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Comparison of in vitro and in vivo performance of a colonic delivery system

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ABSTRACT

The aim of this work is to compare in vitro to in vivo performance of a colonic drug delivery system, made of small pectin-ethylcellulose coated drug beads. The delivery system was evaluated in vitro by conducting drug release studies in different dissolution media to mimic transit times and pH conditions in the stomach, small intestine and colon and in vivo by using gamma-scintigraphic studies in dogs and absorption studies in human volunteers under fed and fasted conditions. In vitro release studies indicated that drug release rate depended on the ratio of the pectin to ethylcellulose in the coat and the thickness of the coat. In vivo release studies obtained by deconvolution of biostudy data did not correlate with in vitro results obtained from most coat formulations. Beads showing ideal release profiles in vitro showed very poor performance in vivo and only those beads showing colonic premature drug release in vitro might be able to deliver the drug to the colon. Scintigraphic studies of a selected formulation showed that the labeled beads had an estimated gastric emptying time of 3 h, an estimated small intestine transit time of 2 h and an estimated colonic transit time of 36 h. Average in vivo lag times of the selected formulation from absorption studies in humans were found to be 6.1 h and 4.8 h under fed and fasted conditions, respectively. The C_{max} was also observed at 6.8 h and 5.5 h on average, under fed and fasted conditions, respectively, which might indicate that release of drug from the beads, resulted from degradation of pectin in the coat by enzymatic action in the colon rather than by simple diffusion. Deconvolution of biostudy data showed that drug absorption continued on average for at least 12 h under both fed and fasted conditions.

1. Introduction

Design and evaluation of colonic delivery systems requires knowledge of the drug, the delivery system and the targeted organ. Physicochemical properties of the drug molecule and other materials in the delivery system in relation to known physiological variables such as gastro-intestinal pH gradients and transit times must be well understood. A large number of colonic drug delivery systems have been developed by several different approaches; however only few studies were able to correlate in vitro/ in vivo performance of these systems (Schellekens et al., 2008; Ueda et al., 1994; Sangalli et al., 2001). Colonic delivery systems include the temporal control of delivery (Steed et al., 1997), pH-based systems (Ibekwe et al., 2006a,b,c; Klein et al., 2005), pressure-based systems (Jeong et al., 2001), enzyme-based systems (Xi et al., 2005) and others. Although pH based systems are the only proven technology used already in commercial products yet it has been reported that these systems are not very reliable because of intestinal physiology where a pH larger than 7 is encountered in a short intestinal segment where the drug system usually resides for a short time which may result in too early or too slow drug release at the wrong moment or at the wrong site in the intestinal tract (Schellekens et al., 2007; Ibekwe et al., 2006a,b,c; Ibekwe et al., 2008). The universal polysaccharide systems, on the other hand, appear promising because of their practicality and simplicity as they depend on the most distinctive property of the colon which is the abundant microflora (Basit, 2005). From the natural polysaccharides, pectin (Xu et al., 2005; Ashford et al., 1993; Ashford and Fell, 1994), amylose (Milojevic et al., 1996; Bloor et al., 2002; Siew et al., 2004), guar gum (Tugcu-Demiro et al., 2004) and chitosan (Norihito et al., 2002) have been heavily investigated for their potential use as colonic delivery systems. Pectin has a pKa-value of 3.5 and has been especially useful compared to other enzyme-based systems as it is unionized in acid media and it has been reported that even a short time exposure of pectin beads in simulated gastric fluid media drastically decreased drug release in higher pH simulated intestinal media such as no enteric coating would be needed and more drug could reach the colon (Ahmed, 2005). Pectin is also reported to be very useful when used with water-insoluble polymers as film coatings (Macleod et al., 1999; Wakerly et al., 1996, 1997; Ahmed, 2005; Marianne et al., 2003; Liu et al., 2003) or in the form of matrix tablets (Ahrabi et al., 2000; Fernandez-Hervas and Fell, 1998). Although

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single unit dosage forms are easier to prepare, multi-particulate systems have been useful especially with film-coatings. Multiple-unit systems have many advantages for colonic delivery when compared to single unit dosage forms such as decreased variability in gastric emptying time, are less influenced by the presence of food in the stomach (Hardy et al., 1985), reduced risk of high local concentrations of released drugs, stagnation at the ileo-cecal junction is less likely to occur than with larger single units (Feely et al., 1985), slower transit of small particles through the colon which prolongs contact between the formulation and the absorptive surface resulting in a greater proportion of drug being absorbed (Adkin et al., 1993) and higher surface area of multiple-unit systems leads to higher area being exposed to bacterial attack in the colon with subsequent rapid drug release (Fernandez-Hervas and Fell, 1998).

In pH-based and temporal systems it is often reported that in vitro characteristics of these systems correlate with in vivo performance (Schellekens et al., 2008; Ueda et al., 1994; Sangalli et al., 2001). In this work in vitro and in vivo performances of selected pectin-ethylcellulose coat formulations on small drug loaded beads as enzyme-based colonic delivery systems have been evaluated in vitro using release studies in different dissolution media to mimic transit times and pH conditions in the gastrointestinal tract and in vivo by using gamma-scintigraphic studies in dogs and absorption studies in human volunteers under fed and fasted conditions. The inter-relationship between in vitro release studies, gamma-scintigraphic studies in dogs and in vivo absorption studies in human volunteers is explored and investigated. Acetaminophen was chosen as a model drug to incorporate into the colonic delivery system because it is known to be absorbed from the colon (Forrest et al., 1982), and it can be easily assayed in saliva. Saliva acetaminophen concentrations have been reported to be proportional and virtually equivalent to serum concentrations (Adithan and Thangam, 1982).

2. Materials and methods

2.1. Materials

Acetominophen (Spectrum Chemical Mfg Corp., Gardena, CA) was used as the model drug, ethylcellulose in the form of Surelease® of 24.8% (w/w) solids content (Colorcon Ltd., West Point, PA), pectin USP from apple (Sigma Chemicals, St. Louis, MO), microcrystalline cellulose (Avicel® PH101, FMC Corp., Newark, DE), polyethylene oxide, mol. wt. 200,000 (Polyox N-80, Union Carbide Corp., Danburg, CT), pectinolytic enzymes (Pectinex Ultra SP-L, Novo Nordisk Biochem., North America, Inc., Franklinton, NC) were obtained from the indicated sources. Samarium III oxide (Sigma Chemicals, St. Louis, MO) was used as a radiotracer in scintigraphic studies.

Tylenol® Extra Strength 500 mg caplets (McNeil, Fort Washington, PA) were used as a control treatment.

2.2. Methods

2.2.1. Bead preparation

Beads (1.5–2 mm in diameter) containing 80% acetaminophen, 15% Avicel PH101 and 5% polyox N-80, were prepared by extrusion and spheronisation using a bench top laboratory extruder model 20/25 and spheronizer model 120 (Caleva Process Ltd., England). The beads were left to dry overnight in an oven at 50 °C. 100 g of acetaminophen beads were prepared for each batch. The beads met established requirements for drug content. The mean % drug content was found to be more than 95% from all batches.

2.2.2. Coat preparation

Three coat formulations containing different amounts of pectin and Surelease® were prepared and coating solutions were coated onto the drug beads to different film thickness. Coat formulation one (F1) was prepared by mixing a 2% (w/v) solution of pectin USP in distilled water with Surelease and distilled water to result in a ratio of pectin (P) to Surelease® (S) of 1:12 by weight. Coat formulation two (F2) was prepared in a similar way but by using a 3% (w/v) solution of pectin USP in distilled water with Surelease and distilled water to result in a ratio of pectin (P) to Surelease® (S) of 1:6 by weight. Coat formulation three (F3) was prepared by mixing a 5% (w/v) solution of pectin USP in distilled water with Surelease and distilled water to result in a ratio of pectin (P) to Surelease® (S) of 1:3 by weight. 25 g of acetaminophen beads were used for each batch of coating and film coat thickness.

2.2.3. Coating process

Coating was performed using a laboratory Aeromatic Strea-1 fluidized bed coater (Niro-Aeromatic, Columbia, MD). Coating was performed at 50 °C inlet temperature and coating solution was applied through a 1.0 mm spray nozzle at a spray rate of 2 ml/min using a rabbit peristaltic pump (Rainin Instrument Co. Inc., Woburn, MA) and 25 psi atomizing air pressure. Coating solutions were coated onto drug beads to result in a different coat thickness. The film thickness is expressed as the theoretical percentage of the weight gained (TWG) used relative to the weight of the uncoated beads. Beads were coated with 9%, 14%, 16% and 18% weight gains for coat formulation F1, 12%, 25%, 30% and 35% weight gains for coat formulation F2, and 25%, 35%, 45% and 55% weight gains for coat formulation F3. Coated beads were cured for 30 min at 50 °C. The coated beads met established requirements for drug content.

2.2.4. Dissolution studies

Drug release from coated beads was determined in a dissolution tester (VK 7000 Dissolution Testing Station, Vankel Industries, Inc., NJ), following the USP paddle method. All tests were conducted in 900 ml dissolution medium maintained at 37 ± 0.5 °C with a paddle rotation speed at 50 rpm. Dissolution studies were carried out under conditions simulating pH and times likely to be encountered during transit in the GI tract. Testing was carried out using simulated gastric fluid (SGF) for 2 h at pH 1.4, followed by simulated small intestinal fluid (SSIF) for 4 h at pH 7.4, followed by simulated cecal fluid (SCF) at pH 6 for at least 6 h (or until 100% drug release) with or without the addition of 3 ml enzymes. The buffer pH 6 was used to compromise between the mean pH of the cecum and the optimum pH for the activity of the enzymes. Acetaminophen concentrations were determined at 242–244 nm using a Beckman DU 640 Spectrophotometer (Beckman Instruments, Inc., CA). For each dissolution experiment, a duplicate was run at the same time under the same conditions. After the 4 h in SSIF, enzymes were added to one of the dissolution vessels but not to the other. Thus, one is a release study with enzymes and the other one is a release study without enzymes. Each dissolution experiment was repeated 3 times ($n = 3$).

2.2.5. Preliminary in vivo absorption studies in humans

Seven coat formulations were selected according to dissolution results to be tested each in 2 human volunteers under fed conditions to see if in vitro dissolution results correlate with in vivo absorption results. These preliminary in vivo studies were performed similar to the methodology described below under in vivo absorption studies. From the seven coat formulations, one coat formulation (55% F3) was chosen to be tested in vivo in dogs by conducting gamma scintigraphic studies and also tested in six human volunteers under fasted and fed conditions for determination of colonic in vivo performance.

2.2.6. Gamma scintigraphic studies

GI behavior of a single multiparticulate capsule containing acetaminophen beads coated with a selected coat formulation was observed by gamma scintigraphy in two dogs under fed conditions. The radioactive beads were prepared as described previously but such as to contain 13% Sm oxide. Beads containing Sm oxide were irradiated in a TRIGA type reactor at Oregon State University with a neutron flux of 3.0×10^{12} n cm^{-2} s^{-1} for 10 min. Such irradiation transformed stable Sm-152 into radioactive Sm-153, a radionuclide with a half-life of 46.7 h and gamma ray emitting at 103 keV. This provided a radioactivity level of 10 μCi per bead 24 h after irradiation. Each capsule included five beads radiolabeled with neutron-activated Sm-153. The residence time of radiolabeled beads in different regions of the GI tract was determined and image displays were observed throughout a 85-h period. The Radiation Safety Review Committee and the Institutional Animal Care and Use Committee (IACUC) at Oregon State University approved the protocol of the study.

2.2.6.1. Dosage form preparation. A hard gelatin capsule (size # 0, Capsugel) was filled with beads equivalent to a 100 mg acetaminophen dose. The capsule comprised approximately 50 coated beads. On the study day the capsule was opened and five of the beads were replaced with five Sm-153 radiolabeled beads with a total radioactivity dose of 50 μCi.

2.2.6.2. Gamma camera imaging. On the study day, one capsule was administered to each dog with 60 ml water. The five radiolabeled beads were visualized using the gamma camera. An external anatomical marker containing 0.1 MBq Technetium-99m (Tc-99m) was taped to the shaved skin on the right-hand side of the body at the level of thoracic number 12 to mark the stomach and the colon and to allow correct alignment of dogs during imaging. Anterior scintigraphic images were recorded at frequent intervals for up to 72 h. Images of 60 s were recorded at approximately 15 min intervals up to 1.5 h post dose and then at approximately 1 h intervals until 12 h post dose and then at different time intervals until the release of the dogs.

2.2.6.3. Scintigraphic data analysis. Anterior and posterior images were recorded by computer for subsequent analysis. The anatomical position of the tracer was established by viewing the full sequence of images and by reference to the external marker taped to the skin of the dog to the right of the stomach and to X-rays of the dog's abdomen taken after taping the external marker. The transit behavior of the multiparticulate system has been expressed in terms of the time for two-thirds of the beads to leave the stomach, or to arrive to the colon. A small intestine transit time has been calculated as the difference between these two figures. Once the position of the beads was determined, representative figures of the GI tract were drawn on transparent sheets. The images from the gamma camera were then overlaid to the transparent sheets to place the beads in their predetermined position.

2.2.7. In vivo absorption studies

2.2.7.1. Study design. The study was carried to compare the pharmacokinetics of acetaminophen from a selected coat formulation (55% F3) to a conventional commercially available immediate release tablet (Tylenol® Extra Strength 500 mg caplets, McNeil, Fort Washington, PA) following administration of single doses of 500 mg each using a non-blind, two-treatment, two-period, randomized crossover design under both the fasted and fed states (2×2 crossover). Six healthy subjects (3 males and 3 females) participated in the study after giving informed written consent. The study protocol was approved by the Oregon State University Institutional Review Board (IRB) for the protection of human subjects and the protocol complies with the declarations of Helsinki and Tokyo for humans. All subjects were regular in their bowel habits and had no known history of intestinal disease. All subjects fasted for at least 10 h before the study day. The subjects were randomly assigned to each of the two groups of equal size under the fasted conditions with a one-week washout period in a crossover design. After a two-week washout period the study was repeated under the fed conditions. The first group received the commercially available immediate release caplets, Tylenol® with 200 ml of water (treatment A), the second group received the colonic capsule (size # 0 Capsugel filled with beads equivalent to a 500 mg acetaminophen dose) with 200 ml of water (treatment B). No food was allowed for 4 h after dosing. In the fed study all subjects received a high fat meal consisting of 2 eggs fried in butter, 2 beef sausages, 2 slices of toast with butter, 4 ounces of hash brown potatoes and 8 ounces of whole milk, i.e. approximately 150 protein calories, 250 carbohydrate calories, and 500–600 fat calories half an hour before receiving the treatment (FDA, 2002). The subjects were then divided into the two treatment groups in a similar way described under the fasted conditions and then crossed over the groups with a one week washout period.

2.2.7.2. Collection of saliva samples. Subjects provided a zero time saliva sample prior to dosing and then ingested a formulation. Saliva samples were collected by chewing squares (1 in. × 1 in.) of Parafilm® (American National Can, Menasha, WI) for 2 min with continuous spitting into a 15-ml polypropylene centrifuge tube. Saliva samples were frozen until delivered. Subjects collected saliva at 0.0, 15.0, 30.0, 45.0 min, and at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 12.0 h for Tylenol® caplets. Saliva was collected at 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0 and 24.0 h for the colonic capsules.

2.2.7.3. Chromatographic conditions. Concentrations of acetaminophen in saliva were determined using a modification of an HPLC procedure used by Borin and Ayres (1989). The column used was a reverse-phase micro-particulate C_{18} (Microsorb MV C_{18}, particle size 5 μm, 25 cm × 4.6 mm, pore size 100 Å, Varian Analytical Instruments, Walnut Creek, CA). Mobile phase was water:methanol (85:15) at a flow rate of 1 ml/min. The detector was a fixed-wavelength UV detector set at 254 nm (Waters model 440, Waters Associates, Milford, MA).

2.2.7.4. Standard solutions. Standards were prepared by spiking 900 μl of blank saliva with 100 μl of stock solutions and 100 μl (50 μg/ml) of internal standard (β-hydroxyethyl-theophylline) to contain 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 15.0, 18.0 and 20.0 μg/ml of acetaminophen. Mixtures were vortexed for 10 s and frozen at −20 °C for 24 h. Retention times of acetaminophen and internal standard were 10 and 22 min, respectively. A standard curve was constructed by plotting the peak-area ratios of acetaminophen to internal standard against acetaminophen concentrations in saliva. The lower limit of quantification was 0.2 μg/ml and linear response across the full range of concentrations from 0.5 to 20.0 μg/ml ($r^2 = 0.999$) was obtained. During the assay of the study samples, the intra-batch precision and accuracy of the analytical procedure were evaluated after replicate analysis ($n = 6$) of control saliva samples spiked at four concentration levels: 0.5, 2, 8 and 15 μg/ml. The analysis of quality control samples showed a precision below 3% relative standard variation and accuracy below ±5% for intra-batch analysis. The coefficient of variation for inter-batch analysis was less than 10%.

2.2.7.5. Saliva analysis. Approximately 2.5 ml of saliva samples were centrifuged at 7000 rpm for 5 min. One ml of the salivary supernatants was then transferred to 1.5-ml microcentrifuge tubes

and 100 μl of internal standard was added. The mixtures were vortexed for 10 s and then frozen at −20 °C for 24 h. The samples were thawed and centrifuged at 14,000 rpm for 5 min before analysis. Portions of supernatant (100 μl) were transferred to HPLC tubes and assayed as described above.

2.2.7.6. Pharmacokinetic analysis. Pharmacokinetic characteristics from saliva data following administration of all treatments were estimated for each subject by using a computer program, WinNonlin® (version 1.5, Scientific Consulting, Inc., Cary, NC, USA). Non-compartmental analysis was used. C_{max} (μg/ml) and t_{max} (h) were the observed maximal drug concentration and its time, respectively. AUCs were calculated using trapezoidal rule to determine relative bioavailability. AUC $_{0-\infty}$ is the area under the saliva concentration vs. time curve calculated from 0 to ∞ and the relative bioavailability (f_{rel}) was the percentage of the $AUC_{0-\infty}$ of acetaminophen from the colonic capsule (OSU formulation) compared to the AUC $_{0-\infty}$ of acetaminophen from Tylenol Extra Strength 500 mg caplet. In vivo lag times were observed before any appreciable amount of acetaminophen could be detected.

2.2.7.7. Statistical analysis. An analysis of variance (ANOVA) was performed for untransformed data for the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using the software SPSS 11.0 (SPSS Inc., Chicago, USA). The nonparametric Signed Rank Test was used to compare the t_{max} for the colonic capsule under fasted and fed conditions. The level of significance was $\alpha = 0.05$. A p-value of ≤ 0.05 was considered statistically significant. In vitro and in vivo lag times were checked for statistical significance using the one-way analysis of variance (ANOVA) F-test for testing the equality of several means. A p-value > 0.05 was considered statistically insignificant.

2.2.7.8. Deconvolution of acetaminophen saliva concentration data. In vivo release/absorption of acetaminophen from the delivery system under fed and fasted conditions were estimated by a model independent numerical deconvolution of pharmacokinetic data. Deconvolved input functions from biostudy data were determined using computer software Kinetica™ 2000 (Innaphase Corporation, PA). Because of the instability of numerical algorithms when there is noise in the supplied raw data (Langenbucher and Moller, 1983), as was observed in the present study due to multiple peaks, mean saliva concentrations obtained in fed and fasted conditions were used as input response functions because mean values gave a smoother curve. Intravenous data for acetaminophen, required to perform model independent deconvolution, were obtained from previous published data (Rawlins et al., 1977). In vitro/in vivo correlation is described using linear regression model and correlation coefficient for describing the degree of linear association between the two variables was calculated.

3. Results and discussion

3.1. In vitro dissolution studies

Drug release from beads coated with F1, F2 and F3 coat formulations, at all weight gain applied, after 2 h in SGF followed by 4 h in SSIF followed by different hours in SCF (until almost 100% drug release from all coats) with and without pectinolytic enzymes are grouped in Figs. 1–3, respectively.

Release profiles from F1, F2 and F3 coats showed that the percentage of drug released depended on the ratio of pectin in the coat and the thickness of the coat. The more pectin in the coat (F3 > F2 > F1) and the thinner the film (smaller weight gains vs. higher weight gains), the faster the drug was released in SGF, SSIF and SCF in presence and absence of enzymes. For example, in the absence of enzymes, 50% drug was released in about 28 h from

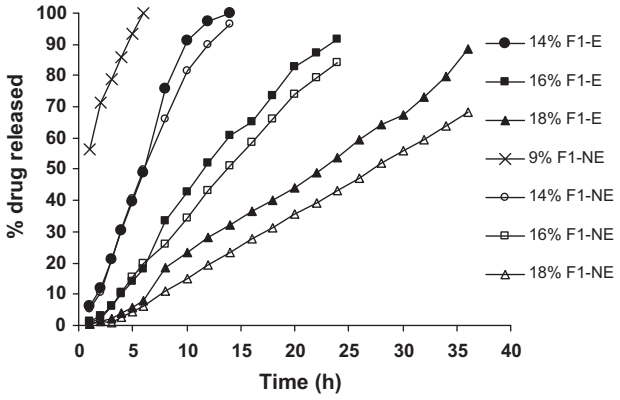

Fig. 1. Release of acetaminophen from beads coated with F1 coat formulation at different percentage weight gains in the presence of enzymes (E) and absence of enzymes (NE) ($n = 3$).

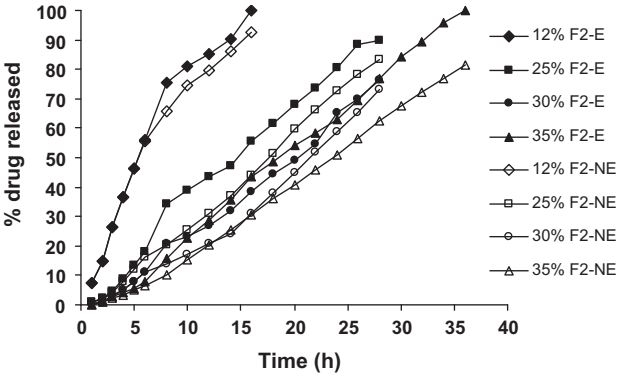

Fig. 2. Release of acetaminophen from beads coated with F2 coat formulation at different percentage weight gains in the presence of enzymes (E) and absence of enzymes (NE) ($n = 3$).

18% F1 coat (highest coat thickness used in F1 coats) compared to 18 h from 25% F2 coat and only to less than 5 h from 25% F3 coat. Also, 50% drug was released in 24 h from 35% F2 coat compared to less than 5 h from 35% F3 coat. The difference in the rate of drug release is related in this case to the amount of pectin in the coat. While for the same coat formulation the percentage drug released depended on the thickness of the coat, for example, in 6 h 50% drug was released from 12% F2 coat compared to less than 7% drug released from 35% F2. Also, after 6 h 70% drug was released from 25% F3 coat compared to only 37% drug released from 55% F3. The slow release observed with thicker coats is typical of rate

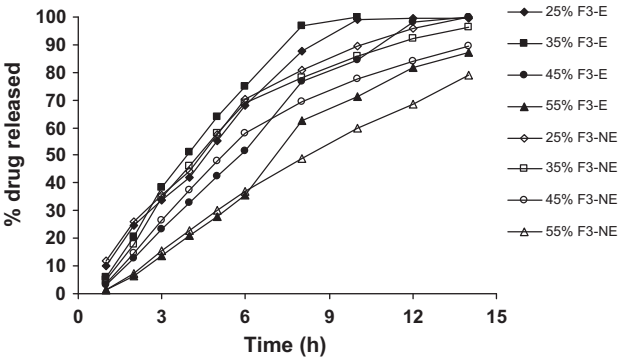

Fig. 3. Release of acetaminophen from beads coated with F3 coat formulation at different percentage weight gains in the presence of enzymes (E) and absence of enzymes (NE) ($n = 3$).

release coating membranes. Ethylcellulose, which is a hydrophobic sustained-release material, is known to form non porous films. However, a non porous film will not be formed in the current case due to the leaching of the water-soluble pectin even after a complete film is formed (Tang et al., 2000). It was found that for F3 coats, it took a relatively high coat thickness to start to slow drug release in both SGF and SSIF which could be due to the relatively small amount of ethylcellulose in these coats and/or incomplete film forming at low coat thickness. However, some studies reported that in vitro release experiments showed a significantly faster release of the model drug from capsules coated with enzyme-dependent polymer when compared with in vivo results (Van den Mooter et al., 1994). Thus, due to possible slower dissolution in vivo compared to in vitro, these coats should also be tested in vivo for site specific drug release.

The percentage of drug released in presence of pectinolytic enzymes was always higher from the different coats when compared to no enzymes. As the ratio of pectin in the coat increased, the effect of pectinolytic enzymes on drug release also increased. It could be that pectinolytic enzymes accelerate pore formation by attacking the pectin in the coat, but the coat remained intact and did not breakdown in most formulations and showed some cracks in formulations containing higher amounts of pectin (F3 coats). Dissolution studies also showed that coated beads resisted release in SGF for 2 h. Thus it can be suggested that pectin in the coat did not dissolve during 2 h in SGF for most formulations.

Drug release from all formulations in the absence of enzymes shows that the three pectin/ethylcellulose coat formulations give a nearly zero-order (linear) release over time. The release rate depended on the amount of the pectin in the coat; the more pectin in the coat the faster the release is while maintaining linearity. For example, release profile from the 55% F3 coat formulation showed linear 100% release over 14 h, while release from 25% F2 coat formulation showed 75% linear release over 24 h indicating the possibility of obtaining different linear release profiles over any period of time that can be tailored to specific dosage form for individual drugs.

In summary when the ratio of pectin to Surelease® was high (i.e. 1:3, w/w), drug release was relatively rapid and low percentage coat thicknesses were accompanied by cracking of the coat. However, there was a significant decrease in drug release when pectin was mixed with Surelease® in 1:6 and 1:12 (w/w) ratio which was expected since Surelease® controls the swelling of pectin and some of the coats thicknesses showed what could be considered as ideal drug release profiles for colonic delivery.

3.2. Preliminary in vivo absorption studies in humans

In vitro dissolution tests can provide essential information on the mechanism of drug release but are not necessarily good predictors of in vivo results. For this reason seven coat formulations showing different dissolution profiles and containing different amounts of pectin and Surelease® and which were coated onto drug beads to result in different percentage weight gains, were tested each in two subjects. The seven coat formulations represented all three coat formulations tested in this study and only coat formulations showing very fast release (9%F1 and 25%F3) or very slow release (30%F2 and 35%F2) were excluded from being tested in vivo. All formulations were tested under fed conditions because this is the condition where it is most difficult to provide protection against pre-colonic drug release. Saliva drug concentration–time curves from the different formulations were obtained to determine the lag time before any appreciable drug absorption could be detected and to determine relative bioavailability compared to the reference formulation (Tylenol). In vitro lag time was calculated as the time elapsed until a % drug release of more than 5% was detected in dissolution media while in vivo lag time was calculated as the time elapsed until a concentration of >0.1 mg/l of acetaminophen was detected in saliva. Table 1 shows a summary of in vitro and in vivo findings for the tested formulations.

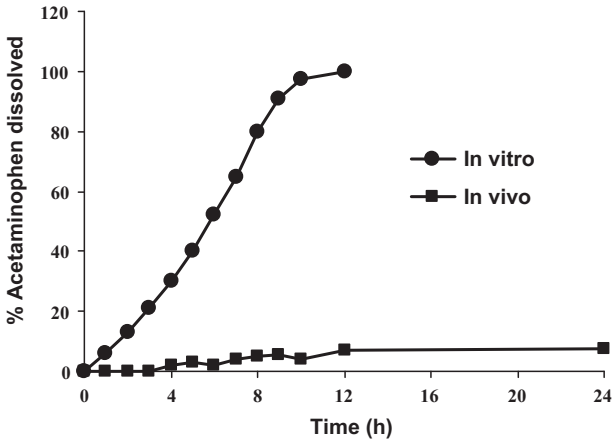

Fig. 4. In vitro and in vivo dissolution from 14% F1 coat formulation.

Results show that in vitro behavior of all coat formulations did not correlate with in vivo results except for 12%F2 and 35%F3 coats which showed good correlation with in vivo results (correlation coefficients were 0.84 and 0.92, respectively) and which could be due to the rapid dissolution of these coats. Comparison of in vitro and in vivo dissolution from one of the F1 coat formulations (14%F1) is shown in Fig. 4 after deconvolution of corresponding in vivo saliva concentration vs. time data from biostudy. Data presented in this figure indicates that in vitro dissolution did not predict in vivo dissolution, showing much faster in vitro dissolution compared to in vivo dissolution from the same coat. The correlation coefficient calculated was only 0.41. All other coats showed correlation coefficients of less than 0.5. It was also noticed that coat formulations showing very little drug release in SSIF in vitro did not show any appreciable amount of drug absorbed in vivo up to 24 h indicating very poor bioavailability from these coat formulations when compared to Tylenol. In vitro lag times from most formulations were also significantly shorter than in vivo lag times. Differences between in vitro performances when compared to in vivo performances could be due to several factors such as differences in

Table 1
In vitro and in vivo average data relevant to the different formulations tested each in 2 subjects in vivo.

Formulation	In vitro lag time (h)	In vitro release (%)	In vivo lag time (h)	Relative bioavailability
14% F1	1 ± 0.0	60	4.5 ± 0.7	Less than 5%
16% F1	4.3 ± 0.57	52	–	No drug absorption
18% F1	6 ± 0.63	32	–	No drug absorption
12% F2	1 ± 0.0	85	2 ± 0.0	91%
25% F2	4 ± 0.0	55	–	No drug absorption
35% F3	1.16 ± 0.28	100	1 ± 0.0	84%
55% F3	2.2 ± 0.57	87	5.5 ± 0.7	61%

In vitro release is the % drug release in presence of enzymes within 8 h following lag time in vitro.

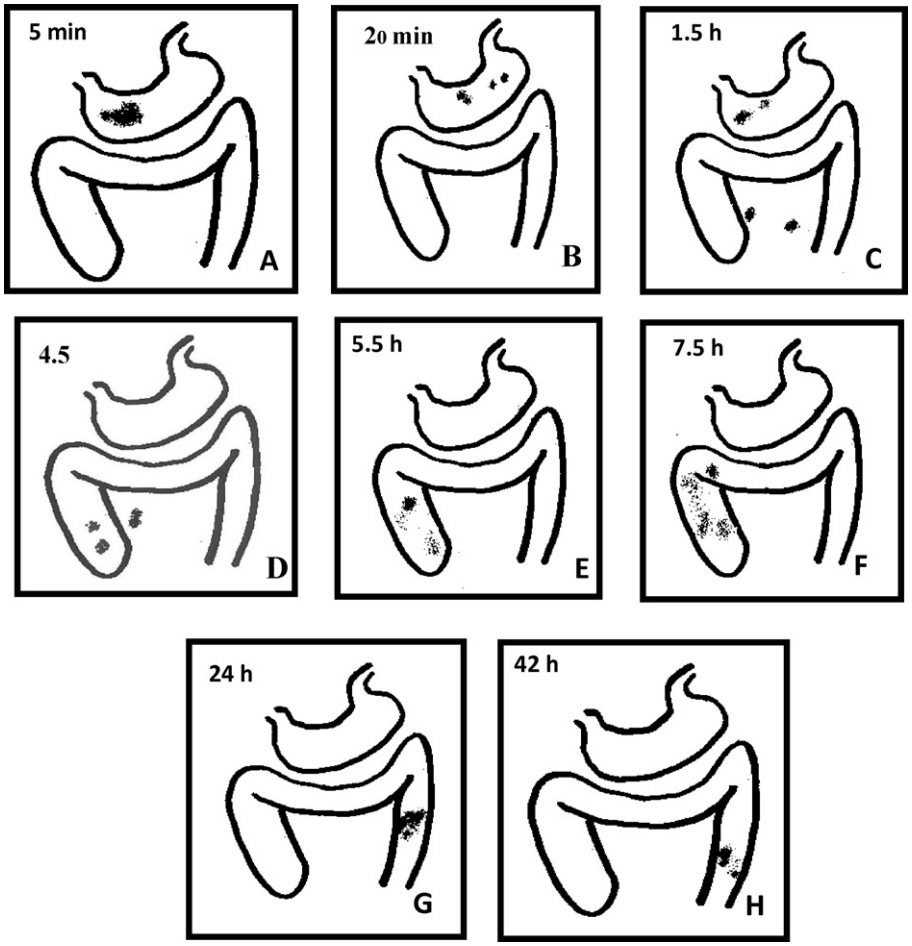

Fig. 5. Representative scintigraphs showing the GI transit of labeled beads in the stomach, small intestine and colon.

hydration rates, bacterial contents, enzymatic activities, viscosities, fluid contents and different physiologic conditions. Although the number of subjects used in this preliminary study is small, the results obtained were further investigated by selecting one of the coat formulations to be tested for GI behavior in two dogs and further in six healthy volunteers for its colonic delivery potential. The selected coat formulation was the 55% F3 because preliminary results in two subjects of this coat showed a lag time of about 5.5 h before any appreciable amount of drug was detected in saliva. This coat formulation consisted of a ratio of pectin (P) to Surelease® (S) of 1:3 by weight and was coated onto drug beads to result in a weight gain of 55%.

In summary, results indicate that coats containing larger amounts of pectin relative to ethylcellulose but at high coat thickness might be able to deliver drug specifically to the colon compared to thinner coats containing larger amounts of ethylcellulose. Thick coats containing a high ratio of pectin could be able to prevent premature release of drug from the beads before reaching the colon while being hydrophilic enough to ensure a good fraction of drug being released under colonic conditions. Studies have shown that pectin must swell enough to be able to absorb enzyme-rich fluids in the colon. Therefore, hydration or swelling of pectin is a major requirement so that colonic enzymes obtain access to the glycosidic linkage in the polysaccharide. On the other hand, thinner coats containing high ratio of ethylcellulose slow down the release in vivo to a large degree and control the swelling of pectin resulting in a very small fraction of drug being released.

3.3. Gamma scintigraphic studies

Anterior and posterior images were taken at different time intervals between 5 min and 85 h post dosing. Representative scintigraphs showing the GI transit of labeled beads in one of the dogs are shown in Fig. 5. At 5 min post dosing a cluster of beads was clearly seen in the stomach (A). At 20 min post dosing beads could be distinguished and were spread in the different parts of the stomach which indicate complete release from the capsule shell (B). At 1.5 h post dosing individual beads could be seen in the stomach and in the small intestine which indicate that beads were emptying from the fed stomach (C). At 4.5 h the beads were moving down and some of the beads reached the ascending colon (D). At 5.5 h beads could be only seen in the ascending colon with probably some beads disintegration (E). At 7.5 h beads could be seen in the ascending and transverse colon (F) and dispersion of radioactive material in the ascending colon was very noticable which might indicate the disintegration of most of the beads in the colon. At 24 h radioactivity could only be seen in the descending colon and remained there until 42 h (G and H). The dog was released at 85 h post dosing after a last image showing no radioactivity in his body. A similar pattern of beads movement through the GIT was also observed with the second dog (scintigraphs are not shown). Analysis of scintigraphic results of both dogs suggests that the labeled beads had an estimated gastric emptying time of 3 h, an estimated small intestine transit time of 2 h and an estimated colonic transit time of 36 h.

3.4. In vivo absorption studies

Considering the aim of this work and the system under investigation, the duration of the lag phase before appearance of drug in biological fluid (saliva) and the relative bioavailability are the fundamental parameters to evaluate in vivo behavior of this delivery system. The IR Tylenol formulation is thought to be valuable in this study because comparing pharmacokinetic parameters between the IR tablet and the colonic capsule (such as C_{max} and t_{max}) would indicate that there was enough delay for the colonic beads to reach the colon and release its drug content in the colon. Since acetaminophen absorption in the colon is expected to be less compared to upper GI tract, a reduced bioavailability is also to be expected.

When the 55% F3 beads were administered to six healthy volunteers, acetaminophen appeared on average after 6.16 h (±2.14 h) in saliva when administered after a standard breakfast and at 4.83 h (±2.23 h) when administered after an overnight fast, however, the difference in time was not statistically significant ($p = 0.223$) which is expected since small particles (less than 7 mm) were reported to empty from the stomach even though it is in the digestive phase (Davis, 1989). These results suggest that beads coated with 55% F3 might successfully deliver drugs to the colon. These findings could not have been predicted from in vitro release studies which showed an in vitro lag time of 2.2 h and 35% drug release in SSIF. Similar findings were observed with in vivo published data in rats using capsules coated with azo-polymers and containing ibuprofen as a model drug (Nubuchi et al., 1986). In this study the results of in vitro release experiments with ibuprofen in sonicated rat cecal contents (cell-free extract) showed a significantly faster release of the model drug from capsules coated with azo-polymers that are susceptible to bacterial azo reductase activity when compared to in vivo results.

It has to be mentioned that pharmacokinetic measurements indicate solely drug absorption and not the mechanism responsible for drug release. Average C_{max} estimated from the tested delivery system was found to be 2.14 and 2.45 mg/l under fed and fasted conditions, respectively compared to 5.96 and 6.35 mg/l under fed and fasted conditions, respectively from Tylenol caplet. C_{max} was also observed at 6.8 h and 5.5 h on average, under fed and fasted conditions, respectively from the colonic capsule, and since small drug beads are known to show small variability in gastric emptying time, are less influenced by the presence of food and are less likely to stagnate at the ileo-cecal junction this might indicate that most of the drug release from the beads resulted from degradation of pectin in the coat by enzymatic action in the colon rather than by simple diffusion. Averages of acetaminophen saliva concentration–time curves under fed and fasted conditions are shown in Figs. 6 and 7.

Table 2
Mean pharmacokinetic parameters of acetaminophen after oral administration of 500 mg in the tested colonic capsule in 6 subjects under fed and fasted conditions.

Parameter	Fed	Fast	Statistical test (p)
In vivo lag time	6.16 ± 2.14	4.83 ± 2.23	$p = 0.223$
C_{max} (mg/l)	2.14 ± 0.84	2.45 ± 0.76	$p = 0.125$
t_{max} (h)	6.83 ± 2.31	5.51 ± 1.74	$p = 0.345$
$AUC_{(0-t)}$ (mg h/l)	12.32 ± 4.34	13.68 ± 3.10	$p = 0.309$
f_{rel} (%)	62.19	67.80	$p = 0.203$

Data are mean values ($n = 6$) ± SD.

Table 2 also summarizes some pharmacokinetic data relevant to the tested formulation under fed and fasted conditions.

The multiple peaks observed in the biostudy data under fed conditions could be due to the gradual emptying of the beads from the fed stomach which was also reported for small beads up to 3 mm in diameter (Khosla and Davis, 1989). Such multiple peaks were not observed under fasted conditions indicating bolus emptying of the beads from the fasted stomach. These results are consistent with scintigraphic studies in dogs. A constant saliva drug concentration was also maintained for 7 h with minimal fluctuations under fasted conditions (Fig. 7). These results suggest that the beads transit time in the ascending colon (where most of the pectinolytic enzymes are located) could be as long as 7 h which is also consistent with published work on the slow transit of small particles through the colon (Adkin et al., 1993).

The relative bioavailability of the colonic delivery system formulation to Tylenol under fed and fasted conditions was 62.19 and 67.80%, respectively (Table 2). Relative bioavailability from the delivery system under fasted conditions was slightly higher than under fed conditions but not statistically different (p-value = 0.203). The reduced bioavailability, as assessed by AUC, compared to Tylenol could be due to several factors such as incomplete drug release from the beads and hence the drug was unavailable for absorption and would then be excreted along with the non-disintegrating beads. Assuming that the drug was released from the beads, the reduced AUC may be due to erratic and incomplete absorption in the colon as reported for many drugs (Koch-Weser and Schechter, 1981). Another contributing factor could be incomplete drug dissolution due to less water in the colon especially in the more distal regions. Although drugs that are well absorbed from the intestines are expected to have lower bioavailability from the colon, the colonic delivery system formulation developed in this study might be useful in improving bioavailability of drugs destroyed or metabolized in upper GI tract such as peptides drugs or deliver-

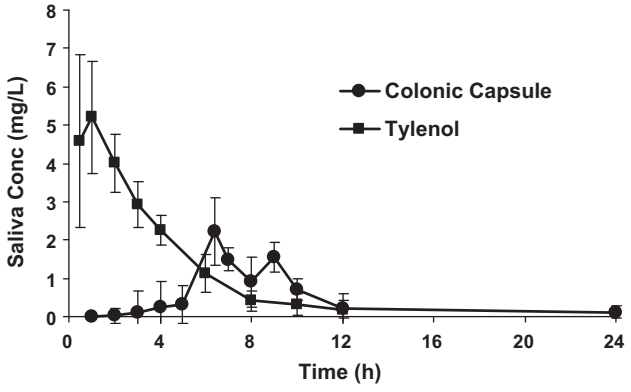

Fig. 6. Mean saliva concentrations of acetaminophen following single 500-mg oral doses under fed conditions in six subjects. Error bars represent standard deviations ($n = 6$).

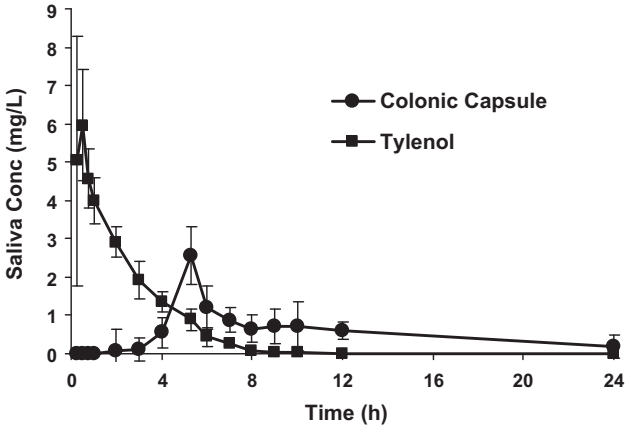

Fig. 7. Mean saliva concentrations of acetaminophen following single 500-mg oral doses under fasted conditions in six subjects. Error bars represent standard deviations ($n = 6$).

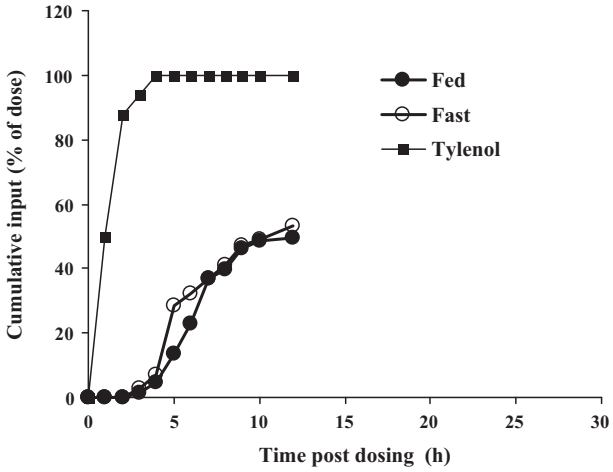

Fig. 8. Mean in vivo acetaminophen release from the colonic delivery system under fed and fasted conditions in six healthy volunteers.

ing drugs to the colon to exert mainly a local action to treat some colonic diseases especially in the ascending and transverse sections.

The obtained results, therefore, indicate that even though the ratio of pectin to ethylcellulose in the coat was high (1:3), there was still very little drug release up to almost 6 h and 5 h (lag times in vivo) under fed and fasted conditions, respectively.

The obtained t_{max} on the other hand (6.8 h and 5.5 h on average, under fed and fasted conditions, respectively) indicate that drug release might had occurred in the colon where there was enough pectin available in the coat with a sufficient degree of swelling to be accessible to bacterial pectinolytic activity resulting in more pore formation with subsequent diffusion of drug through the polymer coating. Increasing the coat thickness in this case was therefore a means to control premature drug release in the small intestine. These findings could not have been predicted from in vitro release studies.

3.5. Deconvolution

Mean percentage input or in vivo dissolution profiles obtained after deconvolution of saliva response data under fed and fasted conditions are presented in Fig. 8. Comparing in vivo release profiles under fed and fasted conditions, drug release was somewhat slower under fed conditions up to 6 h. The mean percentage drug dissolved in vivo at 4 h only amounted to 4.7 and 7.1% under fed and fasted conditions, respectively. Mean percentage drug dissolved in vivo at 5 h was 13.6% and 28.4% under fed and fasted conditions, respectively. This difference may be attributable to the effect of colonic enzymes on coated beads that are estimated to arrive faster and as a bolus to the colon under fasted conditions. There was a significant difference ($p < 0.05$) between in vivo and in vitro drug release (49.5% and 53.4% of the dose was released in vivo under fed and fasted conditions, respectively compared to 100% in vitro release after 12 h). These results are consistent with previous results obtained under preliminary in vivo absorption studies in two subjects. These results also suggest that drug release occurred in the proximal enzyme-rich part of the colon.

Although results of in vitro release experiments showed what could be classified as "ideal release profiles" of the model drug from beads coated with several F1 and F2 coat formulations and "poor release profiles" with F3 coats, the in vitro characteristics of most of these systems did not predict in vivo performance. Based on in vitro data, gamma scintigraphic studies in dogs and absorption studies in human volunteers, it is most likely that 55%F3 beads release its drug content in the colon, a result that could not be predicted from in vitro studies. However, scintigraphic studies in human volunteers would be necessary to confirm these findings.

4. Conclusions

In vitro performance of small pectin-ethylcellulose coated drug beads for colonic delivery in simulated GI fluids did not predict or correlate with in vivo performance. Beads showing ideal release profiles in vitro resulted in no or little drug absorption in vivo while beads showing premature drug release in vitro might successfully deliver the drug to the colon as indicated by performing gamma scintigraphic studies in dogs and deconvolution and absorption studies in humans under fed and fasted conditions.

Conflict of interest

The authors report no declarations of interest.

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