International Journal of Drug Delivery 2 (2010) 242-250

http://www.arjournals.org/ijdd.html

Research article

ISSN: 0975-0215

Investigations on chitosan-carboxymethyl guar gum complexes interpolymer complexes for colon delivery of fluticasone

Vikas Kumar[1], A.K.Tiwary[2], Gurpreet Kaur*[2]

***Corresponding author:**

Gurpreet Kaur
[1]Ranbaxy Research Labs, Gurgaon, India
[2]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147002, India
Tel: 919814724622
Fax +91-(0175)-3046255
Email: kaurgpt@gmail.com

Abstract

The present study was designed to formulate colon release tablets of fluticasone by employing cross linked chitosan (CH) and carboxymethyl guar gum (CMG) interpolymer complexes (IPC). Matrix tablets were prepared by wet granulation method using IPC as binder and coating agent. The IPC were characterized by Fourier transform infrared spectroscopy (FTIR). The uncoated and coated tablets were tested for their suitability as colon specific drug delivery system by *in vitro* dissolution studies. The coated tablets were evaluated for their pharmacodynamic performance after oral administration to TNBS induced ulcerative colitic rats. FTIR studies demonstrated that the IPC was formed through an electrostatic interaction between $-COO^-$ groups of CMG and $-NH_3^+$ groups of CH. Tablets formulated with 50:50 CH:CMG as binder and coated with the respective ratio of IPC was capable of protecting the drug release in stomach and small intestine and delivering the drug in the colon. Histopathology of the rat colon after oral administration of these IPC film coated tablets revealed significantly greater ($p<0.05$) reduction in TNBS-induced ulcerative colitis The study confirmed that selective delivery of fluticasone to the colon can be achieved using cross-linked CH and CMG polysaccharides.

Keywords: Chitosan; Colonic delivery; Carboxymethyl guar gum; Cross-linking; Guar gum; Fluticasone

Introduction

Inflammatory bowel disease (IBD) refers to two related but different diseases: ulcerative colitis (UC) and Crohn's disease (CD). These diseases cause chronic inflammation of the intestinal tract. IBD is a lifelong disease with periods of active disease alternating with periods of disease control (remission). It is widely estimated that between 1 and 1.4 million people in the United States have IBD. Currently no curative therapeutic agent is available and treatment of IBD relies heavily on non-steroidal anti-inflammatory drug, glucocorticoids and immuno-modulators. The primary objective of anti-IBD therapy is to reduce the inflammation of colon. This requires frequent administration of anti-inflammatory drugs at high doses, which may lead to gastric ulceration, bleeding and other gastric complications [1]. Corticosteroids are the most effective agents in the treatment of acute UC because of their broad and nonspecific anti-inflammatory effects, including lymphocyte toxicity, inhibition of cytokines, and reduction of arachidonic acid metabolites [2].

doi:10.5138/ijdd.2010.0975.0215

Fluticasone is a fluorinated steroid which is both poorly absorbed and susceptible to high first pass metabolism. This compound has been primarily developed for topical use in asthma but its pharmacokinetic profile is very promising for inflammatory bowel disease [3].

Delivery of the drugs to the colon via the oral route is valuable in treating diseases related to colon (Crohn's disease, ulcerative colitis, irritable bowel disease, carcinomas and infections) whereby high local concentration of drugs can be achieved at the site of inflammation. Colon targeting can be achieved by pH-dependent systems or pH independent systems. Drug release in pH dependent systems is easily influenced by nature of diet. Further, physiologically, a highly alkaline pH of 7.4 of the small intestine often contributes to premature drug release and failure of the pH-dependent release systems before reaching the colon [4]. The pH-independent release systems suffer from the drawback of incomplete drug release and have to be combined with other polymers that are either soluble at colonic pH or are capable of being degraded by colonic bacteria [5].

Many researchers have reported the use of natural or modified polysaccharides for sustained or colon delivery of drugs. However, when employed in their putative form, these polysaccharides are required to be used in large quantities [6] for achieving colon drug delivery. This is probably due to high solubility of non-cross linked molecules in the acidic pH. Therefore, the recent emphasis is on the use of biodegradable polymer combinations that are cross linked with each other or with ions in order to render them insoluble in acidic pH. Chitosan-chondroitin sulphate interpolymer complexed film coated tablets have been reported for colon targeting [7]. Similarly, carboxymethyl tamarind kernel powder and chitosan interpolymer complexed film coated tablets have been reported for colon specific delivery of budesonide [8].

Chitosan (CH) carries a net positive charge due to $-NH_3^+$ groups and can be easily cross-linked with other anions, oppositely charged drugs and polymers [9]. CH is easily degraded by lysozyme, by non specific cellulases and enzymes secreted by intestinal bacteria [10]. Guar gum has also been examined for use in colonic drug delivery [11]. However, natural gums being hydrophilic swell in the presence of dissolution media. Thus, there is a possibility of the entrapped drug leaking out prior to arrival of the dosage form at the site of absorption. Thus, there is a need to reduce the enormous swelling of the gums by cross linking.

Carboxymethyl guar gum (CMG), a derivative of guar gum has been investigated for colon drug delivery. CMG microbeads were prepared by dropping a solution of CMG in a solution of divalent and trivalent metal ions. The Ba^{2+} cross-linked products were able to protect the drug under gastric pH conditions while Ca^{2+} ions cross-linked products were found to release the encapsulated drug when exposed to pH 7.4 i.e. intestinal pH [12].

In the light of the above, it was proposed to formulate tablets of fluticasone by using CH-CMG IPC as binder and coating them with aqueous mixtures containing respective ratios of CH-CMG IPC. The characterization of the IPC films was done by FTIR and water uptake studies. The effect of varying the composition of CH:CMG in the coating solution on the *in vitro* release of fluticasone from tablets was investigated. Further, the coated fluticasone tablets were evaluated for their pharmacodynamic performance after oral administration to TNBS induced colitic rats.

Materials and methods

Fluticasone propionate was gift sample from Cipla pharmaceuticals ltd. Mumbai (India). Guar gum was purchased from S.D fine chemicals, Mumbai (India). Chitosan (CH) was purchased from Indian sea foods, Cochin, India. Chitosanase was purchased from Sigma Aldrich, USA. Methanol used in analysis was of HPLC grade. All other chemicals used were of AR grade.

Procedure for carboxymethylation of guar gum and characterization by FTIR

Carboxymethylation was carried out using the method described by Ragheb *et al.,* 1994 [13]. 100g of guar gum was added in a mixture of 630 ml of ethanol and 554 ml of toluene. To this 44.8% w/v NaOH was added gradually and mixed thoroughly. This mixture was kept at room temperature for 30 min. Monochloro-acetic acid (120g) was gradually added with agitation to this mixture and kept overnight. The excess alkali was neutralized with glacial acetic acid using phenolphthalein indicator. The product was filtered, washed with ethanol and dried. The degree of substitution of CMG was determined by titrimetric method [14].

Preparation of CH-CMG interpolymer complexed films

CH (300 mg) was dissolved in 15 ml solution of 3%, v/v acetic acid. To this mixture 8 ml of 5 M-ammonium acetate was added. CMG (300 mg) was separately dissolved in 7ml distilled water and slowly added with stirring to CH solution. This mixture was poured in petri plates and dried at 50 °C for 48 h. Films with a total polymer content of 2.5% w/v containing 60:40, 50:50 or 40:60 ratio of CH:CMG were prepared using this method. The dried films were stored in a desiccator until use.

Characterization of IPC films

FTIR studies

CH, CMG and IPC films formed by drying admixtures containing different ratios of CH:CMG were subjected to FTIR analysis (Perkin Elmer RXI, USA). The fresh films were sequentially exposed to pH 1.2 buffer IP [15] for 2h and pH 7.4 buffer IP for 22 h. The exposed films were dried at 50ºC for 24 h and subjected to FTIR analysis.

Swelling Index Measurement

The swelling index of the IPC films after exposure to different pH was determined by sequentially immersing the films in pH 1.2 for 2 h and pH 7.4 for 22 h. The swelling index was calculated according to the formula

$$SwellingIndex = \frac{W2 - W1}{W1}$$

Where, W1 is the initial weight of the film and W2 is the weight of the swollen film.

Formulation of fluticasone tablets

Preparation of core tablets

Tablets (average weight 25 mg) containing 3 mg of fluticasone were prepared by wet granulation technique. Fluticasone and Avicel® pH 102 were granulated using CH:CMG solution (10% w/w) as binder. The granules were passed through #16 and dried at 50 ± 2ºC to 2-3% w/w residual moisture content. The dried granules were passed through #20 sieve and fines were retained on #44 sieve. 10% w/w of fines was mixed with the granules. Magnesium stearate (1% w/w) was added to the granules. Tablets were compressed using biconvex 4mm convex punches in a six station rotary tablet compression machine (A K Industries, M207, Nakodar, India). These tablets were tested for dimensions (axial and radial diameters), hardness, friability and weight variation [16].

Coating of fluticasone tablets

The formulated fluticasone tablets containing CH:CMG solution as binder were coated with aqueous solutions containing respective CH:CMG ratio (composition same as IPC film) to obtain a weight gain of 10% w/w. The total polymer concentration was kept constant at 2.5% w/v. The coating solution was sprayed at a rate of 5 ml/min with the help of peristaltic pump using a spray gun of 1 mm nozzle (Electrolab, PP201V, Mumbai, India) in a coating pan (12"diameter) being rotated at 18 rpm (AK Industries, M1107, Nakodar, India). Compressed air was introduced at a pressure of 1.5 kg/cm^2. The inlet air temperature was maintained at 60°C. The inner surface of coating pan was modified by attaching inert tubes (8 mm diameter) from the centre to the periphery for easy rolling of tablets thereby ensuring efficient mass transfer of polymer. The coated tablets were also evaluated for weight variation, disintegration time. Further, the axial and radial diameters were measured as described above.

***In vitro* release kinetics of fluticasone from coated tablets**

In vitro release of fluticasone from coated tablets was evaluated out using USP 30-NF25 (Dissolution Apparatus 1-basket method) utilizing temperature of 37 ± 0.5°C with constant stirring rate of 50 rev/min in a pH progression media containing 2% v/v Tween 20 to maintain sink conditions. pH progression consisted of exposing the tablets to buffer pH 1.2 IP for 2 h followed by buffer pH 7.4 IP for 3 h and buffer pH 6.8 for further period of 19 h. Dissolution studies were also carried out in pH 1.2 (2 h), pH 7.4 (3 h) and pH 6.8 (containing 0.05 units of chitosanase solution in sodium acetate buffer pH 5.0) for a further period of 19 h. Aliquots (5 ml) were withdrawn at predetermined intervals and immediately analyzed by spectrophotometer at 232 nm.

Mechanism for Drug Release

The mechanism of drug release during dissolution studies in pH progression media or in the presence of chitosanase was evaluated by using the Korsmeyer equation [17].

$$Mt/M_\infty = Kt^n$$

Where, Mt/M_∞ = fractional release of drug, t = release time, k = kinetic constant, which incorporates structural and geometric characteristics of the device.

n = release exponent, which indicates the kinetic release.

A value of 0.45 for 'n' indicates the case of diffusion-controlled drug release (Fickian release). Case II transport or relaxation controlled delivery is indicated by a value of 0.89. Values of 'n' between 0.45 and 0.89 are regarded as an indicator for the non-Fickian release or anomalous transport. The non-Fickian kinetics is suggestive of a combination of diffusion and polymer relaxation. In addition, Super Case II kinetics is indicated when the values of 'n' are greater than 0.89.

Pharmacodynamic evaluation

Sprague Dawley rats (200-250g) of 8-12 weeks age were used in the study. They were fed with standard laboratory chow diet and given water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee of the Punjabi University, Patiala, India and the care and handling of the animals were in accordance with Committee for purpose of control and supervision of experimental animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No.- 107/1999/CPCSEA).

Induction of colonic inflammation

The rats were fasted for 48 h before the induction of ulcerative colitis. 2,4,6 Trinitro benzene sulphonic acid (TNBS) (20mg) was dissolved in 0.25 ml of 50% v/v ethanol. This solution was instilled into the colon (7 cm from the anus) of rats with the help of a catheter in order to induce ulcerative colitis [18]. The rats were monitored for three days without treatment to allow for the development of ulcerative colitis.

Treatment of ulcerative colitis

The rats that weighed 80-100% of their initial weight (before TNBS instillation) were selected after 3 days as the ulcerative colitis positive animals. These rats were divided into two groups. Rats of Group I and Group II received oral administration of, respectively, normal saline (0.5 ml) or tablets coated with IPC films comprising 50:50 ratio of CH:CMG. The treatments were given once daily for five consecutive days.

Assessment of colonic inflammation

Colonic inflammation following rectal administration of TNBS as well as after different treatments (as detailed above) was assessed by the following parameters.

Colon/ body weight (C/B) ratio

The rats were sacrificed on the sixth day (after five days of tablet administration) and distal colon segments (6 cm length) were resected, opened longitudinally and rinsed with iced phosphate buffer. The ratio of wet weight of the colon specimen to the body weight was calculated for each rat.

Histopathological studies

Tissue segments (1 cm in length) were fixed in 10% buffered formalin for histopathological studies. Histopathological studies were carried out using haematoxylin and eosin stains at Department of Pathology, Government Rajindra Hospital, Patiala, Punjab.

Results and discussion

Preparation and Characterization of CMG

The prepared CMG was characterized by FTIR spectroscopy. The appearance of a peak at 1424 cm^{-1} indicated the presence of $–COO^-$ ions (representing C=O stretch of $–COO^-$) in the CMG specimen (Figure 1C). This peak was absent in guar gum sample (Figure 1A) thus, indicating that carboxymethylation had been successfully carried out resulting in formation of CMG. The degree of substitution was calculated to be 0.27.

Characterization of IPC films

FTIR analysis

The presence of peaks at 1560 cm^{-1} and 1422 cm^{-1} in the IR spectrograph of CH indicated the presence of $–NH_3^+$ ions [19] and $–COO^-$ ions. These peaks could have arisen due to 15% acetylation of CH powder (Figure 1B). The presence of peaks at 1402.5 cm^{-1} (due to $–NH_3^+$) and 1573.5 cm^{-1} (due to $–COO^-$) in all CH-CMG IPC films suggested interaction between

$–COO^–$ groups of CMG and $–NH_3^+$ groups in CH (Figs. 1D-F).

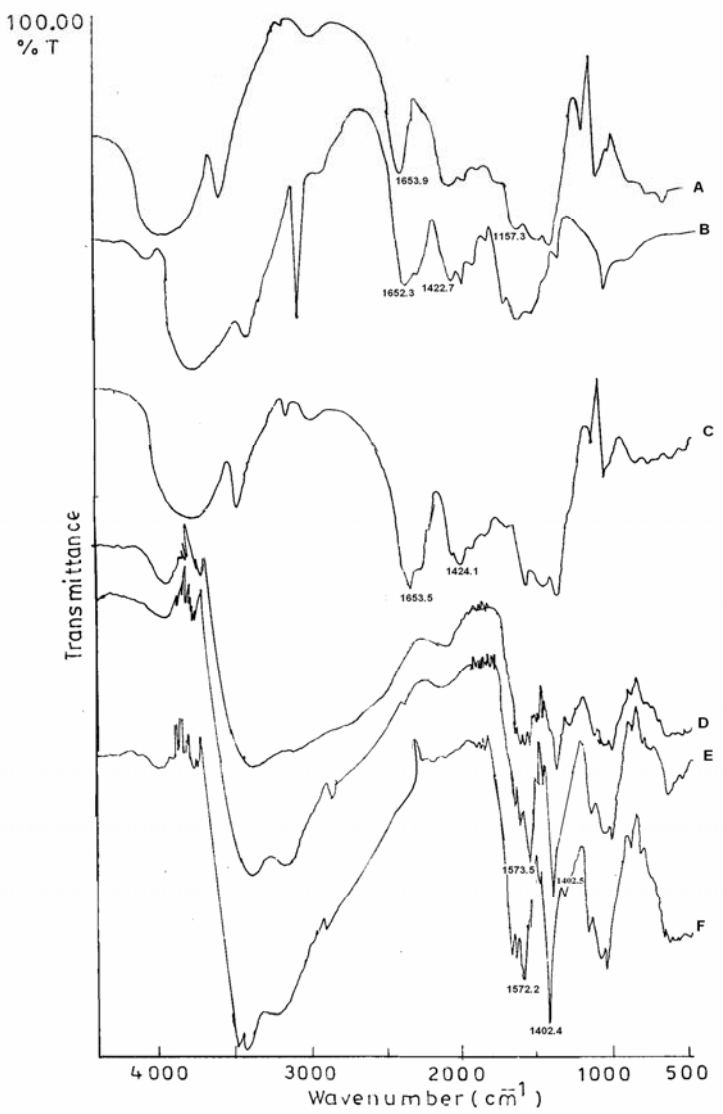

Figure 1: FTIR spectra of Guar gum (A); Chitosan (B); Carboxymethyl Guar gum (C); complex formed by interacting CH and CMG in the ratio of 60:40 (D); 50:50 (E); 40:60 (F).

The spectrographs of IPC films containing 40:60 ratio of CH:CMG showed reduced intensity of peaks at 1565 cm^{-1} and 1410 cm^{-1} after exposure to pH 1.2, thus indicating weakening of $NH_3^+COO^–$ interaction after exposure to acidic pH (Figure 2A). The spectrographs were observed to be similar in nature after exposure of these films to buffer pH 7.4 (Figure 2B). The peak at 1410 cm^{-1} corresponding to $–COO^–$ was observed to considerably decrease in intensity and the peak at 1565 cm^{-1} corresponding to $–NH_3^+$ ions was found to be completely abolished in the IPC films comprising of 60:40 ratio of CH:CMG after exposure to pH 1.2. This suggested total breakdown of $NH_3^+COO^–$ linkages in these IPC films (Figure 1E and F). However, the IR spectrograph of film comprising 50:50 ratio of CH:CMG did not reveal any change in the intensity of peaks at 1402.4 cm^{-1} and 1572.2 cm^{-1} (Figure 2 C and D). This indicated that the interaction in IPC films formed by interacting 50:50 ratio of CH:CMG was not cleaved after exposure to pH 1.2 or pH 7.4.

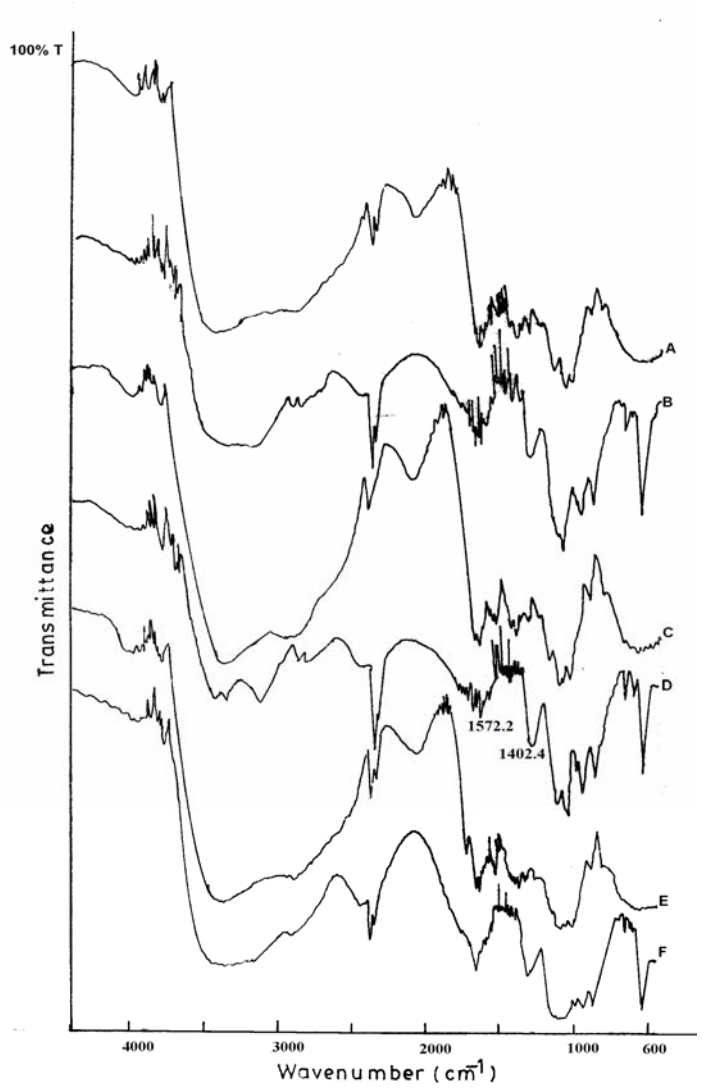

Figure 2 FTIR spectra of IPC films (CH:CMG) after treatment with pH 1.2 and 7.4 buffers respectively: 40:60 (A) and (B); 50:50 (C) and (D); 60:40 (E) and (F).

Swelling Index studies

The CH-CMG IPC films were found to exhibit swelling in acidic medium as well as in basic medium (Table 1). Swelling is favored by the protonation and repulsion of chitosan free ammonium groups. In acidic media, the polyacid is neutralized and due to the free ammonium groups of CH, excess positive charges appear inside the gel resulting in fast drug release [20]. However, IPC films containing any ratio of CH:CMG exhibited considerably less swelling in pH 7.4 as compared to pH 1.2. This can be ascribed to the fact that prolonged immersion in water produced segmental mobility of the interpolymer chains in the swollen state, which allowed the completion of interpolymer reaction between CH and CMG eventually leading to shrinkage after exposure to pH 7.4. A decrease in swelling was observed after exposure to pH 1.2 as well as pH 7.4 with an increase in the CMG concentration except in the IPC films comprising 30:70 or and 20:80 ratio of CH:CMG, which showed a greater swelling. Similar results have been reported earlier where carboxymethyl tamarind kernel powder and chitosan IPC films were observed to exhibit greater swelling with an increase in the concentration of carboxymethyl tamarind kernel powder [8].

Physical evaluation of tablets

The average weight of uncoated core tablets was 29.72 ± 0.43 mg. The acceptance value calculated was 11.98% which was well below the maximum 15% USP tolerance limit [16] Hence, the tablets passed the weight variation test. Hardness of the tablets was 4.5 ± 0.5 kg/cm^2 and friability was found to range from 0.41 to 0.52% w/w. The axial and radial diameters, respectively, ranged from 1.75 to 1.80 mm and 3.98 to 4.02 mm. The uncoated tablets prepared by using 10% w/w CH:CMG as a binder started showing signs of cracking within 30 min of exposure to 0.1M HCl.

The average weight of coated tablets was 33.09 ± 0.17 mg. The acceptance value calculated was 11.31%. Hence, the tablets passed the weight variation test. The axial and radial diameters of coated tablets, respectively, ranged from 2.02 to 2.08 mm and 4.01 to 4.15 mm. Although, these tablets exhibited swelling, they didn't soften or crack after exposure to 0.1M HCl for 2 h.

Table 1. Swelling studies of CH:CMG IPC films

CH:CMG ratio	Swelling Index	
	pH 1.2	pH 7.4
80:20	5.27 ± 0.37	3.58 ± 0.60
70:30	4.58 ± 0.15	2.83 ± 0.14
60:40	3.02 ± 0.15	1.35 ± 0.16
50:50	2.12 ± 0.21	0.83 ± 0.49
40:60	3.65 ± 0.19	1.02 ± 0.15
30:70	4.44 ± 1.05	2.84 ± 0.53
20:80	5.32 ± 0.41	3.89 ± 0.38

***In vitro* dissolution studies**

The *in vitro* drug release profile is depicted in Figure 3. The uncoated tablets started releasing fluticasone within 1 h in pH 1.2. Since, the drug release from uncoated tablets after 2 hrs was found to be more than 10% hence uncoated tablets could not be used for colon targeting [16]. Therefore, these batches were coated with respective ratio of CH:CMG to yield IPC films. The tablets coated with 60:40, 50:50 or 40:60 ratio of CH:CMG released, respectively, 5.60 ± 0.29%, 2.34 ± 0.14% and 5.05± 0.25% fluticasone in pH 1.2. The observation of <10% drug released in acidic media[16] indicated fulfillment of drug release criteria expected of enteric tablets These batches, respectively, released 15.11 ± 0.58%, 8.01 ± 0.17% and 13.08 ± 0.021% fluticasone in pH 7.4 after 5 hr. Further, these tablets released 53.87 ± 0.33%, 37.86 ± 0.42% and 49.63 ± 0.61% fluticasone, respectively in pH 6.8 after 24 h. This was supported with the FTIR spectra of IPC films prepared from 50:50 ratio of CH:CMG that did not reveal any decrease in the intensity of peaks characteristic of CH-CMG interpolymer complexation (Figure 3C and D).

***In vitro* dissolution studies in presence of chitosanase**

The presence of chitosanase increased the amount of fluticasone released from 50:50 (CH:CMG) IPC film coated tablets. The final exposure of these tablets to pH 6.8 containing chitosanase enzyme for 19 h eventually released 92.43% fluticasone. Chitosanase enzyme activity requires the presence of free amino group in CH [21]. However, since the amino group of CH molecule is ionically complexed with –COO$^-$

groups of CMG, complete hydrolysis of CH can not be expected. This seems to be responsible for the release of only 92% fluticasone from IPC film coated tablets.

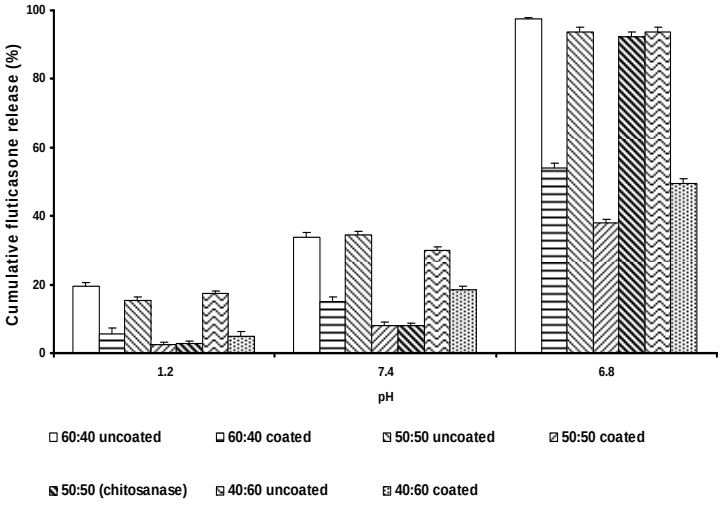

Figure 3. Cumulative fluticasone released (%) from uncoated tablets and tablets coated with IPC films comprising 40:60, 50:50 or 60:40 ratio of CH:CMG containing respective ratio of CH:CMG as binder.

Mechanism of drug release

The release kinetics of fluticasone from coated tablets containing CH-CMG solution as binder and coated with 50:50 ratio of CH:CMG was analyzed by Korsmeyer–Peppas model [17]. The value of r^2 was found to lie above 0.9 and the value of release exponent was more than 1.0. This indicated that the drug release can be ascribed to a Super Case II transport. Super Case II transport is reported to be exhibited when diffusion and relaxation rates are comparable [22]. Therefore, it can be suggested that the interpolymer complexation between CH and CMG was resistant to different pH media and release of fluticasone occurred due to slow erosion of polymer.

Pharmacodynamic evaluation

Colon/Body weight (C/B) ratio

The Colon/Body weight (C/B) ratio was determined for quantitative evaluation of the inflammatory colitis of different groups. The C/B ratio after the intracolonic administration of TNBS was significantly ($p<0.05$) increased as compared to that after administration of saline. The C/B ratio after oral administration of fluticasone tablets coated with CH:CMG (50:50) IPC films significantly decreased in the TNBS-induced colitis rats. Figure 4 represents the C/B ratio observed in different treatment groups.

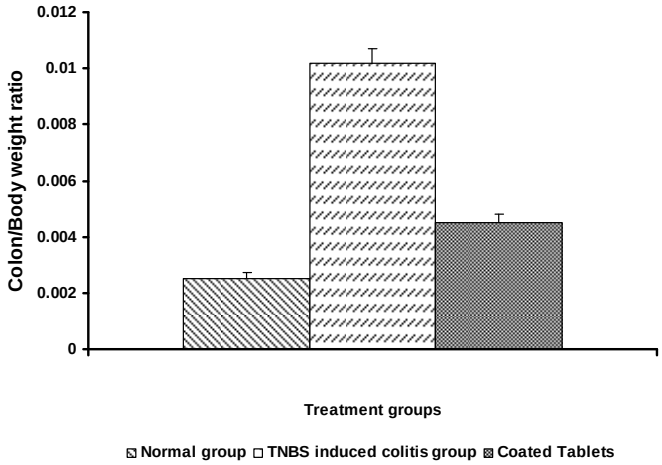

Figure 4 Colon/Body weight (C/B) ratio determined after 5 days of drug administration. Data presented is Mean ± SD (n= 6 animals/ group).

Histopathological studies

The histology of normal colon is depicted in Figure 5. The four layers of colon wall are mucosa, submucosa, muscularis externa and serosa. The section of the colon shows temporary folds of the mucosa and submucosa (Figure 5A). As is evident from Fig 5B, the ulcerative colitis induced colon showed shedding of epithelium and lymphocytic infiltration in lamina propria. The inflammation was spread over the mucosa, submucosa, muscle layer and serosa. Oral administration of coated fluticasone tablets resulted in a marked decrease in the extent and severity of colonic damage. The histopathological features of colon clearly indicated that the morphological disturbances associated with TNBS administration were corrected after five days of oral administration of tablets coated with 50:50 CH:CMG (Figure 5C). The tablets coated with 50:50 ratio of CH:CMG can be envisaged to offer a great promise for colon delivery of fluticasone, thereby providing high local drug concentration in the distal part of gastrointestinal tract for longer durations for effective therapy of IBD.

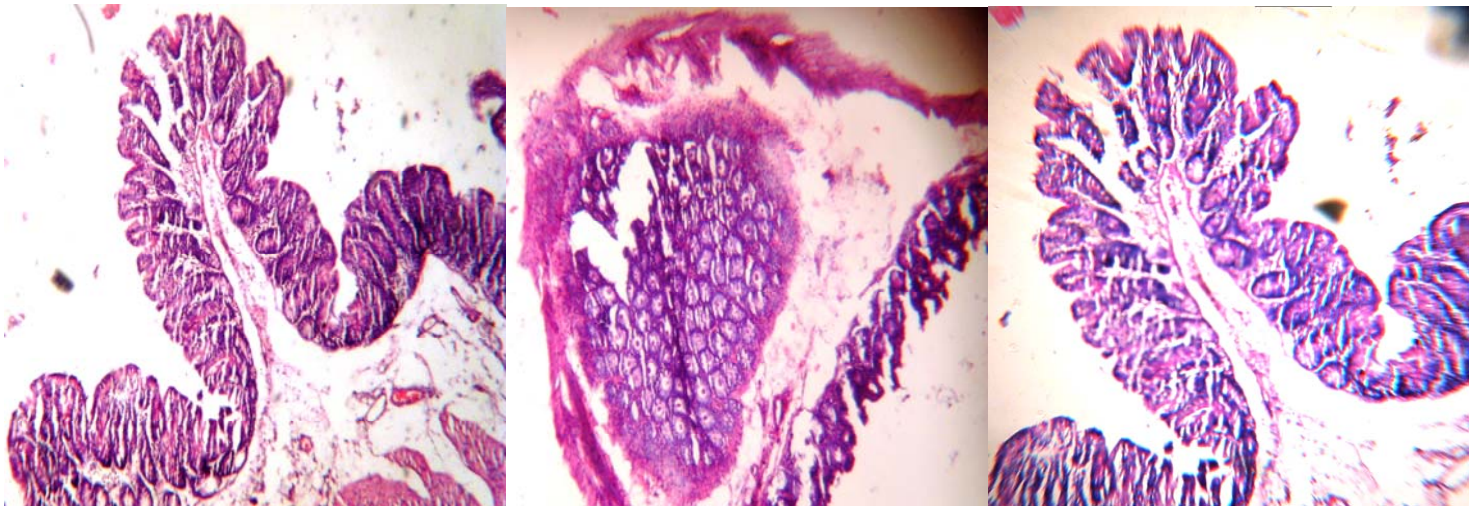

Figure 5. Light microscopic photographs of colon: normal colon (A); TNBS induced ulcerative colitis (B); after treatment with coated fluticasone tablets (C).

Conclusion

The results of the present study indicated that the tablets coated with IPC films containing 50:50 or 40:60 ratio of CH:CMG were effectively restraining the *in vitro* release of fluticasone under conditions mimicking stomach and small intestine as compared to uncoated tablets. The ability of the IPC films in providing the observed release characteristics to fluticasone core tablets was correlated with the ability of $-NH_3^+$ groups of CH to form complex with $-COO^-$ groups of CMGTKP and the stability of these complexes in acidic media. The tablets coated with 50:50 ratio of CH:CMG can be envisaged to offer a great promise for colon delivery of fluticasone, thereby providing drug concentration in the distal part of gastrointestinal tract for longer durations for effective therapy of IBD.

Acknowledgements

The authors are thankful to UGC, Delhi for providing financial assistance for carrying out these studies (Scheme No. F.32-140/2006 (SR). The authors thank Cipla pharmaceuticals Ltd. Mumbai (India) for gift sample of fluticasone propionate.

References

1. Dhaneshwar SS, Gairola N, Kandpal M, Vadnerkar G, Bhatt L. Colon-specific, mutual azo prodrug of 5-aminosalicylic acid with l-tryptophan: Synthesis, kinetic studies and evaluation of its mitigating effect in trinitrobenzenesulfonic acid-induced colitis in rats Bioorg. Med Chem. 2007; 15: 4903-4909.
2. Inoue N, Kinouchi, Y. Lack of common NOD2 variants in Japanese patients with Crohn's disease. Gastroenterol. 2002; 123: 86-91.
3. Crotty B, Jewell DP. Drug therapy of ulcerative colitis. Br. J. Clin. Pharmacol. 1992; 34: 189–198.
4. Rubinstein A. Colonic drug delivery. Drug Dis. Today: Tech. 2005; 2: 33–37.
5. Sangalli ME, Maroni A, Zema L, Busetti C, Giordano F, Gazzanica A. *In vitro* and *in vivo* evaluation of an oral system for time- and/ or site-specific drug delivery. J. Control. Rel. 2001; 73: 103-110.
6. Wong D, Larrabee S, Clifford K, Tremblay J, Friend DR. USP Dissolution Apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations. J. Control Rel. 1997; 47: 173–179.
7. Kaur G, Rana V, Jain S, Tiwary AK. Colon delivery of budesonide: Evaluation of chitosan-chondroitin sulphate interpolymer complex. AAPS Pharm Sci Tech. 2009; 11: 36-45.
8. Kaur G, Jain S, Tiwary AK. Chitosan-Carboxymethyl Tamarind Kernel Powder Interpolymer Complexation: Investigations for Colon Drug Delivery Sci. Pharm. 2010; 78; 57-

78.
9. Muzzarelli C, Vesna Stanic V, Gobbi L, Tosi G, Muzzarelli R. Spray-drying of solutions containing chitosan together with polyuronans and characterisation of the microspheres. Carbohyd. Polym. 2004; 57: 73–82.
10. Xia W, Liu P, Liu J. Advance in chitosan hydrolysis by non-specific cellulases. Biores Technol. 2008; 99: 6751–6762.
11. Sinha VR, Mittal BR, Bhutani, KK, Kumria, R. Colonic drug delivery of 5-fluorouracil: an *in vitro* evaluation. Int. J. Pharm. 2004; 269: 101-108.
12. Thimma RT, Tammishetti S. Barium chloride crosslinked carboxymethyl guar gum beads for gastrointestinal drug delivery**.** J. Appl. Poly. Sci. 2001; 82: 3084-3090.
13. Ragheb AA, Kamel M, El-Tolouth A, Nassar SH, Cairo D. Chemical Modification of Guaran Gum. Part 3: Carboxymethylation in non-Aqueous Medium. Starch, 1994; 46: 443-446.
14. Green JW. In: Whistler RL. Methods in Carbohydrate Chemistry. New York, Academic Press. 3, 107 (1963).
15. Indian Pharmacopeia. Indian Pharmacopeia Commission, Ghaziabad, 2007.
16. United States Pharmacopeia US Pharmacopeial convention, Rockville, 2007.
17. Korsmeyer RW, Gurny R, Docler E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 1983; 15: 25-35.
18. Tozaki H, Odoriba T, Okada N, Fujita T, Terabe A, Suzuki T, Okabe S, Muranishi S, Yamamoto A. J. Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. Control. Rel. 2002; 82: 51–61.
19. Kemp W. Infrared spectroscopy, in organic spectroscopy. London: MacMillan; 1991.
20. Berger J, Reist M, Felt O, Gurny R. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur J Pharm Biopharm. 2000; 57: 35–52.
21. Trimukhe KD, Bachate S, Gokhale DV, Varma AJ. Metal complexes of crosslinked chitosans Part II. An investigation of their hydrolysis to chitooligosaccharides using chitosanase. Int J Biol Macromol. 2007; 41: 491–496.
22. Singh B, Sharma N, Chauhan N. Synthesis, characterization and swelling studies of pH responsive psyllium and methacrylamide based hydrogels for the use in colon specific drug delivery. Carbohyd. Polym., 2007; 69: 631–642.