

**Preparation and Evaluation of Dual Modified Ethiopian Yam
(*Dioscorea abyssinica*) Starch for Sustained Release Tablet Formulation**

Yohaness Mulualem

**A Thesis Submitted to the Department of Pharmaceutics and Social
Pharmacy, School of Pharmacy, Addis Ababa University**

**Presented in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Pharmaceutics**

**Addis Ababa University
Addis Ababa, Ethiopia
January, 2013**

Addis Ababa University
School of Graduate studies

This is to certify that the thesis prepared by Yohaness Mulualem, entitled: *Preparation and Evaluation of Dual Modified Ethiopian Yam (Dioscorea abyssinica) Starch for Sustained Release Tablet Formulation* 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.

Signed by the Examining Committee:

Name	Signature	Date
Prof. Tsige Gebre-Mariam (Advisor)	_____	_____
Dr. Anteneh Belete (Advisor)	_____	_____
Dr. Fistum Feleke (Examiner)	_____	_____
Ato Adamu Zegeye (Examiner)	_____	_____

Head, Department of Pharmaceutics and Social Pharmacy

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, *Dioscorea abyssinica* (*boyna*) starch has been chemically modified with the aim of determining its potential for sustained release application. It was chemically modified by cross-linking using sodium hexametaphosphate (SHMP), acetylating with acetic anhydride and dual modified - cross-linking followed by acetylation. Cross-linked starches (CLSs) with degree of cross-linking (DC) of 54.46, 62.20 and 68.88 (%), CLS-A, CLS-B, CLS-C respectively, were obtained while cross-linking the native starch (NS) with 5, 10, and 15% SHMP for 6 h. By reacting *boyna* starch with acetic anhydride (AA) in ratios of 1:1 and 1:2, starch acetates (SAs) with degrees of substitution of 0.339 (SA-A) and 0.546 (SA-B), were obtained, respectively. In an attempt to prepare a dual modified starch (DMS), the CLSs were acetylated in a similar condition as the NS. The physicochemical, material, and tablet forming properties of the modified starches were investigated to determine potential usages in sustained release applications. In moisture sorption study, CLSs exhibited the lowest water-uptake and SAs notably increased the sorption capacity of the NS and CLS. Cross-linking decreased the swelling power of the NS as the level of SHMP was increased while acetylation showed the opposite effect. The reduction observed in viscosity values indicated the effectiveness of cross-linking in reducing swelling of the NS. On the basis of powder properties analyses, it was evident that acetylation improved the poor flow properties of CLSs and NS. SAs were found to be free flowing with angles of repose of 25.11° and 23.02° and flow rate of 3.09 and 10.54 g/sec, respectively for SA-A and SA-B. The Hausner ratio of the SAs was 1.18 and 1.25 and Carr's index of 22.36 and 20.24, respectively for SA-A and SA-B. Tablets were prepared and evaluated for hardness, tensile strength, friability, and disintegration time. Results indicated that both cross-linking and acetylation processes improved tablet forming properties of the NS with more significant effect in the dual modified starches. The Fourier transform infrared (FTIR) spectra revealed that a structural change occurred in the NS because of cross-linking and acetylation. Correspondingly, the spectral analysis proved that the dual modified starches are compatible with theophylline.

Dissolution studies of theophylline loaded modified starches were performed for 12 h. Matrix tablets containing Ac-CLS-F, where CLS-C reacted in a 1:2 ratio with AA, loaded with 20, 30 and 40% theophylline and Ac-CLS-E, where CLS-C reacted in a 1:1 ratio with AA, loaded with 20% theophylline were studied for drug release. And 80% of theophylline was released in about 10.62, 9.35, and 8.18 h from 20, 30 and 40% theophylline loaded Ac-CLS-F, respectively, whereas 20% theophylline loaded Ac-CLS-E released its drug content within about 12 h of the dissolution study. The dissolution data was subjected to the various drug release kinetic models and the data best fitted Higuchi model with $R^2 > 0.994$. The drug release diffusional exponent (n), with goodness of fit > 0.988 obtained for Korsemeyer-Peppas model for 20, 30 and 40% drug loaded Ac-CLS-F varied between 0.452-0.530 indicating deviation from Fickian diffusion mechanism whereas a quasi-Fickian diffusion behavior was exhibited ($n = 0.31$) for 20% loaded Ac-CLS-E. From the foregoing, it can be concluded that Ac-CLS-F could have a potential for use as a sustained release excipient.

Key words: *Dioscorea abyssinica*, *Boyna* starch, Cross-linking, Acetylation, Dual modification, Sustained release.

ACKNOWLEDGEMENTS

Let praise be to the **Almighty God** who has been looking after me all the way.

I wish to express my sincere thanks to my supervisors, Prof. Tsige Gebre-Mariam and Dr. Anteneh Belete. With their guidance, continuous encouragement, and insightful comments at different stages of my research, they helped me to understand and enrich my ideas and not to lose the focus from my work. It is because of them that this thesis is possible and my graduate experience has been delightful and memorable part of my life.

I would like to share this achievement with my colleagues whose presence made my research environment stimulating and full of fun apart from sharing constructive ideas. I am especially grateful to Shimelis Kumbi and Abreham Degaga for helping me get through the difficult times and providing me with the care I needed.

It is a pleasure to extend my thanks to the staff members of the Department of Pharmaceutics and Social Pharmacy for the various forms of generous support they provided me with. Mr. Fekade Tefera, Efreem Nigussu and Abraham Temesgen deserve special mention for their unreserved assistance.

I would like to acknowledge the scholarship and financial support I received from Haramaya University and Addis Ababa University for my graduate study. I am also grateful to the Ethiopian Pharmaceutical Share Company (EPHARM) for donating theophylline raw material for the study.

Lastly, and most importantly, I am grateful to my Family. In many moments of distress throughout the journey of my study, my brothers', Zelalem and Mekuaninet, moral support and counseling to keep firm with my faith and hope in **God** always have been with me. Thank you all!

Yohaness Mulualem

June, 2012

TABLE OF CONTENTS

TABLE OF CONTENTS.....	vi
LIST OF FIGURES	ix
LIST OF TABLES	x
ACRONYMS	xi
1. INTRODUCTION	1
1.1. Starch	1
1.1.1. Structure and organization in starch granules	1
1.1.2. Amylose and Amylopectin	2
1.1.3. Sources of starch	3
1.1.4. Yam tubers and their starches	4
1.1.5. <i>Dioscorea abyssinica</i> plant and its starch	4
1.2. Starch modification	5
1.2.1. Physical modification.....	6
1.2.2. Chemical modification.....	6
1.2.2.1. Cross-linking.....	7
1.2.2.2. Acetylation	9
1.2.2.3. Dual modification	11
1.3. Modified starches in sustained release formulations	12
1.4. The present study	13
1.5. Objectives	15
1.6.1. General objective	15
1.6.2. Specific objectives	15
2. EXPERIMENTAL	16
2.1. Materials	16
2.2. Methods.....	16
2.2.1. Isolation of starch from <i>D. abyssinica</i> tuber.....	16
2.2.2. Preparation of modified starches	17
2.2.2.1. Preparation of cross-linked <i>D. abyssinica</i> starch.....	17
2.2.2.2. Preparation of acetylated <i>D. abyssinica</i> starch	17
2.2.2.3. Preparation of dual-modified <i>D. abyssinica</i> starch.....	18

2.2.3.	Physicochemical characterization of modified starches	19
2.2.3.1.	Determination of peak viscosity and degree of cross-linking (DC)	19
2.2.3.2.	Determination of degree of substitution (DS).....	19
2.2.3.3.	Determination of moisture content	20
2.2.3.4.	Determination of solubility (S) and swelling power (SP).....	20
2.2.3.5.	Determination of moisture sorption pattern.....	20
2.2.4.	Characterization of powder properties of the modified starches	21
2.2.5.	Fourier transform infrared spectroscopy (FTIR) studies	22
2.2.6.	Tablet preparation and evaluation.....	22
2.2.7.1.	Tablet preparation	22
2.2.7.2.	Tablet evaluation.....	23
2.2.7.2.1.	Crushing strength	23
2.2.7.2.2.	Tensile strength.....	23
2.2.7.2.3.	Friability.....	23
2.2.7.2.4.	Disintegration time.....	24
2.2.7.2.5.	Calibration curve.....	24
2.2.7.2.6.	<i>In vitro</i> dissolution study	24
2.2.7.2.7.	Analysis of drug release kinetics	26
2.2.7.	Data analysis	27
3.	RESULTS AND DISCUSSION	28
3.1.	Characteristics of modified starches	28
3.1.1.	Degree of cross-linking (DC).....	28
3.1.2.	Degree of substitution (DS)	29
3.1.3.	Solubility (S) and Swelling power (SP).....	30
3.1.4.	Moisture sorption pattern.....	32
3.1.5.	Fourier-transform infrared (FTIR) spectra.....	34
3.1.6.	Powder properties	35
3.2.	Evaluation of tablets	39
3.2.1.	Crushing strength and friability	41
3.2.2.	Tensile strength.....	42
3.2.3.	Disintegration time.....	42

3.2.4. <i>In vitro</i> dissolution tests	42
3.2.5. Release kinetics.....	45
4. CONCLUSION.....	49
5. SUGGESTIONS FOR FURTHER WORK.....	50
REFERENCES	51

LIST OF FIGURES

	Page No.
Figure 1.1: Structure of linear amylose and branched amylopectin	3
Figure 1.2: <i>Dioscorea abyssinica</i> plant and its tuber	5
Figure 1.3: A scheme for chemical reactions involved during formation of starch phosphates	8
Figure 1.4: Structure of sodium hexametaphosphate (SHMP)	9
Figure 1.5: A scheme for chemical reactions involved during acetylation of starch with acetic anhydride	10
Figure 2.1: Standard calibration curve of pure theophylline	25
Figure 3.1: Solubility of cross-linked, acetylated, and dual-modified starch samples	32
Figure 3.2: Swelling power of cross-linked, acetylated, and dual-modified starch samples	33
Figure 3.3: Moisture sorption patterns of cross-linked, acetylated, and dual-modified starch samples	34
Figure 3.4: FTIR spectrum of native starch, dual modified starch pure theophylline and physical mixture of theophylline and dual modified starch	37
Figure 3.5: Release of theophylline by dual modified <i>boyana</i> starch from tablets	45
Figure 3.6: The release data from the different formulations fitted to various release kinetic models	46

LIST OF TABLES

	Page No.
Table 2.1: Reaction composition for cross-linking of <i>boyna</i> starch	17
Table 2.2: Reaction composition for Acetylation of <i>boyna</i> starch	18
Table 2.3: Reaction composition for dual-modified <i>boyna</i> starch	18
Table 2.4: Compositions of tablet formulations used in drug release studies	23
Table 3.1: Peak viscosities and degrees of cross-linking of cross-linked <i>boyna</i> starch	29
Table 3.2: Acetyl content and degree of acetylation of acetylated <i>boyna</i> starch	30
Table 3.3: Powder properties of the native and modified <i>boyna</i> starch	38
Table 3.4: Crushing strength, tensile strength, and friability and disintegration time of plain tablets	40
Table 3.5: Crushing strength, tensile strength, friability, and disintegration time of matrix tablets	43
Table 3.6: Interpretation of drug release mechanisms from release exponent values (n) in Korsemeyer-Peppas model	47
Table 3.7: Parameter and statistical estimates of the dissolution data from the different formulations	48

ACRONYMS

AA	Acetic anhydride
Ac-CLS-A	Dual modified starch where cross-linked starch with 5% sodium hexametaphosphate reacted in a 1:1 ratio with AA
Ac-CLS-B	Dual modified starch where cross-linked starch with 5% sodium hexametaphosphate reacted in a 1:2 ratio with AA
Ac-CLS-C	Dual modified starch where cross-linked starch with 10% sodium hexametaphosphate reacted in a 1:1 ratio with AA
Ac-CLS-D	Dual modified starch where cross-linked starch with 10% sodium hexametaphosphate reacted in a 1:2 ratio with AA
Ac-CLS-E	Dual modified starch where cross-linked starch with 15% sodium hexametaphosphate reacted in a 1:1 ratio with AA
Ac-CLS-F	Dual modified starch where cross-linked starch with 15% sodium hexametaphosphate reacted in a 1:2 ratio with AA
CLS	Cross-linked starch
CLS-A	Cross-linked starch with 5% Sodium hexametaphosphate
CLS-B	Cross-linked starch with 10% Sodium hexametaphosphate
CLS-C	Cross-linked starch with 15% Sodium hexametaphosphate
DC	Degree of cross-linking
DMS	Dual modified starch
DS	Degree of substitution
NS	Native starch
RPM	Revolution per minute
S	Solubility
SA	Starch acetate
SA-A	Starch acetate where native starch reacted in a 1:1 ratio with AA
SA-B	Starch acetate where native starch reacted in a 1:2 ratio with AA
SHMP	Sodium hexametaphosphate
SP	Swelling power
SR	Sustained release

1. INTRODUCTION

1.1. Starch

Starch has been the subject of intensive research over many decades occupying a vast body of published literature reporting its preparative and analytical methods, molecular structure, physical, chemical and biochemical properties, functionality and uses (Copeland *et al.*, 2009). The manifestation of diverse native starches (NSs) and numerous starch modification techniques allowed different starches to be used for different applications (Ellis *et al.*, 1998).

The world-wide market for industrial starches is expanding and current 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 (Ellis *et al.*, 1998; Schwartz and Whistler, 2009).

Approximately, 60 million tons of starch is extracted annually worldwide from various cereal, tuber and root crops, of which roughly 60% is used in foods and 40% in pharmaceuticals and for non-edible purposes (Copeland *et al.*, 2009).

1.1.1. Structure and organization in starch granules

After thousands of studies over many decades, starch remains a fully undiscovered mysterious substance. This mysterious nature of starch emanates from its exclusive behavior that each starch is unique in terms of granule organization and structures of constituent polymers. Starches from different sources are different, and that not all granules of a single starch behave identically (BeMiller, 1997). The knowledge of the internal organization helps the researcher to understand the functionalities and the transformation behavior of starch, and improve the properties and stability of starch products (Schwartz and Whistler, 2009).

Starch granules are composed of two types of α -glucan, amylose and amylopectin, which represent approximately 98 - 99% of the dry weight. They also contain non-starch

components such as lipids, proteins and phosphate groups (Ellis *et al.*, 1998; Tester *et al.*, 2004). Although a minor component by weight, lipids can have a significant role in determining the properties of starch. The high amount of lipids in starches has the following unfavorable effects: a) Lipids reduce the water-binding capacity, the swelling and the solubilization of starches; b) The oxidation of lipids results in the formation of undesirable flavors; c) The presence of amylose-lipid inclusion compounds makes starch pastes and starch films opaque or cloudy (Swinkels, 1985; Copeland *et al.*, 2009). The nature of phosphorous is also believed to affect the starch performance. It occurs on the starch molecules as negatively charged group. The ionic repulsion generated by these groups weakens the association forces between the molecules thereby increasing the water binding capacity, swelling power (SP) and the paste clarity (Riley *et al.*, 2006).

1.1.2. Amylose and Amylopectin

Amylose influences the packing of amylopectin into crystallites and the organization of the crystalline lamellae within granules. A better understanding of the location of amylose in starch granules may improve our ability to relate structure to properties that involve water absorption, for example, swelling, gelatinization, and susceptibility to enzymatic attack. Thermal properties and gel formation appear to be influenced by both amylose content and amylopectin architecture (Copeland *et al.*, 2009).

Amylose, a relatively long and linear α -glucan (Fig. 1.1 (A)), exists in an amorphous state in starch granule but becomes crystalline from self-association after dissolved in solution because of its linear structure. In comparison, amylopectin, a heavily branched structural unit (Fig. 1.1 (B)), is present in a semi-crystalline state in granules but becomes less organized when dissolved in solution and then undergoes slower self-association than does amylose. The strong self-association of amylose molecules might reduce their accessibility to reagents, while the less ordered amylopectin molecules were more receptive to modification, leading to higher reaction efficiency (Tolstoguzov, 2003; Tester *et al.*, 2004; Onofre *et al.*, 2009).

In high amylopectin starches, the highly branched structure of amylopectin may promote extensive interaction among amylopectin molecules and form a strong and viscous matrix

capable of sustaining drug release. In contrast, the essentially linear nature of amylose could not support the formation of a network structure unless amylose molecules were linked together through extensive cross-linking. It has been reported that moderate crystallinity or a more balanced ratio between order/disorder of starch chains lead to better sustained release because both crystalline and amorphous structures were involved in network formation upon swelling (Onofre *et al.*, 2009).

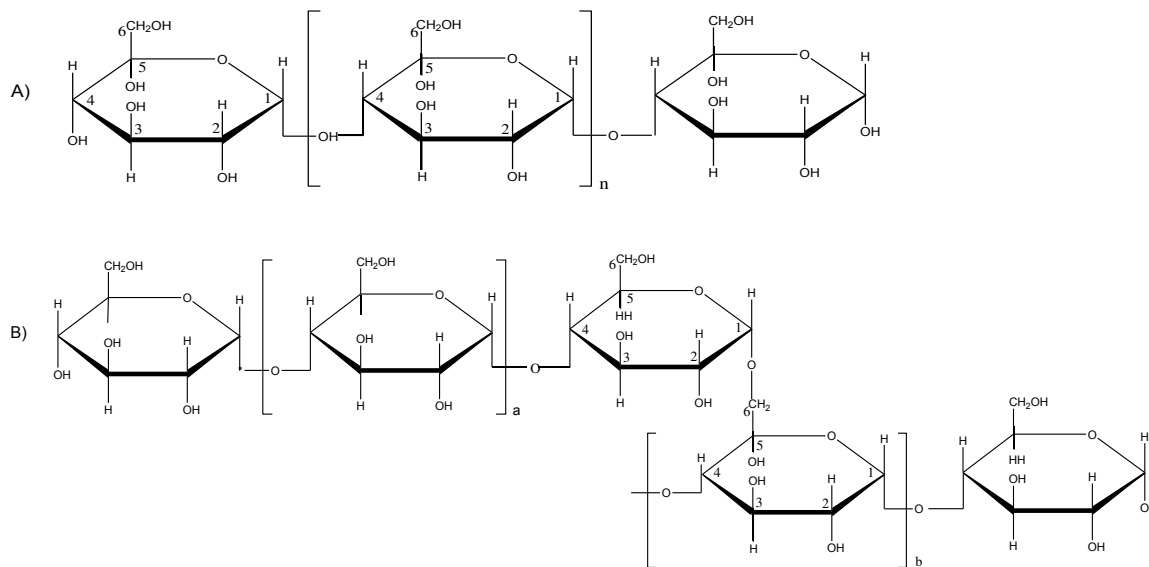


Figure 1.1: Structure of linear amylose (A) and branched amylopectin (B) (On average $n = \text{ca. } 1000$ for amylose, and $a = \text{ca. } 12\text{-}23$ for exterior chains and $b = \text{ca. } 20\text{-}30$ for interior chains of amylopectin).

1.1.3. Sources of starch

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 (Swinkels, 1985; Schwartz and Whistler, 2009).

Commercial starches are obtained from cereals and from tubers and roots. Starches especially from cereals have received very extensive attention. Corn starch, for example, makes up more than 80% of the world market. Although, a wide range of tuber crops are grown worldwide, only five species account for almost 99% of the total tuber starch production. These are potato (46%), cassava (28%), sweet potato (18%), yams (6%) and taro (1%) (Jayakody *et al.*, 2007; Salwa *et al.*, 2010).

1.1.4. Yam tubers and their starches

The yam is a monocotyledonous tuber bearing plant, belonging to the family *Dioscoreaceae* within the genus *Dioscorea* with over 600 species (Riley *et al.*, 2006). Yams are staple tuber crops cultivated in many parts of Africa and South East Asia. Their high starch content, ranging from 70 - 80% of the dry weight, and cheap cost have made them potential source of industrial starch, which could be explored commercially in the food and pharmaceutical industries (Okunlola and Odeku, 2011).

The high starch content of yam tubers readily presents an opportunity for exploitations. In recent years, more attention has been given to yams as potential source of NSs whose functional characteristics, if sufficiently exploited, could find some applications in the food and pharmaceutical industries (Odeku and Picker-Freyer, 2007).

In their report, Odeku *et al* (2008) reported that yam starches provide much better compaction properties. However, native *Dioscorea* starches generally exhibited poor flow properties characteristic of other NSs. Native yam starch and carboxymethyl yam starch were evaluated as tablet disintegrants (Nattapulwat *et al.*, 2008). Starches obtained from four *Dioscorea* species namely *D. dumetorum* (Bitter), *D. oppositifolia* (Chinese), *D. alata* (Water), and *D. rotundata* (White) have been evaluated as binding agents (Okunlola and Odeku, 2011).

1.1.5. *Dioscorea abyssinica* plant and its starch

Dioscorea abyssinica (Fam. *Dioscoreaceae*) is a climber plant twining to the right, with herbaceous stem and a large tuber (Fig. 1.2). It is cultivated during the rainy season in the south, west and the south-west highlands of Ethiopia. Tubers of *dioscorea* have long been

used for food, as they are rich in starch. Locally, dioscorea is commonly known by its vernacular name “*boyna*”. When the tuber is horizontally directed under the soil, it can grow up to 30 cm in length and 20 cm in thickness. Due to its high yield it could be one of the potential alternative sources of commercial starch. The major constituent of dioscorea tuber is starch accounting about 80% on dry weight basis. The proximate compositions of the *boyna* starch are: 0.1% ash, 0.5% protein, 1% fat and 98.4% starch. *Boyna* starch show an X-ray diffraction pattern that is typical of B-type with a distinctive maximum peak at around $17^{\circ} 2\theta$ (Gebre-Mariam and Schmidt, 1998).



Figure 1.2: *Dioscorea abyssinica* plant (A) and its tuber (B) (taken by Mulualem Y)

1.2. Starch modification

Its availability and low cost have allowed starch to be integrated into a wide variety of applications. However, inferior characteristics of NSs such as poor flowing properties, stability limitations, and negligible cold-water swelling have limited its application in solid dosage forms as a sustained release agent. Many petroleum-derived products, such as polymethacrylates (Eudragit) as well as the semi-synthetic cellulose derivatives have shown success in sustaining drug release. Nonetheless, there is a growing interest in

improving the functionality of polysaccharides in order to use them in oral drug delivery systems because of their non-toxicity and biodegradability (O'Brien *et al.*, 2009).

Modified starches have been developed for a very long time and their industrial applications are really significant nowadays (Chiu and Solarek, 2009). Improvements in the properties of starches for industrial uses can be achieved through chemical and physical modification of extracted starch and through the manipulation of starch biosynthesis in the plant itself (Ellis *et al.*, 1998). The properties required for a particular application, availability of the starch and economics play a role in selecting a particular NS for subsequent chemical and physical modification (Chiu and Solarek, 2009).

The distinguishing factors that affect the efficiency of modification are the starch source, amylose to amylopectin ratio, granule morphology, and type and concentration of the modifying reagent. The extent of alteration in the starch properties reflects the resistance or the susceptibility of a starch towards different chemical modifications (Singha *et al.*, 2007).

1.2.1. Physical modification

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 (Wadchararat *et al.*, 2006; Bhat and Karim, 2009; Chung *et al.*, 2009; Zuo *et al.*, 2009).

1.2.2. Chemical modification

The wide variability of starches in form and functionality provides starches of diverse properties, but it can also cause problems in processing due to inconsistency of raw materials. As a result, chemically modified starches are used extensively to overcome the variability of NSs and their lack of versatility over a wide range of processing conditions (Copeland *et al.*, 2009). The chemically modified starches have markedly altered physicochemical properties as compared to their parent starches. Acetylation and cross-

linking are two widely used methods for making modified starches (Liu *et al.*, 1999; Chiu and Solarek, 2009).

1.2.2.1. Cross-linking

Cross-linking is the most important chemical modification in the starch industry. It involves replacement of the hydrogen bonding between starch chains by stronger, more permanent, covalent bonds (Ellis *et al.*, 1998). Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds that act as a bridge between the starch molecules which alters not only the physical properties, but also the thermal transition characteristics of starch (Mirmoghtadaie *et al.*, 2009).

Cross-linking is performed by treating granular starch with bi-functional or multi-functional reagents which are capable of forming ether or ester linkages with hydroxyl groups in starch. These bi-functional or multi-functional reagents include Sodium trimetaphosphate, sodium tripolyphosphate (STPP), sodium monophosphate (SMP), epichlorohydrin (EPI), phosphoryl chloride (POCl_3), a mixture of adipic and acetic anhydrides, and a mixture of succinic anhydride and vinyl acetate. Among them, Sodium trimetaphosphate is one of the most important food additives and a solid of low toxicity. Sodium trimetaphosphate is reported to be an efficient cross-linking agent at high temperature with semidry starch and at warm temperature with hydrated starch in aqueous slurry (Gui-Jie *et al.*, 2006).

Important factors in the cross-linking reaction include reagent type, reagent concentration, pH, reaction time, temperature, and botanical source of the starch (Mirmoghtadaie *et al.*, 2009). During cross-linking small-sized granules have been reported to be derivatized to a greater extent than large-sized granules (Odeku and Picker-Freyer, 2009). At a reaction pH above 10, starch ionized hydroxyls can attack the polyphosphates central phosphate to form starch pyrophosphates, which can be further attacked by starch hydroxyl groups to give distarch phosphate (Fig. 1.3A and B). At a reaction pH below 9.0, the terminal phosphate groups of polyphosphates are protonated and produce monometaphosphates, which can react rapidly with starch hydroxyl groups to produce monostarch phosphates (Fig. 1.3C) (Lim and Seib, 1993). Monostarch

phosphates exhibit increased viscosity and water binding capacity, which would help the formation of a gel barrier to control water penetration and drug diffusion. On the other hand, the formation of distarch phosphates may help maintain the granule integrity when starch is exposed to severe processing conditions such as extrusion (O'Brien *et al.*, 2009).

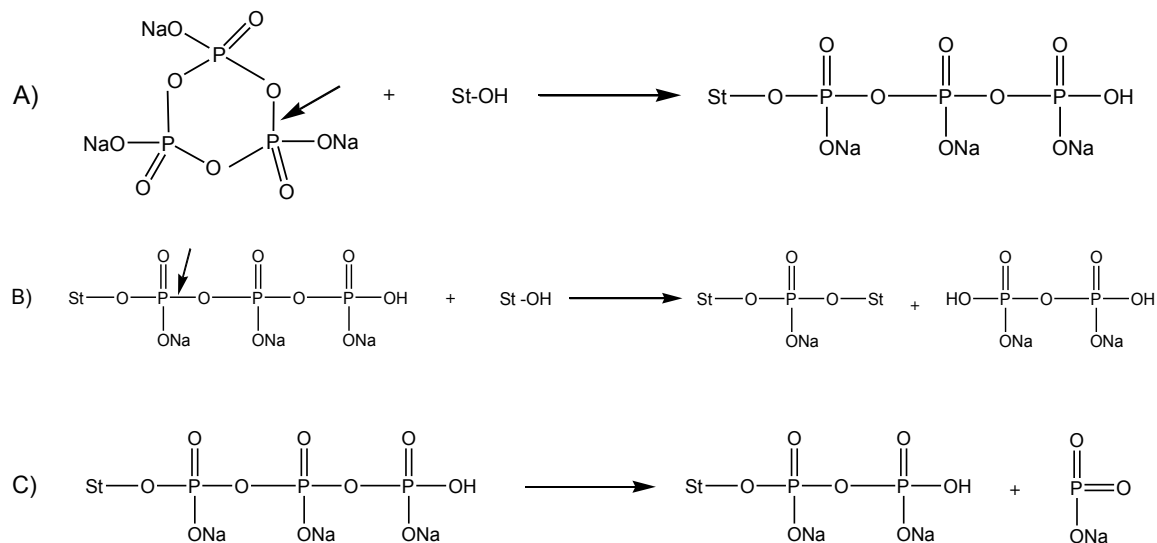


Figure 1.3: A scheme for chemical reactions involved during formation of starch phosphates (A = Ring opening reaction, B = Distarch phosphate, C = Monostarch phosphate).

It is suggested that the presence of neutral salts like sodium carbonate will have the following effects: it builds water structure and allows deeper penetration of reagent into the granule; ionic strength promotes reaction between a starch alkoxide ion and an ionic phosphoryl reactant; ionization of starch hydroxyls is also promoted by ionic strength; sodium ions increase the alkali adsorbed by starch granules (Woo and Seib, 1997).

In the present study sodium hexametaphosphate (SHMP) is used as a cross-linking agent. Also known as sodium polymetaphosphate, SHMP is a white, odorless, crystalline powder and a mixture of polymeric metaphosphates (NaPO_3)₆ (Fig. 1.4). It is generally used as phosphorylating agent of starches for food applications. SHMP hydrolyses to Sodium trimetaphosphate and sodium orthophosphate in aqueous solution.

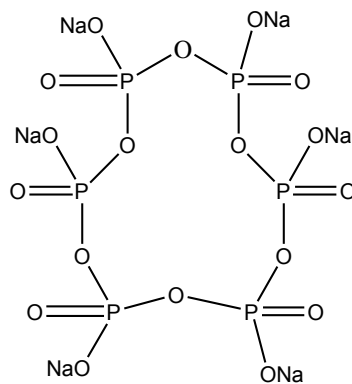


Figure 1.4: Structure of sodium hexametaphosphate (SHMP).

1.2.2.2. Acetylation

Acetylated starch is commonly obtained by the esterification of NS with acetic anhydride (AA) in the presence of an alkaline catalyst (Singha *et al.*, 2007). Chen *et al* (2004) reported that acetylation occurs in all the amorphous regions and also at the outer lamellae of crystalline regions. This has been suggested to be due to the poor penetrating ability of AA in starch granules. Studies on acetylated potato starches suggest that the small size granule population with lower amylose content favors the introduction of acetyl groups and hence results in higher degree of substitution (DS) (Singh *et al.*, 2004).

The acetylation of starch takes place by an addition-elimination mechanism (Fig. 1.5A-C). The three free OH groups of the starch have different reactivities. The primary C-6 OH is more reactive and is acetylated more readily than the secondary ones on C-2 and C-3 due to steric hindrance. Of the two secondary OH groups, the C-2 OH is more reactive than the C-3, mainly because the former is closer to the hemi-acetal and more acidic than the later (Garga and Jana, 2011).

It was observed that the acetylation treatment causes granule fusion. The fusion of starch granules after acetylation is proposed to be due to the introduction of acetyl groups on the starch molecules, which leads to increased hydrogen bonding. Therefore, starch molecules coalesce together which results in fusion of granules (Singh *et al.*, 2004).

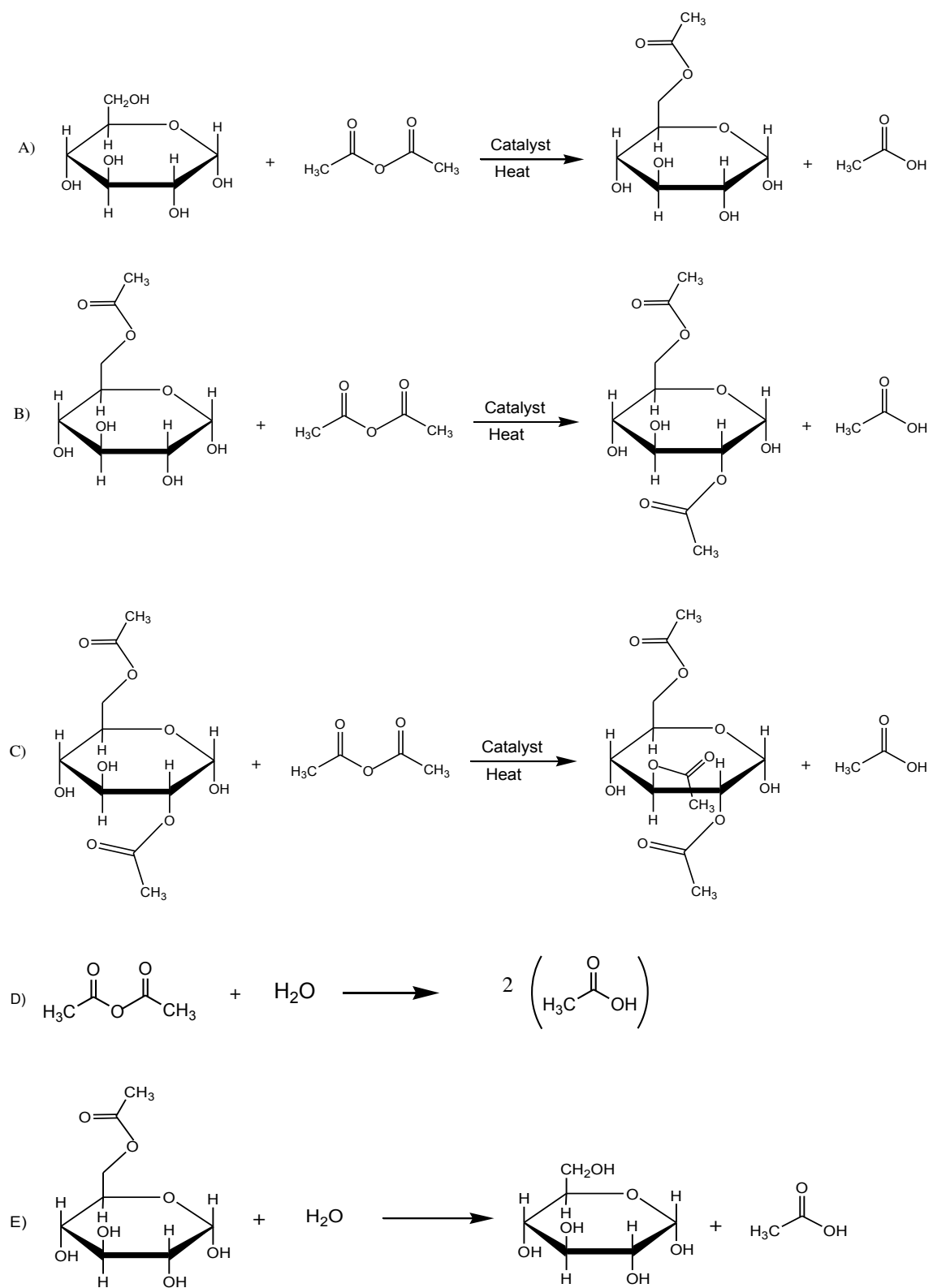


Figure 1.5: A scheme for chemical reactions involved during acetylation of starch with acetic anhydride, main reactions (A-C) and side reactions (D and E).

1.2.2.3. Dual modification

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 (Jyothi *et al.*, 2006; Singha *et al.*, 2007). The chemical modification is commonly done by partial substitution of hydroxyl groups of cross-linked starches (CLSs). By derivatization of CLSs, ionic (aminoalkyl and carboxymethyl) groups as well as less polar (acetate) groups will be introduced on the polysaccharide chains of polymeric matrix (Mulhbacher *et al.*, 2001).

Strengthening bonding between starch chains by cross-linking will increase the resistance of the granule to swelling. Cross-linking greatly increases the stiffness of the gels. Acetylation reduces interactions between starch chains and, by loosening granule structure, increases the ability of granules to swell. Hardness, adhesiveness and cohesiveness of gels made from starches are increased by acetylation (Liu *et al.*, 1999).

Hydroxyl groups are shown to play an important role in the organization of the matrix network from CLSs, which is an important parameter in the control of drug release. It will be of interest to see the impact of various polar and less polar functional substituents on the performances of the CLS through replacing hydroxyl groups of the CLS matrix. In terms of interacting forces, the carboxyl or amino groups can be involved, for instance, in new hydrogen bonds with the hydroxyl groups of the matrix, enhancing its stability, while acetate groups could contribute to hydrophobic interactions within the matrix and thus limit the water access (Mulhbacher *et al.*, 2001).

The quantity of cross-linking reagent required to prepare a dual modified starch (with desirable properties) vary with the source of starch, the type of cross-linking reagent, the efficiency of the cross-linking reaction, the degree of substitution required and the specified range of final modified starch properties (Wattanchant *et al.*, 2003).

Starches of different sources and compositions were cross-linked and substituted to determine the effect of structure and chemical modification on the sustained release properties of the resultant modified starches. Substitution efficiency was overall higher

for high amylopectin (waxy) and normal starches than for high amylose starch, and was higher for starches at low cross-linking levels than those at high cross-linking ones. Waxy starch displayed better sustained release properties when cross-linked to a lower level, whereas high amylose starch showed better performances when cross-linked to a higher level (Onofre *et al.*, 2009).

Dual modification results in starch with improved qualities than those of NSs. However, the effect of dual modification depends upon the preparation procedure. Cross-linking followed by substitution has been reported to yield starches that are more shear and heat stable than the NS. This may be due to the structural change in the granules after the first modification (cross-linking). For example, cross-linked and then hydroxypropylated starch exhibited lower pasting temperature and viscosity than the hydroxypropylated and then cross-linked starch samples. The cross-links in cross-linked and then hydroxypropylated starch were also found to be more resistant to attack by enzymes and chemicals (Reddy & Seib, 2000; Singha *et al.*, 2007).

1.3. Modified starches in sustained release formulations

Drug release modification is a technique by which the delivery pattern of a therapeutic agent is altered via engineering of physical, chemical, and/or biological components into delivery systems for achieving desired plasma drug levels defined by clinical pharmacology. A sustained release (SR) dosage form is formulated to make the drug available over an extended period after ingestion, thus allowing a reduction in dosing frequency compared to a drug presented as a conventional dosage form (Qiu, 2009).

Polymers are often used in SR formulations to provide diverse functionality to the formulation in which they are employed. Derivatives of cellulose such as hydroxypropylmethyl cellulose (HPMC), ethylcellulose (EC), and their combinations with other polymers have been extensively studied in SR formulations (Onofre *et al.*, 2009).

Starch is known to produce low toxicity products that are biodegradable and quite stable in the biological environment. In addition to these, as starch based products are cost

effective, it is wise to apply them in drug delivery (Mundargi *et al.*, 2008). However, NSs are unsuitable for controlled drug delivery system due to various reasons; including fast release properties which results from substantial swelling in aqueous media along with rapid enzymatic degradation. Such unsuitability of starch can be tailored using physical, chemical or enzymatic modification in order to improve the applications of starch as excipient for sustained drug delivery (Lemieux *et al.*, 2009).

Chemically modified starches have shown promising results in the pharmaceutical industry when applied as SR matrices. Cross-linked starches possess unique features that suggest their use as an excipient for the manufacture of controlled release solid oral dosage forms of drugs (Lenaerts *et al.*, 1998). Cross-linked high amylose corn starch is the most extensively studied one. Mulhbach *et al.* (2001) the SR properties of cross-linked and substituted high amylose corn starch matrices.

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).

1.4. The present study

Modified starches made for human use are required to contain only small amounts of substituent groups and have been used as safe food ingredients. During chemical modification of starches, the level of substitution groups introduced should be relatively low. According to Food Additives and Contaminants Committee (FAC, 1980), the maximum permitted levels of substitution for starch acetates (SAs) and starch phosphates are 2.5 and 0.4%, respectively. Similarly, cross-linked starches containing one substituent crosslinking group per 1000 or more anhydroglucose units are considered safe (Singha *et al.*, 2007; Das *et al.*, 2010). Chemically modified starches, to be used in SR tablet formulations, require higher degrees of substitution. This, in turn, requires the starches to be treated with higher concentration of modifying agents at elevated temperature with

appropriate catalyst or methods to be combined. Higher temperatures could damage starch and lead to poor properties of the materials developed (Korhonen *et al.*, 2002; Reddy and Yang, 2010).

Cross-linked starches have been developed as excipients for the formulation of SR solid dosage forms for the oral delivery of drugs (Lenaerts *et al.*, 1998). However, the chemicals used for cross-linking starch are relatively toxic, expensive or do not provide the desired improvement in properties. Drug release rate from SAs decreases as the degree of substitution increases from near 0 to 3 (Korhonen *et al.*, 2002). Obtaining SAs with the required higher degree of substitution for sustaining drug release has its own limitations including high cost, laborious, time consuming process and is associated with degradation (Wurzburg, 1964; Ayoub and Rizvi, 2009). Dual modification, a combination of cross-linking and substitution (acetylation), has been demonstrated to provide starch derivatives with prolonged drug release properties and high drug loading (Mulhbacher *et al.*, 2001).

Literature available on the dual modification of starches and their application as SR polymers is sparse and hence the purpose of this investigation was to study the modification of *Dioscorea abyssinica* starch with cross-linking and acetylation, and to investigate the effect of dual modification on the sustained drug release properties of the starch.

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), 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; Yoon *et al.*, 2009).

1.5. Objectives

1.6.1. General objective

- To evaluate the sustained release properties of dual modified (cross-linked and acetylated) *Dioscorea abyssinica* (boyna) starch.

1.6.2. Specific objectives

- To produce cross-linked, acetylated, and cross-linked-acetylated *Dioscorea abyssinica* (boyna) starch,
- To determine the degree of cross-linking and degree of substitution of the modified starches,
- To characterize the powder properties of the modified starches,
- To evaluate the tableting properties of the modified starches,
- To analyze sustained release properties of the modified starches, and
- To analyze the release kinetics/drug release mechanism from tablets of the modified starches.

2. EXPERIMENTAL

2.1. Materials

Fresh tubers of *boyana* were obtained from local farmers (Chichu Kebele, around Dilla, Gedio Zone). Sodium metabisulphite and Sodium carbonate (Guangzhou Jinhaunda Chemical Reagent Co. Ltd., Guangzhou, China), Sodium hexametaphosphate (Uni-chem Chemical reagent Ltd., Wuhan, China), Hydrochloric acid, Potassium hydroxide and Magnesium stearate (BDH Chemicals Ltd., Poole, England), Sodium hydroxide (Sigma-Aldrich, Stockholm, Sweden), Acetic anhydride (Riedel-de Haen, Sweden), Potassium dihydrogen phosphate (Sorensen, Leuren, Germany), Sodium chloride (E. Merk, Stockholm, Sweden) were obtained from local market. Anhydrous theophylline (Shandong Xinhua Pharmaceutical Co. Ltd, Zibo, China) was kindly donated by Ethiopian Pharmaceutical Share Company (EPHARM).

2.2. Methods

2.2.1. Isolation of starch from *D. abyssinica* tuber

The starch was isolated and purified following the procedure described by Gebre-Mariam and Schmidt (1998). *Boyana* tubers were washed, trimmed to remove defective parts and peeled. Immediately after peeling the tuber flesh were chopped and suspended in large quantities of distilled water containing 0.075% (w/v) of sodium metabisulphite. The suspension was then allowed to settle overnight, and the supernatant was decanted. The sediment was repeatedly treated with sodium metabisulphite solution until the supernatant was clear. The suspension was then passed through fine muslin to remove cell debris and the translucent suspension was collected and allowed to settle. The sediment was washed several times with distilled water by filtering and re-suspending until the wash water was clear and free of suspended impurities. The resulting starch was air-dried at room temperature, and stored in airtight container, after milling and sieving, for further use.

2.2.2. Preparation of modified starches

2.2.2.1. Preparation of cross-linked *D. abyssinica* starch

Cross-linking of *boyna* starch was performed using a method described by Atichokudomchai and Varavinit (2003) with slight modifications. Native starch (100 g, dry weight basis) was suspended in 200 ml of distilled water containing sodium hydroxide and heated to 50 °C. After 15 min of heating, sodium carbonate, and SHMP at three different concentrations (Table 2.1) dissolved in minimal amount of distilled water were added to the medium while stirring. The starch suspension was maintained at 50 °C while stirring and held at this temperature for 6 h. Then, the pH of the suspension was adjusted to 6.5 with 1.5 N hydrochloric acid, after cooling to room temperature, to terminate the reaction. The cross-linked starch slurry was then filtered in a Buchner funnel by vacuum filtration and washed several times with distilled water, filtered and dried in hot air oven (Kottermann® 2711, Germany) at 40 °C for 24 h. The material was then powdered using mortar and pestle, passed through a 224 µm mesh sieve, and packed in air tight container.

Table 2.1: Reaction compositions for cross-linked starches (CLSs) of *boyna* starch

CLS Batches	SHMP (% w/w)	NaOH (% w/w)	Na ₂ CO ₃ (% w/w)	Reaction time (h)	Reaction temperature (°C)
CLS-A	5	0.6	3	6	50
CLS-B	10	0.6	3	6	50
CLS-C	15	0.6	3	6	50

2.2.2.2. Preparation of acetylated *D. abyssinica* starch

Methods described by Das *et al.* (2010), and Mulhbachter *et al.* (2001), were used in combination to prepare SA with slight modifications. The acetylation reaction was carried out by mixing the required amounts of dried *boyna* starch, acetic anhydride (AA) and sodium hydroxide (NaOH) in a 500 ml round bottom flask (Table 2.2). To start with, *boyna* starch (50 g, dry weight basis) was placed in a flask and about 70 ml distilled water was added to obtain a fluid starch suspension. The starch slurry was then placed on hot plate with constant stirring using magnetic stirrer until homogeneous slurry was

obtained. The required amounts of AA and NaOH were slowly added drop wise over a period of 20 min. NaOH was added as a 50% aqueous solution. The reaction vessel was sealed and reaction mixtures were heated to 50 °C, and held at this temperature for 2 h while stirring. The pH was adjusted to 4.5 with hydrochloric acid; then the slurry was filtered on a Buchner funnel by vacuum filtration and washed several times with distilled water. The resulting filter cake was dried in a hot air oven at 40 °C for 24 h. The material was then powdered using mortar and pestle, passed through a 224 µm mesh sieve, and packed in air tight container.

Table 2.2: Reaction compositions for *boyna* starch acetates (SAs)

SA Batches	Starch/AA ratio	NaOH (% w/w)	Reaction time (h)	Reaction temperature (°C)
SA-A	1:1	11	2	50
SA-B	1:2	11	2	50

2.2.2.3. Preparation of dual-modified *D. abyssinica* starch

A two-step procedure was followed for the preparation of dual modified *boyna* starch. A similar method described above for the preparation of SA was followed for acetylation of the cross-linked *boyna* starches as shown in Table 2.3 below.

Table 2.3: Reaction compositions for dual-modified *boyna* starch (DMS)

DMS Batches	CLS	CLS/AA ratio	NaOH (%w/w)	Reaction time (h)	Reaction temperature (°C)
Ac-CLS-A	CLS-A	1:1	11	2	50
Ac-CLS-B	CLS-A	1:2	11	2	50
Ac-CLS-C	CLS-B	1:1	11	2	50
Ac-CLS-D	CLS-B	1:2	11	2	50
Ac-CLS-E	CLS-C	1:1	11	2	50
Ac-CLS-F	CLS-C	1:2	11	2	50

2.2.3. Physicochemical characterization of modified starches

2.2.3.1. Determination of peak viscosity and degree of cross-linking (DC)

The peak viscosities of the CLS and NS samples were determined using a rotational viscometer (VISCOSTAR Plus, KINEMATICA, AG, Switzerland) using spindle number 4 at a shearing stress of 200 rpm. Starch suspensions at 10% (w/v) concentration were prepared by shaking the starch in a 50 ml beaker for 3 min. The suspensions were then heated from 50-90 °C in a water bath and meanwhile, their peak viscosities were recorded at 50, 70 and 90 °C from the digital display on the viscometer. After maintaining the samples at 90 °C for 3 min, the peak viscosities of the same were read as they were cooled from 90 back to 50 °C at 90, 70 and 50 °C. The maximums of the peak viscosities in the entire heating-cooling cycles were taken and used in the estimation of the DC in the CLS samples. The DC was calculated using the formula:

$$\text{Degree of cross - linking} = \frac{(A - B)}{A} \times 100 \quad (2.1)$$

where A is the peak viscosity of the control sample (NS) and B is the peak viscosity of CLS. Results were expressed as a mean of triplicate determinations.

2.2.3.2. Determination of degree of substitution (DS)

A procedure described by Wurzburg (1964) was employed to determine the DS. First, 1 g sample of SA was placed in a 250 ml flask, and 50 ml of distilled water was added. The flask was agitated, heated to 90 °C over water bath, held at this temperature for 30 min, and cooled; 40 ml of 0.5 N potassium hydroxide was added while swirling. The flask was stoppered, and then allowed to stand for 72 h with occasional swirling. The excess alkali was then back titrated with standard 0.5 N hydrochloric acid using phenolphthalein as indicator. A blank (NS) was treated the same way, substituting the SA. The DS was determined using eq. 2.3 below. Results were expressed as a mean of triplicate determinations.

$$\%Acetyl = \frac{[ml. (blank) - ml. (sample)] \times Normality of acid \times 0.043 \times 100}{Sample\ wt. (g)(dry\ basis)} \quad (2.2)$$

$$Degree\ of\ substitution = \frac{162 \times \%Acetyl}{4300 - (42 \times \%Acetyl)} \quad (2.3)$$

2.2.3.3. Determination of moisture content

Moisture content of the powders was measured by gravimetric method. The powder samples (2 g each) were spread over petri dishes (pre-dried and weighed) uniformly and dried in an oven at 120 °C. The weight loss was obtained by accurately weighing the samples after 4 h. Results were expressed as a mean of triplicate determinations.

2.2.3.4. Determination of solubility (S) and swelling power (SP)

A method described by Odeku and Picker-Freyer (2007) was used to determine S and SP with slight modifications. Starch suspensions were prepared by dispersing 0.5 g of starch samples in 10 ml distilled water in pre-dried and weighed centrifuge tubes and heated to 20, 37, 50, 65, 75 and 85 °C, respectively, for 30 min with shaking every 5 min and then left to cool at room temperature. The suspensions were centrifuged for 15 min at 3000 x g. The supernatant was decanted and dried in an oven for 4 h at 120 °C. The residue obtained after drying the supernatant (W_s) represents the amount of starch solubilized in water at that particular temperature. The residue obtained was weighed (W_r) to obtain the swelling of the starch. The S and SP were determined using Eq. 2.4 and 2.5, respectively. Results were expressed as a mean of triplicate determinations.

$$S (\%) = \frac{W_s}{Wt. \ of \ sample \ (dry \ weight \ basis)} \times 100 \quad (2.4)$$

$$SP = \frac{W_r \times 100}{Wt. \ of \ sample \ (dry \ weight \ basis) \times (100 - S)} \quad (2.5)$$

2.2.3.5. Determination of moisture sorption pattern

A methods described by Gebre-Mariam and Schmidt (1996) was followed to study the moisture sorption properties. Saturated solutions of different salts were prepared to

provide different percentage relative humidity (RH). Pyrex desiccators containing distilled water and saturated salt solutions were prepared to provide different RH chambers and kept at room temperature. 2 g of pre-dried starch samples were spread over Petri dishes and transferred to particular RH chamber, and allowed to equilibrate for four weeks. The moisture content was determined based on the weight difference of the starches before and after equilibration.

2.2.4. Characterization of powder properties of the modified starches

Powder properties of modified starches were characterized by flow rate, angle of repose, Carr index, and Hausner's ratio. Bulk density was determined by placing 30 g starch powder in a 250 ml measuring cylinder and volume occupied was read after light tapping and bulk density was calculated. The cylinder was then tapped at a constant velocity using tapped densitometer (ERWEKA, Germany) till a constant volume was obtained, and then tap density was determined. Hausner's ratio and Carr index were calculated using Eq. 2.6 and 2.7 respectively.

$$\text{Hausner's ratio} = \frac{\rho_t}{\rho_b} \quad (2.6)$$

$$\text{Carr's index} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad (2.7)$$

where ρ_t = tapped density, ρ_b = bulk density.

Flow rate and Angle of repose were determined by fixed height funnel method. In this, 30 g starch powder was placed and allowed to flow through a stemless funnel having 10 mm aperture from a fixed height of 10 cm. The duration of flow was recorded and used to calculate the flow rate. Angle of repose was determined from the height and radius of powder pile according to Eq. 2.8.

$$\theta = \tan^{-1} \left(\frac{h}{r} \right) \quad (2.8)$$

where θ = Angle of repose, h = Height of granule, r = Radius of circle formed by the starch powder pile. Results were expressed as a mean of triplicate determinations.

2.2.5. Fourier transform infrared spectroscopy (FTIR) studies

The starch samples were characterized by Fourier transform infrared spectroscopy (FTIR-8400S, SHIMADZU, Japan) to check for structural changes due to the cross-linking and acetylation reactions, as well as to confirm chemical stability of theophylline in the mixture. About 5-10 mg of the samples were equilibrated at 50 °C for 24 h and finely ground in a mortar to produce uniform particle size. The samples were diluted with an oily mulling agent (Nujol) in a mortar and pestle. FTIR spectra of NS, modified starches, pure theophylline and binary mixtures of theophylline and modified starch samples were recorded at a resolution of 4 cm⁻¹ and wave number ranged between 4000 and 400 cm⁻¹ with average of 20 scans using potassium bromide (KBr) plate. A background spectrum corresponding to pure KBr plate was employed. IR solution software was used for data treatment.

2.2.6. Tablet preparation and evaluation

2.2.6.1. Tablet preparation

For matrix tablet formulation, theophylline and modified starch powders were dry mixed in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) for 5 min. Then, the mixtures were lubricated with 0.5% magnesium stearate for 3 min. Tablets were compressed in a single punch tablet press (Korsch EKO, Berlin, Germany), fitted with 10 mm flat faced punches, at a fixed compression pressure. Tablets of 500 mg containing theophylline (20%, 30% and 40% (tablets above 40% loading were more friable and unable to sustain the drug release)), magnesium stearate and starch powders (Table 2.4) were obtained by introducing a weighed amount of the powder mixtures in the die. Plain tablets of 400 mg of native and modified starches were also compressed using the same condition at compression pressure set for the NS.

Table 2.4: Compositions of tablet formulations of modified starches

Excipients	Formulations			
	F1	F2	F3	F4
Theophylline (% w/w)	20	30	40	20
Ac-CLS-F (% w/w)	79.5	69.5	59.5	-
Ac-CLS-E (% w/w)	-	-	-	79.5
Mg Stearate (% w/w)	0.5	0.5	0.5	0.5

2.2.6.2. Tablet evaluation**2.2.6.2.1. 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.

2.2.6.2.2. Tensile strength

The radial tensile strength (σ) was calculated using equation (2.13):

$$\sigma = \frac{2 \cdot F}{\pi \cdot D \cdot T} \quad (2.9)$$

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

2.2.6.2.3. 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.

2.2.6.2.4. 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 0.1 N HCl solution maintained at 37 ± 2 °C as the immersion fluid. The tablets were considered completely disintegrated when all particles passed through the wire mesh. The average disintegration time and standard deviation of six tablets was determined.

2.2.6.2.5. Calibration curve

Aliquots of stock solution (50 µg/ml) of anhydrous theophylline in 0.1 N HCl and phosphate buffer solution pH 6.8 were pipetted into a series of 100 ml volumetric flask and diluted to volume to provide different concentrations. The UV absorbance readings were measured at λ_{max} of 271 nm using UV-Visible spectrophotometer (CECIL, CE 1021, England). The calibration curve was constructed by plotting absorbance against concentration. The linearity or Beer's Curve ranged between 4 - 13 µg/ml (Fig. 2.1), with regression equations $Y = 0.0545X + 0.0058$ ($R^2 = 0.9998$) and $Y = 0.0573X + 0.0127$ ($R^2 = 1$) in 0.1 N HCl and phosphate buffer pH 6.8, respectively (Y = Absorbance, X = Concentration).

2.2.6.2.6. *In vitro* dissolution study

The *in vitro* dissolution study was carried out using USP Type II dissolution apparatus (ERWEKA, DT600, Germany) (USP30/NF25, <711>, 2007), and the test was performed on six tablets. The dissolution medium was kept in thermostatically controlled water bath, maintained at 37 ± 0.5 °C. Paddle rotation was adjusted to 50 rpm. At definite intervals (15 and 30 min, and then 1, 2, 3, 4, 6, 8, 10 and 12 h), 10 ml aliquots were withdrawn and analyzed (after proper filtration and dilution) spectrophotometrically at 271 nm for the drug release. Equal volumes of fresh corresponding medium were replaced into the dissolution flask following withdrawal of each sample. Concentration was determined from the standard calibration curve of theophylline and finally the results were plotted as cumulative % drug release versus time.

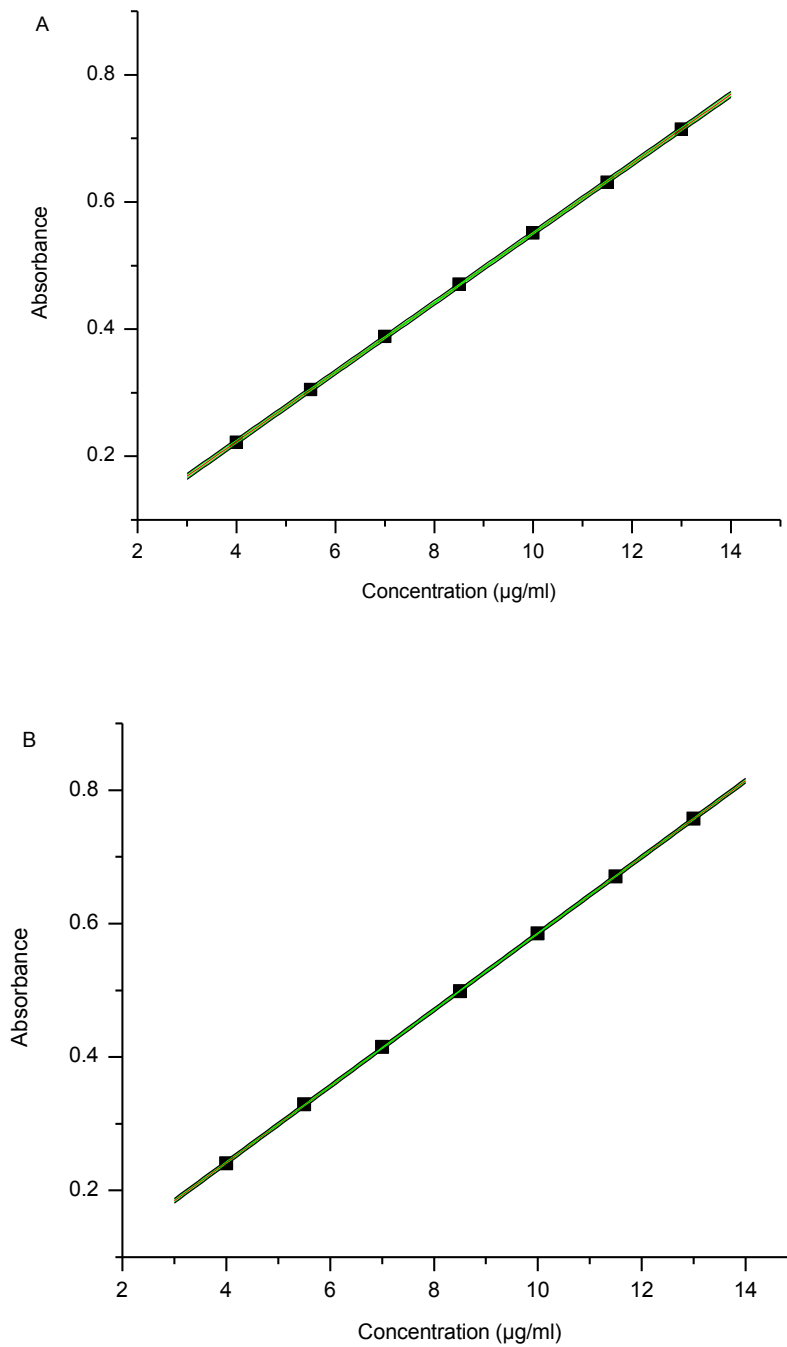


Figure 2.1: Standard calibration curve of pure theophylline in 0.1 N HCl solution (A) and phosphate buffer pH 6.8 (B) with 95% confidence bands for the mean.

2.2.6.2.7. Analysis of drug release kinetics

To analyze the mechanism of drug release rate kinetics, the results of *in vitro* release profile were fitted into various kinetic models. The zero order rate (Eq. 2.10) describes the systems where the drug release rate is independent of its concentration and follows a 'steady-state release', running at a constant rate. The first order (Eq. 2.11) describes the release from systems where release rate is concentration dependent and is assumed to decline exponentially. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Eq. 2.12). The Hixson–Crowell cube root law (Eq. 2.13) describes the release from systems where there is a change in surface area and diameter of the particles or tablets (Dredan *et al.*, 1996; Sood and Panchagnula, 1998).

$$Q_t = Q_o - k_o t \quad (2.10)$$

$$\ln Q_t = \ln Q_o - k_1 t \quad (2.11)$$

$$\frac{Q_t}{Q_o} = k_H \sqrt{t} \quad (2.12)$$

$$\sqrt[3]{Q_o} - \sqrt[3]{Q_t} = k_{HC} t \quad (2.13)$$

where, Q_t is the amount of drug released in time t , Q_o is the initial amount of the drug in tablet, and k_o , k_1 , k_H and k_{HC} are release rate constants for zero order, first order, Higuchi and Hixson-Crowell rate equations, respectively.

In order to define a model which will represent a better fit for the formulations, dissolution data can be further analyzed using Korsemeyer- Peppas equation (Eq. 2.14).

$$\frac{Q_t}{Q_o} = k t^n \quad (2.14)$$

where, $\frac{Q_t}{Q_o}$ is the fraction of drug released at time t, k is kinetic constant, and n is the diffusional exponent. The value of exponent can be used to characterize the mechanism of drug release.

2.2.7. Data analysis

The data was analyzed to compare individual differences in physicochemical and tablet properties using Analysis of Variance (ANOVA) on Origin 8.0 (OriginLabTM Corporation, USA) statistical software. At 95% confidence interval, p-values of ≤ 0.05 were considered statistically significant. All the data measured and reported are averages of a minimum of triplicate measurements and the values are expressed as mean $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1. Characteristics of modified starches

The starch isolated from *D. abyssinica* (*boyna*) was chemically modified by cross-linking, acetylation, and combined cross-linking and acetylation. Some physicochemical properties of *boyna* starch and its proximate compositions may be found in a previous work by Gebre-Mariam and Schmidt (1998).

3.1.1. Degree of cross-linking (DC)

Boyna starch was cross-linked with SHMP in the presence of sodium carbonate at pH 11 at a temperature of 50 °C. The pH of the starch slurry influences the DC significantly. Formation of distarch phosphate is favored by alkalinity above pH 10. Increasing alkalinity of cross-linking medium increases starch anion concentration, and this is expected to accelerate the bimolecular reaction between starch and the cross-linking agent. The reaction of metaphosphate is faster with alkoxy anions than non-ionized starch. Woo and Seib (1997) reported a much faster cross-linking with Sodium trimetaphosphate at alkaline pH 11. Sodium carbonate in the cross-linking reaction functions as a catalyst by weakening the hydrogen bond between molecules of starch, and activates the hydroxyls of starch. Its presence and amount affects the DC. The DC increased with increasing amount of sodium carbonate. However, when sodium carbonate reached a certain amount, the decomposition temperature of starch could be lower than the temperature of the reaction system, and the starch starts to decompose at that temperature (Woo and Seib, 1997; Gui-Jie *et al.*, 2006).

Quantification of phosphodiester cross-links in the cross-linked starches is measured by changes in physical properties (such as viscosity). Measurement of phosphodiester cross-links by chemical means is difficult. Attempts to determine the number of cross-links by increase in total phosphorous level in starch are confounded by endogenous phospholipids, and by the formation of phosphomonoesters (Woo and Seib, 1997). For this reason, the DC of starch is measured physically by viscosity measurement.

Table 3.1 shows the mean peak viscosity values and the respective DC values. The amount of SHMP apparently affects the DC. It is evident from the table that the DC increased significantly with increased SHMP ratio. The reason for exhibiting of such behavior would be the increase in effective collision between SHMP and starch with the increasing amount of SHMP (Gui-Jie *et al.*, 2006).

Table 3.1: Peak viscosities and degrees of cross-linking of cross-linked *boyna* starch (mean $\pm$ SD, n=3).

CLS Samples	Peak Viscosity (cP)	Degree of Cross-linking (%)
CLS-A	1179.00 $\pm$ 29.14	54.46 $\pm$ 0.81
CLS-B	978.67 $\pm$ 15.53	62.20 $\pm$ 0.73
CLS-C	805.67 $\pm$ 19.04	68.88 $\pm$ 0.95

After cross-linking, the strengthened starch granule structure would resist rupture at high temperatures. Cross-linking is effective in inhibiting granular breakdown, and it results in a generally more thermally and mechanically stable starch. This behavior is observed from the decrease in peak viscosity values (Table 3.1) (Gunaratne and Corke, 2007).

3.1.2. Degree of substitution (DS)

The properties of starch acetates are a function of the acetyl content, type of starch, non-starch components, and method of pretreatment. And measurement of the acetyl content is a prime method for characterization of SAs (Wurzburg, 1964). The extent of physicochemical property changes in the acetylated starch compared to the NS is proportional to the degree of acetylation incorporated into the starch molecules (Sodhi and Singh, 2005). The DS of acetylated starches determined by the saponification titration method are summarized in Table 3.2.

It has been indicated that the degree of acetylation is apparently affected by reaction conditions - reaction time, pH, catalysts and AA concentration, and starch source (González and Pérez, 2002).

Table 3.2: Acetyl content and degree of acetylation of acetylated *boyna* starch (mean $\pm$ SD, n=3).

Acetylated starch samples	Starch/AA ratio	Acetyl content (%A)	Degree of substitution (DS)
SA-A	1:1	9.87 $\pm$ 0.559	0.339 $\pm$ 0.027
SA-B	1:2	16.88 $\pm$ 0.372	0.546 $\pm$ 0.022
Ac-CLS-A	1:1	7.22 $\pm$ 0.599	0.254 $\pm$ 0.027
Ac-CLS-C	1:1	5.22 $\pm$ 0.412	0.187 $\pm$ 0.018
Ac-CLS-E	1:1	4.52 $\pm$ 0.493	0.163 $\pm$ 0.021
Ac-CLS-B	1:2	12.91 $\pm$ 0.592	0.432 $\pm$ 0.028
Ac-CLS-D	1:2	10.51 $\pm$ 0.310	0.359 $\pm$ 0.014
Ac-CLS-F	1:2	8.79 $\pm$ 0.248	0.305 $\pm$ 0.011

Starch granules possessing surface pores or inner channels large enough to facilitate the physical access of the AA inside the granule, will enhance the acetylation reactions. In addition, intragranular packaging of starch granules could have an effect too, because the arrangement of amylose and amylopectin chains could affect the chemical substitution reaction in the glucose units of starch macromolecules (González and Pérez, 2002). It was observed that the DS increased linearly when the concentration of AA in the reaction medium increased (Shogren and Biswas, 2006).

The acetyl group content in acetylated native starch is slightly higher than those of cross-linked and acetylated starch. Thus, it can be inferred that the presence of cross-links in the cross-linked starch granules limited the reaction with AA. Considering the same proportion of AA, the DS of cross-linked and acetylated starch obtained in this study appears to be higher than that obtained for cross-linked and acetylated high amylose starch by Mulhbachter *et al.* (2001). This difference could be attributed to differences in the starch sources and variation in reaction conditions.

3.1.3. Solubility (S) and Swelling power (SP)

SP and S are important parameters to understand how modification affects drug release of starch matrices. The mechanism of water uptake and drug release by the gel matrix is strongly affected by the structural features of the network. These properties provide

evidence of the magnitude of interaction between starch chains within the starch granules (Lee *et al.*, 2005; O'Brien *et al.*, 2009).

As can be seen from Fig. 3.1 and 3.2, the S and SP of the starches are generally low at low temperatures (may be as a result of extensive and strongly bonded micellar structures) but increase significantly ($P < 0.05$) at higher temperature. It is likely that, as the temperature of the medium increased, starch molecules become more thermodynamically activated. As a result, granular mobility increases probably, due to macromolecular disorganization and enhanced water penetration (Mirmoghtadaie *et al.*, 2009). Moreover, when starch is heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling and solubility (Lee *et al.*, 2005).

All the CLSs exhibited lower S and SP values than the native and acetylated starches, and the S and SP decreased further with an increase in SHMP ratio (Fig. 3.1 and 3.2), which could be attributed to the increase in the DC. Cross-linking reinforces the structure of starch granules and limits water absorption by restricting the mobility of starch chains in the amorphous region (Gunaratne and Corke, 2007). It is also possible that as the cross-linking process evolves there may be a reduction of available hydroxyl groups, which are important to the swelling process (Curya *et al.*, 2009). Jyothi *et al.* (2006) pointed out that cross-linking reduces the soluble fractions which leach out of the swollen granules and the extent of solubilization is affected by the DC.

Acetylation increased the S and SP of both the NS and CLS (Fig. 3.1 and 3.2). Introduction of (bulky) acetyl group reduces the bond strength between starch molecules causing the opening up of the starch structure. This leads to structural reorganization owing to steric hindrance, which results in repulsion between starch molecules, thus rendering it more accessible to water and consequently increase in SP. It has been reported that the structural disorganization probably enhances amylose leaching from the starch granule, thus increasing starch solubility (Liu *et al.*, 1999; Singha *et al.*, 2007; Garga and Jana, 2011).

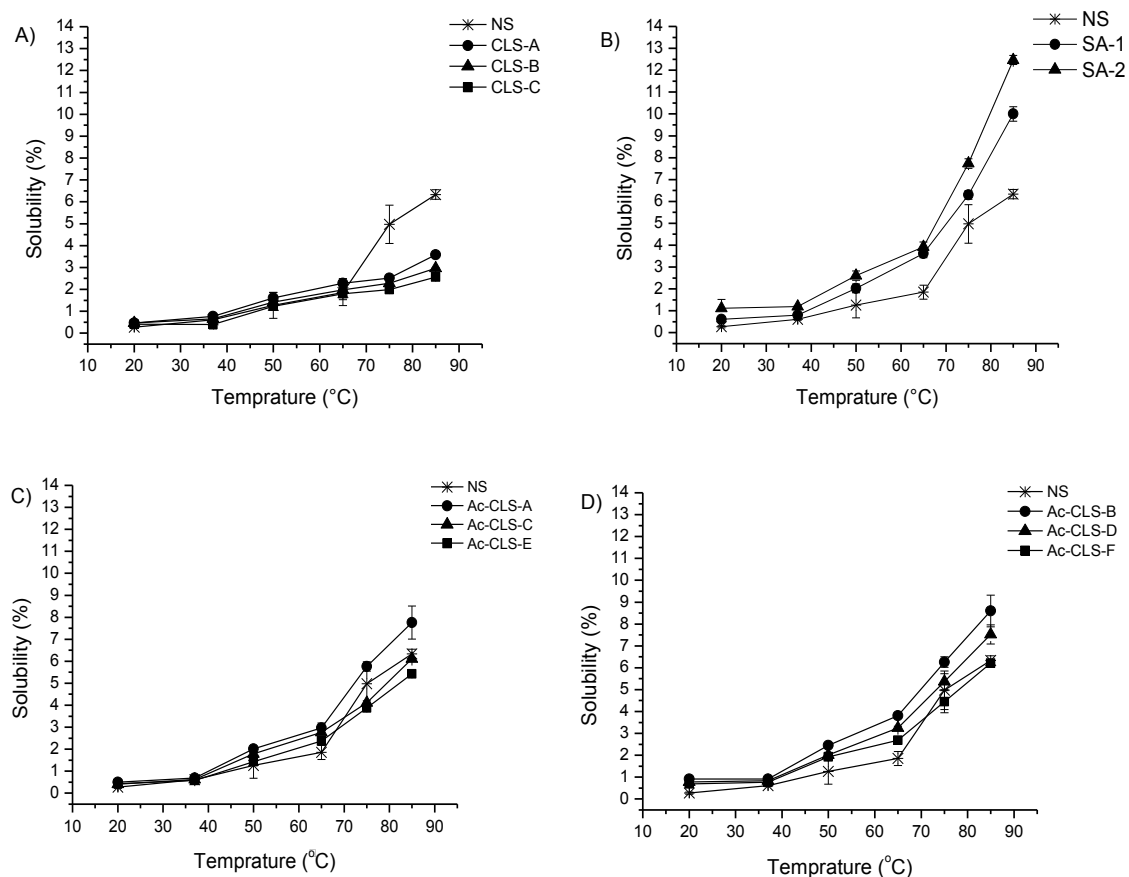


Figure 3.1: Solubility of cross-linked (A), acetylated (B), and dual-modified (C and D) starch samples at different temperatures in comparison to native *boyna* starch.

3.1.4. Moisture sorption pattern

Generally, the initial moisture level as well as the inherent tendency of the active ingredients and excipients for water uptake from the surrounding environment governs the moisture sorption pattern of the final product. The hygroscopic nature of excipients and active ingredients should be considered in designing formulations. Therefore, knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction is critical.

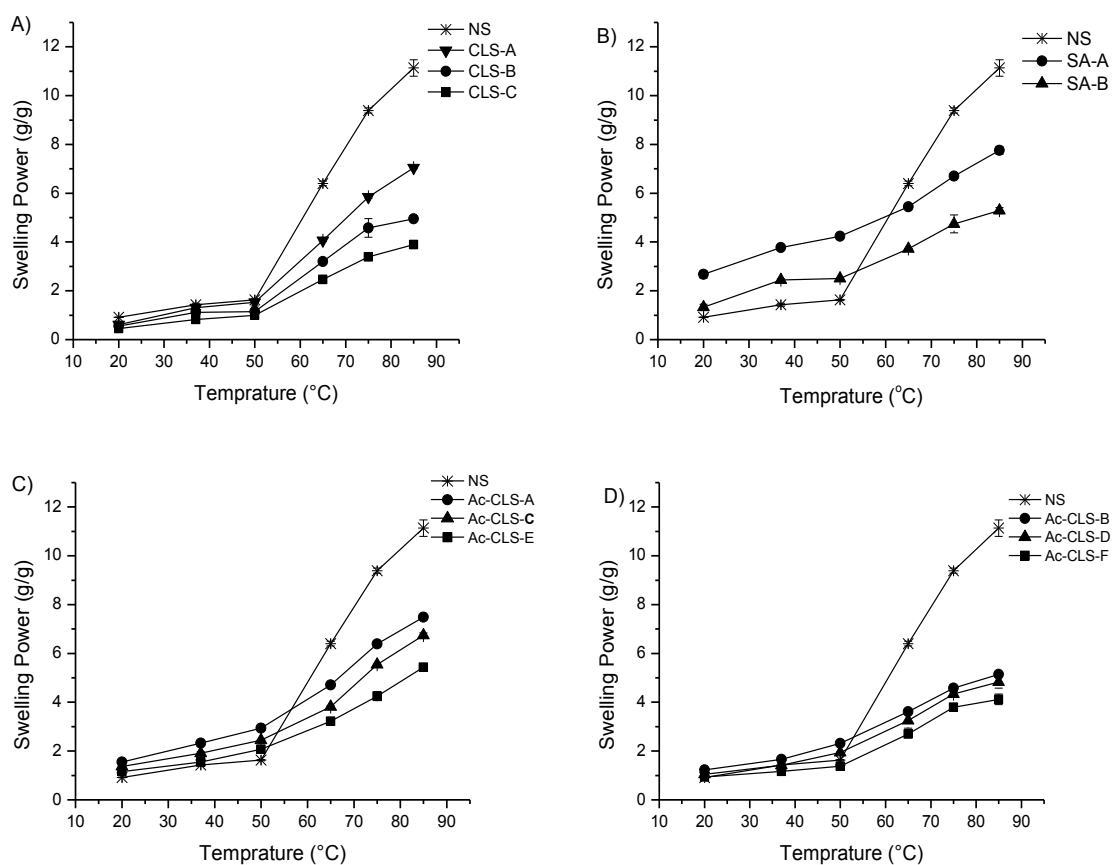


Figure 3.2: Swelling power of cross-linked (A), acetylated (B), and dual-modified (C and D) starch samples at different temperatures in comparison to native *boyna* starch.

The sorption isotherms show that the water uptake increased with the relative humidity (RH) for all samples (Fig.3.3). For the cross-linked samples, the water uptake is lower than the NS and decreases further with increasing SHMP ratio (Fig. 3.3A). A decrease in water sorption capacity with increasing Sodium trimetaphosphate concentration has been reported by Kulicke *et al.* (1990) which was attributed to an increase in the formation of diester phosphate linkages and a corresponding decrease in mesh width and water uptake. The mechanism of reduction of starch swelling and viscosity by cross-linking also may play a role in reducing water sorption of starch by cross-linking (Seker and Hanna, 2006). The acetylated starch samples showed significantly higher ($P < 0.05$) water uptake than the native, cross-linked and dual modified samples (Fig. 3.3). The introduction of acetyl

groups in starch could have facilitated the access of water to amorphous areas and increased water uptake (Sodhi and Singh, 2005).

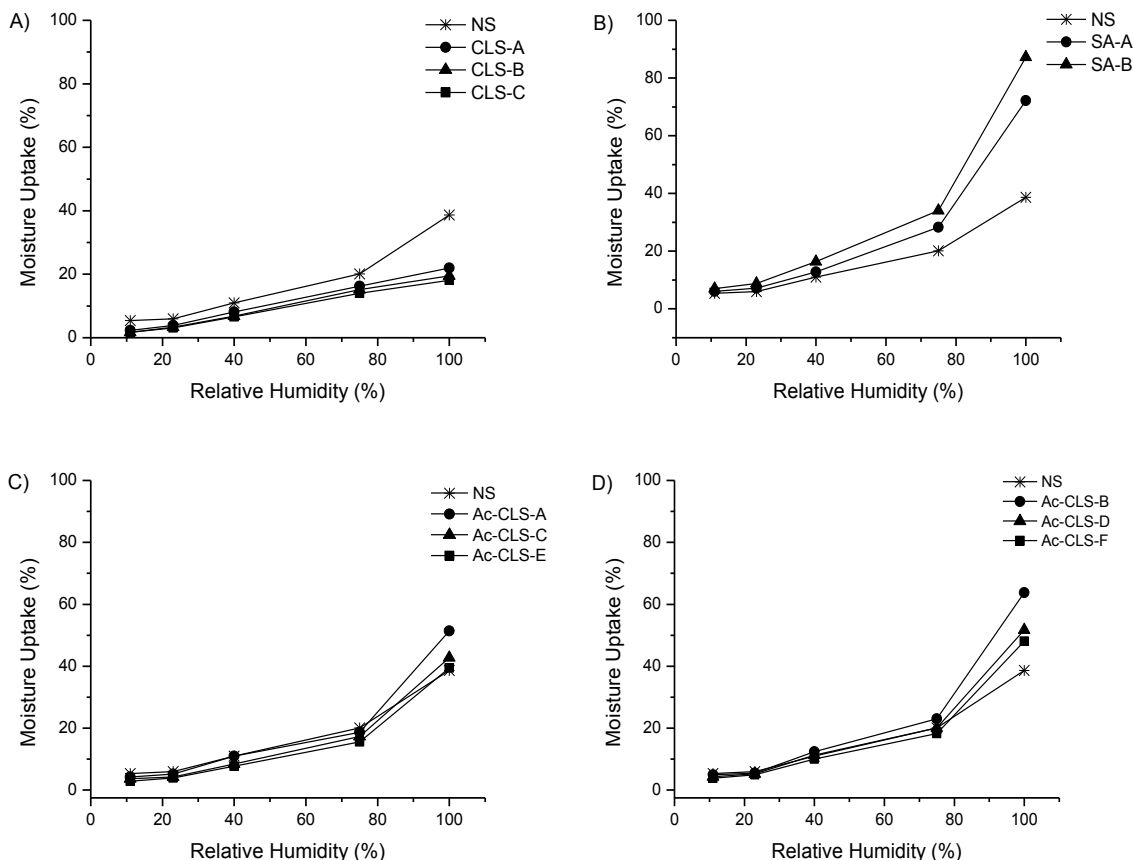


Figure 3.3: Moisture sorption patterns of cross-linked (A), acetylated (B), and dual-modified (C and D) starch samples at different RH in comparison to native *boyna* starch.

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.

3.1.5. Fourier-transform infrared (FTIR) spectra

Fig. 3.4 presents the FTIR spectra of the native and modified *boyna* starches. The spectra of the native starch (Fig. 3.4A) displays the typical profile of polysaccharides in the range 1100 - 920 cm^{-1} , characteristic peaks attributed to C-O/C-C bond stretching. Additional

characteristic absorption bands, appeared at 1000 to 700 cm^{-1} , which were due to anhydroglucose ring stretching vibrations. An extremely broad band due to hydrogen bonded-OH groups appeared at 3400 - 3000 cm^{-1} . A new small peak at 1266 - 1244 cm^{-1} , which is characteristic of P=O bonds in cross-linked starch, in the spectra of dual modified starch (Fig. 3.4B) indicated the formation of phosphate cross-links. This spectra also showed new band at 1750 - 1700 cm^{-1} , assigned to carbonyl C=O vibration confirming acetylation of the cross-linked starch (Stuart, 2004; Chi *et al.*, 2008; Shalviri *et al.*, 2010).

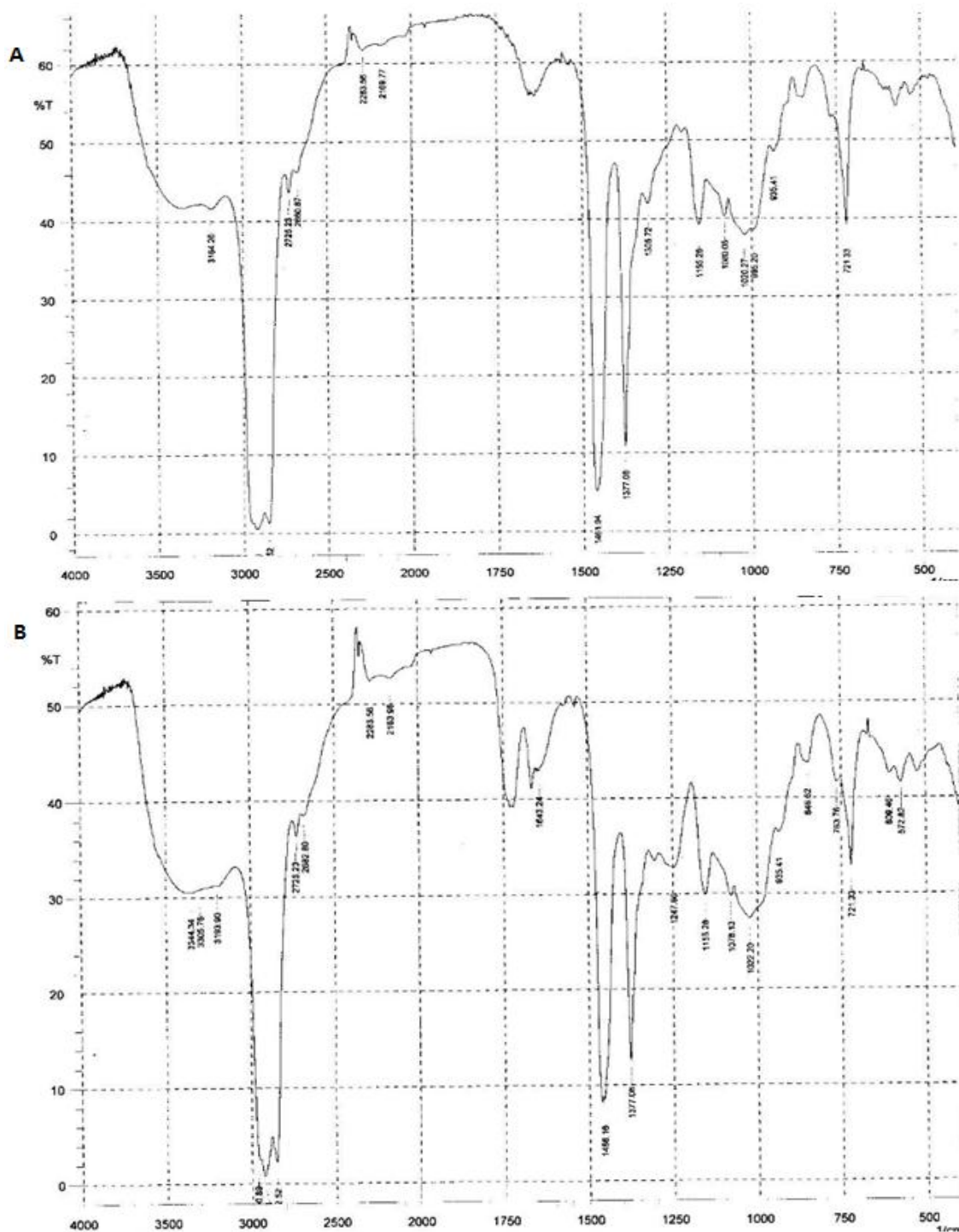
The IR spectra of theophylline and mixture of theophylline and dual modified starch are shown in figure 3.4C and 3.4D, respectively. A characteristic IR spectrum of theophylline (Fig. 3.4C) shows the following bands. The band around 1717 cm^{-1} is characteristic of the imide stretching of the heterocyclic moiety. A sharp band at 1666 cm^{-1} is due to tertiary amide group stretching vibrations. The N-H bending vibration is shown at 1568 cm^{-1} , whereas the band at 1242 cm^{-1} can be attributed to C-N stretching vibrations. All these prominent peaks appear in the mixture indicating the chemical compatibility of theophylline with the dual modified starch.

3.1.6. Powder properties

Powder properties are important in manufacturing of solid dosage forms. The properties are determined by a combination of powder characteristics (like particle size, size distribution, density, and surface properties) and operating conditions (like moisture) (Sinka *et al.*, 2004). For good tablets to be formed, the powder blend has to flow uniformly and form firm compaction. Good flowability ensures uniformity in die fill and thus uniformity in tablet weight. It also facilitates blending of fine powders encountered in direct compression blends (Atichokudomchai and Varavinit, 2003).

Some powder properties of the native and modified starches are presented in Table 3.3. The angle of repose could be used as a qualitative measure of the cohesiveness or the tendency of powder to flow. Angles of 30° or below are usually indicative of free flowing materials while an angle of 40° or above indicates a poor flow. Angle of repose is affected by the particle size distribution and it usually increases with a decrease in

particle size. The values of the bulk and tapped densities provide information on the flowability of powders and are used to calculate the Carr index and Hausner ratio, which are a measure of the flowability and compressibility of a powder (Odeku and Picker-Freyer, 2009).



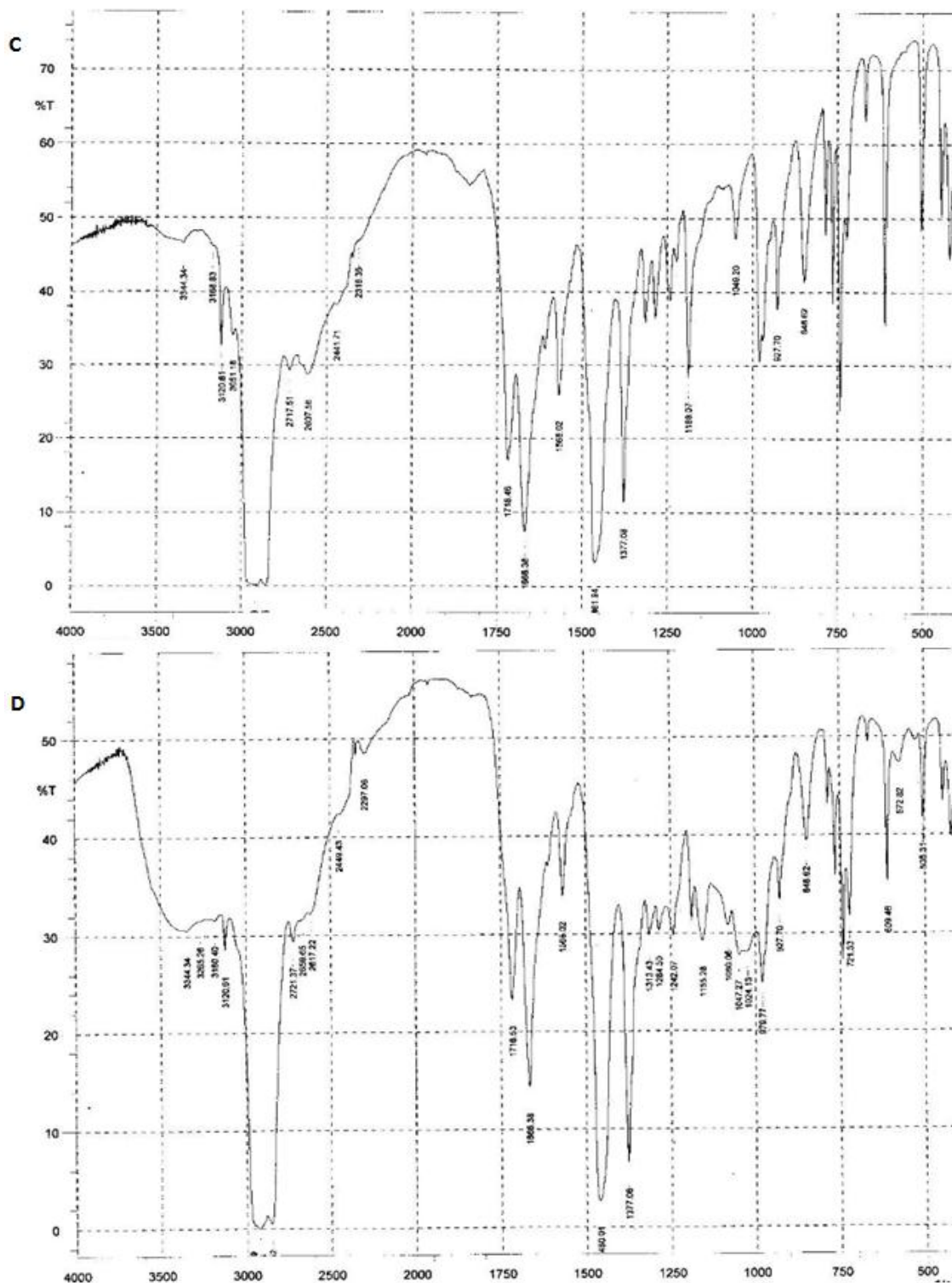


Figure 3.4: FTIR spectra of native starch (A), dual modified starch (B) pure theophylline (C) and physical mixture of theophylline and dual modified starch (D).

Table 3.3: Powder properties of the native and modified *boyana* starch (Mean $\pm$ SD)

Sample	Moisture Content (%)	Angle of Repose ($^{\circ}$)	Flow Rate (g/sec)	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Hausner Ratio	Carr Index (%)
NS	13.00 $\pm$ 0.500	*	*	0.60 $\pm$ 0.007	0.75 $\pm$ 0.000	1.26 $\pm$ 0.014	20.52 $\pm$ 0.905
CLS-A	8.17 $\pm$ 0.577	*	*	0.64 $\pm$ 0.008	0.81 $\pm$ 0.000	1.26 $\pm$ 0.016	20.71 $\pm$ 0.988
CLS-B	7.33 $\pm$ 0.288	*	*	0.66 $\pm$ 0.008	0.85 $\pm$ 0.014	1.28 $\pm$ 0.005	22.06 $\pm$ 0.279
CLS-C	5.67 $\pm$ 0.288	*	*	0.68 $\pm$ 0.009	0.88 $\pm$ 0.000	1.30 $\pm$ 0.017	23.85 $\pm$ 1.939
SA-A	11.17 $\pm$ 0.577	25.11 $\pm$ 0.493	3.09 $\pm$ 0.148	0.58 $\pm$ 0.006	0.69 $\pm$ 0.009	1.18 $\pm$ 0.002	22.36 $\pm$ 0.242
SA-B	10.33 $\pm$ 0.763	23.02 $\pm$ 1.106	10.54 $\pm$ 1.092	0.57 $\pm$ 0.006	0.71 $\pm$ 0.000	1.25 $\pm$ 0.014	20.24 $\pm$ 0.880
Ac-CLS-A	9.33 $\pm$ 0.577	24.85 $\pm$ 0.347	2.94 $\pm$ 0.184	0.62 $\pm$ 0.007	0.74 $\pm$ 0.011	1.12 $\pm$ 0.027	16.43 $\pm$ 1.894
Ac-CLS-C	8.70 $\pm$ 0.500	26.56 $\pm$ 0.618	2.64 $\pm$ 0.035	0.63 $\pm$ 0.008	0.78 $\pm$ 0.012	1.24 $\pm$ 0.017	19.58 $\pm$ 1.106
Ac-CLS-E	7.67 $\pm$ 0.763	27.53 $\pm$ 0.431	2.43 $\pm$ 0.060	0.63 $\pm$ 0.008	0.80 $\pm$ 0.012	1.26 $\pm$ 0.018	20.42 $\pm$ 1.122
Ac-CLS-B	8.83 $\pm$ 0.288	23.17 $\pm$ 0.574	8.84 $\pm$ 0.521	0.60 $\pm$ 0.007	0.72 $\pm$ 0.010	1.12 $\pm$ 0.027	16.42 $\pm$ 1.894
Ac-CLS-D	8.20 $\pm$ 0.500	25.89 $\pm$ 2.087	7.16 $\pm$ 0.513	0.62 $\pm$ 0.007	0.76 $\pm$ 0.011	1.24 $\pm$ 0.017	19.17 $\pm$ 1.084
Ac-CLS-F	7.30 $\pm$ 0.500	28.46 $\pm$ 0.545	4.42 $\pm$ 0.239	0.62 $\pm$ 0.007	0.77 $\pm$ 0.000	1.24 $\pm$ 0.015	19.30 $\pm$ 0.957

*Powder did not flow through the funnel

A good flow of the powder to be compressed is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets. The native and cross-linked samples displayed higher bulk and tapped densities than acetylated starches. The differences observed in the bulk and tapped density values could be due to the different particle size and shape which affecting the packing arrangement of the powder particles. All the cross-linked starches did not flow through a funnel, indicating poor flowability similar to the native starch. This is further confirmed by the Hausner ratio, which was generally higher than 1.25. The flow properties show no differences ($P > 0.05$) between the native and cross-linked starches. This is in agreement with results reported for cross-linked *Dioscorea* species by Odeku and Picker-Freyer (2009). The acetylated starches, on the other hand, showed better flowability than the native and cross-linked starch samples.

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). The moisture content reflects the affinity of the material for moisture (Parrott, 1989). As it can be seen from Table 3.1, cross-linking significantly reduced the moisture content of the NS. Carmona-Garcia *et al.* (2009) reported that moisture content of cross-linked banana starch decreased up to 100% compared with its native sample. They pointed out that this pattern is related to the reaction between the OH groups of glucose units of starch and the cross-linking reagent used, decreasing the possibility of reaction between OH of starch chains and the water molecules. A similar explanation has been given to the slight decrease in water content of acetylated starches by Raatikainen *et al.* (2002). They pointed out that replacing the hydroxyl groups in the starch with acetate moieties decreased the hydrophilicity of the material.

3.2. Evaluation of tablets

The crushing strength, friability, tensile strength and disintegration time of plain tablets are shown in Table 3.4.

Table 3.4: Crushing strength, tensile strength, friability and disintegration time of plain tablets of native, cross-linked, acetylated and dual modified *Dioscorea abyssinica* starch

Formulation	Crushing strength (N)	Tensile strength (Kg/cm ²)	Friability (%)	Disintegration time (min)	Weight (mg)
NS	46.90 ± 5.108	7.59 ± 0.639	*	< 5	395.00 ± 5.477
CLS-A	63.40 ± 6.186	9.96 ± 1.265	1.28	6	403.33 ± 5.163
CLS-B	78.40 ± 7.229	12.58 ± 1.573	1.26	11	396.66 ± 8.165
CLS-C	86.80 ± 6.812	13.99 ± 1.891	0.84	27	391.66 ± 9.832
SA-A	82.60 ± 9.979	10.91 ± 0.719	1.09	11	446.66 ± 5.164
SA-B	163.60 ± 6.785	18.64 ± 1.133	0.37	15	443.33 ± 5.164
Ac-CLS-A	96.40 ± 6.850	13.45 ± 0.831	0.47	23	418.33 ± 7.528
Ac-CLS-C	116.90 ± 7.489	15.44 ± 1.004	0.43	34	415.00 ± 5.477
Ac-CLS-E	121.40 ± 10.616	18.22 ± 0.952	0.38	53	403.67 ± 6.653
Ac-CLS-B	102.60 ± 9.857	19.56 ± 1.443	0.40	37	413.33 ± 8.165
Ac-CLS-D	177.70 ± 10.914	21.35 ± 1.393	0.21	65	401.67 ± 7.528
Ac-CLS-F	201.60 ± 8.884	24.08 ± 1.300	0.08	> 120	397.16 ± 4.021

*Some tablets broke during friability testing.

3.2.1. Crushing strength and friability

As can be seen in Table 3.4, the cross-linked starches showed improved compaction than the native form resulting in higher crushing forces. It has been indicated that cross-linking mainly takes place in amylopectin. Therefore, the reinforcement of amylopectin may lead to stronger (closer) packing within the crystalline regions. Thus tablets from cross-linked starches would likely have stronger packing than the native form. However, cross-linking alone cannot increase the crushing strength of the tablets since the amorphous regions still disrupt crystalline packing (Atichokudomchai and Varavinit, 2003). The table also displays an increased crushing force in tablets prepared from SAs. According to the literature the acetate moiety is found to be a very effective bond-forming substituent. Due to acetylation, the formation of strong molecular bonds, like van der Waal's forces, could increase several-fold (Raatikainen *et al.*, 2002). 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. The crushing strength was significantly higher for the dual modified starches as compared to the cross-linked and acetylated starches. This high crushing strength of dual modified starches could be attributed to the combined effects of cross-linking and acetylation which increase the bonding strength in the crystalline and amorphous regions, respectively.

The friability profiles of the tablets are in line with the crushing strength measurements. In general, increase in tablet hardness 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).

Increasing the theophylline ratio from 20% to 40% reduced the crushing strength of all the starch samples while increasing the friability (Table 3.5). An increase in friability with a decrease in hardness of tablets is expected.

3.2.2. Tensile strength

Tensile strength can be used to characterize the compactibility of pharmaceutical powders. Tensile strength of tablets may indicate bonding strengths of the tablets. As it can be seen from tensile strength values in Table 3.4, both cross-linking and acetylation appear to improve the compactibility of the native starch powder- their effect being notably higher in dual modified samples.

3.2.3. Disintegration time

All tablets made of plain cross-linked and acetylated starches disintegrated within a few minutes. However, the disintegration time showed dramatic increase in tablets made from plain dual modified starches. This can be explained by the significant increase in the mechanical strength of the dual modified starch tablets. Generally, disintegration times are related to hardness (Özyazici and Sevgi, 2003). The swelling and solubility characteristics could also have a role.

3.2.4. *In vitro* dissolution tests

Tablets made from cross-linked and acetylated starches were not selected for further dissolution studies because of their complete disintegration within a few minutes. The disintegration test revealed that those tablets made from CLS failed to swell and to form a gel of good physical strength while tablets from acetylated starch samples exhibited significant swelling and disintegrated within a few minutes. The dissolution tests were thus performed only on tablets containing dual modified starch samples (Ac-CLS-E and Ac-CLS-F) (Table 2.3 and 2.4). These dual modified starch matrices appear to absorb enough water to hydrate the polymer matrix, resulting in a gel of good physical stability, making them ideal candidates for sustaining drug release.

Table 3.5: Crushing strength, tensile strength, friability, and disintegration time of matrix tablets used for drug release studies

Formulation	Hardness (N)	Tensile Strength (Kg/cm ²)	Friability (%)	Disintegration time (min)
F1	171.47 ± 8.342	29.77 ± 1.970	0.10	> 120
F2	153.02 ± 7.101	25.32 ± 1.152	0.34	> 120
F3	140.74 ± 9.603	23.55 ± 1.448	0.39	90
F4	133.34 ± 6.032	20.09 ± 1.811	0.78	45

Even though covalent cross-linking and physical association (closely related to the cross-linking degree) are expected to be critical parameters for the matrix cohesion and for the SR properties, the network properties are also likely to be related to the hydration state of OH groups and retention of water by the polymer (Dumoulina *et al.*, 1998). The CLS could not absorb enough water to hydrate the polymer matrix which is important to form a gel of good physical stability. Onofre and Wang (2009) argued that introduction of substituent groups to starch chains improves their water holding capacity, probably leading to the formation of a better matrix. Dumoulina *et al* (1998) also showed that water has a role in the matrix organization in the cross-linked starch tablets. Network organization and water access are supposed to be determinants for the control of the drug release kinetics.

At low temperatures, acetylation probably occurs in the amorphous regions of the starch granules, leading to swelling in those regions (Shogren and Biswas, 2006). Cross-linking that strengthens the starch granules will allow stronger rigid granules to remain in the gel matrix. It is also possible that creation of more junction zones by the covalent cross-linked bonding could increase gel hardness (Gunaratne and Corke, 2007). A modification of the swelling property is expected to influence the diffusion properties of drugs through the tablet. An increase of the swelling volume of the matrix would enhance the drug permeability due to a larger space between the matrix chains whereas decreasing swelling will reduce it (Mulhbacher and Mateescu, 2005).

Dissolution studies for F1, F2 and F3 were performed in 0.1 N HCl for the first one and half hours and in phosphate buffer solution (pH 6.8) for the rest of the 12 h study period. F4 was more liable to erosion in 0.1 N HCl solution and thus the dissolution study was performed only in phosphate buffer solution. During the dissolution study, all the tablets remained at the bottom of the dissolution flask without sticking or flotation irrespective of the polymer composition and loading.

Fig. 3.5 illustrates the drug release profiles from these matrix tablet formulations. A higher percentage of drug release was observed from Ac-CLS-E (F4) compared with Ac-CLS-F based tablets (F1-F3). Longer release times from the later formulations can probably be explained by a limited water access into the Ac-CLS-F matrices due to the less hydrophilic character imparted by the increased acetyl moieties. Mulhbacher *et al.* (2001) reported a decrease of drug release rate with the increase in the ratio of acetylating agent for cross-linked and acetylated high amylose starch. They also pointed out a higher drug loading capacity of high DS derivatives. The swelling and gel forming properties of the polymers could also be related to the difference in release profiles.

The retarded drug release and non-disintegration behavior of F1, F2 and F3 tablet matrices could also be attributed to the better binding properties of the Ac-CLS-F polymer. 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).

From the release profiles, it can also be observed that polymer-to-drug ratio affected the release rate of the matrices. It appears that the drug-to-polymer ratio is the most important factor affecting the rate of the drug release from the matrix-based tablets (Jamzad *et al.*, 2005). When polymer concentration is low, the hydrated matrix would be highly porous with a low degree of tortuosity leading to low gel strength, rapid erosion of the matrix, and rapid diffusion of the drug from the matrix. On the other hand, an increase in the polymer concentration within the matrix causes an increase in viscosity of the gel consequently decreasing drug release rate. Furthermore, the presence of drug among starch molecules might disrupt the entanglement of chains, thus resulting in

weaker network structure and subsequently increased susceptibility to erosion (Pillay and Fassihi, 1999; Velasco *et al.*, 1999; Bettini *et al.*, 2001).

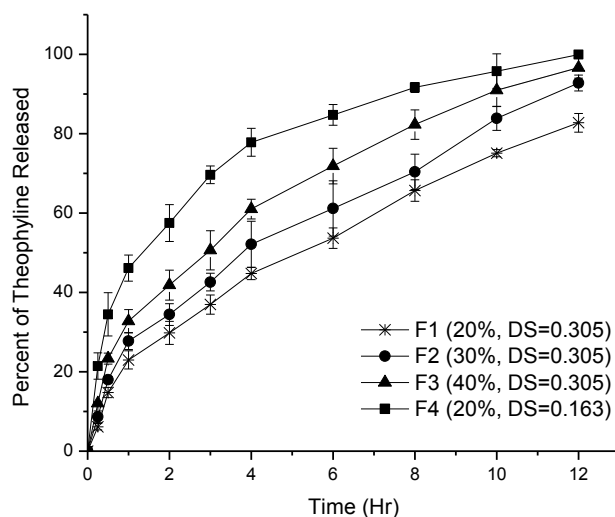


Figure 3.5: Release of theophylline from dual modified *boyna* starch tablets (500 mg) containing 20%, 30% and 40% drug with higher DS – Ac-CLS-F (0.305), and 20% drug with lower DS – Ac-CLS-E (0.163).

3.2.5. Release kinetics

Quantitative comparison of dissolution profiles can be carried out using model-dependent and model-independent methods. The model-dependent methods rely on selecting the proper mathematical model and comparing the goodness of fit and changes in fitted parameters. Model-independent approaches provide direct comparison of a test profile against a reference profile, without transforming to another mathematical expression (Long and Chen, 2009).

The drug release data obtained were subjected to different drug release models in order to establish the drug release mechanisms and kinetics (Fig. 3.6). The release data were treated according to zero-order, first order, Higuchi's and Hixson-Crowell cube root law models.

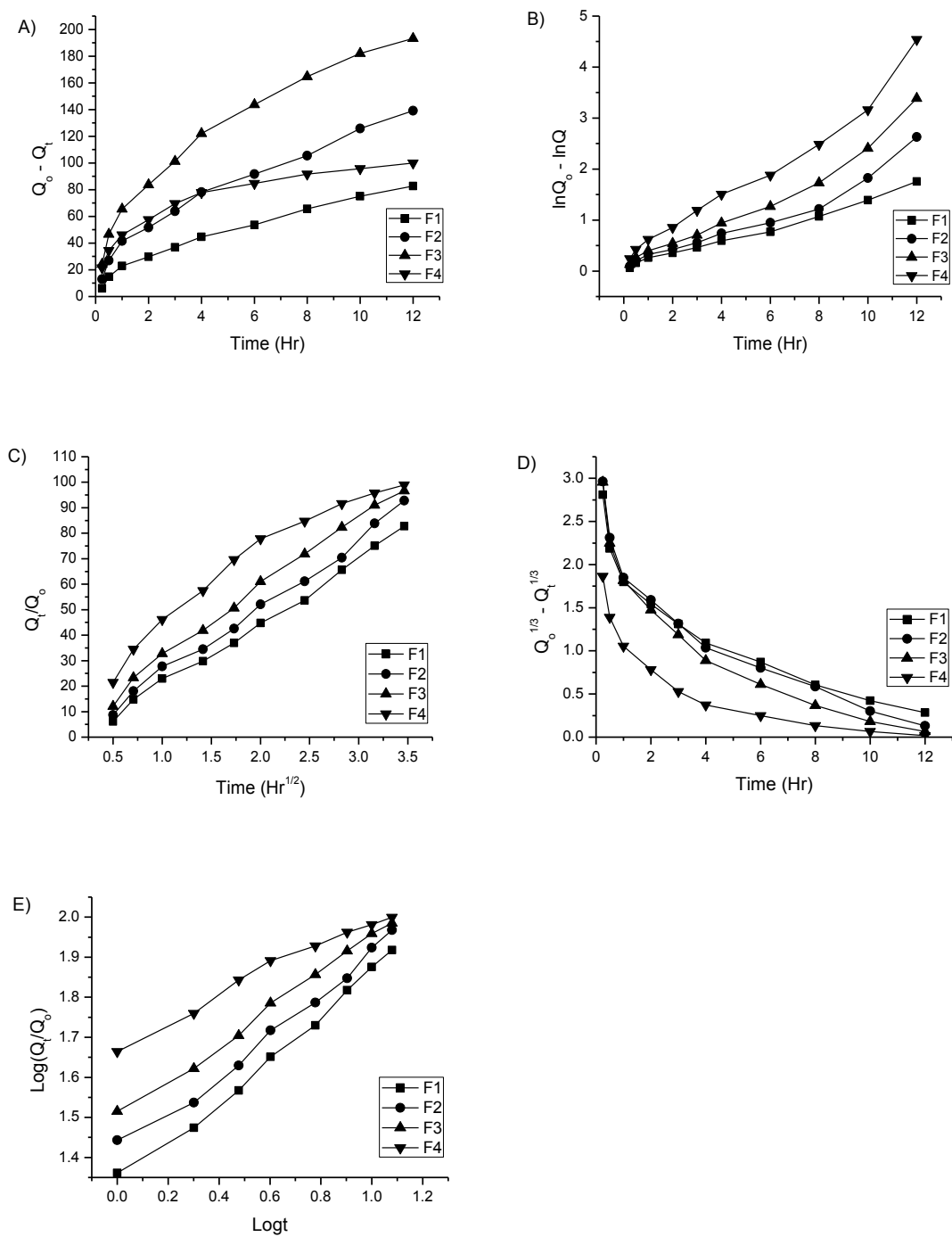


Figure 3.6: The release data from the different formulations fitted to various release kinetic models; zero order (A), first order (B), Higuchi (C), Hixson-Crowell (D) and Korsmeyer-Peppas (E) models.

Dissolution data were also fitted to the well-known exponential equation, Korsmeyer-Peppas model, which is often used to describe the drug release mechanism from polymeric systems (Table 3.6).

Table 3.6: Interpretation of drug release mechanisms from release exponent values (n) in Korsmeyer-Peppas model (Siepmann, and Peppas, 2001).

Release exponent (n)			Drug transport mechanism
Thin film	Cylindrical	Spherical	
0.5	0.45	0.43	Fickian diffusion
$0.5 < n < 1.0$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Anomalous transport
1.0	0.89	0.85	Case-II transport
> 1.0	> 0.89	> 0.85	Super Case-II transport

The curvilinear nature of the cumulative amount of drug released versus time plots (Fig. 3.6A) 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, non-linearity of the Hixson-Crowell plot (Fig. 3.6D) suggests non-applicability of Hixson-Crowell model.

Table 3.7 shows the parameters and statistical estimates of the dissolution data from the different formulations fitted to the different mathematical models, of these, the best fit to Higuchi model ($R^2 > 0.994$), and the n values from Korsmeyer-Peppas equation (0.452 - 0.530) suggest that drug release is controlled mainly by diffusion from F1, F2 and F3. Several authors have also postulated a diffusion controlled mechanism when evaluating the drug release mechanism from matrices obtained from polymeric systems (Tahara *et al.*, 1995; Colombo *et al.*, 1996; Jamzad *et al.*, 2005). The n values from Korsmeyer-Peppas equation for F1, F2 and F3 also confirmed that these products followed non-Fickian kinetics. In case of F4, a quasi-Fickian diffusion behavior was exhibited ($n = 0.31$).

Table 3.7: Parameter and statistical estimates of the dissolution data from the different formulations fitted to the different mathematical models

Models	Parameters	Formulations			
		F1	F2	F3	F4
Zero order	R^2	0.965	0.962	0.934	0.831
	$K_o(\mu\text{gh}^{-1})$	6.102	9.891	13.506	5.956
First order	R^2	0.990	0.950	0.966	0.973
	$K_1(\text{h}^{-1})$	0.134	0.188	0.248	0.324
Higuchi	R^2	0.996	0.994	0.995	0.951
	$K_H(\text{h}^{-1/2})$	24.879	26.904	27.976	25.383
Hixson-Crowell	R^2	0.839	0.845	0.822	0.739
	$K_{HC}(\text{h}^{-1/3})$	0.178	0.200	0.206	0.128
Korsmeyer-Peppas	R^2	0.992	0.988	0.996	0.982
	$K(\text{h}^{-n})$	1.333	1.412	1.502	1.678
	n	0.530	0.497	0.452	0.311

4. CONCLUSION

Dual modified *boyna* starch was prepared with cross-linking followed by acetylation with the aim to provide the starch with more desirable functional properties for sustained release applications. The results obtained indicate that the physicochemical and material properties of the *boyna* starch were modified by the chemical processes employed. Properties of the CLS and SAs suggest that these derivatives could not be used for SR applications alone with the level of cross-linking and acetylation achieved in this study. The properties of the CLS showed poor flow properties but slightly improved compressibility. They effectively inhibited swelling of the native starch and showed lower solubility and moisture uptake. In contrast, SAs improved the flow and compressibility of the starch samples with increased swelling (at lower temperatures), solubility and moisture uptake. The cross-linking and acetylation improved bonding observed during tableting. Improved bonding due to cross-linking and acetylation led to increased compaction which in effect resulted in higher crushing strength. The degree of substitution of CLS by acetate functional groups was found to greatly modulate drug release, and the dual modified starch was able to extend the release for more than 12 h. FTIR spectral analysis verified a change in the chemical structures of the native starch molecules resulting from cross-linking and acetylation. It also verified that the excipients are compatible with the active drug used, theophylline. Based on the above findings, it is possible to conclude that the new dual modified starch-based polymeric excipient can be a potential candidate for the development of new sustained release matrices.

5. SUGGESTIONS FOR FURTHER WORK

The results of this study provided an insight into the sustained release properties of dual modified *Dioscorea abyssinica* starch. Based on these promising results the followings are suggested:

- Characterize the particle size distribution and morphological properties of the dual modified starch;
- Investigate the compressibility and deformation characteristics;
- Investigate sustained release properties of the dual modified starch with anionic, cationic and nonionic drugs;
- Modify the CLS into carboxymethyl and aminoethyl derivatives and study their sustained release properties.

REFERENCES

- Abdullah EC, Geldart D (1999). The use of bulk density measurements as flowability indicators. *Powder Technology*, **102**: 151-165.
- Apu AS, Pathan AH, Kibria G, Jalil R (2009). *In vitro* release kinetic study of theophylline from Eudragit RS PO and Eudragit RL PO matrix tablets. *Dhaka Univ. J. Pharm. Sci.*, **8**: 1-6.
- Atichokudomchai N, Varavinit S (2003). Characterization and utilization of acid-modified cross-linked Tapioca starch in pharmaceutical tablets. *Carbohydr. Polym.*, **53**: 263-270.
- Ayoub A, Rizvi S (2009). An Overview on the Technology of Cross-linking of Starch for Non-food Applications. *J. Plastic Film Sheet*, **25**: 25-45.
- BeMiller JN (1997). Starch modification: challenges and prospects. *Starch/Stärke*, **49**: 127-131.
- Bettini R, Catellani PL, Santi P, Massimo G, Peppas NA, Colombo P (2001). Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. *J. Control. Release*, **70**: 383-391.
- Bhat R, Karim A (2009). Impact of radiation processing on starch, *Comp. Rev. Food Sci. Food Saf*, **8**: 44-58.
- Carmona-Garcia R, Sanchez-Rivera MM, Méndez-Montealvo G, Garza-Montoya B, Bello-Pérez LA (2009). Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisiaca*). *Carbohydr. Polym.*, **76**: 117-122.
- Chen Z, Schols A, Voragen G.J (2004). Differently sized granules from acetylated potato and sweet potato starches differ in the acetyl substitution pattern of their amylose populations. *Carbohydr. Polym.*, **56**: 219 - 226.
- Chi H, Xu K, Wu X, Chen O, Xue D, Song C, Zhang W, Wang P (2008). Effect of acetylation on the properties of corn starch. *Food Chem*, **106**: 923 - 928.
- Chiu C, Solarek D (2009). Modification of Starches. In: BeMiller J, Whistler R eds, *Starch: Chemistry and Technology*, 3rd edn, Elsevier Inc., USA, pp 629-655.

- Chung H, Liu Q, Hoover R (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches, *Carbohydr. Polym.* **75**: 436-447.
- Colombo P, Bettini R, Santi P, De Ascentiis A, Peppas NA (1996). Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. *J. Control. Release*, **39**: 231-237.
- Copeland L, Blazek J, Salman H, Tang MC (2009). Form and functionality of starch. *Food Hydrocol.*, **23**: 1527-1534.
- Curya BSF, Castroa AD, Kleinb SI, Evangelista RC (2009). Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 2: Physical parameters, cross-linking degrees and drug delivery relationships. *Int. J. Pharm.*, **371**: 8-15.
- Das AB, Singh G, Singh S, Riar CS (2010). Effect of acetylation and dual modification on physicochemical, rheological and morphological characteristics of sweet potato (*Ipomoea batatas*) starch. *Carbohydr. Polym.*, **80**: 725-732.
- Dredan J, Antal I, Racz I (1996). Evaluation of mathematical models describing drug release from lipophilic matrices. *Int. J. Pharm.*, **145**: 61-64.
- Ellis R, Cochrane P, Dale F, Dupus C, Lynn A, Morrison I, Prentice D, Swanston S, Tiller S (1998). Starch Production and industrial use. *J. Sci. Food Agric.*, **77**: 289-311.
- Garga S, Jana AK (2011). Characterization and evaluation of acylated starch with different acyl groups and degrees of substitution. *Carbohydr. Polym.*, **83**: 1623-1630.
- Gebre-Mariam T, Schmidt PC, (1996). Isolation and physicochemical properties of enset starch. *Starch/Stärke*, **48**: 241-246.
- Gebre-Mariam T, Schmidt PC, (1998). Some Physico-chemical properties of *dioscorea* starch from Ethiopia. *Starch/Stärke*, **50**: 241-246.
- González Z, Pérez E (2002). Effect of acetylation on some properties of rice starch. *Starch/Stärke*, **54**: 148-154.

- Gui-Jie M, Peng W, Xiang-Sheng M, Xing Z, Tong Z (2006). Crosslinking of corn starch with Sodium trimetaphosphate in solid state by microwave irradiation. *J. App. Pol. Sci.*, **102**: 5854-5860.
- Gunaratne A, Corke H (2007). Functional properties of hydroxypropylated, cross-linked, and hydroxypropylated cross linked tuber and root starches. *Cereal Chem.*, **84**: 30-37.
- Jamzad S, Tutunji L, Fassihi R (2005). Analysis of macromolecular changes and drug release from hydrophilic matrix systems. *Int. J. Pharm.*, **292**: 75-85.
- Jayakody L, Hoover R, Liu Q, Donner E (2007). Studies on tuber starches. II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea* spp.) starches grown in Sri Lanka. *Carbohydr. Polym.*, **69**: 148-163.
- Jyothi AN, Moorthy SN, Rajasekharan KN (2006). Effect of cross-linking with epichlorohydrin on the properties of cassava (*Manihot esculenta* Crantz) starch. *Starch/Stärke*, **58**: 292-299.
- Korhonen O, Kanerva H, Vidgren M, Urtti A, Ketolainen J (2004). Evaluation of novel starch acetate-diltiazem controlled release tablets in healthy human volunteers. *J. Control. Release*, **95**: 515-520.
- Korhonen O, Pohja S, Peltonen S, Suihko E, Vidgren M, Paronen P, Ketolainen J (2002). Effects of physical properties for starch acetate powders on tableting. *AAPS Pharm. Sci. Tech*, **3**: 1-9.
- Kulicke W, Aggour Y.A, Elsabe M. Z (1990). Preparation, characterisation, and rheological behaviour of starch-Sodium trimetaphosphate hydrogels. *Starch/Stärke*, **42**: 134-141.
- Lee JS, Kumar RN., Rozman HD., Azemi BMN (2005). Pasting, swelling and solubility properties of UV initiated starch-graft-poly (AA). *Food Chem.*, **91**: 203-211.
- Lemieuxa M, Gosselinb P, Mateescu MA (2009). Carboxymethyl high amylose starch as excipient for controlled drug release: Mechanistic study and the influence of degree of substitution. *Int. J. Pharm.*, **382**: 172-182.
- Lenaertsa V, Moussab I, Dumoulin Y, Mebsouta F, Chouinarda F, Szabod P, Mateescu MA, Cartilier L, Marchessault R (1998). Cross-linked high amylose starch for controlled release of drugs: Recent advances. *J. Control. Release*, **53**: 225-234.

- Lim S, Seib PA (1993). Preparation and pasting properties of wheat and corn starch phosphates. *Cereal Chem.*, **70**: 137-144.
- Liu H, Ramsden L, Corke H (1999). Physical properties of cross-linked and acetylated normal and waxy rice starch. *Starch/Stärke*, **51**: 249-252.
- Long M, Chen Y (2009). Dissolution testing of solid products. In: Qiu Y, Chen Y, Zhang G, Liu L, Porter WR eds, *Developing solid oral dosage forms: pharmaceutical theory and practice*. Elsevier Inc., USA, pp 469-499.
- Mirmoghtadaie L, Kadivar M, Shahedi M (2009). Effects of cross-linking and acetylation on oat starch properties. *Food Chemistry*, **116**: 709-713.
- Mulhbacher J, Ispas-Szabo P, Lenaerts V, Mateescu MA (2001). Crosslinked high amylose starch derivatives as matrices for controlled release of high drug loadings. *J. Control. Release*, **76**: 51-58.
- Mundargi RC, Shelke NB, Rokhade AP, Patil SA, Aminabhavi TM (2008). Formulation and in-vitro evaluation of novel starch-based tableted microspheres for controlled release of ampicillin. *Carbohydr. Polym.*, **71**: 42-53.
- Nattapulwat N, Purkkao N, Suwithayapanth N (2008). Evaluation of native and carboxymethyl yam (*Dioscorea esculenta*) starches as tablet disintegrants. *Silpakorn U Science & Tech J*, **2**: 18-25.
- O'Brien S, Wang Y-J, Vervaet C, Remon J.P (2009). Starch phosphates prepared by reactive extrusion as a sustained release agent. *Carbohydr. Polym.*, **76**: 557-566.
- Odeku O, Picker-Freyer K (2007). Analysis of the material and tablet formation properties of four *Dioscorea* Starches. *Starch/Stärke*, **59**: 430-444.
- Odeku O, Picker-Freyer K (2009). Evaluation of the material and tablet formation properties of modified forms of *Dioscorea* starches. *Drug Dev. Ind Phar*, **35**: 1389-1406.
- Odeku O, Schmidt W, Picker-Freyer KM (2008). Material and tablet properties of pregelatinized (thermally modified) *Dioscorea* starches. *Eur. J. Pharm. Biopharm.*, **70**: 357-371.
- Okunlola A, Odeku OA (2009). Compressional characteristics and tableting properties of starches obtained from four *Dioscorea* species. *FARMACIA*, **57**: 756-770.

- Okunlola A, Odeku OA (2011). Evaluation of starches obtained from four *Dioscorea* species as binding agent in chloroquine phosphate tablet formulations. *Saudi Pharm. J.*, **19**: 95-105.
- Onofre F, Wang YJ (2010). Hydroxypropylated starches of varying amylose contents as sustained release matrices in tablets. *Int. J. Pharm.*, **385**: 104-112.
- Onofre F, Wang YJ, Mauromoustakos A (2009). Effects of structure and modification on sustained release properties of starches. *Carbohydr. Polym.*, **76**: 541-547.
- Özyazici M, Sevgi F (2003). Formulation and evaluation of salbutamol sulphate tablets prepared by direct compression using various excipients: statistical comparison of dissolution methods and tablet properties. *FABAD J. Pharm. Sci.*, **28**: 1-11.
- Parrott E.L (1989). Comparative evaluation of a new direct compression excipient, soludex. *Drug Dev. Ind. Pharm.*, **15**: 561-583.
- Pillay V, Fassihi R (1999). Electrolyte-induced compositional heterogeneity: a novel approach for rate-controlled oral drug delivery. *J. Pharm. Sci.*, **88**: 1140-1148.
- Qiu Y (2009). Rational design of oral modified-release drug delivery systems. In: Qiu Y, Chen Y, Zhang G, Liu L, Porter WR eds, *Developing solid oral dosage forms: pharmaceutical theory and practice*. Elsevier Inc., USA, pp 469-499.
- Raatikainen P, Korhonen O, Peltonen S, Paronen P (2002). Acetylation enhances the tableting properties of starch. *Drug Dev. Ind. Pharm.*, **28**: 165-175.
- Raja S.T, Palanichamy S, Shanmuganathan S, Tamilvanan S, Thanga T.A (2009). Formulation and evaluation of theophylline controlled release matrix tablets using guar gum. *Ars. Pharm.*, **50**: 205-214.
- Reddy I, Seib PA (2000). Modified waxy wheat starch compared to modified waxy corn starch. *J. Cereal Sci.*, **31**: 25-39.
- Reddy N, Yang Y (2010). Citric acid cross-linking of starch films. *Food Chem.* 118: 702-711.
- Riley CK, Wheatley AO, Asemota HN (2006). Isolation and characterization of starches from eight *Dioscorea alata* cultivars grown in Jamaica. *Afr. J. Biotechnol.*, **5**: 1528-1536.

- Salwa M, Hanan M.A, Nessrien M.N (2010). Physicochemical properties of starch extracted from different sources and their application in pudding and white sauce. *World J. Dairy and Food Sci.*, **5**(2): 173-182.
- Schwartz D, Whistler R (2009). History and future of starch. In BeMiller J, Whistler R "Starch: Chemistry and Technology" 3rd Ed., *Elsevier Inc.* pp. 1-10.
- Seker M, Hanna MA (2006). Sodium hydroxide and trimetaphosphate levels affect properties of starch extrudates. *Ind. Crops Prod.*, **23**: 249-255.
- Shogren RL, Biswas A (2006). Preparation of water-soluble and water-swellaable starch acetates using microwave heating. *Carbohydr. Polym.*, **64**: 16-21.
- Siepmann, J, Peppas, NA (2001). Modeling of drug release from delivery systems based on hydroxypropyl methyl cellulose (HPMC). *Adv. Drug Del. Rev.*, **48**: 139-157.
- Singh J, Kaur L, Singh N (2004). Effect of acetylation on some properties of corn and potato starches. *Starch/Stärke*, **56**: 586-601.
- Singha J, Kaurb L, McCarthy OJ (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications-A review. *Food Hydrocol.*, **21**: 1-22.
- Sinka IC, Schneider LCR, Cocks ACF (2004). Measurement of the flow properties of powders with special reference to die fill. *Int. J. Pharm.*, **280**: 27-38.
- Sodhi N.S, Singh N (2005). Characteristics of acetylated starches prepared using starches separated from different rice cultivars. *J. Food Eng.*, **70**: 117-127.
- Sood A, Panchagnula R (1998). Drug release evaluation of diltiazem CR preparations. *Int. J. Pharm.*, **175**: 95-107.
- Stuart B (2004). Experimental method. In: Stuart B ed, *Infrared spectroscopy: fundamentals and applications*, John Wiley and Sons, New York, pp 15 - 44.
- Swinkels JJM (1985). Composition and properties of commercial native starches. *Starch/Stärke*, **37**(1): 1-5.
- Tahara K, Yamamoto K, Nishihata T (1995). Overall mechanism behind matrix sustained release (SR) tablets prepared with hydroxypropyl methylcellulose 2910. *J. Control. Release*, **35**: 59-66.
- Tester R, Karkalas J, Qi X (2004). Starch-composition, fine structure and architecture. *J. Cereal Sci.*, **39**: 151-165.

- Velasco MV, Ford JL, Rowe P, Rajabi-Siahboomi AR (1999). Influence of drug:hydroxypropylmethylcellulose ratio, drug and polymer particle size and compression force on the release of diclofenac sodium from HPMC tablets. *J. Control. Release*, **57**: 75-85.
- Wadchararat C, Thongngam M, Naivikul O (2006). Characterization of pregelatinized and heat moisture treated rice flours, *Kasetsart J. Nat. Sci.*, **40**: 144-153.
- Wattanachanta S, Muhammad K, Hashim DM, Rahman RA (2003). Effect of cross-linking reagents and hydroxypropylation levels on dual-modified sago starch properties. *Food Chem.*, **80**: 463-471.
- Woo K, Seib PA (1997). Cross-linking of wheat starch and hydroxypropylated wheat starch in alkaline slurry with Sodium trimetaphosphate. *Carbohydr. Polym.*, **33**: 263-271.
- Wurzburg OB (1964). Esters: acetylation. In: Whistler R ed, *Methods in carbohydrate chemistry*, 4th edn, Academic Press, New York, pp 286-288.
- Yoon HS, Lee JH, Lim ST (2009). Utilization of retrograded waxy maize starch gels as tablet matrix for controlled release of theophylline. *Carbohydr. Polym.*, **76**: 449-453.
- Zuo JY, Knoerzer K, Mawson R, Kentish S, Ashokkumar M (2009). The pasting properties of sonicated waxy rice starch suspensions. *Ultrason. Sonochem.*, **16**: 462-468.