Formulation, Evaluation and Optimization of Pectin- Bora Rice Beads for Colon Targeted Drug Delivery System

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ABSTRACT

Purpose: The purpose of this research was to established new polysaccharide for the colon targeted drug delivery system, its formulation and in vitro and in vivo evaluation.

Methods: Microspheres containing pectin and bora rice were prepared by ionotropic gelation technique using zinc acetate as cross linking agent and model drug used was glipizide. A 32 full factorial design was employed to study the effect of independent variables, polymer to drug ratio (A), and concentration of cross linking agent (B) on dependent variables, particle size, swelling index, drug entrapment efficiency and percentage drug release.

Results: Results of trial batches indicated that polymer to drug ratio and concentration of cross linking agent affects characteristics of beads. Beads were discrete, spherical and free flowing. Beads exhibited small particle size and showed higher percentage of drug entrapment efficiency. The optimized batch P2 exhibited satisfactory drug entrapment efficiency 68% and drug release was also controlled for more than 24 hours. The polymer to drug ratio had a more significant effect on the dependent variables. In vivo gamma scintigraphy study of optimized pectin-bora rice beads demonstrated degradation of beads whenever they reached to the colon.

Conclusion: Bora rice is potential polysaccharide for colon targeted drug delivery system.

Introduction

Colonic drug delivery is intended for the local treatment of ulcerative colitis, irritable bowel syndrome and can potentially be used for colon cancer or the administration of drugs that are adversely affected by the upper gastro-intestinal (GI) tract.1 The colon is an ideal site for protein and peptide absorption.2 Acidic and enzymatic degradation are major obstacles in the oral administration of peptide drugs, but by targeting to the colon the proteolysis can be minimized. There has been considerable research in the design of colonic delivery systems and targeting has been achieved by several ways.3 The primary approaches to the colonic delivery of the drugs included prodrugs, coating with pH-sensitive and time-dependent polymers. Nevertheless, these parameters i.e. pH and time can vary from one individual to the next and also according to the pathological and dietary conditions. So these systems can lead to premature and non-specific drug delivery in the colon and they have limited success. Precise colonic drug delivery requires that the triggering mechanism in the delivery system only response to the physiological conditions particular to the colon. Polysaccharides are widely used in oral drug delivery systems because of the simplicity to obtained the desired drug delivery system and drug release profile, by the control of cross-linking, insolubility of crosslinked beads in gastric environment and and broad regulatory acceptance. The includes sodium alginate, pectin, chitosan, xantan gum, guar gum, starch, dextran and gellan.4

Pectin is a predominately linear polymer of mainly α- (1-4)- linked to D-galacturonic acid residues interrupted by 1, 2-linked L-rhamnase residues. Pectin is a polysaccharide found in the cell walls of plants.5 The rationale for the development of a polysaccharide based delivery system for colon is the ability of the colonic microflora to degrade various types of polysaccharides that escape small bowel digestion. Pectins are polysaccharides and consist of linear polymers of d-galacturonic acid residues with varying degrees of methyl ester substituents. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are important means to classify pectin. It is totally degraded by colonic bacteria but is not digested in the upper GI tract.6 One

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interesting approach is to use calcium salts of pectin because calcium binding reduces the solubility and induces non-covalent associations of carbohydrate chains through “egg – box” complex.\(^{13}\) Bora rice is a natural polysaccharide having higher concentration of amylopectin (>98%) than that of the starch due to which it is more resistant to the gastric fluid of the upper GIT and at the same time it is degraded in the colon. Though bora rice can be used as a very good substitute for other starch in the development of colon targeted drug delivery system, it becomes important to establish the bora rice polysaccharide as controlled release polymer. Glipizide is a second-generation sulfonylurea that can acutely lower the blood glucose from the pancreas and is typically prescribed to treat type II diabetes (non-insulin dependent diabetes mellitus). Its short biological half-life (2-4 hr) necessitates it to be administered 2.5 to 10 mg per day in 2 to 3 doses.\(^{7}\) It shows pH dependent solubility thus, the development of controlled release dosage form would be much more advantageous than the conventional tablets.\(^{10,11}\)

Materials and Methods

Materials

Glipizide was obtained as a gift sample from Standmed Pharmaceuticals, Kolkata. Amidated low methoxy pectin (PT) (DE=25% and DA=21%) was purchased from Loba chem., Mumbai. Bora rice (BR) was purchased from the local market of Dibrugarh, Calcium chloride dehydrated (CaCl\(_2\)), Barium chloride (BaCl\(_2\)) and Zinc acetate [Zn(CH\(_2\)COO)\(_2\)] was purchased from SD fine chemicals, Mumbai. All other chemicals and reagents used were of analytical grade.

Methods

Formulation of trial batches of Pectin-Bora Rice (PT-BR) hydrogel beads of glipizide

The standard ionotropic gelation technique\(^{12,13}\) was used for the preparation of the hydrogel beads with slight modification as described below:

Aqueous dispersion of Pectin-Bora Rice (1:2) was prepared and kept overnight. Appropriate amount of the model drug glipizide (2:1 polymer:drug) was dispersed in the PT-BR dispersion until an uniform dispersion was obtained. The bubble free dispersion was added drop wise, through a disposable syringe (nozzle of 1.0 mm inner diameter) to a 200 ml of a gently agitated solution of the crosslinking agents i.e. [CaCl\(_2\), BaCl\(_2\) and Zn (CH\(_2\)COO)\(_2\)] at room temperature separately as shown in Table 1. The distance of falling of the drops was 5 cm. The gelled particles thus formed were allowed to remain in the crosslinking solution up to different duration of time period. The particles were subsequently washed with purified water, in order to remove Cl\(^-\) and excess of Ca\(^{2+}\), Ba\(^{2+}\) and Zn\(^{2+}\) ions and separated by filtration. The particles were air dried for 24 hr and stored in a desiccator at room temperature. Similarly pectin beads (PB) were also prepared by using pectin alone without bora rice by the above gelation technique.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>PT-BR ratio (%w/w)</th>
<th>Polymer:Drug ratio (%w/w)</th>
<th>Cross linking agent</th>
<th>Conc. of cross-linking agent (%w/v)</th>
<th>Curing time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1:2</td>
<td>2:1</td>
<td>CaCl(_2)</td>
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</tr>
<tr>
<td>T2</td>
<td>1:2</td>
<td>2:1</td>
<td>CaCl(_2)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>T3</td>
<td>1:2</td>
<td>2:1</td>
<td>CaCl(_2)</td>
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<td>6</td>
</tr>
<tr>
<td>T4</td>
<td>1:2</td>
<td>2:1</td>
<td>BaCl(_2)</td>
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<td>2</td>
</tr>
<tr>
<td>T5</td>
<td>1:2</td>
<td>2:1</td>
<td>BaCl(_2)</td>
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<td>4</td>
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<tr>
<td>T6</td>
<td>1:2</td>
<td>2:1</td>
<td>BaCl(_2)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>T7</td>
<td>1:2</td>
<td>2:1</td>
<td>Zn (CH(_2)COO)(_2)</td>
<td>2</td>
<td>2</td>
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<tr>
<td>T8</td>
<td>1:2</td>
<td>2:1</td>
<td>Zn (CH(_2)COO)(_2)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>T9</td>
<td>1:2</td>
<td>2:1</td>
<td>Zn (CH(_2)COO)(_2)</td>
<td>6</td>
<td>6</td>
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<tr>
<td>PB</td>
<td>3:0</td>
<td>2:1</td>
<td>Zn (CH(_2)COO)(_2)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Formulation of factorial design batches of PT-BR hydrogel beads of glipizide

The response surface approach involving \(^3\)\(^2\) randomized full factorial design was adopted for optimization purpose. In this design, two factors each was evaluated at three levels and experimental trial were performed at all nine possible combinations, as presented in Table 2. Polymer:Drug ratio (A) and % of zinc acetate (B) were selected as independent variables. The percentage of drug release, entrapment efficiency, particle size and swelling index were selected as four dependent variables.

Evaluation of trial and factorial design batches of PT-BR beads

Entrapment efficiency (EE):\(^{14}\) Hydrogel beads equivalent to 5 mg of glipizide were crushed in a glass mortar-pestle and the powdered beads were suspended in 50 ml phosphate buffer (pH 7.4). After 24 hr the solution was sonicate for 1 hr, filtered and the filtrate was analyzed by a UV spectrophotometer (Shimadzu UV-1601 UV/VIS double beam spectrophotometer) method at the wavelength of 276 nm for the drug content. The drug entrapment efficiency was calculated as per the following formula:

\[
\% \text{ EE} = \left( \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \right) \times 100
\]
The drug entrapment efficiency of the beads of T7 to T9 of trial batches and formulations P1 to P9 of factorial batches have been determined.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Variable level (A)</th>
<th>Variable level (B)</th>
<th>Polymer to Drug ratio (% w/w)</th>
<th>Conc. of cross-linking agent (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>-1</td>
<td>-1</td>
<td>1:1</td>
<td>2</td>
</tr>
<tr>
<td>P2</td>
<td>-1</td>
<td>0</td>
<td>1:1</td>
<td>4</td>
</tr>
<tr>
<td>P3</td>
<td>-1</td>
<td>1</td>
<td>1:1</td>
<td>6</td>
</tr>
<tr>
<td>P4</td>
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<td>P5</td>
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<td>P7</td>
<td>1</td>
<td>-1</td>
<td>3:1</td>
<td>2</td>
</tr>
<tr>
<td>P8</td>
<td>1</td>
<td>0</td>
<td>3:1</td>
<td>4</td>
</tr>
<tr>
<td>P9</td>
<td>1</td>
<td>1</td>
<td>3:1</td>
<td>6</td>
</tr>
</tbody>
</table>

Micromeritic properties of drug loaded PT-BR beads:15

**Bulk density:** Apparent bulk density (D_b) was determined by pouring the beads into a graduated cylinder. The bulk volume (V_b) and weight of the beads (M) was determined. The bulk density was calculated using the formula

\[ D_b = \frac{V_b}{M} \]

**Tapped density:** The measuring cylinder containing a known mass of beads was tapped for 100 times. The minimum volume (V_t) occupied in the cylinder and weight (M) of the beads was measured. The tapped density (D_t) was calculated by following formula

\[ D_t = \frac{V_t}{M} \]

**Angle of repose:** Angle of repose was determined using funnel method. The beads were poured through a funnel that was raised vertically on the plane surface until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and the angle of repose (θ) was calculated using following formula

\[ \theta = \tan^{-1}\left(\frac{h}{r}\right) \]

**Swelling Index:** A known weight (100 mg) of various glipizide loaded PT-BR beads were placed in phosphate buffer, pH 7.4 and allowed to swell at 37 °C ± 0.5 °C in the USP type II dissolution rate test apparatus (Electrolab TDT-06T, India). The beads were periodically removed and blotted with filter paper; then gain in weight (after correcting for drug loss) of the beads were measured until attainment of constant weight. The swelling index (SI) was calculated using the following formula:

\[ SI = \frac{(w_f - w_0)}{w_0} \]

\[ SI = \text{Swelling index} \]

\[ w_f = \text{Final weight of beads} \]

\[ w_0 = \text{Initial weight of beads} \]

**Particle size analysis:** Particle size of beads was determined by optical microscopy method. Approximately 100 particles were counted by using calibrated ocular micrometer in an optical microscope (Nikon, DR-06M, Japan).

**Kinetics of drug release:** Different mathematical models i.e. zero order, first order and Higuchi equations were applied for describing the kinetics of the drug release process from controlled released colon targeted glipizide beads, the most suited being the one which fitted best the experimental results. The data obtained from in-vitro drug release studies were used to calculate the correlation coefficient (R) value between ‘cumulative amount of drug released and time’ for zero order, ‘log cumulative percentage of drug remaining and time’ for first order and ‘cumulative percentage of drug released and square root of time’ for Higuchi’s model. The best fit model was considered to that one which had maximum ‘R’ value (~ 1).

In vitro dissolution rate study of trial and factorial design batches beads:

**Preparation of 4.0% w/v rat cecal material:** Male albino rats were taken weighing 200 - 250 g and maintained at normal diet and administered orally 1 ml of 2.0% w/v aqueous dispersion of PT-BR (1:1) daily for 7 days for induction of reductive and hydrolytic enzymes like β-glucoronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase in the colonic bacteria of the animals. The experiment was conducted in the laboratory of Jay Research Foundation (JRF), Vapi, Gujarat on the permission of the IAEC of JRF with their CPCSEA registration number 35/1999 CPCSEA. Six rats were asphyxiated using carbon dioxide. Their abdomens were opened, the cecal were traced, legated at both ends, dissected, and immediately transferrred into previously weighed beaker containing 100 ml of phosphate buffer PBS, pH 7.4 previously bubbled with CO₂. The cecal content in the beaker, was weighed and required volume of PBS, pH 7.4 was added to provide desired concentration (4.0% w/v) of the rat cecal material in the buffer. Then the suspension was filtered through cotton wool and was used as simulated colonic fluid. Because the cecum is naturally anaerobic, all of these operations were carried out under anaerobic condition i.e. in presence of CO₂.

**In vitro drug release (DR) study of beads of trial and factorial design batches in 4.0% w/v rat cecal medium:** In the in vitro drug release study was carried out using USP XXIV paddle type apparatus (Electrolab, TDT-06T, India) at 37 ± 0.5 °C and at 75 rpm using 500 ml of phosphate buffer (pH 7.4) containing 4.0% w/v rat cecal fluid.
cecal material as a dissolution medium. Hydrogel beads equivalent to 5 mg of glipizide were used for the test. Samples (5ml) were withdrawn at predetermined time intervals replacing equal volume of fresh dissolution medium. The samples were transferred into a series of 10 ml volumetric flask, diluted suitably with PBS, pH 7.4 and centrifuged. The supernatant was filtered through 0.45 μm membrane filter and the filtrate was analyzed in a UV spectrophotometer at 276 nm wavelength using a blank prepared exactly the same way without the drug.

Solid state characterization of factorial design batches:
FTIR study: Mixed glipizide, BR, PT-BR beads powder and drug loaded PT-BR beads powder with KBr individually. KBr pellets were prepared by using KBr press. FTIR spectra of Glipizide, PT-BR beads and drug loaded PT-BR beads were obtained in KBr pellets using a JASCO model 5300, Italy FTIR spectrophotometer in the range of 4000 to 400 cm⁻¹.

XRD study: X-ray powder diffractograms of glipizide, bora rice, pectin-bora rice placebo beads and drug loaded PT-BR beads were recorded on powder X-ray diffractometer (Bruker AXS D8 Advance, Germany). The samples were irradiated with monochromated Cu Kα radiation (1.54060 Å) and analyzed between 10 and 40, 20 (Degree). The voltage and current used were 30 kV and 30 mA, respectively. The range and chart speed were 2 X 10⁸ cps and 10mm/20, respectively.

Thermal Studies: Thermograms of samples were obtained by a Perkin-Elmer Differential Scanning Colorimeter. Samples of 10mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10 °C/min over a temperature range of 50 to 250 °C under the flow of nitrogen gas at a rate of 20 ml/min.

Shape and Surface morphology: Surface and shape morphology of PT-BR beads were evaluated by means of Tabletop microscopy (TM) (HITACHI TM1000). The samples of TM were prepared by lightly sprinkling the beads on a double adhesive tape, which struck to an aluminum stub.

In vivo Gamma Scintigraphy study of the optimized factorial design batch formulation of glipizide: Three Wistar rats, weighing 200 – 250 g were taken for the study. The animals were fasted for 12 hr prior to commencement of the experiment. Radiolabeled (>90%) beads (50 mg) of formulation P9 was administered orally to the animals with the help of feeding tube, followed by sufficient volume of drinking water. All four legs of rat were tied over a piece of plywood and the location of the formulation in GI tract was monitored keeping the subject in front of gamma camera. The total radiation dosimetry for each rat was 0.1 mSv. Scintigraphy image was captured using a Siemens E-Cam gamma camera fitted with a LEHR collimator. The image schedule was as follows: 1 minute, 15 minutes and 450 minutes after dosing. During the gamma scintigraphy scanning, the animals were freed and allowed to move and carry out normal activity. The experiment was conducted in the laboratory of Bombay Veterinary College (BVC), Mumbai, (Maharashtra) permitted by the IAEC of BVC with the CPCSEA registration number BVC/IAEC/23/2010.

Stability study of the optimized factorial design batch formulation of glipizide: Accelerated stability study was conducted on the optimized formulation P9. The samples were stored at 40 °C ± 2 °C and 75% ± 5% RH for 3 months period. The sample was withdrawn periodically and subjected to dissolution rate determination.

For the comparison of release profiles of the samples of stability studies, “difference factor”, f₁ and “similarity factor”, f₂ were calculated. The difference factor (f₁) measures the percent error between the two curves over all time points and was calculated using following equation.

\[ f_1 = \sum_{j=1}^{n} \left[ \frac{R_j - T_j}{\sum_{j=1}^{n} R_j} \right]^2 \times 100 \]

Where, ‘n’ is the number of sampling points, Rj and Tj are the percent dissolved of the reference and test samples at each time point j respectively. The two release profiles are considered to be similar, if f₁ value is lower than 15 (between 0 and 15).

The similarity factor (f₂) is a logarithmic transformation of the sum of squared error of differences between the test Tj and the reference sample Rj over all time points. It was calculated as follows.

\[ f_2 = 50 \log \left[ 1 + \left( \frac{1}{n} \sum_{j=1}^{n} \frac{W_j}{R_j} |R_j - T_j| \right)^2 \times 100 \right] \]

Where, w_j is an optional weight factor and other terms are as defined earlier.

Statistical analysis and modeling: Analysis of variance (ANOVA) was used for the analysis of regression coefficient, predicted equations and case statistics. The experimental results of response surface methodology (RSM) were fitted via the response surface regression procedure, using the following second order polynomial equation:

\[ Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \]

Where Y is the predicted response, X_i and X_j are independent variables, β₀ is the intercept term, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient.

However, in this study, the independent variables are coded as A and B. Thus the second order polynomial equation is represented as follows

\[ Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 \]

The main effects (A and B) represent the average result of changing one factor at a time from its low to high value. The interaction terms A and B show how the response changes when 2 factors were simultaneously changed. The polynomial terms (β_{12} and β_{22}) are included to investigate non-linearity.
Polysaccharide based colon targeted drug delivery system

Model graphs were obtained by using the Design Expert software (Design Expert 8.0) to analyze the effect of variables individually and its interaction to determine their optimum level. The point prediction method was used for optimization of the levels of each variable for maximum response.

Results and Discussion

Formulation of PT-BR hydrogel beads of glipizide

The formulae of the trial batches and the factorial design batches of drug loaded PT-BR hydrogel beads have been shown in Table 1 and 2 respectively. Calcium chloride, barium chloride and zinc acetate were selected as cross-linking agent for PT-BR beads for trial batches formulation. Cross-linking time selected was 4 hr. The curing time was selected as more the time showed better cross linking and hence the release was retarded. The cross-linking agent calcium chloride did not give proper strength to the beads which might be due to the addition of bora rice. In case of barium chloride, PT-BR cross-linked with Ba++ ions where pallets were formed instead of beads. This effect might be due the presence of bora rice in pectin gel suspension, pectin loses its cross-linking property to some extend as calcium and barium ions forms the weak linkage with carboxyl groups in the pectin chain during “egg-box” formation. Zinc cations produced an extensive cross-linking and less permeable pectinate matrix than calcium and barium cations. Thus, it can be concluded that zinc forms stronger network due to its interaction with pectin with a higher binding affinity and selectivity than calcium and barium. Therefore zinc cross-linked PT-BR beads are more suitable than calcium and barium cross-linked beads for the use as a colonic delivery carrier. Also, zinc formed spherical beads with PT-BR. Hence for factorial design batches zinc acetate was selected as cross-linking agent.

Evaluation of trial batch PT-BR hydrogel beads of glipizide

Evaluation parameters of the trial batch PT-BR beads have been produced in the Table 3 and Figure 1. Formulation T7 showed 100 % drug release within 20 hr. T7 showed larger particle size, and lesser entrapment efficiency as compared to formulation T8 and T9. The complete release of the drug within 20 hr from the formulation T7 might be due to less concentration of cross-linking agent (2.0 %w/v) and lesser exposure time (2 hr) to the cross-linking solution. On other hand as cross-linking concentration and exposure time was increased in the formulation T8 to 4.0 %w/v and 4 hr respectively 100% drug released was recorded within 24 hr and drug entrapment efficiency was found to be 52.65 % which is an acceptable value. In case of formulation T9 where the concentration of cross-linking agent was 6.0 %w/v with the exposure time of 6 hr the drug release has been recorded as 100% within 24 hr similar to the formulation T8. Therefore, as the same results have been obtained out of the formulation T8 and T9 we have selected the formulation T8 for the factorial design batches with a lesser concentration of cross-linking agent (4.0 %w/v) and exposure time 4 hr than that of the formulation T9 which contained more amount of cross-linking agent.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>% Drug Release (24 hr) (± SD)</th>
<th>Particle size (mm) (± SD)</th>
<th>Drug Entrapment Efficiency (%) (± SD)</th>
<th>Swelling index (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>100.23 ± 1.25 (20 hr)</td>
<td>1.289 ± 0.23</td>
<td>41.46 ± 0.25</td>
<td>0.743 ± 0.074</td>
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<tr>
<td>T8</td>
<td>100.65 ± 1.57 (24 hr)</td>
<td>1.135 ± 0.12</td>
<td>52.65 ± 0.16</td>
<td>1.068 ± 0.018</td>
</tr>
<tr>
<td>T9</td>
<td>100.42 ± 1.43 (24 hr)</td>
<td>1.132 ± 0.11</td>
<td>51.21 ± 0.20</td>
<td>1.077 ± 0.012</td>
</tr>
<tr>
<td>PB</td>
<td>100.16 ± 1.16 (12 hr)</td>
<td>0.864 ± 0.43</td>
<td>51.67 ± 0.83</td>
<td>0.732 ± 0.047</td>
</tr>
</tbody>
</table>

Evaluation of factorial design batches of PT-BR beads of glipizide

On the basis of the preliminary trials a 3² full factorial design was adopted to study the effect of independent variables (i.e polymer to drug ratio [A] and cross-linking concentration [B] on dependent variables, percent drug release, entrapment efficiency, particle size and swelling index. The result is presented in Table 4 and Figure 2. From the result it is clear that the release profile of factorial design batch beads were in the order of P1>P2>P3>P4>P5>P6>P7>P8>P9. The concentration of cross-linking agent and polymer influence the control of drug release for 24 hr. The concentration of cross-linking agent is inversely proportional to drug release, particle size and swelling index but it is directly proportional to the drug entrapment efficiency which might be due the
formation of more dense network with pectin and bora rice. On the other hand the effect of polymer concentration is also inversely proportional to the drug release and directly proportional to the particle size, drug entrapment efficiency and swelling index which might be due to the presence of the bora rice in the formulation. Formulation P9 showed 91.06% of drug release within 24 hr and highest drug entrapment efficiency i.e. 68.21% among all other formulations (P1 to P9), hence formulation P9 was selected for the further studies.

### Table 4. Evaluation parameters of factorial design batch PT–BR beads of glipizide.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>% Drug Release (± SD) (12 hr)</th>
<th>% Drug release (± SD) (24 hr)</th>
<th>Particle size (mm) (± SD)</th>
<th>Entrapment Efficiency (%) (± SD)</th>
<th>Swelling index (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>76.01 ± 1.25</td>
<td>100.89 ± 1.48 (20 hr)</td>
<td>1.117 ± 0.21</td>
<td>43.23 ± 0.18</td>
<td>0.843 ± 0.034</td>
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<tr>
<td>P2</td>
<td>71.67 ± 1.32</td>
<td>99.40 ± 1.34 (22 hr)</td>
<td>1.113 ± 0.14</td>
<td>44.15 ± 0.13</td>
<td>0.723 ± 0.026</td>
</tr>
<tr>
<td>P3</td>
<td>69.03 ± 1.56</td>
<td>99.60 ± 1.22 (22 hr)</td>
<td>1.107 ± 0.12</td>
<td>45.22 ± 0.15</td>
<td>0.657 ± 0.031</td>
</tr>
<tr>
<td>P4</td>
<td>66.4 ± 1.12</td>
<td>100.85 ± 1.63 (20 hr)</td>
<td>1.142 ± 0.17</td>
<td>50.07 ± 0.26</td>
<td>1.172 ± 0.017</td>
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<tr>
<td>P5</td>
<td>64.17 ± 1.57</td>
<td>100.65 ± 1.14 (22 hr)</td>
<td>1.135 ± 0.13</td>
<td>52.65 ± 0.16</td>
<td>1.068 ± 0.018</td>
</tr>
<tr>
<td>P6</td>
<td>60.85 ± 1.32</td>
<td>100.10 ± 1.58 (20 hr)</td>
<td>1.128 ± 0.22</td>
<td>54.23 ± 0.12</td>
<td>0.947 ± 0.014</td>
</tr>
<tr>
<td>P7</td>
<td>63.22 ± 1.61</td>
<td>94.38 ± 1.32 (20 hr)</td>
<td>1.163 ± 0.21</td>
<td>66.14 ± 0.2</td>
<td>1.343 ± 0.018</td>
</tr>
<tr>
<td>P8</td>
<td>59.11 ± 1.16</td>
<td>91.06 ± 1.40 (20 hr)</td>
<td>1.156 ± 0.13</td>
<td>68.21 ± 0.14</td>
<td>1.278 ± 0.022</td>
</tr>
</tbody>
</table>

### Figure 2. In Vitro dissolution study of factorial design batches PT–BR beads (P1 to P9)

**Micromeretic properties of PT–BR beads**

The values of bulk density, tapped density and angle of repose have been depicted in Table 5 for trial and factorial design batches of PT–BR beads. The flow properties of material considered as excellent, good, poor, very poor if the angle of repose (θ) value is <20°, 25° to 30°, 30° to 40° and >40° respectively. The angle of repose, 31.37° to 36.54° of trial and factorial design batch beads indicated that the beads possess poor flow property which might be due to less spherical structure of the PT–BR beads.

### Table 5. Micromeretic properties of trial and factorial design batch PT–BR beads.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Bulk Density (g/cc) (± SD)</th>
<th>Tapped Density (g/cc) (± SD)</th>
<th>Angle of Repose (θ°) (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>0.986 ± 0.072</td>
<td>1.05 ± 0.068</td>
<td>36.54 ± 2.56</td>
</tr>
<tr>
<td>T8</td>
<td>0.854 ± 0.034</td>
<td>1.10 ± 0.076</td>
<td>32.34 ± 1.95</td>
</tr>
<tr>
<td>T9</td>
<td>0.861 ± 0.044</td>
<td>1.19 ± 0.061</td>
<td>33.24 ± 1.78</td>
</tr>
<tr>
<td>P1</td>
<td>0.842 ± 0.065</td>
<td>1.45 ± 0.12</td>
<td>33.54 ± 2.47</td>
</tr>
<tr>
<td>P2</td>
<td>0.835 ± 0.09</td>
<td>1.36 ± 0.086</td>
<td>32.55 ± 2.13</td>
</tr>
<tr>
<td>P3</td>
<td>0.865 ± 0.057</td>
<td>1.49 ± 0.083</td>
<td>33.74 ± 1.95</td>
</tr>
<tr>
<td>P4</td>
<td>0.851 ± 0.078</td>
<td>1.47 ± 0.08</td>
<td>32.62 ± 2.12</td>
</tr>
<tr>
<td>P5</td>
<td>0.854 ± 0.034</td>
<td>1.10 ± 0.076</td>
<td>32.34 ± 1.95</td>
</tr>
<tr>
<td>P6</td>
<td>0.852 ± 0.019</td>
<td>1.124 ± 0.036</td>
<td>31.56 ± 1.87</td>
</tr>
<tr>
<td>P7</td>
<td>0.859 ± 0.045</td>
<td>1.120 ± 0.033</td>
<td>31.42 ± 2.06</td>
</tr>
<tr>
<td>P8</td>
<td>0.856 ± 0.025</td>
<td>1.123 ± 0.023</td>
<td>32.12 ± 1.76</td>
</tr>
<tr>
<td>P9</td>
<td>0.843 ± 0.015</td>
<td>1.118 ± 0.034</td>
<td>31.37 ± 2.09</td>
</tr>
</tbody>
</table>

**Kinetics of drug release**

The release rate constant was calculated from the slope of appropriate equations and the correlation coefficient (R) was determined for all the formulations (Table 6). The release profile and the entrapment efficiency of formulation P9 was found to be satisfactory in comparison to other formulation, the discussion on the kinetics of other formulations was not considered further.

**In vitro** drug release of P9 was best explained by k-peppas equation with highest linearity ($R_p=0.9985$), followed by Higuchi’s equation, ($R_h=0.9836$) and First order ($R_f=0.981$). This indicates that the drug was diffused from polymeric matrix. The drug release was found to be very closed to Higuchi kinetics which indicates that the drug diffuses at a comparatively slower rate as the distance of diffusion increases.
Mechanism of drug release

The $K_p$ value of P1 to P9 decreases and $n_p$ value increases (Table 6) as the concentration of PT-BR polymer and cross-linking agent increases which revealed that the rate of drug release decreases as the concentration of PT-BR polymer and cross-linking agent increase in the formulation (Table 4).

The $n_p$ value indicated the mechanism of drug release from beads of P1 to P9. All the formulation showed $n_p$ value >0.5 and <1 during the entire period of drug release (24 hr) as shown in Table 6. This indicated that the drug release from the beads followed non-fickian diffusion. Anomalous diffusion of drug release mechanism signifies a coupling of the diffusion and erosion mechanism i.e. the drug release is controlled by more than one process. Hence the drug release from formulation P9 ($n_p = 0.701$) is controlled by both diffusion and erosion process in 24 hr study.

Solid state characterization of factorial design batches of PT–BR beads

FTIR study

FTIR spectra of glipizide, bora rice, PT-BR beads and drug loaded PT-BR beads are shown in Figure 3. Glipizide showed prominent peaks at 1651, 3480, 3030, 1690, and 2943 due to the presence of C=N aliphatic groups, N-H stretching, aromatic –CH stretching, C=O stretching and C-H$_2$ aliphatic respectively. The same peaks were also observed in the formulation of drug loaded PT-BR beads indicating the stable nature of the drug during encapsulation.

XRD study

X-ray diffraction analysis of the PT-BR placebo beads and drug loaded PT-BR beads were performed to characterize the physical state of the loaded drug in the polymeric matrix.20 The characteristics X-ray diffraction spectra of pure drug (glipizide), bora rice, placebo PT-BR beads and drug loaded PT-BR beads are shown in Figure 3.
are presented in the Figure 4. Characteristic crystalline peaks of glipizide were observed at 2θ of 14.5, 16.8, 19.5, 23.3, 26.7 and 29.5, indicating the presence of crystalline glipizide. XRD spectra of Bora rice and placebo PT–BR beads did not show any peaks which indicates its amorphous nature. Glipizide loaded PT–BR beads shows some peaks but not as the intensity of glipizide. Hence it revealed that the glipizide was present in the drug loaded pectin-bora rice beads in dispersed form.

**Figure 4.** XRD spectra of Glipizide, Glipizide loaded PT–BR beads (Gl.PM), PT–BR beads without drug (PBM), Bora Rice (BR)

**DSC study**

DSC study was performed to check the possible interaction of glipizide with other excipients the PT–BR beads. The comparison of DSC thermogram of placebo PT–BR beads and drug loaded PT–BR beads along with glipizide and pectin are presented in Figure 5. Glipizide gave a sharp endothermic peak at 212.90 °C and the drug loaded PT–BR beads (Gl.PM) shows a peak at 209.65 °C which indicated that there were no any interaction between the glipizide and PT–BR polymer.

**Figure 5.** DSC thermogram of Glipizide, Glipizide loaded PT–BR beads (Gl.PM), PT–BR beads without drug (PBM), Pectin (PCT)
Polysaccharide based colon targeted drug delivery system

The broad hemp of Gl.PM, PBM and PCT in the range of 100 to 120 °C indicated the presence of moisture in the formulation. PBM showed two peaks at 147.63 °C and 162.38 °C, and PCT showed one peak at 146.43 °C which may be due to presence of impurity in the pectin.

Shape and Surface Morphology
As shown in the Figure 6 Tabletop microscopy revealed that PT-BR beads were discrete and spherical in shape with rough and porous outer surface at higher magnification because of adherence of drug crystals on the surface of beads.

Figure 6. TM Photograph of glipizide loaded PT-BR beads

In vivo gamma scintigraphy study of the optimized factorial design batch formulation of glipizide
As shown in Figure 7 gamma scintigraphy study in rat showed that the beads were intact in the hostile environment of the stomach but whenever they reached to the colonic region they start degradation due to presence of anaerobic bacteria present in the colon.

Stability study of the optimized factorial design batch formulation of glipizide
The optimized formulation (P9) was evaluated for difference factor ($f_1$) and similarity factor ($f_2$) of dissolution rate study after 3 months of storage at accelerated condition (40 °C ± 2 °C and 75% ± 5% RH), the results of which are shown in Table 7 and Figure 8. The dissolution profile of the formulation at initial stage was considered as reference for calculation of dissimilarity factor ($f_1$) and similarity factor ($f_2$). When the value of $f_2$ lies between 50 to 100 and $f_1$ is less than 15, the two dissolution profiles (test and reference) are considered to be similar. The results obtained (Table 7) revealed that the dissolution profile of formulations after 3 months of storage at accelerated condition was similar with the initial dissolution profile of formulation. Based on the results it was considered that the formulation is stable after 3 months of storage at accelerated stability conditions.

Figure 7. Gamma scintigraphy showed p9 instead of P9

Figure 8. In vitro dissolution profile of the optimized formulation (P9) after subjected to stability study (Initial, Month 1, Month 2 and Month 3)

Table 7. Evaluation of PT-BR beads (P9) after 3 months of storage at 40 °C ± 2 °C and 75% RH ± 5% RH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial</th>
<th>One month</th>
<th>Two months</th>
<th>Three months</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_1$ value*</td>
<td>---</td>
<td>2.43</td>
<td>3.21</td>
<td>4.65</td>
</tr>
<tr>
<td>$f_2$ value*</td>
<td>---</td>
<td>85.32</td>
<td>78.56</td>
<td>73.48</td>
</tr>
</tbody>
</table>

*Initial sample (0 month) was taken as reference to calculate $f_1$ and $f_2$ values

Statistical Analysis and Modeling
The application of response surface methodology (RSM) offers an empirical relationship between the response variable DR$_{12hr}$, Entrapment Efficiency (EE), Particle Size, Swelling Index and the test variables under conditions. Quadratic model (partial sum squares type-III) was selected for all the RSM studies. By applying multiple regression analysis on the experimental data, the response variable DR$_{12hr}$ and the test variables A (Polymer to Drug ratio) and B (% of Zinc acetate) were related by second order polynomial equation.

Final equation in terms of coded factors:

Drug Release (12 hr) = $63.81 - 5.34A - 2.77B + 3.09A^2$

Final equation in terms of actual factors:

Drug Release (12 hr) = $+92.38034 -17.69509(PT-BR) -1.38462(CLA)+3.08898(PT-BR)^2$

It is observed from the coefficient estimation table and above equations that coefficient of ‘A’ and ‘B’ bears a negative sign. It signifies that on increasing the
concentration of PT-BR and Zn(CH₃COO)₂ the DR₁₂hrs was decreased.
By applying multiple regression analysis on the experimental data, the response variable EE and the test variables A (Polymer to Drug ratio) and B (% of Zinc acetate) were related by second order polynomial equation.

**Final equation in terms of coded factors:**

\[
EE = +52.00 + 1.17A + 1.50B + 0.25AB + 3.17A²
\]

**Final Equation in Terms of Actual Factors:**

\[
\text{Entrapment Efficiency (EE)} = +40.333 + 2.000 (PT-BR) + 0.50000 (PT-BR x CLA) + 3.166 (PT-BR)²
\]

It is observed from the coefficient estimation table and above equations that coefficient of ‘A’ and ‘B’ bears a positive sign. It signifies that on increasing the concentration of PT-BR and Zn(CH₃COO)₂, the EE was increased.
By applying multiple regression analysis on the experimental data, the response variable Particle Size and the test variables A (Polymer to Drug ratio) and B (% of Zinc acetate) were related by second order polynomial equation.

**Final equation in terms of coded factors:**

\[
\text{Particle size (mm)} = +1.13 + 0.027A - 8.333E - 003B - 4.000E - 003AB + 4.667E - 003A²
\]

**Final Equation in Terms of Actual Factors:**

\[
\text{Particle size (mm)} = +1.09967 + 0.01667 (PT-BR) + 2.00000E - 003 (PT-BR x CLA) + 4.66667E - 003 (PT-BR)²
\]

It is observed from the coefficient estimation table and above equations that coefficient of ‘A’ bears a positive sign indicating that when the PT-BR polymer concentration increased, particle size was also increased. Coefficient of ‘B’ bears negative sign indicated that concentration of Zn(CH₃COO)₂ is inversely proportional to particle size. It signifies that on increasing the concentration of PT-BR the particle size was decreased.
By applying multiple regression analysis on the experimental data, the response variable Swelling Index and the test variables A (Polymer to Drug ratio) and B (% of Zinc acetate) were related by second order polynomial equation.

**Final equation in terms of coded factors:**

\[
\text{Swelling Index} = +1.06 + 0.31A - 0.098 B + 2.000E - 003 AB - 0.012A²
\]

**Final Equation in Terms of Actual Factors:**

\[
\text{Swelling Index} = +0.59933 + 0.35433 (PT-BR) - 0.051083 (CLA) + 1.00000E - 003 (PT-BR x CLA) + 0.012333 (PT-BR)²
\]

It is observed from the coefficient estimation table and above equations that coefficient of ‘A’ bears a positive sign indicating that when the PT-BR polymer concentration increased, swelling index also increased. Coefficient of ‘B’ bears negative sign indicated that concentration of Zn(CH₃COO)₂ is inversely proportional to swelling index. It signifies that on increasing the concentration of PT-BR the swelling index was increased and on increasing the concentration of Zn(CH₃COO)₂ the swelling index was decreased.

**Conclusion**

The results of study clearly indicate that there is a great potential in delivery of glipizide to the colonic region. Study showed that the manipulation of polymer concentration and cross linking agent influence particle size of beads, sphericity and flow property of beads. From the above study it concluded that high concentration of Bora Rice will retard the drug release, may be due to high content of amylopectin present in the bora rice. Formulation P9 is the best formulation for controlling the drug release to the colon. Hence from the above study it concluded that high amylopectin containing bora rice, natural polysaccharide showed potential for controlled release colon targeting drug delivery.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**


