Biomatter 1:2, 182-188; October/November/December 2011; © 2011 Landes Bioscience

Factorial design-based development of measlamine microspheres for colonic delivery

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Key words: microspheres, colonic delivery, pectin, diffusion, Eudragit S-100

For treating colonic diseases, conventional oral drug delivery systems are not effective, as they fail to reach the appropriate site of action. Thus, there is a need to develop effective and safe therapy for the treatment of colonic disorders. The aim of the present study was to design a colon-specific delivery system for an anti-inflammatory drug, mesalamine, with minimal degradation and optimum delivery of the drug with relatively higher local concentration, which may provide more effective therapy for inflammatory bowel disease including Crohn disease and ulcerative colitis. Factorial designs (four factors and two levels) for eudragit S-100 (pH-dependent polymer)-coated, pectin (natural polysaccharides)-based microspheres of mesalamine were constructed and conducted in a fully randomized manner to study all possible combinations. Based on the desirability function formulation, F14 was found to be the best formulation. The overall desirability coefficient of formulation F14 was found to be 0.825. The formulation F14 was subjected to in vitro release studies, and the results were evaluated kinetically and statistically. The microspheres started releasing the drug at the beginning of 7th hour, which corresponds to the arrival time at proximal colon. The cumulative percent drug release for formulation F14 at the end of 16 h was found to be 98%. The release kinetics showed that the release followed the Higuchi model, and the main mechanism of drug release was diffusion. The study presents a new approach for colon-specific drug delivery.

Introduction

Drug delivery to the colon is beneficial for many drug molecules, like proteins and peptide drugs, drugs used to treat ulcerative colitis, Crohn syndrome, diarrhea and colon cancer. Colon as a site offers various advantages on account of a far from neutral pH, a longer transit time, reduced digestive enzymatic activity and a much better responsiveness to absorption enhancers.[1] A colon-specific drug delivery system is expected to prevent drug release in the upper gastrointestinal tract and effect an rapid onset of drug release upon entry into the colon.[2]

There are several approaches utilized in achieving colon targeting, including the use of prodrugs, coating with pH-sensitive polymers, design of time-release dosage forms, utilization of biodegradable polymers, such as azopolymers and polysaccharides (e.g., pectins and dextrans) that degrade exclusively by the colonic bacteria.[3,4] Each system has advantages as well as disadvantages. The poor site-specificity of pH-dependent and time-release systems due to the large variations in the pH of the gastrointestinal tract and variations in gastric emptying times across the ileo-cecal junction, respectively, is well-documented.[5] Non-starch polysaccharides-based, microflora-activated systems are very promising, because they are degraded by the vast anaerobic microflora present in the colon. Furthermore, they are not digested in the human stomach and intestine.[6] This strategy exploiting the abrupt increase of the bacteria present in the colon and corresponding enzyme activities will also accomplish better site specificity. Moreover, the polysaccharides for colonic delivery are also naturally occurring, reasonably priced and available abundantly.[7,8]

Single-unit dosage forms for colonic delivery may suffer from the disadvantage of unwarrented disintegration of the formulation due to high inter- and intra-subject viability and poor reproducibility, which may lead to loss of local therapeutic action in the colon. Therefore little emphasis is being laid on the preparation of multi-particulate delivery systems in comparison to single-unit dosage forms due to their possible benefits, like better bioavailability, decreased risk of local irritation and predictable gastric emptying.[9]

The microspheres are characteristically free-flowing powders composed of synthetic polymers, which are biodegradable in nature, and ideally, having a particle size less than 200 μm.[10] Microspheres may spread out on their arrival in the colon, with a sharp increase of surface area exposed to bacterial breakdown that produces a rapid drug release and thereby improves drug availability and gives site-specific action to the colon.

In order to treat inflammatory bowel diseases, oral administration of anti-inflammatory agents such as 5-amino salicylic acid, chemotherapy agents, antibiotics and corticosteroids to the colon is required. Mesalamine is a drug of choice to treat

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Submitted: 05/10/11; Revised: 09/16/11; Accepted: 10/18/11
http://dx.doi.org/10.4161/biom.1.2.18461

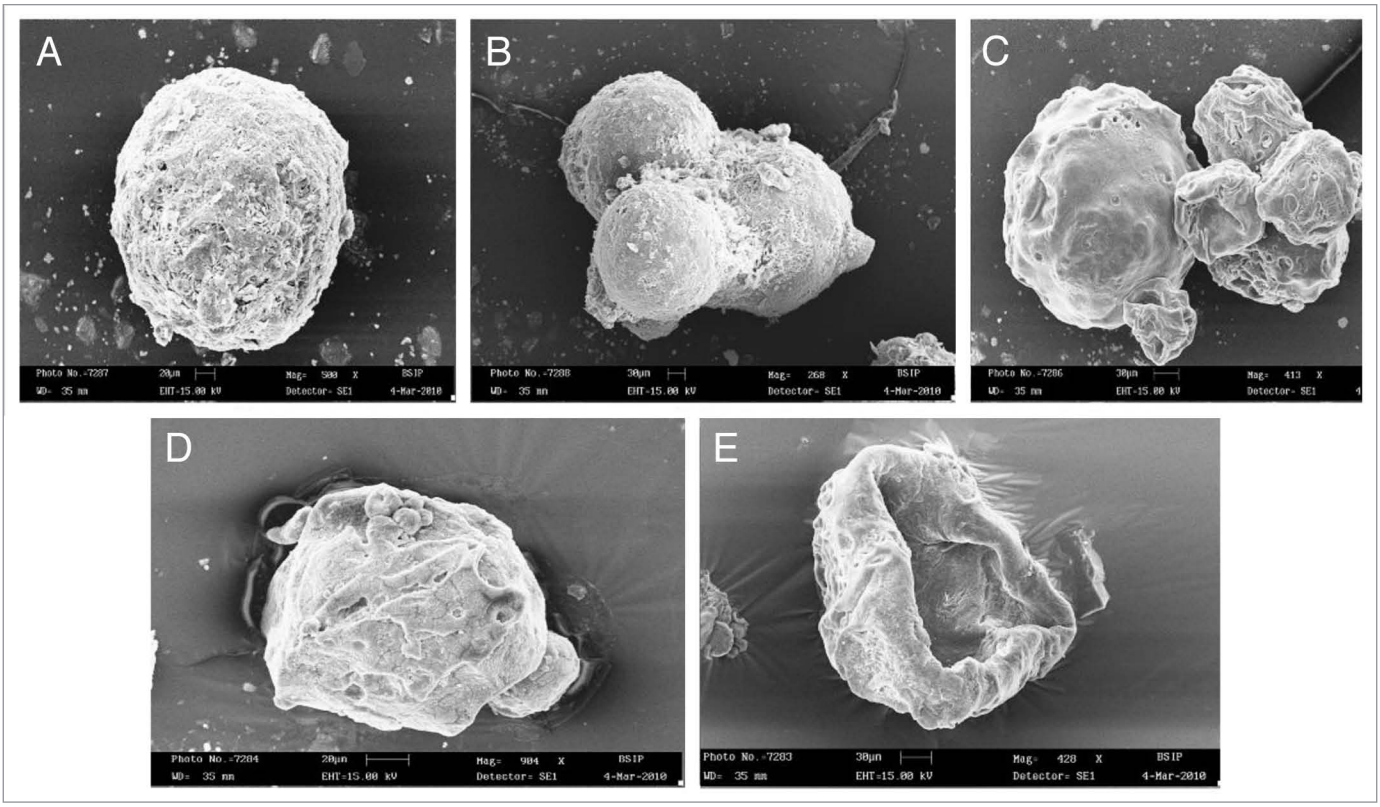

Figure 1. SEM photomicrographs of (A) uncoated microspheres (B) uncoated microspheres in groups (C) enteric-coated microspheres (D) T.S. of enteric-coated microspheres (E) enteric-coated micerospheres after dissolution.

inflammatory bowel disease. It is unstable in acidic environments and is also absorbed by the small intestine and produces side effects. The aim of present study was to design a mesalamine-loaded, colon-specific delivery system to minimize its degradation and to achieve optimum delivery of the drug with relatively higher local concentration to provide more effective therapy for inflammatory bowel diseases, including Crohn disease and ulcerative colitis. A four-factor, two-level full factorial design was used in a fully randomized order to study all possible combinations of all the factors at all levels. Such an experiment allows studying the effect of each factor on the response variable as well as the effects of interactions between factors on the response variable. The response surface was visualized graphically, as the graphs are useful to determine the shape of a response surface; hills, valleys and ridge lines.

Results and Discussion

A four-factor, two-level full factorial designs was conducted and constructed to study all possible combinations of all the factors at all levels in fully randomized order. In order to estimate the relationship between the independent variables and the response variables, a statistical model was used.

Before application of the design, a number of preliminary trials were conducted to establish the control factors and their levels. The statistical evaluation of the results was performed by analysis of variance (ANOVA) using Microsoft Excel Version 2000. The significant parameters in the equations for the calculation of regression analysis can be selected using a step-wise forward and backward elimination. In this study, the full model with a non-significant p value ($p > 0.05$) was used in obtaining dependent variables, and thus, the results of the study were neglected.

Interaction plots were obtained for the deliberate response on the basis of the model using the software (Design-Expert 8) to further explain the relationship between the independent variables and the response.

Effect of drug-polymer ratio. The surface appearance and inner structure of the microspheres were examined using scanning electron microscopy (SEM), which is shown in **Figure 1**. The microspheres were found to be uniform without any drug crystal of free or unentraped drug on the surface. **Figure 1** shows that the drug-to-polymer ratio has considerable effect on the size of microspheres. It was noted that, as the ratio of drug-to-polymer was increased, the particle size was also increased. As depicted in **Table 2**, in case of formulation F4, with low drug-polymer ratio (1:3), the smaller microspheres (130.2 ± 1.62 μm) were obtained, while in formulation F12, with high drug-polymer ratio (1:6), larger microspheres (185.5 ± 2.31 μm) were observed. This might be due to the fact that higher concentrations of polymer produced more viscous dispersion, which formed larger droplets and, consequently, larger microspheres. It was also observed that as the ratio of drug-to-polymer was increased, entrapment efficiency was increased. This might be due to the fact that a high drug-to-polymer ratio produces large microspheres, which,

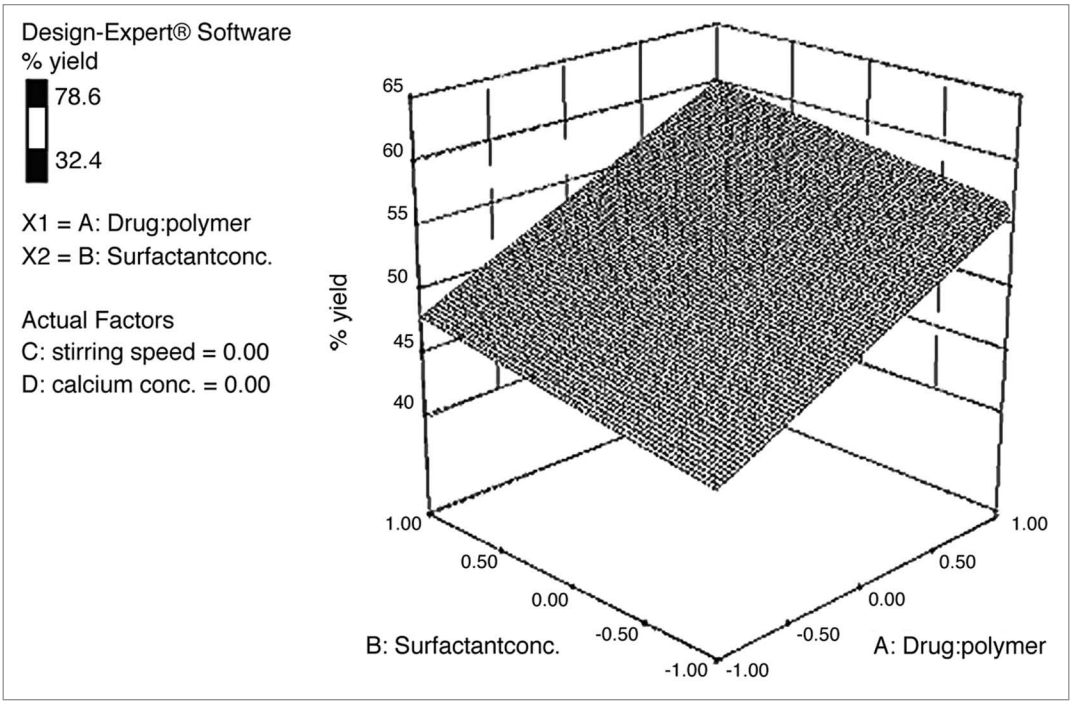

Figure 2. 3D graph of effect of drug polymer ratio and surfactant concentration on % yield.

in turn, shows higher drug loading efficiency. Production yield, entrapment efficiency and mean particle size of formulations F1–F16 are given in **Table 3**. The entrapment efficiency and production yield of the formulations F1–F16 were found to be between 32–78% and 45–86%, respectively. Statistical analysis using software (Design-Expert®) for production yield of microspheres was done. The model F-value of 3.41 implies that the model is significant. A value of "Prob > F" less than 0.05 indicates that model terms are significant. In this case, significant model terms are A (drug:polymer ratio), B (surfactant concentration), C (stirring speed) and D (calcium chloride concentration) (**Fig. 2**). Statistical analysis for entrapment efficiency was also done. The Model F-value of 24.97 implies the model is significant. A value of "Prob > F" less than 0.05 indicates that model terms are significant. In this case, significant model terms are A (drug:polymer ratio), B (surfactant concentration), C (stirring speed) and D (calcium-ion concentration) (**Fig. 3**).

Effect of surfactant concentration on microspheres. An increased amount of surfactant resulted in increased particle size. As shown in **Table 2**, in formulation F4 with a low concentration of surfactant (0.75% w/v), smaller microspheres (130.2 ± 1.62 μm) were obtained, while in formulation F15 at higher concentrations of surfactant (1.5% w/v), larger microspheres (280.5 ± 2.45 μm, respectively) were obtained. This could be due to the increased viscosity, where larger emulsion droplets formed in larger microspheres.

Effect of stirring rate on morphology of microspheres. The mean diameter of microspheres decreased on increasing agitation speed from 1,000 rpm to 2,000 rpm. As shown in **Table 2**, in case of formulation F4 at low stirring speed (1,000 rpm), larger microspheres (130.2 ± 1.62 μm) were obtained, while in formulation F5 at high stirring speed (2,000 rpm), smaller microspheres (112.3 ± 0.89 μm) were obtained. This result was expected, because high stirring rates provide the necessary shearing force required to separate the oil phase into smaller globules.

The model F-value of 4.81 implies the model is significant. A value of "Prob > F" less than 0.05 that indicated that model terms are significant. In this case, significant model terms are B (surfactant concentration) and D (calcium-ion concentration). The effect of independent variables on particle size (responcse variables) is shown in **Figure 4**.

In vitro release studies. Desirability function was used to find out the best formulation; among all formulations, F14 showed the highest overall desirability of 0.825. Therefore, this formulation was considered to be the best formulation, and the values of independent variables of this formulation were considered to be optimum values for the preparation of enteric-coated microspheres.[16]

In vitro drug release studies of the developed microspheres were performed by the Souder and Ellenbogen extraction[15] technique using USP XXIII a type II, i.e., paddle type, dissolution apparatus with a stirring rate of 100 rpm at 37 ± 0.5°C. The release plot obtained for the formulation F14 is shown in **Figure 5**. No drug release was observed during the first six hours. After a lag time of 6 h, the drug started releasing at the 7th hour due to the dissolution of Eudragit S-100 in the presence of the pectinex Ultra-SPL. The overall cumulative percent release for formulation F14 at the end of 16 h was found to be 98%.

The analysis of variance for percent drug release (partial sum of squares type III) was determined using factorial design. The model F-value of 4.15 implies the model is significant. The release data was analyzed using various mathematical models (**Table 4**). The correlation coefficient and release rate constant

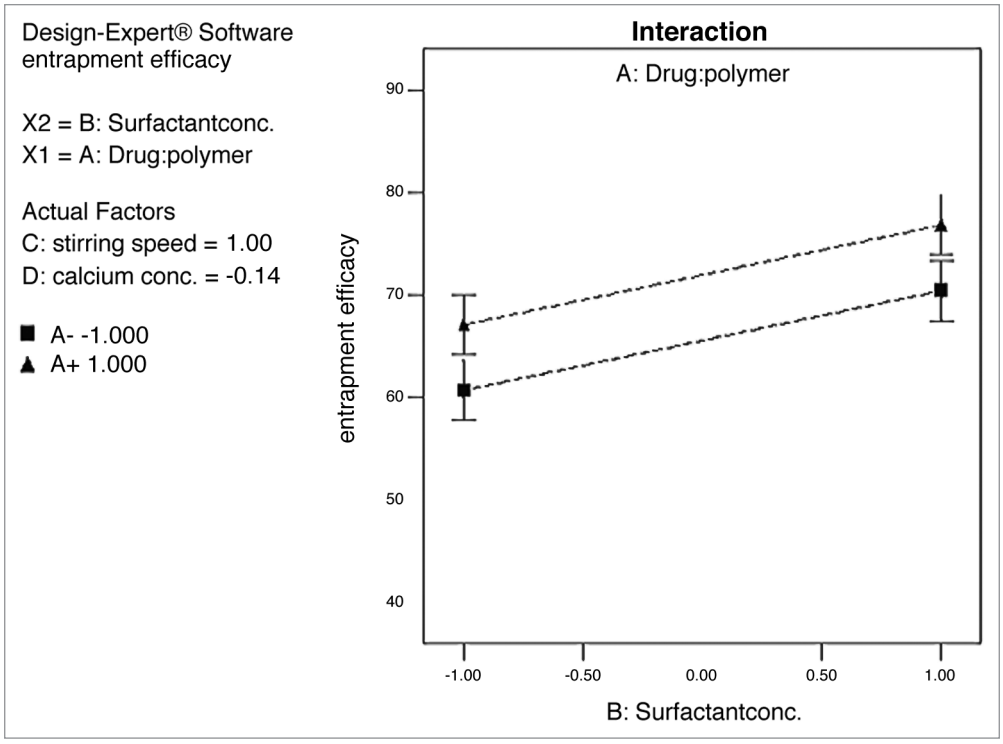

Figure 3. Interaction graph between drug polymer ratio and surfactant concentration.

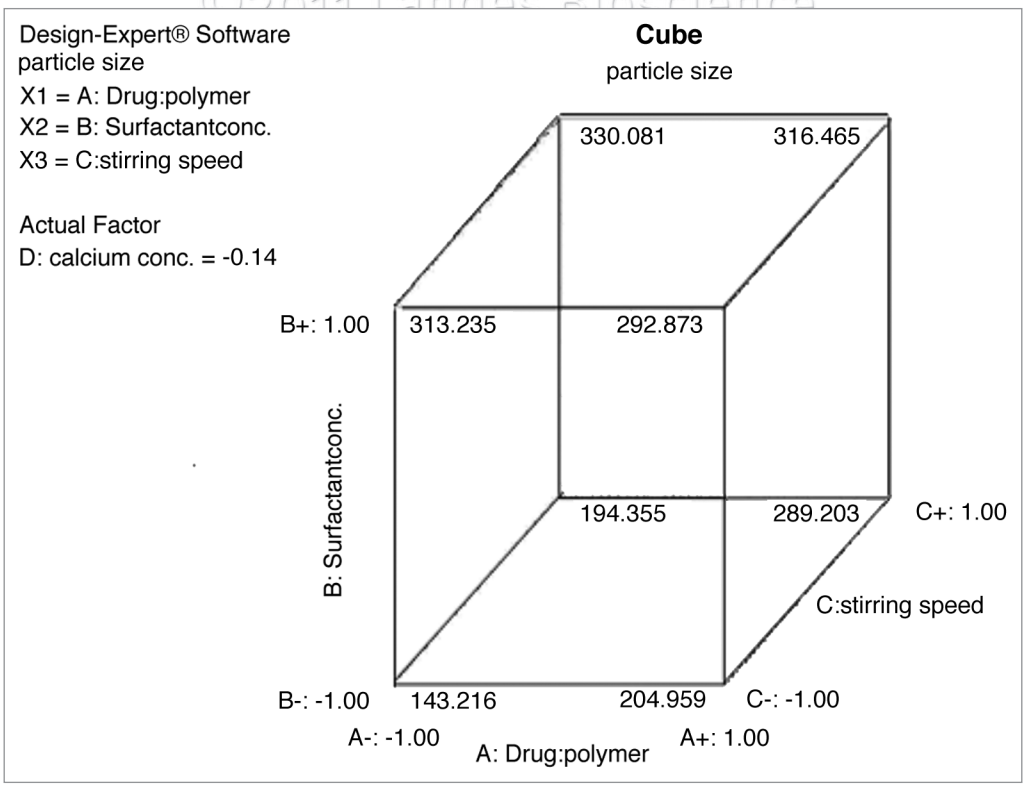

Figure 4. 3D graph of effect of drug: polymer ratio, stirring speed and surfactant on particle size.

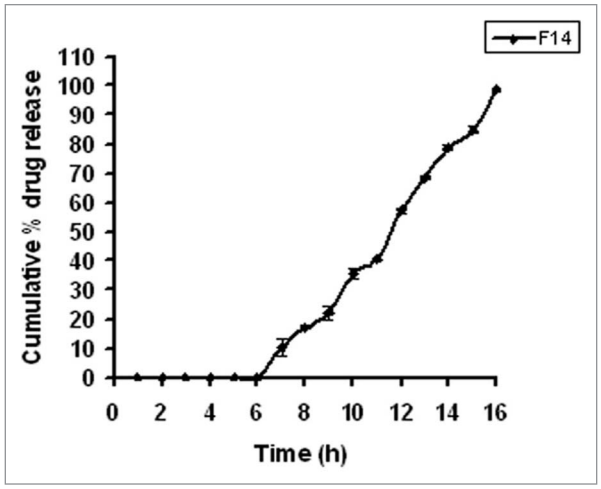

Figure 5. In vitro drug release profile of F14 formulation.

values for zero, first, Higuchi, Matrix, Peppas and Hixson-Crowell models were computed. The correlation coefficient values of F14 formulation for different models were found: 0.9683 for zero order, 0.9522 for first order, 0.9983 for Higuchi matrix, 0.9741 for Peppas and 0.9808 for Hixson-Crowell respectively. The R values were much closer to 1 for the Higuchi matrix kinetics. From the correlation coefficient values, it was concluded that drug release from the F14 microspheres formulation followed the Higuchi model. The Higuchi model explained the matrix diffusion mechanism of drug release for F14 microspheres formulation. Based on highest regression value, the best fit was observed for Higuchi matrix. The n value for the Peppas model was found to be between 0.5–1, indicating non-Fickian diffusion.

Materials and Methods

Materials. Mesalamine was purchased from Sigma Aldrich. Pectin Classic with low methoxylated grade (AU701) and Pectin Amid, Amidated with low methoxylated grade (CF020) were a generous gift from Herbstreith and Fox. Eudragit S-100 was obtained as a gift sample from Evonik India Pvt Ltd. Pectinase ultra was obtained from HiMedia Laboratories Ltd. Methanol and potassium di-hydrogen phosphate were procured from Qualigens Fine Chemicals, Mumbai. Sodium hydroxide pellets, n-octanol, hydrochloric acid and calcium chloride were procured from Central Drug House (P) Ltd.

General method of preparation of enteric coated microspheres. Factorial design and desirability function. A four-factor, two-level full factorial design was constructed and conducted in a fully randomized order to study all possible combinations of all the factors at all levels (**Table 1**). The dependent variables included particle size, yield of microspheres, entrapment efficiency and percent drug release. The concentration of drug and polymer (A), the concentration of surfactant (B), stirring speed (C) and concentration of calcium chloride (D) were set at two different levels i.e., high and low (**Table 2**), which was coded as +1 and -1,

Table 1. Independent variables and levels[16]

Independent Variable	Level	
	Low (-1)	High (+1)
A (drug: polymer)	1:3	1:6
B (surfactant %w/v)	0.75	1.50
C (stirring Speed, rpm)	1000	2000
D (calcium conc. %w/v)	10	20

Table 2. Design of experiment

Formulation Code	Drug: Polymer	Surfactant	Stirring Speed	Calcium ion Concentration
F1	-1.00	1.00	1.00	1.00
F2	-1.00	-1.00	1.00	1.00
F3	1.00	1.00	1.00	1.00
F4	-1.00	-1.00	-1.00	-1.00
F5	-1.00	-1.00	1.00	-1.00
F6	-1.00	-1.00	-1.00	1.00
F7	1.00	1.00	-1.00	1.00
F8	-1.00	1.00	-1.00	1.00
F9	-1.00	1.00	1.00	-1.00
F10	1.00	-1.00	1.00	-1.00
F11	1.00	1.00	-1.00	-1.00
F12	1.00	-1.00	-1.00	-1.00
F13	1.00	-1.00	-1.00	1.00
F14	1.00	1.00	1.00	-1.00
F15	-1.00	1.00	-1.00	-1.00
F16	1.00	-1.00	1.00	1.00

respectively. The range of a factor was chosen so as to adequately measure its effects on the response variables. This design was selected, as it provides sufficient degrees of freedom to resolve the main effects as well as the factor interactions. Step-wise regression analysis was used to find out the control factors that significantly affect response variables.[11] By using full factorial design, 16 formulations were prepared.

Preparation of enteric coated microspheres. Mesalamine microspheres were prepared by emulsion dehydration technique. Both pectins (AU701, 2% w/v and CF020, 8% w/v) were homogeneously dispersed in 20 ml of distilled water using a high speed homogenizer (Remi RQ 1217-D). 200 mg of mesalamine was added to this under stirring.[12] The drug polymer dispersion was added in 50 ml isooctane containing span 80 and stirred at variable speed continuously to obtain stable w/o emulsion. The emulsion was rapidly cooled to 15°C, and then 50 ml of acetone was added in order to dehydrate the pectin droplets. The system was maintained under mechanical agitation for 30 min at 1,000 rpm at 25°C to effect complete evaporation of the solvent. Microspheres were filtered and dispersed in calcium chloride solution for cross-linking for 20 min, separated and washed with distilled water and consequently suspended in hardening agent, glutaraldehyde solution. The microspheres were filtered, rewashed and dried.

Table 3. Characterization of microspheres

Formulation code	Particles Size (μm)	% Entrapment efficiency	% Water content	% Yield	Overall desirabiliy	% Drug release (8 hr)
F1	390.3 ± 2.31	74.2 ± 3.41	62.5 ± 3.12	45.7 ± 4.23	0.000	72.67 ± 1.23
F2	285 ± 2.82	72.5 ± 2.75	58.6 ± 0.23	56.4 ± 4.92	0.605	59.16 ± 0.66
F3	435.2 ± 1.23	86.1 ± 2.92	71.2 ± 0.64	78.6 ± 3.28	0.000	61.25 ± 1.33
F4	130.2 ± 1.62	45.6 ± 3.45	52.4 ± 1.34	43.4 ± 1.49	0.000	65.63 ± 1.57
F5	112.3 ± 0.89	56.4 ± 2.81	49.7 ± 2.66	38.9 ± 4.22	0.000	69.46 ± 0.89
F6	160.3 ± 1.67	53.6 ± 1.34	62.7 ± 3.77	50.4 ± 3.22	0.000	74.01 ± 0.34
F7	223.8 ± 1.82	68.8 ± 2.23	68.5 ± 2.11	60.4 ± 1.53	0.600	68.34 ± 1.04
F8	356.2 ± 0.98	61.2 ± 1.42	54.5 ± 1.75	53.2 ± 2.13	0.000	66.34 ± 1.2
F9	284.2 ± 2.56	66.4 ± 0.64	56.9 ± 3.39	48.9 ± 2.67	0.768	73.70 ± 1.22
F10	250.5 ± 2.34	58.6 ± 2.56	53.2 ± 2.34	51.4 ± 4.67	0.410	68.42 ± 0.453
F11	345.5 ± 1.15	66.8 ± 3.90	58.8 ± 2.21	43.1 ± 4.24	0.000	73.04 ± 0.921
F12	185.5 ± 2.13	54.5 ± 2.31	60.2 ± 4.36	58.5 ± 3.81	0.000	64.14 ± 1.42
F13	230.5 ± 1.92	58.3 ± 2.11	52.1 ± 1.43	48.6 ± 3.67	0.265	61.28 ± 1.75
F14	226 ± 0.86	71.2 ± 0.34	64.9 ± 2.22	70.1 ± 1.78	0.825	78.92 ± 0.97
F15	280.5 ± 2.45	52.4 ± 2.33	48.3 ± 3.13	32.4 ± 3.46	0.000	57.38 ± 0.67
F16	340 ± 3.21	69.4 ± 1.55	61.8 ± 2.26	55.9 ± 2.61	0.000	62.81 ± 1.32

Characterization of microspheres. *Scanning electron microscopy.* The morphology, surface appearance and inner structure of the microspheres were examined using scanning electron microscopy (LEO 430 SEM analyzer). The samples were fixed on a brass stub using double-sided tape and coated with gold-palladium alloy under vacuum. The photographs were then taken using an excitation voltage of 20 kV.

Particle size analysis. The microspheres were examined under photomicroscope RXLr-3T (Radical Instrument, Ambala). Dried powder of microspheres was homogenously mixed in glycerol and was examined to measure the size of microspheres. Around 250 microspheres for each formulation were measured.

Determination of microspheres water content. The water content of the microspheres was determined by weighing the microspheres before[13] and after drying at 50°C for 24 h. The mean water loss (WL) was calculated according to the following equation:

$$WL\% = \frac{W_O - W_D}{W_O} \times 100$$

where W_o is the initial weight of the microspheres before drying, and W_D is the weight after drying.

Production yield of microspheres. The weight of the microspheres after drying was divided by the total dried weight of polymers and drug used to obtain the production yield.[14]

Entrapment efficiency. The weighed amount of drug-loaded microspheres containing mesalamine (100 mg) was kept in phosphate buffer pH 6.8 (100 ml) for 12 h with continuous stirring. The samples were filtered using a membrane filter (0.45 micron) and analyzed at 230 nm using UV spectrophotometer model UV 1700E. The entrapment efficiency was calculated using the following formula:[7] Entrapment efficiency (%) = $M_a / M_t \times 100$, where M_a is the actual drug content in microspheres and M_t is the amount of drug added to the microspheres.

Coating of microspheres. The enteric coating solution (12% w/v) was prepared by dissolving Eudragit S100 in acetone.[13] Coating was affected by immersion of microspheres in the coating solution followed by drying in a rotary evaporator. The process was repeated until the desired amount of coating was achieved. Microspheres were coated at different levels (weight gain ranging from 5 to 100% w/w). Coated microspheres were dried, weighed, and the mean coating weight was calculated. The mean coating thickness was determined by the difference between the mean diameter of microspheres before and after the coating.

Table 4. Kinetic modeling of drug release of F14 formulation

Curve fitting with model/equation	Coefficients of determinations	
	r2	K
Zero order	0.9683	97.351
	73.420	(Passes)
1st order	0.9522	-1.6929
	10.596	(Passes)
Matrix	0.9983	101.3972
	75.470	(Passes)
Peppas	0.9741	0.6783
	11.166	(Passes)
Hixson-Crowell	0.9808	-0.4716
	15.076	(Passes)

In vitro drug release. Coated pectin microspheres were evaluated for the in vitro drug release in the media solution of different pH values using USP XXIII type II dissolution apparatus (Electrolab TDT-08L).[14,15] Microspheres containing 200 mg drug with a magnetic bead were kept in a dialysis bag (Sigma Aldrich; molecular weight cut off, 12,000 Da). The bag was tied with a nylon thread to avoid the escape of microspheres. The dissolution test was performed at 100 rpm in 0.1 N hydrochloric acid for first hour, phthalate buffer pH 4.5 for second and third hour, phosphate buffer pH 6.8 for fourth and fifth hour, phosphate buffer pH 7.4 for sixth hour and, afterwards, in a mixture of phosphate buffer pH 6.8 and pectinex Ultra-SPL (1% v/v) in order to simulate the enzymatic action of the colonic bacteria.

At specified intervals, 5 ml aliquots were withdrawn, filtered, diluted appropriately with the same medium and assayed at 230 nm using a UV double-beam spectrophotometer (Shimadzu UV-1700). Samples withdrawn were replaced with equal volume of the dissolution medium. All the experiments mentioned above were conducted in triplicate.

Conclusion

The purpose of this work was to a design mesalamine-loaded, colon-specific delivery system. To achieve site-specific drug delivery to the colon, eudragit S100-coated low methoxylated amidated pectin (CF020) and low methoxylated pectin (AU701) microspheres were prepared. The combination of both pectins provided microspheres with greater stability due to the formation of stiffer gel as compared with low methoxylated pectin alone. The microspheres protected the drug from being released in the stomach and small intestine and provided more uniform distribution of the drug in the colon. It was concluded that eudragit S100 microspheres prepared using the combination of low methoxylated amidated pectin (CF020) and low methoxylated pectin (AU701) can successfully be deliver the drug to the proximal colon.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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