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DESIGN AND *IN VITRO* EVALUATION OF A BIPOLYMERIC DELIVERY DEVICE FOR THE AMELIORATION OF COLONIC DISEASES USING A POPULAR GLUCOCORTICOID AS A MODEL DRUG

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Abstract: The present investigation concerns with the development and optimization of a bipolymeric delivery device for the treatment of colonic diseases. Prednisolone – a popular glucorticoid was used a model drug. The formulations were designed with an objective to deliver the drug to the large bowel with a minimal release in the upper part of the gastrointestinal tract. Amount of Eudragit RS100 (X_1) and amount of Guar gum (X_2) were the two variables used to characterize and optimize the formulation. Three dependant variables – cumulative drug release at 60 min (Y_{60}), at 240 min (Y_{240}) and at 480 min (Y_{480}) were considered as optimization factors. The results were analyzed statistically and a $p < 0.01$ was considered to be statistically significant. Three dimensional response surface plots were drawn and the impacts of the independent variables on the chosen variables were analyzed and optimized.

Keywords: factorial design, Eudragit RS100, Guar gum, prednisolone

Delivery of active pharmaceutical ingredient to the specific site of action has many advantages such as increasing the bioavailability of the drug and also reducing the amount of drug to be administered, thereby reducing the multifarious side effects. A delivery device able to deliver the drug to the large bowel can treat local pathologies of the colon such as: inflammatory bowel disease (IBD), colorectal cancer and spastic colon that are common among the urban population (1–3). Aminosalicylates and glucocorticoids are used for the treatment of these diseases in their active phase (4–8). Glucocorticoid prednisolone (PD) remains the drug of choice in IBD (9–12). Though the biological t_{50} for PD is 2.5 h (13, 14), the drug pharmacokinetics reportedly follows a nonlinear pattern (15, 16) and the biological absorption and pharmacokinetics are influenced by multiple factors including food intake through out the GIT (17). PD is also rapidly absorbed in stomach leading to gastric ulceration (18). In systemic circulation PD remains predominantly protein bound leading to a series of side effects (19, 20). To alleviate these problems both natural and synthetic polymers have been used as they are expected to release the drug to the target site that could avoid high peaks of plasma PD. Guar gum (GG) remains a potential excepient of choice for colonic delivery in the form of matrix and compression-coated tablets as well as microspheres (21–27).

GG is a polysaccharide obtained from the seeds of *Cyamopsis tetragonolobus* consisting of linear chains of (1-4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by 1-6 linkages. It undergoes enzymatic degradation by the enzymes produced by colonic microflora (21). Eudragit RS 100 (EU) is an amino methacrylate copolymer containing about 6% of quaternary ammonium groups (28, 29). Various grades of EU having applications as a coating material (30–32) for colon specific drug release are available on the market.

We have combined the properties of biodegradable polysaccharides with those of polyionic water insoluble pH-independent polymers with low porosity and inert to endogenous digestive secretions and enzymes (33) to device colon targeted matrix for delivery of PD. Here GG has been used as an enzyme dependent polymer and EU as the time dependent polymer that can provide a localized release of PD. A factorial design approach has been utilized to achieve a stable bipolymeric combination for PD delivery.

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EXPERIMENTAL

Materials

PD (Batch no. PD050601) was received as a gift sample from Dey's Medical Stores (Mfg.) Ltd. (Kolkata, India), GG (mannose to galactose ratio 1:2) was purchased from Merck India Ltd. (Mumbai, India), and EU from Evonik India Ltd. (Mumbai, India). Methanol, tetrahydrofuran, water each of HPLC grade were purchased from Spectrochem India Ltd. All other reagents used were of analytical reagent grade and purchased locally.

Formulation of prednisolone tablets

Nine different batches were prepared by varying the amount of GG and EU for each formulation as presented in Table 1. Largely, 166 mg of PD and different quantities of EU were dissolved together in 5 mL of 95% ethanol. Measured quantities of GG were mixed thoroughly with this solution. The mixture was dried for 30 min in a vacuum drier kept at 60 ± 1°C at 1 mmHg. Two mL of 5% aqueous GG solution was then added to it as binder and the mass was kept for 4 h at room temperature (22°C). The dough was then passed through a No. 20 sieve and dried for 15 min at

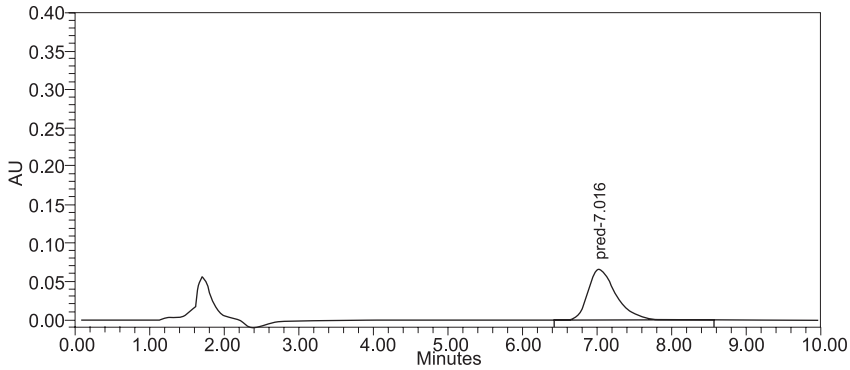

Figure 1. Representative chromatogram for prednisolone (pred-7.016)

Table 1. Formulation of PD tablets and evaluation of factorial design batches [a].

	Variable levels in coded forms[b]					Percent recovery[d]
Batch code	X_1	X_2	Y_{60}[c]	Y_{240}[c]	Y_{480}[c]	%
F1	–1	–1	28.56 ± 1.39	65.48 ± 1.28	90.29 ± 1.25	96.14
F2	0	–1	9.96 ± 1.99	32.53 ± 1.23	64.11 ± 2.59	98.12
F3	1	–1	5.59 ± 2.60	20.45 ± 1.39	57.38 ± 1.89	97.92
F4	–1	0	18.23 ± 1.02	44.99 ± 1.01	82.98 ± 1.45	96.74
F5	0	0	2.89 ± 1.02	20.39 ± 1.36	56.29 ± 1.25	98.01
F6	1	0	1.59 ± 1.01	17.36 ± 1.59	49.52 ± 1.34	98.32
F7	–1	1	12.88 ± 1.98	32.56 ± 1.99	75.21 ± 1.61	97.32
F8	0	1	1.29 ± 0.93	10.23 ± 1.36	48.99 ± 1.25	96.99
F9	1	1	0.89 ± 0.56	9.99 ± 1.02	42.99 ± 0.56	98.49

Coded values	Actual values [b]	
	X_1	X_2
–1	0	570
0	160	720
1	320	870

[a] All batches contain 166 mg of prednisolone. [b] X_1 indicates the amount of Eudragit RS100 and X_2 the amount of Guar gum in mg. [c] Y_j denotes cumulative drug release at j^{th} min with standard deviation values of three replicates. [d] Evaluated on the basis of mass of prednisolone used at the time of preparation and the mass of prednisolone found entrapped in each formulation.

105 ± 5°C. The granules thus obtained were compressed into tablets with fillers in a Minipress II MT tablet punching machine (Kalweka, Rimek, India).

Drug content estimation

Finely powdered formulation was accurately weighed and dissolved in 1:1, v/v mixture of 95% ethanol and water to obtain a final concentration of 400 µg/mL. This solution was allowed to stand for 8 h with intermittent sonication to ensure complete solubility. The content was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was used for the estimation of total drug content. A binary gradient HPLC system (Waters Dual Pump Binary Gradient, Waters Corporation, USA) and a reverse phase C18 column were used. The mobile phase was a mixture of water, tetrahydrofuran and methanol in the ratio of 68.8:25:6.2, v/v/v, respectively, with a flow rate of 0.5 mL/min. The eluent from the column was analyzed using PDA detector (2996 Waters Corporation, USA) and the chromatogram at λ = 254 nm was analyzed. The method was validated for linearity, precision and accuracy. A representative chromatogram is presented in Figure 1.

FT-IR

FT-IR spectra for PD, GG, EU, polymer combination and the formulations were recorded in Spectrum One FT-IR spectrometer (Perkin Elmer, USA) in pressed KBr pellets. The spectra were stacked in Biorad KnowIt ALL software (Biorad, USA) to determine any drug polymer interactions.

X-Ray powder diffraction studies

Powder diffraction studies were carried out in XPer'TPRO console (PANalytical, Netherlands) at 45 kV to study the crystallinity of PD with the polymer matrix. The measurement temperature was kept at 25°C throughout the experiment and the scanning was continued from $2\Theta = 0$ to 40°.

Atomic Force microscopy studies

The Atomic Force microscopic studies were carried out to observe the distribution of PD within the matrix. Base biopolymer GG and formulation batches F1 and F9 were dispersed by shaking in 2 mL of Millipore water. Each sample drop was taken over a mica plate, air dried and imaged under an Atomic Force microscope (Veeco probe NSIIIA, USA) in tapping mode (Software: Nanoscope Ver.5.3).

Preparation of 4% rat cecal content medium

Male Sprague-Dawley rats weighing 125–150 g were used in the test. The protocol was approved earlier by the Institutional Animal Ethics Committee (IAEC) and CPCSEA with registration number 506/01/a/CPCSEA. The rats were asphyxiated by excessive inhalation of carbon dioxide (CO_2) gas (34). The abdomens were opened and the cecum were traced, legated at both ends, dissected and immediate-

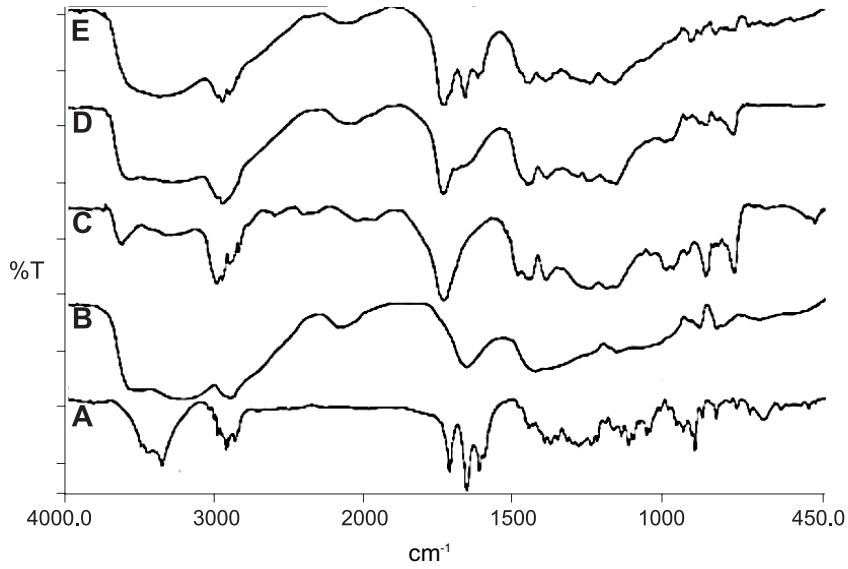

Figure 2. FT-IT spectrum of prednisolone (A), Guar gum (B), Eudragit (C), polymer combination (D), and formulation F9 (E)

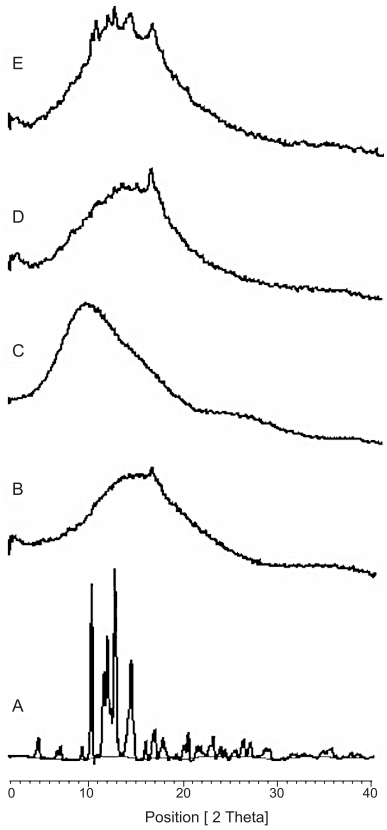

Figure 3. XRD of prednisolone (A), Guar gum (B), Eudragit (C), polymer combination (D), formulation F9 (E)

ly transferred to simulated colonic fluid (SCF) (35) previously bubbled with CO_2. The cecum was opened, weighed separately and pooled and a 4% solution was prepared. The solution was then centrifuged at 5000 rpm for 15 min. The supernatant was used as rat cecal content medium in the dissolution studies.

In vitro PD release testing

The gastrointestinal transit time varies from individual to individual depending on various factors. The normal gastric emptying takes place within 120 min and the colonic arrival occurs after 362 min (36, 37). The drug release study was designed accordingly. The release of PD (n = 3) from the matrix tablets was performed in a USP type I dissolution tester (Electrolab TDL-08L, India) at a rotational speed of 50 rpm and at temperature of 37 ± 0.5°C.

This study was carried out in 400 mL simulated gastric fluid (SGF) for 2 h, followed by simulated intestinal fluid (SIF) for the next 4 h and finally, in simulated colonic fluid (SCF) containing rat cecal contents medium, for next 18 h. A continuous supply of CO_2 was maintained for dissolution study in rat cecal content medium in order to maintain the anaerobic environment of the large bowel. Aliquots of 1 mL were periodically withdrawn and filtered through 0.45 µm polypropylene membrane filter and equal volumes of fresh dissolution medium were returned to the dissolution bath to maintain the sink condition. The aliquots were then analyzed using the previously mentioned HPLC system. No adsorbed PD was recorded onto the filter paper used. A time *versus* percent cumulative drug release curve was drawn for each formulation.

Swelling studies

The degree of swelling was investigated under simulated conditions of the colon. Each tablet of weight W_i was taken in a pre-weighed perforated basket (W_a). The whole basket was dipped in a beaker containing 250 mL SCF maintained at 37 ± 0.5°C. The basket was then taken out from the beaker at regular time intervals, kept over a tissue paper to remove unabsorbed water and was reweighed (W_b). The swelling ratio was obtained using the following equation (34):

$$\text{Swelling ratio} = \left(\frac{W_b - W_a}{W_i}\right) \quad (1)$$

where i = formulations 1 to 9.

Optimization

A 3^2 (three level two factors) full-fledged factorial design (38) was applied to optimize the two independent variables, namely the amount of EU (X_1) and the amount of GG (X_2). The factorial design layout for nine different batches is presented in Table 1. The drug release was monitored first at 30 min and then at every hour. The release at 60 min, 240 min and 480 min were analyzed as response parameters in the factorial design studies. Results were expressed as the second order polynomial equation:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (2)$$

where b_0 denotes the arithmetic mean response for nine runs, b_i (i = 1, 2) denotes the estimated coefficient for the factors X_i (i = 1, 2). X_i (i = 1, 2) denotes the effect of changing one factor at a time from its lowest to highest level. The interaction terms X_1X_2 denote the effect when both the factors were changed simultaneously. The polynomial terms X_i^2 (i = 1, 2) were used to explain non-linearity (39–42). Y was the measured response parameter in each experiment. The coefficient corresponding to the linear effects, b_1 and b_2, interaction b_{12} and the quadratic effects, b_{11} and b_{22} were determined from the experimental results.

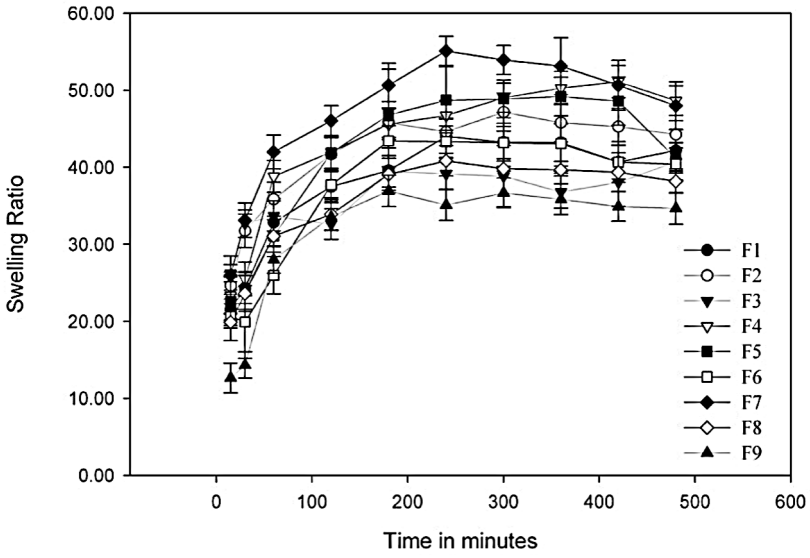

Figure 4. Swelling studies for formulation F1 to F9

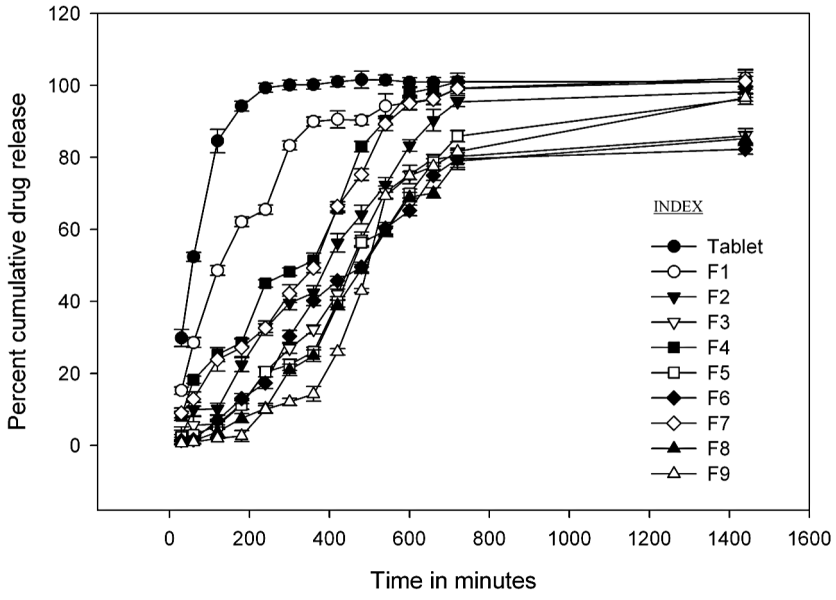

Figure 5. Cumulative drug release profile for formulation F1 to F9 and prednisolone tablet obtained from the market, first for 120 min in SGF, then for 240 min in SIF and finally for 1080 min in SCF containing rat cecum contents

Statistical analysis

Statistical analysis of the drug release studies were carried out using the Sigma plot Software (Version 8.02 SPSS Inc., USA).

RESULTS AND DISCUSSION

Drug content estimation

The percentage recovery for PD in each formulation was evaluated from the mass of PD originally taken and the mass of PD observed entrapped in each formulation and was observed in the range of 96.14 to 98.32% of the total drug load in each formulation. The results are tabulated in Table 1. Therefore, the steps involved in the formulation of the batches as well as the variation of the independent parameters for formulating the factorial design batches incurred an acceptable amount of drug loss.

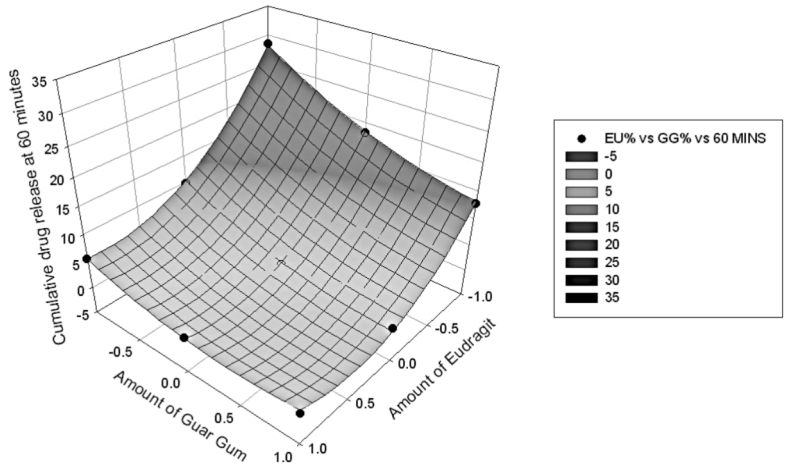

Figure 6. Response surface plot at 60 min of drug release study

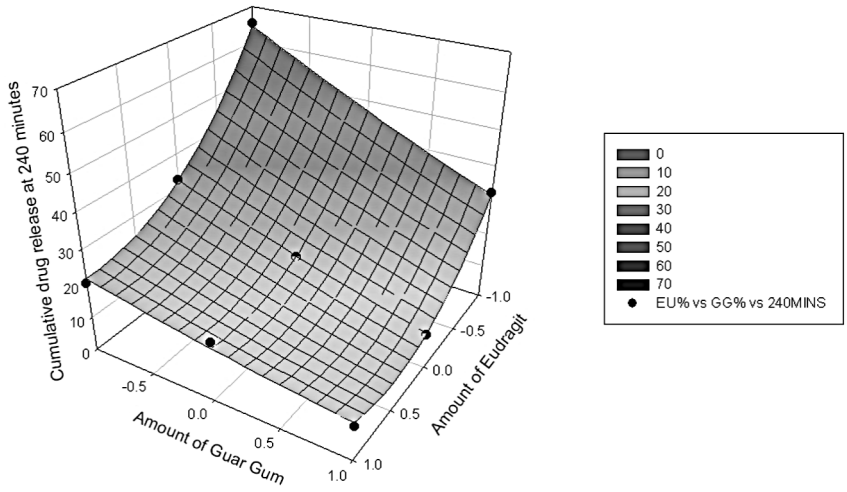

Figure 7. Response surface plots at 240 min of drug release study

FT-IR spectroscopic studies

FT-IR spectroscopy was used for observation of any drug polymer interactions (43). Pure PD was largely crystalline. PD provided sharp absorption peaks at 1707 cm^{-1} due to the presence of six member ring ketone and at 1655 cm^{-1} due to α,β-unsaturated ketone. Absorbance at 1614 cm^{-1} denotes the presence of aliphatic carbonyl group.

GG alone showed a broad band spreading at 3200–3400 cm^{-1} which was due to its galactomannan structure, characteristic of hydroxyl groups. A band at 1151 cm^{-1} was possibly due to the presence of cyclic ether linkages of the sugar backbone.

EU showed a sharp band at 1729 cm^{-1} and 1246 cm^{-1} which was due to the presence of ester linkage, absorbance at 1481 cm^{-1} and 1447 cm^{-1} was due to alkyl stretching vibration and absorbance at 1383 cm^{-1} was due to the presence of tertiary amine group. The representative formulation of PD doesn't reveal any shift in the sharp peaks of PD at 1707, 1655 and 1614 cm^{-1}, respectively. The spectra are presented in Figure 2. It therefore appeared that none of the PD

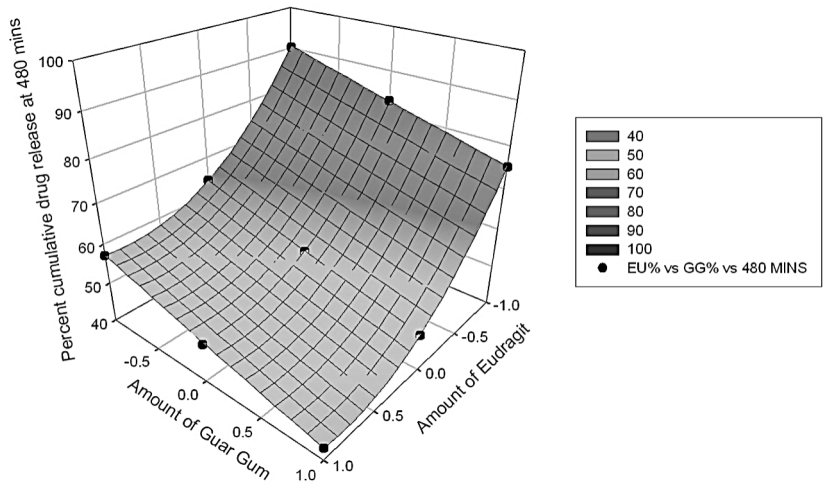

Figure 8. Response surface plot at 480 min of drug release study

major functional groups have interacted with any of the two biopolymers indicating physical entrapment in formulation F9 without any drug polymer interactions. Absence of drug polymer interaction implies that the drug retains its structural integrity within the selected bipolymer matrix. Its biological activity and potency is therefore expected to remain unaltered.

XRD studies

Pure PD is crystalline in nature. The XRD was carried out to study the crystallinity of PD within the matrix. The X-ray diffraction patterns are presented in Figure 3. In XRD studies EU appeared as an amorphous material, when added with GG a spike at 2Θ 20.06 degree was observed. Crystalline PD can be matched at 2Θ 16.15 degree when entrapped in formulation. The crystallinity was determined by comparing representative peak heights in the diffraction pattern of the sample with that of the reference standard. The relationship used for the calculation of relative degree of crystallinity (RDC) (44) is:

$$RDC = \frac{I_{sample}}{I_{reference}} \quad (3)$$

where I_{sample} was the peak height of the sample under consideration and $I_{reference}$ was the peak height at the same angle of the reference with the highest intensity. The pure drug peak at 2Θ 16.13^{o} was used for calculating RDC of the solid dispersion. The RDC value was 0.263 for formulation F9. Therefore, PD was entrapped in EU-GG matrix in the form of crystalline deposits.

AFM studies

Atomic Force microscopy (AFM) studies carried out in tapping mode revealed a 3D hydrated gel surface structure of the base matrix GG. In formulation F1 where no EU was used, solid drug material remained unevenly distributed over the GG matrix. Matrices containing increasing amount of EU resulted in much improved distribution of the drug over the base gum matrix. Since the drug particles were distributed in the crystalline form within the base polymer matrix, it may be a major determinant for controlled dissolution of PD from the prepared formulations.

Swelling studies

The swelling studies revealed a considerably slower swelling rate for formulations containing EU as one of the matrix materials. For formulation F9 containing the maximum amount of both the polymers it was shown a considerably slow swelling rate, which by two hours time attained equilibrium and continued in constancy throughout the period of study (Figure 4). The formulations F1, F4, and F7 showed a faster swelling rate and a higher swelling ratio, which at four hours showed a visible loss of polymer and a rapid fall in swelling parameter. Thus the presence of EU in the matrix may have prevented the early swelling and subsequent drug loss.

***In vitro* PD release**

Release of PD from the tablets was investigated under the simulated condition of the gastro-intestinal tract. The cumulative drug release profile is presented

Table 2. Analysis of Variance.

Responces	Regression	DF	SS	MS	R^2	p
Y_{60}	FM	5	711.5591	142.3118	0.9983	0.0002
	Residual					
	FM	3	1.2019	0.4006		
Y_{240}	FM	5	2589.7320	517.9464	0.9951	0.0012
	Residual					
	FM	3	12.8146	4.2715		
Y_{480}	FM	5	2148.8784	429.7757	0.9998	< 0.0001
	Residual					
	FM	3	0.3172	0.1057		

Note : FM denotes full model

Table 3. Quantitative factor effects and the associated p values for all three responses.

	Y_{60}		Y_{240}		Y_{480}	
Factor	Factor effects	p value	Factor effects	p value	Factor effects	p value
X_1	–8.6000	< 0.0001	–15.8717	0.0003	–16.4317	< 0.0001
X_2	–4.8000	0.0003	–10.9467	0.0010	–7.4317	< 0.0001
X_1X_2	+2.7450	0.0032	+5.6150	0.0122	+0.1725	0.3666
X_1X_1	+6.5767	0.0007	+10.7549	0.0052	+9.9316	< 0.0001
X_2X_2	+2.2917	0.0144	+0.9599	0.5582	+0.2316	0.3879

in Figure 5. For formulation F1, F4, F7 containing increasing amount of GG only the amount of drug release after first two hours was 48.59, 25.33 and 23.87%, respectively, which after 6 h was 65.48, 44.99 and 32.56%, respectively, which in itself corresponds to ileum clearance time meaning that drug loss was mainly in the small bowel. This early drug release may be attributed to the early swelling of the matrix and the consequent polymer loss. The large cobweb-like structure of GG probably failed to prevent the initial burst release of the drug load. However, GG may possibly retain cationic polymer EU for six other formulations which, in turn, can prevent release of the test drug PD through gastric lumen. At 12 h it was 99.23, 100.96 and 99.09%, respectively and at 24 h was 102.00 %, 100.99 % and 101.22 % respectively. For formulations F2, F5 and F8 containing moderate amount of EU along with increasing amount of GG released 10.12, 5.52 and 3.69% of the drug load in the first 2 h followed by 32.53, 20.39 and 10.23% at the 6th h, 95.36, 85.88 and 78.96% at the 12th h and 98.23, 96.33 and 85.23% at the 24th h of the drug release study, respectively. Thus when EU was used along with GG as in formulations F2, F5 and F8, the drug release was retarded considerably in the first 6 h of study. For formulations F3, F6 and F9 containing the highest amount of EU and increasing amount of GG the drug release was minimal at the first 2 h of drug release study, which was 6.01, 6.99 and 1.98%, respectively. The drug release was also low at the 6th h with values of 20.45, 17.36 and 9.99%, respectively, but increased substantially at the 12th h to 80.22, 79.56 and 81.56%, respectively. After 24 h the drug release was optimal for formulation F9 among the three, which was 96.58% whereas for formulations F3 and F6 it was 85.97 and 82.33%, respectively. The optimized formulation F9 is able to prevent the release of the greater part of the drug load in the upper part of the gastrointestinal tract, which was only 9.99% after 6 h and increased sharply to 81.56% at the 12th h. By this time, the formulation was expected to cross the distal colon, reflecting its capability to carry the drug to the diseased site of the colon and prevent the unwanted side effects. The dissolution profile data also present the release profile of a commercially available tablet (Wysolone, 5 mg, Batch No. O7620, Mfg. Dt. 10/2009, Exp. Dt. 09/2012.).The marketed formulation was not a sustained release formulation and therefore failed to retain the drug load and released almost the whole of it in the gastric duodenal zone, whereas our formulation is able to show a sustained profile.

Table 4. Observed and predicted responses and residual values of optimized formulation (F9).

	Formulation F9		
Response	Observed	Predicted	Residual
Y_{60}	0.8900	1.3572	–0.4672
Y_{240}	9.9900	10.9216	–0.9316
Y_{480}	42.9900	42.7814	0.2086

From cumulative drug release profile of all batches (Fig. 5) it is apparent that both GG and EU concentrations are important for retarded *in vitro* release of PD.

Analysis of the above results envisages a possible mechanism for combining the properties of a biodegradable polymer and a synthetic sustained release polymer in designing a drug delivery device for the colon.

Factorial design

The response obtained from *in vitro* release studies was evaluated using a statistical model which involved a number of polynomial terms as explained previously. It was observed that for all nine formulations, after 1 h, the percentage cumulative drug release varied from 0.89 to 28.56% and at the 8th h of study it varied from 42.99 to 90.29%. The values fitted for quadratic model (equation 2) for responses Y_{60}, Y_{240} and Y_{480}.

The high values of correlation coefficients denote a good fit (Table 2). The significance of correlation coefficients was studied using Student's *t*-test. A co-efficient was considered significant if the calculated p value is less than 0.01, 99% confidence limit. Detailed response surface plots were drawn on *in vitro* drug release experiment data at 60, 240 and 480 min in order to understand contribution of each independent variable over each other.

The resulted equation for all three dependent variables Y_{60}, Y_{240} and Y_{480} – in terms of their coded factors were as follows:

$$Y_{60} = +3.18 - 8.60X_1 - 4.80X_2 + 2.74X_1X_2 + 6.58X_1^2 + 2.29X_2^2 \quad (4)$$

$$Y_{240} = +20.41 - 15.87X_1 - 10.95X_2 + 5.61X_1X_2 + 10.75X_1^2 + 0.96X_2^2 \quad (5)$$

$$Y_{480} = +56.31 - 16.43X_1 - 7.43X_2 + 0.17X_1X_2 + 9.93X_1^2 + 0.23X_2^2 \quad (6)$$

It was found that for response Y_{60} the linear contribution of both X_1 and X_2 and quadratic contribution of X_1 were significant with $p < 0.01$, respectively. The Y_{240} also showed significant linearity ($p < 0.01$). A similar highly significant linear contribution was observed for Y_{480} as well. The response surface regression analysis was performed using coded values of factor levels (–1, 0, +1) for each factor to determine the significance. Table 3 shows the respective p values and response surface plots are presented in Figures 6, 7 and 8 corresponding to release at 60, 240 and 480 min, respectively. A positive value indicates a synergistic effect that favors optimization, while a negative sign represents an antagonistic effect or an inverse effect of the factor on the selected response. It was observed that for all three dependent variables Y_{60}, Y_{240} and Y_{480}, the linear contribution of both coded factors has got antagonistic effect ($p < 0.01$). The three dimensional plots gives us an idea about the change in the response surface. The effect of the response surface can also be understood by the plots. Based on the model, quadratic equations were developed; the formulation was optimized on the basis of observed and predicted values of the responses. The optimized factors were determined as GG 64.44%, EU 23.70% and PD 11.85% and the formulation F9 is the optimized formulation since the observed values for F9 showed significant correlation with the statistically predicted values as presented in Table 4.

CONCLUSIONS

The designed matrix formulation provided a suitable bipolymeric combination for colonic delivery of PD. The XRD and AFM studies revealed a uniform PD distribution consistent with higher proportion of polyionic polymers. From IR studies it was revealed that the steroidal drug PD has not interacted with the biopolymer matrix. Among the nine designed formulations those containing higher amounts of both GG and EU showed controlled release profile suitable for colonic delivery. The 3^2 full factorial design has been successfully utilized to study and optimize the two independent concentration parameters, that is, the percentage of GG and EU to obtain the optimized formulation F9 for con-

trolled release to the colon with minimal drug loss in the upper part of gastrointestinal tract.

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