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Research Article

Design, development and in vitro evaluation of Mesalamine tablets containing Pectin and Chitosan for colon-specific drug delivery

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ABSTRACT

The potential of matrix, multilayer and compression coated tablets of Mesalamine to reach the colon intact has been investigated *in vitro*, using Pectin as a carrier. Matrix tablets containing various proportions of Pectin were prepared by wet granulation and direct compression techniques. Multilayer tablets were formulated using Pectin as release controlling layers, on either side of Mesalamine matrix tablets. Mesalamine core tablets were prepared and compression coated with Pectin. The effect of the coat: core ratio as well as the incorporation of different percentages of Chitosan in the Pectin coat on drug release was investigated. *In vitro* release studies indicated that matrix and multilayer tablets failed to control the drug release in the physiological environment of stomach and small intestine. Compression coated formulations were able to protect the tablet cores from premature drug release, but at high Pectin coat: core ratios 4: 1 (F13) and 5: 1 (F14). Inclusion of Chitosan 3% and 5% w/w (F11 and F12) in the Pectin coat offered better protection at a lower coat: core ratio (3: 1). Selective delivery of Mesalamine to the colon could be achieved using a Pectin or Pectin/Chitosan mixture in the form of compression coated tablets.

Keywords: Mesalamine; Pectin; Chitosan; Rat ceacal content; Compression coated tablet; Colon-Specific Drug Delivery.

INTRODUCTION

Since from last decade a novel oral colon-specific drug delivery system (CDDS) has been developing as one of the site-specific drug delivery systems. This delivery system, by means of combination of one or more controlled release mechanisms, hardly releases drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the colon following oral administration. (Kinget 1998, Watts 1997 and Yang 2002) CDDS is convenient for treating localized colonic diseases, i.e. ulcerative colitis, Crohn's disease and constipation *etc.*, CDDS, also selectively deliver drug to the colon, but not to the upper GI tract. (Kinget 1998) Colon is referred to as the optimal absorption site for protein and polypeptide after oral administration, because of the existence of relatively low proteolytic enzyme activities and quite long transit time in the colon. CDDS would be advantageous when a delay in absorption is desirable from a therapeutically point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis. (Halsas 1999 and Halsas 2001)

Various systems have been developed for colon-specific drug delivery. These include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, time dependent release systems, and enzymatically controlled delivery systems. Enteric coated systems are the most commonly used for colonic drug delivery, but the disadvantage of this system is that the pH difference between small intestine and colon is not being very pronounced. These delivery systems do not allow reproducible drug release. The limitation of time dependent release system is that it is not able to sense any variation in the upper gastro-intestinal tract transit time, any variation in gastric emptying time may lead to drug release in small intestine before arrival to colon. Apparently, the most convenient approach for site-specific drug delivery to colon is enzymatically controlled delivery systems. No drug release can occur unless the system arrives to the colon. (Basit 2005 and Chourasia 2005)

The potential of Pectin as carriers for colonic drug delivery has been demonstrated previously. Pectin is heterogeneous polysaccharides composed mainly of galacturonic acid and its methyl ester. (Hiorth 2006, Mura 2003 and Ofori-Kwakye 2003) They are refractory to

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Table 1: Composition of Mesalamine matrix tablet formulations

Technique	Formulation	Ingredients (mg.)					
		Pectin	Avicel PH 101	HPMC	Starch Paste	Talc	Magnesium stearate
Wet Granulation	F1	140	62	----	50	10	5
	F2	185	17	----	50	10	5
	F3	150	17	40	50	10	5
	F4	95	17	95	50	10	5
Direct Compression	F5	140	120	----	----	----	----
	F6	185	75	----	----	----	----

host gastric and intestinal enzymes, but are almost completely degraded by the colonic bacterial enzymes to produce a series of soluble oligogalacturonates. (Macfarlane 1990) Depending on the plant source and preparation; they contain varying degrees of methyl ester substituents. The degree of methoxylation determines many of their properties, especially solubility and requirements for gelation. High methoxy Pectins (HM) are poorly soluble and require a minimum amount of soluble solids and a pH around 3 to form gels. Low methoxy Pectin is more hydrophilic and soluble than High Methoxy Pectin in pH 7.4 buffer, due to the larger number of ionized carboxyl groups. They require the presence of a controlled amount of calcium ions for gelation. Chitosan is a partially deacetylated polysaccharide obtained by alkaline treatment of chitin, one of the most abundant biopolymers in nature. Chitosan has been widely researched for biomedical applications such as wound healing, drug delivery systems, coatings and tissue engineering, as well as applications in food, cosmetics and agricultural industries. Chitosan has been gaining increasing importance in the pharmaceutical field owing to its good biocompatibility, low toxicity and biodegradability. The degradation products of Chitosan are nontoxic, nonimmunogenic, and noncarcinogenic. (Agnihotri 2004)

Mesalamine is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis. However, when Mesalamine is administrated orally, a large amount of the drug is absorbed from the upper gastrointestinal tract, and causes systemic side effects. Free Mesalamine undergoes rapid and nearly complete systemic absorption from the proximal intestine depending on concentration and local pH, followed by extensive metabolism. (Carceller 2001 and Jung 2001) It is thus of tremendous importance to deliver Mesalamine locally in order to reduce influences by systemic drug absorption causing adverse effects and drug loss lowering the probability for a therapeutic success. Hence, selective delivery of Mesalamine into the colon is required.

The aim of the present study was to investigate the development of various Mesalamine colon-specific delivery systems that could be formulated by application of the usual simple tabletting techniques, and by using the natural polysaccharide High Methoxy Pectin as a carrier.

MATERIALS AND METHODS

Materials

Mesalamine was gifted from BEC Chemicals Ltd., Ankleshvar, India. High Methoxy Pectin (Spec. degree of esterification 65% to 70%, Galacturonic acid 65 %, Min. gel. Degree 150) was gifted from WJF Chemicals Co, China. Cellulose was obtained from Mahtani Chitosan Pvt. Ltd, Gujarat, India. Hydroxypropyl methyl cellulose, Avicel PH 101, Corn Starch, Talc and Magnesium stearate was obtained from National chemicals, Baroda, India.

Methods

Preparation of matrix tablets

Matrix tablets, each containing 200 mg Mesalamine were prepared by wet granulation and direct compression techniques using High methoxy Pectin (less than 160 µm) alone or in combination with HPMC as matrices (Table 1). Tablet formulations (F1–F4) were blended and granulated with 10% Corn Starch paste. The wet mass was passed through a mesh (1000 µm) sieve and the granules were dried in Hot air Oven at 50°C for 2–3 h. The dried granules were sieved (600 µm), lubricated with magnesium stearate: talc (1: 2) mixture and compressed on a single-punch tablet machine, (CMD3-16, M/S. Cadmach Machinery Co. Pvt. Ltd, Ahmadabad, INDIA) using 12 mm round slightly concave punches. In tablet formulations (F5, F6), powder ingredients were sieved (smaller than 250 µm), blended and directly compressed with compression force 20 KN on a single-punch tablet machine, (CMD3-16, M/S. Cadmach Machinery Co. Pvt. Ltd, Ahmadabad, INDIA) using 12 mm round, slightly concave punches that had been surface lubricated with magnesium stearate talc mixture. Talc and magnesium stearate were not included in formulations F5 and F6, as they adversely affected the hardness of the tablets.

Preparation of multilayer tablets

Mesalamine multilayer tablets composed of matrix tablet formulation (F2) with an upper and a lower layer of High methoxy Pectin (less than 160 µm) were prepared. Moderately compacted granules of F2 were

compressed with either 50 mg or 100 mg of Pectin powder (p.s. < 160 µm) on each side, using 12 mm round slightly concave punches with compression force 20 KN to produce multilayer tablets F7 and F8, respectively.

Preparation of compression coated tablets

Rapidly disintegrating core tablets (average weight 125 mg) of Mesalamine were prepared by the direct compression technique. Each core tablet consisted of Mesalamine (200 mg), Avicel PH101 (30 mg), croscarmellose sodium (12 mg), talc (5 mg) and magnesium stearate (2.5 mg). All ingredients were passed through a 120 µm sieve, thoroughly mixed and compressed on a single-punch tablet machine, (CMD3-16, M/S. Cadmach Machinery Co. Pvt. Ltd, Ahmedabad, INDIA), using 8 mm round flat faced punches. After passing the quality control tests of drug content uniformity, hardness, friability, disintegration and dissolution rate, core tablets were compression coated using High methoxy Pectin (less than 160 µm) at different coat: core ratios, with and without the addition of different percentages of Chitosan (Table 2). Half the quantity of the coating material was placed in a 14 mm die cavity, the core tablet was carefully positioned in the centre of the die cavity, the other half of the coat was added and compressed using 14 mm round concave punches that have been surface lubricated with magnesium stearate talc mixture. Coating pressures was 20 KN which was measured with help of laboratory hydraulic press that was attached with tablet machine. The thickness of the core and coated tablets was determined using a micrometer. The coat thickness was taken as half the difference between the core and coated tablet thickness. All the tablet formulations under study were assessed for their drug content uniformity, hardness and *in vitro* drug release.

Preparation of rat ceacal content medium

Six rats weighing (150–250 g) and maintained on a normal diet were used. To induce enzymes acting specifically on Pectin and Chitosan in the caecum, 1 ml of 2% w/v Pectin and Chitosan (3:1) aqueous dispersion was directly administered to the rats daily for 5 days. Forty-five minutes before the drug release experiment, the rats were sacrificed by cervical dislocation. The abdomen was opened; the caecum was traced, ligated at both ends, dissected and immediately transferred into phosphate buffered saline (PBS), pH 6.8, previously bubbled with nitrogen to maintain anaerobic conditions. The ceacal bags were opened; their contents were weighed and suspended in PBS to give a final ceacal content concentration of 1.5% w/v. (Sinha 2004, Krishnaiah 2002 and Prasad 1998)

In-vitro drug release studies

Drug release studies were conducted under conditions mimicking mouth-to-colon transit. The dissolution medium consisted of 900 ml 0.1 mol/l HCl for 2 h, replaced by 900 ml phosphate buffer, pH 7.4 for 3 h, kept at 37 ± 0.5°C and stirred at 100 rpm, using USP dissolution apparatus 1. Samples were withdrawn at the end of the specified periods (2 h and 5 h), filtered and assayed spectrophotometrically (UV-3600 UV-VIS-NIR, Shimadzu, USA) for Mesalamine, at 301.5 nm in 0.1 mol/l HCl and 334.5 nm in pH 7.4 buffer. To assess the susceptibility of the prepared Mesalamine delivery systems to the enzymatic action of colonic bacteria, drug release studies were continued in PBS pH 6.8 in the absence (control) and presence of rat ceacal contents since these are known to have similar contents to those of human intestinal microflora. (Macfarlane 1990) The studies were carried out using USP dissolution apparatus 1 (100 rpm, 37°C) with slight modifications. A glass beaker (250 ml) containing 100 ml PBS medium was immersed in water maintained in the flask of the dissolution apparatus. After completing the dissolution in 0.1 mol/l HCl (2 h) and phosphate buffer, pH 7.4 (3 h), baskets containing the tablets under study, were immersed in the PBS medium and the release study was continued for up to 24 h. Samples were withdrawn at different time (6, 8, 12 and 24 h), filtered using membrane filters (0.45 µm) and assayed spectrophotometrically for Mesalamine at 334.5 nm. The same volume of fresh dissolution medium was added to restore the initial volume of the dissolution medium after each sample withdrawal. Experiments were carried out in triplicate.

Drug Release Kinetics

The following kinetic models were fitted to the raw release data:

(a) The zero order model, describing release from porous (erodible) matrices: (Hadjiioannou 1998)

$$M = K_0 t \quad \text{(1)}$$

(b) The square root of time or Higuchi model, describing release by Fickian diffusion through a porous matrix: (Higuchi 1963)

$$100 - M = K_2 t^{1/2} \quad \text{(2)}$$

(c) The cube root law, or Hixson-Crowell model, describing release from monolithic drug particles: (Hixson 1931)

$$100^{1/3} - M^{1/3} = K_3 t \quad \text{(3)}$$

(d) The power law model of Peppas, which for certain values of the exponent (1 and 0.5) converts to the zero order or square root of time model: (Korsmeyer 1983)

$$M/M_\infty = K_p t^n \quad \text{(4)}$$

where M is the percentage of undissolved drug, $M_{infinitive}$ is the drug dissolved after infinite time, kp is the release rate constant and n is a characteristic exponent (n acquires values between 0.43 and 1.0 depending on matrix geometry and release mechanism, in cases of

coupling diffusion and polymer relaxation phenomena or anomalous transport), and finally

(e) the theoretical model describing drug release by diffusion and matrix degradation: (Siepmann 2001)

$$M = A\sqrt{\frac{e^{k_C t} - 1}{k_C}} \quad \text{(5)}$$

Where M is the percentage of dissolved drug, A is the Higuchi constant, calculated by fitting the square root model to the initial part of the curve, and k_C is the matrix erosion constant, calculated by fitting Eq. (5) to the final part of the dissolution curve. The model assumes first order degradation kinetics and it was chosen due to the apparent analogies between degradation of the polymer matrix and erosion by the action of mechanical forces in a disintegration-like manner.

The goodness of fit was compared on the basis of the correlation coefficients (R) and lag times (intercept or theoretical times at which the fraction of drug remaining is 100%, practically representing the time needed for hydration of the matrix in order to initiate drug transport to the solution). Models with best fit are considered those of highest (closest to 1) correlation coefficients and lag times least deviating from zero. Eq. (5) cannot be directly compared with the rest of the models since it is fitted by a two-stage procedure involving non-linear regression. It is used to evaluate the contributions of diffusion and erosion processes on total drug release by comparing the ratio of the erosion over diffusion kinetic constants.

Statistical analysis

The percentages Mesalamine released from the selected compression coated tablets F12, F13 and F14, after 24 h of dissolution testing in the presence and absence of rat ceacal contents were compared, using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant. (Bourne 2002)

RESULTS AND DISCUSSION

Mesalamine matrix (F1-F6), multilayer (F7, F8) and compression coated (F9-F14) tablets prepared in the present study complied with the official requirements for the drug content uniformity test according to B.P. and showed an acceptable mechanical strength (friability < 1%, hardness values in the range of 4–8 kg, Table 2). The ability of the various delivery systems, under study, to protect the drug in the physiological environment of the stomach and small intestine and allow its release into the colon was assessed by carrying out drug release studies in 0.1 mol/l HCl for 2 h, pH 7.4 buffer for 3 h and PBS pH 6.8 in the absence (control) and presence of rat ceacal contents for 19 h.

In vitro release studies

Matrix and multilayer tablets

Fig. 1 illustrates the effect of Pectin concentration on the release of Mesalamine from matrix tablets (F1 and F2). As the concentration of Pectin increased from 30% (F1) to 40% (F2) w/w, the rate of drug release fell relatively, where *t50%* was found to be about 15 min and 2 h, respectively. Also a relative reduction in the extent of drug release was observed. The percentage drug released after 5 h of testing was 100% ± 0.37% and 75.2% ± 0.29% using 30% and 40% Pectin respectively. After 5 h, F1 tablets were found to be completely disintegrated whereas residues of the slightly swollen F2 tablets were detected in the dissolution fl ask. The initial drug release from F2 tablets may be explained by the high solubility of Mesalamine, present on the surface of the tablets, in 0.1 mol/l HCl. (Sinha 2004) The lag-time required for the hydration of Pectin to form the gel layer around the tablets was also an important factor in this respect. Drug diffusion through the gel layer and erosion of the gel may be regarded as the rate- limiting steps for further drug release occurring in simulated intestinal fluid, pH 7.4. The use of mixtures of polymers represents a potential way of achieving the required release properties. Mixtures of non ionic and ionic polymers have been used previously to give different viscosity efficiencies and provide delivery systems with modified drug release. (Shrivestava 1985 and Ranga 1988) In the present study, Pectin: HPMC (80: 20 and 50: 50) mixtures were used to prepare Mesalamine matrix tablets (F3 and F4, respectively) with a total polymer content of 40% (Table 1). The prepared tablets showed a significant ($P < 0.005$) delayed drug release, as compared with matrix tablets F2 containing Pectin alone. The cumulative percentage drug release after 5 h was 75.2% ± 0.29%, 64.99% ± 3.73% and 67.37% ±1.67% for F2, F3 and F4, respectively (Fig. 1).

Table 1: Physical Properties of Mesalamine compression coated tablet formulations

Tablet formulation	Pectin coat: core ratio	Coat weight (mg.)	% W/W Chitosan incorporated in Pectin coat	Coat thickness (mm) ± S.D	Tablet Hardness (Kg.)± S.D
F9	3:1	385	----	0.76 ±0.12	7.2 ± 0.2
F10	3:1	385	2	0.82 ±0.16	6.5 ± 0.6
F11	3:1	385	3	0.75 ±1.2	6.8 ± 0.1
F12	3:1	385	5	0.76 ±0.6	7.8 ± 0.8
F13	4:1	510	----	1.28 ±1.35	7.2 ±1.0
F14	5:1	635	----	1.38 ±0.3	7.0 ± 0.4

The integrity of the tablets F3 and F4 was maintained during the dissolution study and a thick hydrated gel layer was formed. Mesalamine release profiles can be considered as being composed of two parts. The first part involves the drug release during establishment of a fully hydrated gel layer and the second involves the release through this hydrated layer. At the beginning, the matrix tablets allowed the free dissolution of Mesalamine directly in contact with the dissolution medium (0.1 mol/l HCl). The percentage drug released from F3 and F4, after 2 h, was 48.96% ± 3.35% and 51.82% ± 2.75% respectively, in comparison with 51.8% ± 1.51% for F2 tablets (no statistically significant differences). In pH 7.4 medium, F3 and F4 tablets showed lower release profiles, compared with F2 tablets, indicating hydration of the mixed polymers and formation of a stable gel layer. A combination of the anionic Pectin with the non ionic HPMC seems to produce a synergistic increase in viscosity. This may be attributed to the stronger hydrogen bonding between the carboxyl groups of Pectin and the hydroxyl groups of HPMC, leading to stronger physical cross linking between the polymers. Interaction between non ionic and ionic polymers has been reported to be greater than between molecules of the same species. Fig. 1 also shows that F3 composed of Pectin: HPMC (80: 20) exhibited a lower release profile than F4 with a higher HPMC content (Pectin: HPMC = 50: 50).

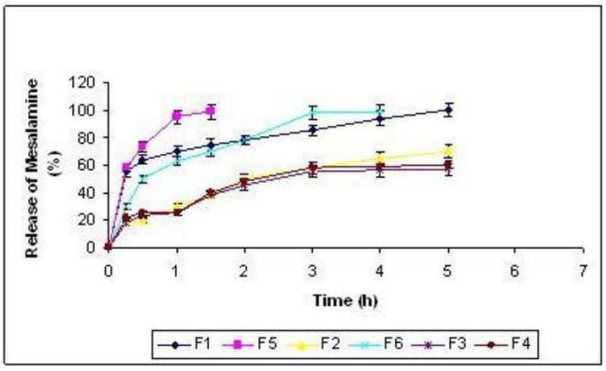

Figure 1: Release profiles of Mesalamine matrix tablets containing 30% pectin (F1, F5), 40% pectin (F2, F6), 40% pectin/ HPMC (80: 20)-F3 and 40% pectin/ HPMC (50: 50)-F4. Dissolution media were 0.1 mol/l HCl (2 h) and pH 7.4 buffers (3 h). Tablets were prepared by wet granulation (F1–F4) and direct compression (F5, F6) techniques

Pectin and HPMC are hydrophilic materials. The systems made from a mixture of these polymers will swell and form a hydrogel layer when they are placed in an aqueous medium. Tablets formulated in a matrix of a more swellable, erodible polymer, such as HPMC and optionally include Pectin. The more swellable erodible polymer has a diffusion rate coefficient which is greater than the diffusion rate coefficient of the relatively less swellable polymer. It is this difference in diffusion rate coefficients between the first and second polymers which controls the rate of drug release and allows the system to approach zero order drug delivery over the drug release period. (Purushotham 2003 and USP 6337091) Results were obtained in a previous study demonstrating that the optimum system for controlled delivery of Mesalamine consisted of 20% HPMC and 80% Pectin as a compression coating material. (Traconis 1997) Although matrix tablets F3 and F4 showed retarded drug release during the first 5 h of testing, they were unable to prevent the drug release in the physiological environment of stomach and small intestine. Furthermore, preparation of Mesalamine matrix tablets (F5 and F6), using the direct compression technique, also failed to control the drug release in the upper gastrointestinal simulated medium. They disintegrated and released 100% of their drug content after 1.5 and 3 h of the dissolution run, respectively (Fig. 1).

Mesalamine multilayer tablets containing 50 mg (F7) or 100 mg (F8) Pectin on either side of the matrix tablets (F2), as release controlling layers, showed a significant ($P < 0.0001$) delayed drug release, compared with the matrix tablets (F2). The cumulative percentage Mesalamine released after 5 h was 75.2% ± 0.29%, 63.1% ± 0.05% and 54.28% ± 0.64% for F2, F7 and F8, respectively (Fig. 2).

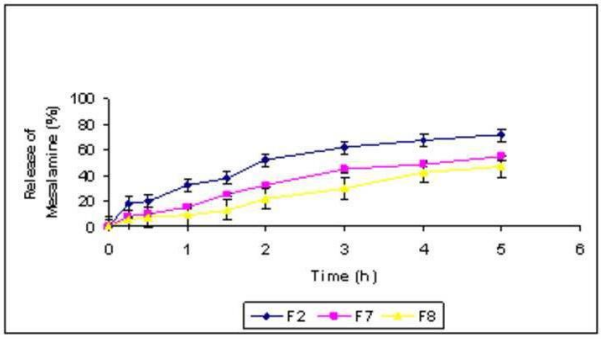

Figure 2: Release profiles of Mesalamine matrix tablets (F2) containing 40% pectin and multilayer tablets containing 50 mg (F7) and 100 mg pectin (F8) as release controlling layers on either side of F2 tablets. Dissolution media were 0.1 mol/l HCl (2 h), pH 7.4 buffer (3 h)

The reduction in the amount of drug released, on increasing the proportion of Pectin in the multilayer tablets, may be due to the increase in the gel strength of the swollen Pectin layers. On exposure to the aqueous dissolution medium, being a hydrophilic polymer, Pectin hydrated, swelled and formed a hydrogel layer. Drug release from hydrophilic polymers occurs by diffusion through the gel layer. Mechanical erosion of the swollen layer then occurs, allowing further hydration and swelling of the polymer and further drug release. (Turkoglu 2002) However; multilayer tablets are unable to protect the tablet cores from premature drug release in the physiological environment of the stomach and small intestine. Hence, further *in vitro* release stu-

dies in simulated colonic fluids for the matrix and multilayer tablets developed in the present study were not carried out.

Compression coated tablets

Mesalamine core tablets, prepared as described above, showed good mechanical strength (hardness: 5 kg), rapid disintegration (50s) and a high dissolution rate (t_{100}, 5 min in PBS, pH 6.8). The ability of Pectin as a compression coat over the fast disintegrating core tablets to specifically deliver the drug into the colon was assessed. Table 2 and Fig. 3 show the effect of the Pectin coat to core ratio on the physical properties of the tablets and the drug release profiles. Tablet formulations with a higher coat: core ratio 4: 1 (F13) and 5: 1 (F14) were able to prevent the tablet cores from premature drug release in the physiological environment of the stomach and small intestine, in contrast to tablets F9 (coat: core, 3: 1) which released 42.65% ± 3.58% after 5 h of the dissolution run (Fig. 3). At the end of 19 h of the dissolution study in simulated colonic fluid without rat ceacal contents (control), tablets F13 and F14 were found to be intact with considerable swelling and the mean percentage drug release was 40.2% ± 2.02% and 7.5% ± 1.25%, respectively, whereas 100% ± 4.32% drug was released from tablets F9 (Fig. 3). As the thickness decreased, the Pectin coat integrity decreased and the release was, therefore, time dependent. The drug release from the intact Pectin coats in media of different pH occurred by different mechanisms. In acidic medium (pH 1.2), 99% of the acidic groups on the Pectin molecules (pKa ≈ 3) are in the unionized form. At pH 7.4, the dissolution of Pectin increased due to the complete ionization of the galacturonic acid groups. However, as High Methoxy Pectin

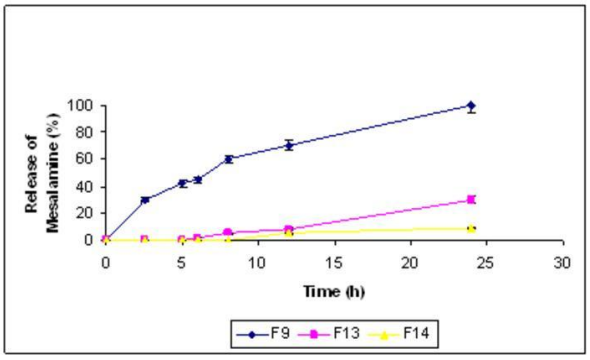

Figure 3: Release profiles of Mesalamine tablets compression coated with pectin at different coat: core ratios 3: 1 (F9), 4: 1 (F13) and 5: 1 (F14). Dissolution media were 0.1mol/l HCl (2 h), pH 7.4 buffer (3 h) and pH 6.8 PBS without rat cecal contents (19 h)

has a low number of free carboxyl groups and, accordingly, a low electrostatic repulsion between the molecules, gelation was more likely to occur. (Ashford 1994) The presence of a surface gel layer delayed both liquid penetration into and diffusion of drug out of the Pectin coat. It should also be noted that under conditions pertaining to the gastrointestinal tract, a gel would be formed at lower pH values.

It has been reported that Pectin: Chitosan physical mixture acts as an efficient matrix for retarding drug release in tablets.(Meshali 1993) In the present study, various proportions of Chitosan were included in the Pectin compression coat to improve the protective ability of the coat at the lowest coat: core ratio 3: 1 (Table 2). Fig. 4 shows that the cumulative mean percentage of Mesalamine released from tablets with Pectin coats containing 0% (F9), 2% (F10), 3% (F11) and 5% (F12) w/w Chitosan were 42.65% ± 3.58%, 37.5% ± 2.96%, 6.0% ± 6.30% and 0.0%, respectively, after 5 h of testing. This implies that the mixed polymer coat was able to protect the core from premature drug release in the physiological environment of the stomach and small intestine, especially at 3% and 5% w/w Chitosan levels. After 24 h of testing, the percentage drug released from F9, F10, F11 and F12 was found to be 100% ± 4.32%, 64.37% ± 3.19%, 32.04% ± 4.76% and 11.46% ± 0.67%, respectively, indicating that the mixed polymer coat substantially retarded the drug release as the Chitosan content increased. The inter polymer complex that could be formed between the carboxyl groups of Pectin and the amino groups of Chitosan, during the dissolution process may be responsible for such delayed drug release. (Fernandez-Hervas 1998) The susceptibility of Chitosan and/ or Pectin coatings, to the enzymatic action of colonic bacteria, was assessed by continuing the drug release studies in rat ceacal content medium for 19 h after 5 h of testing in simulated gastric and intestinal fluids. The importance of pre exposure of polysaccharides compression coated tablets to conditions in the upper gastro-intestinal tract, prior to exposure to the enzymatic action, has been reported. (Ashford 1993).

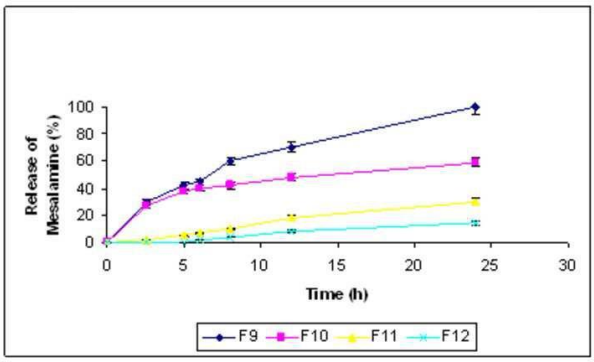

Figure 4: Release profiles of Mesalamine tablets compression coated with pectin containing different proportions of Chitosan 0% (F9), 2% (F10), 3% (F11) and 5% (F12) w/w, at a coat: core ratio 3: 1. Dissolution media were 0.1 mol/l HCl (2 h), pH 7.4 buffer (3 h) and pH 6.8 PBS without rat ceacal contents (19 h)

Tablets, having a Pectin coat: core ratio of 5: 1 (F14), 4: 1 (F13) and 3: 1 with 5% Chitosan in the Pectin coat

(F12), were selected for study. Fig. 5 shows that the presence of rat ceacal contents in the dissolution medium resulted in a significant ($P < 0.0001$) increase in drug release, when compared with the control. The cumulative percent drug released after 24 h from F14 and F13 increased from 7.5% ± 1.25% and 40.2% ± 2.02% in the absence of rat ceacal contents to 51.3% ± 5.45% and 70.25% ± 9.9% in presence of ceacal matter, respectively, indicating that polysaccharides metabolizing Pectin are present in rat ceacal contents. The ability of the enzyme to degrade the coat sufficiently and allow the drug release from the core is highly dependent on the hydration of Pectin. As the coat: core ratio decreased from 5: 1 to 4: 1, the coat might have been more hydrated and subsequently degraded by the ceacal enzymes at a faster rate, explaining the relatively higher drug release from F13, compared with F14. Tablets coated with Pectin containing 5% Chitosan at the lower coat: core ratio 3: 1 (F12) also exhibited a significant ($P < 0.05$) increase in drug release, in the presence of rat ceacal matter. However, the cumulative percent drug released after 24 h was only 20.14% ± 5.01%, compared with 11.46% ± 0.67% in the absence of enzymes (Fig. 5).

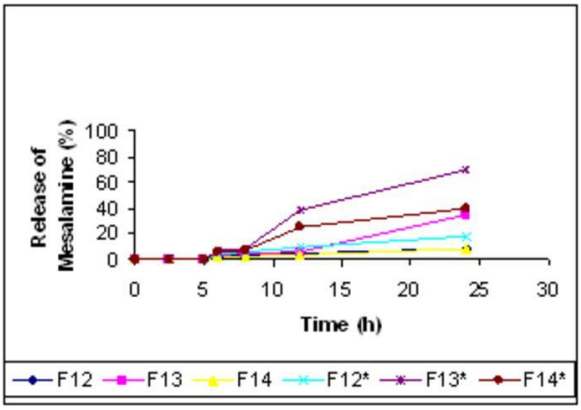

Figure 5: Release profiles of Mesalamine tablets compression coated with pectin containing 5% w/w Chitosan at a coat: core ratio 3:1 and pectin alone at coat: core ratios 4: 1 and 5: 1. Dissolution media were 0.1 mol/l HCl (2 h), pH 7.4 buffer (3h) and pH 6.8 PBS (19h)

The swollen Pectin/Chitosan coat, observed at the end of the study, might be too high and hence reduced the drug release from the tablets. A rat ceacal content concentration higher than 1.5% w/v might be required to provide the bacterial population necessary for using this polysaccharide mixture as a substrate and carrying out its hydrolysis. However, considering that the quantity of ceacal matter in humans, to which the delivery system is supposed to be exposed, is much greater than that generally used in the experimental studies (2% or 4% w/v), a higher drug release from the compression coated tablets (F12, F13 and F14), developed in the present study, would be expected *in vivo*.

Drug release

The zero-order rate describes the systems where the drug release rate is independent of its concentration. The first order describes the release from systems where the release rate is concentration dependent. Higuchi's model describes the release of drugs from an insoluble matrix as a square root of a time-dependent process based on Fickian diffusion. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (r^2) was determined. It was found that the in vitro drug release of batch F11- F14 was best explained by Higuchi's equation, as the plots showed the highest linearity (r^2 values greater than 0.99), followed by zero order (r^2 value greater than 0.982). This explains why the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (or Higuchi's kinetics). However, drug release was also found to be very close to zero-order kinetics, indicating that the concentration was nearly independent of drug release. The dissolution data were also plotted in accordance with the Hixson-Crowell cube root law. The applicability of the formulation to the equation indicated a change in surface area and diameter of the tablets with the progressive dissolution of the matrix as a function of time.

Mechanism of Drug Release

The corresponding plot (log cumulative percent drug release vs. time) for the Korsmeyer-Peppas equation indicated a good linearity. The release exponent n were found in between 045 to 0.89, which appears to indicate a coupling of the diffusion and erosion mechanism—so-called anomalous diffusion—and may indicate that the drug release is controlled by more than one process. Reddy et al observed similar results with a matrix tablet of nicorandil with an n value of 0.71, (Reddy 2003) and Fassihi and Ritschel with a matrix tablet of theophylline with an n value of 0.7. (Fassihi 1993) Both these groups of researchers also considered the corresponding n values to indicate an anomalous release mechanism.

CONCLUSION

The study discusses the formulation of controlled colon targeted Mesalamine tablets for the treatment of Ulcerative colitis. The colon targeting and controlled release of drug was a promising approach for the formulation of such dosage form. This study showed that Pectin and Chitosan was used to deliver the drug specifically to the colon. From the above results, it can be concluded that matrix and multilayer Mesalamine tablets containing various proportions of Pectin failed to control drug release in the physiological environment of the stomach and small intestine. On the other hand, compression coated formulations were able to protect the tablet cores from premature drug release, but at high pectin coat: core ratios 4: 1 (F13) and 5: 1 (F14). Inclusion of Chitosan 3% (F11) and 5% w/w (F12) in the

Pectin coat offered better protection at the lowest coat: core ratio 3: 1. Selective delivery of Mesalamine to the colon could be achieved using F13, F14 and, to a lesser extent, F12 tablet formulations which released 70.25% ± 9.9%, 51.3% ± 5.45% and 20.14% ± 5.01% drug, respectively, at the end of 24 h in simulated colonic fluid containing 1.5% w/v rat ceacal contents. Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as these plots showed the highest linearity (r^2 = 0.9979), but a close relationship was also noted with zero-order kinetics (r^2 = 0.9950). Korsmeyer's plots indicated an n value of 0.45 to 0.89, which was indicative of an anomalous diffusion mechanism or diffusion coupled with erosion; hence, the drug release was controlled by more than one process. Storage of these tablets for 12 months at 25°C/40% RH showed no change either in physical appearance or in dissolution profiles, pointing to the potential of pectin or pectin- Chitosan mixture, as compression coats, for providing targeted delivery of Mesalamine to the colon. The prepared 24 h controlled-release compression coated tablets would provide an extended duration of therapeutic effect of Mesalamine with minimum potential for side-effects.

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