

# **INNOVATIVE DRUG DELIVERY SYSTEMS FOR COLON TARGETING**

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# **1. Introduction**

## **1.1. GENERAL**

The site specific delivery of a drug to the colon can provide major advantages for a pharmaco treatment [Meissner and Lamprecht, 2008], for instance if: (i) inflammatory bowel diseases are to be treated locally, or (ii) protein drugs are to be administered orally with the aim to be absorbed into the systemic circulation [Haupt and Rubinstein, 2002]. In the first case, conventional dosage forms lead to a rapid and complete drug release within the stomach and – generally – to subsequent absorption into the blood stream. Consequently, the systemic drug concentrations and related undesired side effects can be considerable. At the same time, the resulting drug concentrations at the site of action (the inflamed colon) are low, resulting in poor therapeutic efficacies [Bondesen, 1997; Lamprecht et al., 2002; Qureshi et al., 2005; Fedorak et al., 2005]. In the case of protein drugs a premature release within the *upper* gastro intestinal tract (GIT) results in the rapid loss of their biological activity due to denaturation at low pH and enzymatic degradation. Thus, in both cases, an ideal dosage form should effectively suppress drug release/protect the drug in the stomach and small intestine [Klotz et al. 2005]. But once the colon is reached, drug release should set on and be time-controlled (including – if desired – rapid and complete release). In the case of proteins, the drugs should subsequently be absorbed into the blood stream. In the case of inflammatory bowel disease treatments (e.g., Crohn's disease and ulcerative colitis), the drug is, thus, released at its target site, providing optimal therapeutic effects and minimized undesired side effects.

The colon is also considered as an attractive area for the absorption of proteins and peptides due to the less proteolytic activity, than in the upper gastrointestinal tract (GIT) [Haupt and Rubinstein, 2002]. Moreover, the residence time in the colon (more than 24 h) facilitates the absorption of drugs from this area [Basit, 2005]. In contrast to the small intestine ( $10^4$ - $10^7$  CFU/g), the colon is a home to large numbers of bacteria of many kinds, which are anaerobic and facultative aerobic ( $10^{11}$ - $10^{12}$  CFU/g) [Sinha and Kumira, 2003; Eckburg, et al., 2005].

Different types of advanced drug delivery systems have been described in the literature in order to provide such site-specific drug delivery to the colon [Yang et al., 2002; Watts and Illum, 1992; Ashford and Fell, 1993a]. Generally, the drug is embedded within a polymeric matrix, or a drug reservoir (e.g., drug loaded pellet, capsule or tablet) is surrounded by a polymeric film coating [Cummings et al., 1996; Milojevic et al., 1996a, b; Siew et al., 2000 a, b; Basit et al., 2004]. The ideal polymers used for this purpose are poorly permeable



for the drug in the *upper* GIT, but become permeable as soon as the colon is reached. In order to allow for such an increase in drug permeability different types of systems have been proposed, for instance based on: (i) changes in the pH along the GIT, (ii) polymer degradation by enzymes that are preferentially located in the colon [Leong et al., 2002; Siew et al. 2000a, 2004], or (iii) structural changes occurring in the polymeric networks, such as crack formation in poorly permeable film coatings. Alternatively, drug release might already start in the stomach, but at a rate that is sufficiently low to assure that drug release still continues in the colon [Gazzaniga et al., 1994a, b, 2006; Sangalli et al., 2001].

However, great care has to be paid when using these colon targeting approaches, because the *pathophysiological* conditions in the GIT of a patient suffering from Crohn's disease or ulcerative colitis might significantly differ from those in the physiological state. For instance, it is well known that the pH of the contents of the GIT (**Table 1**) and transit times in the various GIT segments as well as the quality and quantity of the (enzyme secreting) microflora in the colon of these patients can fundamentally vary from those in a healthy subject [Friend, 2005; Watts and Illum, 1997; El Yamani, 1992; Carette et al., 1995; Favier et al., 1997]. For instance, considerable amounts of bacteria (e.g., bifidobacteria and bacteroides) are generally present in the colon of healthy subjects and able to degrade complex polysaccharides due to multiple extracellular glycosidases [Sinha et al., 2001a, 2003]. However, in the disease state their concentrations can be significantly reduced [Friend, 2005; El Yamani, 1992]. For example, it was shown that the fecal glycosidase activity (especially that of  $\beta$ -D-galactosidase) is decreased in patients suffering from Crohn's disease and that the metabolic activity of the colonic flora is strongly disturbed in the active disease state [Carette et al., 1995; Favier et al., 1997]. Commonly, ulcerative colitis patient's exhibit diarrhea (accelerated transit). This difference is due largely to mucosal inflammation and the disturbances it produces [Sandborn and Phillips, 1995].

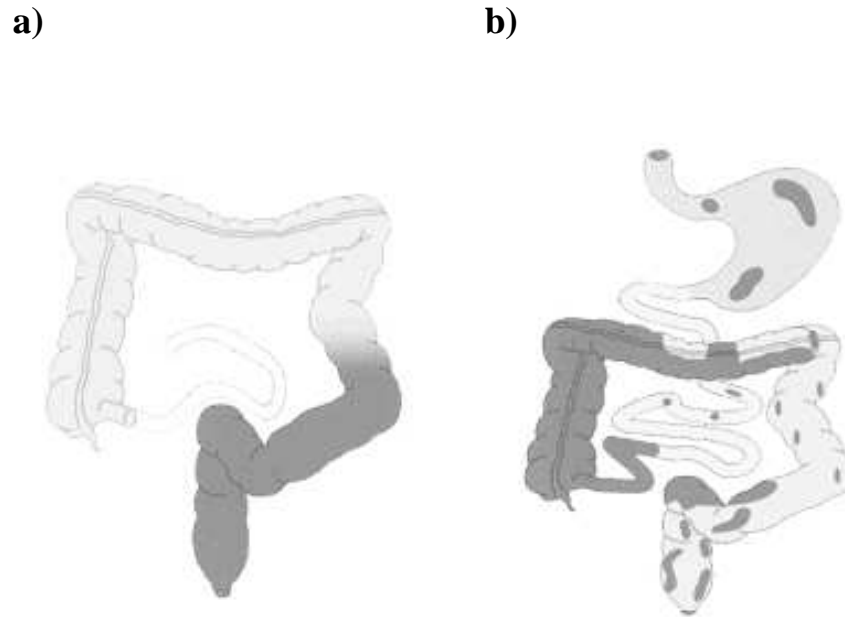
Thus, the impact of the *pathophysiology* can be crucial and lead to the failure of the pharmaco-treatment [Siccardi et al., 2005]. Importantly, these alterations are generally neglected, and the influence of the disease on the performance of the drug delivery system is often ignored [McConnell et al., 2008]. A delivery system which successfully delivers the drug to the colon in a healthy subject might fail in a patient. Also, the inter- and intra-individual variability of the therapeutic effects might be considerable if the dosage form is not appropriately adapted to the disease state [McConnell et al., 2008]. To avoid these major disadvantages, the drug delivery system should be adapted to the disease state of the patient. For instance, if the onset of drug release in the colon is induced by enzymatic degradation, the

responsible enzymes must be present in the colon of the patients in sufficient quantities. To properly address this fundamental aspect, the use of fecal samples from Crohn’s disease and ulcerative colitis patients offers an interesting possibility for the identification of novel polymeric film coatings allowing for *colon targeting in the disease state*.

The main forms of IBD are Crohn’s disease (CD) and ulcerative colitis (UC). The main difference between Crohn’s disease and ulcerative colitis are the location and nature of inflammatory changes. Crohn’s disease can affect any part of the GIT from mouth to anus, but in most cases attacks the terminal ileum (**Figure 1**). In contrast, ulcerative colitis is restricted to the colon and the rectum. Both are chronic diseases that involve inflammation of the colonic mucosa. Current therapy aims to reduce the symptom burden of the disease and maintain disease quiescence. The pathogenesis of inflammatory bowel disease involves interactions between the host susceptibility, mucosal immunity and intestinal microflora (e.g., Adherent-invasive E-coli, AIEC) [Rolhion et al., 2007].

**Table 1:** *pH in the small and large intestine from healthy, ulcerative colitis and Crohn’s disease subjects.*

Subjects	Proximal intestine	Ileum	Proximal colon	Terminal colon	References
<b>Healthy</b>					
66	6.6	7.5	6.4	7.0	Evans 1988
39	6.4	7.3	5.7	6.6	Fallingborg 1989
15	6.4	7.6	6.2	7.4	Schwartz 1997
<b>Ulcerative colitis</b>					
3 (active)	6.4	7.4	6.8	-	Fallingborg 1993
3 (severe state)	6.4	7.4	2.3-3.4	-	Raimundo 1992
7(acute untreated)	-	-	4.7	-	
7 (acute treated)	-	-	5.5	-	
<b>Crohn’s disease</b>					
15 (active)	6.5	7.5	6.2	6.4	Schwartz 1997
12 (active)	6.5	7.5	6.2	6.5	Ewe 1999



**Figure 1:** Location of inflammation in the mucosa of the GIT (dark areas = lesions) in the case of a) ulcerative colitis and b) Crohn's disease (Reprinted from <http://www.sanfte-chirurgie.at/erkrankungen/dickdarm.html>).

Chronic inflammation in inflammatory bowel diseases is usually treated by anti-inflammatory drugs, the most frequently used is 5-aminosalicylic acid (Mesalazin), other drugs like antibiotics have also shown therapeutic efficiency. The anti-inflammatory effects of 5-aminosalicylic acid in the colon has been found to be dependent on the activity of peroxisome proliferators- activated receptor- $\gamma$  (PPAR- $\gamma$ ) which is expressed at high levels in the colonic epithelium and regulates the colonic inflammation. PPAR- $\gamma$  is a nuclear receptor which forms a heterodimer with retinoid X receptor (RXR) regulating gene expression which is involved in the control of the inflammation being in the colon. The synergic effects of PPAR- $\gamma$ /RXR heterodimer on the attenuation of colon inflammation have been reported by Desreumaux. Furthermore, the ability of 5-aminosalicylic acid to bind and activate PPAR- $\gamma$  revealed the effects via direct activation of this receptor [Desreumaux et al., 2001; Rousseaux et al., 2005; Dubuquoy et al., 2006].

## 1.2. COLON-SPECIFIC DRUG DELIVERY SYSTEMS

The large intestine is still considered as an ideal site for the delivery of agents to cure

the local diseases of the colon [Davis, 1990; van den Mooter and Kinget, 1995] or to be absorbed from the colon (e.g. proteins and peptides). Delivery systems for targeted delivery in the GIT could be categorized into four categories: (i) pH-based systems, (ii) Time-based delivery systems, (iii) Pressure-based systems, and (iv) Enzyme-based systems (prodrugs/coatings and matrices).

### **1.2.1. pH-controlled drug delivery systems**

Use of pH-dependent polymers is based on the difference in pH-levels along the GIT. The polymers described as pH-dependent in colon specific delivery are insoluble at low pH-levels but become increasingly soluble as pH rises [Ashford and Fell, 1993b; Leopold et al., 1999].

The pH in the GIT varies between and within individuals and also between healthy and patients [Friend, 1991; Ashford and Fell, 1993c; Kinget et al., 1998; McConnell, 2008], which could lead to the failure of the system in the treatment of inflammatory bowel diseases. Moreover, during acute stage of inflammatory bowel disease colonic pH has been found to be significantly lower than the physiological pH [Leopold and Eikener, 2000]. It must be also taken into consideration that, between the terminal ileum and the distal colon, there is a slightly acidic region in the proximal colon, due to the fermentation of poly and oligosaccharides to short-chain fatty acids, which might affect drug release profiles and the reproducibility of drug release.

Most commonly used pH-dependent coatings polymers are copolymers methacrylic acide and methyl methacrylate containing carboxyl groups (Eudragit<sup>TM</sup>). Eudragit S which is soluble above pH 7 and Eudragit L above pH 6 are mostly used polymers in targeted drug delivery to the colon. Eudragit S coatings have been used to target the anti-inflammatory drug 5-aminosalicylic acid in single-unit formulations on the colon [Dew et al., 1982; Kinget et al., 1998; Zahirul et al., 1999]. Eudragit L coatings have been used in single unit tablets to target 5-aminosalicylic acid on the colon in patients with Crohn's disease and ulcerative colitis [Hardy et al., 1987]. Formulations based on pH-responsive polymers (Eudragit S, Eudragit L, Eudragit FS 30D and Eudragit P4135) have been investigated in order to target the Ileum-colon [Ibekwe et al., 2006; Schellekens et al., 2007; Rudolph et al., 2001]. The failure of enteric coated dosage forms, especially single-unit dosage forms has been reported as a lack of disintegration [Bussemer et al., 2001]. Eudragit S has been also used with another methacrylic acid copolymer (Eudragit L 100-55) in colon targeted systems to regulate drug delivery [Zahirul et al., 1999]. Dissolution data has shown that drug release profiles from

enteric-coated single-unit tablets could be altered in vitro by changing the ratios of the polymers, in the pH range 5.5 to 7.0. Hydroxypropyl methylcellulose acetate succinate (HPMCAS) has been included in outer layers of single-unit press-coated tablets in order to prevent drug release in the stomach and small intestine [Fukui et al., 2001].

Recently, a new type of delivery system has been developed to delivery drug to the colon for the treatment of ulcerative colitis. EUDRACOL™ which is a combined pH- and time-based multi-unit dosage form is already available for targeting drug to the colon [Gupta, et al., 2001a, b; Rudolph et al., 2001]. EUDRACOL™ consists of 5-aminosalicylic acid containing core which is then first coated with an aqueous dispersion of Eudragit RL:RS (2:8), and secondly, with a new pH-dependent anionic polymer Eudragit FS. The latter dissolves rapidly at pH above 7, triggering the onset of drug release in distal GIT. Eudragit RL/RS produce a slow release of drug from the pellets. The performance of this new designed drug delivery system has been investigated in vitro as well as in vivo, and compared with solely pH-dependent system (Eudragit FS- coated pellets) [Klein et al. 2008].

The colon-specific drug delivery system CODES™ Technology is designed to reduce the variability associated with time or pH-dependent drug delivery. The conversion of lactulose (in tablet-cores) to organic acids by colonic bacterial enzymes makes the microenvironment of the tablet acidic which permit the dissolution of Eudragit E. The outer coating of the CODES formulation is composed of an enteric polymer Eudragit L. Once the formulation passes into the duodenum, Eudragit L dissolves exposing the undercoating, which is composed of Eudragit E. This coating will not dissolve in the small and large intestine due to the high pH levels, but permits the lactulose within the formulation core to be released into the environment. Lactulose is metabolized to short chain fatty acids, which decrease the local pH required to dissolve Eudragit E [Kattsuma et al., 2002; Yang et al., 2003]. The coating thickness of Eudragit E has been found to play a decisive role in drug delivery of CODES system. Eudragit E could also limit the rate of the availability of lactulose in the colon for bacterial degradation. The dissolution of eudragit S was, however, dependent on the quantity of lactulose released in the colon (less lactulose released triggered slow Eudragit E dissolution). It was found that  $T_{max}$  was significantly increased from the formulation prepared with 38 % lactulose compared with the 58 % and 78 % lactulose loaded formulations.

### **1.2.2. Time controlled drug delivery systems**

Other physiological characteristics that can be taken advantage of to target the colon is the transit time in the small intestine (approximately 3-5 h). It has been found that both single-

unit formulations and small-unit formulations take three to four hours to pass through the small intestine [Davis et al., 1986; Parker et al., 1988; Wilson et al. 1989; Adkin et al., 1993]. However, the arrival time of formulations into the colon can not be previsible due to the great variation of transit time in the stomach. After a gastric emptying, a time-controlled drug delivery system is intended to release the drug after a predetermined lag time. It has been observed in patients with irritable bowel syndrome and ulcerative colitis that transit times through the colon are faster than in healthy subjects (diarrhoea). Systems based on time-controlled release are identified as unsuitable for drug delivery in the colon for the treatment of inflammatory bowel diseases [Yang et al., 2002]. Polymers used in this concept have a slow or pH-dependent rate of swelling, dissolution or erosion that take advantage of the short constant small intestinal transit time. In time-dependent formulations the drug concerned is released during the period of gastrointestinal transit time. However, drug release could already start in the stomach or small intestine, and be absorbed into blood stream causing serious side effects [Bondesen, 1997].

In the case of coated dosage forms designed for time controlled drug release, the onset of drug release influenced by the coating level, and drug release can be triggered by: (i) a change in pH, (ii) a change in the osmotic pressure or (iii) disruption of the coating by swelling of the core. Time controlled drug release with pH-induced drug delivery is a targeting approach that does not depend on changes in the luminal pH of the GIT but on a pH change within the dosage form itself.

The oral Chronotopic® drug delivery system consists of hydroxypropyl methylcellulose (HPMC)-coated drug core, which is protected by the enteric coating Eudragit L. The enteric coating dissolves in the intestinal fluid and the high- viscosity hydroxypropyl methylcellulose layer starts to swell and slowly erodes over time [Gazzaniga et al., 1994a, b]. After dissolution of the enteric coating, drug release from this system is pH-independent, however a rapid eroding and swelling can be observed. Pulsincap® is an enteric capsule formulation, in which the water-insoluble capsule body is closed by a swellable hydrogel plug. The soluble cap dissolves in the intestinal juice, allowing the hydrogel plug to swell and expand. Ejection of the swollen plug occurs after a lag time that depends on the hydrogel materials, the length of the plug and the fit ratio (diameter plug to diameter body) [Hegarty and Atkins, 1995; Wilding et al. 1991]. A formulation that involves a plug that erodes rather than hydrogel plug has also been developed [Krögel and Bodmeier 1998].

The TIME-CLOCK™ system is characterized by pH-independency, the lag time observed is caused by slow hydration of the hydrophobic coating layer, which consists of

wax, Tween 80, and combined with the hydrophilic hydroxypropyl methylcellulose [Pozzi et al., 1994; Wilding et al. 1994]. In vivo studies of such tablets have shown that the disintegration of such tablets occurred in the proximal colon after a lag time of 5.5 hours. Hydroxypropyl methylcellulose and hydroxypropylcellulose (HPC) have been used as swellable polymers in delayed release formulations [Gazzaniga et al., 1994a, b; Vandelli et al., 1996]. The in vivo behaviour of tablets with drug-containing core coated with hydroxypropyl methylcellulose and an enteric polymer (Eudragit L 30D) has also been investigated using gamma scintigraphy [Sangalli et al. 2001]. The formulations disintegrated in the colon in all six volunteer subjects. However, the lag-time was found to be  $7.3 \pm 1.2$  hours when the thickness of the polymer layer was greatest. Time-controlled formulations have also been prepared using water-insoluble ethylcellulose and swellable polymer hydroxypropylcellulose [Hata et al. 1994, Takaya et al. 1995]. The swelling agent hydroxypropylcellulose absorbed liquid and the ethylcellulose coat disintegrated as the core swelled.

### **1.2.3. Pressure controlled drug delivery systems**

Pressure-sensitive drug formulations release the drug as soon as a certain pressure limit is exceeded. Polymers used for this topic form firm layers that are destroyed by an increase of the luminal pressure in the colon caused by peristaltic waves.

A pressure-controlled drug delivery system that relies on the high pressure in the distal colon has been reported by Niwa et al., 1995. Disintegration of this system, which consists of a gelatin capsule with an inner ethylcellulose coating, triggered by peristaltic waves destroying the ethylcellulose film. As water ingresses into the core the low substituted hydroxypropylcellulose swells. The cap which made of the water-insoluble ethylcellulose (EC) cannot persist the swelling pressure. The ethylcellulose cap disintegrates releasing the active drug from the container within the capsule. The most important factor for disintegration of the formulation is the thickness of the water-insoluble ethylcellulose film [Muraoka et al., 1998; Jeong et al., 2001].

Pressure-controlled colon delivery capsule (PCDC) containing 5-aminosalicylic acid for the treatment of inflammatory bowel diseases has been prepared and evaluated in vivo experiment using beagle dogs. It has been also examined in both animals and humans [Takada et al. 1995; Hu, et al. 1998; Muraoka et al., 1998; Jeong et al. 2001; Takaya et al., 1995]. When comparing this formulation with the prodrug sulfasalazine in gelatin capsule, the time of the appearance of 5-aminosalicylic acid into the systemic circulation was almost the same,

longer  $T_{\max}$  was observed from sulfasalazine capsule than from PCDC. It was concluded that this formulation is suitable for the treatment of inflammatory bowel diseases avoiding the side effect of sulphapyridine (metabolite of sulphasalazine) [Takaya et al., 1995].

As mentioned above, ethylcellulose coatings have also been used for time-controlled drug delivery, therefore the disintegration of the formulation can occur after administration, even in the stomach.

#### **1.2.4. Bacterially triggered drug delivery systems**

The colonic microflora produces a variety of enzymes that are not present or different from those in the stomach and the small intestine and could therefore be used to deliver drugs to the colon after enzymatic cleavage of degradable formulation components or drug carrier bonds. Most bacteria in the colon are anaerobic (95%) and facultative aerobic (5%) [Cummings, 1984; Rubinstein, 1990, Watts and Illum, 1997; Kinget et al., 1998]. More than 400 bacterial species have been found in colon able to ferment complex polysaccharides [Cummings, 1984]. Most bacteria inhabit in the proximal colon, where energy sources are greatest. The carbohydrates are fermented into short chain fatty acids, carbon dioxide, hydrogen, methane and other products by the enzymes glycosidase and polysaccharidase. In the proximal colon the pH is lower than in the distal part of the colon due to the presence of the short chain fatty acids (acetate, propionate and butyrate) and other fermentation products. However, diet can also affect colonic pH [Rubinstein, 1990, Watts and Illum, 1997; Kinget et al. 1998].

Various aspects of the microbially triggered drug delivery to the colon have been published [Sinha and Kumria, 2003]. However, enzymatically degradable polymers have an interesting application providing colon-specific drug delivery. This concept could be divided into (i) the use of prodrugs breakdown by bacterial enzymes within the colon and (ii) use of tools (coatings/matrices) susceptible to colonic bacteria.

##### 1.2.4.1. Prodrugs

A prodrug is a pharmacological substance (drug) that is administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolized in vivo into an active metabolite. Prodrugs are usually designed to improve oral bioavailability, with poor absorption from the gastrointestinal tract usually being the limiting factor, often due to the chemical properties of the drug. Thus, the promoiety is used to increase the hydrophilicity of



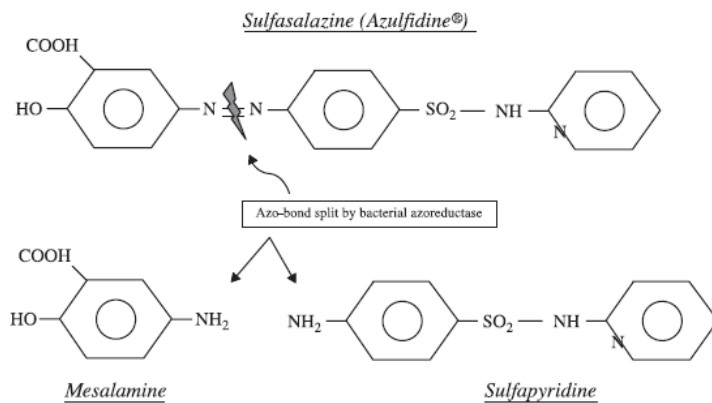
the parent drug, increase molecular size, or both, thus minimizing absorption of the drug prior to reaching the target site [Sinha and Kumria, 2001b]. Additionally, the use of a prodrug strategy increases the selectivity of the drug for its intended target (e.g. the colon) [van den Mooter et al., 1992, 1997].

The oldest of the drugs used in ulcerative colitis therapy is sulphasalazine [Svartz et al., 1941], which consists of sulphapyridine and 5-aminosalicylic acid joined by a diazo bond. It has been used for over 50 years in the treatment of inflammatory bowel disease [Klotz, 1985]. The cleavage of the azo bond is by bacterial enzymes (azoreductases) in the colon (**Figure 2**), releasing the active moiety, 5-aminosalicylic acid, which possesses the anti-inflammatory effect [Azad Khan et al., 1977, Desreumaux et al., 2001, 2006; Rousseaux et al., 2005; Dubuquoy et al., 2006]. The other component, sulphapyridine has been found, however, to have adverse effects [Das et al., 1973]. Another prodrug, olsalazine has been also developed and marketed, consists of two of 5-aminosalicylic acid linked by an azo-bond (**Figure 2**) [Travis, et al., 1994]. In order to eliminate the undesirable effect of sulfapyridine in sulphasalazine, the latter was replaced by 5-aminosalicylic acid.

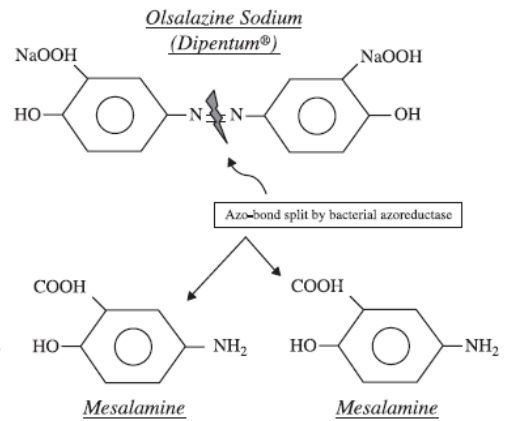
In general, enzymatic degradation of such systems may be excessively slow [Yang et al., 2002]. Mesalamine linked to another polymer via an azo bond have been also developed. The advantages of a polymer-based prodrug for GI delivery over, low molecular weight carriers is the ability to target specific sites in the GIT and the excretion of carrier releasing the active drug, however, side effects can be minimized by maximizing local drug concentrations at the target (e.g., inflamed regions in the case of IBD) [McLeod et al., 1992; Brown et al.1983, Garretto et al. 1983].

A recent variation on the azo polymer approach based on dendrimers as the carriers [Wiwattanapatapee et al., 2003] has been proposed. 5-Aminosalicylic acid was released from these carriers slower in rat cecal contents although at a rate considerably slower than that observed from sulphasalazine [Wiwattanapatapee et al., 2003]. The disadvantage of such drug-carrier based systems is that they have to be administered in high dose size, which is sometimes not feasible and acceptable. In the case of 5-ASA (1 g per day) the weight of the dosage form would be 10 g or more. Thus, this concept will be very useful for potent drugs rather than 5-ASA.

a)



b)



**Figure 2:** Chemical structure and biological degradation of a) sulfasalazine and b) Olsalazine (Qureshi and Cohen, 2005).

#### 1.2.4.2. Coatings and matrices

Polysaccharide-based formulations represent a relative simple formulation concept because of its safety (most can be used without additional safety testing) if there are no chemical modifications to the polysaccharide. Moreover, polysaccharides are inexpensive and readily available in a variety of structures with a variety of properties [Hovgaard and Brondsted, 1996]. They can be easily modified chemically and biochemically and are highly stable, safe, non-toxic, hydrophilic and gel forming and in addition biodegradable, which suggests their use in targeted drug delivery systems to the colon. A broad range of drug delivery systems based on polysaccharides has been investigated. Due to the high hydrophilicity polysaccharides possess high solubility and swelling in aqueous medium which lead to premature drug release in the upper GIT when using polysaccharides solely as coating materials for colon drug delivery systems [Milojevic et al., 1996a, b]. To control the high swelling of polysaccharides hydrophobic polymers should be added in order to reduce the swelling, and subsequently to ensure that no/very low drug is released until it reaches the colon. On the other hand polysaccharides used for this topic should be resistant to the upper GIT conditions with respect to digestive enzymes, but degradable by bacterial enzymes within

the colon. **Table 2** illustrates some polysaccharide-based oral delivery systems for targeted release in the lower intestine.

The polysaccharides naturally occurring in plant (e.g., pectin, guar gum, inulin), animal (e.g., chitosan, chondroitin sulphate), algal (e.g., alginates) or microbial (e.g., dextran) origin were studied for colon targeting.

**Table 2:** *Polysaccharide-based materials used to deliver drugs to the lower intestine*

<i>Polysaccharide</i>	<i>Dosage forms investigated</i>	<i>References</i>
Calcium Pectinate	Matrices, compression coated tablets, enteric coated matrix tablets	Rubinstein et al., 1993, 1995 ; Adkin et al., 1997
Chitosan	Coated capsules and microspheres, matrices	Tozaki et al. 1997, 1999; Aiedeh et al., 1999
Guar gum	Matrix tablets, compression coated tablets,	Krishnaiah et al., 1998, 1999, 2002, 2003 ; Rama et al., 1998 ; Wong et al., 1997
Amylose	Coated pellets, tablets, capsules	Milojevic et al., 1996a, b; Cummings et al., 1996; Siew et al., 2000a, b ; Vilivalam et al., 2000
Chondroitin sulfate	Matrix tablets	Rubinstein et al., 1992a, b
Calcium alginate	Swellable beads	Shun et al., 1992
Inulin	Tablet and bead coatings	Vervoort et al., 1996; Akhgari et al., 2006
Dextran	Hydrogels	Simonsen et al., 1995 Brondsted et al., 1995

*Pectin* is a non-starch linear polysaccharide that consists mainly of  $\alpha$ -(1,4) D-galactorunic acid and  $\alpha$ -(1,2) L-rhamnose , found in the cell walls of plants. It is completely degraded by colonic bacteria but is not digested in the upper GIT [Rubinstein et al., 1993; Salyers et al., 1977; Liu et al., 2003]. The disadvantage of pectin is its solubility. To

overcome this restriction the degree of its methoxylation has been modified and also calcium pectinate has been prepared in order to make pectin resistant in the upper GIT [Rubinstein et al., 1993]. Combination of pectin and ethylcellulose was used to film coat paracetamol tablet cores [Wakerly et al., 1996, 1997]. Drug release was depended on the nature and characteristics of the mixed film as well as the composition of the dissolution medium. 5-Aminosalicylic acid beads coated with pectin/ethylcellulose were prepared and evaluated for drug delivery to the colon. Simulated gastric fluid was found to influence drug release (Hydration and swelling characteristics of pectin), and also the ratio of pectin to ethylcellulose in the coat [Ahmed, 2005]. Pectin has also been investigated in combination with chitosan [Munjari et al., 1997] and hydroxypropyl methylcellulose (HPMC) [Turkoglu et al. 1999]. Using gamma camera pectin-coated tablets disintegrate during transit in the colon (Ashford et al., 1994). Pectin/chitosan and HPMC mixtures have been investigated as a film coating system for colonic delivery, forming in situ polyelectrolyte complex between pectin and chitosan [Macleod et al., 1999a]. In vitro and in vivo investigations were carried out using such systems. In vitro dissolution of the tablets using pectinolytic enzyme showed that the release rate was faster than in the absence of this enzyme. It has also been found that the tablets coated with pectin:chitosan:HPMC were able to pass the stomach and small intestine intact, but once the tablets arrive into the colon started to disintegrate when administered to human volunteers [Macleod et al., 1999b; c]. Eudragit S-coated pectin microspheres of 5-fluorouracil have been prepared and evaluated for colon targeting in order to reduce side effects of the drug caused by its absorption from the upper part of the GI tract. As expected, drug release could be suppressed in simulated gastric fluid and triggered at pH 7.4. In vitro drug release study in the presence of rat cecal content have shown that there are no/slightly difference between the release profile in the presence and absence of cecal content [Paharia et al. 2007].

*Chitosan* is the second most abundant polysaccharides in nature after cellulose, obtained by the alkaline N-deacetylation of chitin. Chitosan molecule is a copolymer of N-acetyl-D-glucosamine and D-glucosamine [Hejazi and Amiji, 2003; Hoppe-Seiler, 1994; Illum, L., 1998]. Chitosan was used in oral drug formulations to provide colonic drug delivery. Chitosan is also considered as a promising candidate for colon targeting because of its favorable biological properties (e.g., non-toxicity, biocompatibility and biodegradability). Chitosan is degraded by the colonic microflora [Tozaki et al., 1997], and it is not digested in the upper part of the GIT by human digestive enzymes [Chourasia and Jain, 2004; Jain et al., 2007]. Drug delivery systems utilizing chitosan is discussed by various researchers [Friend,

2005; Tozaki, 1997]. Insulin and 5-aminosalicylic acid have been administered to rats in enteric-coated chitosan capsules. Recently, a tablet formulation was developed using chitosan, guar gum as carriers in the matrix-tablet, and then was coated firstly with inulin as inner coat, and secondly with shellac as outer coat [Ravi et al., 2008]. The investigated tablet has controlled the drug release in gastric and intestinal fluids, however, drug release was found to be enhanced in the presence of rat cecal contents. Chitosan-Ca-alginate microparticules have been prepared and characterized to deliver 5-aminosalicylic acid to the colon after oral administration [Mladenovska et al. 2007a]. Dissolution and biodistribution studies of <sup>131</sup>I-labelled 5-aminosalicylic acid after peroral administration of these microparticles to rats have shown an intensive mucoadhesion and controlled colon-specific delivery [Mladenovska et al. 2007b]. Chitosan-prednisolon conjugate microspheres were coated with Eudragit L 100 and evaluated in vitro at different pH levels [Onishi et al., 2007]. Microspheres coated with Eudragit are able to protect drug in simulated gastric fluid but once the pH increased to 6.8 the release rate of the microspheres increased significantly.

*Guar gum*, obtained from the ground endosperms of *Cyamopsis tetragonolobus*, is a galactomannan material composed of linear chains of (1,4)- $\beta$ -D-mannopyranosyl units with  $\alpha$ -galactopyranosyl units linked by (1,6) [Yu et al., 1998]. Crosslinked guar gum has been used as a drug matrix tablets [Gliko-Kabir et al., 1998; Rama Prasad et al., 1998]. However, the guar gum formulations mentioned were investigated only in vitro.

*Starch*, a polysaccharide which occurs as microscopic granules in the tissues of many plants species, is degraded by many bacterial species (e.g., bacteroides, bifidobacteria). Starch is composed of two polysaccharides: amylose and amylopectin. Amylose is an essentially linear  $\alpha$ -glucan containing  $\alpha$ -(1,4) bonds. Amylopectine has a much higher molecular weight than amylose and is much more heavily branched, with about 95 %  $\alpha$ -(1,4) and 5 %  $\alpha$ -(1,6) bonds [Biliarderis, 1998]. The amount of amylose usually present in starch is between 20 % and 35 %. Breeders have developed starches which contain amylose between 50 % and 80 % [Biliarderis, 1991]. Resistant starch to digestive enzymes (e.g., pancreatin enzymes within the small intestine) can be made by the formation of an amorphous structure (amorphous amylose) though can be degraded by colonic bacteria [Miles et al. 1985; Ellis and Ring, 1985; Englyst and Macfarlane, 1986]. However, not all forms of amylose are resistant to digestion in the upper GIT. For this reason, *glassy amylose* was chosen to provide colonic drug delivery, besides, only retrograded amylose resists upper GIT digestion by pancreatic enzymes [Englyst and Cummings, 1987; Ring et al., 1988; Leloup et al., 1992] and also due to its microstructure. Amylose has been used in coatings of colon-specific formulations

[Milojevic et al., 1996a, b; Cummings et al., 1996]. A disadvantage of amylose in film form is its swelling in aqueous media and subsequent accelerated drug release. Pure amylose films take up considerable amounts of water upon exposure to aqueous media. They become very permeable and the drug is already released in the upper GIT before the distal GIT is reached. To control this swelling commercially available controlled release polymers (e.g., ethylcellulose) have been mixed with amylose in order to prevent drug in the stomach and small intestine. Coated 5-aminosalicylic acid pellets with amylose:ethylcellulose in a ratio of 1:4 (w/w) have been shown to be resistant to gastric and intestinal fluids but fermentable by colonic bacterial enzymes [Milojevic et al., 1996a]. Mesalazine-tablets coated with amylose:ethylcellulose blends have been also investigated exploiting gastrointestinal bacteria to trigger mesalazine release from amylose-based systems [Wilson and Basit, 2005]. It has been concluded that the ratio of the amylose to ethylcellulose and the coating level play a major role in controlling drug release from this system. Moreover, this system was susceptible to colonic bacteria. The performance of amylose:ethylcellulose coated formulation (ratio 1:3 and 25 % coating level) has been evaluated in vitro and in vivo using gamma scintigraphy, and compared with immediate release pellets formulations as well as with enteric polymer poly vinyl acetate phthalate coated pellets [Basit et al., 2004]. From the results of in vitro studies it was concluded that amylose/ethylcellulose coatings could suppress drug release in the upper GIT depending on the coating thickness and also on the polymer:polymer ratio. Contrary to immediate release formulation in which the drug rapidly released and absorbed into the blood stream enteric formulation delayed drug until they come into the small intestine (most of them), but the amylose based coating retarded the drug release until the pellets had reached the colon. A formulation which provides improved controlled targeted release of an oral administration of prednisolone sodium metasulphobenzoate to the colon has been developed in order to decrease systemic absorption and consequently low risk of systemic adverse events of corticosteroides. The formulation comprises prednisolone sodium metasulphobenzoate surrounded by glassy amylose:ethylcellulose (ratio from 1:3.5 to 1:4.5) plasticized with dibutyl sebacate [Palmer, et al., 2005]. The formulation has shown that the drug delivery starts by the arrival of the dosage form in the colon. An ethylcellulose/glassy amylose surrounded formulation is now available as COLAL<sup>®</sup>, which has been used to coat pellets containing the corticosteroid prednisolone sodium metasulphobenzoate (COLAL-PRED<sup>®</sup>; Alizyme Therapeutics Ltd, Cambridge, UK). This product has achieved successful Phase II clinical trial results [Thompson et al., 2001] and is now in phase III clinical trials for the treatment of moderate to severe ulcerative colitis. Mixed amylose /Eudragit coating

dispersion has also been used to delay drug release and target the colon [Basit et al., 2007]. Another technique, to reduce the hydrophilicity of amylose is the coupling of amylose with hydrophobic polymers. Ethyl methacrylate (EMA) was grafted onto a high amylose starch in order to make amylose hydrophobic increasing its resistant to digestive enzymes [Alias et al., 2007]. To obtain high enzymatic resistant was necessary large quantities of Ethyl methacrylate. In spite of the Ethyl methacrylate coating around the amylose, the carbohydrate of amylose-ethyl methacrylate was susceptible to fermentation in the human colon.

*Chondroitin sulphate* is a soluble mucopolysaccharide that is used as a substrate by the bacteroides (e.g., *Bacteroides. ovatus*) of the large intestine [Toledo and Dietrich, 1977]. Chondroitin sulphate could be used as a carrier for colon targeted delivery of bioactive agents. In contrast to natural chondroitin sulphate, which is readily water-soluble and not able to prevent drug release in the upper GIT, crosslinked chondroitin sulphate would be less hydrophilic and thus would provide a better drug controlling in the stomach and small intestine. Crosslinked chondroitin sulphate in matrix formulations with indomethacin as a drug carrier was investigated to control drug release in the colon (Rubinstein et al., 1992a, b). In vitro indomethacin release upon exposure to phosphate buffer with and without rat cecal content has shown that the faster drug release depended on the biodegradation action of bacterial enzymes.

*Inulin* is a naturally accruing glucofructan found in many plants. It consists of  $\beta$ -(1-2) linked D-fructose molecules having a glucosyl unit at the reducing end. Inulin is not significantly hydrolyzed by digestive enzymes in the upper GIT, however, colonic bacteria and more specifically bifidobacteria can degrade this polysaccharide [van den Mooter et al., 2003]. It can serve as a biodegradable compound with Eudragit RS if an inulin-type with a high degree of polymerization is used to lower its water solubility [Vervoort and Kinget, 1996]. Mixed films of inulin and Eudragit RS withstand gastric and intestinal fluids which indicate that this coating system could also serve as coating materials for colon targeting. The bacterial degradation has been shown to depend on the hydrophilicity of the plasticizer. However, Eudragit RS and RL in combination with inulin made free films have been shown more swelling and permeation of drug in colonic medium rather than in gastric and intestinal fluids [Akhgari et al., 2006].

*Alginates*, natural hydrophilic polysaccharide derived from seaweed, is a linear polymer which consist of (1-4)- $\beta$ - D mannuronic acid and  $\alpha$ -L glucuronic acid residues. The gelation of alginates can be induced by adding  $\text{Ca}^{++}$  ions because alginates do not gel since they have poly (L-glucuronic acids) which are rigid. 5-aminosalicylic acid has been sprayed

on calcium alginate cores for the use in targeted drug delivery system [Shun and Ayres, 1992]. These beads were coated with different percentages of enteric coating polymer and/or sustained release polymer (Eudragit L 30D, Aquacoat). Alginate beads were also coated with dextran acetate [Kiyoungh et al., 1999]. Drug release was significantly faster in the presence of dextranase than in the absence of this enzyme.

*Dextrans* are a class of polysaccharides with a linear polymer backbone with mainly 1,6- $\alpha$ -D-glucopyranoside linkages with side chains of additional  $\alpha$ -(1,4) and  $\alpha$ -(1,3) bonds. Dextran has been found to be degraded in human feces due to bacterial action [Aberg, 1953]. Various drug-dextran prodrugs in which the drug molecule is linked to the polar dextran macromolecule remain intact and unabsorbed from the stomach and small intestine but when the prodrug enters into the colonic environment is degraded by dextranases. Dextran and 5-aminosalicylic acid conjugates were synthesized and evaluated for drug delivery to the colon [Ahmad et al., 2006].



## **1.3. COMERCIAL PRODUCTS USED FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES**

### **1.3.1. Pentasa (Time-controlled drug release)**

Pentasa pellets/tablets consist of 5-aminosalicylic acid loaded starter cores coated with ethylcellulose. Drug release already starts in the *upper* GIT [Wilding et al., 1999]. The release rate of both Pentasa tablets and Pentasa granules, which have similar release profiles, is three to five times faster in simulated stomach than in simulated small intestine and large intestine [Schellekens et al., 2007]. The higher release rate of Pentasa-products in the stomach is best explained by a diffusion-controlled release mechanism in aqueous environment, and also due to cracks formed on the coat.

### **1.3.2. Asacol/Salofalk (pH-controlled drug release)**

Asacol capsules are filled with 5-aminosalicylic acid loaded granules, which are coated with Eudragit S: a poly(acryl methacrylate), which is insoluble at low pH, but becomes soluble at  $\text{pH} > 7$ . Salofalk tablets or granules are coated with Eudragit L: a poly(acryl methacrylate), which is insoluble at low pH, but becomes soluble at  $\text{pH} > 6$ . Both Asacol tablets and Salofalk tablets can prevent drug release in the stomach. However, they showed a pulsatile release profile in the small intestine. Furthermore, the pulsatile release leads to high local concentrations, which are related to increased absorption into the systemic circulation [Zhou et al., 1999; Shellekens]. Also, the failure of pH-sensitive systems has been reported with Asacol tablets [Schroeder et al., 1987; Safdi, 2005], and with other single unit dosage forms based on Eudragit S coatings [Ibekwe et al., 2006; 2008; McConnell et al., 2008]. The failure of Eudragit S coated dosage forms to disintegrate *in vivo* is often attributed to the threshold pH not being reached.

### **1.3.3. Lialda/ Mezavant (pH and time controlled drug release)**

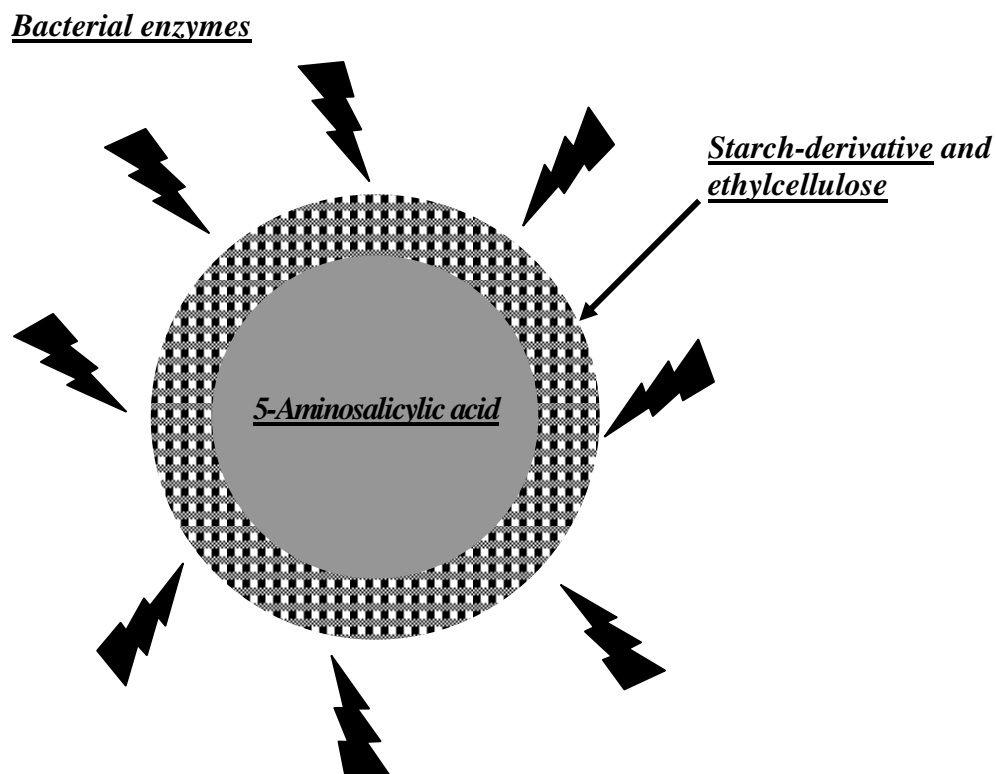
Lialda/Mezavant tablets are matrices consisting of hydrophilic and lipophilic compounds [sodium-carmellose, sodium carboxymethylstarch (type A), talc, stearic acid, and carnauba wax], in which the drug is incorporated. These controlled release matrix tablets are coated with a blend of Eudragit L and Eudragit S: two poly (acryl methacrylates). The Multi Matrix System (MMX<sup>TM</sup> tablet) contains 1.2 g of 5-aminosalicylic acid and indicated for the

treatment of ulcerative colitis. Lialda, based on the multi matrix system technology, is the first oral-once-daily or twice daily mesalamine which utilizes the Multi Matrix technology to release 5-aminosalicylic acid throughout the colon. The film delays release of the 5-aminosalicylic acid until the pH is greater than 7. The tablet course swells due to a hydrophilic matrix, and then a viscous gel mass is formed. As this goes through the colon, fragments of the gel mass break off. They release 5-aminosalicylic acid in proximity to the colonic mucosa to which the hydrophilic matrix will then adhere [Sandborn et al., 2007]. Please not that the tablets remain intact at pH 6.8 which could lead to the failure of the medication when the pH of the colon drops (e.g., in the case of inflammatory bowel diseases).

#### **1.4. INVESTIGATED SYSTEMS**

In this work, *coated pellets* have been studied as advanced drug delivery systems (**Figure 3**). The use of small, *multiparticulate* dosage forms (e.g., pellets and mini-matrices) provides the following major advantage compared to single unit dosage forms (e.g. tablets or capsules): (i) The all-or-nothing effect can be avoided: If a tablet gets accidentally damaged within the *upper* GIT, the entire drug dose is lost. (ii) The gastric emptying time is less variable, because the pylorus can be passed even in the fed state. (iii) The dosage forms are more homogeneously distributed within the contents of the GIT. (iv) The stagnation at the ileo-cecal junction is less likely to occur than with larger single units. (v) The larger surface area by the enzymatic attack. (vi) Slower transit of small particles through the colon which prolongs the contact between the formulation and the absorptive surface. Thus, the entire inflammation area can be more easily reached. Furthermore, coated dosage forms can generally contain higher drug doses than matrix systems (in which the drug is distributed throughout the matrix former).

This is particularly important for high dose drugs, such as 5-aminosalicylic acid, which is the standard drug for the local treatment of inflammatory bowel diseases (Crohn's disease and ulcerative colitis) [Desreumaux et al., 2001; Rousseaux et al., 2005; Dubuquoy et al., 2006].



**Figure 3:** Schematic representation of the principle of the investigated colon targeting approach.

Different types of starch derivatives (being partially acetylated and/or pre-gelatinized) have been studied for this purpose. As they are water-soluble, a second (water-insoluble) polymer was added: ethylcellulose is water-insoluble and avoids premature film dissolution in the *upper* GIT [Milojevic et al., 1996a; Siew et al., 2000a, b; McConnell et al., 2007]. Ethylcellulose is non-toxic, non-allergenic and non-irritant. Thus, the investigated polymeric networks consist of two compounds: (i) a polysaccharide, which should be preferentially degraded by the enzymes present in the colon of inflammatory bowel disease patients, and (ii) ethylcellulose assuring that the film coatings do not spontaneously dissolve in the contents of the stomach and small intestine.

Different types of polymeric blends have been investigated and a combination of Nutriose (a water-soluble, branched maltodextrin with high fiber contents obtained from wheat starch) with ethylcellulose was shown to be particularly promising. Due to the presence of  $\alpha$ -1,6 linkages and non digestible glycoside linkages (e.g.,  $\alpha$ -1,2 and  $\alpha$ -1,3), Nutriose is

only incompletely hydrolyzed and absorbed in the small intestine (approximately 10-15 %). But this starch derivative is progressively fermented to about 85 % in the colon [Van den Heuvel et al., 2004, 2005; Passman et al., 2006]. Furthermore, Nutriose is known to exhibit a significant pre-biotic activity, normalizing the microflora and enzyme patterns in the colon of the patients [Van den Heuvel et al., 2004, 2005; Passman et al., 2006; Lefranc-Millot et al., 2006]. This is of major clinical benefit for this type of GIT diseases [Velazquez et al., 1997; Wachtershauser et al., 2000; Cummings et al., 2001; Macfarlane et al., 2006].

5-Aminosalicylic acid-loaded beads were prepared by extrusion-spheronisation and coated with different types of starch-derivative:ethylcellulose blends. 5-Aminosalicylic acid release from coated pellets was monitored in the presence and absence of fecal samples from inflammatory bowel disease patients. For reasons of comparison, also drug release from commercially available products was determined.

## **1.5. PURPOSES OF THIS WORK**

The major objective of this work was to identify novel polymeric film coatings allowing for the site-specific delivery of drugs to the colon. This type of advanced pharmaceutical dosage forms (multiparticulate systems) is of great practical importance for instance for the treatment of inflammatory bowel diseases, e.g. Crohn's disease. Importantly, the identified new polymeric films are adapted to the pathophysiological conditions in inflammatory bowel disease- patients and provide additional pre-biotic effects, normalizing the patients' microflora. Particular aims included:

- (i) The Preparation and physicochemical characterization of novel types of polymer coated pellets and thin, free polymeric films of identical composition as the pellets coatings, allowing for the site-specific delivery of drugs to the colon.
- (ii) The identification of efficient tools that can be used to easily adjust the crucial film coating properties of novel polymeric film coatings allowing for this purpose
- (iii) The investigation of the effects of various formulations (e.g., polymer blend ratio, content of the plasticizer and type of the polysaccharide) on drug release.
- (iv) The evaluation of the ability of starch derivative:ethylcellulose blends to provide site specific drug delivery to the colon.
- (v) The optimization of the properties of novel polymeric films based on blends of ethylcellulose and a second polysaccharide (a water-soluble, modified branched

dextrin).

- (vi) The elucidation of these aspects to be able to easily adapt film coatings' properties to the specific needs of a particular type of drug treatment (e.g., osmotic activity of the drug and administered dose).

Blends of ethylcellulose and different types of starch derivatives (partially being pregelatinized, acetylated and/or hydroxypropylated) were studied and the effects of the polymer blend ratio on the resulting systems' water uptake and dry mass loss kinetics as well as on their mechanical properties in the dry and wet state monitored. *In vitro* drug release from 5-aminosalicylic acid coated pellets with these blends was measured under various conditions, including the exposure to fecal samples from inflammatory bowel disease patients under *anaerobic* conditions.

## **2. Materials and Methods**

## **2.1. MATERIALS**

The following chemicals were obtained from commercial suppliers and used as received:

### **Drug**

5-Aminosalicylic acid (5-ASA; Sigma-Aldrich, Isle d'Abeau Chesnes, France).

### **Commercial products**

Pentasa granules, Asacol capsules and Lialda.

### **Polymers**

Nutriose FB<sup>®</sup> 06 (Nutriose, a water-soluble, branched dextrin with non digestible glycoside linkages:  $\alpha$ -1,2 and  $\alpha$ -1,3 and high fiber contents obtained from wheat starch; Roquette Freres, Lestrem, France), Peas starch N-735 (peas starch), Lycoat<sup>®</sup> RS 780 (Lycoat, pregelatinized hydroxypropyl pea starch), Glucidex<sup>®</sup> 1 (Glicidex, a maltodextrin), Eurylon<sup>®</sup> 7 A-PG [an acetylated and pregelatinized high amylose maize starch; (70 % amylose)], Eurylon<sup>®</sup> 6 A-PG [an acetylated and pregelatinized high amylose maize starch (60 % amylose)] and Eurylon<sup>®</sup> 6 HP-PG [a hydroxypropylated and pregelatinized high amylose maize starch (60 % amylose)] (Roquette Freres, Lestrem, France); aqueous ethylcellulose dispersion (Aquacoat ECD 30; FMC Biopolymer, Philadelphia, USA).

### **Plasticizer**

Triethylcitrate (TEC; Morflex, Greensboro, USA).

### **Digestive enzymes**

Pancreatin (from mammalian pancreas = mixture of amylase, protease and lipase) and pepsin (Fisher Bioblock, Illkirch, France); extract from rat intestine (rat intestinal powder, containing amylase, sucrase, isomaltase and glucosidase; Sigma-Aldrich, Isle d'Abeau Chesnes, France).

### **Additives**

Microcrystalline cellulose (Avicel PH 101; FMC Biopolymer, Brussels, Belgium); bentonite and polyvinylpyrrolidone (PVP, Povidone K 30) (Cooperation Pharmaceutique Francaise, Melun, France).

### **Ingredients for culture medium preparation**

Columbia blood agar, extracts from beef and yeast as well as tryptone (= pancreatic digest of casein) (Becton Dickinson, Sparks, USA); L-cysteine hydrochloride hydrate (Acros Organics, Geel, Belgium); McConkey agar (BioMerieux, Balme-les-Grottes, France); cysteinated Ringer solution (Merck, Darmstadt, Germany).

### **Organic solvents**

Methanol HPLC grade (Fisher Bioblock, Illkirch, France), acetic acid glacial (Fisher Bioblock, Illkirch, France).

### **Buffer components**

Potassium dihydrogen phosphate (Fisher Bioblock, Illkirch, France), sodium hydroxide (Fisher Bioblock, Illkirch, France), sodium hydrogen phosphate (Fisher Bioblock, Illkirch, France), sodium chloride (Fisher Bioblock, Illkirch, France).



## **2.2. EXPERIMENTAL METHODS**

### **2.2.1. Preparation of thin films**

Thin polymeric films were prepared by casting blends of different types of aqueous starch derivatives and aqueous ethylcellulose dispersion into Teflon moulds and subsequent drying for 1 d at 60 °C. The water soluble polysaccharide was dissolved in purified water (5 % w/w, in the case of Eurylon 7 A-PG, Eurylon 6 A-PG and Eurylon 6 HP-PG in hot water), blended with plasticized aqueous ethylcellulose dispersion (25 % w/w TEC, referred to the ethylcellulose content, overnight stirring) at a ratio of 1:2, 1:3, 1:4, 1:5 (polymer:polymer, w:w).

Furthermore, Nutriose was dissolved in purified water (5 % w/w), blended with plasticized aqueous ethylcellulose dispersion (25.0, 27.5 or 30.0 % TEC, overnight stirring; 15 % w/w polymer content) at a ratio of 1:2, 1:3, 1:4, 1:5 (polymer:polymer w:w), as indicated. The mixture was stirred for 6 h prior to casting.

### **2.2.2. Preparation of drug-loaded pellet cores**

Drug-loaded pellet cores (diameter: 710-1000 µm; 60 % 5-ASA, 32 % microcrystalline cellulose, 4 % bentonite, 4 % PVP) were prepared by extrusion and spheronization. The powders were blended in a high speed granulator (Gral 10; Collette, Antwerp, Belgium) and purified water was added until a homogeneous mass was achieved. The wetted powder mixture was passed through a cylinder extruder (SK M/R; Alexanderwerk, Remscheid, Germany). The extrudates were subsequently spheronized at 520 rpm (Spheronizer Model 15; Calveva, Dorset, UK) and dried in a fluidized bed (ST 15; Aeromatic, Muttenz, Switzerland) at 40°C for 30 min.

### **2.2.3. Preparation of coated pellets**

Nutriose was dissolved in purified water (5 % w/w), blended with plasticized aqueous ethylcellulose dispersion (25 % TEC, overnight stirring; 15 % w/w polymer content) at a ratio of 1:2, 1:3, 1:4, 1:5 (w/w) and stirred for 6 h prior to coating. The drug-loaded pellet cores were coated in a fluidized bed coater equipped with a Wurster insert (Strea 1; Aeromatic-Fielder, Bubendorf, Switzerland) until a weight gain of 5, 10, 15 and 20 % (w/w) was achieved. The process parameters were as follows: inlet temperature = 39 ± 2 °C, product

temperature =  $40 \pm 2$  °C, spray rate = 1.5-3 g/min, atomization pressure = 1.2 bar, nozzle diameter = 1.2 mm. After coating, the beads were further fluidized for 10 min and subsequently cured in an oven for 24 h at 60 °C.

#### **2.2.4. Film characterization**

##### 2.2.4.1. Water uptake and weight loss

The thickness of the films was measured using a thickness gauge (Minitest 600; Erichsen, Hemer, Germany). The mean thickness of all films was in the range of 300-340  $\mu\text{m}$ . The water uptake and dry mass loss kinetics were measured gravimetrically upon exposure to:

- (i) simulated gastric fluid (0.1 M HCl)
- (ii) simulated intestinal fluid [phosphate buffer pH 6.8 (USP 30) with or without 1 % pancreatin or 0.75 % extract from rat intestine]
- (iii) culture medium inoculated with feces from healthy subjects
- (iv) culture medium inoculated with feces from inflammatory bowel disease patients
- (v) culture medium free of feces for reasons of comparison.

Culture medium was prepared by dissolving 1.5 g beef extract, 3 g yeast extract, 5 g tryptone, 2.5 g NaCl and 0.3 g L-cysteine hydrochloride hydrate in 1 L distilled water (pH  $7.0 \pm 0.2$ ) and subsequent sterilization in an autoclave. Feces of patients with Crohn's disease or ulcerative colitis as well as feces of healthy subjects were diluted 1:200 with cysteinated Ringer solution; 2.5 mL of this suspension was diluted with culture medium to 100 mL. Film pieces of  $1.5 \times 5$  cm were placed into 120 mL glass containers filled with 100 mL pre-heated medium, followed by horizontal shaking at 37 °C (GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany). The incubation with fecal samples was performed under anaerobic conditions (5 %  $\text{CO}_2$ , 10 %  $\text{H}_2$ , 85 %  $\text{N}_2$ ). At predetermined time points samples were withdrawn, excess water removed, the films accurately weighed (wet mass) and dried to constant weight at 60 °C (dry mass). The water content (%) and dry film mass (%) at time t were calculated as follows:

$$\text{water content (\%)} (t) = \frac{\text{wet mass (t)} - \text{dry mass (t)}}{\text{wet mass (t)}} \cdot 100 \% \quad (1)$$

$$\text{dry film mass (\%)} (t) = \frac{\text{dry mass (t)}}{\text{dry mass (t = 0)}} \cdot 100 \% \quad (2)$$

#### 2.2.4.2. Mechanical properties

The mechanical properties of the films in the dry and wet state were determined with a texture analyzer (TAXT.Plus, Winopal Forschungsbedarf, Ahnsbeck, Germany) and the puncture test. Film specimens were mounted on a film holder (n= 6). The puncture probe (spherical end: 5 mm diameter) was fixed on the load cell (5 kg), and driven downward with a cross-head speed of 0.1 mm/s to the center of the film holder's hole. Load versus displacement curves were recorded until rupture of the film and used to determine the mechanical properties as follows:

$$\text{puncture strength} = \frac{F}{A} \quad (3)$$

Where F is the load required to puncture the film and A the cross-sectional area of the edge of the film located in the path.

$$\% \text{ elongation at break} = \frac{\sqrt{R^2 + D^2} - R}{R} \cdot 100 \% \quad (4)$$

Here, R denotes the radius of the film exposed in the cylindrical hole of the holder and D the displacement.

$$\text{energy at break per unit volume} = \frac{\text{AUC}}{V} \quad (5)$$

Where AUC is the area under the load versus displacement curve and V the volume of the film located in the die cavity of the film holder.

### 2.2.5. Bacteriological analysis

For the bacteriological analysis of *fecal samples*, the latter were diluted 1:10 with cysteinated Ringer solution. Eight further tenfold dilutions in cysteinated Ringer solution were prepared and 0.1 mL of each dilution was plated onto non-selective, modified Columbia blood agar [Neut et al., 2002] (for total cultivable counts) and on McConkey agar (being selective for enterobacteria). Columbia blood agar plates were incubated during 1 week at 37 °C under anaerobic conditions (5 % CO<sub>2</sub>, 10 % H<sub>2</sub>, 85 % N<sub>2</sub>). Colonies were outnumbered, predominant colonies subcultured and identified based on phenotypic identification criteria [Neut et al., 2002]. McConkey agar plates were incubated during 48 h at 37 °C in air. The colonies were outnumbered and identified using the API 20E system (BioMerieux, Balme-les-Grottes, France). Counts were expressed as log CFU/g (Colony Forming Units per gram) of fresh feces.

For the bacteriological analysis of the microflora developed upon *film incubation with fecal samples*, photomicrographs were taken after Gram-staining with an Axiostar plus microscope (Carl Zeiss, Jena, Germany), equipped with a camera (Unit DS-L2, DS camera Head DS-Fi 1; Nikon, Tokyo, Japan). Incubation was performed in a glucides-free culture medium containing only small amounts of polypeptides (thus, favoring the use of the investigated polysaccharides as substrates) under *anaerobic* conditions.

### 2.2.6. In vitro drug release from coated pellets

Drug release from the coated pellets was measured using 3 different experimental setups, simulating the conditions in the:

(i) Upper GIT: The pellets were placed into 120 mL plastic containers, filled with 100 mL dissolution medium: 0.1 M HCl (optionally containing 0.32 % pepsin) during the first 2 h, then complete medium change to phosphate buffer pH 6.8 (USP 30) (optionally containing 1 % pancreatin). The flasks were agitated in a horizontal shaker (80 rpm; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At pre-determined time points, 3 mL samples were withdrawn and analyzed UV-spectrophotometrically ( $\lambda = 302.6$  nm in 0.1 M HCl;  $\lambda = 330.6$  nm in phosphate buffer pH 6.8) (Shimadzu UV-1650, Champs sur Marne, France). In the presence of enzymes, the samples were centrifuged for 15 min at 11,000 rpm and subsequently filtered (0.2  $\mu$ m) prior to the UV measurements. Each experiment was conducted in triplicate.

(ii) Entire GIT, without feces: To simulate the gradual increase in the pH along the GIT, drug release was measured using the USP Apparatus 3 (Bio-Dis; Varian, Paris, France). Pellets were placed into 250 mL vessels filled with 200 mL 0.1 M HCl. The dipping speed was 10, 20 or 30 dpm (as indicated). After 2 h the pellets were transferred into phosphate buffer pH 5.5 (Eur. Pharm). **Table 3** indicates the subsequent changes and exposure times to the different release media. At pre-determined time points, 3 mL samples were withdrawn and analyzed UV-spectrophotometrically ( $\lambda = 306.8/328.2/330.6/330.2/330.2$  at pH = 5.5/6.0/6.8/7.0/7.4) as described above.

(iii) Entire GIT, with feces: To simulate the transit through the *upper* GIT, the pellets were exposed to 0.1 M HCl for 2 h and subsequently to phosphate buffer pH 6.8 or 7.4 (USP 30) for 9 h in an USP Apparatus 3 (Bio-Dis). Afterwards, the pellets were transferred into 120 mL flasks filled with 100 mL culture medium inoculated with feces from inflammatory bowel disease patients, culture medium inoculated with a specific type of bifidobacteria, culture medium inoculated with a mixture of bifidobacteria, bacteroides and E-coli, or culture medium free of feces and bacteria for reasons of comparison. The samples were incubated at 37 °C under anaerobic conditions (5 % CO<sub>2</sub>, 10 % H<sub>2</sub>, 85 % N<sub>2</sub>) and gentle agitation. Culture medium was prepared as mentioned before. Feces of patients with Crohn's disease or ulcerative colitis as well as feces of healthy subjects were diluted 1:200 with cysteinated Ringer solution; 2.5 mL of this suspension was diluted with culture medium to 100 mL. At pre-determined time points, 2 mL samples were withdrawn, centrifuged at 13,000 rpm for 5 min, filtered (0.22  $\mu$ m) and analyzed for drug content using high performance liquid chromatography (HPLC; ProStar 230; Varian, Paris, France). The mobile phase consisted of 10 % methanol and 90 % of an aqueous acetic acid solution (1 % w/v) [Siew et al., 2000b]. Samples were injected into Pursuit C18 columns (150  $\times$  4.6 mm; 5  $\mu$ m), the flow rate was 1.5 mL/min. 5-Aminosalicylic acid was detected UV-spectrophotometrically at  $\lambda=300$  nm.

*Table 3: Dissolution media used to simulate the gradual increase in pH along the GIT.*

<b>Simulated GI segment</b>	<b>Exposure time</b>	<b>Release medium</b>	<b>pH</b>
Stomach	2 h	0.1 M HCl	1.2
Duodenum	0.5 h	Phosphate buffer (Eur. Pharm. 5)	5.5
Jejunum- Ileum	9 h	Phosphate buffer (USP 30)	6.8
Caecum	0.5 h	Phosphate buffer (USP 30)	6.0
Proximal Colon	6 h	Phosphate buffer (USP 30)	7.0
Distal Colon	18 h	Phosphate buffer (USP 30)	7.4



## **3. Results and Discussion**

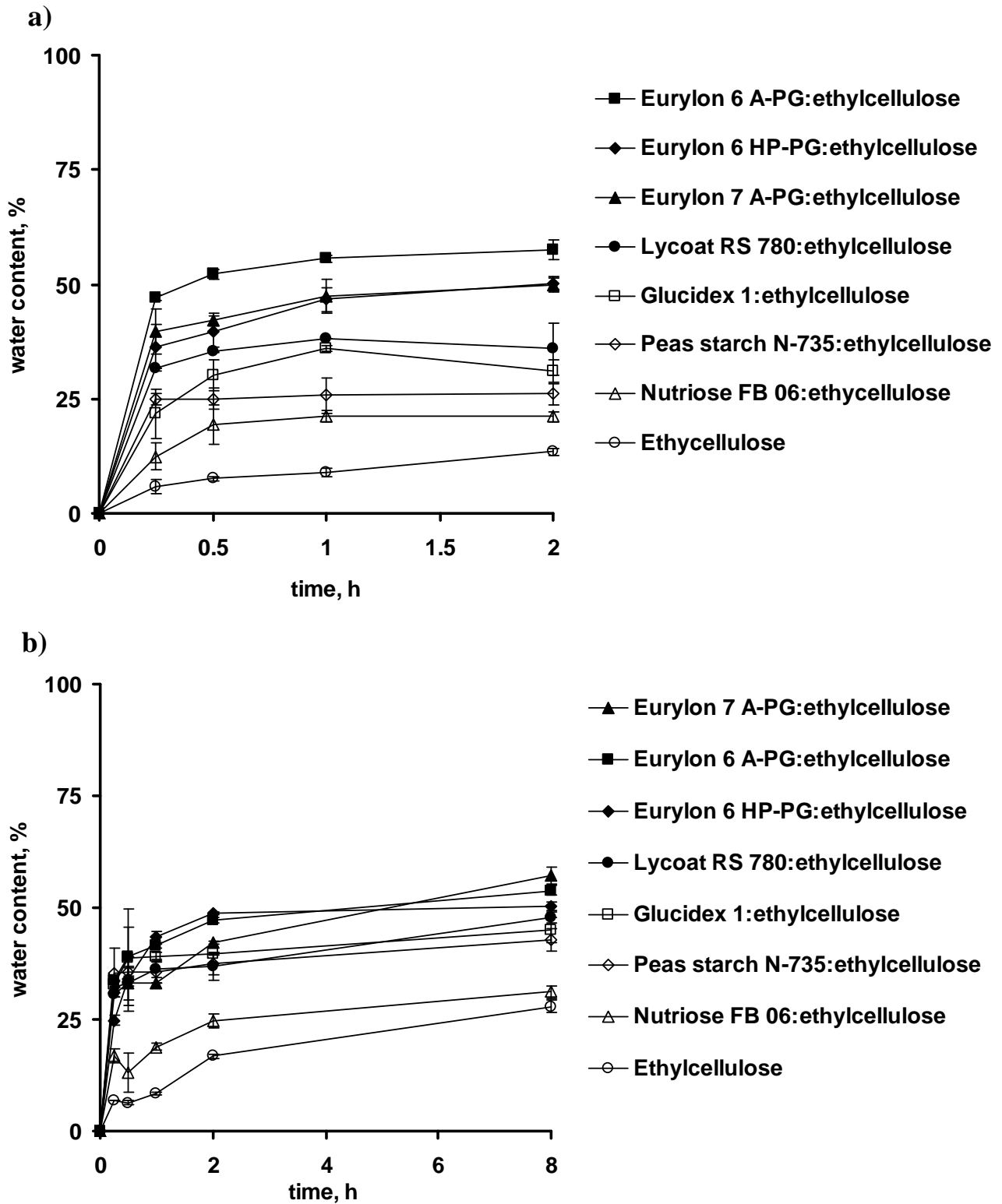


### 3.1. EFFECTS OF THE TYPE OF POLYSACCHARIDE

#### 3.1.1. Film properties in the upper GIT

The permeability of a polymeric system for a drug strongly depends on its water content and dry mass, which determine the density and mobility of the macromolecules [Crank and Park, 1968]. For instance, in dry hydroxypropyl methylcellulose (HPMC)-based matrix tablets the apparent diffusion coefficient of a drug approaches zero, whereas in a completely hydrated HPMC gel diffusivities can be reached, which are in the same order of magnitude as in aqueous solutions [Siepmann and Peppas, 2000]. With increasing water content the macromolecular mobility significantly increases and, thus, the free volume available for diffusion [Fan and Singh, 1989]. In some systems, the polymer undergoes a glassy-to-rubbery phase transition as soon as a critical water content is reached. This leads to a significant, stepwise increase in polymer and drug mobility. Thus, the water content of a polymeric film coating can give important insight into the macromolecular mobility and, hence, permeability for a drug. **Figures 4a and 4b** show the water uptake kinetics of thin films consisting of various types of starch derivative:ethylcellulose blends in 0.1 N HCl and phosphate buffer pH 6.8, respectively. The presence of ethylcellulose in all films allows avoiding premature dissolution in the upper GIT. The investigated starch derivatives are all water-soluble and aim at providing the sensitivity of the coatings' drug permeability to the surrounding environment: Once the colon is reached, the starch derivatives are to be enzymatically degraded and drug release to be started. The starch derivative:ethylcellulose blend ratio in **Figure 4** is constant: 1:3. Clearly, the water uptake *rates* and *extents* significantly depend on the type of starch derivative. The ideal film coating allowing for colon targeting should take up only small amounts of water at a low rate in both media in order to prevent premature drug release in the *upper* GIT. As it can be seen, blends of ethylcellulose and Nutriose or peas starch are most promising for this purpose. Plasticized ethylcellulose films without water-soluble polysaccharide take up only minor amounts of water (empty circles).

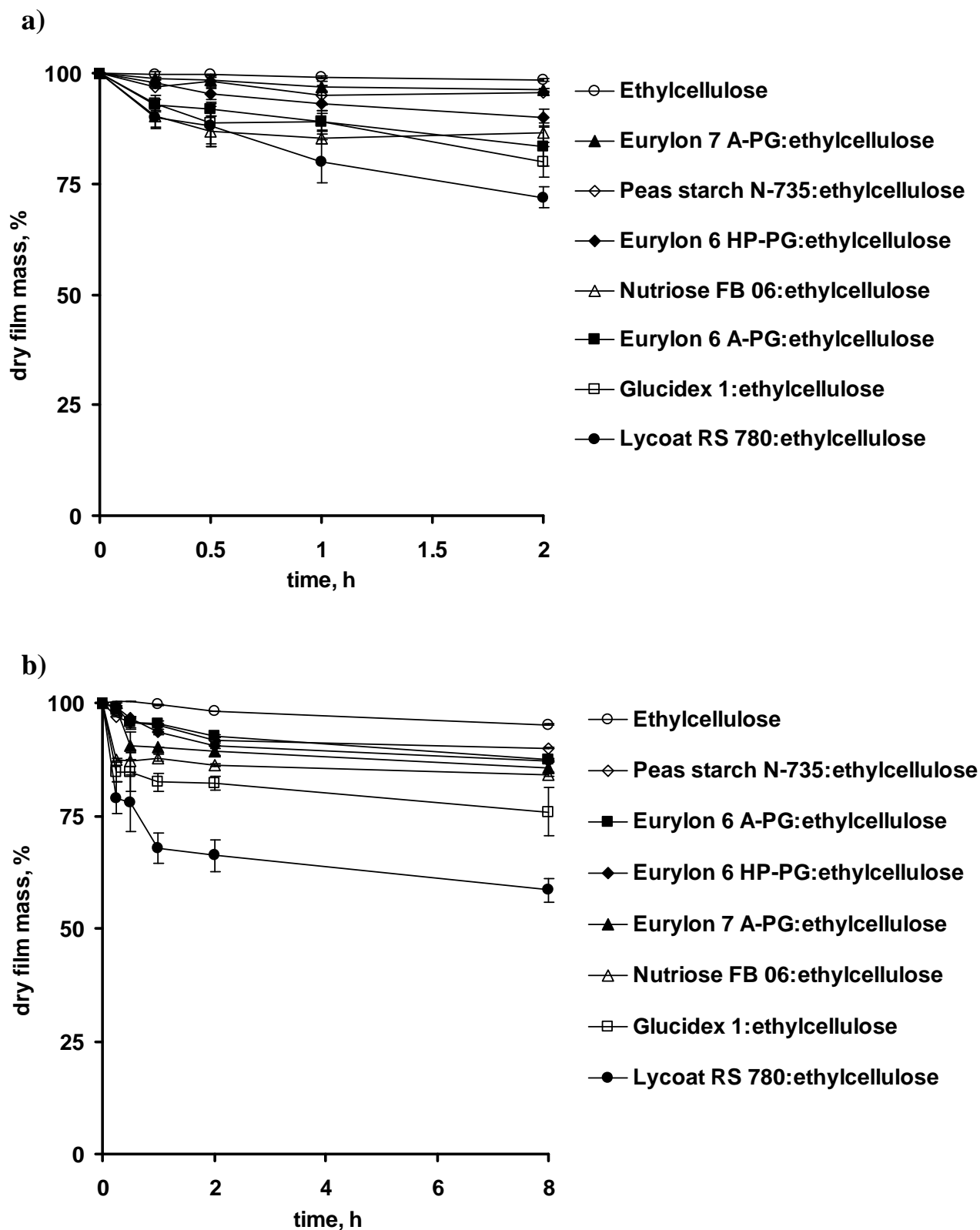
In addition to the water uptake kinetics also the *dry mass loss behavior* of thin polymeric films serves as an indicator for the coatings' permeability for the drug [Lecomte, et al., 2003; 2005] and, hence, potential to suppress premature release within the *upper* GIT. If the films loose significant amounts of dry mass upon exposure to the release media, the coatings can be expected to become permeable for many drugs, in particular those with a low



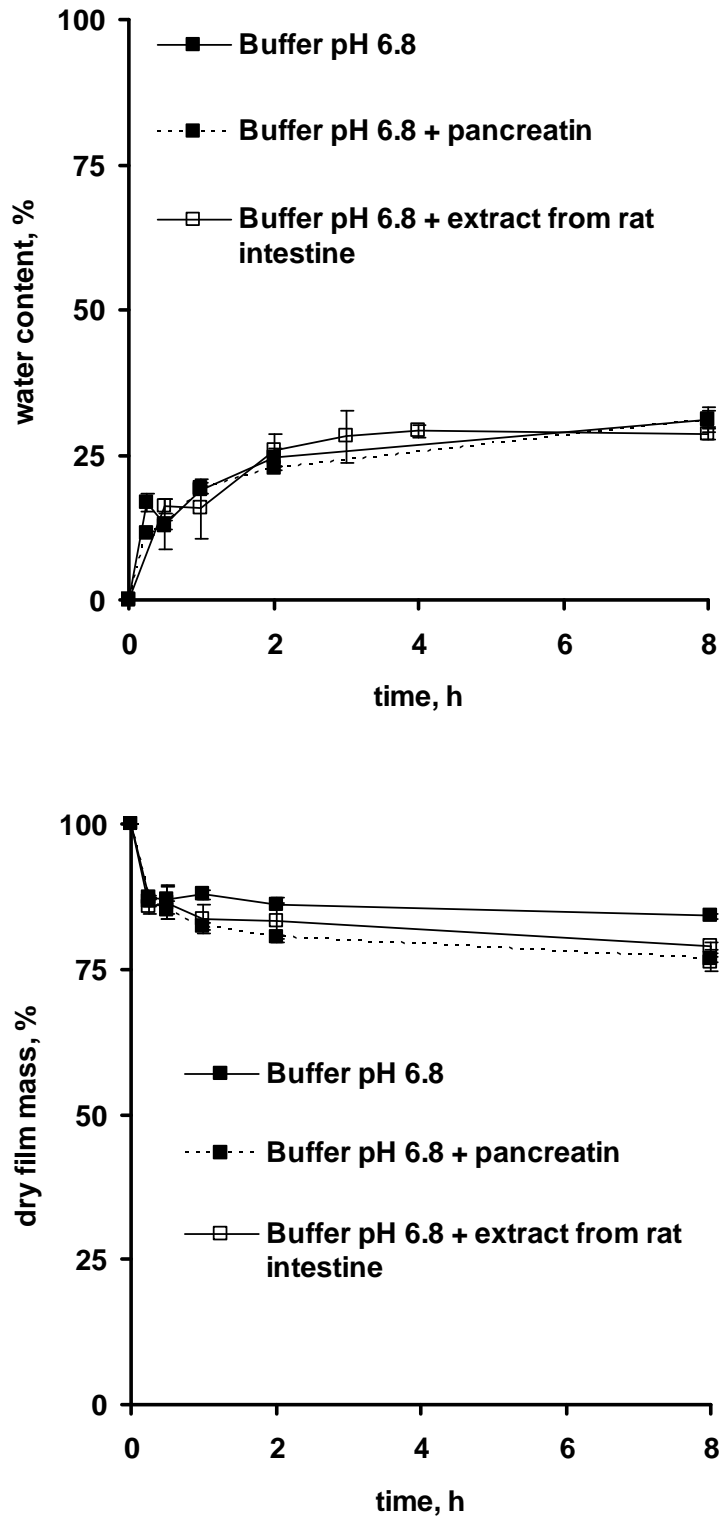
**Figure 4:** Water content of thin films consisting of different types of polymer blends (indicated in the figures) upon exposure to: (a) 0.1 M HCl, and (b) phosphate buffer pH 6.8. Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.

molecular weight such as 5-aminosalicylic acid (5-ASA, 153.1 Da). **Figures 5a and 5b** illustrate the experimentally determined dry mass loss of thin films consisting of various starch derivative:ethylcellulose blends (constant ratio = 1:3) upon exposure to 0.1 N HCl and phosphate buffer pH 6.8, respectively. The ideal film loses only minor amounts of dry mass at a low rate (or no mass at all), assuring dense polymeric networks which are poorly permeable for the incorporated drug under these conditions. As it can be seen, the dry mass loss of peas starch- and Nutriose-containing films is very low, even after up to 8 h exposure to these release media. The observed decrease in dry mass can at least partially be attributed to the leaching of the water-soluble plasticizer triethyl citrate (TEC, used to plasticize the aqueous ethylcellulose dispersion) into the bulk fluid. In addition, parts of the water-soluble starch derivative might leach out of the films. Plasticized ethylcellulose films without water-soluble polysaccharide lose only very small amounts of water, irrespective of the type of release medium (empty circles). However, the permeability of intact ethylcellulose films is known to be very low for many drugs [Lecomte, et al., 2003; 2005], which can at least partially be attributed to the low water-uptake rates and extents of these systems. For this reason, intact ethylcellulose films are also used as moisture protective coatings. Please note that the loss of the water-soluble plasticizer TEC into the bulk fluids can be expected to be much more pronounced in films containing 25 % (w/w) water-soluble polysaccharides compared to pure (plasticized) ethylcellulose films, because the increased water uptake rates and extents (**Figure 4**) of the blended systems lead to much higher polymer chain mobility and, thus, also increased TEC mobility.

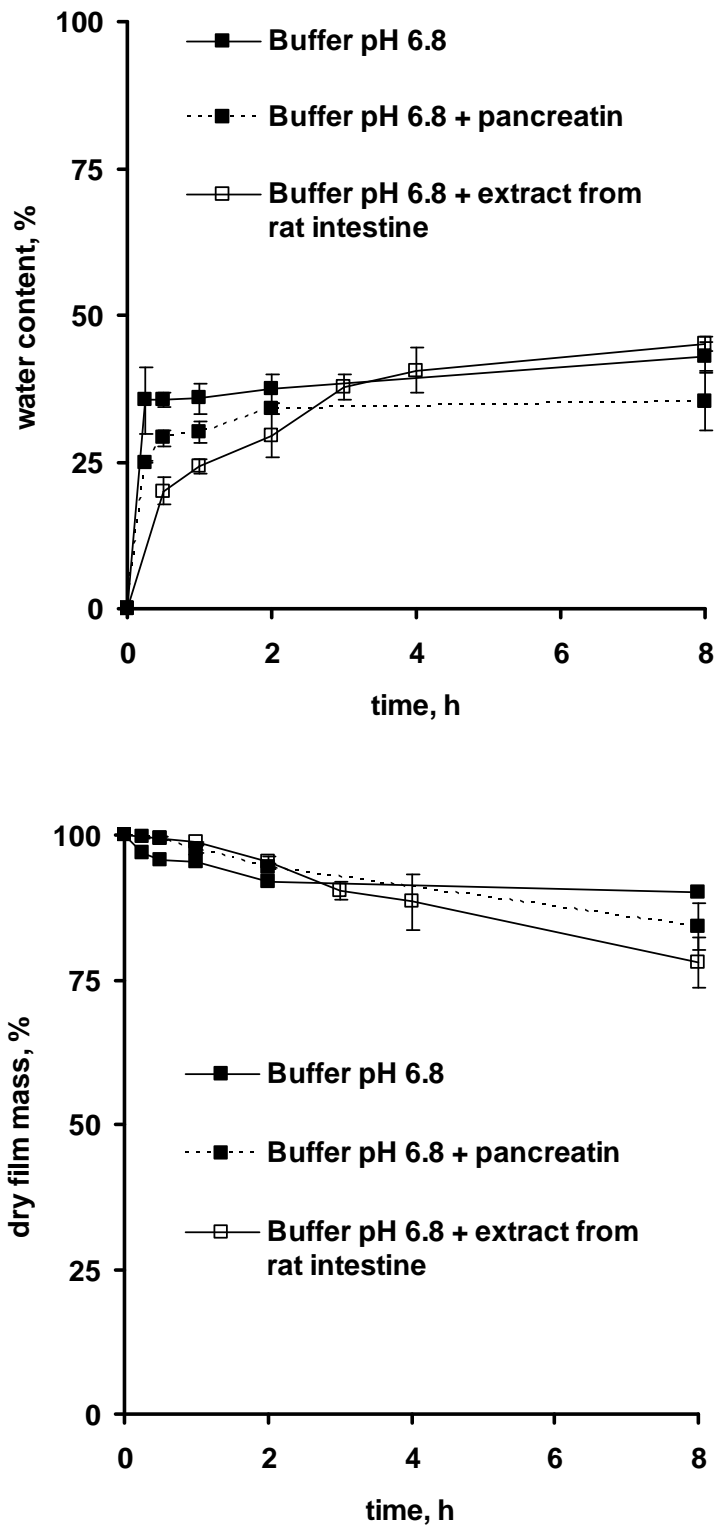
It has to be pointed out that the results shown in **Figure 5** were obtained in the *absence* of any enzymes. It is well known that pancreatic enzymes can degrade certain polysaccharides and, thus, potentially induce significant mass loss and water uptake under *in vivo* conditions, resulting in increased film permeability for the drug. To clarify the importance of this phenomenon, the water uptake kinetics and dry mass loss behavior of the thin films were also measured in the *presence* of pancreatin (= mixture containing amylase, protease and lipase) and of an extract from rat intestine (containing amylase, sucrase, isomaltase and glucosidase) in phosphate buffer pH 6.8 (**Figure 6 and 7**). Clearly, the addition of these enzymes did not significantly affect the resulting water uptake and dry mass loss kinetics of the investigated films. Thus, the latter do not serve as substrates for these enzymes.



**Figure 5:** Dry mass of thin films consisting of different types of polymer blends (indicated in the figures) upon exposure to: (a) 0.1 M HCl, and (b) phosphate buffer pH 6.8. Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.



**Figure 6:** Water mass content and dry mass of thin films consisting of Nutriose blended with ethylcellulose upon exposure to phosphate buffer pH 6.8 containing or not pancreatin or extract from rat intestine.



**Figure 7:** Water content and dry mass of thin films consisting of peas starch blended with ethylcellulose upon exposure to phosphate buffer pH 6.8 containing or not pancreatin or extract from rat intestine.

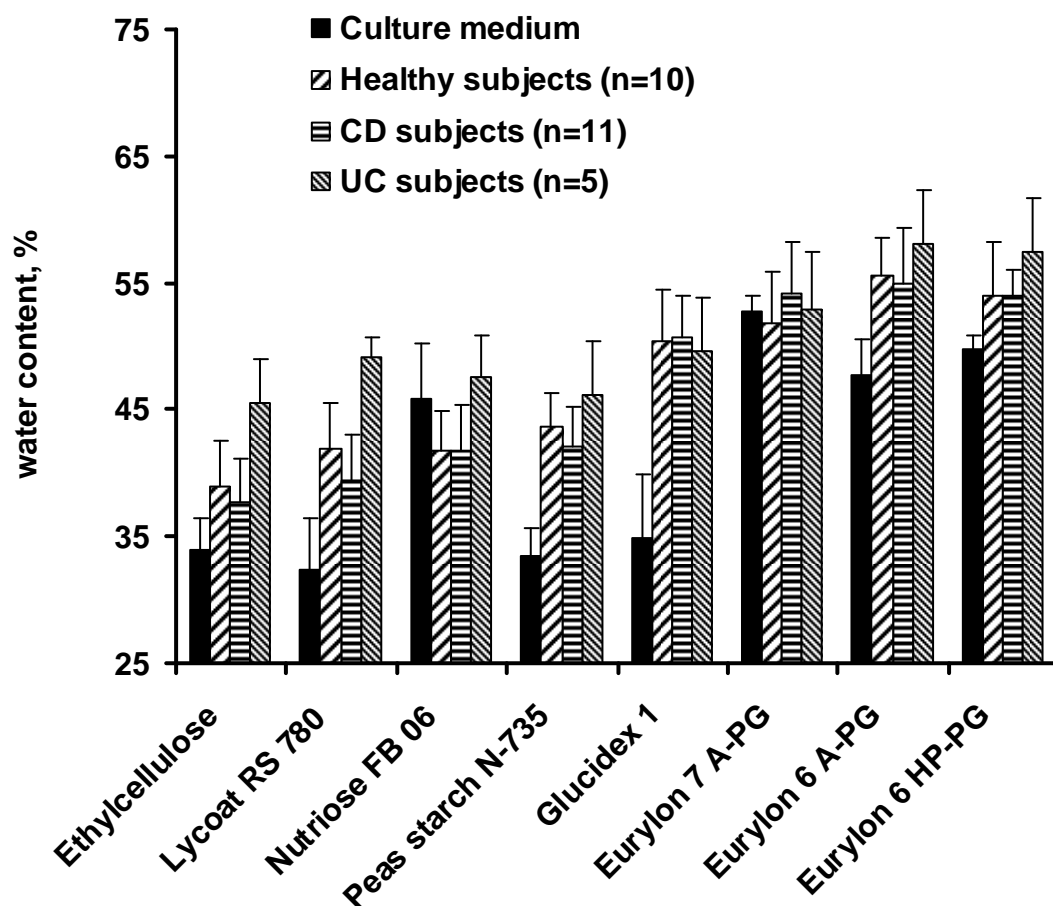
### 3.1.2. Film properties in the colon

Once the colon is reached, the polymeric film coatings should become permeable for the drug. This can for instance be induced by (partial) enzymatic degradation. Importantly, the concentrations of certain enzymes are much higher in the colon than in the *upper* GIT. This includes enzymes, which are produced by the natural microflora of the colon (this part of the GIT contains much more bacteria than the stomach and small intestine). However, great caution must be paid when using this type of colon targeting approach, because the microflora of patients suffering from inflammatory bowel diseases can be significantly different from the microflora of healthy subjects. Thus, the drug delivery system must be adapted to the disease state of the patient. **Table 4** shows for instance the concentrations of the bacteria determined in the fecal samples of the healthy subjects as well as of the Crohn's disease and ulcerative colitis patients included in this study. Importantly, there were significant differences, in particular with respect to the concentrations of *Bifidobacterium* (being able to degrade complex polysaccharides due to multiple extracellular glycosidases) and *Escherichia coli*, which were present at much higher concentrations in the feces of healthy subjects compared to the feces of the inflammatory bowel disease patients. In contrast, the fecal samples of the Crohn's disease and ulcerative colitis patients contained *lactose negative E. coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter cloacae*, which were not detected in healthy subjects. Thus, there are fundamental differences in the *quality* and *quantity* of the microflora, which must be taken into account: Polymeric film coatings, which allow for colon targeting under physiological conditions in a healthy volunteer, might fail under the pathophysiological conditions in the disease state of a patient. To address this very crucial point, which is very often neglected, the water uptake and dry mass loss of thin films consisting of various types of starch derivative:ethylcellulose blends were determined upon exposure to fecal samples from Crohn's disease and ulcerative colitis patients as well as to the feces of healthy subjects and to pure culture medium for reasons of comparison (**Figure 8 and 9**). Appropriate films should take up considerable amounts of water and show significant dry mass loss upon exposure to patients' feces in order to induce drug release at the site of inflammation in the colon. As it can be seen in **Figures 8 and 9**, films based on ethylcellulose:Nutriose and ethylcellulose:peas starch (which are the two most promising types of polymer blends based on the above described results obtained in media simulating the contents of the *upper* GIT) show significant water uptake and dry mass loss

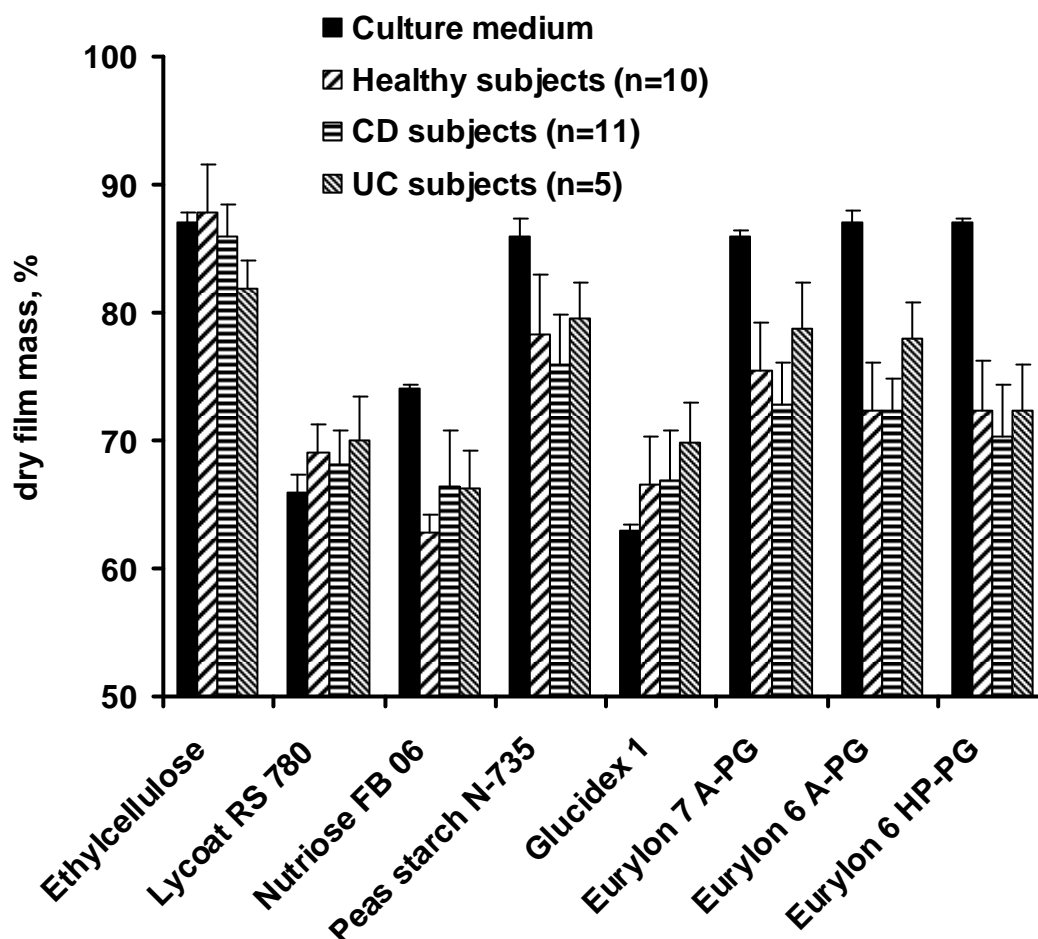
**Table 4:** Concentrations of bacteria [log CFU/g] in the investigated fecal samples of healthy subjects and inflammatory bowel disease patients.

	Healthy subjects	Crohn's disease	Ulcerative colitis
Number	10	11	5
Mean age	40 +/-15	32+/-12	36+/-20
Mean total counts [log UFC/g]	9.88+/-0,48	9.15+/-1.30	9.88+/-0.57
Number of strains	28	34	14
Mean	2.8	3.1	2.8
<b>Anaerobes</b>			
<i>Bacteroides</i>	9	10	3
<i>Prevotella</i>	2	2	2
<i>Fusobacterium</i>	3	3	2
<i>Veillonella</i>	0	0	1
<i>Clostridium</i>	0	5	1
<i>Bifidobacterium</i>	9	3	1
Other Gram + rods	3	2	2
Gram + cocci	1	2	0
<b>Aerobes</b>			
Enterobacteria	1	3	2
<i>Escherichia coli</i>	1	2	1
<i>Citrobacter freundii</i>	0	2	1
<i>Lactobacillus</i>	0	2	0
<i>Streptococcus</i>	0	2	0
Mean counts McConkey agar	6.30+/-1.19	7.16+/-1.48	8.01+/-1.06
Number of strains	10	14	8
<i>Escherichia coli</i>	10	6	4
<i>E. coli lac-</i>	0	1	0
<i>Citrobacter freundii</i>	0	3	1
<i>Klebsiella pneumoniae</i>	0	1	1
<i>Klebsiella oxytoca</i>	0	2	0
<i>Enterobacter cloacae</i>	0	1	0
Other Gram - rods	0	0	1





**Figure 8:** Water content and of thin films consisting of different types of polysaccharides blended with ethylcellulose upon exposure to culture medium, culture medium inoculated with feces of healthy subjects and culture medium inoculated with feces of Crohn's disease (CD) patients and ulcerative colitis (UC) patients (as indicated in the figures). Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.



**Figure 9:** Dry mass of thin films consisting of different types of polysaccharides blended with ethylcellulose upon exposure to culture medium, culture medium inoculated with feces of healthy subjects and culture medium inoculated with feces of Crohn's disease (CD) patients and ulcerative colitis (UC) patients (as indicated in the figures). Films consisting only of plasticized ethylcellulose are shown for reasons of comparison.

upon exposure to the feces of Crohn's disease patients, ulcerative colitis patients as well as of healthy subjects. Please note that also other types of polymer blends look promising with respect to the presented films' water uptake and dry mass loss behavior upon exposure to fecal samples (or even more appropriate than ethylcellulose:Nutriose and ethylcellulose:peas starch blends). However, these systems already take up considerable amounts of water and remarkably loose in dry mass upon contact with media simulating the contents of the *upper* GIT (**Figures 4 and 5**).

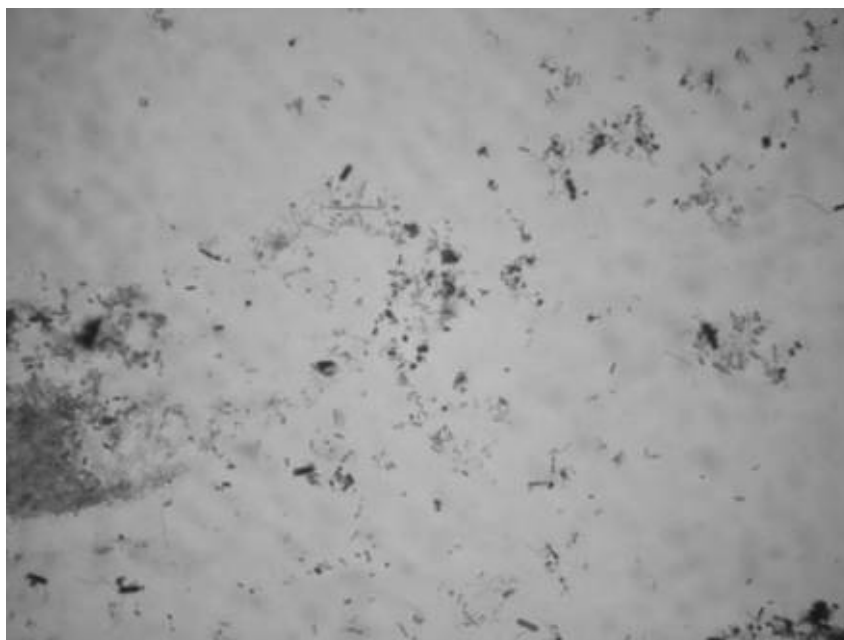
The fact that the investigated polymeric films serve as substrates for the bacteria in feces from inflammatory bowel disease patients could be further confirmed by the analysis of the microflora developed upon film exposure to fecal samples under anaerobic conditions at 37 °C (**Figures 12-15**). Clearly, specific types of bacteria proliferated upon incubation with the blended films. Importantly, this phenomenon can be expected to be highly beneficial for the ecosystem of the GIT of the patients in the disease state, normalizing the microflora in the colon. This very positive, pre-biotic effect comes in addition to the drug targeting effect. Biological samples incubated without any polymeric films or with pure (plasticized) ethylcellulose films showed much less bacterial growth (**Figures 10 and 11**).

#### **No film**



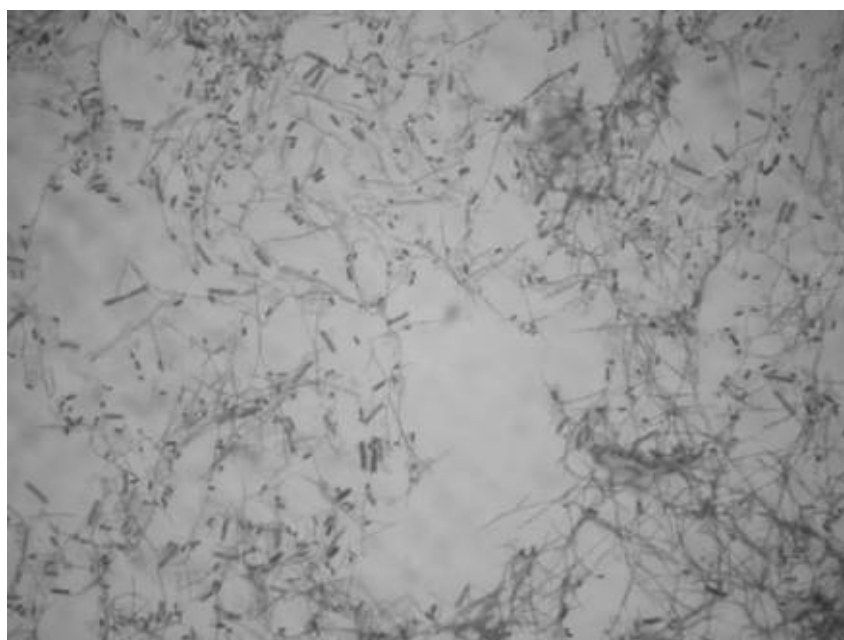
**Figure 10:** *Picture of the microflora developed upon incubation without thin, polymeric film with fecal samples of inflammatory bowel disease patients.*

### Ethylcellulose



*Figure 11: Picture of the microflora developed upon incubation of thin, polymeric film of ethylcellulose with fecal samples of inflammatory bowel disease patients.*

### Nutriose:ethylcellulose

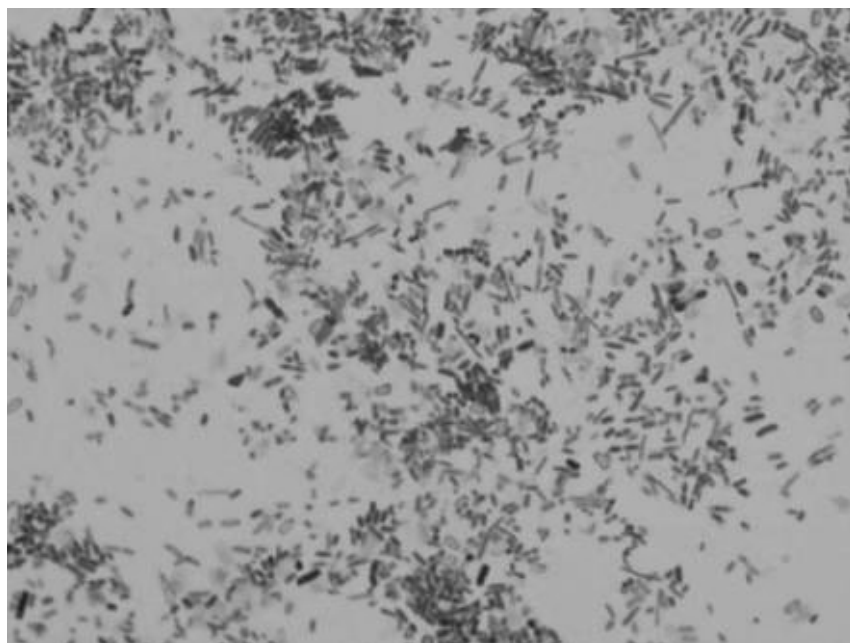


*Figure 12: Picture of the microflora developed upon incubation of thin, polymeric film of Nutriose composition (indicated in the figure) with fecal samples of inflammatory bowel disease patients.*

**Peas starch:ethylcellulose**

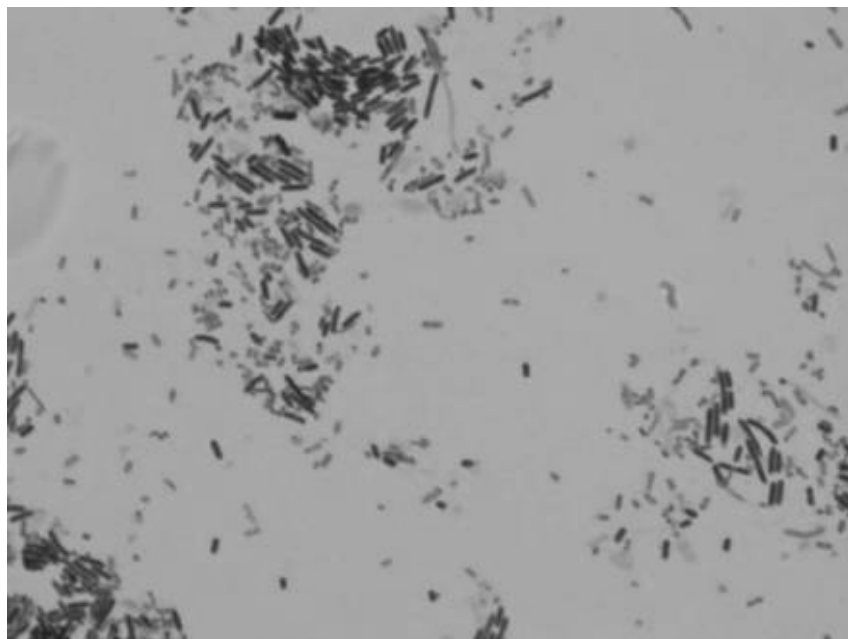


**Lycoat:ethylcellulose**

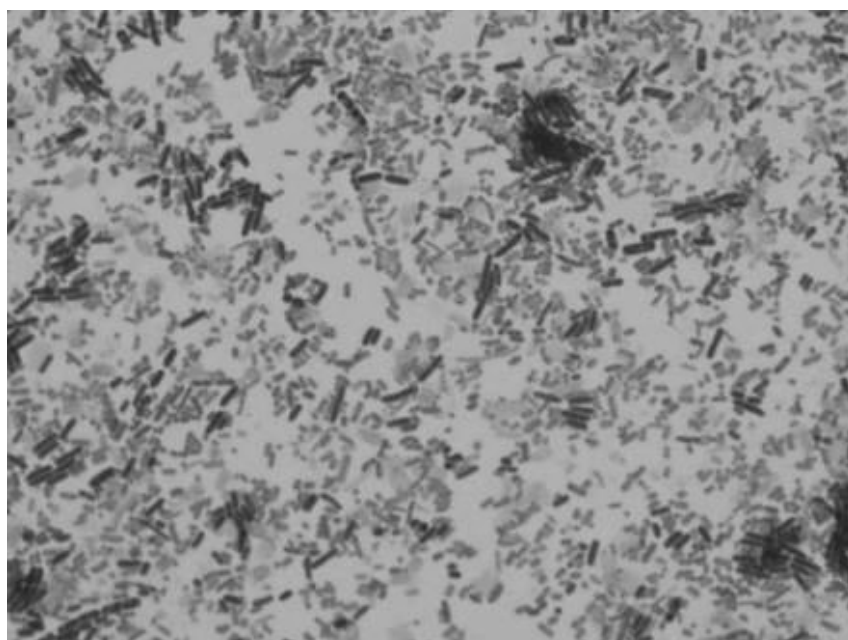


**Figure 13:** Pictures of the microflora developed upon incubation of thin, polymeric films of different composition (indicated in the figure) with fecal samples of inflammatory bowel disease patients.

**Glucidex:ethylcellulose**

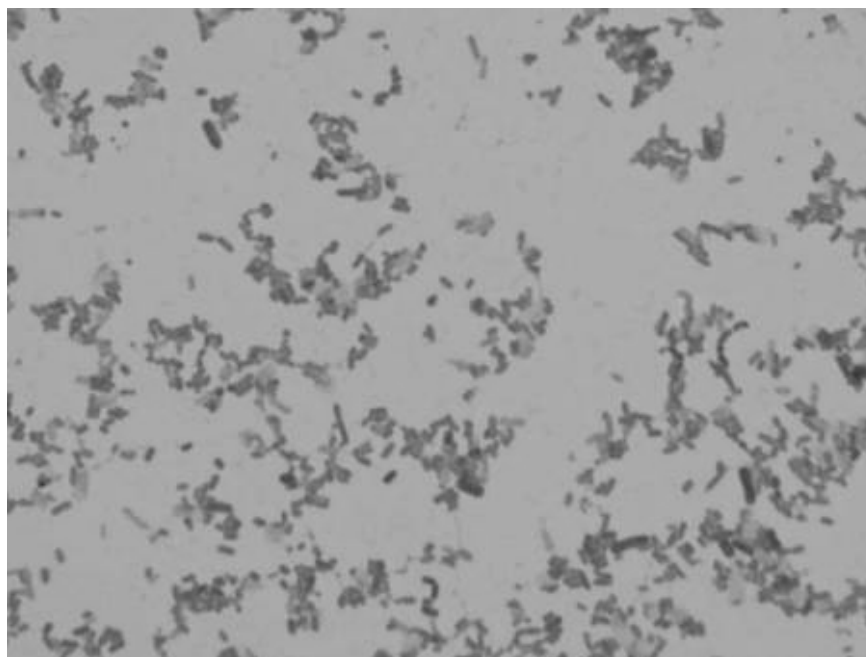


**Eurylon 7 A-PG:ethylcellulose**

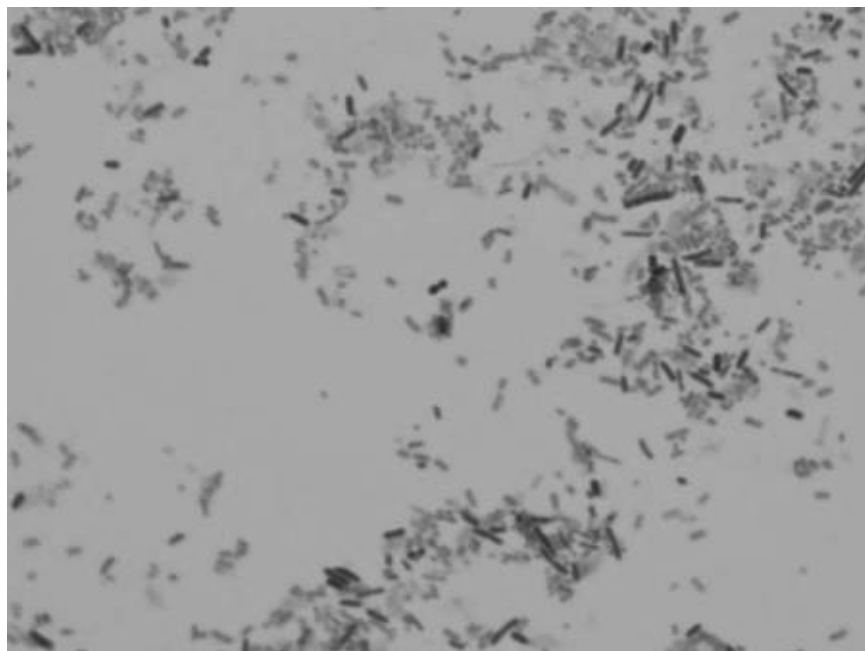


**Figure 14:** Pictures of the microflora developed upon incubation of thin, polymeric films of different composition (indicated in the figure) with fecal samples of inflammatory bowel disease patients.

**Eurylon 6 A-PG:ethylcellulose**



**Eurylon 6 HP-PG:ethylcellulose**



**Figure 15:** Pictures of the microflora developed upon incubation of thin, polymeric films of different composition (indicated in the figure) with fecal samples of inflammatory bowel disease patients.

### **3.1.3 Conclusions**

Novel polymeric film coatings for colon targeting have been identified, which are adapted to the disease state of the patients. Importantly, low water uptake and dry mass loss *rates* and *extents* in media simulating the contents of the *upper* GIT can be combined with elevated water uptake and dry weight loss upon contact with feces from inflammatory bowel disease patients. Changes in the composition of the flora in the colon of patients indicate that these polysaccharides serve as substrates for colonic bacteria in the *disease state* and are likely to exhibit beneficial effects on the ecosystem of the GIT of the patients. The obtained new knowledge, thus, provides the basis for the development of novel polymeric film coatings able to deliver drugs specifically to the colon. Importantly, these polymeric barriers are adapted to the conditions at the target site in the disease state.



## 3.2. EFFECTS OF THE POLYMER BLEND RATIO (THIN FILMS)

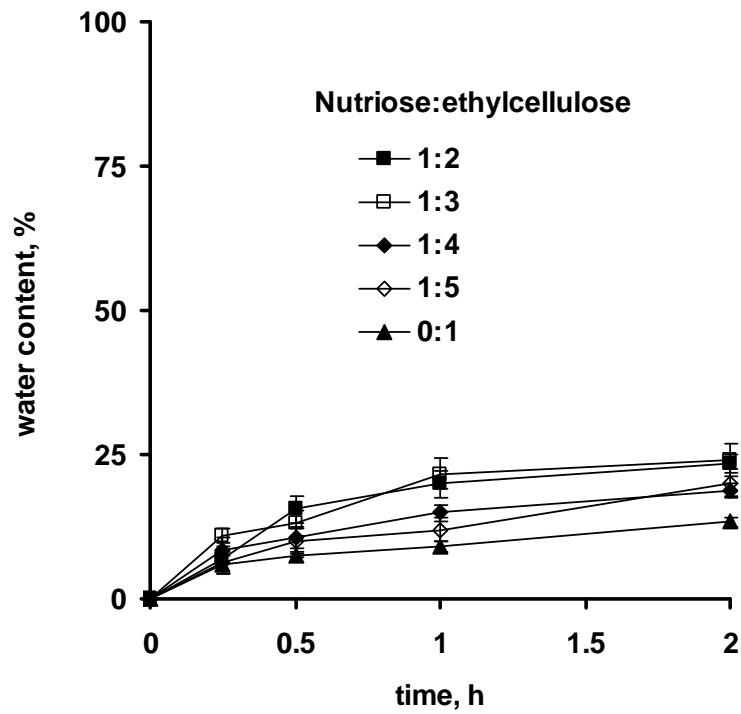
### 3.2.1. Water uptake and dry mass loss of thin films

The permeability of a polymeric film coating strongly depends on its water content (Siepmann and Peppas, 2000). In a dry system, the diffusion coefficients approach zero. With increasing water content, the mobility of the macromolecules increases and, thus, also the mobility of incorporated drug molecules. **Figures 16a and 16b** show the gravimetrically measured water uptake of thin, polymeric films based on different Nutriose:ethylcellulose blends upon exposure to 0.1 M HCl and phosphate buffer pH 6.8 at 37 °C. Clearly, the polymer blend ratio significantly affected the resulting water penetration *rates* and *extents*. With increasing Nutriose content the amount of water taken up as well as the rate of this mass transport step increased. This phenomenon can be attributed to the more hydrophobic nature of ethylcellulose compared to the water-soluble starch derivative Nutriose. Thus, it can be expected that the mobility of a drug within this type of polymeric films significantly increases with increasing Nutriose contents. Interestingly, the water uptake rates and extents of the investigated films were higher in phosphate buffer pH 6.8 than in 0.1 N HCl (**Figure 16b versus Figure 16a**). This can be attributed to the presence of the emulsifier sodium dodecyl sulfate (SDS) in the aqueous ethylcellulose dispersion Aquacoat ECD. At low pH, SDS is protonated and neutral, whereas at pH 6.8 it is de-protonated and negatively charged. Thus, the ability to decrease interfacial surface tensions is more pronounced at pH 6.8, resulting in facilitated water penetration into the system. Importantly, even the highest water uptake rates and extents of the investigated systems (up to a blend ratio of 1:2 Nutriose:ethylcellulose) are relatively low (**Figure 16**). Thus, premature drug release within the upper GIT can be expected to be limited with this type of polymeric films, irrespective of the polymer:polymer blend ratio in the investigated range.

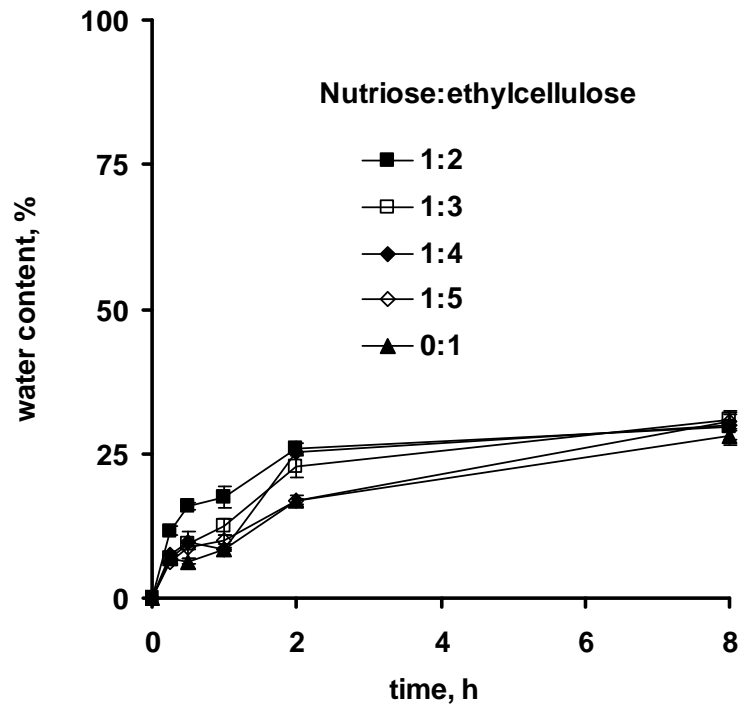
In addition to the water uptake kinetics, also the *dry mass loss* behavior of thin polymeric films offers important insight into the latter's ability to suppress or allow drug release. The effects of the Nutriose:ethylcellulose blend ratio on the resulting dry mass loss of thin films upon exposure to 0.1 M HCl and phosphate buffer pH 6.8 are illustrated in **Figures 17a and 17b**, respectively. Clearly, both, the rate and the extent of the dry mass loss increased with increasing Nutriose contents. This can at least partially be attributed to the leaching of this water-soluble compound out into the bulk fluids. However, also the diffusion of the water-soluble plasticizer TEC (which is used to facilitate the fusion of the ethylcellulose

nanoparticles during film formation) into the release media can be expected to be significantly facilitated: Due to the increasing water contents of the systems (**Figure 16**), the mobility of the polymer chains increases and, thus, also the mobility of the low molecular weight plasticizer. Please note that the dry mass loss of pure (plasticized) ethylcellulose films can primarily be attributed to such TEC leaching and that a (slight) pH dependence of this phenomenon is observed (due to the SDS effect discussed above). Importantly, the dry mass loss is limited in all cases, and the presence of the water-insoluble ethylcellulose in the films effectively hinders the leaching of the water-soluble starch derivative into the bulk fluids. Again, premature drug release within the *upper* parts of the GIT is likely to be limited, irrespective of the polymer:polymer blend ratio in the investigated range (up to 1:2 Nutriose:ethylcellulose).

a)

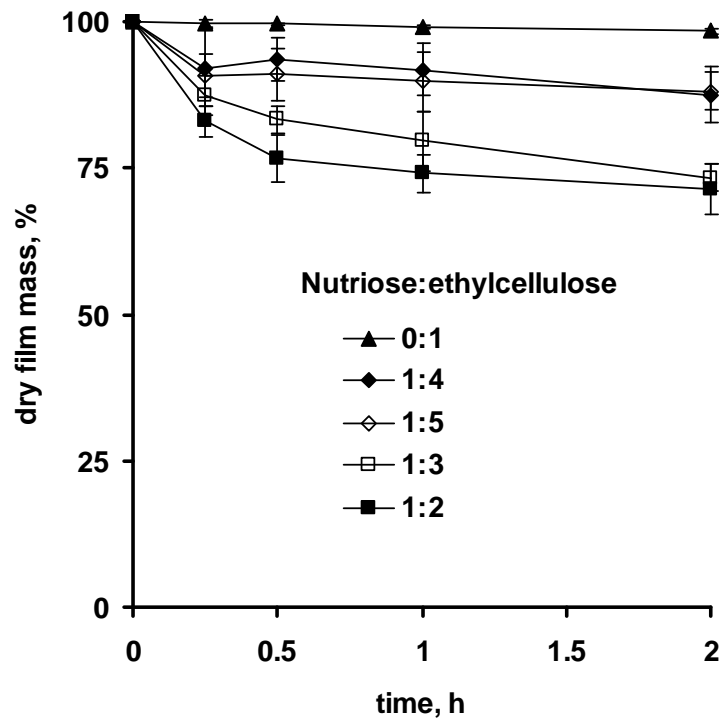


b)

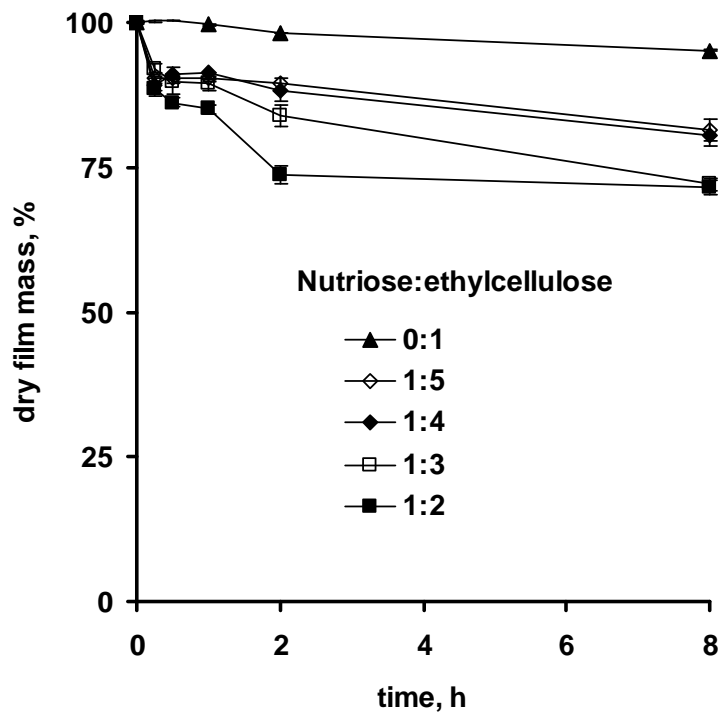


**Figure 16:** Water uptake of thin films consisting of Nutriose:ethylcellulose blends (the ratio is indicated in the figures) upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8 (TEC content, referred to the ethylcellulose mass: 25 % w/w).

a)



b)



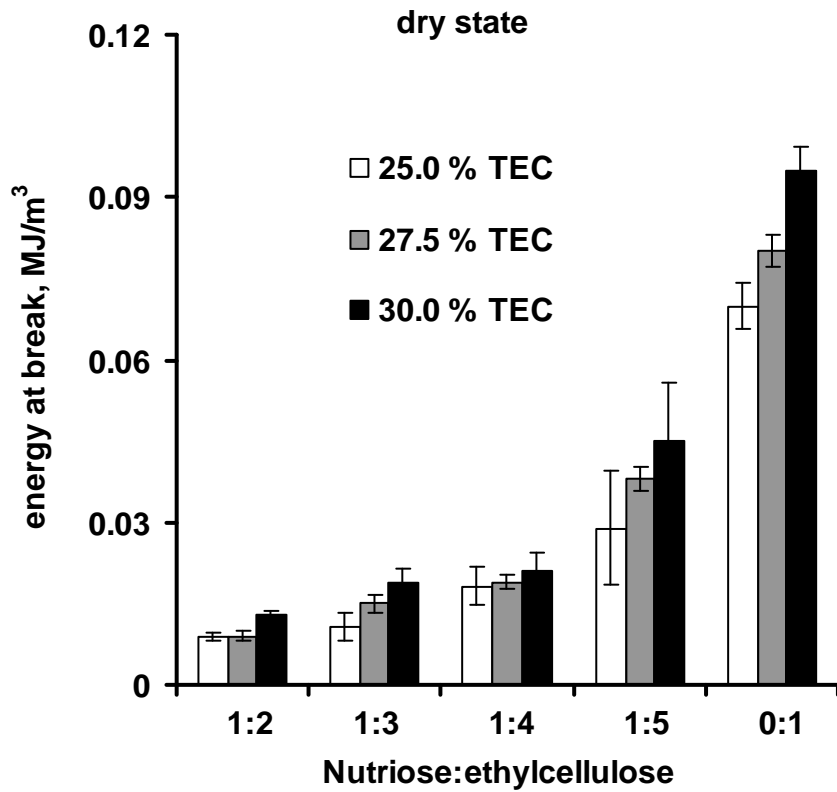
**Figure 17:** Dry mass loss of thin films consisting of Nutriose:ethylcellulose (the ratio is indicated in the figures) upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8 (TEC content, referred to the ethylcellulose mass: 25 % w/w).

### 3.2.2. Mechanical properties of thin films

In addition to limited water uptake and dry mass loss in the upper GIT, a polymeric film coating providing site-specific drug delivery to the colon must be sufficiently (mechanically) stable in order to avoid accidental crack formation due to the shear stress encountered in the stomach and small intestine *in vivo*. In addition, significant hydrostatic pressure might be built up within a coated dosage form due to the penetration of water into the system upon contact with aqueous body fluids. The presence/absence of osmotically active drugs and/or excipients in the core formulation can strongly affect the importance of this phenomenon. Fragile film coatings are likely to rupture because of such shear forces from *outside* (caused by the motility of the GIT) and hydrostatic pressures from *inside* (caused by water penetration) they are exposed to. In order to be able to estimate the risk of such accidental crack formation, the energy required to break the investigated Nutriose:ethylcellulose films was measured using a texture analyzer and the puncture test before and upon exposure to 0.1 N HCl and phosphate buffer pH 6.8, respectively. The white bars in **Figure 18** indicate the mechanical stability of thin Nutriose:ethylcellulose films (plasticized with 25 % w/w TEC, referred to the ethylcellulose content) in the *dry* state at room temperature as a function of the polymer blend ratio. Clearly, the energy at break of the films significantly increased with increasing ethylcellulose content, indicating that this compound mainly contributes to the mechanical stability of the system under these conditions. Importantly, all the investigated films showed a mechanical stability that is likely to be sufficient to withstand the shear stress and hydrostatic pressure they are exposed to within the *upper* GIT at appropriate coating levels. This was confirmed by the experimentally determined puncture strength and % elongation at break of the films (data not shown). However, it must be pointed out that the penetration of water into the polymeric systems significantly changes the composition of the films (**Figures 16 and 17**) and, thus, their mechanical properties. In particular the fact that water acts as a plasticizer for many polymers and that the water-soluble TEC and starch derivative (at least partially) leach out of the polymeric networks can be expected to lead to time-dependent changes in the mechanical stability of the films. In addition, the results shown in **Figure 18** were obtained at room temperature, and not at 37 °C body temperature. It is well known that the temperature of a polymeric network can strongly affect its mechanical properties, e.g. due to glassy-to-rubbery phase transitions.

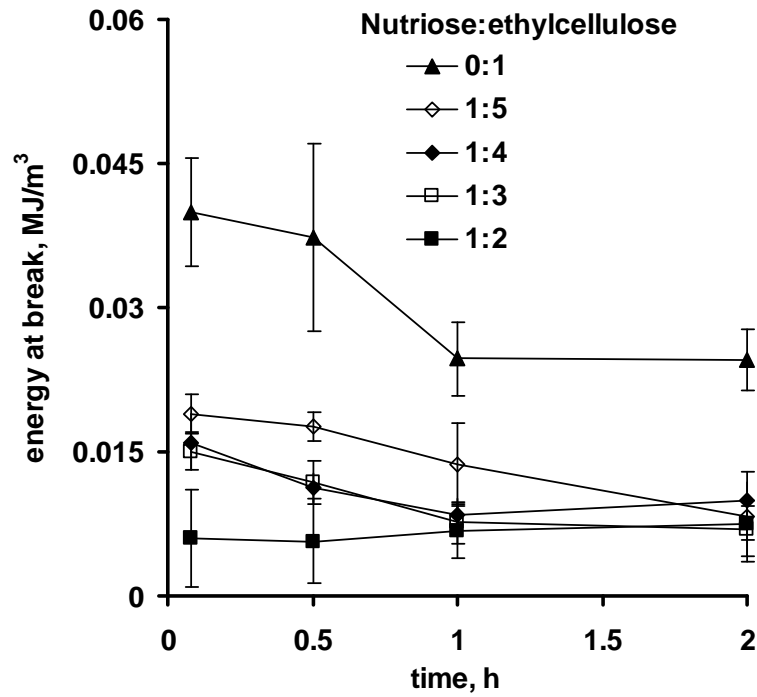
For these reasons the energy required to break the investigated Nutriose:ethylcellulose films was also measured upon exposure to 0.1 N HCl for up to 2 h and upon exposure to

phosphate buffer pH 6.8 for up to 8 h at 37 °C (**Figure 19**). As it can be seen, the mechanical stability of the polymeric networks decreased with time, irrespective of the polymer blend ratio and type of release medium. This can at least partially be attributed to the leaching of the water-soluble plasticizer TEC and of the starch derivative into the bulk fluids. Importantly, even the lowest observed values indicate that accidental crack formation due to external shear stress and/or internal hydrostatic pressure encountered *in vivo* is unlikely (at appropriate coating levels). Again, this was consistent with the experimentally determined puncture strength and % elongation of the films, irrespective of the polymer blend ratio, exposure time and type of release medium (data not shown).

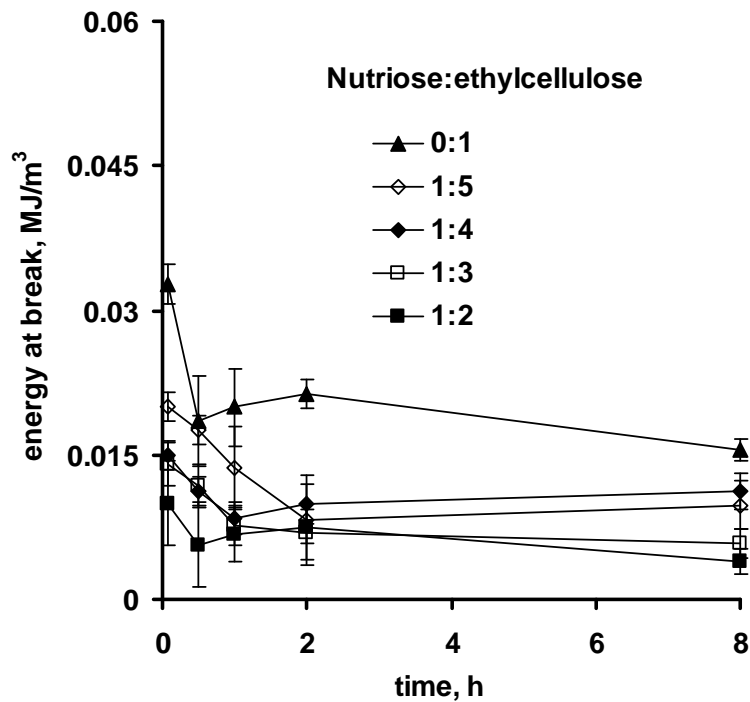


**Figure 18:** Effects of the Nutriose:ethylcellulose blend ratio and initial plasticizer content on the energy required to break thin, polymeric films in the dry state at room temperature.

a)



b)



**Figure 19:** Changes in the energy required to break thin Nutriose:ethylcellulose films (the blend ratio is indicated in the figures) upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8 at 37 °C (TEC content, referred to the ethylcellulose mass: 25 % w/w).

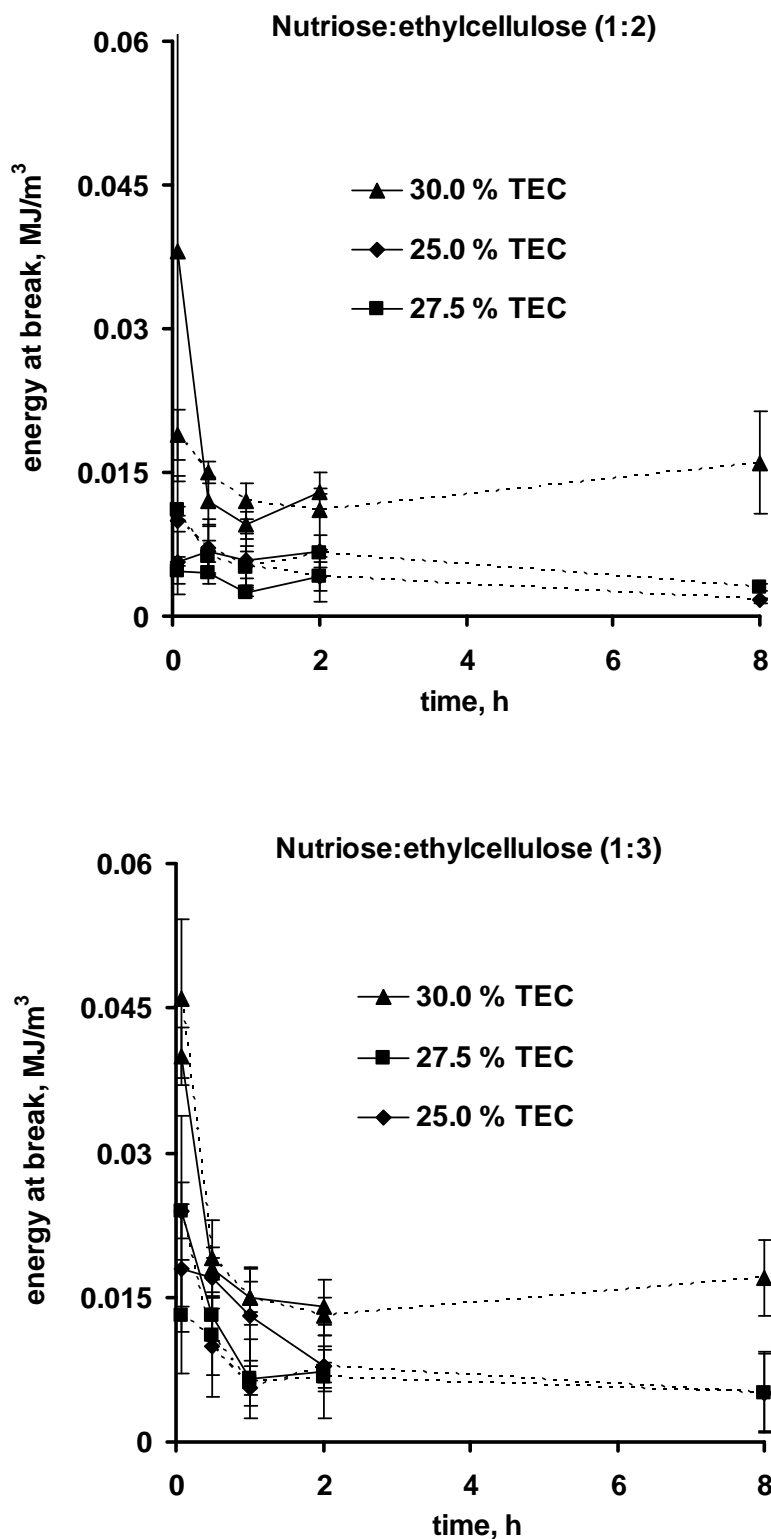


### 3.2.3. Effects of the plasticizer content

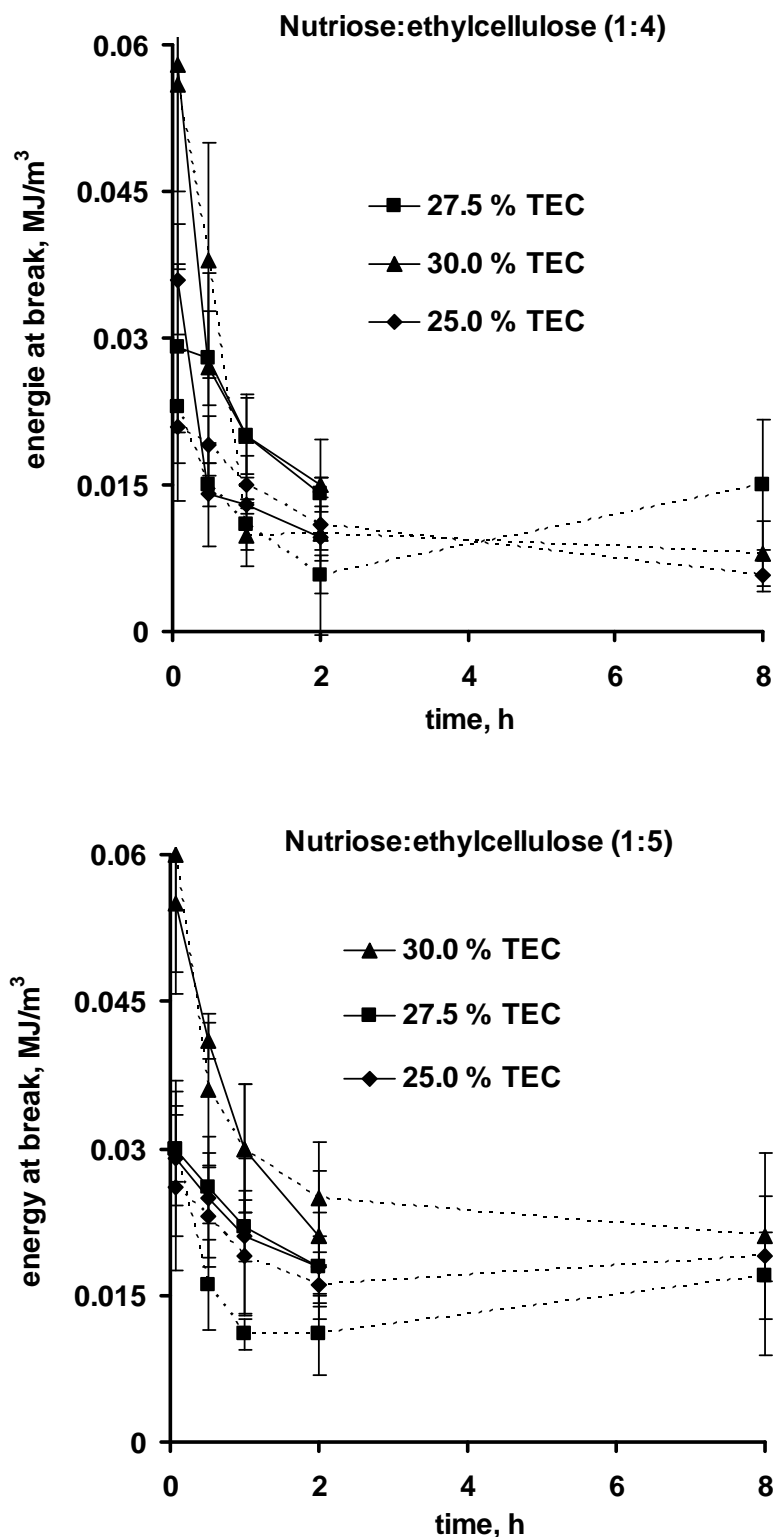
It is well known that the plasticizer content can significantly affect the mechanical properties of polymeric films. In order to evaluate the importance of this phenomenon for the investigated Nutriose:ethylcellulose blends, the percentage of incorporated TEC was increased from 25 to 30 % w/w (referred to the ethylcellulose content). TEC contents below 25 % w/w would render the fusion of the ethylcellulose nanoparticles during film formation difficult, the mobility of the polymer chains being crucial for this step. TEC contents higher than 30 % w/w significantly increase the sticking tendency during coating and curing and should, thus, be avoided. As it can be seen in **Figure 18**, the mechanical stability of the Nutriose:ethylcellulose films significantly increased with increasing TEC content, irrespective of the polymer blend ratio. This was consistent with the experimentally determined puncture strength and % elongation of the films (data not shown). Thus, in case of osmotically highly active core formulations (resulting in significant hydrostatic pressure built up within the dosage forms upon water penetration), the required coating levels (avoiding accidental crack formation) can be decreased by increasing the TEC content. Again, it was important to monitor the effects of the time-dependent changes in the composition of the polymeric networks upon exposure to 0.1 N HCl and phosphate buffer pH 6.8 as well as of the increase in temperature to 37 °C. As it can be seen in **Figures 20 and 21**, the energy required to break the films decreased upon exposure to the release media for the reasons discussed above, irrespective of the polymer blend ratio, initial plasticizer content and type of release medium. Importantly, in all cases an increase in the initial TEC content from 25 to 30 % w/w (referred to the ethylcellulose content) led to increased mechanical stability at all time points.

However, when increasing the percentage of the water-soluble plasticizer TEC in the polymeric films, also the *rates and extents* of the systems' *water uptake* and *dry mass loss* upon exposure to aqueous media can be expected to increase. This might potentially lead to significantly increased drug permeability of the polymeric films, resulting in potential premature drug release within the *upper GIT*. To estimate the importance of these phenomena, the water uptake and dry mass loss kinetics of the investigated films were monitored upon exposure to 0.1 N HCl for 2 h and upon exposure to phosphate buffer pH 6.8 for 8 h. The highest TEC content (30 %) was selected as well as the two most critical Nutriose:ethylcellulose blend ratios: 1:2 and 1:3 (**Figure 22 and 23**). Importantly, the resulting changes in the water uptake and dry mass loss kinetics were only minor when increasing the initial TEC content from 25 to 30 %, irrespective of the polymer blend ratio

and type of release medium. Thus, the mechanical stability of Nurtiose:ethylcellulose films can efficiently be improved by increasing the plasticizer level, without losing the systems' capability to suppress drug release within the *upper* GIT.

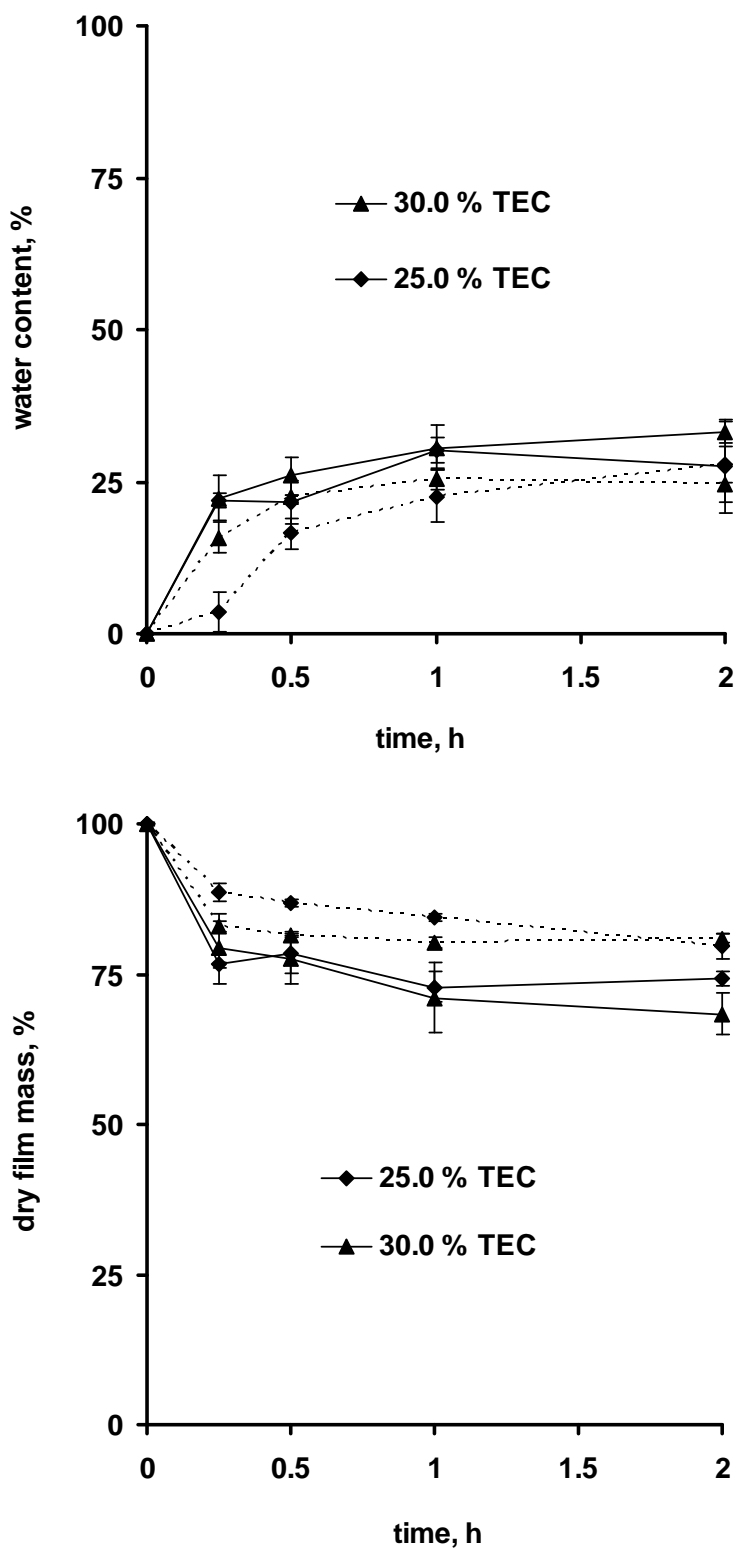


**Figure 20:** Changes in the energy required to break thin films consisting of Nutriose:ethylcellulose (the blend ratio is indicated on the top of figures) plasticized with different amounts of TEC (the percentages refer to the ethylcellulose mass) upon exposure to 0.1 M HCl for 2 h (solid curves) and phosphate buffer pH 6.8 for 8 h at 37 °C (dotted curves).



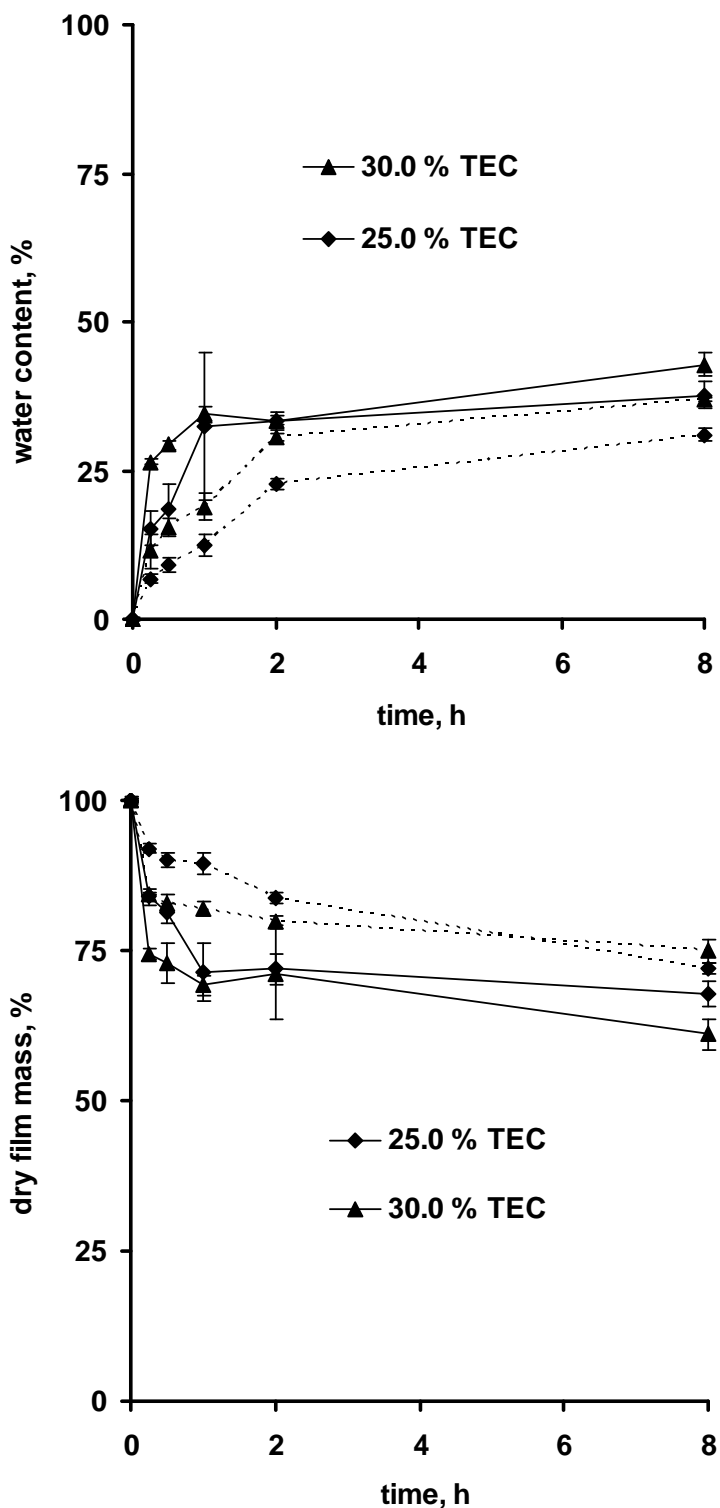
**Figure 21:** Changes in the energy required to break thin films consisting of Nutriose:ethylcellulose (the blend ratio is indicated on the top of figures) plasticized with different amounts of TEC (the percentages refer to the ethylcellulose mass) upon exposure to 0.1 M HCl for 2 h (solid curves) and phosphate buffer pH 6.8 for 8 h at 37 °C (dotted curves).

0.1 M HCl



**Figure 22:** Effects of the plasticizer content (indicated in the figures, referred to the ethylcellulose mass) on the water uptake and dry mass loss of Nutriose:ethylcellulose films upon exposure to 0.1 M HCl. The solid and dotted curves represent results obtained at the blend ratios 1:2 and 1:3, respectively.

## Phosphate buffer pH 6.8



**Figure 23:** Effects of the plasticizer content (indicated in the figures, referred to the ethylcellulose mass) on the water uptake and dry mass loss of Nutriose:ethylcellulose films upon exposure to phosphate buffer pH 6.8. The solid and dotted curves represent results obtained at the blend ratios 1:2 and 1:3, respectively.

#### **3.2.4. Conclusions**

Nutriose:ethylcellulose blends are highly promising film coating materials for advanced drug delivery systems allowing for colon targeting. Importantly, desired system properties, being adapted to the specific needs of a particular treatment (e.g., osmotic activity and dose of the drug) can easily be adjusted by varying the polymer:polymer blend ratio as well as the plasticizer content.

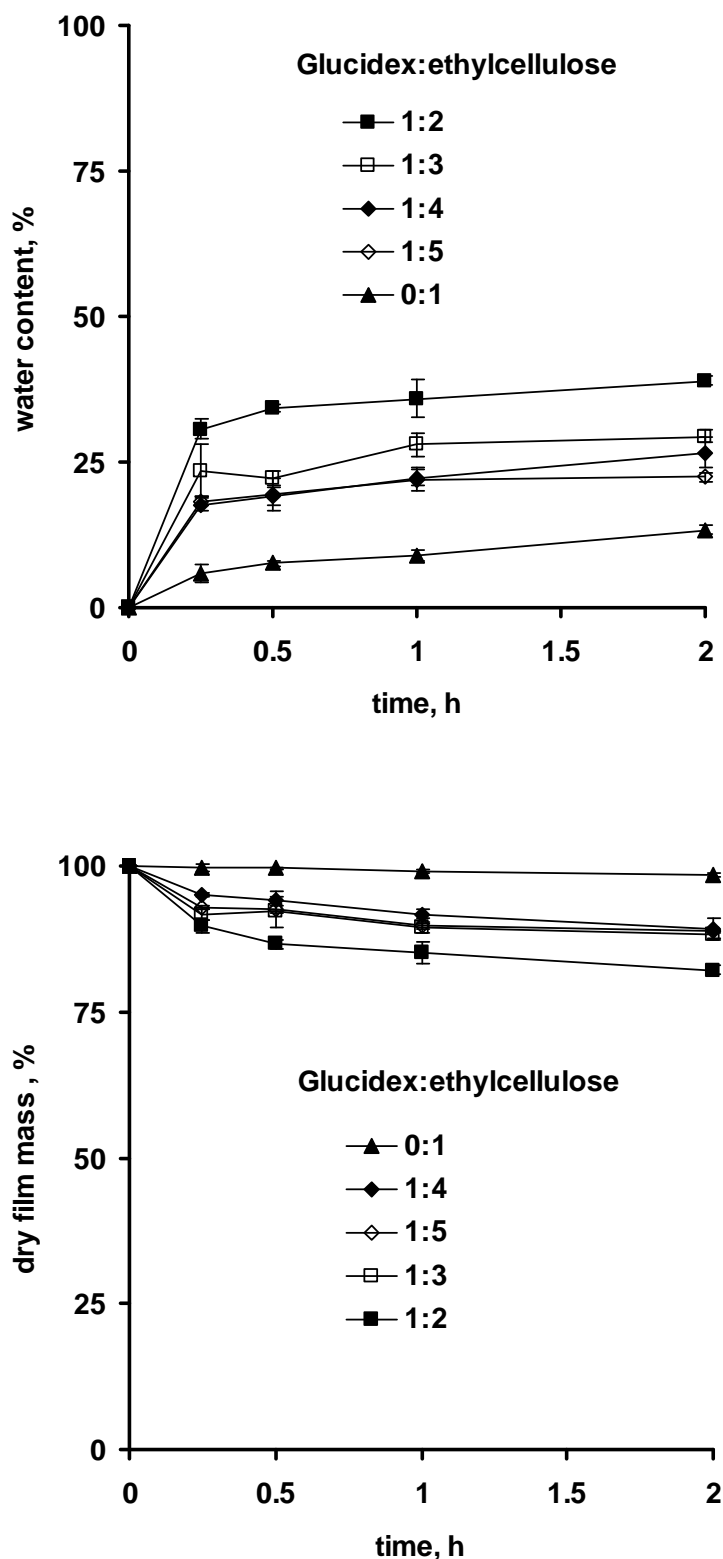
### 3.3. EFFECTS OF THE TYPE OF POLYMER BLEND

#### 3.3.1. Glucidex:ethylcellulose blends

**Figure 24 and 25** show the effects of the composition of Glucidex:ethylcellulose films on the resulting water uptake kinetics and dry mass loss behavior upon exposure to 0.1 M HCl and phosphate buffer pH 6.8, respectively. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown. Clearly, the water uptake rates and extents significantly increased when increasing the Glucidex:ethylcellulose blend ratio from 1:5 to 1:2. This can be attributed to the fact that Glucidex is a maltodextrin and much more hydrophilic than ethylcellulose. At high initial Glucidex contents the water content became significant, e.g. about half of the films consisted of water in the case of 1:2 blends after 1 h exposure to phosphate buffer pH 6.8. This can be expected to render an efficient suppression of the release of freely water-soluble, low molecular weight drugs in the *upper* GIT challenging, because the mobility of the macromolecules significantly increases with increasing water content, resulting in increasing drug mobility. Elevated coating levels are likely to be required. However, the permeability for *larger* drug molecules (e.g., proteins) can be low in polymeric networks, even at elevated water contents. In this case the mobility of the drug essentially depends on the ratio “drug molecule size:average mesh-size of macromolecular network”. Advanced drug delivery systems with site specific delivery to the colon might for instance be attractive to allow for the systemic delivery of proteins after oral administration: If the proteins are effectively protected against the low pH and enzymatic degradation in the *upper* GIT, they might get absorbed upon release in the colon. Furthermore, the relative release rate of a *poorly water-soluble drug* might be very low, even if the film coating contains significant amounts of water, as long as the dosage form remains

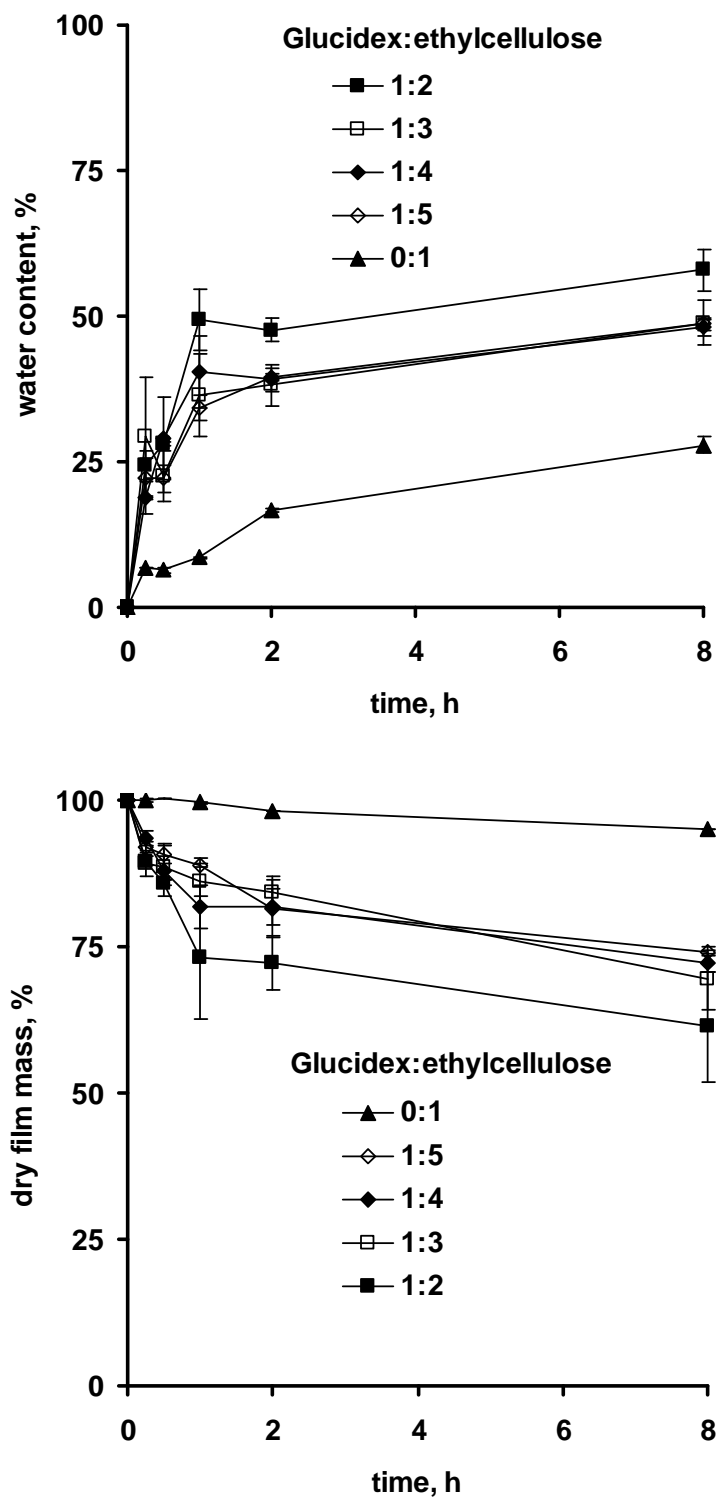


0.1 M HCl



**Figure 24:** Water uptake and dry mass loss of thin films consisting of Glucidex:ethylcellulose blends upon exposure to 0.1 M HCl. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

## Phosphate buffer pH 6.8



**Figure 25:** Water uptake and dry mass loss of thin films consisting of Glucidex:ethylcellulose blends upon exposure to phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

intact.

Interestingly, both, the water uptake *rates* and *extents* were higher in phosphate buffer pH 6.8 than in 0.1 M HCl, irrespective of the polymer blend ratio (**Figure 24 and 25**, top row). This can be attributed to the presence of sodium dodecyl sulfate (SDS) in the aqueous ethylcellulose dispersion (acting as a stabilizer) used for film preparation. At low pH, this emulsifier is protonated and neutral, whereas at pH 6.8 it is deprotonated and negatively charged. Thus, its ability to decrease interfacial tensions is increased, facilitating water penetration into the polymeric networks.

Furthermore, the rates and extents of the *dry films' mass loss* significantly increased with increasing Glucidex content (**Figure 24 and 25**, bottom row). This can at least partially be explained by the leaching of this water-soluble maltodextrin into the bulk fluids. However, also the (partial) leaching of the water-soluble plasticizer TEC into the release media is responsible for this phenomenon. TEC is required for the plasticization of the ethylcellulose nanoparticles to allow for the film formation from aqueous dispersions. Even Glucidex free films lose some dry mass, in particular at pH 6.8. The considerable water contents of the polymeric systems containing high initial Glucidex contents can be expected to facilitate the leaching of the low molecular weight, water-soluble plasticizer TEC. Again, the observed effects were more pronounced upon exposure to phosphate buffer pH 6.8 than to 0.1 M HCl (**Figure 24 and 25**), because of the presence of SDS.

In addition to appropriate water uptake and dry mass loss kinetics, polymeric film coatings which are intended to allow for site specific drug delivery to the colon must also provide sufficient *mechanical stability* in order to withstand the various mechanical stresses encountered *in vivo*. This concerns in particular: (i) the shear forces resulting from the motility of the *upper* GIT, and (ii) the hydrostatic pressure acting against the film coating from the core of the dosage form, caused by the osmotically driven water influx into the system upon contact with aqueous body fluids. In order to estimate the capacity of the investigated Glucidex:ethylcellulose blends to withstand such external and internal stresses, the mechanical properties of thin films were measured with a texture analyzer and the puncture test. The puncture strength, % elongation at break as well as the energy required to break the films in the dry state at room temperature are shown in **Table 5**. Clearly, the mechanical stability of the systems increased with increasing ethylcellulose content. Thus, the latter compound is the stabilizing agent in these polymeric networks.

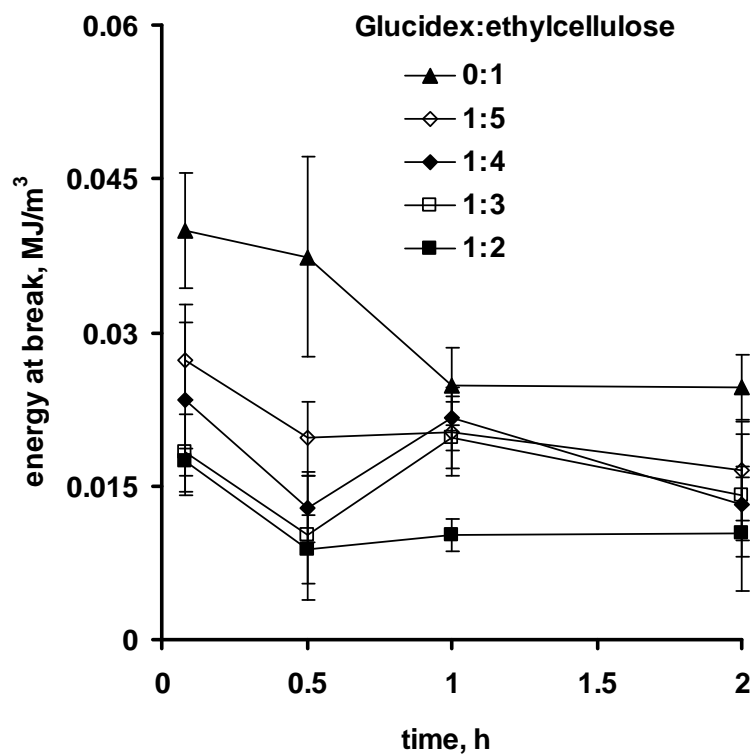
It has to be pointed out that the mechanical properties shown in **Table 5** were obtained with *dry* films at *room temperature*. It is well known that water acts as a plasticizer

**Table 5:** Effects of the type of starch derivative blended with ethylcellulose and of the starch derivative:ethylcellulose blend ratio on the mechanical properties of thin films in the dry state at room temperature.

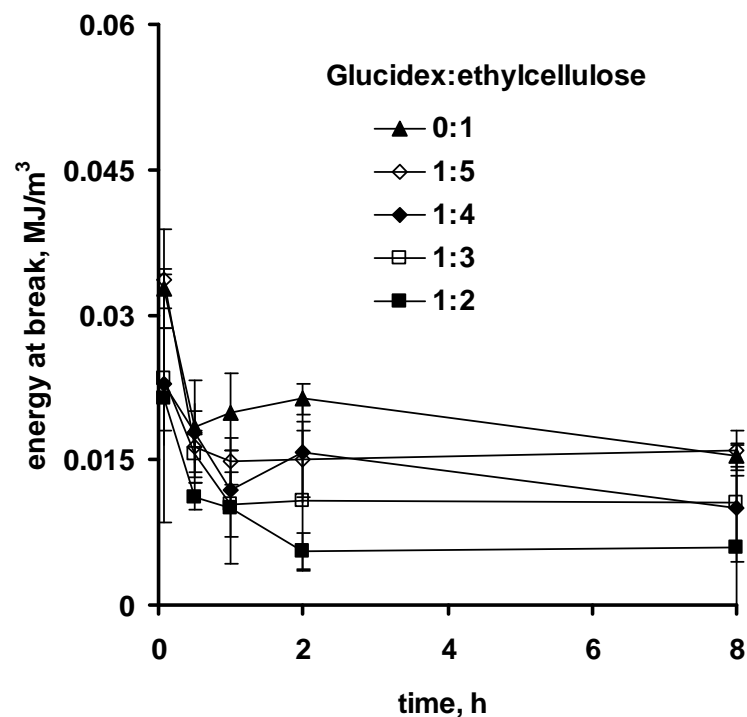
	Blend ratio	Puncture strength $\pm$ (s), MPa	Elongation at break $\pm$ (s), %	Energy at break $\pm$ (s), MJ/m <sup>3</sup>
Glucidex	1:2	0.34 $\pm$ (0.05)	0.43 $\pm$ (0.08)	0.012 $\pm$ (0.005)
	1:3	0.36 $\pm$ (0.09)	0.57 $\pm$ (0.05)	0.014 $\pm$ (0.006)
	1:4	0.43 $\pm$ (0.07)	0.53 $\pm$ (0.04)	0.011 $\pm$ (0.003)
	1:5	0.42 $\pm$ (0.11)	0.58 $\pm$ (0.07)	0.015 $\pm$ (0.009)
Lycoat	1:2	0.45 $\pm$ (0.04)	0.55 $\pm$ (0.09)	0.016 $\pm$ (0.008)
	1:3	0.40 $\pm$ (0.03)	0.53 $\pm$ (0.07)	0.012 $\pm$ (0.007)
	1:4	0.42 $\pm$ (0.09)	0.60 $\pm$ (0.09)	0.016 $\pm$ (0.008)
	1:5	0.50 $\pm$ (0.08)	0.60 $\pm$ (0.05)	0.020 $\pm$ (0.004)
Eurylon 7 A PG	1:2	0.78 $\pm$ (0.09)	0.63 $\pm$ (0.02)	0.061 $\pm$ (0.005)
	1:3	0.84 $\pm$ (0.05)	0.67 $\pm$ (0.08)	0.065 $\pm$ (0.009)
	1:4	0.85 $\pm$ (0.04)	0.66 $\pm$ (0.07)	0.070 $\pm$ (0.011)
	1:5	0.87 $\pm$ (0.05)	0.75 $\pm$ (0.02)	0.073 $\pm$ (0.006)
LAB 3874 6 A PG	1:2	0.60 $\pm$ (0.01)	0.50 $\pm$ (0.07)	0.052 $\pm$ (0.002)
	1:3	0.52 $\pm$ (0.05)	0.75 $\pm$ (0.10)	0.068 $\pm$ (0.008)
	1:4	0.76 $\pm$ (0.02)	0.82 $\pm$ (0.04)	0.077 $\pm$ (0.006)
	1:5	0.77 $\pm$ (0.03)	0.81 $\pm$ (0.06)	0.075 $\pm$ (0.010)
LAB 3877 6 HP PG	1:2	0.53 $\pm$ (0.07)	0.72 $\pm$ (0.05)	0.053 $\pm$ (0.010)
	1:3	0.64 $\pm$ (0.03)	0.81 $\pm$ (0.07)	0.066 $\pm$ (0.009)
	1:4	0.63 $\pm$ (0.02)	0.82 $\pm$ (0.07)	0.062 $\pm$ (0.009)
	1:5	0.87 $\pm$ (0.03)	0.77 $\pm$ (0.05)	0.070 $\pm$ (0.010)

for many polymers and as it can be seen in **Figure 24 and 25**, significant amounts of water penetrate into the films upon exposure to 0.1 M HCl and phosphate buffer pH 6.8. Furthermore, the composition of the polymeric systems significantly changes upon contact with the release media, due to (partial) Glucidex and TEC leaching. In addition, the mechanical resistance of the polymeric films might significantly depend on the temperature. Polymers can for instance undergo glassy-to-rubbery phase transitions when increasing the temperature to 37 °C. For these reasons, the mechanical properties of the investigated Glucidex:ethylcellulose blends were also determined upon up to 2 h exposure to 0.1 M HCl and for up to 8 h exposure to phosphate buffer pH 6.8. As it can be seen in **Figure 26**, the mechanical stability of the polymeric films decreased with time due to partial Glucidex and TEC leaching, irrespective of the polymer blend ratio and type of release medium. Importantly, appropriate mechanical stabilities can effectively be adjusted by varying the polymer:polymer blend ratio (and eventually by varying the coating thickness).

a)



b)

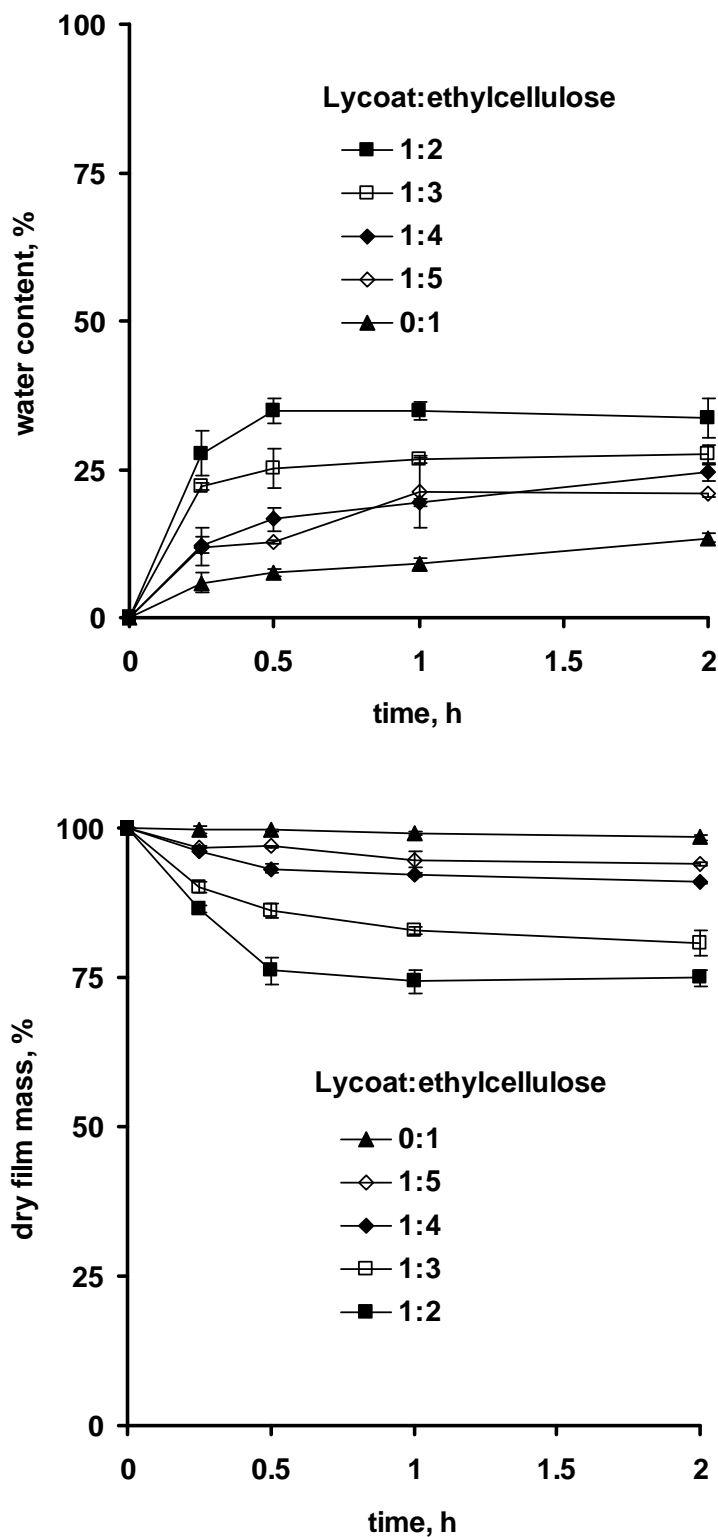


**Figure 26:** Changes in the energy at break of thin Glucidex:ethylcellulose films upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown.

### 3.3.2. Lycoat:ethylcellulose blends

**Figure 27 and 28** show the gravimetrically determined water uptake and dry mass loss kinetics of thin films consisting of different types of Lycoat:ethylcellulose blends upon exposure to 0.1 M HCl and phosphate buffer pH 6.8, respectively. Lycoat is a pregelatinized modified starch. As in the case of Glucidex, the resulting extent and rate of the water penetration into the systems significantly increased when increasing the starch derivative:ethylcellulose ratio from 1:5 to 1:2 (**Figure 24 and 25, top row**). This can again be attributed to the higher hydrophilicity of the starch derivative compared to ethylcellulose. Appropriately elevated coating levels are likely to be required to suppress the premature release of freely water-soluble, small molecular weight drugs in the *upper* GIT at high initial Lycoat contents. Also the rate and extent of the films' *dry mass loss* significantly increased with increasing Lycoat contents, due to partial TEC and starch derivative leaching. In all cases, the rates and extents of the water penetration and dry mass loss were higher in phosphate buffer pH 6.8 compared to 0.1 M HCl, because of the pH-dependent ionization of SDS as discussed above. As in the case of Glucidex:ethylcellulose blends, the mechanical stability of Lycoat:ethylcellulose films could effectively be adjusted by varying the initial ethylcellulose content. This was true for the puncture strength, % elongation at break and energy at break in the dry state at room temperature (**Table 5**) as well as for the mechanical resistance in the wet state upon exposure to 0.1 M HCl and phosphate buffer pH 6.8 (**Figure 29**). The decrease in the energy at break with time can again be attributed to partial plasticizer and starch derivative leaching into the bulk fluids, irrespective of the type of release medium.

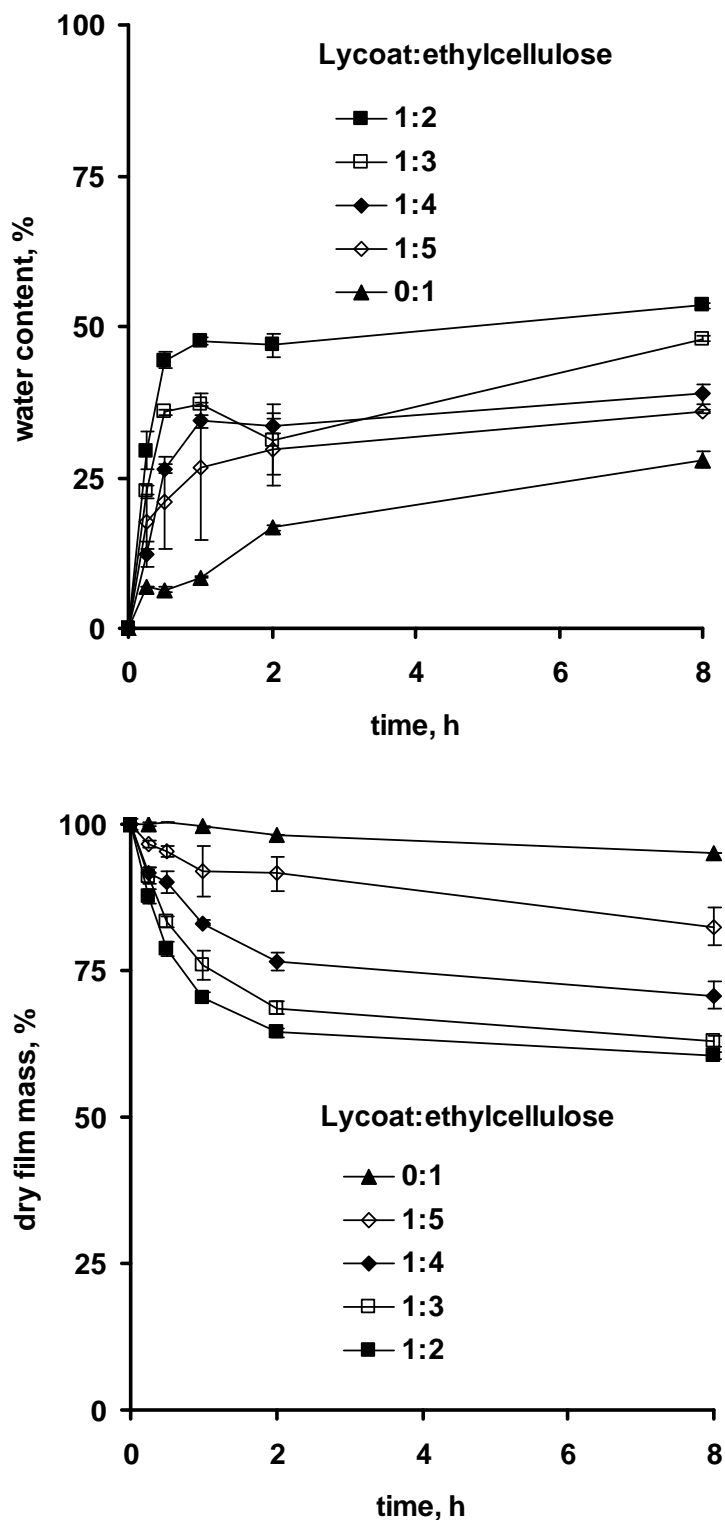
0.1 M HCl



**Figure 27:** Water uptake and dry mass loss of thin films consisting of Lycoat:ethylcellulose blends upon exposure to 0.1 M HCl. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

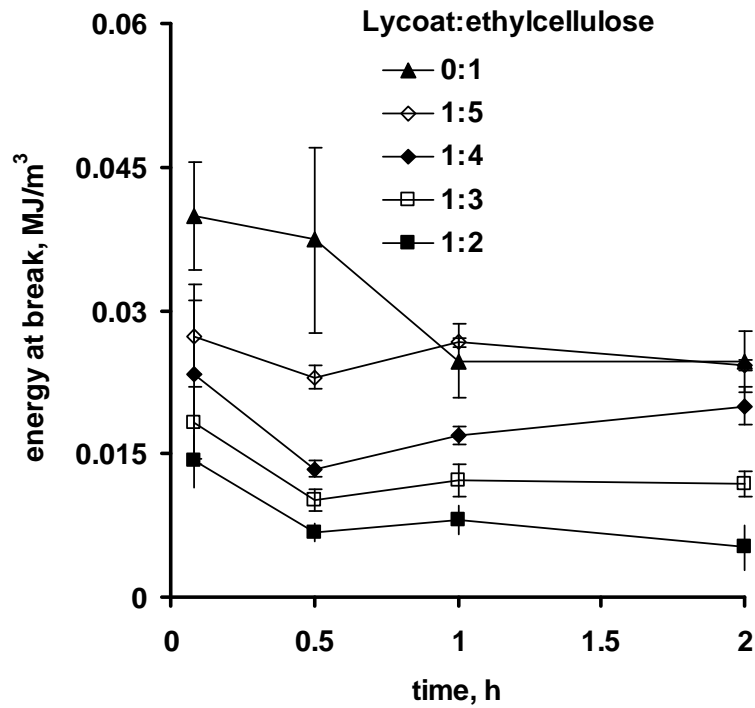


Phosphate buffer pH 6.8

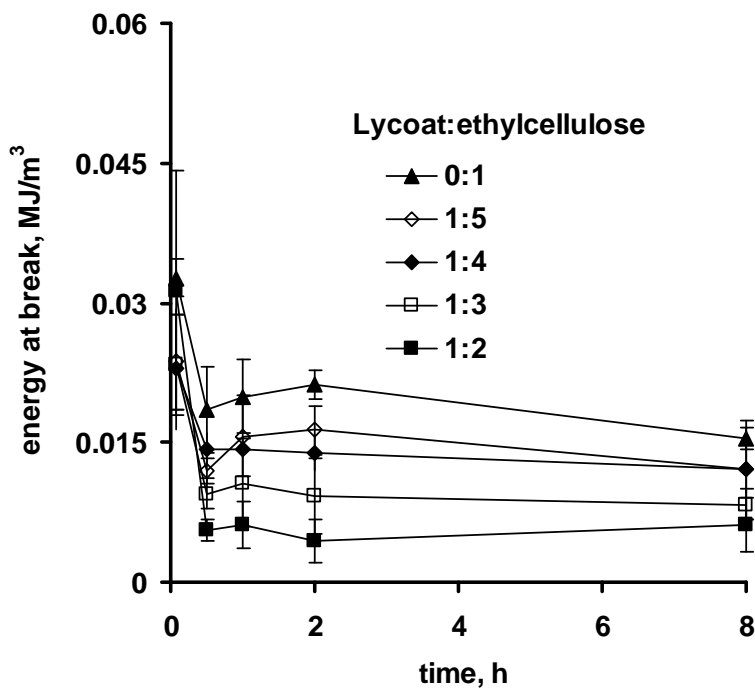


**Figure 28:** Water uptake and dry mass loss of thin films consisting of Lycoat:ethylcellulose blends upon exposure to phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

a)



b)

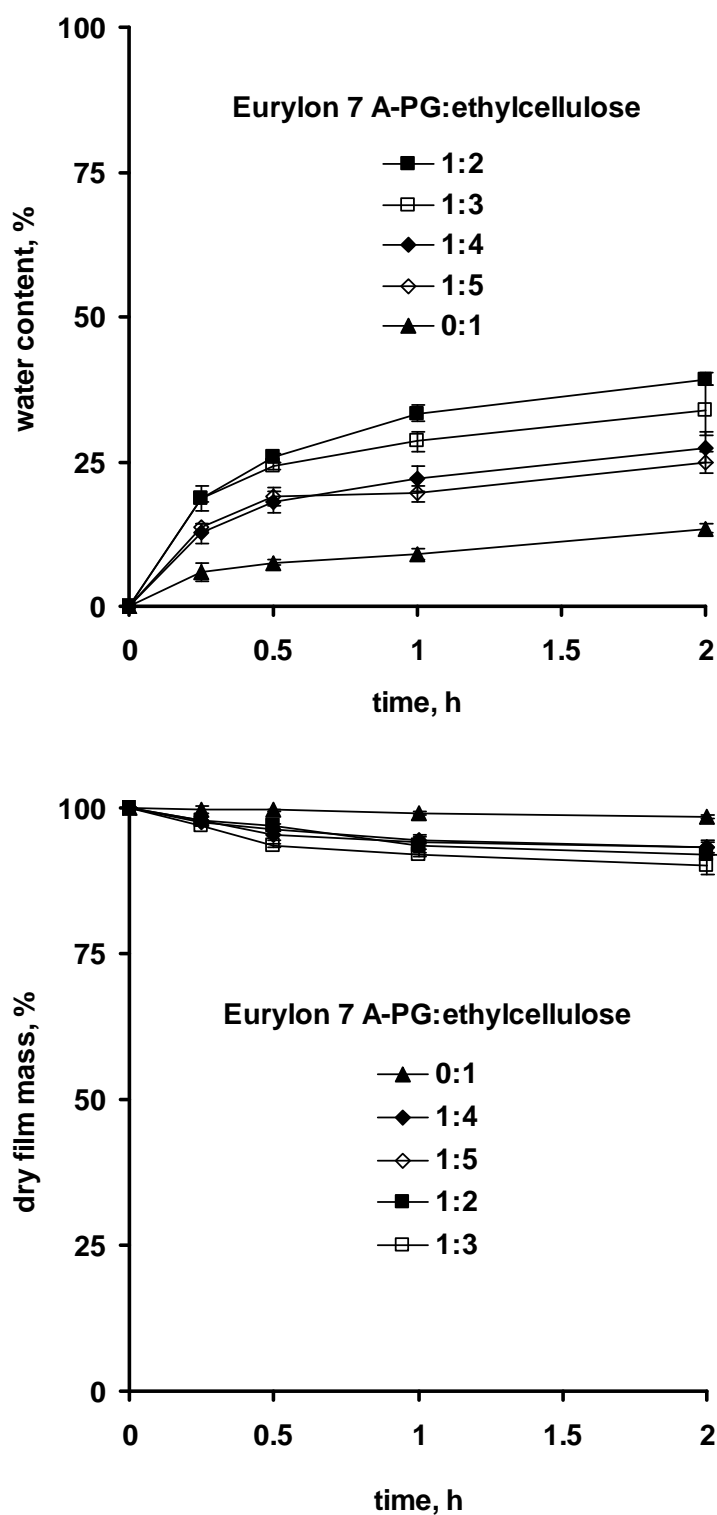


**Figure 29:** Changes in the energy at break of thin Lycoat:ethylcellulose films upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown.

### 3.3.3. Eurylon 7 A-PG:ethylcellulose blends

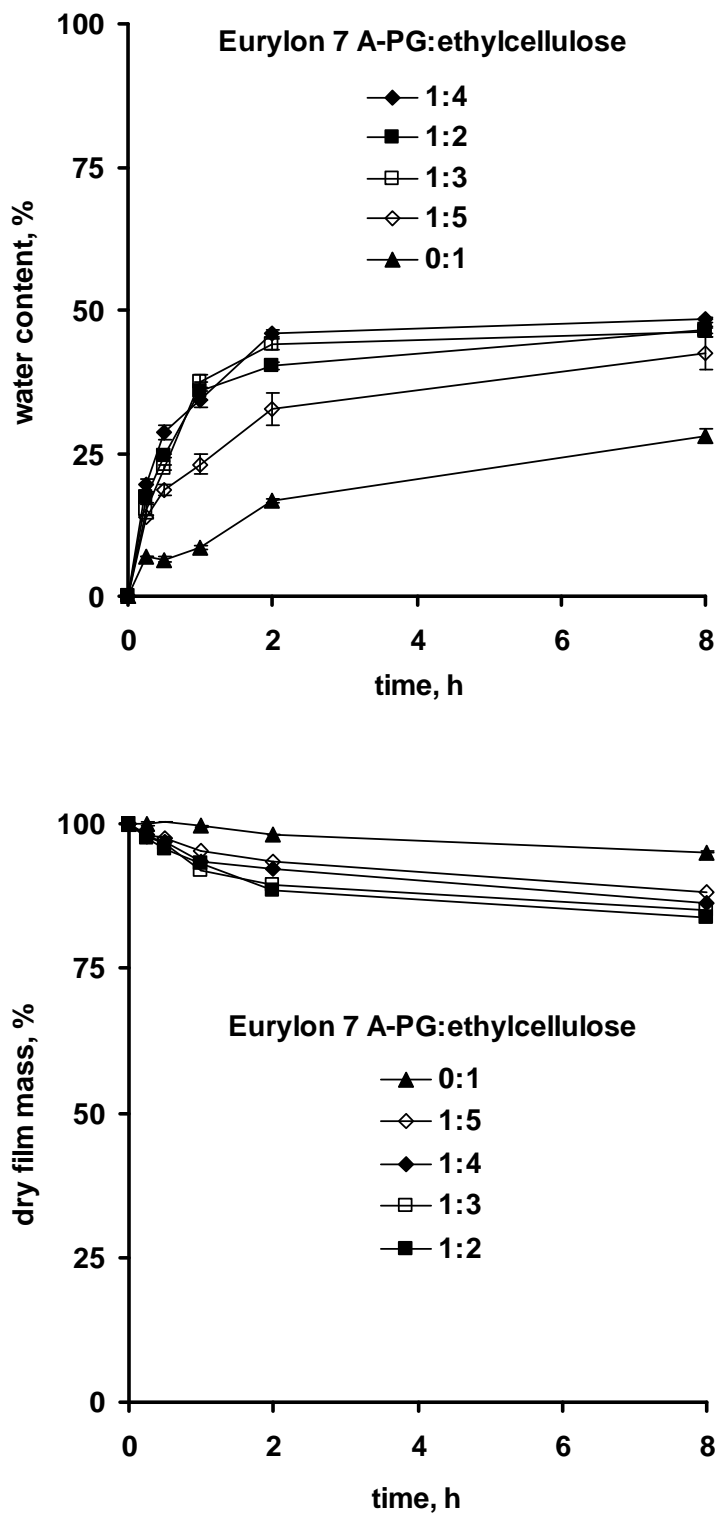
The water uptake and dry mass loss kinetics of thin films consisting of 1:2 to 0:1 Eurylon 7 A-PG:ethylcellulose blends in 0.1 M HCl and phosphate buffer pH 6.8 are shown in **Figures 30 and 31**. Eurylon 7 A-PG is an acetylated and pregelatinised high amylose starch. As it can be seen, the same tendencies as with Glucidex:ethylcellulose and Lycoat:ethylcellulose blends were observed: (i) the water uptake rates and extents increased with decreasing ethylcellulose contents, (ii) the dry mass loss rates and extents increased with increasing starch derivative contents, (iii) these effects were more pronounced in phosphate buffer pH 6.8 than in 0.1 M HCl. Importantly, the water contents of the films upon 2 h exposure to phosphate were considerable: about 50 % w/w. Thus, also at high initial Eurylon 7 A-PG contents, elevated coating levels are likely to be required in order to suppress the premature release of freely water-soluble, low molecular weight drugs in the *upper* GIT. Importantly, the mechanical resistance of the Eurylon 7 A-PG:ethylcellulose based films was significantly higher than that of films consisting of Glucidex:ethylcellulose and Lycoat:ethylcellulose blends in the dry state at room temperature (**Table 5**). However, these differences became minor when the films were exposed to 0.1 M HCl and phosphate buffer pH 6.8, irrespective of the type of release medium (**Figure 32**). Importantly, the variation of the polymer blend ratio again allowed for an efficient adjustment of the mechanical stability of the films.

0.1 M HCl



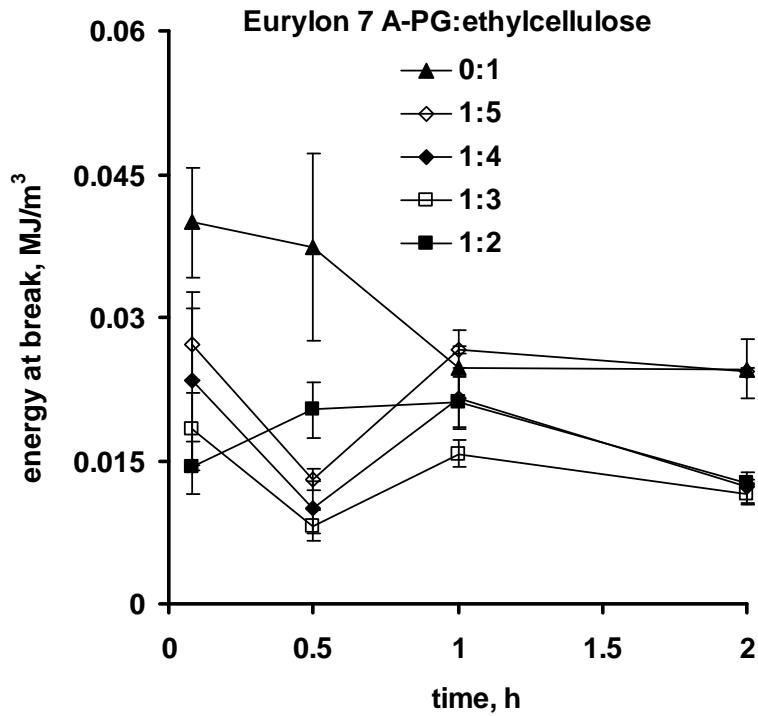
**Figure 30:** Water uptake and dry mass loss of thin films consisting of Eurylon 7 A-PG:ethylcellulose blends upon exposure to 0.1 M HCl. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

## Phosphate buffer pH 6.8

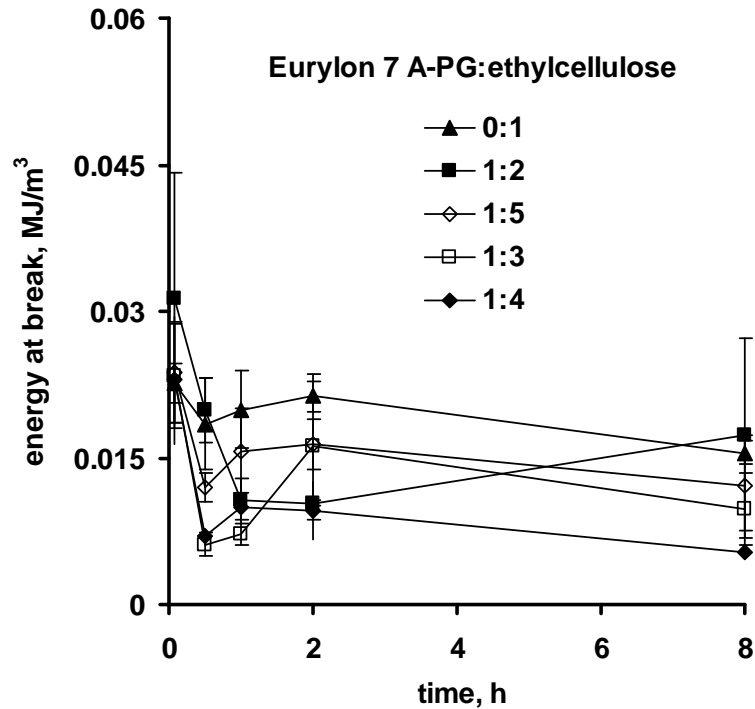


**Figure 31:** Water uptake and dry mass loss of thin films consisting of Eurylon 7 A-PG:ethylcellulose blends upon exposure to phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

a)



b)

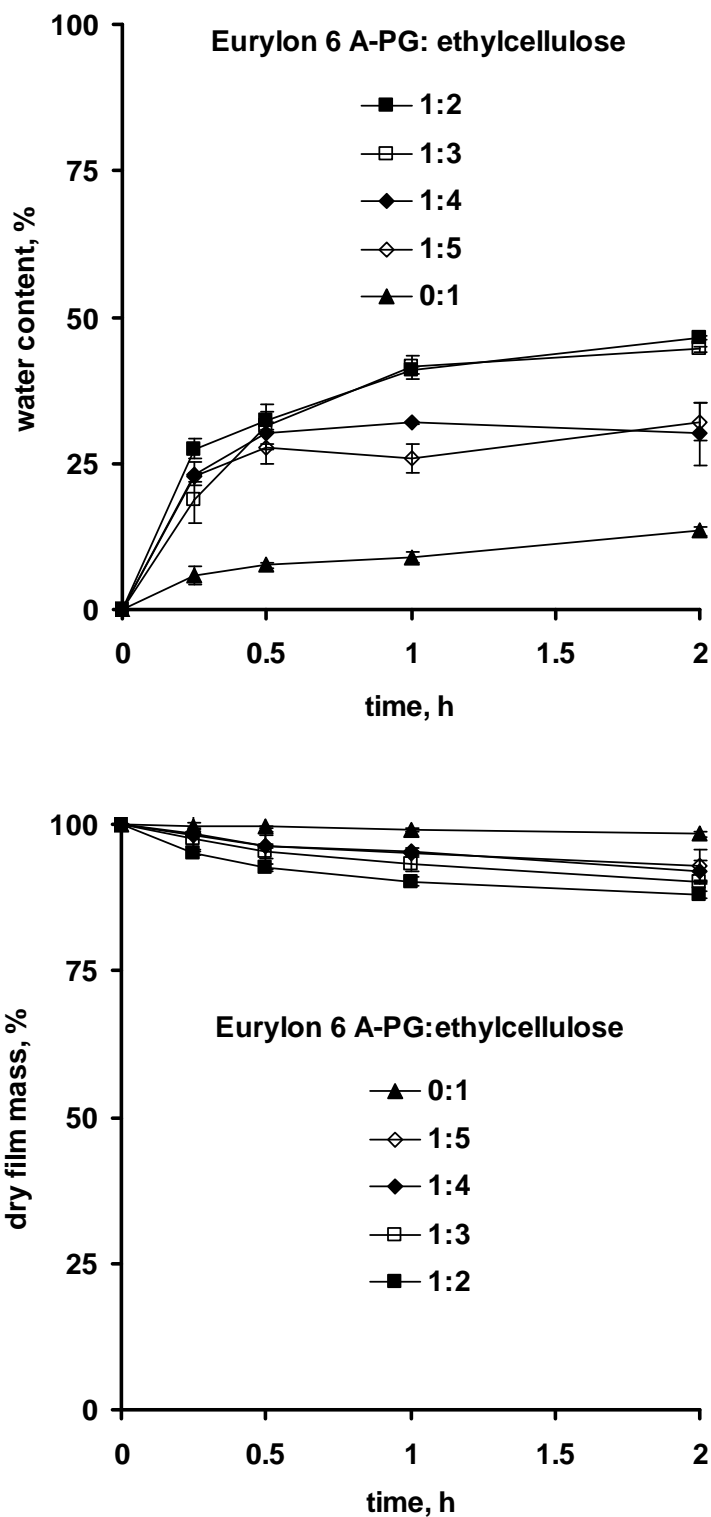


**Figure 32:** Changes in the energy at break of thin Eurylon 7 A-PG:ethylcellulose films upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown.

### 3.3.4. Eurylon 6 A-PG:ethylcellulose and Eurylon 6 HP-PG:ethylcellulose blends

*Eurylon 6 A-PG* is an acetylated and pregelatinised high amylose starch, and *Eurylon 6 HP-PG* a hydroxypropylated and pregelatinised high amylose starch. Interestingly, the dry mass loss of thin films consisting of *Eurylon 6 A-PG*:ethylcellulose and *Eurylon 6 HP-PG*:ethylcellulose blends was much less pronounced than that of the other investigated polymer blends upon exposure to 0.1 M HCl and phosphate buffer pH 6.8, respectively (**Figures 33, 34, 36 and 37**, bottom rows). This was true for both, the *rates* and the *extents* of the dry mass loss and for all the investigated polymer blend ratios. In contrast, the *water uptake* rates and extents of these films upon exposure to the different release media were similar to those of the other starch derivative:ethylcellulose blends, reaching water contents of approximately 50 % w/w after 1-2 h exposure to phosphate buffer pH 6.8 in the case of high initial starch derivative contents (**Figures 33, 34, 36 and 37**, top rows). Thus, also for *Eurylon 6 A-PG*:ethylcellulose and *Eurylon 6 HP-PG*:ethylcellulose blends elevated coating levels are likely to be required to suppress premature release of freely water-soluble, low molecular weight drugs in the *upper* GIT at low initial ethylcellulose contents. As it can be seen in **Table 5**, the mechanical properties of thin films consisting of these types of polymer blends in the dry state at room temperature are similar to those of *Eurylon 7 A-PG*:ethylcellulose blends at the same blend ratios. As in the case of the latter blends, exposure to 0.1 M HCl or phosphate buffer pH 6.8 resulted in a decrease in the mechanical stability of the macromolecular networks, irrespective of the type of release medium and polymer blend ratio (**Figures 35 and 38**). Importantly, desired system stabilities can again effectively be adjusted by varying the polymer blend ratio.

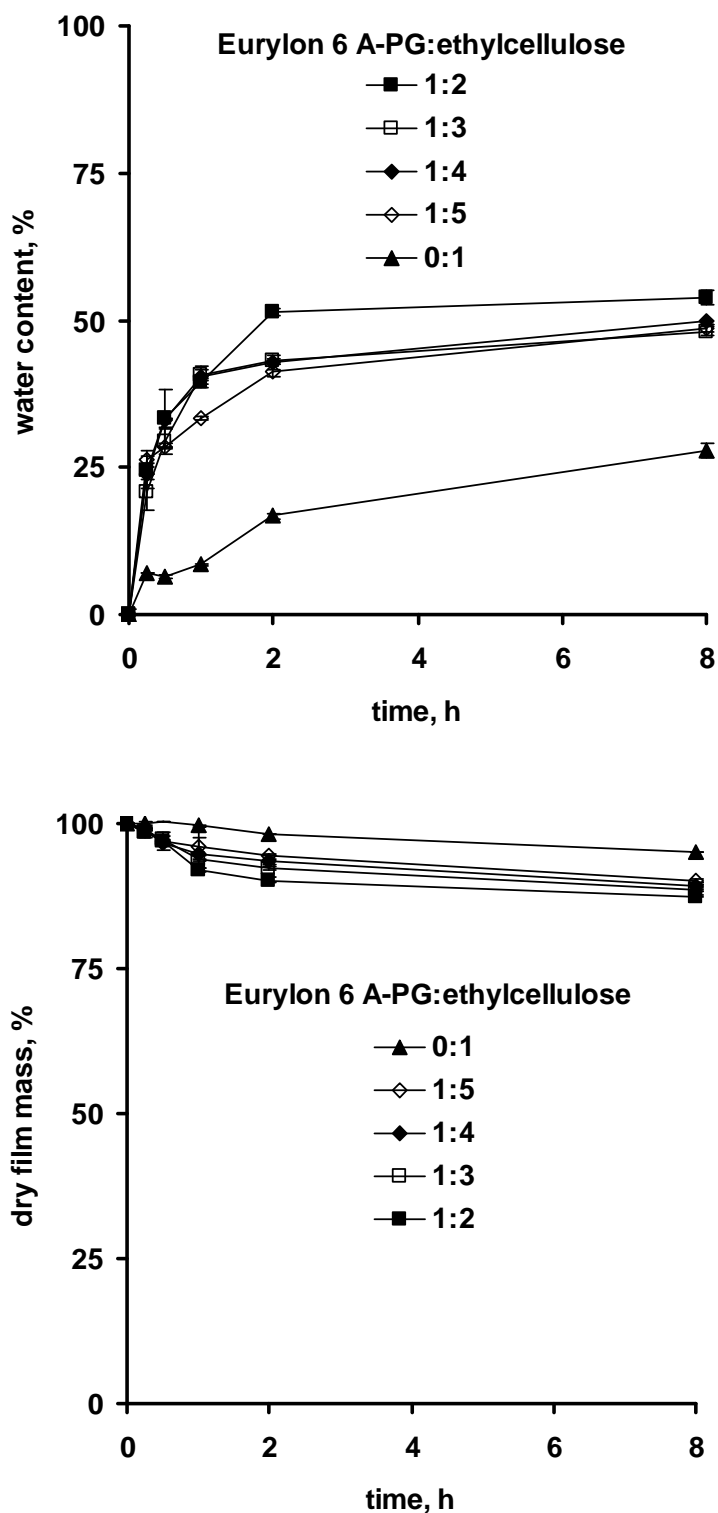
0.1 M HCl



**Figure 33:** Water uptake and dry mass loss of thin films consisting of Eurylon 6 A-PG:ethylcellulose blends upon exposure to 0.1 M HCl. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

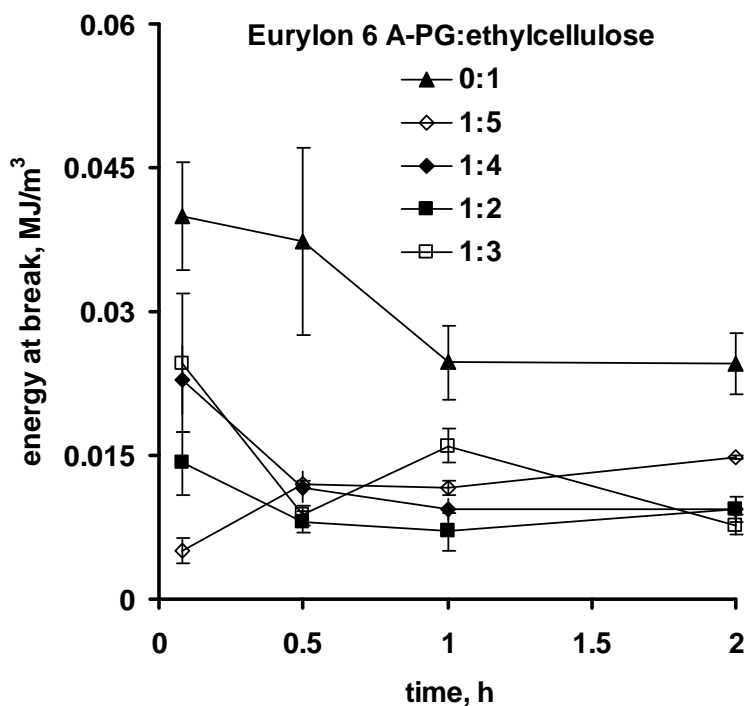


## Phosphate buffer pH 6.8

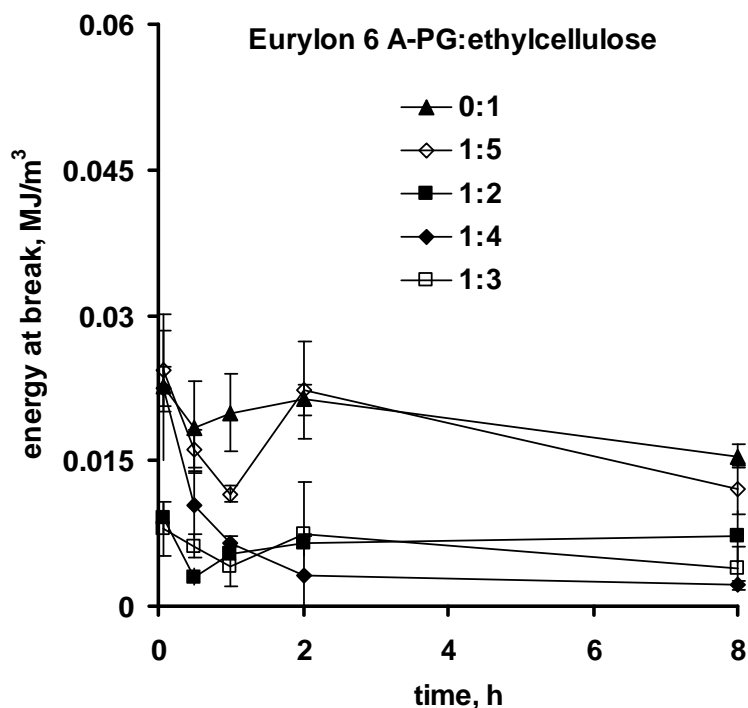


**Figure 34:** Water uptake and dry mass loss of thin films consisting of Eurylon 6 A-PG:ethylcellulose blends upon exposure to phosphate buffer pH 6.8, respectively. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

a)

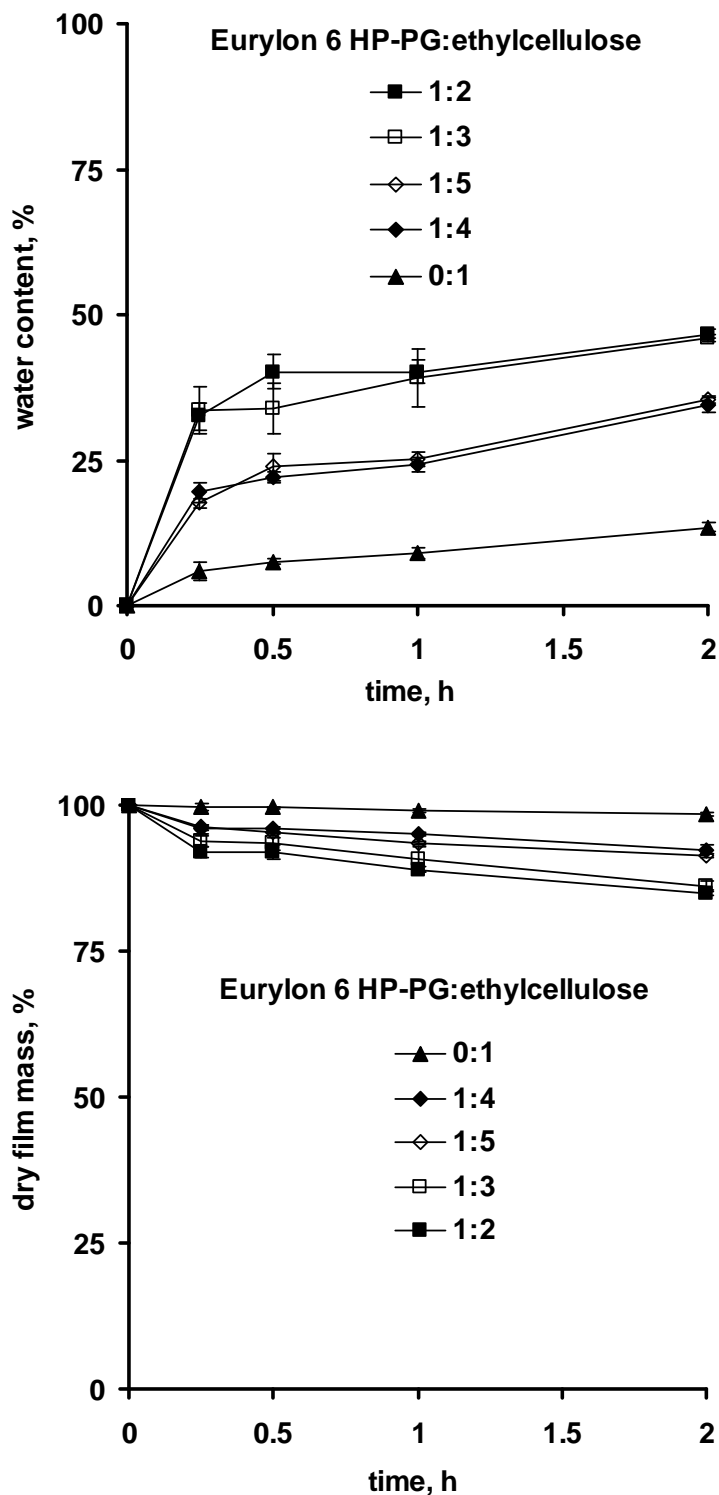


b)



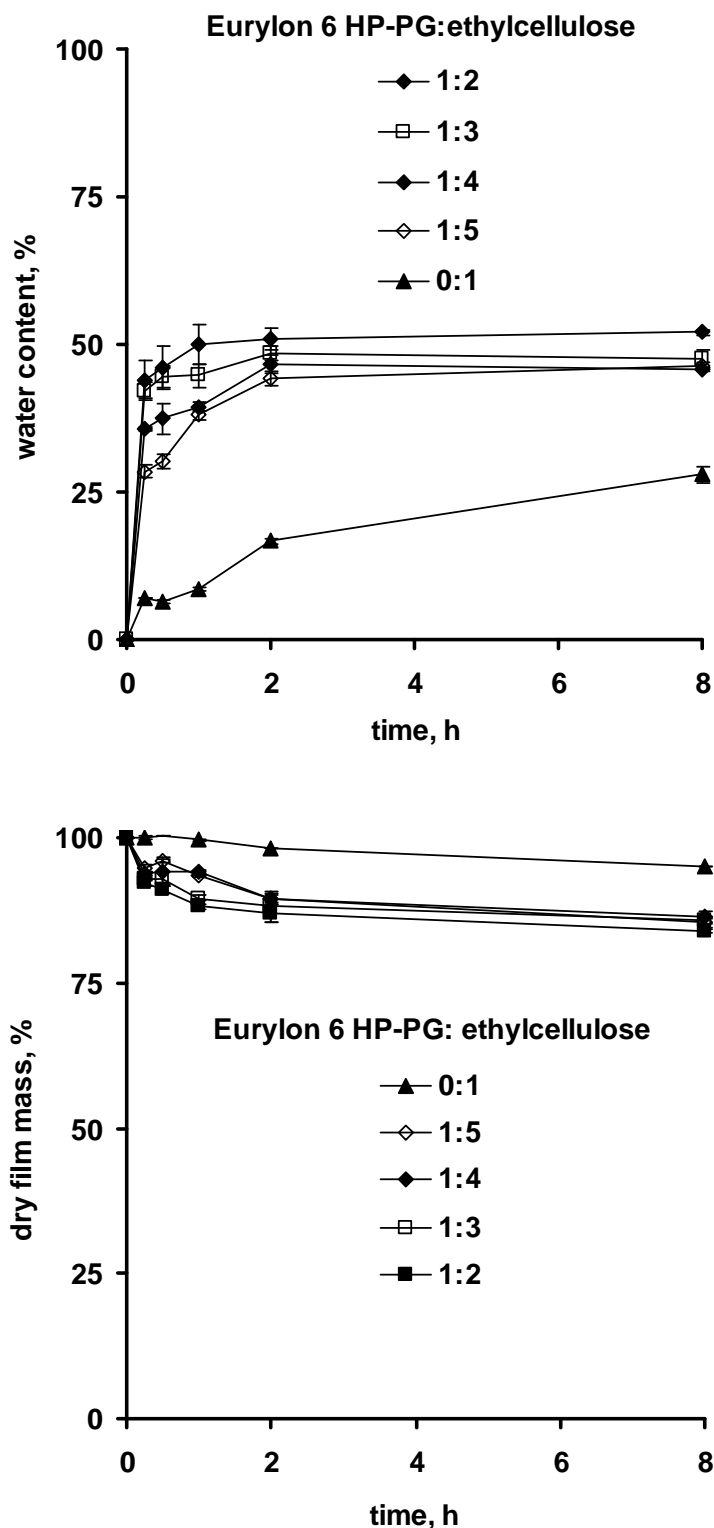
**Figure 35:** Changes in the energy at break of thin Eurylon 6 A-PG:ethylcellulose films upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown.

0.1 M HCl

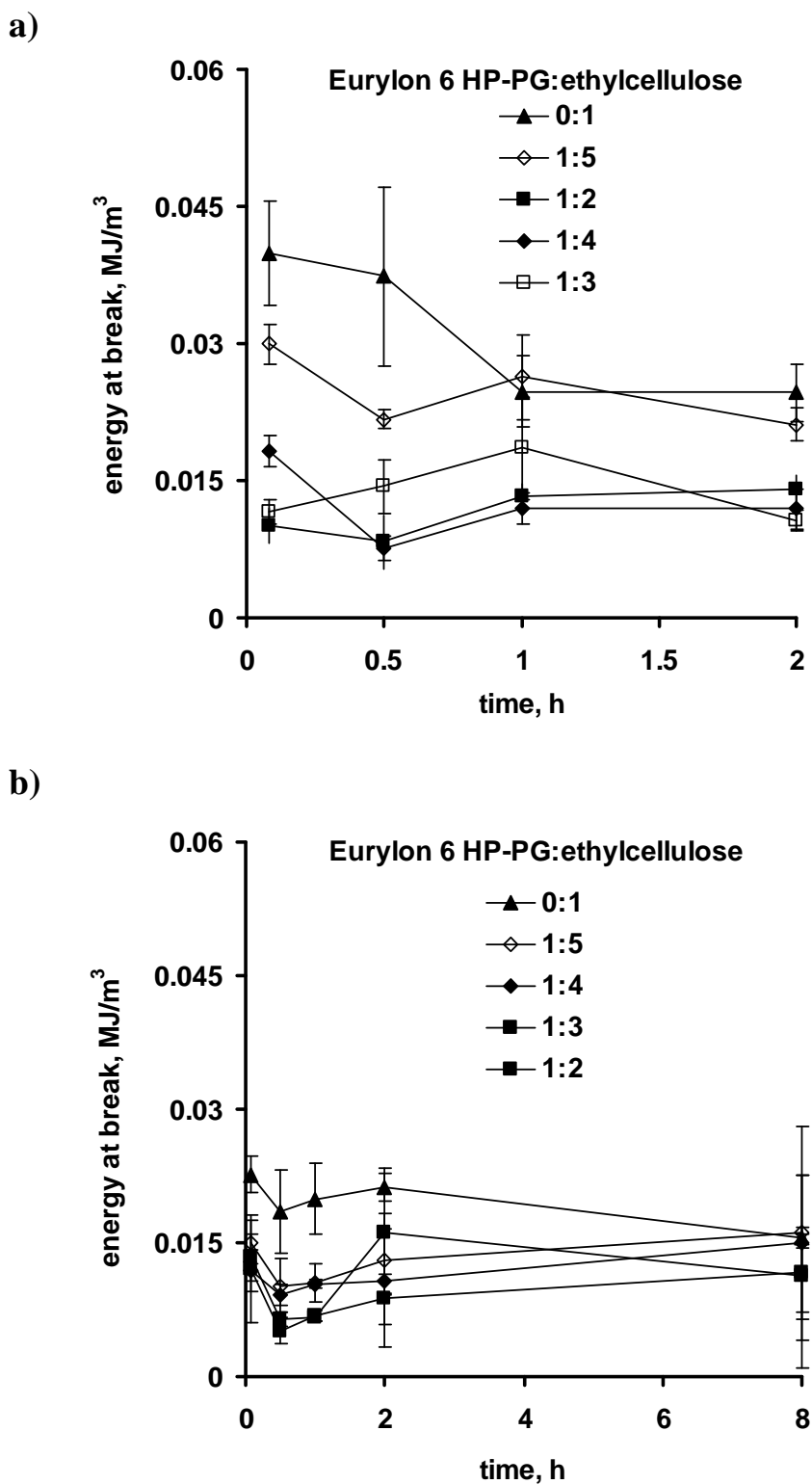


**Figure 36:** Water uptake and dry mass loss of thin films consisting of Eurylon 6 HP-PG:ethylcellulose blends upon exposure to 0.1 M HCl. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.

## Phosphate buffer pH 6.8



**Figure 37:** Water uptake and dry mass loss of thin films consisting of LAB Eurylon 6 HP-PG:ethylcellulose blends upon exposure to phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the behavior of pure (plasticized) ethylcellulose films is shown.



**Figure 38:** Changes in the energy at break of thin Eurylon 6 HP-PG:ethylcellulose films upon exposure to: (a) 0.1 M HCl and (b) phosphate buffer pH 6.8. The polymer blend ratio is indicated in the figures. For reasons of comparison also the results obtained with pure (plasticized) ethylcellulose films are shown.

### **3.3.5. Conclusions**

The key properties of thin polymeric films consisting of starch derivative:ethylcellulose blends exhibiting an interesting potential to provide site specific drug delivery to the colon (and being adapted to the pathophysiology of inflammatory bowel disease patients) can effectively be adjusted by varying the polymer blend ratio and type of starch derivative. This includes the water uptake and dry mass loss kinetics as well as the mechanical properties of the films before and upon exposure to aqueous media simulating the contents of the *upper* GIT. Thus, broad ranges of film coating properties can easily be provided, being adapted to the needs of the respective drug treatment (e.g., osmotic activity of the core formulation and administered dose).

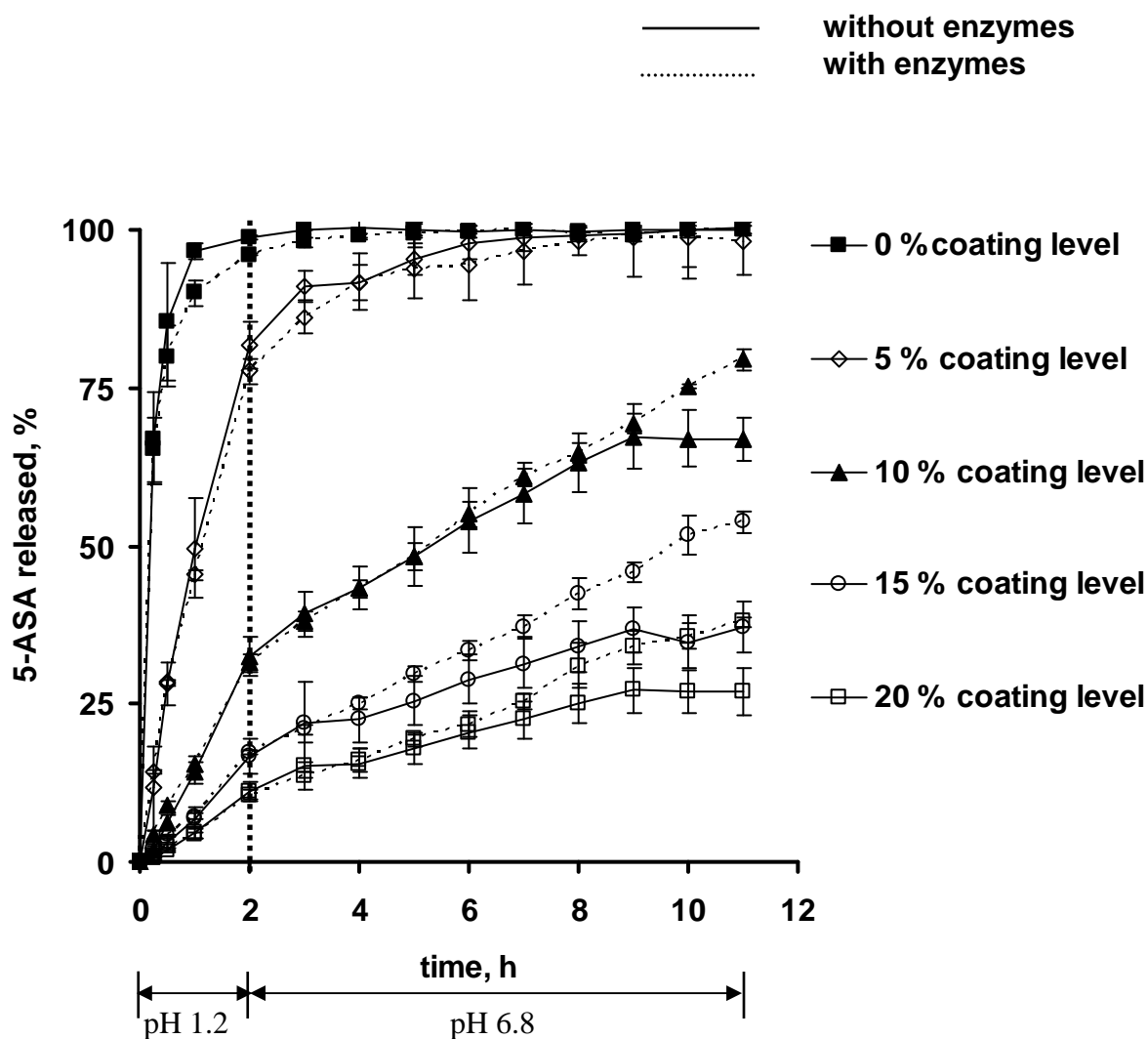
### 3.4. EFFECTS OF THE POLYMER BLEND RATIO (COATED PELLETS)

#### 3.4.1. Drug release in the upper GIT

An ideal film coating allowing for the site specific delivery of a drug to the colon should completely suppress drug release in the *upper* GIT. However, once the colon is reached, drug release should be time-controlled (this may include rapid and complete release). Recently, promising novel polymeric films have been identified (blends of the starch derivative Nutriose and ethylcellulose), which show low water uptake and dry mass loss rates and extents upon exposure to release media simulating the transit through the stomach and small intestine. However, once the colon is reached, they serve as substrates for the microflora in inflammatory bowel disease patients and lose significant dry mass and take up considerable amounts of water [Karrout et al., 2008a; b]. Yet, it was unknown whether these novel polymeric films are able to adequately control drug release from *coated* solid dosage forms.

**Figure 39** shows *in vitro* drug release rate of 5-ASA from pellets coated with Nutriose:ethylcellulose 1:2 blends at different coating levels upon exposure to 0.1 M HCl for 2 h and subsequent complete medium change to phosphate buffer pH 6.8 (USP) in agitated flasks at 37 °C (solid curves). Clearly, the relative drug release rate decreased with increasing coating level, due to the increasing length of the diffusion pathways. However, even at 20 % w/w coating level, drug release was still significant under these conditions, with approximately 20 % of the 5-ASA being released after 11 h. It has to be pointed out that these results were obtained in release media free of enzymes. This does not appropriately reflect the conditions *in vivo*: The presence of digestive enzymes potentially alters the film coating properties and might result in much faster drug release. To estimate the importance of this phenomenon, 0.32 % pepsin were added to the 0.1 M HCl and 1 % pancreatin to the phosphate buffer pH 6.8. The *dotted* curves in Figure 35 show the respective experimentally measured drug release kinetics under these conditions. Importantly, there was only a slight increase/no effect in all cases, indicating that the enzymes cannot degrade this polymeric film coating to a considerable extent under these conditions (e.g., in the presence of ethylcellulose). Nevertheless, the observed drug release rates even at higher coatings levels were too high.

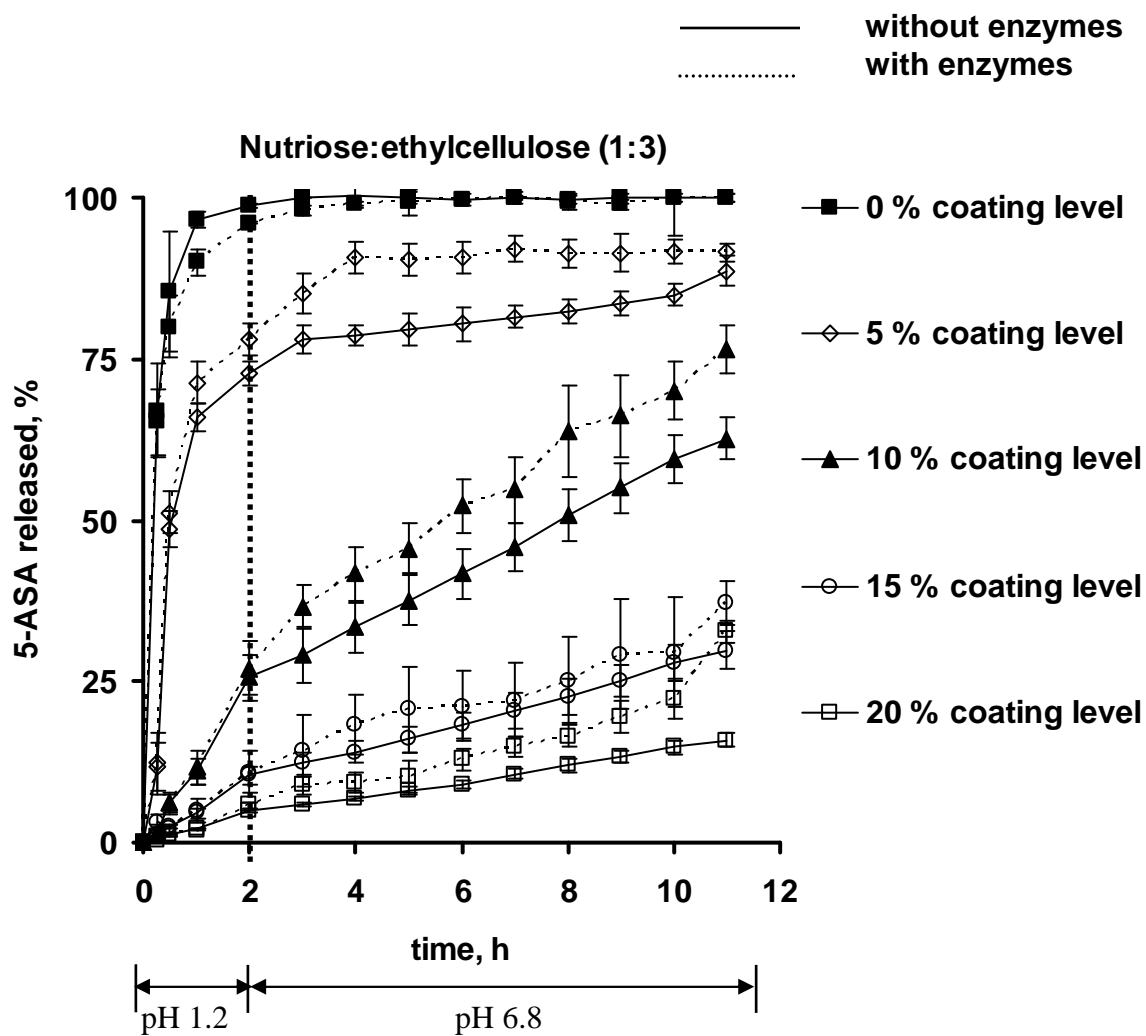
In order to decrease the release rate of 5-ASA from the investigated pellets, the initial ethylcellulose content in the film coating was increased. It has recently been shown, that with



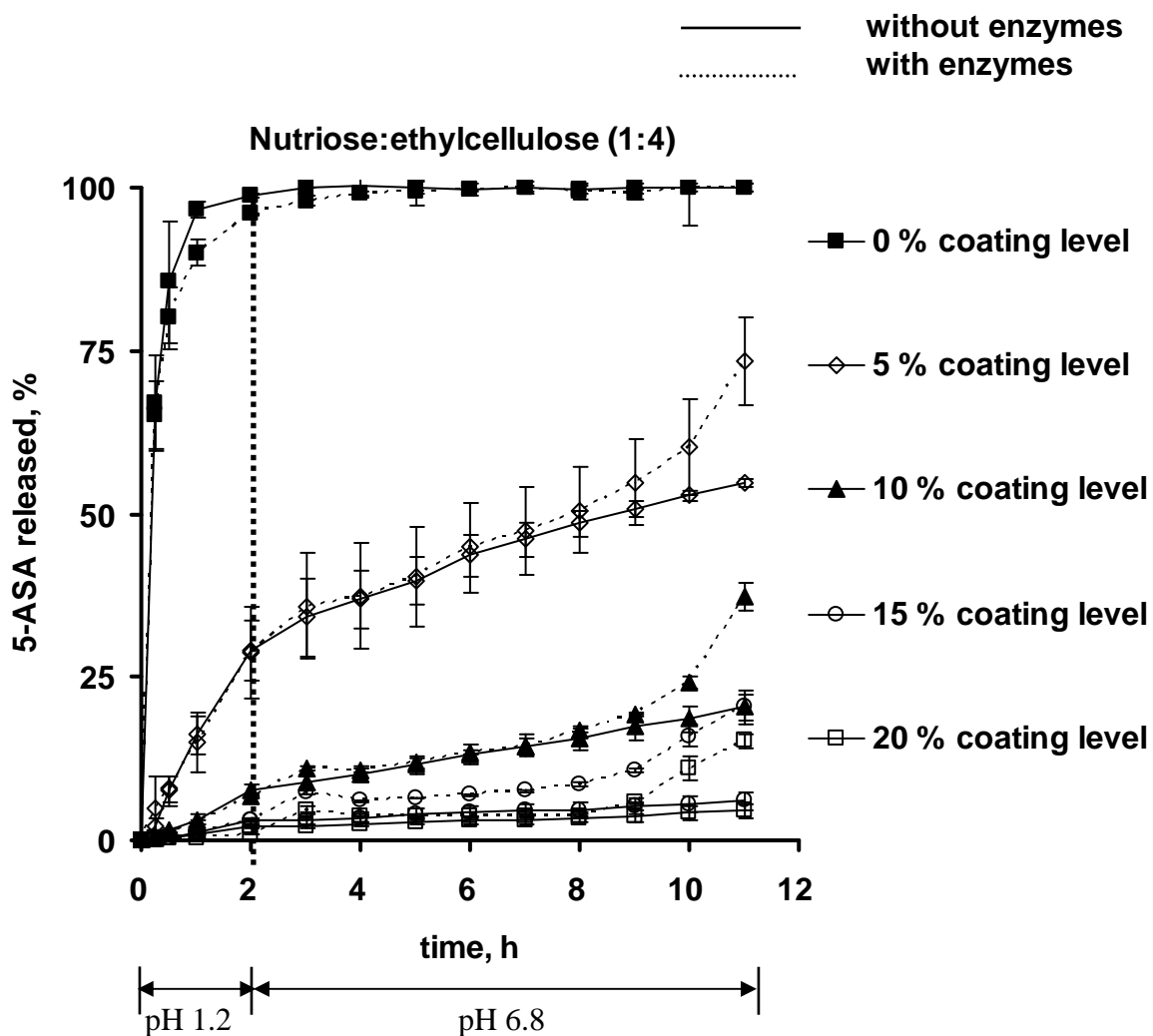
**Figure 39:** *In vitro* release of 5-ASA from pellets coated with Nutriose:ethylcellulose blends (1:2) under conditions simulating the transit through the upper GIT. The coating level is indicated in the figure as well as the presence (dotted lines) and absence (full lines) of enzymes.



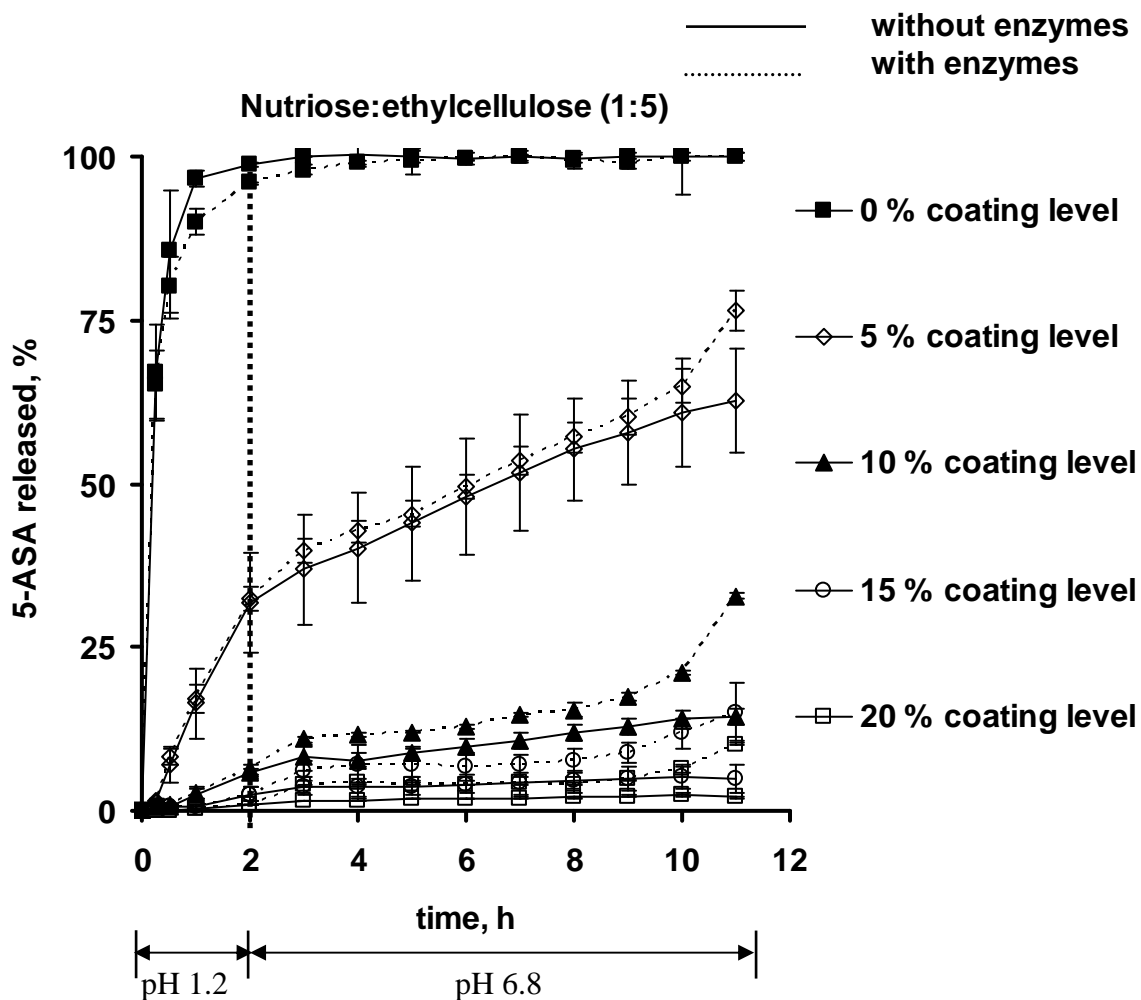
decreasing initial Nutriose contents, the water uptake rates and extents as well as the dry film mass loss rates and extents decreased if free films were exposed to 0.1 N HCl and phosphate buffer pH 6.8, respectively [Karrout et al., 2008b]. **Figures 40-42** show the effects of the Nutriose:ethylcellulose blend ratio on the resulting 5-ASA release kinetics from the investigated pellets. Clearly, the relative drug release rate significantly decreased when decreasing the polymer:polymer blend ratio from 1:2 to 1:5. Furthermore, in all cases the release rate decreased with increasing coating level. As it can be seen in **Figures 40-42**, a coating level of 15-20 % at a Nutriose:ethylcellulose blend ratio of 1:4 or 1:5 is sufficient to almost completely suppress drug release under these conditions, simulating the transit through the *upper* GIT. Please note that all transit times were chosen in such a way that they can be expected to be well above the real transit time *in vivo* (worst case conditions) [Watts and Illum, 1997; Davis et al., 1986]. Thus, the *in vivo* performance of the pellets can be expected to be even better. Importantly, little to no effect was observed when adding 0.32 % pepsin and 1 % pancreatin to the release media, irrespective of the coating level and polymer blend ratio (dotted curves in **Figures 40-42**). However, in these experiments the gradual increase in the pH of the release medium throughout the *upper* GIT was very much simplified. Furthermore, the mechanical stress the pellets were exposed to was not very important (horizontal agitation in flasks at 80 rpm). *In vivo*, significant mechanical shear forces (caused by the motility of the *upper* GIT) might induce crack formation within the polymeric film coatings, resulting in much higher drug release rates. To better simulate these two important aspects, pellets coated with 20 % Nutriose:ethylcellulose at a blend ratio of 1:4 and 1:5 were also released in a USP apparatus 3 using the release media and transit times listed in **Table 3**. Three different dipping speeds were studied: (i) high: 30 dpm for 11.5 h, then 20 dpm, (ii) medium: 20 dpm for 11.5 h, then 10 dpm, and (iii) low: 10 dpm for 11.5 h, then 5 dpm. Clearly, 5-ASA release was effectively suppressed also under these more harsh conditions, in particular at the Nutriose:ethylcellulose blend ratio 1:5 (**Figure 43 and 44**). Again, please note that the chosen release periods are non-physiological and represent extreme (worst case) conditions. The *in vivo* performance of these polymeric blends can be expected to be better. Thus, the mechanical stability of these film coatings is sufficient even upon exposure to considerable shear forces for prolonged periods of times.



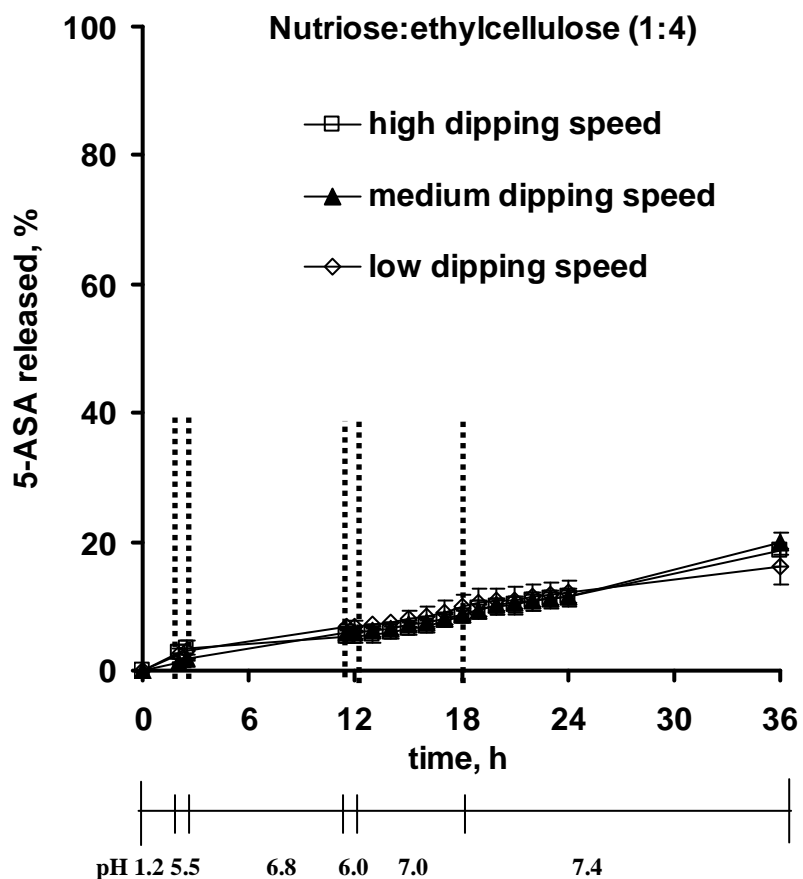
**Figure 40:** Effects of the Nutriose:ethylcellulose blend ratio and coating level (indicated in the figures) on the *in vitro* release of 5-ASA from the investigated pellets under conditions simulating the transit through the upper GIT. Full/dotted lines indicate the absence/presence of enzymes.



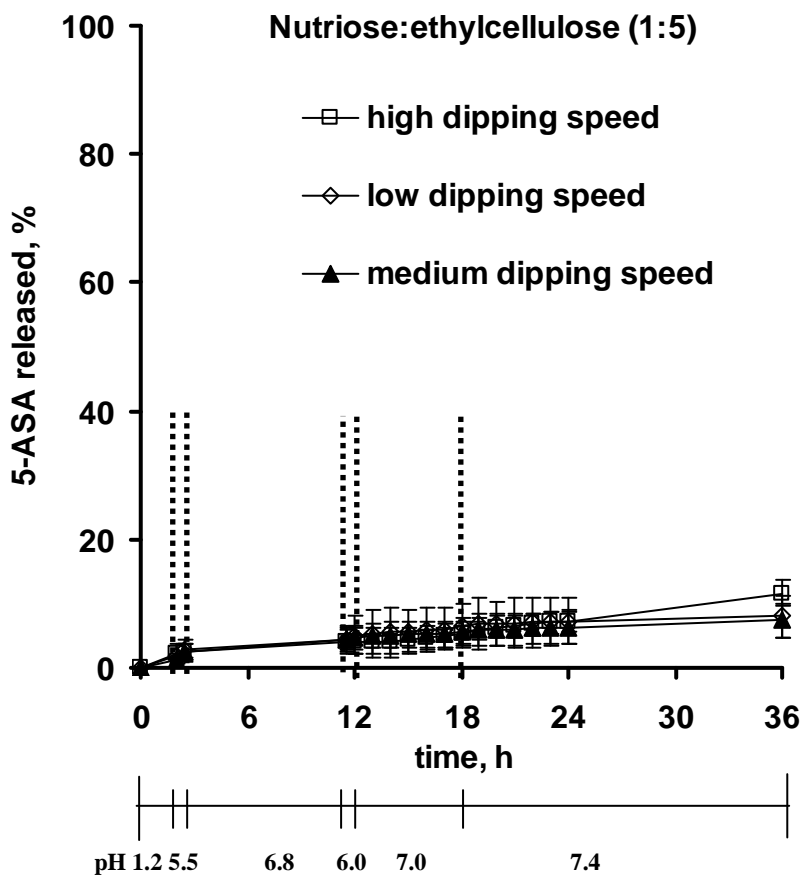
**Figure 41:** Effects of the Nutriose:ethylcellulose blend ratio and coating level (indicated in the figures) on the *in vitro* release of 5-ASA from the investigated pellets under conditions simulating the transit through the upper GIT. Full/dotted lines indicate the absence/presence of enzymes.



**Figure 42:** Effects of the Nutriose:ethylcellulose blend ratio and coating level (indicated in the figures) on the *in vitro* release of 5-ASA from the investigated pellets under conditions simulating the transit through the upper GIT. Full/dotted lines indicate the absence/presence of enzymes.



**Figure 43:** Drug release from pellets coated with Nutriose:ethylcellulose blends (the ratio is indicated in the figure) at 20 % coating level under conditions simulating the transit through the entire GIT (without fecal samples). High dipping speed: 30 dpm for 11.5 h, then 20 dpm. Medium dipping speed: 20 dpm for 11.5 h, then 10 dpm. Low dipping speed: 10 dpm for 11.5 h, then 5 dpm (USP Apparatus 3).



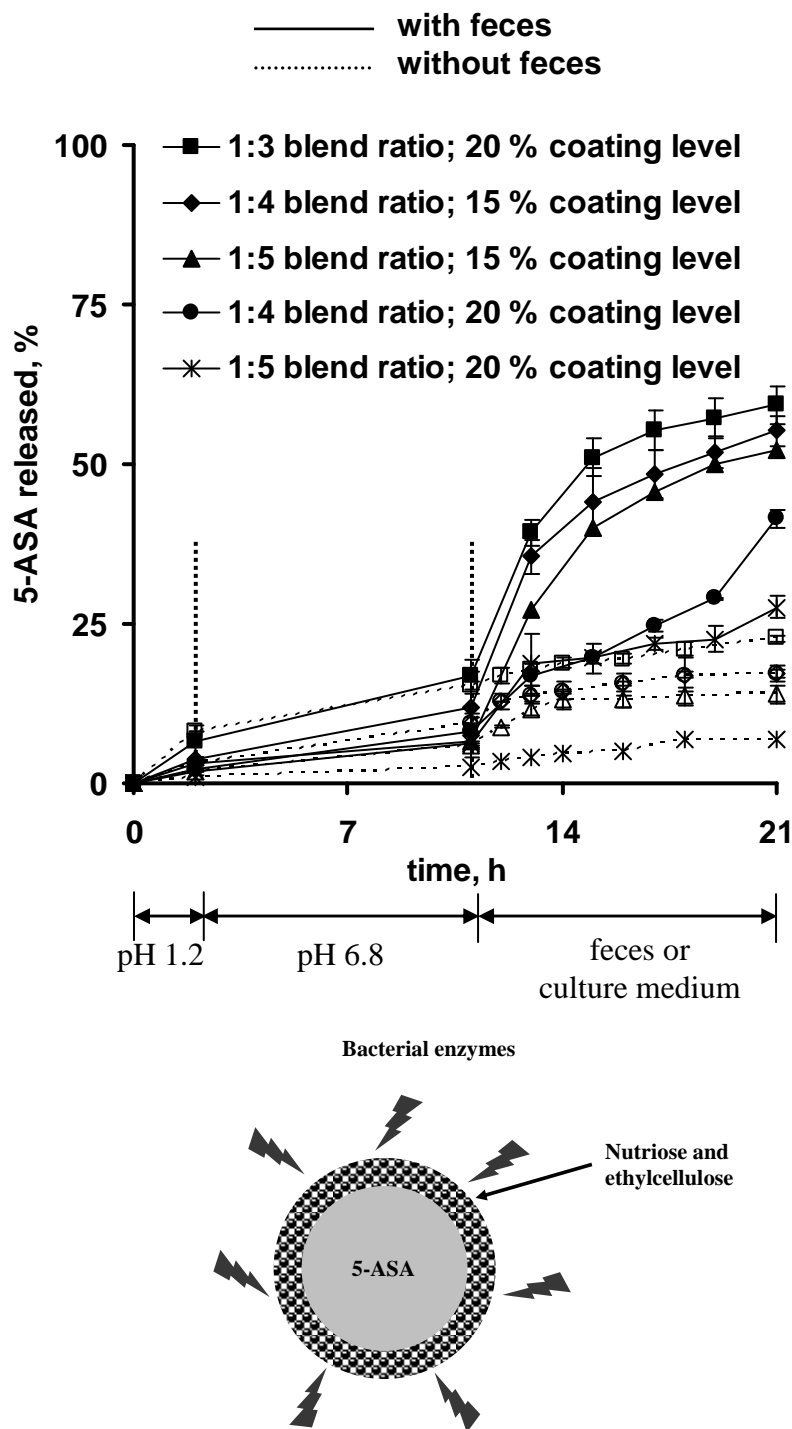
**Figure 44:** Drug release from pellets coated with Nutriose:ethylcellulose blends (the ratio is indicated in the figure) at 20 % coating level under conditions simulating the transit through the entire GIT (without fecal samples). High dipping speed: 30 dpm for 11.5 h, then 20 dpm. Medium dipping speed: 20 dpm for 11.5 h, then 10 dpm. Low dipping speed: 10 dpm for 11.5 h, then 5 dpm (USP Apparatus 3).

### 3.4.2. Drug release in the colon

Once the colon is reached, the polymeric film coating (which effectively suppressed drug release in the *upper* GIT) should become permeable for the drug. **Figure 45** shows the release 5-ASA from the investigated pellets coated with 15 % and 20 % w/w Nutriose:ethylcellulose at the following three blend ratios: 1:3, 1:4, or 1:5. The release medium was 0.1 M HCl during the first 2 h, which was subsequently completely replaced by phosphate buffer pH 6.8 for 9 h. For the last 10 h the pellets were exposed to feces from inflammatory bowel disease patients and incubated under *anaerobic* conditions (solid curves). Clearly, 5-ASA release in the media simulating the transit through the *upper* GIT was effectively suppressed, whereas a significant increase in the release rate was observed once the pellets were exposed to the patients' feces. This sudden increase in the drug permeability can be attributed to the fact that Nutriose:ethylcellulose serve as substrates for the enzymes secreted by the microflora in patients suffering from Crohn's disease and ulcerative colitis (cartoon in **Figure 45**) [Karrout et al., 2008a]. Please note that the viability of this microflora is limited *in vitro*. Thus, the enzymatic activity is likely to be *underestimated* under the given experimental conditions. *In vivo* the bacteria continuously produce the respective enzymes, which are able to degrade the starch derivative in the film coatings. Thus, the leveling of effects of drug release below 100 % as observed in this study is unlikely to occur *in vivo*.

For reasons of comparison, 5-ASA release was also measured upon exposure to the release media simulating the conditions in the *upper* GIT followed by exposure to culture medium *without patient's feces* under *anaerobic* conditions (dotted curves in **Figure 45**). Importantly, no sudden increase in the drug release rate was observed after 12 h. This confirms the hypothesis that the significant increase in the film coatings' permeability is caused by the (partial) enzymatic degradation of this type of polymeric systems by the enzymes present in the feces of inflammatory bowel disease patients.

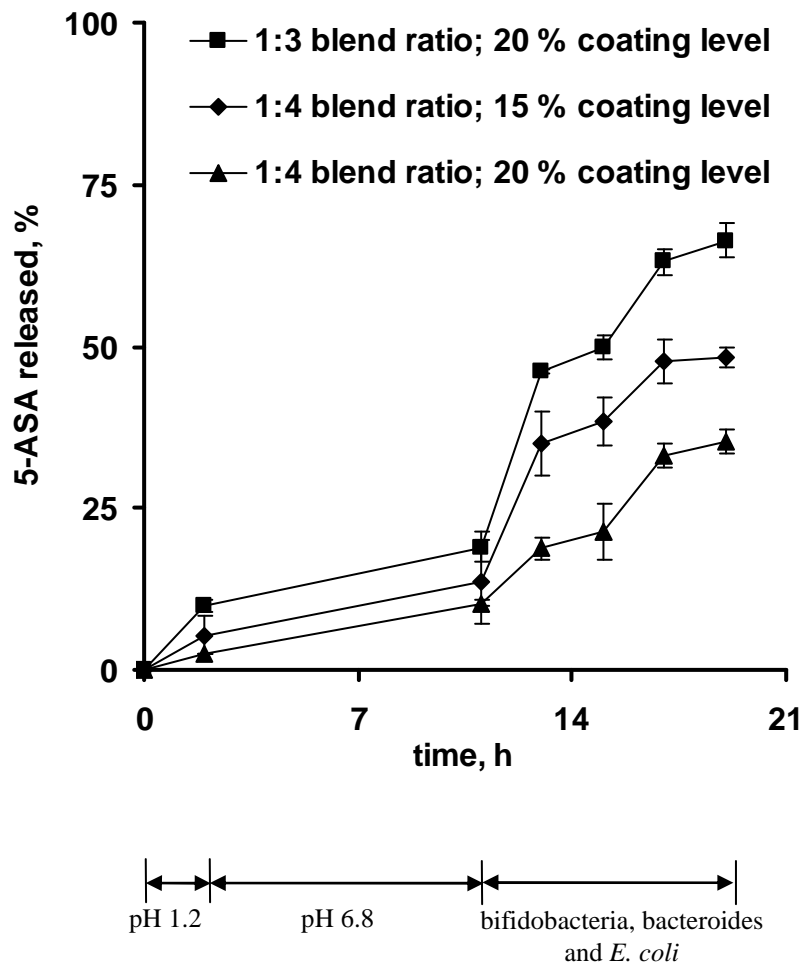
It has to be pointed out that *only fresh* fecal samples can be used for the *in vitro* drug release measurements (due to the limited viability of the complex microflora). As the availability of such samples is likely to be restricted in practice, in particular for applications in routine use, the most important bacteria in the fecal samples were to be identified and two alternative release media simulating the conditions in the colon of a subject to be developed. **Figures 46 and 47** show the experimentally determined 5-ASA release rates from pellets coated with 15 or 20 % Nutriose:ethylcellulose at a blend ratio of 1:3, 1:4 or 1:5, respectively. The pellets were exposed to 0.1 M HCl for the first 2 h, subsequently to phosphate buffer



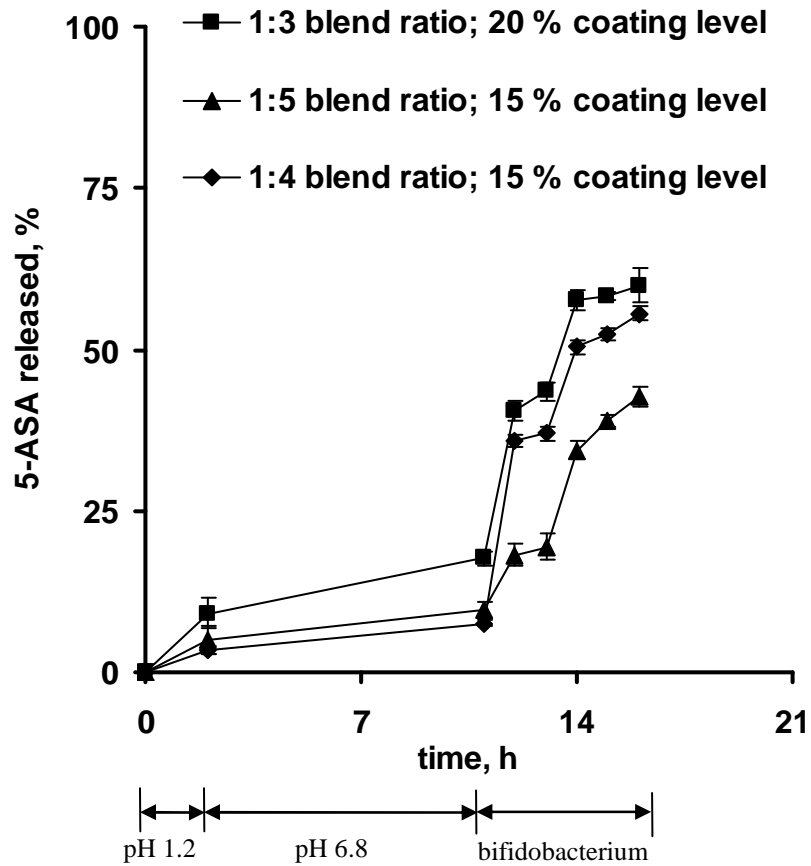
**Figure 45:** 5-ASA release from pellets coated with Nutriose:ethylcellulose blends (the ratio is indicated in the figure) at 15 or 20 % coating level under conditions simulating the transit through the entire GIT, with fecal samples from inflammatory bowel disease patients. The dipping speed was 10 dpm. For reasons of comparison also drug release in culture medium without fecal samples is shown (dotted lines). The cartoon illustrates the principle of the investigated colon targeting approach.



pH 6.8 for 9 h, and finally to either culture medium containing a mixture of bifidobacteria, bacteroides and *Escherichia coli* (**Figure 46**), or to culture medium containing *Bifidobacterium* (**Figure 47**). Clearly, the sudden increase in the relative release rate upon exposure to these “alternative” drug release media simulating colonic conditions was similar to the one observed in feces from inflammatory bowel disease patients (**Figure 46 and 47** versus **Figure 45**). Thus, these media might be good substitutes for real fecal samples.



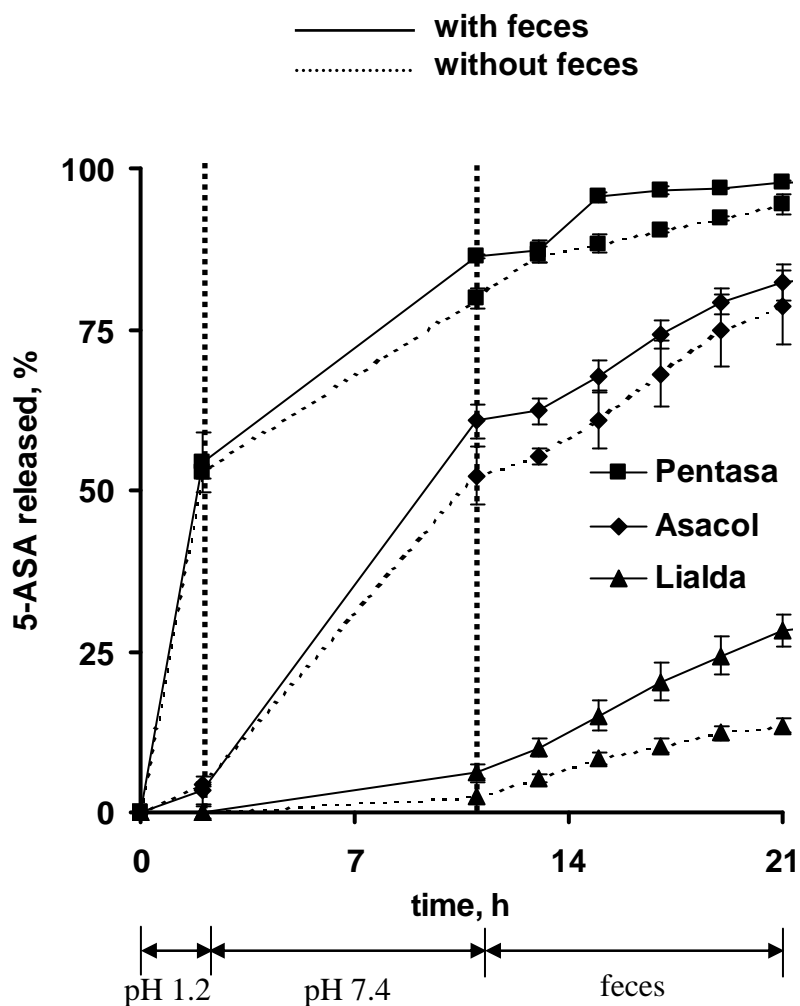
**Figure 46:** 5-ASA release from pellets coated with Nutriose:ethylcellulose blends (the ratio is indicated in the figures) at 15 or 20 % coating level under conditions simulating the transit through the entire GIT, with: a mixture of bifidobacteria, bacteroides and *Escherichia coli*. The dipping speed was 10 dpm.



**Figure 47:** 5-ASA release from pellets coated with Nutriose:ethylcellulose blends (the ratio is indicated in the figures) at 15 or 20 % coating level under conditions simulating the transit through the entire GIT, with: (a) *Bifidobacterium*. The dipping speed was 10 dpm.

**Figure 48** illustrates the experimentally determined 5-ASA release kinetics from three commercially available products: Pentasa pellets, Asacol capsules filled with coated granules and Lialda tablets. Pentasa pellets consist of 5-ASA loaded starter cores coated with ethylcellulose. As it can be seen, drug release already starts in the *upper* GIT, which is consistent with reports in the literature [Wilding et al., 1999]. Asacol capsules are filled with 5-ASA loaded granules, which are coated with Eudragit S: a poly(acryl methacrylate), which is insoluble at low pH, but becomes soluble at  $\text{pH} > 7$ . In order to be able to provide sink conditions using the Bio-Dis release apparatus and selected time schedule for media changes, hard gelatine capsules were opened and 0.05 g granules placed into each vessel. As it can be seen in **Figure 48**, 5-ASA release is already significant in the *upper* GIT under the investigated conditions. Please note that the performance of this type of drug delivery system essentially depends on the pH of the environment the pellets are exposed to. Lialda tablets are matrices consisting of hydrophilic and lipophilic compounds [sodium-carmellose, sodium carboxymethylstarch (type A), talc, stearic acid, and carnauba wax], in which the drug is incorporated. These controlled release matrix tablets are coated with a blend of Eudragit L and Eudragit S: two poly (acryl methacrylates). As it can be seen in **Figure 48**, 5-ASA release is effectively suppressed in the release media simulating the contents of the *upper* GIT under the investigated conditions. Once the systems are exposed to the colonic media, drug release starts. Interestingly, the presence/absence of fecal samples under these conditions did not show a very pronounced effect in any of the investigated formulations.

The newly developed Nutriose:ethylcellulose coated pellets provide the major advantage: (i) to be a multiple unit dosage form, allowing for less variability in the gastric transit times, a more homogeneous distribution throughout the contents of the GIT and the avoidance of the “all-or-nothing” effect of single unit dosage forms, (ii) to effectively suppress drug release in the *upper* GIT, (iii) to provide time-controlled drug release in the colon, the onset of which is induced by enzymes that are present in the colon of inflammatory bowel diseases, (iv) to contain the starch derivative Nutriose, which is known to exhibit a significant pre-biotic activity, normalizing the microflora in the patients’ colon.



**Figure 48:** 5-ASA release from different commercially available products under conditions simulating the transit through the entire GIT, with fecal samples from inflammatory bowel disease patients. The dipping speed was 10 dpm. For reasons of comparison also drug release in culture medium without fecal samples is shown (dotted lines).

### **3.4.3. Conclusions**

Novel polymeric films coatings are proposed based on Nutriose:ethylcellulose blends allowing for the site specific delivery of drugs (e.g., 5-ASA) to the colon. Importantly, these new polymeric barriers are adapted to the conditions at the target site, especially with respect to the microflora in the disease state and pH of the environment. Furthermore, Nutriose is known to exhibit significant pre-biotic effects, normalizing the microflora in the colon, which is particularly beneficial for patients suffering from inflammatory bowel diseases.



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## **5. Summary**



The site specific delivery of drugs to the colon can be highly advantageous for various applications, including: (i) the local treatment of inflammatory bowel diseases, and (ii) the oral administration of protein drugs, which are to be absorbed into the blood stream. In the first case, premature drug release into the stomach is likely to lead to complete and rapid drug absorption into the systemic circulation. Thus, the risk of undesired side effects can be considerable, and at the same time the resulting drug concentrations at the site of action (in the colon) are low, leading to poor therapeutic efficacies. In the second case, fragile protein drugs need to be effectively protected against the low pH and enzymatic degradation within the *upper* gastro intestinal tract (GIT). Thus, in both cases, premature release into the contents of the stomach and small intestine must be avoided. In contrast, once the colon is reached, the drug should be released (in a time-controlled manner) to allow for local drug action in the case of inflammatory bowel diseases or to allow for drug absorption into the blood stream in the case of protein drugs with systemic effects.

Several strategies have been reported in the literature in order to provide such site specific drug delivery to the colon. Most of them are based on the incorporation of the drug within a polymeric matrix or on the coating of a drug reservoir with a polymeric film. In both cases, the macromolecular networks should be poorly permeable for the drug in the *upper* GIT, but become permeable once the colon is reached. To provide this change in drug permeability, the delivery system might: (i) be sensitive to the changes in the pH along the GIT, (ii) be preferentially degraded by enzymes, which are located in the colon, or (iii) undergo significant structural changes, e.g. crack formation in poorly permeable coatings once the colon is reached. Alternatively, the dosage form might release the drug right from the beginning (in the stomach), but at a rate that is sufficiently low to allow for drug release throughout the GIT, including the colon. However, great caution must be paid, because the conditions in a patient's colon might significantly differ from those in the physiological state. For instance, it is well known that the pH and transit times in the various GIT segments as well as the types and concentrations of enzymes in the colon of patients suffering from Crohn's disease and ulcerative colitis can fundamentally vary from those in a healthy subject. Thus, a dosage form might reliably deliver the drug to the target site in a healthy subject, but fail in a *patient*. Furthermore, considerable inter- and intra- individual variability in the therapeutic efficacy might be observed. To avoid these major disadvantages, the drug delivery system needs to be adapted to the disease state of the patient.

In this work, novel types of polymeric film coatings have been developed, which allow for colon targeting *under the pathophysiological conditions* in patients suffering from

inflammatory bowel diseases. These films consist of blends of different types of starch derivatives and ethylcellulose. The starch derivative is water-soluble and preferentially degraded by enzymes secreted by the microflora present in the colon of Crohn's disease and ulcerative colitis patients. Ethylcellulose is water-insoluble and avoids premature film dissolution in the *upper* GIT. Based on the water uptake and dry mass loss kinetics as well as on the changes in the mechanical properties of thin polymeric films upon exposure to release media simulating the contents of the GIT the following starch derivatives could be identified as being most promising for this type of advanced drug delivery systems: Nutriose FB 06 (a branched dextrin with non digestible glycoside linkages:  $\alpha$ -1,2 and  $\alpha$ -1,3), Lycoat RS 780 (a pregelatinized modified starch), Glucidex 1 (a maltodextrin), Eurylon 7 A-PG (an acetylated and pregelatinised high amylose starch), Eurylon 6 A-PG (an acetylated and pregelatinised high amylose starch) and Eurylon 6 HP-PG (a hydroxypropylated and pregelatinised high amylose starch).

Importantly, it could further be shown how desired membrane properties (in particular the water uptake and dry mass loss kinetics as well as the mechanical stability) can effectively be adjusted to the specific needs of particular drug treatments. Different highly efficient and easy to apply tools were identified allowing to alter the membranes' properties, especially their mechanical resistance required to withstand the shear forces resulting from the motility of the upper GIT and the hydrostatic pressure built up within the devices upon contact with aqueous media. This includes the variation of the starch derivative:ethylcellulose blend ratio and initial plasticizer content.

Furthermore, 5-Aminosalicylic acid (5-ASA)-loaded beads were prepared by extrusion-spheronisation and coated with different types of Nutriose:ethylcellulose blends. *In vitro* drug release from these systems was measured under various conditions, including the exposure to fecal samples from inflammatory bowel disease patients under *anaerobic* conditions. Interestingly, the release of 5-ASA (which is commonly used for the local treatment of inflammatory bowel diseases) could effectively be suppressed upon exposure to release media simulating the conditions in the *upper* GIT, irrespective of the degree of agitation and presence or absence of enzymes. In contrast, drug release started as soon as the pellets came into contact with fecal samples of inflammatory bowel disease patients and continued in a time-controlled manner.

Thus, this novel type of colon targeting system is adapted to the pathophysiology of the patient. In addition, the starch derivative Nutriose also exhibits significant pre-biotic

activity, normalizing the microflora in the patients' colon, which is of major clinical benefit in the case of inflammatory bowel diseases.

## **6. Zusammenfassung**

Die örtlich kontrollierte Freisetzung eines Wirkstoffes im Dickdarm kann für diverse Anwendungen erhebliche Vorteile bieten, unter anderem: (i) die lokale Behandlung von entzündlichen Dickdarmerkrankungen, und (ii) die orale Administration von Protein-basierten Arzneistoffen, die systemisch wirken sollen. Im ersten Fall führt eine frühzeitige und vollständige Freisetzung im Magen in der Regel zu schneller Aufnahme in den Blutkreislauf. Daher kann das Risiko von unerwünschten Nebenwirkungen erheblich sein. Außerdem sind die resultierenden Arzneistoffkonzentrationen am Wirkort (im Dickdarm) gering, was zu geringer therapeutischer Effizienz führt. Im zweiten Fall müssen Protein-basierte Arzneistoffe vor dem niedrigen pH und enzymatischem Abbau im *oberen* Gastro Intestinal Trakt (GIT) geschützt werden. Das heißt in beiden Fällen muss eine vorzeitige Freisetzung im Magen und Dünndarm vermieden werden. Sobald die Arzneiform den Dickdarm erreicht, sollte der Wirkstoff zeitlich kontrolliert freigesetzt werden, um eine lokale Arzneistoffwirkung im Falle von entzündlichen Dickdarmerkrankungen zu gewährleisten oder um die Resorption von Protein-basierten Arzneistoffen mit systemischer Wirkung zu erlauben.

Mehrere Strategien sind in der Literatur beschrieben, um eine derartige, örtlich-kontrollierte Wirkstofffreisetzung zu gewährleisten. Die meisten basieren auf dem Prinzip, dass der Arzneistoff in eine Polymermatrix eingebettet wird, oder ein Arzneistoffdepot von einem polymeren Film umgeben wird. In beiden Fällen sollte das makromolekulare Netzwerk im *oberen* GIT wenig permeabel für den Arzneistoff sein. Sobald der Dickdarm erreicht wird, sollte die Arzneistoffpermeabilität zunehmen. Um diese Veränderung in der Arzneistoffpermeabilität zu gewährleisten, kann das System: (i) auf pH-Wertänderungen entlang des GIT reagieren, (ii) von Enzymen degradiert werden, die hauptsächlich im Dickdarm lokalisiert sind, oder (iii) strukturelle Veränderungen (z.B. Rissbildung in wenig permeablen Überzügen) zeigen, sobald der Dickdarm erreicht ist. Alternativ kann die Wirkstofffreisetzung gleich im Magen beginnen, allerdings mit einer ausreichend niedrigen Freisetzungsgeschwindigkeit, die garantiert, dass noch genügend Wirkstoff in der Arzneiform vorhanden ist, sobald der Dickdarm erreicht wird.

Jedoch ist große Vorsicht geboten, da sich die Bedingungen im Dickdarm von *Patienten* sehr von denen in gesunden Probanden unterscheiden können. Zum Beispiel ist bekannt, dass sich der pH-Wert als auch die Verweildauern in den einzelnen GIT Abschnitten sowie die Arten und Konzentrationen der Enzyme im Dickdarm von Patienten, die unter Morbus Crohn oder Colitis Ulcerosa leiden, fundamental unterscheiden können von denen im Dickdarm gesunder Probanden. Demzufolge kann eine Arzneiform, die in der Lage ist, den Wirkstoff gezielt im Dickdarm unter *physiologischen* Bedingungen freizusetzen in *Patienten*

versagen. Darüber hinaus kann die intra- und inter-individuelle Variabilität bezüglich der therapeutischen Effizienz sehr groß sein. Um diese fundamentalen Nachteile zu vermeiden, muss die Arzneiform an die pathophysiologischen Bedingungen der Patienten angepasst sein.

In dieser Arbeit wurden neue polymere Filmüberzüge entwickelt, die eine örtlich-kontrollierte Freisetzung im Dickdarm unter *pathophysiologischen Bedingungen* in Patienten, die unter entzündlichen Dickdarmerkrankungen leiden, ermöglichen. Diese Filmüberzüge bestehen aus Mischungen unterschiedlicher Stärkederivate und Ethylcellulose. Das Stärkederivat ist wasserlöslich und wird bevorzugt im Dickdarm von Enzymen degradiert, die in Morbus Crohn und Colitis Ulcerosa Patienten in ausreichender Menge von der Mikroflora in das Darmlumen sekretiert werden. Ethylcellulose ist wasserunlöslich und verhindert eine vorzeitige Filmauflösung im *oberen* GIT. Basierend auf den Wasseraufnahme- und Trockengewichtsverlust-Kinetiken sowie auf den Veränderungen der mechanischen Eigenschaften von dünnen Polymerfilmen nach Exposition zu Freisetzungsmitteln, die den Inhalt des GIT simulieren, konnten folgende Stärkederivate als die vielversprechendsten für diese Art von innovativen Arzneiformen identifiziert werden: Nutriose FB 06 (ein verzweigtes Dextrin mit nicht verdaulichen Glycosidbindungen:  $\alpha$ -1,2 und  $\alpha$ -1,3), Lycoat RS 780 (eine pregelatinierte, modifizierte Stärke), Glucidex 1 (ein Maltodextrin), Eurylon 7 A-PG (eine acetylierte, pregelatinierte Stärke mit hohem Amyloseanteil), Eurylon 6 A-PG (eine acetylierte, pregelatinierte Stärke mit hohem Amyloseanteil) und Eurylon 6 HP-PG (eine hydroxypropylierte, pregelatinierte Stärke mit hohem Amyloseanteil).

Es konnte weiterhin gezeigt werden, dass gewünschte Filmüberzugseigenschaften (insbesondere Wasseraufnahme- und Trockengewichtsverlustkinetiken sowie mechanische Stabilität) effizient eingestellt werden können, um den spezifischen Anforderungen einer bestimmten Arzneistofftherapie zu entsprechen. Verschiedene, einfach anwendbare und hochwirksame Methoden wurden identifiziert, um die Membraneigenschaften zu ändern, insbesondere deren mechanische Resistenz, die erforderlich ist, um den Scherkräften, die durch die Motilität des GIT verursacht werden, sowie den hydrostatischen Kräften, die durch einströmendes Wasser verursacht werden, zu widerstehen. Dazu gehören die Veränderung des Stärkederivat:Ethylcellulose Mischungsverhältnisses sowie der initiale Weichmachergehalt.

Darüber hinaus wurden 5-Aminosalicylsäure-haltige Pellets durch Extrusion-Sphäronisation hergestellt und mit verschiedenen Nutriose FB 06:Ethylcellulose Mischungen überzogen. Die *in vitro* Arzneistofffreisetzung aus diesen Systemen wurde gemessen unter den verschiedensten Bedingungen, unter anderem nach Exposition zu Fäkalproben von Patienten, die unter entzündlichen Dickdarmerkrankungen leiden unter *anearoben*

Verhältnissen. Interessanterweise konnte die Freisetzung der 5-Aminosalicylsäure (dem Standardarzneistoff zur lokalen Behandlung von entzündlichen Dickdarmerkrankungen) im *oberen* GIT effizient unterdrückt werden, unabhängig von der Intensität und Art der Agitation des Freisetzungsmediums und der Anwesenheit/Abwesenheit von Enzymen. Im Gegensatz dazu setzte die Arzneistofffreisetzung ein, sobald die Pellets mit fäkalen Proben von Patienten mit entzündlichen Dickdarmerkrankungen in Kontakt kamen und war zeitlich kontrolliert.

Somit sind die neu entwickelten Arzneiformen, die eine örtlich-kontrollierte Wirkstofffreisetzung im Dickdarm erlauben, angepasst an die Pathophysiologie der Patienten. Darüber hinaus besitzt das Stärkederivat Nutriose eine prä-biotische Aktivität, die die Mikroflora der Patienten normalisiert. Dies ist von besonderer klinischer Bedeutung im Falle von Morbus Crohn oder Colitis Ulcerosa Patienten.

## **7. Publications & Presentations Resulting from this work**



### Original Research Articles

1. **Karrout, Y.**, Neut, C., Wils, D., Siepmann, F., Deremaux, L., Dubreuil, L., Desreumaux, P., Siepmann, J., Novel polymeric film coatings for colon targeting. *submitted*.
2. **Karrout, Y.**, Neut, C., Wils, D., Siepmann, F., Deremaux, L., Desreumaux, P., Siepmann, J., Novel polymeric film coating for colon targeting: How to adjust desired membrane properties. *submitted*.
3. **Karrout, Y.**, Neut, C., Wils, D., Siepmann, F., Deremaux, L., Desreumaux, P., Siepmann, J., Ethylcellulose:starch-based film coatings for colon targeting. *submitted*.
4. **Karrout, Y.**, Neut, C., Wils, D., Siepmann, F., Deremaux, L., Flament, M-P, Dubreuil, L., Desreumaux, P., Siepmann, J., Novel polymeric film coatings for colon targeting: Drug release from coated pellets. *submitted*.

### Patent Application

1. Siepmann, J., **Karrout, Y.**, Wils, D., Water insoluble polymer: modified starch derivative-based film coatings for colon targeting. EP Application 2008

### Oral Presentation

1. **Karrout, Y.**, Neut, C., Wils, D., Deremaux, L., Desreumaux, P., Flament, M.P., Siepmann, F., Dubreuil, L., Siepmann, J., Novel polymeric film coatings for colon targeting. 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, Spain, Proceedings # 83, 2008.

## **Poster Presentations**

1. **Karrout, Y.**, Neut, C., Wils, D., Deremaux, L., Cherifi, H., Parmentier, E., Desreumaux, P., Flament, M.P., Siepmann, F., Dubreuil, L., Siepmann, J., Novel polymeric film coatings for colon targeting. Innovation in drug delivery: From biomaterials to devices, (ADRITELF/APGI), Naples, Italy, Proceedings # 149, 2007.
2. **Karrout, Y.**, Neut, C., Wils, D., Deremaux, L., Cherifi, H., Desreumaux, P., Flament, M.P., Siepmann, F., Dubreuil, L., Siepmann, J., Physicochemical characterisation of thin free polymeric films for site specific delivery in the GIT. 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, (APV/APGI) Barcelone, Espagne, Proceedings # 204, 2008.
3. **Karrout, Y.**, Neut, C., Wils, D., Deremaux, L., Cherifi, H., Desreumaux, P., Flament, M.P., Siepmann, F., Dubreuil, L., Siepmann, J., Mechanical stability of novel polymeric film coatings for colon targeting. 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, (APV/APGI) Barcelone, Espagne, Proceedings # 205, 2008.
4. **Karrout, Y.**, Neut, C., Wils, D., Deremaux, L., Desreumaux, P., Flament, M.P., Siepmann, F., Dubreuil, L., Siepmann, J., Novel polymeric film coatings for colon targeting. Coating Workshop, (APGI), College of Pharmacy, University of Lille, Lille, France, Proceedings # 183, 2008.

## **8. Curriculum Vitae**

Aus Datenschutzgründen kann der Lebenslauf nicht veröffentlicht werden.