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RESEARCH ARTICLE

Coated hard capsules as the pH-dependent drug transport systems to ileo-colonic compartment

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Abstract

Purpose: The aim of this study was to investigate the suitability of hard capsules of different composition (gelatin—G, gelatin coated with hydroxypropyl cellulose—G/HPC, and hypromellose—H) for a coating with aqueous dispersion of pH-dependent synthetic polymer Eudragit® FS (E_{FS}) and to evaluate *in vitro* the coated capsules as transport systems for ileo-colonic drug delivery.

Methods: Three sets of hard capsules with increasing coating levels (5–30%) were obtained by Wurster technique. The release of model drug (caffeine) from prepared samples was tested using paddle dissolution method with continual pH change (pH 1.2–2 h, 6.8–4 h and 7.5–2 h).

Results: During the coating process, no problems occurred and similar suitability of capsules materials for E_{FS} application was observed in contrast to some published reports. The application of HPC subcoating onto gelatin capsules surface was shown as the redundant step. The samples G/E_{FS} 10–15% and H/E_{FS} 15–20% with 6 h lag time and fast drug release after the pH adjustment to 7.5 corresponded with the requirements for ileic drug delivery. Samples releasing the drug after the pH change to 7.5 in 2-h interval such as G/E_{FS} 20%, G/HPC/E_{FS} 25% and H/E_{FS} 25% are considered as promising transport systems to ileo-colonic area. Samples G/E_{FS} 25–30%, G/HPC/E_{FS} 30% and H/E_{FS} 30% with 7 h lag time could be used for colon delivery.

Conclusion: The desired intestinal part could be targeted without significant formulation changes only by the selection of capsules shell forming material and suitable E_{FS} coating thickness.

Keywords: Gelatin hard capsules, hypromelose hard capsules, Eudragit® FS, ileum delivery, colon delivery, dissolution test

Introduction

Gastro intestinal tract (GIT) is a very hostile environment, with regions of lower and higher pH, high enzymatic activity and lipid solubilizing ability[1]. For ileic and colonic drug delivery, it is necessary to overcome relatively long part of GIT without any drug activity and its stability changes. For this reason, many laboratories have investigated the transport systems which would protect the administered drug and deliver it to the desirable GIT compartment. Transporting the time-dependent systems to a specific compartment of GIT could be very problematic, as GIT transit time depends on many physiological factors including fluid volume, fluid composition, bacterial flora, GIT motility and pH values, which are further influenced by food ingestion, the type of meal-caloric content, volume, viscosity, physical state, gender and age[2–4]. Pressure-dependent systems and microbial-triggered systems are also highly sensitive to physiological parameters[5]. The development of gastro- and intestine-resistant transport systems with pH-dependent coatings seems to be more effective. These systems utilize polymeric carriers that are insoluble in the low pH media of the upper gastrointestinal tract, but dissolve at a higher, near neutral pH of the distal gut. Such polymers will begin to dissolve in the ileum and as such are more appropriately defined as the materials for ileo-colonic delivery systems. The pH-dependent approach for colonic drug delivery is based on the pH differences along the gastrointestinal

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tract with values increasing from about 1 to 2.5 in the stomach through 6.6 in the proximal small bowel to a peak of about 7.5 in the terminal ileum followed by a fall in pH to 6.4 in the colon[6,7]. The pH in the middle part of colon is reported to be 6.4 and that one in the left-colon 7.0. The decrease of pH value in the beginning of colon is due to the presence of short chain fatty acids arising from the bacterial fermentation of polysaccharides[8]. For the evaluation of pH-dependent coated solid dosage forms intended for ileo-colonic delivery, their mean gastric residence time is considered to be 0.25–2 h[9,10] and small intestinal residence time between 3 and 4 h at the minimum. These conditions are valid for single solid dosage forms and for fasting state of patients. In summary, the essential presumption for coated dosage form intended for distal ileic or proximal colonic delivery is the total GIT resistance and the solubility of polymer coating material in pH corresponding to the ileum. Fast drug release in dissolution medium of pH value above 7.0 could predict drug absorption in the ileic compartment, whereas sustained release of active substance could move the drug absorption to the lower part of GIT, to the colon. Table 1 shows the pH values and transit times of human GIT parts.

The release of active substance in ileic compartment could be useful for oral vaccination. Critical point is the drug delivery near to the Peyer's patches[11]. In humans, the number of Peyer's patches along the length of the gastrointestinal tract increases to 300 at puberty and then declines thereafter. They comprise 10–1000 individual follicles organized into discrete lymphoid structures overlaid by a follicle associated epithelium and are an example of organized gut-associated lymphoid tissue[12]. Lymphoid follicles are also found in the human colon but their function remains unknown.

Terminal ileum and proximal colon could be also primarily affected by several colonic diseases, mainly chronic inflammatory bowel disease—Crohn's disease and ulcerative colitis with unknown origin[13], irritable bowel syndrome[14] and colon cancer[7]. Colonic delivery can be used for the improvement of oral bioavailability of peptides and proteins such as insulin, calcitonin or vasopressin[15], for colonic polyp therapy[5], the prophylaxis of colon cancer, and the treatment of nicotine addiction[7]. The long colonic residence time provides a significant opportunity for the drug absorption[8].

Coated hard capsules could act as primary transport systems for a variety of dosage forms such as powders[16], granulates, pellets[17], microparticles etc. The area of Peyer's patches is permeable for the molecules with higher molecular weight (e.g. peptides) as well as for micro- or nanoparticles. Currently, upper size limit of particulate absorption is controversial. The total uptake of 2.5 μm polystyrene microparticles on one hand and effective absorption of 380-nm sized titanium oxide particles on the other hand were published in scientific reports[18]. Higher permeability of this compartment is caused by the presence of specific receptors with endocytosis activity and by lower quantity of lysozymes inside of the cells[19].

Table 1. Human intestine physiology.

Small intestine region	pH range	Transit time (h)
Duodenum[31]	6.0–6.1	1
Jejunum[31]	6.1–7.2	1
Ileum[31]	7.0–7.8	2
Proximal colon[2]	6.4	6–48
Distal colon[2]	7.0	

Methyl acrylate, methacrylic acid and methyl methacrylate ester copolymer marketed as Eudragit® FS (E_{FS}) which is soluble at pH above 7.0, is commonly used pH-responsive polymer to facilitate drug delivery to the ileo-colonic region. E_{FS} is an anionic copolymer available as the 30% aqueous dispersion; the ratio of the free carboxyl groups to the ester groups is approximately 1:10. The average molecular weight is close to 220,000. The dispersion contains 0.3% of sodium laurylsulphate and 1.2% of Polysorbate 80 as emulsifiers[20].

Aqueous coating dispersions have several advantages over the organic polymer solution systems, such as the lower raw material costs; avoidance of capital cost for solvent recovery and explosion proof equipment, with a safer working environment in the development and production; they are environmentally friendly, have faster processing time, while still providing reliable coating performance, faster development and scale up process[3,21,22]. Aqueous dispersions coating process could be very sensitive especially if gelatin capsules are used. The coating problems such as shell softening and capsule sticking were published[23]. Huyghebaert et al. reported that above-mentioned problems occurred due to the physical properties of gelatin especially due to its solubility in water[24]. To eliminate these problems, the application of hydroxypropyl- or hydroxypropyl methyl cellulose as a subcoat was reported[25]. This step could avoid water evaporation from gelatin shell capsules and thus increasing their brittleness.

Although there are some articles describing the application of E_{FS} 30 D on HPMC capsules, there is no article dealing with direct coating of gelatin capsules with this polymer and focusing on the comparison of coated gelatin and HPMC capsules. Therefore, the aim of this study was to compare suitability of gelatin, gelatin with hydroxypropyl cellulose subcoating and hypromellose capsules for the coating with aqueous dispersion of E_{FS} and to evaluate *in vitro* potential usage of coated capsules for ileum-colonic delivery of incorporated model substance.

Experimental methods

Materials

Caffeine (Jilin Province Shulan Synthetic Pharmaceutical Co. Ltd., Shulan City, China; the particle size range was 2–41μm and the mean particle diameter 9.5 μm) as the

model substance together with soluble filler α-lactose monohydrate (Cerapharm, Vienna, Austria; the particle size range was 5–85 μm and the mean particle diameter 35.9 μm) were filled into hard gelatin capsules of size 4 (Capsugel, Bornem, Belgium) or HPMC capsules of size 4—Vcaps® (Capsugel, Colmar, France). Declared surface area value of this capsule size is 235 mm². E_{FS} 30 D (Evonik Röhm GmbH, Darmstadt, Germany) and hydroxypropyl celullose (HPC)—Klucel EF®—Mr-80 kDa (Aqualon, Wilmington, DE) were used as coating materials. Glycerol monostearate—GMS, Polysorbate 80 and triethyl citrate sulphate (Sigma-Aldrich, Prague, Czech Republic) were added into E_{FS} dispersion to improve the film quality. All materials were of Ph. Eur. quality.

Capsules filling and evaluation

Of 800 hard gelatin capsules and 400 hard HPMC capsules were filled in manual filling machine with caffeine (10.0 g for 100 capsules) and lactose monohydrate (2.3 g for 100 capsules to adjust the volume) mixture. The powder mixture was prepared by drug and excipient homogenization in a mortar. Uniformity of mass and content were evaluated according to Ph. Eur. 6 for each capsules batch (200 of hard capsules) prior to the coating. Caffeine was measured spectrophotometrically at 275 nm. Characterization of hard capsules batches is presented in Table 2.

Hard capsules coating

The prepared batches were marked according to the capsule types, i.e. G—gelatin capsules, G/HPC—gelatin capsules coated with hydroxylpropyl cellulose subcoating and H—hydroxypropyl methyl cellulose hard capsules. Filled capsules (200 pieces) of G, G/HPC and H batches were coated in Wurster-M 100 coater (Medipo ZT, s.r.o., Brno, Czech Republic) with final coating liquid prepared using the technique recommended by the producer. The dispersion contained 61.0% of E_{FS} 30 D aqueous dispersion, 36.8% of distilled water, 0.6% of GMS, 0.7% of Polysorbate 80 and 0.9% of triethyl citrate, the solid polymer content was 18% (w/w)[26]. Polysorbate 80, triethyl citrate and GMS were homogenized in 50% needed water (70–80°C) using an Ultra-Turrax (T25 basic, IKA-Werke, Staufen, Germany) at 10,000 rpm for 10 min. The prepared emulsion was mixed with remaining amount of water and then cooled down to room temperature while stirring with a conventional stirrer. The emulsion was added slowly into E_{FS} dispersion under the stirring (30 min, 300 rpm) and final product was passed through a 0.5-mm sieve.

Caffeine (100 mg/capsule) was selected because of its relatively independent solubility in media of different pH values (1.2, 6.8, and 7.5). To avoid the above-mentioned problem (poor film adhesion) within gelatin hard capsules coating process[23], the HPC intermediate layer was used in batch G/HPC. HPC coating solution contained 9.9 g of HPC in 100.0 g of deionized water. Approximately, 3% coating level of HPC interlayer was formed.

The coating dispersion was continuously stirred to ensure its homogeneity, and it was fed by peristaltic pump to the 1-mm nozzle port. The coating laboratory equipment with small Wurster column was used for this experimental study (the length of the Wurster tube—110 mm, the diameter—40 mm). The process parameters were: inlet air temperature 30°C, air pressure 100 kPa, flow rate 1.0 mL/min. Coating process was interrupted every 15 min, and capsules were dried for 2 min at the same temperature. When approximately 17% (34, 51, …%) of the coating dispersion was applied, the sample of 20 coated capsules was withdrawn and weighed. Coating level was calculated as the percent weight gain between coated and uncoated capsules. If the weight gain was not sufficient because of the coating material loss, the coating process continued to achieve required weight of capsules. All capsules withdrawn for coating thickness measurement and dissolution testing were replaced by uncoated capsules of approximately same weight and colored content, and at the end of the coating process extended. Thus coated hard capsules with 5, 10, 15, 20, 25 and 30% E_{FS} (dry polymer) were obtained for evaluation. The total coating time was 141, 131 and 128 h for G, G/HPC and H set, respectively. Coating of each batch was carried out twice. The curing was performed in an oven for 24 h at 40°C.

Determination of E_{FS} coating thickness

The thickness of E_{FS} film was determined by an optical analysis using the optical microscope (DN 45, Lambda, Prague, Czech Republic) connected to the CCD camera (Alphaphot, Nikon, Tokyo, Japan) and operated by Ia32 software. Ten different positions were measured for each from 10 tested capsules to obtain a mean thickness and a standard deviation of measurement. In the case of samples from G/HPC set, only E_{FS} layers were measured; clearly visible HPC subcoatings were not included in the results.

Drug dissolution profiles

To evaluate the applied coating quality, the dissolution profiles of prepared capsules batches were determined (SOTAX AT 7 On-Line System—Donau Lab,

Table 2. Capsules batches characteristics.

Batch	Film coating material	Hard capsules material	Subcoating	Theoretical content of caffeine (mg)	*Practical content of caffeine (mg)
G/E_{FS}	Eudragit® FS 30 D	gelatin	No	100	106.12 ± 5.23
$G/HPC/ E_{FS}$	Eudragit® FS 30 D	gelatin	HPC	100	102.05 ± 4.58
H/ E_{FS}	Eudragit® FS 30 D	HPMC	No	100	102.98 ± 4.15

*Drug content was determined in uncoated hard capsules.

Zurich, Switzerland) by dissolution test with continual pH change. Sodium triphosphate was used as the pH increasing agent. Medium volume was 900 mL, its temperature was 37 ± 0.5°C, and a stirring rate of 50 rpm was employed. Efficiency of E_{FS} coatings was determined by the released amount of caffeine analyzed in a UV spectrophotometer (Lambda 25, Perkin Elmer, Wellesley, MA) at 275 nm. Dissolution tests were carried out from six hard capsules obtained from both coating processes, respectively. Presented results were calculated as the average value and standard deviation (SD) for caffeine release from 12 coated hard capsules.

Results and discussion

Four batches of hard gelatin capsules and two batches of hypromellose capsules (200 capsules per batch) were filled with the homogenized mixture of caffeine and lactose prior to the coating. Their content uniformity is shown in Table 2 and the mass uniformity in Table 3. Practical content of caffeine was found 102.05–106.12 mg with SD values less than 5.2. The average weight of filled uncoated capsules were laid in the interval 156.7–158.7 mg. Both parameters of prepared samples corresponded to the required pharmacopoeia limits.

Thereafter, coated capsules batches of three types were prepared, i.e. gelatin capsules and hypromellose capsules coated with E_{FS} 30 D aqueous dispersion and gelatin capsules with 3% HPC subcoating and with E_{FS} final coating. In our experiment, we did not observe any of previously published problems associated with direct coating of gelatin capsules using aqueous dispersion of E_{FS}, i.e. shell softening or capsules sticking[23]. The E_{FS} 30 D dispersion applied on the surface of capsules formed transparent, smooth and opaque films. The gap between capsules body and cap was perfectly covered when sufficient (see later) coating level was used (Figure 1B, 1D, and 1E). No film quality differences, no increase in film brittleness were observed in all types of appropriately coated hard capsules. These results differed from our previous study, where G, G/HPC and H capsules were coated with isopropyl alcoholic solution of Eudragit L and S[27]. Here the film adhesion on smooth surface of gelatin capsules was insufficient. Increased brittleness of these capsules was probably due to capsule humidity loss caused by isopropyl alcohol. The application of HPC subcoating brought significant improvement of Eudragit L and S film quality. This might be related to the use of HPC water solution and therefore no capsule humidity loss.

Determination of E_{FS} coating thickness

Theoretical coating amount of prepared samples was calculated as the total weight gain. The summary of prepared coated capsules samples and their average weights is in Table 3. Accurate average thickness of E_{FS} film was determined by an optical analysis using the optical microscope, and the obtained values are summarized in Table 4 together with the amount of dry coating material (mg) applied on 1 cm^2 of capsule surface. In gelatin capsules the coating thickness varied in range 32.0–173.5 µm for E_{FS} (G/E_{FS} set). In gelatin capsules with HPC sublayer the thickness of E_{FS} film (G/HPC/E_{FS} set) was in range 38.1–179.6 µm. The thickness of E_{FS} film applied onto HPMC capsules (H/E_{FS} set) was in range 36.7–198.7 µm (coating amount of each set—5.0–30.0%).

In vitro dissolution studies

The dissolution test with continual pH change was selected to simulate pH values in different GIT parts[28,29]. First 2 h of dissolution test were carried out in 900 mL of dissolution medium with pH 1.2 (artificial gastric juice—AGJ) for 2 h. After this interval, the pH value was changed to 6.8 by adding of 18.7 g of sodium triphosphate. After 4 h in pH 6.8, the value was increased again to pH 7.5 by adding of 5.8 g of sodium triphosphate. E_{FS} coating, as reported, should release no drug within the interval of 6 h in pH 1.2 and 6.8, and it should respond to pH change above its solubility, i.e. 7.0 by fast drug release[28,30].

Six samples of coated gelatin hard capsules (G/E_{FS}) with different coating level (weight gain 5–30%) were prepared. Figure 2 shows the release profiles of caffeine obtained from continual dissolution tests, and Table 5 summarizes standard deviations of average released drug amount.

Sample G/E_{FS} 5% released all the drug within 3 h (Figure 2). The coating was untouched; however, the connection areas of capsules after 4 h in phosphate buffer (PB) were opened as shown in Figure 1A. Due to the capsule opening fast drug release independent on pH with high SD value (20.17%; Table 5) was observed. The applied coating level (thickness 32.0 µm) of this capsule sample was not sufficient for drug transport to the desired, i.e. ileo-colonic, area. The samples G/E_{FS} 10% and G/E_{FS} 15% (51.9 µm; Figure 3A, and 79.5 µm, respectively) were resistant to pH 1.2 for 2 h and to pH 6.8 for 4 h (Figure 2). After the pH change to 7.5 corresponding to the upper part of ileum, the drug was rapidly released from coated capsules within 1 h. As the total transit time through the ileic compartment is considered 2 h[31], drug absorption

Table 3. Capsules with increasing different amount of Eudragit® FS coating.

Batch	Average uncoated capsules weight ± SD (g)	Labeling of coated hard capsules samples and average coated capsules weight ± SD (g)					
Theoretical coating level (%)		5.0	10.0	15.0	20.0	25.0	30.0
G/EFS	0.1582 ± 0.0040	0.1668 ± 0.0017	0.1738 ± 0.0032	0.1818 ± 0.0023	0.1904 ± 0.0041	0.1987 ± 0.0049	0.2051 ± 0.0037
G/HPC/EFS	0.1587 ± 0.0035	0.1673 ± 0.0054	0.1750 ± 0.0046	0.1824 ± 0.0057	0.1907 ± 0.0039	0.1991 ± 0.0039	0.2072 ± 0.0037
H/EFS	0.1567 ± 0.0025	0.1654 ± 0.0026	0.1732 ± 0.0031	0.1808 ± 0.0051	0.1921 ± 0.0062	0.1978 ± 0.0071	0.2088 ± 0.0046

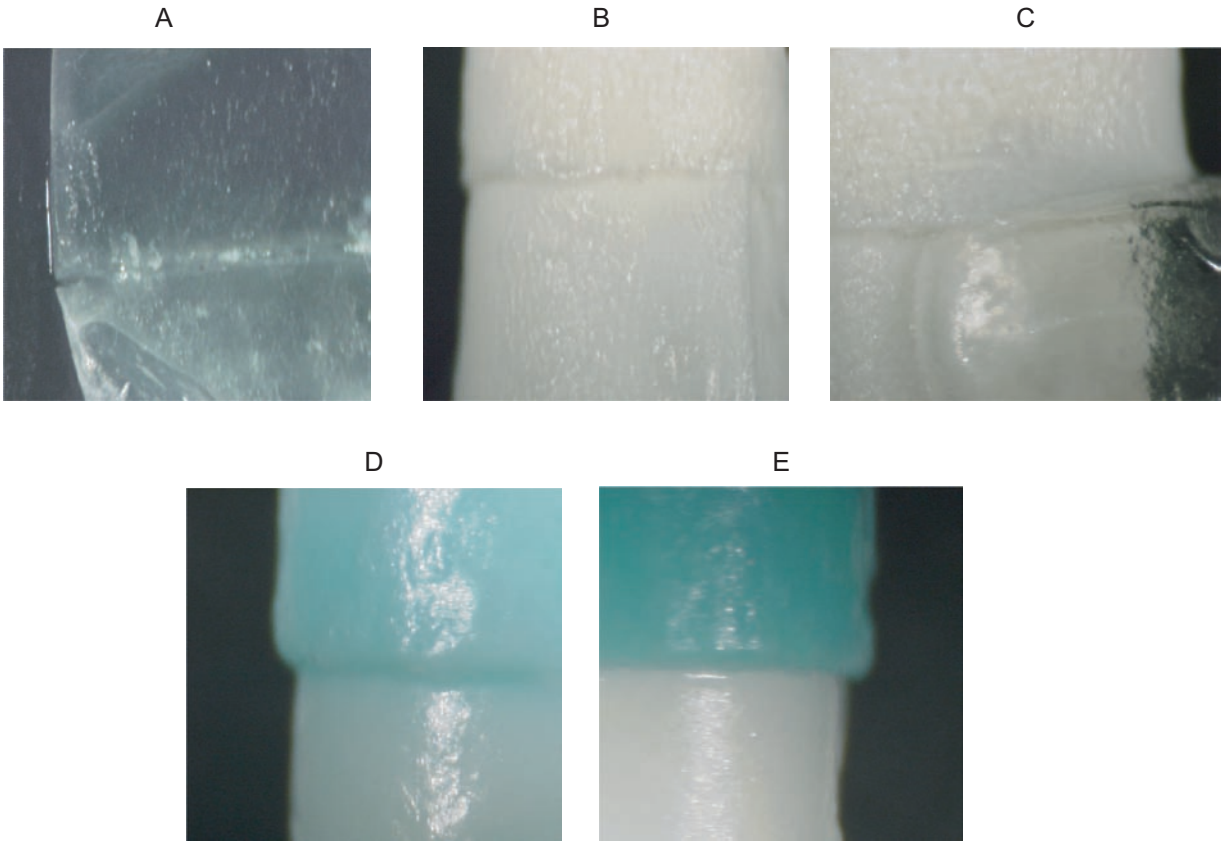

Figure 1. Hard capsules connection areas of selected samples after 4 h in phosphate buffer pH 6.8. (A) G/EFS 5 % (release of capsule filling), (B) G/EFS 20 %, (C) G/HPC/EFS 25 % (blister of coating), (D) H/EFS 20 %, E – H/EFS 25 %.

Table 4. Thickness of Eudragit® $_{FS}$ coating layer.

Batch	Thickness of Eudragit® layer (μm)					
Theoretical coating level (%)	5.0	10.0	15.0	20.0	25.0	30.0
G/EFS	32.0 ± 4.3	51.9 ± 6.3	79.5 ± 8.1	122.3 ± 11.1	145.2 ± 12.6	173.5 ± 7.2
Dry coating material applied on capsules surface (mg/cm^2)	3.6	6.7	10.0	13.7	17.2	20.0
G/HPC/EFS	38.1 ± 5.5	64.7 ± 8.7	86.6 ± 11.0	115.9 ± 13.9	153.3 ± 10.3	179.6 ± 9.1
Dry coating material applied on capsules surface (mg/cm^2)	3.7	7.0	10.1	13.6	17.2	20.6
H/EFS	36.7 ± 12.5	53.1 ± 7.0	88.9 ± 14.9	115.8 ± 9.6	155.3 ± 14.1	198.7 ± 10.5
Dry coating material applied on capsules surface (mg/cm^2)	3.7	7.0	10.3	15.1	17.5	22.2

in the distal ileum is expected. These samples of coated capsules could be used for oral vaccination or hormones delivery undergoing the absorption via Peyer's patches concentrated in distal ileum. Similar trend was found in caffeine drug release from sample G/E_{FS} 20% (coating thickness 122.3 μm; Figure 3B). The drug release after pH change to 7.5 was, however, more prolonged for 2 h (Figure 2). Thus more sustained drug release of sample G/E_{FS} 20% may extend the drug absorption also to the proximal colon with pH value under the E_{FS} solubility[7] as it can be presumed that drug release from disrupted coating will continue. The appearance of G/E_{FS} 20% capsules line between the cap and body after 4-h interval in PB (pH 6.8) is shown in Figure 1B. From this figure, it is obvious that the juncture is intact without any abnormalities. The drug release with the longest lag time was found for samples G/E_{FS} 25% and G/E_{FS} 30% (Figure 2). E_{FS} coating remained untouched in pH 1.2, pH 6.8 and for 1 h also in pH 7.5. Thicker coating (sample G/E_{FS} 25%; 145.2 μm, sample G/E_{FS} 30%; 173.5 μm) caused 1 h lag time extension after dissolution media alkalization to pH 7.5. Therefore, these capsules could be used in the therapy of inflammatory bowel disease in ileo-colonic or colonic areas.

E_{FS} coating applied onto gelatin capsules was effective for drug delivery to ileo-colonic area from relatively low coating level, i.e. 10%. This fact could be probably explained by polyelectrolyte interaction between anionic polyacrylate and ambivalent gelatin after the coating hydration as described by Attama between Eudragit® L and gelatin. Similar interaction could occur between

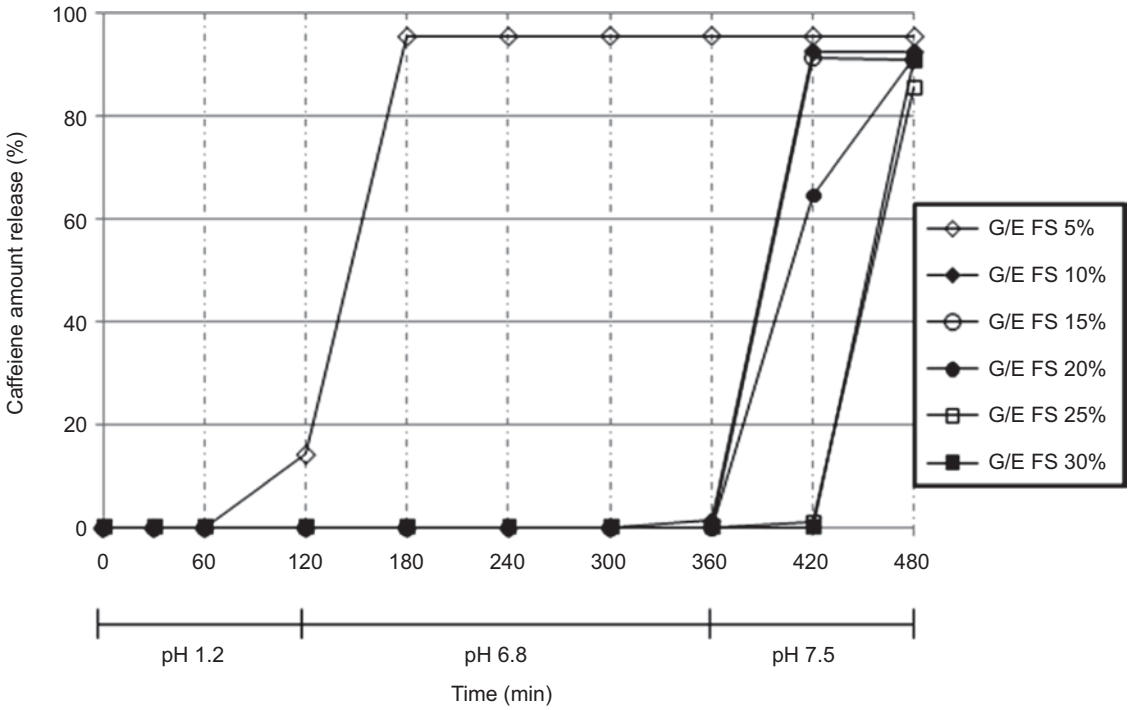

Figure 2. Dissolution profiles of Eudragit® FS coated gelatin hard capsules.

Table 5. Standard deviation values of caffeine released amount from Eudragit® FS coated hard capsules of G, G/HPC and H sets within dissolution test with continual pH change.

Standard deviation values of caffeine released amount (%) within dissolution test										
Time (min)		30	60	120	180	240	300	360	420	480
G/E_{FS}	G/ E_{FS} 5%	0.00	0.00	20.17	0.43	0.59	0.59	0.49	0.63	0.64
	G/ E_{FS} 10%	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.88	1.03
	G/ E_{FS} 15%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.81	2.89
	G/ E_{FS} 20%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.15	2.51
	G/ E_{FS} 25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.36	1.89
	G/ E_{FS} 30%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.50
G/HPC/E_{FS}	G/HPC/E_{FS} 5%	1.57	18.59	30.51	22.56	10.13	12.56	9.63	6.22	6.12
	G/HPC/E_{FS} 10%	0.00	0.00	0.00	0.00	8.45	7.02	10.15	6.54	1.87
	G/HPC/E_{FS} 15%	0.00	0.00	0.00	0.00	8.45	7.02	9.89	1.82	1.87
	G/HPC/E_{FS} 20%	0.00	0.00	0.00	0.02	0.08	2.69	5.88	5.25	5.24
	G/HPC/E_{FS} 25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.36	3,96
	G/HPC/E_{FS} 30%	0.00	0.16	0.00	0.16	0.62	0.45	0.66	2.75	1.21
H/E_{FS}	H/E_{FS} 5%	0.00	28.15	50.18	19.62	9.89	2.13	3.55	5.69	4.12
	H/E_{FS} 10%	0.00	0.00	0.00	28.91	17.23	13.76	10.30	3.48	3.92
	H/E_{FS} 15%	0.00	0.00	0.00	0.13	0.25	2.16	2.21	2.75	3.85
	H/E_{FS} 20%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.21	2.55
	H/E_{FS} 25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.36	3.96
	H/E_{FS} 30%	0.00	0.00	0.00	0.10	0.07	0.03	0.03	3.53	3.62

E_{FS} and gelatin as this polymer contains also anionic carboxylic groups in its molecule[32].

Different results were found in dissolution behavior of G/HPC/E_{FS} batch of gelatin capsules firstly coated with 3% HPC interlayer and subsequently coated with 5–30% E_{FS} final coating. Release profiles of caffeine obtained from continual dissolution tests are shown in Figure 4, and standard deviations values are summarized in Table 5.

Samples G/HPC/E_{FS} 5%, 10%, 15%, 20% (coating thicknesses 38.1–115.9 µm) showed 0.5 h, 1 h, 3 h and 4 h lasting lag time, respectively (Figure 4). HPC interlayer resulted in less delayed caffeine release followed by its sustained release in contrast to gelatin capsules coated with E_{FS} only. One possible explanation is its preventing effect on E_{FS} a gelatin interaction. These samples could not be used as the ileo-colonic transport systems because

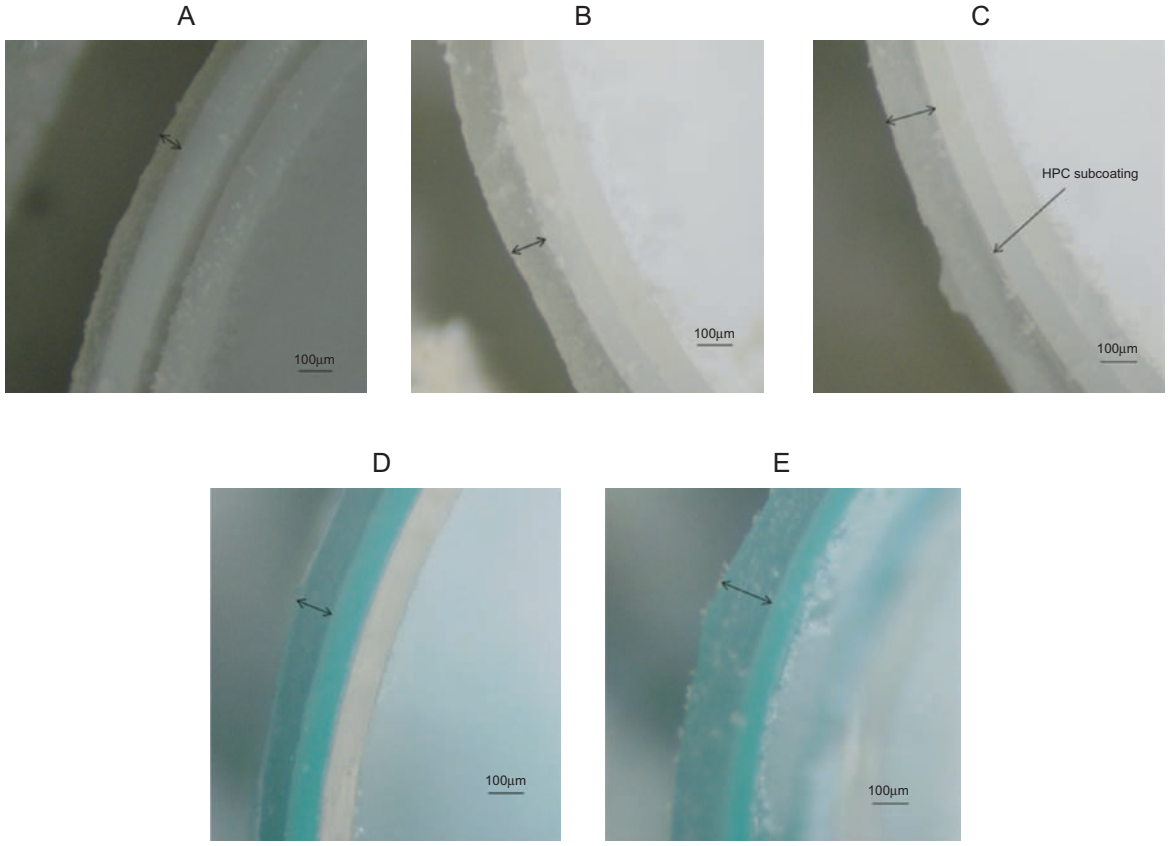

Figure 3. Cross-section of selected Eudragit® FS coated capsules samples. (A) G/EFS 10.0%, (B) G/EFS 20.0%, (C) G/HPC/EFS 25.0%, (D) H/EFS 15.0%, (E) H/EFS 25.0%.

of faster and pH-independent drug release; in G/HPC/E_{FS} 5% sample in addition the SD values were very high. With increasing coating level, on one hand longer lag time could be observed, on the other hand the following drug release was faster. Probably after a longer lag time, the E_{FS} coating was more swollen and therefore coating defects were manifested faster; swollen HPC interlayer can also contribute to this process. The sample G/HPC/E_{FS} 25% (coating thickness 153.3 µm, 17.2 mg/cm^2; Figure 3C) showed the gastro- and small intestine resistance and released caffeine after pH change to 7.5 completely in 2-h interval (Figure 4). Therefore, the incorporated drug could be delivered into ileo-colonic compartment. The appearance of capsules connection areas of this sample after 4-h interval in PB is shown in Figure 1C. Within dissolution test, the medium penetrated through the film, which could be proved by a visible blister structure observed in the coating. Sample G/HPC/E_{FS} 30% (coating thickness 179.6 µm, 20.6 mg/cm^2) with the lag time of 7 h (1-h resistance in pH 7.5; Figure 4) could be suitable for potential colonic area drug delivery.

Six HPMC capsules samples were prepared when coated with the increasing amounts of E_{FS} (H/E_{FS} set). The release profiles of caffeine obtained from continual dissolution tests are shown in Figure 5 and standard deviations are summarized in Table 5.

Samples H/E_{FS} 5% (thickness 36.7 µm) and H/E_{FS}10% (thickness 53.1 µm) showed 30 min and 2 h lag time, respectively. After capsules opening, the drug release was prolonged with great standard deviation up to 50.18% for sample H/E_{FS} 5% and 28.91% for sample H/E_{FS} 10%, respectively (Table 5). Prolonged caffeine release was caused probably due to an inclusion of a part of the drug into the pocket of coating polymer observed; it did not dissolve under the gentle conditions of the dissolution test. This pocket could act as a reservoir. Similar dissolution behavior of coated HPMC capsules at the same dissolution conditions was described also by Cole et al.[23].

Only 10% of drug released from sample H/E_{FS} 15% within 6 h and no drug released from sample H/E_{FS} 20% in both dissolution media (1.2, 6.8) before pH adjustment to 7.5 value were detected (Figure 5). After pH increasing to 7.5, caffeine was released rapidly and completely in 1 h due to polymer coating dissolution[7]. Samples H/E_{FS} 15% (88.9 µm; Figure 3D) and H/E_{FS} 20% (115.8 µm) fulfilled thus the basic parameters for ileic drug delivery as reported in the literature[33,34], saying that the sample is still considered as satisfactory if 10% of the model drug is released within 6-h interval in pH 1.2 and 6.8 at the maximum. The different coating thickness of these two samples had only a little influence on the dissolution profile of caffeine confirming the robustness of the formulation[21]. The drug was released from sample H/E_{FS} 25% (thickness 155.3 µm; Figure 3E) after 6 h lag time within 2-h interval (70% in the first hour after pH adjustment to 7.5; Figure 5) and

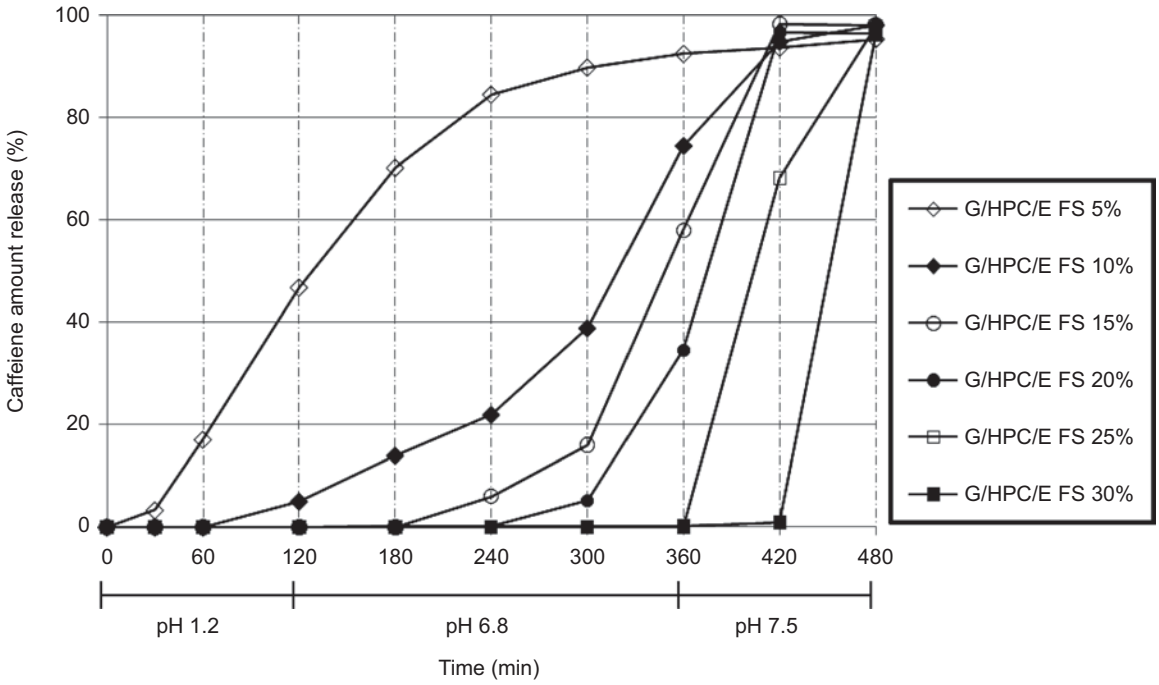

Figure 4. Dissolution profiles of gelatin hard capsules with HPC subcoating and Eudragit® FS final coating.

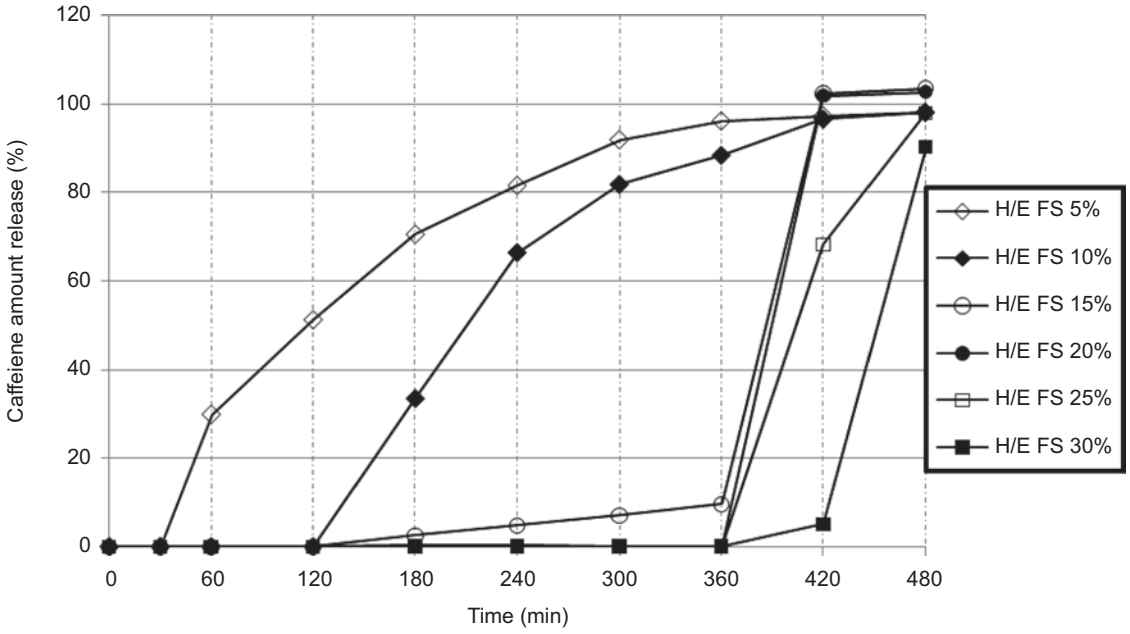

Figure 5. Dissolution profiles of HPMC hard capsules coated with Eudragit® FS.

thus *in vitro* dissolution characteristics could indicate the drug transport to distal ileum and upper colon. The intact capsules connection areas of samples H/E_{FS} 20% and H/E_{FS} 25% after 4-h interval in PB are shown Figure 1D and 1E, respectively. Lower color contrast between capsules cap (initially blue) and body (initially white) in sample H/E_{FS} 20% was caused by a weak water penetration and a partial dissolution of the cup colorant. The same lag time, i.e. 6 h, was observed for sample H/E_{FS} 30% (thickness 198.7 µm), followed by 5% drug release within next hour and its fast release in 8th h of dissolution test (Figure 5). From these results, it is obvious that the main absorption or the site of drug action could be moved further to the colon compartment.

After this evaluation, the selected coated capsules samples (G/E_{FS} 10–30%, G/HPC/E_{FS} 25–30% and H/E_{FS} 15–30%) were placed into stability boxes for their stability evaluation, i.e. for 3, 6, 9 and 12 months under conditions of 25°C/60% of relative humidity (RH) and 30°C/65% of RH, and for 3 and 6 months at 40°C/75% of RH, respectively. Preliminary results showed that caffeine dissolution profiles at 25°C/60% RH after 9 months and at

40°C/75% of RH after 6 months did not change much from the profiles at the time zero, i.e. the values difference was 10% at the maximum. Thus the samples fulfilled the criteria described in the literature mentioned before (*in vitro* dissolution studies).

Conclusion

Coated hard capsules for ileic resp. colonic drug delivery were prepared. The transport of drugs (powders, granulates and coated granulates or multiparticle dosage forms such as pellets, micro- or nanoparticles) to desired intestinal parts could be realized without significant formulation changes just by the selection of capsules material and suitable E_{FS} coating thickness.

Some capsules samples, for instance G/E_{FS} 10–15%, and H/E_{FS} 15–20% are suitable for drug delivery to lower part of small intestine, i.e. to ileic compartment as indicated by 6 h lag time following fast drug release within an hour after the pH change to 7.5. Capsules of thicker E_{FS} coating, i.e. G/E_{FS} 20%, G/HPC/E_{FS} 25% and H/E_{FS} 25% releasing caffeine within 2 h after the pH change to 7.5 would be usable for drug transport to ileo-colonic part of GIT. Samples G/E_{FS} 25–30%, G/HPC/E_{FS} 30% and H/E_{FS} 30% showing 7 h lag time and consequent fast drug release are predetermined for upper colon drug delivery.

Gelatin capsules directly coated with E_{FS} fulfilled the requirements for desired drug delivery starting from the lowest coating level (10%).

The process in the case of coated hard gelatin capsules with two layers—HPC subcoating and final E_{FS} coating—was more complicated and time consuming than the simple coating procedure. With respect to the dissolution data, it is apparent that HPC subcoating applied onto gelatin capsules was considered as the needless complication.

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Declaration of interest

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References

1. Foster N, Hirst BH. (2005). Exploiting receptor biology for oral vaccination with biodegradable particulates. Adv Drug Deliv Rev, 57:431–450.
2. McConnell EL, Fadda HM, Basit AW. (2008). Gut instincts: explorations in intestinal physiology and drug delivery. Int J Pharm, 364:213–226.
3. Mohamad A, Dashevsky A. (2006). pH-independent pulsatile drug delivery system based on hard gelatin capsules and coated with aqueous dispersion Aquacoat ECD. Eur J Pharm Biopharm, 64:173–179.
4. Goole J, Amighi K, Vanderbist F. (2008). Evaluation and floating enhancement of levodopa sustained release floating minitablets coated with insoluble acrylic polymer. Drug Dev Ind Pharm, 34:827–833.
5. Gao C, Huang J, Jiao Y, Shan L, Liu Y, Li Y et al. (2006). *In vitro* release and *in vivo* absorption in beagle dogs of meloxicam from Eudragit FS 30 D-coated pellets. Int J Pharm, 322:104–112.
6. Ibekwe VC, Fadda HM, Parsons GE, Basit AW. (2006). A comparative *in vitro* assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery. Int J Pharm, 308:52–60.
7. Singh BN. (2007). Modified-release solid formulations for colonic delivery. Recent Pat Drug Deliv Formul, 1:53–63.
8. Shimono N, Takatori T, Ueda M, Mori M, Higashi Y, Nakamura Y. (2002). Chitosan dispersed system for colon-specific drug delivery. Int J Pharm, 245:45–54.
9. Marvola T, Marvola J, Kanerva H, Ahonen A, Lindevall K, Marvola M. (2008). Neutron activation based gamma scintigraphic evaluation of enteric-coated capsules for local treatment in colon. Int J Pharm, 349:24–29.
10. Ibekwe VC, Fadda HM, McConnell EL, Khela MK, Evans DF, Basit AW. (2008). Interplay between intestinal pH, transit time and feed status on the *in vivo* performance of pH responsive ileo-colonic release systems. Pharm Res, 25:1828–1835.
11. van der Lubben IM, Verhoef JC, van Aelst AC, Borchard G, Junginger HE. (2001). Chitosan microparticles for oral vaccination: preparation, characterization and preliminary *in vivo* uptake studies in murine Peyer's patches. Biomaterials, 22:687–694.
12. Brayden DJ, Jepson MA, Baird AW. (2005). Keynote review: intestinal Peyer's patch M cells and oral vaccine targeting. Drug Discov Today, 10:1145–1157.
13. Krishnamachari Y, Madan P, Lin S. (2007). Development of pH- and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon. Int J Pharm, 338:238–247.
14. Sangallia ME, Maronia A, Zemaa L, Busettib C, Giordanoc F, Gazzanigaa A. (2001). *In vitro* and *in vivo* evaluation of an oral system for time and/or site-specific drug delivery. J Control Rel, 73:103–110.
15. Ishibashi T, Harumi H, Kobayashi M, Mizobe M, Yoshino H. (1998). Design and evaluation of a new capsule-type dosage form for colon-targeted delivery of drugs. Int J Pharm, 168:31–40.
16. Lin YK, Ho HO. (2003). Investigations on the drug releasing mechanism from an asymmetric membrane-coated capsule with an in situ formed delivery orifice. J Control Release, 89:57–69.
17. Scala-Bertola J, Gajdziok J, Rabisková M, Bonneaux F, Lecompte T, Sapin A et al. (2009). Pellets for oral administration of low-molecular-weight heparin. Drug Dev Ind Pharm, 35:1503–1510.
18. Hussain N, Jaitley V, Florence AT. (2001). Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev, 50:107–142.
19. Dostálek M., et al. (2006). Farmacokinetics. 1st edn. GRADA Publishing: Praha, 221 s.
20. Kwol GS. (2005). pH-sensitive polymers for drug delivery. In: Na K, Bae YH. Polymeric drug delivery systems, 1st edn. New York: Taylor & Francis Group, pp. 129–180.
21. Porter SC, Felton LA. (2010). Techniques to assess film coatings and evaluate film-coated products. Drug Dev Ind Pharm, 36:128–142.
22. Siepmann F, Muschert S, Leclercq B, Carlin B, Siepmann J. (2008). How to improve the storage stability of aqueous polymeric film coatings. J Control Release, 126:26–33.

23. Cole ET, Scott RA, Connor AL, Wilding IR, Petereit HU, Schminke C et al. (2002). Enteric coated HPMC capsules designed to achieve intestinal targeting. Int J Pharm, 231:83–95.
24. Huyghebaert N, Vermeire A, Remon JP. (2004). Alternative method for enteric coating of HPMC capsules resulting in ready-to-use enteric-coated capsules. Eur J Pharm Sci, 21:617–623.
25. Thoma K, Bechtold K. (1986). Enteric coated hard gelatin capsules. Capsugel Technical Bulletin, 1–16.
26. Scott RA, Cole ET. (2006). Enteric and colonic delivery using HPMC capsules. US patent 7094425, www.pharmcast.com, 03. 04. 2010.
27. Dvorácková K, Rabisková M, Gajdziok J, Vetchý D, Muselík J, Bernatoniene J et al. (2010). Coated capsules for drug targeting to proximal and distal part of human intestine. Acta Pol Pharm, 67:191–199.
28. Akhgari A, Sadeghi F, Garekani HA. (2006). Combination of time-dependent and pH-dependent polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets. Int J Pharm, 320:137–142.
29. Franc A, Sova P. Oral pharmaceutical composition for targeted transport of a platinum complex into the colorectal region, method for producing and use as medicament thereof European Patent Office, http://v3.espacenet.com, 20.1.2009.
30. Chourasia MK, Jain SK. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci, 6: 33–66.
31. Mercier GT, Nehete PN, Passeri MF, Nehete BN, Weaver EA, Templeton NS et al. (2007). Oral immunization of rhesus macaques with adenoviral HIV vaccines using enteric-coated capsules. Vaccine, 25:8687–8701.
32. Attama AA. (2007). Polyelectrolyte complexes of Eudragit l30 d-55 and gelatin: antinociceptive activity of entrapped piroxicam. Drug Deliv, 14:155–162.
33. Schellekens RC, Stellaard F, Mitrovic D, Stuurman FE, Kosterink JG, Frijlink HW. (2008). Pulsatile drug delivery to ileo-colonic segments by structured incorporation of disintegrants in pH-responsive polymer coatings. J Control Release, 132:91–98.
34. Akhgari A, Afrasiabi Garekani H, Sadeghi F, Azimaie M. (2005). Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int J Pharm, 305:22–30.