# ADDIS ABABA UNIVERSITY
# COLLEGE OF HEALTH SCIENCES

# SCHOOL OF PHARMACY
# DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY


# EVALUATION OF RESISTANT STARCH FROM *ERAGROSTIS TEF* AS A FILM COATING MATERIAL FOR COLON-TARGETED TABLETS


YOHANNES TESHOME (B. Pharm.)



February, 2020

Addis Ababa, Ethiopia

# EVALUATION OF RESISTANT STARCH FROM *ERAGROSTIS TEF* AS A FILM COATING MATERIAL FOR COLON-TARGETED TABLETS

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

Yohannes Teshome (B. Pharm.)

Under the supervision of Prof. Tsige Gebre-Mariam and Dr. Anteneh Belete,

Department of Pharmaceutics and Social Pharmacy, School of Pharmacy,

College of Health Sciences, Addis Ababa University

February, 2020

Addis Ababa, Ethiopia

## Addis Ababa University School of Graduate Studies

This is to certify that the thesis investigated by Yohannes Tehsome, entitled: "*Evaluation of Resistant Starch from Eragrostis Tef as a Film Coating Material for Colon-Targeted 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.

| Name | Signature | Date |
|---|---|---|
| Prof. Tsige Gebre-Mariam (Advisor) | _____ | _____ |
| Dr. Anteneh Belete (Advisor) | _____ | _____ |
| Dr. Nisha Mary Joseph (Examiner) | _____ | _____ |
| Dr. Getahun Paulos (Examiner) | _____ | _____ |

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

## ACKNOWLEDGMENTS

First of all, I would like to thank the almighty God for making every single unimaginable dream real. If not for His mother's, Virgin Mary's help that keeps me strong nothing I have done were possible.

My deepest and sincere gratitude and appreciation goes to my advisers, Prof. Tsige Gebre-Mariam and Dr. Anteneh Belete for their support towards the accomplishment of this work and for giving me a timely hope when there was almost none.

It’s my special wish to thank the AAU and its staff for giving me the platform of this research and the whole post graduate study.

I cordially thank EPHARM for the provision of chemicals and solvents and access to their laboratory facilities. It was such an honor to meet and work with the R&D department and the QC department throughout my stay there.

My deepest gratitude also goes to AHRI and its staff for providing me with chemicals, solvents, and access to their laboratory facilities and also for their very interesting working environment.

I am highly indebted to the Holeta Agricultural Research Institute who gave me the four different teff grain varieties for the experiment.

Finally, I would like to share this achievement with all my family and friends for the moral and material supports they extended to me while I was doing the research.

## ABSTRACT

Targeting drug delivery into the colon is highly desirable with many advantages including local treatment of a variety of bowel diseases and systemic delivery of protein and peptide drugs. Research studies propose the use of resistant starch to serve as part of a targeted drug delivery system to the colon where it gets digested by the local bacterial enzymes.

Considering this natural phenomenon, in this study Teff's resistant starch has been evaluated as a film coating material for colon targeted drug delivery system (CTDDS) for the first time. Teff (*Eragrostis tef*) is a native cereal crop widely grown in Ethiopia. It has 73 % carbohydrates and out of the total starch around 30 % is resistant starch.

The whole work includes the main steps of extraction of starch from teff and resistant starch from the total starch, preparation of a film coating material using the resistant starch and coating a sample tablet with the film-forming material, finally testing the film-coated tablet if it could pass the upper GIT intact to release the drug in the colon under simulated *in vitro* conditions.

Different methods were used to achieve the above objectives including The methods by Bultosa *et al.* (2002) and Gebre-Mariam and Schmidt, (1998) for total starch isolation. While in the isolation of RS the AOAC official method 2002.02 and for the tablet coating process the method described by Siew *et al,* (2000) was used.

In the preparation of the film coating material from the resistant starch for CTDDS, because of resistant starch dominant part amylose's property of swelling when it gets in contact with water, a water-insoluble polymer Ethylcellulose (EC) was used and managed to control the premature film dissolution before reaching the colon.

To get the optimum combination of amylose and EC for a film coating solution, their different ratios and film thickness (expressed in percentage total weight gain of the tablet) have been prepared and tested in a simulated gastrointestinal condition as per the standard pharmacopoeia of the model drug, Metronidazole tablet.

In the study, as per the result of dissolution and fermentation data, the best film material proportions of amylose to EC and the corresponding thicknesses in percentage total weight gain identified were; the ratio of 1:1 with thickness 6%, ratio of 1:2 with thickness 4 % and 6%, and finally ratio of 1:3 with thickness 2% and 4%. These were found to be the optimum film thicknesses and combination of the film coating materials to release the drug in the colon but not in the upper GIT.

The reason behind the site selected (targeted) drug release of the film material is due to bacterial enzyme digestion of the RS component of the film-coat in the colon. The digestion of RS produces pores through the EC scaffold of the film which brings a release of the drug content of the coated tablet only in the colon where these bacteria reside.

Based on the above result, a film coating material from the local Teff's resistant starch could be isolated, prepared and evaluated to use it as a CTDDS in the pharmaceutical industry.

***Key words:*** Amylose, Colon targeted drug delivery, Ethylcellulose, Optimization of amylose, Resistant starch, Teff (*Eragrostis tef*)

**TABLE OF CONTENTS**

ACKNOWLEDGMENTS ..... III

ABSTRACT ..... IV

LIST OF TABLES ..... IX

LIST OF FIGURES ..... X

ACRONYMS ..... XI

1. Introduction ..... 1

1.1 Teff Plant (*Eragrostis tef*) ..... 1

1.2 Starch and Resistant Starch ..... 3

1.3 The physiologic effects of RS ..... 7

1.4 Starch and RS as Pharmaceutical Excipients ..... 8

1.5 Targeted Drug Delivery ..... 9

1.5.1 Colon targeted drug delivery system ..... 10

1.5.2 Tablet Coating and Polymeric Film Coating ..... 18

1.6 The Present Study ..... 23

1.7 Research Questions ..... 24

1.8 Objectives ..... 25

1.8.1 General objective ..... 25

1.8.2 Specific objectives ..... 25

2. Experimental ..... 26

2.1 Plant Material ..... 26

2.2 Methods ..... 27

2.2.1 Isolation of total starch from Teff ..... 27

2.2.2 Isolation of resistant starch from the total starch ..... 28

2.2.3 Test for starch ..... 29

2.2.4 Test for resistant starch ..... 29

2.2.5 Optimization of film forming property of amylose (resistant starch) with ethylcellulose ..... 29

2.2.6 Preparing film coated colon-targeted metronidazole tablet using teff's resistant starch ..... 30

2.2.7 Evaluation of film coated tablets ..... 38

2.2.8 Data analysis ..... 40

3. Results and Discussion ..... 41

3.1 Evaluation of the Extracted Total Starch and Resistant Starch ..... 41

3.1.1 Total starch and resistant starch ..... 41

3.1.2 Quantitative test (Teff grain yield) of total starch and resistant starch ..... 41

3.2 Evaluation of Granule Properties of the Formulated Blend ..... 42

3.3 Evaluation of Core-tablets ..... 44

3.3.1 Diameter, thickness and weight variation of core tablets ..... 44

3.3.2 Crushing strength and friability of core tablets ..... 45

3.3.3 The disintegration of core tablets ..... 45

3.3.4 Content uniformity of core tablets ..... 45

3.4 *In vitro* Evaluation of Drug Release Characteristics of the Film-coated Tablets ..... 46

3.4.1 Dissolution studies ..... 46

3.4.2 Fermentation studies ..... 50

3.4.3 Analysis of drug release kinetics ..... 55

3.4.4 Dissolution and fermentation result Summary ..... 56

4. Conclusion ..... 58

5. Suggestions for Further Work ..... 59

6. References ..... 60

Appendix I ..... 70

Appendix II ..... 71

## LIST OF TABLES

Table 1.1: Nutritional value of Teff (By USDA, United States Department of Agriculture Research Service) (USDA, 2015) ..... 2

Table 1.2: Colon targeting diseases, drugs and sites. (Philip et. al., 2010; Sreelatha and Brahma, 2013). ..... 11

Table 1.3: Different GIT parts and varying factors. (Kolt et al., 2012; Amritpal et al., 2014)..... .....15

Table 1.4: Criteria for selection of drugs for CTDDS (Philip et al., 2010; Kolt et al., 2012; Prasanth et al., 2012) .....16

Table 1.5: The amount of EC used in coating (Dahl, 2009). .....22

Table 2.1:Metronidazole Tablet (200 mg) reduced formula of 1000 tablets. ..... 30

Table 2.2: Coating process parameters that were maintained during the coating of tablets. .....38

Table 3.1: Quantitative values (yields) for the isolation of total starch and resistant starch from 100 g of teff grain ..... 41

Table 3.2: Physicochemical properties of the granule ..... 43

Table 3.3: Core tablet properties of Metronidazole formulation ..... 44

Table 3.4: Summery of dissolution and Fermentation studies for different ratio of amylose to EC with six sample groups each having thickness of 2%, 4% and 6% in a simulated gastric, small intestine and colonic environment..... 56

## LIST OF FIGURES

Figure 1-1: Teff (Eragrostis tef): The grass plant (a), harvesting (b), its white (c) and brown seeds (d) (UCSC, 2015)..... 1

Figure 1-2: Chain structure of Amylopectin ..... 4

Figure 1-3 Chain structure of Amylose ..... 4

Figure 1-4: Diagram of various regions in gastrointestinal tract..... 13

Figure 1-5: The mechanism of film formation (Hegyesi et al., 2014)..... 19

Figure 1-6: Structural formula of EC (Dahl, 2009). ..... 22

Figure 3-1: Standard calibration curve of metronidazole in 0.1 N HCl, and 0.32 % pepsin (pH 1) at 278 nm with 95 % confidence interval; ($R^2$ = 0.9998). ..... 47

Figure 3-2: Standard calibration curve of metronidazole in phosphate buffer and 1% pancreatin (pH 6.8) at 278 nm with 95 % confidence interval; ($R^2$ = 0.9997). ..... 48

Figure 3-3: The dissolution study showing the cumulative percentage release of coated tablets with different coating ratios of amylose to EC for each thicknesses in a simulated gastric (from t=0 to t=3 h) and in a simulated intestinal conditions (from t=3 to t=6 h) ..... 49

Figure 3-4: Standard calibration curve of metronidazole in phosphate buffer (pH 7.2) at 278 nm with 95% confidence interval; ($R^2$ = 0.9998). ..... 51

Figure 3-5: The colon simulated fermentation studies on the release of film coated tablet showing the influence of the coating thickness and the ratios of the film forming materials in three dimension ..... 52

Figure 3-6: The test on control for a colon simulated fermentation studies showing the influence of the ratios of the film forming material and the coating thickness on the drug release of the film coated tablets in the three dimensional graph (from t= 0 to t=8 h)..... 54

## ACRONYMS

| | |
|---|---|
| AHRI | Armauer Hansen research institute |
| ANOVA | Analysis of Variance |
| AOAC | The Association of official analytical chemists |
| CD | Crohn's disease |
| CTDDS | Colon targeted drug delivery system |
| DS | Digestible starch |
| EC | Ethylcellulose |
| EPHARM | Ethiopia pharmaceutical share company |
| IBD | Inflammatory bowel diseases |
| IBS | Irritable bowel syndrome |
| RDS | Rapidly digestible starches |
| RS | Resistant starch |
| SDS | Slowly digestible starches |
| SSG | Sodium starch glycolate |
| TS | Total starch |
| TWG | Total Weight Gain |
| UC | Ulcerative colitis |

# 1. INTRODUCTION

## 1.1 Teff Plant (*Eragrostis tef*)

Teff is a tropical cereal that originated in Ethiopia between 4000 and 1000 BC. Teff is a low-risk cereal that grows over a wider ecology and can tolerate harsh environmental conditions where most other cereals are less viable, and it shows better tolerance to many plant diseases and pests (Bultosa *et al.,* 2003; Assefa *et al.,* 2015; Gizaw *et al.,* 2018).

The seeds of the teff plant are among the smallest of cereals (an average length of ~ 1 mm and width of ~ 0.6 mm). The average thousand kernel weight of 13 varieties of teff grain is 0.264 g which is only 0.6-0.8% of the total mass of a wheat grain. Because of this, teff grains are difficult to decorticate rather it is milled into whole-grain flour. This results in a much higher content of fiber and other nutrients such as minerals, vitamins and bioactive phenolic compounds than most other cereals (Belay *et al.,* 2009; Gebremariam *et al.,* 2014). The color of teff can vary from white (ivory) to dark brown (black) depending on the variety, Fig. 1.1. In Ethiopia, three major categories can be identified: white (*nech*), red (*quey*) and mixed (*Sergegna*). It is also common for the locals to further subdivide white teff into very white (*magna*) and white (*nech*) although it is exposed to subjectiveness (Bultosa, 2007; Baye, 2014; Gebremariam *et al.,* 2014).

*(a)* *(b)* *(c)* *(d)*

*Figure 1-1: Teff (Eragrostis tef): The grass plant (a), harvesting (b), its white (c) and brown seeds (d) (UCSC, 2015)*

Unlike other cereals such as maize and wheat, the productivity of teff is very low. In spite of the low yields, Teff has the largest share of area (23.42%, 2.6 million hectares) under cereal

cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia in 2014. The principal use of teff grain for human food is the Ethiopian bread *enjerra*, a soft porous thin pancake with a sour taste and for the traditional alcoholic drink *tela*. *Enjerra* is made from flour, water, and starter *ersho*. *Ersho* is a fluid saved from a previously fermented dough (Bultosa *et al.,* 2002; Assefa *et al.,* 2015; Mezemir S, 2015).

Teff is also gaining popularity as a health food in the western world, notably in the Netherlands and the USA, and its flour is becoming a preferred ingredient in gluten-free diets and as a sport food, and also for its content of the slowly digestible starches (Gebremariam *et al.,* 2013). Miller D, (2010) and Girma *et al.,* (2015) have shown that teff is gluten-free that can provide an alternative food source for people with coeliac disease also known as coeliac sprue or gluten-sensitive enteropathy.

Teff has an attractive nutritional profile (Table 1.1), being high in dietary fiber, iron, calcium and carbohydrate. It also has high levels of phosphorus, copper, aluminum, barium, thiamine and excellent composition of amino acids essential for humans (Hager *et al.,* 2013; Girma *et al.,* 2015; Gizaw *et al.,* 2018).

*Table 1.1: Nutritional value of Teff (By USDA, United States Department of Agriculture Research Service) (USDA, 2015)*

| Nutrients | Unit | One value per 100 g |
|---|---|---|
| Carbohydrate | g | 73.13 |
| Protein | g | 13.3 |
| Total Lipid (fat) | g | 2.38 |
| Minerals (Ca, Mg, P, K, Na, Fe & Zn) | g | 1.25 |
| Vitamins | g | 0.005 |
| Water | g | 8.82 |
| Energy | Kcal | 367 |

Of the total starch in teff, approximately 20-40% is referred to as 'resistant starch' because it is not converted into glucose by the digestive system. However, it has been found that this resistant carbohydrate is used as a food by colonic flora, Bifidobacterium, hence it gets digested ultimately (US PAP 2006).

## 1.2 Starch and Resistant Starch

Starch is a polymer made by green plants to store energy. It is not only the most vital carbohydrate in the human diet but the abundant one in the plant world next to cellulose. This carbohydrate type is made up of glucose units (monosaccharides) linked together by a glycosidic bond. Plants store glucose as a polysaccharide starch. They do not store all of their starch in the same way, however, and for this reason, whole food sources of starch have a variety of starches that is made up of two polymers of D-glucose (structural components) namely; Amylopectin and Amylose. Depending on the plant source the two forms present at different ratios, mostly starch contains 20 to 25% amylose and 75 to 80% amylopectin (Tharanathan, 2005; Karmakar *et al.,* 2014).

The first of these varieties, amylopectin with a molecular mass $10^7$ to $10^9$ g/mole, is typically the most abundant one. Amylopectin has chains of α [1→ 4] linked glucoses arranged in a highly branched structure with α [1→ 6] branching links like the arms of a tree (Fig. 1.2). Branching occurs every 24 to 30 glucose units. Tree branches serve the purpose of creating more surface area, and the branching arms of amylopectin do the same. All that surface area exposes the molecule to digestive enzymes which, in both plants and humans, allows amylopectin to be easily converted back to sugar.

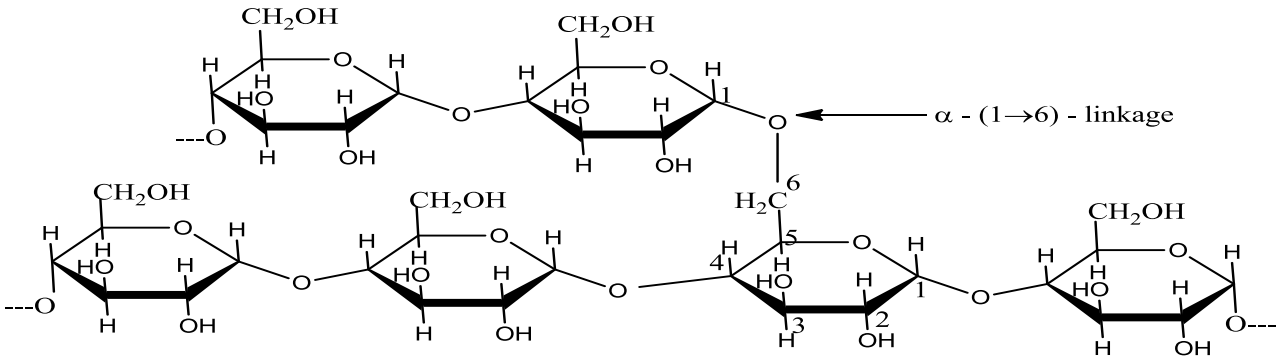



*Figure 1-2: Chain structure of Amylopectin*

The second primary form of starch, amylose with a molecular mass of $10^5$ to $10^6$ g/mol., lacks the branches possessed by its sister molecule amylopectin. Except for some amylose molecules that may have about 0.3-0.5% of α [1→ 6] linkages (branches). The amylose unbranched α [1→ 4] linked glucan is more similar to a bare tree trunk (Fig. 1.3). Tree trunks are solid and strong, as is amylose. The tight structure of this molecule presents less surface area for digestive enzymes to attach to and because of this, amylose is called resistant starch (RS) to mean it resists enzymatic digestion. Both forms of these polymers exist in varying proportion in starch bearing plants. Hence, in those types of starch which are resistant to digestion, amylose is the portion that makes them a RS (Sajilata *et al.,* 2006; Karrout Y, 2008).

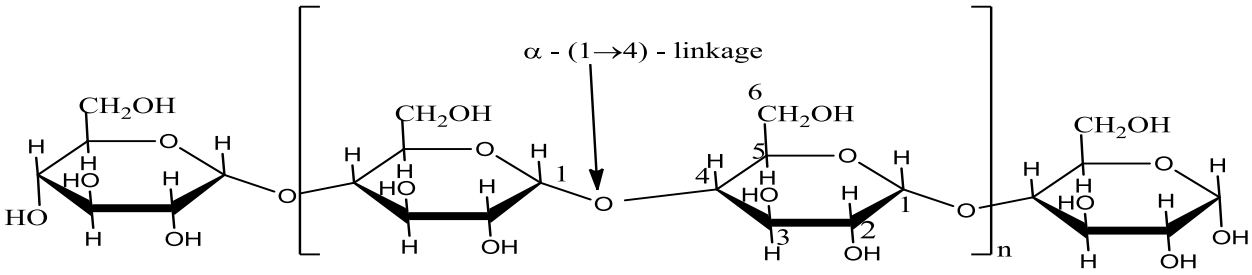



Figure 1-3 Chain structure of Amylose

In water, at increased temperatures, starch undergoes gelatinization followed by amylase-induced hydrolysis. Owing to this, it is completely digested in the gastrointestinal tract of humans. Also, a raw starch of some plant species, e.g. cereals, is subject to complete but slow

digestion. As mentioned above starch may occur in the form incapable of enzymatic hydrolysis, referred to as “resistant starch”. Yet, Amylose is not the only type of RS and not all forms of amylose are resistant to digestion in the upper GIT.

Based on the extent of digestibility of the starch, the following classification gives the two main groups of starch; Digestible or nonresistant starch and RS which themselves have their subgroups.

**Digestible starches:** These include the starches digestible by body enzymes, namely the rapidly digestible starches (RDS) and the slowly digestible starches (SDS) (Eq. 1.1). RDS consists mainly of amorphous and dispersed starch, found in high amounts in starchy foods cooked by moist heat. It is measured chemically as the starch, which is converted to the constituent glucose molecules in 20 min of enzyme digestion. Like RDS, SDS is expected to be completely digested in the small intestine, but for one reason or another, it is digested more slowly. It is measured chemically as starch converted to glucose after a further 100 min of enzyme digestion.

$$DS = RDS + SDS \quad \text{..... Eq. 1.1.}$$

**Resistant starch:** The term “resistant starch” was first coined by Englyst *et al.,* (1982) to describe a small fraction of starch that was resistant to hydrolysis by exhaustive α-amylase and pullulanase enzymes treatment *in vitro*. RS is the starch not hydrolyzed after 120 min of incubation. However, because starch reaching the large intestine may be more or less fermented by the gut microflora, RS is now defined as “The sum of dietary starch and product of starch degradation, which escapes digestion in the small intestine of healthy individuals.” It is measured chemically as the difference between total starch (TS) obtained from homogenized and chemically treated sample and the sum of RDS and SDS (Eqs. 1.2, 1.3), generated from non-homogenized food samples by enzyme digestion (Leszczyñski W, 2004; Sajilata *et al.,* 2006).

$$RS = TS – (RDS + SDS) \quad \text{..... Eq. 1.2.}$$

RS = TS - DS…… Eq. 1.3.

Four main subtypes of RS have been identified based on the structure or the source as described below.

**Resistant starch Type I –** Physically inaccessible starch i.e., starch bound by indigestible plant cell walls; found in beans, in not completely ground cereal grain and seeds. It is unavailable to amylolytic enzymes since the gastrointestinal tract lacks enzymes capable of degrading cellulose, hemicelluloses, lignin, and other constituents of plant cell walls. Therefore, such starch together with fragments of plant tissue passes the small intestine in the intact form. RS 1 is heat stable in most normal cooking operations (Homayouni *et al.,* 2014).

**Resistant starch Type II –** Starch that is intrinsically indigestible granule in the raw state or and due to its high amylose content with highly crystalline structure; found in potatoes, bananas, and plantains. RS 2 may be divided into two sub-types; RS 2a the uncooked starch and RS 2b the high amylose starch group. Type 2a RS becomes accessible upon heating.

The phenomenon of raw starch resistant to the activity of amylolytic enzymes has not been fully explored. Yet, in raw starch granules, starch is tightly packed in a radial pattern and is relatively dehydrated. This compact structure limits the accessibility of digestive enzymes, various amylases, and accounts for the resistant nature of RS ungelatinized starch (Rose *et al.,* 2010; Homayouni *et al.,* 2014).

**Resistant starch Type III** – Retrograded starch, i.e., spontaneously- or artificially-precipitated from starch paste or starch gel occurring in the form of water-insoluble semi-crystalline structures in the retrogradation process. when some starches have been cooked, cooling them (fridge or freezer) changes the structure and makes it more resistant to digestion; found in cooked and cooled potatoes, grains, and beans. RS 3 represents the most RS fraction of all RS and is mainly retrograded amylose formed during the cooling of gelatinized starch.

Amylose in solution crystallizes readily, the phenomenon known as retrogradation and higher resistance is displayed by retrograded amylose than by the products of amylopectin

retrogradation. It results from considerably higher thermo-stability of the crystalline structures of retrograded amylose, compared to those formed upon retrogradation of amylopectin.

RS 3 resists both dispersion by boiling and enzyme digestion. It can only be dispersed with KOH or dimethyl sulphoxide. It is also entirely resistant to digestion by pancreatic amylases (Homayouni *et al.,* 2014).

**Resistant starch Type IV** – Industrial RS; type 4 RS does not occur naturally and has been chemically modified; commonly found in "hi-maize RS."

RS 4 is the RS where novel chemical bonds other than α-(1-4) or α-(1-6) are formed. Modified starches obtained by various types of chemical treatments are included in this category. For example; acetylated starch of papilionaceous plants (a leguminous plant) is characterized by a relatively high degree of resistance to the activity of amylolytic enzymes. Similar properties are displayed by the starch of papilionaceous plants modified by hydroxypropylation (Leszczyñski W, 2004; Hartzell and Rose, 2011).

## 1.3 The physiologic effects of RS

The physiologic effects of RS have been studied during the past 30 years in animals and human beings and include health effects in the large intestine and some systemic effects.

RS's health benefits in the large intestine include: enhanced fermentation and laxation, increased uptake of minerals such as calcium, changes in the microflora composition that includes increased bifidobacterium and reduced pathogen levels (Murphy *et al.,* 2008). Bifidobacteria are human colonic bacteria that ferment RS to short-chain fatty acids mainly, acetate, propionate, butyrate and to gases. Short-chain fatty acids stimulate colonic blood flow and fluid and electrolyte uptake. Butyrate is crucial because it's the prime energy source of our colonic cells and reduced pathogen levels, which are responsible for the favorable selection of intestinal microflora, reduce the levels of cholesterol, triglycerides, and urea in

blood, as well as prevent the formation of gut cancer. It also reduces symptoms of diarrhea (Topping and Clifton, 2001; Leszczyñski, 2004).

Systemic effects involve plasma glucose and insulin, insulin sensitivity, and fatty acid oxidation. Consumption of a meal high in RS decreases peak insulin and glucose concentrations. RS supplementation influences blood lipid concentrations and glucose control in overweight subjects. RS has a "second meal effect", a meal's ability to diminish the glucose response to carbohydrates eaten during the following meal. It also improves insulin sensitivity in subjects with the metabolic syndrome, Type II Diabetes (Murphy *et al.,* 2008; Johnston *et al.,* 2010).

## 1.4 Starch and RS as Pharmaceutical Excipients

Excipients are pharmaceutical additives, the inactive ingredients used to make up a medication. According to the International Pharmaceutical Excipient Council, excipient is "Any substance other than active drug or pro-drug that is included in the manufacturing process or is contained in finished pharmaceutical dosage forms". The US Pharmacopoeia -National formulary USP/NF. (2007) categorizes excipients according to the functions they perform in the formulations e.g. binders, disintegrants, etc. Choosing the right excipients can make all the difference in the efficient production of robust pharmaceutical dosage forms (Abrantes *et al.,* 2016; Bari., 2019).

Excipients play a very important role in the design of dosage forms. Though there are number of excipients available, still there is a need for more excipients with varied characteristics. This is because of the introduction of novel drug delivery systems and new drug moieties. Excipients used in the pharmaceutical industry should be Generally Recognized as Safe (GRAS) by FDA, chemically stable and free from viable micro-organisms including pathogens (Kusuma *et al.,* 2014).

Starch granules produced by each plant have specific structures and compositions (for instance the length of glucose chains or the amylose to amylopectin ratio). Starch granules also contain small quantities of proteins, fatty acids, and minerals that influence the properties of the

starch. Therefore, starch can have different industrial uses depending on its source which is the agricultural raw material it was extracted from.

In pharmaceutical technology, starch appears indispensable in its application as an excipient in several dosage forms. Its traditional role as a disintegrant or diluent is giving way towards a more modern role as drug carrier; the therapeutic effect of the starch-adsorbed or starch-encapsulated or starch-conjugated drug largely depends on the type of starch in question. As a natural polymer that can easily be modified, it is highly stable, safe and non-toxic. In addition, starch is biocompatible and biodegradable. These properties of starch make it still suitable for human use in designing advanced drug delivery systems as in some targeted drug delivery systems, in addition to its use in conventional dosage forms (Chen *et al.,* 2007; AGROSYNERGIE, 2011; Vamadevan and Bertoft, 2015).

As it has been mentioned above, starch is chemically composed of two parts, amylose (the dominant part in RS) and amylopectin. In using starch as an excipient, the amylose content is responsible for its film forming, gelling as well as binding properties. whereas amylopectin is responsible for the high viscosity. Both properties are useful in food and pharmaceutical industries (Nutan, 2004; Satyam *et al.,* 2010).

## 1.5 Targeted Drug Delivery

Targeted drug delivery, also known as smart drug delivery, is a method of drug delivery that involves concentrating the drug in one or few body parts in comparison to others. Drug delivery vehicles transport the drug either within or in the vicinity of the target (Aulton, 2007). This improves the efficacy of the drug while reducing side effects. The drug's therapeutic index, as measured by its pharmacological response and safety, relies on the access and specific introduction of the drug with its candidate receptor, whilst minimizing its introduction with non-target tissue. The desired differential distribution of the drug, its targeted delivery, would spare the rest of the body and thus significantly reduce the overall toxicity while maintaining its therapeutic benefits.

Delivering a drug to a specific organ or tissue (i.e. spatial placement) and/ or controlling the rate of drug to be delivered to the acting site (i.e. temporal delivery) are the two main aspects of a targeted drug delivery system (Manish and Vimukta, 2011).

The main reasons that brought the demand for targeted drug delivery system are:

- Pharmaceutical reasons: Drug instability and low solubility
- Pharmacokinetic reasons: Poor absorption and overcoming barriers like blood brain barrier, Short half-life and Large volume of distribution
- Pharmacodynamics reason: Low specificity, Low Therapeutic index and reduction of unnecessary exposure of other body parts where the drug is not required

Targeted drug delivery system gives these advantages; Increased specific localization or the delivery of drug in its intact form as close as possible to the target sites, Increase treatment efficacy, controlled bio-distribution, modulated pharmacokinetics, improved patient compliance, decreased toxic side effects and decreased frequency of administration or a reduced dose as a result a decreased cost of drugs (Florence and Attwood, 2008; Jones, 2008).

Drug targeting to specific sites in different parts of the body requires different delivery systems depending on the drug delivery route selected. Accordingly, based on the various targeting site, targeted drug delivery could be classified as a method that target **the respiratory tract, the brain,** and **the GIT.** Again under GIT, there are drug delivery systems that target stomach or duodenum, the small intestine, the lymphatic systems and the system that **targets the colon,** which is the focus of the present research (Mahajan, *et al.,* 2007; Bae and Park, 2011).

### 1.5.1 Colon targeted drug delivery system

CTDDS is capable of protecting the drug all the way to the colon, i.e., drug release and absorption do not occur in the stomach as well as in the small intestine, and the bioactive agent is not degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon.

The delivery of drugs to the colon might be important; when local effect is valuable in a variety of conditions like in inflammatory bowel diseases-IBD (e.g., Ulcerative colitis-UC and Crohn's disease-CD), Irritable bowel syndrome- IBS, for infectious diseases and colon cancer, for local treatment of colonic pathologies, and even for systemic delivery of protein and peptide drugs as shown in Table 1.2 (Aulton, 2007; Karrout Y, 2008; Gadhave *et al.,* 2017).

*Table 1.2: Colon targeting diseases, drugs and sites. (Philip et. al., 2010; Sreelatha and Brahma, 2013).*

| Target sites | Diseases | Drugs |
|---|---|---|
| **Topical action** | IBD (CD, UC), IBS. | Hydrocortisone, Prednisolone, Sulfasalazine, Mesalazine, Mercaptopurine. |
| | Amebiasis | Metronidazole, Tinidazole, Albendazole, Mebendazole. |
| **Local action** | Pancreatectomy, Cystic fibrosis and Chronic pancreatitis. | Digestive enzyme supplements. |
| | Colorectal cancer | 5- Fluorouracil |
| **Systemic action** | To prevent gastric irritation | NSAIDS |
| | To prevent first pass metabolism of orally administered drugs | Steroids |
| | Oral delivery of peptides | Insulin |
| | Oral delivery of vaccines | Typhoid |

Ingested materials have longer residence time in the colon and colon appears highly responsible to agents that enhance the absorption of poorly absorbable drugs in the upper GIT. Also, it's a suitable absorption site for peptide and protein drugs with the following reasons;

a) Less diversity and intensity of digestive enzymes

b) Comparative proteolytic activity of colon mucosa is much less than that observed in the small intestine, thus CTDDS protects peptide drugs from hydrolysis, and enzymatic degradation in duodenum and jejunum, and eventually releases the drug into ileum or colon which leads to greater systemic bioavailability; and
c) Finally, the colon's ability of allowing longest residence time for materials in the GIT and is highly responsiveness to absorption enhancers (Florence and Attwood, 2008; Karrout Y, 2008; Gadhave *et al.,* 2017).

All the advantages that have been mentioned previously (section 1.5, Targeted drug delivery) are also the advantages of CTDDS. Yet, CTDDS has got the following limitations.

1. Multiple manufacturing steps, a higher need for excipients and a large number of process variables;
2. Lack of manufacturing reproducibility and efficacy;
3. The need for advanced technology and skilled personnel for manufacturing a drug for CTDDS;
4. Incomplete release of drug and low dose loading; and
5. Lowering of bioavailability due to the binding of drugs to intestinal contents (Jones ,2008; Amritpal *et al.,* 2014).

#### 1.5.1.1 Factors influencing the CTDDS

The CTDDS is primarily affected by two main factors: **Anatomical-Physiological factors** and **Pharmaceutical factors**. It would be obligatory to consider both to attain the desired colon targeted formulation.

##### 1.5.1.1.1 Anatomical and Physiological factors

The human large intestine is approximately 1.5 m long and forms the colon (ascending, transverse, descending, and sigmoid colon. Fig. 1.6.), with a small distal part forming the rectum. The colon is 5-7 cm in diameter, and its lumen is lined with mucus. The physiology of the colon differs significantly from other segments of the GIT (Appendix II c.). Moreover, the

physiology and the physical properties of the colonic contents also differ between the ascending, transverse, descending, and sigmoidal colon. Besides, there exists variability in the movement of food and dosage forms across the colon, which may present a challenge in the development of colonic drug delivery systems (Gupta *et al.,* 2011; Amidon *et al.,* 2015).

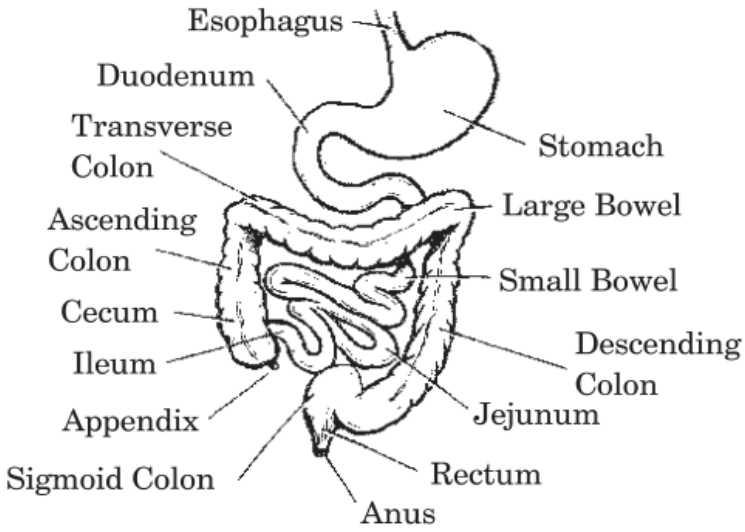



*Figure 1-4: Diagram of various regions in gastrointestinal tract*

The average human food intake is approximately 1.5 kg/day and mainly consists of undigested proteins, carbohydrates, and fats. These food components may serve as substrates for the microbial enzymes in the colon. The colon has a high water-absorbing capacity and can absorb around 90% of the water entering the colon. The colonic fluid volume is calculated to be in the range of 1–44 ml with an average volume of approximately 13 ml. Due to this low volume of colonic fluids, the dissolution of drugs from the dosage forms becomes challenging and may affect the local drug bioavailability (Gupta *et al.,* 2011).

The other determining factors are;

a) Gastric emptying and bowel transit time

One of the major determinants of the absorption of compounds from the colon is the residence of formulation in some particular segment of the colon. The transit time in the small intestine is reported to be quite consistent than the stomach and the colon.

Size, disease condition and food intake are the one that vary the emptying and transit time itself. The size of the particles influences the colon transit when dosage forms reach the colon. Small particles pass through the colonic region more slowly than the larger unit. Diarrhea patients have shorter transit time whereas constipation patients have longer transit times. In a fed state the gastric emptying is larger than fasting state. These are shown in Table 1.3. (Jones 2008; Amritpal *et al.,* 2014).

b) pH of colon

Inter- and intra-subject variations in gastrointestinal tract pH are reported. Diet, diseased state and food intake influence the pH of the gastrointestinal fluid. This change in the pH in different parts of GIT by itself become the basis for the development of pH dependent colon targeted drug delivery systems. Coating with different polymers (responsive to the pH) is used to target the drug to the site. The average pH values in different regions of the human GI tract are given in Table 1.3 (Amritpal *et al.,* 2014; Prathap *et al.,* 2014).

c) Colonic microflora and enzymes

A large number of anaerobic and aerobic bacteria are present in the entire length of the human GI tract. Intestinal enzymes are used to trigger drug release in various parts of the GI tract. Usually, these enzymes are derived from gut microflora residing in high numbers in the colon. These enzymes are used to degrade coatings or matrices as well as to break bonds between an inert carrier and an active agent (i.e.., the release of a drug from a prodrug). Over 400 distinct bacterial species have been found 20-30% of which are of the genus bacteroids. The concentration of bacteria in the human colon is around 1000 CFU/ml (colony-forming units per milliliter). The most important anaerobic bacteria are Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, and Clostridium. The

abundance of bacteroids makes colon a good candidate for Bacterial enzyme dependent targeted drug delivery system (section 1.5.1.2). Table 1.3 compares the estimated number of microorganisms in GIT (Sarangi and Padhi, 2015; Sreelatha and Brahma 2013).

*Table 1.3: Different GIT parts and varying factors. (Kolt et al., 2012; Amritpal et al., 2014)*

| No. | GIT segment Main part | GIT segment Sub part | Length (m) | Surface area (m,2) | pH | Transit time (h) | Micro-organisms |
|---|---|---|---|---|---|---|---|
| 1 | Stomach | | 0.2 | 0.1 | | 1-2 | 10,2 |
| | Fasted condition | | | | 1.5-2 | | |
| | Fed state | | | | 3-5 | | |
| 2 | Small intestine | | 3 | | | 3-4 | |
| | | Duodenum | 0.25 | 0.1 | 5-6.5 | 2 | 10 |
| | | Jejunum | 1 | 6 | 6-7.5 | 1.5 | 10’ 5 |
| | | Ileum | 3 | 6 | 6.4 | 1.5 | 10’7 |
| 3 | Large intestine | 0.5 | 1.5 | 0.3 | | >12 | >10’11 |
| | | Ascending (proximal) colon | 0.20-0.25 | | 6.4 | | |
| | | Transverse colon | 0.40-0.45 | | 6.6 | | |
| | | Descending colon | 0.10-0.15 | | 7 | | |
| 4 | Rectal | Recto Sigmoid | R. 0.12 &R.S 0.40 | | 7 | >12 | |

#### 1.5.1.1.2 Pharmaceutical factors

a) Drug candidates

Drugs which show poor absorption from the stomach and intestine including peptide are most suitable for CTDDS. The drugs used in the treatment of IBD, Diarrhea, and Colon cancer are

ideal candidates for local colon delivery. Table 1.4 shows the criteria to select a drug for CTDDS (Mukesh *et al.,* 2013; Sreelatha and Brahma, 2013).

*Table 1.4: Criteria for selection of drugs for CTDDS* (Philip *et al.,* 2010; Kolt *et al.,* 2012; Prasanth *et al.,* 2012)

| **Criteria** | **Pharmacological class** | **Non-peptide drugs** | **Peptide drugs** |
|---|---|---|---|
| Drugs used for local effects in colon against GIT diseases | Anti-inflammatory drugs | Ibuprofen, 5-Amino-salicylic acid, hydrocortisone | Amylin, Antisense Oligonucleotide |
| Drugs poorly absorbed from upper GIT | Antihypertensive and antianginal drugs | Oxyprenolol, Metoprolol, Nifedipine, Isosorbides, Theophylline | Epoetin, Glucagon |
| Drugs for colon cancer | Antineoplastic drugs | Bromophenaramine, 5-Flourouracil, Doxorubicin | Cyclosporine, Desmopressin |
| Drugs that degrade in stomach and small intestine | Peptides and proteins | Bleomycin | Gonadoreline, Insulin, Interferons |
| Drugs that undergo extensive first pass metabolism | Nitroglycerin and corticosteroids | Nicotine, hydrocortisone | Protirelin, Sermorelin, Saloatonin |
| Drugs for targeting | Antiarthritic and antiasthamatic drugs | Prednisolone, Pseudoephedrine, Theophylline | Somatropin, Urotoilitin |

b) Drug carriers

The selection of carrier for a particular drug candidate depends on the physiochemical nature of the drug as well as the disease for which the system is to be used. The factors such as chemical nature, stability and partition coefficient of drug and the type of absorption enhancers chosen influence the carrier selection. Moreover, the choice of drug carrier depends on the functional groups of the drug molecule. The carriers which contain additives like polymers (may be used as matrices and hydrogels as coating agents) may influence the release properties and efficacy of the systems (Mukesh *et al.,* 2013; Amritpal *et al.,* 2014).

c) Formulation Factors

The formulation factors that influence colonic drug delivery and bioavailability include the physicochemical properties of the drugs, the dose, and the dosage form factors. Due to the lower amount (1 – 44 ml or an average of 13 ml) of colonic fluid available for dissolution, the solubility and the dose of a drug become important factors for its colonic bioavailability.

#### 1.5.1.2 Strategies for CTDDS

In general, **six primary approaches** have been proposed for targeted colon delivery systems, namely;

i. Transit time-dependent CDDS
ii. pH-dependent CDDS
iii. pH- and time-dependent CDDS
iv. Bacterial enzyme-dependent CDDS
    a. Prodrug based system
    b. Coating and matrices Based system
v. pH and bacterial enzyme-dependent colonic DDS
vi. Osmotic pressure controlled CDDS (Sarangi and Padhi, 2015).

The present work is a bacterial enzyme dependent CTDDS based on RS as a coating material.

#### 1.5.1.3 Resistant starch as CTDDS

Many research studies suggest the expanded utilization of starch into the pharmaceutical industry where RS can serve as a part of a drug delivery system to the colon. Chen *et al. (*2007) formulated a novel tablet of protein drug matrix for colon targeting using RS as a carrier prepared by pre-gelatinization and cross-linking of starch. An oral colon-targeting controlled release system based on RS acetate as a film-coating material has been developed by Pu *et al. (*2011). Films from RS-pectin dispersions intended for colonic drug delivery by Meneguin *et al. (*2014) was the other experiment that blend retrograded starch with pectin as a dispersion film-forming material. Also, various other uses of starch-based drug delivery system for colon

targeting has been reported (Satyam *et al.,* 2010; Pu *et al.,* 2011; Kittipongpatana O and Kittipongpatana N, 2014)

In each of the findings, some modification of the RS's amylose or optimization with other additives like acetate and ethylcellulose have been mandatory. Because using amylose alone in a film coating has the disadvantage of swelling in aqueous media and subsequent accelerated drug release (Karrout Y, 2008).

Similarly, in this work of designing a film-coated tablet as a CTDDS with Teff's RS polymer, an optimization of the amylose of the RS with another polymer (ethylcellulose) was obligatory and had been done.

### 1.5.2 Tablet Coating and Polymeric Film Coating

#### 1.5.2.1 Tablet coating

Tablet coating is the application of coating material to a moving bed of tablets with the concurrent use of heated air to facilitate the evaporation of the solvent. The coating is done to perform a specific function or to get the benefits and properties to the dosage form over the uncoated variety (Hussan *et al.,* 2012).

In a modern tablet coating system, the coating equipment combines several components. These are: a coating pan, a spraying system, an air handling unit and a dust collector.

Though, the main coating process types are three: Film coating, Sugar coating, and Press coating; there are also other methods like Microencapsulation, Dry coating, Melting coating, Dip coating, etc. (Ankit *et al.,* 2012).

#### 1.5.2.2 Film coating

Film coating is an approach to coat the surface of a solid dosage form, such as tablets, capsules, or pellets by surrounding them with a thin layer of stable polymeric film-forming materials. A great number of polymers available for coating ensure different dissolution profiles. With the optimal choice of film-forming materials, the possibility that the coating could dissolve or the

drug to be revealed in the desired part of the GIT is provided, therefore the rate and the place of the drug release can be influenced (Hegyesi et al., 2014).

Film coating has the advantage of becoming a single step process in a relatively short period of time. This single step process involves mixing the film-forming solution (polymer, solvent, plasticizer, and colorant) and spraying it on to a rotating tablet bed followed by drying, which facilitates the removal of the solvent leaving behind the deposition of a thin film of coating materials (Fig. 1.5). The film formation mechanisms may depend on the type of coating formulation i.e., aqueous versus organic solvent used (Melegari, 2016).

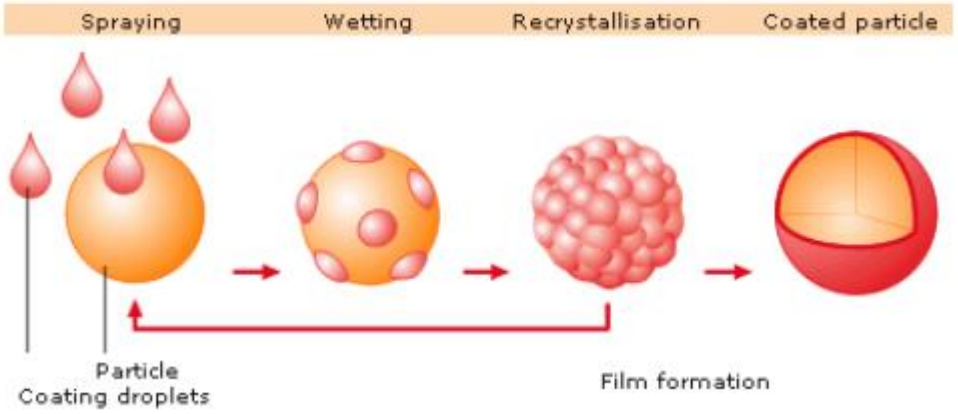



*Figure 1-5: The mechanism of film formation (Hegyesi et al., 2014)*

In addition to the content of the film-forming solution, Film coating is also governed by the following film coating process parameters: rotating speed of the pan, flowrate of the coating solution or spraying air pressure, the distance and angle of the spray nozzle from the tablet bed, the inlet air flow rate, its humidity and its temperature, also. These factors help to obtain an ideal coated tablet that ought to be free from any visual illness or functional defect. The general requirement of the final film coat is to be smooth, uniform and to have a good appearance (Osman, 2012; Ruotsalainen, 2003; Griesser, 2016).

#### 1.5.2.3 Film forming polymers

Polymers are substances whose molecules have high molar masses and composed of a large number of repeating structural units (mers) joined by some type of linkage, form into a chain-like structure. They usually contain more than five monomers, and some may contain hundreds or thousands of monomers in each chain. Most polymers are organic and formed from hydrocarbon molecules (Semwal *et al.,* 2015).

Polymers are both naturally occurring and synthetic. Naturally occurring polymers, include proteins, latex, cellulose, guar gum, inulin, pectin, shellac, cyclodextrin, starches, dextran, amylose, chitosan, chondrotin sulfate, locust bean gum; while synthetic polymers include ethylcellulose, polyethylene, cellulose acetate phthalate, hydroxypropyl methylcellulose, eudragit, and polyvinyl acetate phthalate (Amritpal *et al.,* 2014; Priya *et al.,* 2016).

Advances in polymer science have led to the development of novel delivery systems. The introduction of new polymers has resulted in the development of polymers with unique properties. Initially, polymers were used as solubilizers, stabilizers and mechanical supports for sustained release of drugs. But through time, the functionalities of polymers have changed. The polymers have been synthesized to suit specific needs or rather solve specific problems associated with the development of drug delivery systems.

Polymers can form particles of solid dosage form and can change the flow property of liquid dosage form, too. Polymers have been also used as important tools to control the drug release rate from the formulations. One of the common uses of polymers is as coating formulations (Karrout Y, 2008; Hussan *et al.,* 2012)

The selection and design of a polymer for specific drug delivery system is a challenging task because of the inherent diversity of structures and require a thorough understanding of the surface and bulk properties of the polymer that can give the desired chemical, interfacial, mechanical, and biological functions. The choice of polymer, in addition to its physicochemical properties, is dependent on the need for extensive biochemical characterization and specific preclinical tests to prove its safety. Surface properties such as hydrophilicity, lubricity,

smoothness, and surface energy govern the biocompatibility with tissues and blood, in addition to influencing physical properties such as durability, permeability, and degradability (Aulton, 2007; Semwal *et al.,* 2015).

#### 1.5.2.3.1 RS in polymeric film coating

At the beginning of Section 1.3, it has been mentioned that out of the two forms of starches, the amylose content (the dominant one in RS form) is responsible for its film forming, gelling as well as binding property (Satyam *et al.,* 2010). The starch films can be used for different drug delivery system. But, in designing it for sustained (prolonged) or targeting drug delivery systems, like the CTDDS, it has this disadvantage of swelling in aqueous media and subsequent accelerated drug release. Pure amylose films take up considerable amounts of water upon exposure to aqueous media. They become very permeable and the drug is already released in the upper GIT before the distal GIT is reached. To control this swelling, commercially available controlled-release polymers such as PVP (Polyvinyl pyrrolidone), chitosan, sodium alginate or ethyl cellulose have to be mixed with amylose to prevent drug release in the stomach and small intestine (Karrout Y, 2008; Satyam *et al.,* 2010).

In this study, the polymer used with the RS for the film-coated formulation was ethylcellulose. Siew *et al.,* (2000b) studied the physico-mechanical and digestibility properties of water-miscible organic solvent-based amylose–ethylcellulose films as potential coatings for colonic drug delivery. Their resultant mixed films were characterized in terms of tensile strength and elasticity, polymer miscibility, permeability, and digestibility under simulated colonic conditions. Films containing higher concentrations of amylose displayed increasing weakness and softness and faster permeation to hydrogen ions compared to films with lower amylose content. No apparent miscibility was detected between the amylose and ethylcellulose, regardless of film composition. The films were found to be susceptible to digestion by bacterial enzymes within a simulated colonic environment. The extent of digestion was directly proportional to the amount of amylose present within the film.

#### 1.5.2.3.2 Ethylcellulose

Ethylcellulose, an ethyl ether of cellulose, is a long-chain polymer of β-anhydroglucose units joined together by acetal linkages. $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_n C_{12}H_{23}O_5$ where n can vary to provide a wide variety of molecular weights with structural formula as shown in Fig. 1.6.

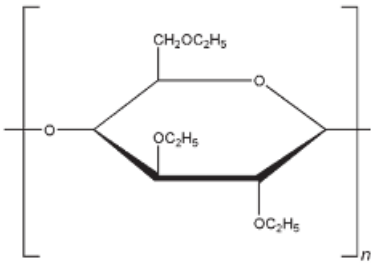



*Figure 1-6: Structural formula of EC (Dahl, 2009).*

Functionally, it could be categorized as coating agent, flavoring agent, tablet binder, tablet filler and viscosity-increasing agent. EC is widely used in oral and topical pharmaceutical formulations. EC is chosen in this study due to its availability and its use in small concentration in tablet coating (1 to 3%) that allows other excipients (like RS) to take part in the film coating solution (see Table 1.5).

*Table 1.5: The amount of EC used in coating (Dahl, 2009).*

| **Use** | **Concentration (%)** |
|---|---|
| Microencapsulation | 10.0-20.0 |
| Sustained-release matrix tablet | 3.0-20.0 |
| Tablet coating | 1.0-3.0 |
| Tablet granulation | 1.0-3.0 |

The main use of EC is in oral formulations as a hydrophobic coating agent for tablets and granules. EC coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. Safety-wise, it is generally regarded as a non-toxic, non-allergenic, and non-irritant material. As EC is not considered to be a health hazard, the WHO has not specified an acceptable daily intake. In addition to using it widely in oral and topical pharmaceutical formulations, it is also used in food products. Because EC is not metabolized, it is not recommended for parenteral products; parenteral use may be harmful to the kidneys.

EC, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films. An aqueous polymer dispersion (or latex) of EC such as Aquacoat ECD (FMC Biopolymer) or Surelease (Colorcon) may also be used to produce EC films without the need for organic solvents. Drug release through EC-coated dosage forms can be controlled by diffusion through the film coating (Ozturk and Dressman, 1990; Narisawa *et al.,* 1994; Dahl, 2009).

## 1.6 The Present Study

The main purpose of this work is to evaluate Teff's RS as a film coating material for colon-targeted tablets. Such kind of advanced pharmaceutical dosage form (in this case targeted drug delivery system) has great importance in increasing the efficacy and decreasing the side effect of drug treatments (Karrout Y, 2008). Targeting a colon, as mentioned previously, has many practical benefits; in the drug treatment of IBD (UC and CD), colonic cancer and other diseases. Also, it has a great significance in the delivery of peptide and protein oral drugs via colon to the systemic circulation. Hence, the development of such a film coating material would be a problem-solving piece of work.

Specifically, for Ethiopia developing a film coating material using the native teff's RS has greater benefit. The work will promote the indigenous Teff and will add some knowledge towards having a full understanding and use of the local grain. Economically, it saves valuable currency in importing film-coating materials for colon targeting. Even, it may generate revenue

if exported as a film-coating material/excipient or as a finished prepared colon targeting drug delivery system.

To show how the film coating material from teff RS could be used for colon targeting, Metronidazole tablet is used for this study as a model drug because of its availability and a wider use in the country. Metronidazole, $C_6H_9N_3O_3$ a white or yellowish crystal powder with a slight solubility in water and alcohol, is an imidazole antibacterial indicated in the treatment of intestinal and hepatic amebiasis, trichomoniasis, acute and ulcerative gingivitis, prevention and in the treatment of infection due to anaerobic bacteria, for pre-and post-operative prophylaxis in gynecological, and GI surgery. Besides increasing its efficacy, delivering it directly to the colon minimize its well-known disturbing side effect; nausea, vomiting, and diarrhea (BP, 2009).

## 1.7 Research Questions

This study attempted to address the following questions:

- Can teff starch be a potential source of resistant starch?
- Can teff resistant starch be used as a film forming-material in CTDDS?
- What modification and/ or optimization does teff resistant starch requires to attain a CTDDS?
- What are the optimum amylose to EC ratios in the preparation of the best film-forming solution?

Consequently, in this study, RS (dominantly amylose) obtained from Teff (*Eragrostis tef*) was evaluated as a film coating material for its potential application as a colon targeted drug delivery system using metronidazole as a model drug.

## 1.8 Objectives

### 1.8.1 General objective

To isolate Teff's RS and evaluate its property as a film coating material for colon targeted dosage forms.

### 1.8.2 Specific objectives

The specific objectives are:

- To isolate teff's starch from teff grain and the resistant starch from the total starch;
- To optimize the resistant starch hydrophilic property with EC, and to obtain the best ratios of amylose to EC in the preparation of a film-forming solution;
- To prepare tablets coated with the optimized RS solution and evaluate this *in vitro* dissolution and fermentation in simulated gastric, intestinal and colonic fluids.

# 2. EXPERIMENTAL

## 2.1 Plant Material

Teff grain (two white varieties (DZ-01-196 and DZ-CR-37) and two brown varieties (DZ-01-1681 and DZ-01-99)) teff grains were obtained from Holeta Agricultural Research Institute. A teff variety between white and brown called "*Sergegna*" was obtained from the local market.

**CHEMICALS & SOLVENTS**

Pancreatic $\alpha$-amylase (SOLARAY® Dietary Supplements, Park City, UT 84098 USA), Amyloglucosidase (AMG ASIN: B01EKFX8J0, LD Carlson Co. Ohio, USA), Propylene glycol (Horst G.F. von Valtier GmbH and Co. KG, Germany), Pepsin (BCBR 3132v, Sigma-Aldrich/Switzerland), Pancreatin (Caelo Ch.-B:15348703 Caesar & Loretz GmbH Pharma. Hilden, Germany), Iodine Resublimed (Reagent Chemical Services Ltd., UK), potassium iodide (Loba Chemie Pvt. Ltd.), potassium chloride (BDH Chemicals Ltd Poole, England), distilled water, Hydrochloric acid (HCL 36.46 BH15 1TD, BDH Laboratory supplies, Poole, England) freshly prepared simulated gastric fluid (0.1 N hydrochloric acid containing 0.32% w/v pepsin), freshly prepared simulated intestinal fluid (phosphate buffer of pH 6.8 containing 1% w/v pancreatin), simulated colonic fluid (phosphate buffer pH 7.2 environment with colonic bacteria, like that of bifidobacterium, from human feces) were used.

The followings were kindly donated from EPHARM SC. and they were used as received: EC (BDH Chemicals Ltd Poole England), Metronidazole API (BN 0181801001, Hubei Hongyuan Pharmaceutical Technology CO, Ltd. China), absolute ethanol (Carlo Erba Reagents, Italy), Sodium metabisulphite and sodium hydroxide (BDH Laboratory Supplies, England), monobasic potassium phosphate (ERBA Pharma Reagents Group, Italy), corn starch (Roquette, Orkila, France), Erythrosine/Red No.3 (Kronos International Inc., Germany), Sodium starch glycolate /SSG (Huzhou Zhanwang Pharmaceutical Co. Ltd, China), Povidone K 29-32 (China Associate Group co., Ltd., China), aluminum foil (Billcare, India).

## 2.2 Methods

### 2.2.1 Isolation of total starch from Teff

The teff grains were sieved to remove extraneous/foreign materials. It was then milled in a laboratory mill (FRITSCH Pulverisette 2, RoHS, industriestr 8, 55743 idar-Oberstein, Germany) to pass through a sieve size of 315 µm to produce a whole grain meal. Small scale extraction of teff's starch was conducted by the method described by Bultosa *et al.* (2002) with a little modification, while larger-scale extraction used the method described by Gebre-Mariam and Schmidt, (1998).

In brief, for the small-scale starch isolation, 100 g of teff flour was weighed (ADAM digital Balance, Wagtech international Ltd, England) and mixed with 500 ml of distilled water to form a slurry. The slurry was left on a shaker (Heidoph instruments, unimax 1010 DT D-91126 Schwabach, made in Germany) at 170 rpm for 2 h. Removed from the shaker, it was immediately passed through a sieve size of 115 µm then through fine muslin to wash it with some more distilled water (300 ml) until a fibrous residue was left on the muslin surface. A vacuum filter system was used to facilitate the process. The liquid containing the starch as a suspension was retained while the fibrous residue on the muslin was discarded. The filtrate was then centrifuged (BECKMAN COULTER, Allegra® 64R centrifuge machine) in 50 ml centrifuge tubes at 8000 rpm for 10 min. After the supernatant was decanted, the brown protein layer was scraped off from the top of the solid residue unveiling the starch at the bottom. This procedure was repeated by adding distilled water to the remaining starch pellet until a white starch pellet was obtained. The TS obtained was then air-dried, milled and stored in an airtight container after sieving with a 224 µm sieve (Bultosa *et al.,* 2002; D'Silva *et al.,* 2011; Teklehaimanot *et al.,* 2013).

For larger amount of starch extraction, the following procedure was used; the teff flour (2 kg) was suspended in a bucket of distilled water (10 liters) containing 0.075% (w/v) of sodium metabisulphite to form a slurry. The slurry was then allowed to stay overnight. It was then passed through a fine muslin adding more distilled water on it with continuous stirring until

an insoluble fibrous component remained on the surface of the muslin. The fibrous residue on the muslin was discarded and the translucent suspension was allowed to settle. After hours, the pure supernatant was decanted. On the bottom of the container, a hard cake of white starch was uncovered when a brownish protein layer was scraped off from its surface. It took some more re-suspending, filtrations and allowing settling to obtain a snow-white starch by getting rid of all the impurities and a protein layer above it. The resulting starch was air-dried at room temperature, and stored in an airtight container, after milling and sieving (224 µm), for further extraction of RS (Gebre-Mariam and Schmidt, 1998).

### 2.2.2 Isolation of resistant starch from the total starch

The isolation of RS was done as per the AOAC official method 2002.02.

Non-resistant starch/digestible starch was solubilized and hydrolyzed to glucose by the combined action of pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37 $^{o}$C. The reaction was terminated by the addition of ethanol and RS was recovered as a pellet by centrifugation.

In brief, the TS sample (100 g) was suspended in a distilled water (250 ml) to form a slurry. Then, pancreatic α-amylase and amyloglucosidase (AMG) were added and left on a magnetic stirrer for 16 h at 37 $^{o}$C during which the non-resistant starch was solubilized and hydrolyzed to D-glucose by the combined action of the two enzymes. The reaction was terminated by the addition of ethanol (96%) and RS was recovered as a pellet by centrifugation (8000 rpm for 10 min). It was then washed again by suspending it in ethanol (96%) followed by centrifugation. The free liquid was removed by decantation. This process of enzymatic digestion was done three times to re-confirm the complete digestion of the digestible starch /non-resistant starch.

The amount of pancreatic $\alpha$-amylase and AMG needed for the enzymatic digestion of starch was calculated as shown in Appendix I a. and I b., respectively.

### 2.2.3 Test for starch

A test for TS was done using iodine test for starch to confirm the isolation of TS from the teff grain (section 2.1.1). Five test tubes were prepared, four test tube D1 to D4 containing the claimed starch powders from the four different teff varieties in a distilled water and the other as a blank, a distilled water only. Then, the Iodine solution (0.2 g and 2 g potassium iodide in 100 ml of distilled water) was dropped into the five test tubes. If the samples contain starch, a color change to blue black occurs.

### 2.2.4 Test for resistant starch

There are two types of test for RS; a direct method that quantify RS in the residues obtained after removing digestible starch using AOAC Official Method 2002.02 or an indirect method that determine RS as the difference between TS and digestible starch as it was mentioned in section 1.2, Eq. 1.3 (Goni *et al.,* 1995).

Since the methods of isolation of RS in section 2.2.2 above is digestion of the digestible starch with digesting enzymes, the remaining pellets are RS as per the mentioned formula. Hence, the indirect method of the test of RS has been done already. And the result is shown in section 3.1.2, "isolation of RS from TS".

### 2.2.5 Optimization of film forming property of amylose (resistant starch) with ethylcellulose

The disadvantage of amylose in film-formation is its swelling property in aqueous media and subsequent accelerated drug release. To control this, a commercially available controlled release polymer (e.g., EC) was mixed with amylose to prevent premature drug release in the stomach and small intestine (Karrout Y, 2008). In this study, the proportion of amylose (hydrophilic) to EC (hydrophobic) were optimized and evaluated as film-forming solutions.

To prepare the coating formulations as per the method described by Siew *et al,* (2000); the EC was dissolved in ethanol (96%) to produce five separate 400 ml solutions for one batch with various quantities of EC present. Then, a plasticizer, Propylene glycol, 2.5 % V/V was added to each solution of EC and mixed for 3 h using a magnetic stirrer. On each plasticized EC solutions,

100 ml aqueous dispersion of amylose (10 % W/V) in ethanol (96 %) was then added and stirred for another 1 h to produce coating formulations with different solid ratios of amylose to EC (1:1, 1:2, 1:3, 1:4, 1:5 and 1:0 as a control).

### 2.2.6 Preparing film coated colon-targeted metronidazole tablet using teff's resistant starch

#### 2.2.6.1 Formulation of the core tablets

A batch of one thousand tablets were prepared using the formula shown in Table 2.1.

*Table 2.1: Metronidazole Tablet (200 mg) reduced formula of 1000 tablets.*

| No. | Scale (mg/tablet) | Ingredient | Quantity (g) |
|---|---|---|---|
| 1 | 200.00 | Metronidazole | 200.00 |
| 2 | 75.00 | Lactose monohydrate | 167.00 |
| 3 | 18.75 | Starch (corn) | 42.00 |
| 4 | 15.00 | Povidone K 29-32 | 33.50 |
| 5 | 18.75 | Starch (corn) | 42.00 |
| 6 | QS | Water, purified | QS |
| 7 | 6.50 | SSG | 14.00 |
| 8 | 0.63 | Magnesium stearate | 1.50 |

#### 2.2.6.2 Preparation of granules and the core tablets

Wet granulation method was employed for the preparation of metronidazole core tablets.

The granulation was done making first a paste using corn starch (item 3 as a binder) and purified distilled water (item 6 as a solvent) in a stainless-steel container. A starch paste was prepared in a boiling water and stirred until a translucent paste was found. Each of the

ingredients, metronidazole (API), lactose (diluents), and corn starch (Item 5 as a diluents) were passed through a 500-µm aperture screen and subsequently transferred to a mixer (Machines Collette, Belgium). Povidone (binder) was then added to the mixer containing the mixture and mixed for 5 min. Then, the starch paste was added to the mixer and mixed with the mixtures until suitable consistency mass was obtained. Extra water was added as required. The wet mass was then passed through a 2.5-mm screen to form a wet granulate. The granulate obtained was spread on paper-lined tray oven (KOTTERMAN®) and dried at 50 $^0$C until the moisture content was not more than 5.5%. Weigh were taken for moisture content checking at this point and after drying. Finally, the dried granules were passed through a 1.5-mm aperture screen. The dried granules were then transferred to a blender (Mixer Machines Collette, Belgium) for 10 min. SSG (disintegrant) and magnesium stearate (lubricant) were screened through a 600-µm aperture screen to the blender. These were blended for 5 min. The granules were discharged into polyethylene-lined drums, sealed, and weighed.

The blended granules were compressed using a 10 station rotary tablet press machine (Rimek Mini Press-II, India) with 14/32-inch round, standard concave punches at a pressure of 6.5 to 10.5 kg/cm$^2$. Different batches of core tablets were produced containing 200 mg of metronidazole with an average tablet weight of 500 mg.

#### 2.2.6.3 Determination of flow, density and related properties of the granules

Bulk and tapped densities were determined by taking a 30 g of granule mix, before tableting, in a 250 ml measuring cylinder. Bulk volume occupied was read after light tapping. The cylinder was then tapped at a constant velocity using tapped densitometer (ERWEKA® GmbH, Germany) for 500 times. The tapped volume was then recorded. Bulk and tapped densities were determined based on Eq. 2.1 and 2.2, respectively. Both measurements were done in triplicate and respective standards deviations were calculated.

$$Bulk\ Density\ (\rho_b) = \frac{m}{V_b} \quad \text{..... Eq. 2.1.}$$

where, m is the weight of the granule/ powder and $V_b$ is bulk volume

$$Tapped\ Density\ (\rho_t) = \frac{m}{V_t}$$ …… Eq. 2.2.

where m is the weight of the granule/ powder and $V_t$ is the tapped volume

Carr's index and Hausner ratio were also determined using the calculated density values.

$$HR = \frac{\rho_t}{\rho_b}\ or\ \frac{V_b}{V_t}$$ …… Eq. 2.3.

$$CI = 100\text{x}\left(\frac{\rho_t - \rho_b}{\rho_t}\right)\ \text{or}\ \text{CI} = 100\text{x}(V_b - V_t)/\ V_b$$ …. Eq. 2.4.

Where $\rho_b$ is bulk density, $(\rho_t)$ is tapped density, HR is Hausner ratio and CI Carr's index.

The angle of repose was determined by the funnel method. In this, a 30 g of granule was placed and allowed to flow through a stem-less funnel having a 10 mm orifice from a fixed height of 10 cm. The duration of flow was recorded and used to calculate the flow rate. The powder raised vertically making a pile until a maximum cone height (h) was obtained.

The angle of repose and flow rate were calculated from Eq.2.5 and Eq. 2.6, respectively.

$$\theta = \tan^{-1}(\frac{h}{r})$$ …… Eq. 2.5

where θ = angle of repose, h = height of granules, r = radius of circle formed by the granules.

$$Flow\ rate = \frac{m}{t}$$ …… *Eq. 2.6*

Where $m$ is mass in gram and $t$ is time in seconds.

These two measurements were done in triplicate, and from the results the respective standards deviations were calculated.

### 2.2.6.4 Evaluation of core tablets

The metronidazole core tablets were evaluated for physicochemical parameters, namely, weight variation, friability, hardness, diameter, thickness, and disintegration time.

#### 2.2.6.4.1 Hardness

The tablet crushing strengths of 10 tablets were measured using hardness tester (Model 2E/205, Dr. K Schleuniger & Co, CH-8033, Zurich, Switzerland). In this, a tablet was placed between the anvils and the crushing strength, which causes the tablet to break, was recorded. The average crushing of the 10 tablets was calculated.

#### 2.2.6.4.2 Thickness and Diameter

The diameters reading of the tablets were recorded concurrently while doing the test of hardness on ten sample tablets. The thickness reading was taken with the same apparatus by placing ten tablets vertically one at a time.

#### 2.2.6.4.3 Friability

Tablet strength was tested by a friability tester (ERWEKA® GmbH, TAR 20, Heusenstamm, Germany). Twenty tablets were accurately weighed and placed in the revolving chamber of the apparatus then operated at 25 rpm for 4 min. The tablets were dedusted and the percentage weight loss was calculated by reweighing the tablets. Tablets that lose less than 1% weight were considered acceptable.

$$\%\ \text{Friability} = \left(\frac{\text{Intial Weight} - \text{Final Weight}}{\text{Intial Weight}}\right) \times 100 \quad \text{.... Eq. 2.7}$$

#### 2.2.6.4.4 Weight variation

Twenty tablets were selected at random and the individual weights were recorded using an electronic balance, and the average weight was determined and the standard deviation calculated.

#### 2.2.6.4.5 Disintegration time

Disintegration time was determined using the disintegration apparatus (ERWEKA DT 700 GmbH, Germany). Six tablets were placed in each tube of the apparatus and the basket rack assembly was positioned in a 900 ml beaker of water at 37± 2 $^0$C and run at 29 to 32 cycles per min for 15 min as per USP 30-NF25<701>, (2007). At the end of 15 min, the basket was lifted from the fluid. The tablets were considered disintegrated if no particles remained on the mesh of the tubes. Otherwise, the test was to be repeated for additional 12 tablets. Where the requirement is 16 tablets of the total 18 need to be disintegrated.

#### 2.2.6.4.6 Drug content uniformity

Twenty metronidazole core tablets were randomly selected and crushed into powder. Exactly 503.88 mg of powder (the average weight of the tablets from the three batches, as in Table 3.3, claimed to contain 200 mg of metronidazole API) transferred to a 250-ml volumetric flask, and about 100 ml of dilute hydrochloric acid (0.001N) was added, then the sample was shaken for 30 minutes on a shaker. It was then diluted with hydrochloric acid to volume, mixed and filtered. The first 15 ml of the filtrate was discarded.

From the filtrate 25 ml was diluted with 100 ml of diluted hydrochloric acid, to obtain a solution having a concentration of about 0.2mg of metronidazole per ml. A 10 ml of this solution was transferred into a 100-ml volumetric flask, diluted with dilute hydrochloric acid to volume giving 0.02mg/ml of drug solution, and mixed to make it ready for UV-reading.

Concurrently, the absorbance of this test solution was determined in reference with that of a similarly prepared standard solution of USP Metronidazole reference standard, having a known concentration of about 20 µg per ml (0.02mg/ml as that of the final concentration of the test solution for reading), in 1-cm matched cells, at the wavelength of maximum absorbance at about 278 nm using dilute hydrochloric acid of 0.001N as a blank. The quantity was calculated in mg and the amount of metronidazole ($C_6H_9N_3O_3$) in the whole tablet using the formula in Eq. 2.8.

$$(\frac{TC}{D})(\frac{Au}{As}) \quad \text{..... Eq. 2.8}$$

In which $T$ is the labeled quantity, in mg, of the metronidazole in the Tablet; $C$ is the concentration, in µg per ml, of USP Metronidazole RS in the Standard solution; $D$ is the concentration, in µg per ml, of metronidazole in the test solution, on the basis of the labeled quantity per Tablet and the extent of dilution; and $Au$, and $As$, are the absorbance of the test solution and the standard solution, respectively (USP 30-NF25 <903>, 2007). The experiment was done in triplicate.

#### 2.2.6.4.7 UV calibration curve of metronidazole

Calibration curves were plotted for simulated gastric environment (pH 1, 0.1 N HCl and 0.32 % pepsin), simulated small intestinal environment (in a phosphate buffers solution with pH 6.8 and 1% pancreatin) and simulated colonic environment (in a phosphate buffers solution with pH 7.2 in the presence of human feces 10% (W/V)).

Three calibration curves were constructed by using three stock solutions prepared by dissolving 0.0555g of metronidazole in 250 ml of the corresponding media, which provided a stock solution of concentration 222 µg/ml which was an equivalent concentration to the drug in the dissolution media (i.e. one 200 mg metronidazole tab in 900 ml of the media).

Using the stock solutions, a series of dilutions were done to get ten different concentrations (from $C_1$ to $C_{10}$ which are 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, and 25 µg/ml) for the calibration curve in gastric and small intestinal environments while twelve different concentrations (from $C_1$ to $C_{12}$ which are 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 µg/ml) for the calibration curve of simulated colonic environment. Then, the corresponding absorbance readings of each of the dilutions were measured at the $\lambda_{max}$ (278 nm) with a UV-Visible spectrophotometer (Model SHIMADZU UV-1800, Japan). Each experiment was done in triplicate.

The USP 30-NF25, (2007) method was used to prepare a phosphate buffer solution with pH 6.8 and pH 7.2 (for small intestine and colon media, respectively) using Chemicals: Potassium phosphate monobasic ($KH_2PO_4$) and Sodium hydroxide.

#### 2.2.6.5 Film coating

##### 2.2.6.5.1 Preparation of a film coating solution

Various quantities of the Polymer: Polymer-Plasticizers blend (Amylose:EC-Prpylene glycol blend) with a ratio of 1:1, 1:2, 1:3,1:4, 1:5 were prepared using the procedure described by Siew *et al.,* (2000); Wilson and Basit (2005); Karrout Y, (2009). For a comparison purpose, a control film coating material in the absence of EC in the blend (Only the amylose solution with plasticizer and colorant) was also prepared and named as a 1:0 ratio. For each of the six groups, 200 tablets were allocated to be coated.

1. On one hand, an amylose solution (10% W/V) was prepared using ethanol (96%) of 100 ml solvent.

A 10 g of amylose in 100 ml of ethanol solvent was stirred for 1 h for each five different film coating solutions that were intended to coat the five different groups of amylose to EC ratios.

2. Polymer-Plasticizers blend solution:

On the other hand, various amounts of EC solutions each in 400 ml of ethanol (96 %) were prepared to make five different solid ratios of amylose to EC (1:1, 1:2, 1:3, 1:4 and 1:5). i.e., 10 gm of EC was used for the 1:1 amylose to EC ratio coating solution preparation while a 20 g of EC was used for the 1:2 ratio of amylose to EC, etc. Then, a 10 g of propylene glycol plasticizer (2.5 % W/V of the blend solution) was added to each of the five EC solutions. These EC polymer-plasticizer blend solutions were stirred for 3 h with a magnetic stirrer.

3. The above two separate groups of solutions; five amylose solution and five EC-Plasticizer blends solution were mixed according to their specific ratio of Amylose to EC ratio. By then

each group made a 500 ml of blend. Then, these blend were stirred for another 6 h before coating started. The colorant (Erythrosine) was also added at this mixing stage.

The sixth group or the control film coating solution (1:0 ratio of amylose to EC) was prepared in the absence of EC. Yet, in a similar fashion (500 ml of amylose solution of 10% W/V with the plasticizer and colorant).

#### 2.2.6.5.2 Film coating of core tablets

Using the procedure described by Siew *et al., (*2000) and Wilson and Basit (2005); the tablets were coated using a pan coater (Model ERWEKA TYPE AMD, Germany). A series of coated samples with different film thicknesses were obtained by weighing and removing the sample from the pan in between.

The film thickness was expressed in terms of the percentage total weight gain (%TWG, Eq. 2.9.) to obtain formulations with three film thicknesses (i.e. 2%, 4% and 6% of the average weight of the uncoated tablet).

The steps followed to produce these three groups of different thicknesses of a coating levels for all the six ratios of amylose to EC (1:0, 1:1, 1:2, 1:3, 1:4 and 1:5) were:

Tablets were initially dried (pre-warmed) in coating pan for 10 min with a bed temperature around 40-45 °C. Sample tablets were randomly taken from different locations inside the pan. Then, the total weight of 50 tablets was determined and the average weight of the tablets was calculated. This was used as a baseline for the calculation of TWG of the coated tablet in the process.

The coating process then continued for the batch with the specific coating solution prepared (starting from a 1:0 to 1:5 amylose to EC ratio). Each coating batch was divided into three and 2%, 4% and 6% coating were done, respectively. The coating level was calculated using Eq. 2.9. After completion of the spray coating process in each sub-batch, the tablets were again dried

in the coating pan for an additional 10 min with hot air spray till the bed temperature reached and maintained at around 40-45 °C. The coating parameters are shown in Table 2.2 below.

*Table 2.2: Coating process parameters that were maintained during the coating of tablets.*

| No. | Fixed parameters | Specific Values |
|---|---|---|
| 1 | Number of spray gun | 1 |
| 2 | Coating pan speed | 15 rpm |
| 3 | Atomizing pressure | 2 bar |
| 4 | Inlet air temperature | 50 -60 °C |
| 5 | Spray rate application | 5 g/min/kg |
| 6 | Distance tablet bed to-spray gun | 15 cm |

### 2.2.7 Evaluation of film coated tablets

#### 2.2.7.1 Percentage weight gain of coated tablets

Percent weight gain or % TWG was calculated using the following equation during the coating process with the coating solutions.

$$TWG = \left(\frac{Wf-Wi}{Wi}\right) x100\% \text{.....} \quad \text{Eq. 2.9}$$

Where $Wi$ and $Wf$ are tablet weight initial and final, respectively.

Three groups of thickness; 2%, 4%, and 6% TWG were studied for each of the six different Amylose: EC ratios, totally eighteen sample groups were prepared for the study.

#### 2.2.7.2 *In vitro* dissolution test

Metronidazole release from the film-coated tablets was assessed by *in vitro* dissolution tests using USP type I basket dissolution apparatus (Model: LABINDIA DS 8000). The tests were performed using a basket rotation speed of 100 rpm in 900 ml dissolution medium that was varied at different courses of the experiment (USP 30/ NF25, 2007):

First, the test was done in a simulated gastric fluid condition (0.1 N hydrochloric acid with 0.32% pepsin) for the first 3 h followed by pH 6.8 phosphate buffer with 1% Pancreatin for the next 3 h to simulate the condition of the small intestine (Wilson and Basit, 2005; Karrout Y, 2008; Karrout *et al.,* 2010).

At pre-determined time points, ¼ h, ½ h, ¾ h, 1 h, 2 h, 3 h in gastric simulated medium and 4 h, 5 h, and 6 h in small intestine simulated medium, 5 ml of samples were withdrawn and analyzed by UV spectrophotometry at $\lambda_{max}$ of 278 nm. But, the enzyme present turned the sample turbid in case of intestinal medium. Hence, before the UV visible study, the samples were filtered with a filter paper (Double rings® 102 Medium, 9 cm) and suitably diluted with the solvent. For each sample group (ratio of Amylose to EC; 1:0 to 1:5), with 3 different thicknesses (2%, 4% and 6%) the experiments were conducted in triplicate.

#### 2.2.7.3 Fermentation study

Metronidazole release from the film-coated tablets was assessed following the dissolution test from the 6th to the 14th h in *in vitro* condition simulating the human colon with and without colonic bacteria extracted from fecal matters.

One tablet was introduced into individual 100 ml batch culture fermenters inoculated with human feces (10% w/v). The fermenters were prepared by homogenizing freshly voided human feces from three healthy subjects in a phosphate-based buffer medium of pH 7.2 (Silvester *et al.,* 1995; Basit *et al.,* 2002; Wilson and Basit, 2005).

The fermenters containing one batch of test samples were sealed in an airtight jar that was made anaerobic previously with anaerobic kit with aerobic condition indicators (Anaerobic

container system with indicator; BD GasPakTm EZ, Bacton Dickinson and company, 7 Loveton circle, spark, MD 21152 USA). Placed in shaker-incubator (Stuart®, orbital incubator / SI500/ BIBBY SCIENTIFIC LIMITED, STONE, STAFFORDSHIRE, ST15 0SA, UK), the jar was kept at 37 $^{o}$C and shaken at 100 rpm. The established anaerobic environment was maintained while opening the jar to take samples with the old candle jar method (letting the candle to burn out the oxygen in the closed jar), this also controlled by the anaerobic indicator that turned to blue when exposed to oxygen (Appendix II b.).

"A control experiment", a buffer medium without the presence of feces, was also running in parallel. Each experiment was done in triplicate.

Two milliliters of the samples were removed every 15 min for the first 1 h then at hourly intervals for the next 8-h period. The collected sample filtered through 0.2 µm filters prior to analysis for drug concentration by UV at its $\lambda_{max}$ (Siew *et al.,* 2000b).

The results were plotted to extend the cumulative percentage drug release versus time profiles of gastric and small intestine dissolutions up to the end of the 14$^{th}$ h.

### 2.2.8 Data analysis

Statistical analysis was done using Analysis of Variance (ANOVA) with a statistical software Origin 6.0 (Origin Lab ™ Corporation, USA). Tukey multiple comparison tests were used to compare the individual difference. All the data measured and reported are averages of a minimum of triplicate measurements and the values are expressed as mean ± standard deviation (SD) at 95 % confidence interval, and p-values of $< 0.05$ were considered statistically significant.

# 3. RESULTS AND DISCUSSION

## 3.1 Evaluation of the Extracted Total Starch and Resistant Starch

### 3.1.1 Total starch and resistant starch

The starch test with iodine solution in test tubes containing sample solutions of the four varieties of teff (labeled D1 to D4) turned to blue-black confirming the presence of starch (Appendix II a).

The indirect test (Table 3.1; RS a mean percentage yield of 19.10 %) showed that the claimed RS was obtained from teff grain after the enzymatic digestion of the TS that was previously isolated (as per Eq. 1.3).

### 3.1.2 Quantitative test (Teff grain yield) of total starch and resistant starch

The yield and other properties of teff grains of different traits were characterized elsewhere (Bultosa *et al.,* 2002, Bultosa, 2007). In the present study, the TS and RS yields obtained from varieties of teff grains are presented in Table 3.1.

*Table 3.1: Quantitative values (yields) for the isolation of total starch and resistant starch from 100 g of teff grain*

| No. | The specific types of teff grain used | Teff flour (passing through 315 µm) Percentage yield | TS Percentage yield | RS Percentage yield |
|---|---|---|---|---|
| 1. | DZ-01-1681 | 81.28±1.4 | 37.20±1.2 | 18.07± 0.8 |
| 2. | DZ-01-99 | 79.04±1.3 | 39.10±1.1 | 19.54±1.1 |
| 3. | DZ-01-196 | 84.16±0.9 | 40.40±1.3 | 19.74±0.9 |
| 4. | DZ-CR-37 | 83.60±1.1 | 34.90±0.9 | 18.04±0.6 |
| *Mean % yield of the four traits* | | *82.02* | *37.90* | *19.10* |
| *Standard Deviation of the mean (SD)* | | *2.35* | *2.39* | *0.76* |
| *Standard error of the Mean (SE)* | | *1.17* | *1.20* | *0.38* |

The present findings show that the mean of the TS yield from different varieties of teff grains is 37.90±2.39% while the mean of the RS yield from the TS obtained from these different varieties is 19.10±0.76% (Table. 3.1). For each of the four varieties, the means were compared at $P < 0.05$ and were not significantly different for RS yield. But, the means were significantly different for the TS yield at 0.05 level. This indicates the yield of starch may vary significantly between the four varieties chosen for the present work while their RS content variation is insignificant.

Comparing the result with those similar researches, the teff yield of TS and RS (Amylose), is lower in the present study. The alleged reason for this could be the modification that was made on the methods of isolation. Because of the lack of laboratory apparatus of a shaking water bath that precisely maintains the extraction temperature by ±0.1 $^{o}$C. (AOAC, 2002), a magnetic stirrer was used where agitation speed and only an approximate temperature of 37 $^{o}$C were preset. Also, the milling was done with the laboratory mill rather than the wet milling machine according to the extraction method used in Taylor *et al.,* (1997) and Bultosa *et al,* (2002). Consequently, nearly twenty percent of the total grain removed in the sieving process (through 315 μm) to get the teff flour (82.02%). But, it was also expected to contain some amount of TS and RS. The pericarp of teff grain contains starch grain (Bultosa *et al.,* 2002). These could be one of the reasons for the underreported percentage yield of TS and RS here. Hence, in this part, the focus of the research is briefing the convenience method that was used and the results obtained in the process of obtaining the RS in question than reporting quantitative maximum yield. Yet, comparing the RS yield of the different varieties, the result of each variety, all in all, seems consistent with other research results vis-à-vis Bultosa, (2007). In that study, it was reported as a mean yield of 28.88±0.62 but the individual verities (verity No.1 to 4 as in Table 3.1) reported as 28.8, 30.1, 31.7 and 24.9 % of amylose, respectively. Those with relatively higher and relatively lower yield keeps their property in this study, also.

## 3.2 Evaluation of Granule Properties of the Formulated Blend

For tablets to be uniform in weight and content, the powder has to be blended well and has to flow uniformly to have steady compaction during tableting process. Good followability

ensures uniformity in die fill and thus uniformity in tablet weight (as it is an indirect weighing of the powder volumetrically). These are the parameters (shown in table 3.2) that need to be measured quantitatively before tableting process to have an ultimate "good" tablet.

*Table 3. 2: Physicochemical properties of the granules*

| Parameters➔ | Angle of repose (°) | Flow rate (g/sec.) | Bulk density(g/ml) | Tapped density(g/ml) | Carr's index (CI) | Hausner ratio (%) |
|---|---|---|---|---|---|---|
| Measured Values➔ | 27.46±1.61 | 16.90±1.15 | 0.37±0.01 | 0.43±0.01 | 13.08±1.48 | 1.15±0.02 |

The angle of repose has been used to characterize the flow properties of solids. The angle of repose is characteristic related to inter-particulate friction or resistance to movement between particles. It's an indirect method of quantifying powder flowability; because of their relationship with inter-particle cohesion. A static heap will slide when the angle of inclination is large enough to overcome frictional forces and stop when gravitational forces balance the forces. The sides of the heap that make an angle with horizontal line is called the angle of repose (Hussan *et al.,* 2012).

Here, the angle of repose was found to be 27.46±1.6 $^{o}$. According to USP30-NF25 <1174>, (2007) the angle of repose as classified by Carr; if the angle is in the range of 25$^{o}$ to 30$^{o}$, it has an excellent flow property.

The Compressibility index (Carr's index) and the related Hausner ratio are the simplest, fastest, and popular methods of predicting powder flow characteristics. The Compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of materials because of these can influence the observed compressibility index.

In the present study, the CI and HR were found to be 13.08±1.48 and 1.15±0.02, respectively. These values fall in the range of "GOOD" flow character as per the generally accepted scale of flowability, i.e., a CI value of 11 – 15 and a HR value of 1.12 -1.18 (USP 30-NF25 <1174>, 2007).

As the results of the pre-compression study of characteristics of the granule have shown, all the above parameters were within the acceptable range which allows to proceed to the tableting process.

## 3.3 Evaluation of Core-tablets

Table 3.3 depicts the properties of the core tablets (for all the three batches produced) such as diameter, thickness, weight variation, hardness, friability, disintegration time, and content uniformity.

*Table 3. 3: Core tablet properties of Metronidazole formulation*

| Batch Code | Diameter (mm) N=10 | Thickness (mm) N=10 | Weight-variation (mg) N=20 | Hardness (Kg/cm2) N=10 | Friability (%) N=20 | Disintegration time (min) N=6 | Content uniformity N=20 |
|---|---|---|---|---|---|---|---|
| **01** | 11.74±0.02 | 4.47±0.09 | 503.64±2.85 | 128.80±5.18 | 0.36±0.01 | 2.19±0.23 | 107.10 |
| **02** | 11.71±0.06 | 4.46±0.03 | 503.63±3.50 | 128.10±8.30 | 0.31±0.02 | 1.92±0.19 | 106.27 |
| **03** | 11.73±0.01 | 4.40±0.04 | 504.41±3.53 | 129.50±5.19 | 0.40±0.01 | 2.30±0.43 | 108.08 |

### 3.3.1 Diameter, thickness and weight variation of core tablets

The mean diameter and thickness of the concave tablets had values ranging from 11.71±0.06 to 11.74±0.01 mm and 4.40±0.03 to 4.47±0.09 mm, respectively. At 0.05 level, the population means are not significantly different for both results (p-value >0.05).

The weight of the tablets ranged from 503.63±3.50 to 504.41±3.53 mg. Here, the average percent deviation of 20 tablets of each batch was less than 15% (less than ±30.0 mg) of the active ingredient (200 mg). As a result, the requirement for dosage uniformity was met according to USP 30-NF25<905>, (2007). These results indicate that the die fill was uniform.

### 3.3.2 Crushing strength and friability of core tablets

The hardness and friability of the core tablets had values that range between 128.10±8.3 to 129.50±5.19 kg/cm2 and 0.31 to 0.40 %, respectively. These show that the hardness of all the batches have acceptable mechanical strength while their friability range shows the tablets are mechanically stable as the range is less than 1% which is the maximum weight loss allowed according to USP 30-NF25<1216>, (2007).

### 3.3.3 The disintegration of core tablets

At the 3$^{rd}$ min, way before the 15$^{th}$ min that is set as maximum disintegration time limit specified in the monograph, all the six tablets were already disintegrated in the three batches of the metronidazole tablets as required in USP 30-NF25<701>, (2007).

### 3.3.4 Content uniformity of core tablets

In the evaluation of core tablets, for all the tablets tested, the values of the content uniformity fall within acceptable range. For the three batches; eq. 2.7 gave the average amount of the active ingredient in the test solution as 214.21, 212.53 and 216.15 mg for batch 01, 02 and 03, respectively.

To ensure the consistency of the dose units, each unit in a batch need to have a drug substance content contained within a narrow range around the labeled claim. The results of the assay revealed 107.10 %, 106.27%, and 108.08 %, for batch 01, 02 and 03, respectively (Table 3.3). According to USP 30-NF25, 2007; Metronidazole tablets should contain not less than 90.0 percent and not more than 110 percent of the labeled amount of metronidazole $C_6H_9N_3O_3$. Hence, for the labeled amount of 200 mg, the percentage obtained is within the limit for all batches.

## 3.4 *In vitro* Evaluation of Drug Release Characteristics of the Film-coated Tablets

### 3.4.1 Dissolution studies

The drug release profile studies were carried out *in vitro* in a simulated gastric, intestinal and colonic fluids to test the film-coated tablet for a CTDDS. But, the environmental conditions for drug release *in vivo* are highly complex and it is not possible to fully simulate them *in vitro* (Siew *et al.,* 2004). Still, the use of different types of *in vitro* setups (exhibiting for instance different chemical (pH)and mechanical (agitation) stresses on the dosage form) can provide important information on the sensitivity of the investigated drug delivery systems to the environmental conditions (Karrout Y, 2008). This information from the *in vitro* test that simulates the *in vivo* condition is well enough to approximate the *in vivo* result, hence the result of the *in vitro* test is fairly acceptable.

#### 3.4.1.1 UV Calibration curve for dissolution in GIT

*Calibration curve for simulated gastric environment (pH 1, 0.1 N HCl and 0.32% pepsin):*

Using the different diluted concentrations from the stock solution (from $C_1$=2.50 µg/ml to $C_{10}$=25.00 µg/ml), the corresponding successive UV readings were found and absorbance (at 278 nm) versus concentration of the drug were plotted. A calibration curve with a linear regression equation of: Y = 0.0375X + 0.0455 (where Y is the absorbance and X is the concentration in µg/ml) and correlation coefficient ($R^2$) of 0.9998 was obtained showing good correlation between absorbance and the concentration (Fig. 3.1).

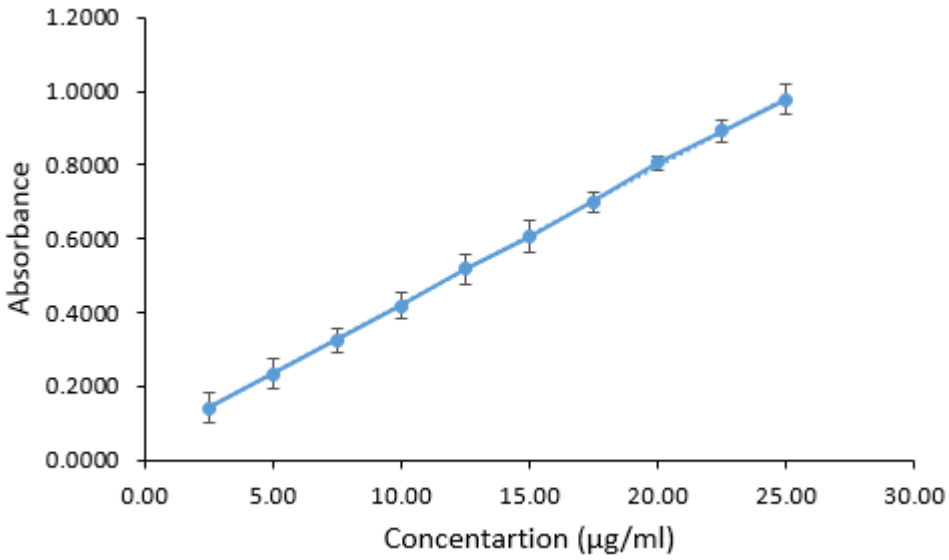



*Figure 3-1: Standard calibration curve of metronidazole in 0.1 N HCl, and 0.32 % pepsin (pH 1) at 278 nm with 95 % confidence interval; ($R^2$ = 0.9998).*

## *Calibration curve for simulated small intestinal environment (a phosphate buffers solution with pH 6.8 and 1% pancreatin):*

Using the different diluted concentrations from the stock solution (from $C_1$=2.50 µg/ml to $C_{10}$=25.00 µg/ml), the corresponding UV readings were found and absorbance (at 278 nm) versus concentration of the dilution was plotted. A calibration curve with a linear regression equation of: Y = 0.0455X + 0.0187 (where Y is the absorbance and X is the concentration in µg/ml) and correlation coefficient ($R^2$) of 0.9997 was obtained showing good correlation between absorbance and the concentration (Fig. 3.2).

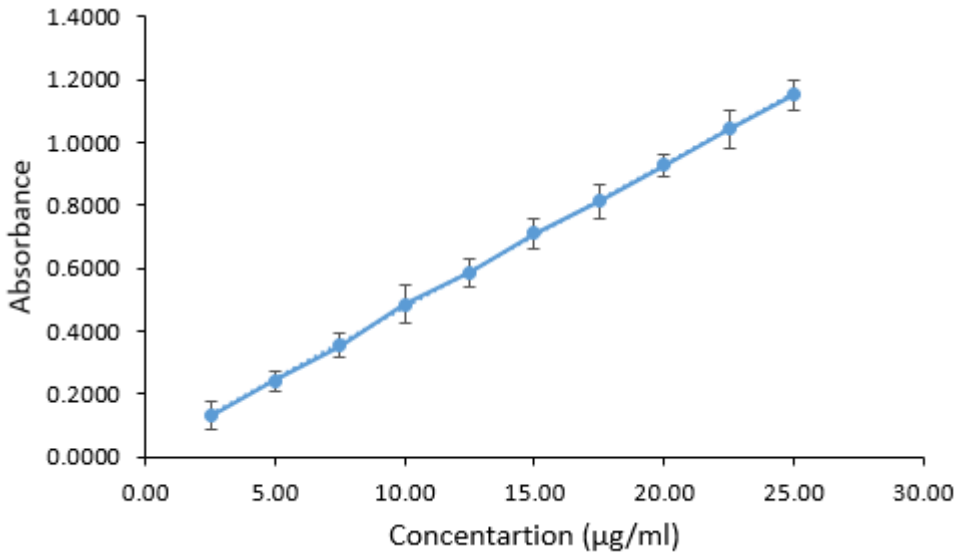



*Figure 3-2: Standard calibration curve of metronidazole in phosphate buffer and 1% pancreatin (pH 6.8) at 278 nm with 95 % confidence interval; ($R^2$ = 0.9997).*

### 3.4.1.2 Dissolution

In a simulated gastric environment (pH 1, 0.1 N HCl and 0.32 % pepsin from t=0 to t= 3 h) and simulated intestinal environment (pH 6.8 phosphate buffers with 1% pancreatin from t=3 to t= 6 h) dissolution was carried out and the UV absorbance reading for each timely withdrawn sample data was interpreted with the calibration curve drawn.

The result is depicted in Fig. 3.3, as a three dimensional graph (X, Y, Z) with coordinates; Z = the % TWG (2%, 4% & 6%) for each ratio of Amylose to EC (1:0, 1:1, 1:2, 1:3, 1:4 & 1:5), Y= cumulative percentage drug release of each sample (0 to 100%) and X= the time taken for the total dissolution process.

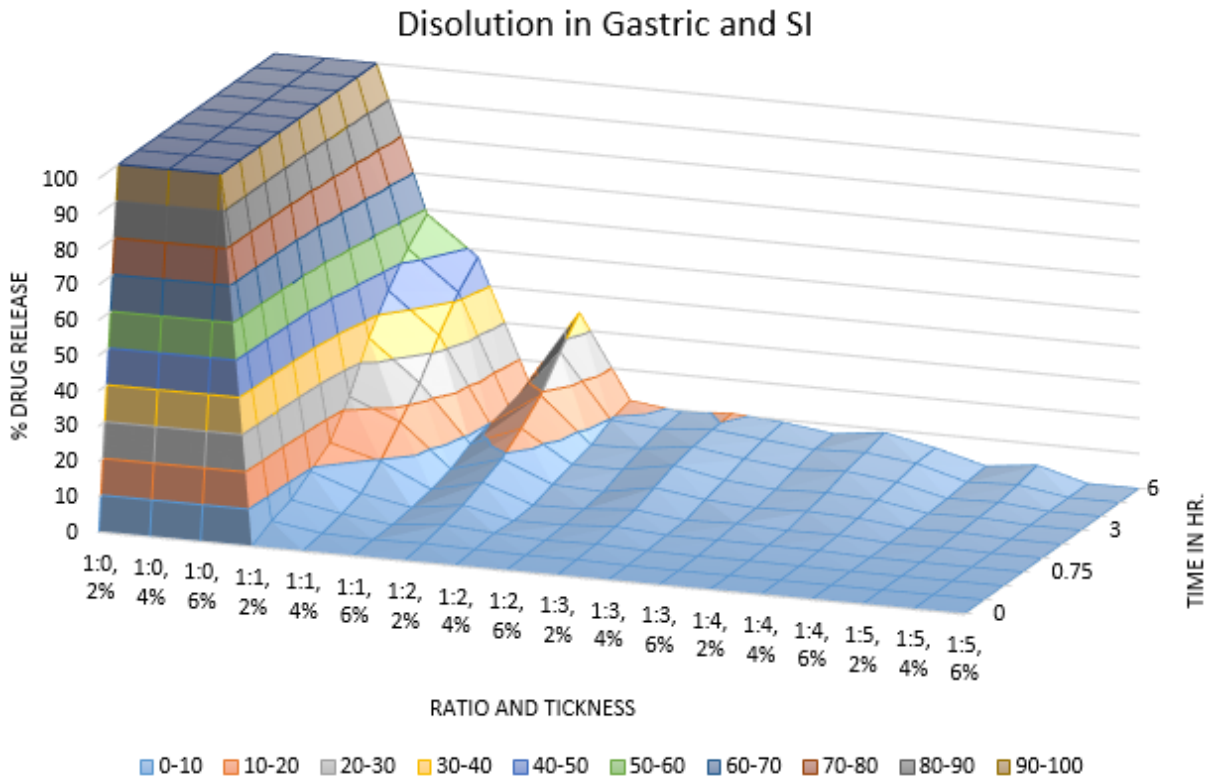



*Figure 3-3: The dissolution study showing the cumulative percentage release of coated tablets with different coating ratios of amylose to EC for each thicknesses in a simulated gastric (from t=0 to t=3 h) and in a simulated intestinal conditions (from t=3 to t=6 h)*

Of the 18 formulations, a candidate for colon targeted drug delivery is a formulation that has not released more than 15% of its content in the dissolution test of the upper GIT, gastric and the small intestine fluids. This 15% cut off is calculated from USP30-NF25<711> (2007) that claims in the dissolution test not less than 85% of the labeled amount of metronidazole need to be dissolved in 60 min for normal releasing tablet. In this special dosage form or a CTDDS, the main focus is in delivering enough amount of the drug in the colon within the normal GIT transit time. Hence, to release 85% of the drug ultimately in the colon, the formulation need not release more than 15% of its content in the upper GIT in its 6 h transit time.

The following formulations released more than 15% of their content in the gastric and small intestine before they reach their destination. Hence, they were disqualified from candidacy as a colon targeting formulations.

These were: The 1:0 ratio of amylose to EC (or the formulation that contains no EC but only amylose in the film material) all its thickness group i.e., 2%, 4% 6% TWG, from the ratio of 1:1 amylose to EC those thickness of 2% and 4 % TWG and, from the ratio of 1:2 formulations; formulation with thickness of 2% TWG. The test was continued on the remaining 12 formulations as a candidate formulation for colon targeting system.

The 100 % immediate release of its content in the stomach is expected for the formulation of 1:0 ratio of amylose to EC. It shows the water-insoluble EC polymer incorporation in the film component was mandatory to improve the amylose's hydrophilic property.

The other formulations (1:1 and 1:2 ratio of amylose to EC), the premature release of their content here in gastric and small intestine media is due to its very thin coating thickness and larger amount of amylose proportion in the film forming material.

### 3.4.2 Fermentation studies

Metronidazole release from the film-coated tablets was assessed in conditions simulating the human colon (starting from the $6^{th}$ h to the $14^{th}$ h at a pH around 7.2 with and without a bifidobacterium cultured from a fecal matter of three healthy individuals). The results are expressed as cumulative percentage drug release versus time profiles (Figure 3.6 and 3.7 then 3.8 and 3.9 in the subsequent subtitles).

Similarly, a calibration curve was drawn to estimate the percentage of drug release in the colon.

#### 3.4.2.1 UV Calibration curve for fermentation in colon:

From the stock solution containing 222 µg/ml of metronidazole in phosphate buffer of pH 7.2, a series of dilutions were prepared to get twelve different concentrations (ranging from $C_1$ =5.00 µg/ml to $C_{12}$=60.00 µg/ml). The corresponding successive UV readings were found and the mean absorbance (Â) was taken to draw the calibration curve. The experiment was done three times.

The absorbance (at 278 nm) versus concentration of the solutions was plotted and a calibration curve with a linear regression equation of: Y = 0.0163X + 0.0207 (where Y is the absorbance and X is the concentration in µg/ml) and correlation coefficient ($R^2$) of 0.9998 were obtained showing good correlation between the absorbance and the concentration (Fig. 3.5).

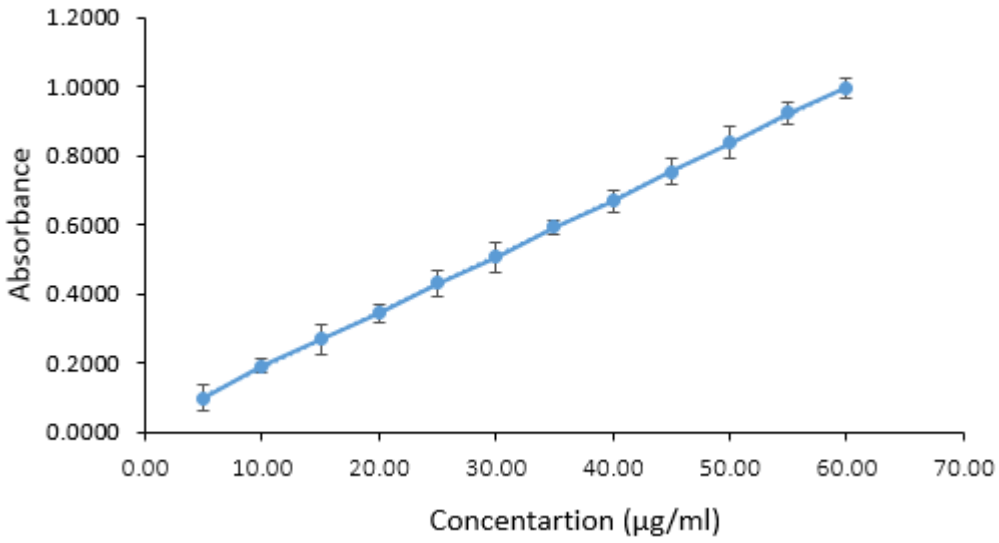



*Figure 3-4: Standard calibration curve of metronidazole in phosphate buffer (pH 7.2) at 278 nm with 95% confidence interval; ($R^2$ = 0.9998).*

### 3.4.2.2 Fermentation

Colon simulated fermentation study was done in the presence of colonic bacteria (in a phosphate buffers solution of pH 7.2). At predetermined time intervals samples were withdrawn and the absorbance reading under UV-spectrophotometer at a wavelength of $\lambda_{max}$ 278 nm was read.

As mentioned in section 3.4.1, some formulations had been already disqualified from candidacy as a CTDDS due to their release of more than 15% of their content in the upper GIT before reaching the colon. Hence, their value is nil in the three-dimensional graph in Fig. 3.5.

Below is the three-dimensional (X, Y, Z) graph for the remaining 12 samples demonstrating the result of the fermentation process through time (X-axis), the progression of the cumulative

percentage drug release process (Y-axis) that was continued from dissolution to this fermentation process for each ratio amylose to EC (from 1:0 to 1:5) and the thicknesses (2%, 4% and 6%) in % TWG of the film coat (Z-axis).

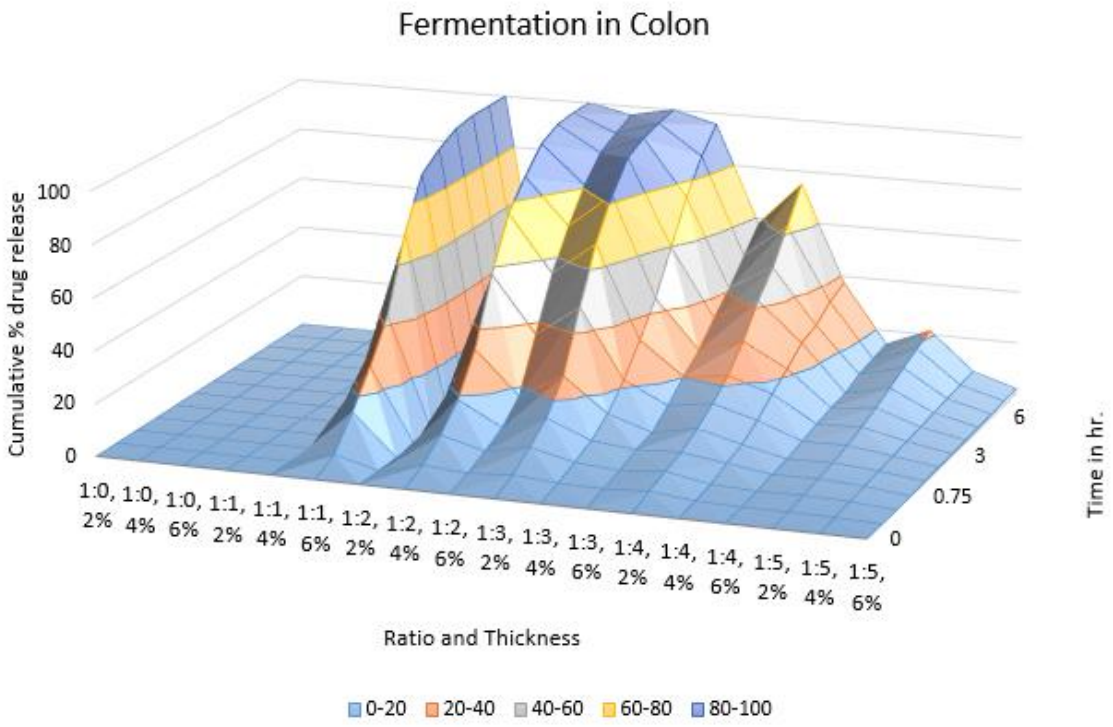



*Figure 3-5: The colon simulated fermentation studies on the release of film coated tablet showing the influence of the coating thickness and the ratios of the film forming materials in three dimension*

For the drug metronidazole, according to the monograph, the release of Q+5 or 85% +5% amount of drug release from a formulation is enough amount of drug available for a treatment (USP30-NF25<711>, 2007). Hence, in the above graph (fig. 3.5), those five formulations with more than 90% drug release in the colon are shown in blue (the upper half of blue) and they are; 1:1-6%, 1:2-4%, 1:2-6%, 1:3-2%, and 1:3-4%.

Hence, the results depicted that the drug content with these formulations released only in the colon. It passed through the upper GIT intact or it lost only an insignificant amount (≤10 %) of their drug content. These spatial drug release or local drug release is because of the presence of the colonic bacteria which makes it different from the previous two media. As it was

mentioned earlier, the RS content of the film coat is a substrate for the colonic bacteria and that is the one which is digested by the local bacteria. This leaves an opening in the film coat of the core tablet which paves the way for the drug delivery to the colon.

In the same medium, the extreme decline of the release of the drug as the proportion of EC increases in the film shows, EC is rather a barrier for the release of the drug. It's not digested by the bacteria nor could it be dissolved in the medium as it is a water-insoluble polymer which acts only as a structuring agent of the film.

The five formulation; the ratio of 1:1 with thickness 6%, ratio of 1:2 with thickness 4 % and 6%, and the ratio of 1:3 with thickness 2% and 4% are observed to be the best combinations for the design of a film-coated CTDDS. Although the role of RS and EC in the drug-release process explained well, the extent of the influence of the film thickness for this CTDDS is not determined even at this point. Which one, the film thickness or the film content is the primary factor? and which one is the subordinate? These could only be answered in the next section of a fermentation test with the absence of human fecal bacteria in the medium which is equivalent to the absence of the RS effect in the film material. If the presence of the RS is the center of the CTDDS, the drug release will not occur at all or may occur insufficiently (< 90 %) because of the absence of the digesting bacteria. Otherwise, regardless of the content of the film, only the optimization of the film thickness could give an independent CTDDS somehow at some point and that shall be the focus.

#### 3.4.2.3 Fermentation test on controls

The whole experiment of fermentation was similarly done on a control (in the absence of human feces i.e. in the absence of colonic bacteria that null the effect of the RS on the drug release) and the result is depicted in the following two graphs; first as cumulative percentage drug release versus time profile and second as a three dimensional graph showing how the percentage release relate with the ratio and TWG of the film coat against the running time.

Here, the following graph (Fig. 3.6), shown that the cumulative percentage release (Y-axis) for each sample thickness in % TWG (2%, 4% and 6%) with different content of the film coating

solutions (1:0 to 1:5) ratios of amylose to EC (Z-axis) against the running time (X-axis) for the fermentation test on control.

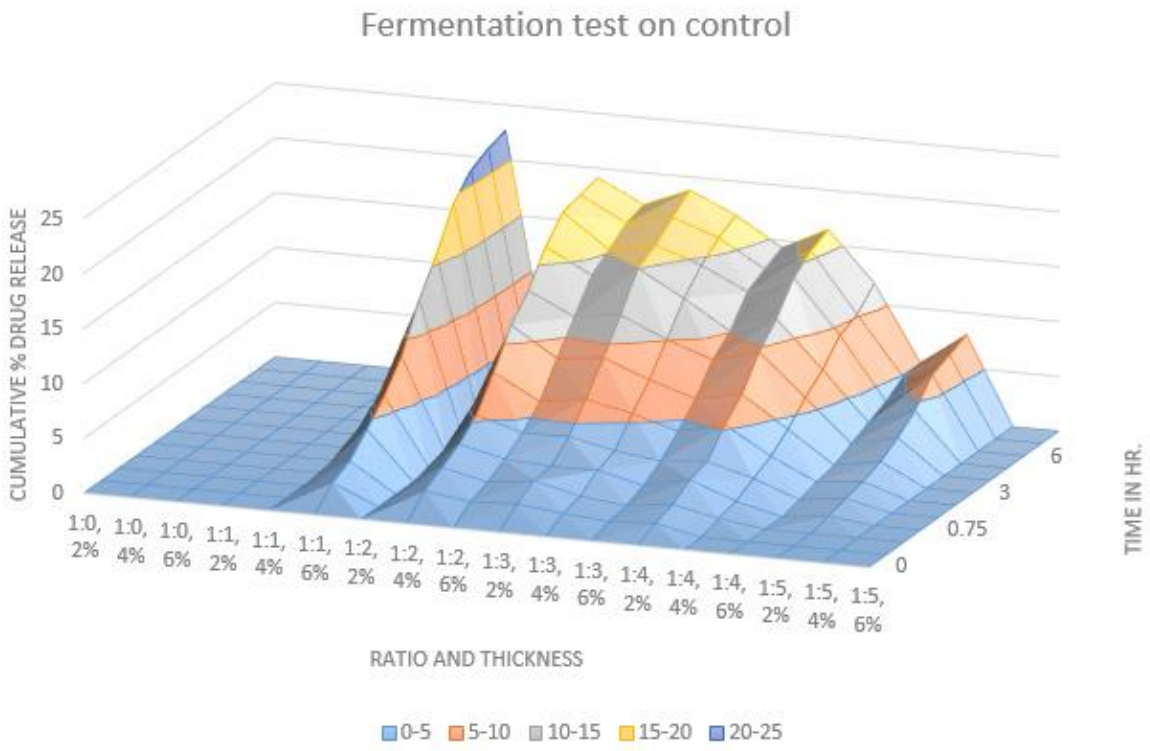



*Figure 3-6: The test on control for a colon simulated fermentation studies showing the influence of the ratios of the film forming material and the coating thickness on the drug release of the film coated tablets in the three dimensional graph (from t= 0 to t=8 h).*

As can be seen from the above graphs, all the tested 12 formulations could not release more than 25% of their content in colon regardless of the same film thickness and amylose to EC ratio were used as in the main fermentation test (section 3.4.2.2) where five of its formulation released more than 90 percent of their content in the colon only.

The absence of the fecal matter in the colonic medium is the absence of the colonic bacteria which is responsible for digestion of the RS that could open pores and cracks on the surface of the film-coated tablet for a drug release by the process of diffusion and erosion, respectively.

This makes the amount of amylose in the film, the bacterial substrate, rather a governing factor for the drug release than the thickness of the film which is just the path length for the diffusion to take place shortly.

Subsequently, the thickness is the subordinate factor for the drug release in the process of pore opening for diffusion or cracking for erosion to reveal the core tablet's content. And, it has the potential to delay the drug release. The reason is, the thicker the layer the longer it takes for the colonic bacteria to digest RS to uncover the core drug and it is also the longer the diffusion path to become for the drug release.

### 3.4.3 Analysis of drug release kinetics

The drug release of such a film-coated tablet is expected to be controlled by the superposition of diffusion and erosion. In the present experiment; First, in a simulated gastric and intestinal environment; the drug release is mainly controlled by diffusion as the film cracks due to the larger numbers of pores not yet occurred. Still, a faster and higher drug release kinetics was observed for some of the formulations because of the coating thickness and the larger amount of amylose in the film coat. The higher the amylose portion the larger the number of pores to be opened due to the hydrophilicity of amylose while the thinner the film the shorter the path for the diffusion of the drug to take place through the EC polymer scaffold. These were shown on all of the formulations of 1:0 and some of 1:1 ratio of Amylose to EC with a thinner film with higher portion of amylose. Second, the releasing mechanism is by the superposition of diffusion and erosion in that of the simulated colonic environment. Ultimately, it rather becomes by erosion chiefly. The possible explanation for this is, the smaller volume (1 to 44 ml or an average of 13ml) of the colonic fluid contribution to dissolve the drug through the pore for diffusion might be insignificant and the role of diffusion is very small. But once the film coat containing the natural polysaccharides get fermented by the colonic microflora and the film get cracked letting the drug eroded by the colonic fluid and consequently making the dominant releasing kinetics for the system to be an erosion (Chen *et al.,* 2007; Kosaraju, 2007; *Pu et al.,* 2011).

### 3.4.4 Dissolution and fermentation result Summary

There are many factors like GI motility, disease condition and the number of colonic bacteria that might affect the drug release from a bacterially triggered CTDDS (Karrout Y, 2008). But, if we consider the above factors to be constant or change slightly, the whole dissolution and fermentation data in this study indicate that the ratio of amylose to ethylcellulose in the film coat and the thickness of the coating are the key parameters for CTDDS developed here. The reasons behind this are the proportion of the RS that affects the availability of the substrate for digestion by the colon bacteria for opening a path for drug release. And thickness of the film is the one that delays or prevents the diffusion of the drug through the pores.

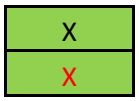
A range of TWG from 2% to 6% and a ratio of amylose to EC from 1:0 to 1:5 were tested in the process of dissolution and fermentation. But, as can be seen from the table (Table 3.4) the optimum percentage drug released for a robust film-coated CTDDS were found somewhere between the two extreme values, rather exactly lining the junction of the two extreme ratios and thicknesses. In Table 3.4, it is shown that the largest amount of amylose with the smallest thickness is found on top left corner which release the drug abruptly while the largest amount of EC with the larger thickness is found on right bottom corner that release the drug barely. The five best formulations chosen for CTDDS are located about the center of these two extreme values (in yellow).

*Table 3. 4: Summary of dissolution and fermentation studies for different ratio of amylose to EC with six sample groups each having thickness of 2%, 4% and 6% in a simulated gastric, small intestine and colonic environment.*

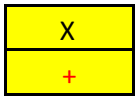
| | | Ratio of Amylose: EC | | | | | |
|---|---|---|---|---|---|---|---|
| TWG | Release site | 1:0 | 1:1 | 1:2 | 1:3 | 1:4 | 1:5 |
| 2% | G & S.I | + | + | + | X | X | X |
| | Colon | + | + | + | + | X | X |
| 4% | G & S.I | + | + | X | X | X | X |
| | Colon | + | + | + | + | X | X |
| 6% | G & S.I | + | X | X | X | X | X |
| | Colon | + | + | + | X | X | X |

**Key**:


An area that released both in upper GIT and in colon




An area that released neither in upper GIT nor in colon




An area that did not release in the upper GIT but only in a colon ➔ i.e. Colon targeted!

# 4. CONCLUSION

The whole dissolution and fermentation data in this study indicate that the drug release from the coated products was accelerated in the fermentation environment of the colon. This is attributed to bacterial digestion of the RS component of the film-coat producing pores through the EC polymer for drug diffusion and the ultimate breaking of the film coat for the drug to be released by erosion. Still, only five formulations managed to release a sufficient amount of the drug content in the colon area but not in the upper GIT.

The results of this experiment indicate that the ratio of amylose to EC in the film coat and the thickness of the coating are the key parameters in controlling drug release from the system. When the amount of RS in the film coating material increases drug release increases and vice versa. The reason is a larger amount of RS in the blend means the bioactive component create larger numbers of pores for diffusion and erosion of the drug. On the contrary, the increasing of the film-coating thickness decreased drug release. This is because the increase of film-coating thickness increases the length of the diffusion pathway for the drug whereas decreasing thickness increases the release.

To compare the influence of the film coating material content versus the coating thickness on the extent of the overall drug release, a control test was done omitting the RS effect from the formulation. The result was a significant decrease in drug release. This makes the amount of RS in the film, the bacterial substrate, rather the basic factor for the drug release than the thickness of the film.

Consequently, after the optimization of amylose, the film coating material, amylose with varied proportion of EC in the coating formulation, and the adjustment of the film thickness; the ratio of amylose to EC of 1:1 with film thickness 6% TWG, ratio of 1:2 with thicknesses 4 % and 6% TWG, and ratio of 1:3 with thicknesses 2% and 4% TWG were found to be the best combinations to release the drug content sufficiently (≥ 90% in the colon only). This justifies the evaluation of a film coating material from the local Teff's RS, which gives all the benefit of the required CTDDS, was successful in this study.

# 5. SUGGESTIONS FOR FURTHER WORK

- Direct quantification and identification test of the RS found from the total starch, using AOAC Official Method 2002.02 or any other better method found.

- *In vivo* dissolution and fermentation study method in the test of the film-coated tablets for CTDDS.

# 6. REFERENCES


Abrantes C G, Duarte D, Reis C P (2016). An Overview of Pharmaceutical Excipients: Safe or Not Safe? *J Pharm Sci* 105 :2019-2026

AGROSYNERGIE (2011). European commission DG agriculture and rural development final report: Evaluation of Common Agricultural Policy Measures applied to the Starch Sector. Available at: http://ec.europa.eu/agriculture/eval/reports/starch/toc_en.pdf [Accessed 25 September, 2017].

Amidon S, Brown J E, Dave S V (2015). Colon-targeted oral drug delivery systems: Design trends and approaches. AAPS *Pharm Sci Tech* 16(4):731-741.

Amritpal S, Ankush S, Anju P (2014). Novel approaches for colon targeted drug delivery system. *Int J Res Dev Pharm L Sci* 3 (2): 877-886.

Ankit G, Ajay B, Kumar K M, Neetu K (2012). Tablet coating techniques: Concepts and recent trends. *Int Res J Pharm* 3(9):50-58.

AOAC international (2002). Association of analytical chemist official method 2002.02: Resistant starch in starch and plant materials. Enzymatic digestion first action 2002. *J AOAC Int* 85,1103(2002).

Assefa K, Cannarozzi G, Girma D, Kamies R, Chanyalew S, Plaza-Wüthrich S, Blösch R, Rindisbacher A, Rafudeen S and Tadele Z (2015). Genetic diversity in tef [*Eragrostis tef* (Zucc.) Trotter]. *Front Plant Sci* 177(6):1-13

Aulton ME (2007) Aulton's Pharmaceutics – The Design and Manufacture of Medicines. Edinburgh: Churchill Livingstone.

Bae H Y and Park K (2011). Targeted drug delivery to tumors: Myths, reality and possibility. *J Controlled Rel* 153: 198–205.

Bari M A (2019). The Excipients between Effects and the Side Effects. J Med Care Res Rev.5(2): 178-187

Basit AW, Newton JM, Lacey LF (2002). Susceptibility of the H2-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora. *Int. J Pharm* 237: 23–33

Baye K (2014). Teff: nutrient composition and health benefits. Center for food science and nutrition, college of natural sciences, Addis Ababa University. Ethiopian strategy support program, working paper 67. Accessible at : https://www.researchgate.net/profile/Kaleab_Baye2/publication/266316373_Teff_Nutrient_Composition_and_Health_Benefits/links/542c24320cf277d58e8b0b42.pdf [ Accessed 20 February. 2017].

Belay G, Zemede A, Assefa K, Metaferia G, and Tefera H (2009). Seed size effect on grain weight and agronomic performance of tef (Eragrostis tef). *Afr J Agric Res* 4(9):836-839.

BP, (2009). British Pharmacopeia: The pharmaceutical press. Her Majesty‘s Stationary Office, London, vol. I-VI.

Bultosa G (2007). Physicochemical Characteristics of Grain and Flour in 13 Tef [Eragrostis tef (Zucc.) Trotter] Grain Varieties. *J Appl Sci Res* 3(12): 2042-2051.

Bultosa G, Hail AN, Taylor J (2002). Physicochemical characterization of grain teff [*Eragrostis teff*(Zucc.) Totter]. *Starch-starke* 54 (10):461-468.

Bultosa G, Taylor J R N (2003). Chemical and Physical Characterization of Grain Tef [Eragrostis tef (Zucc.) Trotter] Starch Granule Composition. *Starch/Stärke* 55:304–312

Catholic University of the Sacred Heart-UCSC, (2015), Milano Italy. Production of appropriate food: sufficient, safe, suitable: TEFF *Eragrostis tef* [Zucc.] Trotter. Available at: https://dipartimenti.unicatt.it/diana-15._INV_SC_AGR_15_TEFF.pdf [ Accessed 8 February 2017].

Chen L, Li X, Pang Y, Li L, Zhang X, Yu L (2007). Resistant starch as a carrier for oral colon – targeting drug matrix system. *J Mater Sci Mater Med* 18(11): 2199-2203.

D'Silva TV, Taylor J RN, Emmambux MN (2011). Enhancement of the pasting properties of teff and maize starches through wet–heat processing with added stearic acid. *J Cereal Sci* 53: 192–197.

Dahl TC (2009). Ethylcellulose. In: Rowe RC, Sheskey PJ and Quinn ME eds, Hand book of pharmaceutical Excipients, 6th edn, Pharmaceutical Press and the American Pharmacists Association, London and Chicago, pp 262-267.

Englyst HN, Wiggins HS, Cummings JH (1982). Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107:307–18.

FDA (2014). U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER): SUPAC: Manufacturing Equipment Addendum Guidance for Industry, pp 19-24. Accessible at: https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs or at : https://www.fda.gov/media/85681/download [ Accessed 20 December. 2019].

Florence AT, Attwood D (2008). FASTtrack: Physicochemical Principles of Pharmacy. London: Pharmaceutical Press.

Gadhave MV, Shevante TB, Takale AA, Jadhav SL, Gaikwad DD (2017). Formulation and evaluation of colon targeted drug delivery of mesalamine. *Int J Pharm Chem Res* 9(1): 26-34.

Gebremariam M M, Zarnkow M, Becker T (2013). Effect of teff (*Eragrostis tef*) variety and storage on malt quality attributes. *J Inst Brew* 119: 64–70.

Gebremariam M M, Zarnkow M, Becker T (2014). Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. *J Food Sci Technol* 51(11): 2881-2895.

Gebre-Mariam T, Schmidt PC, 1996. Isolation and physicochemical properties of enset starch. *Starch/Staerke* 48: 208–214.

Girma D, Tadele Z, Assefa K (2015). Tef: Cultivating a healthy lifestyle. *New Food* 18(4) Available at: https://www.newfoodmagazine.com/article/19311/tef-cultivating-a-healthy-gluten-free-lifestyle/ [ Accessed 18 February 2017].

Gizaw B, Tsegay Z, Tefera G, Aynalem E, Abatneh E and Amsalu G (2018). Traditional Knowledge on Teff (Eragrostis tef) Farming Practice and Role of Crop Rotation to Enrich Plant Growth Promoting Microbes for Soil Fertility in East Showa: Ethiopia*. Agri Res & Tech: Open Access J* 5(16):139-155

Goni I, Garcia-Diz L, Mans E, Saura-Calixto F (1995). Analysis of resistant starch: a method for foods and food products. *Food Chem* 56(4): 445-449.

Griesser HJ (2016). Thin film coatings for biomaterials and biomedical applications. *Woodhead Publ. Ser. Biomater* 110: 10-19

Gupta A, Mittal A, Gupta AK (2011). Colon targeted drug delivery systems- a review. *Biofarm Zh* 3(4):3-13.

Hager A S (2013). Cereal products for specific dietary requirements. Evaluation and improvement of technological and nutritional properties of gluten free raw materials and end products. PhD Thesis, University College Cork. Available at: https://cora.ucc.ie/bitstream/handle/10468/1274/phdthesis_hager_final_corrected.pdf?sequence=1 [ Accessed on 10 November, 2016].

Hager A S, Lauck, F, Zannini E, Arendt EK (2012). Development of gluten-free fresh egg pasta based on oat and teff flour. *Eur Food Res Technol* 235(5): 861-871.

Hartzell A and Rose DJ (2011). Polysaccharides for colon–targeted drug delivery: Improved drug–release specificity and therapeutic benefits. University of Nebraska- Lincoln, United States of America. Available at: http://cdn.intechopen.com/pdfs/22858/InTech-Polysaccharides_for_colon_targeted_drug_delivery_improved_drug_release_specificity_and_therapeutic_benefits.pdf [ Accessed 12 November, 16].

Hegyesi D, Sovány T, Berkesi O, Pintye-Hódi K, Regdon Jr. G (2013). Study of the effect of plasticizer on the structure and surface characteristics of ethylcellulose free films with FT-IR spectroscopy. *Microchem J* 110: 36-39

Hegyesi D, Süvegh K, Kelemen A, Pintye-Hódi K, Regdon Jr. G (2014). Characterization of ethylcellulose free films by positron annihilation spectroscopy and mechanical testing. *Microchem J* 115: 47-50

Homayouni A, Amini A, Keshtiban A K, Mortazavian A M, Esazadeh K, Pourmoradian S (2014). Resistant starch in food industry: A changing outlook for consumer and producer. *Starch/Stärke* 66:102–114

Hussan SD, Santanu R, Verma P, Bhandari V (2012). A review on recent advances of enteric coating. *IOSR J Pharm* 2(6): 05-11.

Johnston KL, Thomas EL, Bell JD, Frost GS, Robertson MD (2010). Metabolism Resistant starch improves insulin sensitivity in metabolic syndrome. *Diabetic Med* 27: 391-397

Jones D (2008). FASTtrack: Pharmaceutics: Dosage Form and Design. London: Pharmaceutical Press.

Karmakar R, Ban DK, Ghosh U, (2014). Comparative study of native and modified starches isolated from conventional and nonconventional sources. *Int Food Res J* 21(2): 597-602

Karrout Y (2008). Innovative Drug Delivery Systems for colon targeting, Freien Universität Berlin, Germany. Available at: https://refubium.fu-berlin.de/bitstream/handle/fub188/232/Karrout.pdf?sequence=1&isAllowed=y [ Accessed on 25 November, 2016].

Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Desreumaux P, Siepmann J (2009). Characterization of ethylcellulose: starch-based film coatings for colon targeting. *Drug Dev Ind Pharm* 35(10): 1190–1200.

Karrout Y, Neuta C, Siepmanna F, Wilsd D, Ravauxa P, Deremauxd L, Flamenta M, Dubreuila L, Lemdania M, Desreumauxc P, Siepmanna J (2010). Enzymatically degraded Eurylon 6 HP-PG:

Ethylcellulose film coatings for colon targeting in inflammatory bowel disease patients. *J Pharm Pharmacol* 62: 1676-1684.

Kittipongpatana OS and Kittipongpatana N (2014). Resistant starch contents of native and heat-moisture treated jackfruit seed starch. *Sci World J* Volume 2015, Article ID 519854.

Kolte B P, Tele K V, Mundhe V S, Lahoti S S (2012). Colon Targeted Drug Delivery System – A Novel Perspective. *Asian J Biomed Pharm Sci* 2(14): 21-28.

Kosaraju S L, (2007). Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. *Rev Food Sci Nutr* 45:251-258

Kusuma R, Reddy VP, Samba RA, (2014). Evaluation of physicochemical character of ipomoea batata starch as a pharmaceutical excipient. *Int J Res Pharm Sci* 5(4): 275-281.

Leszczyñski W, 2004. Resistant Starch – classification, structure, production. *Pol J Food Nutr Sci* 13(54): 37-50.

Mahajan H S, Patil S B, Gattani S G, Kuchekar B S (2007). Targeted drug delivery systems. Available at: https://www.researchgate.net/publication/292926440_Targeted_drug_delivery_systems [ accessed on 21 March, 2018].

Manish G and Vimukta S (2011). Targeted drug delivery system: A Review. *Res J Chem Sci* 1(2):135-138.

Melegari C (2016). Study of different technologies for film coating of drug layered pellets using ethylcellulose as functional polymer. University of Bologna. Available at: http://amsdottorato.unibo.it [Accessed 10 November, 2018]

Meneguin A B, Cury B S, Evangelista R C (2014). Films from resistant starch-pectin dispersions intended for colonic drug delivery. *Carbohydr Polym* 99: 140– 149.

Mezemir S (2015). Probiotic Potential and Nutritional Importance of Teff (Eragrostis tef (Zucc) Trotter) Enjerra - A review. *Afr J Food Agric Nutr Dev* 15(2):9964-9971.

Miller D, (2010). Teff Guide 3rd edition. Available at: http://www.calwestseeds.com.product/teff [accessed on 12 November, 2016].

Mukesh PR, Fattesingh US, Sham AP, Shilpa PC, Jyoti PG, Kalyani AB (2013). Colon targeted drug delivery system. *Int J Pharma Res Rev* 2(8):33-42

Murphy Mm, Douglass JS, Birkett A (2008). Resistant starch intakes in the united states. *J Am Diet Assoc* 108(1): 67-78.

Narisawa S, Yoshino H, Hirakawa Y, Noda K (1994). Porosity-controlled ethyl cellulose film coating evaluation of mechanical strength of porous EC film. *Chem Pharm Bull* 42(7): 1491–1495.

Nutan MTH (2004). Starch acetate as a film forming excipient in controlled drug delivery. PhD dissertation, Graduate Faculty of Texas Tech University Health Sciences Center. Available at: https://ttu-ir.tdl.org/handle/2346/21349 [ Accessed on 19 September, 2018].

Osman Z, 2012. Investigation of different shellac grades and improvement of release from air suspension coated pellets. The Johannes Gutenberg university, Maniz, Germany. Available at: https://publications.ub.uni-mainz.de/theses/frontdoor.php?source_opus=3325 [Accessed 10 November, 2018].

Ozturk AG and Dressman JB (1990). Mechanism of release from pellets coated with an ethyl cellulose-based film. *J Control Release* 14(3): 203-213.

Philip AK and Philip B (2010). Colon targeted drug delivery systems: A review on primary and novel approaches. *Oman Med J* 25: 70-78

Prasanth VV, Jayaprakash R, Sam T. Mathew S T (2012). Colon specific drug delivery systems: A Review on various pharmaceutical approaches. *J Appl Pharm Sci* 02 (01): 163-169

Prathap M, Gulshan MD, Rao NR (2014). Colon: targeted drug delivery system – a review. *Int J Res Pharm Nano Sci* 3(5): 429-437.

Priya VS, Roy HK, Jyothi N, Prasanthi NL (2016). Polymers in drug delivery technology, Types of polymers and applications. Scholars Acad J Pharm 5(7):305-308.

Pu H, Chen L, Li X, Xie F, Yu L, Li L (2011). An oral colon-targeting controlled release system based on resistant starch acetate: Synthetization, characterization, and preparation of film-coating pellets. *J Agric Food Chem* 59(10):5738-5745.

Rose DJ, Patterson JA, Hamaker BR (2010). Structural differences among alkali-soluble arabinoxylans from maize (Zea mays), rice (Oryza sativa), and wheat (Triticum aestivum) brans influence human fecal fermentation profiles. *J Agri Food Chem* 58(1):493–9.

Ruotsalanine M, (2003). Studies on aqueous film coating tablets performed in a side-vented pan coater. University of Helsinki, Finland. Pharmaceutical technology division department of pharmacy. Available at: http://ethesis.helsinki.fi/julkaisut/mat/farma/vk/ruotsalainen/studieso.pdf [ Accessed on 11 October, 2018].

Sajilata MG, Singhal RS, Kulkarni P R (2006). Resistant Starch: a review. Comprehensive reviews in food science and food safety. *CRFSFS* 5(1):1-17.

Sarangi MK and Padhi S (2015). Colon targeted drug delivery system-an approach for treating colonic ailments. *J Crit Rev* 4(2):12-18

Satyam G, Shivani S, Shivam G, Shobhit J, Sanjeev V, Arvind K (2010). Starch as a material for drug delivery. Int J Bio Phar Res 1(2):56-60.

Semwal R, Semwal RB, Semwal DK (2015). Drug delivery systems: Selection criteria and use. Encyclopedia of Biomedical Polymers and Polymeric Biomaterials. Pp 2938-2949. Available at: https://www.researchgate.net/publication/280734889 [Accessed on 19 November, 2018].

Siew LF, Basit AW, Newton JM (2000). The potential of organic-based amylose-ethylcellulose film coatings as oral colon-specific drug delivery systems. *AAPS Pharm Sci Tech* 1 (3): article 22

Siew LF, Basit AW, Newton JM (2000b). The properties of amylose-ethylcellulose films cast from organic-based solvents as potential coatings for colonic drug delivery. *Eur J Pharm Sci* 11:133-139.

Silvester KR, Englyst HN, Cummings JH (1995). Ileal recovery of starch from whole diets containing resistant starch measured *in vitro* and fermentation of ileal effluent. *Am J Clin Nutr* 62:403–411.

Sreelatha D and Brahma C K (2013). Colon targeted drug delivery – A review on primary and novel approaches. *J Global Trends Pharm Sci* 4 (3):1174-1183.

Taylor JRN, Dewar J, Taylor J, von Ascheraden RF (1997). Factors affecting the porridge-making quality of South African sorghums. *J. Sci. Food Agric.* 73: 464–470

Teklehaimanot WH, Duodu KG, Emmambux MN (2013). Maize and teff starches modified with stearic acid as potential fat replacer in low calorie mayonnaise-type emulsions. Melkassa agricultural research center, Ethiopian, institute of agricultural research, Adama, Ethiopia Department of Food Science, University of Pretoria, Hatfield, South Africa. *Starch/Staerke* 65:773-781.

Tharanathan RN (2005). Starch: Value addition by modification. *Crit Rev Food Sci Nutr* 45: 371 - 384.

Topping DL and Clifton PM (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and non-starch polysaccharides. *Physiol Rev* 81(3):1031-1064.

USP 30-NF 25, (2007). United States Pharmacopeia: The United States Pharmacopeial Convention, Inc., Rockville, MD.

US PAP (2006). United states patent application publication: Processing of teff flour. Pub. No.: US 2006/0286240 A1. Pub. Date: Dec. 21, 2006. Available at: https://patentimages.storage.googleapis.com/fb/6d/ea/ffec0672ea5930/US20060286240A1.pdf [ accessed on 25 December 2016].

USDA (2015). United states national nutrient database for standard reference release. Basic Report 20142, Teff, uncooked Report Date: October 05, 2015 04:58 EDT. Available at : https://ndb.nal.usda.gov/ndb/foods/show/305324 [ Accessed on 28 November 2016].

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

Wilson PJ and Basit AW (2005). Exploiting gastrointestinal bacteria to target drugs to the colon: An *in vitro* study using amylose coated tablets. *Int. J Pharm (Amsterdam, Neth.)* 300: 89-94.

# APPENDIX I

a) *Calculation for the amount of Pancreatic α-amylase in the enzymatic digestion of starch:*

The preparation was presented in capsule form.

1 Capsule Contains → 1,300mg of Pancreatic α-amylase

Then, 1,300 mg of Pancreatic α-amylase→ 32 gm starch

Accordingly, the # of capsule (amount of Pancreatic α-amylase) needed to digest a given amount of starch could be obtained easily as: -

1,300 mg (1 Cap.) of Pancreatic α-amylase→ 32 gm starch

X? → 100 gm starch

X=4062.5 gm

1 cap. → 1,300 gm ; 4,062.5 ➔ 3 cap.

b) *Calculation for the amount of Amyloglucosidase (AMG) in the enzymatic digestion of starch:*

The preparation was presented in powder form.

75gm of AMG could digest → 100 lb. (453.59gm ) of starch

Accordingly, the amount of AMG needed to digest a given amount of starch could be obtained easily as: -

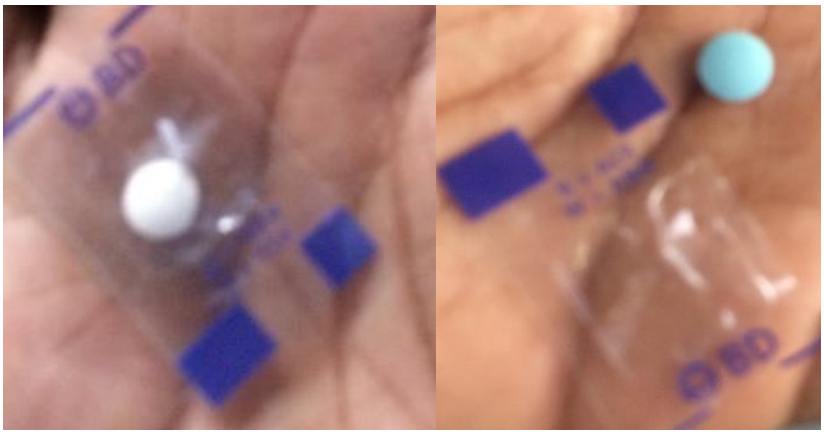
75gm of AMG for → 453.59gm of starch

Y? → 100 gm of starch. Y=16.5 gm of AMG.

# APPENDIX II

a) Color change result of the test for TS

b) Color Change Indicator for the Anaerobic Tester; without (a) and with Oxygen (b)



(a) (b)

c) Variations in the Physiology of Human Gastro-intestinal Tract (Amidon *et al.,* 2015)

| **Organ** | **Contents** | **pH** |
|---|---|---|
| Stomach | Thin soluble mucus, HCl, intrinsic factor, pepsin, lipases, gastrin, histamine, serotonin, somatostatin | 1-1.5 |
| Small intestine | Chyme (from stomach), alkaline mucus, intestinal juice which is mostly water, motilin, cholecystokinin, brush border enzymes (maltase, sucrose, lactase, enterokinase and carboxypeptidase) Bile (which contains electrolytes, fatty substances, bile salts and pigments), pancreatic juice (a bicarbonate-rich fluid containing enzymes) | 5-.7.5 |
| Cecum | Mucus, enteric bacteria, vitamins, food residue, gases such as carbon dioxide and methane | 5.5-7 |
| Ascending colon | Mucus, enteric bacteria, vitamins, food residue, gases such as carbon dioxide and methane | 5.7-6.9 |
| Transverse colon | Mucus, enteric bacteria, vitamins, food residue, gases such as carbon dioxide and methane | 5.8-7.4 |
| Descending colon | Mucus, enteric bacteria, vitamins, food residue, gases such as carbon dioxide and methane | 6.3-7.7 |
| Rectum | Undigested food residues, mucus, epithelial cells from the intestinal lining, numerous bacteria (millions), some remaining water | ~7 |