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In vitro evaluation of coating polymers for enteric coating and human ileal targeting

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Abstract

Recombinant interleukin-10 producing *Lactococcus lactis* is an alternative therapy for Crohn's disease. For in vivo interleukin-10 production, thymidine, the essential feed component of these recombinant bacteria should be coadministered. Different coating polymers were evaluated in vitro for enteric properties and targeting suitability to the ileum, the major site of inflammation in Crohn's disease. To guarantee ileal delivery, the polymer must dissolve from pH 6.8 and allow complete release within 40 min. Aqoat® AS-HF coated pellets (15%) showed poor enteric properties and thymidine was released below pH 6.8. Eudragit® FS30D coated pellets (15%) showed good enteric properties, but no thymidine was released within 40 min at pH 6.8. Eudragit® S coated pellets (15%) showed good enteric properties after curing at elevated temperature while no thymidine was released within 40 min at pH 6.8. In another approach to pass the proximal small intestine intact, pellets were coated with 30% Eudragit® L30D-55. At pH 6.0, they showed a lag-phase of 20 min. No influence of layer thickness was seen above pH 6.5. Alternatively, pellets were coated with a mixture of Eudragit® FS30D/L30D-55 but they showed poor enteric properties and thymidine was released below pH 6.8. In conclusion, none of the tested polymers/mixtures ensured enteric properties and ileal targeting.

Keywords: Thymidine; Ileum; Crohn; Coating; Eudragit; Aqoat

1. Introduction

Genetically modified *Lactococcus lactis* that secrete human interleukin-10 (hIL-10) provide a novel therapeutic approach for Crohn's disease, as IL-10 plays a central role in down-regulating inflammatory cascades (van Deventer et al., 1997). Steidler et al. (2000) showed that by the administration of IL-10 secreting *L. lactis* to the intestine of mice, in which experimental enterocolitis was installed, the intestinal inflammation was either cured or prevented. As the biosafety issue raises on the use of genetically modified organisms, a strain was constructed in which the essential gene encoding for thymidilate synthetase is knocked out to assure self-containment of the bacteria (Steidler et al., 2003). For in vitro growth until saturation, 5×10^6 cfu

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of this strain requires 2.45 μg thymidine. When this strain is devoid of thymidine, its viability (cfu) drops six orders of magnitude in approximately 60 h. This system of biological containment was found to be functional in vivo in pigs (Steidler et al., 2003). To enable in vivo hIL-10 production and hence effective treatment of Crohn's disease, a formulation of both hIL-10 producing *L. lactis* and thymidine should be developed.

Although Crohn's disease can occur in any area of the gastro-intestinal tract, the site of inflammation is mainly localised in the more distal regions of the small intestine i.e. the ileum (Both et al., 1983). For effective delivery of IL-10, the viability and involving metabolic activity of *L. lactis* at the target site must be ensured. Klijn et al. (1995) showed that only up to 2% of the amount of *L. lactis* consumed are recovered in the faeces indicating that the gastrointestinal environment negatively influences its viability. This implies development of a formulation that protects the bacteria from the detrimental gastric fluid and the bile salts. To avoid associated risk of penetration of the detrimental gastric fluid into the formulation because of prolonged gastric residence time, pellets were chosen as a multiple-unit delivery system to obtain fast gastric emptying (Krämer and Blume, 1994).

Because of their active ileal absorption, the bile salt concentration is at lowest in the distal part of the small intestine (Northfield and McColl, 1973). Therefore, the development of a formulation for targeting *L. lactis* to the ileum is required. Besides, specific ileum targeting of thymidine is also required to ensure the availability of thymidine for the survival and hence hIL-10 production by *L. lactis*. From literature it is clear that the pH in the ileum ranges from 6.6 to 8.3 (Evans et al., 1988; Fallingborg et al., 1989, 1998; Sasaki et al., 1997; Friend, 1998; Press et al., 1998; Ewe et al., 1999). The mean transit in the small intestine is relatively constant and ranges from 3 to 4 h (Friend, 1998; Abrahamsson et al., 1996; Gupta et al., 2001). Davis et al. (1986) reported that the residence time of pellets in the jejunum is 2 h, and approximately 1.5 h in the ileum. In patients with Crohn's disease, transit time can be dramatically decreased because of diarrhoea. In this study, a pH-dependent approach will be examined in an attempt to reach ileum targeting. To guarantee ileal delivery of thymidine and *L. lactis* in all patients, the pH-sensitive polymer must dissolve from pH 6.8 and allow fast and complete release in 40 min to ensure at any time a sufficient amount of thymidine and *L. lactis* released and a quick start of its hIL-10 secretion at the site of inflammation. Three pH-sensitive polymers, dissolving from pH 6.8, available as and/or applicable as aqueous dispersion and pharmaceutically approved were tested: Aqoat® AS-HF (a polymer consisting of hydroxypropyl methylcellulose acetate succinate and available as a fine powder), Eudragit® FS 30 D (an anionic copolymer of methyl acrylate, methyl methacrylate and methacrylic acid and available as a 30% aqueous dispersion) and Eudragit® S (an anionic copolymer of methacrylic acid and methyl methacrylate (1:2), available as a fine powder and redispersable in water by partial neutralisation with NH_4OH). In another attempt to obtain ileal targeting, pellets were coated with a thicker coat of Eudragit® L30D-55 (an anionic copolymer of methacrylic acid and ethylacrylate (1:1) and available as a 30% aqueous dispersion) in order to ensure intact passage through the proximal small intestine (Harris and Ghebre-sellassie, 1997). Next, the pellets were coated with a mixture of Eudragit® FS 30 D/L30D-55 to manipulate release profile (Khan et al., 1999).

The main objective of this study was to evaluate the suitability of the available coating polymers for enteric coating and ileal targeting of thymidine and *L. lactis*. However, as the formulation of *L. lactis* is still under development, thymidine pellets will be used for adequate evaluation of the coating polymers as this molecule is easier to monitor and quantify (UV-spectrophotometry). Moreover, because of its pH-independent (pK_a 9.94) and high water-solubility (5.5 g/100 ml) (80% thymidine released within 20 min from microcrystalline cellulose pellets at all pH values ranging form 2.5 to 7.4), thymidine (MW 242) has excellent properties for adequate evaluation of enteric properties of the pellets, coated with different coating polymers. On the basis of the thymidine release data, the polymers will be discussed for their gastric protection and ileal targeting properties of *L. lactis* in order to ensure its viability at the target site if administered in the same pellet formulation.

2. Materials and methods

2.1. Production of pellets

Pellets were prepared with a thymidine concentration of 1% (w/w). Thymidine (7 g) (Alkemi, Lo-

keren, Belgium) and microcrystalline cellulose (693 g) (Avicel® PH 101, FMC, Brussels, Belgium) were preblended and granulated with 700 ml demineralized water in a planetary mixer (Kenwood Major Classic, Hampshire, UK). Extrusion was performed in a single screw extruder (Dome extruder lab model DG-L1, Fuji Paudal, Tokyo, Japan) at 45 rpm, through a 1 mm perforated screen. A 600 g extrudate was spheronized on a spheronizer (Caleva model 15, Sturminster Newton, UK), using a cross-hatched friction plate, operating at 1000 rpm with a residence time of 5 min. A 600 g wet spheres were dried in a fluid bed dryer (GPCG1, Glatt, Binzen, Germany) for 90 min at an inlet air temperature of 35 °C. The 700–1250 μm fraction was separated using a vibratory sieve (VE 1000, Retsch, Haan, Germany) for 20 min at amplitude 2.

2.2. Preparation of coating dispersions

2.2.1. Aqoat® AS-HF

Three different coating dispersions of Aqoat® were prepared using Aqoat® AS-HF powder (Shin-Etsu Chemical Co., Tokyo, Japan) (Table 1). The coating dispersion A was prepared by first dissolving triethyl citrate (TEC, plasticiser) (Sigma–Aldrich, Bornem, Belgium) and sodium lauryl sulphate (wetting agent) (Federa, Brussels, Belgium) in water according to the producers guidelines. Next, Aqoat® AS-HF and than talc (glidant) (Alpha pharma, Nazareth, Belgium) were gradually added while stirring. The dispersion was mixed with a high speed mixer (Silverson, Bucks, England) for additionally 10 min. The coating dispersions B and C were prepared by first dissolving TEC in water (Nykänen et al., 1999). Next, Aqoat® AS-HF and than magnesium stearate (glidant) (Alpha pharma, Nazareth, Belgium) were gradually added while stirring. The dispersion was mixed with a high speed mixer for additionally 10 min.

2.2.2. Eudragit® FS 30 D and Eudragit® L30D-55

The composition of the coating dispersions containing Eudragit® FS 30 D and Eudragit® L30D-55 is shown in Table 2. To prepare the Eudragit® FS 30 D coating dispersion a 30% (w/w) aqueous Eudragit® FS 30 D dispersion was used (Röhm, Darmstadt, Germany). Polysorbate 80 (wetting agent) (Tween® 80, Alpha pharma, Nazareth, Belgium) and glyceryl monostearate (glidant) (Federa, Braine-l'Alleud, Belgium) were added to water and stirred for 10 min with a high-speed mixer until a fine, homogenous dispersion was obtained. This dispersion was gently added to the Eudragit® FS 30 D dispersion and mixed by magnetic stirring. For the Eudragit® L30D-55 coating dispersion, the preparation was identical, except that TEC was used as a plasticiser. For the Eudragit® FS 30 D coating dispersions no plasticiser was needed in the formulation since Eudragit® FS 30 D exhibits a minimum film-forming temperature (MFT) of 14 °C (Röhm, Germany).

2.2.3. Eudragit® S

The composition of the coating dispersions containing Eudragit® S is shown in Table 3. The three dispersion differ in plasticiser type and content: 60% TEC in A, 40% TEC in B and 60% dibutyl sebacate (DBS) (Sigma–Aldrich, Bornem, Belgium) in C. To prepare the Eudragit® S coating dispersion A and B, Eudragit® S powder (Röhm, Darmstadt, Germany) was dispersed in water. Addition of 1 M ammonia drop-wise to the aqueous suspension over 5 min resulted in neutralisa-

Table 1
Composition of coating dispersions containing Aqoat® AS-HF

	A		B		C	
	Total (g)	Dry (g)	Total (g)	Dry (g)	Total (g)	Dry (g)
Aqoat® AS-HF powder	10	10	10	10	10	10
Mg-stearate	–	–	3	3	3	3
Talc	2	2	–	–	–	–
Triethyl citrate	3.5	3.5	3.5	3.5	5	5
Sodium lauryl sulphate	0.2	0.2	–	–	–	–
Water	83.5	–	83.5	–	83.5	–
Polymer content (%, w/w)	10.1			10.0		9.9
Solid content (%, w/w)	15.8			16.5		17.7

Table 2
Composition of coating dispersions containing Eudragit® FS 30 D, Eudragit® L30D-55 and a mixture of Eudragit® FS 30 D/L30D-55

	Eudragit® FS 30 D		Eudragit® L30D-55		Mixture	
	Total (g)	Dry (g)	Total (g)	Dry (g)	Total (g)	Dry (g)
Eudragit® FS 30 D 30% (w/w) aqueous disp.	55	16.5	–	–	42.4	12.7
Eudragit® L30D-55 30% (w/w) aqueous disp.	–	–	51	15.3	10.6	3.2
Glyceryl monostearate	1.3	1.3	1.3	1.3	1.3	1.3
Tween® 80 (33% aq. sol.)	1.6	0.5	1.6	0.5	1.6	0.5
Triethyl citrate	–	–	3.1	3.1	0.6	0.6
Water	41.7	–	43	–	45	–
Polymer content (%, w/w)	16.4		15.3			15.6
Solid content (%, w/w)	18.4		20.2			18.0

tion of 15% of the carboxyl groups of the polymer. A milky latex was formed. After additional stirring for 1 h, TEC was added. After overnight stirring, a glyceryl monostearate (GMS)-dispersion, prepared by homogenising GMS for 10 min in a polysorbate 80–water mixture using a high-speed mixer, was added to the polymer dispersion. To prepare the Eudragit® S coating dispersion C, DBS was mixed with the Eudragit® S powder in a mortar by pestle. After overnight standing, the powder mixture was dispersed in water.

2.2.4. Mixture Eudragit® FS 30 D/L30D-55

The composition of the coating dispersion containing a mixture of Eudragit® FS 30 D/L30D-55 (80/20, w/w) is shown in Table 2. The preparation of the dispersion is identical to the method described in Section 2.2.2. The plasticiser amount is calculated as 20% (w/w) on Eudragit® L30D-55.

2.3. Coating of pellets with different coating dispersions

The coating dispersions were passed through a 0.3 mm sieve before use. Throughout the coating process the coating dispersions were stirred using a magnetic stirrer. A 300 g of pellets were coated in a fluid bed coating apparatus (GPCG 1, Glatt, Binzen, Germany), used in the bottom spray mode with the Wurster setup (nozzle diameter 0.8 mm; atomising pressure 1.5 bar). The spray rate and the product temperature during the coating process with Aqoat® AS-HF were 8.5 g/min (first 30 min), 10.5 g/min (next 30 min), 11.4 g/min (till the end of the process) and 30 °C and for coating with Eudragit® FS 30 D, Eudragit® L30D-55, a mixture of Eudragit® FS 30 D/L30D-55 and Eudragit® S 4 g/min and 23–25 °C. Before coating, the pellets were preheated to the desired product temperature during coat-

Table 3
Composition of coating dispersions containing Eudragit® S

	A		B		C	
	Total (g)	Dry (g)	Total (g)	Dry (g)	Total (g)	Dry (g)
Eudragit S® powder	15	15	15	15	15	15
Water	97.3	–	97.3	–	97.3	–
Ammonia 1 M	7.6	–	7.6	–	7.6	–
Triethyl citrate	9.2	9.2	6	6	–	–
Dibutyl sebacate	–	–	–	–	9.2	9.2
Glyceryl monostearate	1.2	1.2	1.2	1.2	1.2	1.2
Tween® 80 (33% aq. sol.)	1.5	0.5	1.5	0.5	1.5	0.5
Water	15	–	15	–	15	–
Polymer content (%, w/w)	10.2		10.4			10.2
Solid content (%, w/w)	17.6		15.8			17.6

ing. After coating, the pellets were cured standard for 15 min at the same conditions as the coating process. Thereafter, they were cured for 2 or 5 days on trays at room temperature, 40 or 60 °C, depending on the polymer and coating dispersion used. The pellets were coated with 15–25% (w/w) Aqoat® AS-HF, depending on the coating dispersion used, 15% (w/w) Eudragit® FS 30 D, 10–30% (w/w) Eudragit® L30D-55, 15% (w/w) of the mixture Eudragit® FS 30 D/L30D-55 and 15–20% (w/w) Eudragit® S.

2.4. Dissolution testing

Dissolution testing ($n = 3$) was performed using the reciprocating cylinder method (USP apparatus 3) (Bio-Dis, Vankel, NJ, USA) at a dip rate of 21 dpm using 1 g pellets per vessel (250 ml) with two consecutive media: 0.1N HCl (2 h) and consequently a buffer solution (phosphate buffer 0.2 M) at pH 5.5, 6.0, 6.5, 6.8, 7.0, 7.2 or 7.4, depending on the polymer tested, with a drain time of 10 s in between the two dissolution media. The concentration of thymidine was measured spectrophotometrically (Perkin-Elmer, Zaventem, Belgium) at 267 nm.

2.5. Scanning electron microscopy

The morphology of the coating surface and the coating thickness were examined by scanning electron microscopy (SEM) (Jeol JSM 5600 LV, Jeol, Tokyo, Japan). Pellets were radially sheared and platina coated using a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan). The coating thickness of five pellets was measured at five sites per pellet.

2.6. Modulated differential scanning calorimetry

Films of Eudragit® FS 30 D, Eudragit® L30D-55 and the mixture of Eudragit® FS 30 D/Eudragit® L30D-55 (80/20, w/w) were prepared by casting a thin layer of pure Eudragit® FS 30 D dispersion (30% (w/w) aqueous dispersion), pure Eudragit® L30D-55 dispersion (30% (w/w) aqueous dispersion) and Eudragit® FS 30 D/L30D-55 mixture in a recipient. After drying for 2 days at RT, the films were cured for two more days at RT.

T_g of the films was determined using a model 2920 modulated DSC (TA Instruments, Brussels, Belgium). Approximately 25 mg of sample was placed in an aluminium pan that was hermetically sealed. The sample was heated from −40 to 80–120 °C with an underlying heating rate of 2 °C/min, a modulation period of 60 s and modulation amplitude of 0.5 °C. T_g was reported as the midpoint of the transition. The analysis was performed in duplicate.

3. Results and discussion

Interest in specific targeting to the human ileum is increasing for the treatment of Crohn's disease, but also in the domain of mucosal vaccination. Recent advances in biotechnology resulted in the use live micro-organisms, genetically engineered to express foreign antigens and/or immune stimulating cytokines at the mucosal target site (Mielcarek et al., 2001). Mucosal immunity can best be obtained by local exposure of the antigens to the Peyer's Patches of the gut-associated lymphoid tissue, most prominent in the terminal ileum (Chen, 2000; Kato and Owen, 1994).

3.1. Release of thymidine from pellets coated with Aqoat® AS-HF

Pellets coated with coating dispersion A (15%, w/w) showed sub-optimal enteric properties: the release in 0.1N HCl after 2 h (13.6%) was slightly above the limits indicated in USP/Eur. Pharm. (maximally 10%) (Fig. 1). Increasing the amount of polymer applied to 20% (w/w) did not improve the enteric properties (13.1% release in 0.1N HCl after 2 h). Fig. 2 shows a SEM picture of the cross-section of a pellet coated with 15% (w/w) Aqoat® AS-HF (coating dispersion A). Although the high coating thickness (34.8 ± 6.9 μm ($n = 25$)), the porous appearance of the coating surface could explain the sub-optimal enteric properties. No continuous polymer layer was formed probably due to incomplete coalescence and fusion of the polymer droplets during coating and subsequent curing, despite the fact that, as recommended, triethyl citrate was used as plasticiser in a concentration of 35% (w/w) to the polymer and the product temperature during coating was 30 °C. Curing time and temperature were increased in an attempt to improve film formation and hence coating performance. Increasing curing time to 5 days did not decrease release after 2 h in 0.1N HCl (13.1%). In-

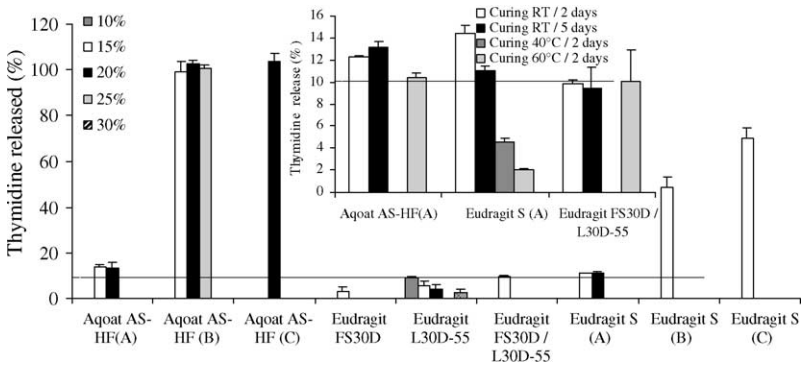

Fig. 1. Thymidine released (mean ± S.D., $n = 3$) after 2 h 0.1N HCl from pellets coated with different amounts of Aqoat® AS-HF (A, B, C), Eudragit® FS 30 D, Eudragit® L30D-55, Eudragit® FS 30 D/L30D-55 mixture and Eudragit® S (A, B, C), cured at room temperature for 2 days. Inserted figure Thymidine released (mean ± S.D., $n = 3$) after 2 h 0.1N HCl from pellets coated with 15% (w/w) Aqoat® AS-HF (A), Eudragit® S (A) and Eudragit® FS 30 D/L30-55 mixture.

creasing curing temperature to 60 °C slightly decreased release after 2 h in 0.1N HCl (10.4%) but the value was still above the limits indicated in USP/Eur. Pharm.

Nykänen et al. (1999) suggested using coating dispersion B (containing triethyl citrate, Aqoat® AS-HF and magnesium stearate (glidant)) for enteric coating of granules containing ibuprofen, but no data were provided on the gastric resistance of the enteric coated granules. When using coating dispersion B, no enteric properties were obtained (Fig. 1). Applying more polymer (up to 25% (w/w)) decreased the release rate of thymidine in 0.1N HCl, but still 100% was released within 2 h. The low solubility of ibuprofen in acidic medium could explain that no problems were reported by Nykänen et al. (1999) in relation to enteric coating efficiency. In contrary, thymidine has a pH independent solubility, so it has excellent properties for evaluating enteric coated formulations and more specifically formulations of acid sensitive compounds such as *L. lactis*. The release of thymidine in HCl 0.1N gives a good indication of the acid permeability of the enteric-coat during passage through the stomach and the possible detrimental effect on *L. lactis*' viability.

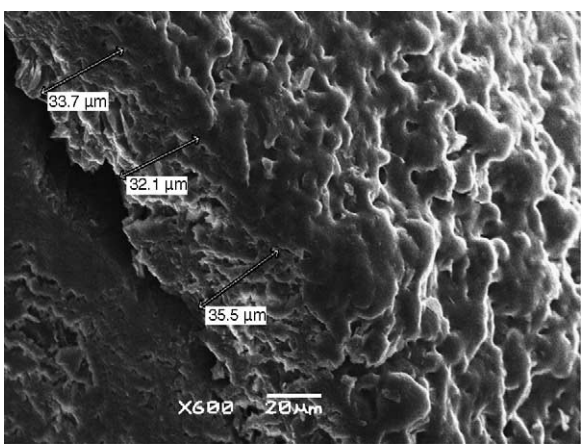

Fig. 2. SEM picture of a cross-section of a pellet coated with 15% Aqoat® AS-HF (w/w) using coating dispersion A (Aqoat® AS-HF 10%, talc 2%, triethyl citrate 3.5%, sodium lauryl sulphate 0.2% and water 83.5%) and cured for 2 days at RT.

On SEM pictures (not shown) the coating surface appeared porous and discontinuous, just as in the case of dispersion A. By varying the plasticiser concentration, the film forming properties of a polymer can be modified. Therefore, the thymidine pellets were coated with coating dispersion C, containing more plasticiser. However, this formulation did neither meet the requirements for enteric-coated dosage forms of USP/Eur. Pharm (Fig. 1).

The poor enteric properties achieved with coating dispersion B and C were probably due to an incompatibility between Aqoat® AS-HF and magnesium stearate as after coating, the pellets were covered with a white and dusty polymer layer.

In this study, the best enteric properties with Aqoat® AS-HF were achieved using coating dispersion A. Before further optimisation of this coating in order to obtain good enteric properties, the suitability of the polymer to obtain ileal targeting was evaluated by studying the release profile (Fig. 3). This release

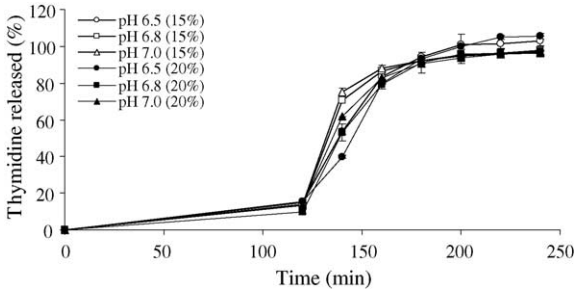

Fig. 3. Release profiles (mean ± S.D., $n = 3$) of thymidine from pellets coated with 15 (- - -, open symbols) and 20% (—, filled symbols) Aqoat® AS-HF (w/w) using coating dispersion A (Aqoat® AS-HF 10%, talc 2%, triethyl citrate 3.5%, sodium lauryl sulphate 0.2%, water 83.5%, cured for 2 days at RT) after 2 h 0.1N HCl and subsequently buffer solution with pH 6.5 (◯), 6.8 (□) or 7.0 (△).

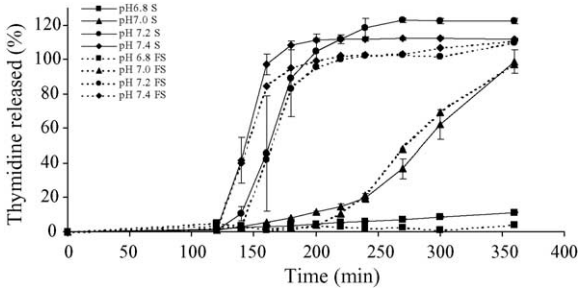

Fig. 4. Release profiles (mean ± S.D., $n = 3$) of thymidine from pellets coated with 15% (w/w) Eudragit® FS 30 D (- - -) and Eudragit® S (—) after 2 h 0.1N HCl and subsequently buffer solution with pH 6.8 (□), 7.0 (△), 7.2 (◯) or 7.4 (◊).

rate increased with increasing pH, while the release rate decreased with increasing amount of coating polymer applied on the pellets. From pellets coated with 15% (w/w), as well as 20% (w/w) polymer, 80% thymidine was released after 40 min dissolution, even at pH values lower than 6.8. The release data indicated the inability of Aqoat® AS-HF to obtain ileal targeting for thymidine incorporated in pellets. This is in contrast to the data of Nykänen et al. (1999) who reported that after 3 h at pH 6.8, only 35% of ibuprofen was released from granules, coated with 20% (w/w) Aqoat® AS-HF. This difference in dissolution profile could be explained by the difference in formulation approach as Nykänen et al. used Aqoat® AS-HF both as binder and coating material to delay drug release from the granules in order to target the colon.

From these data is can be concluded that Aqoat® AS-HF is not suitable neither for enteric coating of thymidine pellets, nor for ileum targeting of thymidine from this pellet formulation.

3.2. Release of thymidine from pellets coated with Eudragit® FS 30 D

Fig. 4 shows the release profiles of thymidine from pellets coated with Eudragit® FS 30 D. After 2 h dissolution in 0.1N HCl less than 10% thymidine (3.0 ± 2.1%) was released, which proves the gastroresistance of the coating at the applied coating thickness (Fig. 1). Fig. 5 shows a SEM picture of the cross-section of a pellet coated with 15% (w/w) Eudragit® FS 30 D. The coating thickness was 25.7 ± 3.1 μm ($n = 25$). This is in agreement with Gupta et al. (2001) who reported a coating thickness of 47 μm on pellets (0.8–1 mm) coated with 30% Eudragit® FS 30 D. Contrary to pellets coated with Aqoat® AS-HF, the coating surface had a smooth appearance and a continuous polymer layer was formed. At pH 6.8 no release was observed after 1.5 h, considered to be the maximal transit time of pellets in the ileum. Release was very slow at pH 7.0: only 11% of thymidine was released after 1.5 h. Only at pH 7.2 and above, 100% thymidine was released within 1.5 h. At pH 7.2, a lag-time of 20 min was observed before the release started. When the requirements of the polymer to allow complete release within 40 min from pH 6.8 are considered, it can be concluded that only at pH 7.4 and above thymidine

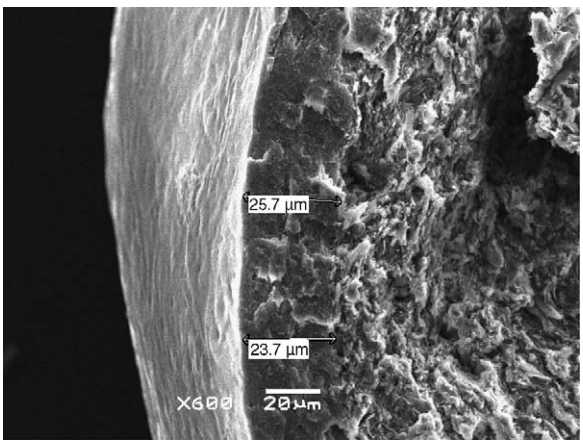

Fig. 5. SEM picture of a cross-section of a pellet coated with 15% (w/w) Eudragit® FS 30 D.

release was completed within 40 min. Below pH 7.0, no thymidine was released. At pH 7.2 and 7.4, 42% and 84% was released within 40 min, respectively.

The pH from which Eudragit® FS 30 D dissolved (pH 7.2) was not in accordance to the value reported in literature (pH 6.8) (Gupta et al., 2001). As the ileal pH can be lower than 7.2, it is evident that in some patients the thymidine release will not occur in the ileum or only at its distal parts. For the co-formulated *L. lactis*, incomplete and/or delayed release will result in a lack of time to become metabolically active and to secrete the hIL-10 at the site of inflammation. This study emphasises clearly the necessity of testing a formulation, developed for specific targeting, at a range of pH-values since little variation in pH leads to remarkable differences in release profiles.

3.3. Release of thymidine from pellets coated with Eudragit® S

Fig. 1 shows that the release from Eudragit® S coated and subsequently RT cured thymidine pellets after 2 h in 0.1N HCl is higher than the limits indicated in USP/Eur. Pharm. (Fig. 1). Increasing the coating thickness to 20% (w/w) could not improve the coating performance. Since Eudragit® S is a tough polymer (T_g 160 °C), contrary to the Flexibel Eudragit® FS 30 D, 60% TEC was added to reach sufficient plastisation of the polymer. But the hydrophilic characteristics of the plasticiser, combined with its high content in the coating layer could lead to pore forming and subsequent release of thymidine in the gastric stage (Frohoff-Hülsmann et al., 1999). Decreasing the plasticiser content (from 60 to 40% TEC) or using a hydrophobic plasticiser (dibutyl sebacate) did not improve the coating performance (Fig. 1). However, increasing the curing temperature to 40 and 60 °C for 2 days markedly improved the coating performance (Fig. 1). This might be due to the fact that because of the toughness of the polymer, higher curing temperatures are required for complete coalescence and hence film formation. Fig. 4 shows that at all pH values, the release profiles of thymidine from pellets coated with 15% Eudragit® S are comparable with the profiles of thymidine from pellets coated with 15% Eudragit® FS 30 D. These results are not in agreement with Rudolph et al. who showed faster release from 5-ASA pellets coated with Eudragit® S than coated with Eudragit® FS 30 D at pH 7.2, both from an aqueous dispersion. About 100% was released within 30 and 360 min, respectively (Rudolph et al., 2001). When the requirements of the polymer to allow complete release within 40 min from pH 6.8 are considered, it can be concluded from our data that only at pH 7.4 and above thymidine release was completed within 40 min. Below pH 7.0, no thymidine was released. At pH 7.0, 7.2 and 7.4, 6%, 45% and 97%, respectively was released within 40 min. Moreover, as *L. lactis* is temperature sensitive, this polymer cannot be used for the production of an enteric coated formulation consisting of this micro-organism because curing has to be performed for 2 days at minimally 40 °C.

3.4. Release of thymidine from pellets coated with Eudragit® L30D-55

The percentage of thymidine released from pellets coated with different amounts of Eudragit® L30D-55 after 2 h in 0.1N HCl is shown in Fig. 1. The amount of thymidine released after 2 h decreased with increasing the coating thickness (9.1 ± 0.7, 5.7 ± 2.0, 4.2 ± 1.8, 2.8 ± 1.1 for 10, 15, 20 and 30%, respectively). For every coating thickness applied, the coated pellets met the requirements of the USP/Eur. Pharm. concerning enteric coated dosage forms. Fig. 6 shows a SEM picture of the cross-section of a pellet coated with 15% (w/w) Eudragit® L30D-55. The coating thickness was 29.5 ± 2.0 μm ($n = 25$), and 61.3 ± 8.6 μm ($n = 25$) for pellets coated with 30% (w/w) polymer (picture not shown). The surface of the pellets coated

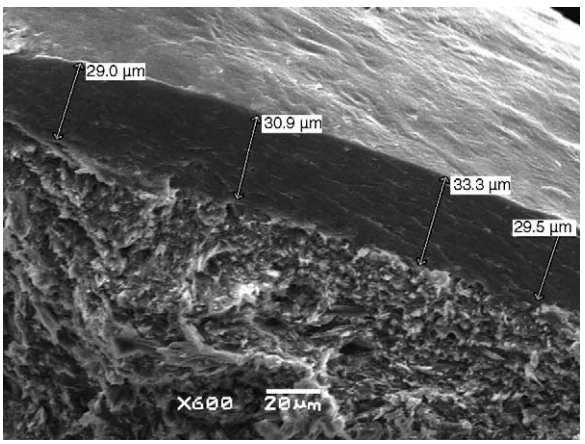

Fig. 6. SEM picture of a cross-section of a pellet coated with 15% (w/w) Eudragit® L30D-55.

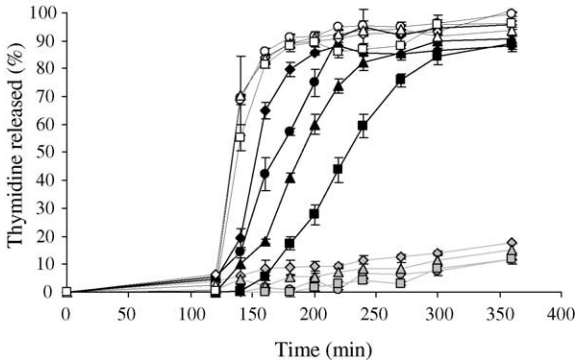

Fig. 7. Release profiles (mean ± S.D., $n = 3$) of thymidine from pellets coated with 10% (◊), 15% (◯), 20% (△) and 30% (□) Eudragit® L30D-55 after 2 h 0.1N HCl and subsequently buffer solution with pH 5.5 (—, grey symbols), 6.0 (—, black symbols) and 6.5 (—, open symbols).

with Eudragit® L30D-55 was smooth. Fig. 7 shows the release profiles of thymidine from pellets coated with different amounts of Eudragit® L30D-55 at pH 5.5, 6.0 and 6.5. At pH 5.5, a very slow thymidine release was observed: 17.5% was released after 4 h from pellets coated with 10% (w/w) Eudragit® L30D-55. The release from pellets coated with a thicker coat was as expected lower (12% after 4 h from pellets coated with 30% (w/w) Eudragit® L30D-55). Fig. 7 clearly shows that at pH 6.0, by increasing the coating thickness, the release rate decreased. However, only a short lag-phase was obtained (20 min) from pellets coated with 30% (w/w) Eudragit® L30D-55. At pH 6.5, increasing the coating thickness did not affect the release rate and in all cases 80% was released within 20 min.

At the generally proposed coating thickness of Eudragit® L30D-55 for multiple unit formulations (10–20% (w/w) polymer weight gain) to reach enteric properties, an in vivo dilution and absorption of thymidine will take place before it reaches the ileum. Moreover, the co-formulated bacteria would be released in the small proximal bowel (pH 6.5 (Evans et al., 1988; Fallingborg et al., 1989, 1998) and this will result in a loss of viability due to the presence of the bile salts. A way to overcome this problem could be the application of a thicker coat of Eudragit® L30D-55 (Harris and Ghebre-sellassie, 1997). A lag-phase of approximately 2 h must be obtained to ensure intact passage through the proximal small intestine.

Klein et al. (2002) studied the dissolution of mesalazine from two types of tablets coated with Eudragit® L30D-55: Claversal® and Salofalk®. A lag-time in buffer pH 6.8 was reported of 30 and 150 min, respectively, due to the difference in coating thickness, which was 100 and 250 μm, respectively. In our study, the coating thickness was only 61.3 ± 8.6 μm for pellets coated with 30% (w/w) Eudragit® L30D-55 and can explain why no lag-time was observed at pH 6.5. Alternatively, the pellets could be coated with Eudragit® L 100 as this polymer dissolves from pH 6.0. However, this polymer exhibits a very high T_g (200 °C), even higher than Eudragit® S. Most likely, this polymer will also require high curing temperatures for adequate film formation and hence good enteric properties. However, this is inappropriate for the *L. lactis*' viability.

3.5. Release of thymidine from pellets coated with a mixture Eudragit® FS 30 D/L30D-55

Fig. 1 shows that pellets coated with a mixture Eudragit® FS 30 D/L30D-55 have no good enteric properties, even after 5 days curing (9.4 ± 2.0% release after 2 h in 0.1N HCl) or curing at 60 °C (10.1 ± 2.9% release after 2 h in 0.1N HCl). However, pellets coated with Eudragit® FS 30 D or L30D-55 only showed good enteric polymers i.e. 3.0 ± 2.1 and 5.7 ± 2% release after 2 h in 0.1N HCl, respectively. Although the similarity in chemical structure of both polymers, it was investigated if these polymers showed any incompatibility by determination of the T_g of several polymer films, prepared by casting the coating dispersions (pure or mixture) and subsequently drying and curing (Fig. 8). The pure Eudragit® FS 30 D and Eudragit® L30D-55 films showed a T_g of 32.0 ± 0.6 °C ($n = 2$) and 56.4 ± 0.4 °C ($n = 2$). A physical mixture of both films showed two T_g signals i.e. the first at 34.8 °C and the second at 54.9 °C. These values are in accordance with the values obtained from the pure films and can be attributed to the Eudragit® FS 30 D and Eudragit® L30D-55 polymer, respectively. The films, prepared by casting a mixture of Eudragit® FS 30 D and Eudragit® L30D-55 (80/20, w/w) showed only one signal (33.3 ± 4.5 °C; $n = 6$). From these results it can be concluded that these polymers show no incompatibility. A hypothesis for the explanation of the increased release after 2 h in 0.1N HCl may be that the plasticiser included in the polymer mixture before coating results

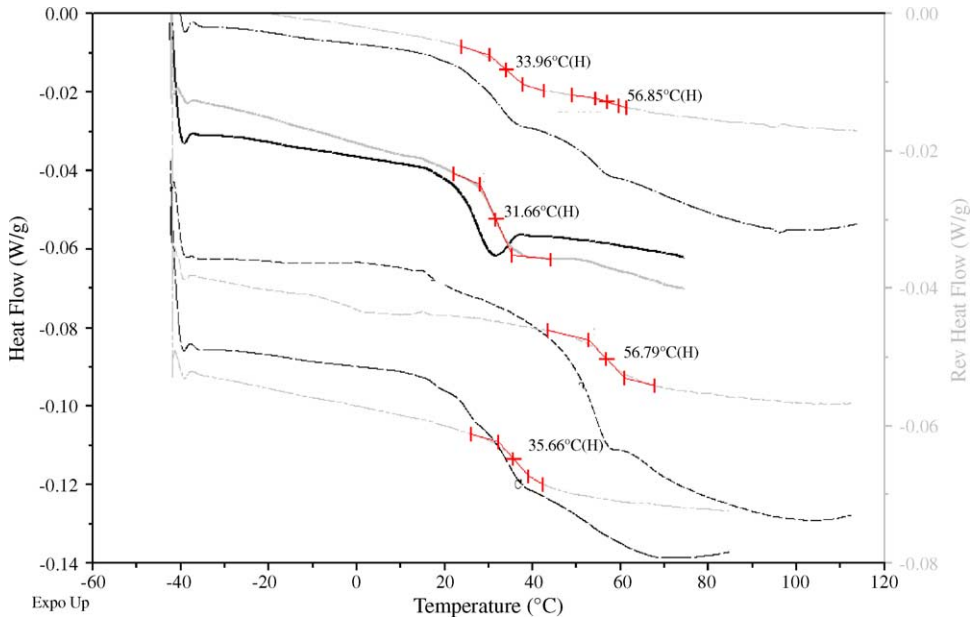

Fig. 8. MDSC curves (Heat Flow (black lines) and Rev Heat Flow (grey lines)) of pure Eudragit® FS 30 D (——), pure Eudragit® L30D-55 (- - -), physical mixture of Eudragit® FS 30 D/L30D-55 (80/20) (—·—·) and of Eudragit® FS 30 D/L30D-55 (80/20) film (—·—) with T_g mentioned on the curves.

in an increased permeability of the film. This may be the case for Eudragit® FS 30 D as this polymer requires no plasticizer because of its inherent flexibility.

Release profiles show a decrease in release rate at pH 6.0, which is comparable with the release rate from pellets coated with 30% (w/w) Eudragit® L30D-55 (data not shown), but no lag-phase was seen. At pH 6.5 and higher, release is completed within 40 min. A hypothetical explanation for these results can be that although the polymers show compatibility, individual spots of pure Eudragit® FS 30 D and Eudragit® L30D-55 can be identified on the surface of the coated pellets. An Eudragit® L30D-55 spot dissolves from 6.0 resulting in the formation of pores, from which the thymidine can be released.

Khan et al. (1999) coated mesalazine tablets using combinations of Eudragit® L100-55 and Eudragit® S100 and showed that the release profile of mesalazine from tablets could be manipulated by changing the Eudragit® L100-55 and Eudragit® S100 ratios within the pH range of 5.5–7.0. In this study, a combination of Eudragit® L30D-55 and Eudragit® FS 30 D was used as they result separately both in good enteric properties after curing at room temperature. Eudragit® S was not used in the mixture as this polymer requires high curing temperatures, which is inappropriate for the viability of *L. lactis*. The polymers were used in a ratio of 1/4 as with this ratio, Khan et al. showed a lag-time of 45 min at pH 6.5. As Khan et al. (1999) showed only a slight increase in lag-phase from 45 to 60 min by changing the ratio of both polymers from 1:4 to 1:5, other ratio's have not been tested in this study.

From this study, it is clear that Aqoat® AS-HF, Eudragit® L30D-55 and a mixture of Eudragit® FS 30 D/L30D-55 dissolve at a pH lower than the pH at the target site (6.8) and consequently thymidine will be diluted and absorbed in the proximal small intestine and that the co-formulated *L. lactis* will be subjected to the detrimental bile salts present in the jejunum. Eudragit® FS 30 D and S dissolve at a pH above the pH at the target site. Consequently, thymidine and the co-formulated *L. lactis* will not be released or will only be released in the most distal parts of the ileum. This implies that the hIL-10 production will not occur in the ileum but in the colon. It can be concluded that none of the tested polymers or polymer mixtures can

guarantee ileal targeting on his own. To circumvent the issue of high gastrointestinal pH variability among individuals, a combination of doses could be used: one dose coated with Eudragit® L30D-55 while another dose coated with Eudragit® FS 30 D. In patients with a high GI-pH profile, thymidine and *L. lactis* will be released in the ileum from the formulation coated with Eudragit® FS 30 D, while in patients with a low GI-pH profile, thymidine and *L. lactis* will be released in the ileum from the formulation coated with Eudragit® L30D-55.

4. Conclusion

It can be concluded that using different polymers and varying the coating thickness allow modulation of the thymidine release from pellets. Only the pellet formulations coated with Eudragit® FS 30 D and L30D-55 met the requirements of USP/Eur. Pharm. concerning enteric-coated formulations (maximally 10% release after 2 h in 0.1N HCl) without any additional rise in curing temperature, while pellets coated with Aqoat®, Eudragit® S, or the mixture of Eudragit® FS 30 D/L30D-55 did not. None of the tested polymers and used mixtures ensured specific targeting to the ileal mucosae. A possible solution for this problem could be the administration of one dose coated with Eudragit® L30D-55 and another coated with Eudragit® FS 30 D. This approach is now used to deliver biological contained hIL-10 secreting *L lactis* in a safety study in patients suffering from Crohn's disease.

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