In vitro/in vivo evaluation of procera gum-ethylcellulose microspheres for colonic delivery of budesonide.

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ABSTRACT

The objective of the present research was to a develop colonic delivery system for budesonide based on polymer blends of natural polysaccharides from *Albizia procera* and the GI-insoluble polymer ethylcellulose. An emulsion solvent evaporation method was used for the preparation of the microspheres. *In vitro* drug release was studied in a medium simulating gastrointestinal fluid and the mechanism of drug release was determined using the Korsemeyer-Peppas equation. *In vivo* performance of the microsphere was evaluated in acetic acid induced colitis in rats. Drug release studies showed that the microspheres with a procera gum-ethylcellulose coating were able to resist premature drug release in the upper GI tract and yet were susceptible to enzyme effects in the colon. Treatment of rats with a budesonide test formulation for five days significantly attenuated the extent and severity of the cell damage and could thus be a promising system for the treatment of ulcerative colitis.

KEY WORDS: Colon drug delivery, *Albizia procera*, Ethylcellulose, Polymer blends, Ulcerative colitis

INTRODUCTION

Natural carbohydrate polymers such as plant gums and mucilages have been widely explored as devices for controlled delivery of medicinal drugs. These polymers are abundant, inexpensive, safe and available in a variety of structures that can be easily modified chemically and biochemically. In recent years natural polysaccharides have received considerable interest as carriers for the peroral delivery of drugs to the colon. Several polysaccharides such as pectin (1-2), guar gum (3), chitosan (4), konjac glucomannan/xanthan gum (5), amylase (6) and others have been successfully evaluated for colonic delivery. The colonic microflora secretes a number of enzymes that are capable of hydrolytic cleavage of glycosidic bonds including β-glucosidase, β-galactosidase, α-arabinosidase, amylase, pectinase and others. Natural polymer-based colon targeted drug delivery systems rely on anaerobic bacteria in the colon to degrade them with the enzymes resulting in the release of the entrapped drugs (7). In fact, these microflora activated delivery systems are considered to be

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most promising and a preferable means of achieving targeted delivery of drugs to the colon since the marked increase in the bacteria population and its associated enzymes are independent of gastrointestinal (GI) transit time or pH (8).

Biodegradable natural polysaccharides used in colonic drug delivery can have limitations. The main drawback with using natural polymers alone, in their native form, is their inherent solubility and swelling properties in aqueous media (9). This solubility and swelling often results in the absorption or degradation of the active ingredient in the upper GI tract which is the main obstacle that must be circumvented for successful colonic drug delivery (10). A commonly employed approach to alleviate this problem is to structurally modify the polysaccharides or to blend them with other functional polymers such as GI insoluble or pH sensitive polymers.

The present study was carried out to formulate and evaluate a multi-particulate system for colon-specific drug delivery system using a blend of natural polysaccharide from *Albizia procera* and a GI insoluble ethylcellulose. Multiparticulate dosage forms based on pellets, granules, microspheres or nanoparticles offer several advantages over a single unit system for colon specific delivery of drugs. Due to their smaller particle size, these systems are capable of passing through the GI tract more easily, resulting in less inter- and intra-subject variability (11).

Albizia procera (Roxb.) Benth is a fast growing, medium sized tree known to exude gums in small transparent tears and vermiform pieces (12). It has recently been reported that the physicochemical properties of this gum exudate (13). Ethylcellulose, being hydrophobic in nature, does not readily allow water penetration across the coating and avoids premature film dissolution in the upper GI tract (14-15). The main reason for selecting *Albizia procera* gum, an arabinogalactan, in this study was because of the known biodegradation of arabinogalactans in the colon by the colonic microflora since these microorganisms produce a wide range of enzymes including α-arabinosidase, β-galactosidase and so on. (16-17).

Budesonide is a novel gluccocorticoid which is highly effective in the treatment of irritable bowel disease (IBD) due to its greater topical anti-inflammatory activity than many other gluccocorticoids. Due to the rapid, near complete first-pass hepatic conversion to its metabolites, the systemic effects of budesonide are significantly less than for other conventional corticosteroids which make it an ideal candidate for specific delivery to the colon for topical treatment of IBD.

MATERIALS AND METHODS

Materials

Budesonide (Batch No. B110263) was received as gift from Cipla, Baddi, India. Ethylcellulose (18-22 cps grade, Lobachemie) was used as supplied. *Albizia procera* gum exudates (Authenticated at the Department of Forestry, School of Earth Sciences, Mizoram University) were collected by hand picking in Mizoram (India) during the month of January-March and purified as described previously (13). Tween 80 LR was procured from Sd-Fine Chem. Ltd., India and used as received. All other chemicals and reagents used were of analytical grade.

Methods

Compatibility studies

FTIR spectroscopy

FTIR spectroscopy was performed to assess the compatibility of budesonide with the polymer. Samples were taken at 1:100 ratio with KBr and mixed uniformly in a porcelain dish. About 10 mg of the mixed sample was transferred into sample holder and pressed using a hydraulic press to make a smooth surface. The percentage transmittance was recorded between

400 and 4000 cm^{-1} on FTIR spectrophotometer (IR Prestige-21, Shimadzu). FTIR Spectrum for budesonide, the dried procera gum, the drug-procera gum mixture and the microsphere formulation F3 was recorded for analysis.

Thermogravimetric analysis (TGA)

To study the possible interaction between budesonide and polymer thermogravimetric analysis was performed by TGA (Pyris TGA, Perkin Elmer) between 40°C and 855°C at a heating rate of 10°C/min while nitrogen purging was maintained at 20 ml/min. For each analysis, about 6 mg of the sample was put into the aluminium sample pan and sealed. An empty aluminium pan was used as reference, and the thermograms were recorded for the drug, procera gum, drug-procera gum mixture and microsphere formulation F3.

Formulation and in vitro evaluation of microspheres

Preparation of the microspheres

The microspheres were prepared using an emulsion solvent evaporation technique using the formulation shown in Table 1. The required amount of ethylcellulose was dissolved in 20 ml of acetone and a given amount of the drug and procera gum were dispersed in it and stirred for approximately 10 minutes. Then the polymer drug dispersion was poured into 60 ml of light liquid paraffin containing 2% of Tween 80. The whole system was then stirred for about 5 hours at 900 RPM. After stirring was complete, the liquid paraffin (light) was decanted off and the microspheres formed were collected by filtration and washed with n-hexane to completely remove the remaining oil, and tray-dried at 50°C overnight.

Encapsulation efficiency

The amount of budesonide entrapped in the microsphere was determined by extraction in a solvent system comprising of methanol-water (80:20). A sample of microspheres weighing 50mg was taken, crushed and powdered followed by extraction in the solvent by stirring for about 2 hours. The solution was filtered and after suitable dilution, the content of budesonide was determined using a Waters HPLC system with a UV/Visible detector (2489, Waters). The budesonide determination was modified from a previously developed method (18) and validated accordingly. Briefly, the analysis was carried out using a Symmetry C18 Column (dimension = 150x4.6 mm and particle size 5µm, Waters) at a wavelength of 244 nm. 20 µl of the sample was injected and methanol-water (80:20) was used for the mobile phase at a flow rate of 0.8 ml/min. Data acquisition and processing were performed by using Empower 2 software (Waters). The percentage of encapsulation was calculated using equation 1.

$$\text{Encapsulation efficiency} = \frac{\text{Experiment Drug Content}}{\text{Amount of drug added in the formulation}} = \text{x } 100 \quad \text{Eq. 1}$$

Particle size

The particle size of the microspheres was determined by microscopy. The ocular micro-

Table 1 Formulation of microspheres

FORMULATION	BUDESONIDE	PROCERA GUM	ETHYLCELLULOSE	AVERAGE PARTICLE SIZE (µm) ± SD	% ENCAPSULATION (%) ± SD
F1	9 mg	1000 mg	200 mg	566.80 ± 21.91	68.07 ± 1.98
F2	9 mg	900 mg	300 mg	556.66 ± 16.57	69.50 ± 1.02
F3	9 mg	600 mg	600 mg	534.44 ± 12.54	73.42 ± 2.88
F4	9 mg	300 mg	900 mg	488.88 ± 13.02	76.62 ± 4.82
F5	9 mg	200 mg	1000 mg	482.49 ± 06.58	76.60 ± 4.46

meter was calibrated using stage micrometer and each division of the ocular micrometer was measured in micrometers. For each batch of the microspheres, 100 particles were counted at 10x magnification in triplicate from the same field.

Scanning electron microscopy

The shape and surface characteristics of selected microspheres was analyzed using scanning electron microscopy (JSM-6360, Jeol). Samples were mounted on the aluminium stub and photomicrographs of the microspheres were taken after sputter coating with a thin layer of gold. The quality of the microspheres with respect to surface properties, and the nature and size of pores developed on the surface was evaluated.

In vitro release studies

The *in vitro* release of budesonide from the microspheres was performed using USP Apparatus I (rotating basket) at a rotation speed of 50 RPM (USP Dissolution Test Apparatus, ACMAS Technocrat, India). 400 mg of budesonide-containing microspheres from each batch were placed in 250 ml of dissolution medium which was maintained at 37±0.5°C. The release study was performed in 250 ml 0.1 N HCl for the first 2 hours, followed by 250 ml of pH 6.8 Phosphate buffer for another 3 hours and finally 250 ml of pH 7.4 Phosphate buffer to a total of 24 hours to simulate the gastro-intestinal pH condition. About 2 ml of the dissolution medium was withdrawn at predetermined time intervals and replaced with the same volume of fresh dissolution medium and analyzed using the HPLC method described earlier.

In vitro release study in presence of rat cecal content

Rat cecal content was prepared by the method reported previously (19). Dissolution medium containing 2% rat cecal content were prepared in pH 7.4 phosphate buffer which had previously been deoxygenated with N_2 to provide anaerobic conditions. Drug release from the microspheres in the physiological environment of colon was assessed by performing drug release studies in the rat cecal content medium. In this case, dissolution study was performed in 0.1N HCl for the first two (2) hours followed by pH 6.8 phosphate buffer for the next three (3) hours. Finally, pH 7.4 phosphate buffer containing 2% rat cecal content was used and the study was continued for up to 24 hours. At different time intervals, 2 ml of the dissolution medium was withdrawn and replaced with the same volume of the fresh dissolution medium and analyzed by the HPLC method.

In vitro drug release kinetics

To determine the release mechanism from the microspheres, *in vitro* the drug release data were also fitted using Korsemeyer-Peppas equation (20) expressed as:

$$\frac{Q_t}{Q_\infty} = k_k * t^n \qquad \text{Eq. 2}$$

Where, k_k is the kinetic constant, Q_∞ is the amount release at time t = ∞, thus Q_t / Q_∞ is the fraction of drug released at time t. The value *n* is the diffusion exponent which can be used to characterize both mechanism for both solvent penetration and drug release. The value of n=0.5 indicates a Fickian Diffusion, 0.5<n<1.0 indicates anomalous (non-Fickian) diffusion, n=1.0 indicates case II transport (zero-order release) and n>1.0 indicates super case II transport. Determining the correlation coefficient assessed fitness of the data into various kinetic models. The rate constants, for respective models were also calculated from the slope.

In vivo studies

Animals

Both male and female wistar rats (150 ± 50 g) were used in the *in vivo* studies. They were

housed under environmentally controlled conditions with free access to water and standard diet. The animal studies were carried out according to the guidelines of CPCSEA, Ministry of Environment & Forests, Government of India and the study protocol was approved by the Institutional Animal Ethics Committee of Dibrugarh University, India (Reg. No. 1576/GO/a/11/CPCSEA, India).

Induction of colitis

Colitis was induced according to the method described previously (21-22). Briefly, following a 24 hour fast, the rats were slightly anaesthetized with ether, and then a medical-grade polyurethane tube (external diameter 2 mm) was inserted into the anus and the tip was advanced to 8 cm proximal to the anus verge. Acetic acid (2 ml, 4% v/v in 0.9% saline) was instilled into the colon through the tube for 30 seconds, after which the fluid was withdrawn. The animals were left with free access to water and pellets until the experiment was finished.

Administration of microspheres

The animals were divided into 6 groups of six as follows:

1. A normal group (No induction of colitis) which received 2 ml of 0.9 % saline by rectal route.
2. A control group which did not receive any treatment after induction of colitis.
3. A placebo group which received an oral placebo dose of procera gum-ethylcellulose microspheres without drug (blank microspheres).
4. A vehicle group which received only 0.5 ml of the vehicle, 1% Sodium Carboxymethyl cellulose (Na CMC) orally.
5. A standard group which received marketed product of budesonide microspheres (Budez CR®) orally at 300 µg/kg dose of budesonide.
6. A the test group which received formulation F3 of procera gum-ethylcellulose coated budesonide microspheres at 300 µg/kg dose of budesonide.

The placebo (Blank), standard (Budez CR®) and test microspheres (F3) were suspended in 0.5 ml of 1% Na CMC as per the dose calculated (300 µg/kg) and administered orally to the rats by feeding tube. Drug treatment was started 48 hours after colitis was induced where sufficient time was given for the full development of colitis and treatment was given once daily for 5 consecutive days.

Assessment of colitis

Twenty four hours after administration of the last dose of the formulations, the rats were sacrificed using a high dose of ether and a midline incision was made in the abdomen. The 8 cm distal segment of the colon was removed, washed and wet weight/length (g/cm) ratios were obtained as criterion of injury (23). The macroscopic appearance of the colonic mucosa was scored according to an arbitrary scale ranging from 0 – 3 as follows: 0 = no macroscopic change, 1 = erythema & inflammation without ulcer, 2 = inflammation & ulcer, 3 = ulcer with necrosis. Inflammation and ulcer surface area were measured and ulcer index was calculated from the macroscopic evaluation using the following equation:

$$\text{Ulcer Index} = \text{Ulcer area (sq.cm)} + \text{Macroscopic Score} \quad \text{Eq. 3}$$

Histopathological study was performed by fixing sections of colon specimens in phosphate buffered formalin solution (10 %), embedded in paraffin stained with haemotoxylin and eosin and evaluated by light microscopy at suitable magnification to observe and study the morphological changes.

Statistical analysis

Statistical analysis was performed using computer software SigmaStat 2.03 (SPSS, USA). Analysis of variance /Tukey Test was performed to compare the effects of different

polymers on physical and drug release properties of the microspheres. The susceptibility of procera gum coating to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in a medium with 2% rat cecal content after completing 5 hours of study in simulated gastric and small intestinal media. The same software was also used for the statistical analysis of the *in vivo* studies.

RESULTS AND DISCUSSION

The microspheres were prepared following the emulsion solvent evaporation method using Tween 80 to help stabilize the emulsion. The dried microspheres were obtained as spherical, free flowing particles.

Drug-polymer interaction studies

FTIR spectroscopy

Figure 1 shows the FTIR spectra of budesonide, procera gum, the drug-procera gum mixture and the microsphere formulation F3. A typical FTIR spectrum of budesonide should showed characteristic O-H stretching peak at 3378 cm^{-1}, C-H stretching peak at 2935 cm^{-1}, and C=O stretching peaks at 1720 and 1659 cm^{-1} (24). These typical peaks were observed for the budesonide sample (A) at 3483.44 cm^{-1}(O-H stretch), 2935.66 cm^{-1} (C-H stretch), 1716.65 cm^{-1} and 1658.78 cm^{-1} (C=O stretch). In the IR spectrum of budesonide with the polymer physical mixture (C), typical peaks for budesonide at 3321.42 cm^{-1} (O-H stretch), 2935.66 cm^{-1} (C-H stretch), 1716.65 cm^{-1} and 1658.78 cm^{-1} (C=O stretch) were still observed confirming the presence of these functional groups in the physical mixture and the compatibility of the drug with the procera gum. The spectrum for microsphere formulation F3 also showed budesonide peaks at 3312.56 cm^{-1} (O-H stretch), 2935.66 cm^{-1} (C-H stretch) and 1710.33 cm^{-1} and 1643.35 cm^{-1} (C=O stretch).

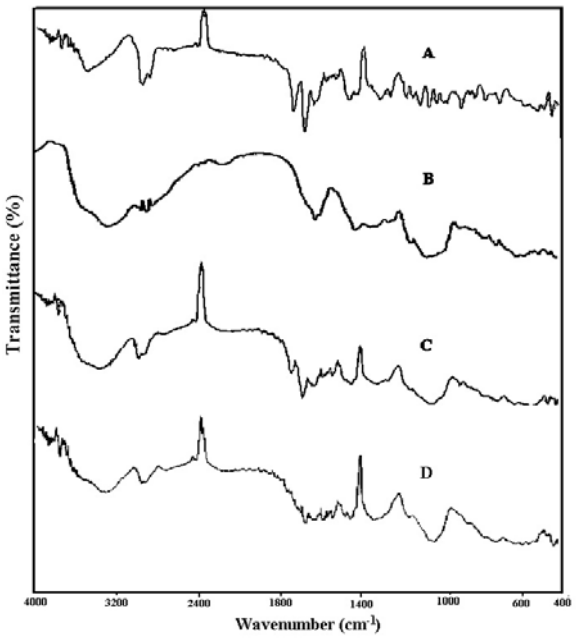

Figure 1 FTIR spectra of (A) Budesonide, (B) Procera gum, (C) Solid admixture of the drug with procera gum and (D) Microsphere formulation F3

Thermogravimetric analysis

TG curves for the samples are shown in Figure 2. The thermogram for budesonide (A) showed a single endothermic weight loss occurring in between 235.81°C and 486.59°C. There was a significant weight loss of 82.54% during this event. The melting point of budesonide is around 220-235°C which correlates well with the TGA thermogram. The thermogram for procera gum (B) showed two endothermic events. The first event occurred in between 49.15°C and 145.73°C with 7.08% weight loss and may be attributed to evaporation of water. The second one occurred

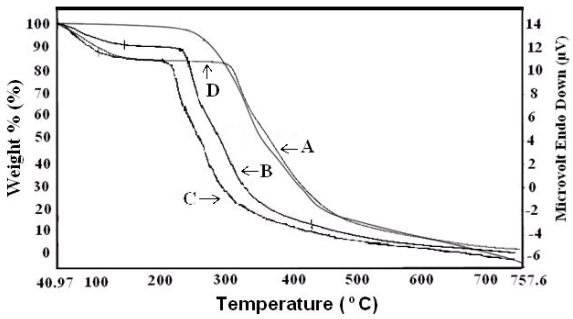

Figure 2 TG curves of (A) Budesonide, (B) Procera gum, (C) Solid admixture of the drug with procera gum and (D) Microsphere formulation F3

between 224.04°C and 406.77°C was assigned to the characteristic decomposition of the polysaccharide. There was a total weight loss of 56.08% during this second event. The TG pattern of the drug and the polymer mixture (C) also showed that two stages of degradation occurred between 45.47°C and 110.55°C with 9.72% weight loss which could be attributed to the evaporation of water from the polymer. The second event between 219.01°C and 405.57°C with 52.4% weight loss could be attributed to a composite of the events observed for budesonide and procera gum. The TG thermograms for microsphere formulation F3 (D) also showed two stages of weight loss occurring between 42.80°C and 112.21°C with 10.48% weight loss and 221.85°C and 395.27°C with 54% weight loss. The thermogram obtained supported well the results of the FTIR spectroscopic studies confirming compatibility.

Characterization of microspheres

Encapsulation efficiency

The drug content in all the formulations was between 6.13 mg in F1 and 7.02 mg in F5 as given in the Table 1. The encapsulation efficiency was in between 68.09 ± 4.51% and 78.02 ± 2.89 % with no statistically significant difference ($p>0.05$) observed in all of the above formulations with encapsulation efficiency.

Scanning electron microscopic studies

The emulsion solvent evaporation method is the second most frequently used technique for microencapsulation involving ethylcellulose (25) and this method was used successfully here for the production of the microspheres. Figure 3 shows the scanning electron micrographs of the microspheres. It was observed that most of the microspheres were spherical in shape and had pores in the surface which may be due to the gradual evaporation of the solvent during stirring. However, these pores may be beneficial for the release of budesonide from the microspheres. It was observed that, as the amount of ethylcellulose was increased in the formulations from F1 to F5, the surface became much smoother. The microsphere formulations with higher proportions of procera gum possessed a rougher surface. Increased ethylcellulose concentration in the coatings (accompanied by a corresponding reduction in proportion of procera gum) resulted in microspheres with better, smoother surfaces. A possible reason for this may be the disturbance of the continuity of ethylcellulose coating membrane by the presence of procera gum particles as reported for Eudragit S coated pectin microspheres (26), a different polymer system, which, however is prepared using the same emulsion solvent evaporation method.

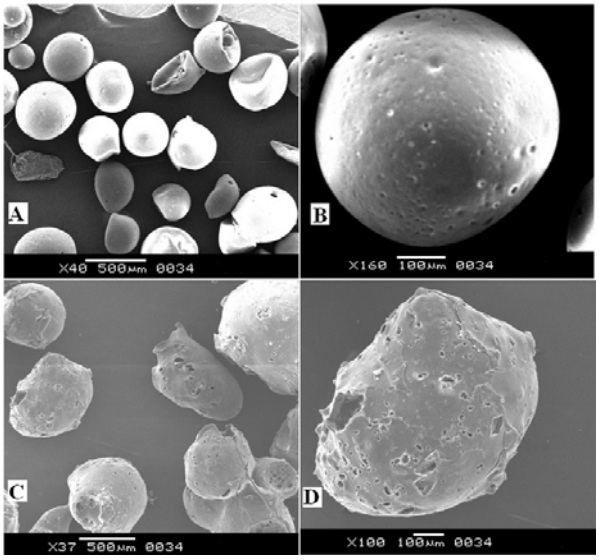

Figure 3 Scanning electron micrographs of formulation F5 (A & B) and F2 (C & D)

Average particle size

The average particle size was determined by microscopic method using ocular eye piece calibrated through a standard stage micrometer. As given in Table 1, the average particle sizes were between 567.88 ± 11.39 µm in F1 and 462.16 ± 06.05 µm in F5. The average particle sizes tended to become smaller as the level of ethylcellulose was increased. Significant changes were observed ($p<0.05$) between formulations F2 and F4, but not between F1 and F2 or F4

and F5. Normally, an increase in the viscosity of the polymer solution results in an increase in the size of emulsion droplets which ultimately produces larger size microspheres (27). However, the trend observed in this study indicated that an increase in the amount of ethylcellulose and the resultant increase in viscosity, due to more ethylcellulose being dissolved in the same volume of acetone, subsequently produced smaller microspheres with improved surface properties. This may be attributed to the presence of procera gum in the dispersed phase which may have affected the emulsification process and altered the continuity of the coating membrane as indicated previously.

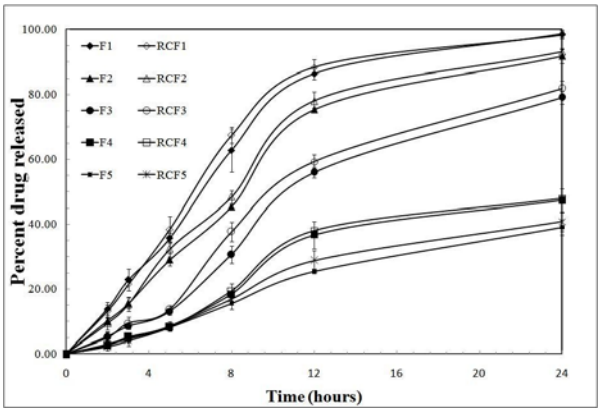

Figure 4 Graph of % drug release versus time for different formulations in dissolution medium containing rat cecal contents (RCF1, RCF2, RCF3, RCF4 & RCF5) and without rat cecal contents (F1, F2, F3, F4 & RCF5)

In vitro drug release

Simulated GI fluid

For successful colonic drug delivery, absorption or degradation of the active ingredients in the upper GI tract should be prevented or minimized (10). The main drawback of using natural polymers alone for colon specific drug delivery is their inherent swelling and solubility in aqueous media which results in the drug being released before the colon is reached (2). Blending natural polysaccharides, such as procera gum with GI tract insoluble ethylcellulose is therefore expected to produce polymeric films with reduced drug permeability in the stomach and small intestine (28). To evaluate the performance of the developed systems in achieving specific delivery of drugs to the colon, *in vitro* dissolution testing was performed with dissolution medium simulating the pH conditions in the GI tract. Drug release profiles for the different formulations are shown in Figure 4. Release of budesonide in acidic medium after 2 hours varied between 13.89% in F1 and 2.78% in F5. After 5 hours, drug release was 35.80%, 29.03%, 13.13%, 8.58% and 8.15% in F1, F2, F3, F4 and F5 respectively. There were significant differences in the dissolution time of budesonide ($p<0.001$) between the different formulations after 24 hours in the simulated GI fluid. Previous studies have indicated that, when the amount of drug released in the initial 5 hours, is not more than 10% (29) or less than 20% in 6 hours (15), the system is considered to be capable of functioning as a colonic drug delivery system. After 24 hours dissolution study, the total amount of drug released was more than 90% in F1 and F2, 79% in F3 and around 40% in F4 and F5. The percentage of drug released in the target area of the simulated colonic environment over 24 hours was 62.82% for F1, 62.73% for F2, 65.99% for F3, 38.73% for F4 and 30.95% for F5. From the percent of budesonide released after 5 hours, it is apparent that formulations F3, F4 and F5 are capable of preventing much of the drug from being released in the physiological environment of the stomach and intestine. However, the percentage drug released after 24 hours from formulations F4 and F5 was too low, or not sufficiently rapid, for topical colonic delivery of budesonide. To avoid the possible systemic effects of budesonide and harness its superior anti-inflammatory activity for treating IBDs, the delivery system should ideally prevent the release of the drug in the upper GI tract, while providing a rapid release once it reaches the colon. Formulations F3 and F4 appear to achieve this target for colonic drug delivery better than the other microsphere formulations.

Medium with 2% rat cecal content

The susceptibility of procera gum-ethylcellulose coating to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 2% rat cecal content medium after 5 hours study in simulated gastric and intestinal fluids. The dissolution medium was changed at the 6th hour to simulate the colon arrival time under normal conditions. The presence of 2% rat cecal content in simulated colonic fluid showed evidence of slightly faster drug release at different time periods when compared with the release study without rat cecal content. However, the difference in drug release with or without rat cecal content was not found to be significant in different formulations of the microspheres ($p=0.9605$ for F1, $p = 0.9498$ for F2, $p = 0.9122$ for F3, $p = 0.9535$ for F4 and $p = 0.8563$ for F5) when they are treated statistically. This could be attributed to the fact that the colonic bacterial action of the rat cecal medium may not be sufficient to degrade the gel barrier developed in the system or the 2% rat cecal content used may be too low in the *in vitro* system. With other polysaccharide-based colonic drug delivery formulations, such as tablets or cross-linked polysaccharide microspheres, the whole system is usually accessible to the colonic fluids. In the present formulation, where a polymer blend was used, the presence of ethylcellulose in the coating also restricted to certain extent the entry of the simulated colonic fluid, and this may have led to reduced degradation of the polysaccharide. However, the slightly increased drug release observed with the medium containing 2% rat cecal contents can be considered as evidence that degradation of the microspheres will take place in the colonic environment. Also, as reported earlier, the human cecal contents would be far better at degrading this gel barrier (30). In the present study, 2% rat cecal content was used to obtain an overview on the susceptibility of the system to the colonic enzymes, and a standard protocol was available in the literature for its preparation (19).

Release kinetics

The mechanism of drug release was determined using the Korsemeyer-Peppas model and the results are given in Table 2. The release exponent 'n' calculated from the equation, shows that in all the batches the 'n' values were between 1.047 and 1.216. The 'n' value does not differ significantly between the two dissolution media taken for the *in vitro* release study. The 'n' value for all the *in vitro* drug release study was larger than 1.0 which indicates that drug release follows super case II transport where more than one specific mechanism was involved in release of the drug from the microspheres (31). For this type of transport, the polymer relaxation is the rate-limiting step to water transport (32). In general, the contribution of polymer relaxation was higher for the formulations with higher 'n' values. The mechanism also explains the initial slow release phase where only 13.89%, 10.10%, 5.39%, 2.65% and 2.78% of the drug were released in F1, F2, F3, F4 and F5 respectively. This is probably due to the slow hydration of the polymer which resulted in incomplete relaxation of the polymer chains. Insufficient polymer hydration could be attributed to the presence of ethylcellulose where pores/ channels did not develop quickly enough during the initial stage. However, with time more pores developed resulting in increased hydration of the procera gum and subsequent relaxation. The effect of procera gum relaxation became more prominent which ultimately controlled the release of the drug from the microspheres.

Table 2 Drug release kinetics

FORMULATION	n	r^2	K
F1	1.047	0.981	1.170
F2	1.112	0.981	1.353
F3	1.084	0.966	1.558
F4	1.216	0.963	1.884
F5	1.103	0.984	1.855

In vivo studies

Acetic acid-induced colitis was chosen as a method for ulcer induction because the formed

ulcer resembles human ulcerative colitis in histology, eicosanoid production and excessive oxygen-derived free radicals release by inflamed mucosa (33). The histopathological features of acetic acid treated rats included necrotic destruction of mucosa and sub-mucosa, areas of hemorrhage and diffuse inflammatory cell infiltration in the mucosa. After 4% v/v acetic acid was instilled into the colon, the animals started to developed bloody diarrhea, the animals were weak and their food intake was also decreased which resulted in weight loss. Macroscopic examination as shown in Figure 5 showed signs of severe hemorrhagic lesions and inflammation as assessed by macroscopic scores. Parameters such as colon wet weight/length ratio, ulcer area, ulcer index and macroscopic damage score were used to evaluate the macroscopic damage of the colon and these data are summarized in Table 3. Results of the colon wet weight/length ratio from the colitis induced groups showed that the group treated with formulated test microspheres (F3) exhibited the minimum value of 0.269 g/cm. However, since there was no statistically significant difference (p=0.255) between the groups, it would not be appropriate to use as an index of colonic inflammation. Ulcer area, ulcer index and macroscopic score were significantly different between the normal and negative control groups. As shown in Table 3, animals treated with the test formulation showed an attenuated extent of the colonic injury with significantly reduced ulcer area (p = 0.0026) when compared to the control group. There was also slight reduction of ulcer area in the Budez CR®, blank microspheres, and vehicle treated groups when compared to the control, but the reduction was not statistically significant (p=0.0557 for Budez CR® versus the control, p=0.1081 for blank microspheres versus the control and p=0.1936 for vehicle versus the control groups). Treatment of rats with the test budesonide formulation for five days reduced the severity of the colonic damage. The ulcer index, which is calculated from the summation of the ulcer area and the macroscopic score, was also significantly reduced from 10.86±1.35 in the untreated control group to 4.05 ± 0.63 in the test group (p<0.001). However, the reduction in ulcer area between control and other groups was not statistically significant (p=0.0756 for Budez CR® versus the control, p=0.0865 for blank microspheres versus the control and p=0.1964 for vehicle vs control).

Table 3 Results of macroscopic evaluation of the colonic damage

TREATMENT GROUP	WEIGHT/LENGTH RATIO (g/cm) AVERAGE ± SD	ULCER AREA (cm^2) AVERAGE ± SD	ULCER INDEX AVERAGE ± SD
Normal	0.239±0.002	0.00	0.00
Control	0.323±0.015	9.40±1.69	10.86±1.21
Vehicle treated	0.396±0.016	6.18±1.20	8.38±1.04
Placebo (Blank microspheres)	0.342±0.011	5.08±0.99	6.48±1.18
Reference product	0.375±0.016	3.58±0.84	5.46±1.52
Test drug	0.269±0.002	2.75±1.11	4.06±1.24

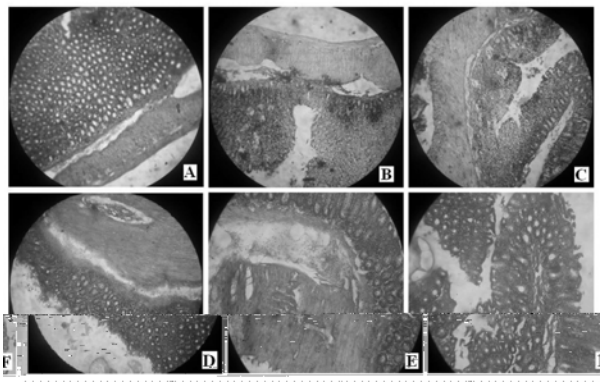

Figure 5 Histopathological examination of the colon (x45) in (A) Normal group, (B) Control group, (C) Placebo group, (D) Vehicle group, (E) Market product group and (F) Test group

CONCLUSION

A microsphere system for colonic delivery of budesonide based on polymer blends of natural polysaccharides from *Albizia procera* and ethylcellulose was developed successfully. The compatibility between the drug and the polymer was established through FTIR and TGA analysis. The appropriate blends of the

polymers have the ability to limit the release of drug in simulated gastric and small intestinal conditions while maximizing drug release in the colonic environment indicating its potential for colonic drug delivery. *In vitro* analysis showed that 1:1 ratio of procera gum-ethylcellulose (F3) was the optimum formulation. The data generated as an outcome of this research demonstrates that ethylcellulose-procera gum coated budesonide microspheres reduced the severity of ulcerative colitis in acetic acid-induced colitis in rats.

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