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RESEARCH ARTICLE

NOVEL DRUG DELIVERY SYSTEM

IN-VITRO EVALUATION OF PECTIN AS A COMPRESSION COATING MATERIAL FOR COLON TARGETED DRUG DELIVERY

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ABSTRACT

Colon targeted delivery of satranidazole (STZ), which immediately releases the drug as soon as the drug delivery system reaches the colon was formulated. Different grades of pectin were used as a compression coat and the effect of degree of esterification (DE) of pectin on swelling and drug release property was evaluated. STZ is a sparingly soluble drug, so to obtain maximum effect of drug at the site of action the solubility enhancement was carried out using Hydroxypropyl-β-cyclodextrin (HP-β-CD). Pectin as a compression coat was unable to direct STZ containing core tablets to the colon, so HPMC was added to increase the tensile strength of the coat. STZ core tablets compression coated with High DE pectin: HPMC in 1:1 ratio imparted the lag time of 5 h and burst release in colon.

KEYWORDS

Crohn's Disease, Degree of esterification, HP-β-CD, HPMC, swelling study.

INTRODUCTION

Drug delivery systems to the colon are being extensively investigated in order to treat local colonic diseases like irritable bowel syndrome, crohn's disease and ulcerative colitis [1]. Satranidazole (STZ) is a novel 5-nitroimidazole possessing superior activity against anaerobes as compared to metronidazole, tinidazole and ornidazole. STZ can be used as an ideal therapy for crohn's disease [2]. Colon specific drug delivery of STZ will not only increase the availability of the drug at the target site, but also may reduce the dose requirement and the side effects. Amongst different methods developed for targeting drugs to the colon, polysaccharide-based delivery systems rely upon the enzymatic degradation of the carrier in the colon, thereby resulting in drug release [3]. The enzyme trigger mechanism in such delivery systems makes them site specific [4, 5].

Of all the different polysaccharides, pectin is one of the most extensively investigated for its suitability for targeting drugs to the colon. Pectin is normally classified according to its degree of esterification. Pectin in which less than 50% of the carboxyl acid units occur as the methyl ester is normally referred to as low ester or low DE pectin, whereas pectin with 50% or more carboxyl acid units as the methyl ester is referred to as high DE pectin. Some of the carboxyl groups may be converted to carboxamide groups, when ammonia is used in the process of de-esterification, producing amidated pectin [6].

Many researchers have explored usefulness of pectin for directing drug to the colon. Pectin is used alone [7] or in combination chitosan [8] as a compression coat, and as a film coat in combination with ethyl cellulose [9] and Eudragit RS/NE [10]. The aim of the present work is to formulate colon-targeted tablets of STZ. STZ is very sparingly soluble in water (0.01mg/ml). Since the aim is to treat the local pathologies of colon, it is essential for drug to be in soluble form at the site of action to provide maximum effect. Thus, the study was divided in two parts, a) To increase the solubility of STZ, and b) To formulate pectin based colon targeted drug delivery system for satranidazole. Previously, STZ solubility has been increased by complexation with β-cyclodextrin [11]. In the present study Hydroxypropyl-β-cyclodextrin (HP-β-CD) was tried to increase the solubility of STZ.

MATERIALS AND METHODS

Pectin Classic CU 201 (Non Amidated High methoxy pectin, Degree of esterification (DE): 71, High DE Pectin), Pectin Classic CU 701 (Non Amidated Low methoxy pectin, DE: 38, Low DE Pectin), and Pectin Amid CU 020 (Amidated Low methoxy pectin, DE: 30, Degree of amidation: 19, Amidated Low DE Pectin) were a kind gift from Herbstreith and Fox (Neuenburg, Germany). Pectinex Ultra SP-L® (pectinolytic enzymes, extracted from Aspergillus niger and having an activity of 26,000 PG/ml at pH 3.5) was kindly supplied by Novo Nordisk Ferment Ltd. (Dittingen, Switzerland). STZ was obtained from Alkem Laboratories (Mumbai, India). Colorcon (Mumbai, India) kindly provided HPMC K4M (Methocel® K4M). HP-β-CD was a generous gift from Roquette (France). Flowcel® 301 and Cross Carmellose Sodium were supplied generously from Gujarat Microwax Ltd. (Ahmedabad, India). Double distilled water was used throughout the study. All other materials used were of analytical reagent grade.

(i) Solubility enhancement of STZ

STZ was triturated with HP-β-CD in molar ratio of 1:0.1, 1:0.2 and 1:0.3, using water-methanol (1:2 v/v) in a quantity sufficient to form thick paste. The kneading time for

mixture was optimized to 60 min. The kneaded mass was dried at 45 ºC till the moisture content of sample comes between 4-5 %. The dried mass was sifted through 40 # sieve.

(ii) X-Ray Diffraction (XRD) study

The powder XRD patterns of STZ and STZ-HP-β-CD complex (STZ-CD) were recorded by using automated Philips Holland –PW 1710 scanner with filter Cu radiation over the interval 5-60°/2θ. The operation data were as follows: voltage 35 kV, current 20 mA, filter Cu and scanning speed 1° / min. The XRD study was carried out to check the conversion of reduction in crystallinity of STZ after kneading it with HP-β-CD.

(iii) Preparation of core and compression coated tablets

Initially, Flowcel® 301 (diluent), PVP K30 (binder, 8%), Croscarmellose Sodium (disintegrant, 5%) and Talc (glidant, 2%) were sifted through 20 # sieve and was blended with, whereas Magnesium stearate (lubricant, 1%) was sifted through 40 # sieve. Thereafter, STZ-CD equivalent to 300 mg of STZ/tablet was blended with Flowcel®, PVP, Cross Carmellose Sodium and Talc for 15 min. in a bin blender (Inweka Multi-Purpose instrument, Gujarat, India). This mixture was blended with Magnesium Stearate for 5 min and then compressed into 700 mg tablets using 10 station Rotary tablet machine (Minipress-II, Karnavati Engineering Limited, Gujarat, India), equipped with 13 mm concave punches. The core tablets were tested for hardness, thickness, content uniformity, friability, and disintegration.

The compression coat material was prepared using either pectin or a combination of HPMC K4M: Pectin in ratios of 1:3, 1:1 and 3:1 at 200 mg compression coat weight. Core tablets were compression coated with different compression coating mixtures as shown in Table 1. For compression coating, exactly 50% of the coat powder was first placed in the die cavity of the compression machine. Then, the core tablet was carefully positioned at the center of the die cavity, which was filled with the remainder of the coat powder. The coating material was compressed around the core tablet at an applied force of 5000 kg using 15 mm round concave punches using a 10 station Rotary tablet machine.

Table 1
Compression coat combinations

Pectin type	Batch code	HPMC (mg)	Pectin (mg)
Pectin Classic CU 201	P2	-	200
	HP231	150	50
	HP211	100	100
	HP213	50	150
Pectin Classic CU 701	P7	-	200
	HP731	150	50
	HP711	100	100
	HP713	50	150
Pectin Amid CU 020	P0	-	200
	HP031	150	50
	HP011	100	100
	HP013	50	150

(iv) In Vitro Drug Release Studies

In-vitro drug release studies were carried out using USP XXIII dissolution test apparatus Type II, paddle apparatus (100 rpm/min, 37 ± 0.5 ^{0}C). Compression coated colonic tablets were evaluated by exposing them to 900 ml 0.1 N HCl (simulated gastric fluid, SGF) for 2 h, which was then replaced with 900 ml pH

7.4 phosphate buffer solution (simulated intestinal fluid, SIF) wherein it was kept for 3 h and lastly SIF was replaced with 900 ml pH 6.8 phosphate buffer solution (simulated colonic fluid, SCF), and tested for release for the rest of the dissolution run. The drug release at different time intervals was analyzed by UV double beam spectrophotometer (Shimadzu UV 2450, Japan) at 319 nm. Each test was performed in triplicate.

(v) Swelling studies

The compression coated pectin/ pectin-HPMC tablets were accurately weighed (W0) and placed in the USP paddle apparatus (Electrolab, India) in a manner similar to method described under in-vitro drug release studies for compression coated tablets. The changes in weight and swelling were recorded on hourly basis from the beginning and continued until one time point before (n-1) the erosion of the tablet. The n-1 time point was selected on the basis of in-vitro drug release study carried out in presence of pectinolytic enzymes. After each time point tablets were withdrawn from the medium and lightly blotted with tissue paper to remove excess test liquid and then reweighed (W1). The experiment was performed in triplicate. The percentage increase in weight due to absorbed medium was estimated at each time point from the following equation:

% weight gain = W1-W0/W0 x 100……...(1)

RESULTS AND DISCUSSION

(i) Solubility enhancement of STZ

Aim of the present investigation was deliver STZ to colon for treatment of local pathologies of colon. For the local treatment drug needs to be in soluble form so as to attain its maximum therapeutic effect. STZ shows pH dependent solubility with HP-β-CD complex (Figure 1). 100% drug release was obtained in phosphate buffer pH 7.4 within 25 min. at 1:0.2 ratio of STZ: HP-β-CD.

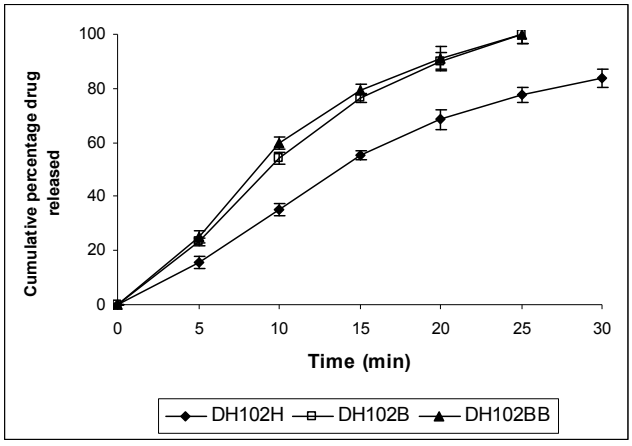

Figure 1

Effect of pH on solubility of STZ (data shown as mean ± SD, n=3)

(ii) X-Ray Diffraction (X-RD) study

Crystallinity was determined by comparing peak heights in the diffraction patterns of the STZ-CD (Sample B) with those of a STZ (Sample A). The powder XRD patterns for the STZ and STZ-CD are presented in Figure 2. Characteristic diffraction peaks of STZ and STZ-CD between at 0° and 40° (2θ) were used for conformation studies. The STZ-CD complex showed all characteristics peaks corresponding to the drug, but with lower intensity. The possible reduction in crystallinity may be due to complex formation.

The relationship used for the calculation of crystallinity was relative degree of crystallinity (RDC)

RDC= Hsam / Href……..(2)

Where, Hsam = the peak heights of the sample investigated and Href = the peak heights of STZ with the highest intensity at 2θ values of 15, 21, 25.

The RDC values of corresponding STZ-CD were 2.66, 2.37 and 3.17 at 2θ values of 15, 21 and 25 respectively. RDC values higher than 1 indicate reduction in crystallinity of the STZ-CD.

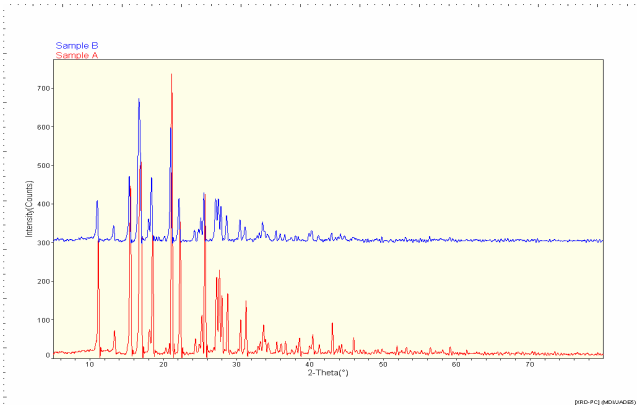

Figure 2
XRD pattern of STZ (Sample A) and STZ-β-cyclodextrin complex (Sample B)

(iii) Core tablets

The hardness of the core tablets was found to be in the range of 4.2–5.5 kp. These tablets were found to comply with the friability test since the weight loss was found to be 0.13%. Due to the presence of cross-carmellose sodium in the core tablets, the disintegration time of the core tablets was found to be 2 min ±10 sec. The matrix tablets contained 97.8% to 102.9 % of STZ in each batch.

(iv) Effect of different grades of pectin on release property in different media

Core tablets compression coated with Pectin 701 and 020 showed 100% drug release within 1 h and 0.5 h respectively, in both SGF and SIF (Figure 3a and 3b). On the contrary, Pectin 201, a High DE Pectin, delayed the drug release to 3 h in acidic medium as compared to 2 h in basic medium (refer Figure 3a and 3b). This finding is probably due to the lack of gel formation of High DE pectin in phosphate buffer. High DE pectin requires a relative low pH for gel formation, while Low DE pectin requires the presence of divalent cations [12]. Higher the pectin hydration-gel forming ability, the lesser the drug was released.

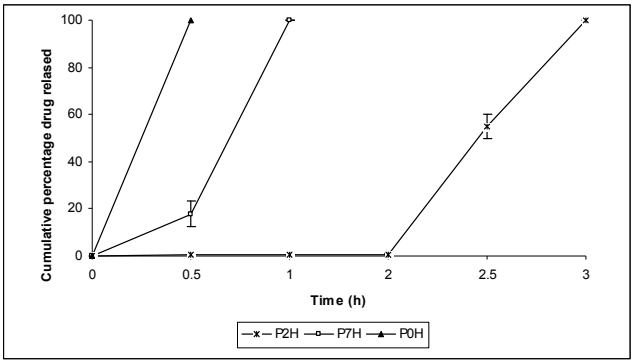

Figure 3a
Release in 0.1 N HCl (data shown as mean ± SD, n=3)

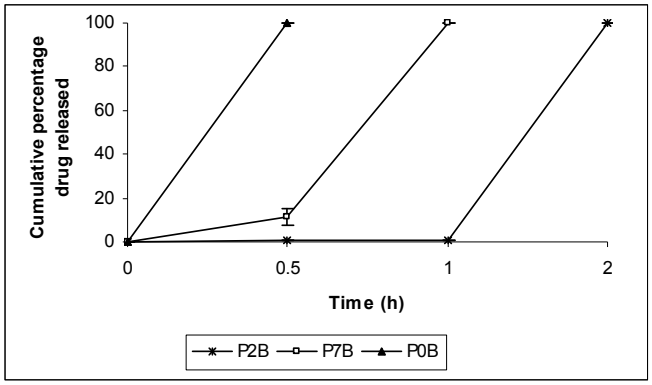

Figure 3b
Release in Phosphate buffer pH 7.4 (data shown as mean ± SD, n=3)

(v) Effect of addition of HPMC to the compression coat

From the above results it was clear that pectin alone as a compression coat was unable to prepare colon targeted formulation since it showed premature drug release. To provide sufficient lag time to the system to reach colon, HPMC was added to the compression coat containing pectin. The lag time for a formulation to reach colon was taken as 5 h. Compression coats containing HPMC:Pectin in the ratio of 1:3 were not able to reach colon. Pectin 201 delayed drug release to the higher extent compared to other grades of pectin used. Batch HP211 containing HPMC:Pectin 201 in the ratio of 1:1 delayed drug release to 7.5 h whereas batch HP231 containing HPMC:Pectin 201 in the ratio of 3:1 delayed drug release to 10 h (Figure 4).

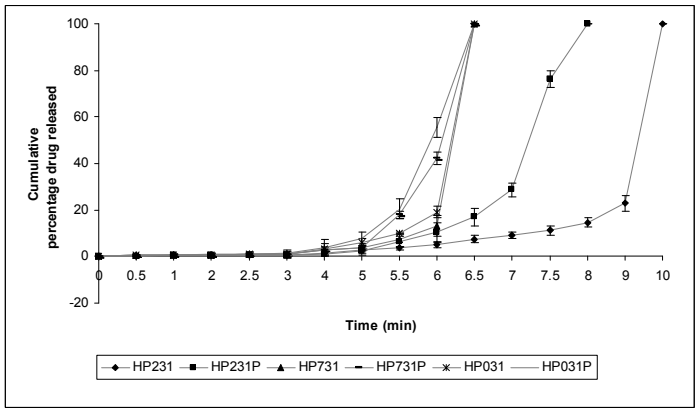

Figure 4
Effect of HPMC: Pectin ratio (data shown as mean ± SD, n=3)

Delay in drug release by HP211 is attributable to, (a) strong gel formation of Pectin 201 in SGF, and (b) high swelling capacity. Batch HP211 showed highest weight gain of 135% (Figure 5) at n-1 time point. This study acted as a proof of concept for proving high DE pectins have the highest swelling and water uptake capacity.

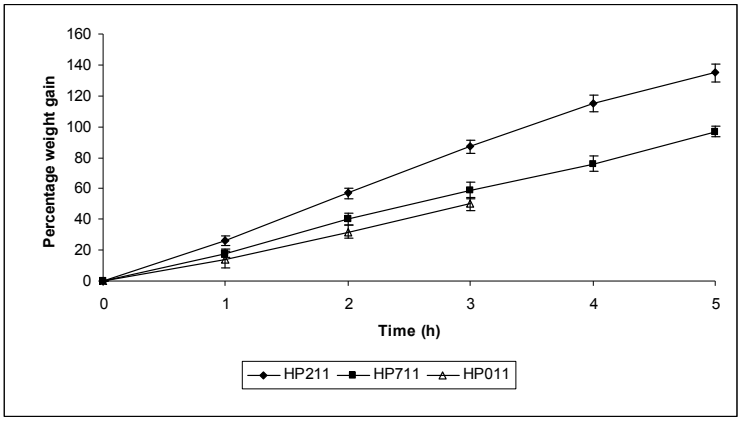

Figure 5
Water uptake study (data shown as mean ± SD, n=3)

(vi) Effect of the Presence of Pectinolytic Enzymes

With the aim of evaluating the influence of the pectin biodegradation process on the drug release profile, we performed a series of experiments by adding in the SCF a commercially available mixture of specific enzymes (Pectinex Ultra SP-L), whose pectinolytic activity showed to be closely correlated with that of the Bacteroides ovatus, the main producer of pectinolytic enzymes in colon [6].

The drug release curves obtained from experiments in the presence of pectinolytic enzymes were clearly different from those previously obtained from the same tablets in the absence of enzymes (see Figure 6a and 6b), thus confirming the actual potential of pectins in colon specific delivery. This result is probably attributable to the different degradation rates of the various pectin types, which, in this case, were directly related to their hydration and gel forming properties. Amongst different grades of pectin tried, High DE pectins, the pectin with the highest viscosity among those examined, was the most sensitive to the action of pectinolytic enzymes, showing the greatest improvement in drug release rate in comparison with the results obtained without enzymes. It is supposable that High DE pectin, being the pectin with the highest swelling hydration power, allows a faster penetration of colonic enzymes which, consequently, leads to a faster degradation of the pectin barrier. The percent of drug release from High DE pectin containing tablets changed from 11% after 6 h (Batch HP211), in the absence of enzymes, to 100% (Batch HP211P) in the presence of enzymes. As expected, it was also observed that effect of enzyme addition was more pronounced in batches containing higher amount of pectin. In presence of enzymes, comparing drug release profile of batch HP211P with HP231P it was observed that batch HP211P containing HPMC:Pectin 201 in the ratio of 1:1 shows 100% drug release in colon within 1 h after the system reaches the colon, whereas batch HP231P containing containing HPMC:Pectin 201 in the ratio of 3:1 takes 3 h for the same. The delay in drug release indicates that higher the amount of HPMC in compression coat lesser is the effect of enzyme on degradation of compression coat.

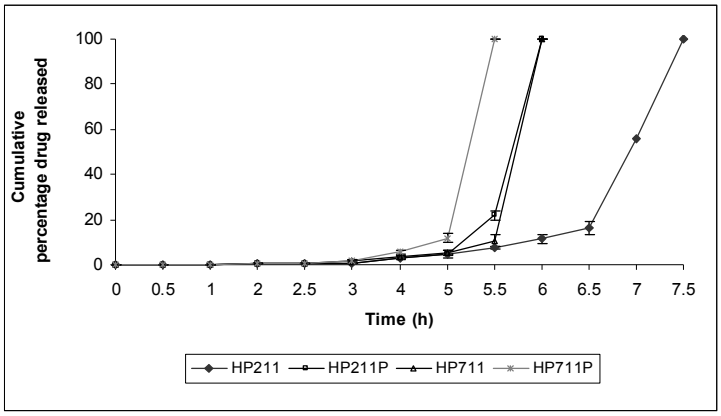

Figure 6a

Effect of Pectinolytic enzymes at 1:1 ratio HPMC: Pectin (data shown as mean ± SD, n=3)

In-vitro dissolution study carried (a) in absence of pectinolytic enzymes (HP211, HP711) and (b) in presence of pectinolytic enzymes (HP211P, HP711P).

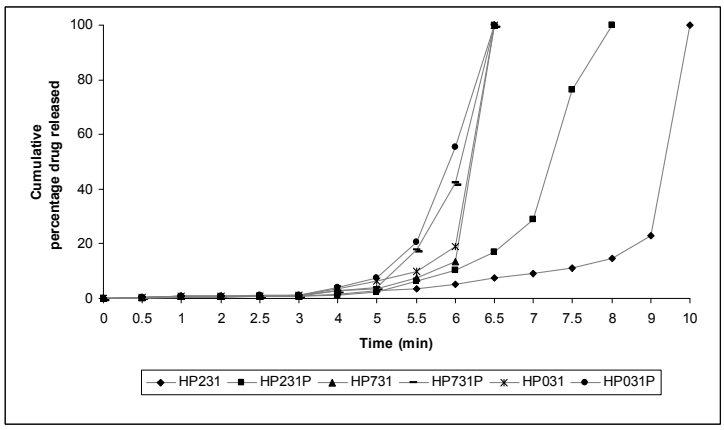

Figure 6b

Effect of Pectinolytic enzymes at 3:1 ratio HPMC: Pectin (data shown as mean ± SD, n=3)

In-vitro dissolution study carried (a) in absence of pectinolytic enzymes (HP231, HP731 and HP031) and (b) in presence of pectinolytic enzymes (HP231P, HP731P and HP031P).

(vii) Selection of best batch

The aim of this study was to develop CTDDS for STZ with an intent to have <10% release at the end of 5 h (which is considered as lag time for the system to reach colon) and 100% release within 1 after the system reaches the colon. Moreover to make the system more colon specific it was desirable to choose those batches which are more prone to pectinolytic enzyme degradation. Core STZ tablet compression coated with HPMC:Pectin in the ratio of 1:3 showed premature drug release, whereas core tablets compression coated with HPMC:Pectin in ratio 3:1 showed colon targeting ability but with a disadvantage of delayed drug release. Only batch HP211P compression coated with HPMC:Pectin in ratio 1:1 met the desired criteria. Thus, batch HP211P was considered as best batch.

CONCLUSION

Complexation of STZ with HP-β-CD reduces the crystallinity of STZ and thus increases the solubility. STZ solubility increases with the increase in pH. Pectin alone as a compression coat cannot direct STZ to the colon. Addition of HPMC increases the tensile strength of the compression coat and helps in achieving desired lag time for the formulation

to reach colon. The results of this study enable us to state that DE plays an important role in swelling mechanism of the pectin. High DE pectins show higher swelling capacity when exposed to the acidic medium. The presence of the pectinolytic enzymes in the dissolution media results in the increase of the STZ release rate from the compression coated systems. Systems compression coated with high DE pectins are more susceptible to pectinolytic enzymes since than the low DE pectins.

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