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Selective drug delivery to the colon using pectin:chitosan:hydroxypropyl methylcellulose film coated tablets

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Abstract

A study has been carried out to assess the potential of pectin:chitosan:hydroxypropyl methylcellulose (HPMC) (P:C:H) films for colonic drug delivery. Radiolabelled (^{99m}Tc) tablets were coated with a 3:1:1, P:C:H film and administered to human volunteers. The gastro-intestinal transit of the tablets was assessed by gamma scintigraphy. The results showed that in all cases ($n = 4$), the tablets were able to pass through the stomach and small intestine intact. Break up of the tablets commenced once they were in the colon, due to degradation of the coat by colonic bacteria. The study has highlighted the potential of this coating system for colonic drug delivery. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Colonic delivery; Pectin; Chitosan; Film coating; Gamma scintigraphy

1. Introduction

The utilisation of enzymes produced by the bacteria residing exclusively in the colon is a means of obtaining site specific delivery to this region. Dosage forms have been prepared from (Rubinstein and Radai, 1995), or coated with (Milojevic et al., 1996a; Milojevic et al., 1996b) selected polysaccharides to achieve this aim. The polysaccharide, pectin, has been extensively investigated (Ashford et al., 1993a; Ashford et al., 1993b). To overcome the problem of dissolution of pectin in the upper gastrointestinal tract (GIT), relatively thick compression coats have been used (Ashford et al., 1993a; Ashford et al., 1993b; Fernandez-Hervas and Fell, 1998) or the pectin has been combined with an insoluble polymer, such as ethylcellulose, to produce a film coat (Wakerly et al., 1997).

More recently, 1-4, 2-amino-2-deoxy-β-D-glucan (chitosan), obtained by the partial *N*-deacetylation of chitin, a naturally occurring polymer

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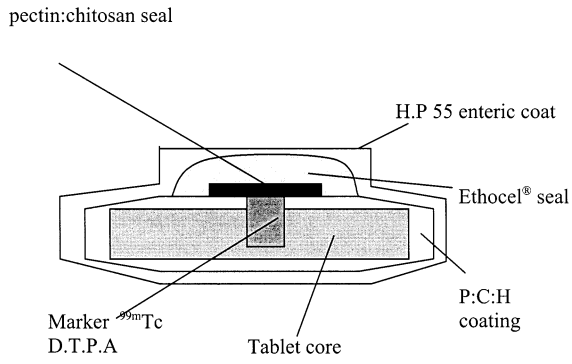

Fig. 1. A schematic representation of the labelled tablets.

present in the exoskeleton of many crustaceans, has been investigated as a material for selective colonic targeting (Fernandez-Hervas and Fell, 1998; Lorenzo-Lamosa et al., 1998). Tozaki et al. (1997) suggested that chitosan per se was degraded in the colon by the bacterial enzymes, which triggered the release of insulin from enteric coated chitosan capsules. In addition, there exists the potential of polyelectrolyte complex (PEC) formation between pectin and chitosan (Meshali and Gabr, 1993). We recently reported (Macleod et al., 1999) that films composed of pectin:chitosan:hydroxypropyl methylcellulose (P:C:H) were insoluble and swelled to different

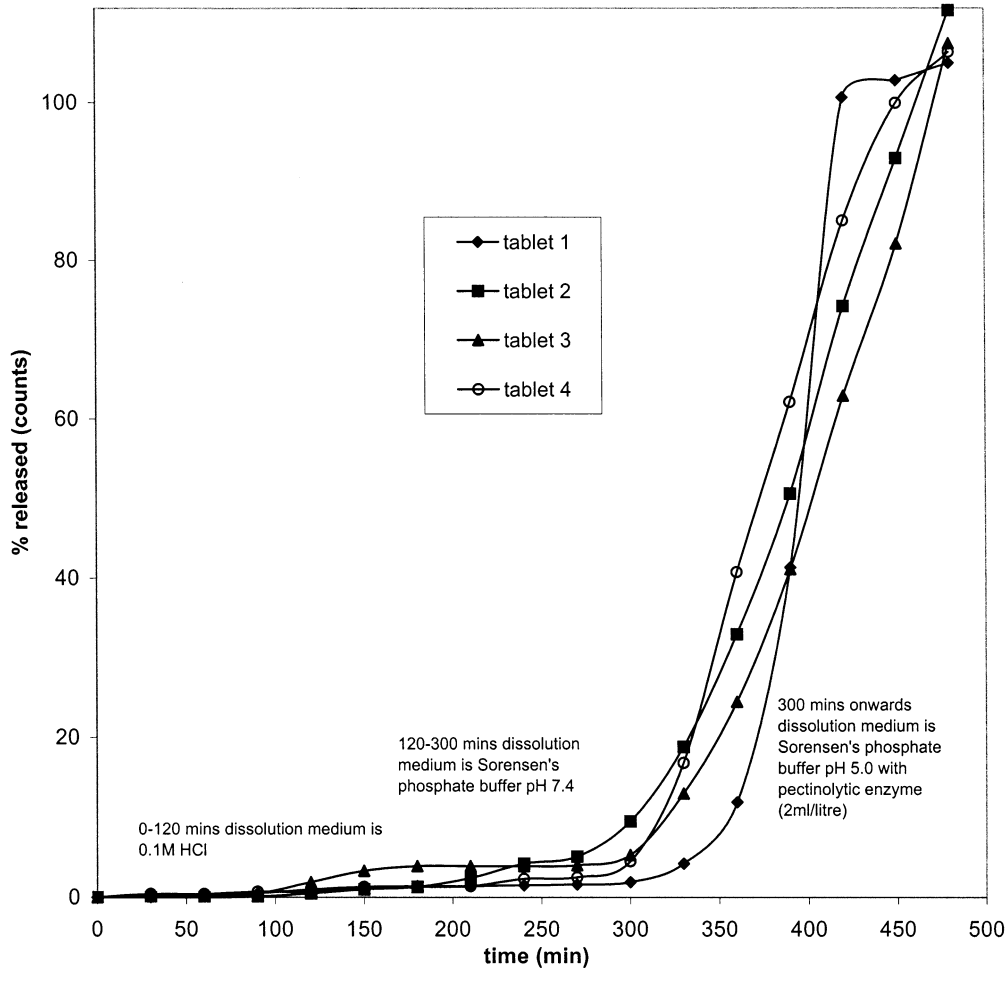

Fig. 2. In vitro radioactivity released from the coated tablets under simulated GI conditions.

Table 1
Gastrointestinal transit data for the volunteers

Subject	Gastric emptying time (min)	Small Intestinal transit time (min)	ICJ lag time (min)	Mouth to colon transit time (min)
1	15	142	58	215
2	67	150	38	255
3	113	202	175	490
4	53	182	33	268

extents in phosphate buffer according to the ratio of pectin to chitosan. This gave rise to films with different permeabilities to a model drug, the permeabilities increasing on exposure to pectinolytic enzymes intended to mimic colonic conditions. These films thus have the potential for bimodal or colonic delivery.

This paper reports the results of an in vivo study, using gamma scintigraphy, of a radiolabelled tablet formulation coated with a 3:1:1 ratio of P:C:H, a combination which has the potential to afford colon selective delivery.

2. Materials

The materials used together with the suppliers were as follows — Pectin USP (DM 70%) (Citrus Colloids, UK); chitosan (high molecular weight, DA 86.2%) (Sigma Chemical, UK); HPMC (Methocel E4M, Colorcon, UK); glycerol BP (Macarthy's, UK); colloidal silicon dioxide (Aerosil 200® USNF) and powdered cellulose (Elcema F150® USNF) both (Degussa, Germany); lactose EP (Meggle); magnesium stearate BP (SKF, UK); Starch 1500® USNF (Colorcon, UK); sodium fluorescein and hydrochloric acid (both Fison's, UK); hydroxypropyl methylcellulose phthalate (HPMCP 55 USNF, Shin Etsu, Japan); diethylphthalate USNF (Eastman Kodak, USA); ethylcellulose (Ethocel 7Cp® USNF, Dow, UK), acetone, disodium hydrogen orthophosphate and potassium dihydrogen orthophosphate (all general reagent grade, BDH, UK); DTPA (Amersham International); Technetium-99m (as pertechnetate in saline, Mallinckrodt Medical) and Pectinex Ultra SP-L, 30925 PG ml^{-1} at pH 3.5 (Novo Nordisk Ferment, Switzerland). The Pectinex Ultra SP-L solution contains a mixture of pectinolytic enzymes, mainly polygalacturonases, pectinesterases and pectin lyases and was used to mimic the conditions in the colon.

3. Methods

3.1. Tablet manufacture and coating

Core tablets of 150 mg were prepared from lactose, 69.4%; powdered cellulose, 15%; starch 1500, 15%; magnesium stearate, 0.5% and colloidal silicon dioxide, 0.5%. The excipients

Table 2
Tablet position and radioactivity released with time after administration (volunteer 4)

Time after tablet administration (min)	Estimated radioactivity remaining (%) in tablet	Estimated tablet position
2	100.0	Stomach
15	101.2	Stomach
30	102.7	Stomach
60	105.0	Small intestine
120	103.3	Small intestine
180	105.7	Small intestine
260	90.7	Ileo caecal junction
275	66.4	Ascending colon
290	63.5	Ascending colon
305	49.0	Ascending colon
335	38.0	Ascending colon
365	16.7	Spread into transverse colon
405	11.3	Spread into transverse colon

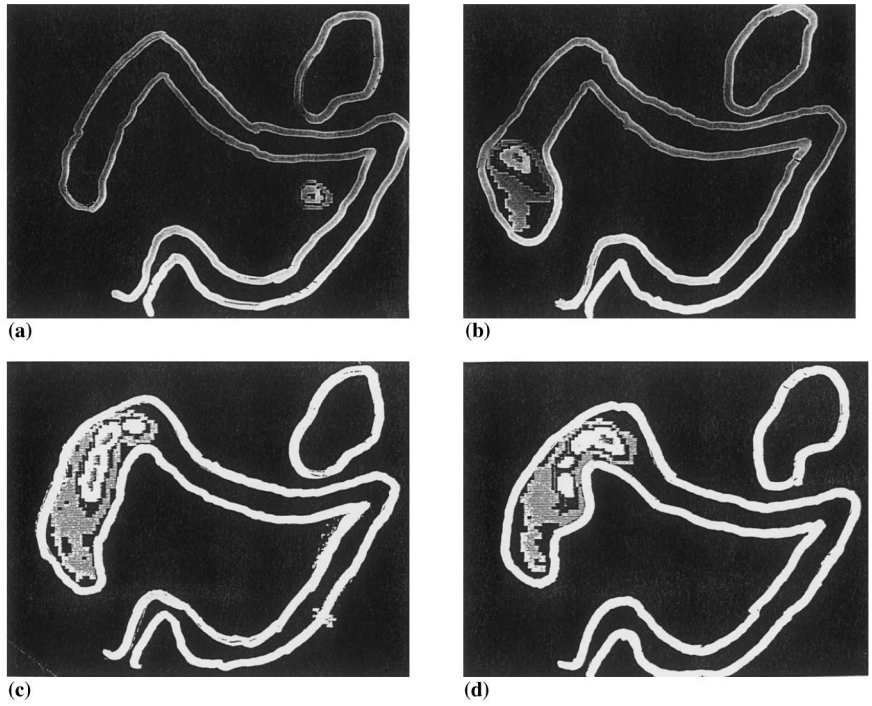

Fig. 3. (a) A scintiscan showing the tablet (intact) in the small intestine of subject 4. Time after tablet administration = 60 min. (b) A scintiscan showing the tablet having disintegrated and starting to spread in the ascending colon of subject 4. Time after tablet administration = 305 min. (c) A scintiscan showing the tablet having extensively spread in the ascending colon of subject 4. Time after tablet administration = 365 min. (d) A scintiscan showing the tablet having extensively spread toward the transverse colon of subject 4. Time after tablet administration = 405 min.

were mixed for 20 min on a Turbula® mixer prior to manufacture of the tablets. Using a rotary tablet machine (B3B, Manesty, UK), 3 kg of 10 mm diameter bevelled edged tablets were manufactured . The tablets were coated with a formulation consisting of 0.1 M HCl, 97.9% w/w; pectin, 1%; chitosan, 0.34%; HPMC, 0.34% and glycerol, 0.42% using a 16 inch diameter stainless steel coating pan (Skermans, UK). Drying air (inlet air) was introduced into the front of the pan approximately perpendicular to the tablet bed and the extract was located at the top of the coating pan. Coating was carried out at an atomising pressure of 3.5 bar, an inlet temperature of 64°C, a bed temperature of 30°C, a pan rotation speed of 16 rpm and a spray rate of 10–12 g min^{-1} (Binks 460 spray gun, Binks and Bullows, UK). Spraying was stopped after the tablets had acquired a 6.5% weight gain. The coated tablets were stored in a sealed polythene bag until use.

3.2. Tablet labelling

The tablets used for both the in vitro exploratory work and the in vivo study were prepared in an identical manner. A 1.0 mm diameter hole was drilled in the centre of the tablet to a depth of approximately 2.0 mm. The required amount of marker was then added by measurement of the radioactivity (^{99m}Tc DTPA). A drop of 3:1 pectin:chitosan in 0.1 M HCl was used to seal the tablet. This sealing process was repeated a total of five times, drying in between applications. A secondary seal was achieved using Ethocel 7Cp's dissolved in acetone (12% w/w). This secondary sealing procedure was repeated three times in total and was undertaken to ensure marker release occurred via the intact coating and not via the sealed region An enteric coating was applied to the tablets by careful painting of a 12% w/w

solution of HPMCP 55 (in acetone with 10%, as a percentage of the polymer, DEP as plasticiser) onto the tablet. A total of five enteric coats were applied, each coat being allowed to dry before the new one was applied. Fig. 1 shows a schematic representation of the labelled tablets. Testing of the tablets for leakage in 0.1 M HCl for 6 h showed less than 3% release of radioactivity, confirming the effectiveness of the seal.

3.3. Dissolution testing

The labelled tablets were tested for release of the marker using a Caleva ® dissolution apparatus following the BP 1998 Apparatus II (Paddle apparatus) method at 50 rpm and 37°C. Simulation of GIT transit was achieved by using different dissolution media. 0.1 M HCl was used for the first 2 h, pH 7.4 Sorensen's phosphate buffer for 3 h and finally, pH 5.0 Sorensen's phosphate buffer with pectinolytic enzyme (Pectinex Ultra SP-L 2 ml l^{-1}) was used to mimic the colon. The pH of 5.0 was chosen as a compromise between pH values of around 7.0 found in the colon (Evans et al., 1988) and the optimal pH for enzyme activity of 3.5. A sodium iodide crystal scintillation counter was used to measure the radioactivity (counts per 20 s) in each of the tablets prior to the dissolution experiment. The tablet with the lowest number of counts was assigned the reference tablet. The radioactivity (counts per minute) of each of the samples removed during the dissolution experiment together with that of the reference tablet were measured using a gamma counter (Cobra II, Auto-gamma, Packard, Canberra) at the end of the experiment. This allowed the percentage release (counts per minute) to be calculated for each tablet.

3.4. In vivo study

Four healthy male volunteers (age range 22–55 years; height range 1.78–1.91 m; weight range 73–92 kg) took part in the study that was approved by the University of Manchester Ethics Committee. Subjects 2 and 4 fasted overnight, whereas subjects 1 and 3 had a light breakfast 2 h prior to commencing the study. The breakfast consisted of two slices of toast and a cup of tea or coffee. Once it was apparent that the tablet had emptied from the stomach, the subjects were allowed to eat a lunch consisting of four sandwiches with a canned drink and 30 g of potato crisps.

Each subject was given 200 ml of fresh orange juice labelled with approximately 0.4 MBq of ^{99m}Tc DTPA that allowed the outline of the stomach to be visualised. Fifteen minutes later each subject swallowed a ^{99m}Tc DTPA labelled tablet (with 2.6–3.7 MBq of ^{99m}Tc DTPA), manufactured and labelled as outlined above, along with 150 ml of water. The relatively high amount of radioactivity in the tablet relative to that in the drink effectively swamps the signal from the latter allowing visualisation of the tablet only. Scintigraphic images were taken for 120 s at 15 min or appropriate intervals following the administration of the labelled orange juice. The images were taken from both the anterior and posterior position for subject 1 and 4 but from only the anterior position for the other two subjects as subsequent analysis showed that inclusion of the count data from posterior imaging made little difference to the overall count profile of the tablet as it passed down the GIT (posterior counts averaged between 7.6 and 8.4% of the total counts for subjects 1 and 4). The subjects were in the standing position for all images. An Ohio Nuclear Sigma 410 gamma camera (Packard Instruments) with a 40 cm parallel hole collimator was used to take the images. The camera was set to detect 140 keV gamma radiation with a 20% energy window. The camera was linked to a computer and the images were stored on disc for subsequent analysis using MAPS 2000 software (Link Systems). The position of the stomach, together with the high frequency of images allowed the progress of the tablet as it travelled along the GIT to be monitored. The software allowed calculation of the total counts in a region of interest corrected for a set number of pixels. It was therefore possible to draw a region of interest around the tablet which allowed an estimation of the activity of the tablet (corrected for background counts) to be made. These estimations were made at various time points (and corrected for decay) as the tablet progressed down the GIT. An estimate of the

position of the tablet within the GIT and residence time in each of the stomach, small intestine, ileo-caecal junction (ICJ) could be made. Image analysis also allowed an observation as to whether or not a spreading of the radiolabel had occurred.

4. Results and Discussion

4.1. In vitro study

Fig. 2 shows the release profiles of ^{99m}Tc DTPA from four individual tablets during the dissolution testing at different pH values. Under conditions simulating the stomach and small intestine there is minimal loss of drug confirming the value of this system for colonic delivery. The release increases in the presence of pectinolytic enzymes.

4.2. In vivo study

Table 1 summarises the GIT transit times for each of the subjects involved in the in vivo study. The time for the tablet to leave the stomach was judged against the initial outline of the stomach. The entry into the colon was judged by reference to the change from a stationary position at the ICJ to one of continuous upward movement. The results quoted are therefore subject to the discrepancies of the imaging time and the time between images. The range in gastric emptying times (15–113 min) for the tablet in this current study is similar to those reported elsewhere for fairly large (10 mm) non-disintegrating tablets (Kholsa and Davis, 1989; Wilding et al., 1995).

The results for small intestinal transit times (SITT's) show a range of 142–202 min. SITT's have been shown to be fairly reproducible and average around 3 h being independent of dosage form size and fed state of the subject (Davis et al., 1986). The results shown in Table 1 for SITT's therefore are in agreement with these observations.

The ICJ lag times shown in Table 1 range from 33–175 min. It is thought that the ICJ may act as a valve (Phillips et al., 1988) and therefore retention of large single unit dosage forms for extended periods may be expected (Adkin et al., 1993). The results in Table 1 show that the tablet was retained for at least 30 min in all four subjects and therefore concur with the theory that the ICJ may afford a retaining function.

The range in colon arrival times shown in Table 1 is 215–490 min. Again these results are similar to colon arrival times reported elsewhere for large non-disintegrating tablets (Abrahamsson et al., 1996).

Table 2 shows the data for radioactivity remaining in the tablet relative to the tablet position for volunteer 4. In this and in all subjects, release of radioactivity is minimal when the tablet is in the stomach, small intestine or ICJ of the GIT. It is only once the tablet enters the ascending colon that a significant decrease in the percentage activity remaining in the tablet is seen in all four subjects. Fig. 3a shows that when the tablet is in the small intestine the radioactivity is concentrated in a very small area which is indicative that little release has occurred. Once the tablet enters the ascending colon (Fig. 3b–d) there is considerable spreading of the radioactivity up the ascending colon toward the transverse colon. This spreading is most likely caused by the action of the bacterial enzymes in the colon degrading the film coat and accelerating the release of the radioactivity.

5. Conclusion

The delivery of drugs directly to the colon via the oral route has several useful therapeutic advantages. The results from this study clearly show the potential of mixed P:C:H films for colonic drug delivery. These coatings are capable of retarding the release of tablet core materials until they reach the colon, an environment rich in bacterial enzymes, which degrade the coating allowing drug release to occur.

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