Investigation of the native starch-ethyl cellulose film coating for colon targeting

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The purpose of this study

The aim of this study was to optimize a function starch-ethyl cellulose film for targeting the therapeutically active substance to the colon in the disease state for patients suffering from inflammatory bowel diseases.

This study includes:

- The preparation of the polymeric films and the investigation of the characterization (for example mechanical properties, water uptake, dry mass loss, and microscopy analysis) for these prepared film coatings.
- Coating of the pellets with 3-1 as well as 3-2 and the study of the release through the coated pellets.
- The study of the film formulation factors such as (formulation temperature, amount of the TEC, and polymer blends ratio) and their impact on the properties of the final prepared film as well as on the drug release from the coated pellets at healthy as well as disease state before and after upon exposure to:
  - Gastric fluid with pepsin
  - Intestinal fluid with and without pancreatin
  - Colonic fluid at different PH (6.8, 5.6, and 2.8) with different concentration of bacterial amylase.

Utveckling och optimering av en läkemedelsförmulerings teknik för kolon

De besvärliga symptomen såsom Diarré och blod i avföringen är mesta tydliga tecken för Kolons sjukdomar Inflammatory bowel disease (IBD). Därför utvecklingen av ett läkemedel som botar, lindrar, behandlar, eller förebygga sjukdomen är jätte viktigt för både patienterna och läkemedelsindustrierna. Minsta biverkningar och bekväm leveransrutt är de mest önskade egenskaper för det ideala läkemedlet.

Orala läkemedel som t.ex. suspensioner, tabletter och lösningar som riktar sig mot kolon måste ha en specifik formulering teknik som kallas (filmbeläggning).
Filmbeläggning som täcker aktiva substanser bör ha förmåga att skydda och förhindra lösningen av aktiva substanser som är benägna att lösas i övre delen av mag-tarmkanalen.

Denna typ av formulering kan utvecklas med hjälp av beläggning av aktiva substanser med vanliga och billiga polymersblandningar såsom stärkelse och cellulosa.
Dessa polymerer, speciellt cellulosa tål sura miljön d.v.s. låg pH -värdet samt matsmältningsenzymer i den övre delen av mag-tarmkanalen. Olika polymersblandning förhållande av stärkelse-cellulosa kan användas för att belägga tabletter eller piller med specialutrustning som kallas (fluidized bed).

Dessa polymerer kan användas för att kontrollera frisättning av aktiv substans i tarmen beroende av polymer blandningsförhållande d.v.s. stärkelse- cellulosa.

Många studier har redan genomförts på många typer av polymerer, polymer blandning, mjukgörande substans halt samt med olika blandningsförhållande. Resultatet från dessa studier har bevisat att polymerstyp och blandningsförhållande har stor betydelse för frisättning av en aktiv substans som leder till minskning av dosseringsintag av läkemedel som i sin tur öka patients bekvämhet, speciellt hos äldre och barn.

Vår studie fokuserades på utveckling, analys och optimering av filmbeläggning.
Olika analyser för filmbeläggningar med två olika polymer blandningsförhållande 3-1 och 3-2 etylcellulosa- stärkelse genomfördes i friska samt som sjukdomstillstånd.
Analyserna har omfattat mekaniska egenskaper, vattenupptagning, torr massa förlust och mikroskopi analys. Dessutom studerades frisättning av en aktiv substans.
Dessa analyser genomfördes i magsaft med pepsin, tunntarms vätska med pankreatin.
Analysen i kolonsvätska genomfördes med olika koncentration av amylas enzymen och pH-värde.

Resultatet har visat att filmbeläggning av polymersblandning 3-1 etylcellusa-stärkelse med 25 % mjukgörande substans trietylcitrat (TEC) beräknas enligt den totala torrvikten för båda polymererna.
Med 60 ^{o}C som en filmbildnings temperatur resulterar i funktionsfilm med de acceptabla mekaniska egenskaperna och vattenupptag samt torrvikt förlust.
Dessutom den 3-1 filmbeläggning blandningsförhållande ger kontrollerade frisättning av aktiv substans i kolonen samt bevisat mer motstånd mot sura miljön d.v.s.
låg pH -värdet samt matsmältningsenzymer i den övre delen av mag-tarmkanalen.

Slutligen den typen av formuleringsteknik kan utformas praktiskt ekonomisk samt med hög kvalitet. Mer forskning och analys behövs för att garantera vilka faktorer som kan förbättra och minska kostnad är viktigt för läkemedelsindustrier.

Contents

1-Background

Generally, the manufacture and development of colonic drug delivery is an issue for pharmaceutical industry essentially, the manufacture of drug with the least possible side effects and with convenient delivery route. Colon specific controlled release drug is one of the most important techniques to achieve this aim. In general, many attempts have been designed and developed to achieve the best and most desired oral controlled release drug which can be targeted to desired organ or tissue. For example, the film coated tablets and pellets are widely used formulation technique for oral controlled-release. (K.V. Vinaykumar*, T. Sivakumar T. Tamizhmani1, T. Sundar Rajan, I. Sarath Chandran, 2004)

1.1. Oral colon–specific controlled-release drug

Colon specific controlled release is used for the delivery of the therapeutically active substances to a specific site in colon for local treatment of different types of colon diseases such as ulcerative colitis, colorectal cancer, and amebiasis1 (K.V. Vinaykumar*, T. Sivakumar T. Tamizhmani1, T. Sundar Rajan, I. Sarath Chandran, 2004), as well as for oral administration of different types of proteins and lipids drug for systematic circulation in order to avoid the denaturation of proteins by low pH and enzymes activity in the upper GIT. All these references (Malin E. V. Johansson, 2013), (Q.W. Yang a,b,c, M.P. Flament a, F. Siepmann a, V. Busignies c, B. Leclerc c, C. Herry b P. Tchoreloff c, J. Siepmann a, 2010) and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009) pointed out that the colon specific controlled release has many advantages compared with other drug formulation techniques. For instance, to maintain the drug concentration within therapeutic window, deliver of therapeutic substance to site of action for local treatment, minimize undesirable side effects, and improve efficiency of treatment with leas amount of drug. In addition, this type of formulation reduces administrations frequent which in turn increase patient convenience and compliance especially in the older people and child as well as provides a better pharmacokinetic behaviour than other types of formulation. However, there are many problem associated with this type of formulation for example the active substance content is higher than content in conventional dosage which at damage of this formulation could lead to that the concentration of active substance exceed the acceptable and safe level value. In addition, the variations of gastric emptying time can with difficulty be predicted. This cause poor drug availability in the colon if one has not predicted correctly the desired therapeutic dosage.

1.2. Gastrointestinal physiology, pH, enzymes, and transit time

The gastrointestinal tract is an approximately six meter muscular digestive tube which stretches from the mouth to anus with varying diameter. Gastrointestinal tract (GIT) consists of four main anatomical parts: the esophagus, the stomach, the small intestine, and the large intestine (E.Aulton, 2011).

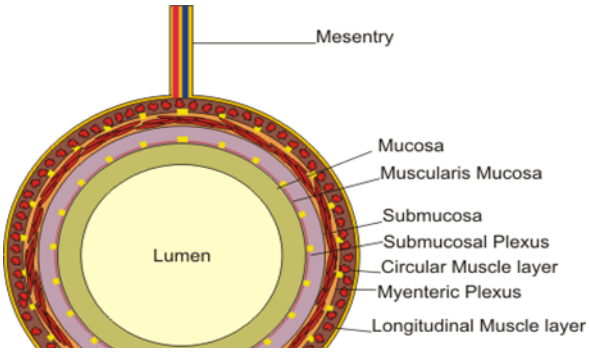


Figure 1 cross-section through the wall of the GIT tract

The wall of gastrointestinal tract is composed of four histological layers. As showed in the figure 1. These layers are from inside to outside mucosa, submucosa, muscularis externa, and serosa. Approximately, these layers are the same along the GIT (E.Aulton, 2011) and (Wikibooks.org/w/index.php?oldid).

The gastrointestinal epithelium is covered by a layer of mucus which is a viscoelastic translucent aqueous gel. This layer is secreted by the gastrointestinal mucous membrane and the thickness of this layer ranges from 5 μm to 500 μm along the gastrointestinal tract. Additionally, this layer is continuous in the stomach and duodenum; on the other hand, it is not in the rest of the GIT. Significantly, this layer is very vital in the GIT as result to it is protection and lubricant function. This layer can be divided into two layers: an inner attached layer to the mucosa and an outer unattached layer which is removable. The mucus layer in the esophagus lubricates the digested food and pharmaceuticals through the esophagus. The stomach and the colon have the attached and unattached layers; in contrast to, the small intestine which it has only thin unattached layer. Importantly, this layer protects the stomach and duodenum from the digestive enzymes as well as acidic environment. However, the mucus layer functions in the small intestinal and colon functions as a lubricant facilitating the digested matter and to protect the epithelial cell layer from the mechanical stress. Moreover, this mucus layer has very significant role in the colon by providing a protective barrier against the pathogens but at the same time provide an essential environment to enteric microflora (E.Aulton, 2011), (Digestive System,CHAPTER 5 and CHAPTER 7) and (Wikibooks.org/w/index.php?oldid).

1.2.1. Stomach

The stomach is the muscular dilated part of the GIT which is located between the esophagus and the small intestine as showed in the figure 2. Generally, the stomach has many important functions in digestive system. These functions are that the stomach acts

as a reservoir for the ingested food and controlled delivery of digested as well as ingested material to the duodenum by the pyloric sphincter. Furthermore, it digests the ingested material to chime (semifluid partially digested material) by its enzymatic and acid action. This digestion improves contact with the mucous membrane in the small intestine which in turn facilitates the absorption. In addition, the stomach reduces the anxious material which may be reached the small intestines. The volumetric capacity of stomach ranges between 50 ml to approximately 1.5 l in fasting and feeding state respectively. Especially, the stomach contains important gastric secretion fluids which are responsible of its digestion ability (E.Aulton, 2011), (Digestive System,CHAPTER 5 and CHAPTER 7) .

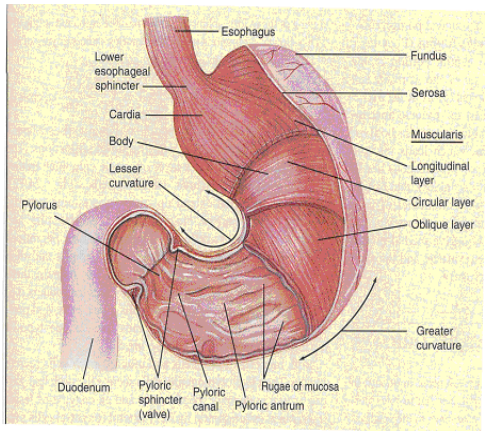


Figure 2 the anatomay of stomach

These secretions comprise of (E.Aulton, 2011), (Digestive System,CHAPTER 5 and CHAPTER 7), and (Wikibooks.org/w/index.php?oldid):

- Acid that is secreted from the parietal cells to maintain the PH-value in the stomach between 1 and 3.5 in fasted state.
- The hormone gastrin that is responsible for production of gastric acid.
- Pepsins are peptidases which break down the proteins to peptides. The pepsin enzyme functions at low PH and denatures at high PH.
- Mucus can be secreted by the mucosal cells. This layer is essential for the protection of the gastric mucosa from enzymatic and acidic digestion.

1.2.2. Small intestinal

It is the longest (4-5) part of the GIT which is located between the stomach and the large intestinal. The small intestine consists of three parts duodenum, jejunum, and ileum. Considerably, the small intestinal is an organ where the most important enzymatic digestion process and absorption of nutrients and other digested materials take place (Ulrich Klotz*, 2005) and (E.Aulton, 2011).

Generally, the enzymatic digestion of proteins, fats, and carbohydrates in small intestine can be done as result to presence of bile and many digestive enzymes such as proteolytic enzymes, pancreatic lipase, and pancreatic amylase which are secreted in the pancreas where inter the small intestine by the pancreatic duct. Importantly, the pancreatic amylase is one of the enzymes that is responsible for digestion of starch into oligosaccharides is important used enzyme for this study (E.Aulton, 2011), (Digestive System,CHAPTER 5 and CHAPTER 7), and (Wikibooks.org/w/index.php?oldid).

Essentially, the other considerable function of the small intestines is absorption of many important nutrients as result to its large area which is approximately 200 m^2 in adult as well as to the presence of a rich network of blood and lymphatic vessels in wall of small intestine. These networks play an important role in absorption of nutrients and drug. Subsequently, these networks deliver the blood with absorbed digested materials and drug to the hepatic portal vein to the liver which in turn metabolizes the drug before delivery into the circulation system in process which is called the first-pass metabolism. This process is called the first-pass metabolism.

This huge absorptive surface area which is packed into small space is according to three essential features. Firstly, the submucosal of small intestinal is circular folds, which contribute in the regulation of transport of digestive materials. Secondly, the existences of villi which are the tiny finger- like projections into the lumen. These are covered with the cells that absorb the digested materials. Additionally, these villi are supplied with blood and lymphatic vessels that can be used to absorption and transport of nutrients. Finally each villus is covered by the 600-1000 hair like structures which is called microvilli. Enormously, these microvilli increase the inner surface area of each cell which in turn increases of the absorption of nutrients and drug (E.Aulton, 2011).

1.2.3. Colon

This is the last 1.5 m part of the GIT which stretches from ileocecal junction to anus. The colon consists of a proximal colon (which consists of the cecum, the ascending colon, the hepatic flexure, the transverse colon, and the splenic), the distal colon (which includes descending colon and the sigmoid colon) and the rectum. As showed in the figure 3 (E.Aulton, 2011) and (Hull, Colonic Physiology). The inner surface area of the colon is less than that in the small intestine which in turn decreases the absorptive capacity of the colon. This decrease in the inner surface area of the colon results to the fact that colon does not have a specialized villi. On the other hand, the mucosal layer of the colon is irregular as well as contains deep crypts which participate in increase of the inner surface area of the colon. Even though, this small absorption area the colon has a large capacity of absorption such as the absorption of water, chloride ions, and sodium ions from the lumen. The absorption of these ions and water provide considerable homeostatic role in the body (Wikibooks.org/w/index.php?oldid), and (E.Aulton, 2011).

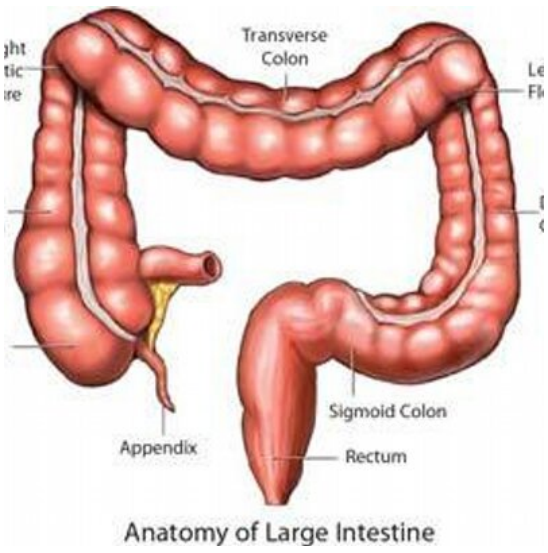


Figure 3 the anatomy of colon

Generally, the human colon can be divided into three functional parts. The first part is the cecum and proximal colon which is important site for the metabolism of carbohydrates by bacterial fermentation. Secondly, the transverse colon which is responsible for many functions such as keeping the materials in the proximal colon for fermentation, control the emptying of the proximal colon, and it functions as a site for absorption of water as well as for the formation of faeces. Finally, the distal colon and rectum act as a reservoir for faeces and control sites for the removal of faeces material in convenient way (E.Aulton, 2011) and (Christine Edwards, 96).

The characteristics and mechanism of absorption differs between the distal and the proximal colon as results to the differences in the structure of luminal layer, motility pattern and the transit time. The absorption of drug in the colon depends on different factors for instance transit time, particle size and charge, site of absorption, pH, viscosity, blood flow, absorptive epithelium, and the movement of drug along the colonic lumen layer or in the bulk phase. Essentially, the proximal colon has a greater of the blood flow than the distal colon which, for this reason the proximal colon has a better absorptive capacity than the distal colon (E.Aulton, 2011) , (Christine Edwards, 96)and (N. V. Satheesh Madhav1, 2012).

As stated above there are many important materials which can be absorbed in the colon. Particularly, the chloride and the sodium ions can be absorbed in colon as result to electrochemical gradients with the potassium and bicarbonate which are secreted in the colon. In addition, the colon is the major site for the water absorption in the proximal and transverse colon (Friend*, 2005).

The colonic epithelial is less permeable than that in the small intestine therefore the water can be retained from the colonic luminal. The retained water from the colonic luminal should be taken into account when the colonic control release drug is designed. This retained water may dilute the drug concentrations which in turn delay the absorption of the drug. However, this retention improve the absorption by mixing of materials with the colonic lumen layer which facilitate the contact with colonic epithelial. Finally, the colon is a site for absorption of other molecules which contain drug such as lipids, amino acids, glucose, sucrose, organic acids, and the carbohydrates. These molecules can be absorbed by different grads and various mechanisms. On one hand, the lipid soluble molecules and the organic acids can be absorbed rapidly. On the other hand, the absorption of the glucose, sucrose, and amino acids is very poorly. Finally, the carbohydrate such as starch is fermented by the colonic bacteria to short chain fatty acids SCFA. These SCFA can be in ionized and un-ionized form depending on the microclimate pH (Hull, Colonic Physiology) and (Christine Edwards, 96).

These SCFA can be absorbed easily by passive diffusion. Mostly, the absorption of these SCFA can be occurred in the ionized as well as un-ionized form. However, the absorption

of these SCFA can be occurred in the ionized form in the distal part. This difference can be motivated according to the pH difference between the proximal and distal part.

As result to the high water absorption in the colon which can be estimated to 90% the available amount of the water ranges between 1-44 ml. For this reasons, the solubility of the drug is limited which in turn impact the bioavailability of the drug in the colon. Furthermore, this low water availability can increase the viscosity gradually from the ascending colon toward the descending colon. Considerably, this increase can result in reduced drug dissolution, absorption as well as the penetration into the colonic layer to reach the colonic pathogens (Hull, Colonic Physiology) and (Christine Edwards, 96).

1.3. pH of the gastrointestinal tract

Considerably, the luminal pH of GIT tract is rapidly changed from the high acid which is approximately between 1 and 3.5 in the stomach to the pH of approximately 6 in the duodenum. Due to the secretion of the bicarbonate from pancreas the pH along the small intestine from the duodenum to the ileum gradually rises to about 7.4 in the ileum. The pH drops in the colon as result of the fermentation of undigested carbohydrate such as cellulose and starch to acids SCFA by the enzymes which are secreted from the colonic bacteria. Increasingly, the pH value in the colon rises from 5.4 in the caecum to nearly 6.7 in the rectum (E.Aulton, 2011), (Friend*, 2005), and (Wikibooks.org/w/index.php?oldid).

The fed and fasted state significantly influences the pH of the stomach, the small intestine, and the colon. For example the pH value in the stomach at fed state is approximately ranged between 3 and 7. The pH of the colon can be influenced by the carbohydrate rich food as well as drug which are coated with polysaccharides. These materials decrease the pH in the colon as result to the fermentation of these carbohydrates into fatty acids by the colonic bacteria. Furthermore, many diseases such as ulcerative colitis (UC) and Crohn's disease (CD) decrease the pH of the colon which in turn impacts the concentration of colonic bacteria and enzymatic activity in the colon. Importantly, this impact of enzyme activity has a crucial impact on the stability of the polymer film coating as well as on the release of therapeutically active substance. Thus, the controlled formulation should be adapted to the patient state (E.Aulton, 2011)and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009).

1.4. The enzyme of GIT

The GIT tract has many important digestive enzymes that are responsible of digesting of nutrients as well as drugs in the appropriate condition such as pH. Particularly, the enzymes that digest food in the stomach like pepsin functions best at pH around 2. Conversely, the intestinal digestive enzymes such as

pancreatin function best at pH (6-7). Initially, the pepsin is secreted from the stomach is responsible for breaking down protein into small peptides and amino acids. Moreover, there are many enzymes which can be secreted from the pancreas into small intestine for instance pancreatin which consist of lipase, amylase, and protease. The protease enzyme is responsible for digesting of protein and peptide drug. The lipase is essential for hydrolyses of fats (lipids) drug. Finally, the pancreatic amylase is responsible for digestion of carbohydrates such as starch into sugars. Additionally, the small intestine has many enzymes which can be produced from gut microflora. The concentration of microflora increases obviously from the terminal ileum to the ascending colon (E.Aulton, 2011), (Christine Edwards, 96), and (Friend*, 2005).

Considerably, there are a various types of natural aerobic as well as anaerobic colonic bacterial microflora such as *Escherichia coli* and *Clostridium* species, respectively. Importantly, these bacteria can be associated with the secretion of many hydrolytic and reductive enzymes such as α-amylase which works best between 32 ^{0}C and 40 ^{0}C and at a pH between 6.5 and 7.5. These enzymes can catalyse many reactions such as metabolism of drug and bio molecules such as bile and acids, deactivation of harmful metabolites, and fermentation of undigested carbohydrates and proteins. As mentioned above the fermentation of the carbohydrates by these bacteria can be resulted in the SCFA which facilitate their absorption. Additionally, these bacteria can be converted the prodrug into the active therapeutic form as well as metabolism of the drug into harmful metabolites. For this reason these bacteria impact the pharmacodynamics as well as pharmacokinetic characteristics of drug (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009), and (High-amylose starch-based coatings for colonic delivery Cristina Freire1, 2010)

1.5. Transit time of the gastrointestinal tract

The investigation of the transit time for pharmaceuticals (tablets, particles, and liquids) along the GIT is essential for designing of oral-controlled release drug delivery system. Considerably, the transit time is different along the GIT tract and can be impacted by different factors such as fed and fasted state, food types, as well as diseases (E.Aulton, 2011) and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009).

1.5.1. Gastric transit

Normally, the gastric emptying time ranges between 5 minutes to two hours. The gastric emptying time of pharmaceutical is dependent on many factors such as

dosage form dosage size, the fed as well as fasted state, and the type of food especially fatty food (E.Aulton, 2011).

During the fasted state the myoelectric activity is governed the stomach activity which in turn can be governed the transit of pharmaceuticals. However, during the fed state there are two characteristic activities that is governed the transit time of the pharmaceuticals (E.Aulton, 2011).

Initially, the gradual contraction of the proximal stomach can be contributed to move the contents to distal stomach. Subsequently, the peristalsis contraction of the distal stomach is functioned to mix and break down food particles and move these towards the pyloric sphincter.

On one hand the pyloric sphincter controls the emptying of the liquids and small particles to the small intestine. On the other hand, the larger particles retained to the stomach to further digestion (E.Aulton, 2011) and (Digestive System,CHAPTER 5 and CHAPTER 7).

Consequently the liquid, pellets, and the disintegrated tablets be discharged to the small intestine rapidly while the controlled-release drug and large dosage is retained to the stomach and take more time for emptying. Finally, the gastric emptying time for dosage from the stomach to small intestine during the fasted state is fast (E.Aulton, 2011).

1.5.2. Intestinal transit

The small intestine is often the primary site for absorption of the drug due to the different factors that was stated above. Thus, the residence time of the drug in the small intestine is essential when the oral- controlled release is designed to target the colon and small intestine. There are two intestinal movements propulsive and mixing. The propulsive is very vital for determination of the residence time and the transit time of drug. Generally, the small intestine transit time ranges between 2.5 to 3 hours regardless the dosage forms, fed-fasted state, and between the liquid and solid dosage (E.Aulton, 2011) and (Friend*, 2005).

Normally, the transit time in the colon can be varied from 2-48 hours in the healthy state. The main movement of the colon is to move away the content toward the anus. This movement comprises two important activities which control the colonic transit time. Primary, the propulsive contractions which are done by the longitudinal muscles can transfer the contents toward the anus. However, the segmental contractions which are caused by the circular muscles can be contributed to mix the luminal content. There are different influences which can be impacted the colonic time such as type of dosage form, diet, presence and

absence of food, and diseases state. For instance the large dosage such as capsules transited faster than smaller dosage and coated particles. The diseases state has a significant impact on the colonic transit. Obviously, the transit time in inflammatory bowel diseases patients especially as ulcerative colitis (UC) and Crohn's disease CD patients can be varied from the healthy issues. Commonly, the transit time in the UC patient is shorter than the normal transit time. This abnormality is a result of the mucosal inflammation which can cause diarrhea (E.Aulton, 2011), (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009), (High-amylose starch-based coatings for colonic delivery Cristina Freire1, 2010) (Mitja Pišlar, 2014)and (Friend*, 2005).

1.6. Polymer blends for oral colon–specific controlled-release drug

The polymer blends for this type of formulation should be investigated according to the colonic environment to provide appropriate pharmacokinetic as well as pharmacodynamics characteristics. Importantly, the polymer blends for this type of formulation must be prepared from at least one polymer which has a low permeability of molecular network at the low pH-value as well as resistance to the human digestive enzymes in upper GIT. However, these polymers should be permeable and digestible when exposure to high pH-value and to digestion of bacterial enzymes in the colon (M. Tarvainen, 2003) and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009). This type of polymer blends can be adjusted to control and target the release of active substance especially in the colon. Particularly, the starch and ethyl cellulose are the most common used polymers for this type of formulation due to their availability and non-toxicity (F. Siepmanna, 2005).

Ethyl cellulose (EC) is a widely used polymer in the food and pharmaceutical industries. It is a hydrophobic organic polymer which prevents premature film dissolution in the upper GIT. It consists of glucose units. These units are joined by b-1.4 links. As result to its hydrophobicity the EC have been used widely in aqueous coating dispersions. In addition this polymer provides stability for coated tablets and pellets during storage.

On the other hand, the starch is a white, semi-crystalline and tasteless organic chemical compound, which can be biosynthesized by most of green plants. It can be used widely in food and pharmaceutical industry as well as other applications in order that it is a renewable, inexpensive, non-toxic and biodegradable material.

Starch is a polysaccharide which consists of two glucose polymers (20-30%) amylose which is a long linear chain of α-D glucose units linked together by α-1, 4

glycoside linkages and (70-80%) amylopectin which is branched chain of α-1, 4 linkages of glucose units linked by 1,6 linkages. These two polymers give the starch the most important functional properties such as swelling, gelatinization, retrogradation, pasting and susceptibility to enzymatic digestion (KARROUT", 2008)and (M. Tarvainen, 2003).

The amylose is difficult to digest and absorbed in contrast the amylopectin is easy to digest and absorb. As result a small portion of starch digests and absorbed in the small intestine but this effect does not influence the stability of the film. Roughly, 85% of starch is fermented and digested in the colon which functions as a substrate to the useful bacteria such as microflora in the colon (Y. Karrout, 2009), (C.W. Leonga, 2002), and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009).

As stated above during the inflammatory bowel the pH-value, beneficial bacteria, and enzyme amount decrease for different values depending on the type of the disease and the disease stage for example active, severe state and acute untreated state with pH value 6.8, 2.3 and 4.7 respectively (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009). This changing is very important to take into consideration when such formulation is designed because of that this variation impact the stability, permeability, water uptake, mechanical properties and digestibility of polymeric film which in turn impacts the release of the active substance. As result of this alteration in condition, the optimization of film with right polymers blend ratio, plasticizer amount, mechanical properties and stability at different condition is very essential for this study (High-amylose starch-based coatings for colonic delivery Cristina Freire1, 2010).

The polymer blends ratio is one of the formulation aspects that might impact on the film properties essentially on the homogeneity, stability, permeability as well as on water uptake and dry mass loss behaviour of the coating. Consequently, the polymer blends ratio should be investigated in to ensure the desirable release of the therapeutic active substance.

Moreover, a plasticizer is added to promote flexibility, plasticity and reduce brittleness of the polymeric film as well as reduce the stress at coating process. The hydrophilicity, amount as well as the affinity for polymer blends of the plasticizer might have a considerable impact on the stability, permeability and characteristic of the coating. For example the tri-ethyl citrate (TEC) can be used with an appropriate ratio aqueous ethyl cellulose and starch to prepare a functional polymeric film coating for colonic control drug release (M. Tarvainen, 2003).

Practically, many characteristics of the polymeric film such as mechanical properties, water uptake, microscopy study and dry mass loss can be investigated to determine the most important formation factors as well as to develop and optimize the desired functional properties of the polymeric film (C.W. Leonga, 2002).

1.7. Coating of the pellets

The coating of pellets with functional polymeric film can be prepared by using a fluidized bed. Importantly, the coating level and coating process parameters such as pressure, temperature of bed as well as air, and the air velocity should be adjusted to ensure appropriate coated particles. Failure of coating process for example the imbalance between the delivery of coating dispersion and the evaporation rate can be resulted in over wetting which in turn lead to that the pellets stuck together or this imbalance may result in over drying which lead to erosion. On the other hand, the failure of the ability of pellets to withstand the corrosion which can be caused from the coating process result in the reduction of the mechanical properties of film coating as well as lead to cracking. This cracking can decrease the film adhesion to the pellets which in turn cause the film exfoliation (Caroline De´sire´e Kablitz *, 2007) and (E.Aulton, 2011).

Coating of pellets can provide many advantages compared to traditional spray coating of tablets (Caroline De´sire´e Kablitz *, 2007) and (E.Aulton, 2011).

- The transit time of pellets shorter than the tablet along the GIT tract as well as these pellets can homogenously be distributed throughout the contents of the small and large intestines as result to its small dosage size.
- The use of these coated pellets can be avoided the lodge of large dosage such as tablets and capsules in the GIT which in turn reduces the premature drug release and the damage to mucosal GIT.
- Reduce the toxicity which may be associated with imperfection of the film coating process and formulation. Due to the failure of coating of some pellets has less impact on the release of the active substance in comparing with the failure with large dosage such as tablets.

1.8. Mechanism of drug release from the coated pellets

The release behaviour of the coated pellets can be studied in gastric fluid, intestinal fluid, and colonic fluid. This release profile can be prepared with enzyme and free enzyme fluid at different pH values for each fluid by using USB-bad.

The release mechanism of the coated pellets consists of four important mechanisms (E.Aulton, 2011) and (Y.Karrouta, C. Neutb, D. Wilsc, F.

Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009):

- *Diffusion* is the initial process of drug release which includes the partition of drug into the film coat membrane as well as the permeation of drug through membrane. This process can be impacted by different factors such as polymer types of film, polymer blends ratio, solubility of drug into the membrane, drug particle size, drug concentration gradient across this membrane, thickness of membrane. And the permeability coefficient.
- *Osmosis* process is the second mechanism for release. During this process the osmotic pressure inside the core can be generated as result of diffusion of water into the core of the formulation which in turn can dissolve the drug as well as excipients which leads to a osmotic pressure driven transport through the membrane of drug.
- *Dialysis* is the third process of the drug release mechanism. This process can occur as result to the formation of water- filled channels in the film coat membrane. These channels can be formed due to the imperfection of the coating process. In addition, these channels participated in the transient of the drug. The dialysis process can be affected by different factors for instance the length of water-filled channels, drug solubility in water, and the number of channels into the membrane.
- *Erosion* is the process which is associated with the corrosion of the film coating. This corrosion can result in the drug release especially with natural pH- sensitive coated polymers which designed to target the GIT tract.

2. Material and method

2.1. Material

2.1.1 Polymers

- Starch(which was a kind gift from Spexímo AB- Medicon Village)
- Aqueous ethyl cellulose (Ethoxy content of Ethyl cellulose 49.2 %, Made in USA).
- Active substance (Metoprol pellets gift from AstraZeneca)

2.1.2. Plasticizer

- Tri-ethyl citrate (TEC).

2.1.3. Digestive enzymes

- Pancreatin (from porcine Pancreas)
- Amylase (from *Bacillus* species. 51 units/mg solid)
- Pepsin

2.1.4. Buffer components

All components of PA quality

- Monobasic potassium phosphate
- Sodium hydroxide
- Sodium chloride
- Hydrochloric acid

2.2.5. Equipment

- Teflon moulds in size of 10 x 10 cm.
- Microscope with light board
- Fluidized bed
- Texture analyser
- Digital Measuring Instrument
- USB bad.

2.2 EXPERIMENTAL METHODS

All tests were done in triplicate and the average as well as the standard deviation were calculated and summarized in the results chapter.

2.2.1 Preparation of films

The starch- aqueous ethyl cellulose films with different polymers blend ratio and different amount of plasticizer TEC were prepared by diluting of 30% aqueous ethyl cellulose with deionised water to 15%. Thereafter, the desired amount of TEC was added to the 15% EC overnight. Then, the starch was added to the plasticized EC dispersion gradually and mixed for one hour. Finally, these polymeric blends were casted into 10 x 10 cm Teflon moulds and dried at different drying temperature (40, 60, and 80 ºc).

2.2.2. Preparation of gastric fluid, stimulated intestinal fluid and colonic fluid

Simulated gastric fluid is an artificial dissolution medium that is intended to represent stomach acid. It is prepared by dissolving 2.0 g of sodium chloride and 3.2 g of purified pepsin (800 to 2500 units per mg of protein), in 7.0 ml of hydrochloric acid and water up to 1000 ml. This test solution has a pH of about 1.2.

The simulated Intestinal Fluid can be prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 mL of water and then adding 77 mL of 0.2 N sodium hydroxide and 500 mL of water. 10.0 g of pancreatin is added and the resulting solution is adjusted with (0.2) N sodium hydroxide or (0.2) N hydrochloric acid to a pH of 6.8 and finally diluted to 1000 ml.

The simulated colon Fluid prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 mL of water and then adding 77 mL of 0.2 N sodium hydroxide

and 500 mL of water. 10.0 g of amylose added and the resulting solution can be adjusted with (0.2 N) sodium hydroxide or (0.2 N) hydrochloric acid to a pH of 6.8. Finally, the solution was diluted to 1000 ml. These steps repeated to get simulated colon fluid with pH 5.6 and 2.8 as expected colon have thus values at colon at different disease state.

Commonly, the recipes of fluids recipes were prepared according the used dissolution media described in United States Pharmacopeia 33-28 NF (2010) and European Pharmacopeia 7.0 (2010).

2.2.3. Pellets coating

Aqueous ethyl cellulose dispersion blended with plasticizer (25 % TEC, 15% EC-solution; overnight stirring) in cold room, after that starch was added at different polymer ratio of 1-2, and 1-3 starch: cellulose. Subsequently, the solution with added starch was stirred for 1 h. The drug-loaded pellet was coated in a fluidized bed (Procept processing concept, model 4M8-TriX) by spraying of the solution.
The fluidized bed parameters were as follows:

- product temperature = 25 °C
- spray rate = 2.5 g/min
- Air speed= 0.3$m3/mi$

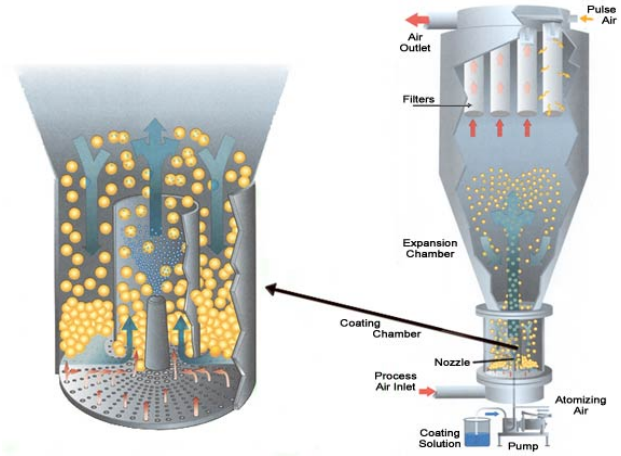


Figure 4 fluidized bed

2.2.4. Water uptake and dry mass:

Film pieces of 2 * 3 cm were placed into 80 mL tubes filled with 40 mL pre-heated medium, followed by horizontal shaking at 37 °C by using plate shakers apparatus. The medium was used as:

(i) Simulated gastric fluid (sodium chloride, hydrochloric acid and purified pepsin), it has a pH of about 1.2.

(ii) Simulated Intestinal fluid (monobasic potassium phosphate, 0.2 N sodium hydroxide and pancreatin), solution is adjusted to pH 6.8.

(iii) Simulated colon fluid (monobasic potassium phosphate, 0.2 N sodium hydroxide and amylose), solution is adjusted to different PH value which is related to the condition of diseases. The pH used was 2.8, 5.6, and 6.8.

Pieces of different polymeric films in size of 2 x 3 cm were prepared and weighted. Thereafter, these pieces were immersed into the 50 ml tubes with colonic enzyme and enzyme- free buffer solutions with horizontal shaking for 18 hour at 37 °C. Finally, these pieces were weighted directly after immersing and after drying at 60º for 2 h. Water uptake and dry loss mass were calculated according to equations below

$$\text{water content } (\%)(\text{t}) = \frac{\text{wet mass (t)} - \text{dry mass (t)}}{\text{wet mass (t)}} . 100\ \%$$

$$\text{dry film mass } (\%)\ (\text{t}) = \frac{\text{dry mass (t)}}{\text{dry mass (t} = 0)} . 100\ \%$$

2.2.5. Microscopy

After withdrawn of the films from the pre-heated medium exposed to examination of the film`s surface by used the microscopy with light board (Olympus from instrument AB models CHS/CHT)

2.2.6. Mechanical properties:

The mechanical properties of the films at wet state were determined with a texture analyser (TAXT2i from stable micro system LTD) by the puncture and needle test. Film specimens were mounted on the puncture probe which has spherical shape with 50 mm in diameter. The force apply on the films was fixed by use a shaft with load cell (2 kg) and the needle at the ended. The shaft driven downward to the centre of the film holder's hole. When the needle touches the film, results can be obtained as a curve and the top of the curve shows the maximum force film suffer before it damaged. The results were obtained as a plot which show the puncture force that need to puncture the film surface VS time as shown in the figure 5.

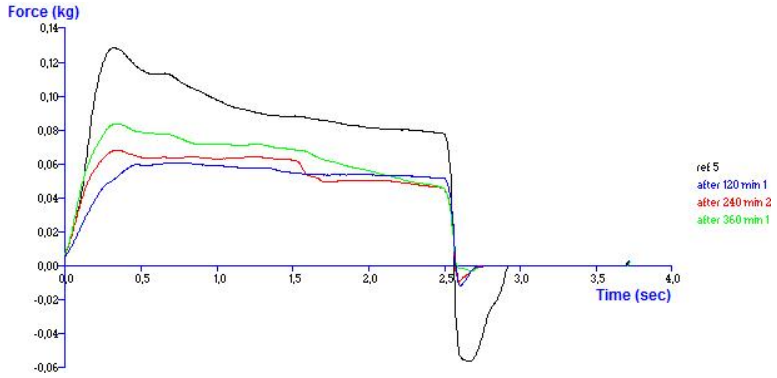


Figure 6 result from the texture analyzer

2.2.7. In vitro drug release from coated pellets:

In vitro drug release studies were undertaken according to the USP method (paddle method) by used USB bad (from Vankel technology group). All tests were conducted in double test using 1000 mL of dissolution media maintained at 37 °C with a paddle rotation speed of 100 rpm and samples were withdrawn and analysed UV-spectrophotometrically (λ = 274 nm). The wave length as selected related to testing which was done in spectrophotometer by used dissolution with dilute range to get the calibration curve with absorption under 2 as took into consideration as appropriate value with the reading of the USP bad.
Coated and uncoated formulations were first tested in enzyme-free simulated gastric fluid (pH 1.2), enzyme-free simulated Intestinal fluid (pH 6.8), and enzymes' simulated intestinal colon (PH 2.8, 5.6, and 6.8) over a period of 18 hours. Samples were withdrawn at predetermined intervals for every 10 minutes for first hours, 15 min for second hours, 30 minutes for 4 hours, and the last sample predetermined after 18 hours.

Tables 1 and 2 summarize all parameters that be used in this study.

Table 1 this table shows GIT organs, enzyme as well as pH which was used in this study

GIT	pH	enzyme	Transit time
stomach	1—3.5	pepsin	5 min—2h
Small intestines	6—7.4	pancreatin	2.5h —4h
colon	5.4—6.7	Bacterial α-amylase	2h—48h

Table 2 this table shows pH values and the enzyme concentration in the colon in the healthy and different disease states

State of colon	pH	Concentration of *Bacterial α-amylase*
Healthy state	6.8	500, 1500, and 2500 U
Acute disease state	5.6	500, 1500, and 2500 U
Severe state	2.8	500, 1500, and 2500 U

3. Result and discussion

3.1. Film characterization

The water uptake, dry mass loss, microscopy analysis, texture analysis and camera images of prepared polymeric films was investigated along the GIT tract especially in the colon at the healthy as well as disease state, using simulated fluids. This investigation provides an insight into properties of the prepared films such as stability, permeability, digestibility, diffusion, residence time in the different stimulated fluids of GIT tract as well as the behaviour of drug release from the prepared coated pellets. The study and understanding of these properties and their relationship to condition in disease state as well as factors that impact these properties provide information which can be used to optimize a function film that can ensure the delivery of therapeutically active substance to colon with a desired dose in convenient and safe way. The results from these investigations are presented in chapter 3.1, 3.2, 3.3, and 3.4.

3.1.1. Water uptake, dry mass loss, and microscopy analysis

As mentioned above the water uptake and dry mass loss have a considerable influence on the permeability and stability of the film coating which in turn impact the release of drug through it. In addition, this investigation was prepared at different pH values as well as with pepsin, pancreatin, and different concentration of *B. licheniformis* α-amylase. Moreover, these measurements were also plotted with transit time to illustrate the behaviour of these results along the GIT at stated transit time as well as at different colonic disease states which were showed in figures (6-20) below.

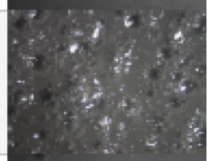
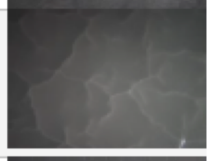
The results of the water uptake and dry mass loss in tables and more clearly in plots can be provided insight of how these polymeric films can be impacted along the GIT tract at different states. Moreover, these results can provided an understanding of the influence of the most important factors such as polymer

blend ratio, pH, enzyme concentration, and buffer type on the water uptake and dry mass loss.

3.2. Investigation of the film coating

In table 1 and 2 the initial pre-studies on the effect of formulation and production conditions on film formation is presented. These tables summarize the formulation parameters, properties of film as well as the impact of these parameters on the prepared film.

Table 3 summery of film properties as well as microscopy images

Ratio (Starch: Cellulose)	features	Properties achieved	
3:1	Plasticizer added to total dry weight. Low drying temperature 40°C.	Almost homogenous film. Good to characterize.	
3:1	Plasticizer added to total dry weight. Middle drying temperature 60°C.	Most homogenous film. Good to characterize	
3:1	Plasticizer added to total dry weight. High drying temperature 80°C.	Not homogenous film. with evenly distributed small grains. Good to characterize	
3:1	Extra plasticizer added to total dry weight. Middle drying temperature 60°C.	homogenous structure with evenly distributed small grains.	
3:1	Less plasticizer added to total dry weight. Middle drying temperature 60°C.	Homogenous film. with some distributed small grains.	
3:2	Plasticizer added to total dry weight. Middle drying temperature 60°C.	No intactness and no homogenous. Not as good to characterize.	
4:1	Plasticizer added to total dry weight. Middle drying temperature 60°C.	brittle film, phase separation occurred when drying, can only remove segments of film from Teflon pan.	
0:3	Plasticizer added to total dry weight. Middle drying temperature 60°C.	Cellulosic film, most homogenous structure. Good to characterize.	

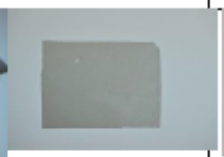
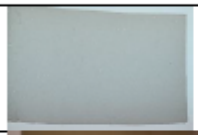
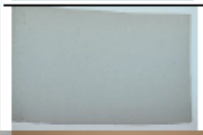
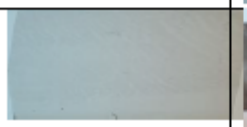
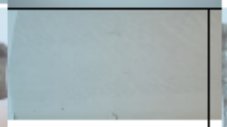
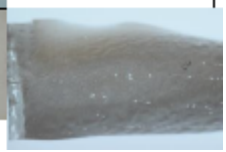
			Without Enzyme		With Enzyme	
Starch	TEC	Temp.	Before	After	Before	After
3:1	1	40				
3:1	1	80				
3:1	1	60				
3:1	0.75	60				
3:1	1.25	60				
3:2	1.25	60				
4:1	1	60				
0	1	60				

Table 4 impact of the buffer solution with and without enzyme on the film surface

3.2.1 Effect of the film formation temperature

From the table 3 and 4, the temperature does not have a significant impact on the water uptake and dry mass loss. However, it does affect the visual appearance of the film as can be seen in table 1 and 2 .The films prepared at 60 °C are more homogenous than the ones prepared at 80 °C which shows more roughness of the prepared film. This could be due to that the interactions between the polymers as well as TEC can be facilitated with high temperature.

In table 4 the effect on the films to exposure to solutions can be seen. It can be noted that the enzyme solution has a distinctly different effect on the films compared to buffer solutions leading to more loss of materials from the films as seen by more brittle and curved films. This can be due to that the buffer solution impacts entire surface of the polymeric film equally which in turn increase the exposure area to contact with buffer solution as well as increase the starch availability to buffer and enzyme. Moreover, the pieces of films with high temperature were curved more than pieces with lower temperature (40 and 60 ºc) after that these pieces were exposure to the buffer solution.

This increase of impact leads to that all starch and TEC, which are uniformly distributed on the film surface is attacked by buffer solution. However, the high film formation temperature decreases adhesion between polymers, which in turn reduce the homogeneity, and uniformity for the surface as well as influence the TEC role on fusion of EC particles. This minimizes exposure surface area, which in turn could impact the water uptake and diffusion of buffer solution and drug release.

This phenomenon can be attributed to the swelling of starch and this phenomenon can be seen obviously at higher drying temperature because of the inhomogeneity. However, this impact does not occur with cellulose and with small grade with high cellulose- starch ratio blend according to the minimizing of swelling which is associated with starch. Obviously, images on table 3 and table 4 describe these results, phenomena, and factors which impact the films.

3.2.2. Effect of the plasticizer (TEC) amount

TEC is a water soluble plasticizer which can be used to improve the fusion of the nanoparticles of aqueous ethyl cellulose dispersion. Plasticizer should be partition from aqueous phase into polymer phase when they are added to solvent- EC. As can be seen in table 3 and 4 the amount of plasticizer affects the appearance of the dry film. This partition of TEC leads to cracks on the film surface during the drying process which explains the small cracks on the surface of cellulose film or film with high cellulose- starch ratio as showed in (table 3) (M. Tarvainen, 2003). It has been shown that, plasticizer amount of 25% for the total dry weight of starch-EC provides a function film with good mechanical properties, stability and water uptake (Y. Karrout, 2009). In our work we see that TEC content below this value effects the films probably by reducing the fusion of ethyl cellulose nanoparticles. This could be due to reduction in polymer chain mobility during the film formation. This impact the homogeneity and uniformity of the film surface as showed in the (table 3). Increasing of TEC amount above this value gives anti-plasticizer effect such as increasing of sticking tendency which impacts the coating process and the adhesion of two polymers.

Significantly, the water uptake and dry mass loss increase with increasing of plasticizer amount irrespective of the presence or absence of the enzymes. For example the films of 3-2 polymer ratio have more TEC than 3-1 polymeric films for 3:1 ratio as showed in the tables (5-30) and figures (6-20). This performance can be attributed to the influence of the water solubility of plasticizers on the release mechanism. This means that the water soluble plasticizers such as TEC migrate from the coating after contact with water and that water can also act as a plasticizer which in turn increases the permeability and diffusion of water into coating system. This diffusion increases the water uptake, dry mass loss which led to increasing drug release.

As showed in the tables (5-30) the surface for film piece after exposure to gastric as well as buffer solution with and without enzyme was different for films produced with low and high amounts of TEC. Increase of TEC content gave more damaged surface of film pieces and resulted in many pores probably due to the leaching of the TEC as well as starch. Observably, this impact was increased with exposure time to solution (table 6-30).

3.2.3 Effect of the starch-aqueous EC dispersion ratio

Clearly, the polymer-starch ratio has the most impact on the film properties both on the homogeneity tables 3 and 4 as well as on water uptake and dry mass loss behaviour (tables 5-30). As showed in the (table 3) films with higher ratio of starch relative cellulose results in starchy, brittle, non-homogenous film surface and not and a much weaker film as seen by puncture force measurements in texture analyses as showed in the figures (21-25) . This can be rationalized to that the starch has a poor mechanical properties. Results from tables (4-15) showed that the water uptake and dry mass loss increase with the increasing starch ratio independently of the presence or absence of enzyme and the pH value investigated. The increase in water uptake can be attributed to that the starch is hydrophilic polymer in contrast to EC which is hydrophobic polymer.

The increasing of dry mass loss can be attributed to that the leaching of hydrophilic compounds such as TEC and starch into buffer solution. The premature of leaching occur but in small grad in upper GIT as result to the stated reason but EC prevent completely leaching of these hydrophilic components(9). (figure 4) shows also that the cast aqueous ethyl cellulose film has a significant water uptake and dry mass loss and the reason for this behaviour may be occurred as result to that the leaching of TEC and in smaller grade to the sodium dodecyl sulphate (SDS) in aqueous EC which becomes de-protonated at PH 6.8-

7.2 at the same time, this can be justified the increase of the water uptake as well as the dry mass loss in the intestinal fluid as well as colonic fluid at high PH.

3.3 Effects of simulated GI fluids on EC: starch films

3.3.1 Effect of low pH- simulated gastric fluid

As can be seen in tables (5-7) and figures 6 and 7 there are some initial effect on the films by exposing them to simulate gastric fluids. However, the dry mass loss does not increase over time while there is a small gradual water uptake that was more pronounced over time for the 3:2 EC: starch films. Pepsin only has minor effects on the results seen in table 5. This is as expected since pepsin is a protease and should not influence the main components of the film.

Table 5, behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at gastric fluid with pepsin

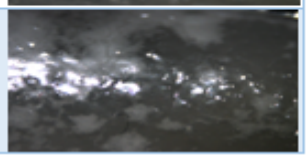
Stomach with pepsin			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscope
0.5	10.5±0.6%	3.94±1%	
1	14.4±0.8%	3.68±1.4%	
1.5	16.7±1.1%	3.63±0.6%	
2.0	17.1±0.8%	4.50±0.8%	

Table 6 behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at gastric fluid without pepsin

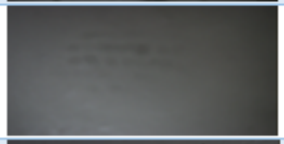
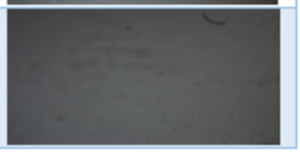
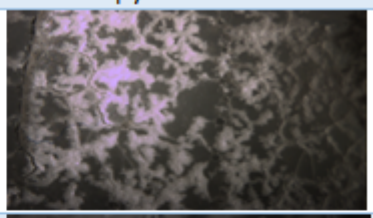
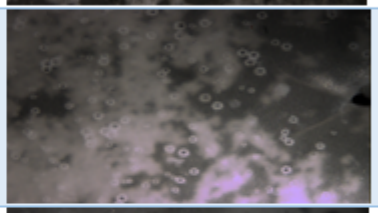
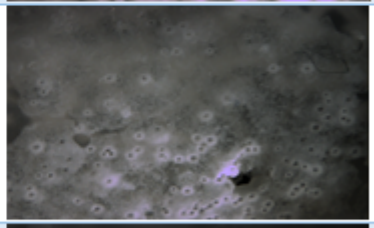
Stomach without pepsin			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscope
0.5	18.6±1.5%	5.7±0.8%	
1	17.0±1.2%	6.4±0.2%	
1.5	17.8%1.3%	6.9±0.4%	
2.0	16.0±2.0 %	8.4%0.7%	

Table 7 behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at gastric fluid with pepsin

Stomach with pepsin			
Time (h)	Water uptake	Dry mass loss	Microscopy
0.5	3.5 ±0.9%	5.4± 0.3%	
1	5.7 ±1%	7.8 ±0.7%	
1.5	17.8 ±2%	7.8 ±0.5%	
2.0	22.6±3%	9.1 ±0.2%	

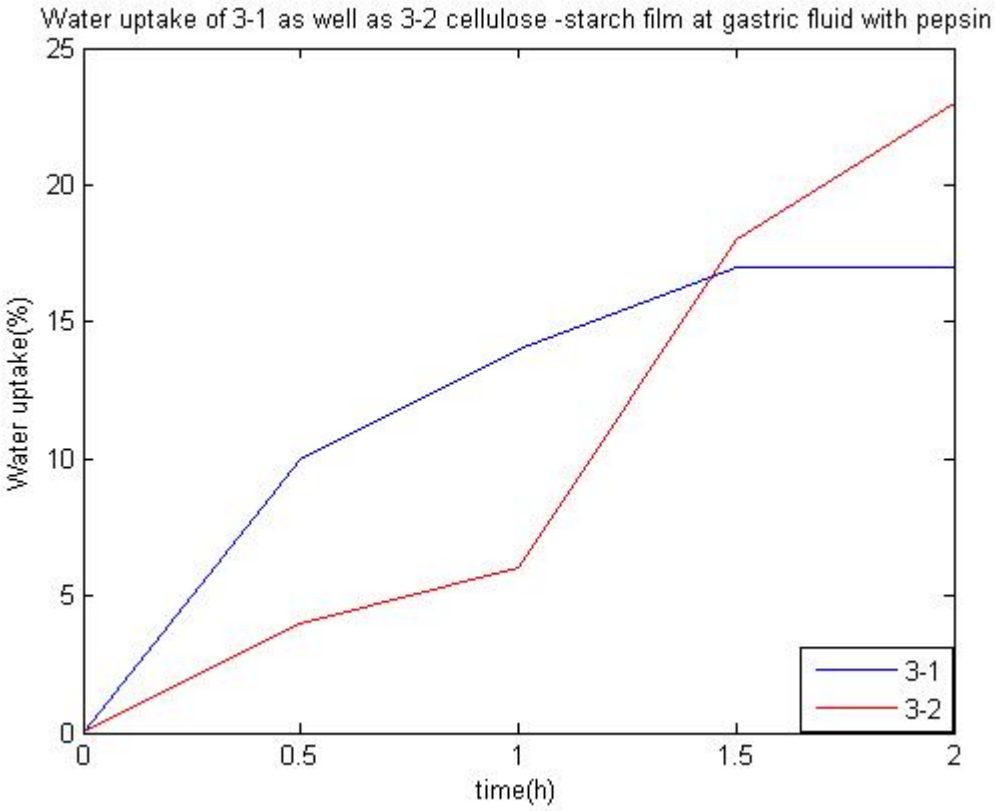


Figure 6 Water uptake of the 3-1 as well as 3-2 aqueous ethyl cellulose- starch at stimulated gastric fluid with pepsin

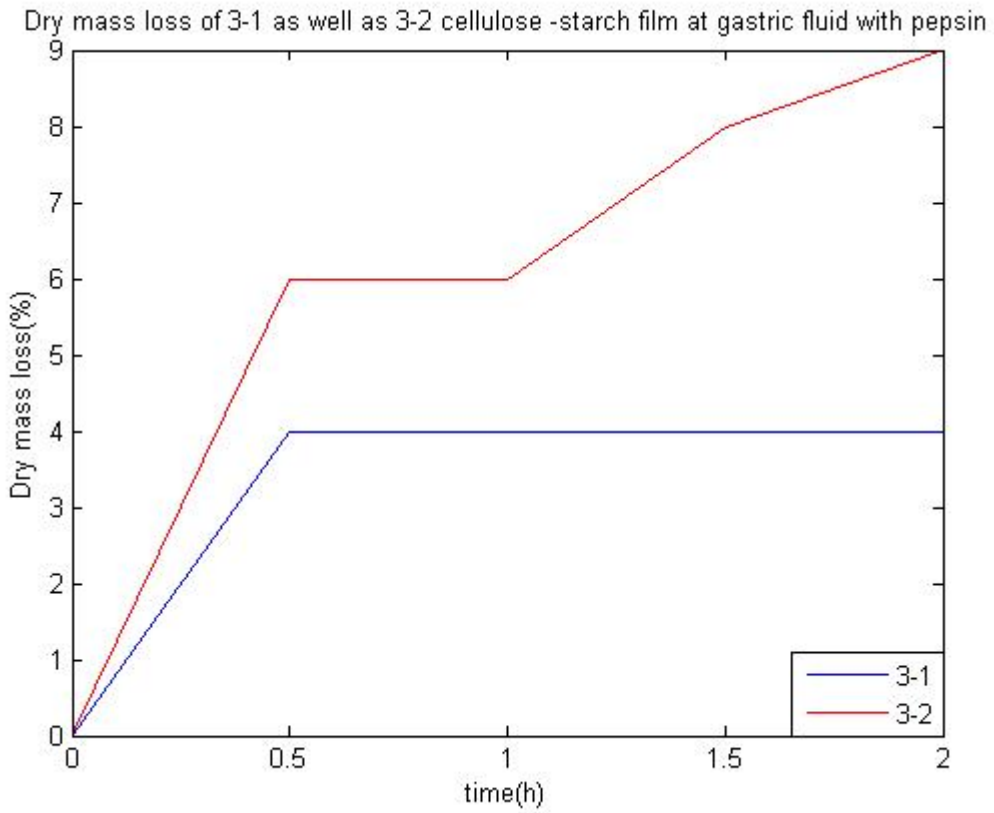


Figure 7 Dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the gastric fluid with pepsin

3.3.2. Simulated intestinal fluid-effect of α-amylase

As stated above, the pancreatin is a complex enzyme mixture which comprises lipases, proteases, and amylases. Considerably, the α-amylase has a significant impact on the starch in the upper GIT. However, this enzyme has lower activity than the bacterial α-amylase on the digestion of starch.

Table 8 and table 9 illustrate the impact of this enzyme on the water uptake, dry mass loss, and the surface of 3-1 as well as 3-2 EC-Starch films respectively. Additionally, the (figure 8) and (figure 9) shows the water uptake and the dry mass loss rate with transit time respectively. These results showed that simulated intestinal fluid with pancreatin has more obvious impact on the dry mass loss than water uptake for both polymer blends´. In difference from simulated gastric solution there are a dry mass loss with time both for solution with and without pancreatin but the effect is much larger with pancreatin. The water uptake is in the same range as for gastric fluid and as for gastric fluid it is more pronounced for the 3:2 EC-Starch films.

Table 8 Behaviour of the water uptake dries mass loss, and microscopy of 3-1 ethyl cellulose- starch at intestinal fluid

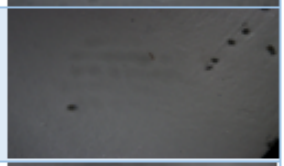
Intestinal fluid with pancreatin film 3-1			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV±SD)	Microscope
1.5	9.0±0.8%	19.5±2.0%	
3	6.8±1.0%	24.1±3.5%	
4.5	11.1±2.0%	26.3±2.8%	
6	13.1±1%	29.2±2.0%	

Table 9 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at intestinal fluid

Small intestines			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV ± SD)	Microscopy
1.5	12.9± 1.1%	21.6 ±1.2%	
3	13.3±2%	24.8 ±1%	
4.5	18.7±1.1 %	26.31.3 ±%	
6	18.9 ±3%	29.7±0.3%	

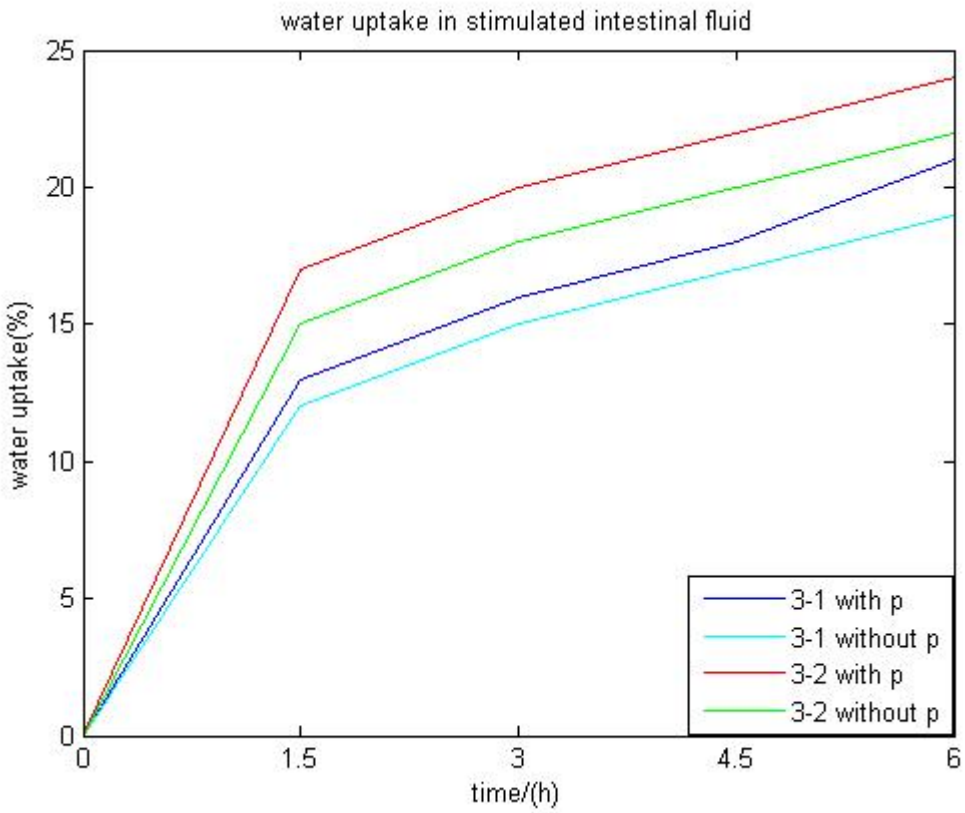


Figure 8 water uptake of the 3-1 as well as 3-2 aqueous ethyl cellulose- starch at stimulated intestinal fluid with pancreatin.

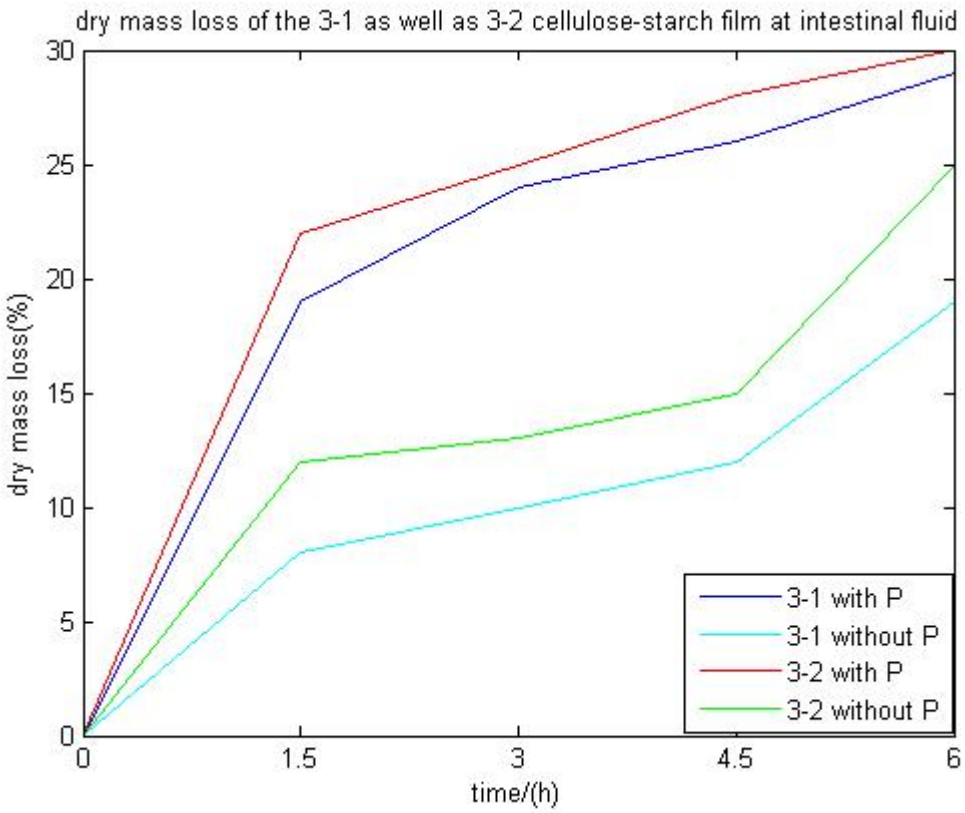


Figure 9 dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the stimulated fluid with pancreatin

The increase of the dry mass loss with time can be attributed that α-amylase digest the starch by cleaving the α-1, 4 glycoside linkages of amylose to yield dextrin, maltose, or maltotriose. The increase of the water uptake can be attributed to the leaching of the water soluble starch and TEC as described above. Moreover, this increase may be caused by the digestion of the starch by the pancreatic amylase. This digestion can be resulted in formation of pores on the film surface which in turn increase the penetration of the buffer into the film formulation.

The microscopy analysis was provided an appropriate investigation of the impact on the surface of film formulation. Noticeably, this impact can be observed more clearly on the surface of film with 3-2 EC-Starch films due to that more starch are available to pancreatic amylase. The increasing of this impact with time can be rationalized by increasing of penetration of buffer with pancreatic enzyme into the film formulation which in turn facilitates the contact of starch with this enzyme.

3.3.3. Stimulated colonic fluid-Effect of bacterial α-amylase

As discussed previously the diseased and healthy colon have different pH and bacterial amylase content and thus effect of pH and bacterial α-amylase content was investigated

In tables (10-15) and the figures (10-12) the effects of pH without any bacterial α-amylase was shown. As can be seen the loss of dry mass and water uptake was only affected by to a minor extent by the pH although there are an increasing mass loss and water uptake with time. This is further discussed in chapter 3.5.

Table 10 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid pH 6.8

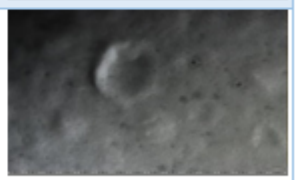
Colon PH 6.8			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV±SD)	Microscope
2	14.9±0.4%	7.8±0.7%	
4	19.6±1.1%	9.6±1.5%	
6	20.02±2.0%	11.6±0.6%	
18	22.4±2.6%	18.5±1.4%	

Table 11 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid 5.6

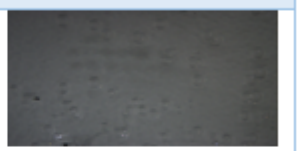
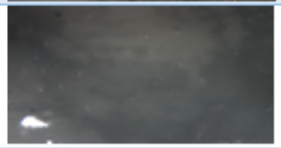
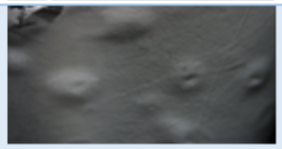
Colon PH 5.6			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV ± SD)	microscope
2	19.5±1.4%	6.2±0.5%	
4	19.4±0.6%	9.0±0.6%	
6	19.6±2.7%	10.8±0.5%	
18	19.9±1.2%	17.2±1.2%	

Table 12 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid pH 2.8

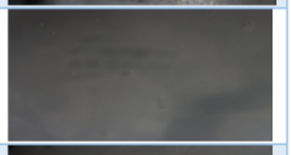
Colon PH 2.8 film 3-1			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV ± SD)	microscope
2	17.0±0.8%	8.3±1.5%	
4	18.0±1.4%	7.6±0.3%	
6	24.0±1.9%	9.1±0.6%	
18	18.4±2.1%	15.1±0.5%	

Table 13 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid pH 6.8

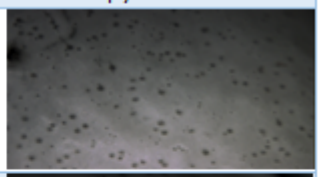
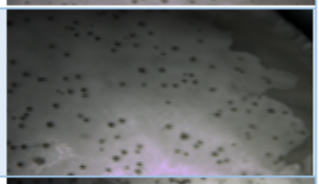
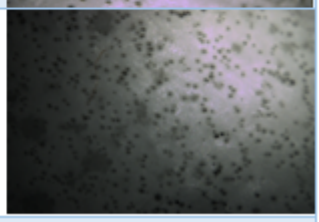
Colonic pH 6.8			
Time (h)	Water uptake(AV ± SD)	Dry mass loss(AV ± SD)	Microscopy
2	19.7±2,7%	12.0±0,3%	
4	15.0±2,5%	13.0±0,5%	
6	23.4±3,2%	14.9±0,2%	
18	19.7±1,3%	24.6±0,8%	

Table 14 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid pH 5.6

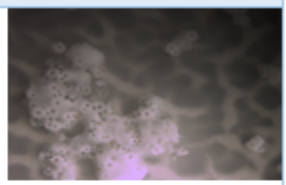
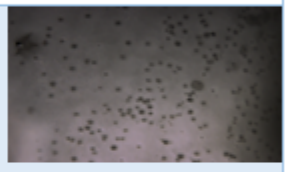
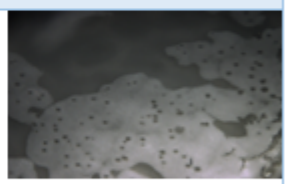
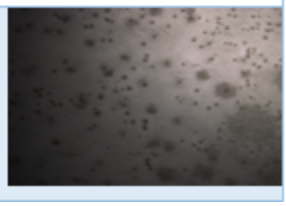
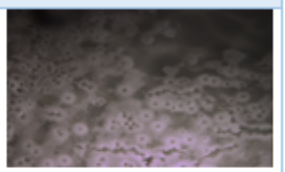
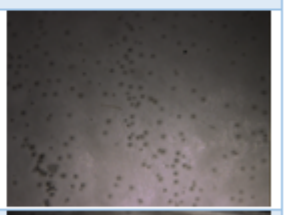
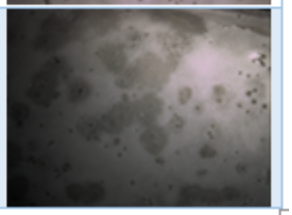
Colonic pH 5.6			
Time (h)	Water uptake (AV ± SD)	Dry mass loss(AV ± SD)	microscopy
2	18.6±0.8%	8.9±0.7%	
4	25.3±2.9%	12.6±0.5%	
6	25.5±3	15.5±0.4%	
18	23.1±2.1%	19.9±0.9	

Table 15 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid

Colonic pH 2.8			
Time (h)	Water uptake (AV ± SD)	Dry mass loss (AV ± SD)	Microscopy
2	24.0±3.2%	9.0±0.7%	
4	24.4±1.8%	11.9±0.3%	
6	20.8±3%	13.7±1.1%	
18	19.8±1.4%	18.8±1.5%	

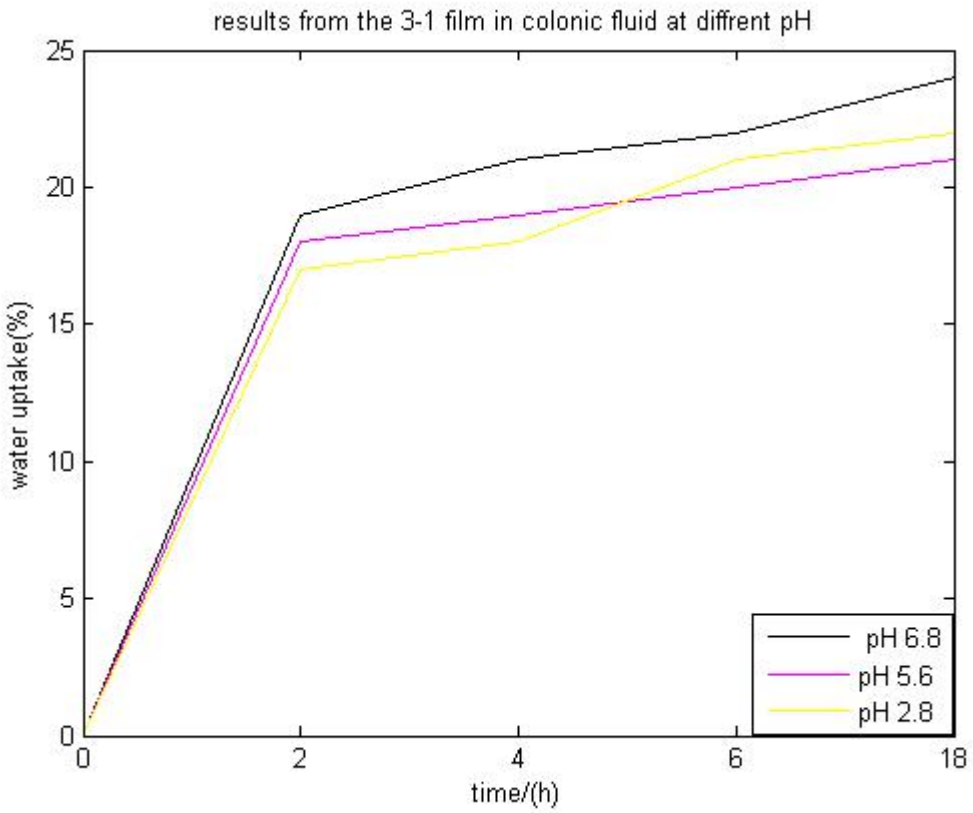


Figure 10 water uptake release of the 3-1 aqueous ethyl cellulose- starch at stimulated colonic fluid at different PH.

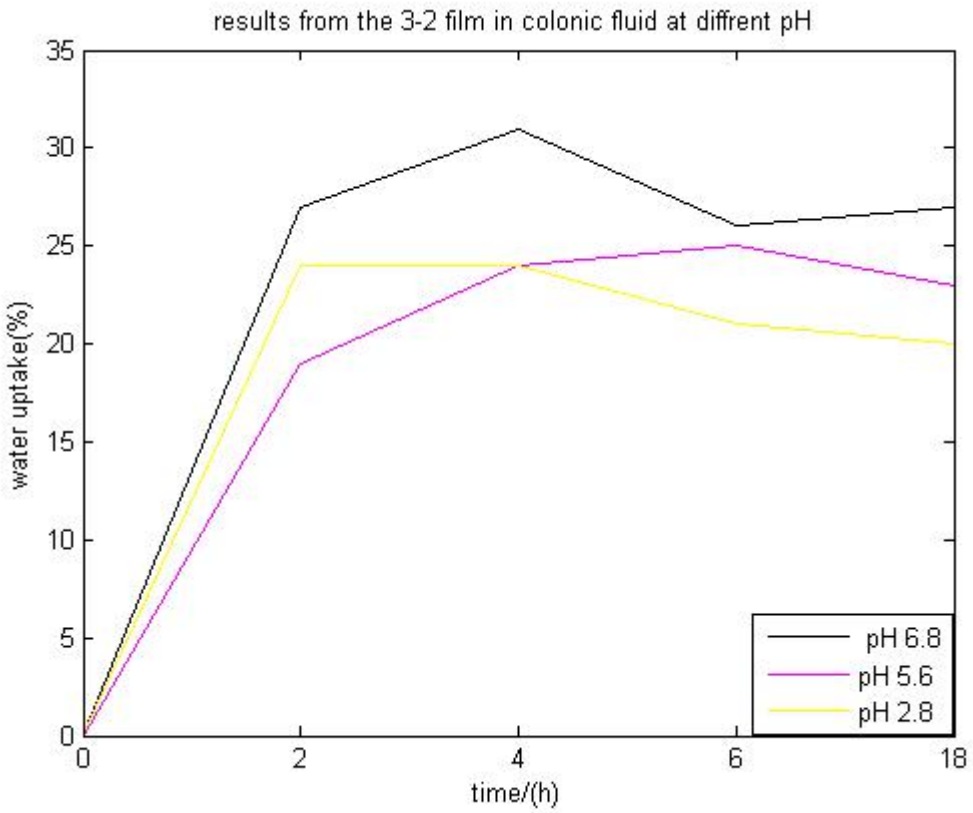


Figure 11 water uptake release of the 3-2 aqueous ethyl cellulose- starch at stimulated colonic fluid at different PH.

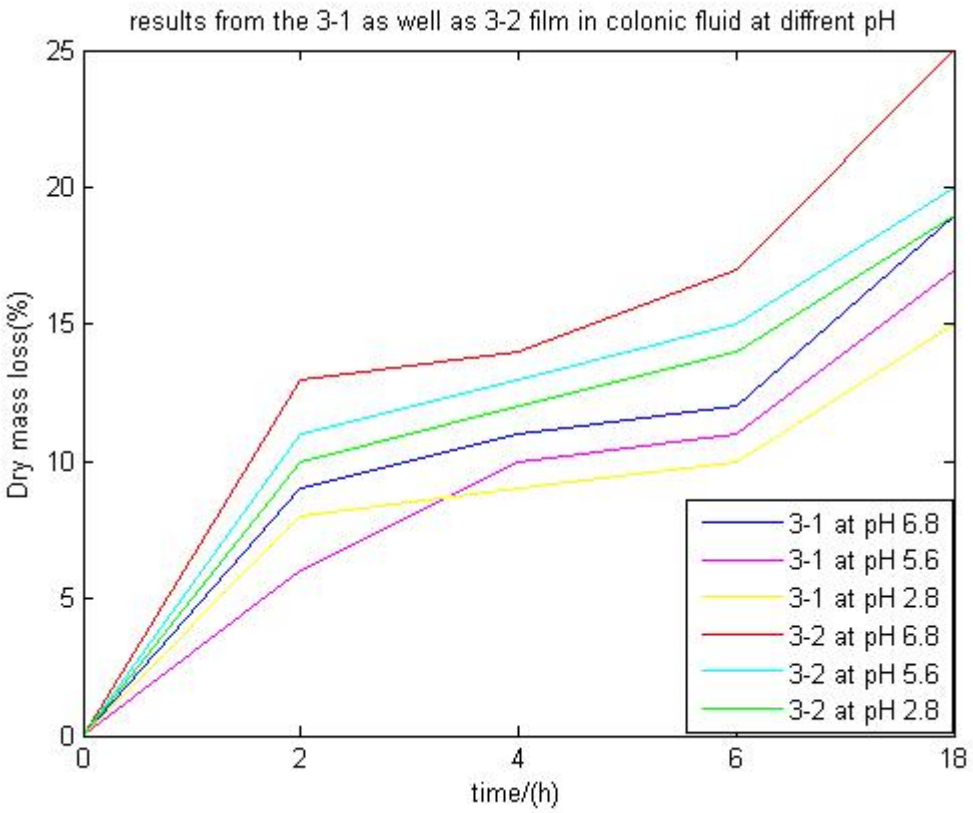


Figure 12 Dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the colonic fluid at different PH

To assess the effect of bacterial α-amylase this was added to the colonic fluids. This simulated colonic fluid and thus bacterial α-amylase has a very significant impact in water uptake and dry mass loss behaviour for investigated polymeric films. The dry mass loss is slightly higher than for simulated intestinal fluid with pancreatin and it is higher for the 3:2 EC-starch film than the 3:1 one.

Table 16 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

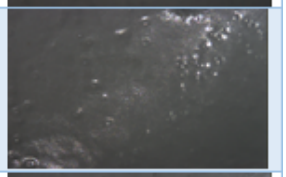
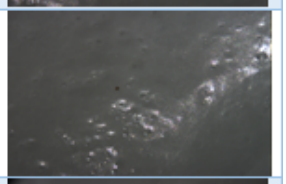
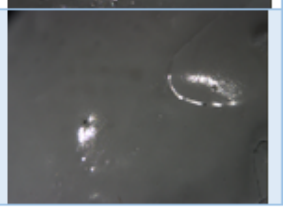
Colon PH=6.8 500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	19.5±0.6%	8.7±0.6%	
4	20.5±1.5%	17.0±1.9%	
6	16.2±4.7%	17.3±2.4%	
18	13.2±1.1%	32.6±0.2%	

Table 17 behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

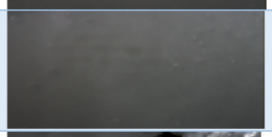
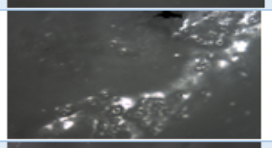
Colon PH=6.8 1500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	20.6±2.2%	9.1±0.9%	
4	20.9±0.1%	15.3±3.5%	
6	16.2±6.8%	20.4±0.8%	
18	17.1±1.2%	35.4±0.7%	

Table 18 behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

Colon PH=6.8 2500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	17,7±0.9%	10,2±0.9%	
4	25,2±0.8%	20,6±4.4%	
6	17,4±3.1%	23,2±1.8%	
18	13,4±0.3%	36,2±1.4%	

Table 19 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

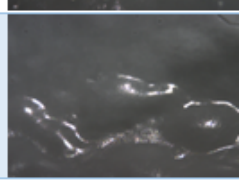
Colon PH=5,8 500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	17.4±4.1%	10.3±0.5%	
4	12.3±3.6%	14.8±2.7%	
6	17.3±3.4%	16.6±2.2%	
18	11.5±0.8%	23.7±1.8%	

Table 20 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

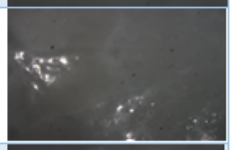
Colon PH=5.6 1500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	13.4±0.7%	11.4±0.6%	
4	12.2±1.7%	16.1±0.6%	
6	17.0±0.7%	20.1±0.1%	
18	9.0±1.0%	29.1±0.9%	

Table 21 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

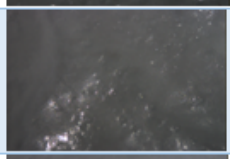
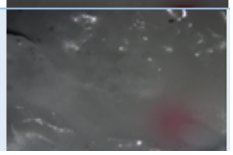
Colon PH=5.6 2500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	14.5±0.7%	17.5±0.9%	
4	13.0±1.9%	13.3±4.7%	
6	16.5±2.9%	20.7±0.6%	
18	12.1±2.6%	29.6±0.8%	

Table 22 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

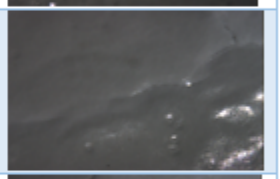
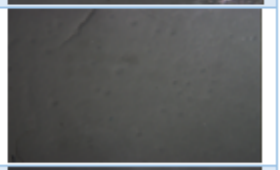
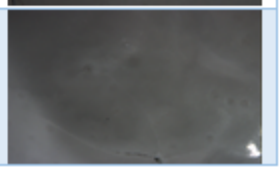
Colon PH=2.8 500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	16.6±0.1%	7.5±0.4%	
4	18.3±0.1%	8.7±0.0%	
6	11.4±1.4%	12.2±1.9%	
18	17.2±7.3%	15.3±1.9%	

Table 23 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

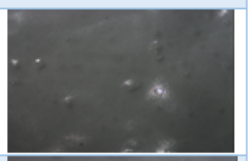
Colon PH=5.6 1500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	20.1±4.4%	6.0±0.8%	
4	17.8±2.2%	8.6±0.8%	
6	14.8±4.9%	9.9±2.1%	
18	20.7±1.0%	13.6±0.4%	

Table 24 Behaviour of the water uptake, dry mass loss, and microscopy of 3-1 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

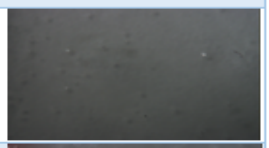
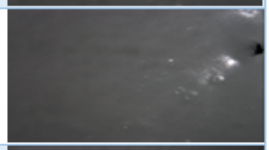
Colon PH=2.8 2500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	18.7±5.1%	6.7±0.5%	
4	20.6±8.1%	10.6±1.8%	
6	24.7±5.9%	15.9±2.5%	
18	15.1±4.9%	14.8±0.9%	

Table 25 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

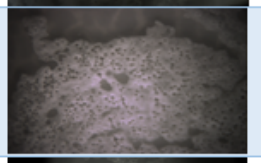
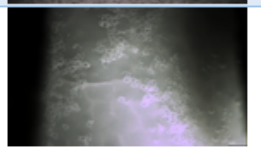
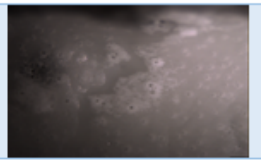
Colon PH=6.8 500 U			
Time (h)	Water uptake(AV ± SD)	Dry mass loss (AV±SD)	Microscopy
2	18.1±1.3%	19.0±1.3%	
4	15.6±0.4%	23.3±2.6%	
6	19.2±5.7%	24.1±2.5%	
18	11.4±0.6%	37.9±1.0%	

Table 26 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

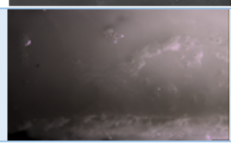
Colon PH=6.8 2500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	20.4±1.4%	18.9±1.1%	
4	14.2±0.5%	29.7±0.0%	
6	16.5±1.7%	29.7±1.2%	
18	29.2±1.5%	38.1±1.1%	

Table 27 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

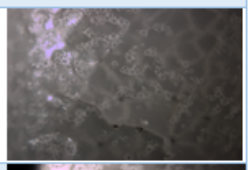
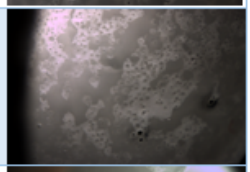
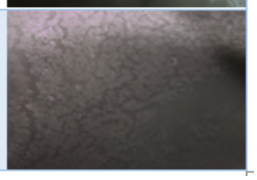
Colon PH=5,8 500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	18.4±1.9%	15.6±2.0%	
4	22.2±1.2%	20.1±0.7%	
6	16.7±0.8%	22.6±0.4%	
18	8.6±5.4%	40.2±1.4%	

Table 28 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

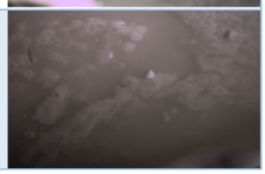
Colon PH=5.6 2500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	18.0±3.6%	18.4±2.0%	
4	13.8±1.7%	25.3±1.7%	
6	17.2±5.9%	33.7±5.1%	
18	10.8±0.4%	41.4±2.3%	

Table 29 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

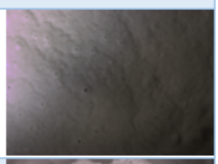
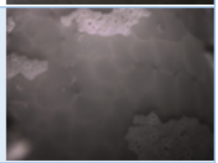
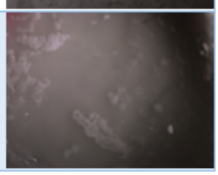
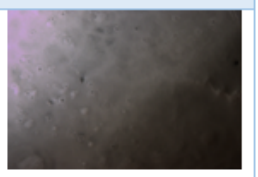
Colon PH=2.8 500 U			
Time (h)	Water uptake(AV±SD)	Dry mass loss (AV±SD)	Microscopy
2	15.0±1.7%	15.2±3.5%	
4	17.6±1.2%	15.5±0.4%	
6	20.3±1.1%	15.9±0.2%	
18	20.3±1.9%	21.2±0.4%	

Table 30 Behaviour of the water uptake, dry mass loss, and microscopy of 3-2 ethyl cellulose- starch at colonic fluid with bacterial α-amylase

Colon PH=2.8 2500 U			
Time (h)	Water uptake	Dry mass loss (AV±SD)	Microscopy
2	21.4±2.8%	14.2±2.1%	
4	19.5±0.3%	15.0±0.3%	
6	20.0±2.2%	16.2±0.1%	

Obviously, tables (16-24) and tables (25-30) show impact of different concentration (500 U, 1500 U, and 2500 U) of this enzyme on water uptake, dry mass loss, and structure of 3-1 and 3-2 polymeric film respectively at stimulated colonic fluid with different PH value (6.8, 5.6, and 2.8). The results of dry loss mass and water uptake were plotted and showed in the figures (13-15) and (16-20) respectively.

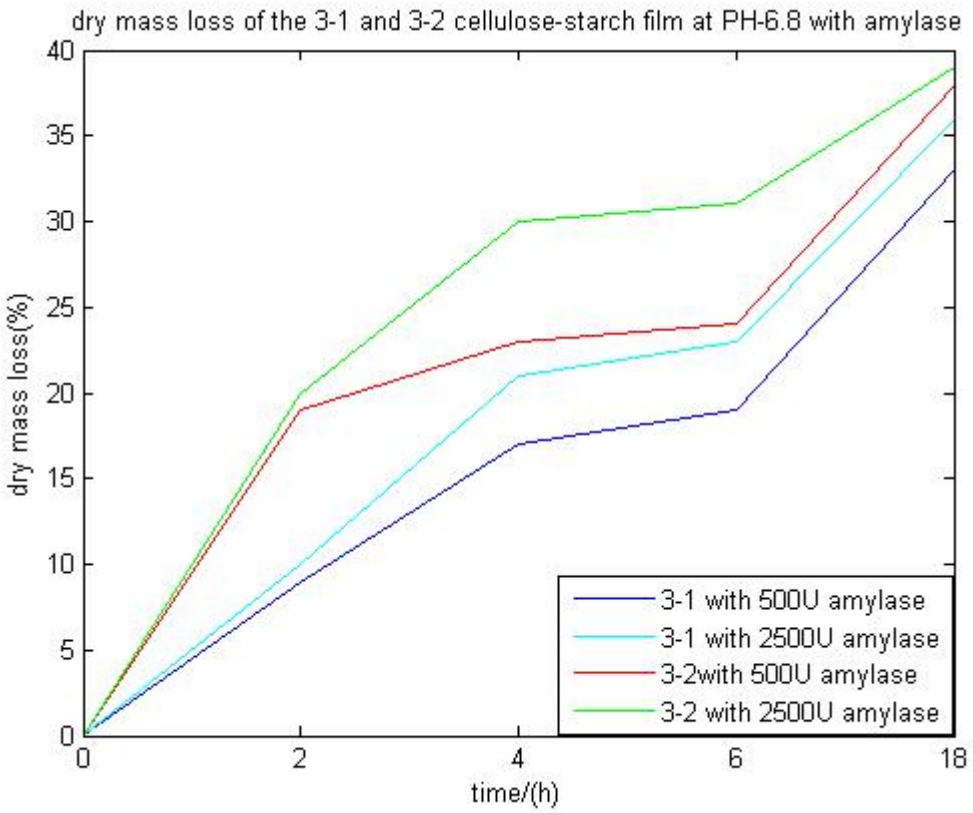


Figure 13 dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the stimulated colonic fluid at PH 6.8 with different concentration of amylase.

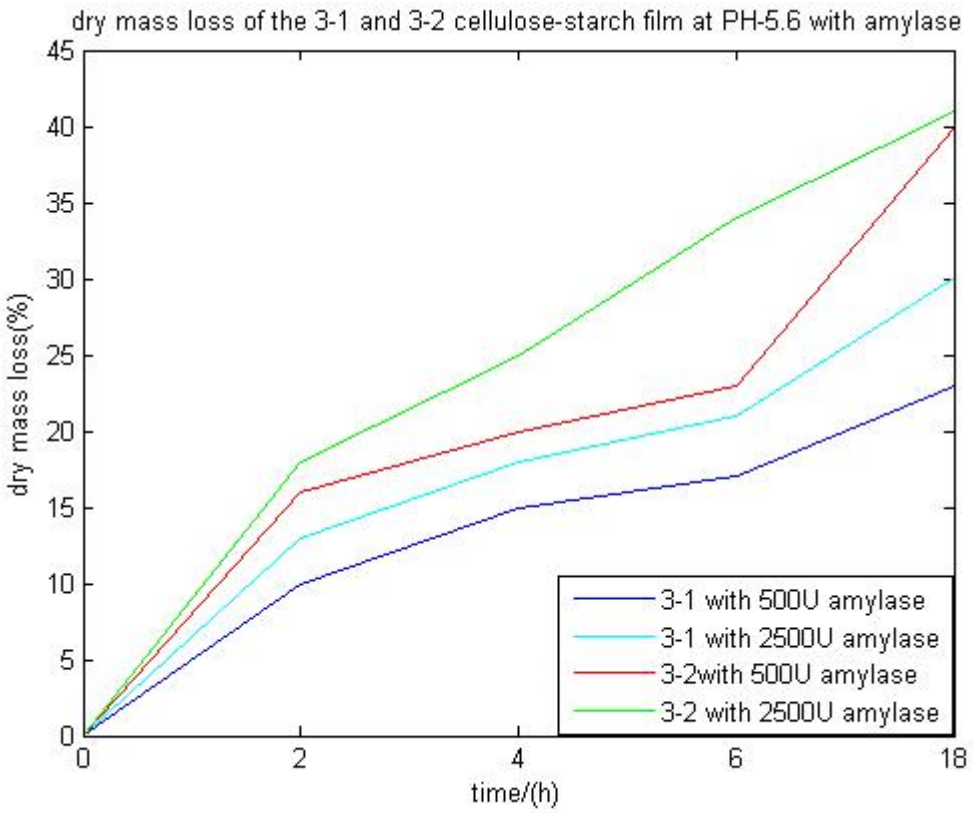


Figure 14 dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the stimulated colonic fluid at PH 5.6 with different concentration of amylase.

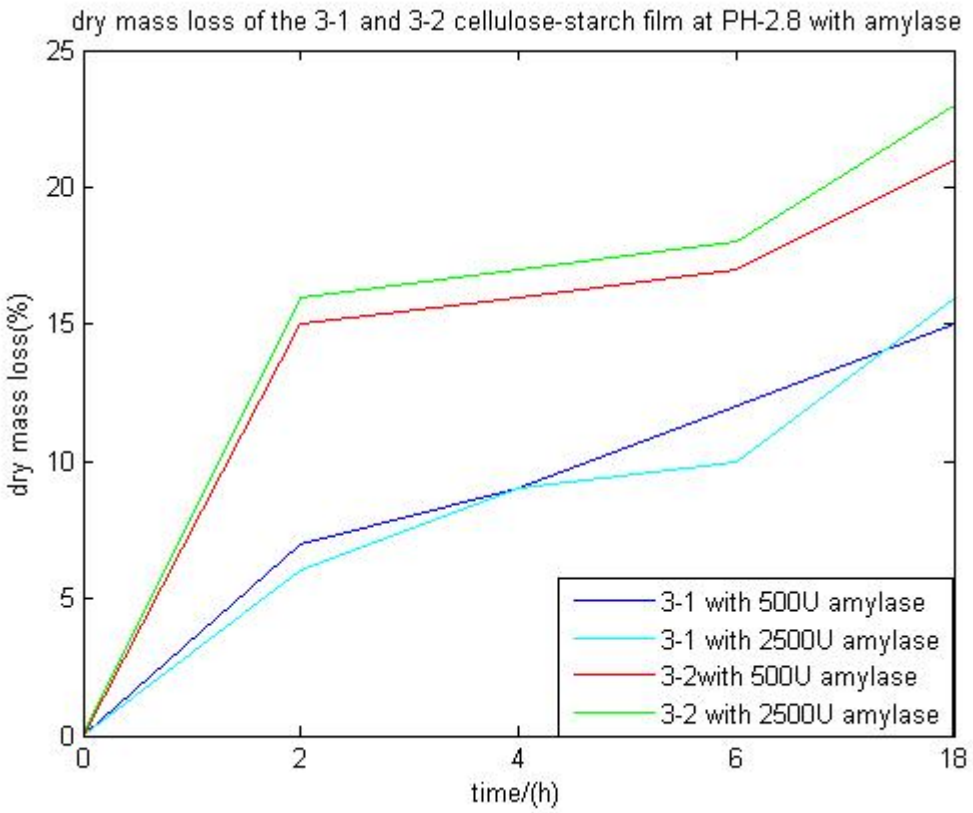


Figure 15 dry mass loss of the 3-1 as well as 3-2 aqueous ethyl cellulose-starch film in the stimulated colonic fluid at PH 2.8 with different concentration of amylase.

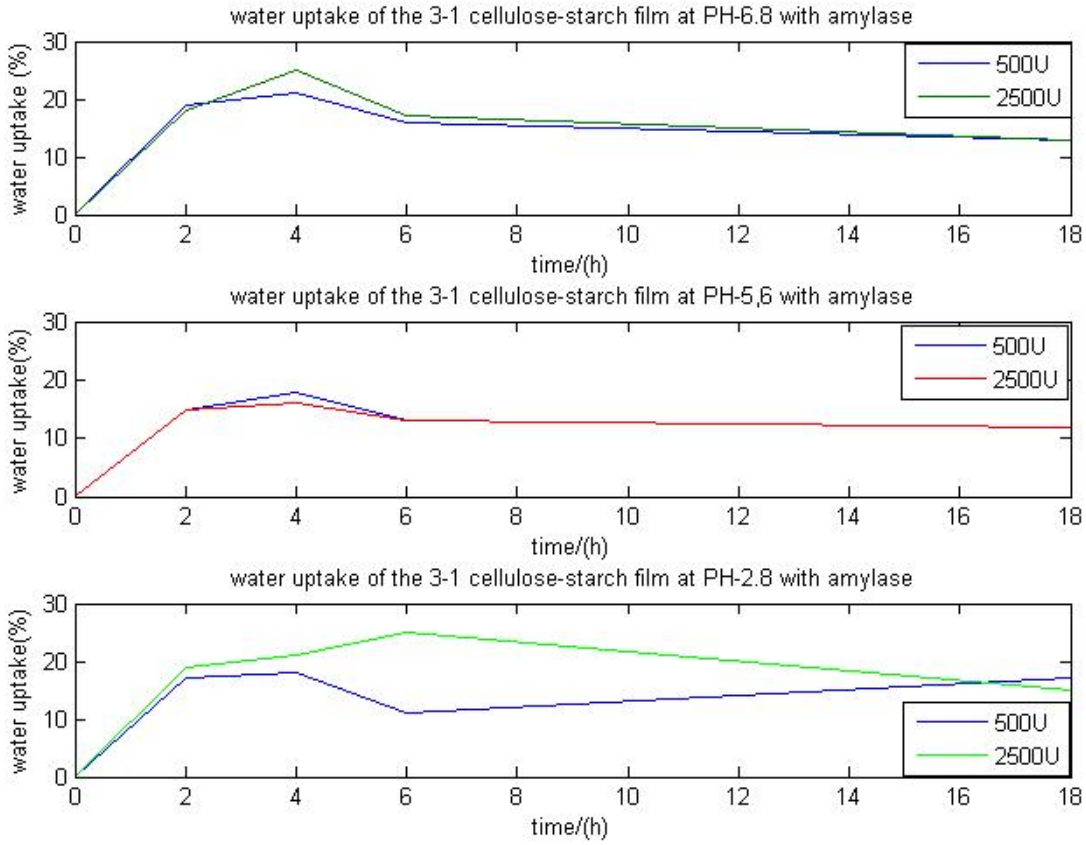


Figure 16 water uptake release of the 3-1 aqueous ethyl cellulose- starch at stimulated colonic fluid with amylase.

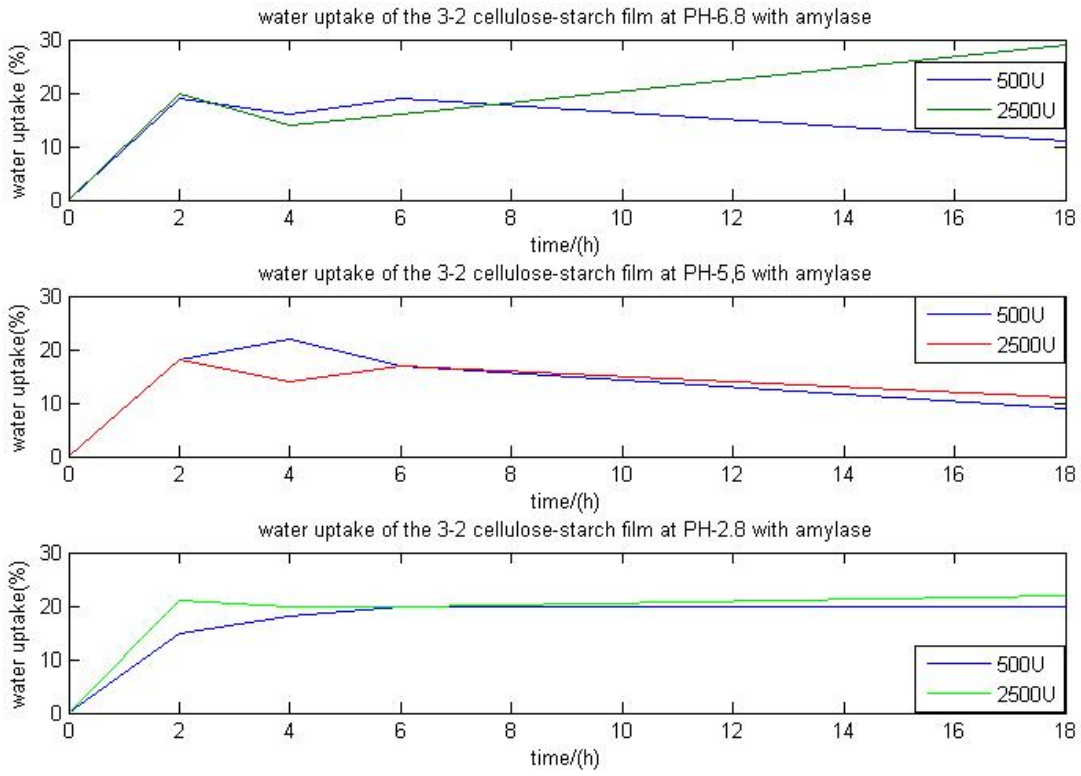


Figure 17 water uptake release of the 3-2 aqueous ethyl cellulose- starch at stimulated colonic fluid with amylase.

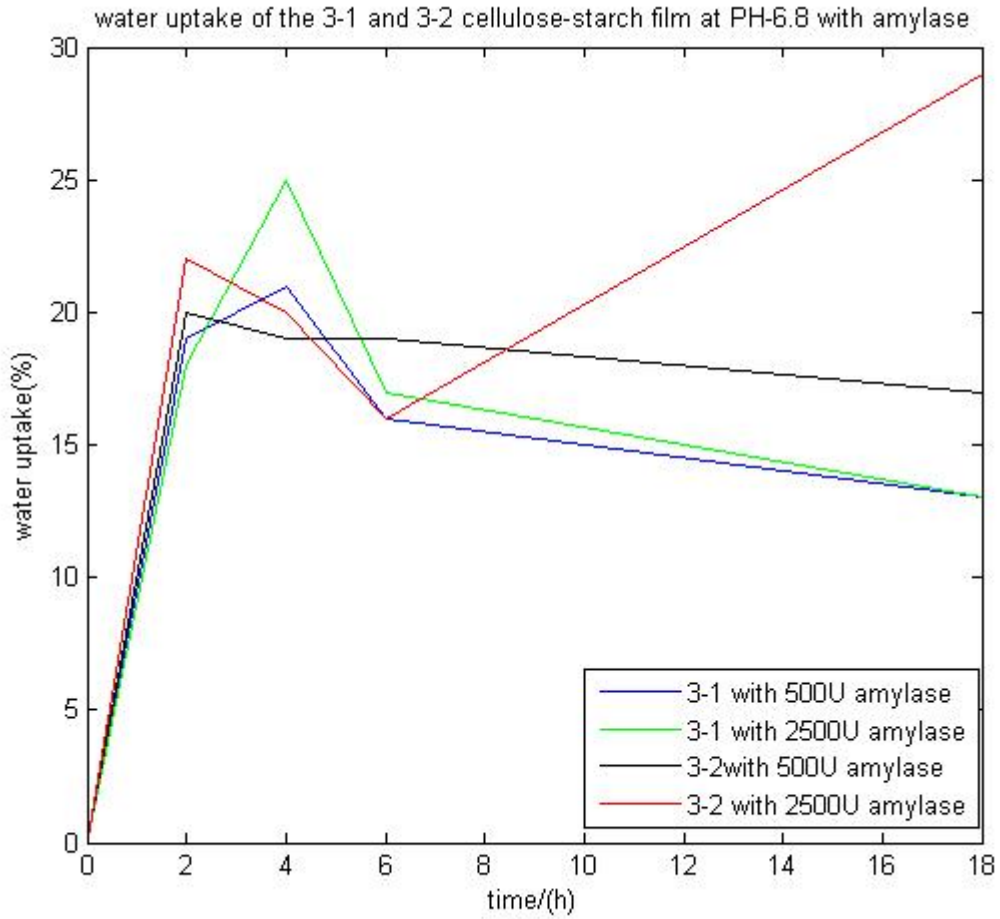


Figure 18 water uptake of the 3-1 as well as 3-2 aqueous ethyl cellulose- starch at stimulated colonic fluid at PH 6.8 with different concentration of bacterial amylase.

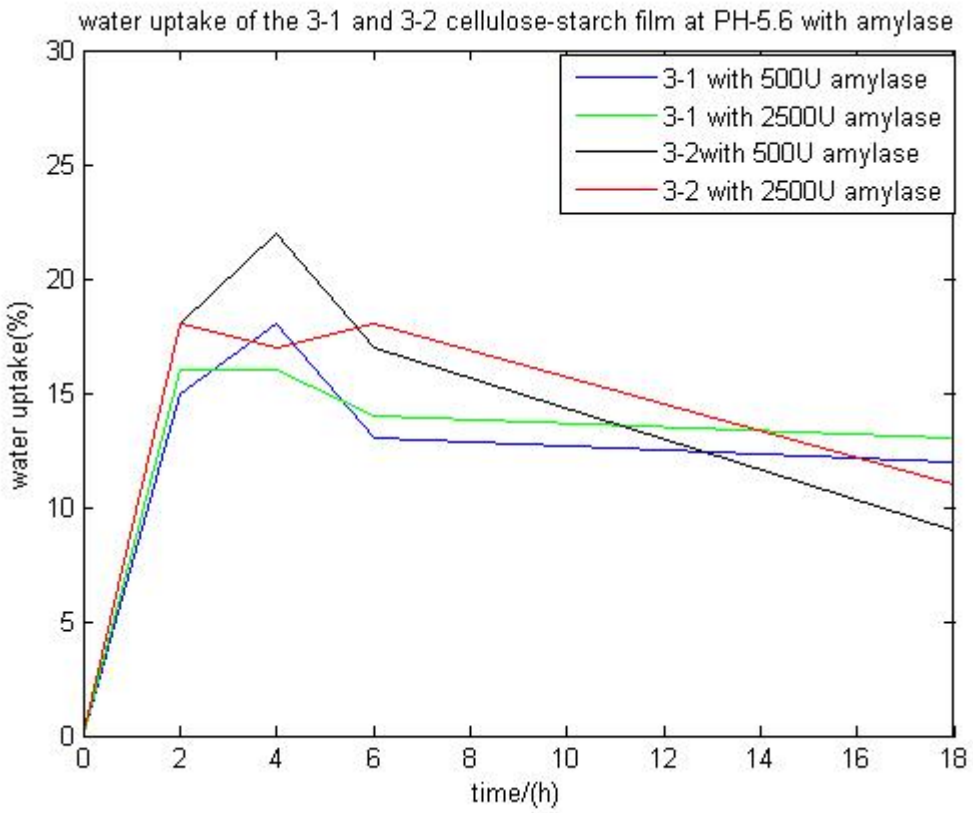


Figure 19 water uptake of the 3-1 as well as 3-2 aqueous ethyl cellulose- starch at stimulated colonic fluid at PH 5.6 with different concentration of bacterial amylase.

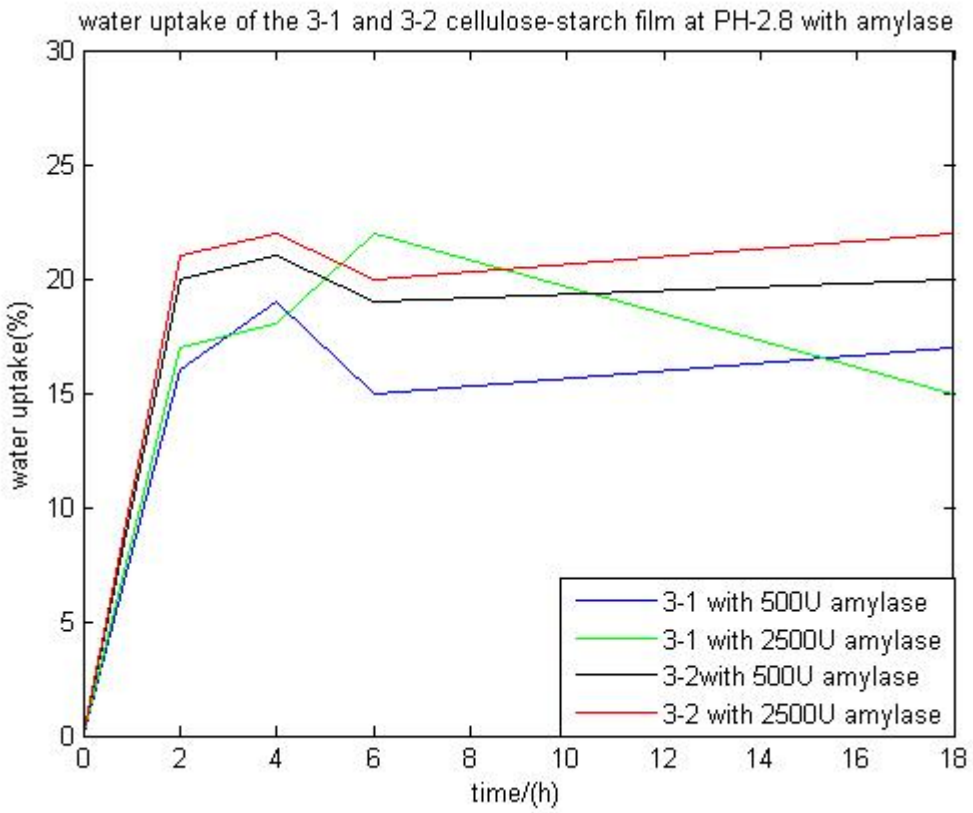


Figure 20 Water uptake of the 3-1 as well as 3-2 aqueous ethyl cellulose- starch at stimulated colonic fluid at PH 2.8 with different concentration of bacterial amylase.

This initial increase of water uptake and increase in dry mass loss with increasing enzyme concentration can be attributed to that this enzyme which can be secreted from the colonic microflora essential for digestion of starch as stated above. The digestion ability of starch by this enzyme depends on many factors such as pH, enzyme concentration, starch-cellulose ratio, and TEC amount and exposure time.

The influence of this enzyme was higher in the 3-2 starch-cellulose blend ratio than 3-1 polymer ratio as result to that the higher starch ratio the more starch are available to enzyme.

The impact which is associated with increasing of the concentration of enzyme from 500 U to 2500 U can be attributed to that there is enough available starch for digestions and thus the enzyme concentration is the rate limiting factor in this concentration region. In addition, the effect of this enzyme depends on the pH value for example this enzyme works best at the range (5.5-7.5). For this reason, the impact of this enzyme on dry mass loss, water uptake, and structure was higher at pH 6.8 and 5.6 than 2.8. This effect can be observed clearly in tables (16-30) as well as figures (13-20).

From the previous study, increasing of amount of TEC as well as starch can increase the leaching of hydrophilic components from the film which in turn could increases the diffusion of buffer solution with amylase and thus increases the effect on the structure of polymeric film leading to increase in water uptake and dry mass loss with time.

The decrease of water uptake at long exposure could be due to the degradation of starch, and dissolution of hydrophilic components starch, TEC as well as (SDS) in aqueous EC to buffer. If there is less hydrophilic components in the film there could be a decrease in the water adsorption.

The surface image for different investigated films was summarized after exposure to buffer solution with and without this enzyme. Moreover, homogeneous film surface has the most impact as showed in table 3 and 4 at 40 ºC. This can be justified by that the entire film surface is equally influenced. Visibly, at higher temperature the studied pieces become more curved. This is because the surface inhomogeneity and the impact of enzyme are not equally for the whole surface.

3.3.4. Effect of the pH

The results for the pH impact on the polymeric film with 3-1 and 3-2 starch-ethyl cellulose were summarized in tables (10, 11, and 13) and (12, 14, and 15) respectively. Additionally, these results were plotted versus exposure time and presented in figures (10-12). The reason for the impact of the pH requires more study.

3.4. Mechanical properties of the prepared films

The study of mechanical properties for the polymeric film is important to ensure that the prepared film coating has a sufficient mechanical stability during the transportation along the GIT especially in the upper part (stomach and small intestines).

These films should be tolerated mechanical shear stress which can be resulted from the motility of the stomach and small intestines. Additionally these film coating should be tolerated a hydrostatic pressure which can be occurred due to the penetration of the water into formulation system upon exposure to the stimulated fluids (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009),(High-amylose starch-based coatings for colonic delivery Cristina Freire1, 2010), and (Youness Karrouta, 2009).

Figures 21-25 shows the puncture force for the films as dry and after exposure to simulated GI liquids. Evidently, the most important factors that impact the mechanical properties are the polymers ratio and the amount of TEC. The amount of TEC has an important role on improvement of the mechanical properties such as flexibility and elasticity for the brittle nature of EC -starch film which was showed from our previous study. This mechanism for improvement can be explained by that the plasticizer molecules increase the space between the polymer chains which reduce the rigidity of starch- cellulose polymers structure. However, plasticizers make films more deformable as result to the interference into the polymer structure (Youness Karrouta, 2009).

Additionally, the higher starch ratio relative to EC has very significant impact because of that the starch has very poor mechanical properties as compared to EC, irrespective of the type and the pH of the buffer used as showed in the figures 21-23

Importantly, the penetration of the water into the polymeric formulation system can be altered the composition of the polymeric film coating which in turn impact the mechanical properties of the prepared films. This alteration can be described by that the water acts as a plasticizer for many polymers. To investigate this the mechanical properties of the films was investigated upon exposure to the gastric fluid with and without pepsin, intestinal fluid with and without pancreatin, and colonic fluid at different PH with different concentration of bacterial amylase. Figures (21-25) illustrates the impact of these factors on the mechanical properties.

Figure 23 illustrates the impact of the pancreatin on the puncture force of the 3-1 and 3-2 polymer blends ratio. These results show that the pancreatin has a significant impact especially on the 3-2 films. This can be attributed as result to the digestion ability of pancreatin on the starch as described above in the impact of the pancreatic amylase. The same reason can explain the influence of the bacterial amylase but with more significant impact due to the higher activity of the bacterial enzyme as shown in the figures 24 and 25 below. As expected the effects were not seen for simulated gastric fluid figure 22.

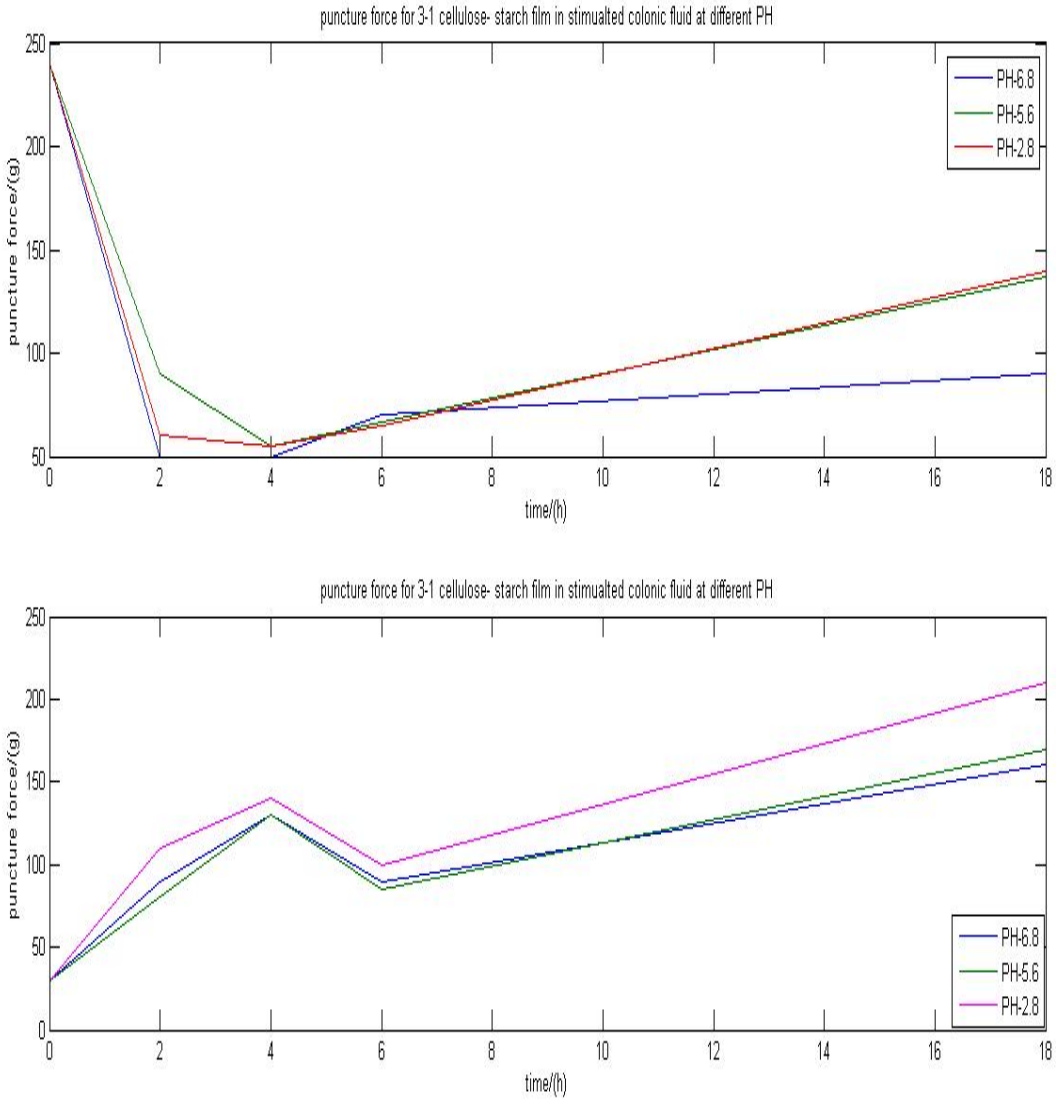


Figure21 the effect of the PH on the puncture force for 3-1 as well as 3-2 starch-ethyl cellulose polymeric film

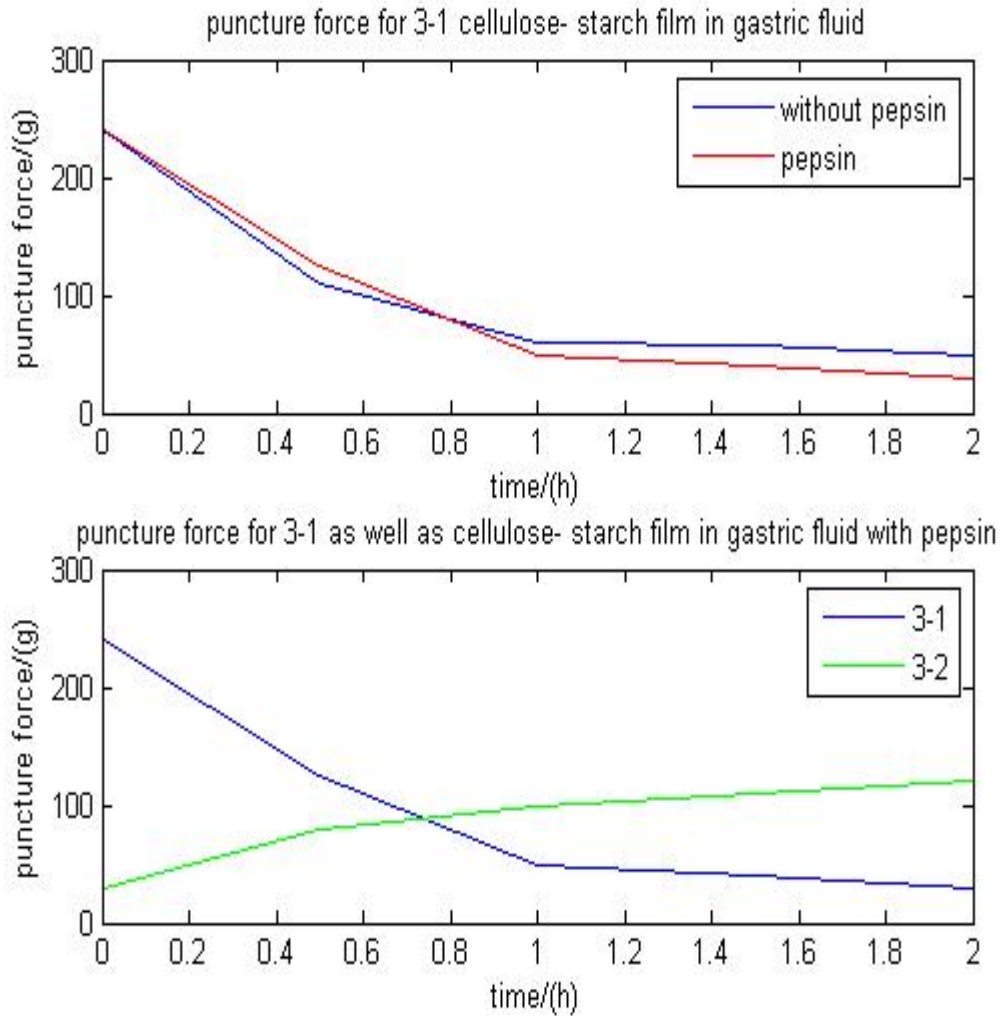


Figure 22 Impact of the polymer blend ratio on the puncture force at gastric fluid with and without pepsin

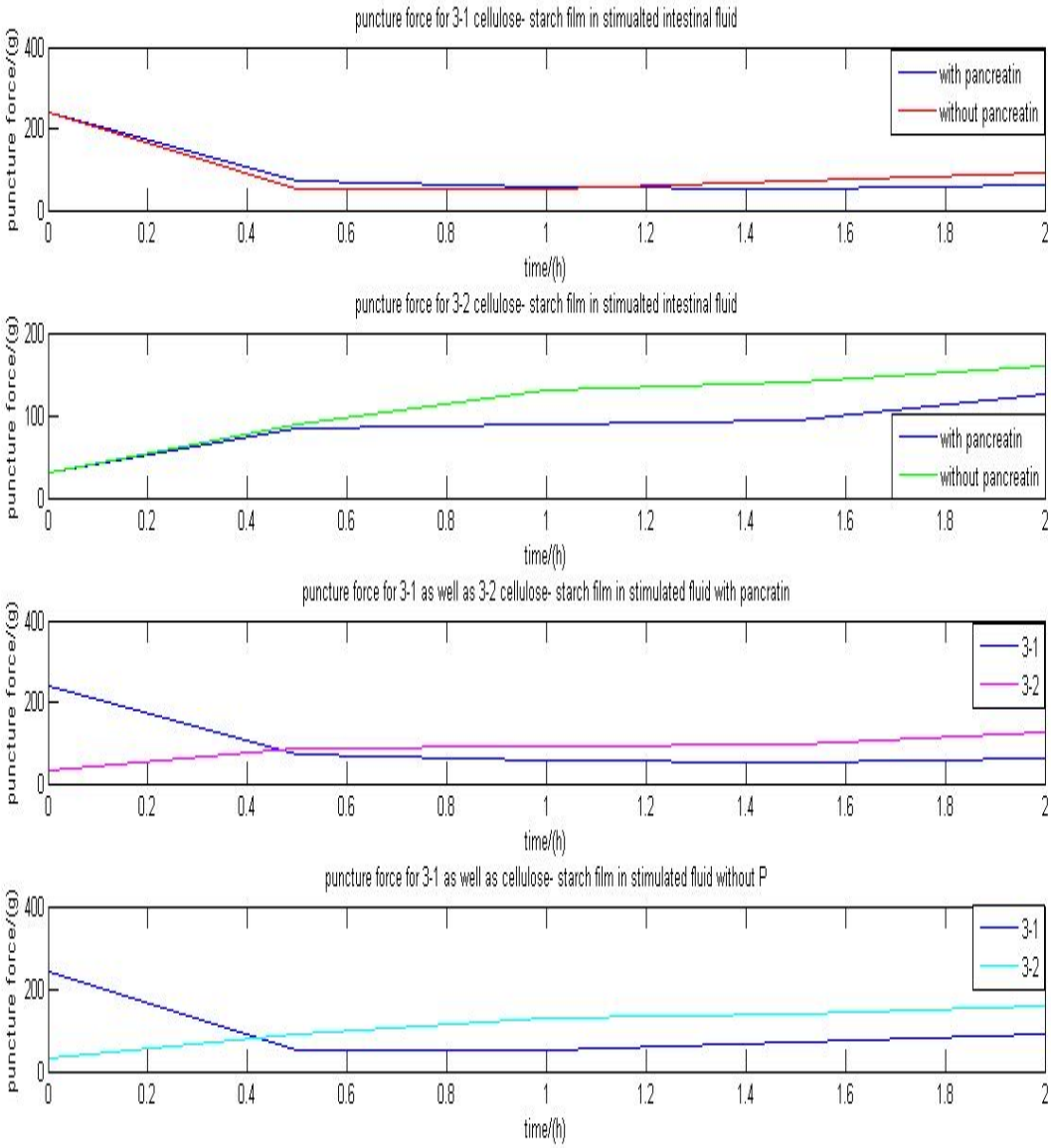


Figure 23 Impact of the polymer blends ratio as well as pancreatin on the puncture force

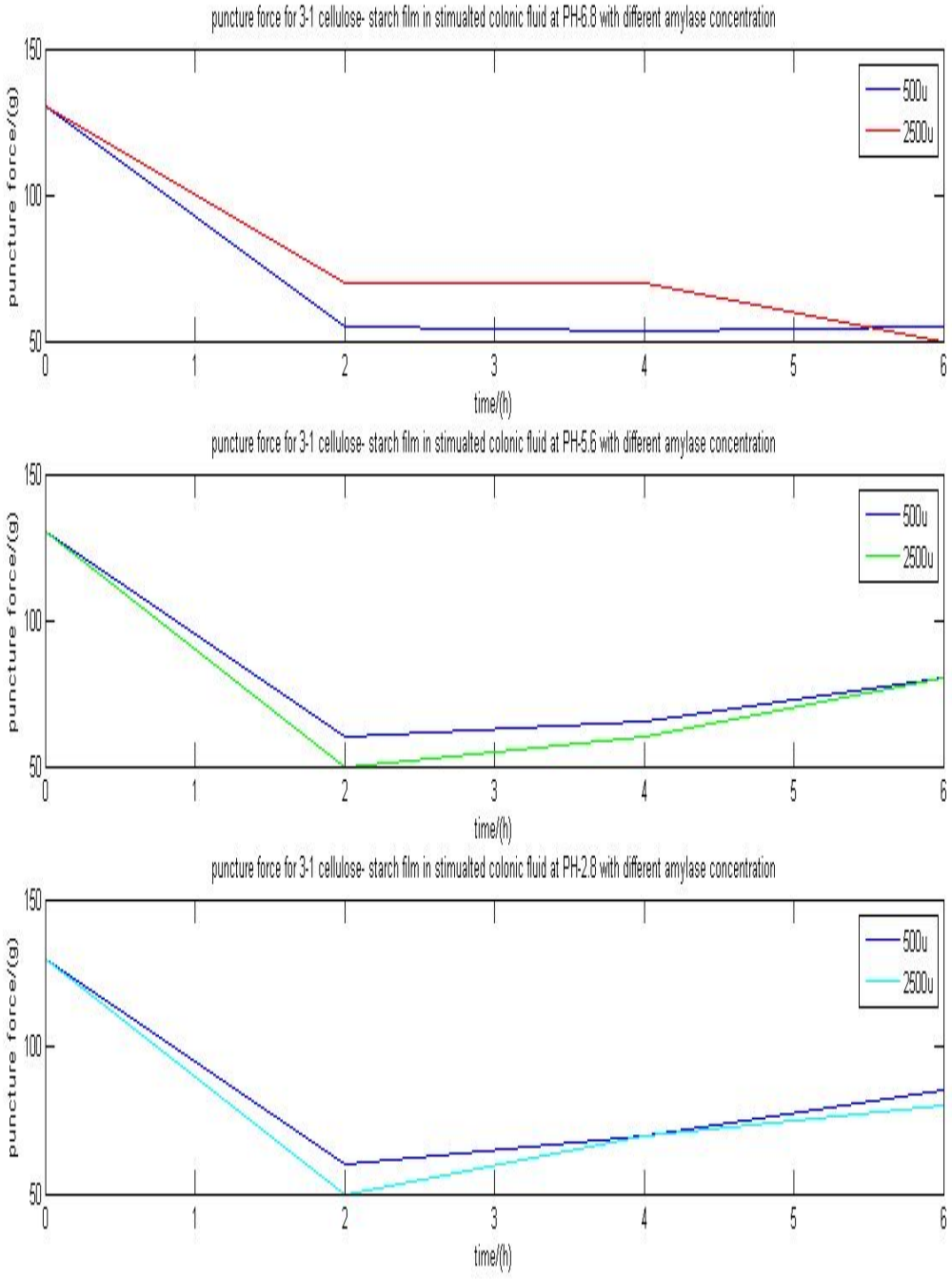


Figure 24 influence of the bacterial colonic enzyme on the puncture force for 3-1 starch. Ethyl cellulose film

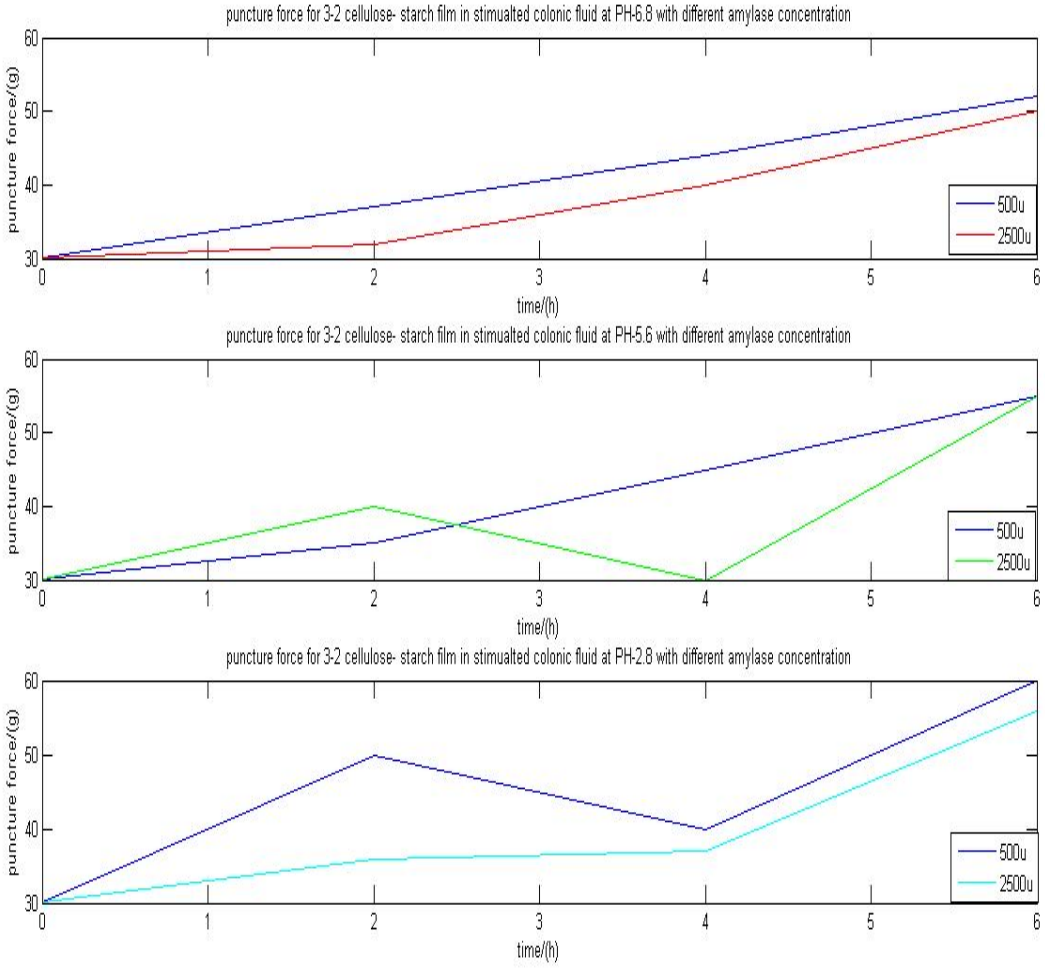


Figure 25 influence of the bacterial colonic enzyme on the puncture force for 3-2 starch. Ethyl cellulose film

3.5. Investigation of the release behaviour of the active substance (Metoprolol pellets)

The ideal film coating formulation for colon targeting should prevent the premature dissolution/rupturing of the coating as well as release of the active substance in the stomach and small intestines. However, the film coating must be permeable upon exposure to the colonic fluid to enabling the release of the drug.

The 3-1 as well as 3-2 starch- ethyl cellulose polymer blends ratio was prepared to obtain these properties. The studied coating films of these polymers were provided a slight dry mass loss and water uptake in the upper GIT especially the 3-1 polymer blend ratio.

The release of active substance from coated pellets can be due to different mechanisms (diffusion, subsequently, dialysis, and erosion) as described in the

introduction. Normally, in the upper GIT the release of the drug from starch-Ethyl cellulose coated pellets occurred due to the swelling of the film and to the impact of pancreatic amylase. However, in the colon this release occurs due to the digestion of the starch by colonic microflora.

The results of the release from the coated metoprolol pellets were presented in the figures (26-29) below. These figures show the impact of the polymer ratio, pH, the type of buffer, as well as the concentration of bacterial amylase.

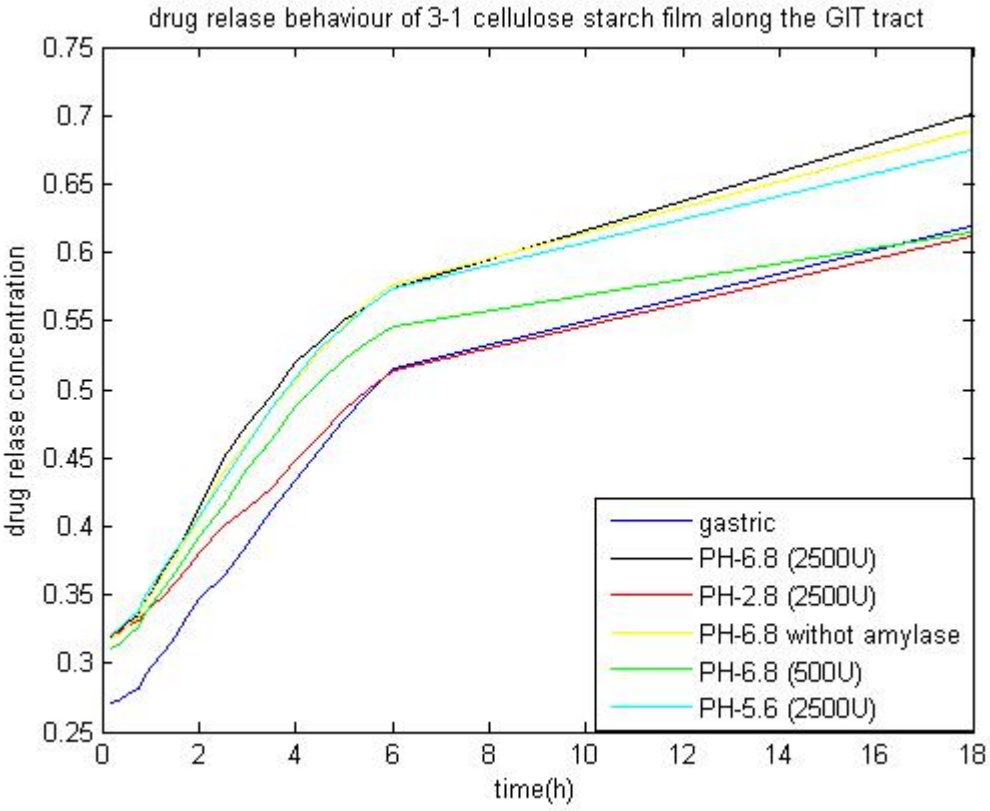


Figure 26 Release behaviour of the 3-1 starch-ethyl cellulose coated pellets in the gastric fluid, colonic fluid at different PH as well as with different concentration of bacterial enzyme

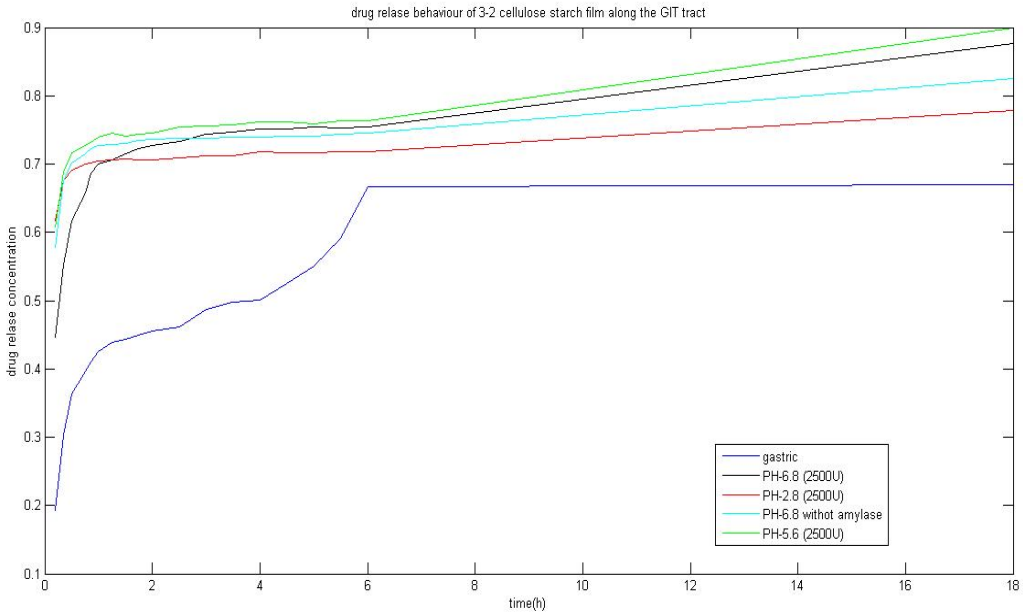


Figure 27 Release behaviour of the 3-2 starch- ethyl cellulose coated pellets in the gastric fluid, colonic fluid at different PH as well as with different concentration of bacterial enzyme

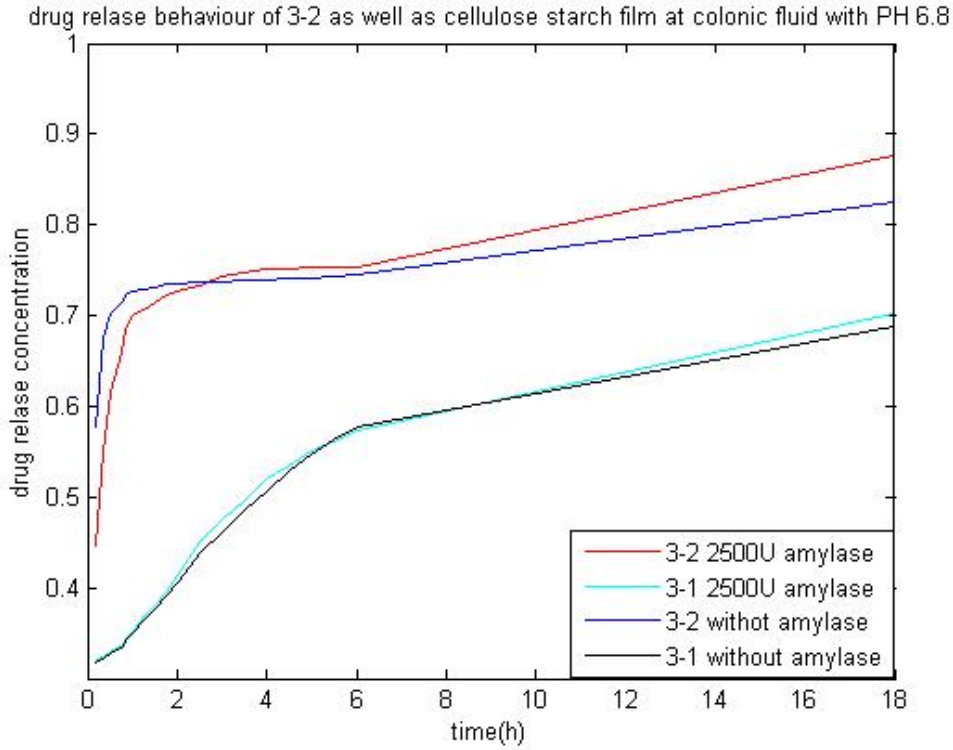


Figure 28 Release of the 3-1 and 3-2 starch-ethyl cellulose at the colonic fluid with PH 6.8 at different concentration of bacterial amylase

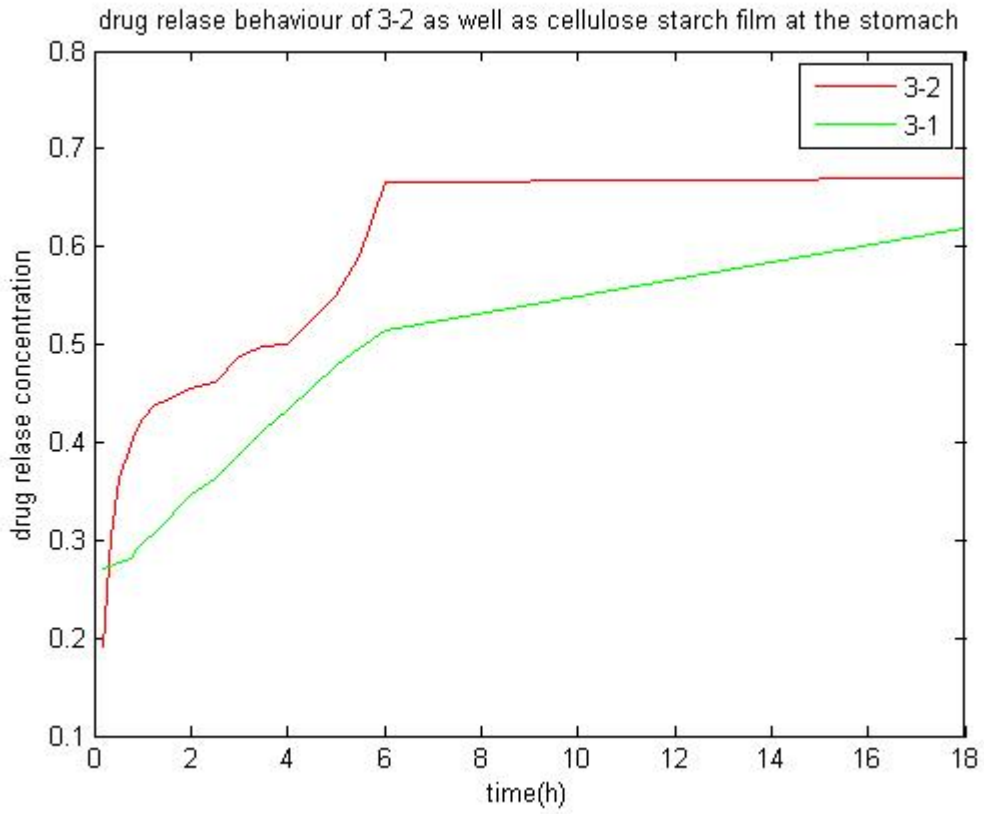


Figure 29 Release of the 3-1 and 3-2 starch-ethyl cellulose at the gastric fluid without pepsin

The 32 EC-starch film coating was showed higher release of these pellets than the 3:1 EC-starch film coating irrespective of the buffer type, PH, as well as enzyme concentration. This can be attributed by that the leaching of the water soluble or pore former such as starch and TEC as well as to the swelling and solubility of the amylopectin in the starch. The solubility and swelling of starch can be enabled the penetration of the buffer in the formulation system which in turn increase the mobility of the investigated pellets.

Interestingly, figures 28 and 29 shows that the release of these coated pellets were higher in the colonic buffer at all studied pH state than the gastric fluid.

Figures (26-28) were showed the impact of the concentration of bacterial amylase on the release of the coated pellets. The release was increased with increasing of enzyme especially with higher starch content. This can be attributed due to the digestion of the starch by the bacterial amylase which in turn can be raptured the film and facilitated the penetration of water as well as release of drug.

4. Conclusion

The study of the formulation factors of the starch-ethyl cellulose film as well as the characterization of these films with different polymer blends ratio can provide a very significant understanding of the performance of these films along the GIT. Furthermore, the investigation of the characterization of these film before and after exposure to the gastric, intestinal, as well as colonic fluid especially at condition of disease state can be enabled the design and optimization of the ideal film coating. This ideal film must be showed an appropriate mechanical stability. In addition, this film should be prevented the premature digestibility and the release of active substance in the upper GIT.

Generally, the drug release mechanism of this coating mechanism can be explained by leaching of the hydrophilic components such as TEC and plasticizer and digested starch. This leaching acts as a pore former. These pores can be facilitated the diffusion of buffer solution into the system which in turn facilitates the release of drug. According to this mechanism and the study of factors impact on this formulation, the adjusting of these parameters was achieved. Significantly, the film with 3:1 EC- starch ratio with 25 %TEC content was calculated according to the total dry weight for both polymers 60 ºC as a film formation temperature results in function film with the acceptable mechanical properties and water uptake as well as dry mass loss.

Finally, the effect that results from changing of more than one or all factors such as TEC amount, polymers ratio and film formation temperature led to different influence if just one factor change with keeping other factors constant. Therefore, this effect should be taken into consideration before designing and formulation of coating system. Additionally, impact of the GIT condition at healthy as well as disease state on these factors should be investigated carefully. Finally, the study of all these factors and their relationship with GIT condition such as PH, enzyme type and concentration, share stress, and residence and exposure time provide very significant information which can be used to optimize a function film (Friend*, 2005) and (Y.Karrouta, C. Neutb, D. Wilsc, F. Siepmanna, L. Deremaux c, M.-P. Flamenta, L. Dubreuil b, P.Desreumaux d, J. Siepmanna, 2009).

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