Evaluation of Haricot Bean Retrograded Resistant Starch as a Film Forming Excipient in Oral Colon-Targeted Delivery of Mesalamine

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November, 2020

EVALUATION OF HARICOT BEAN RETROGRADEDRESISTANT STARCH AS A FILM FORMING EXCIPIENTINORAL COLON-TARGETED DELIVERY OF MESALAMINE

A THESIS SUBMITTED TO THE SCHOOL OF PHARMACY, DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICS

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ADDIS ABABA, ETHIOPIA
November, 2020

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ACKNOWLEDGEMENTS

I am very much grateful to my advisers DrAntenehBelete and Dr.Nisha Mary Joseph for their unreserved support and guidance throughout this research.

I would like to express my sincere gratitude to the following institutions:EPHARM for supplying me with different substances & allowing me to use their facilities, Ethiopian Leather Industry Institute, Department of Chemistry College of Natural Science and NAHDICfor allowing me to use their facility. I would also like thank Melkasa Agricultural Research Center for supplying me with the four different varieties of Haricot beans. I wouldalso like to thank VDFACA for sponsoring me this Postgraduate study and AAU for sponsoring the research work. Mr TsegayeDichasaand Mr MersiAduegna, EPHARM personnels, are acknowledged for their wholehearted support and care they renderedto me throughout my two months stay in EPHARM.

I am also grateful to my Chinese friendsMr FuJin and Mrs LeiYun who had helped me to get many chemicals and Enzymes from abroad and all of my classmates from whom I have learntseveral things during our study.

TABLE OF CONTENTS

List of figures

List of Tables

List of Acronyms

AAU	Addis Ababa University
AEZ	Agro-ecological Zone
AFM	Atomic Force Microscopy
ASA	Aminosalicylic Acid
AV_m	Average mass
CRC	Colorectal Cancer
DNA	Deoxyribonucleic Acid
DP	Degree of Polymerization
DSC	Differential scanning Calorimetry
DT	Disintegration Time
DTA	Differential Thermal Analysis
EC	Ethyl cellulose
EPHARM	Ethiopian Pharmaceutical Manufacturing Share Company
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
GI	Gastro Intestinal
GRAS	Generally Regarded as Safe
HMT	Heat Moisture Treatment
HPMC	Hydroxy Propyl Methyl Cellulose
IBD	Inflammatory Bowel Diseases
IR	Infrared
IU	International Unit
LGP	Length of Growing Period
NAHDIC	National Animal Health Diagnostic Investigation Center
NF	National Formulary
NIR	Near Infrared
NPU	New Pullulanase Unit

NS	Native Starch
PVP	Poly Vinyl Pyrrolidone
RDS	Rapidly Digestible Starch
RVA	Rapid Visco Analysis
RS	Resistant Starch
SAXS	Small Angle X-ray Scattering
SCFAs	Short-chain Fatty Acids
SDS	Slowly Digestible Starch
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SP	Swelling Power
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
TMG	Total Mass Gains
TPA	Texture Profile Analysis
XRD	X-ray Diffraction
XRPD	X-ray Powder Diffraction
US	United States
USP	United States Pharmacopeia
UV	Ultra Violet
VDFACA	Veterinary Drug & Feed Administration & Control Authority
WAXD	Wide Angle X-ray Diffraction
WSI	Water Solubility Index

ABSTRACT

The colon has been evaluated intensively for about three decades as a site of drug delivery, not only for local colonic pathologies but also for systemic delivery of small therapeutic molecules, protein and peptide drugs. As a site for drug delivery, colon offers a near neutral pH, reduced digestive enzymatic activity, a long transit time and an increased responsiveness to absorption enhancers. Targeting drugs to the colon has two major implications in colonic disorders, explicitly reduced systemic toxicity and increased local therapeutic efficacy.

The main objective of this study is to prepare haricot bean retrograded resistant starch and evaluate its potential as film forming excipient in tablet formulation for oral colon-targeted delivery of mesalamine.

To prepare retrograded resistant starch, haricot bean starch was dispersed in distilled water in the ratio of 1:4 and autoclaved (121 °C) for about 1 h until complete disruption of starch granules and then the gelatinized starch de-branched using pullulanase enzyme. The product obtained was autoclaved (121 °C) for about 1 h, cooled to room temperature and then stored in a refrigerator for 24 h at 3 °C. This step was repeated 10 times. The slurry obtained in the previous stage was treated with heat stable alpha amylase, amyloglucosidase and then with pepsin to obtain enzyme resistant starch type 3 (RS3). The product obtained was washed four times with plenty of hot water (90 °C) and the residue was left to dry in an oven overnight. Gravimetric method was used to determine the RS3yield.*In-vitro* methods, dissolution and fermentation studies were used to determine the release profiles of mesalamine in the GI tract.

The yields of haricot bean RS3 were in the range of 74.7- 78.0% per dry mass of haricot bean starch. The inherent nature of haricot bean and extrinsic factors (processing conditions) may have contributed for such high yield. Haricot bean native starch had showed CA-type starch polymorphism. The *in-vitro* mesalamine cumulative percentage releases of all coating formulations, which differ either in RS3: EC (Ethyl cellulose) ratio or in their film thickness, in simulated gastrointestinal fluids were less than 4% during the 7 h study period. The cumulative percentage releases of mesalamine of the different coating formulations in the fermentation study were in the range of 57-82% in 12 h of the study period.

The results indicate that haricot bean resistant starch has a good potential to be used as a film forming excipient in oral colon-targeted delivery of drugs.

Keywords: Colon-targeted delivery, Haricot bean, Resistant starch, Meslamine, Fermentation study

1. Introduction

1.1. Starch

The carbohydrate starch is an important energy reserve of plants. It is synthesized as microscopic granules in the tissues of many plant species, and has provided dietary energy for animals and man for several millennia. Chemically, starches are polysaccharides, composed of a number of glucose molecules linked together with Ŭ-D-(1-4) andŬ D-(1-6) linkages. Starch consists of two structural components, the amylose, which is essentially a linear polymer in which glucose residues are Ŭ-D-(1-4) linked, typically constituting 15% to 20% of starch and amylopectin (Ratnaningsih *et al.*, 2016), which is a larger branched molecule with Ŭ-D-(1-4) and Ŭ-D-(1-6) linkages and normally is the major component of starch. Amylose has a degree of polymerization up to DP 6000, and has a molecular mass in the range of 10^5 to 10^6 g/mol. The chains can easily form single or double helices. Amylopectin (10^7 to 10^9 g/mol) (Cai *et al.*, 2010; Hartesi *et al.*, 2016) has an average DP of 2 million, making it one of the largest molecules in nature. Chain lengths of 20 to 25 glucose units between branch points are typical (Wang*et al.*, 2015; Hartesi *et al.*, 2016). Native starch is a semi-crystalline entity; composed of loosely packed amorphous and densely packed crystalline regions (Vasanthan *et al.*, 1998).

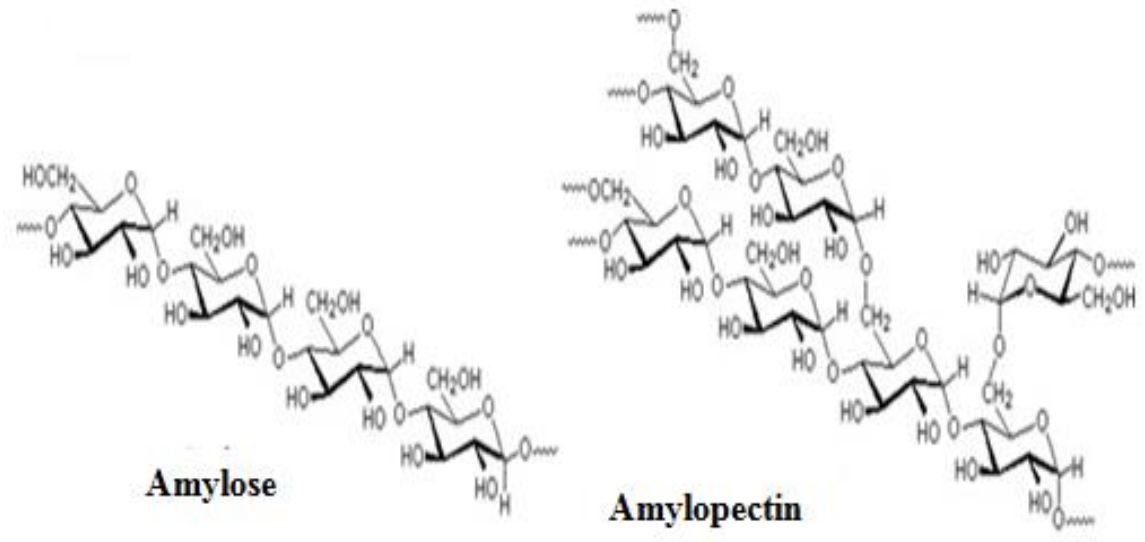

Fig.1.1.The Chemical Structures of Amylose and Amylopectin (Adapted from Sajilata et al., 2006)

Though the size and morphology of starch granules is specific for each plant species, their internal structures have remarkably similar architecture, consisting of growing rings, crystalline and amorphous lamellae (Fig. 1.2).Starch granules are generally composed of an amorphous bulk core area surrounded by concentric semi-crystalline growth rings alternating with amorphous growth rings. The amorphous core, as observed by SEM and TEM, is composed mainly of amylose and few amylopectin chains disordered at the reducing end. The size of the amorphous core is related to the amylose content of starch; waxy maize starch granules have the smallest core compared to normal and high amylose maize starch, with the latter having the largest core (Wang*et al.*, 2015).

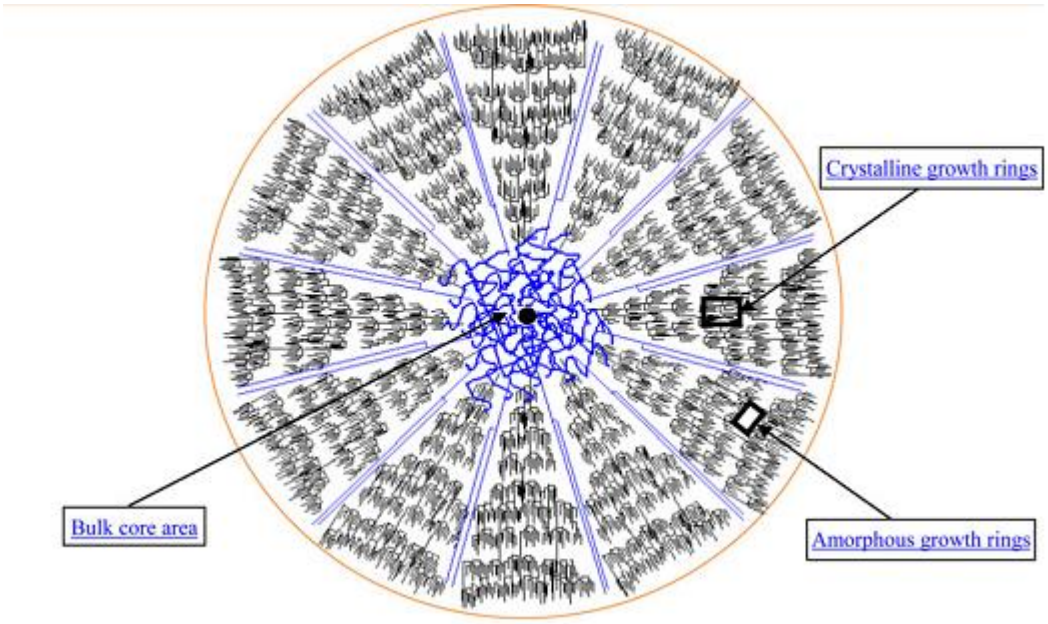

Fig. 1.2.Amodel representing the distribution of amylose and amylopectin molecules in microscopic granules of starch. The blue lines represent amylose molecules, and the black lines represent amylopectin molecules (adapted from Wang *et al.* 2015)

Based on their X-ray diffraction patterns three types (polymorphs) of starches, designated as type A, type B, and type C have been identified. Although type A and type B are real crystalline modifications, type C is a mixed form(a combination of A and B). Recently the C-type polymorph has been further sub-classified in to C_A and C_B depending on their proximity in characteristics or/and in their distinct polymorph form to A or B types, respectively. Type A is a characteristic of cereals, whereas, type B is a characteristic of tubers, and type C is a characteristic of legume starches (Ratnaningsih *et al.*, 2016). Intrinsically, among others they

differ in their density of packing and crystal lattice size. The B polymorph is the most densely packed form and has the largest crystal lattice size and the A polymorph is the loosely packed form and has the smallest crystal lattice size than the other two (Wang *et al.*, 2015). Functionally, they differ in their capability to tolerate different processing techniques and conditions (Shimelis *et al.*, 2005; Ratnaningsih *et al.*, 2016).

1.2.Haricot bean (*Phaseolus vulgaris L.*)

Legumes belong to the Leguminosae (Fabaceae) family, which comprises about 16,000ï 19,000knownspeciesand approximately 750 genera (Zhang *et al.*, 2017).They are ranked fifth globally in terms of annual grain production after wheat, rice, corn, and barley (Wani*et al.*, 2016). Legumes play a major role in the human diet, because they are rich in proteins, carbohydrates, fiber, essential amino acids, minerals, and vitamin (especially the B-complex vitamins) (Fabbri *et al.*, 2016). The protein and carbohydrates content of legumes are in the range of 19-40%and 50-65%, respectively (Carrera *et al.*, 2006). The carbohydrate of legumes is composed ofreducingand non-reducing sugars, oligosaccharides of the raffinose family, etc (Shimelis *et al.*, 2005; Carrera *et al.*, 2006; Zhang *et al.*, 2017). Starch is one of the most abundant substances in legumes, accounts for 30 ï 50%on the dry weight basis (Zhang *et al.*, 2017).

Haricot bean plays an important role in the economy of small farmers of Ethiopia. It is the most important traditional pulse crop (legume) in lowland areas of Ethiopia, particularly in the Rift Valley. Its grain is used for food and making money. Moreover, since it is short maturing (75-100 days),and has moderate drought tolerance (Melkasa Agricultural Research Centre, 2017), it is used as the main or the only food in short growing seasons and poor annual harvest areas. It plays a vital role in farmersô risk aversion strategies in Ethiopia (Bekele *et al.*, 2005).

Greater attention is being paid to the exploitation of haricot beans released from research centres in Africa, such as Melkasa Agricultural Research Centre, Ethiopia. This is in addition to the program for development of high-yielding, disease resistant varieties through adaptation, selection and hybridization (Shimelis *et al.*, 2006).

There are eight varieties of Haricot bean currently being adapted from abroad in Melkasa Agricultural Research Centre/Ethiopia. The research centre is located between 8º24ôN and

39º21ðEand at altitude 1550meter above sea level. Its Agro-ecological zone is arid to semi-arid; temperature is between 14ºC and 28.4ºC; average rain fall is 763 mm per annum; length of growing period (LGP) is 3-6 months and soil type is Andosol of volcanic origin with pH ranging from 7 to 8.2 (Melkasa Agricultural Research Center, 2017).

Fig.1.3.Someof varietiesof haricot bean currentlybeing adapted from abroad in Melkasa Agricultural Research Center(Photo by Abebe Y.).

Looking for new physicochemical and functional properties now a days it is a common practice to explore starch from non-conventional starch sources such as Legumes including Haricot beans. Polesi (2011) had extracted starch from chickpea & pea and characterized them in terms of the swelling power, amylose content, resistant starch content, total dietary fiber, thermal properties. Shimelis*et al.* (2006) had isolated starch from three improved varieties of haricot bean grown in Ethiopia and characterized them in terms of color, pH, chemical composition, swelling profile, etc. The improved haricot bean varieties that were used in this study had been released from Melkasa Agricultural Research Centre and identified

by their vernacular name as Roba, Awash and Beshbesh. The varieties had been popular food and export type of beans in the central rift valley of Ethiopia and East African countries at the time (Shimelis *et al.*, 2006).The crude starch yield of Roba, Awash and Beshbesh were 46.95 ± 0.89, 46.53 ± 0.68 and 48.77 ± 0.45,respectively. In the present study four varieties of eight currently being adapted in Malkasa research centre had been used namely: GLP-2, KAT-B9 (Dandesu), Dicta 105 (Nasir) and Awash-2.Wani *et al.* (2016) had summarized the composition and physicochemical properties of varieties legume starches.

1.3. Legume starch and its isolation

Moisture content, lipid, ash, and nitrogen content of the legume starches have been reported varying from 3.12ï 16.00%, 0.04ï 1.40%, 0.03ï 0.65%, and 0.00ï 0.43%, respectively (Wani *et al.*, 2016; Zhang *et al.*, 2017). The amylose content of legume starches varies from normal to high in the range of 17.00ï 51.69% (Wang*et al.*, 2015). The amylose content of Haricot bean starch in particular varies from 43-49% (Wani *et al.*, 2016).

The onset (T_o), peak(T_p) and end set (T_c) transition temperature of legume starches are in the ranges of 58.73 -61.96 °C, 64.07ï 68.35 °C, and 71.30ï 78.99°C, respectively (Zhang *et al.*, 2017). Scanning electron microscopy has revealed that legume starch granules have smooth surface and exhibit typically large oval to spherical shape. Low swelling, high solubility, and their resistant to retrogradation process of several legume starches indicate their higher functional properties as compared to cereal and potato starches (Ratnaningsih *et al.*, 2016).

Starch isolation from legume seeds follows a different procedure from a well established procedure of extraction of starch from cereals and tubers mainly due to the co-precipitation of flocculent proteins and fine fibers constituents along with the starch sediment (Wani*et al.*, 2016).Three wet milling methods had been devised and described by Schoch and Maywald(1968) for the extraction of starch from legumes. Thus, one needs to select the optimum method from these methods by trial and error for a particular legume first from the easiest method.

Method A: this is the simplest of the three methods and involve steeping of legumes for some time (for example, overnight) in warm (cold) water (first wetting with toluene is usually employed, toluene is basically employed to prevent fermentation and thereby avoiding

theacidic conditions which ultimately lead to acid hydrolysis of starch), grinding, and sedimentationin water medium. Then, the legumes are screened. The method is basically employed for legumes which are easily processed such as Mung beans, Garbanzos, Dehulled split yellow peas.

Method B:method B is the same as method A except that 0.2% sodium hydroxide solution is employed to digest insoluble flocculent protein and highly hydrated fine fiber present in some legumes (such as Lentils, lima beans and white navy beans) which will slow down sedimentation and co-settle with starch to give a light lose deposit. In method B the legumes will be steeped in water, ground, and screened as method A. Then the aqueous slurry will be allowed to settle overnight, the supernatant liquid siphoned off, and the sediment will be re-suspended in 0.2% sodium hydroxide solution to digest out any protein or fiber left. The final alkaline deposit of starch will be suspended in distilled water; the pH will be adjusted to 6.0 with 0.1 N HCl and then thoroughly washed with water. Some legumes such as wrinkled-seed peas because they will not be soften by steeping in water they will be steeped in alkali solution (0.3% NaOH solution) at the start. This method is known as method C. Method C is otherwise the same as method A and B (Schochand Maywald, 1968).

1.4.Colorectal diseases

Inflammatory bowel disease is the most disabling disease entity and CRC is the most killer disease among the diseases that occur in the colonic & rectal segments of the gastrointestinal tract (Thornton et al., 1981).

Inflammatory bowel disease (IBD) is usually characterized as Crohn's disease and Ulcerative colitis which involve spontaneous and uncontrolled inflammation of intestinal mucosa once they are activated. While UC is limited in Colon area, CD affects the whole part of GIT. In 2012, global prevalence of IBD had reached to 36.9 per million persons. The highest annual incidence as well as prevalence was also reported in Europe (incidence: 2.43 per million persons for UC; 1.27 per million persons for CD, prevalence: 50.5 per million persons for UC; 32.2 per million persons for CD) (Kondamudi *et al.*, 2013).

Globally, CRC is the third most commonly diagnosed cancer in males and the second in females, with 1.4 million new cases and almost 694,000 deaths estimated to have occurred in

2012(Kondamudi *et al.*, 2013). Global, country-specific incidence and mortality rates are available in the World Health Organization website. WHO (2014) had reported that CRC mortality rate in male (11.2%) is the second after Leukemia (12.7%) whereas in females (4.8%) is the fifth after Breast cancer (24.4%), cervix-uteri cancer (17%), ovarian cancer (7.2%) and Leukemia (5.3%) in Ethiopia. Whereas, as per Addis Ababa Cancer Registry of Ethiopia (2014) colorectal cancer is the most killer cancer disease (19%) in males and is the fourth most common (5%) in females after Breast (33%), Cervix-uteri (17%)and ovarian (6%) cancers in Addis Ababa.

1.5. Controlled drug delivery systems

Controlled release delivery is available for many routes of administration and offers many advantages over immediate release delivery. In order to achieve efficient disease management, the concentration of released drugs from a dosage form should be within the therapeutic window; with minimal fluctuation over prolonged periods of time at the intended site of action (Mansour *et al.*, 2010). Generally, two different approaches are used to achieve the desired controlled (modified) drug release profile, i.e., matrix and reservoir (film coated) systems, the latter forming the target of this work (Grund *et al.*, 2013).

Matrix tablets are tablets in which the drug is embedded in a functional carrier (Chen *et al.*, 2007). Matrix tablet is one of the most convenient approaches for the preparation of the sustained release dosage forms. In actual practice, mixture of drug, retardant material, and additives is directly compressed to form a tablet in which drug particles are embedded in the matrix core of the retardant. Dry or wet granulation technique may also be employed for the preparation of this type of tablets. Among the different strategies to prolong the drug action, formulation of matrix tablet has gained immense popularity now days because it has the advantage of simple processing and a low cost of fabrication (Bose *et al.*, 2013).

The polymeric carriers used for matrix system drug delivery can be grouped into: water soluble/erodible, water insoluble/ swellable or water insoluble/ non-swellable polymers by considering the solubility and swelling characteristics of the carrier materials. Generally, the dissolution medium penetrates the matrix tablets more or less hindered by the functional

excipient that makes the carrier. The tablets deliver the drug in a sustained fashion due to their barrier-free structure(Grund *et al.*, 2013).

Film system consists of a drug containing core and a release controlling barrier. Film coatings have routinely been applied to multi-unit systems such as pellets. By virtue of their small size and divided properties, coated pellets provide a large surface area for enzymatic attack, which should lead to rapid and consistent drug release. This has been confirmed by a number of studies both *in-vitro* and *in-vivo* (Chen *et al.*, 2007).Film coated single-unit systems, tablets, provide an alternative and more common platform for oral modified-release drug delivery, primarily because of their ease and low cost of manufacture, and can be made to release high molecular weight compounds such as peptides and proteins (Wilson and Basit, 2005; Chen *et al.*, 2007).

1.5.1. Film coated tablets as drug delivery systems

Film Coated tablets consist of a drug containing tablet core and a release controlling barrier. According to the nature of the film coating polymer two types of release mechanisms can be distinguished(Fig. 1.4).In case of soluble/erodible polymers, the coating prevents medium penetration to the core until it is dissolved or eroded, liberating the drug containing core. In case of insoluble polymers, medium penetration and drug diffusion through the coating are limiting factors. Drug is released by diffusion through pores and cracks in the polymer network, which initially exist or form due to dissolution of soluble compounds such as Hydroxy Propyl Methyl Cellulose (HPMC) (Grund *et al.*, 2013).

Formulation and manufacturing of film coated tablets are well known and established processes resulting in highly reproducible drug release. Film coating almost always utilize a spray atomization technique. In the spray process, coating liquid is finely atomized and delivered in droplets sufficiently fluid to wet the surface of the core being coated, spread out and coalesce to form film. Film-coating solutions may be non aqueous (organic solvent based) or aqueous (water solvent based) (Allen LV *et al.*, 2011).

Many quality aspects of the final film coated products are greatly influenced by the combined effect of process parameters. Coating process parameters that at most affect efficiency of coatings, that is, the spreading, penetration and drying of the coating liquid on the tablet

surface and subsequently, the surface roughness and residual moisture of coated tablets are Atomizing/ spray air pressure, inlet air temperature, flow rate of the coating solution and pan speed.

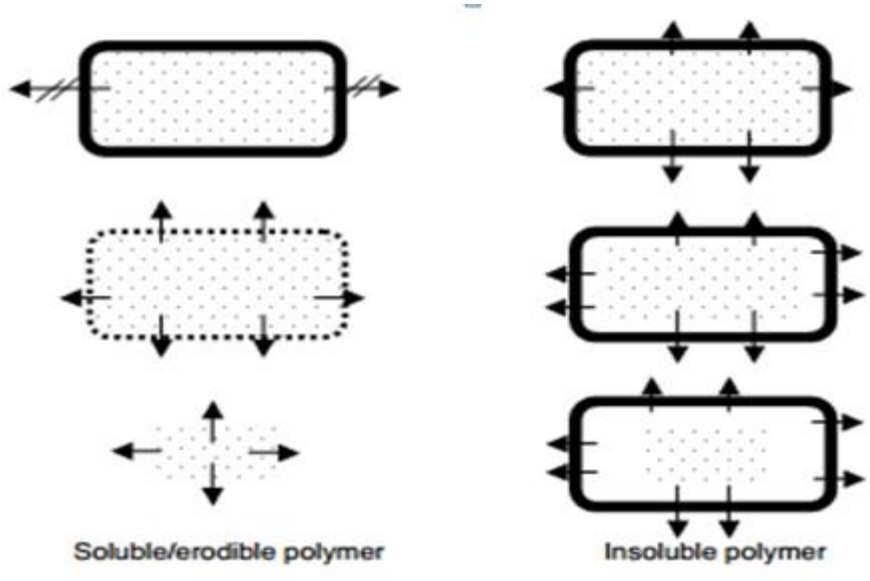

Fig.1.4. Release behaviour of coated tablets with soluble/erodible or insoluble polymers (adapted from Grund *et al.*, 2013)

1.5.2. Colon as a drug delivery site

Colon is being evaluated as a site for drug delivery, not only for local colonic pathologies but also for systemic delivery of small molecules and for delivery of protein and peptide drugs(Sinha and Kumria, 2003; Madhu *et al.*, 2012; Deore *et al.*, 2013).Oral colon targeted controlled release delivery systems have been proven useful for systemic action of bioactive polypeptides such as insulin, calcitonin, and metenkaphalin (Freire *et al.*, 2009; Karrout*et al.*, 2009; Chen*et al.*, 2011).This site may be useful in treatment of diseases susceptible to diurnal rhythm such as asthma, arthritis, etc. As a site for drug delivery, colon offers a near neutral pH, reduced digestive enzymatic activity, a long transit time and an increased responsiveness to absorption enhancers (Sinha and Kumria, 2003; Madhu *et al.*, 2012).

Targeting drugs to the colon has two major implications in a number of colonic disorders, including the direct treatment of conditions such as IBD. Targeting drugs to the colon has two major implications in colonic disorders. If for example, mesalamine, a drug that used to treat

colonic pathologies by a surface action, is orally administered using a conventional pharmaceutical dosage form, the drug is rapidly released in the GIT and likely to be absorbed into the blood stream. This leads to elevated systemic drug concentrations and, thus, to an increased risk of undesired side effects and at the same time to low drug concentrations at the site of action in the colon, resulting in poor therapeutic efficiency (Freire *et al.*, 2009; Karrout*et al.*, 2009; Chen*et al.*, 2011).

Three approaches have been used for oral delivery of drug(s) to the colon: time dependent, pH dependent and bacteria-dependent delivery (Madhu *et al.*, 2012).In this study bacteria-dependent delivery was chosen.

1.5.3. Commercial strategies for oral colon-targeted delivery of mesalamine

Mesalamine or 5-aminosalicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammation of the digestive tract, ulcerative colitis and mild to moderate Crohn's disease. The mechanism of action of mesalamine is unknown, but appears to be topical rather than systemic. Mucosal production of arachidonic acid (AA) metabolites is increased in patients with chronic inflammatory bowel disease, and it is possible that mesalamine diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandins (PG) production in the colon (Food Drug Administration, 2015).

The current strategies to "protect" orally administered 5-ASA(Mesalamine)from absorption until it reaches the colon include the use of pro-drugs (such as Azulfidine (5-ASA linked to sulfapyridine by azo bond; available as a tablet) and Dipentum (5-ASA dimmer linked by azo bond; available as a gelatine capsule), delayed-release formulations, sustained-release formulations and more recently, sophisticated formulations that combine both delayed-release and sustained-release mechanisms. Both delayed and sustained release formulations currently in use for oral-colon targeted delivery are based on two polymers: Ethyl cellulose and Eudragit S/L polymers (Sandborn, 2018).

1.5.3.1 . Ethyl cellulose film coated system for colon delivery of mesalamine

Ethyl cellulose, a cellulose derivative where the hydroxyl group of cellulose is substituted with ethoxy groups, is an insoluble, non-swellable polymer and by itself impermeable to water. It is

generally regarded as safe, listed and monographs exist in the European, Japanese and United States Pharmacopoeia. Ethyl cellulose film coated products exhibits time dependent release profile by design or by formulation(Grund *et al.,* 2013).

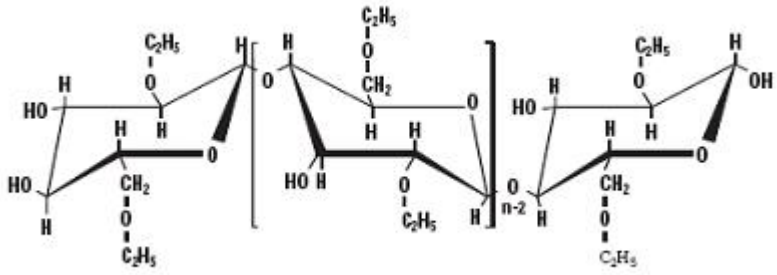

Fig. 1.5.The chemical structure of ethyl cellulose (Adapted from Grund *et al.,* 2013)

It is frequently used as coating material for delayed release or matrix polymer for sustained release because it forms a water penetration barrier. Drug is released by diffusion through pores and cracks in the polymer network, which initially exist by design or form due to dissolution of soluble compounds such as Hydroxy Propyl Methyl Cellulose (HPMC)(Grund *et al.*, 2013,).

Currently, Pentasa®, Ethylcellulose-coated mesalamine micro granules, that exhibit a time dependent release profile are commercially available as a tablet (250-mg and 500-mg), capsule (250-mg and 500-mg), or sachet (1000-mg). The sites of delivery of Pentasa® are Duodenum, jejunum, ileum and colon (Sandborn, 2018; Food and Drug Administration, 2015). Pentasa® is indicated for the induction of remission and for the treatment of patients with mildly to moderately active ulcerative colitis. The dissolution profile of Pentasa® is as follows: 5-25% dissolves at 1 h; 30-50% dissolves at 2h, 60-90% dissolves at 4 h and not less than 80% dissolves at 8h (Food and Drug Administration, 2015).

1.5.3.2. Eudragit Sand L film coated colon targeted delivery of mesalamine

Eudragit L and Eudragit S are anionic copolymers based on methacrylic acid and methyl methacrylate. The ratio of the free carboxyl groups to the ester groups is approx. 1:1 in Eudragit L and approx. 1:2 in Eudragit S. The monomers are randomly distributed along the copolymer chain. Both are available as ammonium salts. They are pH responsive, permeable

and swellable polymers. They are generally regarded as safe and monographs exist in the European, Japanese and United States Pharmacopoeia (Grund *et al.*, 2013).

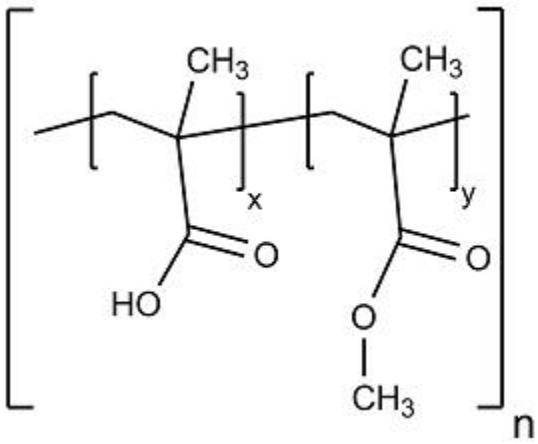

Fig.1.6. The chemical structure of Eudragit Land S (adapted from Evonik Industrial AG, 2012)

Currently three Eudragit coated mesalamine products are commercially available. Asacol®, Eudragit-S-coated tablet (400 mg) (release at pH Ó 7.0), sites of delivery terminal ileum and colon; Salofalk®, Mesasal® or Claversal®, Eudragit-L-coated tablets (250mg and 500mg) (release at pH Ó 6.0), sites of delivery distal jejunum, proximal ileum and Salofalk® or Granustix®, Eudragit-L-coated pellets (500mg sachets) with an additional retarding polymer in the pellet core (delayed and sustained release), sites of delivery distal small bowel and colon, are commercially available (Sandborn, 2018).

1.6. Starch as a pharmaceutical excipient

Starch is one of the traditional excipients in the pharmaceutical industry (Hartesi *et al.*, 2016). Relatively low cost, well-established standard manufacturing procedures of extraction, accessibility, biocompatibility, good *in-vivo* performance, their multi-functionality and amenability for modification make starch a good pharmaceutical excipient. Starches have been very useful in tablet production due to their utilization as fillers, binders and disintegrants (Olayemi *et al.*, 2008; Rashid *et al.*, 2011).Depending on the application, specific starches are available for use as disintegrants, fillers or binders (Hartesi *et al.*, 2016). Despite its vast commercial value as excipient, native starch has also some inherent weaknesses when it comes

to pharmaceutical applications, which include: poor compressibility, elastic compression behaviours and low flowability values. Since starch paste is recrystallized in a tablet during storage, there is also currently arising concern on the use of starch in tablets planned for immediate drug delivery (Hong *etal.*, 2016).

The main problem encountered with the use of native starches as a target delivery excipient is their easy digestibility by digestive enzymes and acid in the GI tract, so the limitation can easily be overcome by physical and chemical modifications and expand the application of starch as an excipient (Wilson and Basit, 2005; Karrout*et al.*, 2009; Chen*et al.*, 2011; Hong*et al.*, 2014).

1.7. Resistant starch

The term ñresistant starchò 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 treatment *in-vitro*(Sajilata *et al.*, 2006). Resistant starch (RS), thus, is the starch not hydrolyzed after 120 min of incubation with Ŭ-amylase and pullulanase. RS is now defined as the portion of starch and starch products that resist digestion, passing directly through the small intestine (Shukri *et al.*, 2015; Fabbri *et al.*, 2016).

Resistant starches have been classified in most literatures into four categories: RS1, RS2, RS3 and RS4.These are also called as type I, II, III, and IV resistant starches (Shi and Jeffcoat, 2003; Wilson and Basit, 2005; Dundar and Gocmen, 2014; Reddy *et al.*, 2014). In some literatures amylose-lipid complex has been considered as RS5 (Shukri *et al.*, 2015).

RSl: represents mainly physically inaccessible starches (those ñlockedò in plant cells) (e.g., starch embedded in a protein or fiber matrix, such as starch found in a whole grain and partly milled grains (Fabbri *et al.*, 2016). And also the large size of the granules (the small surface-to-volume ratio) of some starches has been used to explain portion of resistance of some native starches as RS1 such as high amylomaize starch (Sajilata *et al.*, 2006).

RS2: refers to starch that are intact, tightly packed crystalline native starch granules (e.g., raw potato or raw banana starch) (Sajilata *et al.*, 2006; Fabbri *et al.*, 2016). It is a characteristic of type B polymorph starches. The enlarged crystal lattice and compact structure of type B

polymorph limits the accessibility of starch to digestive enzymes (Vasanthan *et al.*, 1997; Fabbri *et al.*, 2016; Babu and Parimalavalli, 2018). Un-gelatinized native high-amylose starch is known to be high in RS2 content**.** In some literatures retrograded amylopectin has also been considered as RS2 (Vasanthan *et al.*, 1997). RS1 and RS2 represent residues of starch forms, which are digested very slowly and incompletely in the small intestine **(**Sajilata *et al.*, 2006).

RS3 is mainly retrograded amylose formed during cooling of gelatinized starch (Vasanthan *et al.*, 1997; Fabbri *et al.*, 2016). It is the most resistant of the natural resistant starches to hydrolyzing enzymes and moist-heat (Fabbri *et al.*, 2016).

RS4 is the RS where novel chemical bonds other than Ŭ-(1-4) or Ŭ-(1-6) are formed **(**Sajilata *et al.*, 2006). Chemical (Babu and Parimalavalli, 2018) or physical modification (particularly those involving thermal treatment) will create these additional bonds **(**Sajilata *et al.*, 2006). Modified starches obtained by chemical modification include for example, Di starch phosphate ester, acetyl, or hydroxyl-propyl substituted starch **(**Sajilata *et al.*, 2006).

Resistant starch is associated with many of the health benefits attributed to its dietary fiber nature or the production of short-chain fatty acid in the colon (EEerlingen, 1993; Fabbri *et al.*, 2016). Further, enzyme resistant starches are non-caloric ingredients due to their fiber nature and do not contribute to increase in blood glucose. The metabolism of RS occurs in 5 to 7 h after consumption, in contrast to normally cooked starch, which is digested almost immediately (Vasanthan *et al.*, 1997). Digestion over a 5 to 7 h period reduces postprandial hyper glycemia and insulinemia and has the potential for increasing the period of satiety. Therefore, consumption of a diet rich in RS is beneficial in the reduction of type II diabetes risk (Fabbri *et al.*, 2016). Resistant starches have also additional benefits such as inhibiting fat accumulation and allow greater apparent absorption of calcium and iron when compared to digestible starch (Szczodrak and Pomeranz, 1991; Polesi, 2011).

Some undesirable manifestations of RS have been also seen in clinical studies. One of the most alarming emergences is the cecal enlargement, especially in rats. But, in humans, it is said to be of little relevance because of the considerably smaller size and weight of cecum as well as its smaller role in the human physiological function. Pelvic nephrocalcinosis is another

phenomenon observed in rats especially those fed with mono-substituted cross-linked starches (RS4) (Sajilata *et al.*, 2006).

1.7.1. Type three resistant starch

RS3 resistant starch, a semi crystalline solid, has a crystalline region interspersed with amorphous region (Fig. 1.7). Both the crystalline and amorphous regions are integral part of a molecule. Originally, it was thought that only the crystal region is enzyme resistant. Later, it was realized that the amorphous region is also resistant to enzymes at least partially. Treating retrograded starch with enzymes such as Ŭ-amylase and amyloglycosidase decreases or at most removes enzyme sensitive portion of amorphous region and the residue left becomes highly/totally resistant to digestion (Iyengar*et al.*, 1991).

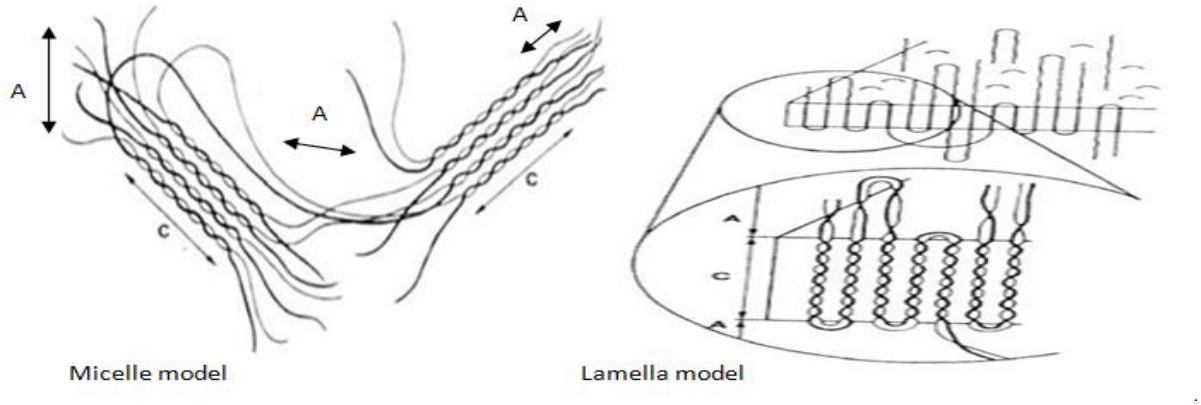

Fig. 1.7.The schematic presentations of RS3 (adapted from Sajilata *et al.*, 2006)

It is resistant to hydrolyzing enzymes because its densely packed crystal double helices and amorphous regions inhibit the diffusion of starch hydrolyzing enzymes into the region and their binding to starch chains (Vasanthan *et al.*, 1997). It is resistant to both boiling and pancreatic amylase enzyme attack, but it is fully or partially soluble in 2M KOH or Dimethyl sulphoxide and subsequently rendered accessible to amylolytic enzymes (Sivert and Pomeranz, 1989). This research work basically deals with this resistant starch.

1.7.2. Type three resistant starch preparation

Based on the current theory of RS3 formation, RS3preparation can be summarized under the term ñstarch retrogradationò or ñstarch re-crystallizationò (Sivert and Pomeranz, 1989). Formation of RS3 is achieved by gelatinization and subsequent retrogradation of starch (Kim and Kwak, 2009). Starch retrogradation is a process in which disaggregated amylose and amylopectin chains in a gelatinized starch paste re-associate to form more ordered structures (Wang *et al.*, 2015).

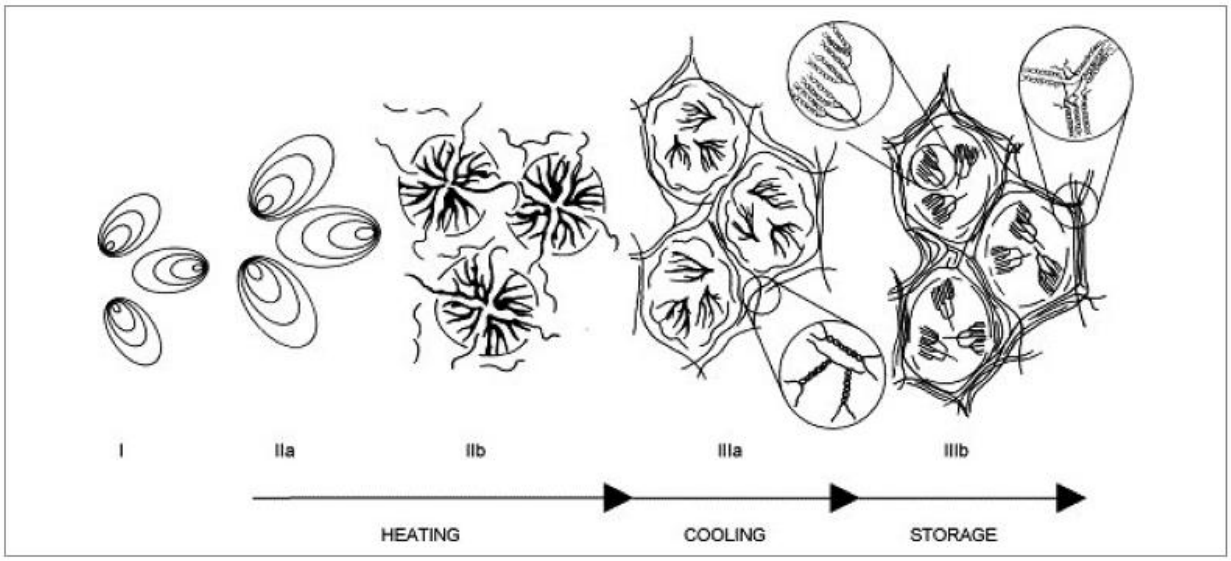

Fig.1.8.The schematic representation of the retrogradation process: (I) Native starch granules; (IIa &IIb) gelatinization, associated with swelling and amylose leaching and partial granule disruption, resulting in the formation of a starch paste; (IIIa) retrogradation: association of amylose chains to form double helix during cooling of the starch paste and & (IIIb) formation of ordered or crystalline amylopectin molecules (amylopectin retrogradation) during storage (Adapted from Sajilata *et al.*, 2006)

As illustrated in Fig. 1.8 above, in the formation of RS3, at start the starch granule is completely hydrated, amylose leaches from the granules into the solution as a random coil polymer, which upon cooling begin to re-associate as double helices, stabilized by hydrogen bonds. More precisely, when starch is heated to about 50 °C, in the presence of water, the amylose in the granule swells; the crystalline structure of the amylopectin disintegrates and the granule ruptures. The polysaccharide chains take up a random configuration, causing swelling of the starch and thickening of the surrounding matrix, this process is called gelatinization, a

process that renders the starch easily digestible. On cooling re-crystallization (retrogradation) occurs. This takes place very fast for the amylose moiety as the linear structure facilitates the hydrogen bonds between separate chains. However, the branched nature of amylopectin inhibits its re-crystallization to some extent and it takes place over several days (Sajilata *et al.*, 2006).

RS3 can be prepared by heat treatment, enzyme treatment and combined heat treatment & enzyme treatment, and chemical treatment. Studies have shown that acid (2.2 N HCl) preferentially hydrolyses starch chains in the amorphous regions of the native starch granule. This is because a conformational change (from chair to half chair) in anhydroglucose unit of starch is necessary for the acid hydrolysis of glycosidic bond. The anydroglucose unit in the crystalline regions would require a very high energy of activation for such conformational change, therefore, would resist acid hydrolysis. Since the branch points of amylopectin molecules exist in amorphous region of a starch gel, such conformational change will be possible.

RS3 formation, crystallization in amorphous matrix of amylose, involves three steps: 1) nucleation, i.e., formation of critical nuclei; 2) propagation, i.e., growth of crystals from the nuclei formed and 3) maturation, i.e., crystal perfection or continuing slow growth. The extent to which these processes occur is clearly dependent upon temperature (Fig. 1.9). The nucleation rate is 0 (zero) at the melting temperature of the crystals (T_m); it increases with decreasing temperature. The propagation rate is 0 (zero) at the temperature less than the glass transition temperature (T_g) of the amorphous matrix because diffusion does not occur at such temperatures. At higher temperatures, diffusion increases and so does the rate of propagation. At temperatures above Tm, the propagation rate is also 0 (zero). The maturation rate is dependent on temperature in a way similar to that of the propagation rate. The overall crystallization rate depends mainly on the nucleation and propagation rates. For a partially crystalline polymer system, therefore, crystallization can occur only in the temperature range between T_g and T_m, i.e, in the rubbery state. B-type polymorph starch gels containing more than 27% water (by weight) for example have a T_g of about -5 °C and Tm of the amylose is about 150 °C, therefore, crystallization of amylose can occur only between these temperature limits (EEerlingen, 1993).

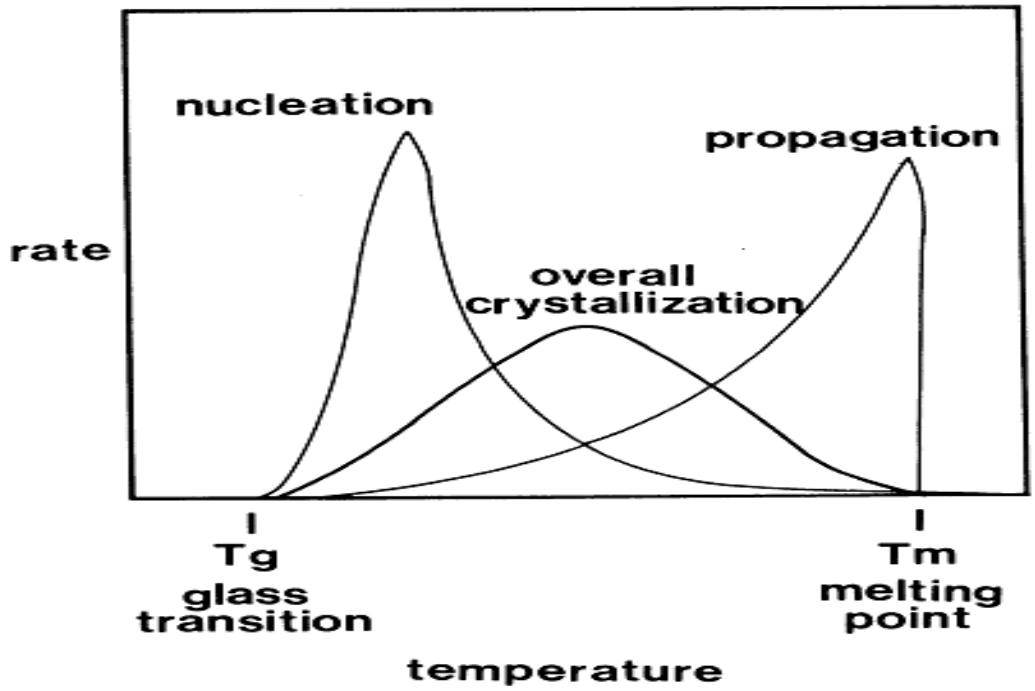

Fig.1.9. Dependence on temperature of the nucleation, propagation, and overall crystallization rates of partially crystalline polymers (Adapted from EEerlingen *et al.*, 1993)

The nucleation temperature also determines the nature of the resulting resistant starch. A nucleation temperature above the melting point of the amylopectin crystals avoid substantial production of lower melting amylopectin crystals and lower melting amylose crystals, and lower melting amylose-lipid complexes. The propagating temperature should also be above the melting point of any amylose-lipid complexes but below the melting point of the RS3. Many combinations of time and propagation temperature treatments had been used by different researchers to make RS3 from various sources of native starch. The temperatures that had been used by researchers for autoclaving (for propagation purpose) of starch includes 110 °C, 121 °C, 127 °C, 134 °C and 148 °C for periods ranging from 30 min to 1 h.

In general factors that determine nature of RS3 and yield of RS3 can be categorized in two: the inherent nature of the original starch and extrinsic factors (processing conditions) (Sievert and Pomeranz, 1989; EEerlingen *et al.*, 1993; Sajilata *et al.*,2006; Kim and Kwak, 2009).

1.7.3. Methods used to characterize type three resistant starch

As starch retrogradation is a complex process involving a series of molecular (including conformational change) and physicochemical events, a diversity of physical and chemical methods have been applied to investigate the changes that take place in starch properties (Table 1.1). These methods include various thermal, rheological, spectroscopic, chromatographic, X-ray diffraction (XRD), microscopic imaging techniques and others. These various techniques provide information on specific molecular characteristics or transitions, as well as on changes in the material as a whole. No single method can give a complete picture of retrograded starch at both macroscopic and molecular levels (Wang *et al.*, 2015).

Table 1.1 Methods currently in use to characterize type 3 resistant starch (adapted from Wang *et al.*, 2015, with some modifications).

Method type	Techniques	Properties measured
Thermal analysis	Differential Scanning Calrometery, (DSC)	Transition temperature, enthalpy changes (qH) of crystallite melting, changes in heat capacity of amorphous phase
	Differential Thermal Analysis (DTA)	Temperature differences between the sample and a reference during a thermal program
	Thermogravimetry Analysis (TGA)	The mass loss of the sample
Rheological analysis	Rapid Visco analysis (RVA)	Pasting viscosities during programmed heating and cooling of starch suspension
	Texture Profile Analysis (TPA)	Hardness, cohesiveness, adhesiveness, elasticity, and brittleness
Spectroscopic analysis	Fourier Transform Infrared (FTIR)	Order in crystalline regions and the state of organization of the double helices localized inside crystallites.
	Near Infrared (NIR)	Molecular changes of starch gel during retrogradation
	Raman	Internal Vibration of molecules
	Nuclear Magnetic Resonance (NMR)	Continuous monitoring of starch retrogradation at the molecular level
		Hydrogen-1Nuclear Magnetic Resonance (NMR) (^{1}H NMR) analyzes the mobility of starch polymer chains
		Solid state Carbon-13 Cross ï Polarisation /Magic angle Spinning Nuclear Magnetic Resonance (^{13}CCP/ MAS NMR) investigates molecular organization of starch granule at a short distance scale (double helices) and amorphous single chains
X-ray difraction	Wide Angle X-ray Diffraction (WAXD)	Long range ordered structure of starch
	Small Angle X-ray Scattering (SAXS)	Repetitive crystalline and amorphous lamellae
Microscopic	Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM)	Surface morphology of starch granules and retrograded starch gels
Physical methods	Turbidity Syneresis	Changes in density distribution of gelatinized starch paste
Other methods	Blue value	Amylose content of starch and RS3
	Resistant of starch to hydrolysis	Resistance of starch to enzymatic hydrolysis during retrogradation
	Swelling and Solubility	Swelling power measures the ability of the starch to hydrate, whereas, solubility measures the ability of starch solids to dissolve in an aqueous solution

1.8. Significance of the study

The relatively low cost of Haricot bean (short maturing that requires 75-100 days Melkasa Agricultural Research Center (2017), the simple extraction procedure of starch from Haricot bean and the reasonably high yield of Haricot bean starch (47-49%) (Shimeliset *et al.*, 2006) make the production of RS3 from Haricot bean and using RS3 as a film forming excipient practical. On the other hand the polymers currently in use for the same purpose are expensive as their manufacturing process is complex. Strip of 10 tablets of delayed/ sustained released formulation of mesalamine based on these polymers currently costs up to 220USD (Sandborn, 2018).

For the reason that it is physically modified starch, has high-specific surface area owing to its small particle size (Hong *et al.*, 2014) and has been used as food ingredient for different purposes such as for its aesthetic and health values for many years RS3 is presumably safe both to the environment and users.

It has been also documented that inter and intra subject pH variability is the major limitation of pH dependant target dosage forms. And abrupt (burst) release is a characteristic limitation of time dependent dosage forms due to poor in design of a polymer or coating formulation. Since retrograded starch based drug delivery exploits micro-biota that exists only in the colon it is expected that it will avoid such limitations.

1.9. Objectives of the study

1.9.1. General objective

To evaluate the potential of Haricot bean resistant starch for oral-colon targeted delivery of mesalamine as a film forming excipient in tablets.

1.9.2 Specific Objectives

- To prepare RS3Haricot bean resistant starch and characterize it; and
- To manufacture RS3 film coated Mesalamine tablets and evaluate their *in-vitro* release profiles in simulated GI environments.

2. Materials and methods

2.1.Materials

Four different varieties of Haricot bean (Dicata-105 (Nasir), GLP-2, KAT-B9 (Danedsu), Awash-2) were kindly donated by Melkasa Research Centre, Ethiopia. Pullulanase (Batch number: SLBK 7548v, Sigma-Aldrich/USA), Ŭ-Amylase (Batch number: SLBN2925v, Sigma-Aldrich/USA), amyloglucosidase (Batch number: SLBR2692v, Sigma-Aldrich/USA) and Pepsin Porcine Gastric mucosa (Batch number: BCBR 3132v, Sigma Aldrich/Switzerland), Meslamine (model drug) (Batch number: 20170601, Hubei Kangbaotai Fine Chemical Co., Ltd/China) and Meslamine Reference standard (Lot number: E1515005, Aladdin/China) were purchased from China. Hydrochloric acid, Acetic acid, Sodium acetate , Sodium hydroxide, Ethanol, Whatman no 41 filter paper and Mono basic potassium phosphate were purchased from the local market. Polyvinyl Pyrrolidone (PVP), Lactose monohydrate, Crospovidone, Aerosil, Magnesium stearate, Ethyl cellulose and Propyl glycol and Monobasic potassium phosphate were kindly supplied by Ethiopian Pharmaceutical Manufacturing Share Co (EPHARM). Pancreatin (Batch no: 15348703, Germany) was kindly shared by a friend, Mr Yohannes Teshome. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Starch extraction

The first four varieties of Haricot beans in terms of their relative yield per hectare were selected among the eight Haricot beans currently being adapted in Ethiopia from abroad in Melkasa Agriculture Research Centre. After the preliminary study, method C of the three methods devised by Schoch and Maywald (1968) was used to extract starch from the four selected varieties of Haricot beans. One hundred grams of each of the four varieties were washed, stepped in 0.3% sodium hydroxide solution to wet beans for 6 h and then the pulp (the upper cover) was removed. Haricot beans without pulp were washed three times with plenty of distilled water to remove the pigment and left to dry in open air, weighed and were stepped to wet again in 0.2% sodium hydroxide solution for about 5 h. Wet milled, filtered and were stepped in 0.2% Sodium hydroxide solution overnight (to digest out the flocculent proteins

and fine fibers).The filtrates were siphoned-off three to four times using 0.2% sodium hydroxide solution, the pH adjusted to neutral using 0.1N HCl and were siphoned-off three to four times using distilled water. The sediments were dried overnight and weighed. The gravimetric method was used to determine the crude starch content according to the Eq. 2.1.The extraction was done for each of the varieties in triplicate. Haricot bean with high starch yield was selected for additional starch extraction, which then was used for RS3 preparation.

$$\% \text{ of Crude Starch Yield} = \frac{\square\square\square\ \square\square\square\square\square\square}{\square\square\square\ \square\square\square\square\square\square\square\square\square\square\square} \times 100\% \qquad (2.1)$$

2.2.2. Resistant starch (RS3)preparation and characterization

2.2.2.1.Resistant starch (RS3) preparation

Sievert and Pomeranz (1989) method with some modification was used to prepare and isolate RS3 from KAT-B9 (Danedsu) haricot bean starch. The main modifications were gelatinization (disruption of starch granules) at start to make 1, 6-Ŭ glycosidic bond accessible for the enzyme (Pullulanase), de-branching to enhance the yield and rate of formation of RS3 and the 24 h incubation employed instead of 12 has was in Sivert and Pomeranz (1989) method.

Briefly, 200 g of (KAT-B9 (Danedsu)) haricot bean starch was dispersed in distilled water in the ratio of 1:4 and autoclaved for about 1 h until complete disruption of starch granules (P= 1.05kg/cm^2/15 psi, T= 121°C). The pH (to 5, using acetate buffer) and Temperature (to 60°C) were adjusted and starch was de-branched using Pullulanase (1500 new pullulanase unit (NPU)/kg starch). The enzyme was deactivated by heating at 75°C in water bath. The product obtained in the preceding stage was autoclaved for 1 h (T=121°C), removed from the Autoclave, cooled to room temperature and then stored in refrigerator for24 h at 3°C. This step was repeated 10 times. The pH and temperature of RS3 containing slurry formed at the preceding stage was adjusted (pH 5, using acetate buffer, T= 100°C) and treated with heat stable alpha amylase (0.5 International unit (IU)/1g starch, time= 30min); then the temperature

adjusted to 60°C and treated with amyloglucosidase (132IU/kg of starch)to digest out enzyme sensitive starch (starch residue & enzyme sensitive portion of RS3(portion of the amorphous region of RS3); and then the pH (to 7.5, using phosphate buffer) and temperature (to 60°C) were adjusted and treated with pepsin (0.1IU/mg starch) to digest out protein that might be present. The enzyme was deactivated(using 80% ethanol), then washed five times with hot water (90°C) and the residue left to dry in oven (40°C) overnight. In all events the temperatures were adjusted in water bath. The acetate and phosphate buffer were prepared according to USP 30/NF 25 (2007). The dried residue material had been considered as RS3 (resistant starch) (Sivert and Pomeranz, 1989). The preparation was done in triplicate. The yield was calculated according to the Eq. 2.2.

$$\% \text{ of RS3 Yield} = \frac{\text{[illegible]}}{\text{[illegible]}} \times 100\% \qquad (2.2)$$

2.2.2.2.Fourier transform infra-red spectroscopy

KBr-pelletized KAT-B9 starch and its resistant starch samples were analyzed using FTIR spectrometer (Shimadzu-model FT-IR-8400S, Japan) in the range of 4000-400 cm^{-1} at 22ºC. The number of scans and resolution of FTIR spectroscopy of both samples were 20 and 4($1cm^{-1}$), respectively.

2. 2.2.3. X-ray powder diffraction

KAT-B9 native starch and its RS3 (resistant starch) powder samples were analyzed using X-ray diffractometer (Miniflex - model 600, Japan).The X-ray was Cu Kα radiation; samples were run from 5º to 40º (2d) Bragsô angle at 40 kV and 15 mA; at a scanning speed of 5º/min. Match! 3, crystal impact software was used to record the XRPD pattern.

2.2.2.4. Scanning electron microscope

Morphology of particles of Haricot bean RS3 was determined by scanning electron microscope (SEM) (JEOL-model JSM-IP 300 LV, USA).An accelerating potential of 20 kV

was used during micrography. Images were taken at 250, 500, 1000 and 2500 times magnifications.

2.2.2.5. Swelling power and solubility index

Swelling power (SP) and water solubility index (WSI) of both starch and its resistant starch were determined following the method adapted by Weldegiorgis*et al* (2016).A 0.5 g dry powder sample was mixed with 10 ml distilled of water in pre-dried and weighed centrifuge tubes. After heating for 30 min in water bath to room temperature (22°C), 37°C, 55°C, 65°C, and 85°Cwith shaking every 5 min, the heated solutions were centrifuged at 3,000g for 15 min. The supernatant decanted in pre-dried and weighed plate and dried in an oven for 2h at 130°C. The residue obtained after drying the supernatant (W1) represents the amount of starch solubilised in water at that particular temperature. The residue left in centrifuge tube (Ws) represents the precipitate at that particular temperature.Values was determined in triplicate. The water solubility index (WSI) and solubility power (SP) were determined according to Equations 2.3 and 2.4, respectively.

$$\text{WSI (\%)} = \frac{W1\ X\ 100}{Dry\ sample} \qquad (2.3)$$

$$\text{SP} = \frac{Ws\ X\ 100}{Dry\ sample\ (100-WSI)} \qquad (2.4)$$

2.2.3.Preparation of RS3 – EC film coated Mesalmine tablet

2.2.3.1. Preparation and characterization of Mesalamine granules

i. Preparation of Mesalamine granule by wet granulation

Mesalamine (Active substance) (1000g, 67.8%) powder was blended with (357.3 g, 24.2%)of lactose monohydrated (filler)powder. A viscous binder-disintegrant suspension of Poly vinyl pyrrolidone (PVP) (84 g, 5.7%)as a binder and Crospovidone as disintegrante (33 g, 2.3%) was made by mixing them and continuously stirring in a sufficient quantity of ethanol. Wet mass of Mesalamine was made by adding bit by bit the binder-disintegrant suspension into

previously prepared powder blend in planetary mixer. The wet mass was passed through sieve (US mesh number 10, sieve size of 2 mm) to prepare wet granules of Mesalamine. The resulting wet granules of Mesalamine were dried in an oven dryer at 50°C for about 2 h until the moisture content was 3 ± 0.2%.Then, the dried granules were passed through sieve (US mesh number 16, sieve size of 1.18 mm).

ii. Characterization of Mesalamine granules

Bulk and Tapped densities

Bulk and tapped densities were determined by taking thirty grams of granules in a 250 ml measuring cylinder and bulk volume occupied was read after light tapping. The cylinder was then tapped at a constant velocity using tapped densitometer (ERWEKA, Germany) for 500 times and then tapped volume was recorded. Bulk and tapped density were determined using Eq. 2.5 and 2.6, respectively. The experiments were run in triplicate.

$$\text{Bulk density} = \frac{Weight\ of\ granules}{Volume\ of\ packing} \qquad (2.5)$$

$$\text{Taped density} = \frac{Weight\ of\ granules}{Tapped\ volume\ of\ packing} \qquad (2.6)$$

iii. Hausner' ratio and Carr's index

Hausnerôratio and Carr's index were calculated using Eq. 2.7 and 2.8, respectively

$$\text{Hausnerôratio} = \frac{\square\square}{\square\square} \qquad (2.7)$$

$$\text{Carr's index} = \frac{\square\square\square\square}{\square\square} \times 100 \qquad (2.8)$$

Where$\square\square$= tapped density, $\square\square$ = bulk density

iv. Angle of repose

The angle of repose of the granule was measured by fixed height funnel method. Thirty grams of granule was placed and allowed to flow through a stemless funnel having 10 mm aperture from a fixed height of 10 cm. The Angle of repose was determined according to Eq. 2.9

$$\theta = \tan^{-1}(h/r) \qquad (2.9)$$

Where h = height of the granule cone, r = radius of the granule cone

2.2.3.2. Fabrication and characterization of Meslamine core tablets

i. Fabrication of Mesalamine core tablets

Aerosil (Glidant) (16 g, 1%) and Magnesium stearate (Lubricant) (16 g, 1%) were passed separately through a sieve (US mesh no = 50, sieve size of 300 µm) and blended with Mesalmine granules. The blend was compressed in to tablets using 16 stations laboratory scale rotary compression machine with concave 8/32 inch punch size to have tablets of 220-230 N hardness.

ii. Weight variation test

USP weight variation test was run by weighing 20 tablets individually in an electronic balance, average weight was calculated and standard deviation was determined as per USP <905>.The measurement was run in triplicate.

iii. Friability test

Ten tablets (with average weight = 670mg) were evaluated by placing at the same time in Friabilator (Model ERWWEKA TDR-100, Germany) which was operated at 25 rpm for 100 revolutions as per USP < 1216> . The tablets were de-dusted and weighed. Percent friability was calculated using Eq. 2.10. The test was run in triplicate.

$$\% \text{ Friability} = \frac{Initial\ weight - Finalweight}{Intial\ weight} X\ 100 \qquad 2.10$$

iv. Crushing strength, Thickness and Diameter test

Crushing strength, Thickness and Diameter of ten tablets were measured by ERWEKA Hardness tester (Model ERWEKA TBH-120, Germany) each at a time. Each measurement was done in triplicate.

v. Tensile strength

The radial tensile strength was calculated using the data obtained from hardness, thickness and diameter of the tablets using Eq. 2.11.

$$\sigma_x = \frac{2F}{\pi DT} \qquad 2.11$$

Where σ_x =tensile strength, F = Hardness, D = Diameter, and T = thickness.

vi. Disintegration test

Six tablets were placed in each of the six open ended transparent tubes of the basket-rack assembly of USP Disintegration test apparatus (Model ERWEKA DT 700 GmbH, Germany) and the basket - rack assembly was placed in a 900ml beaker of water at 37 ± 2 °C and run at 28-32 cycles per min for 10min as per USP < 701> .

2.2.3.3. Preparation of RS3–Ethylcellulose film formulation

Three different formulations of coating suspensions in terms of RS3 and Ethyl cellulose ratio were prepared according to the following formula (Table 2.1). Each of the coating formulations was made first by mixing ethyl cellulose, RS3 and propylene glycol in accordance with their corresponding formula in80mlof ethanol, stirring for 6 h using a magnetic stirrer and milling using Colloid mill(type N 60, Germany) for 5 min and were used

instantly for film coating. Each of the coating formulations was used to coat previously prepared tablets in two different film thickness (4% and 8% mass gain).

Table 2.1.RS3 ï EC Film Coating Formulations

Formulation	RS3:EC[1]	Propylene glycol[2]	Total mass gain (%)	Weight of film formulation in 80 ml ethanol (g)
F_{11}	2:1	10% of the formulation	4	29
F_{12}			8	38
F_{21}	1:1		4	29
F_{22}			8	38
F_{31}	1:2		4	29
F_{32}			4	38

[1] Resistant starch to Ethyl cellulose ratio (sum of which make 90% of total weight of the formulation)

[2]Density 1.04g/ml (plasticizer, making 10% of total weight of a formulation)

2.2.3.4. Film coating of Meslamine core tablets

Tablets weighing 190 g were placed in coating pan which was previously and continuously heated during the coating process with an inlet air temperature of 60 °C and rotating at4 rpm/min. Film coating suspension was sprayed directly in to the tumbling tablets (with spry air pressure = 55 psi and flow rate = 150 ml/min).Six batches of film coated tablets were made, which differ either in RS3/EC ratio or film thickness (Table 2.1). Then, uniformly coated tablets from each batch were selected, with a mass of 698mg±1%(for 4% total weight gain) and 720mg±1% (for 8% total weight gain) for subsequent *in-vitro* GI and fermentation drug release profiling.

2.2.4. *In vitro* drug release study

2.2.4.1. UV calibration curve of Meslaminein Hydrochloric acid media (pH 1.1)

A stock solution of 100ml containing200 µg/ml Meslamine reference standard in 0.1N HCl solution (pH 1.1) was prepared. From this stock solution, six50 ml of different concentrations (4, 8,12,16,20 and 24 µg/ml) were prepared by diluting 1, 2, 3, 4, 5, and 6ml of the stock solution to 50 ml, respectively. The UV absorbance readings of these solutions were measured at 331 nm using UV/Visible spectrophotometer (Shimadzu-model UV-1800,Japan). 0.1N

Hydrochloric acid solution (pH1.1) was used as a blank. The absorbance versus concentration of the solutions was plotted, and a linear regression equation was obtained.

2.2.4.2. UV calibration curve of Meslamine in phosphate buffer media (pH 7.2)

A stock solution of 100 ml containing 150 µg/ml Mesalamine reference standard solution in phosphate buffer (pH 7.2) was prepared. From this stock solution, five 50 ml of different concentrations (3, 6,9, 12, 15,18 µg/ml) were prepared by diluting 1, 2, 3, 4, 5, and 6 ml of the stock solution to 50 ml, respectively. The UV absorbance readings of these solutions were measured at 331 nm using UV/Visible spectrophotometer (Shimadzu- model UV-1800, Japan). Phosphate buffer (pH 7.2) was used as a blank. The absorbance versus concentration of the solutions was plotted, and a linear regression equation was obtained.

2.2.4.3. *In-vitro* GI profiling

In-vitro drug release from the film-coated tablets was assessed using USP type II paddle dissolution apparatus. The tests were performed at a paddle rotation speed of 50 rpm in 900 ml dissolution medium at 37.0±0.5 ŮC. The pH and nature of the dissolution medium were varied over the course of the experiment: pH 1.1, hydrochloric acid with and without 0.32% Pepsin for 3 h, followed by pH 7.2, phosphate buffer with and without 1% Pancreatin for 4 h. The dissolution media were prepared according to USP 30/NF 25 (2007). The quantity of drug released from the dosage form was investigated at 30 min interval in 0.1 N HCl (for 3 h)and at hourly interval in simulated intestinal medium (for 4 h) by UV spectrophotometer(Shimadzu UV-model UV-1800, Japan).2ml of sample was taken at each sampling time which was diluted to 50ml to determine concentration against blank medium (0.1 N HCl in the first 3 h and in pH 7.2, for the next 4 h).Absorbance readings were taken in triplicates at each sampling point. The results were expressed as cumulative percentage drug release versus time profiles. Only four extreme formulations in terms of resistant starch/ethyl cellulose content were evaluated in the presence of enzymes. Photographs were taken at 3 and 6 h of the dissolution process.

2.2.5. Fermentation study

The colon release profiles of four extreme formulations (F11, F12, F31 and F32) in-terms of RS3/EC ratio from the six formulations under the study were assessed in condition simulating the human colon by the method described by Wilson and Basit (2005).One tablet was introduced into previously prepared individual 100 ml batch culture fermenter 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 media, pH7.2. The fermenters were first placed in an aerobic jar (a jar equipped with a candle) and the jar was sealed. Then, when the ignited candle was extinguished the anaerobic jar was placed in an incubator with shaker at37°C and shaken at 100rpm. Two millilitre samples were withdrawn at hourly intervals over a 12h period, centrifuged at 13,000 rpm for 5 min, the supernatant filtered through Whatman No 41filter paper, then 100µl of the filtrate was diluted to 50ml. The absorbance of each of the formulations at each sampling points was measured at 331 nm using UV/Visible spectrophotometer (Shimadzu- model UV-1800, Japan). The concentration of each of the formulations at each sampling point was determined using previously prepared Concentration versus Absorbance calibration curves. Each experiment was run in triplicate. The results were expressed as cumulative percentage drug release versus time.

2.2.6. Statistical analysis

Statistical analysis was performed using Analysis of Variance (ANOVA) and Student T test with statistical software Origin 8 (Origin LabTM Corporation, USA)

3. Results and Discussions

3.1.Native Starch Yield

Very fine white powders of starch were obtained from all four varieties of Haricot bean currently being adapted in Ethiopia from abroad. The yields were in the range of 30.8 -44.2% per dry mass of haricot beans (Table 1), which were lower than what had been reported by Shimelis*et al.* (2006), 46.5-49-9%, for the other varieties of Haricot bean. KAT-B9 (Danedsu) variety Haricot bean had the highest yield. Accordingly, further starch extraction was made from KATï B9 (Danedsu) Haricot bean, for the production of Resistant starch.

Table 3.1The percentage starch yields of the four varieties of Haricot bean

Variety	[a]Seed Size	[b]Seed Color	[c]Yield per hector(t)	Starch (%)
Dicta-105 (Nasir)	Small	Red	2.0-3.2	30.9 ± 0.1^{e}
GlP-2	Large	Red mottled	2.0-3.0	36.9 ± 0.3^{f}
KAT-B9 (Dandesu)	Medium	Red	2.2-3.0	43.0 ± 1.2^{g}
Awash-2	Small	White	2.8-3.1	35.2 ± 0.3^{h}

- [a-c] superscripts refer the source of the data is Melkassa Agriculture Research Centre
- [e-h] different superscript letters indicate the mean yields are significantly different at P = 0.05 level, Values are in mean ± standard deviation, n=3,unpaired student t-test was used for statistical analysis of the different starch yields

3.2.Resistant starch Preparation and Characterization

3.2.1. Resistant starch yield

There is cumulated scientific evidence that the percentage yield of Resistant starch (RS3) depends on the inherent nature of the native starch and extrinsic factors (processing conditions).

However, the quality (characteristics) of the prepared Resistant starchis largely depends on the processing condition than the inherent nature of the native starch.

The inherent natures of the native starch that have a considerable influence on the percentage yield of RS3 include: the amylose content of the starch, the degree of polymerization of amylose present and the polymorph form of the original starch used for the purpose. Sievert and Pomeranz (1989) had reported that the RS3 percentage yield correspondingly increases with amylose content. Between 10 and100 degree of polymerization (DP) of amylose is necessary to form the double helix and hence RS3 formation (Sajilata *et al.*, 2006). The polymorph form of the original starch also influences the yield of RS3 upon retrogradation. For example, the starch polymorph of beans (type C) is more stable compared to the polymorph of cereal grains (type A), processing cereal grains accordingly results in a large decrease in RS3 content, while legumes (type C) are excellent sources of resistant starch (Ratnaningsih *et al.*, 2016).

Extrinsic factors (processing conditions) that have a significant effect on RS3 extent of formation include: starch-water ratio in the slurry, nucleation and propagation temperature, incubation (storage) time, the number of freeze-thaw cycle and the drying method used at the end stage of RS3 production. Sievert and Pomeranz (1989) had reported that the optimum starch to water ratio in the slurry and autoclaving temperature for retrogradation process are ~1:3.5 and 121 °C, respectively. They had also reported that the RS3 yield increases first rapidly up to 10, then slowly up to 20 freeze-thaw cycles and then level off. Kim and Kwak (2009) had reported that the RS content increased linearly with increasing the number of autoclaving-cooling cycles at an autoclaving temperature of 127°C. Szczodrak and Pomeranz (1991) had reported that the freeze drying process increases the yield of RS3.EEerlingen *et al.* (1993) had reported that in the first 200 min of incubation at relatively low temperature (0 °C) better yield resulted than at higher temperatures (68 and 100 °C), and after 200 min the result was otherwise.

The processing conditions also have a considerable impact on the characteristics (qualitative properties) of the produced RS3. For example, on its thermal properties, on its SEM image feature, and on the polymorph form of RS3. Degree of compactness increases and porosity decreases with increasing the number of freeze-thaw cycle (Sievert and Pomeranz, 1989, EEerlingen *et al.*; 1993).EEerlingen *et al.*(1993) had reported that incubation at relatively low

temperature (0 or 68 °C) favours formation of B-polymorph resistant starch whereas incubation at relatively high temperature (100 °C) favours formation of A-polymorphic, which is the most stable polymorph form of RS3,regardless of the nature of the original native starch.

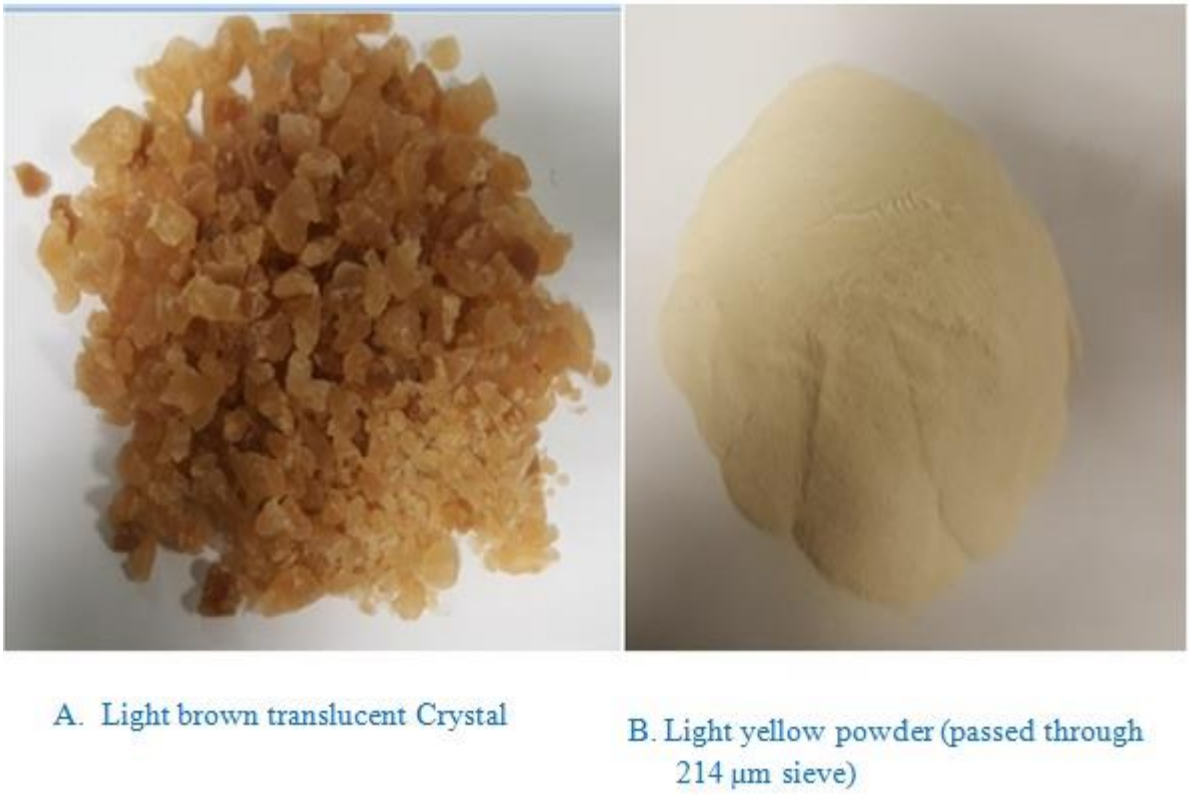

Fig. 3.1. RS3 resistant starch of Haricot Bean (Photo by Abebe Y.)

In this study light brawn translucent semi-crystals of RS3 were formed (Fig. 3.1A). The percentage yields were from 74.7-78.0%. The inherent characteristics of Haricot bean starch(for example, its C_A polymorph form, as we will see it afterward in this study)and the process conditions such as the de-branching step employed, the 1:4 proportion of starch and water in the starch slurry, the relatively large number of freeze-thaw cycles (10 cycles), the low storage (nucleation)temperature (3°C), the long storage time (24h) and the relatively high autoclave (propagation) temperature employed (~121°C) may have contributed for such high yield of RS3(Sievert and Pomeranz, 1989; EEerlingen *et al.*, 1993; Sajilata *et al.*,2006; Kim and Kwak, 2009). This was also in agreement with a lot of United States Patent claims, such as Iyengar *et al.* (1991), Chiu *et.al.* (1994), Henley and Chiu (1995), Seib and Woo (1999), Thompson and

Brumovsky (2002) and Shi and Jeffcoat (2003).They had claimed percentage yields in the range of 25-80%, depending on methods and conditions of their production.

The resistant starch contents reported in this study could only be RS3 with few amounts of low melting crystals of Amylopectin &crystals of Amylose and Amylose lipid-complex. As RS1 is unlikely to be present in the purified starches (Shi and Jeffcoat, 2003; Wilson and Basit, 2005; Dundar and Gocmen, 2014; Reddy *et al.*, 2014) and both RS1 and RS2 could not survive the high autoclaving temperature (up to 121 °C) employed during the retrogradation process. In fact, both RS1 and RS2 lose the potential of RS if gelatinized (Dundar and Gocmen, 2014).

3.2.2. FTIR Characteristics of the starches

The FT-IR spectra characteristic peaks of starch can be divided into four main regions: 3600-3000cm^{-1}(O-H stretching region), 3000-1500 cm^{-1} and 1500-800 cm^{-1} (the fingerprint region) and below 800 cm^{-1} region (Ratnaningsih *et al.*, 2016).

In the FT-IR spectra of haricot bean starch (Fig. 3.2),the weak broad band that has been observed from 3392-3080 cm^{-1}may be attributed to ï OH stretching vibration of free hydroxyl groups of starch or/and with ï OH stretching vibration of water adsorbed on the amorphous region of starch.

Triplet peaks that were observed at2954, 2923 and 2852cm^{-1}-may be associated with ï CH_2symmetrical stretching vibration of anhydroglucose ring with interference from starch, lipid, or protein. The peak observed at 1612 cm^{-1} (Fig. 3.2) may be attributed to ï OH bending and twisting peak of water adsorbed on the amorphous region of starch.

In the finger print region, the weak band that wasobservedat1436may be attributed to ï CH twisting and bending of anhydroglucose ring. Several weak peaks that had been observed from 1375ï 929 cm^{-1}spectral region may be attributed to C-O stretching of starch/anhydroglucose ring or C-C stretching of anhydroglucose ring. They are very sensitive to conformational& order/crystal lattice sizechange and change of the amount of water adsorbed on amorphous region of starch (Wang *et al.*, 2015; Ratnaningsih *et al.*, 2016). In simple terms they are the most affected by the retrogradation process. The weak peaks that has been observed at

1155and 1020 cm^{-1} and assigned to the C-O stretch of C-O-H of anhydroglucose ring are very sensitive to conformational & order/crystal lattice size change.

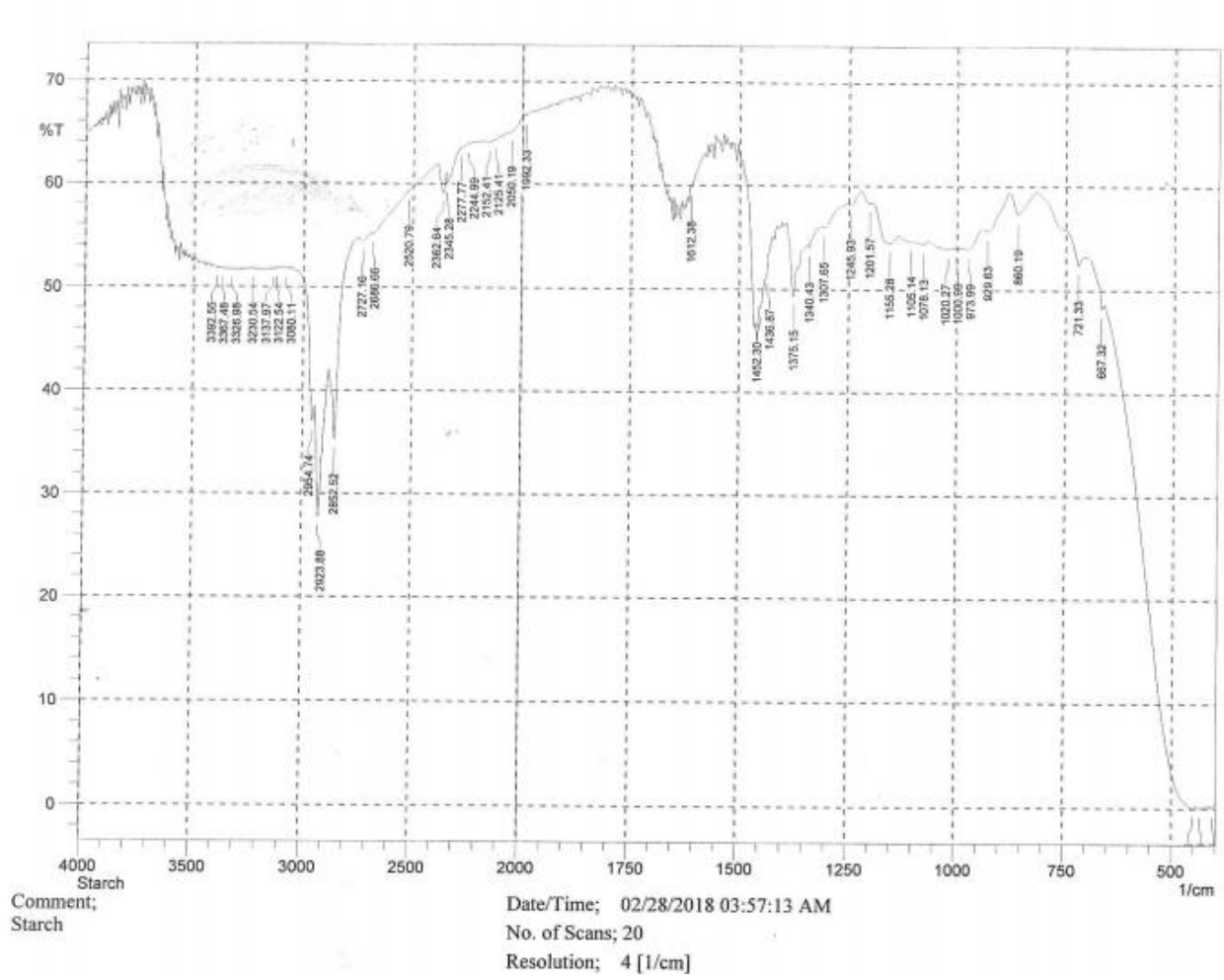

Fig. 3.2.The FTIR spectrum of KAT-B9 Haricot bean native starch

In the FTIR spectrum of RS3 (Fig. 3.3),the broad band from 3392-3080 cm^{-1}that has been observed in haricot bean native starch was no longer observed. This may be because the free -OH groups of haricot bean native starch were involved in the formation of the double helixes and hence in conformational change of amylose from single helix to double helixes or and due to lose of water as a consequence of the retrogradation process.

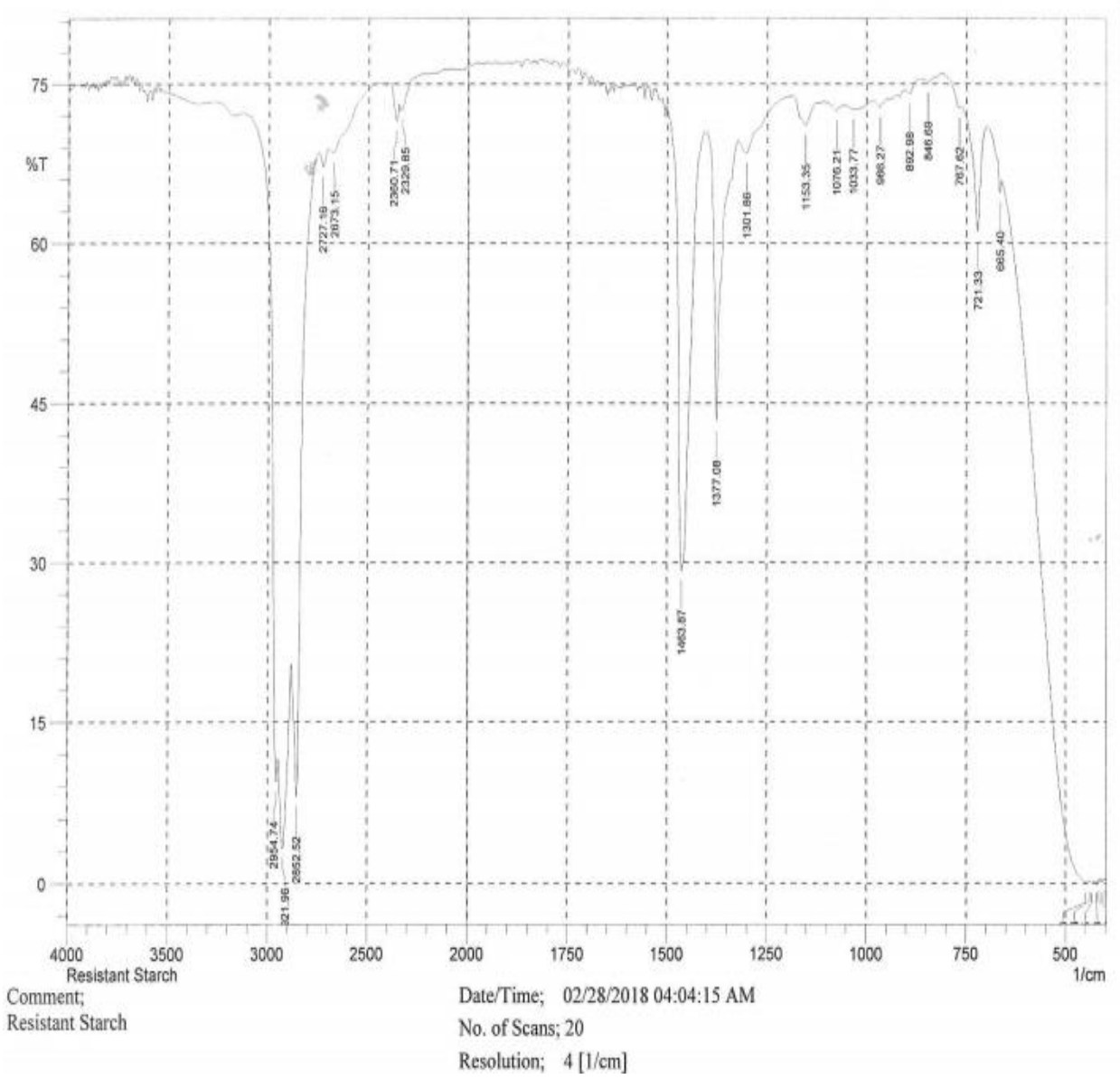

Fig. 3.3. The FTIR spectrum of Haricot bean RS3

Relatively very strong intense bands, in the FTIR spectrum of RS3, were observed compared to Haricot bean native starch at 2954, 2921 and 2852 cm^{-1} which may be associated with increased ï CH_2 symmetrical stretching vibration of anhydroglucose ring (Ratnaningsih *et al.*, 2016), as a consequence of conformational change or/and formation of amylose-lipid complex (Sajilata *et al.*, 2006). Unlike as in Haricot bean native starch there was no band at 1612 cm^{-1}

in resistant starch (RS3) FTIR spectrum. This may be because the process involves exudation of water (syneresis) (Sajilata *et al.*, 2006; Ratnaningsih *et al.*, 2016).

The fingerprint region (1500-800cm^{-1}) spectrum of Haricot bean resistant starch (RS3), except that two adjacent very strong peaks were observed, in general it had few peaks compared to double helixes.

3.2.3. Crystalline properties of the starches

The XRD pattern of A-type starch gives singlet peaks at about 15º and 23º 2ɗ and an unresolved doublet at about 17º and 18º2ɗ; whereas, B-type starch, gives strong diffraction peak at 17º 2ɗ and a few small peaks at around 15º, 20º, 22º, and 24º 2ɗ, and a characteristic peak at about 5.6º 2ɗ and C-type gives intense peaks at about 17º and 23º 2ɗ and two peaks located at 5.6º and 15º 2ɗ (Zhang *et al.*, 2017; Babu and Parimalavalli, 2018).The XRD patterns of $C_A$and C_B-type starches are similar to that of C- type, but there is a peak at about 18º 2ɗ for C_A-type starch, and two shoulder peaks at about 22º and 24º 2ɗ for C_B-type starch(Ratnaningsih *et al.*, 2016).

In this study the XRPD difractogram of Haricot bean (KAT-B9 variety) native starch had weak peak at 5.6° and strong peaks at 15º, and unresolved doublet peak at 17º and 18° and 23º (2ɗ) (Fig 3.4). From the observed pattern we may deduce, thus, Haricot bean (KAT-B9 variety) native starch has exhibited C_A-type starch polymorphism (Ratnaningsih *et al.*, 2016). Further, since Haricot bean native starch show C_A type starch polymorphism, it may be considered as a good source RS3 EErlingen *et al.*(1993).

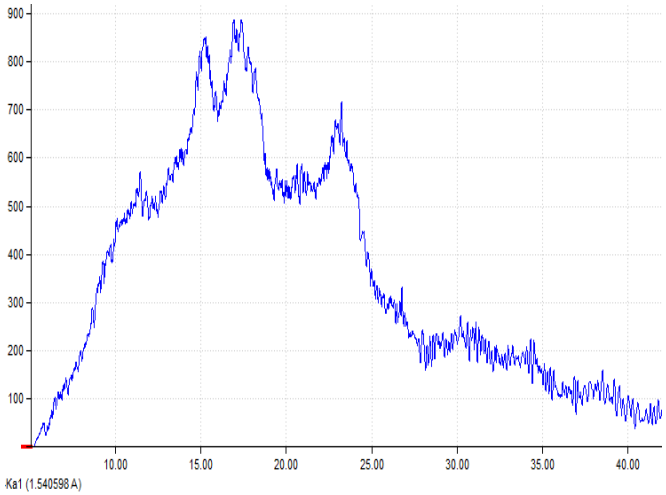

Fig. 3.4.The XRPD difractogram of KAT-B9 Haricot bean native starch

The XRPD pattern of Haricot bean resistant starch obtained in this study was not well resolved (Fig. 3.5). Nevertheless, somehow strong peak at 17°2ɗ was observed. However, the expected characteristic small peaks of B-type polymorph at about 5.6^{o}, 15^{o}, 20^{o}, 22^{o} and 24^{o} 2ɗ were not observed. Thus, it is unreasonable to deduce the polymorph of Haricot bean resistant starch obtained in this study as B-type polymorph as established in literature (Ratnaningsih *et al.*, 2016).

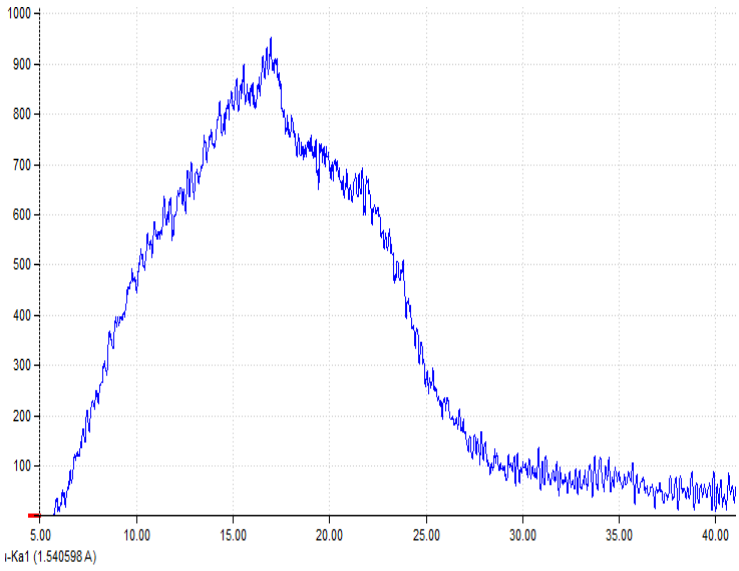

Fig. 3.5.The XRPD difractogram of KAT-B9 Haricot bean resistant starch

3.2.4. Morphologic properties of Resistant starch (RS3)

As observed by Scanning Electron Microscopy (SEM), legume native starches exhibit typically large oval to spherical shape granular structures with a characteristics smooth surface (Ratnaningsih *et al.*, 2016).

The SEM images of RS3 obtained in this study at different magnification scales (250, 500, 1000 &2500 times) (Fig. 3.6) had showed aggregates of irregularly shaped particles with continuous spongy (cell wall like network). This may suggest the starch had lost its granular nature by the retrogradation process. This is understandable as mobility of particles had been restricted due to the low (nucleation) temperature employed during the production of the resistant starch (Sievert and Pomeranz, 1989). Further, from the SEM image we may also infer that RS3 particles were compactly packed. Visible cracks (fractures) and hole like structures were also observed. The holes seem shallow whereas the cracks were profound.

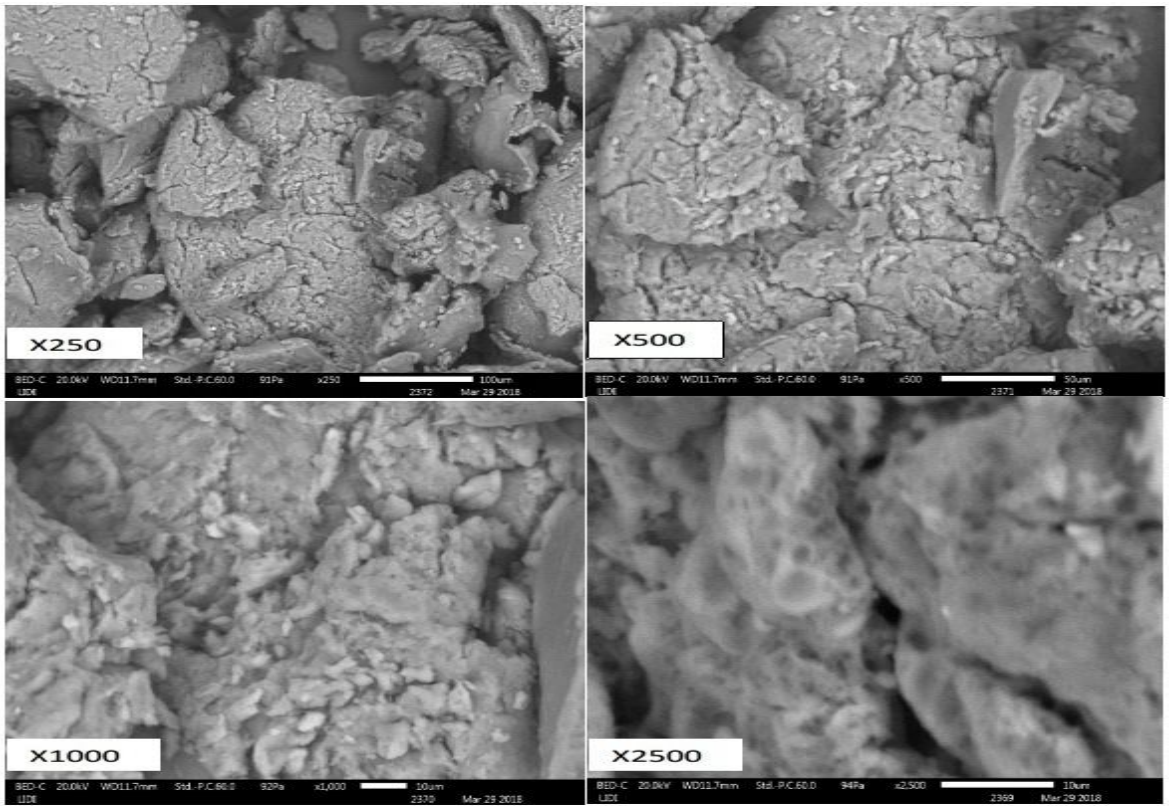

Fig.3.6. Scanning electron micrographs of RS3,at 250X, 500X, 1000X, and 2500Xmagnification

3.2.5. Swelling Power and Solubility Power of the starches

The swelling power of starch is a measure of the ability of the starch to hydrate under specific conditions such as temperature and water content, and the greater the swelling capacity of the starch, the weaker is the intermolecular forces. The swelling power of a given starch in general is related to its amyloseï lipid complexes, the intra- and intermolecular interactions, and the ratio and molecular weights of amylose and amylopectin. And amylose always acts as an inhibitor of starch swelling. However, solubility is generally related to the presence of soluble molecules, such as amylose, that indicates the ability of starch solids to dissolve or disperse in an aqueous solution under specific conditions such as temperature and water content (Zhang *et al.*, 2017).

In this study the swelling power of Haricot bean native starch was increased sharply from 55°C onwards (Fig. 3.7A), almost similar to what had been reported by Zhang *et al.*(2017) for other varieties of Legume starches. This rapid increase in swelling power from 55°C on wards may be due to an increase in molecular mobility of the amorphous region as a result of decreased intermolecular force from this temperature on wards.

The solubility of Haricot bean native starch (Fig. 3.7B), though lately, increased sharply in the same fashion as its swelling power, from 65°C onwards. This is almost in agreement with what had been reported by Zhang et al.(2017) for other legumes. Rapid increase in solubility from 65 °Con wards may be due to the higher disorganization of starch granules and hence increased solubility of amylose around their gelatinization temperature (Zhang *et al.*, 2017).

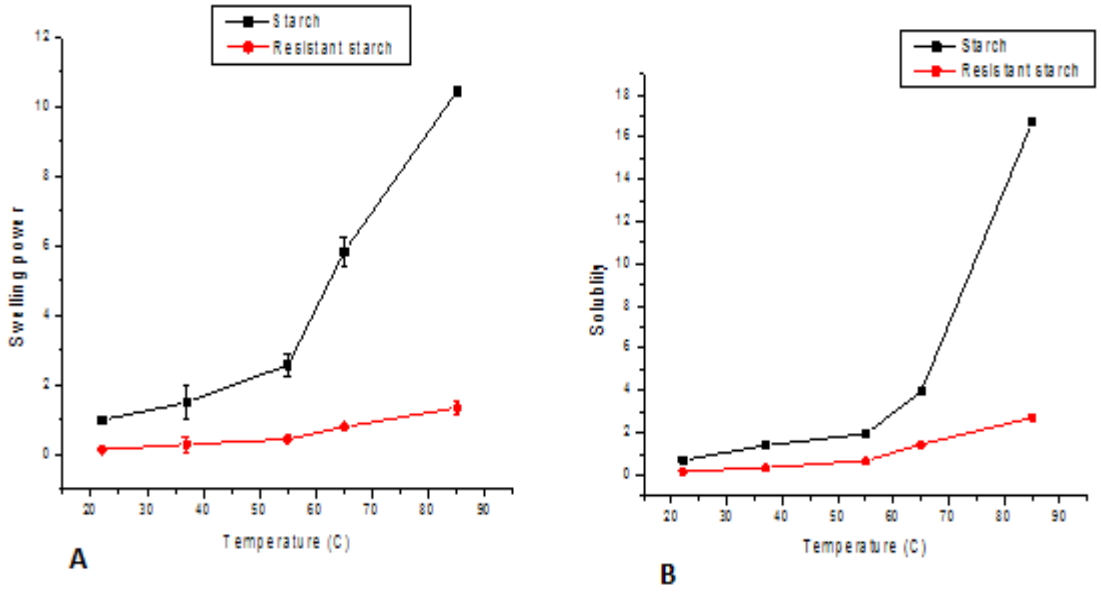

Fig.3.7. Swelling power of Haricot bean Starch & its RS3 (A) and Solubility power of Haricot bean Starch & its RS3 (B)

The swelling power and solubility of RS3 of Haricot bean (Fig 3.7 A and B) were less than 1% and 2%, respectively, in the same temperature range of the study. Further, both the swelling power and solubility of RS3 of Haricot bean resistant starch had been less responsive to change in temperature unlike its native counterpart. Clearly, the swelling power and solubility of RS3 of Haricot bean (Fig 3.8 A and B) were significantly lower at (P = 0.05) than that of Haricot bean native starch. Similar reduced swelling power and solubility of resistant starch than native starch were reported by Babu and Parimalavalli (2018) for other legumes. These are understandable as RS3 has strong intermolecular force, relatively high amylose lipid complex, lower amylose content and are more hydrophobic than the native starch (Zhang *et al.*, 2017).

Then negligible swelling power and solubility of resistant starch at normal body temperature (37 °C) may have its own implication on the use of resistant starch as controlled release excipient, both as matrix and film forming agent.

3.3. Characteristics of RS3–EC film coated Mesalmine tablets

3.3.1. Characteristics of Mesalamine granules

The production of uniform tablets heavily relies on free granule/powder flow, which in return depends on granule/powder characteristics. Granule parameters that have been used to demonstrate granule/powder free flow include: Moisture content, Hausner ratio, Carrôs index and Angle of repose. The limits of moisture content, Hausner ratio, Carrôs index and angle of repose (d) are less than 3%, 1.25, 15 and 40°, respectively, for uniform tablet manufacturing (Florence and Siepmann, 2009; Allen LV*et al.*, 2011).

In this study, the values obtained are presented in Table 3.2. It seems the granules that were prepared in this study had the required property for uniform tablet manufacturing.

Table 3.2.Characteristics of Mesalamine granules.

Parameters	**Values**
Bulk density (g/ml)	0.65 ± 0.20
Tapped density (g/ml)	0.75 ± 0.30
Hausnerôratio	1.14
Carrôs index (%)	12.5
Angle of repose (º)	36. 4 ± 0.10
Moisture content (%)	2.7 ± 0.21

3.3.2. Characteristics of Meslamine core tablets

Most of tablet physical characterization tests are required either to demonstrate the mechanical strength of a tablet for further process operations, for instance coating, blistering or/and provides an idea of robustness of a tablet till it reaches a patient. These include: Friability and Crushing strength (Hardness). Or they are required because they are prerequisite for dissolution-bioavailability, for example, the Disintegration test and Crushing strength. Under normal conditions a plain tablet for a film coating should have: Crushing force in the range of

120 - 300 N; Tablet disintegration no longer than 15 minutes; Tablet friability in the range of 0.3-0.5%. On the other hand, weight variation, which is both official and in-process parameter, demonstrates uniformity of content. Its limit of variation for tablets weighing more than 250 mg is less than 5%. There are also other physical characterization tests such as diameter and thickness which serve as a marker for the most functional parameters mentioned above, Friability and Crushing strength (Florence and Siepmann, 2009). The values obtained in this study are been present in Table 3.3. It seems the Core tablet has the required feature for subsequent film coating.

Table 3.3. Characteristics of Mesalamine core tablets

Characteristics	**Values**
Shape and appearance	Smooth elegant biconvex tablets
Average mass (mg)	670 ± 4.5
Hardness (N)	220 ± 4.2
Thickness (mm)	2.9 ± 0.03
Diameter (mm)	6.4 ± 0.02
Tensile strength (N/mm^2)	7.6
Friability (%)	0.42
Disintegration time (min)	Ò10

3.3.3. Characteristics of Mesalamine film-coated tablets

Off-white to light-yellow film coated Mesalamine tablets were obtained. F11 and F12 coated tablets have yellow colour where as F31 and F32 coated tablets have almost off-white color. The color of F21 and F22 coated tablets was halfway between the two. The yellow feature increases with increasing proportion of RS3 in the film formulation compared to ethyl cellulose and film thickness. And also the roughness of the film increases in the same fashion as the color of film of the coatings.

3.4 . *In vitro* GI Mesalamine release study

3.4.1. UV Calibration Curves of Mesalamine in 0.1N Hydrochloric Acid and Phosphate buffer media

Calibration curves of Mesalamine in Hydrochloric acid (pH=1) and Phosphate buffer (pH=7.2) media are presented in Fig. 3.8 and Fig. 3.9, respectively.

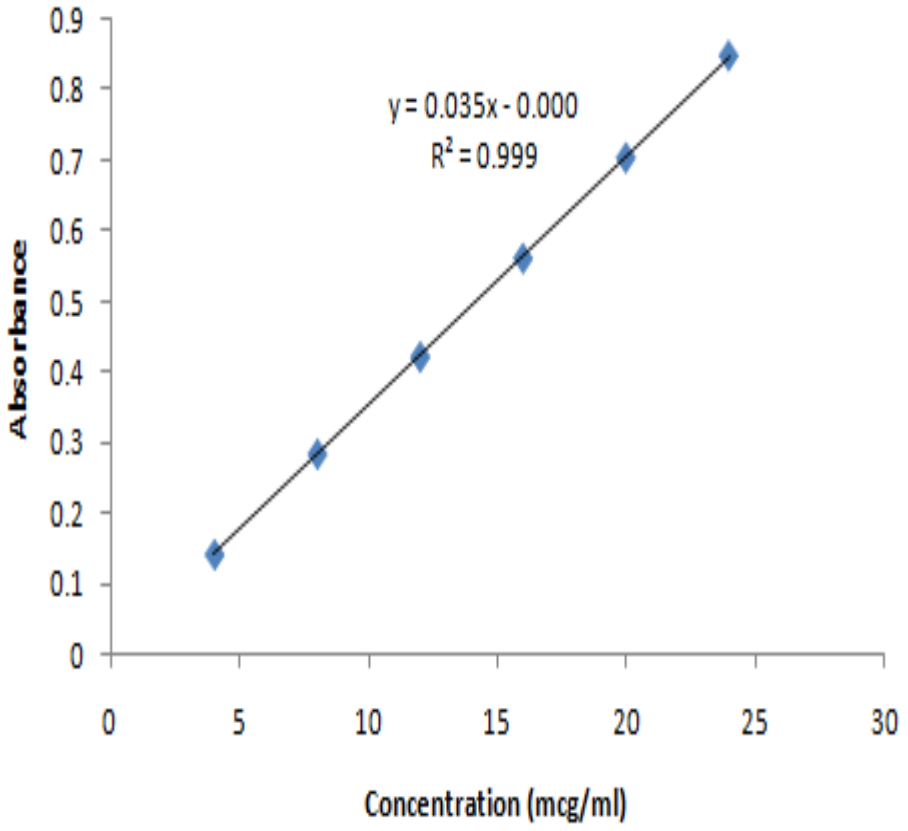

Fig. 3.8.Six points calibration curve of Mesalamine in 0.1 N Hydrochloric acid medium.

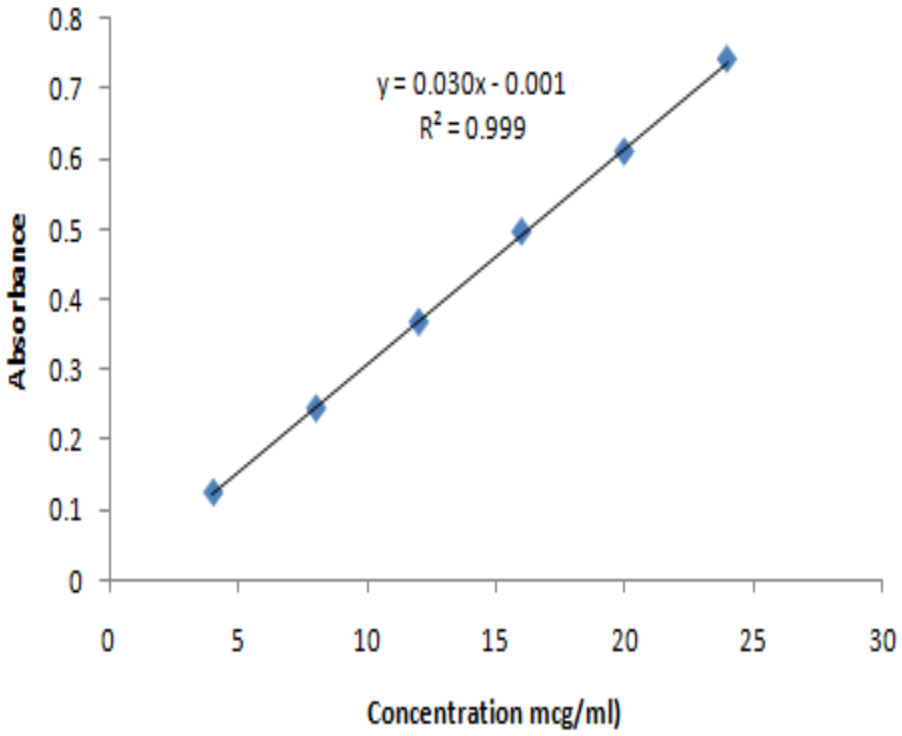

Fig. 3.9.Six points calibration curve of Mesalamine in Phosphate buffer medium.

3.4.2. *In-vitro* release profile Mesalamine tablets

In the 7 h *in-vitro* study (Fig. 3.10 A and B**),** the releases of Mesalamine from all six coating formulations in simulated gastrointestinal fluid were almost negligible both in the absence (Fig 3.10 A) (max ~2.5%) and presence of enzymes (Fig. 3.10 B) (max ~3.5%). There were no significance differences between the Mesalamine release profiles of all of the different coating formulations (P = 0.05), both in the absence and presence of enzyme. Further (Fig. 3.11), all coated tablets remained intact, as swellings or ruptures of coatings were not observed. From this, we may deduce that RS3 is as resistant as Ethylcellulose. And 4% weight gain of film coating seems at least sufficient. The results were different from what had been reported by Wilson and Basit (2005) and Freire *et al.* (2009).

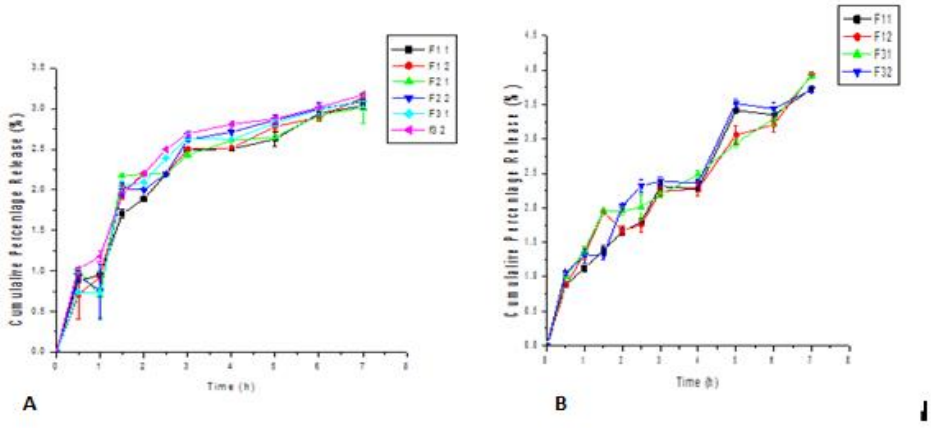

Fig. 3.10.*In-vitro* dug release profile, (A) in the absence of enzymes and (B) in the presence of enzymes

Wilson and Basit (2005) had used pure Amylose and Ethylcellulose in different ratios in their film coating suspension formulations to evaluate the potential of Amylose for colon targeted delivery, instead of RS3 as in this study. They had claimed that RS3 had been formed *in-situ* while coating, consequently had resisted the simulated environment. They had reported that the Mesalamine release in gastrointestinal fluid was progressively suppressed with increasing Ethylcellulose ratio. Five to Sixty percent Mesalamine release had been reported by them depending on film thickness of coatings and the relative ratio of amylose and Ethyl cellulose of their coatings formulations.

Freire *et al.* (2009) had used different brand high amylose maize starches and Ethylcellulose with the aim to make film coating suspension which differ either in amylose or/and Ethylcellulose content to evaluate the potential of the formulations for colon targeted delivery. Freire *et al.* (2009) had reported that the Mesalamine release in gastrointestinal fluid was progressively suppressed with increasing Ethylcellulose ratio as Wilson and Basit (2005) and also as amylose content of the high amylose maize starches increases. Fifteen to Eighty

percent Mesalamine release had been reported by Freire *et al.* (2009), depending on film thickness and the relative ratio of amylose and Ethyl cellulose of the their coatings.

The finding of the present study was different from what had been reported by Wilson and Basit (2005) and Freire *et al.* (2009) probably because the film coating suspension prepared in this study had pre-prepared RS3 unlike the previous studies. On the other hand, Wilson and Basit (2005) and Freire *et al.* (2009), had claimed RS3 formed *in situ* while coating. Further, the pre prepared RS3 produced in this study is expected to be in its most state if not at all resistant, as hydrolyzing enzymes were employed in the isolation stage of the retrogradation process.

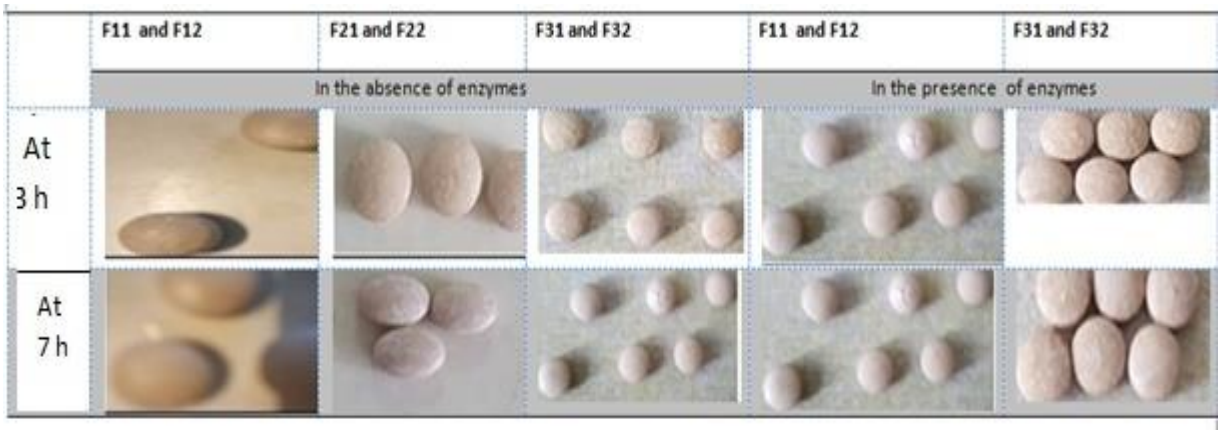

Fig.3.11.Photographs of film coated tablets taken after 3 h exposure in gastric medium and additional 4 h exposure in intestinal medium (Photo by Abebe Y.)

3.5. Fermentation study

As the study progresses, two types release profiles had been observed, in the 12 h of the fermentation study (Fig. 3.12**).**In the first 4 h of the study, all of the different four film formulations coated tablets had exhibited almost negligible Mesalamine release and then from 4-12 exhibited releases that increase gradually. In this time segment of the study F11 and F12 have better release profiles than F31 and F32 and had started releasing earlier. This may be because they had higher RS3/EC ratio than F31 and F32.The cumulative release in 12 h of the study of F11, F12, F31, and F32 were 82.2%, 76.3%, 60.2% and 56.9%, respectively.

On the other hand Wilson and Basit (2005)had reported up to 10% and 70% cumulative releases in their first 1 h and 6 h of their fermentation study of two coatings, respectively that vary depending on the relative film thickness of the coatings, unlike the present study where the observed releases are very small if any in the same period (6 h) of the study. Still the small release that were observed in the first 6 h of the present study may not have a considerable impact on the potential of the use of RS3 as film coating excipient, as the colon transit time is generally much longer than 12 h, which assures almost complete release.

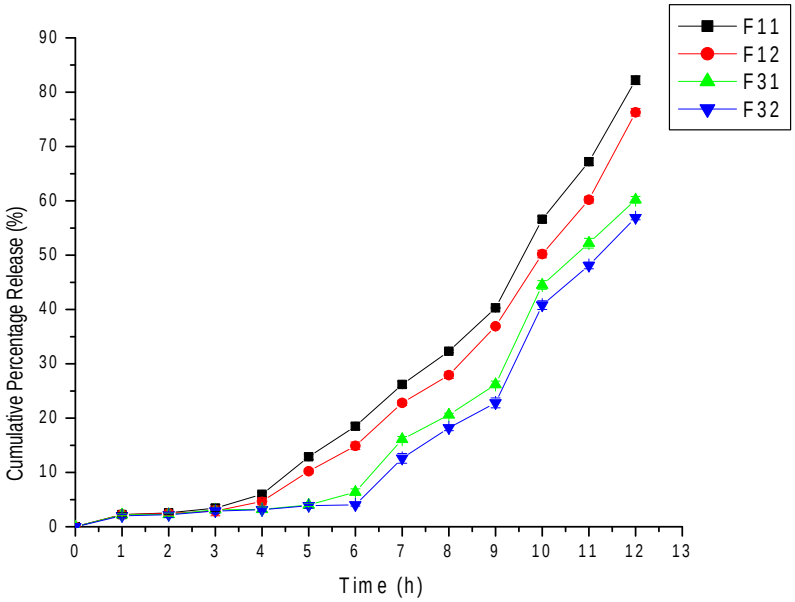

Fig. 3.12. Release Profiles of the four extreme formulations in simulated colon environment.

Further, the late Mesalamine release that was seen in this study compared to Wilson and Basit (2005)may be improved by formulating as film coated granules tablets or by manufacturing small size tablets in contrast to the present study as these assures large surface area and hence better releases.

4. Conclusions

The cost of manufacturing of Haricot bean starch is sound because on average 1.25 kg of starch can be extracted from 3 kilograms of Haricot Bean.

Haricot bean starch has desirable characteristics and the processing condition also appears to be appropriate to produce resistant starch (RS3) because the conversion rate of Haricot bean starch to resistant starch on average is 78%.

Resistant starch of Haricot bean produced in this study has desirable features to be used as a film forming excipient for oral-colon targeted delivery. As the *in-vitro* drug release in gastrointestinal medium is less than 4% in 7 h and up to 82.2% releases observed in the simulated colon medium within 12 h of the study.

5. Suggestion for further work

The results of this study suggest:

- detailed *in-vitro* investigation on formulation design to overcome the current limitation of late release of Mesalamine in the colon environment.
- examining the potential of resistant starch film coated pellet, as their large surface area may enable to overcome the limitation of the current study in the colon environment and
- *In-vivo* scintigraphy (Gamma Scan) study in laboratory animals (Rabbit).

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