ISOLATION, PHYSICOCHEMICAL CHARACTERIZATION AND EVALUATION OF TRITICUM DECOCUM STARCH AS BINDER AND DISINTEGRANT IN PARACETAMOL TABLETS

BY

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ISOLATION, PHYSICOCHEMICAL CHARACTERIZATION, AND EVALUATION OF *TRITICUM DECOCUM* (ETHIOPIAN OAT) STARCH AS BINDER AND DISINTEGRANT IN PARACETAMOL TABLETS

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This is to certify that the thesis prepared by Balisa Mosisa, entitled *Isolation, Physicochemical Characterization, and Evaluation of Triticum Decocum (Ethiopian Oat) Starch As Binder and Disintegrant in Paracetamol Tablets* submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Background: Oat is one of cereal grain which has about 10-15 species of true grasses (family Poaceae). Even though, Avena sativa L (common oat) is the most important one among cultivated oat in most parts of the globe; four other species are grown as crops of minor or regional importance of which *Triticum decocum* which is cultivated in Ethiopia is one. *Triticum decocum* is a half-weed, half-crop with erect stems which are small and fairly stiff with equilateral panicles and medium size, just very drooping.

Objective: The main purpose of current work is to study the physicochemical properties of *Triticum decocum* starch, and evaluate its binding and disintegrating properties in paracetamol tablets.

Statement of the problem: With the recent global renewed interest of natural polymers for industrial consumptions, the demand for the starch is expected to hit 230 million metric tons per year by 2015 globally. Current starch demand of Ethiopia which is more than 400 tons per year is imported from other countries. The need is escalating with industrial advancement. To cope up with these demands, potential endogenous sources of starches including, *Triticum decocum* starch, should be considered if being economical and sustainable supply is anticipated.

Methods: Different methods previously described elsewhere were used to achieve the above objective with slight modification as its importance.

Result and Discussion: The proximate composition of the starch on dry weight basis was found to be 0.23 % ash, 0.26 % protein, 1.54 % fat and 56.31 % starch. The total amylose content was 28.4 % with 20.11% apparent amylose and 29.19% lipid complexed amylose. SEM showed oval and spherical shaped granules. The particle size distributions of the starch showed monomodal normal particle size distribution with the average volumetric particle diameters being about 23.03µm, and specific surface area 0.7186 m^2/g. XRD showed a pattern that is typical of A-type with a distinctive maximum peak at around 17° 2θ. At all relative humidities, its water sorption properties resemble that of *Zea mays* starch. It has unique swelling power and solubility in water at different temperature. Viscosity curves of 10% starch paste showed a peak viscosity of 1926cP. Its cold paste viscosity and hot paste viscosity were 4236cP and 1720 cP, respectively. The breakdown and set back of *Triticum decocum* starch were 206 and 2647cP, respectively.

Powder mix of the starch with paracetamol has poor flow and compressibility properties. But after wet granulation, the granules have good flow and compressibility properties. Tablet crushing strengths, friabilities, disintegration times and dissolution profiles results had shown promising results.

Conclusion and Recommendation

Based on the above results, *Triticum decocum* starch can be used both as a binding agent and disintegrant. It has a better binding ability than *Zea mays* starch, giving tablets of higher strength and less friability. However, tablets containing *Triticum decocum* starch disintegrate slightly more slowly. Different methods of extraction, modifications, and other use evaluations had better conducted on the starch.

Key Words: *Triticum decocum*, physico-chemical properties, chemical composition, flow and compressibility.

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Balisa Mosisa
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ACRONYMS

API	Active pharmaceutical ingredient
BD	Breakdown
C_v	Cold paste viscosity
FTIR	Fourier-Transform Infrared
H_v	Hot paste viscosity
N	Trial number
P_v	Peak viscosity
RH	Relative humidity
SEM	Scanning electron microscope
SB	Setback
SD	Standard deviation
USPNF	United States Pharmacopoeia / National Formulary
XRD	X-ray diffraction/diffractometry

1. INTRODUCTION

1.1. Starch

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

Starch is by far the most extensively utilized biopolymer for different purposes (BCC Research, 2009). It occurs in the form of granules which varies in shape, size, physicochemical characteristics, and functionalities. These differences have been attributed to the composition, proportion, and structures of the amylose and amylopectin molecules; the amylose-to-amylopectin ratio; and the substitution of the glucose monomers. The genetic makeup and the activities of the plants have also been reported by to contribute to such differences in physicochemical properties of the starch from different origin (Bhat and Karim, 2009).

Starch and its derivatives are used mainly for application of the food, plastics and pharmaceutical industries due to its readily availability, abundant supply, cheapness, inertness, biodegradability, environmental benignness, easy of its physicochemical properties modification through chemical or physical or enzymatic treatment (Adane and Gebre-Mariam, 2004; Wondimu and Gebre-Mariam, 2010).

The world-wide market for industrial starches is expanding and current demand is met by limited range of crops, the most important of which are potato, *Zea mays*, wheat and tapioca. Availability of novel processing techniques and current demand for biodegradable and renewable resources undoubtedly make starch to command more versatile markets (Ellis *et al*., 1998; Schwartz and Whistler, 2009). Approximately, 60 million tons of starch is extracted annually worldwide from various cereal, tuber and root crops, of which roughly 60% is used in foods and 40% in pharmaceuticals and for non-edible purposes (Copeland *et al*., 2009). The demand for the starch is expected to hit 230 million metric tons by 2015 (BCC Research, 2009).

1.2.Sources of Starch

Starch granules are synthesized in a broad array of plant tissues and within many plant species. Cereals, roots and tubers strike top in the list of starch sources. Other sources such as ginger, *Manihot esculenta*, cocoyam, sorghum, plantain, rye, barley, yam, enset, and colocasia have also been researched to cope up with accelerating global needs (Tester *et al.*, 2004).

Most commercially available starches are isolated from cereals (corn and wheat), tubers (potato) and roots (cassava, sweet potato, arrow root) (Hofvander, 2004). Starches especially from cereals have received very extensive attention. *Zea mays* or corn is by far the biggest crop corresponding to 80% of total starch production and most of this is produced in the USA. Europe is the major producer of wheat and potato starches. In Europe, about 7.7 $x10^6$ tons of starch is produced annually. It consists of corn starch (49%); wheat starch (29%); and potato starch (22%) , where as cassava starch is produced in Asia (Jayakody *et al.*, 2007; Salwa *et al.*, 2010).

The chemical composition and the physical characteristics are essentially typical to the biological origin of the starch, i.e. they are unique to each type of starch. Therefore, what has been discovered about the structural features of one type of starch does not necessarily apply to other types of starch (Schwartz and Whistler, 2009).

Some of the Ethiopian plants which have been shown to possess starch, either modestly or abundantly include Enset (Gebre-Mariam and Schmidt, 1996a); Ethiopian Yam (Gebre-Mariam and Schmidt 1998); Godare (Adane *et al*., 2004); Anchote (Nigussie *et al*., 2006); Cassava (Paulos *et al*., 2007); Kottee Harree (Mohammed *et al*., 2007).

1.3. Extraction and Physicochemical Characterization of Starches

1.3.1. Starch Extraction

Maximum recovery of starch with good physicochemical and functional qualities coupled with economical extraction of starches from sources is important (Moorthy, 2002). The procedure for starch extraction depends on the botanical sources, which usually involves the use of wet millers in a large scale. Refining processes differ depending on the type of raw materials. The extraction and purification of starches from cereals is more difficult than is the case for many other botanical sources, presumably because the cereal grains normally contain much less moisture.

Furthermore, most of the starch is found in the endosperm, where it is embedded in the cytoplasmic matrix (Borght, *et al*, 2005).

High purity starch (low protein-lipid content) is desired for industrial application. Alkaline solution of sodium hydroxide is used to achieve this purpose by purifying starch from endosperm. Alkaline solution of sodium hydroxide has also been used to eliminate protein from rice, sorghum, taro, Godare and sweet potato starch (Wang, 2004; Adane, *et al.*, 2006).

1.3.2. Physico-Chemical Characterization of Starches

1.3.2.1. Structure and content of Amylose and Amylopectin

Amylose/amylopectin ratio, molecular weight and molecular fine structure influence the physicochemical properties of starch. After thousands of studies over many decades, starch remains a fully undiscovered mysterious substance. This mysterious nature of starch emanates from its exclusive behavior that each starch is unique in terms of granule organization and structures of constituent polymers. Starches from different sources are different, and that not all granules of a single starch behave identically (BeMiller, 1997).

The knowledge of the internal organization helps to understand the functionalities and the transformation behavior of starch, and improve the properties and stability of starch products (Schwartz and Whistler, 2009). In addition to fine structure, amylose and amylopectin differ in many respects as shown in **Table 1.1** below (Senanayake, 1995).

Table 1.1. Structure and physicochemical properties of Amylose and Amylopectin

Properties	**Amylose**	**Amylopectin**
Molecular structure	Essentially linear- $\alpha(1\rightarrow4)$	Branched- $\alpha(1\rightarrow4)$, $\alpha(1\rightarrow6)$
Molecular Weight	$1.5x(10^5-10^6)$	$(50-500)x10^6$
Iodine complex, λ_{max}(color)	600-640 (Blue)	530-550 (Purple)
Stability of dilute aq. solution.	Unstable (retrogrades)	Stable

Amylose has traditionally been considered as a linear polymer made up of D-glucose units. Although there is now evidence that it is not completely linear (Hizukuri et.al, 1981), its behavior is that of linear polymer in solution. This polysaccharide is one of the two components of starch, making up approximately 20-30% of the structure; the other component is amylopectin, which makes up 70-80% of the structure. Amylose is made up of α (1→4) bound glucose molecules (Nelson et.al, 2008). The structural formula of amylose is shown in **Fig 1.1 (a)** below. The number of repeated glucose subunits (n) is usually in the range of 1000 to 10,000.

There are three main forms that amylose chains can take. It can exist in a disordered amorphous conformation or two different helical forms. It can bind with itself in a double helix (A or B form), or it can bind with another hydrophobic guest molecule such as iodine, fatty acid or an aromatic compounds. This is a form of amylose which allows amylopectin to be bound to it to form a starch.

The α(1→4) structure promotes the formation of a helix structure, making it possible for hydrogen bonds to be formed between the oxygen atoms bound at 2-carbon of one glucose molecule and the 3-carbon of the next glucose molecule (Cohen *et al.*, 2008).

Unlike amylopectin, amylose is insoluble in water. It also reduces the crystallinity of amylopectin and how easily water can infiltrate the starch. The higher the amylose content, the less swelling potential and the lower the gel strength for the same starch concentration. This can be countered partially by increasing the granule size (Li and Yeh, 2001).

Powder X-ray diffraction analysis coupled with computer-based structure refinement has found A-, B-, and C- polymorphs of amylose. Each form corresponds to the A-, the B-, or the C- starch forms. A-and B- structures have different helical crystal structures and water contents, whereas the C- structure is a mixture of A- and B- unit cells, resulting in an intermediate packing density between the two forms (Sarko and Wu, 1978).

Amylose is an important thickener, water binder, emulsion stabilizer, and gelling agent in both industrial and food-based contexts. Loose helical amylose chains have a hydrophobic interior that can bind to hydrophobic molecules such as lipids and aromatic compounds (Chung and Liu, 2009).

Amylose has also units less than 0.5% in α [1→6] linkage, resulting in a low degree of branching (Copeland, 2009).

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

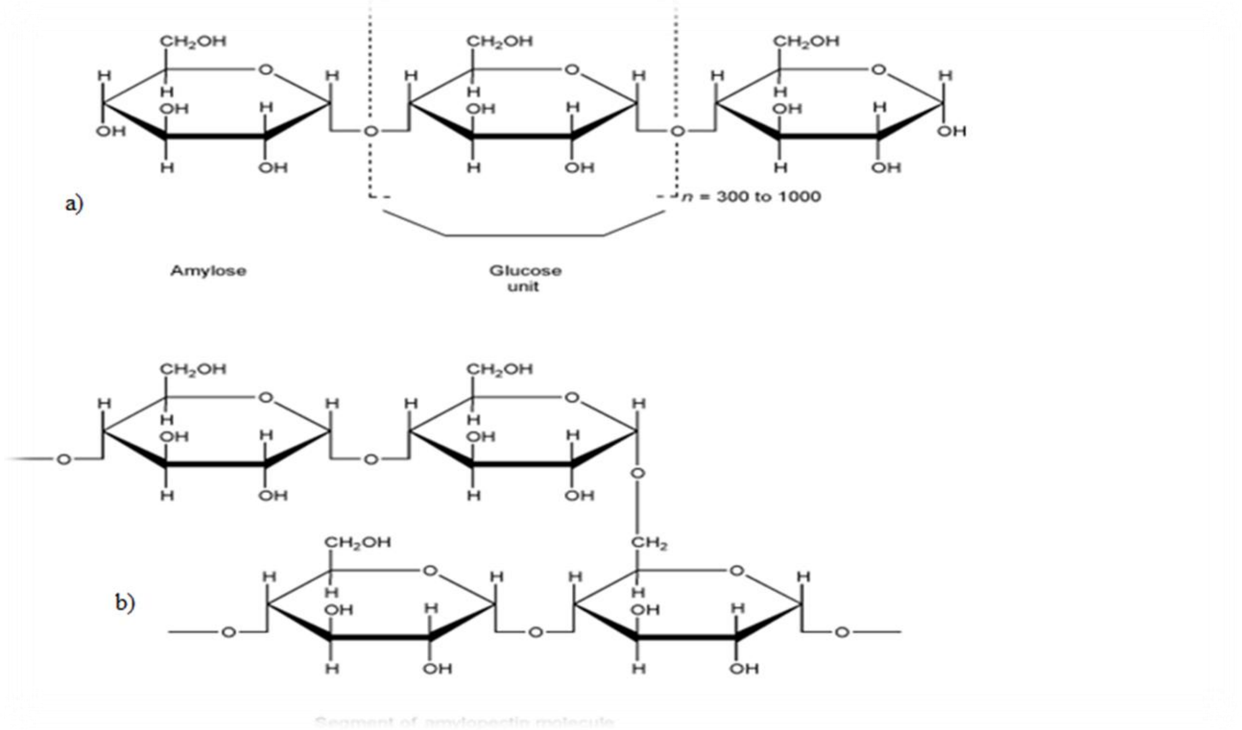

Figure 1.1. Structure of linear amylose (a) and branched amylopectin (b) molecules

Amylopectin is a soluble polysaccharide and highly branched polymer of glucose found in plants. It has a molecular mass greater than 10^8 (Green et.al., 1975; Dayner, *et al.*, 2001).

Glucose units are linked in a linear way with α (1→4) glycosidic bond. Branching takes place with α(1→6) bonds occurring every 24 to 30 glucose units, resulting in a soluble molecule that can be quickly degraded as it has many end points for enzymes to attach onto. In contrast, amylose contains very few α (1→6) bonds or even none at all. Hence, it is hydrolyzed more slowly, and insoluble due to its higher density and particle compaction . Each amylopectin molecule contains a million or more residues, about 5% of which form the branch points. There are usually slightly more 'outer' unbranched chains (called A-chains) than 'inner' branched chains (called B-chains). There is only one chain (called the C-chain) containing the single reducing group (Richardson and Gorton, 2003).

1.3.2.2. Moisture, Fiber, Protein and Lipid Contents

A. Moisture Content

The moisture content reflects the affinity of the material for moisture (Parrott, 1989). The initial moisture level as well as the inherent tendency of the active ingredients and excipients for water uptake from the surrounding environment governs the moisture sorption pattern of the final product. The hygroscopic nature of excipients and active ingredients should be considered in designing formulations. Starch, one of excipients 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. Therefore, knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction and disintegration of tablet are critical. Moisture modifies the flow and mechanical properties of many powders including starches (Gebre-Mariam and Schmidt, 1996b) ; Paulos *et al*., 2009).

The moisture content of air-equilibrated dry starches varies from about 10-12% (cereal) to about 14-18% (some roots and tubers) depending on the starch species (Tester *et al*., 2004).

Climatic factors also play a part in deciding the moisture content. For example, *Dioscorea dumetorum* starch has 12% moisture for starch extracted during the wet season, but 13.5% for that extracted during the dry season (Moorthy, 2002). Lower moisture contents are required for safe storage as higher moisture contents can lead to microbial damage and subsequent deterioration in quality (Mweta *et al*, 2009).

B. Fibers Content

The fibers content in starch varies to a great extent depending on the sieve used for removal of the fibrous material, varietal variation and age of the crop like cassava where fiber content may increases with maturity. Wide variation in fiber and ash contents in different starch sources is evident from various reports (Charalampopoulos, *et al.*, 2002).

C. Protein Content

Starch granules also contain small quantities of other "minor" components, such as proteins, lipids, pentosans, and minerals (e.g. phosphorus and silica) either at the surface or inside the starch granules. Of these, proteins and lipids are by far the most abundant (Vandeputte and Delcour, 2004).

The exact quantities of protein and lipid associated with the starch depends on both the botanical origin of the starch and its degree of purification during extraction, however, a typical well-washed cereal starch sample contains ~0.25% protein and up to ~1.0% lipid, whilst a typical root or tuber starch (e.g. potato) contains 0.05% protein and 0.05 – 0.1% of lipid. Although on a quantitative scale these two constituents are deemed minor, there is increasing awareness that their presence significantly affects both the properties of the granule as a whole and the properties of starch-derived products (Jobling, 2004).

D. Lipid Content

Cereal starches (e.g. corn, wheat, and rice) contain relatively high levels of lipids (0.2-0.8%). Tuber (e.g. potato) and root (e.g. cassava) starches have lower levels of lipids (0.1-0.2%). The starch-lipid interaction is particularly important in cereal starches (Moorthy, 2002).

Presence of higher lipid content can affect some physicochemical properties of Oat starches significantly such as delaying/decreasing of starch granules swelling, reducing the solubility, reducing rate of gelatinization and reduction of water uptake especially if the lipid is medium and long chain compound through interaction with linear amylose chains. It has also the potential to affect the size and shape of the starch granules at higher temperatures (Camire *et al.*, 1990; Gray and Schoch, 1962; Tester and Morrison, 1990).

Also lipid can affect paste viscosity and pasting characteristics of starch. A significant portion of these lipids is found on the surface of the starch granule, especially in cereal starches (Svihus, *et al.*, 2005).

1.3.2. 3. Granule Shape and Size

Granule size, size distribution and shape are among the most important morphologically distinguishing factors of starches from different origins. Granule size and shape influences the physicochemical characteristics of starch, as well as the procedures employed in starch refining. It also influences the physicochemical characteristics of starch including Starch composition, gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling and solubility. For this, spherical granules are assumed, which is seldom correct (Jane et.al., 1994).

The granules sizes, shapes and surfaces characteristics were examined using Scanning Electron Microscope. Monomodal or Bimodal granule size distributions, with differentiation being made between granule fractions with smaller and larger diameters were revealed in different studies for starches of different origin (Morrison and Scott, 1986); (Dengate and Meredith, 1984).

Morphological characteristics of starch granules (shape and size) depend on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant. Starch granules from different botanical origins differ in morphology. Granules of tuber and root starches, for example, are oval, although round, spherical, polygonal, and irregular shaped granules also exist (Lindeboom, *et al*., 2004).

In general, starch granule size may vary from less than 1µm to more than 110µm. No precise categorization of granule size is found in the literature. For this reason, the following classes are defined: large (>25µm), medium (10-25µm), small (5-10µm) and very small (<5µm) granules.

1.3.2.4. Granule Crystallinity

A native starch granule consists of a semi-crystalline structure. The branches of amylopectin form double helices which are arranged in crystalline domains. Contrarily, amylose largely makes up the amorphous regions which are randomly distributed between the amylopectin clusters. The branching regions are constituted of the amorphous layer that separates the crystallites from each other (Tester and Karkalas, 2004).

According to the X-ray diffraction pattern, native starch granules can be classified as A, B and C type. These depend partly on the chain lengths making up the amylopectin lattice, the density of packing within the granules, and the presence of water. Although type A and type B are real crystalline modifications, type C is a mixed form (Sajilata, *et al.*, 2006).

Most cereal starches (e.g. normal corn, rice, wheat) display the A type, while tuber starches (e.g. enset, dioscorea, potato) exhibit the B type (Gebre-Mariam and Schmidt, 1998). Several rhizome and bean starches belong to the C type. Tuber starches have been shown to exhibit a 'B' type X-ray pattern with reflections centered at 5.5-5.6°, 14.1°, 15.0°, 17.0°, 19.7°, 22.2° and 24° 2θ angles. Whereas, 'A' type starches (cereals) exhibit reflections at 15.3°, 17.0°, 18.0°, 20.0° and 23.4° 2θ angles (Jayakody, *et al.,* 2005).

1.3.2.5. Moisture Sorption Pattern

The initial moisture level as well as the inherent tendency of the active ingredients and excipients for water uptake from the surrounding environment governs the moisture sorption pattern of the final product. The hygroscopic nature of excipients and active ingredients should be considered in designing formulations. Starch has been classified as a moderately hygroscopic material. Moisture is known to modify the flow and mechanical properties of many powders including starch. Therefore, knowledge of moisture sorption properties of starch is necessary where controlled powder flow or compaction is critical. The moisture sorption isotherms show the equilibrium amount of water sorbed onto a solid as a function of steady state vapour pressure at a constant temperature. In the natural state, starch granules are at moisture equilibrium with the surrounding atmosphere and under ordinary atmospheric conditions, contain 8-12% moisture. (Whistler and Smart, 1953; Gebre-Mariam and Schmidt, 1998).

1.3.2.6. Swelling Capacity and Solubility

When starch is pasted in hot water, the individual granules swell and a portion of the starch substance dissolves in the aqueous medium. Swelling power provides evidence of non-covalent bonding between starch molecules. Thus, highly associated starch granules with an extensive and strongly bonded micellar structure should display relatively greater resistance towards swelling. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation determine the degree of swelling and solubility (Moorthy, 2002; Paulos et.al, 2009).

When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which cause an increase in granule swelling and solubility (Ratnayakea, *et al.*, 2002).

The swelling power of starch has been reported to depend on water holding capacity of starch molecules by hydrogen bonding. Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and are replaced by the hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch. The granules become increasingly susceptible to shear disintegration as they swell, and they release soluble material as they disintegrate. It has been indicated that starch granules with low amylose content being less rigid, swell freely when heated. The starch granules with higher amylose content, on the other hand, being better reinforced and thus more rigid, probably swells less freely (Schoch, 1994).

1.3.2.7. Starch Pasting Properties

Starch exhibits unique viscosity behavior with change of temperature, concentration, and shear rate. The viscosity parameters during pasting are cooperatively controlled by the properties of the swollen granules and the soluble materials leached out from the granules. Early in the pasting test, the temperature is below the gelatinization temperature of the starch, and the viscosity is low. When the temperature rises above the gelatinization temperature, the starch granules begin to swell, and viscosity increase on shearing when these swollen granules have to squeeze paste each other. The temperature at the onset of this rise in viscosity is known as the pasting temperature. Granule swelling is accompanied by leaching of granular constituents, predominantly amylose, into the external matrix resulting in a dispersion of swollen granules in a continuous matrix. When a sufficient number of granules become swollen, a rapid increase in viscosity occurs, known as peak viscosity. The increase in viscosity with temperature may be attributed to the removal of water from the exuded amylose by the granules as they swell. Peak viscosity occurs at the equilibrium point between swelling and polymer leaching. As the mixture is subsequently cooled, viscosity will increase to a final viscosity. It has been reported that increase in final viscosity might be due to the aggregation of the amylose molecules (Nelles, *et al.,* 2003).

1.4. ***Triticum decocum*** Starch

Oat is one of cereal grain which has about 10-15 species of true grasses (family Poaceae). They are native to Europe, Asia and Africa. One species is widely cultivated elsewhere, and several have become naturalized in many parts of the world. All oats have edible seeds, though they are small and hard to harvest in most species. Even though, Avena sativa L (common oat) is the most important one among cultivated oat in most parts of the globe; four other species are grown as crops of minor or regional importance. These are:

1. *Triticum decocum* (Its ancient name was *Avena abyssinica*)- *Triticum decocum* , "a half-weed, half-crop confined to the highlands of Ethiopia
2. *Avena byzantine* a minor crop in the Near and Middle East
3. *Avena nuda* – Naked Oat or Hulls Oat plays much the same role in Europe as does Triticum decocum in Ethiopia. It is sometimes included in *A. sativa* and was widely grown in Europe before the latter replaced it. As its nutrient content is somewhat better than that of Common Oat, *A. nuda* has increased in significance in recent years, especially in organic farming
4. *Avena strigosa* – Lopsided Oat or Bristle Oat or Black Oat, grown for fodder in parts of Western Europe and Brazil (Watson and Dallwitz, 2008).

Oats are mainly grown in temperate regions. They have a lower summer heat requirement and greater tolerance of rain than other cereals, such as wheat, rye or barley, so are particularly important in areas with cool, wet summers, such as North West Europe; they are even being grown successfully in Iceland. They are an annual plant, and can be planted either in autumn (for late summer harvest) or in the spring (for early autumn harvest). In tropical Africa, it is mainly grown in Ethiopia and Kenya. It is also cultivated in South Africa, Morocco, Algeria and Tunisia (Brik, 2006).

The Food and Agriculture Organization of the United Nations reports that the production of oat in 2007 was 25,991, 961 metric tons, which is up from 22,758,002 metric tons in 2006, but down considerably from the 1961 production of 49,588,769 metric tons (FAO, 2008). Incase of sub-Saharan Africa , the average oat grain production in 1993-2003 has been estimated at 55,000 tons per year from 53,000 hectare almost entirely from Ethiopia (50,000 tones) per year from 49,000 hectare and Kenya 3,500 tons per year from 3,400 hectare and small amount from Zimbabwe (Brik, 2006).

Ethiopia also has a native oats, *Triticum decocum* which is a "half-weed, half-crop confined to the highlands of Ethiopia." Stems are erect, tend to be small, and are fairly stiff, and panicles are equilateral, medium in size, and very drooping (CNCPP, 1999). It has long been used in Ethiopia and is well adapted to the high elevations and other conditions there. It is, however, unknown elsewhere. With a rising international interest in oats this little-known species deserves research attention.

It is used in injera (the flat national bread), local beer (*tela*), and the straw serves as bedding for live stock, fuel and roofing materials for traditional houses. In Kenya, oat is used as food and fodder. Nonetheless, Ethiopian native oats deserves research attention and a chance to prove itself (Baum, 1971; Engels *etal.*, 1991; Ladizinsky *etal.*, 1975).


Ethiopian Oat (*Triticum decocum*)

Figure 1.2. *Triticum decocum* plant and its grain

1.5.Starch Application

1.5.1. Applications in pharmaceutical formulations

As an excipient, starch finds a wide application in the manufacture of pharmaceuticals. Its use is based on its adhesive, thickening, gelling and film forming properties. Native starch has also been recognized as one of the most commonly used excipient in the manufacturing of tablets, which can be used as binders, disintegrants or fillers (Gebre-Mariam, *et al.*, 1996b).

1.5.2. Other Applications

Starch is a very versatile raw material with a wide range of applications from dietary needs for humans to industries (food, plastic, cosmetics, pharmaceutical, confectionery, textile, paper and adhesive).

1.6. The Present Study

Efforts to find alternative sources of starch for the Ethiopian industries have led to the development of starch research in Ethiopia. For years, the Ethiopian industries have relied on imported starches of limited sources for use in various applications. Compiled data from the Central Statistics Agency of Ethiopia revealed that Ethiopia imported an average of 385.32 tons of starches annually during the 1997-2006 fiscal years. The manufacturing industry sectors are thought to consume most of these imported starches and hence, assuming the average annual growth rate of the sector to stand at 7%, the agency projected the demand for the starch by 2022 to be about 1137.52 metric tons for Ethiopia. This importation has led to loss of large amounts of foreign currency and employment opportunities for the local Ethiopians. Though demand for starch is ever increasing, the Ethiopian industries face problems due to increased costs, supply capacity, availability and late deliveries. Ethiopia has variety of plant species of tuber and cereal types which can be used as a source of starch for various purposes (Girma and Gebre-Mariam, 2009).

Therefore, there is a need to explore indigenous crops locally grown by subsistence farmers, as alternative sources of starch. There are such attempts so far focused on indigenous crops which have great potential for this purposes, but not enough to meet the industrial demand of the nation at least. If starch production is to be increased to meet the industrial demand, and bring economic benefit to Ethiopians, there is a need to explore other sources of starch which could supplement the existing sources. *Triticum decocum* offer this opportunity as alternative sources of starch for the local industry since Ethiopia is the leading country in Africa in Oat production (Brik and Belay, 2006).

The present study reports on the, composition, physicochemical properties of *Triticum decocum starch* and its application in paracetamol tablet. Comparison of these data with that of *Zea mays* starch were also performed. Further, a basis for the explanation of difference and similarities were scientifically forwarded. Paracetamol, which is both poorly compressible and sparingly soluble drug, normally requires a binder and disintegrant to form satisfactory tablets. Thus, it was selected as a model drug for this study.

1.6.Objectives

1.6.1. General Objectives

To extract and evaluate the physicochemical properties of *Triticum decocum* starch and assess its binding and disintegrating properties in paracetamol tablets.

1.6.2. Specific Objectives

- To extract *Triticum decocum* starch from *Triticum decocum* grain
- To study the physico-chemical properties of the isolated oat starch
- To evaluate the binding and disintegrating abilities of the starch in paracetamol granules and tablets in comparison with *Zea mays* starch.

2. EXPERIMENTAL

2.1. Materials

Cereal grains of *Triticum decocum* was bought from local market in Addis Ababa, Ethiopia. Different equipments and chemicals used were specified with in the methods section.

2.2. Methods

2.2.1. Extraction and Purification of *Triticum decocum* Starch

Extraction and purification of *Triticum decocum* starch was carried out according to Lim *et al*, (1992) with slight modification. *Triticum decocum* flour was sieved through a fine sieve (224 µm) to remove roughages and unnecessary impurities from the flour. Sieved oat flour of 500 g was mixed with 4L 0.1M NaOH, stirred for 2 h at room temperature, and was allowed to stand for 12 h. The supernatant was then discarded by gentle decantation. After that, the sediment was slurred with 4 liter of distilled water, and stirred for 1 h, and was allowed to stand for 12 h. Then the supernatant was discarded by gentle decantation. This step was repeated three times to remove soluble substances. The sediment was then neutralized with 2L 1 M HCl, and strained to pass through muslin cloth three times to remove cell debris, soluble substances, sugar, and mucilage. The suspension was collected by filters through a fine sieve (224 µm). The filtrate was centrifuged at 4000 rpm. The supernatant was discarded, along with the tailings layered on top of the starch, which were carefully removed by scraping; the sedimented starch was collected to be dried in an oven (Kottermann® 2711, Germany) at 40 ^{o}C over night. Then it was milled to fine powder in grinding machine, sieved using 224 µm mesh size sieve and stored in plastic bags.

2.2.2. Determination of Percent Yield

Percent yield on dry basis was determined according to the method described by Association of Official Analytical Chemists with slight modification, (AOAC, 2000). About one gram of *Triticum decocum* powder was extracted by the method described under **section 2.2.1** three times individually and the average percent yield was calculated.

2.2.3. Determination of Chemical Composition

2.2.3.1. Estimation of Amylose Content

A colorimetric assay method reported elsewhere (Williams *et al.*, 1970; Gebre-Mariam and Schmidt, 1996) was slightly modified and used for estimation of the defatted and native *Triticum decocum* Starch. Similarly it was done for *Zea mays* starch. In this, a stock solution of each of amylose and amylopectin was prepared by dissolving 100 mg of the respective starches and standards in separate tubes with 1 ml of 95% ethanol and 9 ml of 1 M NaOH. The test tubes were covered with foil, thoroughly mixed and heated for 10 min in boiling water bath to gelatinize the starch and were cooled. 0.1 ml of Acetic acid solution and 0.2 ml of Iodine solution was added to 0.5 ml aliquots of each stock solution. These were made up to 10 ml mark with distilled water. The solutions were left for 20 minutes for color development. Mixtures of amylose and amylopectin solutions were prepared to provide starch concentrations of a 50 μg in 10 ml in any mixture. Various mixtures were prepared to contain 100%, 80%, 60%, 40%, 20%, and 0% amylose or amylopectin, respectively. The absorbance reading of the resulting solutions were taken at 600 nm for amylose and 540 nm for amylopectin using JENWAY, model 6505 UV/ VIS spectrometer after staining with Lugol's solution. 100 μg of the diluted Lugol's solution was used for staining 2 ml of the mixture. Similarly, absorbance readings of each of *Triticum decocum* and *Zea mays* starches were taken at the same wave lengths. Blanks were run with stained 95% ethanol, 1 M NaOH, acetic acid solution and distilled water. The amylose content of the starch was estimated from the relationship between concentrations and absorbance of known mixtures of amylose and amylopectin at 600 nm as the interferences from amylopectin at the concentrations used was negligible. The results are the average and standard deviations of five determinations.

2.2.3.2. Moisture Content

Moisture content of the *Triticum decocum* starch and *Zea mays* starch powders was determined by a gravimetric method which was described by Senanayake, (1995). The powder sample (5 g) was spread onto pre-weighed petridishes uniformly and dried in an oven at 105 ^{o}C until constant weight was obtained. The weight loss was obtained by accurately weighing the samples after attainment of constant weight. The moisture content was determined from the loss of weight. The result was expressed as a mean of three parallel determinations.

2.2.3.3. Total Ash Determination

Ash content was determined according to FAO manuals of food quality control (FAO, 1986). The ash content was determined by weighing 2.5 g of *Triticum decocum* and *Zea mays* starch samples into crucible that has been ignited, cooled in a desiccator, and weighed soon after reaching room temperature. The sample was ignited in a furnace (Naber, D-2804, Liliethal, Germany) at about 550 ^{0}C for 1 h. The remaining mass in the crucible is the total ash, which was expressed in percentage.

2.2.3.4. Protein Content

Protein was analyzed by destruction of organic matter by sulphuric acid. Nitrogen is liberated as ammonia, distilled, collected and titrated according to Association of Official Analytical Chemists, (AOAC, 2000). Weighed starch sample (0.5 g) was placed in 500 ml digestion flask. 6 ml acid mixture (2 parts of conc. sulphuric acid and 1 part of conc. ortophosphoric acid) was added. The flask was placed on heater and violent reaction was observed. As soon as violent reaction was ceased, heat was increased and the destruction was continued until the content appears light green for one hour. It was then cooled and diluted with distilled water. The digested and diluted solution was transferred into sample compartment of the distiller. It was then distilled until a total volume of 150 ml was collected (NH_3 as distillate). Finally the excess standard acid in the distillate was titrated with standard NaOH solution.

2.2.3.5. Lipid Content Determinations (Defatting)

Defatting of *Triticum decocum* starch and *Zea mays* starch was conducted using the method described by Bligh and Dyer, (1959), and Goshima, *et al*., (1985). That is 5 g of respective starches were extracted under vigorous shaking with 100ml of chloroform-methanol (2:1 v/v) at 25 ^{o}C for 1 h. This extract was separated into filtrate (Filtrate 1) and residue by decantation. The filtrate was used for further isolation of unbound lipids, while residue were further solvent extracted with 100 ml of n-propanol-water (3:1v/v) at 95 ^{o}C for 7 h to extract mainly bound lipids in water bath. This second extract was decanted after being cooled at room temperature. Its filtrate (Filtrate 2) was used for further isolation of starch-bound lipids. After all these, both filtrates (Filtrate 1 and Filtrate 2) were purified by extraction with chloroform-methanol-water (1:2:0.8) and chloroform-methanol-water (1:1:0.9), respectively. Then, two layers (water and Chloroform) were formed. By gentle decantation, layers were separated. The chloroform layers were diluted with benzene and shaken slightly. Then the organic solvents were evaporated on a rotary evaporator. The weight difference was used to calculate percentage of unbound, bound and total lipids. Results are given as mean and standard deviation of three parallel determinations.

2.2.3.6. pH Determination

The pH of *Triticum decocum starch* was determined using the method described by Singh *et al* (2010) .It was done by shaking a 1% w/v dispersion of the starch in water for 5 min and the pH determined using a digital pH meter (Wagtech 3510, U.K.).

2.2.4. Physicochemical Characterization

2.2.4.1. Densities and Related Properties

Thirty gram of each of *Triticum decocum* and *Zea mays* starch were transferred into two separate 250 ml measuring cylinders; the volume occupied by each starch powder was read to the nearest 0.5 ml and bulk density was calculated as g/ml. The bulk in the cylinder was tapped for 1 min. using tapped volumeter (ERWEKA, type SVM, Germany), which provides a fixed drop of half an inch at a rate of 250 taps/min. The volume occupied by the starch was recorded and tapped density was calculated as g/ml. The true density of the starch was determined by liquid displacement pycnometer using **eq. 2.1** (Beckman GmbH, 8000 Munich, Germany) at room temperature, using xylene as displacement liquid (Anderson, *et al*., 1999). The Carr's index (% compressibility) of the starches was calculated from difference between the tapped and bulk densities divided by the tapped density and the ratio expressed as percentage. The Hausner ratio was calculated by dividing the tapped density by bulk density of the starch powder (Kumar, *et al.*, 2001).

Absolute (True) density $=\left(\frac{\mathbf{axd}}{(\mathbf{a}+\mathbf{b})-\mathbf{c}}\right)$ ……………………………………………….Eq. 2.1

where *a* is the original weight of the sample starch (in gram), *b* the weight of pycnometer filled with xylene (in gram), *c* the weight of the pycnometer plus sample plus xylene (in gram) and *d* is the density of xylene which is 0.87 g/ml at room temperature (25 $^{\circ}$C).

Angle of repose and flow rate

Flow rate and Angle of repose were determined by fixed height funnel method. In this, 30 g starch powder was placed and allowed to flow through a stem less funnel having 10 mm aperture from a fixed height of 10 cm. The duration of flow was recorded and used to calculate the flow rate. Angle of repose was determined from the height and radius of powder pile according to Eq. 2.2 below.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right) \qquad \mathbf{2.2}$$

Where, θ=Angle of repose, h = Height of granule, r = Radius of circle formed by the

2.2.4.2. Morphological Characterization

The starch granule morphology was studied as described by Stevenson, *et al., (*2006). The starch sample was mounted and sputter coated with gold to a thickness of about 30 nm (Sputter Coater Type E 5100, Biorad GmbH, Munich Germany). The scanning electron micrographs were taken with a DSM 940 SEM (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 4.5 KV. The granules sizes were estimated by the use of calibrated scale bar on the SEM micrograph.

2.2.4.3. Crystallinity of the Starch

X-ray powder diffraction of the starch sample was taken with X-Ray diffractometer (Bruker AXS, Bavart Zulassung, BW/508/98/Rö, Germany) operating in the 2θ modes. A Cu target tube operated at a power setting of 40 kV (30 mA) in the range of 4-60^o of 2θ with single crystal graphite monochromator equipped with a microprocessor to analyze peak position and intensities was utilized. A standard polycrystalline silicon powder was used to calibrate the equipment. The type of oat and *Zea mays* starch crystallinity was determined based on the major diffraction peaks as described by (Cheetham and Tao, 1998).

2.2.4.4. Granule size and size distribution

Granule size and size distribution and the specific surface area of the starch sample were measured with Malvern Mastersizer 2000 laser diffraction spectrometer (Malvern Instruments Ltd. Malvern, UK). Volume and surface weighted means as well as the uniformity of the particle size distributions were determined (Gebre-Mariam and Schmidt, 1998).

2.2.4.5. Moisture Sorption Pattern

Moisture sorption pattern was studied using the method described by Odeku and Picker-Freyer (2009). Pyrex desiccators containing distilled water (100% RH), saturated solution of NaCl (75.6%), and appropriate concentrations (40% NaOH, 31.58% NaOH, 24.66% NaOH) sodium chloride solution were prepared to obtain different relative humidity (10, 20, 40, 60, 75.5, and 100), respectively and stored in relative humidity chamber at room temperature in . *Triticum decocum* and *Zea mays* starch powder samples were pre-dried in an oven for 4 h at 120 ^{o}C. Two grams of *Triticum decocum* and *Zea mays* starch powder samples were spread evenly on each petridish (dried and weighed) and transferred to a particular RH chamber. Samples were equilibrated for four weeks at room temperature. The weights were recorded and moisture uptake of each sample was calculated as the weight difference of the samples before and after equilibrium in a given RH. Water sorption capacity of the starches was expressed as percent moisture uptake.

2.2.4.6. Swelling capacity and Solubility

Water solubility and swelling of *Triticum decocum starch* and *Zea mays* Starch were determined using the method described by Bello-Perez *et a*l., (2000) with slight modification. Powder samples (0.5 g each) were dispersed in distilled water (10 ml) in pre-weighed test tubes. The test tubes were then kept in thermostatically controlled water bath at 25, 37, 50, 65, 75, 85 and 95 ^{O}C for 30 min, with shaking every 5 min and then left to cool to room temperature. The suspensions were centrifuged for 45 min at 4000 rpm in order to facilitate the removal of the supernatant, which was carefully decanted and the weight of the starch paste taken. Then, the supernatant was decanted into a pre weighed watch glass and dried in an oven (Kottermann® 2711, Germany) for 2 hrs at 105 ^{O}C until constant weights were obtained. The residues obtained after drying the supernatant represents the amount of starch solubilized in water. The solubility was calculated as gram per 100 g of samples on dry weight basis. All determinations were done in triplicate. The relative solubility (% RS) and swelling power were determined according to **Eq. 2.3** and **2.4**.

$$\text{RS (\%)} = \left(\frac{W1}{0.5}\right) \text{x} 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{..Eq.2.3}$$

$$\text{SP} = \left(\frac{w2}{0.5x(100 - S(\%))}\right) \text{x} 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots\text{Eq.2.4}$$

Where W_1 is the weight (g) of soluble material in the supernatant, W_2 is the weight (g) of precipitate.

2.2.4.7. Starch Pasting

Pasting properties were studied using the method described elsewhere with slight modifications (Gebre-Mariam and Schmidt, 1996a). Viscosities were measured using spindle number 4 of the viscometer (Viscostar plus L, KINEMATICA AG, Switzerland). A known gram of starch powder was dispersed in portion of distilled water. The dispersions were adjusted to volume to get 10% using distilled water with continuous stirring. The preparations were then kept overnight at room temperature. Viscosity measurements of the dispersion were made at 50, 65, 80, 95 °C then stayed on 95°C for 30 minutes, and then cooled to 50 °C and held at this temperature for 1 min. The speed of the rotor was fixed at 75 rpm and the rate of heating and cooling was 1.5 °C min^{-1} throughout the range of gelatinization, holding and cooling steps. The Pv, the Hv, (the viscosity after 20 min stirring at 95 °C) and the Cv, (the viscosity after cooling to 50 °C) were recorded and the BD and the SB were calculated and expressed as cP. Viscosities recorded are averages of three determinations and standard deviation.

2.2.4.8. Drug- Starch Compatibility Studies

The compatibility studies of drug, polymer and the physical mixture (1:1) of drug and polymer were carried out using Fourier Transform Infrared Spectrophotometer (FTIR 8400-S, SHIMADZU, Japan). The samples were first ground in a mortar to reduce the average particle size. Five milligrams of finely ground powder of each sample was mixed with an oily mulling agent (Nujol) in a mortar and pestle. The sample mixture was then placed onto the face of a potassium bromide (KBr) disk and the second window was placed on top of the first salt plates to form a thin film of the mull by compression between two plates. The sandwiched plates were placed in the infrared spectrometer and the spectra were obtained by scanning the sample in the range of 4000-400 cm^{-1}.

2.2.4.9. Calibration Curve of Paracetamol

Different concentrations of Paracetamol, the model drug used in the study, were prepared to obtain 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16µg/ml and 18 µg/ml from stock solutions containing 200 µg/ml of paracetamol in (PBS - pH = 5.8) dissolution media as per the preliminary scanning results. Then, the absorbance of each of these concentrations were measured at λmax 257 nm in UV-Visible Spectrophotometer (CECIL, CE, 1021, Cambridge, England) using the buffer solutions as blank. The absorbance readings were plotted against concentration (**Fig.2.1** and **Fig.2.2**). The linear regression equations obtained was, $A = 0.04\ C + 0.042$ and $R^2 = 0.9996$ at a 95% confidence interval.

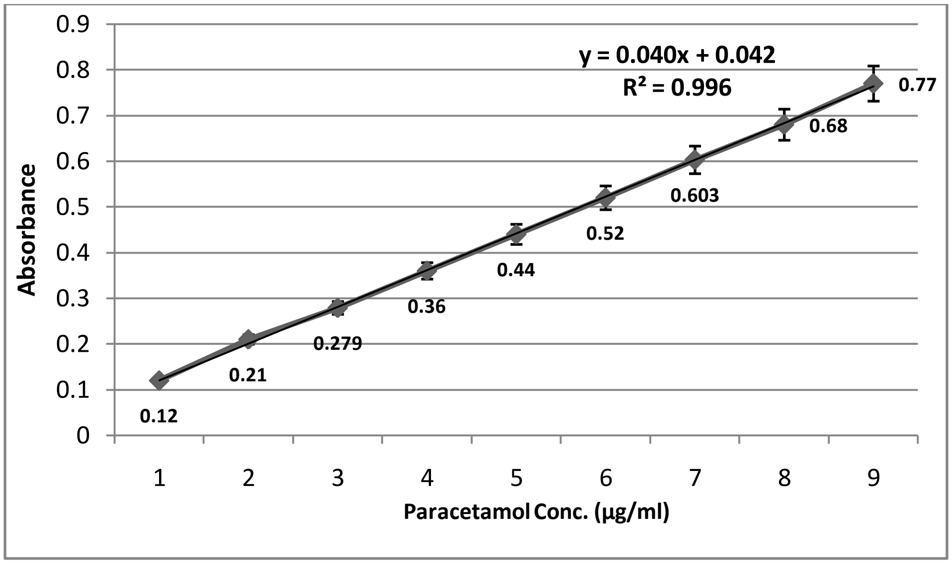

Figure 2.1. Standard linear calibration curve of paracetamol at λmax of 257 nm in PBS- pH= 5.8

2.2.5. Preparation of Paracetamol Granules with *Triticum decocum* and *Zea mays* Starches

Table 2.1. Paracetamol formulation containing *Triticum decocum starch* as binder

Ser. No.	Formulation Ingredients	Amount (%w/w)
1.	Paracetamol	85
2.	Starch	2.5, 5, 7.5, 10
3.	Ac-Di-Sol®	4
4.	Magnesium Stearate	0.5
5.	Lactose	8, 5.5, 3, 0.5

Table 2.2. Paracetamol formulation containing *Triticum decocum starch* as disintegrant

Ser. No.	Formulation Ingredients	Amount (%w/w)
1.	Paracetamol	85
2.	Starch	2.5, 5, 7.5, 10
3.	Povidone	4
4.	Magnesium Stearate	0.5
5.	Lactose	8, 5.5, 3, 0.5

All ingredients, except magnesium stearate, were wet granulated with binder mucilage in mortar and pestle. Then, it was mixed for 10 minutes in Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland). The wet masses were passed through wet granulator with 1.6 mm sieve and dried in an oven at 50 ^{o}C for 12 h. The dried granules were dry screened by passing though a 1 mm sieve. Sieved granules ranging from 224-1000 µm sieve sizes were mixed with specified amount of disintegrant, which is added internally, in a Turbula mixer (Willy A. Bachofen AG, Turbula 2TF, Basel, Switzerland) for 10 min. at 25 rpm. Magnesium stearate (0.5%) was added and the mixture was mixed for further 5 min (Adane and Gebre-Mariam, 2006). 10 % fineness was added to each batch to get uniform drug containing tablet.

2.2.6. Characterization of granules

2.2.6.1. Moisture content of granules

Moisture content of granules was determined gravimetrically by taking 5 g from each batch of the granules and heating the samples in an oven at 120 ^{o}C for 1h. The granules were weighed immediately and the loss in weight was considered as moisture content of the granules.

2.2.6.2. Size distribution of the granules

Granules (10 g) from each batch were put in a set of sieves (ISO 3310-1)) arranged in mesh size from top to bottom. The sieves were shaken at constant intensity for 2 min using the universal drive unit (ERWEKA, Type AR 401, Germany). The granules remaining on each sieve were weighed and percent granules retained on each sieve recorded and mean granule sizes were calculated for each batch. The granule size distributions recorded were averages of three determinations.

The weight retained on each sieve were converted to percentage retention and multiplied by the average of two successive sieves. The sum of these products divided by 100 yielded an average granule size.

2.2.6.3. Determination of densities and related properties of granules

Various density and related granular properties of the formulation such as bulk density, tapped density, angle of repose, Hausner's ratio, Carr's index, and bulkiness were determined according to the procedures described under **section 2.2.4.1.**

2.2.6.4. Determination of granule friability

Each batch of granule (10 g) larger than 315 μm were put in a friability tester and allowed to revolve for 5 min at 20 rpm dropping the granules a distance of 6 inches. The granules were then sieved using the 315 μm sieve and percent losses were calculated as friability. Percent friability recorded in averages of three determinations.

2.2.7. Tablet Compression

The tablets were compressed at a certain fixed compression force (adjusted to give tablets with a crushing strength greater than 70 N with official *Zea mays* starch) on eccentric single punch tablet press (EKO Korsch, 8410-68, Berlin, Germany) which was fitted with 10 mm flat-faced punches. The target weights were 471 mg of total tablet with 400 mg of paracetamol drug weight. The tablets were kept for 24 h at room temperature in glass containers before their properties were evaluated (Adane and Gebre-Mariam, 2004).

2.2.8. Evaluation of Tablets

2.2.8.1. Crushing strength Measurement

Ten tablets were taken from each batch and the crushing strength was determined using hardness tester (Schleuniger, 2E/205, Switzerland). Each tablet was placed between two anvils and force was applied to the anvils, and the crushing strength in Newton (N) that just caused the tablet to break was recorded. A mean hardness was calculated for each batch (USP/NF, 1980).

2.2.8.4. Friability test

Ten tablets of known weight from each batch were subjected to combined effects of abrasion and shock by placing them in the plastic chamber of friability tester (ERWEKA, TAR 20, and Germany) that revolves at 25 rpm for 4 min. The tablets were then dedusted, and reweighed, and the percent loss in weight was calculated for each batch of tablets as friability (USP/NF, 2007).

2.2.8.5. Disintegration time determination

The disintegration time was determined according to USP 30/NF25, <701>, (2007). 1000 ml of distilled water maintained at 37±2 was used as a disintegration medium. Six tablets were selected at random from each batch and placed in a disintegration tester (CALEVA, G.B. Caleva Ltd., UK). The time taken for each tablet to pass through the mesh was recorded. The average disintegration time and standard deviation of six tablets was determined.

2.2.8.6. In Vitro Dissolution Test

The dissolution test was done according to the USP/NF USP30/NF25, <711>, 2007) specification using dissolution apparatus Type II (ERWEKA, DT600, Germany), with 900 ml phosphate buffer (pH 6.8) as the dissolution medium at 37 ± 0.5 ^{o} 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, and 45 min and filtered using Whatman No.1 filter paper. 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 using UV/Visible spectrophotometer (JENWAY, 6505, England) at λ= 257 nm. Phosphate buffer (pH 6.8) was used as a blank. After all the necessary corrections for dilution and sample extraction were made, concentration was determined from the standard calibration curve of paracetamol, and finally the results were plotted as cumulative % of drug released versus time.

2.2.9. Statistical Analysis

Statistical analysis was carried out using one way Analysis of Variance (ANOVA) on computer software SigmaStat$^{®}$ 3.5 for Windows (Systat Software, Inc. Point Richmond, USA). At 95% confidence interval, p values less than or equal to 0.05 were considered statistically significant. The results are reported as mean and standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of *Triticum decocum* Starch

Triticum decocum starch was approved for its identity by appearance of deep blue color with iodine which indicates the presence of starch in the solution, since amylose in solution form helical inclusion complexes with iodine in its cavity. The deep blue color appears immediately after a few drops of iodine solution addition to the starch dispersion, and disappears on heating (Kalaiarasan and Ahmed, 2010).

Knowledge of the pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depends on pH. A 1% w/v solution of oat starch in water gave a pH of 6.50 while *Zea mays* starch measured 6.73 pH. The near neutral pH of the oat starch implies that when it is used in uncoated tablets, it may be less irritating to the gastrointestinal tract. It may also find useful applications in formulation of acidic, basic and neutral drugs (Sarangapani and Rajappan, 2012). This pH value is within USP limit of wheat and *Zea mays* starches (4.0-7.0) (USP30-NF25, 2007).

Starch is the major constituent of *Triticum decocum* grain. The finding showed that more than 56.3% of the *Triticum decocum* grain powder (on dry weight basis) is starch.

The granule chemical composition of starches is dependent on botanical sources and growing conditions (Senanayake, 1995). The chemical composition of *Triticum decocum starch* obtained by the extraction and isolation method described above are given in **Table 3.1**. Corresponding values of *Zea mays* starch determined simultaneously are given for comparison. The exact quantity of protein and lipid associated with the starch depends on both the botanical origin of the starch and its degree of purification during extraction *(*Paulos *et al*., 2007). The total lipid content of *Triticum decocum starch* was found to be 1.56% indicating that the lipid content of oat starch is significantly higher ($P<0.05$) than that of *Zea mays* starch (0.67%). This value is lower than that reported on two oat cultivars, *Avena sativa* and *Avena nuda* oat starches (1.64% and 1.67%), respectively (Senanayake, 1995), but higher than those reported for *Avena nuda* oat starch (1.12%) and wheat (0.68%) (Hoover and Vasanthan, *1992),* but were within the range

(1.3-2.4%) reported for starches isolated from other varieties of oat grains (Morrison *et al*., 1984; Doublier *et al*.,1987a; Doublier *et al*.,1987b; Gudmundsson and Eliasson *et al*.,1989).

Bound fat content of oat starch was found to be 0.56% with 0.98% unbound fat, while that of *Zea mays* starch were 0.33% and 0.34% respectively. Presence of higher lipid content can significantly affect some physicochemical properties of oat starch such as delaying/decreasing of starch granules swelling, reducing the solubility, reducing rate of gelatinization and reduction of water uptake especially if the lipid is medium and long chain compound through interaction with linear amylose chains. It has also the potential to affect the size and shape of the starch granules at higher temperatures (Camire *et al*., 1990; Gray and Schoch, 1962; Tester and Morrison, 1990). Also lipid can affect paste viscosity and pasting characteristics of starch. However, having higher lipid content rendered oat starch probably the potential of becoming resistant to α-amylase attack (Senanayake, 1995).

Protein content of *Triticum decocum starch* (0.26%) was significantly ($P< 0.05$) higher than that found in *Zea mays* (0.13%). It is reported elsewhere that oat starch has 0.3% (Lim W. *etal*., 1992*)* protein contents. The value obtained in this study is within the limit specified in USP for wheat starch which states not more than 0.3% protein content is allowed (USP30-NF25, 2007).

Table 3.1. Composition and Physicochemical properties of *Triticum decocum* and *Zea mays* starch powders

S. No.	Starch Constituents	***Triticum decocum* Starch**	***Zea mays* Starch**
1.	Appearance	White powder	White powder
2.	Protein content	0.26% ± 0.01	0.13% ± 0.02
3.	Un bound fat content	0.98% ± 0.03	0.34% ± 0.04
4.	Bound fat content	0.56% ± 0.02	0.33% ± 0.02
5.	Total fat content	1.56% ± 0.06	0.67% ± 0.03
6.	Apparent amylose content	20.11% ± 0.65	25.26% ± 0.048
7.	Lipid complexed amylose content	29.19% ± 0.75	13.49% ± 0.05
8.	Percent yield (DB) (%)	56.314% ± 2.12	Not extracted
9.	Total amylose content	28.4% ± 1.05	29.2% ± 1.04
10.	Moisture content	10.24% ± 0.83	12.58% ± 0.085
11.	Total ash value	0.23% ± 0.06	0.12% ± 0.02
12.	Limit of Iron	Comply	Comply
13.	pH	6.50 ± 0.01	6.73 ± 0.02
14.	Swelling Index at 37 ^{o}C	1.93% ± 0.19	3.24% ± 0.82
15.	Solubility at 37^{o}C in water	1.65% ± 0.11	2.35% ± 0.10

Oat starch contained significantly ($P<0.05$) higher content of total ash (0.23%) than *Zea mays* starch (0.12%) but with in USP limit of 0.6% (USP30-NF25, 2007).

The moisture content reflects the affinity of the material for moisture (Parrott, 1989). Moisture is known to modify the flow and mechanical properties of many powders including starches. The moisture content of air-equilibrated starches ranges from about 10-12% (cereal) to about 14-18% (some roots and tubers) (Tester *et al.*, 2004). The moisture content of oat starch was 10.2% while that of *Zea mays* starch was 12.58%. The moisture content level of the oat starch was below the maximum limit (<15%) required for prolonged storage of the starches. Lower moisture contents are required for safe storage as higher moisture contents can lead to microbial damage and subsequent deterioration in quality (Mweta *et al.*, 2009). Oat starch has shown swelling index of

1.93 % at 37 ^{o}C while, *Zea mays* has 3.24% in the similar experimental conditions. On the other hand, oat starch has shown solubility of 1.65 % at 37 ^{o}C while, *Zea mays* starch exhibited 6.24%.

The total amylose content of *Triticum decocum* and *Zea mays* starches were estimated to be 28.4% and 29.2% respectively. The accuracy of the method was confirmed by the results obtained for six varieties of oat starch (with mean of 28.38%) which was in agreement with previous work based on calorimetric assay (Tester and Karkalas, *1996).*

After defatting, apparent amylose (amylose before removal of bound lipid by hot propanol-water extended extraction) of *Triticum decocum* starch was found to be 20.11%, while that of *Zea mays* starch was 25.26%. On the other hand, Lipid complexed amylose content ((Total Amylose-Apparent Amylose)/(Total Amylose) x 100) was 29.19% and 11.83% for oat and *Zea mays* starch, respectively. It was reported elsewhere that oat starch has an average of about 19.45% apparent amylose and 14.7% amylose complexed with native lipid for *Avena sativa* and *Avena nuda* varieties (Senanayake, *1995).*

3.2. Morphological Characteristics and Crystallinity of *Triticum decocum* Starch

3.2.1. Morphological Characteristics of *Triticum decocum* starch

Oat starch appears to be confined to the endosperm where individual granules develop in bundles or clusters. The granules sizes, shapes and surface characteristics were examined using SEM. Bimodal granule size distributions, with differentiation being made between granule fractions with smaller and larger diameters were revealed in this study for *Triticum decocum*. This is characteristic of wheat starches as well as those from rye and barley. This somewhat contributes to their unique physicochemical characteristics and end-use potential of the starches. (Morrison and Scott, 1986; Dengate and Meredith, 1984). The SEM (**Fig.3.1** and **3.2**) of *Triticum decocum* shows larger spherical granules with an average of 15.22 ± 4.3 µm particles size, while smaller spherical granules with an average of 5.73 ± 2.2 µm particles, and also there are oval granules. The average size of *Triticum decocum* starch as a whole according to this study was 10.48±6.728 µm which was similar to sizes reported elsewhere as 2-14 µm (Zheng and Sosulski, 1997).

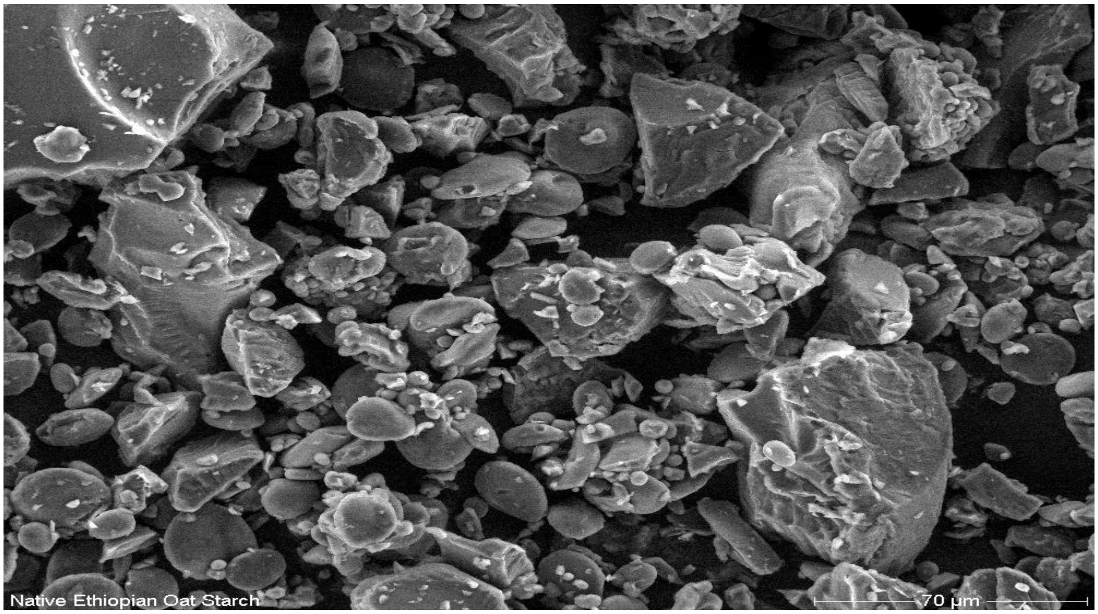

Figure 3.1. SEM of native *Triticum decocum* starch granules with 70 µm scale bars.

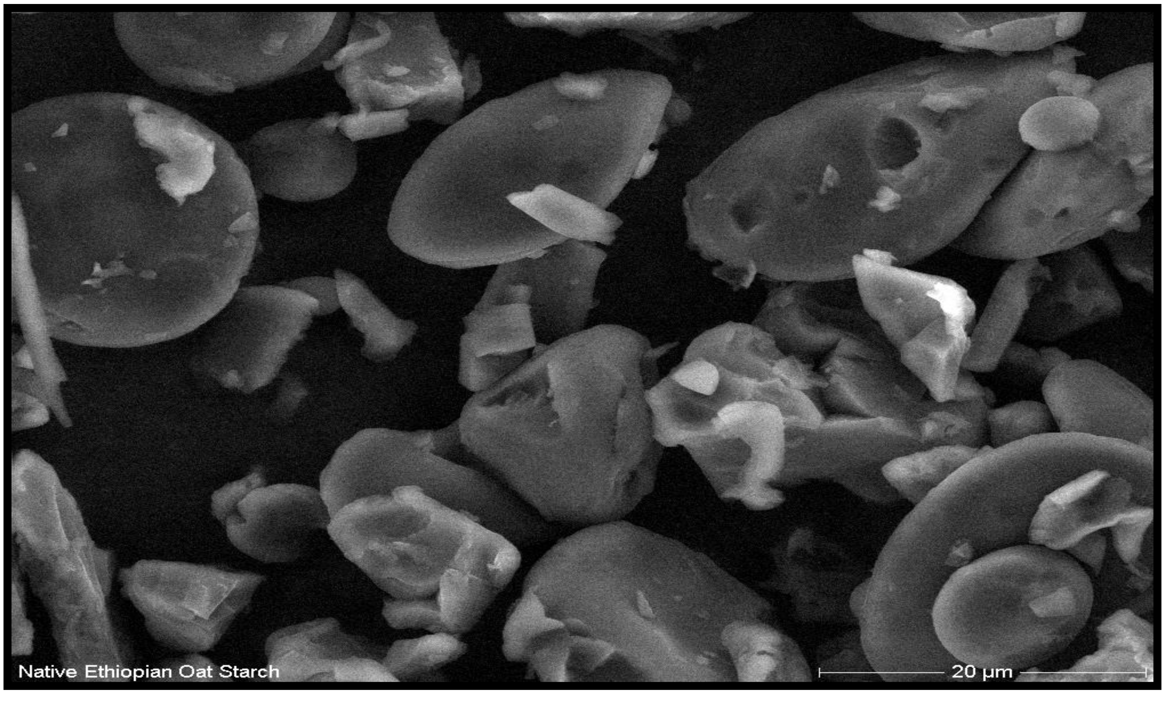

Figure 3.2. SEM of native *Triticum decocum* starch granules with 20 µm scale bars.

3.2.2. Particle Size, Size Distribution and Specific Surface Area

The particle size of a new drug substance is a critical parameter, as it affects every phase of formulation and its effectiveness. Appropriate particle size is required to achieve optimal dissolution rate in solid dosage forms. The content uniformity and compressibility are governed by the particle size. Although, the size characterization is simple for spherical particles, the study of irregular particles requires specialized methods. The Malvern Mastersizer measures particle size by laser diffraction. The use of this technique is based on light scattered through various angles, which is directly related to the diameter of the particle. Thus, by measuring the angles and intensity of scattered light from the particles, a particle size distribution can be deduced. The laser light scattering generally reports broader size distribution compared with image analysis (Naizi, 2007).

The particle size distributions of *Triticum decocum* starch and *Zea mays* starch were shown in **Figs** 3.3 and 3.4, on which both of them showed monomodal normal particle size distribution with the average volumetric particle diameters being about 23.03 and 27.60 µm respectively. *Triticum decocum* has significantly lower volume mean diameter of particle size compared to *Zea mays* starch with higher specific surface area. Surface to volume (or mass) ratio is a fundamental property of a powder since it governs the rate at which the powder interacts with its surroundings. Particles with high surface to volume ratios dissolve or grow more rapidly than large ones (Naizi, 2007).

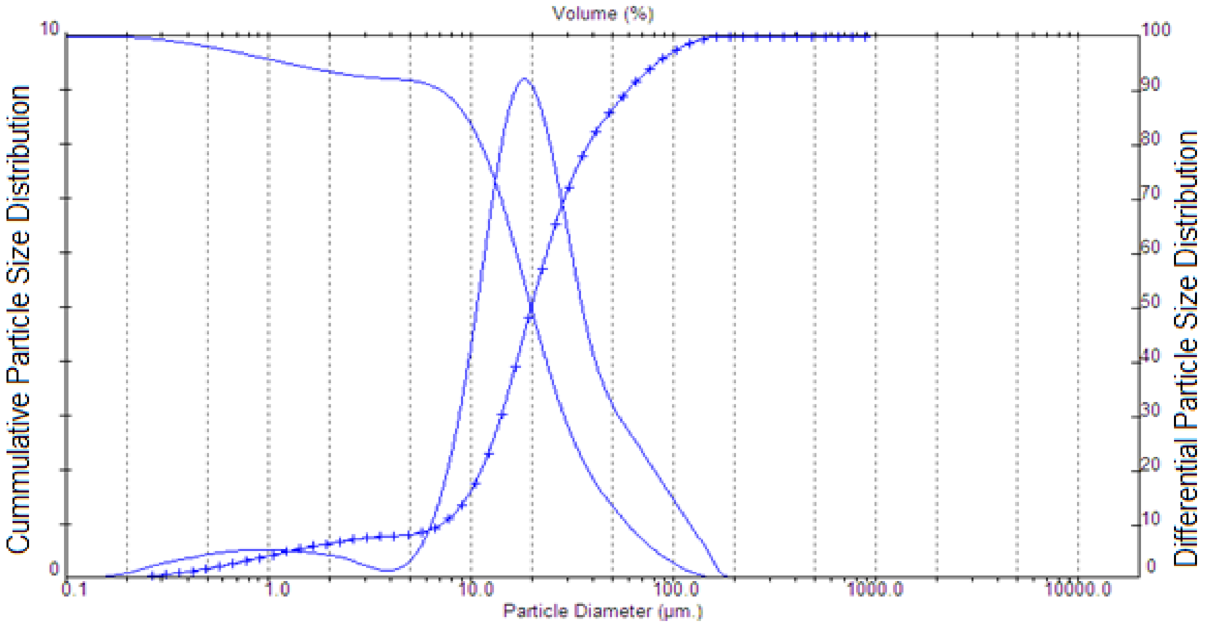

Figure 3.3. Volumetric granule size distribution of *Triticum decocum* starch

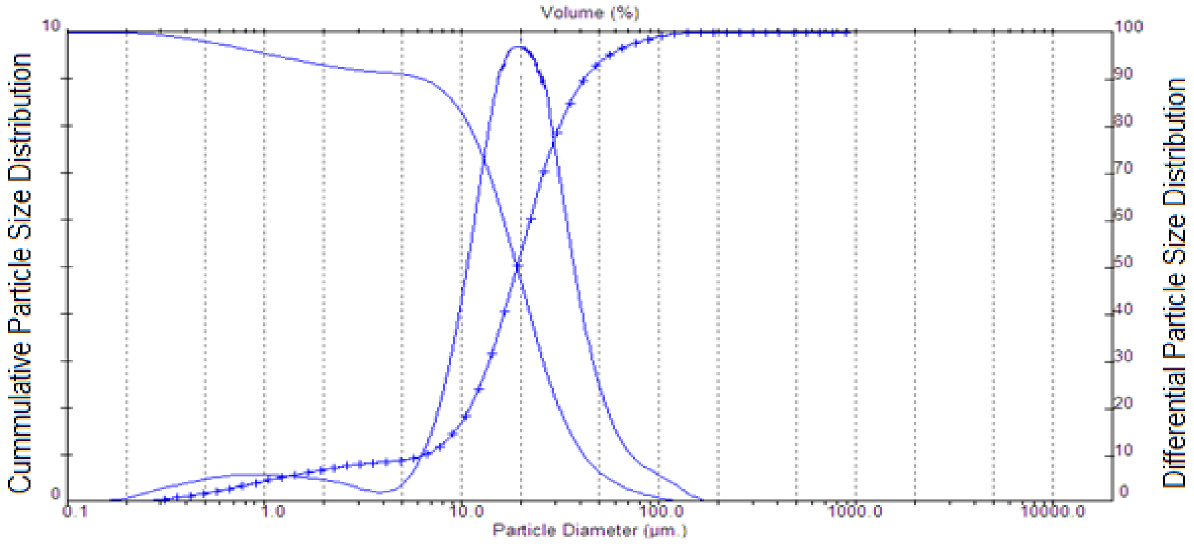

Figure 3.4. Volumetric granule size distribution of *Zea mays* starch

Table 3.2. Particle size and size distribution of *Triticum decocum* and *Zea mays* starches

No.	Parameter of particle size	Type of starch	
		Triticum decocum starch	*Zea mays* starch
1.	Volume mean diameter (µm)	23.03	27.60
2.	Surface area mean diameter (µm)	5.72	5.91
3.	10% of total particles	<6.36 µm	<7.07 µm
4.	50% of total particles	<19.19 µm	<19.89 µm
5.	90% of total particles	<42.05 µm	<59.57 µm
6.	Specific surface area (m^2/g)	0.7186	0.6957
7.	Uniformity (deviation from median)	6.169E-01	8.044E-01
8.	Width of distribution (Span)	1.861E+0	2.640E+0
9.	Residual (freeness from error of work)	<1 (acceptable)	<1 (acceptable)
10.	Volume Concentration (%)	0.0168	0.0228

3.2.3. Crystallinity of *Triticum decocum* Starch

Principally, XRD is used for analysis of crystal structures at the atomic level. Identification of crystalline material can be accomplished by comparison of X-ray powder diffraction patterns obtained for known materials with those of the unknown. The intensity ratio (ratio of the peak intensity of a particular d spacing to the intensity of the strongest maxima in the diffraction pattern) and the d spacing are used in the comparison (USP30-NF25, 2007).

Starch has a definite crystalline nature which is attributed to the well-ordered structure of the amylopectin molecules inside the granules. Using X- ray diffractometry, the crystalline nature of the starches can be determined. Starch molecules exist as helices and these helices can have different packing arrangements giving rise to different crystalline patterns. Starches possess either "A", "B" or "C" patterns. The X-ray powder defractogram of *Triticum decocum* starch is presented in **Fig.3.5**, which is similar to that of *Zea mays* starch, the peaks of oat and corn starches almost overlapping. Both starches possess "A" crystalline pattern with five major peaks around 15, 17, 18, 22 and 23.5 2θ angles, especially showing maximum peaks at around 17° 2θ. It has A-type crystallinity which shows a close-packed arrangement of amylopectin within the granule (Adane and Gebre-Mariam, 2004).

The work of Zobel, (1964) on structure of amylopectin from a variety of starches has indicated that the average chain length of the polymer is the main determinant of crystalline polymorphism of granular starches. Molecular fine structure influences the physicochemical properties of starch.

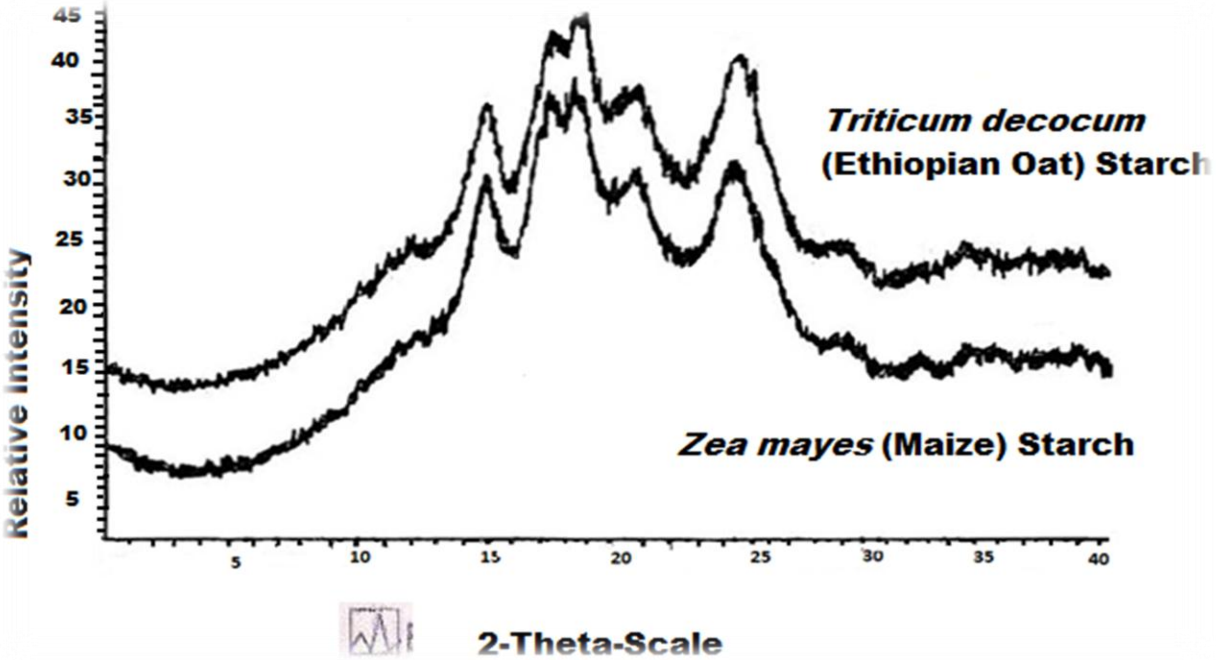

Figure 3.5. X-ray powder diffractogram of native *Triticum decocum* and *Zea mays* starch

3.3. Starch Pasting

In the presence of water and heat, starch granules swell by imbibing water. As the heating temperature is increased the gelatinization temperature is reached after which a paste is formed. The pasting characteristics of oat starch and *Zea mays* starch at 10% concentration on different temperature were compared, **Fig.3.6,** during heating and cooling cycle in excess water. Significant differences were observed between both starches. The pasting properties of starch are affected by the starch granule size, amylose and lipid contents, and amylopectin structure. Amylopectin is primarily responsible for granule swelling, whereas amylose and lipid restrict the swelling (Tester & Morrison, 1990).

Triticum decocum starch had higher values of viscosity at all temperatures when compared to the *Zea mays* starches. This may be due to stronger bonding forces within oat starch granules compared to *Zea mays* starch granules. The characteristic difference between the oat and *Zea mays* starch varieties can also be attributed to their amylose and phospholipid contents. Phospholipids could form helical complexes with amylose and restrict granule swelling to a lower maximum viscosity at a substantially higher pasting temperature (Senanyake, 1995).

The Pv at any concentration is an important distinguishing feature of a starch from other species. Oat starch has shown a Pv value (1926 cP) which is higher than that of *Zea mays* starch (924 cP). During the isothermal holding at 95 ^{o}C, the viscosity of all the starches goes down, but at a relatively lower rate for oat starch as compared to *Zea mays* starch. The Hv has been attributed to the mixed effect of swollen starch granules, granule fragments, colloidally and molecularly dispersed starch molecules, rate of amylose exudation, competition between exuded amylose and the remaining granules for free water (Gebre-Mariam and Schmidt, 1996a)

When hot starch pastes are cooled, the extent of increase in viscosity is governed by the retrogradation tendency of the starch. This behavior is largely determined by the affinity of hydroxyl groups of one molecule to another. The amylose molecules being randomly dispersed can orient themselves in parallel fashion to form aggregates of low solubility, leading to gel formation (Gebre-Mariam and Schmidt, 1996a). Both starch samples showed an increase in viscosity during cooling down to 50°C (Cv), with respect to the holding period at 95°C. This increase is indicative of the tendency of starch to retrogradation. Oat and *Zea mays* starch had the highest viscosity at 50°C (4367cP for oat starch, and 1810 cP for *Zea mays* starch). It is also noteworthy that the maximum viscosities of oat and *Zea mays* starches are attained at higher temperature (95°C) as compared to potato starch (72°C) (Gebre-Mariam and Schmidt, 1996a).

The BD viscosities of the oat starch (206 cP) were considerably higher than that of the *Zea mays* starch (168 cP). The ability of starches to withstand heating at high temperature and shear stress is an important factor in many processes. High values of breakdown are associated with high peak viscosities, which in turn are related to the degree of the starch granules swelling during heating (Šubarić *et al*., 2011). The higher breakdown viscosity of oat starch is also indicative of maintaining its structural integrity under shear and heat more than *Zea mays* starch (Gebre-Mariam and Schmidt, 1996a).

The SB viscosity of oat starch (2647cP) was greater than that of *Zea mays* starch (1054cP). Hoover and Vasanthan (1992) explained that the setback values reflect the extent of water immobilization around the charged centers of the starch components and those of free and helically complexed lipid molecules, rather than starch paste retrogradation. The association of water molecules with these charged centers would decrease the effective water concentration in the continuous phase, resulting in a rise in viscosity during the cooling cycle. The extent of water immobilization (SB) may be greater in oat starch because of the presence of more charged centers (from the lipid) in the continuous phase (BeMiller & Whistler, 2009).

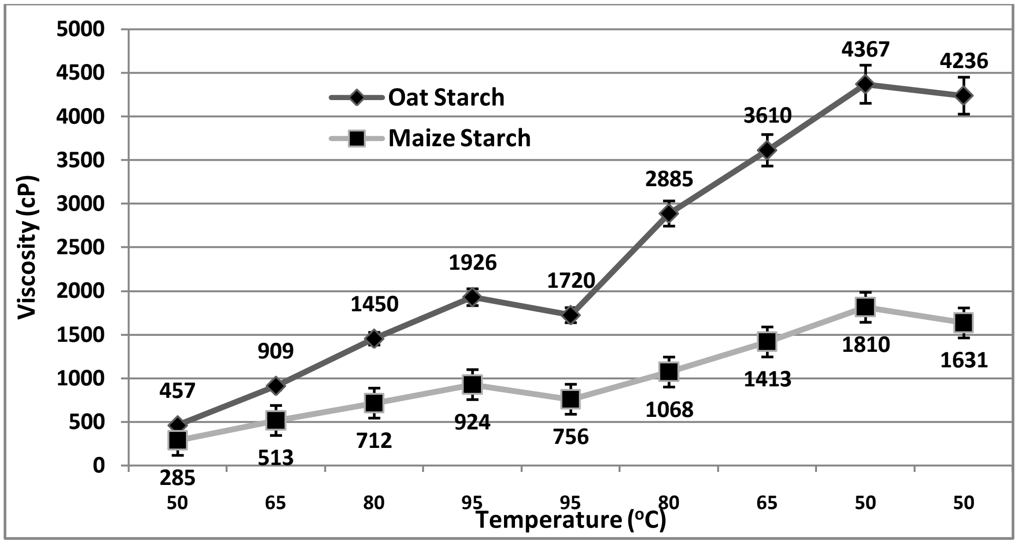

Figure 3.6. Pasting curve of *Triticum decocum* and *Zea mays* starches

Note: The 2nd 95 and 50 °C is after holding for 20 minutes each.

Table 3.3. Viscosity parameters of *Triticum decocum* and *Zea mays* starches

Parameter	Viscosity (cP)	
	Triticum decocum starch	*Zea mays* starch
Peak viscosity	1926± 4.82	924± 6.75
Hot Paste viscosity	1720± 3.66	756± 5.42
Cold Paste viscosity	4236± 6.54	1631± 6.50
Breakdown viscosity	206	168
Setback viscosity	2647	1054

3.4. Drug-Excipient Compatibility Studies of Native *Triticum decocum* Starch

Drug-excipient interactions/incompatibilities are major concerns in formulation development. Drug-polymer compatibility studies are done to evaluate interaction between drug (paracetamol) and the polymers (oat or *Zea mays* starch). FTIR spectrum of pure paracetamol powder, mixture of paracetamol and *Triticum decocum* starch (1:1) were studied and shown in **Figs. 3.7** and **3.8.** The physical mixture of drug and *Triticum decocum* starch showed the characteristic peaks of pure drug at major peaks suggesting roughly absence of incompatibilities between the drug and the starch.

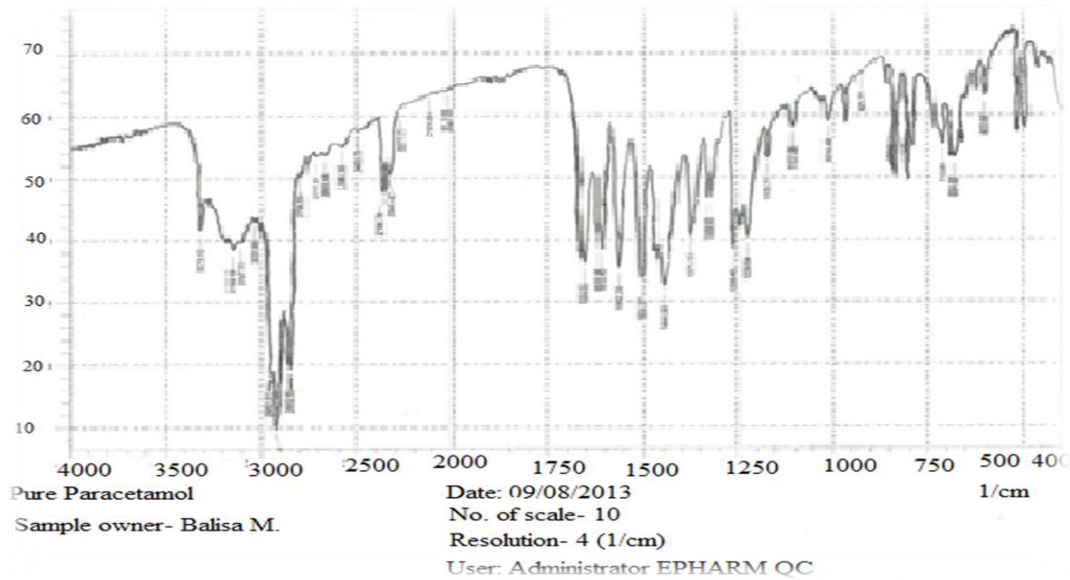

Figure 3.7. FTIR spectrum of pure paracetamol powder

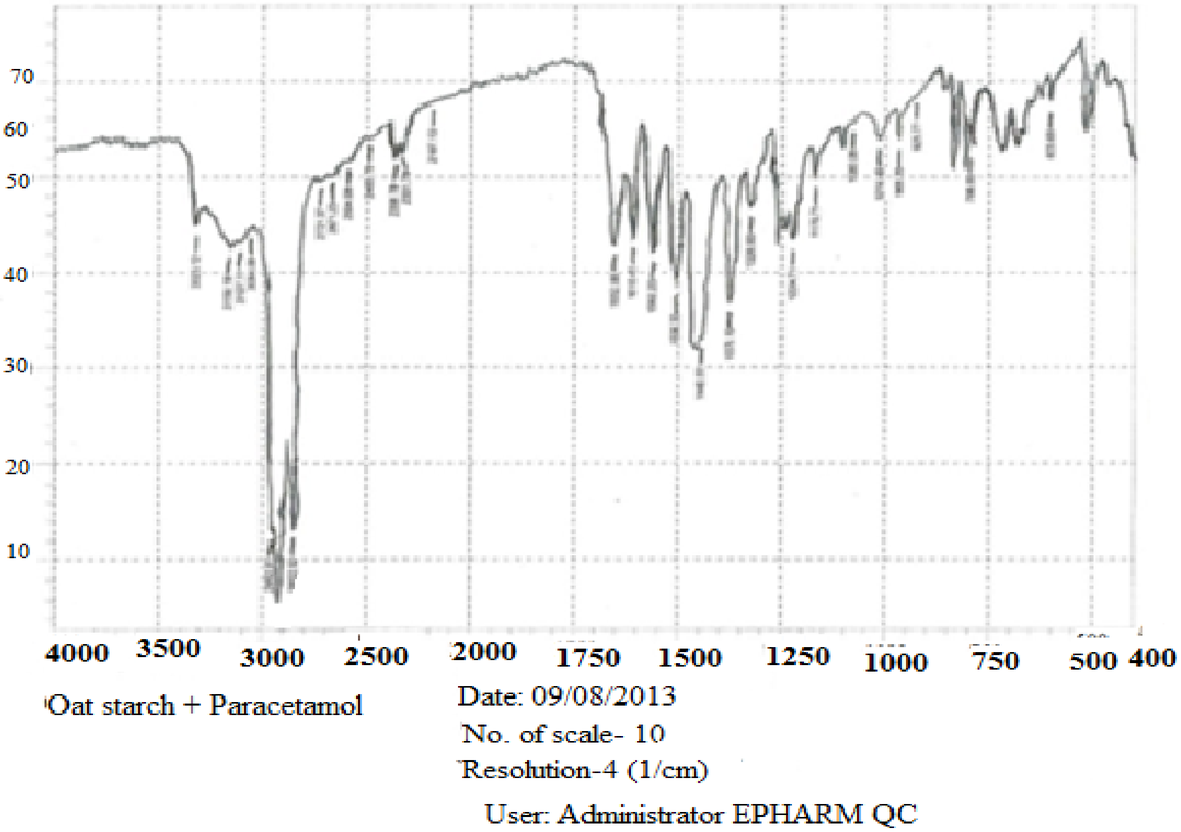

Figure 3.8. FTIR spectrum of native *Triticum decocum* starch and paracetamol 1:1 physical mixture

3.5. Micromeritic Properties of *Triticum decocum and Zea mays* Starch Powders

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

The absolute and relative densities of pharmaceutical solids play an important role in determining their performance (e.g. flow and compaction properties) in tablet dosage forms. The angle of repose could be used as a quantitative measure of the cohesiveness or the tendency of powder to flow. Angles of 30^{o} or below are usually indicative of free flowing materials while an angle of 40^{o} or above indicates a poor flow. Angle of repose is affected by the particle size distribution and it usually increases with a decrease in particle size. Bulk and tapped densities give an insight on the packing arrangement of the particles and the compaction profile of a material (Sarangapani & Rajappan, 2012). So, they can provide information on the flowability of powders and are used to calculate the Carr's index and Hausner ratio, which are a measure of the flowability and compressibility of a powder (Odeku and Picker-Freyer, 2009). Powders with high values of compressibility index are considered as materials with poor compressibility, indicating also relatively high inter-particle interactions. Concerning the Hausner ratio, the higher the ratio, the greater will be the tendency of the powder to densify.

The flow properties and compressibility of the native *Triticum decocum* and *Zea mays* starch powders, including bulk and tapped density, Carr's index, Hausner's ratio, and the bulkiness are given in **Table 3.4.** The true density of *Triticum decocum* starch was significantly higher than that of *Zea mays* starch. The native *Triticum decocum* starch samples displayed higher bulk and tapped densities than *Zea mays* starches. The differences observed in the bulk and tapped density values could be due to the different particle size and shape which affect the packing arrangement of the powder particles. Generally, percent compressibility and Hausner ratio of *Triticum* *decocum starch* was slightly lower compared to that of *Zea mays* starch (Schüssele and Bauer-Brandl, 2003). Both starches did not flow through a funnel, so it was impossible to measure flow rate and angle of repose for both powders indicating poor flowability. This is further confirmed by the Hausner ratio, which was generally higher than 1.25 for both starches.

Table 3.4. Flow properties of *Triticum decocum* and *Zea mays* starch powder

		Types of starch	
No.	**Parameters**	*Triticum decocum* Starch	***Zea mays* Starch**
1.	Bulk density (g/ml)	0.650 ± 0.06	0.457 ± 0.08
2.	Tapped density (g/ml)	0.88 ± 0.08	0.653 ± 0.09
3.	Carr's index (%)	26.14 ± 1.45	30 ± 1.15
4.	Hauser's ratio	1.35 ± 0.11	1.43 ± 0.12
5.	Angle of repose (^{o})	No flow	No flow
6.	Flow rate (g/sec)	No flow	No flow
7.	True density (g/ml)	1.602 ± 0.014	1.462 ± 0.021

3.6. Solubility and Swelling Properties

When starch is pasted in hot water, the individual granules swell and a portion of the starch substance dissolves in the aqueous medium. Swelling power provides evidence of non-covalent bonding between starch molecules. Bonding forces within the granules of a starch affect its swelling power. Thus, highly associated starch granules with an extensive and strongly bonded micellar structure should display relatively greater resistance towards swelling. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation determine the degree of swelling and solubility. In the range of gelatinization temperature, there is limited swelling, and only a small amount of starch is solubilized (Senanyake, 1995).

At higher temperature, there is a large increase in the extent of swelling and solubility, due to disruption of intermolecular hydrogen bonds which maintain the structural integrity of the granules leading to more substantial loss of amylose and amylopectin from the granules. Water molecules solvate the librated hydroxyl groups and the granules continue to swell. As a consequence of severe disruption of hydrogen bonds, the granule will be fully hydrated and finally the micellar network separates and diffuses into the aqueous medium. Starches show different patterns of swelling and solubility when heated at different temperatures (*Hashim et al, 1992). Triticum decocum* starch showed a two stage swelling and solubility pattern (**Fig.3.9** and **Fig.3.10**) just like *Zea mays* and other cereals elucidated elsewhere (Blanshard, 1978; Doublier *et al.*, 1987b*;* Hoover and Vasanthan, 1992).

As expected, the swelling power and solubility of the *Triticum decocum starch* increased with temperature from 25 °C to 95 °C. The same trend was observed with *Zea mays* starch. The swelling power of *Triticum decocum starch* was found to vary considerably from 0.57 to 18.20 g/g; this is significantly lesser than *Zea mays* starch's swelling power (1.46 to 16.44 g/g) with increasing temperature before gelatinization attainment, but significantly higher after gelatinization temperature. The water solubility of the *Triticum decocum starch* was found to vary considerably from 0.98 to 19.02%; this is significantly lesser than *Zea mays* starch solubility (1.06 to 15.15%) with increasing temperature before gelatinization attainment, but significantly higher after gelatinization at higher temperatures. The reasons behind decrease/delay of swelling power and solubility at lower temperature of oat starch when compared to *Zea mays* starch were most probably its higher content of total lipid, and higher content of protein which more or less affect both swelling power and solubility negatively (Senanayake, 1995). This suggestion is supported by evidences reported elsewhere by comparative studies conducted revealing strong positive correlations between starch swelling and amylose content as well as between amylose leaching and amylose content (Zuluaga, *et. al.*, 2007).

According to Zheng and Sosulski (1997), the swelling power of starch is associated more with granule structure and chemical composition, particularly amylose and lipid content, than with granule size. Higher amounts of lipid-complexed amylose would inhibit swelling and gelatinization. However, small granules are associated with a higher rate of water absorption, earlier hydration and more swelling than are larger granules (Chiotelli and Meste, 2002). The reason for this is the less crystallized arrangement of the polysaccharide chains in smaller granules (a higher proportion of amorphous zones more accessible to water).

Greater specific surface area may also contribute to the higher water absorption of small starch granules (Chiotelli and Meste, 2002), though other factors like starch ageing and sorption time might have a larger influence than surface area (Von Hennig *et al*., 1997; Taylor, *et al*., 1961). The degree of swelling and the soluble amount will also depend on the starch species. Again, differences in swelling factor between different cereal species are, in part, due to differences in amylopectin structure (Tester and Morrison, 1990b; Tester and Morrison, 1992). So, it is logical to speculate that, the granules of *Triticum decocum* starch exhibit greater bond strength than *Zea mays* starch.

This pattern was shifted at higher temperature for oat starch due to, most probably the following reasons. Normally, amylose leaves the granule first and it is only at higher temperature stage of the pasting process that the amylopectin is lost for other starches (Senanayake, 1995). However, for oats starch, during heating its dispersions, amylose and amylopectin are co-leached from the granules, leading to swelling power and solubility dramatic rise (Doublier *et al*., 1987*)*. This result is somewhat similar to swelling power of *Avena nuda* varieties (maximum 22.3 g/g) reported elsewhere (Senanayake, 1995).

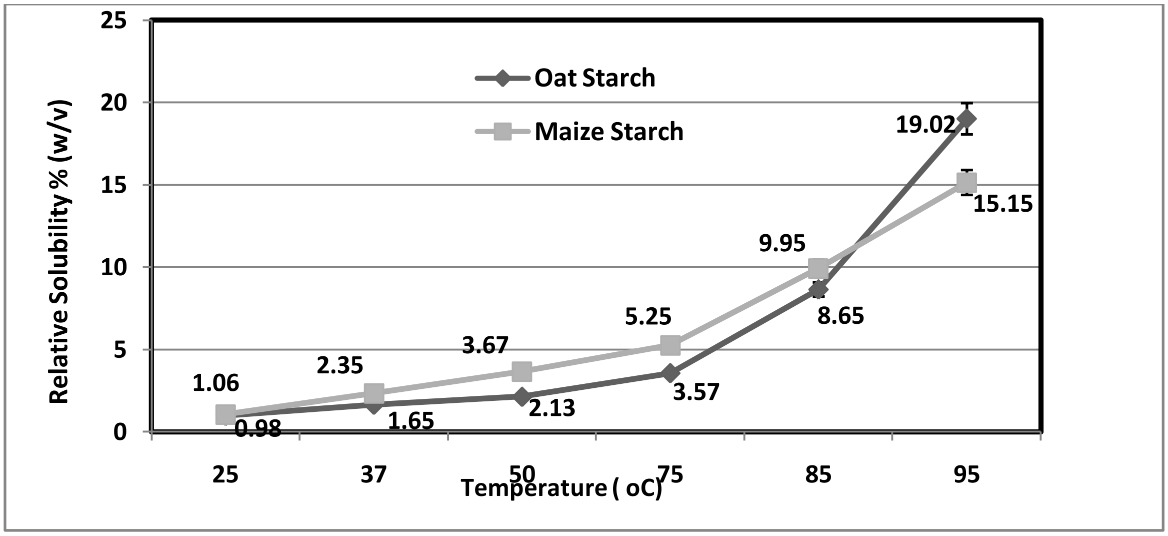

Figure 3.9. The water solubility of *Triticum decocum* and *Zea mays* starches as a function temperature

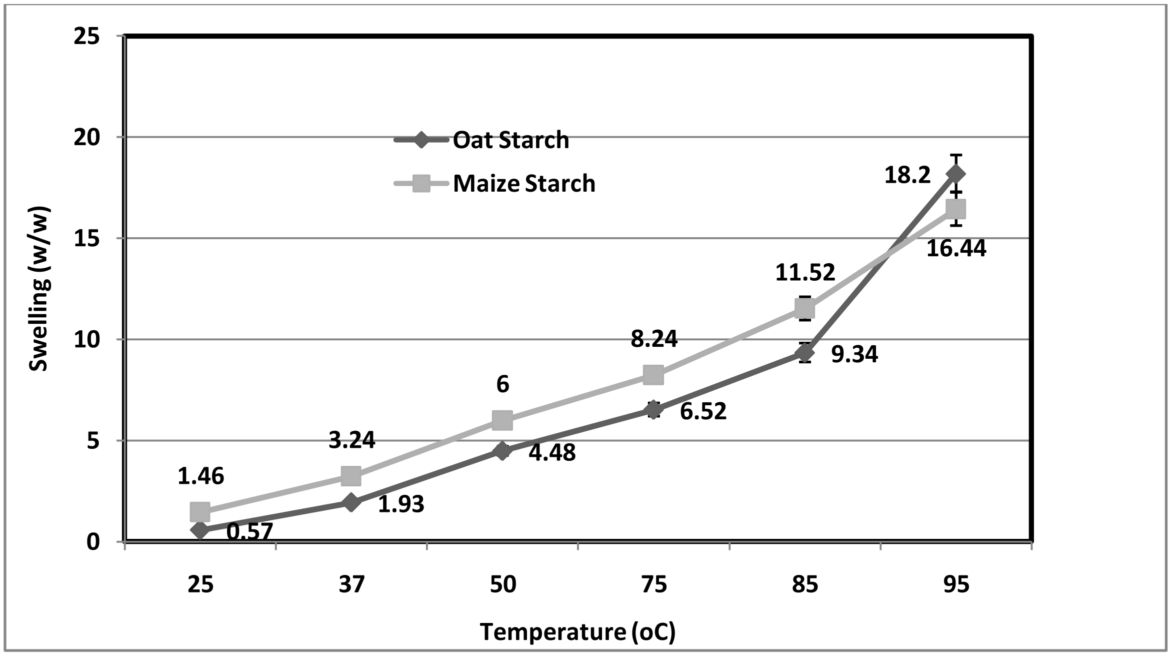

Figure 3.10. The swelling power of *Triticum decocum* and *Zea mays* starches as a function temperature

3.7. Moisture Sorption Property of *Triticum decocum* Starch

The result of moisture sorption isotherms studies showed that the water uptake increased with the relative humidity (RH) for both *Triticum decocum* and *Zea mays* at various moisture levels as it was depicted in **Fig .3.11**. Moisture sorption property increased generally with RH. Percent of moisture sorbed ranged from 6.25% at low (10%) RH to 41.32% at high (100%) RH for *Triticum decocum* starch, while it was 7% and 42.72% for *Zea mays* starch at low and high relative humidities respectively. The moderately hydrophilic nature of these starch molecules is probably responsible for the observed high moisture sorption profile of the powder at high RH, and this enforces the necessity for moisture preclusion during storage owing to higher levels of water can lead to microbial spoilage and subsequent deterioration in the starch quality (Riley *et al., 2006*; Odeku and Picker-Freyer, 2009; Gebre-Mariam and Schmidt, 1998). As long as a range in which the water content remains almost constant can't be defined, during tablet production and storage, the relative humidity should be carefully controlled to obtain powders with optimum flow and compaction properties and also to prevent the deterioration of the tablets.

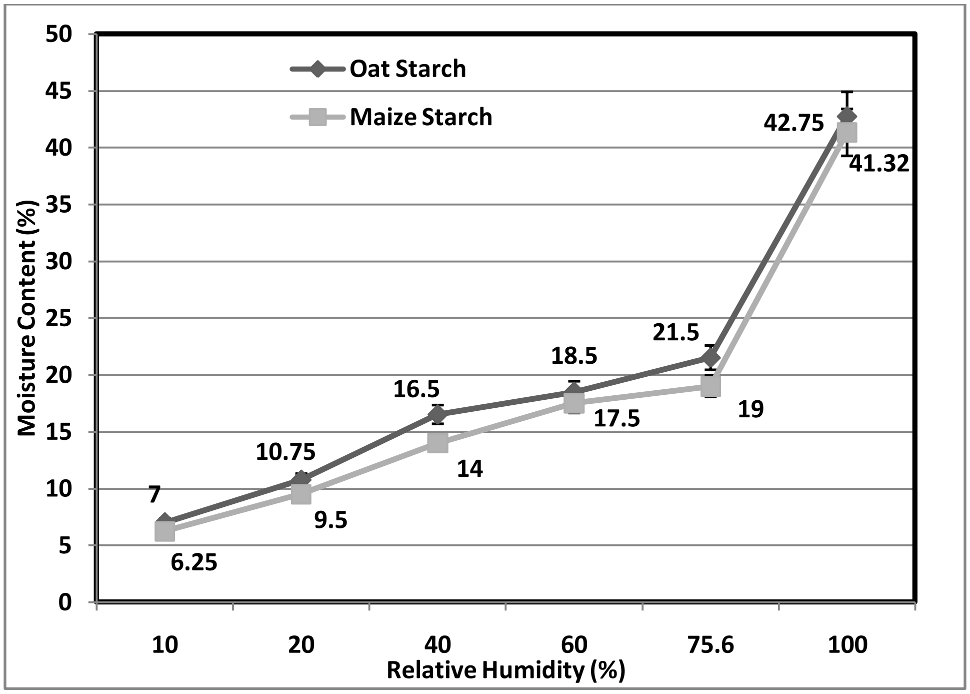

Figure 3.11. Moisture sorption patterns of *Triticum decocum* and *Zea mays* starches at different relative humidities

3.8. Granule Properties

3.8.1. Particle Size and Size Distributions

Despite the technical progress in tableting technology, the pharmaceutical properties of tablets are still controlled by the granules used in the formulation (Miyamoto *et al.*, 1998).

Pharmaceutical granulation is often necessary to overcome the significant compression difficulties and erratic flow properties of many APIs, characteristics that often limit the successful production of acceptable dosage form (Andrews, 2011). **Figs. 3.12** and **13** depict the particle size distributions of paracetamol granules prepared with *Triticum decocum* and *Zea mays* starch at different concentrations as binder and disintegrant. In case of binder effect evaluation, the mean granule size and proportion of larger granules prepared by the entire starches at higher concentrations were higher than those granules prepared at lower concentrations for both starches. But, granule size of paracetamol prepared with *Triticum decocum* starch was significantly larger than that prepared with *Zea mays* starch. The growth in granule size and reduction in fines at high concentration of the starch may be attributed to better binding efficiency of the oat starch. On the other hand disintegrating effect evaluation by both starches revealed that slightly but, not significantly higher granule size was obtained with *Triticum decocum starch* most probably due to better concomitant binding effect with povidone at higher disintegrant concentration.

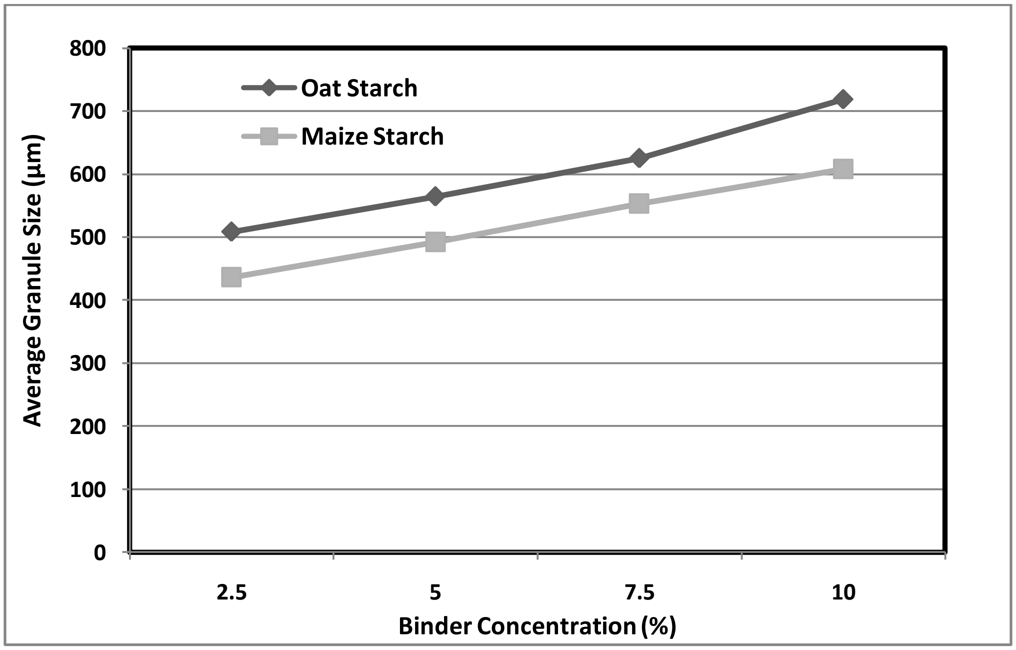

Figure 3.12. Mean granules size distributions of paracetamol formulations prepared with *Triticum decocum* starch and *Zea mays* starch used as a binder

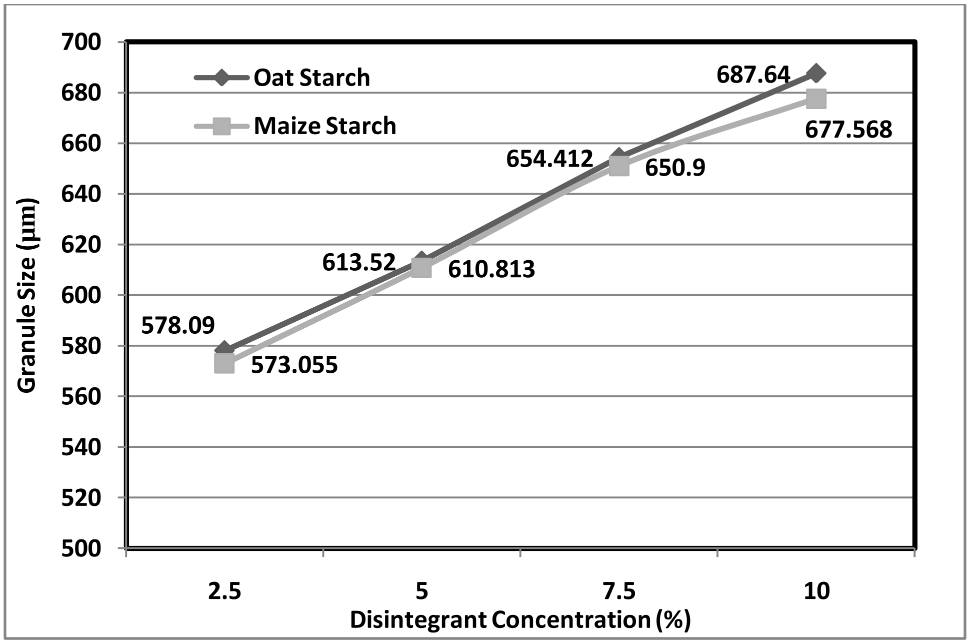

Figure 3.13. Mean granule size distributions of paracetamol formulations prepared with *Triticum decocum* and *Zea mays* starch used as a disintegrant

3.8.2. Micromeritic Properties of Paracetamol Granules

Since densities of granules are known to influence flowability and compressibility of the granules, they can affect the quality of tablets. Density and related properties of the paracetamol granules are presented in **Tables 3.5** & **3.6**. As it can be seen in the table, both bulk and tapped densities of paracetamol granules generally decreased with an increase in binder concentration for all types of starches. This could be attributed to an increase in particle size, which may increase the volume of granules. An increase in bulk volume in turn reduces the bulk density. Significant difference was observed between bulk densities of granules of oat starch and *Zea mays* starches over all binder concentrations ($p<0.05$). All the formulations for both types of granules with both starches as binder and disintegrant have Carr's index below 15 % implying that 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 (Wells, 2002).

Flow rate is a direct method of determining granule flow property. Factors affecting flow property of granules are complex (Kachrimanis *et al*., 2005). It 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). Flow rates of paracetamol granules in this study are ranging from 103.45 to 112 g/min for oat starch at 2.5% and 10%, respectively, and 104.9 to 110.1 g/min for *Zea mays* starch at 2.5% and 10%, respectively. The angles of repose are below 30° for all the formulations, indicating the free flowing property of the granules. Good flow property of the granulation to be compressed is necessary to ensure efficient mixing and acceptable weight uniformity for the compressed tablets (Yüksel *et al*., 2003). Similar argument is true for all parameters when starch is used as disintegrant.

Table 3.5. Densities and related properties of paracetamol granules prepared at different concentrations of oat and maize starch as a binder.

Parameters	Binder Concentration (%)							
	Triticum decocum Starch				*Zea mays* Starch			
	2.5	5	7.5	10	2.5	5	7.5	10
Bulk density (g/ml)	0.470	0.454	0.447	0.441	0.460	0.444	0.437	0.421
Tapped density (g/ml)	0.495	0.475	0.472	0.458	0.490	0.471	0.460	0.442
Carr’s index (%)	5.05	4.42	4.13	3.71	6.12	5.73	5.00	4.75
Hausner’s ratio	1.05	1.05	1.06	1.04	1.06	1.06	1.05	1.05
Angle of repose (°)	29.25	27.55	20.88	17.08	29.87	28.67	22.87	18.98
Flow rate (g/min)	103.45	107.14	109.45	112	104.9	106.00	108.1	110.10

In wet granulation, liquid bridges are formed between particles, and the tensile strength of these bonds increases as the amount of liquid is increased. During drying, inter-particulate bonds result from fusion or re-crystallization and curing of the binding agent (Gebre-Mariam and Nicolayev, 1995). It is therefore assumed that as the amount of starch powder used is increased, a greater amount of bonding takes place that improve granulation.

Table 3.6. Densities and related properties of paracetamol granules prepared at different concentrations of *Triticum decocum* and *Zea mays* starch as disintegrant.

Parameters	Disintegrant Concentration (%)							
	Triticum decocum Starch				*Zea mays* Starch			
	2.5	5	7.5	10	2.5	5	7.5	10
Bulk density (g/ml)	0.484	0.492	0.500	0.505	0.491	0.495	0.504	0.507
Tapped density (g/ml)	0.515	0.522	0.530	0.534	0.518	0.519	0.527	0.530
Carr's index (%)	6.02	5.75	5.66	5.43	5.21	4.62	4.36	4.34
Hausner's ratio	1.06	1.06	1.06	1.06	1.05	1.05	1.05	1.05
Angle of repose (°)	28.35	26.65	19.88	16.24	27.88	25.66	20.77	18.98
Flow rate (g/min)	106.46	109.16	112.55	114.1	105.9	107.09	109.65	112.14

3.8.3. Friability of Paracetamol Granules

The measurement of granule friability is aimed at estimating the relative magnitude of attractive forces seeking to hold the granules together. This strength is dependent upon the nature of the drug, the kind and amount of granulating agent used and the granulating equipment. Granule strength may affect compressibility of the granules into cohesive tablets. **Table 3.7 and 3.8** show friability of paracetamol granules prepared with oat and maize starch mucilage at different concentrations. The result indicates that there is a decrease in granule friability with an increase in formula weight of binder. This is due to more effective wetting and more bonding occurring as the binder concentration increases. Granules prepared with oat starches have lower friability than those prepared with maize starch showing its better binding potential.

Table 3.7. Friability of paracetamol granules prepared at different concentrations of *Triticum decocum* and *Zea mays* as binders. Mean ± SD (n = 3).

Granule Friability	Binder Concentration (%)							
	Ethiopia Oat Starch				*Zea mays* starch			
	2.5	5	7.5	10	2.5	5	7.5	10

	10.2±1.2	8.2±1.6	5.6±0.7	3.17±1	12.3±0.8	9.4±0.2	7.89±0.8	5.67±0.7

Table 3.8. Friability of paracetamol granules prepared at different concentrations of *Triticum decocum* and *Zea mays* starch as a disintegrant. Mean ± SD (n = 3)

	Disintegrant Concentration (%)							
	Ethiopia Oat Starch				*Zea mays* starch			
	2.5	5	7.5	10	2.5	5	7.5	10
Granule Friability (%)	15.2±1.2	12.2±1.6	10.6±0.7	7.16±1	14.62±0.8	11.42±0.2	8.89±0.8	6.67±0.7

3.8.4. Moisture Content of Paracetamol Granules

In order to maintain good compressibility and tablet with optimum tensile strength, the moisture content of paracetamol granules prepared with oat and *Zea mays* starches as binder and disintegrant were found between 1-2.5%. It has been reported that the moisture content of granules influence tablet tensile strength. **Tables 3.9** and **3.10** revealed these results.

Table 3.9. Moisture content of paracetamol granules prepared at different concentrations of *Triticum decocum* and *Zea mays* starch as a binder. Mean ± SD (n = 3).

	Binder Concentration (%)							
	Triticum decocum Starch				*Zea mays* starch			
	2.5	5	7.5	10	2.5	5	7.5	10
Moisture Content (%)	2.1±0.4	2.3±0.3	2±0.3	2.4±0.6	2.1±0.5	2±0.3	2.5±1.2	1.5±0.6

Table 3.10. Moisture content of paracetamol granules prepared at different concentrations of *Triticum decocum* and *Zea mays* starch as a disintegrant. Mean ± SD (n = 3).

	Disintegrant Concentration (%)							
	Triticum decocum Starch				*Zea mays* starch			
	2.5	5	7.5	10	2.5	5	7.5	10
Moisture Content (%)	2±0.5	1.5±0.3	2±0.2	2.5±1.2	1.2±0.6	1.1±0.2	1±0.2	1.2±1.6

3.9. Tablet Evaluations

3.9.1. Crushing strength and friability

While crushing strength indicates the strength (hardness) of the tablet, friability values provide a measure of tablet weakness. The relationship between binder and disintegrant concentration and crushing strength as well as friability for all formulations at a constant disintegrant and binder concentration, and compression condition are shown in **Figs. 3.14** and **3.15,** respectively. There was an increase in crushing strength with corresponding decrease in friability values with increased binder concentration for all the formulations. This is in agreement with previous works reported (Esezobo and Pilpel, 1976; Agrawal and Prakasam, 1988; Zubair *et al*., 1988; Gebre-Mariam and Schmidt, 1996b; Adane *et al*., 2006; Adetunji *et al*., 2006).

It has been established that the presence of high concentration of plasto-elastic binding agent leads to an increase in plastic deformation of the formulation and consequently to the formation of more solid bonds with increase in tablet strength and resistance to fracture and abrasion (Banker and Anderson, 1986; Gebre-Mariam and Schmidt, 1996b). For the paracetamol tablets; therefore, the increased binder concentration resulted in the reduction of elastic recovery of the tablets. Tablets prepared with *Triticum decocum starch* as binder showed significantly higher crushing strength and lesser friability due to the higher inter-particulate bonding strength of its mucilage.

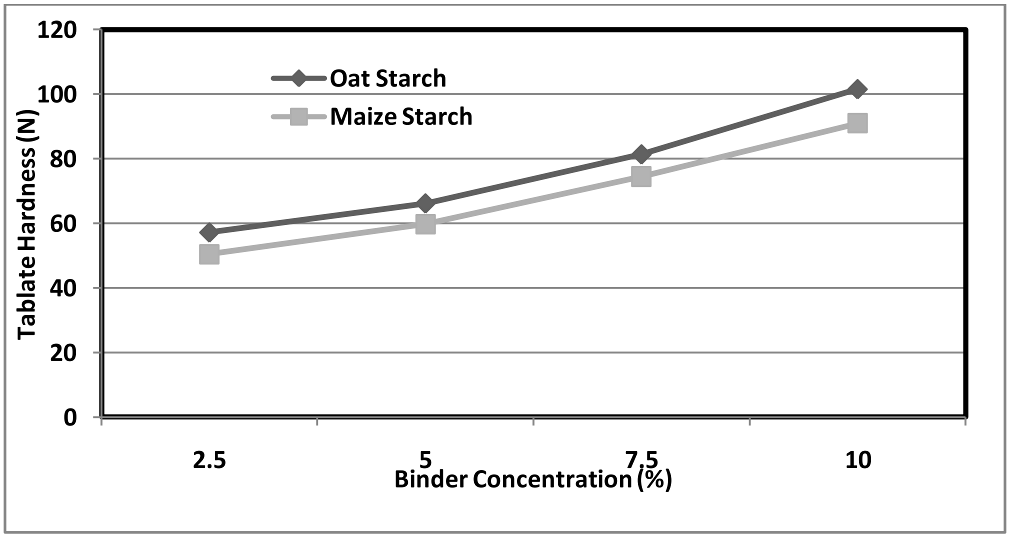

Figure 3.14. Hardness of paracetamol tablets prepared with *Triticum decocum* and *Zea mays* starches as a binder.

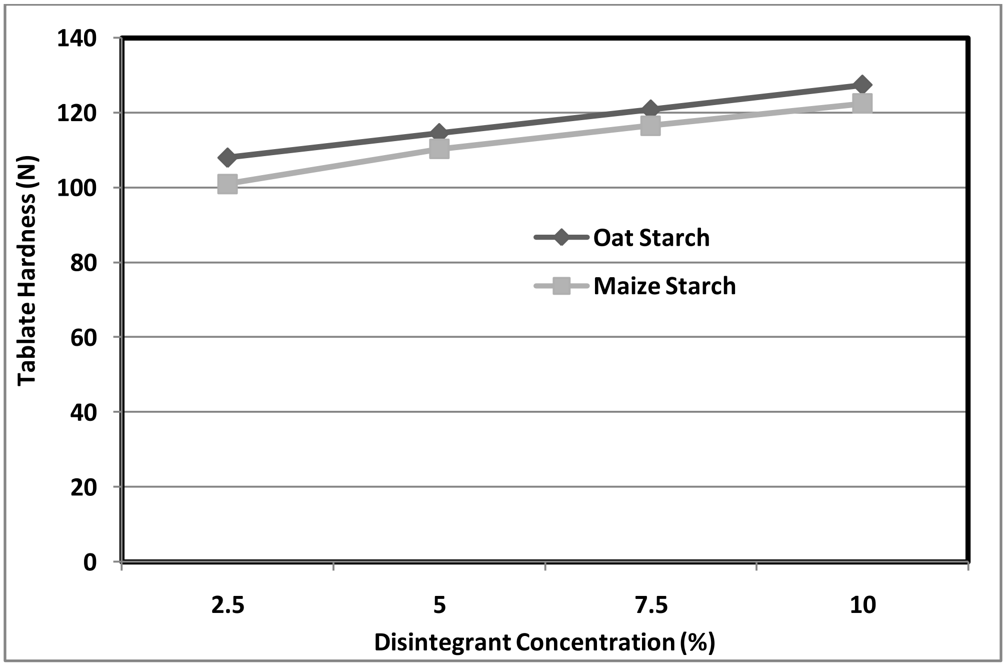

Figure 3.15. Hardness of paracetamol tablets prepared with *Triticum decocum* and *Zea mays* starches as a disintegrant.

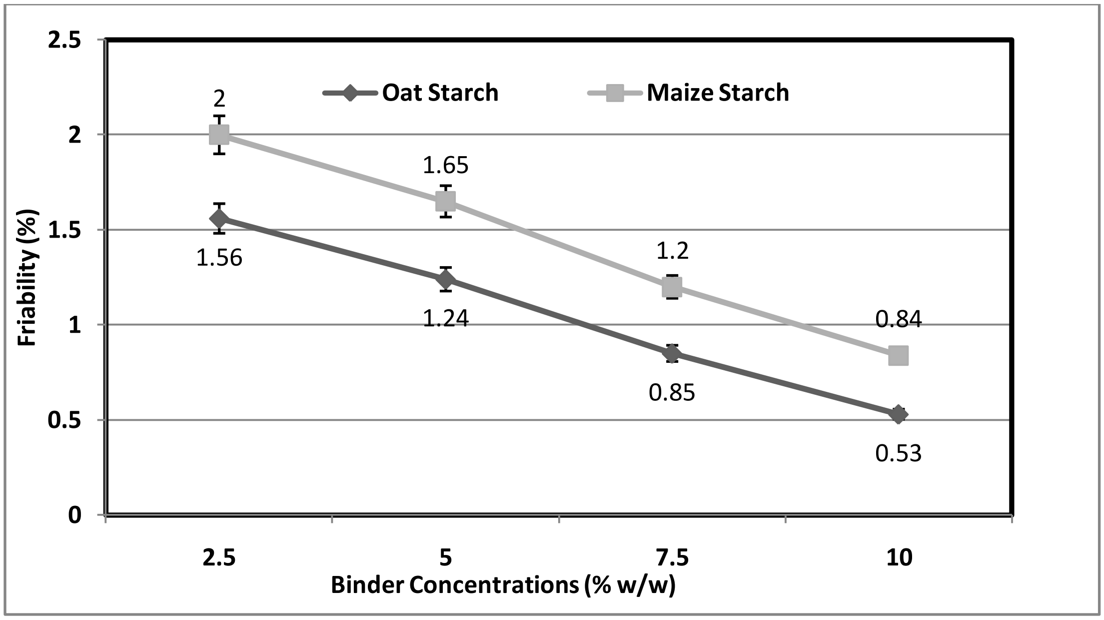

Figure 3.16. Friability of paracetamol tablets with *Triticum decocum* and *Zea mays* starches as a binder

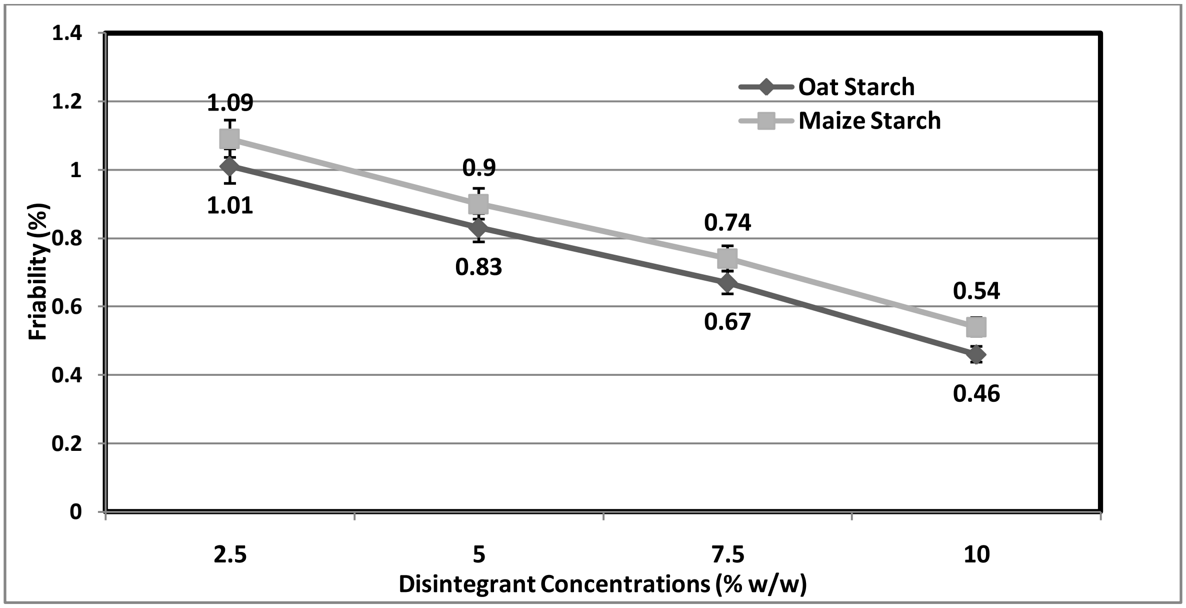

Figure 3.17. Friability of paracetamol tablet with *Triticum decocum* and *Zea mays* starches as a disintegrant

3.9.2. Tablet Disintegration Time

Disintegration time determination was performed to determine whether tablets disintegrate within the prescribed time when placed in a liquid medium of experimental conditions.

The effect of concentration of starches as a binder on the disintegration time of tablets is depicted in **Fig. 3.18**. The disintegration time of the tablets increased as the binder concentration increases. This could be attributable to the reduced liquid penetration into the tablets due to an increase in tablet hardness at higher binder concentration. Moreover, it has been stated by Gebre-Mariam and Schmidt (1996b) that a thin film of mucilage around granules is formed with a thickness depending on the quantity of mucilage used. In the presence of water this thin film is converted into a mucilaginous viscous barrier between the granules and surrounding water. This may act by slowing the rate at which invading water reaches the surface of the powder particles retarding the disintegration of the tablets and delaying the ability of the disintegrant to promote disintegration. The longer disintegration time for tablets prepared with *Triticum decocum starch* at binder concentrations of 7.5 and 10 % w/w could be attributed to the highest crushing strength (**Fig. 3.18**) of the tablets at these binder concentrations.

The relationship between disintegrant concentration and disintegration time at fixed binder concentration is shown in **Fig. 3.19.** One can clearly notice from the figure that the impact of concentration on the disintegration time is disintegrant dependent. The disintegration time of tablets prepared with *Zea mays* starch decreases slightly more than oat starch with insignificant difference. This minor variation might be related to the impact of disintegrant concentration on the crushing strength of tablets. All tablets made of plain *Triticum decocum* and *Zea mays* starches disintegrated within a few minutes. Generally, disintegration times are related to hardness (Özyazici and Sevgi, 2003). The swelling and solubility characteristics could also have a role.

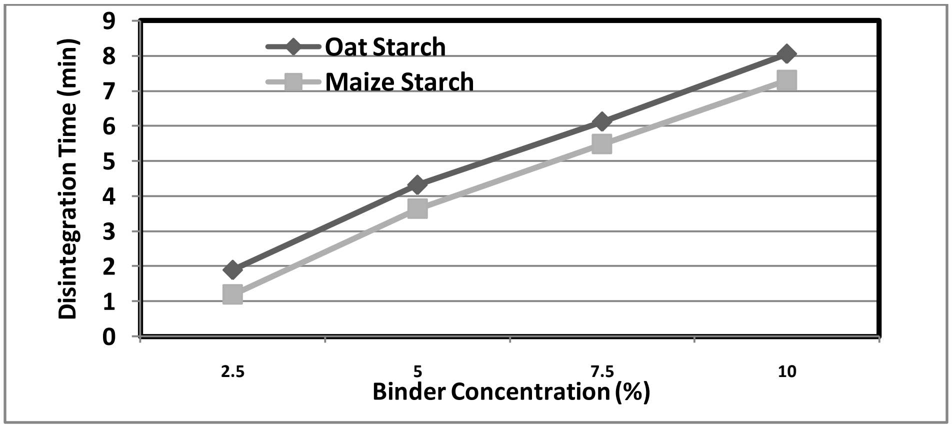

Figure 3.18. Disintegration time of paracetamol tablets with *Triticum decocum* and *Zea mays* starches used as a binder.

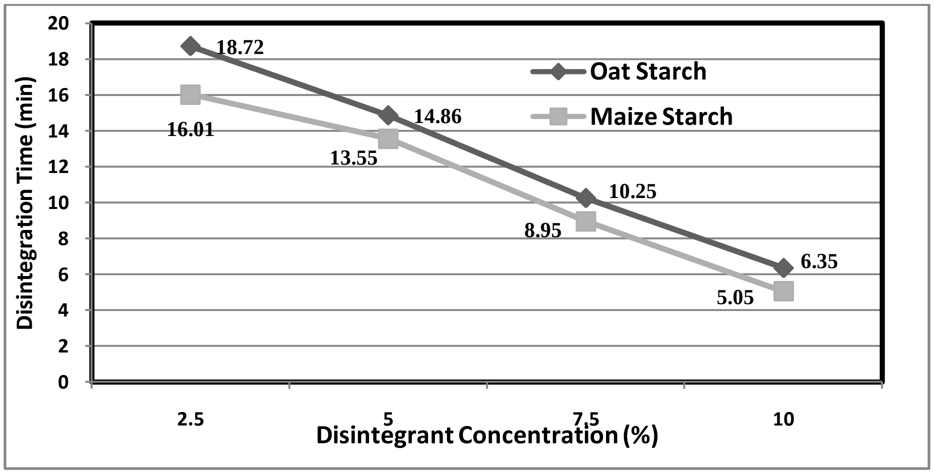

Figure 3.19. Disintegration time of paracetamol tablet with *Triticum decocum* and *Zea mays* starches used as disintegrants.

3.9.3. Drug dissolution study

The dissolution profiles of paracetamol tablets at low (2.5%) and high (10%) starch binder concentrations are shown in **Fig. 3.20**, while, dissolution profiles at low (2.5%) and high (10%) starch disintegrant concentrations are depicted in **Fig.3.21.**

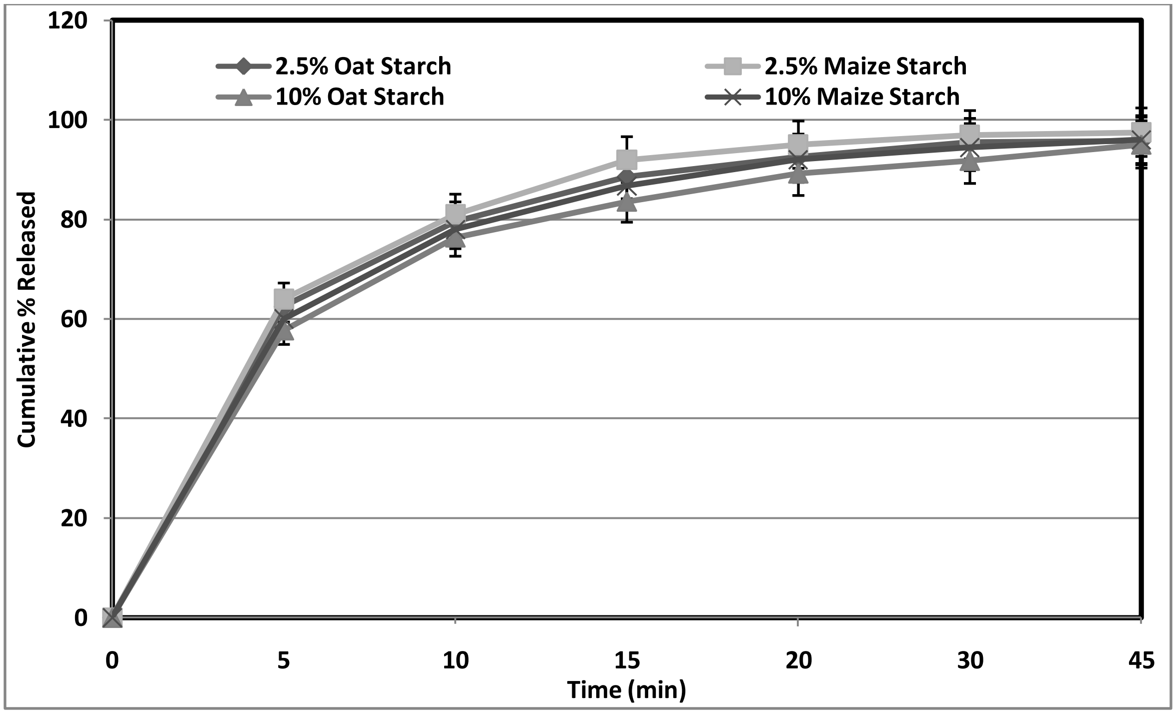

Figure 3.20. Dissolution profiles of paracetamol tablets prepared with *Triticum decocum* and *Zea mays* starches used as binders.

It was observed that the tablets formulated with higher binder concentration (10% w/w) gave lower percent drug release at all time points. The higher bond strength of tablets prepared with binder concentration of 10% w/w that prolonged the disintegration time of the tablets (**Fig.3.20**) could be responsible for the retardation of dissolution. 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). Slightly higher dissolution rates were obtained for formulation containing *Zea mays* starches than when *Triticum decocum starch* was employed as a binder due to stronger bond formation that requires more time for water to dissolve for *Triticum decocum starch* bearing tablet. The amount of drug dissolved from the tablets in 30 min was comparable between both starches both at low (2.5% w/w) and high (10% w/w) binder concentrations.

As it can be observed from dissolution profiles of paracetamol tablets at low (2.5% w/w) and high (10% w/w) disintegrant concentrations in **Fig. 3.21,** the dissolution rate of paracetamol tablet containing oat starch and the *Zea mays* starch increased by increasing the disintegrant concentration. The amounts dissolved from formulations at higher amount of disintegrant (10% w/w) in 30 min were in the order of *Zea mays* starch (95.85%) oat starch (95.65%); however, the variation was not significant ($p > 0.05$). The amount of paracetamol released from tablets when *Zea mays* starch was used as a disintegrant was slightly higher than that of oat starch at their higher concentration ($p > 0.05$). Higher swelling power and higher amylose content of *Zea mays* starch may be the likely reason for the delay of drug release from the tablet containing oat starch as a disintegrant at all concentration (Senanayake, 1995).

Figure 3.20 and **3.21** also show that paracetamol tablets prepared using oat starch and *Zea mays* starch as binder and disintegrant, respectively, have good drug release profile. All formulations released more than 80% in 30 min, complying with USP30/NF25 specification.

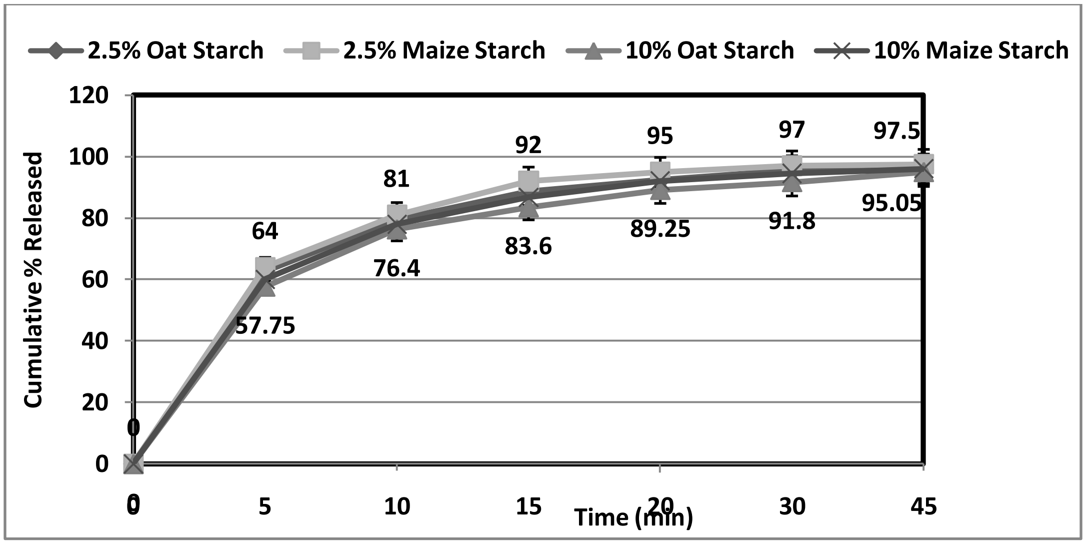

Figure 3.21. Dissolution profiles of paracetamol tablets prepared with *Triticum decocum* and *Zea mays* starches used as disintegrants

4. CONCLUSION

From the foregoing, the following can be concluded about *Triticum decocum* starch. It contains relatively high content of protein and lipid. Some of its granules has oval; others have spherical shape .The particle size distributions of *Triticum decocum* starch showed monomodal normal particle size distribution with the average volumetric particle diameters being about 23.03µm, and specific surface area 0.7186 m^2/g. X-ray powder diffraction pattern indicated that the starch is A-type. At all relative humidities, its water sorption properties resemble that of *Zea mays* starch. Its swelling power and solubility are slightly lower than *Zea mays* starch at lower temperature, while it is significantly higher than *Zea mays* starch at higher temperatures. The peak viscosity is more than twice that of *Zea mays* starch. The hot paste viscosity on the other hand, was more than twice that of *Zea mays* starch. Like *Zea mays* starch, it shows very low breakdown, a positive setback viscosity and remarkable resistance to heat. The presence of relatively strong and homogeneous bonding forces within its granules is evident as suggested by viscosity studies. In general, it shows features, in some cases resembling cereal starch in addition to oat specific properties like high swelling and solubility increment at high temperatures.

Powder mix of the starch with paracetamol has poor flow and compressibility properties. But after wet granulation, the granules showed good flow and compressibility properties. Increase in binder concentration resulted in increased crushing strength and disintegration time and decreased percentage friability of tablets. Increasing the concentration of native starch when used as disintegrant reduced the crushing strength and disintegration time of the tablets and increased the percent friability of the tablets. *Triticum decocum* starch is a better binder than *Zea mays* starch in tablets of paracetamol as it gave less friable tablets with a greater crushing strength and tensile strength, and with higher disintegration time. As a disintegrant, it is less effective than *Zea mays* starch. Nonetheless, *Triticum decocum* starch can be used as a disintegrant when formulated in higher concentration. It is therefore suggested that *Triticum decocum* could be used as an alternative source of starch for use in tablet formulation as binder, and disintegrant.

SUGGESTIONS FOR FURTHER WORK

The results of the study suggest that further investigations on the following directions should be undertaken:

- Comparative studies on different methods of isolation and the chemical composition of the resulting starch;
- Derivatives of *Triticum decocum* starch such as pre-gelatinized starch, sodium starch glycolate and starch acetate in tablet formulations;
- Other applications of the starch (in food and other industries)

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