (i.e. porosity), the ratio of drug and SA concentrations in the formulation, and the physicochemical nature of the drug (Korhonen *et al*. 2004).

Acetylated starches with intermediate DS (0.2-1.5) and high DS (1.5-3.0) have been reported for their solubility in acetone and chloroform and thus, can be used as thermoplastic material (Luo and Shi 2012). As biodegradable carbohydrate polymers, acetylated starches with intermediate or high DS also have many potential uses such as hot melt adhesives, coatings, cigarette filters, biodegradable packaging materials, tablet binders, and other pharmaceutical applications (Fang *et al*. 2002, Shogren 2003). Acetylation of starches is performed to bring about desired changes in physical, functional and chemical properties of starches (Xu *et al*. 2004) and has been used by many researchers (Bello-Perez *et al*. 2000; Diop *et al*. 2011; Garg and Jana, 2011; Huang *et al.* 2007; Mbougueng *et al.* 2012).

Different factors play vital role in the inducement of changes caused by acetylation such as the source, ratio of amylose and amylopectin, the molecular structure of starch and the DS. The

reaction efficiency and the number of acetyl groups induced in the starch molecule during the process of acetylation depend upon the reaction time, presence of catalyst, botanical origin, reagent concentration and structural characteristics of starch granules (Huang *et al*. 2007, Huber and BeMiller, 2001).

Esterification of starch leads to a product with a good thermoplastic processability enhanced mechanical properties and an increased hydrophobicity. Depending on the DS some of the highly hydrophilic hydroxyl groups in the anhydroglucose units of starch are converted to more hydrophobic acetyl groups (Diop *et al*. 2011). The characteristic swelling and enzymatic degradation of NS can be considerably decreased by the acetylation of starch. This is due to the hydrophobicity and steric bulkiness of the acetyl groups of SAs (Tuovinen *et al*. 2004). There has been a trend to combine different kinds of chemical treatments to create new kinds of modifications. Similarly, chemical methods have been combined with physical modifications such as microwave, radiation and extrusion to produce modified starch with specific functional properties. Overall advantages of these modifications were to shorten the time of modification and increase production.

1.5. Microwave-assisted modification

Microwave irradiation is one of starch modification methods that can treat starch and change its functionality. There are several reported studies related to the interaction of microwaves with starch following the investigations undertaken by Muzimbaranda and Tomasik (1994) and Lewandowicz *et al*. (1997) in the 1990s. The application of microwave radiation in numerous organic reactions has been reviewed and compared to the conventional heating technique which results extensive shortening of the reaction time. During ultrafast synthesis using microwave irradiation, reactions are completed in minutes compared to hours and days using the conventional methods. This has been reported with various chemical modifications of polysaccharides (Biswas *et al*. 2008; Bushra *et al*. 2012; Cizova *et al*. 2008 and Gui-Jie *et al.* 2006). Although, microwaves have been used in the cooking of food for many years, it is only in the past decades that their application has been exploited in the chemical laboratory. Microwave techniques find application in many diverse areas of chemistry, including analytical chemistry, organic and inorganic synthesis, and manufacture of ceramics, pharmaceutical chemistry and catalysis (Mingos and Baghurst1991).

Microwave processing have been reported to change gelatinization mechanism and influences both thermal and rheological properties of starches (Lee *et al* 2007; Lewandowicz *et al.* 1998 and Luo *et al.* 2006). Microwave has been used in grafting of vinyl polymers onto starch Singh *et al*. (2006) and Mishra *et al*. (2001), in methylation of starch Singh and Tiwari (2008), or some non-typical starch modification as introducing inorganic moieties like silication (Staroszczyk *et al.* 2009a), boration (Staroszczyk *et al.* 2009b). Corn starch also crosslinked with sodium trimetaphosphate (STMP) in solid state under microwave irradiation to improve the DS and decrease the reaction time. The DS value of crosslinked starches obtained by microwave irradiation was significantly improved and the reaction time was also dramatically decreased to achieve an appropriate DS value by microwave irradiation, when compared with traditional method (Gui-Jie *et al*. 2006).

As a new processing method for the production of modified starch, microwave heating was applied to native arrowroot starch. The effect of processing parameter on the sustained release (SR) properties of the obtained physically modified starches was investigated. The sustained action of tablets made from modified arrowroot starch were explained by the high gel-forming ability of microwave-assisted modified arrowroot starch with high viscosity to prevent the dissolution based on its degree of pregelatinization (Sriamornsak *et al*. 2010).

1.5.1. Microwave-assisted acetylation

It has become increasingly popular to apply microwave field in the esterification of different types of starch. The first method of starch esters synthesis using a microwave field was the reaction of tapioca starch with succinic anhydride and the succinates obtained in this reaction had higher viscosity and higher water absorption capacity as compared to NS (Lewandowicz *et al*. 1997). Acetic anhydride is not only important because of the role of polysaccharide acetates but also due to the fact that acetic anhydride, unlike higher anhydrides, is a liquid. Energy is readily transferred from microwave radiation to the highly polar molecules of acetic anhydride through the characteristic dipolar activation of microwave heating. In a reaction mixture comprised of a polysaccharide, a salt (catalyst), and acetic anhydride, the latter is far more energy-absorbent than the other components. This results in fast evaporation in an open system or rapid pressure buildup in a closed system (Koroskenyi and McCarthy 2002).

A microwave-assisted method to prepare SAs with DS up to 3 is reported. A reaction time of 2 min at 100 ^{o}C in a microwave reactor using 0.16–2.5 mol% I_2 was sufficient to obtain SAs with a DS range of 0.13–3.0. This microwave-assisted synthetic methodology provides a rapid, efficient, and environmentally friendly method of preparing SAs by minimizing energy consumption, minimizing solvent, minimizing toxicity and amount of catalyst needed, increasing reaction efficiency, and minimizing byproducts (Biswas *et al*. 2008).

Most starch esters used commercially such as SAs ranging from low to high DS, are commonly produced by aqueous suspension of starch reacting with acetic anhydrides and NaOH to maintain pH 7–9 for several hours. Preparation of starch esters in non-aqueous systems using organic solvent like heating in formic acid, acetic acid, pyridine, and dimethyl sulfoxide had been extensively studied (Shogren and Biswas 2006). Such procedures and solvents, that is, water or organic solvents, have many drawbacks such as time-consuming procedure, water hydrolysis of acetic anhydride, expensive solvent for commercial use, reduction of the reaction rate by dilution of the modifiers, chemicals recovery after the end of the reaction is problematic owing to the complicated separation of the solvent. In addition, organic solvents are often harmful to humans and environment. Therefore, a solvent-free reaction is preferable to eliminate the use of organic solvents (Li *et al*. 2009).

Bushra *et al.* (2012) prepared mung bean SAs by solvent-free reaction and reduce energy inputs by carefully using the resources in an environmentally friendly way under microwave radiation assistance with vinyl acetate and studied physiochemical properties of mung bean SA and results shows the increment of the DS of SA in solvent-free reaction under microwave assistance in short time to produce SA with low DS. An efficient method of esterifying starch was developed by microwave-assisted esterification to produce starch maleate using the dry method and this give a reaction efficiency of 98% in a reaction time of 5 min (Xing *et al*. 2006). It has been reported elsewhere that octenyl succinate cassava starch was prepared by solid phase reaction by microwave irradiation which permitted to shorten the esterification time to less than 10 minutes in comparison to about 5-6 h in the conventional method. The modified starches were tested as a controlled release matrix for the drug, theophylline by *in vitro* methods. The swelling power of octenyl succinic anhydride (OSA) starch in simulated gastric and intestinal fluids was higher than that of NS and it increased with increase in DS. The *in vitro* drug release rate was found to be in

accordance with the DS of the OSA starch and more SR of drug was observed from OSA starch with 0.02 DS (Athira and Jyothi 2015).

1.5.2. Fundamentals of microwave radiation treatment

Microwaves are electromagnetic waves in the frequency range of 300–300,000 MHz. Polar molecules absorb microwave energy and orient themselves with respect to the electric field. The rapid change in their orientation generates heat by molecular friction (Sumnu 2001), resulting in a bulk heating throughout the sample and a faster heating rate compared with the conventional heating. The most used frequency bands in microwave processing of materials are 915 MHz for industrial/commercial microwave ovens and 2.45 GHz reserved for household microwave ovens (Das *et al*. 2009).

Microwaves are a nonionizing energy that can generate heat deep inside the penetrated medium by the ‘‘molecular friction’’ in an alternating electromagnetic field. There are a number of applications in the food industry to which microwave processing can be applied. Since it is quite competitive in cost compared with other methods of heating, it has been used for thawing of frozen foods, drying, baking, rendering, pasteurization and sterilization (Lewandowicz *et al*. 1997).

Traditionally, organic reactions are heated using an external heat source (such as an oil bath), and therefore heat is transferred by conductance. This is a comparatively slow and inefficient method for transferring energy into the system because it depends on the thermal conductivity of the various materials that must be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture. By contrast, microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the polar molecules (for example, solvents, reagents and catalysts) that are present in the reaction mixture. Microwave-assisted reaction is mainly based on the efficient heating of materials by ‘microwave dielectric heating’ effects (Mingos and Baghurst 1991).

Microwave dielectric heating is dependent on the ability of a specific material to absorb microwave energy and convert it to heat. Microwave irradiation triggers heating by two main mechanisms: dipolar polarization and ionic conduction. Whereas the dipoles in the reaction mixture (for example, the polar solvent molecules) are involved in the dipolar polarization effect, the charged particles in a sample (usually ions) are affected by ionic conduction. When irradiated at microwave

frequencies, the dipoles or ions of the sample align in the applied electric field. As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field and, in the process, energy is lost in the form of heat through molecular friction and dielectric loss. The amount of heat generated by this process is directly related to the capacity of the matrix to align itself with the frequency of the applied field. If the dipole does not have enough time to realign, or reorients too quickly with the applied field, no heating occurs. The allocated frequency of 2.45 GHz used in all commercial systems lies between these two extremes, and gives the molecular dipole time to align in the field but not to follow the alternating field precisely. Under such conditions, rapid heating of chemical reaction mixtures to high temperatures will be observed, particularly if a sealed vessel system is used (Mingos and Baghurst 1991 and Gabriel *et al*.1998).

Theoretically, all reactions that require an energy input of some sort can be activated by microwave radiation as well. The latter offers an advantage over conventional techniques by reducing the required amount of solvent, thus the amount of waste produced, the reaction time even by orders of magnitude (Loupy *et al.* 1993), and by using more efficient energy transfer and heat the contents directly. Because polysaccharides themselves are inherently biodegradable, their modification calls for green chemistry to convert the entire life cycle of the products to be environmentally friendly (Koroskenyi and McCarthy 2002). In thermal reactions, microwave technology has shown a pronounced advantage over conventional heat sources where the energy must first be conducted through the walls of the vessel containing the reactants.

1.5.3. Microwave equipment

Typical microwave heating system consists of three basic components: (1) source (magnetrons and travelling wave tubes) to generate microwave power, (2) waveguide components to transmit microwave energy into (3) a cavity which accommodates the load (Fig 1.4). The microwave cavities are generally classified into two categories: (a) single-mode (monomode) and (b) multimode. Single-mode microwave cavity generates a homogeneous energy field of high density around a small cavity. Instead, multimode cavity is larger with reflective walls to prevent leakage of radiation and to increase the efficiency of the oven (Kappe 2004).

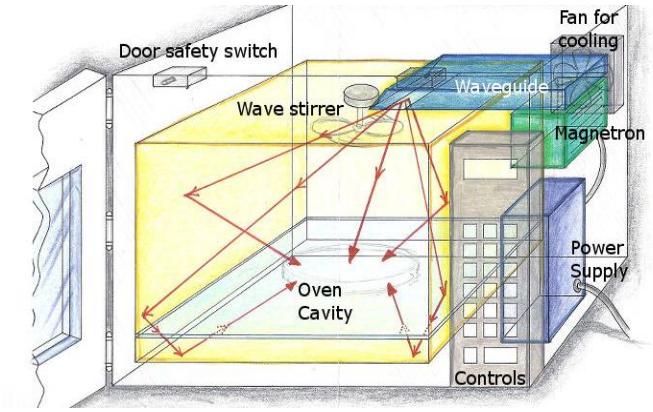


Figure 1.4: Basic Parts of microwave oven (Whelan *et al*. 2014)

The domestic microwave oven is an example of multi-mode cavity; it is an affordable device for preliminary laboratory scale investigations. Common domestic microwave ovens are unable to maintain a constant microwave power and temperature and therefore overheating may occur. Since microwave processing requires very precise temperature control, it is advisable to use laboratory microwave appliance designed and equipped with thermocouple temperature probe and temperature controller for research accuracy. Several dedicated microwave reactors equipped with highly automated and precise control systems of temperature and pressure are currently available. These appliances enable controlled and reproducible operating conditions, optimal for relatively easy scale-up and technology transfer (Kappe 2004). Unfortunately, the majority of research to date on starch using microwave irradiation has employed domestic microwave ovens and the reported data should be rather considered as a part of an initial screening.

1.6. Sustained release (SR) drug delivery

Conventional drug delivery formulations exhibit a fluctuation of drug plasma concentration over time due to a burst drug release from the pharmaceutical form, which is not ideal in a proper pharmacotherapy (Collett and Moreton 2002). Sustained delivery systems offer a better approach to drug therapy because they provide a more constant drug plasma concentration within the therapeutic window Jantzen and Robinson (2002) and Onofre *et al.* (2009) and are important in the administration of drugs with short biological half-life such as theophylline which can lengthen the dosing intervals, thus, enhancing the patient compliance toward drug therapy.

There are many different ways to achieve preparations having sustained drug release properties, but the most common types are tablets. In the production of matrix tablets many different factors contribute to the final properties of the tablet preparation. The factors affecting the formation of the tablet are attributable to the physicochemical properties of the tablets' components, such as particle morphology and deformation properties, and processing, such as the mixing of the powder and compaction. In addition, the drug release mechanism and functionality of the SR preparation has to be optimized. This is most often performed routinely using *in vitro* dissolution tests, which are defined in the Pharmacopoeias (Alderborn 2007 and Hayashi *et al.* 2005).

In sustained-release technology, directly compressed matrix tablets are the most attractive both scientifically and economically. Among the different polymers used for this purpose, EC has been used successfully to obtain appropriate SR matrix formulations of different active materials (Katikaneni *et al*. 1995 and Pather *et al*. 1998). SA with high degree of acetylation is a relatively new polymer used as SR matrix former. For matrix system to be diffusion-controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix (Jantzen and Robinson 2002).

The easiest and, thus, most generally applied method to produce orally administrable SR matrix tablets is the direct compression of a physical mixture consisting of drug compound, matrix forming polymer and, if needed, other excipients (Le Tien *et al*. 2003; Nabais *et al*. 2007 and Sánchez-Lafuente *et al*. 2002). Direct processable matrix formers would be important with respect to time and energy saving as well as to better control the whole manufacturing chain. The manufacture of controlled release formulations using a direct compression process is in principle a simple and easily controllable process. Several undesirable processes, e.g. granulation, drying of granules, usage of organic solvents, drying of solvents, can be avoided (Paronen *et al*. 1997).

1.7. Hydrophobic matrix tablets

Controlled release preparations can also be prepared by compressing formulations containing matrix forming excipients. These systems can be considered as two groups: those with drug particles dispersed in a soluble matrix, with drug becoming available as the matrix dissolves or swells (hydrophilic colloid matrices) and those with drug particles dispersed in an insoluble matrix,

with drug becoming available as a solvent enters the matrix and dissolves the particles (lipid matrices and insoluble polymer matrices) (Collett and Moreton 2002)

Matrix systems are widely used in oral controlled drug delivery because of their flexibility, cost effectiveness, low influence of the physiological variables on its release behavior and broad regulatory acceptance (Salsa *et al.* 1997). Many researchers investigated various natural, semi synthetic and synthetic polymeric materials. Cellulose ethers such as hydroxypropyl methylcellulose, sodium carboxymethylcellulose, Eudragit (polymethacrylate) polymer (Mehta *et al*. 2001), ethyl cellulose (Sanchez *et al*. 2002) and some natural gums like guar gum and xanthan gum are widely used hydrophilic polymers as release retardants (Reddy *et al*. 2003).

Polymers for SR hydrophobic matrix tablets can be either natural or chemically modified natural materials, e.g. starch, cellulose and chitin, and their derivatives, or synthetic products, e.g. acrylate derivatives (Le Tien *et al.* 2003 and Siepmann *et al.* 2008,). The originally swelling or dissolving polymers can be transformed into more hydrophobic derivatives by changing the degree of polymerization, adding cross-linkages or introducing hydrophobic groups into the polymer backbone (Le Tien *et al*. 2003; Nabais *et al*. 2007 and Raatikainen *et al*. 2002). The range of hydrophobic polymers for sustained matrices is wide, but the most commonly used are polyethylene, polypropylene, polyvinyl chloride and polyvinyl acetate (Venktraman *et al*. 2000).

Among various approaches preparation of drug embedded matrix tablets is one of the least complicated approaches for obtaining controlled release and is widely used in industry. 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 (Chowdary and Radha 2011). The type of modification required to produce good SR matrices is strongly affected by starch composition and structural characteristics as well as the type of drug used. The formation of a satisfactory SR matrix is strongly influenced by both the proportion and the structural characteristics of amylose and amylopectin, which determine the nature of the resultant matrix structure, gel strength, and viscoelastic properties from their interactions after hydration (Onofre *et al.* 2009).

1.8. The present study

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 controlled release tablets. One of the potential alternative sources of starch in Ethiopia is the indigenous plant *P. edulis* tuber which is cultivated in many parts of the country. To date, no work has been done to evaluate the usefulness of chemically modified *P. edulis* starches as directly compressible matrix systems for SR applications. Literature available on microwave-assisted modification of starches and their application as SR polymers is sparse. Thus, in the present study, the microwave-assisted acetylated *P. edulis* starch was evaluated as directly compressible matrix systems for application in SR formulations using theophylline as the model drug. Direct compression technology used in this study is scientifically and economically appealing. It entails reduced labor, cost, time, operational space, and equipment and further no heat or moisture is used. It is the preferred method of manufacturing of immediate or SR tablets (Katikaneni *et al*. 1995).

Theophylline was used as a model drug. Theophylline is a methylxanthine derivative and it is very effective in the chronic treatment of bronchial asthma and bronchospastic reaction. Its therapeutic serum concentration range is narrow from 10 - 20 µg/ml while toxicity usually appears at concentrations exceeding 20 µg/ml. Its narrow therapeutic index calls for regular monitoring of serum theophylline concentrations. Therefore, SR forms of theophylline are used to avoid adverse effects and promote its more efficient use. In addition, it has a good thermal stability (melting between 270 and 274 °C), and almost constant solubility (1 g/120 ml) in a wide range of pH (pH 2 and 7.5), which are suitable properties for release tests (Apu *et al*. 2009; Raja *et al*. 2009 and Yoon *et al*. 2007).

1.9. Research questions

- What kind of characteristics will microwave-assisted acetylated *P. edulis* starches have?
- What will be the direct compression properties of *P. edulis* SA powders?
- How is the performance of *P. edulis* SA powders as SR agents in tablet formulations?
- How do the release profiles of the SR preparations fit into various kinetic models?

1.10. Objectives

1.10.1. General objective

- To evaluate the SR properties of microwave-assisted acetylated *P. edulis (Ethiopian potato)* starch.

1.10.2. Specific objectives

- To prepare and characterize microwave-assisted acetylated *P. edulis* starches;
- To evaluate the direct compression properties of *P. edulis* SA powders and to compare with commercially available direct compression excipient;
- To prepare and analyze SR properties of the modified starches with tablet formulations;
- To analyze the release kinetics/drug release mechanism from tablets of the modified starches.

2. EXPERIMENTAL

2.1. Materials

Fresh tuber of *P. edulis* (Ethiopian potato) were obtained from Nekemte, Ethiopia. Anhydrous theophylline was kindly donated by Addis Pharmaceuticals Factory PLC, Ethiopia. Acetic anhydride (May and Baker Ltd Dagenham, England), EC, Hydrochloric acid (HCl), Magnesium stearate, MCC (Avicel® PH 101) and Potassium hydroxide (KOH) (BDH Chemicals Ltd, England), Ethanol (ChangshuYangyuan Chemicals, China), Sodium metabisulphite (Guangzhou Jinhaunda Chemical Reagent Co. Ltd, China), Potassium dihydrogen phosphate (Sorensen, Leuren, Germany), Sodium Chloride (NaCl) (LABMERK CHEMICALS PVT.LTD, India), Sodium Hydroxide (NaOH) (LOBA CHEMIE PVT.LTD, India) were obtained from local suppliers. All reagents and solvents were used as received.

2.2. Methods

2.2.1. Isolation of starch from *P. edulis* tuber

Starch was isolated from tubers of *P. edulis* by the method described elsewhere (Gebre-Mariam and Schmidt 1996). Tubers of *P. edulis* were thoroughly washed to remove all foreign materials (Fig 2.1). The washed tubers were peeled and pulverized using a blender and suspended in large quantities of distilled water containing 0.075% w/v sodium metabisulphite. The material was then allowed to settle overnight, and the supernatant was decanted and the sedimented starch was repeatedly treated with sodium metabisulphite solution and filtered until the supernatant was free of coloring materials and the suspension was translucent. Then the material was passed through fine muslin cloth to remove cell debris and the translucent suspension was collected; re-filtered through the muslin cloth and allowed to settle. The sedimented starch was washed several times with distilled water until the wash water was clear and free of suspended impurities. The resulting starch was air dried at room temperature and sieved through appropriate mesh sieve. The starch was kept in a tight-light resistant container until further work.

Figure 2.1: The tuber of *P. edulis* and dried form of starch granule obtained from the tuber (taken by Mosisa A.)

2.2.2. Microwave-assisted acetylation

After the NS was isolated from the *P. edulis* tuber microwave-assisted SAs were prepared by modifications and combinations of the methods stated in Biswas *et al*. (2008); Shogren and Biswas 2006 and Bushra *et al. (*2013). Before acetylation, *P. edulis* starch was dried in vacuum oven (Kottermann® 2711, Germany) for 24 h at 50 °C to minimize the interference of moisture on the chemical reaction. A sample of native *P. edulis* starch and acetic anhydride in the ratio of 1:4 was placed in a sealed round bottom flask and mixed continuously at room temperature for 5 min by magnetic stirrer, when the mixture was uniformly mixed, the vessel was placed into LG microwave at power level of 100 W, 200 W and 300 W at different time. Maximum microwave power was limited to 300 W to avoid overshooting the temperature. The mixture was heated for 2 min so that acetic anhydride can penetrate the dried starch granules before adding NaOH. Afterwards, the flask was removed from microwave cavity and 50% (w/w) NaOH was added to the mixture as a catalyst drop wise with constant swirling and the sealed flask was placed again in the microwave, for total reaction time of 5, 9 and 13 min. At the end of the reaction, the reactor was removed from the microwave oven and cooled to room temperature. The mixture was transferred to a beaker containing 75% ethanol and stirred for 30 min and washed repeatedly for 3-4 times with distilled water and filtered by Buchner funnel. The samples were dried at 60°C in vacuum oven overnight. The different acetylated starches were then ground to a fine powder and tightly closed and stored under dry condition for further analysis.

2.2.3. Physicochemical characterization of modified starches

2.2.3.1. Determination of acetyl percentage and degree of substitution

The percentage of acetylation (% acetyl) and DS was determined titrimetrically, following the method of Bartz *et al*. (2015) and Bushra *et al*. (2013). Acetylated starch was placed in a flask and 20 ml of 70% ethanol was added, the loosely stoppered flask was shaken, warmed to 50 °C for 30 min in water bath, cooled and 30 ml of 0.5N KOH was added. After occasional swirling for 72hr at room temperature, the excess alkali was back titrated with 0.5 M HCl using phenolphthalein as an indicator. A blank sample with NS was titrated with the same procedure at the same time. Reaction efficiency (RE) measured how effectively the starch samples reacted with acetyl groups. The acetyl %, DS and RE (%) was calculated from equations 2.1, 2.2 and 2.3, respectively,

$$Acetyl\ (\%) = \frac{[V\ in\ blank(ml) - V\ in\ sample\ (ml) \times M\ of\ HCL\ \times 0.043 \times 100]}{Wt\ of\ sample\ (g)} \ldots Eq. 2.1$$

$$DS = \frac{\left(162 \times Acetyl\ (\%)\right)}{4300 - (42 \times Acetyl\ (\%))} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.2$$

$$RE\ (\%) = \frac{DS}{102.1/42} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.3$$

Where, 162 is the molecular weight of the anhydroglucose unit, 42 is the molecular weight of replaceable acetyl group, 4300 is the molecular weight of the acetyl group attached with 100 anhydroglucose units and 102.1 is mole of acetic anhydride.

2.2.3.2. Determination of Fourier transform infrared (FTIR) spectra

FTIR spectra of native *P. edulis* starch and the SAs were acquired at room temperature using FTIR spectrophotometer (FTIR-8400S, SHIMADZU, Japan) in transmittance mode. About 5 to 8 mg of finely ground samples were mixed with an oily mulling agent (Nujol) and sample mixture was 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 spectrometer and the spectra were obtained. Each

IR spectrum was collected with 20 scans and spectral resolution of 2 cm^{-1}. Scanning was performed between wave numbers 4000 and 600 cm^{-1}. IR solution software was used for data treatment.

2.2.3.3. XRD analysis

Powder XRD patterns of native and acetylated *P. edulis* starch were recorded on X-ray diffractometer (Rigaku Miniflex-600, Japan) at ambient temperature at 40 kV and 15 mA, using Cu Kα radiation at a step size of 0.02. Data were collected from 2θ of 5.00–50.00 (θ being the angle of diffraction) which is sufficient to cover all significant diffraction peaks of starch crystallites. Origin 7.0 statistical software was used for analysis.

2.2.3.4. Measurement of gelatinization property

Differential scanning calorimetry (DSC) measurements of the starch-water mixture (starch/water ratio of 1:1 (w/w), on dry basis) were carried out using DSC 200 thermal analyzer (Netzsch, Selb, Germany) from which gelatinization temperatures were obtained. Starch sample of known moisture content was thoroughly mixed with distilled water and allowed to equilibrate in closed glass jars for 24 h at room temperature. Portion of well mixed starch slurry was transferred into aluminum DSC sample pans and sealed hermetically. The sealed pan was placed in the DSC cell and heated from 20 ^{o}C to 200 ^{o}C at a rate of 10 ^{o}C/min. An empty sealed pan was used as a reference and a nitrogen environment. From the DSC trace: onset (To), peak (Tp) and end set (Te) temperature of the gelatinization (^{o}C), and enthalpy of gelatinization (ΔHgel, J/g) were obtained.

2.2.4. Measurement of density and related properties

2.2.4.1. True density

True density was determined by liquid displacement method using xylene as immersion fluid, the method mentioned in Gonzalez and Peret (2002) with slight modification. Two grams of each of native *P. edulis* starch, acetylated starches and MCC (Avicel PH 101) samples were placed in a pycnometer, closed and weighed (the weight of the empty pycnometer and of the pycnometer filled with xylene is known). Sufficient xylene was added to wash down and overlay the sample. After 10 min, the sedimented starch was stirred with a small glass-stirring rod to release entrapped air, the sample equilibrated for a few min and stirred again. When evolution of minute air bubbles

through the supernatant xylene layer had stopped, the stirrer was removed and rinsed into the pycnometer with several milliliters of xylene. The sample was allowed to settle, the pycnometer filled with xylene and the meniscus was adjusted. True density (g/ml) was calculated from Eq. 2.4. True density was measured as a mean of three measurements.

$$True\ density = \frac{W1}{[(W2 + W1) - W3]} \times SG \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.\ 2.4$$

Where, W1 = weight (g) of starch, W2 = weight (g) of the pycnometer filled with xylene, W3= weight (g) of pycnometer plus sample plus xylene, and SG = specific gravity of xylene (0.855g/ml)

2.2.4.2. Bulk density

Bulk densities of NS, SA and MCC (Avicel PH 101) were determined by carefully pouring 30 g powder into a graduated glass measuring cylinder. The cylinder was then lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density. The bulk density (g/ml) was calculated from Eq.2.5 and determined as a mean of three measurements.

$$Bulk\ density(\rho_b) = \frac{W}{V_b} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.\ 2.5$$

Where, W is the weight of the powder and V_b is bulk volume

2.2.4.3. Tapped density

For tapped density 30 g of powder was tapped in graduated measuring cylinder 500 times using tapped densitometer (ERWEKA, Germany) to attain a constant volume reading from the cylinder and the tapped density was calculated from the weight and tapped volume of the powder by using Eq. 2.6. Tapped Density (g/ml) was determined as a mean of three measurements.

$$Tapped\ Density\ (\rho_t) = \frac{W}{V_t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.\ 2.6$$

Where, W is the weight of the powder and V_t is the tapped volume. To assess the flowability of powders, Hausner ratio (HR) and the Carr's index (CI) were calculated from the bulk and tapped densities of the powders using Eq. 2.7 and 2.8 respectively.

$$Hausner's\ ratio = \frac{\rho_t}{\rho_b} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.7$$

$$Carr's\ index = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.8$$

2.2.4.4. Angle of repose and flow rate

Flowability of the samples was determined using the standard funnel method. The angle of repose was determined from the dimensions of the powder pile, which formed when 30 g of powder mass was placed in a funnel and allowed to flow. The orifice of the funnel was fixed at 10 cm above the base. Flowing time was measured. The angle of repose was calculated from the formed powder pile by using Eq. 2.9. Results were expressed as a mean of three determinations.

$$\theta = tan^{-1}\left(\frac{h}{r}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.9$$

Where, h, is height of the pile of powder and r is the radius of the base at the cone. The flow rate (g/sec) was calculated according to Eq. 2.10.

$$Flow\ rate = \frac{W}{t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.10$$

Where, W is the weight in grams and t is flowing time in seconds.

2.2.4.5. Determination of moisture content

Water content of the powders was measured by gravimetric method. The powder samples (2 g each) were spread onto Petri dishes uniformly and dried in an oven at 130 ºC. The weight loss was obtained by accurately weighing the samples after 2 h. The moisture content was determined from the loss of weight. Results were expressed as a mean of three parallel determinations.

$$Moisture\ content = \frac{Wi - Wf}{Wi} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.11$$

Where *Wi* and *Wf* are starch weights before and after drying, respectively

2.2.4.6. Kawakita analysis

Compressibility and cohesiveness were determined using the Kawakita analysis mentioned elsewhere with slight modification (Parakash *et al*. 2011). The method involved pouring 30 g of sample powders into a 250 mL glass measuring cylinder. The heaped particles in the cylinder were then leveled off horizontally with a thin metallic spatula and the bulk volume *Vo* was measured. Tapping was afterwards initiated mechanically and the change in volume of the powder column *Vn* was noted after *N* number of taps.

The Kawakita equation, which is used for assessing the flow properties of powders, is given by:

$$\frac{N}{C} = \frac{N}{a} + \frac{1}{ab} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.12$$

Where *a* and *b* are constants; *a* describes the degree of volume reduction at the limit of tapping and is called compactibility; *1/b* is considered to be a constant related to cohesion and is called cohesiveness, *C* being the degree of volume reduction is calculated from the initial volume *Vo* and tapped volume *Vn* as:

$$C = \frac{(Vo \ - Vn)}{V0} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.13$$

From the graphical presentation of N/C versus N *(N = 5,* 10, 20, 30, 40, 50, 75, 100, 300, 400 and 500*)*, the constants were evaluated. The constant “a”, is given as a reciprocal of the slope from the linear portion of the plot and 1/b represent the product of “a” and intercept (Autamashih *et al.* 2011).

2.2.4.7. Determination of swelling power (SP) and solubility

Swelling power and solubility were determined using the slightly modified method described by Singh *et al*. (2011) and Zhang *et al*. (2014). Effect of temperature on swelling power and solubility was carried out in the temperature range of 20–85°C. A total of 0.5g of starch samples was accurately weighed and quantitatively transferred into a clear dried test tube. 10ml of distilled water was added to the test tube, and the mixture was mixed thoroughly and the resultant slurries were heated at desired temperatures, varied between 20 and 85°C for 30 min in a thermometer-controlled temperature water bath. The mixture was cooled to room temperature and centrifuged

at 2000rpm for 15 min. The supernatant was carefully transferred into a pre-weighed Petri dish and dried at 120 °C for 2hr. The test tube with residue obtained from the above experiment (after centrifugation) was also weighed. All measurements were done in triplicate. Solubility (S) and swelling power (SP) were calculated according to Eq. 2.14 and 2.15.

$$S\% = \frac{A}{W} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.14$$

$$SP = \frac{P \times 100}{W \times (100 - S)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq. 2.15$$

Where, A is the weight of the residues in the supernatant after evaporation, P is the weight of the precipitate, and W is the weight of the starch sample (dry basis). The precipitate weight P was calculated by subtracting the weight of empty tube from the total weight of tube after centrifugation with swelling starch.

2.2.4.8. Determination of moisture sorption pattern

The method described by Odeku and Picker-Freyer (2007) was slightly modified for moisture sorption studies. Pyrex desiccators containing distilled water, saturated solution of NaCl or appropriate concentrations of NaOH were prepared to provide different RH chambers and stored at room temperature. Native and SAs powder samples were predried in an oven for 4 h at 120 ^{o}C. Two grams of the starches were spread evenly on each Petri dish (dried and weighed) and transferred to particular RH chamber. Samples were equilibrated for four weeks at room temperature. The weights after four weeks were recorded and the moisture uptake of each sample was calculated as the weight difference of the starches before and after equilibration in a given RH. Water sorption capacities of the starches were expressed as percent moisture uptake. Results were expressed as a mean of three parallel determinations.

2.2.5. Tablet preparation and evaluation

2.2.5.1. Tablet preparation

To study compressibility of *P. edulis* SA powders plain tablets were prepared and compared with native *P. edulis* starch and references. To prepare plain tablets, native *P. edulis* starch, *P. edulis*

SA DS 1.77 and DS 0.44 and reference excipient powders were lubricated with 0.5% (w/w) magnesium stearate for 3 min using Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) at 45 rpm. The lubricated powders were compressed with an instrumented Eco-press tablet compression machine (PARLE ELIZABETH TOOLS PLC., India) using flat faced punches and a die at fixed compression pressure.

For *invitro* drug release studies, each of the modified starches, EC or MCC (Avicel PH 101) was mixed with the model drug, anhydrous theophylline, under ambient room conditions for 5 min in a Turbula mixer at 45 rpm. The powder mixtures were lubricated with magnesium stearate for 3 min and compressed with an instrumented eccentric tablet press at fixed compression pressure. The matrix tablets contained different theophylline and *P. edulis* SA concentrations were prepared. The compositions (% w/w) of the formulations used for the drug release studies are indicated in Table 2.2. The weight of tablets was fixed to 400 mg.

Table 2.1: The compositions of tablet formulations used in drug release studies.

Ingredients	Formulation number					
	F1	F2	F3	F4	F5	F6
Theophylline (%w/w)	20.0	30.0	40.0	20.0	20.0	20.0
SA DS 1.77 (%w/w)	79.5	69.5	59.5	-	-	-
SA DS 0.44 (%w/w)	-	-	-	79.5	-	-
MCC (%w/w)	-	-	-	-	79.5	-
EC (%w/w)	-	-	-	-	-	79.5
Mg. stearate (%w/w)	0.5	0.5	0.5	0.5	0.5	0.5

2.2.5.2. Tablet evaluation

2.2.5.2.1. Weight, thickness and diameter

From each batch, 10 tablets were randomly selected and weighed individually on an analytical balance (Mettler Toledo, PR 203, Switzerland) and then the average weight and standard deviation were calculated. Tablet thickness and diameter (fixed 11 mm according to punch size) were measured by hardness tester.

2.2.5.2.2. Crushing strength

Crushing strength of the tablets was determined with the crushing strength tester (Schleuniger, 2E/205, Switzerland). Ten tablets were taken randomly from each batch and the crushing strengths of the tablets were measured individually and the means and standard deviations were calculated.

2.2.5.2.3. Tensile strength

The tablets were characterized regarding their dimensions and hardness by calculating the radial tensile strength (σ) according to Fell and Newton (1970) using Eq. 2.15

$$\sigma = \frac{2 \cdot F}{\pi \cdot T \cdot D} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (Eq. 2.15)$$

Where, F is the force required to break the tablets, D and T are the diameter and thickness of the tablets.

2.2.5.2.3. Friability

The friability was conducted on ten tablets of known weight using a friability tester (ERWEKA, TAR 20, Germany). The drum was rotated at 25 rpm for 4 min. The tablet samples were then removed and dedusted and reweighed. Loss of tablet weight with respect to the initial value was then calculated as percent friability.

2.2.5.2.4. Porosity

Porosity (ε) of tablets was calculated according to Equation stated by Korhonen *et al.* (2002)

$$\varepsilon = \left[1 - \frac{m}{v} \Big/ \rho m\right] \times 100 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . (Eq. 2.16)$$

Where m is the weight of the tablets, V is the volume of the tablets that is $V = \pi r^2 h$, where r is the radius, and h is the thickness of the compact, and ρm is the true density of materials.

2.2.5.2.5. 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 tester (CALEVA, G.B. Caleva Ltd., UK) filled with distilled water maintained at 37 ± 2°C as the immersion fluid. The tablets were considered completely disintegrated when all particles passed through the wire mesh screen of the basket rack assembly and if any residue remains, it must have a soft mass with no palpably firm core. The average disintegration time and standard deviation of six tablets was determined.

2.2.5.2.6. Construction of calibration curve

Different volumes of stock solutions of anhydrous theophylline (0.05% w/v) in phosphate buffer pH 6.8 and 0.1 N HCl were sampled and then diluted to 100 ml in volumetric flasks to get seven different concentrations in the range of 4 μg/ml to 13μg/ml. Then, the absorbance of each of these serial dilutions was measured at the λmax (271 nm) by using UV-Visible spectrophotometer (CECIL. CE1021. Cambridge, England). A 0.1 N HCl and phosphate buffer pH 6.8 were used as blank sample. The absorbance versus concentration of the solutions was plotted, and a linear regression equation and correlation coefficient were obtained.

2.2.5.2.7. *In vitro* drug dissolution

The *in vitro* drug release studies were performed in USP Dissolution Apparatus II (Paddle Method) (ERWEKA. DT600. Germany). The paddle was adjusted to rotate at 75 rpm. A dissolution medium (900 ml) of 0.1 N HCL for the first 2 h and phosphate buffer solution of pH 6.8 for remaining 10 h, maintained at 37 ± 0.5 °C, were used. Samples of dissolution medium (5 ml each) were withdrawn at predetermined time intervals (at 15 and 30 min, and then at 1, 2, 3, 4, 6, 8, 10 and 12 h) and immediately replaced with an equal volume of the dissolution medium in order to maintain constant volume in the dissolution vessels. The samples withdrawn were filtered, properly diluted and analyzed for theophylline content in a UV-Vis spectrophotometer at the λmax of 271 nm. Concentration was determined from the standard calibration curve of theophylline and finally the results were plotted as cumulative % drug release versus time.

2.2.5.2.8. Analysis of drug release kinetics

The dissolution data were fitted into the following drug release models (Eq. 2.17- 2.21) to evaluate the *in vitro* drug release kinetics from the hydrophobic SA matrix tablets:

I. Zero order release model:

$$Q = Q_o - Kt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Eq. 2.17)$$

Where, Q is the amount of drug remaining at time t, Qo is the quantity of drug present initially in the dosage form and K is the zero-order release constant. The plots made were cumulative percent drug release versus time.

II. First order release model:

$$lnQ = \ln Q_o - Kt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Eq. 2.18)$$

Where, Q is the amount of drug remaining at time t, Qo is the quantity of drug present initially in the dosage form and K is the first order release constant. The plots made were log cumulative of percent drug released versus time.

III. Higuchi square root model:

$$Mt/_{Mo} = Kt^{1/2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Eq. 2.19)$$

Where, Mt is the amount of drug released at time t, Mo the amount of total drug in tablets, Mt/Mo is the fractional drug release at time t and K is a constant incorporating the matrix structure. The plots made were cumulative percent drug release versus square root of time

IV. Korsemeyer-Peppas release model:

$$Mt/_{Mo} = Kt^{n} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (Eq. 2.20)$$

Where, Mt is the amount of drug released at time t, Mo the amount of total drug in tablets, Mt/Mo is the fractional drug release at time t, K is a constant incorporating the matrix structure and n is a diffusional exponent, which was used to characterize the transport mechanism. The plots made were log cumulative percent drug release versus log time

V. Hixson-Crowell cube root model

$$Qo^{1/3} - Qt^{\frac{1}{3}} = Kt \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . (Eq. 2.21)$$

Where, Qt is the amount of drug remaining in time t, Qo is the initial amount of the drug in tablet and K is the rate constant for Hixson-Crowell rate equation. The plots made were cube root of drug percent remaining in matrix versus time

2.2.6. Statistical analysis

Wherever appropriate, the data were subjected to further statistical analysis to compare the properties of the modified starches with those of NS and/or standard excipient using Analysis of Variance (ANOVA) on Origin 7.0 statistical software. A confidence limit of $P < 0.05$ was fixed for interpretation of the results.

3. RESULTS AND DISCUSSION

3.1. Isolated and acetylated *P. edulis* starch

Some physicochemical properties of *P. edulis* starch and its proximate compositions are reported by Assefa *et al*. 2015. As stated earlier, the starch isolated from *P. edulis* was chemically modified by acetylation, under microwave heating. The acetylation reaction is accelerated under microwave heating mainly due to the high temperatures obtained in the flask. During acetylation, the residual water and NaOH probably cause swelling of the starch and make it more reactive with acetic anhydride. The starch then begins to react and dissolve, forming a viscous mass.

Based on the literature survey, preliminary studies were undertaken to obtain suitable reaction conditions for the synthesis of *P. edulis* SA with high DS for SR application of the polymer in tablets manufactured by direct compression method. The reaction condition that yielded high degree of acetylation was selected and used for further study and low degree of acetylation was used for comparison purpose.

3.2. Characteristics of starch acetate

3.2.1. Degree of substitution (DS)

P. edulis starch was acetylated to different levels by microwave heating and the products were characterized by determining their percentage of acetylation and DS. As shown in Table 3.1, the highest DS of 1.77 was obtained by the acetylation of native *P. edulis* starch having a moisture content of 13%, with acetic anhydride in 1:4 ratio of native *P. edulis* starch to acetic anhydride. The temperatures measured by thermometer was 60 - 100 °C for the exposure times of 5 – 13 min based on the optimum reaction conditions. Different reports indicate that the properties of acetylated starch depend on the botanical starch source, the DS, the reaction time, the PH, the amylose/amylopectin ratio, and how the molecular structure of starch is modified (Bello-Perez *et al.* 2000; Xu *et al*. 2004).

Table 3.1: Acetyl content and degree of acetylation of *P. edulis* SA (mean (SD))

Reaction condition	Starch/Acetic anhydride ratio	Time (min)	Power (watt)	Acetyl content (%)	DS	RE (%)
1	1:4	5	100	10.62 (0.46)	0.44 (0.02)	18.56
2	1:4	5	200	12.90 (0.45)	0.56 (0.05)	23.63
3	1:4	5	300	13.97 (0.47)	0.61(0.07)	25.74
4	1:4	9	100	16.13 (0.59)	0.72 (0.05)	30.38
5	1:4	9	200	17.20 (0.51)	0.78 (0.03)	32.91
6	1:4	9	300	19.35 (0.54)	0.89 (0.01)	37.55
7	1:4	13	100	22.58 (0.49)	1.09 (0.02)	45.99
8	1:4	13	200	25.80 (0.42)	1.29 (0.02)	54.43
9	1:4	13	300	32.25 (0.12)	1.77 (0.03)	74.54

3.2.2. Fourier transform infrared (FTIR) spectra

The FT-IR spectra of native and acetylated *P. edulis* DS 1.77 and DS 0.44 starches are shown in Fig 3.1, 3.2 and 3.3, respectively. Amylose and amylopectin, the two structural units of starch, are composed of identical monomers, which have only one prominent functional group, OH (alcohol) group. There are two types of alcohol groups in those structural units, primary alcohol (–CH2OH) and secondary alcohol (OH, directly attached to ring carbons) (Nutan *et al*. 2007).

In the spectrum of native *P. edulis* starch (Fig.3.1), an extremely broad band appeared around 3,000 - 3,500 cm^{-1} which is attributed to the complex vibrational stretches associated with free, inter and intramolecular bound OH groups which make up the gross structure of starch. The peak of OH group around 2900 cm^{-1} was merged with the peak of the mulling agent, Nujol around 3000 cm^{-1}. The peak at around 1020 cm^{-1} explains the stretching vibration of the secondary cyclic alcohol groups. The presence of polysaccharides is described by the occurrence of absorption bands in the region known as the ‘‘fingerprint region’’ ranging from 1000 to 700 cm^{-1}. The bands are due to the entire anhydroglucose ring stretching vibrations (Bartz *et al*. 2015). The peak at 1618.17 cm^{-1} due to bending of water molecules tightly bound in the starch and 1078.13 cm^{-1}, 1151.42 cm^{-1} and 1244.00 cm^{-1} attributed to the C–O–C stretching vibrations.

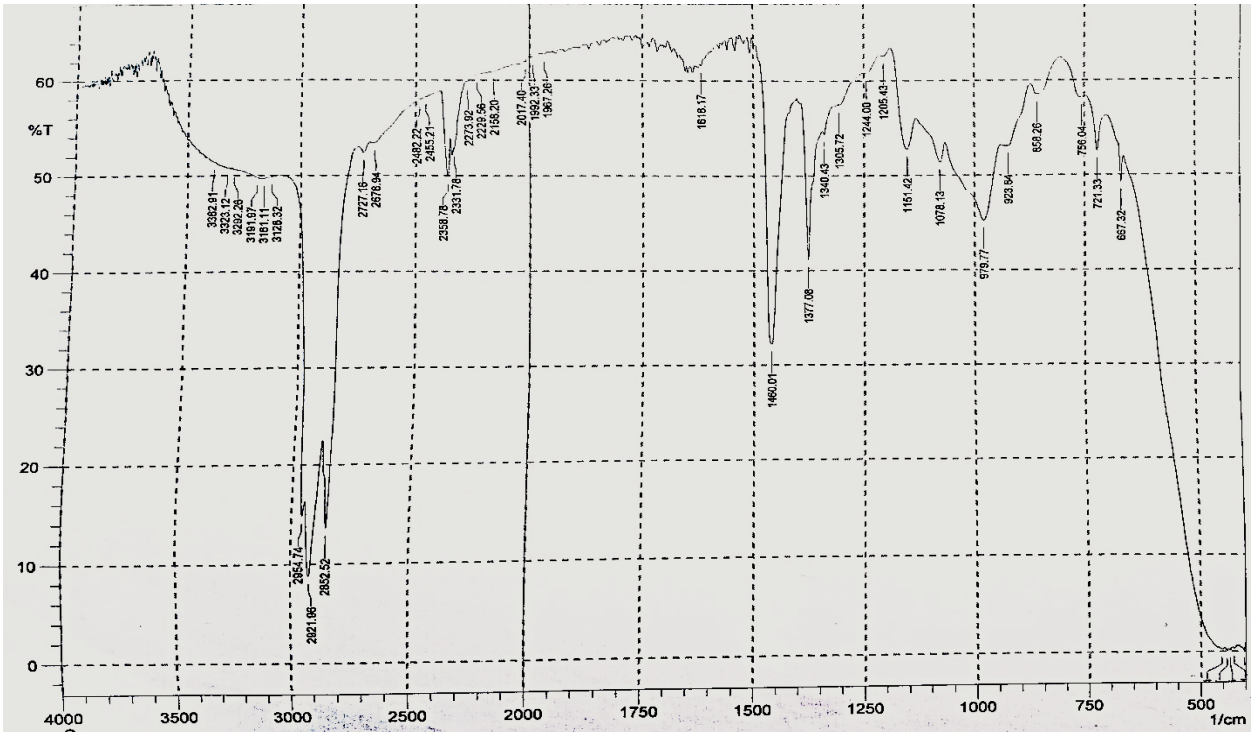


Figure 3.1: FTIR spectrum of native *P. edulis* starch

In the acetylation reaction, the OH moieties of monomers in starch are substituted with acetyl moieties. As shown in Fig. 3.2 after acetylation by acetic anhydride, new absorption band appeared at 1716.53 cm^{-1} was assigned to the carbonyl C=O ester group. These new absorption band suggest that the ester carbonyl groups were formed during the esterification process in starch (Diop *et al*. 2011 and Li *et al*. 2009).

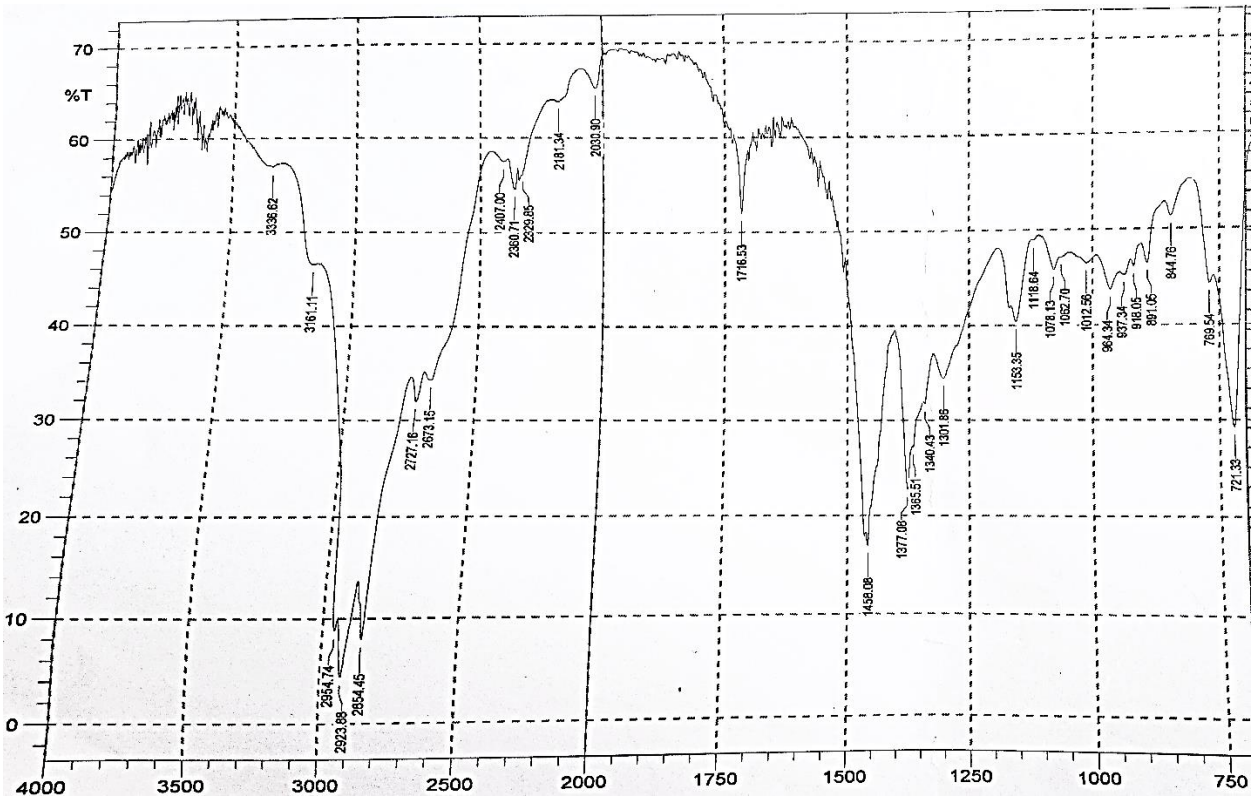


Figure 3.2: FTIR spectrum of *P. edulis* SA DS 1.77

And the intensity of the OH group's peak at 3,000 - 3,500 cm^{-1} decreased up on acetylation of *P. edulis* starch. This suggests that the OH groups in the starch molecules are converted into acetyl groups, resulting in the loss of starch granule crystallinity (Korhonen *et al*. 2000). As DS decreased from 1.77 to 0.44 resulting undetected very broad peak for acetyl group.

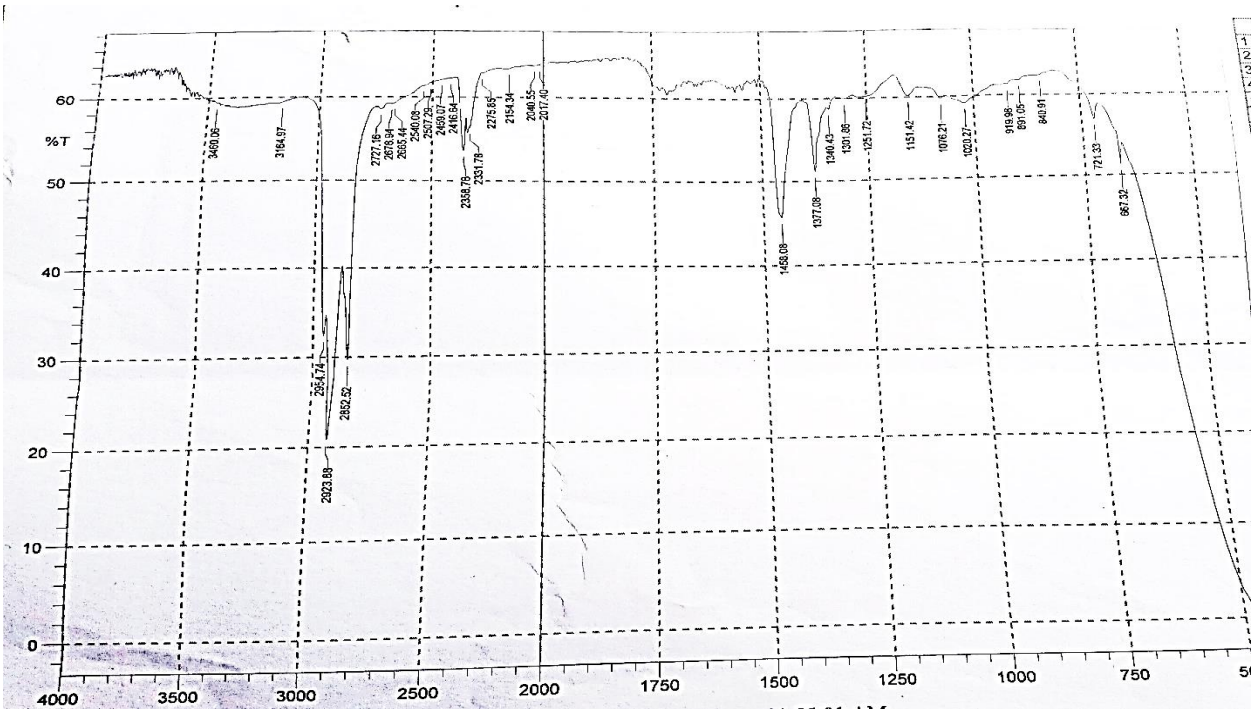


Figure 3.3: FTIR spectrum of *P. edulis* SA DS 0.44

3.2.3. X-ray diffraction

X-ray diffraction measurements were performed to check if chemical modification altered the crystallinity of starch. The X-ray diffraction spectra of NS and acetylated starches are presented in Fig 3.4. The native *P. edulis* starch exhibited the typical B type crystallinity characterized by strong diffraction peaks at 2θ of about 17.32. The other small peaks were 5.96, 15.6, 19.60 and 22.06 which confirmed type B pattern.

As shown Fig 3.4, acetylated starch showed a similar pattern to that of native *P. edulis* starch (NPES); however, the peaks at 5.96°, 17.32° and 19.6° almost disappeared after acetylation for *P. edulis* SA. For the *P. edulis* SA having the lowest DS 0.44, some small reflections were apparent, which indicate that the molecular structure included some highly ordered crystalline regions, although mostly amorphous. These diffraction peaks disappeared in *P. edulis* acetylated starch when the DS was 1.77. Destruction or even loss of the ordered crystalline structure increased with increasing DS. According to Huang *et al*. (2007), the minimal crystallinity changes in the SA with low DS suggests that the acetyl groups are concentrated primarily in the amorphous region of the granules.

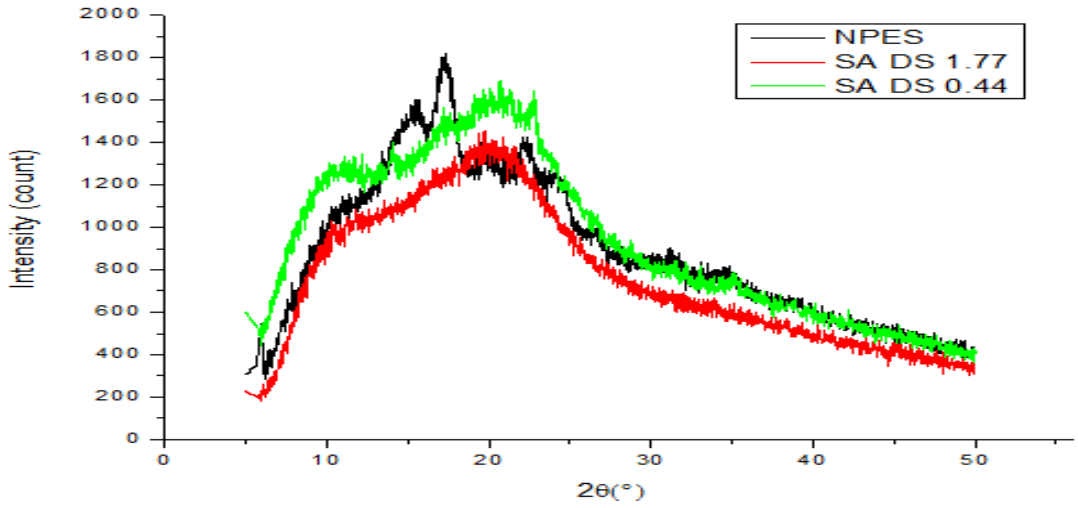


Figure 3.4: XRD pattern of native *P. edulis* starch and *P. edulis* SA

After acetylation, acetyl groups replace some hydroxyl groups on starch, reducing the formation of inter and intramolecular hydrogen bonds, resulting thereby in the destruction of the original ordered crystal structure (Luo and Shi 2012; Xu *et al*. 2004 and Zhang *et al*. 2009).

3.2.4. Differential scanning calorimeter (DSC) study of gelatinization

Starch gelatinization is an important starch functional property that varies with respect to the composition, the molecular structure of amylopectin, crystalline to amorphous ratio, and granule morphology and size distribution of starches (Singh *et al*. 2007). Gelatinization properties of native and acetylated *P. edulis* starches as measured by differential scanning calorimetry (DSC) are summarized in Table 3.2 and Fig 3.5. As shown in Fig 3.5 the To, Tp, and Te value between the *P. edulis* SA DS 0.44 and its native counterpart reflects a structural disorganization produced by the acetylation reaction. More significant changes in the To, Tp, and Te and enthalpy of gelatinization were observed in the *P. edulis* SA DS 1.77, corroborating that acetylation of *P. edulis* starch with high DS produced structural disorganization as was shown in the FTIR and XRD tests.

Table 3.2: DSC Gelatinization Properties of native *P. edulis* starch and *P. edulis* SA

	T_o (°C)	T_p (°C)	T_e (oC)	ΔHgel (J/g)	ΔT (Te-To)
PENS	57.77	68.49	72.12	11.90	14.35
SA DS 1.77	43.56	51.43	52.92	3.82	9.36
SA DS 0.44	47.24	54.56	57.69	6.60	10.45

The higher the DS of the starch the larger the decrease in thermal parameters which is in line with study reports elsewhere (Bello-Pérez *et al*. 2000, Betancur *et al*.1997 and Sodhi and Singh 2005). Weakening of the starch granules by acetylation leads to early rupture of the amylopectin double helices, which accounts for the lower values of To, Tp and Tc (Adebowale and Lawal, 2003).

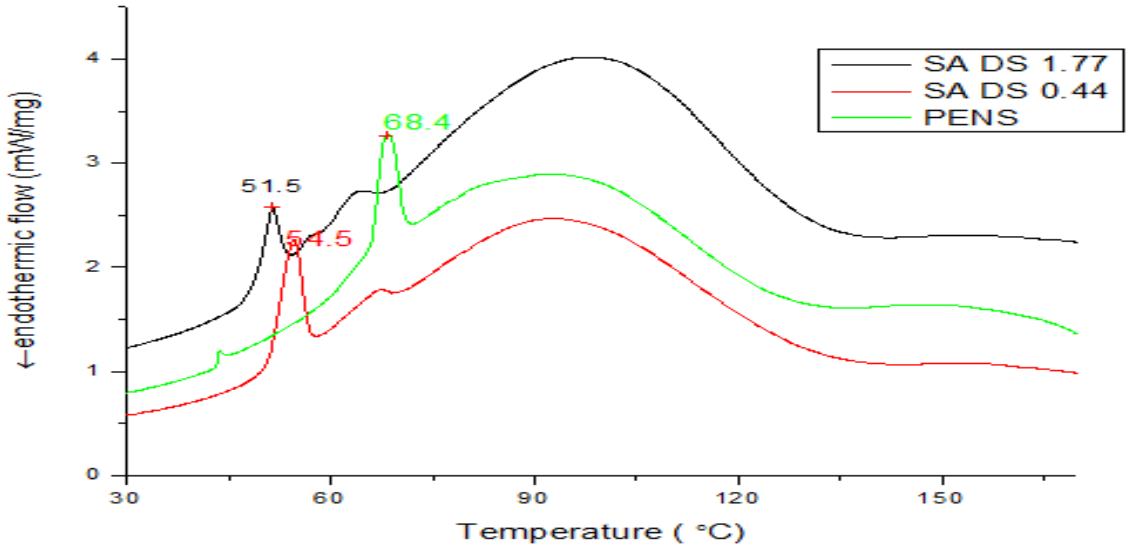


Figure 3.5: DSC thermograms of native *P. edulis* and *P. edulis* SA.

3.2.5. Powder Properties

Some powder properties of the native and modified starches are presented in Table 3.3. The bulk density of a powder describes its packing behavior while the tapped density indicates the rate and extent of packing that would be experienced by a material during the various unit operations of tableting. The greatest bulk density was observed in native *P. edulis* starch whereas MCC (Avicel PH 101) had the lowest bulk density. Acetylation of *P. edulis* starch decreased the bulk density of

the powder significantly. The values of the bulk and tapped densities provide information on the flowability of powders and are used to calculate the Carr's index (CI) and Hausner ratio (HR), which are a measure of the flowability and compressibility of a powder (Odeku and PickerFreyer 2009).

Acetylation of *P. edulis* starch significantly improved its flow property, that is the CI and HR of native *P. edulis* starch reduced significantly upon chemical modification (Table 3.3). The HR for SA DS 1.77, EC and MCC is less than 1.25 which indicates better flowability whereas SA DS 0.44 HR is in passable range. The range of CI is 13.357 (1.486) to 24.258 (2.294) for all powders which is in good to poor range of compressibility for SA DS 1.77 to native *P. edulis* starch. Flowability is an important factor in tableting since a uniform flow ensures minimal weight variations in tablets produced.

The angle of repose is another qualitative measure of the cohesiveness or the tendency of powdered materials to flow. Angle of 30° or below usually indicates that the powder is free flowing. Angles of repose of 40° or above indicate poor flow. As shown in Table 3.3, the angle of repose of native *P. edulis* starch is not determined because the powder did not flow through the funnel. The remaining SA, EC and MCC has angle of repose less than 30 which shows good flowability. These results indicate that the modification remarkably improved the flow property of *P. edulis* starch. Moisture is known to modify the flow and mechanical properties of many powders including starches. The moisture content of air-equilibrated starches ranges from about 10-12% (cereal) to about 14-18% (some roots and tubers) (Tester *et al*. 2004).

Generally, the favorable powder flow properties of SA DS 1.77 and the optimal moisture content (8.667%) decrease the cohesiveness of the powder making it appropriate for direct compression formulations.

Table 3.3: Powder properties of native *P. edulis* starch, *P. edulis* SA, EC and MCC (mean (SD))

Powder properties	Native *P. edulis* starch	*P. edulis* SA DS 1.77	*P. edulis* SA DS 0.44	EC	MCC
Bulk density(g/ml)	0.56 (0.04)	0.49 (0.01)	0.38 (0.02)	0.36 (0.01)	0.34 (0.02)
Tapped density(g/ml)	0.74 (0.01)	0.57 (0.01)	0.48 (0.01)	0.42 (0.01)	0.4 (0.03)
True density (g/ml)	1.39 (0.07)	1.1 (0.61)	1.4 (0.03)	*	1.45 (0.1)
Carr's index (%)	24.26 (2.29)	13.4 (1.49)	20.3 (2.1)	13.65(2.4)	14.8 (0.04)
Hausner's ratio	1.32 (0.03)	1.15(0.02)	1.26 (0.04)	1.16(0.03)	1.2(0.001)
Angle of repose (°)	x	19.7 (3.9)	28.1 (3.5)	21.8(3.2)	27.7 (4.12)
Flow rate (g/sec)	x	2.3 (0.2)	4.04 (0.9)	3.6(0.2)	6.0 (1.732)
Moisture content	13.0 (0.0)	8.7 (0.3)	9.3 (0.3)	0.1 (0.03)	7.7(0.5)

N.B. x indicates the powder did not flow, * means the true density of EC is not determined

3.2.6. Kawakita analysis result

Kawakita constants indicate the behavior of the powder from the bulk density state to the tap density state. In general, small values of constants 'a' (compressibility, or the amount of densification due to tapping) and '1/b' (cohesiveness, or how fast or easily the final packing state was achieved) indicate good flowability and small cohesiveness (Prakash *et al*. 2011). As shown in Fig 3.6 the Kawakita plots of native *P. edulis* starch, SA DS 0.44, SA DS 1.77, EC and MCC, resulted in a linear relationship with equations of $Y = 4.416X + 40.994$, $Y = 5.257X + 27.782$, $Y = 7.695x + 30.552$, $Y = 8.263X+54.618$ and $Y = 5.11X + 56.598$ respectively where Y is N/C and X is N

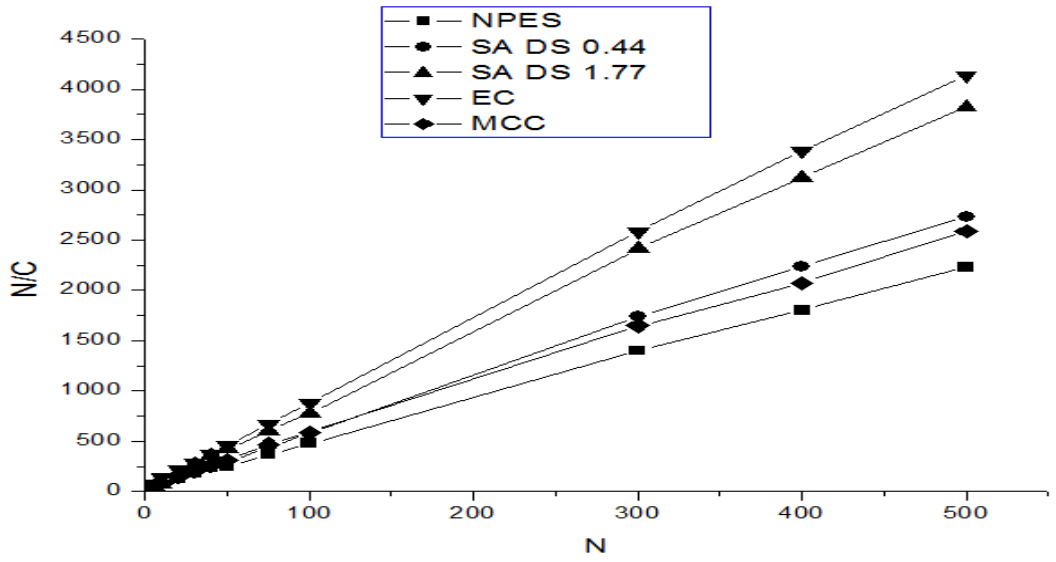


Figure 3.6: Kawakita plots of native *P. edulis* starch, *P. edulis* SA, EC and MCC

The results presented in Table 3.4 indicate that EC, SA DS 0.44 and SA DS 1.77 densified the least (due to small compressibility values) and attained the final packing state slowly (the greater cohesive values). On the other hand, MCC and native *P. edulis* starch densified considerably and achieved the final packing state rather quickly, of which native *P. edulis* starch is the fastest to attain the final packing state. The Kawakita analysis supported the fact that the modification of starch improves the powder flowability. The smaller 'a' values of SA DS 1.77 and EC indicated good flowability which is necessary during tableting by direct compression.

Table 3.4: Kawakita constants of *P. edulis* SA, EC, MCC and native *P. edulis* starch.

	Kawakita compressibility (a)	Kawakita cohesiveness (1/b)	Correlation coefficient (R^2)
SA DS 1.77	0.129	3.941	0.999
SA DS 0.44	0.190	5.279	0.999
EC	0.121	6.609	0.999
MCC	0.196	11.093	0.999
NPES	0.226	9.264	0.999

3.2.7. Swelling power (SP) and solubility

The physicochemical properties of starches such as SP and solubility have been reported to be affected significantly by chemical modification. It is reasonable that as the temperature of the medium increases, starch molecules become more thermodynamically activated, and the resulting increase in granular mobility enhances penetration of water, which facilitates improved swelling capacities (Lawal 2004). The SP and solubility profiles of the native *P. edulis* starch and modified starches are depicted in Fig 3.7 and 3.8. Both swelling power and solubility were temperature dependent, and values increased with an increase in temperature. Native *P. edulis* starch exhibited the typical increase of swelling power with increasing temperature. The SP of native *P. edulis* starch increased dramatically as the temperature raised from 50 °C to 85 ˚C.

The DS introduced after acetylation mainly affects the intensity of change in the swelling power and solubility of starches. Acetylation appreciably increased starch swelling ratio at a lower DS than higher DS due to increased acetyl content. The SP of SA DS 1.77 and SA DS 0.44 powder was higher than those of the corresponding native *P. edulis* starch powder (Fig. 3.7). Bello-Pérez *et al*. (2000) acetylated banana starch and the acetylated sample showed higher swelling value and this was explained by the introduction of acetyl groups, allowing the retention of water molecules because of the remaining OH groups to form hydrogen bonds.

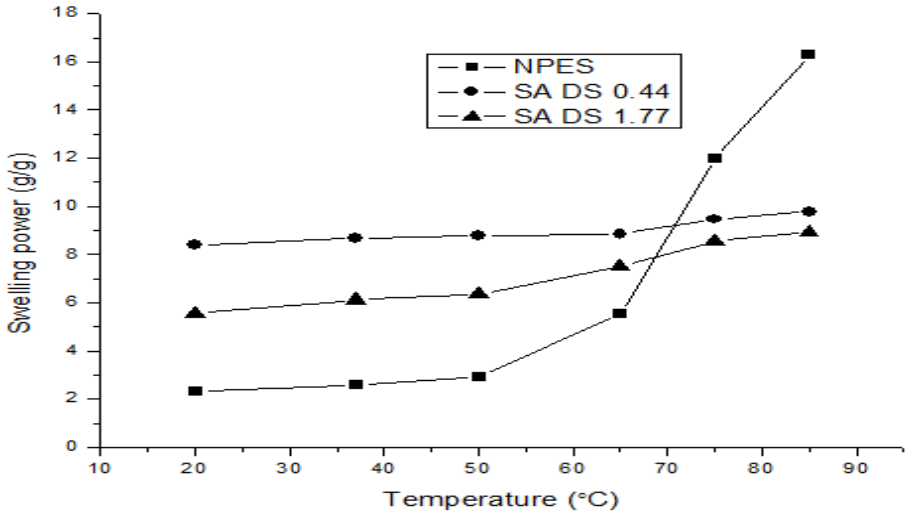


Figure 3.7: SP of native *P. edulis* starch and *P. edulis* SA

The introduction of the acetyl group (with low DS) reduces the interaction between starch molecules and, thereby, increase the SP (Lawal, 2004). SP was reported to have correlation with the amylose content, crystallinity, and intermolecular hydrogen bonding. When starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed OH groups of amylose and amylopectin. This causes an increase in granule swelling and solubility (Bello-Pérez *et al*. 2000).

The acetylated *P. edulis* starches in the present study (Fig 3.8) exhibited slightly higher solubility in water at all temperatures, when compared to that of the NS. The values ranged from 1.48 to 5.69%, whereas it was 0.02 to 4.78 % for the native starch. The structural disintegration probably weakens the starch granules after acetylation, and this enhances amylose leaching from the granule, thus increasing starch solubility. These increases in solubility show better dispersion of starch in aqueous systems because acetyl groups obstruct chain association (Singh *et al.* 2007). Similar observations have been reported earlier for various starches (Mbougueng *et al.* 2012; Singh *et al*. 2004; Sodhi and Singh 2005 and Zhang *et al*. 2014).

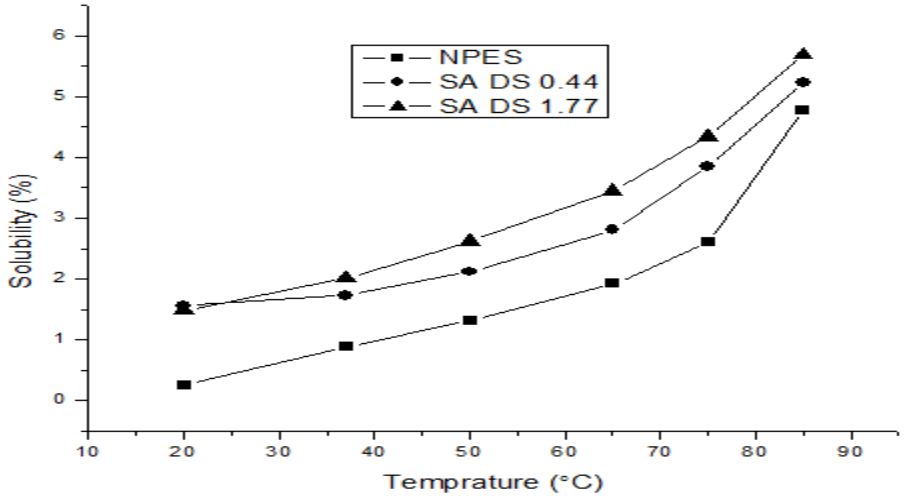


Figure 3.8: Solubility of native *P. edulis* starch and *P. edulis* SA

3.2.8. Moisture sorption pattern

The moisture sorption profiles of native *P. edulis* starch, SA DS 1.77 and SA DS 0.44 equilibrated at various humidity levels (20, 40, 60, 75 and 100% RH) for four weeks are depicted in Fig 3.9. The hygroscopic nature of excipients and active ingredients should be considered in designing

formulations. Higher levels of water can lead to microbial spoilage and subsequent deterioration in starch quality. Furthermore, water has been known to affect the flow and mechanical properties of starches (Gebre-Mariam and Schmidt 1998). Therefore, knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction is critical.

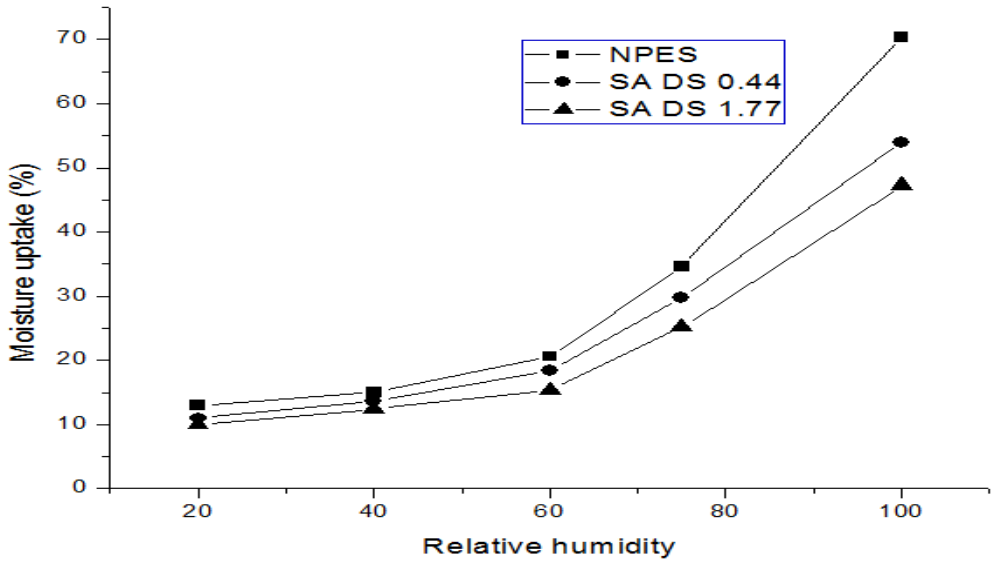


Figure 3.9: Moisture sorption patterns of native *P. edulis* starch and *P. edulis* SA at room temperature.

As can be seen from the sorption isotherms, the water uptake of *P. edulis* SA powders is slightly increased with RH at lower values but increased significantly at about 100% RH. Lower ranges of RH, in which the water uptake of the powders changed slightly. Thus, during tablet production and storage the RH should be carefully controlled to obtain powders with optimum flow and compaction properties and also to prevent the deterioration of the tablets.

3.3. Characteristics of tablets

3.3.1. Compressibility

The crushing strength, friability, tensile strength and disintegration time of plain tablets are shown in Table 3.5. The plain tablets were produced after adjusting the upper and lower punches of single punch tablet press to produce 400 mg MCC (Avicel PH 101) tablets with hardness of about 75 N. The modified starches are then compressed at the same apparent volume of die cavity. Due to

differences in bulk densities among the powders, tablets are clearly expected to have different weights.

The acetylated starches showed improved compression than the native form resulting in higher crushing forces. Due to acetylation, the formation of strong molecular bonds, like van der Waal's forces, could increase several-fold. This, in combination with existing hydroxyl groups, increases the formation of strong molecular bonds. This in turn, will lead to the formation of a very firm and intact tablet structure. As the degree of acetylation increases, SA becomes more hydrophobic, the mechanical strength increases and disintegration does not occur any more (Raatikainen *et al*. 2002).

Plain SA DS 0.44 tablets had larger weight losses which is greater than 1% MCC (Avicel PH 101), SA DS 1.77 and EC which have friability <1% showed smaller weight losses during the friability test (Table 3.5). In disintegration tests, all tablets made of plain SA DS 0.44 and MCC (Avicel PH 101) were completely disintegrated within a few min while tablets made of plain SA DS 1.77 and EC did not disintegrate at all during the measurement time of 2 h. It was clear that the manufactured tablets exhibit acceptable properties in terms of weight uniformity, diameter, thickness, crushing strength, and friability at the studied tablet hardness. The matrix tablets of plain SA DS 1.77 which showed some high tensile strength than SA DS 0.44 had also different porosities, i.e., the latter were more porous than the former ones (Table 3.5).

As shown in Table 3.5, 3.6 and 3.7, the SA DS 1.77 tablets show higher characteristics of tablet than the SA DS 0.44 tablets, which reflected the better compression properties of the former modified starch which can be attributed due to higher extent of acetylation but it is significantly higher than the native *P. edulis* starch tablets. The compression of tablets that gave crushing strength of about 75 N for MCC (Avicel PH 101) tablets can be increased significantly to produce tablets with even larger hardness values.

Table 3.5: The weight, thickness, diameter, crushing strength, friability, tensile strength and disintegration time of plain tablets.

	PENS	SA DS1.77	SA DS0.44	MCC	EC
Weight (mg)	401.46 (3.72)	398.85(4.34)	402.5(8.65)	396.7 (6.45)	395.5 (3.58)
Thickness(mm)	4.47 (0.04)	4.17 (0.31)	4.312 (0.37)	4.33 (0.02)	4.62 (0.39)
Diameter (mm)	10.85 (0.04)	10.86 (0.02)	10.85 (0.02)	10.86 (0.03)	10.87 (0.02)
Crushing strength (N)	31.8 (6.79)	148 (8.87)	119.1 (6.84)	74.6 (8.5)	158.7(10.1)
Tensile strength (Kg/cm^2)	4.09 (0.85)	20.49 (1.98)	15.98 (1.43)	9.90 (1.1)	19.89(1.9)
Friability (%)	1.043(0.03)	0.005(0.07)	0.014(0.02)	0.135(0.04)	0.004(0.05)
Porosity (%)	30.106 (0.79)	5.74(1.14)	29.44 (0.97)	31.836 (0.9)	-
Disintegration time (min)	5	>120	10	15	>120

NB: values are stated in mean and standard deviations are in parenthesis

Raatikainen *et al*. (2002) have shown that the acetate moiety of SA, perhaps in combination with existing OH groups, is a very effective bond-forming substituent. The formation of strong molecular bonds leads to a very firm and intact tablet structure. They also reported that the effect of increasing DS on the strength of SA tablets is due to the slight fragmentation and enhanced plastic flow of the particles under compression. SA with high DS may fragment more than SA with low DS during compression which increases the bonding surface area of the SA powder with high DS and, consequently, tablet strength. They also reported that increasing the compaction force improved the tensile strength of the tablets prepared from all materials.

3.3.2. Crushing strength and friability

Crushing strength is obviously a parameter which can be related directly to the compression force used. When the crushing strength increases, the particles deform plastically and the tablets become harder and less friable. Therefore, the recorded friability values for the formulation were under 1% which can generally be regarded as desirable. The friability profiles of the tablets are in line with the crushing strength measurements. In general, increase in tablet crushing strength results in lower

friability values and longer disintegration times (Özyazici and Sevgi 2003). Conventional compressed tablets that lose less than 1% of their weight during the friability test are generally considered acceptable (Parrott 1989). As Table 3.6 shows, the friability of the matrix tablets of SA DS 1.77 increased as the percentage of anhydrous theophylline increased from 20% to 30% and 40% (w/w) due to reduced crushing strength. *P. edulis* starch SA DS 1.77 tablets containing 40% (w/w) theophylline had the highest weight loss and EC tablets had the smallest weight loss.

Table 3.6: The weight, thickness, diameter, crushing strength, friability, tensile strength and disintegration time of theophylline loaded matrix tablets.

Parameters	Formulations					
	F1	F2	F3	F4	F5	F6
Weight (mg)	400.31(6.9)	400.18 (5.2)	400.58 (3.5)	400.57 (8.94)	401.43 (4.9)	399.2 (9.42)
Thickness (mm)	4.45 (0.09)	4.42 (0.05)	4.61(0.09)	4.51(0.13)	4.56 (0.18)	4.45 (0.15)
Diameter (mm)	11.03 (0.01)	11.04 (0.01)	11.04 (0.01)	11.04 (0.02)	11.05 (0.01)	11.04 (0.02)
Crushing strength (N)	156.2 (3.36)	154.3 (4.73)	150.9 (7.57)	138 (4.89)	138.3 (8.88)	156.2 (7.2)
Tensile strength (Kg/cm^2)	19.86 (1.23)	19.73 (1.2)	18.50(1.1)	17.30 (1.24)	17.17 (1.21)	19.86 (1.2)
Friability (%)	0.002 (0.01)	0.003 (0.01)	0.004 (0.001)	0.002 (0.001)	0.002 (0.01)	0.001 (0.1)
Porosity (%)	14.45 (0.01)	14.55 (0.04)	17.51 (0.01)	35.22 (0.007)	36.75 (0.01)	-
Disintegration time (min)	>120	>120	>120	25	35	>120

NB: values are stated in mean and standard deviations are in parenthesis

3.3.3. Tensile strength

As shown in Table 3.6 above, the tensile strengths of tablets of formulation F1 containing SA DS 1.77 of 79.5 % (w/w) was significantly higher than those of formulation F2 with 69.5% (w/w) of SA DS 1.77 and F3 with 59.5 % (w/w) of SA DS 1.77 which is in line with the fact that the strength of tablet structure of the SA powder increases with its increase in SA concentration in the tablets. Thus, a decrease in SA concentration decreased the compactibility of theophylline/SA powder blends.

Tablets of F1 were prepared with different compression force and compared for their tablet properties. Tensile strength data presented in Table 3.7 illustrated how the tensile strength is correlated to the tablet crushing strength and it shows that when compression force increases tablet crushing strength and tensile strength increases. As the increase in the crushing force is associated to a decrease in the tablet porosity and, hence, in a reduction of water uptake and consequent drug release.

Table 3.7: The weight, thickness, diameter, crushing strength, friability, tensile strength and disintegration time of F1 tablets at different compression force.

Tablet Properties	Compression force				
	CF1	CF2	CF3	CF4	CF5
Weight (mg)	399.6 (5.56)	397.6(3.751)	400.21 (8.17)	398.64 (1.67)	400.3 (7.11)
Thickness (mm)	4.568 (0.13)	4.653 (0.11)	4.357 (0.03)	4.352 (0.037)	4.370 (0.04)
Diameter (mm)	10.87 (0.02)	10.87 (0.02)	10.81 (0.06)	10.80 (0.08)	10.83 (0.04)
Crushing strength (N)	51.1 (3.45)	72.7 (4.32)	103.2 (5.26)	122.3 (5.18)	150.2 (6.47)
Tensile strength (Kg/cm^2)	6.425 (0.4)	8.968 (0.49)	13.672 (0.65)	16.238 (0.64)	19.81 (0.9)
Friability (%)	0.963 (1.02)	0.971 (1.02)	0.097(0.037)	0.068 (0.03)	0.09 (0.06)
Porosity (%)	16.36 (2.22)	14.256 (2.93)	9.673(1.835)	9.154 (1.49)	9.079 (2.78)
Disintegration time (min)	15	30	90	100	>120

NB: values are stated in mean and standard deviations are in parenthesis

3.3.4. Porosity

The release rate of a drug from hydrophobic matrix can be modified by changes in the porosity and tortuosity of the matrix, i.e., its pore structure. Interparticulate porosity is essential for solvent penetration into the matrix and, consequently, for drug release. Crushing strength controls the porosity of the matrix, which in turn controls drug release (Collett and Moreton, 2002). The interparticulate porosity of tablets also increases by void formation during stress relaxation and tablet expansion after the densification process (Pohja *et al*. 2004). As shown in Table 3.7 above,

the matrix tablets with crushing strength of 51.1 N has the highest porosity when compared to tablet with crushing strength of 150.2 N because the highest crushing strength exhibit the smallest porosity of matrix tablets. Generally, a more rigid and less porous matrix will release drug more slowly than a less consolidated matrix.

3.3.5. Calibration curve

Aliquots of stock solution of anhydrous theophylline (0.05% w/v) in 0.1 N HCl and phosphate buffer solution pH 6.8 prepared at different concentrations of 4 μg/ml to 13μg/ml were measured at the λmax (271 nm) by using UV-Visible spectrophotometer (CECIL. CE1021. Cambridge, England). The linear regression equations obtained were Y = 0.0536X + 0.0065 (R^2 =0.9999) and Y = 0.0506X + 0.0096 (R^2 = 0.9997) in 0.1N HCl and phosphate buffer pH 6.8, respectively where Y is the absorbance and X is the concentration in μg/ml (Fig. 3.10 and 3.11).

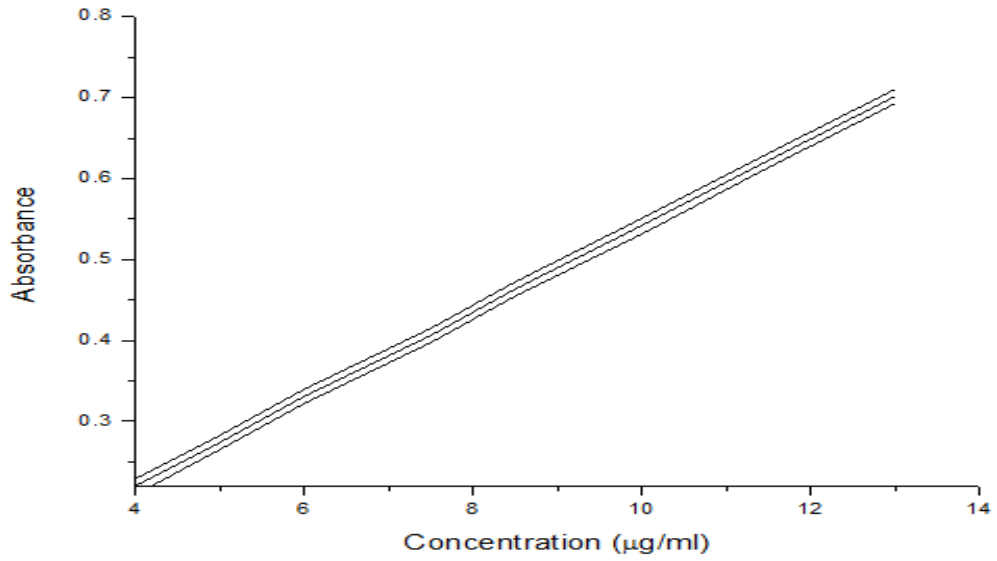


Figure 3.10: Standard calibration curve of pure theophylline in 0.1 N HCl with upper and lower 95% confidence limits.

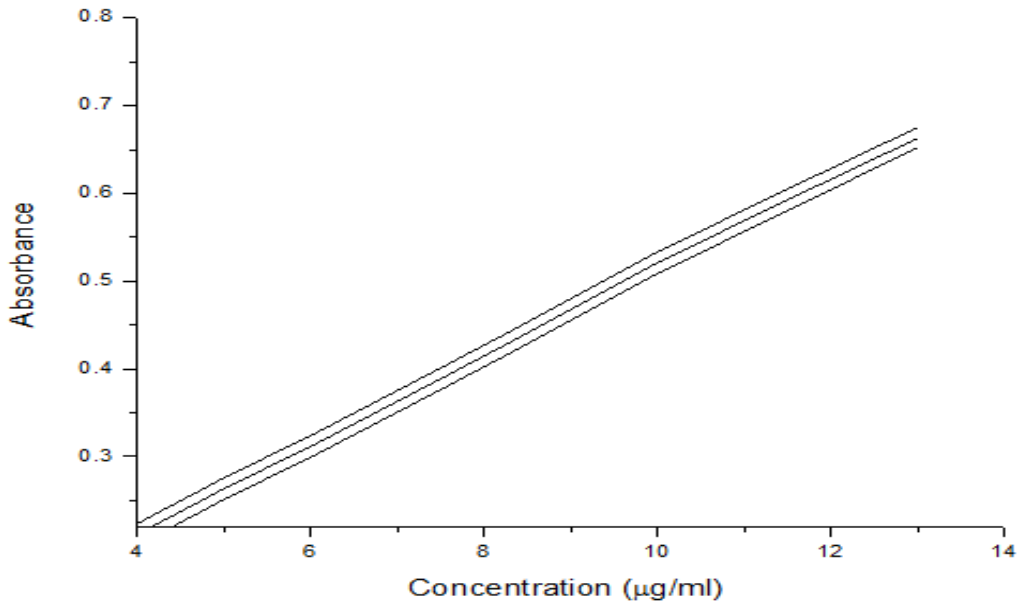


Figure 3.11: Standard calibration curve of pure theophylline in phosphate buffer pH 6.8 with upper and lower 95% confidence limits.

3.3.6. *In vitro* drug release

It is well established that the hardness of a tablet could markedly affect the release rate of drug. Usually, an increase in hardness of a tablet is accompanied by a decrease in release rate, due to a decrease in porosity of the tablet (Apu *et al*. 2009). The dissolution profiles of matrix tablets of F1 containing 20% of theophylline, 0.5% of magnesium stearate and 79.5% of SA DS 1.77 prepared at different crushing strength were depicted in Fig 3.12.

As observed in Fig 3.12, the drug release at crushing strength of 150.2 N have more sustained release whereas tablets with crushing strength of 51.1 N and 72.7 N showed immediate drug release. The retarded drug release and non-disintegration behavior of tablet matrices with crushing strength of 150.2 N could also be attributed to the better binding properties of the *P. edulis* SA DS 1.77 polymer in F1. Tensile strength also affects drug release rate, that is, generally a stronger tablet structure and lower porosity leads to slower drug release due to the inverse exponential relationship between porosity and tensile strength of tablets compressed from neat SA powders (Pohja *et al*. 2004).

When a matrix tablet is placed in the dissolution medium, the initial drug release occurs from the tablet's superficial layers and, consequently, the release rate is relatively fast. As time passes, the external layers of the tablet become depleted of the drug and water molecules must travel through long, tortuous channels to reach the drug remaining in the deeper layers of the tablet. Similarly, the drug solution that is formed within the tablet must diffuse through long capillaries to reach the external dissolution medium. The primary reason for the continuously decreasing rate of drug release is the increasing distance that must be traversed by water and drug molecules into, and out of, the tablet, respectively (Pather *et al*. 1998).

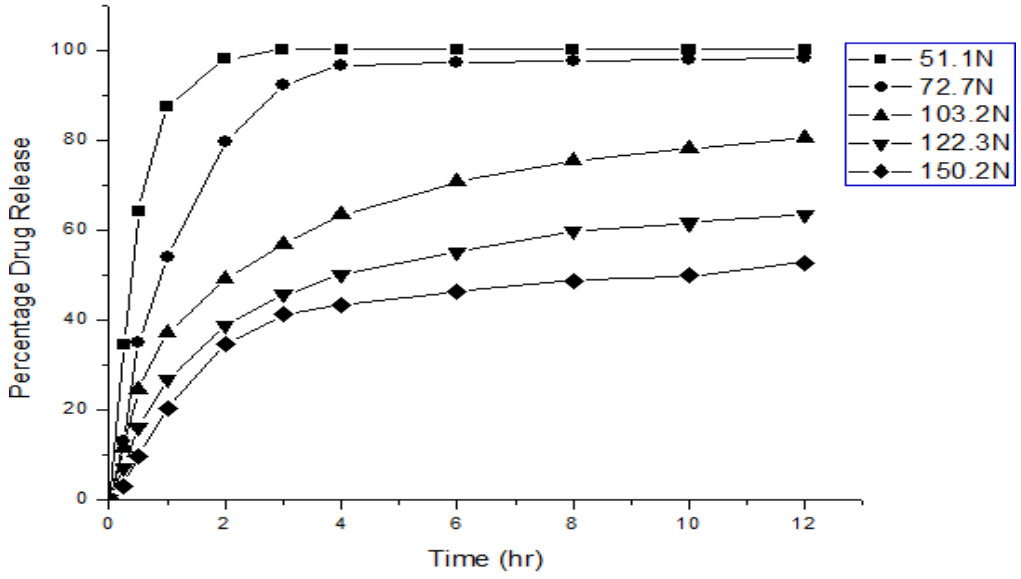


Figure 3.12: Theophylline release profiles from matrix tablet of F1 at crushing strength of 51.1 N, 72.7N, 103.2N, 122.3 N and 150.2 N.

Based on the results obtained tablets of SA DS 1.77 with different rations with model drug, SA DS 0.44 and the standard excipients providing highest crushing strength were used for further drug release kinetics study. The dissolution profiles of theophylline from SA DS 1.77, SA DS 0.44, EC and MCC (Avicel PH 101) tablets were depicted in Fig 3.13. As can be seen from Fig 3.13, the dissolution rate of theophylline changed from rapid release to SR as the DS increased from 0.44 to 1.77. Theophylline was released from the SA tablets having DS value of 0.44 within a few min. It shows that DS of the acetylated *P. edulis* starch had a significant effect on drug release from the tablets. The release was more sustained from the tablets incorporated with acetylated *P. edulis*

starch of higher DS. As the DS of the starch increases, its swelling power also increases and consequently the incorporated drug takes more time to release into the medium.

In addition, when SA DS 1.77 concentration is decreasing, the hydrated matrix would be highly porous results rapid diffusion of the drug from the matrix which is observed from F1 (79.5%), F2 (69.5%) and F3 (59.5%) (Fig 3.13). A significant increase in the release rate and a slight decrease in tablet hardness were observed with decrease in the SA DS 1.77 concentration from F1 to F3. The study done by Pohja *et al.* (2004) also demonstrated that an increase in SA concentration in tablets decreases the drug release rate. In addition, both DS and SA concentration can be varied to control drug release from SA matrices.

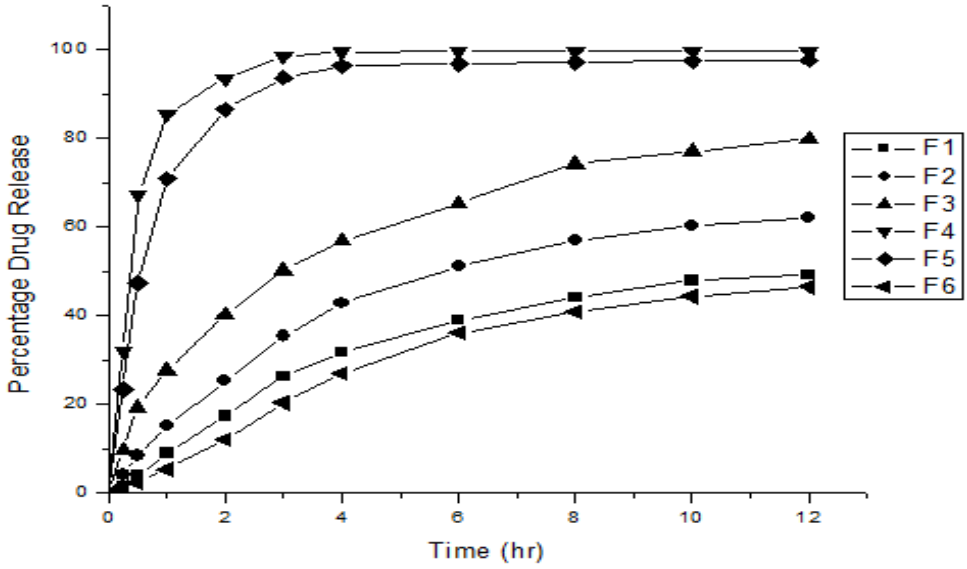


Figure 3.13: Theophylline release profiles from F1, F2, F3 (different concentration of SA DS 1.77), F4 (SA DS 0.44), F5 (MCC) and F6 (EC) tablets.

The drug was released rapidly from the tablets with F4 (SA DS 0.44) and F5 (MCC) reached almost 100% within 1 h and therefore both excipients had no SR property. This is also shown by increase in porosity of tablets which can enhance drug release. The drug release from hydrophobic F1 (SA DS 1.77) and F6 (EC) matrices containing 20% (w/w) of theophylline showed comparable results. Dissolution results showed that SA having the high DS values could act as matrix-forming agents in tablets, where the release of a drug can be significantly sustained.

3.3.7. Drug release kinetics

The release kinetics was evaluated by fitting the drug release data to five kinetic models. The release data were treated according to zero-order, first order, Higuchi's, Korsemeyer–Peppas and Hixson-Crowell cube root law models. The kinetic model with the highest correlation coefficient value (R^2) was selected as the model that best described the dissolution data.

As Table 3.8 shows, formulation F1, F2 and F3 containing different ratios of SA DS 1.77 and F4 containing EC with theophylline have the R^2 value of 0.9050-0.9772 as the zero-order plot shown in Fig 3.14A and first order plot in Fig 3.14B gave 0.9819-0.9883 R^2 describing the drug release rate relationship with concentration of drug. The best linearity was found in Higuchi's equation plot (Fig 3.14C) with R^2 = 0.9710-0.9988 indicating the release of drug from matrix as a square root of time dependent process based on Fickian diffusion. This explains that the drug release from monolithic matrix tablet prepared with *P. edulis* acetylated starch with highest DS value followed primarily diffusion-controlled mechanism.

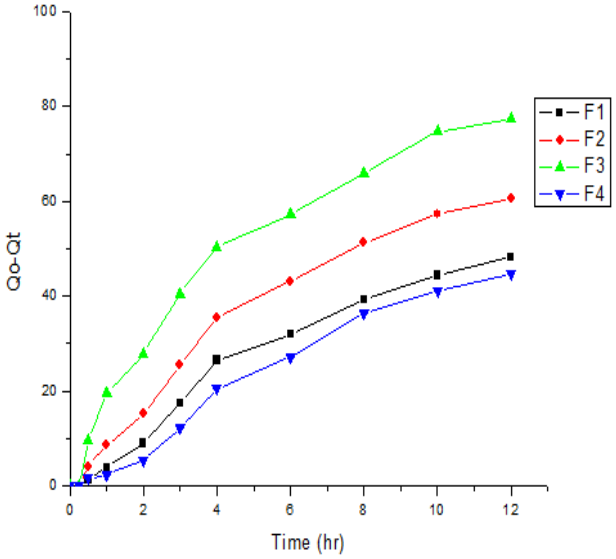
The curvilinear nature of the cumulative amount of drug released versus time plots (Fig 3.14A) suggest that none of the formulations follow zero order drug release kinetics which is confirmed by poor correlation coefficients obtained in all the cases. Similarly, poor correlation coefficients and nonlinearity of the Hixson-Crowell plot (Fig 3.14E) suggests non-applicability of Hixson-Crowell model.

To evaluate the *in vitro* drug release profile, the data at various time points were fitted into the Korsemayer–Peppas equation, where K is the release rate constant and 'n' is characteristics for the mechanism of the drug release. The 'n' value can be used for characterization of different release mechanisms. In the case of a cylinder, the exponent n has values of 0.45 for Fickian diffusion (leading to square root of time release kinetics), from 0.45 to 0.89 for non-Fickian anomalous transport (leading to first-order release kinetics) and 0.89 for non-Fickian Case-II transport (leading to zero-order kinetics) (Collet and Moreton 2002)

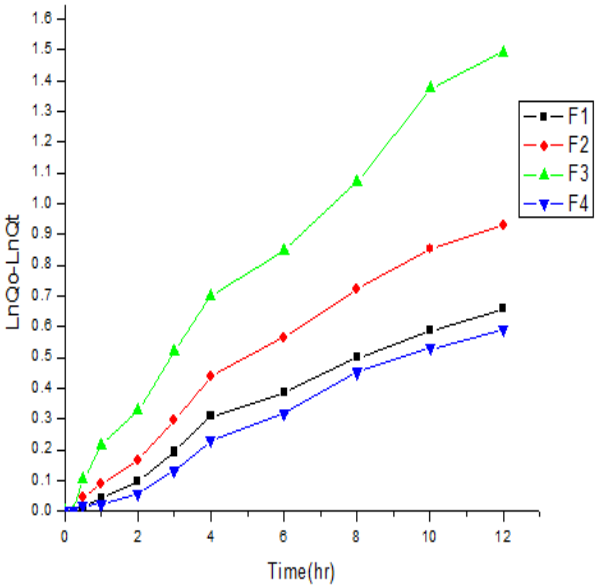
Table 3.8: Drug release parameters and statistical estimates of the zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models fitted to dissolution data from theophylline/SA and theophylline/EC matrix tablets.

Formulation	Zero order		First order		Higuchi		Korsemeyer- Peppas			Hixson-Crowell	
	R^2	K(hr^{-1})	R^2	K (hr^{-1})	R^2	K ($hr^{-1/2}$)	R^2	K (hr^{-n})	n	R^2	K ($hr^{-1/3}$)
F1	0.9050	4.3538	0.9883	0.0586	0.9988	17.561	0.9806	1.008	0.5011	0.9725	-0.0815
F2	0.9434	5.4026	0.9823	0.0826	0.9845	21.724	0.9679	0.8058	0.7808	0.9685	-0.1087
F3	0.9588	6.5524	0.9710	0.1270	0.9819	26.133	0.9573	0.5711	0.9093	0.9637	-0.1511
F4	0.9572	4.093	0.9832	0.0534	0.9955	16.405	0.9703	1.2093	0.5158	0.9841	-0.0758

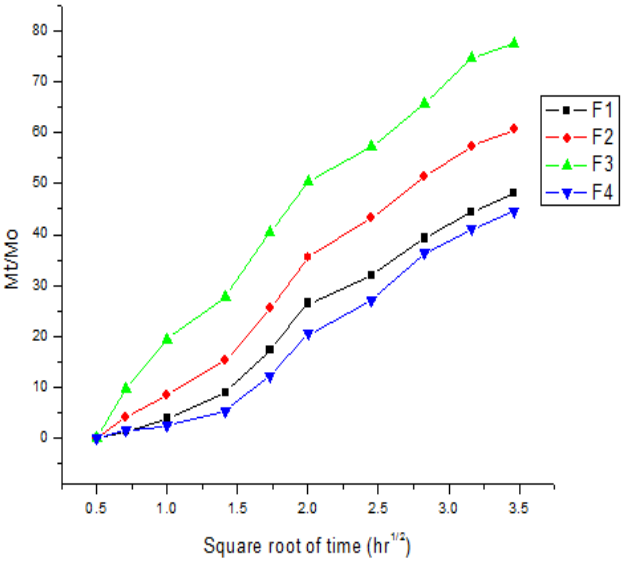
Therefore, drug release from the SR formulations of F1 containing 79.5 % (w/w) of SA DS 1.77 and F4 containing 79.5 % (w/w) EC with 20 % (w/w) of theophylline was primarily by Fickian diffusion (Higuchi model) and anomalous diffusion that is combination of either diffusion or swelling mechanism takes place depending Korsemayer–Peppas model. Same kind of phenomenon has been observed in other studies (Pohja *et al.* 2004 and Van Veen *et al.* 2005). The dissolution profiles plotted in different scales (i.e., normal time and the square root of time) resulted in a non-zero y-intercept due to immediate release of drug present at the surface of the tablets. The burst effect existed for the formulations at the beginning of the dissolution process (0 - 1 h).



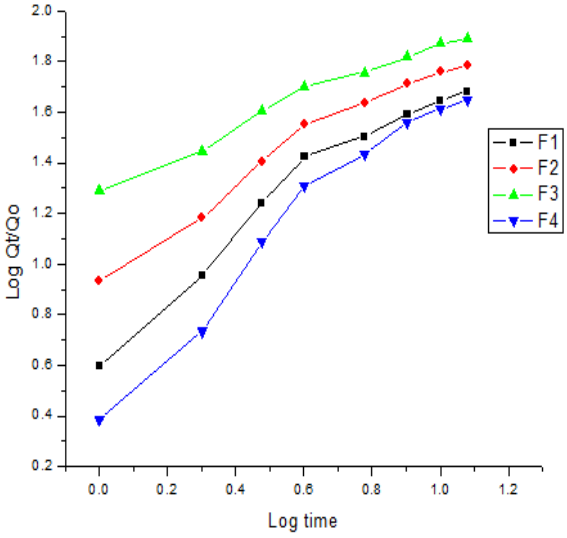
(A) Zero Order Release



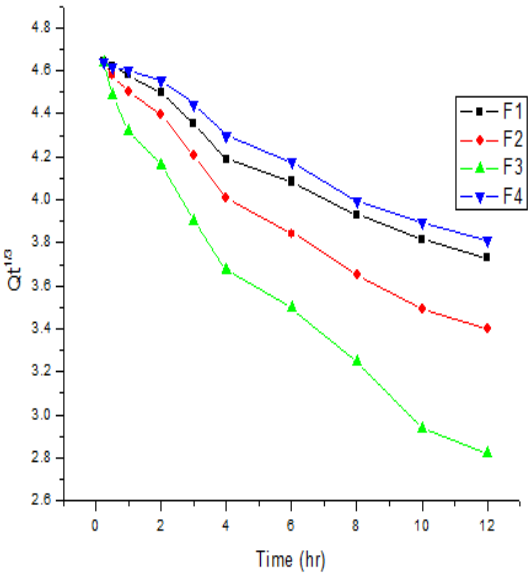
(B) First Order Release



(C) Higuchi release model



(D) Korsmeyer-Peppas release model



(E) Hixson-Crowell model

Figure 3.14: The various drug release kinetic models [zero order (A), first order (B), Higuchi (C), Korsmeyer-Peppas (D) and Hixson-Crowell (E)] of the tablets studied.

The model drug theophylline was assumed to be diffused only through the pores that were formed by dissolution of dispersed drug particles that is the drug release mechanism was leaching of dissolved drugs through the pores and the compacts matrix tablets compressed from a mixture of drug and SA or EC were laminated or cracked during dissolution tests, which increased porosity and the available surface area for dissolution thereby the drug particles from the surface of a tablet were able to dissolve without any control by SA/EC. This has contributed for the deviation from Fickian diffusion.

4. CONCLUSION

P. edulis SA with a DS of 1.77 was synthesized by acetylation of *P. edulis* starch with acetic anhydride using NaOH as a catalyst under microwave heating. The preparation of microwave-assisted SA was rapid, providing high DS within 13 min of esterification. The formation of SA was confirmed by the FTIR spectra. The *P. edulis* SA with high DS exhibited good flowability and bond forming properties appropriate for direct compression. Matrix tablets of theophylline prepared with SA with DS of 1.77 exhibited SR over 12 h. Drug release profiles fitted best Higuchi model followed by first order model. The present study demonstrated that *P. edulis* SA prepared by microwave-assisted acetylation can act both as a direct compression diluent and as a binder in tablet formulations with additional matrix forming property and sustained release characteristics. Thus, *P. edulis* SA is potentially useful as multifunctional direct compression excipient, and may be a promising polymer in sustained release formulations.

5. SUGGESTIONS FOR FURTHER WORK

The results of this study provided an insight into the properties of microwave-assisted *P. edulis* SA. Based on these promising results the followings are suggested:

- Characterize the *P. edulis* SA in solution, film forming and their drug release properties.
- Further characterization of *P. edulis* SA by microwave-assisted modification and other methods.
- Correlate the drug release profile obtained *in vitro* with in vivo results to confirm that *P. edulis* SA is able to control and extend the drug release in vivo and to establish predictive *in vitro-in vivo* correlation (IVIVC).

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