# ADDIS ABABA UNIVERSITY
## COLLEGE OF HEALTH SCIENCES
## SCHOOL OF PHARMACY

### DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY


# PREPARATION AND CHARACTERIZATION OF MODIFIED *DIOSCOREA BULBIFERA* STARCH AND EVALUATION OF ITS USE AS A DISINTEGRANT IN TABLET FORMULATIONS


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# PREPARATION AND CHARACTERIZATION OF MODIFIED *DIOSCOREA BULBIFERA* STARCH AND EVALUATION OF ITS USE AS A DISINTEGRANT IN TABLET FORMULATIONS

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

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# ADDIS ABABA UNIVERSITY

# SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Meseret Adugna, entitled "Preparation *and characterization of modified Dioscorea bulbifera starch and evaluation of its use as a disintegrant in tablet formulations"* submitted in partial fulfilment of the requirements for Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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*Dedicated to my families with love, appreciation and respect*

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## ACRONYMS

| | |
|---|---|
| AGU: | Anhydrous glucose unit |
| APIs: | Active pharmaceutical ingredients |
| BP: | British Pharmacopoeia |
| CM: | Carboxymethyl |
| CMS: | Carboxymethyl starch |
| DBS: | *Dioscorea bulbifera* starch |
| DS: | Degree of substitution |
| DSC: | Differential scanning calorimetry |
| DT: | Disintegration time |
| FTIR: | Fourier transform infrared |
| GRAS: | Generally regarded as safe |
| MCA: | Monochloroacetic acid |
| NDBS: | Native *Dioscorea bulbifera* starch |
| PGDBS: | Pregelatinized *Dioscorea bulbifera* starch |
| PGS: | Pregelatinized starch |
| RE: | Reaction efficiency |
| RH: | Relative humidity |
| RSMR: | Reagent to starch molar ratio |
| SCMDBS: | Sodium Carboxymethyl *Dioscorea bulbifera* starch |
| SCMPGDBS: | Sodium Carboxymethyl pregelatinized *Dioscorea bulbifera* starch |
| SCMRSs: | Sodium carboxymethyl rice starches |
| SEM: | Scanning electron micrograph |
| SMCA: | Sodium monochloroacetic acid |
| SSG: | Sodium starch glycolate |
| USP: | United States Pharmacopoeia |

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## ABSTRACT

Starch is a naturally occurring biodegradable polysaccharide. It is found in different parts of plant organs, including seeds, fruits, tubers, roots and bulbil. Locally, *Dioscorea bulbifera* is a rich source of starch that forms an important dietary supplement. Starch is usually modified either chemically or physically to overcome shortcomings of native starch, making them more suitable for specific uses. This study aims to prepare modified *D. bulbifera* starch (DBS) by pregelatinization and carboxymethylation and evaluate its disintegrant effect in tablet formulations. In this study, DBS was isolated from bulbil and modified by pregelatinization and carboxymethylation. Pregelatinized starch was prepared using 2:1(water to starch ratio) that was heated at 64 ˚C for 15 min. Carboxymethylation was carried out in 80% isopropanol at different molar ratio of monochloroacetic acid: anhydrous glucose unit (MCA:AGU) (0.2:1, 0.6:1 and 1:1) and at 20% and 30% of NaOH using native and pregelatinized *D. bulbifera* starch (PGDBS). The influences of the amount MCA, NaOH and types of starch on degree of substitution (DS) of carboxymethylated starch were investigated. Properties of pregelatinized and carboxymethylated starch such as, densities, flow, swelling power, percentage solubility and moisture sorption were investigated and compared to those of native *D. bulbifera* starch (NDBS), Starch 1500® and sodium starch glycolate (SSG). Tablets were produced by wet granulation method using modified DBS as disintegrant at different concentrations: PGDBS (5%, 7.5%, 10% and 12.5%), sodium carboxymethyl DBS (SCMDBS) and sodium carboxymethyl pregelatinized DBS (SCMPGDBS) (2%, 4%, 6% and 8%) using paracetamol as a model drug. The granules prepared at constant binder concentrations (povidone 3%) were characterized for density related properties and flow properties and the tablets were tested for weight uniformity, crushing strength, friability, disintegration time (DT) and dissolution rate using standard methods. FTIR spectral findings show the presence of the carboxymethylated group on modified starch granule with new band at 1593.09 $cm^{-1}$; and for pregelatinized starch weak absorption at 1610 $cm^{-1}$ due to bound water. The FTIR of a mixture of paracetamol and SCMDBS or PGDBS showed no interaction. Both were found to be compatible with paracetamol. The DS value increased with increasing molar ratio of MCA: AGU from 0.2 to 1 with 30% NaOH. Higher DS obtained with SCMDBS (0.36652+0.021) showed higher swelling power followed by SSG and SCPGDBS. The moisture sorption profiles indicated that the relative humidity during tablet production and storage should be carefully monitored. The shortest DT was obtained for tablets prepared at 4% disintegrant concentration with SCMDBS (0.49$\pm$0.01 min) followed by SSG (0.5$\pm$0.01 min) and SCMPGDBS (0.53$\pm$0.011 min). Swelling power exhibited some direct effect on disintegrant

activity of the starches. The tablets prepared with PGDBS and Starch 1500® exhibit comparable characteristics. The results also indicated that the properties of paracetamol tablets formulated with native and modified starches as disintegrants were affected by their concentration and nature of disintegrant. The dissolution studies of paracetamol tablets fulfilled USP requirements (quantity dissolved in 30 min ≥ 80%). Paracetamol tablets prepared with SCMDBS exhibited shorter DT than others but generally all the tablets containing the NDBS and modified DBS also passed the official disintegration time test.

**Keywords:** *Dioscorea bulbifera* starch, pregelatinization, carboxymethylation, sodium carboxymethyl pregelatinized DBS, tablets disintegrant.

# 1. INTRODUCTION

## 1.1. Starch

Starch is a natural, cheap, available, renewable and biodegradable polymer produced by many plants as a source of stored energy. It is the second most abundant, after cellulose, biomass material in nature. It is widely distributed in the form of tiny granules as the major reserve carbohydrate in plant leaves, stems, roots, bulbs, nuts, stalks, crop seeds, and staple crops such as rice, corn, wheat, cassava, and potato, and fruits of all forms of green leafed plants (Rahman, 2007; Neelam et al., 2012; Ochubiojo and Rodrigues, 2012).

Being a carbohydrate, starch is composed of two types of alpha-glucans: amylose and amylopectin, which represent approximately 98–99% of the dry weight with the remains comprised of small amounts of ash, protein, lipids, minerals, and phosphorus in the form of phosphates esterified to glucose hydroxyls (Gebre-Mariam and Schmidt, 1996; Lindeboom et al., 2004; Tester et al., 2006; Desai et al., 2016).

Amylose is essentially unbranched α [1→ 4] linked glucoses, and amylopectin has chains of α [1→ 4] linked glucoses arranged in a highly branched structure with α [1→ 6] branching links. The structures of amylose and amylopectin are given in Figure 1.1. Amylopectin is a much larger molecule than amylose with a molecular weight and a heavily branched structure built from about 95% (α 1→ 4) and 5% (α 1- 6) linkages. Amylopectin unit chains are relatively short compared to amylose molecules with a broad distribution profile (Tester et al., 2006).

The ratio of the two polysaccharides varies according to the botanical origin of the starch and classified as the ‘waxy’ starches containing less than 15% amylose, ‘normal’ 20–35% and ‘high’ amylose starches greater than 40% (Tester et al., 2006).

## 1.1. Sources of starch

The most important sources of starch are represented by cereals, tubers, legumes, bulbs and other starches (Rahman, 2007). Some fruits are also rich in starch (Li *et al.,* 2014). Cereal grains, such as wheat, maize, rice, sorghum, millet; root and tuber crops as potato, cassava, sweet potato, taro, yams, arrowroot; others as sago palm or plantains and bananas etc., are some of the commercial sources of starch for industrial exploitation (Végh, 2010).

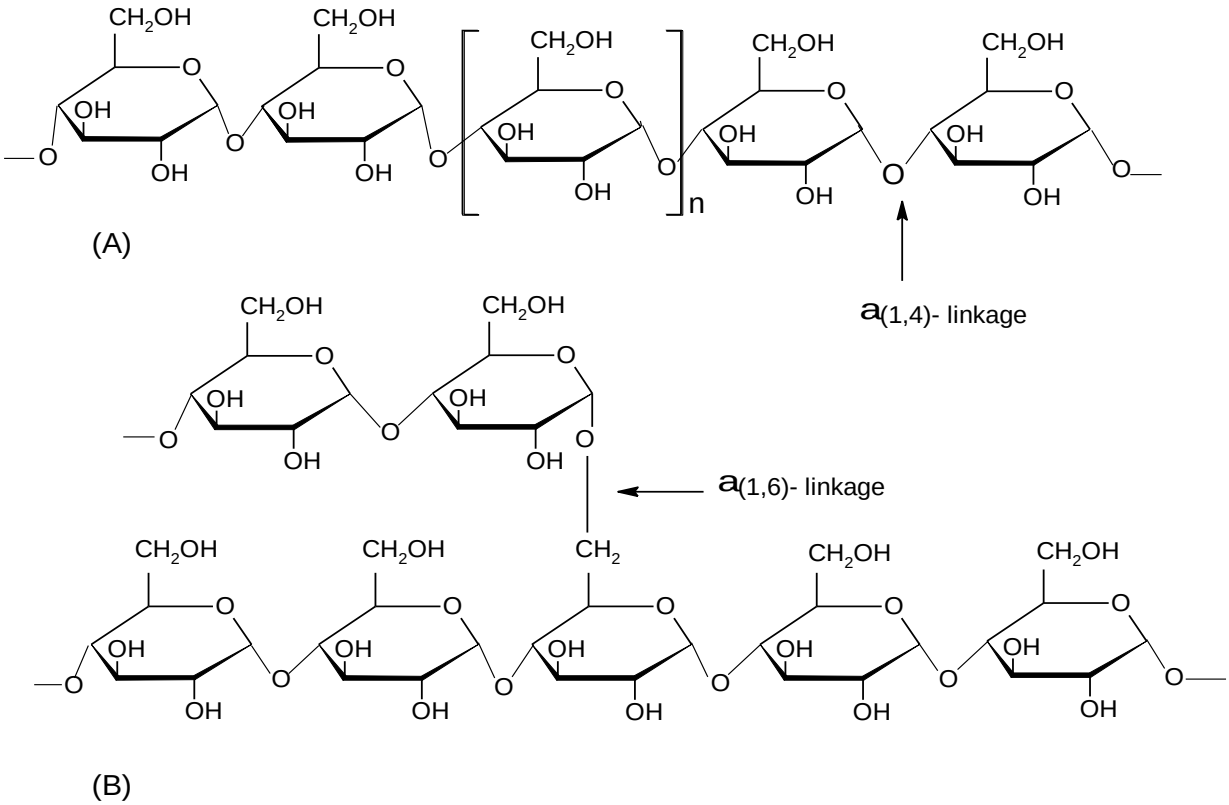



Figure 1.1: Schematic representation of amylose (A) and amylopectin (B).

Root crops are suitable to supply the calorie intake of a growing population. Especially, cultivation and processing of tropical root crops such as cassava, sweet potato and yams are promising in this regard (Végh, 2010).

Yams are staple root crops which include over 600 species; of which about 50-60 species have been cultivated and utilized as food and medicinal plants. In West and Central Africa, the most cultivated and economically important species that have been evaluated as excipients in the food and pharmaceutical industries are *Dioscorea dumetorum*; *D. oppositifolia* Thunb; *D. alata* DIAL2 and *D. rotundata* Poir, *D. bulbifera*, *D. esculenta* Lour and *D. cayenensis* Lamk (Odeku, 2013;Trimanto and Hapsari, 2015).

The tubers of several species of yams (Dioscorea spp.) are edible and are counted just after potato in their food value. In fact, species like *D. alata*, *D. pentaphylla* and *D. bulbifera* are the most worldwide cultivated true yams for their tubers which are of rich source of starch that form an important dietary supplements (Perez *et al*., 2011).

## 1.2. *Dioscorea bulbifera* and its starch

### 1.2.1. Overview of *D. Bulbifera* plant

Yam is a monocotyledonous tuber bearing plant, belonging to the family Dioscoreaceae within the genus *Dioscorea* (Riley *et al.,* 2006). In Ethiopia *Dioscorea* is commonly known by its vernacular name "*boyna*" which is equivalent to the common English name "yam" (Gebre-Mariam and Schmidt, 1998).

*D. bulbifera* is the only species of the genus that originated in Asia, Africa, and Australia and is still found in the wild. It is cultivated in the Southeast Asia, Africa, South America, Central America, pacific Islands and other tropical and subtropical regions (Mohamed, 2007; Hsu *et al*., 2013; Celestine and David, 2015; Silva *et al*., 2016; Ojinnaka *et al*., 2017). The common names for *D. bulbifera* are air potato, air yam, aerial yam, potato yam, bitter yam, bulbil-bearing yam and Kottee harre. It is called air potato because it produces potato-like aerial bulbs in the leaf axils of the twining stems. It is called Kottee hare because it looks like donkey foot (Kay, 1987; Mohamed, 2007; Hsu *et al.,* 2013; Celestine and David, 2015; Ojinnaka *et al.,* 2017).

*D. bulbifera* is a large vine, 6 meters or more in length. It has two types of edible tubers (underground tubers and aerial bulbils) produced by the same plant. They are about the size of potatoes, weighing from 0.5 to 2 kg (Kay, 1987). The plants are characterized by the production of considerable numbers of aerial tubers or bulbils per plant (Ogbuagu, 2008). Both the bulbils and the tubers have bitter taste which disappears after proper boiling or roasting. The plant is known to be a highly invasive plant that will readily overgrow, choke and displace native plant communities via asexual propagation of bulbils that drop from the parent vines to the ground (Celestine and David, 2015). The picture of *D. bulbifera* plant is depicted in Figure 1.2.

*D. bulbifera* is cultivated at the beginning of raining season from March to June (Tamiru *et al.,* 2008). In Ethiopia it is widely cultivated around Jimma and Wollega where it is known by the name of Kottee harre (Mohamed, 2007).

### *1.3.2. Dioscorea bulbifera* Starch

*D. bulbifera* is a rich source of starch that forms an important dietary supplement (Kayode *et al*., 2016). The mean starch content of aerial yam (*D. bulbifera*) accessions was significantly lower than those of accessions with underground tubers, while their amylose fraction was significantly higher than that of the latter group. Aerial yam starch on dry weight base contains 66.9% and its amylose content is 27.0%; the underground starch on dry weight base

comprises 71.2% starch and its amylose content is 16.7%. The late-maturing landraces have significantly lower starch and amylose contents compared to the early-maturing ones (Tamiru *et al.,* 2008).

(A) (B) (C)

Figure 1.2: *Dioscorea bulbifera* leaf and bulbil (Photo by Meseret A. (A); Amenu T. (B&C)).

### *1.4.* Modification of starches

Starch modification is a process whereby the alteration of the starch structure is done by affecting the hydrogen bonds in a controllable manner (Yiu *et al.,* 2008). The shortcomings of native starches, such as the lack of free-flowing properties, insolubility in cold water, uncontrolled viscosity after cooking and low shear resistance, thermal resistance, thermal decomposition and high tendency towards retrogradation limit their use, for example, in the textile, papermaking and pharmaceutical industries (Moorthy, 2002; Kittipongpatana *et al.,* 2006).

In order to overcome these problems, chemical, physical, enzymatic and genetic methods or a combination of these have been employed to prepare "modified starches" with improved properties (Kittipongpatana *et al.,* 2006; Singh *et al.,* 2006; Chiu and Solarek, 2009). These modifications change the granular structure of starch and modify their functional properties to eliminate some of the undesirable properties, making them more suitable for specific uses (Odeku, 2013).

#### 1.4.1. Physical modification of starch

Starch can be altered to improve water solubility and to change particle size (Cui, 2005). Physical methods have received more attention since they are chemical-free and fairly easier than the other methods (Majzoobi *et al.,* 2011). The physical modification methods involve the treatment of native starch granules under different temperature/moisture combinations, pressure, shear and irradiation. It also involves mechanical attrition to alter the physical size

of starch granules (Cui, 2005). Pregelatinization, sonication, ball milling, heat-moisture treatment, and pulsed electric fields treatment are some of the physical methods used for starch modification (Chung *et al*., 2009). Physical modification of starch granules is simple, cheap and safe. These techniques do not require chemical or biological agents, and are therefore preferred when the product is intended for human consumption (Alcázar-Alay and Meireles, 2015).

#### 1.4.1.1. Pregelatinization of starch

Pregelatinized starches also referred to as "pre-gel" or "instant starch" slurries, are those that have been simply precooked to give products that readily disperse in cold water to form moderately stable suspensions (Nakorn *et al*., 2009; Majzoobi *et al.,* 2011). Pregelatinized starch can be produced by various methods such as spray cooking, drum drying and extrusion (Chiu and Solarek, 2009).

Three main processes happen to the starch granule during pregelatinization; granule swelling, crystal or double helical melting, and amylose leaching. Penetration of water thus increases the randomness in the starch granule structure and this pressure caused by this swelling eventually rupture the granule and allows for leaching of amylose molecules to surrounding water (Belitz *et al.*,2004).

The process of gelatinization causes substantial changes in both chemical and physical nature of granular starch due to the rearrangement of intra- and intermolecular hydrogen bonding between water and starch molecules resulting in the collapse or disruption of molecular orders within the starch granules. This causes irreversible changes in the starch properties including loss of organized structure of starch, granule swelling, loss of birefringence and crystallinity (Majzoobi *et al.,* 2011).

### 1.4.2. Chemical modification of starch

Modification of starch chemically implies introduction of functional groups into the starch molecule, resulting in markedly transformed physicochemical properties. Chemical modification of starch generally involves esterification, etherification or oxidation of the available hydroxyl groups on α-D- glucopyranosyl units that make up the starch polymers (Chiu and Solarek, 2009). The chemical and functional properties achieved when modifying starch by chemical substitution depend on starch source, reaction conditions (reactant concentration, reaction time, pH and the presence of catalyst), type of substituent, extent of substitution or degree of substitution (DS); and the distribution of the substituents in the starch molecule (Neelam *et al*.,2012).

### 1.4.2.1. Carboxymethylation of starch

Starches can have hydrogen replaced by a moiety, such as a carboxymethyl group, making carboxymethyl starch (CMS). Adding bulky functional groups like carboxymethyl and carboxyethyl reduces the tendency of the starch to recrystallize and makes the starch less prone to damage by heat and bacteria (Spychaj *et al.*, 2013; Agwamba *et al.*, 2016).

CMS is an important modified starch with unique properties due to the presence of negatively charged functional group (CH2COO-). The introduction of carboxymethyl groups interrupts the ordered structure of native starch and intervenes with the re-association of gelatinized starch. This modification yields starch with decreased gelatinization temperature, increased solubility and improved storage stability (Arul Kumar and Soundararajan, 2016). CMS is cold water soluble material giving viscous, colourless and transparent solutions (Spychaj *et al.*, 2013).

CMS is a green polymer with great importance in pharmacy, medicine, cosmetics, food industry, environmental protection and many other industrial applications (Spychaj *et al.*, 2013).SCMS, under the name sodium starch glycolate (SSG), is used in the pharmaceutical industries as a disintegrant (Gao *et al.*, 2010).

During the carboxymethylation reaction, hydroxyl groups in the starch molecules are substituted with carboxymethyl groups. They are prepared by a reaction of starch and sodium MCA in the presence of sodium hydroxide (NaOH). The process is performed in two steps. The first step is the reaction where the alkalinization of GU–OH group within the PGS molecule are activated and converted into a more reactive alkaline form (GU–$O^{-}$.):

$$GU\text{-}OH + NaOH \leftrightarrow GU\text{-}O\text{–}Na^{+} + H2O \quad (1.1)$$

In the second step, glucopyranose unit is etherified by carboxymethyl groups (Eq. (1.2)):

$$GU\text{-}O\text{–}Na^{+} + ClCH2COONa \rightarrow GU\text{-}O\text{-}CH2COONa + NaCl \quad (1.2)$$

Additionally, two undesirable side reactions can also occur (The hydrolysis reaction leads to loss of sodium MCA in side reactions with formation of sodium glycolate as shown in Eqs. (1.3)) and (1.4):

$$ClCH2COONa + NaOH \rightarrow HO\text{-}CH2COONa + NaCl \quad (1.3)$$

$$ClCH2COONa + HO\text{-}CH2COONa \rightarrow (NaOOCCH2)_2O + HCl \quad (1.4)$$

The amount of carboxymethyl group formed is indicated by the degree of substitution (DS). DS is defined as the average number of substituents per anhydroglucose unit (AGU), the monomer unit of starch. Each AGU contains three hydroxyl groups (C2, C3, and C6), so the DS lies between zero and three in the order of substitution C2> C6> C3. DS increases with increasing reaction time and temperature. An increase in the DS with time is a result of enhanced period of contact of the etherifying reagent and the starch molecules (Nattapulwat *et al.,* 2009; Barrios *et al.,* 2012).

### 1.4.3. Enzymatic modification

Enzymatic modification is modification of starch using enzymes that are found in Eukarya bacteria and archaea representatives, mainly hydrolyzing enzymes. The enzyme used has to be free of enzymatic components that can cause undesirable damage to the starch molecule (Kaure *et al.,* 2012). These are basically four groups of starch-converting enzymes: (i) endoamylase; (ii) exo amylase; (iii) debranching enzymes; and (iv) transferases (Tri Le *et al.,* 2009).

### 1.4.4. Genetic modification

The advancement of genetic engineering technologies has made the genetic modification of starch in plants possible by targeting the enzymes of the starch biosynthetic pathway. This trans-gene technology has a potential to produce novel starches which can reduce or eliminate the use of environmentally hazardous post harvest chemical and enzymatic modification. Genetic modification can be carried out by traditional plant-breeding techniques or through biotechnology (Kaure *et al.,* 2012).

## 1.5. Dual modification of starch

Dual modification of starch has been demonstrated to introduce desirable properties to starch for specific applications (Singh *et al*., 2006). These include methods that involve the chemical reaction in the presence of a specific physical environment or an enzymatic treatment that enhance the rate of derivatization or can enhance the DS in some instances (Xing *et al.,* 2006). To further improve properties and uses of starch, chemical dual modification and other types of dual modifications have been introduced in order to optimize the functionality of modified starches (Ashogbon and Akintayo, 2014).

## 1.6. Pharmaceutical applications of native and modified starches

Both native and modified starches are used as pharmaceutical excipients. Some essential attributes that make native starch attractive for use as a pharmaceutical excipient includes: their white, soft, smooth dryness as well as gelling, and viscosity imparting properties. Also, when they are modified, new attributes are impacted which expand their functions and applications, making them more efficient in both conventional and novel drug delivery systems. Some superior properties of the modified motifs include enhanced flow, disintegration, direct-compression, and formation of stable gels in hot and cold water (Builders and Arhewoh, 2016).

Starch and its derivatives are used mainly for applications in the food, plastic and pharmaceutical industries due to its readily availability, abundant supply, cheapness, inertness, biodegradability, environmental benignness, ease of its physicochemical properties modification through chemical, physical or enzymatic treatment (Adane *et al.*, 2006).

They are used as fillers, glidants, thickeners, binders, disintegrants as well as gelling, bulking, water retention agents, film forming material, microspheres, colon targeting of drugs and nanoparticle (Alebiowu and Itiola, 2002; Odeku, 2013).

Partially pregelatinized starch performs the multiple functions of a binder, disintegrant, flow-aid and self-lubricant and its versatility being effective in a variety of processing methods for solid oral dosage forms describing the multipurpose usage of starch polymers in pharmaceutical applications (Colorcon, 2005).

Non-toxic character and natural origin of CMS allow its application in pharmacy, medicine, cosmetics and food industry. In pharmaceutical industry, CMS is typically applied as tablets disintegrant, controlled release and as a tablet film coating agent (Spychaj *et al.*, 2013).

The function of starch can depend on how it is incorporated into the formulation. Starch functions as a disintegrant when added in the dry state before adding a lubricant. It may exhibit both binding and disintegrant properties when it is incorporated as a paste or dry before granulation with other agents (Newman *et al.*, 1996). It has been also reported that starch can be used for capsule manufacturing, alternative to hard gelatin capsule (Bae *et al.*, 2008).

### 1.7. The present study

Studies indicate that Ethiopia has many plant species which can be used as potential sources of starch for various purposes. These include *Ensete ventricosum*, *Dioscore abyssinica*, *Colocasia esculenta*, *Manihot esculenta*, *D. bulbifera* etc (Gebre-Mariam and Schmidt, 1996; Gebre-Mariam and Schmidt, 1998; Adane *et al.*, 2006; Mohammed, 2007; Paulos, 2007; Tamiru *et al*., 2008). In Ethiopia, the demand for starch is essentially satisfied through imports. In 2012 demand for starch was estimated to be 400 tons. The demand for starch is projected to reach 644 tons and 1,018 tons by the year 2017 and 2022, respectively. It is desirable to exploit starch from cheaper indigenous sources in order to save costs of imports, create job opportunity and earn money from exports market.

The yam species is highly neglected all over the world. It is only consumed in the rural areas during periods of food scarcity (Celestine and David, 2015). *D. bulbifera* is consumed by a small number of communities and is generally underutilized both at subsistence and commercial levels (Ojinnaka *et al*., 2017). The starch content and world wide availability of *D. bulbifera* lends itself as possible alternative source of starch. So, in this study starch from the bulbils of *D. bulbifera* are used.

Native starches have been used for a long time in food and pharmaceutical applications. The shortcomings of native starch, such as the lack of free-flowing properties, insolubility in cold water uncontrolled viscosity after cooking and the sensitivity of cooked starches to shear and low pH, limit their further use (Nattapulwat *et al.,* 2009). Therefore, native starches must be modified chemically and/or physically in order to enhance their positive attributes and/or to minimize their defects (Chiu and Solarek, 2009). Only few polymers possess multiple functionalities especially in terms of good flow, direct compression and enhanced disintegration abilities. The choice of excipients becomes critical in terms of its functionality as rapid disintegration abilities (Philip *et al*., 2010).

The aim of this study is to evaluate disintegrant property of pregelatinized *D.bulbifera* (DBS), carboxymethylated DBS and carboxymethylated pregelatinized DBS in paracetamol tablet formulations. SSG, and partially pregelatinized starch (Starch 1500®) and potato starch were included in the study for comparative purposes. Paracetamol, which is both poorly compressible and sparingly soluble drug, was selected as a model drug for this study.

## 1.8. Objectives of the study

### 1.8.1. General objective

- To prepare modified *D. bulbifera* starch by pregelatinization and carboxymethylation and evaluate its disintegrant property in tablet formulations.

### 1.8.2. Specific objectives

- To prepare pregelatinized, carboxymethylated and dually modified carboxymethyl pregelatinized *D. bulbifera* starch;
- To study the physicochemical properties of the modified starches;
- To evaluate disintegrant properties of the modified starches in paracetamol tablets.

# 2. EXPERIMENTAL

## 2.1. Materials

Bulbils of *D. bulbifera* were collected from Jimma, Oromia region, South West Ethiopia. Hydrochloric acid (BDH Chemicals Ltd, Poole, England), sodium chloride (LAB MERCK CHEMICALS, India), saracetamol powder (China associate Co Ltd, China), Mg stearate (Shandong Head Co., Ltd. China), sodium metabisulphite (Guangzhou Jinhaunda Chemical Reagent Co. Ltd, China), sodium hydroxide (Loba Chemie Pvt. Ltd., Mumbai, India), povidone (K-30) (China associate Co. Ltd, China), sodium starch glycolate, (Starch 1500®), monochloroacetic acid (Hopkin & Williams Ltd, England), isopropyl alcohol (Riedel-de Haėn, Germany), glacial acetic acid (Riedel-de Haėn, Germany), methanol (Carlo Erba Reagents, Italy), potassium phosphate monobasic (FARMITALIA CARLO ERBA, Italia), potato starch BP (BDH LTD., Poole, UK) and lactose were used as received.

## 2.2. Methods

### 2.2.1. Isolation of starch from *D. bulbifera*

Starch was extracted according to the methods described by Subhadhirasaku *et al.,* (2001). Bulbils were washed, peeled, sliced and steeped in 0.1% w/v sodium bisulphite water solution. The slices were ground with blender and the paste recovered in 4% sodium chloride solution and then sieved successively through 315 μm and 224 μm sieve and muslin. The sediment was washed several times with saline solution to remove soluble substances, sugars and mucilage's. The remainder was washed with 0.01M NaOH till the mucilage was removed and then allowed to settle for decantation. The sediment was then washed with distilled water until the pH of the supernatant solution became neutral. The starch obtained was spread over a tray and dried at room temperature. The dried starch was ground with mill (Pulverisette 2, FRITSCH, Germany), sieved with 224 μm mesh sieve and stored in glass bottle.

### 2.2.2. Preparation of pregelatinized *D. bulbifera* starch

Pregelatinized *D. bulbifera* starch (PGDBS) starch was prepared following the methods reported elsewhere (Odeku *et al*., 2008). For preliminary study, eighty grams of starch was dispersed in distilled water in 3:1 or 2:1 water to starch ratio and then heated on thermostated water bath (GFL®, D3006, Germany) at a temperature of 54 ˚C, 59 ˚C or 64 ˚C while stirring for 10, 15, or 20 min to form a paste which was then crisp dried in an oven (Kottermann® 2711, Germany) at 50 $^0$C for 48 h. Following the preliminary study, 500 g starch was pregelatinized at a temperature of 64 ˚C with 2:1 water to starch ratio by heating over

thermostated water bath while stirring for 15 min to form a paste which was crisp dried in an oven (Kottermann 2711, Germany) at 50 ˚C for 48 h. The resultant masses were pulverized in a laboratory pulveriser (Pulverisette 2, FRITSCH, Germany). Each sample was passed through a 224 μm mesh sieve and then stored in airtight bottles for the next use.

### 2.2.3. **Preparation of carboxymethyl *D. bulbifera* starch**

The carboxymethylation process of *D. bulbifera* starch was carried out in a three neck glass reactor which was heated over thermostated water bath. The reflux condenser was used to prevent the loss of organic liquid. Glass stirrer was used to stir the reaction medium. The reactor set-up is depicted in Figure 2.1.

Figure 2.1: Three neck glass chemical reactor vessel over thermostated water bath (GFL®, D3006, Germany) for carboxymethylation of *Dioscorea bulbifera* starch (Picture taken by Meseret A.).

#### 2.2.3.1. Carboxymethylation of native and pregelatinized *D. bulbifera* starch

The synthesis of CMS was carried out following the method of Lazik *et al.* (2002). Eighty grams of PGDBS which was pregelatinized at a temperature of 64 ˚C with water to starch ratio of 2:1 or NDBS was suspended in 400 ml of isopropyl alcohol and the reaction medium was mixed in the glass reactor at room temperature. Then, 100 ml of aqueous NaOH solution

at different concentrations (20% or 30% w/v) was added into the reactor flask over a period of 20 min. The mixture was then stirred for 30 min and monochloroacetic acid (MCA) was then added in order to obtain different MCA /GU molar ratios (0.2:1, 0.6:1 and 1:1) in the reaction mixture. Subsequently, the flask was heated to the reaction temperature of 70 ˚C for 1 h on thermostated water bath. After cooling, the reaction mixture was then neutralized with glacial acetic acid until the pH of about 5.0 was obtained. The liquid supernatant was decanted and the gummy product was washed with a methanol/ water (80:20, v/v) solution several times and finally with analytical grade methanol. The purified sodium carboxymethyl pregelatinized DBS (SCMPGDBS) and sodium carboxymethyl DBS (SCMDBS) were then dried in oven for 24 h at 60 ˚C, milled and passed through sieve size of 224 μm and kept for further analysis. The reactor content was run at twelve different reaction conditions (Table 2.1) at 70 ˚C for 1 h using isopropyl alcohol as reaction medium.

Table 2.1: Reaction conditions for carboxymethylation of native and PGDB starch, reaction time of 1 h at 70 $^0$C.

| Starch source | Reaction media (80% V/V) | Molar ratio of MCA/GU | Concentration of NaOH (%) | Designation |
|---|---|---|---|---|
| PGDBS | ISPA | 0.2:1 | 20 | 0.2:1d20% |
| PGDBS | ISPA | 0.6:1 | 20 | 0.6:1d20% |
| PGDBS | ISPA | 1:1 | 20 | 1:1d20% |
| PGDBS | ISPA | 0.2:1 | 30 | 0.2:1d30% |
| PGDBS | ISPA | 0.6:1 | 30 | 0.6:1d30% |
| PGDBS | ISPA | 1:1 | 30 | 1:1d30% |
| Native DBS | ISPA | 0.2:1 | 20 | 0.2:1n20% |
| Native DBS | ISPA | 0.6:1 | 20 | 0.6:1n20% |
| Native DBS | ISPA | 1:1 | 20 | 1:1n20% |
| Native DBS | ISPA | 0.2:1 | 30 | 0.2:1n30% |
| Native DBS | ISPA | 0.6:1 | 30 | 0.6:1n30% |
| Native DBS | ISPA | 1:1 | 30 | 1:1n30% |

n- Represents native starch carboxymethylated and d- represents dually modified carboxymethyl pregelatinized starch.

### 2.2.4. Degree of substitution

Two grams of SCMS was converted to the H-form by treating with excess 0.1 N aqueous 80% methanolic HCl in a 100 ml beaker with occasional stirring for 1 h. It was then filtered and

washed several times under suction in a sintered glass funnel (OAKTON®, WP-15-1, Japan), with aqueous 80% methanol solution. The resulting sample was dried in the oven at 100 ˚C for 1 h and cooled. A portion of sample weighing 0.25 g was placed into a 250 ml conical flask and to this 100 ml distilled water was added, followed by 10 ml of 0.1 N NaOH solution. The mixture was heated over a boiling water bath (GFL®, D3006, Germany) for 20 min until a clear solution resulted. The hot solution was titrated with a standard 0.1 N HCl solution to a phenolphthalein end-point. Similarly, a blank, without CMC/ CMPGS (H) was also titrated. Each sample analysis was carried out in triplicate and the mean values were taken for DS calculation (Nwokocha and Ogunmola, 2008). The DS was calculated as shown in Equation 2.1.

$$DS = 162\ X\ \%\ Carboxyl/\ [4500-58\ X\ \%\ Carboxyl] \quad (2.1)$$

$$\%\ Carboxyl = [(Vb-V)/wt]\ X\ \ MNaOH\ \ X0.045X100 \quad (2.2)$$

where 162 is the molar mass of AGU (in g/mol); 58 g/mol is the increase in the molecular weight for each CM group substituted; Vb (in mL) is the volume of HCl used for the titration of the blank; V (in mL) is the volume of HCl used for the titration of the sample; wt -is weight in g of sample or native starch and M NaOH -is molarity of NaOH.

$$RE\ (\%) = \frac{DS}{RSMR} X\ 100 \quad (2.3)$$

Where RSMR represents reagent to starch molar ratio which corresponds to molar ratio of MCA/ AGU

### 2.2.5. Physicochemical characterization of *D. bulbifer*a starches

The physicochemical properties of native and modified starch powders were evaluated based on the following parameters:

#### 2.2.5.1. Determination of bulk density, tapped density and related properties

Sixty grams of starch granules were gently poured through a short stemmed glass funnel into a 250 ml graduated glass cylinder. The volume occupied by granules was read and the bulk density was then calculated as g/ml. The bulk in the cylinder was tapped in graduated measuring cylinder 500 times using tapping densitometer (ERWEKAGmbH, Heusenstamm, Germany) to attain a constant volume. The volume occupied by the starch was recorded and tapped density was calculated as g/ml.

$$Bulkdensity(\rho b) = \frac{The\ weight\ of\ the\ powder}{Bulk\ volume\ of\ the\ powder} \quad (2.3)$$

$$Tappeddensity(\rho b) = \frac{The\ weight\ of\ the\ powderm}{Tapped\ volume\ of\ the\ powder} \quad (2.4)$$

To assess the flowability of powders, Hausner ratio (HR) and the Carr's index (CI) were calculated from the bulk and tapped densities of the powders using the following equation (Schüssele and Bauer-Brandl, 2003). The values are the means of triplicate determinations.

$$Carr's\ index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} X\ 100 \quad (2.5)$$

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad (2.6)$$

### 2.2.5.2.Flow rate and angle of repose

Flow rate and angle of repose of the starch powders were determined by using the funnel method. Starch powder (30 g) was allowed to flow through a glass funnel having a 0.8 cm hole from a 10 cm height on a flat surface. The duration of flow was recorded in seconds. The height, h and diameter, 2r (radius), of the powder cone was measured and angle of repose was calculated using the following equation. The average diameter and height of the powder piles formed were recorded.

Flow rate was determined using the relationship;

$$Flow\ rate\ (Vf) = \frac{M}{t} \quad (2.7)$$

Where m is the mass in g and t is time in second.

Angle of repose was calculated according to:

$$Angle\ of\ repose(\Theta) = tan^{-1}\left(\frac{h}{r}\right) \quad (2.8)$$

Where h is the height and r is the radius of the starch powder pile.

All determinations were done in triplicate.

#### 2.2.5.3. Swelling power and solubility

Swelling power and water solubility index of the starches were determined according to the method of Odeku and Picker-Freyer (2007). Starch (0.5 g) was weighed directly into a pre-weighed centrifuge tubes, and 10 ml distilled water was added in each tubes. The tubes were then kept in a thermostatically controlled water bath at 25 ˚C, 37 ˚C, 47 ˚C, 57 ˚C, 67 ˚C and 77 ˚C for 30 min with frequent mixing at 2 min intervals. The tubes were then cooled to room temperature and centrifuged (BECKMAN COULTER, Allegra64R centrifuge, Germany) at

1500 rpm for 20 min, and the supernatant was removed and the sediment weighed (Ws). The supernatant was dried to constant weight (W1) in an oven at 130 ˚C for 2 hrs. The water solubility index (WSI) and swelling power (SP) were calculated as follows:

$$WSI = \frac{W1\ x\ 100\%}{0.5} \quad (2.9)$$

$$SP = \frac{WS\ x\ 100\%}{0.5x\ (100-WSI)} \quad (2.10)$$

#### 2.2.5.4. Determination of moisture content

Moisture content was determined following the method described in USP-30/NF-25 (2007) for modified starches. Accordingly, 2 g of each of the samples was weighed into previously washed, dried and weighed Petri-dish and was heated in an oven at a temperature of 120 ˚C for 4 h. The sample was then weighed, and the moisture content expressed from triplicate determinations using Eq. 2.12

$$\%\ Moisture\ content\ = \frac{Wi-Wf}{Wi} * 100 \quad (2.12)$$

Where Wi and Wf are the weights of sample before and after drying, respectively.

#### 2.2.5.5. Moisture sorption property

The moisture sorption properties of the starches were investigated based on a method described elsewhere (Gebre-Mariam and Schmidt, 1996; Odeku and Picker-Freyer, 2007). Pyrex desiccators containing distilled water, saturated solutions of sodium chloride, sodium hydroxide and potassium chloride were prepared to provide different relative humidity chambers and stored at room temperature. NDBS, PGDBS, SCMDBS and SCMPGDBSs were pre-dried in an oven for 4 h at 120 ˚C. One g (W) of dried and weighed starch was spread evenly on each Petri dish and transferred to particular relative humidity chamber. Samples were equilibrated for four weeks at room temperature.

Water sorption of the starch samples was calculated as the weight difference of the starches before and after equilibration ($W_g$) in a given relative humidity. Moisture sorption capacities ($W_a$) of the starches were expressed as percent moisture sorbed as calculated from Eq.2.13

$$\mathrm{Wa} = \frac{(\mathrm{Wg}-W)}{W} * 100 \quad (2.13)$$

### 2.2.5.6. Analysis of Fourier transform infrared (FTIR) spectra

FTIR spectra of NDBS, PGDBS, SCMDBS, SCMPGDBS, pure paracetamol and paracetamol-starch mixtures were acquired at room temp using FTIR spectrophotometer (FTIR-8400S, SHIMADZU, Japan) in transmittance mode. The samples were first ground in a mortar to reduce the average particle size. About 5 mg of finely ground samples were mixed with liquid paraffin in a mortar and pestle.

The sample mixture was then placed onto the face of a potassium bromide (KBr) plate and the second window was placed on top of the first salt plate to form a thin film of the mull by compression between the two plates. The sandwiched plates were placed in the infrared spectrometer and the spectra were obtained. Each IR spectrum was collected with 20 scans and spectral resolution of 8 $cm^{-1}$. Scanning was performed between wave numbers 4000 and 400 $cm^{-1}$. Background spectrum was collected before running each sample. IR solution software was used for data treatment.

### 2.2.6. Preparation of granules

The compositions of tablet formulations are given in Table 2.2. The filler and paracetamol were mixed and 3% povidone solution were added as granulating agent and mixed for 20 min in mortar. The wet mass was then passed through wet granulator with 1.6 mm sieve and dried in an oven(Kottermann® 2711, Germany). The dried granules were then dry screened by passing them through a 1 mm sieve. The sieved granules were mixed with 2, 4, 6 or 8% (for SCMS); 5, 7.5,10 and 12.5% (for pregelatinized starch) disintegrant in Tubular mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) for 10 min. Finally magnesium stearate (0.5%) was added and the mixture was mixed for further 5 min.

Table 2.2: Paracetamol formulations containing SSG, SCMDBS and SCMPGDBS at different disintegrant concentrations.

| **Formulation ingredients** | **Amount of ingredients (%)** |
|---|---|
| Paracetamol | 84.0 |
| Lactose | q.s |
| Povidone | 3.0 |
| Disintegrant | 2.0, 4.0, 6.0 or 8.0 |
| Magnesium stearate | 0.5 |

Table 2.3: Paracetamol formulations containing PDBS and starch 1500®, at different disintegrant concentrations.

| Formulation ingredients | Amount of ingredients (%) |
|---|---|
| Paracetamol | 84.0 |
| Lactose | q.s |
| Povidone | 3.0 |
| Disintegrant | 5.0, 7.5, 10 or 12.5 |
| Magnesium stearate | 0.5 |

### 2.2.7. Characterization of granules

#### 2.2.7.1. Determination of density and density related properties

Sixty grams of each batch of granules were put into 250 ml measuring cylinder. The bulk density, tapped density, Carr's index and Hausner ratio of the granules were calculated as described for starch powders above (Section 2.2.5.1).

#### 2.2.7.2. Determination of granule flow rate and angle of repose

Flow rate and angle of repose of granules were determined by using the funnel method as described above for starch powders (Section 2.2.5.2).

### 2.2.8. Preparation of tablets

Paracetamol granules were compressed in a 16 station rotary compression machine (B3B®, Manesty, Liverpool, England) fitted with 12 mm diameter flat-faced punches. Compression was carried out at certain fixed compression force (adjusted to give tablets with crushing strengths between 60-100N). Target tablet weight was 450 mg. The tablets were kept for 24 h at room temperature in glass container before their properties were evaluated.

### 2.2.9. Evaluation of tablets

#### 2.2.9.1. Weight and thickness

Twenty tablets were randomly selected from each batch and weighed individually on an analytical balance (Mettler Toledo, Switzerland). Similarly, 20 tablets from the representative batch were randomly taken and individual tablet thickness was measured with tablet thickness tester (ERWEKA GmbH, Heusenstamm, Germany). Average thickness and standard deviation were calculated.

#### 2.2.9.2. Crushing strength

The crushing strengths of ten tablets were measured using a hardness tester (ERWEKA GmbH, Heusenstamm, Germany) and the average values and standard deviation were calculated.

#### 2.2.9.3. Friability

Ten tablets were weighed from each batch and placed in a friability tester (ERWEKA, GmbH, Heusenstamm, Germany). The friability tester was allowed to revolve at 25 rpm for 4 min. The tablets were then dedusted and weighed, and the percent loss in weight was calculated as friability.

$$\%\ Friability = \frac{Weight\ before\ friability - Weight\ after\ friability}{Weight\ before\ friability} \text{x}100 \qquad (2.14)$$

#### 2.2.9.4. Disintegration time

Disintegration test was carried out according to USP 30/NF 25 (2007) specifications. Six tablets from each batch were placed in a disintegration tester (ZT304, ERWEKA GmbH, Heusenstamm, Germany) filled with distilled water at 37 ± 2 ˚C. The tablets were considered completely disintegrated when all the particles passed through the wire mesh.

#### 2.2.9.5. UV calibration curve of paracetamol

Stock solution containing 0.2 mg/ml of paracetamol in phosphate buffer of pH 5.8 was prepared. From this stock solution, six different concentrations (0.002, 0.004, 0.006, 0.008, 0.01, 0.012 mg/ml) were prepared. The UV absorbance readings of these solutions were measured at 243 nm using UV/Visible spectrophotometer (JENWAY, 6505, England). Phosphate buffer (pH 5.8) was used as a blank. Then, the absorbance versus concentration of solutions was plotted.

#### 2.2.9.6. Dissolution test

The dissolution test was done according to the USP30/NF25, <711>, 2007) specification using dissolution apparatus Type II (ERWEKA, D-63150 GmbH, Germany), with 900 ml phosphate buffer (pH 5.8) as the dissolution medium at 37 ± 0.5 ˚C which was stirred at a rate of 50 rpm. Five ml of aliquots of the dissolution medium were removed at 5, 10, 15, 20, 30, 45 and 60 min and filtered using cellulose acetate filter, 0.45µm pore size. Equal amount of fresh medium kept at the same temperature was transferred into the dissolution vessel to keep the sink condition. One ml of the filtered samples was diluted to 100 ml and absorbance

readings were taken with UV/Visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 243 nm. Phosphate buffer (pH 5.8) was used as blank. All the necessary corrections for dilution were made when calculating the drug content.

### 2.2.10. Statistical analysis

Unless and otherwise stated, each test was conducted in triplicate and the results are reported as mean ± standard deviation. The data were subjected to statistical analysis whenever required. At 95% confidence interval, p-values of $\leq 0.05$ were considered statistically significant.

# 3. RESULTS AND DISCUSSION

## 3.1. Pregelatinized *Dioscorea bulbifera* starch

PGDBS was prepared at different condition for preliminary study (2:1 and 3:1 water to starch ratio heated for 10, 15 and 20 min at a temperature of 54 ˚C, 59 ˚C or 64 ˚C. The gelatinization temperature from DSC study was reported by Mohamed (2007) as 58.94 ˚C, 63.7 ˚C and 67.83 ˚C (onset, peak and end set, respectively). It was studied for swelling and solubility, density and related properties and flow properties. Starch pregelatinized at 64 ˚C with 2:1 (water to starch ratio) gelatinized for 15 min showed higher SP than others. So, it was selected and used for further study and modification by carboxymethylation.

## 3.2. Degree of substitution

DS represents the average number of the carboxymethyl groups in the molecular unit of the AGU. Each AGU contains three hydroxyl groups (C2, C3, and C6). In principle, all hydroxyl groups (HO-2, HO-3, and HO-6) in the AGU unit can be substituted. The substitution is in the order C2>C6>C3 and lies between 0 and 3 per AGU (Stojanovic *et al.,* 2005; Spychaj *et al.,* 2013; Lefnaoui and Moulai-Mostefa, 2015).

Several methods including direct titration of the acid form, Cu salt precipitation, back titration, 1H-NMR, HPLC, capillary electrophoresis and flame atomic absorption spectrometry (Lazik *et al.,* 2002; Stojanovic*et al.,* 2005: Lawal *et al*., 2007; Nwokocha and Ogumola, 2008; Nattapulwat *et al*., 2009) have been employed for the determination of DS of CMS. Stojanovic *et al.* (2005) found that direct titration gave lower DS values, while the Cu salt precipitation method gave higher DS values than the back titration method and the value of DS by these methods vary especially at higher value of DS; from comparative study of the three methods mentioned above it was suggested that back titration method was the most accurate one. Back titration method was also recommended as a standard procedure for the determination of DS of CMS by the Work Group 3 of the International Standards Organization Technical Committee 93 (ISO/TC 93/WG3) (Stojanovic *et al.,* 2005). So, back titration method was preferred to determine the DS in this study. Equations 2.1 and 2.2 were used for determinations of DS and % carboxyl.

### 3.3.Factor affecting degree of substitution

**Effect of the concentration of monochloroacetic acid (MCA)**

The influence of molar ratios of MCA to AGU on the values of DS and reaction efficiency (RE) is presented in Table 3.1. The DS value increased with increasing MCA/ AGU molar ratio from 0.2 to1. However, RE decreased with the increasing of DS and reagent to starch molar ratio (RSMR). This is probably due to the competitive reaction of SMCA with NaOH to form sodium glycolate (Heinze *et al.,* 2004). The increase in RSMR reflects an increase of nMCA/nAGU. Consequently, it favours this secondary reaction (Kooijman *et al.,* 2003). The decrease of the RE with the increase of DS, may also be the fact that MCA has difficulty to react with the hydroxyl groups present on the chain of starch because of the steric effects (Sangseethong *et al.,* 2005) or the electrostatic repulsion caused by the carboxylic groups already substituted on PGS backbone (Suriyatem and Kittipongpatana, 2010).

Table 3.1: Impact of starch source, Molar ratio of MCA/AGU and the concentration of NaOH (%) on DS and carboxymethylation process.

| Starch type | % Carboxyl | DS | RE (%) |
|---|---|---|---|
| 0.2:1n30% | 3.600 (0.233) | 0.135(0.011) | 67.953 |
| 0.6:1n30% | 6.660 (0.123) | 0.262(0.020) | 43.712 |
| 1:1n30% | 9.000 (0.230) | 0.366(0.021) | 36.651 |
| 0.2:1n20% | 3.240 (0.320) | 0.121(0.012) | 60.861 |
| 0.6:1n20% | 5.940 (0.140) | 0.231(0.013) | 38.594 |
| 1:1n20% | 7.380 (0.270) | 0.293(0.009) | 29.360 |
| 0.2:1d30% | 3.060 (0.320) | 0.114(0.012) | 57.341 |
| 0.6:1d30% | 5.580 (0.170) | 0.216(0.021) | 36.074 |
| 1:1d30% | 6.840 (0.210) | 0.270(0.014) | 27.004 |
| 0.2:1d20% | 2.880 (0.160) | 0.107(0.030) | 53.838 |
| 0.6:1d20% | 5.400 (0.220) | 0.208(0.020) | 34.823 |
| 1:1d20% | 6.300(0.160) | 0.246(0.021) | 24.684 |

Values in parenthesis indicate standard deviation, n-native carboxymethylated and d-dually modified carboxymethyl pregelatinized starch.

**Effect of the concentration of NaOH**

The NaOH solutions used in this study were 20% (w/v) and 30% (w/v). It was found that DS was higher in 30% (w/v) than 20% (w/v) of NaOH solution. NaOH facilitates the swelling of starch with an increased surface area for the etherification process to form starch alkoxide (Lawal *et al.,* 2008b). At higher NaOH concentrations, the reaction rates will increase and the starch-NaOH equilibrium (Eq. 1) will shift to the right. As a consequence, the DS is expected to increase at higher NaOH concentrations (Jie *et al*., 2004), which is in accordance with the experimental observations.

Percent yield of carboxymethyl starch increased with increasing NaOH concentrations because when native starch reacts with chloroacetic acid in an alkaline condition, the hydroxyl groups of the starch molecule are transformed into an alkoxide form (St-O-) which then react with chloroacetic acid resulting in the St –O -$CH_2$–COO- form (Lawal *et al.*, 2008a). Additionally, a side reaction also occurs which competes with the carboxymethylation process, that being the reaction between sodium hydroxide (NaOH) and sodium monochloroacetate (SMCA) to form sodium glycolate (Rachtanapun *et al.*, 2012)

**Effect of types of starch**

In this study starch used for carboxymethylation was NDBS and PDBS. DS was found higher for native starch than for pregelatinized starch. The characteristics of starch are influenced to a large extent by their amylose fraction constituents (Eliasson and Gudmundsson, 1995). Kittipongpatana *et al.,* (2006) found that the DS showed a significant positive correlation with the amylose content: values of the DS of the carboxymethylated derivatives suggested that the amylose molecules in starch are likely to be more susceptible to chemical modification than the amylopectin molecules. It has been observed that the amylose content affects the physicochemical properties of not only the native starches but also the corresponding carboxymethyl starch. This could be due to the straight-chain nature of the amylose which allows better access of the reacting chemicals to the –OH groups of the glucose units, while the branched-chain and considerably larger amylopectin structure could limit such access (Kittipongpatana *et al.,* 2006).

In pregelatinized starch, the amylose content is less than the amount in native starch due to leaching out of amylose that may be the reason for the decrease of DS in pregelatinized starch (Wadchararat *et al.,* 2006). An increase in amylose content results in an increase in the DS (Kittipongpatana *et al.,* 2006).

Carboxymethylated starch from both native (SCMDBS 1:1n30%) and pregelatinized form (SCMPGS 1:1d30%) has higher DS of 0.366 ±0.021 and 0.270 ± (0.014), respectively. The DS found is presented in Table 3.1.

### 3.4. Powder properties of starches

The powder properties of native and PGDBS, potato starch and Starch 1500® are presented in Table 3.2 and properties of SCMDBS, SCMPGDBS, SSG and NDBS starch are shown in Table 3.3. The ranking for bulk and tapped densities are PGDBS > Starch 1500® > Potato starch >NDBS. The bulk and tapped densities of SCMDBS, SCMPGDBS and SSG are greater than the densities of NDBS. The particle size and shape of the starches may be responsible for the differences in the density values (Garcia *et al*., 2009).

The density values were used to calculate the Hausner ratios and Carr's indices which are measures of the flowability of a powder. Hausner ratio less than 1.25 indicate good flow, whereas greater than 1.25 indicates poor flow and Carr's index values 5 to 10, 12 to 16, 18 to 21, and 23 to 35 represent excellent, good, fair and poor flow properties, respectively according to USP30/NF 25(2007). The Hausner ratio and Carr's index are significantly lower for the PGDBS and Starch 1500® than those of the NDBS and potato starch ($p < 0.05$). The Carr's index and Hausner ratio values of potato starch is higher than that of DBS but not significant ($p>0.05$). And Carr's index and Hausner ratio of PGDBS were comparable with Starch 1500® ($p>0.05$). Hausner ratio and Carr's index values were comparable for SCMDBS, SCMPGDBS and SSG ($p > 0.05$). PGDBS, Starch 1500,® carboxymethylated, SCMPGDBS and SSG possess Carr's compressibility index less than 20 and Hausner ratio of less than 1.25, implying that starch granules have acceptable flow property (Schüssele and Bauer-Brandl, 2003). The Hausner ratio and Carr's index preview the degree of densification which could occur during tableting. The higher the ratio, the greater the tendency of the powder to densify. This phenomenon may cause tablets, which lack uniformity of weight and content to be produced. This study showed that all starches the native and modified have Hausner ratio value less than 1.25 and Carr's index less than 20% which is an indicative of good flow property (USP30/NF 25, 2007).

Angle of reposes for PGDBS, Starch 1500®, SCMDBSs, SCMPGDBS and SSG fall below $30^0$; characteristics of material with good flow property (USP30/NF 25, 2007). Moreover, direct measure of the flow property showed that PGDBS and Starch 1500® have comparable flow rate of 13.74 ± 1.12 and 12.81 ± 1.10 g/sec, respectively ($p > 0.05$). And flow property showed SCMDBSs (9.79±1.22), SCMPGDBS (10.87±1.57) and SSG (10.06±1.58) have

comparable flow rate ($p > 0.05$). NDBS, on the other hand, did not flow through the funnel and therefore no angles of repose could be determined. This had been expected because native starches have poor flow properties (Odeku and Picker-Freyer, 2007). The high bulk and tap densities of PGDBS coupled with its good flowability offer a unique advantage of being used as filler in tablet and capsule formulations. The better flow property of PGDBS powder could be probably due to a lower degree of inter-particle interactions as stated by Zuluaga *et al*., (2007).

Table 3.2: Powder properties of NDBS and PGDBS, potato starch and Starch 1500®.

| **Powder properties** | **NDBS** | **Potato starch** | **PGDBS** | **Starch 1500®.** |
|---|---|---|---|---|
| Bulk density (g/ml) | 0.549 (0.005) | 0.551(0.006) | 0.640 (0.005) | 0.600 (0.004) |
| Tapped density (g/ml) | 0.682 (0.002) | 0.688 (0.003) | 0.724 (0.002) | 0.690 (0.001) |
| Hausner ratio | 1.242 (0.030) | 1.248 (0.031) | 1.130 (0.010) | 1.150 (0.020) |
| Carr's index (%) | 19.501 (1.810) | 19.913 (1.72) | 11.600 (1.630) | 12.280 (1.650) |
| Angle of repose (θ) | * | * | 20.130 (1.010) | 21.550 (1.080) |
| Flow rate (g/sec) | * | * | 13.740 (1.120) | 12.810 (1.100) |
| **Moisture content (%)** | **12.247(1.11)** | **20 (1.19)** | **6.740 (0.320)** | **8.778 (0.190)** |

Values in parenthesis indicate standard deviation, NDBS: Native *D. bulbifera* starch, PGDBS: pregelatinized *D. bulbifera* starch, *There was no flow of the starch in the specified condition.

Table 3.3: Powder properties of NDBS, SCMDBS and SCMPGDBS and SSG.

| **Powder properties** | **NDBS** | **SCMDBS (1:1n30%)** | **SCMPGDBS (1:1d30%)** | **SSG** |
|---|---|---|---|---|
| Bulk density (g/ml) | 0.549 (0.005) | 0.676 (0.008) | 0.642 (0.006) | 0.626(0.004) |
| Tapped density (g/ml) | 0.682 (0.002) | 0.826 (0.003) | 0.790 (0.001) | 0.757(0.002) |
| Hausner ratio | 1.242 (0.030) | 1.220 (0.050) | 1.230(0.040) | 1.210(0.010) |
| Carr's index (%) | 19.501 (1.810) | 18.040 (1.970) | 18.730 (1.760) | 17.310(1.860) |
| Angle of repose (θ) | * | 25.520 (1.630) | 24.050 (1.830) | 22.450 (1.680) |
| Flow rate (g/sec) | * | 9.790 (1.220) | 10.870 (1.570) | 10.060 (1.580) |
| **Moisture content (%)** | **12.247(1.110)** | **10.400 (1.090)** | **10.410(1.080)** | **10.330 (1.070)** |

Values in parenthesis indicate standard deviation, NDBS: native *D. bulbifera* starch, SCMDBS: Sodium carboxymethyl *D. bulbifera* starch, SCMPGDBS: Sodium carboxymethyl pregelatinized *D. bulbifera* starch, SSG: Sodium starch glycolate.

### 3.5. Moisture content

Moisture is known to affect a wide range of physico-mechanical properties of pharmaceutical formulations including powder flow, compressibility/compactibility, hardness of granules and tablets, die-wall friction and stability (physical, chemical and microbiological). Regulation of moisture in formulation is very important as high moisture content may interfere with active ingredient (Hoag *et al.,* 2008). Result of moisture content is given in Table 3.2.and 3.3. The result show that the NDBS had greater value of water content (12.25 ± 1.11%) than the PGDBS (6.740 ± 0.32%) because its air dried, while Starch 1500® had 8.778± 0.19%. Alebiowu and Itiola (2002); Adedokun and Itiola (2010) also reported lower values of moisture content for pregelatinized starches from various sources. The water content of NDBS is also greater than SCMDBS, SCMPGDBS and SSG ($p > 0.05$). The lower moisture content of the modified DBS indicates that it less prone to microbial attack, less ability to interact with drugs that are moisture sensitive and high ability to absorb water to facilitate disintegration, although the moisture content of modified starches were within official limit 4% to 12% (Olayemi *et al.,* 2008).

### 3.6. Swelling power and solubility

Swelling and solubility of NDBS, PGDBS, SCMDBS and SCMPGDBS with various DSs were measured at different temperatures (25 ˚C, 37 ˚C, 47 0˚C, 57 ˚C, 67 ˚C and 77 ˚C). The purpose was to measure the relative capacity of the starch granules to swell at different temperature and the amount of soluble materials produced.

As shown in Figure 3.1, the pregelatinized starch showed higher swelling ability than the unprocessed starch. PGDBS exhibited greater swelling power at all temperatures except at 77 ˚C. Increasing temperature and water in pregelatinization process caused higher swelling ability due to the fact that hydrogen bonds were destroyed with increasing of temperature and water acts as a plasticizer, which diminishes the degradation of starch granules and resulted in increasing capacity of water absorption (Ritruengdech *et al.,* 2011).

Pregelatinization of natural starches often leads to increase in their swellability and water absorptivity (Alebiowu and Itiola, 2002). Due to starch granule disruption, pregelatinized flour can absorb water and increase viscosity immediately even with cold water (Wadchararat *et al.,* 2006). This showed that during pregelatinization the disruption of the starch granules took place (Ritruengdech *et al*., 2011). The swelling capacity of starch is directly associated with the amylopectin content because the amylose acts as a diluent and inhibitor of swelling (Wadchararat *et al.,* 2006; Alcázar-Alay and Meireles, 2015). When starch granules are

heated with water, swelling, rupture, crystallinity loss and amylose leaching occur thus pregelatinized starch powder could absorb water and raise viscosity instantly (Adedokun and Itiola, 2010).

In Figure 3.2, pregelatinized starches showed higher solubility than the native starch almost at all temperature points. The solubility of native starch increased with temperature, a pattern that agrees with its swelling behaviour. These results are due to the lixiviation of amylose from starch granule at high temperature and the increase was rapid after 50 ˚C ($p<0.05$). The external long chain of amylopectin also contributes to those solubility values (Garcia *et al.*, 2009). Solubility increments as a result of gelatinization are expected, because gelatinization causes disruption of the weak associative bonds in the amorphous region of the granules enabling increased hydration of the starch molecules and leaching out of amylose. The results are in agreement with various research reports (Wootton and Bamunuarachchi, 1978; Alebiowu and Itiola, 2002; Adedokun and Itiola, 2010).

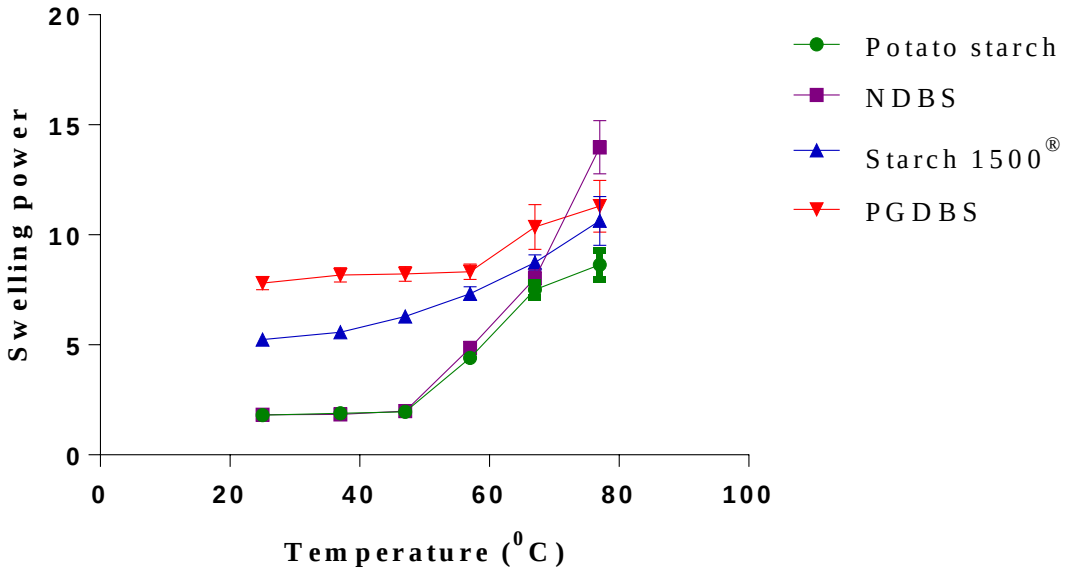



Figure 3.1: Swelling power of NDBS, potato starch, PGDBS and Starch 1500® at different temperatures.

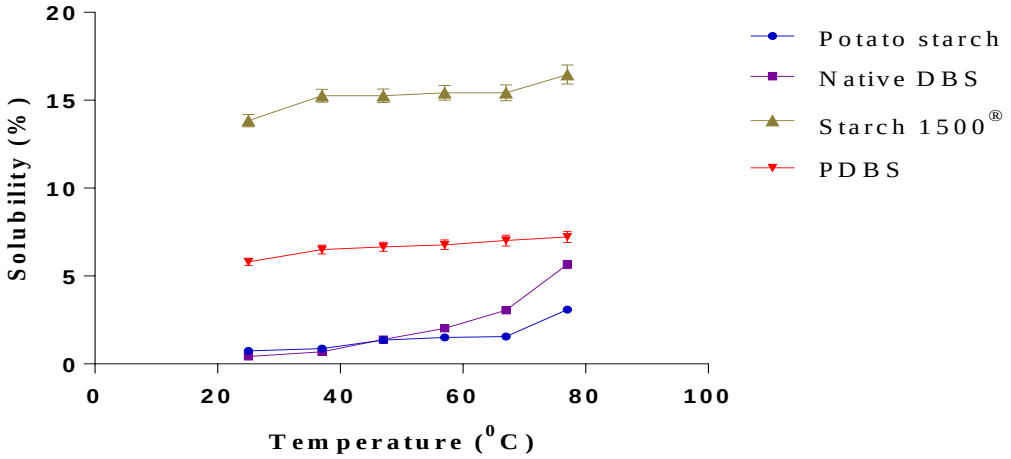



Figure 3.2: Solubility of NDBS, potato starch, PGDBS and Starch 1500® at different temperatures.

Figure 3.3, 3.4, 3.5 and 3.6 show swelling power and solubility of NDBS, SCMDBS and SCMPDBS. Carboxymethylation increased swelling power and solubility of native starch significantly ($p < 0.05$) and generally increasing the DS led to an increase in these values. It is evident that SCMDBS, SCMPMDBS and SSG granules swollen readily, even at 25 ˚C, compared with that of native starch. Further increase in granule swelling at elevated temperature was observed for all starch but in general the SCMS showed a lower percentage increase in swelling than that of native starch. This may suggest that SCMDBS and SCMPGDBS can achieve greater than 80% of their swelling capacity in water at room temperature (25˚C), depending on the DS. Similar trends are reported in carboxymethylation of sago and banana starch (Fadzlina *et al.*, 2005; Afolabi, 2011).

Carboxymethylation is used to improve aqueous dispersibility and cold storage stability of starch pastes. In carboxymethylation, the hydroxyl groups of linear amylose and branched amylopectin molecules of starch granule are derivatized to form CMS ethers. This derivatization interferes with the side-by-side alignment of starch hydroxyl groups such that when the starch is pasted in water, the granules disrupt and the carboxymethyl groups stabilize the aqueous dispersion through improved starch-water interaction (Nabais *et al.*, 2007; Kittipongpatana and Sirithunyalug, 2006).

The ability to dissolve in water of all sodium carboxymethyl rice starches (SCMRSs) is also an indication that amylose portions, which are less soluble in water than amylopectin, were involved in the substitution reaction that converted them into a soluble part (Kittipongpatana *et al.*, 2006). The introduction of carboxymethyl group in to the starch granule appeared to

result in a weakening of the granular structure due to repulsion between neighbouring groups, thus inhibiting inter-chain associations. It is suggested that the structural loosening may occur predominantly in the amorphous region (including the branching point of amylopectin), consequently permitting greater water uptake and an increase in the swelling of the granule (Fadzlina *et al.*, 2005).

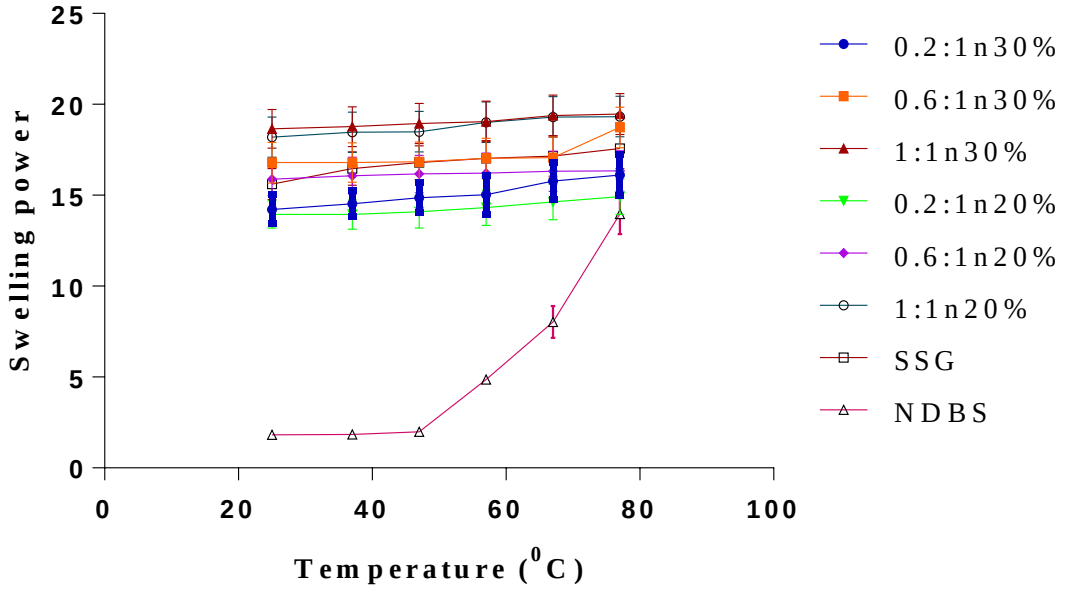



Figure 3.3: Swelling power of NDBS, SCMDBS and SSG at different temperatures.

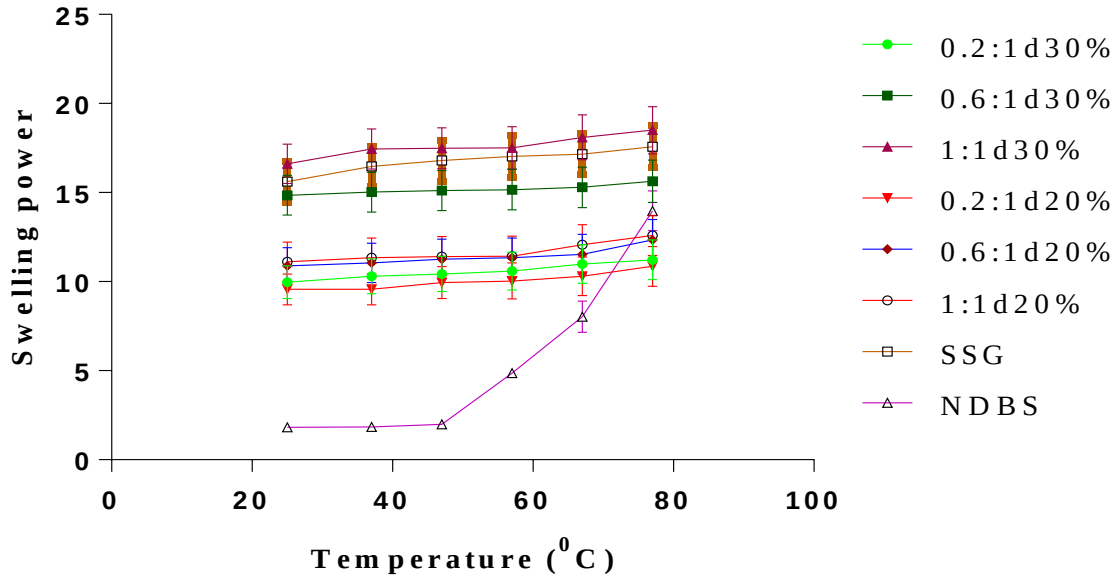



Figure 3.4: Swelling power of NDBS, SCMPGDBS and SSG at different temperatures.

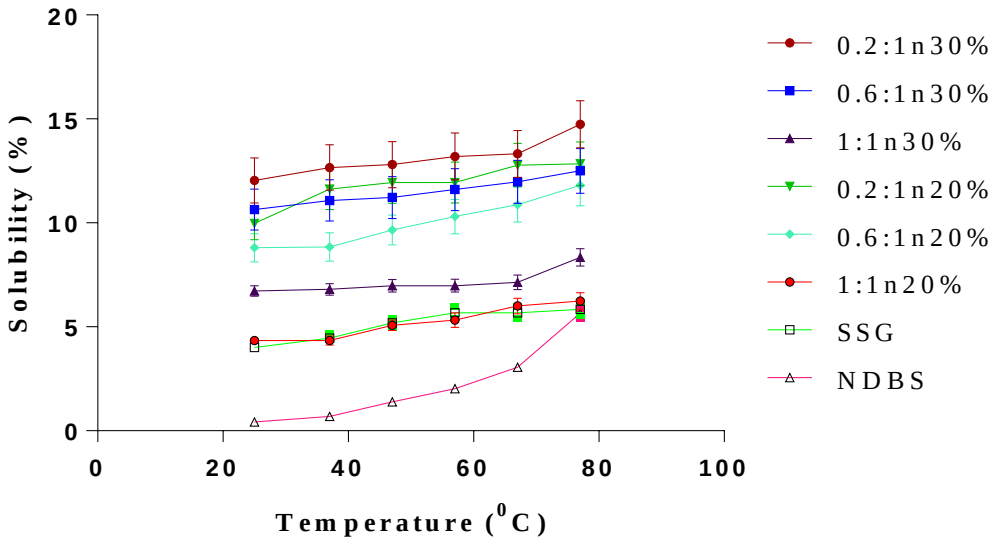



Figure 3.5: Solubility of NDBS, SCMDBS and SSG at different temperatures.

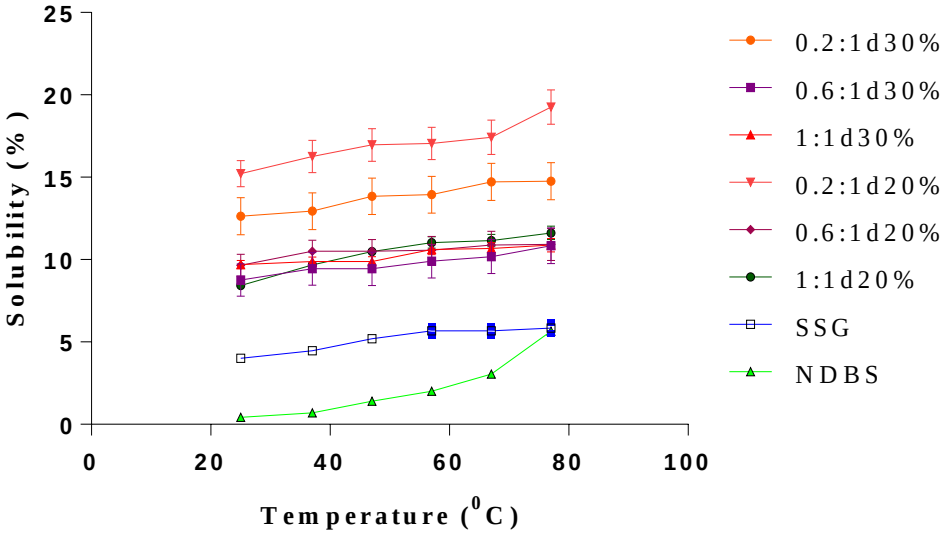



Figure 3.6: Solubility of NDBS, SCMPGDBS and SSG at different temperatures.

### 3.7. Moisture sorption property

An understanding of the moisture sorption characteristics of pharmaceutical excipient is imperative. Since most of physicochemical and functional properties of these materials either depends or are affected by it. Moisture may also induce unpredicted phase transitions in excipients which may also be imparted to the APIs when used in formulation. Generally,

when starch is exposed to a moisture rich environment, the water molecules interact strongly with the polar groups of the amylose and amylopectin units, forming a monomolecular layer (Builders *et al.*, 2013).

Hygroscopic materials can experience significant increase in moisture content when exposed to humid air (Hoag *et al.*, 2008). Starch has been classified as a moderately hygroscopic material. The moisture sorbed has been attributed to intra- and intermolecular hydrogen bonding of water with the hydroxyl groups of the starch molecule. Moisture is known to modify the flow and mechanical properties of many powders including starches (Hancock and Shamblin, 1998). Therefore, knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction and disintegration of tablet are critical.

Moisture sorption profiles of starches are shown in Figure 3.7 and 3.8. Moisture sorption increased generally with relative humidity. Generally, all the starches showed gradual increase in moisture sorption between 20% and 80% RH followed by a sharp increase reaching a maximum at 100% RH. The gradual increase between 20% and 80% RH may be due to the gradual saturation of the monomolecular layer of the polymer powder beds at these RHs. The sharp increase in moisture uptake occurred between 80 and 100% RH which corresponds to the saturation of monomolecular layer and subsequent diffusion of excess moisture (absorption) into bulk powder bed (York, 1981). Percent moisture sorbed ranged between 2.07% at low (8.24%) relative humidity to over 70% at high (100%) relative humidity. SCMDBS had the highest moisture sorption at 100% relative humidity (72.46 ± 0.45 %). This higher affinity to water could be explained by the presence of carboxymethyl group (Fechner *et al.*, 2005). The moisture sensitivity order at 100% RH was SCMDBs>SCMPGDBS > SSG > PGDBS > Starch 1500®>Native DBS>Potato starch. No significant difference was observed between SCMDBS, SCMPGDBS and SSG; PDBS and Starch 1500® but all differ significantly from the NDBS at 100% RH. The hydrophilic nature of the starch molecule is probably responsible for the observed high moisture sorption by the starches and reinforces the necessity for moisture preclusion during storage (Riley *et al.*, 2006). Higher levels of water can lead to microbial spoilage and subsequent deterioration in starch quality. Furthermore, water has been also known to affect the flow properties of starches (Gebre-Mariam and Schmidt, 1998). Higher moisture sorption of starch may indicate a possible higher susceptibility to moisture induced changes in quality. Since no range can be defined in which the water content remains almost constant, during tablet production and storage, the relative humidity should be carefully controlled to obtain powders with optimum

flow and compaction properties (Odeku and Picker-Freyer, 2007). Therefore, native and modified DBS need good control of moisture as compared to potato starch for their functional and mechanical properties to be maintained. The higher capacity of starch to absorb water was indicated to facilitate disintegration (Olayemi *et al.*, 2008). The result of moisture sorption profile together with swelling power reveals that native and modified DBS starch might have promising disintegrant property.

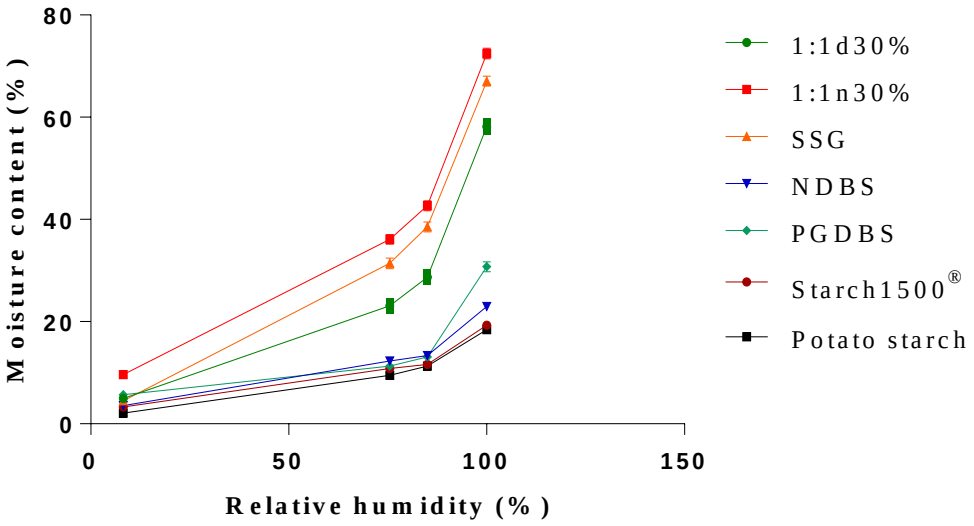



Figure 3.7: Moisture sorption patterns of NDBS, PGDBS, SCMDBS, SCMPGDBS, SSG, potato starch and Starch 1500®.

### 3.8. Fourier transform infrared spectroscopy

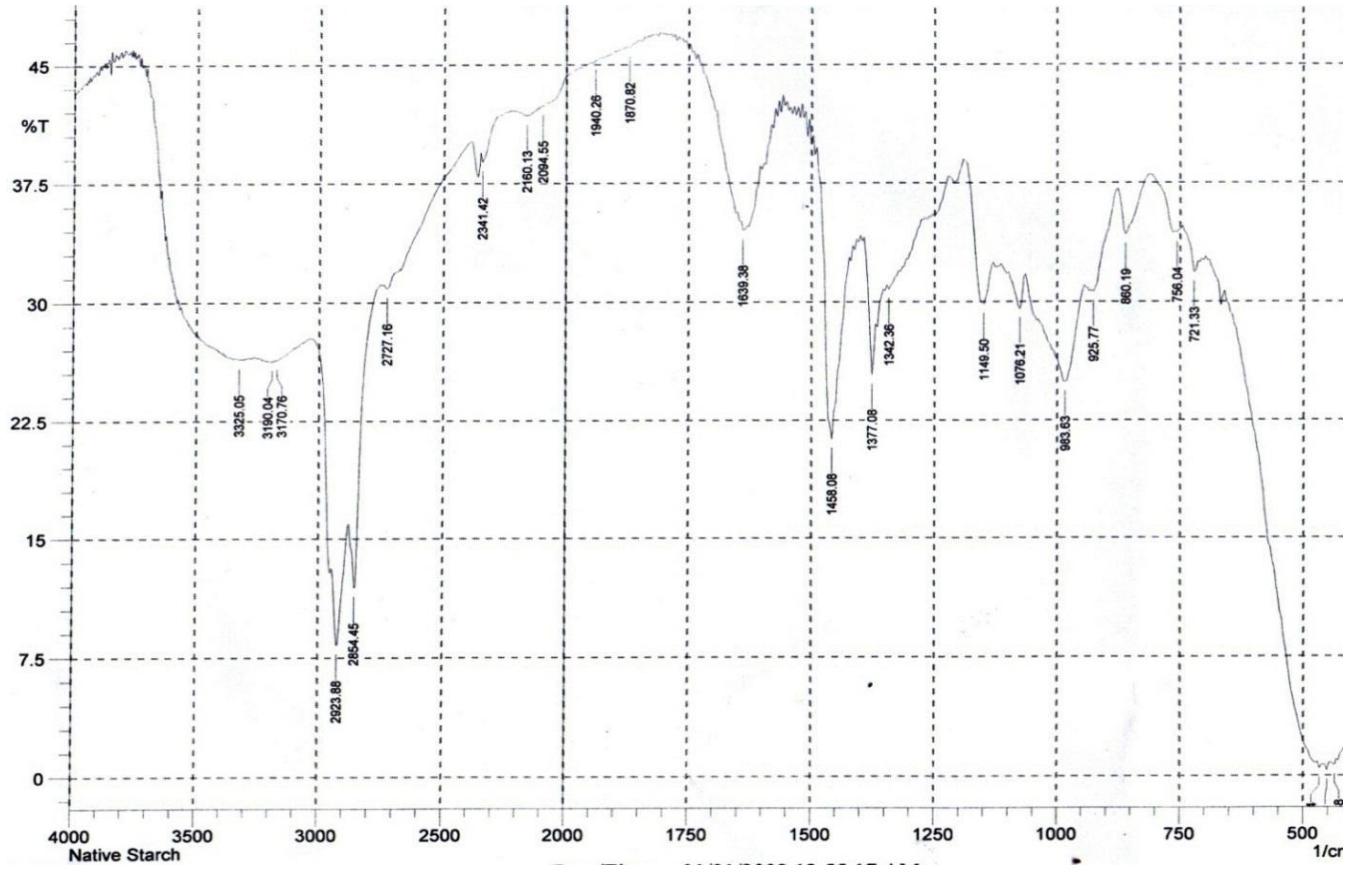
FTIR spectroscopy is a quick and simple technique for identifying compounds. The IR spectrum of a given compound is unique and characteristic. This is because IR spectrum distinguishes between the different kinds of bonds in a molecule (Philip *et al.*, 2010). The FTIR spectral results of NDBS, SCMDBS, PGDBS and SCMPGDBS are displayed in Figure 3.8 (A, B, C & D, respectively).

The FTIR spectra of native DBS (Figure 3.8A) shows broad band at 3000-3700 $cm^{-1}$ because of hydrogen bonded hydroxyl groups that contribute to the vibrational stretches associated with inter- and intramolecular bound hydroxyl group having polymeric association, which make up the gross structure of starch. The peaks at 1076 $cm^{-1}$ are attributed to C-O stretching in NDBS, while the peaks at 2727.16 $cm^{-1}$ are attributed to $CH_2$ asymmetric stretching. The band at 2923.88 $cm^{-1}$ is attributed to CH symmetrical stretching vibrations. The band 1639.36 could be due to tightly bound water molecule in DBS. The bands at 983 $cm^{-1}$, 925 $cm^{-1}$ and

860 $cm^{-1}$ are due to skeletal mode vibrations of α-1, 4-glycosidic linkages (C-O-C) in native starch. The peaks at 1458 $cm^{-1}$ and 1377 $cm^{-1}$ were attributable to the bending modes of H–C–H, C–H and O–H. The two peaks of 1149 $cm^{-1}$ and 1076 $cm^{-1}$ were associated to represent the COC asymmetric stretching. After carboxymethylation of DBS (Figure 3.8B), new peak observed at 1593 are indicative of the presence of carbonyl C=O (asymmetrical COO– stretching). This new absorption suggests that carboxymethylation of the starch has occurred. In addition, the number of hydrogen bonded hydroxyl groups contributing to an extremely broad band at 3200-3700 $cm^{-1}$ in the native starch decreased after carboxymethylation. The reduction in the number of hydroxyl group was because of incorporation of the carboxyl group in to the starch molecules. Similar observations are reported for carboxymethylated kuduzu root starch and mung bean starches (Wang *et al.,* 2010; Zeng *et al.,* 2011; Fathy *et al.,* 2016)

The FTIR spectra of the PGDBS sample are shown in Figure.3.8C. The absorption band at 2923 $cm^{-1}$ shows the C–H symmetric stretching. The –CH2 symmetrical bending is found at 1377 $cm^{-1}$. Peak at 1157 $cm^{-1}$ represents coupling mode of C-C, C-O stretching vibrations. The absorption bands in the range of 1300–860 $cm^{-1}$ are characteristics of C–O stretching in C– O–C and C–O–H in the glycosidic molecule of PGS (Zhou *et al*., 2007). Weak absorption at 1601 $cm^{-1}$ for PGDBS, probably features the tightly bound water molecules present in PGS molecules; assigned to deformational vibrations of water molecules absorbed by the starch samples (Lefnaou and Moulai-Mostefa, 2015).

The substitution of carboxymethyl groups on PGDBS molecules may be revealed by FTIR spectroscopy. The FTIR spectrum of SCMPGS are shown in Figure 3.8D. The typical absorption of PGS backbone as well as the additional peaks occurred corresponding to specific groups after etherification reaction indicating the substitution of carboxylic groups on the PGDBS chains. The absorption bands are identified as the symmetrical carboxylic groups at 1458 $cm^{-1}$ and asymmetric ones at 1593 $cm^{-1}$ (Kittipongpatana *et al*., 2006; Lawal *et al.,* 2008a). The absorption band of O–H stretching is reduced in intensity and has shifted to 3336 $cm^{-1}$. This could be due to the interaction of O–H group with the carboxylic group. The reduction in intensity of this band may also correspond to partly substituted O–H group with carboxymethyl group during the reaction (Fang *et al.,* 2002).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30
22.5
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7.5
0
3325.05
3190.04
3170.76
2923.88
2854.45
2727.16
2341.42
2160.13
2094.55
1940.26
1870.82
1639.38
1458.08
1377.08
1342.36
1149.50
1078.21
983.63
925.77
860.19
756.04
721.33
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3500
3000
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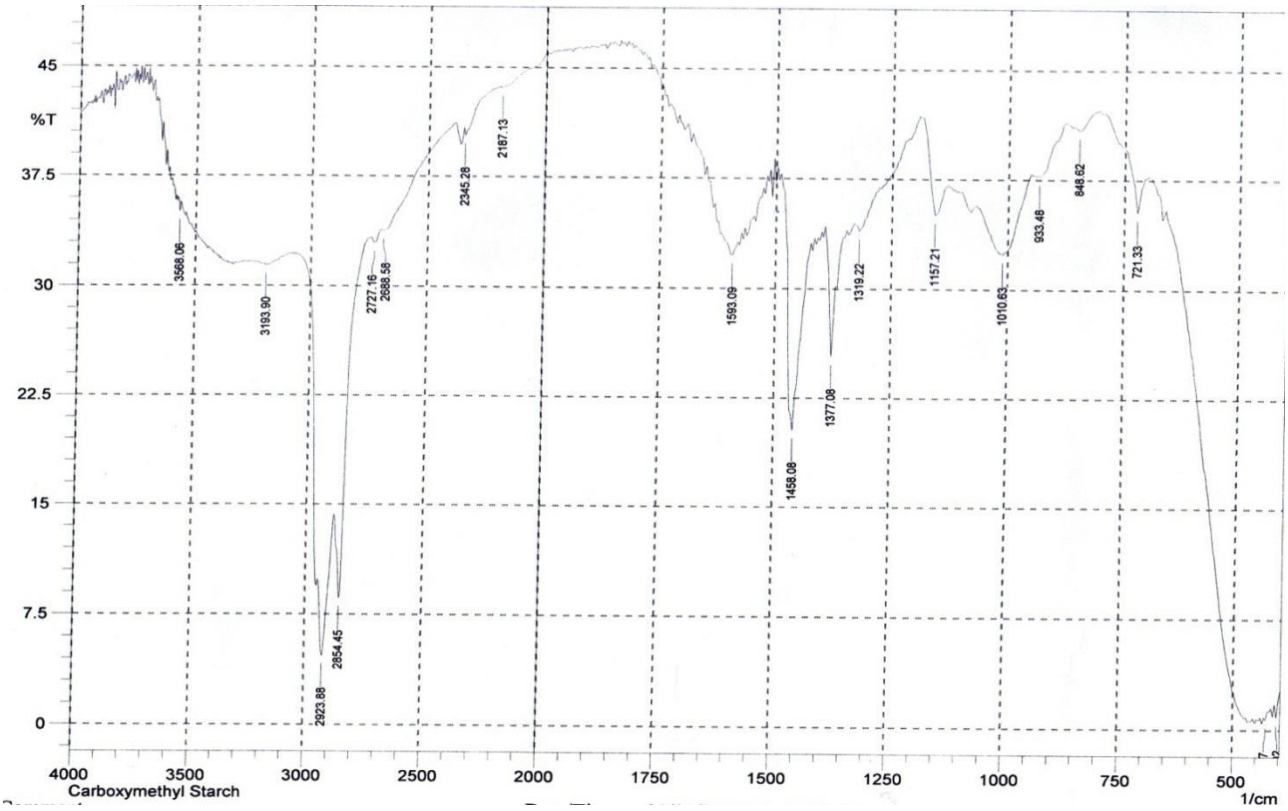
2000
1750
1500
1250
1000
750
500
Native Starch
1/cr

**(A)**


%T
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2854.45
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2688.58
2345.28
2187.13
1593.09
1458.08
1377.08
1319.22
1157.21
1010.63
933.48
848.62
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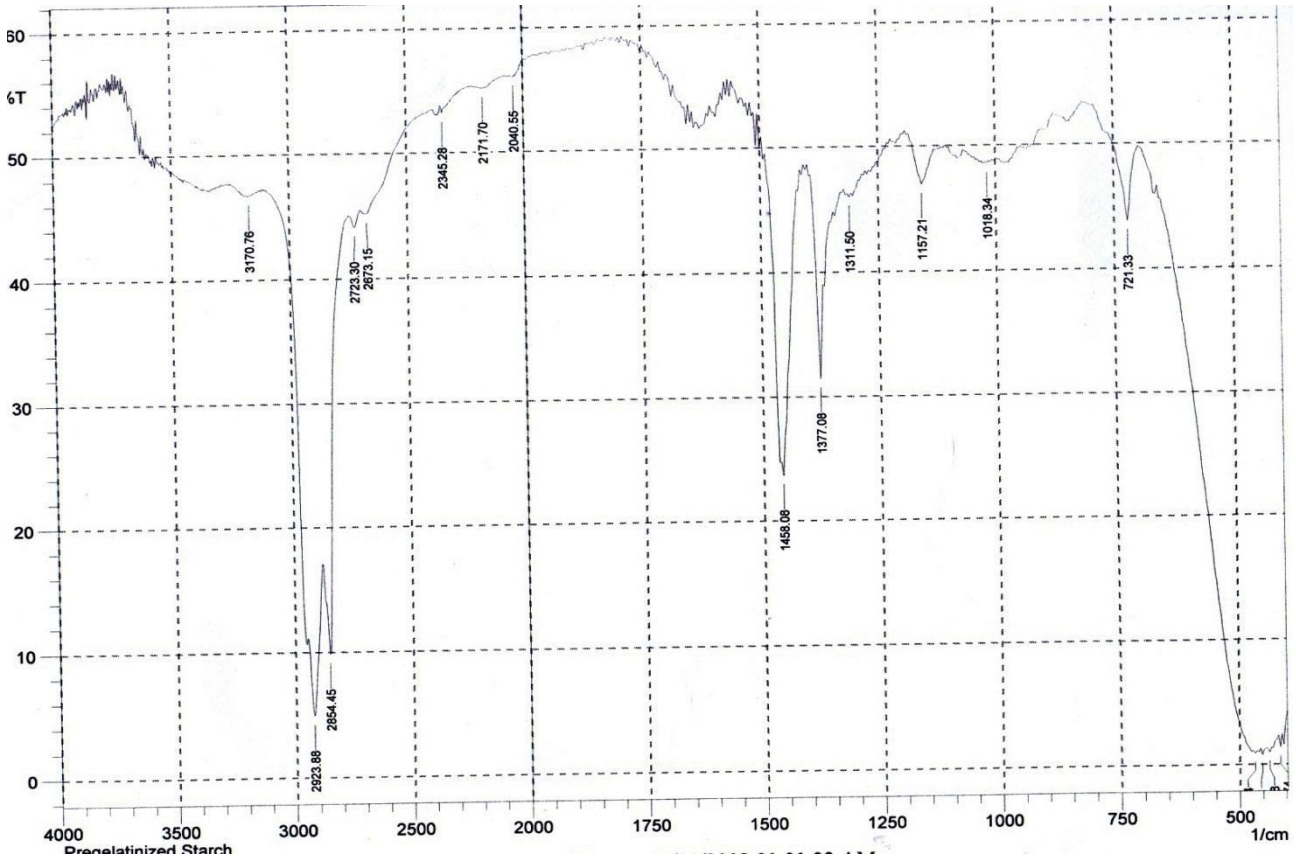
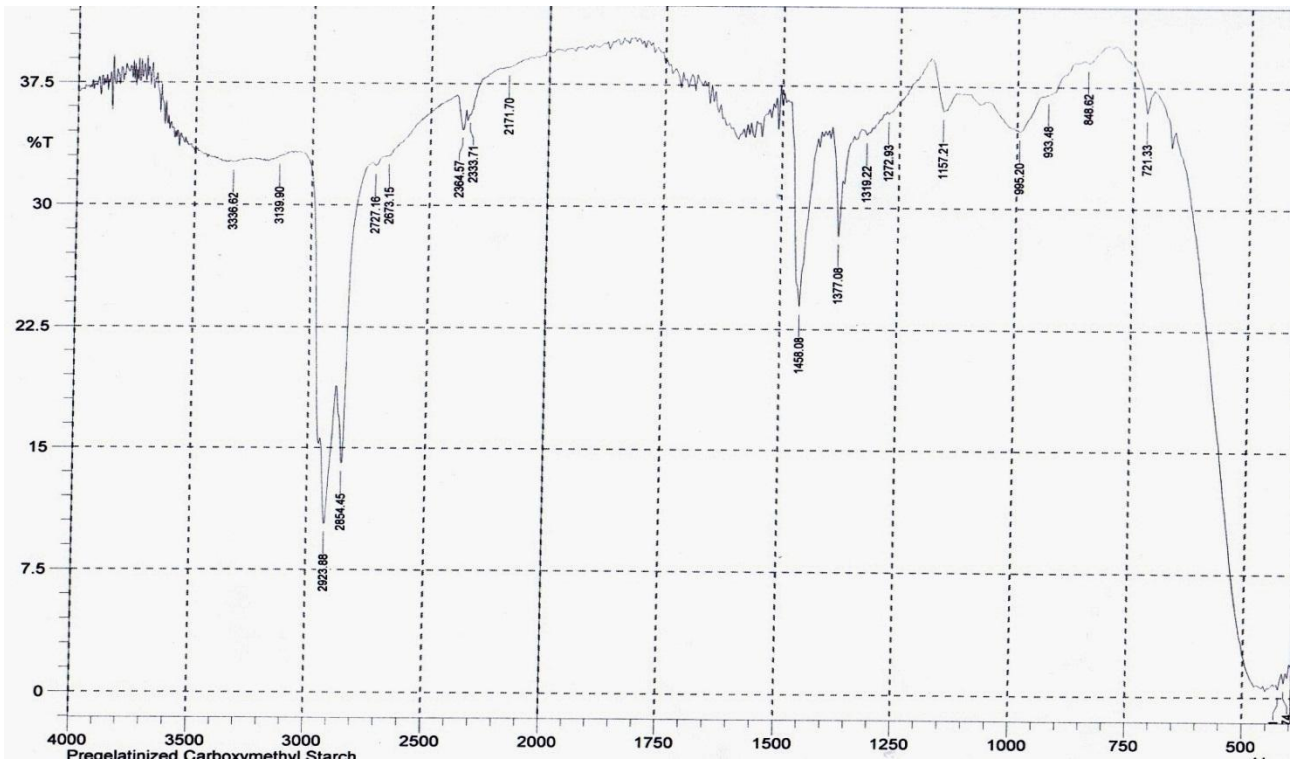
750
500
Carboxymethyl Starch
1/cm

(B)




(C)




(D)

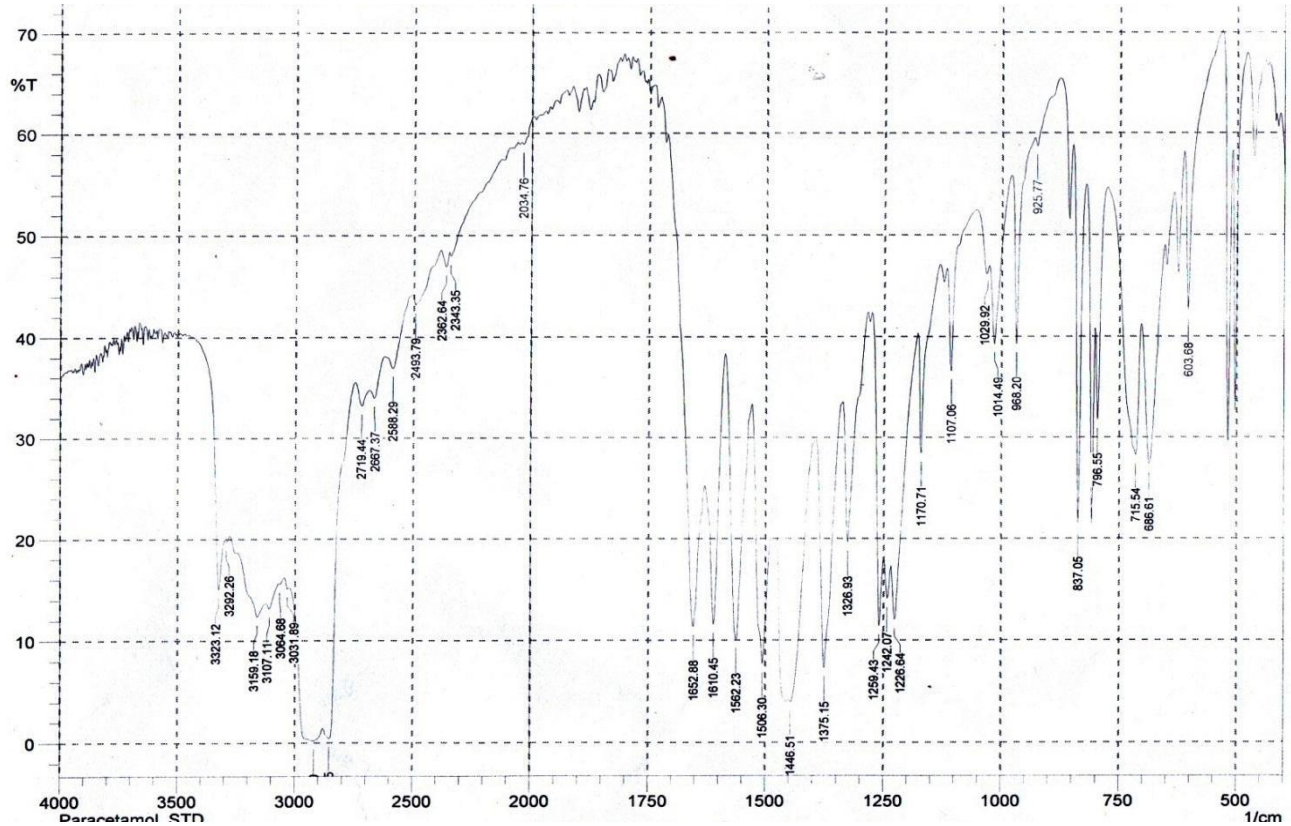
Figure 3.8: FT-IR spectrum of NDBS (A), SCMDBS (B), PGDBS (C) and SCMPGDBS (D).

### 3.9. Drug- excipient interaction study

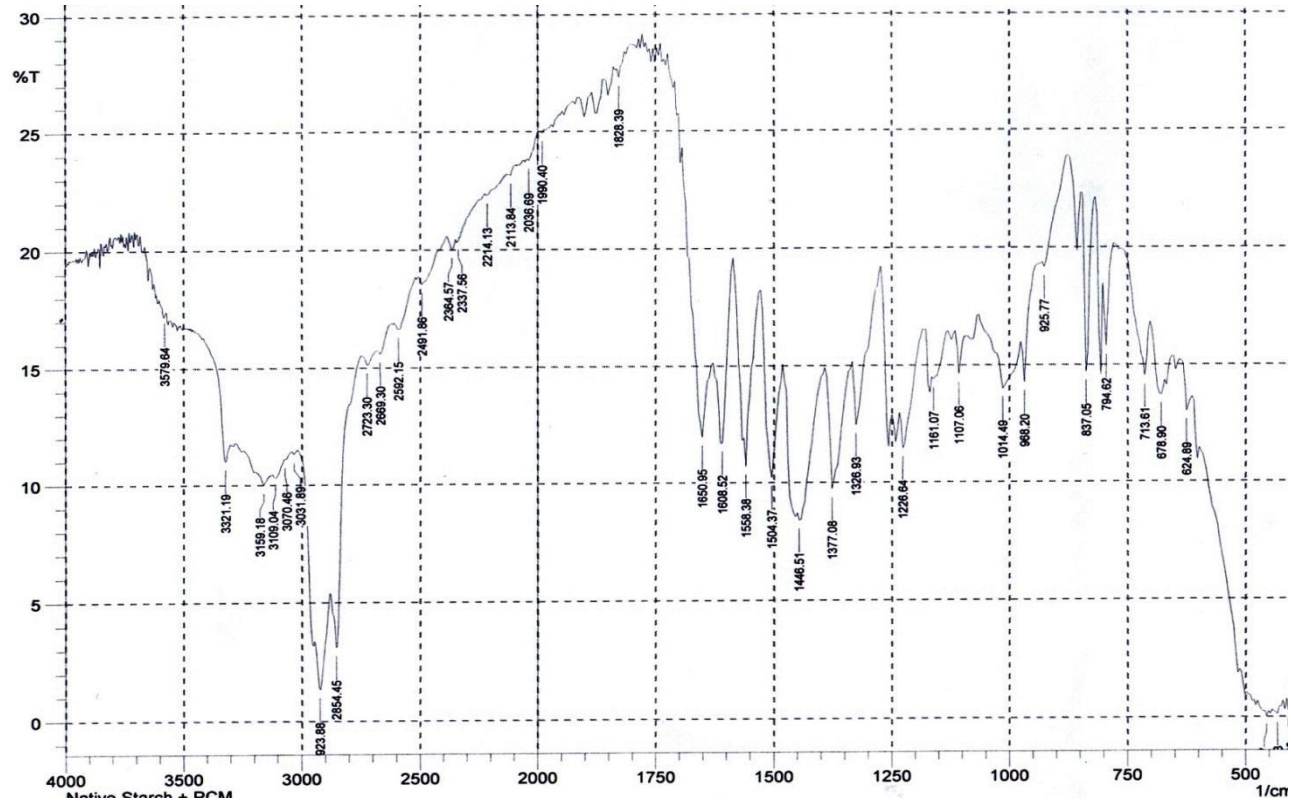
Drug-excipient interactions/incompatibilities are major concerns in formulation development. FTIR spectrum of pure paracetamol powder, mixture of paracetamol and native or modified DBS starch (1:1) were studied and shown in Figures 3.9 (A, B, C, D &E).

FTIR spectra of paracetamol, showed characteristic O-H, N-H, C=O (amide) stretching bands at 3323.12 $cm^{-1}$, 3107.11 $cm^{-1}$, 1652.88 $cm^{-1}$, respectively. The aromatic C= C stretching vibration bands are indicated by absorption bands at 1610.45 $cm^{-1}$ and aliphatic C=C stretch vibrations show band at 1506.3$cm^{-1}$ whereas amide II band, C-N-H group and para-disubstituted aromatic rings at 1562.23 $cm^{-1}$, 1259.43 $cm^{-1}$ and 837.05 $cm^{-1}$, respectively, were also observed (Bashar, 2010).

All characteristic peaks of paracetamol were observed in the FTIR spectra of physical mixture of paracetamol & excipients (native and modified DBS). These results suggest that there may not be possible interactions between paracetamol and starch (native and modified DBS) indicating compatibility of the paracetamol with starch (native and modified DBS).




(A)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3579.64
3321.19
3159.18
3109.04
3070.46
3031.89
2923.86
2854.45
2723.30
2669.30
2592.15
2491.86
2364.57
2337.56
2214.13
2113.84
2036.69
1990.40
1828.39
1650.95
1608.52
1558.38
1504.37
1446.51
1377.08
1326.93
1226.64
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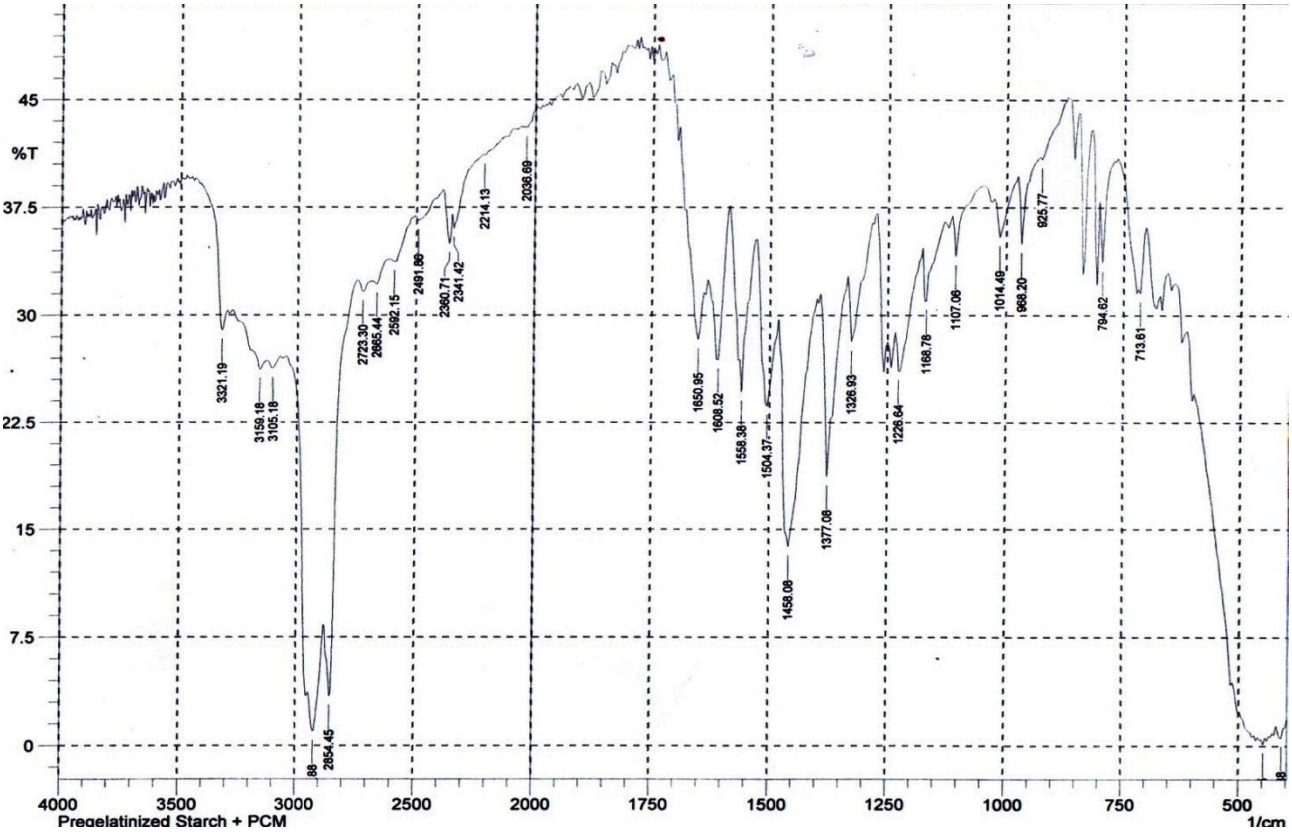
794.62
713.61
678.90
624.89

(B)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22.5
15
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3500
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2500
2000
1750
1500
1250
1000
750
500
Pregelatinized Starch + PCM
1/cm
3321.19
3159.18
3105.18
2923.88
2854.45
2723.30
2665.44
2592.15
2491.86
2360.71
2341.42
2214.13
2036.69
1650.95
1608.52
1558.38
1504.37
1458.08
1377.08
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1107.06
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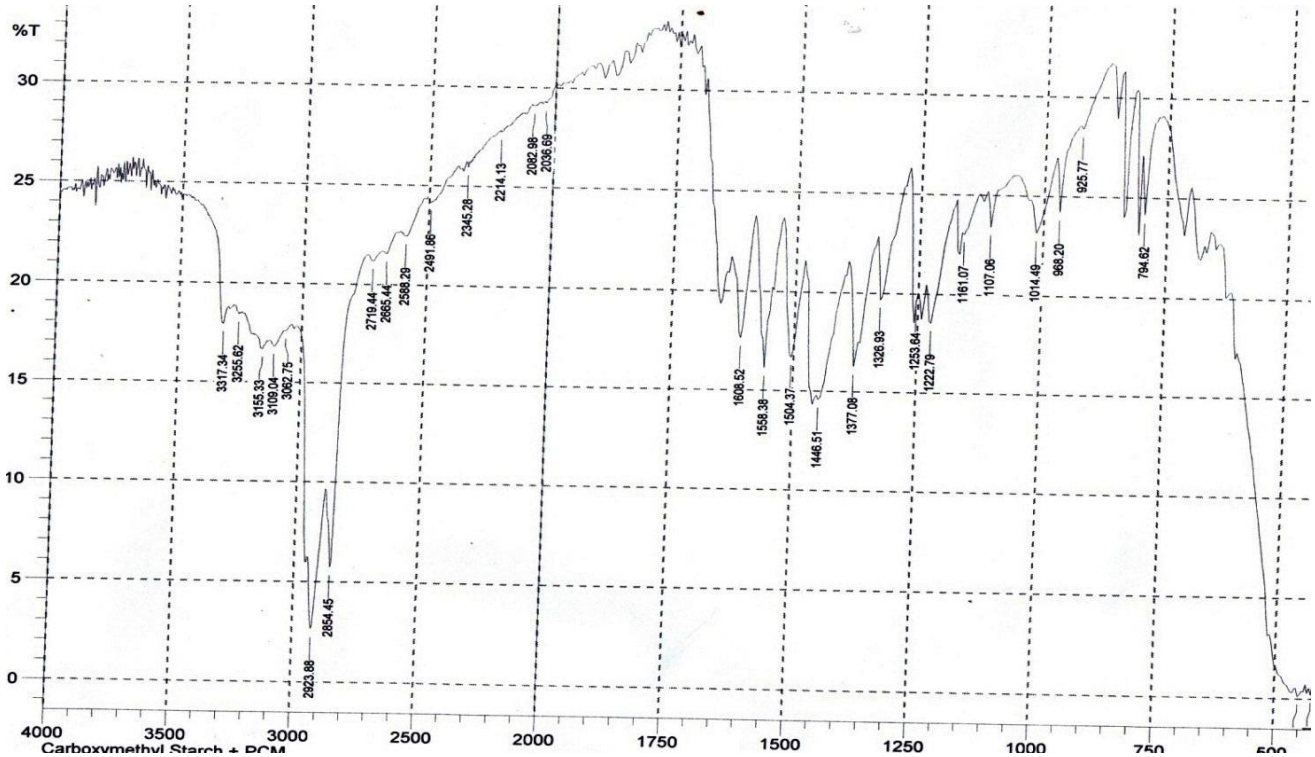
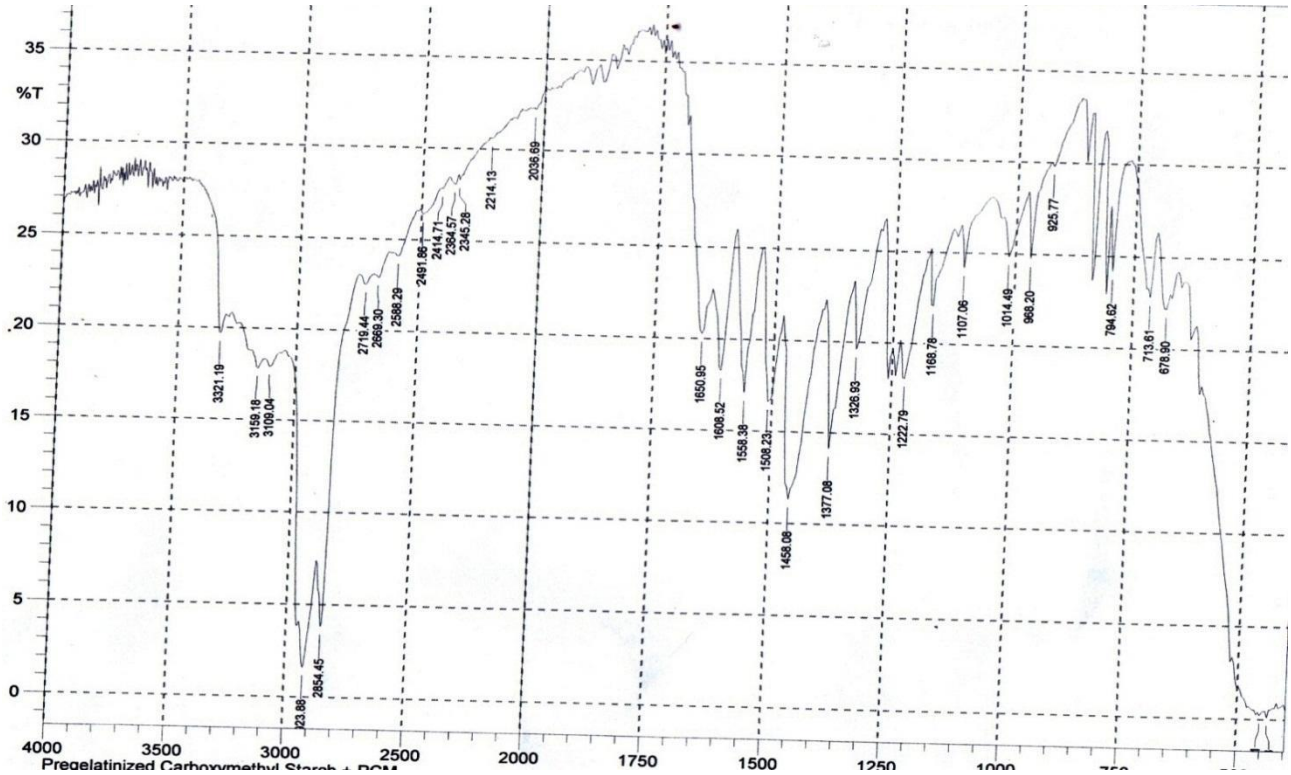
968.20
925.77
794.62
713.61

(C)




**(D)**




(E)

Figure 3.9: FTIR spectra of pure paracetamol powder (A), and 1:1 physical mixture of NDBS and paracetamol (B), PGDBS and paracetamol (C), SCMDBS and paracetamol spectrum(D) and SCMPGS and paracetamol (E) 1:1 physical mixture.

## 3.10. Granule properties

### 3.10. 1 Densities and related properties

Since densities of granules are known to influence flowability and compressibility of granules, they can affect the quality of tablet. Bulk densities of granulation are an indirect measure of granule flow and die fill volume. The Hausner ratio and Carr's index give a preview on the degree of densification, which could occur during tableting; the lower the Hausner ratio, the lesser the tendency for densification to occur. As the values of these indices increase, the flow of the powder decreases and more likelihood of producing tablets with more weight variation (Olayemi *et al*, 2008). Density and related properties of the paracetamol granules are presented in Table 3.4 and 3.5. The table shows that all the formulations have Carr's index below 20% implying the granules have excellent flow property. The Hausner ratios were also observed to be less than 1.25, which also confirmed good flow property of the granules (USP30/NF 25, 2007). Good flow property of the granulation is necessary to ensure efficient mixing and acceptable weight uniformity in compressed tablets (Yüksel *et al*., 2003).

### 3.10. 2 Flow rate and angle of repose

The flow properties of the powder mixture are important for the uniformity of the mass of tablets. The flow rate and angle of repose are shown in Tables 3.4. and 3.5. Flow rate is a direct method of determining granule flowability. Flow rates of paracetamol granules in this study are ranging from 4.22 – 6.983 g/sec and angle of reposes are below 30° for all the formulations, indicating the free flowing property of granules (USP30/NF 25, 2007).

As shown in Table 3.5, the effect of concentration and type of the disintegrant on the flow property was not consistent. This indicates that factors affecting flow property of granules are complex. Kachrimanis *et al*. (2005) stated that flow properties of granules can be affected by a combination of material properties (particle size, size distribution, shape, packing density and surface properties) and operating conditions (moisture, temperature, and static charge).

Table 3.4: Densities and related properties of paracetamol granules prepared with NDBS and PGDBS as disintegrant at different disintegrant concentrations.

| Granule Properties | Disintegrant type | Disintegrant Concentration (% w/w) | | | |
|---|---|---|---|---|---|
| | | 5 | 7.5 | 10 | 12.5 |
| Bulk density (g/ml) | PGDBS | 0.441 (0.002) | 0.442 (0.004) | 0.418 (0.003) | 0.416 (0.002) |
| | Starch 1500® | 0.428 (0.005) | 0.378 (0.003) | 0.418 (0.001) | 0.379 (0.003) |
| | NDBS | 0.386 (0.004) | 0.434 (0.002) | 0.381 (0.002) | 0.389 (0.002) |
| Tapped density (g/ml) | PGDBS | 0.492 (0.002) | 0.508 (0.003) | 0.508 (0.002) | 0.508 (0.003) |
| | Starch 1500® | 0.491 (0.004) | 0.461 (0.005) | 0.508 (0.003) | 0.468 (0.001) |
| | NDBS | 0.471 (0.003) | 0.532 (0.001) | 0.471 (0.003) | 0.483 (0.002) |
| Hausner ratio | PGDBS | 1.115 (0.020) | 1.203 (0.010) | 1.214 (0.010) | 1.220 (0.020) |
| | Starch 1500® | 1.147 (0.030) | 1.220 (0.030) | 1.214 (0.020) | 1.234 (0.020) |
| | NDBS | 1.220 (0.020) | 1.224 (0.010) | 1.235 (0.010) | 1.241 (0.010) |
| Carr's index (%) | PGDBS | 10.310 (0.100) | 16.904 (1.210) | 17.649 (1.110) | 18.056 (1.120) |
| | Starch 1500® | 12.857 (1.760) | 18.064 (1.310) | 17.649 (1.350) | 18.987 (1.291) |
| | NDBS | 18.025 (1.270) | 18.357 (1.220) | 19.073 (1.250) | 19.481 (1.252) |
| Angle of repose ($^0$) | PGDBS | 23.829 (1.850) | 25.099 (1.770) | 27.045 (1.670) | 27.314 (1.451) |
| | Starch 1500® | 24.309 (1.660) | 27.474 (1.420) | 28.213 (1.260) | 28.310 (1.351) |
| | NDBS | 27.980 (0.680) | 27.910 (0.730) | 28.320 (0.980) | 28.511(1.213) |
| Flow rate (g/sec) | PGDBS | 6.343 (0.110) | 6.018 (0.130) | 6.652 (0.120) | 6.983 (0.121) |
| | Starch 1500® | 5.503 (0.110) | 5.923 (0.100) | 5.269 (0.110) | 5.721 (0.102) |
| | NDBS | 4.710 (0.100) | 4.520 (0.040) | 4.357 (0.070) | 4.220 (0.011) |

Values in parenthesis indicate standard deviation, DBS: *D. bulbifera* starch, PGDBS: pregelatinized *D. bulbifera* starch

Table 3.5: Densities and related properties of paracetamol granules prepared with various SCMDBS and SCMPGDBS as disintegrant at different disintegrant concentrations.

| Granule Properties | Disintegrant type | Disintegrant Concentration (% w/w) | | | |
|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 |
| Bulk density (g/ml) | SCMDBS (1:1n30%) | 0.434 (0.004) | 0.438 (0.003) | 0.432 (0.003) | 0.463 (0.004) |
| | SCMPGDBS (1:1d30%) | 0.439 (0.001) | 0.445 (0.003) | 0.434 (0.004) | 0.476 (0.001) |
| | SSG | 0.439 (0.002) | 0.436 (0.004) | 0.432 (0.003) | 0.468 (0.004) |
| Tapped density (g/ml) | SCMDBS (1:1n30%) | 0.526 (0.002) | 0.500 (0.001) | 0.523 (0.002) | 0.555 (0.003) |
| | SCMPGDBS (1:1d30%) | 0.520 (0.001) | 0.517 (0.001) | 0.532 (0.002) | 0.555 (0.003) |
| | SSG | 0.517 (0.001) | 0.505 (0.002) | 0.523 (0.001) | 0.552 (0.001) |
| Hausner ratio | SCMDBS (1:1n30%) | 1.210 (0.020) | 1.166 (0.010) | 1.209 (0.020) | 1.185 (0.010) |
| | SCMPGDBS (1:1d30%) | 1.184 (0.020) | 1.160 (0.010) | 1.225 (0.010) | 1.166 (0.020) |
| | SSG | 1.178 (0.020) | 1.157 (0.010) | 1.209 (0.020) | 1.177 (0.030) |
| Carr's index (%) | SCMDBS (1:1n30%) | 17.391 (1.320) | 14.286 (1.140) | 17.308 (1.220) | 15.628 (1.170) |
| | SCMPGDBS (1:1d30%) | 0.441 (0.002) | 13.857 (1.430) | 18.339 (1.360) | 14.271 (1.23) |
| | SSG | 15.087 (1.110) | 13.591 (1.080 | 17.302 (1.130) | 15.098 (1.220) |
| Angle of repose ($^0$) | SCMDBS (1:1n30%) | 25.816 (1.130) | 24.346 (1.020) | 25.526 (1.110) | 27.574 (1.120) |
| | SCMPGDBS (1:1d30%) | 25.094 (1.110) | 26.413 (1.010) | 24.058 (1.130) | 28.457 (1.120) |
| | SSG | 26.663 (1.12) | 26.962 (1.230) | 22.450 (1.230) | 26.812 (1.120) |
| Flow rate (g/sec) | SCMDBS (1:1n30%) | 5.742 (0.130) | 6.646 (0.110) | 6.360 (0.120) | 6.594 (0.130) |
| | SCMPGDBS (1:1d30%) | 6.151 (0.110) | 6.059 (0.120) | 6.061 (0.010) | 6.166 (0.120) |
| | SSG | 6.396 (0.140) | 6.390 (0.120) | 6.360 (0.130) | 6.106 (0.120) |

Values in parenthesis indicate standard deviation, SCMDBS: Sodium carboxymethyl *D. bulbifera* starch, SCMPGDBS: Sodium carboxymethyl pregelatinized *D. bulbifera* starch, SSG: Sodium starch glycolate.

## 3.11. Tablet properties

### 3.11.1. Weight and thickness

The paracetamol tablets prepared under single compression force adjusted to give crushing strength 60 - 100 N were examined for their weight and thickness uniformity. The results are summarized in Table 3.7 and 3.8. The tablets were within acceptable range of weight variation (± 5%) as per the British Pharmacopoeia for tablets weighing 250 mg or more (BP,

2009).Since the weight of a tablet being compressed is determined by the amount of granulation in the die prior to compression, the weight uniformity could be attributed to the good flow property of the granules described in Section 3.12.2. No significant difference in weight between formulations was observed ($p>0.05$).

At a constant compressive load, tablet thickness varies with changes in die fill, particle size distribution and packing of the particle mix being compressed, and tablet weight, while with a constant die fill, thickness varies with variations in compression force. As can be seen from Table 3.15, tablet thickness falls within 5% variation of the average. No significant difference in thickness of tablets between formulations was observed ($p>0.05$).

Since the drug substance forms the greater part of the tablet mass, the weight uniformity can reflect the uniformity in the content of active ingredient. However, there was slight weight and tablet thickness variation between the various batches of formulations; this might be related to the variations in powder density as well as compression behaviour of the compacted material (Ahmed *et al*., 2001).

Table 3.6: Paracetamol tablet weight and thickness at different NDBS and PGDBS concentrations.

| DC (% w/w) | PGDBS | | Starch 1500® | | NDBS | |
|---|---|---|---|---|---|---|
| | Weight (g) | Thickness (mm) | Weight (g) | Thickness (mm) | Weight (g) | Thickness (mm) |
| 5 | 0.445 (0.010) | 4.580 (0.010) | 0.450 (0.010) | 4.700 (0.010) | 0.457 (0.050) | 4.880 (0.030) |
| 7.5 | 0444 (0.020) | 4.580 (0.010) | 0.445 (0.020) | 4.630 (0.020) | 0.454 (0.030) | 4.740 (0.020) |
| 10 | 0.453 (0.010) | 4.660 (0.020) | 0.456 (0.010) | 4.660 (0.010) | 0.456 (0.020) | 4.760 (0.010) |
| 12.5 | 0.448 (0.010) | 4.630 (0.010) | 0.451 (0.020) | 4.640 (0.010) | 0.456 (0.010) | 4.750 (0.020) |

Values in parenthesis indicate standard deviation, DBS: *D. bulbifera* starch, PGDBS: pregelatinized *D. bulbifera* starch, SSG: Sodium starch glycolate.

Table 3.7: Paracetamol tablet weight and thickness at different SCMDBS and SCMPGDBS concentrations.

| DC (% w/w) | SCMDBS (1:1n30%) | | SCMPGDBS (1:1d30%) | | SSG | |
|---|---|---|---|---|---|---|
| | Weight (g) | Thickness (mm) | Weight (g) | Thickness (mm) | Weight (g) | Thickness (mm) |
| 2 | 0.448 (0.001) | 4.660 (0.010) | 0.447 (0.002) | 4.650 (0.010) | 0.445 (0.002) | 4.530 (0.020) |
| 4 | 0456 (0.001) | 4.760 (0.010) | 0.454 (0.003) | 4.760 (0.020) | 0.453 (0.001) | 4.740 (0.010) |
| 6 | 0.450 (0.010) | 4.700 (0.010) | 0.457 (0.001) | 4.880 (0.010) | 0.458 (0.002) | 4.880 (0.010) |
| 8 | 0.457 (0.002) | 4.740 (0.010) | 0.456 (0.002) | 4.810 (0.010) | 0.450 (0.001) | 4.720 (0.020) |

Values in parenthesis indicate standard deviation, SCMDBS: Sodium carboxymethyl *D. bulbifera* starch, SCMPGDBS: Sodium carboxymethyl pregelatinized *D. bulbifera* starch, SSG: Sodium starch glycolate.

### 3.11.2. Crushing strength and friability

The mechanical properties of the tablet formulations were assessed by the crushing strength and friability of the tablets. While crushing strength indicates the strength of the tablet, friability values provide a measure of tablet weakness.

Tablets require a certain amount of strength and resistance to friability, to withstand mechanical shocks of handling in manufacture, packaging, and shipping. Adequate tablet hardness and resistance to powdering and friability are necessary requisites for consumer acceptance. In this study, hardness between 60-100 N was obtained at constant compression force.

The effect of the starches as disintegrating agent on the crushing strength and percent friability of paracetamol tablets at constant binder concentrations (3% povidone) are shown in Table 3.8 and 3.9, respectively. The effect of starches as disintegrant on the crushing strength and percent friability differ significantly. When the concentration of the binder was constant, an increase in the concentration of NDBS as disintegrant resulted in reduction of crushing strength and increment of percent friability. This may be attributed to the poor compressibility and softening effect of native starch at higher concentrations. On the contrary, the crushing strength increased and percent friability of the tablets decreased gradually as the concentration of PDBS and Starch 1500® increased, indicating better compressibility and *in situ* binding activity of this starch. This could be credited to its better compactability than the native one.

The high friability of tablets causes lack of elegance and consumer acceptance. For tablets weighing up to 0.65 g each, a sample of 20 tablets are tested. A maximum loss of 1% is

acceptable for most products (BP, 2009). In this study, the friability of the tablets ranged from 0.16 - 0.91% (Table 3.9 and 3.10).Conventional compressed tablets that lose less than 1% of their weight are generally considered acceptable. The percentage friability for all the formulations was below 1%, indicating that the friability is within the acceptable limit of USP30/NF25 (2007).

Table 3.8: Paracetamol tablet Hardness and Friability at different NDBS and PGDBS concentrations.

| DC (% w/w) | PGDBS | | Starch 1500® | | NDBS | |
|---|---|---|---|---|---|---|
| | Hardness(N) | Friability(%) | Hardness(N) | Friability(%) | Hardness(N) | Friability(%) |
| 5 | 82 (8.25) | 0.24 | 83 (7.11) | 0.28 | 82 (8.10) | 0.43 |
| 7.5 | 84 (9.1) | 0.22 | 88 (8.09) | 0.22 | 75 (9.11) | 0.66 |
| 10 | 86 (8.13) | 0.21 | 93 (6.14) | 0.21 | 64 (9.01) | 0.87 |
| 12.5 | 91 (8.06) | 0.18 | 95 (8.77) | 0.16 | 61 (8.91) | 0.91 |

Values in parenthesis indicate standard deviation, DBS: *D. bulbifera* starch, PGDBS: pregelatinized *D. bulbifera* starch.

Table 3.9: Paracetamol tablet Hardness and Friability at different SCMDBS and SCMPGDBS concentrations.

| DC (% w/w) | SCMDBS | | SCMPGDBS | | SSG | |
|---|---|---|---|---|---|---|
| | Hardness(N) | Friability (%) | Hardness(N) | Friability (%) | Hardness(N) | Friability (%) |
| 2 | 78 (9.18) | 0.44 | 81 (8.12) | 0.44 | 82 (6.12) | 0.44 |
| 4 | 81 (8.12) | 0.43 | 81 (7.14) | 0.44 | 82 (8.11) | 0.44 |
| 6 | 82 (6.13) | 0.43 | 82 (6.13) | 0.43 | 81 (7.13) | 0.43 |
| 8 | 82 (7.12) | 0.44 | 83 (8.11) | 0.43 | 83 (8.12) | 0.44 |

Values in parenthesis indicate standard deviation, SCMDBS: Sodium carboxymethyl *D. bulbifera* starch, SCMPGDBS: Sodium carboxymethyl pregelatinized *D. bulbifera* starch, SSG: Sodium starch glycolate.

### 3.11.3. Disintegration time

The disintegration times of tablets formulated with NDBS, PGDBS and Starch 1500® at different concentrations of disintegrant are depicted in Figure 3.10 while tablets formulated with SCMDBS, SCMPGDBS and SSG at different concentrations of disintegrant are depicted in Figure 3.11. All formulations generally passed the official disintegration test for uncoated tablets i.e. <15 min (USP 30/NF 25, 2007).

As clearly depicted in the figures the disintegration times of paracetamol tablets decreased with increase in the concentration of excipients as disintegrant for NDBS, PGDBS and Starch 1500® from 5% to 12.5%. Paracetamol tablets formulated with PGDBS and Starch 1500® disintegrants exhibited lower DT values than those formulated with natural starch disintegrants, probably due to the higher swelling power of the pregelatinized starches than the natural ones, thereby promoting active disintegration mechanism in the tablets caused by the generation of swelling force of the starch disintegrants (Adedokun and Itiola, 2010). But as concentration increases from 10% to 12.5%, DT for NDBS decreases more than seen for PGDBS and Starch 1500®. Also, for all the starches, as the concentration of disintegrant increased, DT values of tablets decreased. This effect of increase in disintegrant concentration was less pronounced with pregelatinized starch disintegrants than with natural starch disintegrants. The reduction in DT of tablets between two concentrations of pregelatinized starch disintegrants was lower than the rate for two similar concentrations of natural starch disintegrants. This could be due to very high swelling ability of the pregelatinized starches (Alebiowu and Itiola, 2002), which, as the concentration increases, causes the blockage of the pores and subsequently reduces the water absorption into the core of the tablets. As the concentration of pregelatinized starch disintegrant increased, the expected fast rate of reduction of DT of the tablets would be impeded by the reduced water absorption (Lowenthal, 1973). The reduction in porosity would lead to slower water penetration into tablets, and, consequently, swelling would be reduced and, eventually, development of active mechanism of disintegration would be reduced (Atichokudomchai and Varavinit, 2003; Adedokun and Itiola, 2010).

For tablets prepared with SCMDBS, SCMPGDBS and SSG; the tablets containing 2.0%w/w SCMBDS showed the fastest disintegration time than SCMPGDBS and SSG. For SCMDBS and SSG as the concentration increases to 4% the disintegration times decrease, but as concentration increases to 6% then to 8% the disintegration times increase .This could be due to the formation of viscous gel mass of SCMS after contact with water. Consequently, water relatively slowly penetrated into the tablet, resulting in retardation of tablet disintegration. Hence carboxymethyl yam starch can be used as an excellent tablet disintegrant in low concentrations (Nattapulwat *et al.*, 2009).

One can clearly notice from the figure that the impact of concentration on the disintegration time is disintegrant dependent. Expectedly, CMS and PGS exhibited higher disintegrant activity as they had higher swelling power and water absorption capacity.

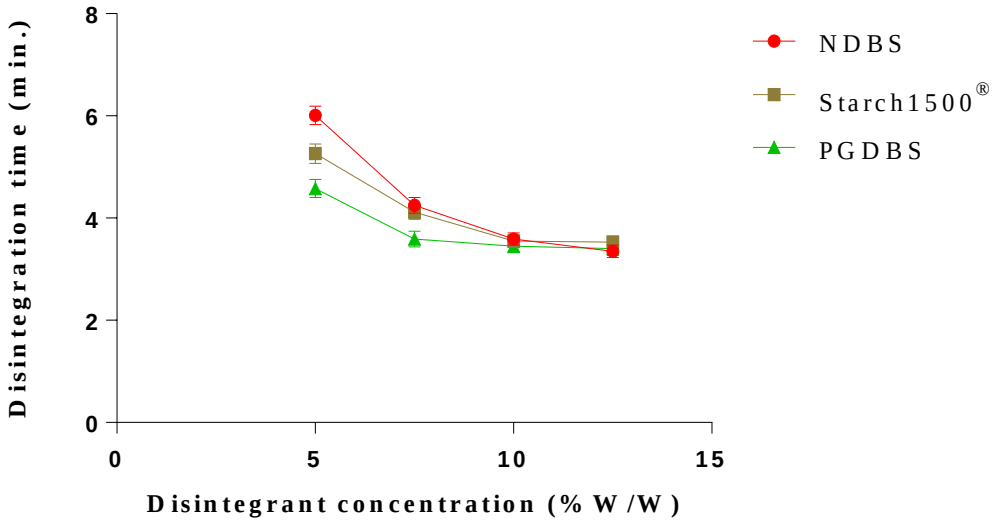



Figure 3.10: Disintegration time of paracetamol tablet with PGDBS, Starch 1500® and NDBS used as disintegrant.

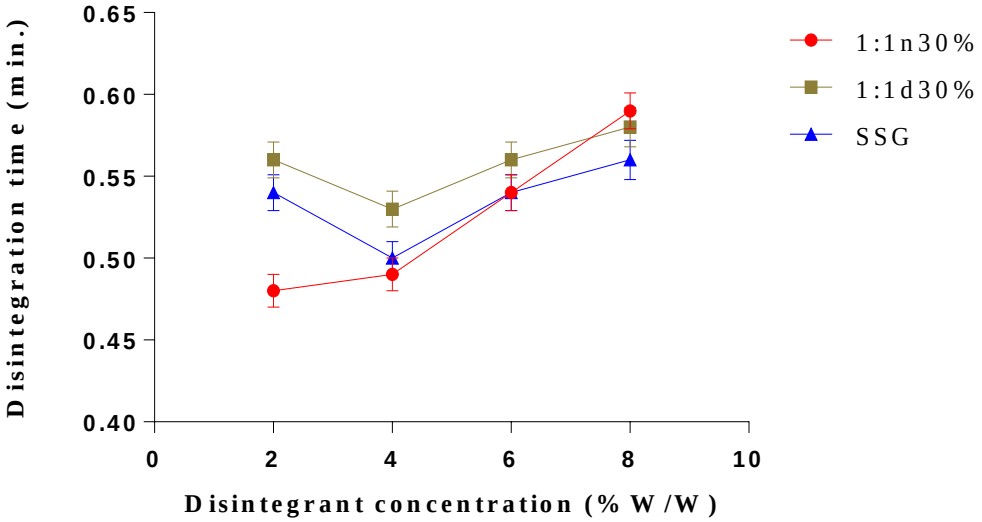



Figure 3.11: Disintegration time of paracetamol tablet with SCMDBS (1:1n30%), SCMPGDBS (1:1d30%) and SSG used as disintegrant.

## 3.12. Calibration curve of paracetamol

Figure 3.12 shows the standard calibration curve of paracetamol. The absorbance of the solution as a function of its concentrations were plotted and a calibration curve with a linear regression equation of Y= 0.077X + 0.01 (where Y is the absorbance and X is the concentration in mg/ml) and correlation coefficient ($r^2$) of 0.9998 was obtained.

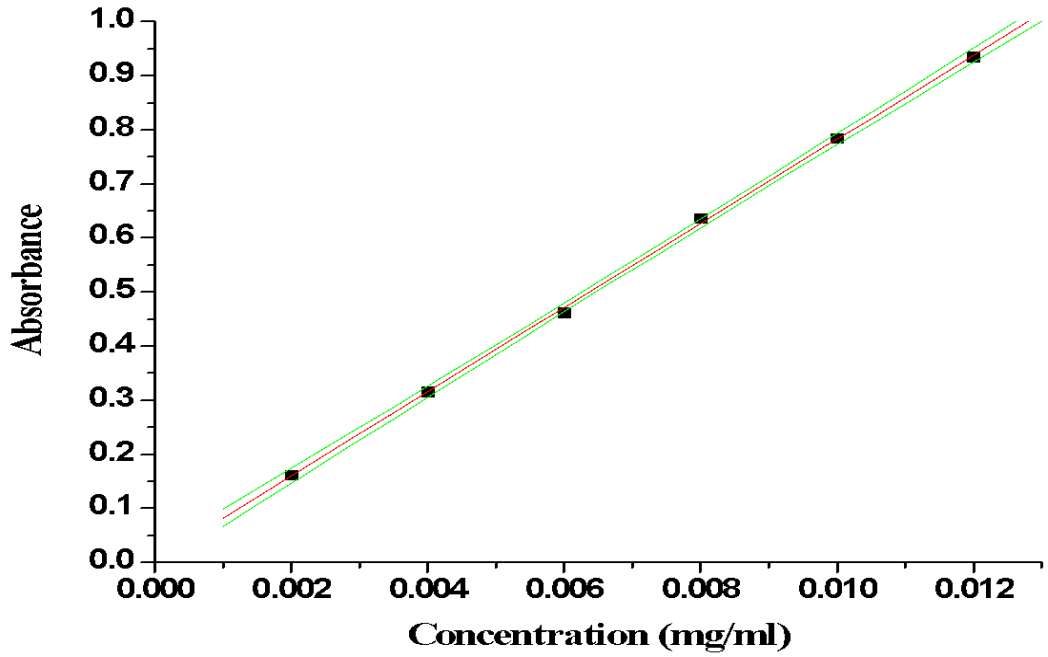



Figure 3.12: The UV absorption calibration curve of paracetamol in phosphate buffer (pH 5.8) at 243nm ($r^2$ =0.9998).

## 3.13. Drug dissolution profile

The dissolution profiles of paracetamol tablets at 10% w/w (PGDBS) and at 4% w/w (SCMDBS and SCMPGDBS) disintegrant concentrations are shown in Figure 3.12.The formulation showed rapid dissolution rate and the percentage cumulative drug release after 5 min was more than 60%.

The disintegrant concentrations used in tablets for dissolution studies are those that gave the shortest disintegration times. It is evident that disintegration usually plays a vital role in the dissolution process since it determines to a large extent the area of contact between the solid and liquid (Odeku and Itiola, 2006).

All formulations released more than 80% in 30 min, complying with the USP-30/NF-25 (2007) specifications.

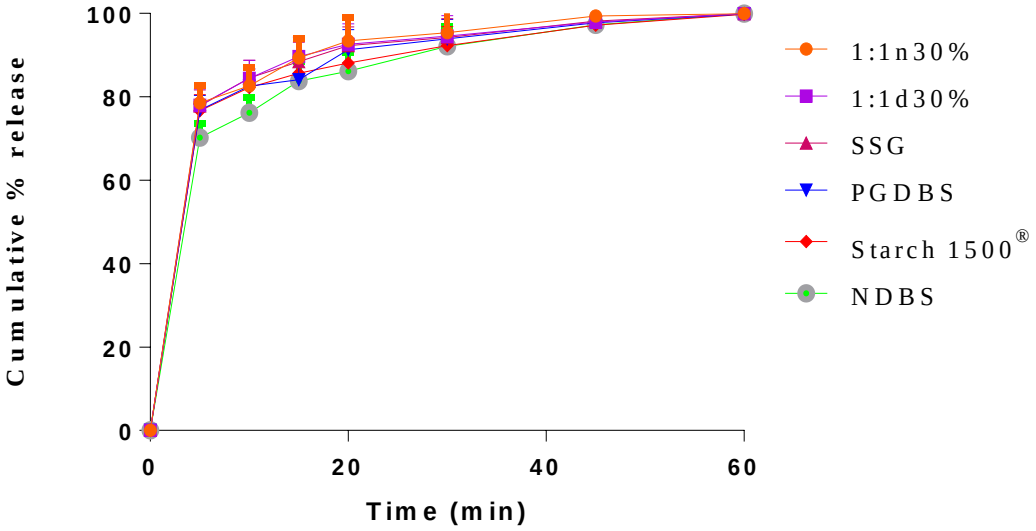



Figure 3.13: Dissolution profiles of paracetamol tablets prepared with SCMDBS (1:1n30%), SCMPGDBS (1:1d30%), SSG, PGDBS, Starch 1500 and native DBS used as disintegrant.

# 4. CONCLUSIONS

Pregelatinization of DBS improved the flowability of the DBS. Pregelatinization increased the percentage solubility, swelling power and water holding capacity of DB starch.

Paracetamol granules containing native and pregelatinized DBSs and Starch 1500® exhibited good flow property as indicated by their flow rate, Carr's index, Hausner ratio and angle of repose.

Increasing the concentration of native starch when used as disintegrant in paracetamol formulations reduced the crushing strength and DT of the tablets and increased the percent friability of the tablets. On the other hand, crushing strength and percent friability of tablets were less sensitive to the increment of the concentration of PGDBS when it is used as a disintegrant, implying that PGDBS has lower softening effect than the native one.

SCMDBS and SCMPGBDS were obtained as products of reaction of starch and different molar ratio of MCA: AGU in the presence of NaOH at 20% or 30%w/v. DS is affected by types of starch , molar ratio of MCA:AGU and concentration of NaOH. Higher DS was obtained at 1:1 molar ratio of MCA: AGU and 30%NaOH. DS in turn affect properties of SCMS. SCMDBS and SCMPGBDS showed better swelling power and more hygroscopic than NDBS.

The study showed that modified DBS (i.e., PGS, SCMDBS & SCMPGDBS) have a better disintegrant property than the unmodified DBS due to their relative high swelling power. Swelling power exhibit some direct effect on disintegrant activity of the starches. SCMDBS shows superior disintegrant activity than dually modified SCMPGDBS. Although it is known that SSG with optimum disintegration properties can be prepared from potato starch, the present study reveals DBS is also a potential alternative candidate.

Paracetamol tablet prepared with SCMDBS exhibited shorter DT than others but generally all the tablets containing the NDBS and modified DBS also passed the official DT test.

# 5. SUGGESTION FOR FURTHER WORK

Further investigation on the following directions is suggested:

- The application of SCMDBS as suspending and thickening agent in liquid dosage form,
- The application of SCMDBS as binder and sustained releasing agent in solid dosage form,
- PGDBS as filler-binder in tablets formulations.

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DECLARATION

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

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This thesis has been submitted for examination with our approval as advisors

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