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# Formulation, Evaluation and Optimization of Controlled Release Hydrogel Microspheres for Colon Targeted Drug Delivery

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**Abstract:**
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. Microspheres containing chitosan and bora rice were prepared by simple emulsification phase separation technique using glutaraldehyde as cross linking agent and model drug used was glipizide. Results of trial batches indicated that polymer to drug ratio and stirring speed affects characteristics of microspheres. Microspheres were discrete, spherical and free flowing. Microspheres exhibited small particle size, higher percentage yield and also showed higher percentage of drug entrapment efficiency. A $3^2$ full factorial design was employed to study the effect of independent variables, polymer to drug ratio (A), and stirring speed (B) on dependent variables, particle size, swelling index, drug entrapment efficiency and percentage drug release. The optimized batch C2 exhibited satisfactory drug entrapment efficiency 63% 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 chitosan-bora rice microsphere demonstrated degradation of microspheres whenever they reached to the colon. Hence bora rice is potential polysaccharide for colon targeted drug delivery system.
**Keywords:** Bora rice, chitosan, glipizide, factorial design, *in vivo* study

## 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[i]. The colon is an ideal site for protein and peptide absorption[ii]. 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[iii]. 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.

Natural polysaccharides such as pectin[iv], chitosan[v], xylan and guar gum[vi] are not digested in the human stomach or small intestine, but are degraded in the colon by resident bacteria. Chitosan is a cationic natural copolymer of glucosamine, obtained from the deacetylation of chitin which is the second most abundant polysaccharide after cellulose in the world. It has been widely used in several pharmaceutical formulations as controlled release carrier systems such as beads[vii], gels[viii], films[ix], sponges[x] and microspheres[xi,xii] for its many unique properties such as low toxicity, biocompatibility and biodegradability[xiii]. Chitin is a polysaccharide composed of β-(1□4) linked N-acetyl-D-glucosamine unit[xiv]. Chitosan is a weak base and is insoluble in water and organic solvents, however, it is soluble in dilute aqueous acidic solution (pH <6.5), which can convert the glucosamine unit to $R\text{-}NH^+_3$ [xv]. In pharmaceutical formulations, chitosan is used as vehicle for directly compressed tablets[xvi], disintegrating, binding and granulating agent[xvii]. The rationale in 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[xviii].

Glipizide is a second-generation sulfonylurea that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas and is typically prescribed to treat type II diabetes (non-insulin dependent diabetes mellitus). Its short biological half-life (2-4 hours) necessitates it to be administered 2.5 to 10 mg per day in 2 to 3 doses[xix]. It shows pH dependent solubility thus, the development of controlled release dosage form would be much more advantageous than the conventional tablets[xx, xxi].Until recently, colon was considered as a site for water reabsorption and residual carbohydrate fermentation. 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. However, it is currently being viewed as a site for drug delivery. Moreover, colon transit time may last for upto 78 hours, which is likely to increase the time available for drug absorption. Bora rice is a rich source of amylopectin which shows sustained action of drug release. Several polysaccharides are being investigated as carriers for colon-specific drug delivery.

## MATERIALS:

Chitosan (CS) was obtained as a gift sample from sigma fisheries, cochin. Bora rice (BR) purchased from upper Assam region. Light liquid paraffin obtained from Rankem. Gluteraaldehyde purchased from Qualigens. All other chemicals/reagents used were of analytical grade.

## METHODS:

**Preparation of microspheres:** Chitosan 3%w/v was dissolved in 20ml of 2% v/v aqueous acetic acid solution and stirred to get uniform solution. This chitosan solution was centrifused at 2000 rpm for 2 min to form bubble free solution, dispersed required quantity of glipizide to form uniform dispersion of drug. Add required amount of bora rice powder (Table - 1) previously passed through sieve no. 100. This dispersion was emulsified into liquid paraffin in presence of 0.5% span 80 using propeller stirrer (Remi, Mumbai, India) at various speed for 20 min. Glutaraldehyde (5 ml) was added slowly and stirring were continue for 3 hours. The hardened microspheres were separated by filtration and washed with n-hexane for several times to remove excess liquid paraffin. The microspheres were dried at 50 $^0$C for 24 hours and stored in desiccator.

**Formulation of factorial design batches of CS–BR microspheres:** The response surface approach involving $3^2$ randomized full factorial design was adopted for optimization purpose. Two independent variables, the amount of CS-BR:Drug ratio (A) and stirring speed (B) were studied at 3 level each. All other formulation and processing variables were kept invariant throughout the study. Percentage drug release, particle size, entrapment efficiency and swelling index were taken as response variables.

**Assay of Glipizide:** Glipizide was estimated by UV/Vis spectrophotometric (Shimadzu UV-1601 UV/Vis double beam spectrophotometer) method (Fig. 1). Aqueous solutions of glipizide were prepared in phosphate buffer (pH 7.4) and absorbance was measured on UV/Vis spectrophotometer at 276 nm. The method was validated for linearity, accuracy and precision. The method obeys Beer's Law in the concentration range of 5-35 µg/ml.

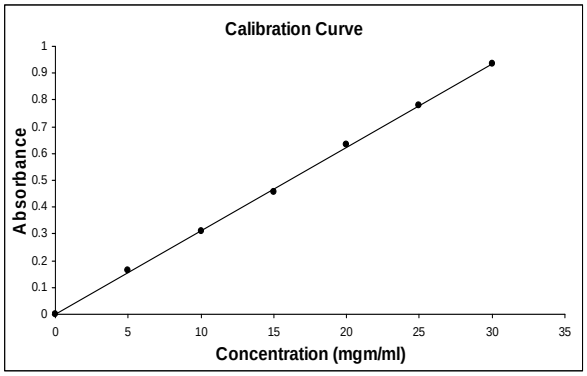


**Fig. 1:** Calibration curve for Glipizide in PBS 7.4

**Particle size analysis:** The particle size of the microspheres was determined by sieving method (Table 2). Microspheres were separated into different size fractions by sieving for 10 min using mechanical sieve shaker (Cuprit Electronic co. India) containing std sieves having aperture of 1000, 710, 500, 355, 250 & 180 µm.

**Micromeritic properties of drug loaded microspheres:** The flow properties of microspheres were investigated by determining the angle of repose, bulk density and tapped density (Table 3). The angle of repose was determine by the fixed base cone method. Bulk and tapped densities were measured in 10 ml graduated cylinder. The sample contained in the cylinder was tapped mechanically, tapped volume was noted down when it showed no change in its volume and bulk density and tapped density was calculated.

**Drug entrapment efficiency:** Microspheres (50 mg) were crushed in a glass mortar-pestle, and the powdered microspheres were suspended in 50 ml phosphate buffer (pH 7.4). After 24 hours the solution was sonicate for 1 hour, filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated as per the following formula:

**Practical drug content/Theoretical drug content x 100.**

The drug entrapment efficiency for batches A1 to B4 and C1 to C9 are reported in Table 4 and 5, respectively.

**Swellability:** A known weight (100 mg) of various glipizide loaded chitosan/bora rice microspheres were placed in phosphate buffer, pH 7.4 and allowed to swell for the required period of time at 37ºC ± 0.5ºC in the dissolution. The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using the following formula:

$$SR = (w_g - w_0)/w_0$$

SR= Swelling ratio
$w_g$ = Final weight of microspheres
$w_0$ = Initial weight of microsphere
The swelling index for microspheres of batches A1 to B4 and C1 to C9 are reported in Table 4 and 5, respectively.

**FTIR Study:** FTIR spectra of Glipizide, blank microspheres and drug loaded microspheres were obtained in KBR pellets using a JASCO model 5300 spectroscope in the ranges 4000 to 400 $cm^{-1}$ (Fig. 2).

**Thermal Studies:** Thermogram of samples were obtained by a Prkin-Elmer Differential Scanning Colorimeter (Fig. 3). 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 $10^0$C/ min over a temperature range of 50 to $250^0$C.

**Shape and Surface morphology:** Surface and shape morphology of CS-BR microspheres were evaluated by means of scanning electron microscopy (HITACHI S-3600N). The samples of SEM were prepared by lightly sprinkling the microspheres on a double adhesive tape, which struck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 $^0$A using a sputter coated and viewed under the scanning electron microscopy (Fig. 4).

***In vivo* Gamma Scintigraphy study:**

Three Wistar rats, weighing 200 – 250 g were taken for the study. The animals were fasted for 12 hours prior to commencement of the experiment. Radiolabled (>90%) microspheres (50 mg) of formulation C2 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, 450 minutes and 540 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. The photograph is depicted in Fig. 5.

**In vitro dissolution study:** The drug release study was carried out using USP XXIV paddle type apparatus (Electrolab, TDT-06T, India) at 37 ± 0.5° and at 75 rpm using 900 ml of phosphate buffer (pH 7.4) as a dissolution medium. Microspheres equivalent to 5 mg of glipizide were used for the test. Five ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analyzed spectrophotometrically. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug released at different time intervals was calculated using the Lambert-Beer's equation (y = 32.0624 - 0.0212, R2=0.9997) described above.

**Preparation of 2% rat cecal material:** Male albino rats weighing 200 – 250 g and maintained at normal diet were used for the study. Six rats were asphyxiated using carbon dioxide. The abdomens were opened, the ceci were traced, legated at both ends, dissected, and immediately transferred into pH 7.4 phosphate buffered saline (PBS), previously bubbled with $CO_2$. The cecal bags were opened and the contents were individually weighed, pooled and the suspended in PBS to provide 2% w/v dilution. Because the cecum is naturally anaerobic, all of these operations were carried out under $CO_2$.

**In vitro drug release study in the presence of colonic fluid containing 2% rat cecal material:** The drug release study of trial and factorial design batches were carried out using USP XXIV paddle type apparatus (Electrolab, TDT-06T, India) at 37 ± 0.5° and at 75 rpm using 900 ml of phosphate buffer (pH 7.4) containing 2% rat cecal material as a dissolution medium. Microspheres equivalent to 5 mg of glipizide were used for the test. 5 ml of sample solution was withdrawn at predetermined time intervals. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. The withdrawn samples were pipetted into a series of 10 ml volumetric flask, diluted suitably and centrifuged. The supernatant was filter through 0.45 µm membrane filter and filtrate was subject to UV analysis as described previously.

**Accelerated Stability study:** Accelerated stability study was conducted on the optimized formulation C2. The samples were stored at 40 $^0$C ± 2 $^0$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", $f_1$ and "similarity factor", $f_2$ were calculated. The $f_1$ and $f_2$ values for formulation C2 is represented in Table 8.

**Table 1:** Trial bach specification for prepared microsphere.

| Batch Code | Polymer Ratio (CS:BR) wt/wt | Drug:Polymer ratio wt/wt | Temperature ($^0$C) | Time of Cross-Linking (min) | Volume of Cross-Linking agent (ml) |
|---|---|---|---|---|---|
| A1 a | 1:2 | 1:2 | 30 - 40 | 10 | 5 |
| A2 a | 1:4 | 1:2 | 30 - 40 | 10 | 5 |
| A3 a | 1:6 | 1:2 | 30 - 40 | 10 | 5 |
| A4a | 1:8 | 1:2 | 30 - 40 | 10 | 5 |
| B1 b | 1:2 | 1:2 | 30 - 40 | 10 | 5 |
| B2 b | 1:4 | 1:2 | 30 - 40 | 10 | 5 |
| B3 b | 1:6 | 1:2 | 30 - 40 | 10 | 5 |
| B4 b | 1:8 | 1:2 | 30 - 40 | 10 | 5 |

Stirring Rate: a = 600 rpm; b = 1200 rpm

**Table 2:** Formulation of $3^2$ full factorial design batches of CS-BR microspheres.

| Batch code | Variable level (A) | Variable level (B) | Polymer to Drug ratio (% w/w) | Stirring speed (rpm) |
|---|---|---|---|---|
| C1 | -1 | -1 | 1:1 | 600 |
| C2 | -1 | 0 | 1:1 | 1200 |
| C3 | -1 | 1 | 1:1 | 1800 |
| C4 | 0 | -1 | 2:1 | 600 |
| C5 | 0 | 0 | 2:1 | 1200 |
| C6 | 0 | 1 | 2:1 | 1800 |
| C7 | 1 | -1 | 3:1 | 600 |
| C8 | 1 | 0 | 3:1 | 1200 |
| C9 | 1 | 1 | 3:1 | 1800 |

| Variable level | Low (-1) | Medium(0) | High (1) |
|---|---|---|---|
| ***Polymer:Drug Ratio (% w/w) (A)*** | 1:1 | 2:1 | 3:1 |
| ***Stirring speed (rpm) (B)*** | 600 | 1200 | 1800 |

**Table 3:** Micromeritic properties of drug loaded microspheres.

| Batch Code | Bulk Density (g/cc) | Tapped Density (g/cc) | Angle of Repose | Sphericity of Microspheres |
|---|---|---|---|---|
| A1 | 0.842 ± 0.065 | 1.45 ± 0.12 | 38.54 ± 2.47 | Very irregular |
| A2 | 0.835 ± 0.09 | 1.36 ± 0.086 | 36.55 ± 2.13 | Very irregular |
| A3 | 0.865 ± 0.057 | 1.49 ± 0.083 | 38.74 ± 1.95 | Very irregular |
| A4 | 0.81 ± 0.078 | 1.47 ± 0.08 | 39.62 ± 2.12 | Very irregular |
| B1 | 0.551 ± 0.034 | 0.610 ± 0.076 | 28.34 ± 1.95 | Spherical free flowing |
| B2 | 0.551 ± 0.019 | 0.614 ± 0.026 | 27.56 ± 1.87 | Spherical free flowing |
| B3 | 0.559 ± 0.045 | 0.620 ± 0.033 | 28.42 ± 2.06 | Spherical free flowing |
| B4 | 0.556 ± 0.025 | 0.612 ± 0.023 | 29.12 ± 1.76 | Spherical free flowing |

**Table 4:** Evaluation parameters of trial batch microspheres

| Batch Code | Yield (%) | Entrapment efficiency | Mean Particle size (µm) | Swelling Index |
|---|---|---|---|---|
| A1 | 90.12 ± 0.23 | 57.2 ± 2.5 | 570 ± 13.92 | 1.35 ± 0.15 |
| A2 | 91.25 ± 0.42 | 55.6 ± 4.8 | 592 ± 11.48 | 1.31 ± 0.15 |
| A3 | 90.68 ± 0.16 | 57.7 ± 3.6 | 661 ± 22.65 | 1.27 ± 0.16 |
| A4 | 92.51 ± 0.34 | 56.4 ± 5.3 | 723 ± 12.33 | 1.21 ± 0.12 |
| B1 | 93.22 ± 0.24 | 56.1 ± 4.5 | 420 ± 4.28 | 0.98 ± 0.04 |
| B2 | 91.35 ± 0.25 | 57.4 ± 1.9 | 445 ± 6.67 | 0.92 ± 0.02 |
| B3 | 90.45 ± 0.54 | 56.8 ± 4.9 | 479 ± 5.42 | 0.86 ± 0.04 |
| B4 | 91.68± 0.61 | 57.3 ± 2.8 | 487 ± 4.33 | 0.73 ± 0.03 |

## RESULT AND DISCUSSION:

Microspheres were prepared by the emulsification method using chitosan/bora rice (Table 1). The fabricated microspheres were spherical in shape and exhibited a range of sizes within each batch. Microspheres were prepared using gradually increasing BR concentration in combination with a fixed concentration of CS to assess the effect of polymer concentration on the size of microspheres. The mean particle size of the microspheres

significantly increased with increasing BR concentration and was in the range of 420 to 723 µm (Table -2). It may be due to high molecular weight. The particle size was reduced as agitation speed increases. Formulation A1 to A4 forms lumps and they are irregular in shape due to low agitation speed i.e 600 so we selected the batches B1 to B4 for the further study.

Tapped density of the formulation batches A1 to A4 was high as compare to B1 to B4 it may be due to the irregular in size and hence it shows very poor flow property. Batches B1 to B4 and all factorial design batches showed good flow property due to its spherical nature. On the basis of the preliminary trials a $3^2$ full factorial design was adopted to study the effect of independent variables (i.e polymer to drug ratio [A] and stirring speed [B] on dependent variables, percent drug release, entrapment efficiency, particle size and swelling index.

**FTIR Study:**

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 CS–BR microspheres indicating the stable nature of the drug during encapsulation.

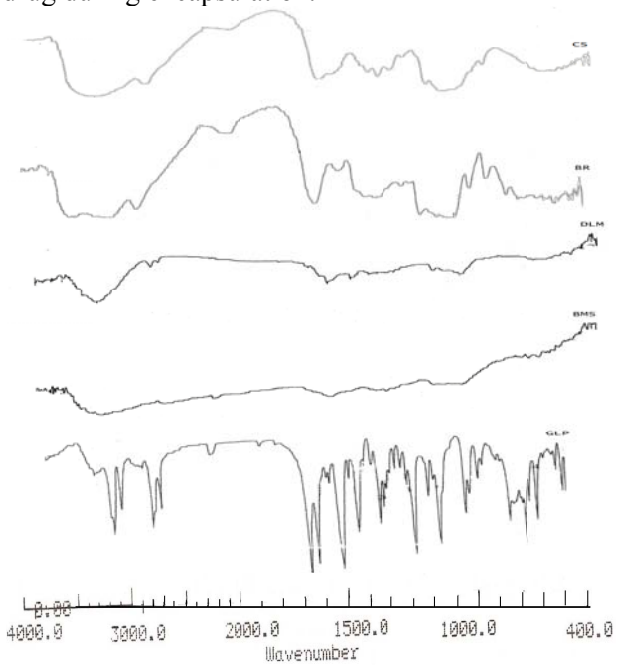


**Fig. 2:** FTIR spectra of pure Glipizide (GLP), Blank Microspheres (BMS), Drug Loaded Microspheres (DLM), Bora Rice (BR), Chitosan (CS).

**DSC Study:**

DSC is very useful in the investigation of the thermal properties of microspheres, providing both qualitative and quantitative information about the physicochemical state of drug inside the microspheres. Prominent melting endotherm of pure glipizide was found at 218.5 $^0$C. Drug loaded microspheres doesn't show any endotherm may be due to the drug was present in the molecular dispersion or solid solution state in the polymeric microspheres loaded with drug.

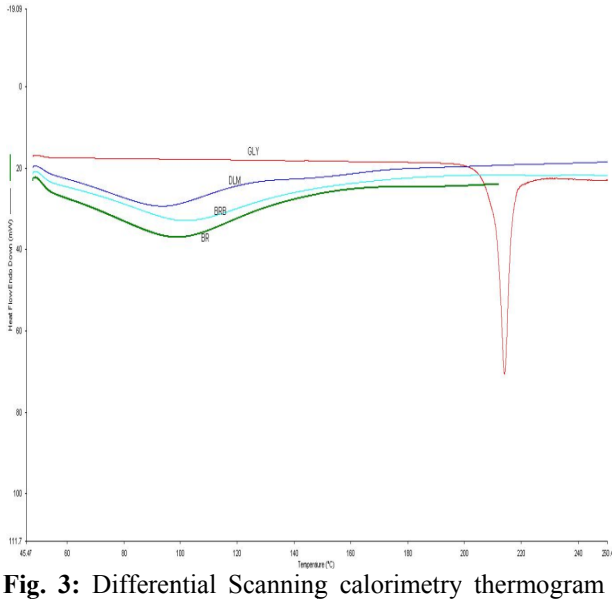


**Fig. 3:** Differential Scanning calorimetry thermogram of (GLY) Glipizide, (DLM) Drug loaded microsphere, (BRB) Blank microsphere and (BR) Bora Rice powder.

**Scanning Electron Microscopy:**

Scanning electron microscopy revealed that CS-BR microspheres were discrete and spherical in shape with rough outer surface because of the surface associated with crystals of drug (Fig. 4C). After dissolution study the surface showed erosion (Fig. 4D).

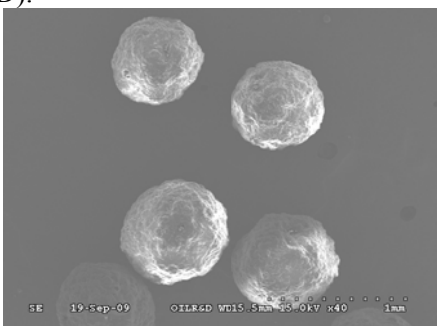

**Fig 4(A)**

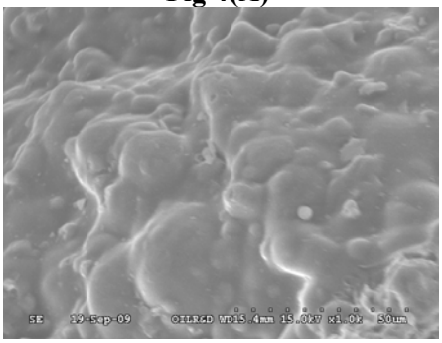

**Fig 4(B)**

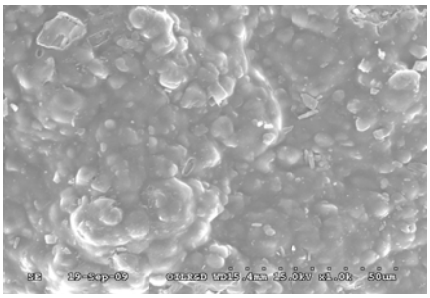

**Fig 4 (C)**

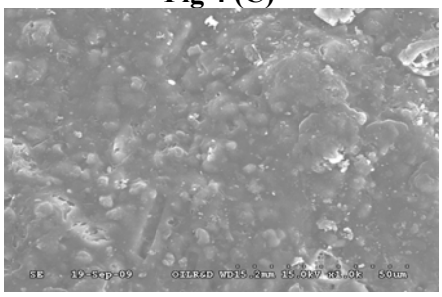

**Fig 4 (D)**

**Fig. 4:** A: Blank CS-BR microspheres, B: Surface of Blank microspheres, C: Surface of Drug loaded microspheres, D: Surface of drug loaded microspheres after dissolution

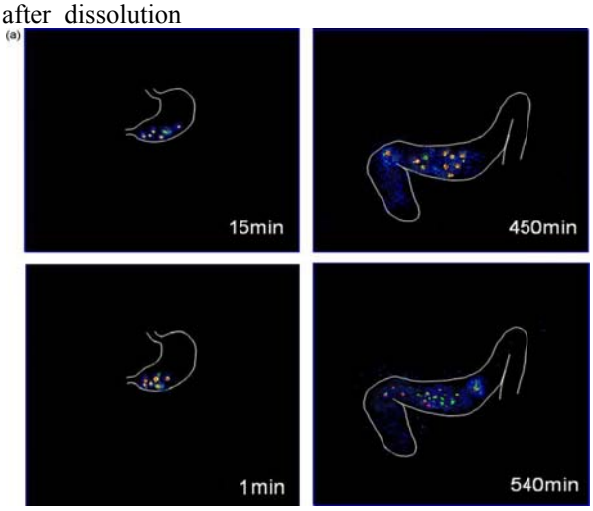


**Fig. 5:** Gamma Scintigraphy photographs of the formulation C2 in rat.

***In vivo* Gamma Scintigraphy Study:**

The gamma scintigraphy study in rat showed that the microspheres were intact in the hostile environment of the stomach at 1 min and 15 min duration (Fig. 5) but whenever they reached to the colonic region they start degradation due to presence of anaerobic bacteria present in the colon.

***In vitro* release Study:**

In vitro glipizide study was performed in PHS 7.4 for 24 hours, the drug release of Glipizide significantly decreased with increasing BR concentration (Fig. 6). This may be due to the higher concentration of amylopectin present in the bora rice which retarded drug release from bora rice matrix.

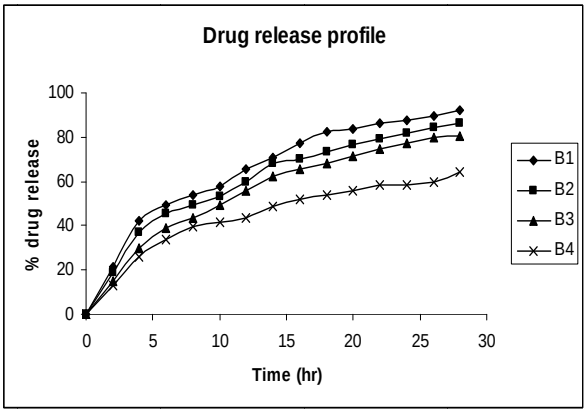


**Fig. 6:** In vitro release study of trial batch CS-BR microspheres.

The presence of rat cecal material in the dissolution medium increased the rate of drug release from CS-BR microspheres. In vitro drug release without rat cecal material was 58.54%, but drug release in simulated colonic fluid containing 2% rat cecal material after 24 hours was 85.5%. Formulation B4 showed higher entrapment efficiency and spericity as compared to other formulations which might be due to higher concentration of bora rice in the formulation. Therefore, formulation B4 was selected for the factorial design batches. The rat cecal material in the dissolution medium had increased the drug release from the microspheres, which may be attributed to chitosan and bora rice degradation by various anaerobic bacteria present in the caecum (Fig. 7).

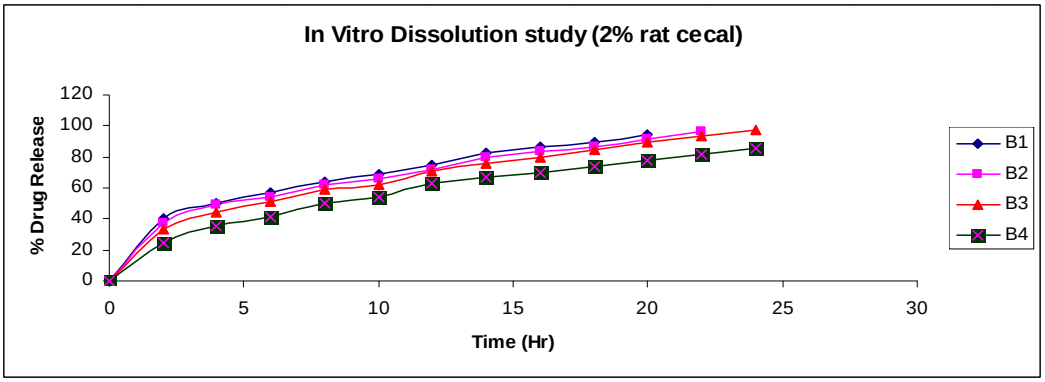


**Fig. 7:** In vitro release study of trial batch CS-BR microspheres in PBS 7.4 in presence of 2% rat cecal material.

**Table 5:** Evaluation parameters of factorial design batch CS–BR microspheres

| Batch code | % Drug Release (± SD) (12 hr) | % Drug release (± SD) (24hr) | Particle size (µm) (± SD) | EE (%) (± SD) | Swelling index (± SD) |
|---|---|---|---|---|---|
| C1 | 82.34 ± 1.26 | 99.54 ± 1.32 | 460.54 ±12.36 | 62.23 ± 0.57 | 0.812 ± 0.042 |
| C2 | 80.25 ± 1.43 | 96.81 ± 1.28 | 438.45 ±15.59 | 63.32 ± 0.53 | 0.788 ± 0.036 |
| C3 | 78.30 ± 1.67 | 94.70 ± 1.21 | 396.56 ±14.37 | 62.63 ± 0.56 | 0.754 ± 0.055 |
| C4 | 74.21 ± 1.73 | 91.25 ± 1.52 | 647.42 ± 7.23 | 68.28 ± 0.46 | 1.238 ± 0.036 |
| C5 | 68.38 ± 1.42 | 89.64 ± 1.67 | 604.34 ± 7.83 | 67.93 ± 0.38 | 1.186 ± 0.049 |
| C6 | 62.17 ± 1.12 | 79.50 ± 1.42 | 561.24 ± 5.18 | 62.56 ± 0.53 | 1.124 ± 0.051 |
| C7 | 60.41 ± 1.76 | 82.44 ± 1.26 | 712.27 ± 4.21 | 83.92 ± 0.42 | 1.453 ± 0.042 |
| C8 | 56.18 ± 1.81 | 77.56 ± 1.42 | 670.38 ± 4.52 | 82.81 ± 0.33 | 1.384 ± 0.033 |
| C9 | 50.06 ± 1.18 | 63.92 ± 1.64 | 634.06 ± 3.86 | 78.36 ± 0.37 | 1.217 ± 0.045 |

**Table 6:** Micromeretic properties of factorial design batch CS–BR microspheres.

| Batch Code | Bulk Density (g/cc) (± SD) | Tapped Density (g/cc) (± SD) | Angle of Repose ($\theta^0$) (± SD) |
|---|---|---|---|
| C1 | 0.534 ± 0.045 | 0.649± 0.023 | 24.36 ± 2.23 |
| C2 | 0.546± 0.032 | 0.618 ± 0.053 | 24.41 ± 2.06 |
| C3 | 0.561 ± 0.048 | 0.659 ± 0.035 | 23.33 ± 2.16 |
| C4 | 0.532 ± 0.042 | 0.663 ± 0.048 | 25.76 ± 1.82 |
| C5 | 0.538 ± 0.039 | 0.672 ± 0.052 | 26.32 ± 1.69 |
| C6 | 0.576 ± 0.046 | 0.649 ± 0.046 | 27.46 ± 2.54 |
| C7 | 0.587 ± 0.043 | 0.675 ± 0.028 | 26.21 ± 2.32 |
| C8 | 0.529 ± 0.032 | 0.661 ± 0.035 | 27.42 ± 2.25 |
| C9 | 0.548 ± 0.052 | 0.652 ± 0.026 | 26.89 ± 2.45 |

Hence the drug release in the simulated colonic fluid with cecal content may be a result of the combined effect of diffusion and erosion. From the result it is clear that the release profile of factorial design batch microspheres were in the order of C1>C2>C3>C4>C5>C6>C7>C8>C9. The concentration of polymer influenced the control of drug release for 24 hours. The stirring speed is inversely proportional to particle size, entrapment efficiency, swelling index and percent drug released. The concentration of polymer is directly proportional to the particle size, swelling index and drug entrapment efficiency which might be due to formation of denser network upon addition of cross-linking agent and presence of higher molecular weight polymer, bora rice in the formulation.

Formulation C2 showed 96.81% of drug release within 24 hours and its drug entrapment efficiency was 63.32%. The formulation C1 though showed higher percent of drug release i.e. 99.54% within 24 hour, its drug entrapment efficiency was 62.23% which is less than that of the formulation C2 for which it was not selected. Other formulations i.e. C3 to C9 showed lower percentage of drug release i.e. from 63.92 to 94.70% due to which they could not be selected as for once a daily dosage form maximum amount of drug should be released within 24 hours. Therefore though the formulation C3 to C9 should higher entrapment efficiency than that of the formulation C2, they could not be selected and the formulation C2 has only been selected for the further studies. The result is presented in Table 5 and Figure 8.

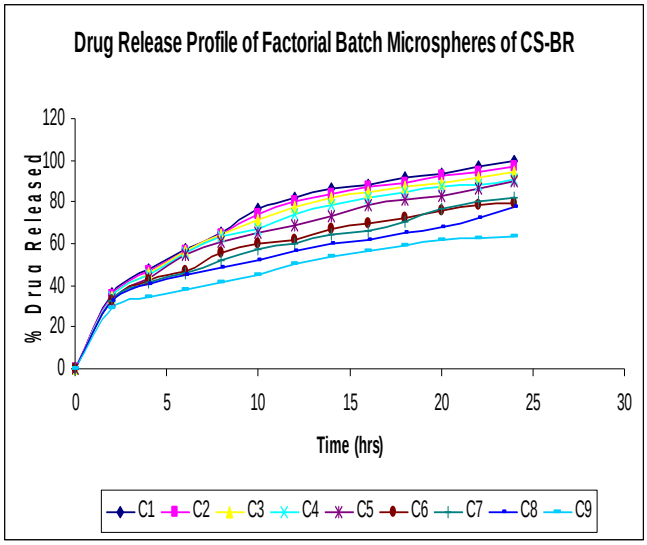


**Fig. 8:** *In vitro* dissolution study of factorial design batch CS–BR microspheres.

**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 7). The release profile and the entrapment efficiency of formulation C2 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 C2 was best explained by k-peppas equation with highest linearity ($R_p$ =

**Table 7:** Analysis of *in vitro* dissolution data of factorial design batch CS–BR microspheres.

| Batch Code | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 |
|---|---|---|---|---|---|---|---|---|---|
| Zero order | | | | | | | | | |
| $R_0$ | 0.7240 | 0.7169 | 0.7146 | 0.6911 | 0.7059 | 0.6512 | 0.7421 | 0.6500 | 0.6574 |
| $K_0$ | 5.2208 | 5.1000 | 5.0961 | 4.8049 | 4.6053 | 4.1718 | 4.1112 | 3.8070 | 3.3455 |
| 1st order | | | | | | | | | |
| $R_1$ | 0.9389 | 0.9944 | 0.9754 | 0.9863 | 0.9807 | 0.9395 | 0.9678 | 0.9242 | 0.8873 |
| $K_1$ | -0.1638 | -0.1338 | -0.1389 | -0.1058 | -0.0947 | -0.0743 | -0.0737 | -0.0628 | -0.0500 |
| Higuchi | | | | | | | | | |
| $R_h$ | 0.9855 | 0.9852 | 0.9850 | 0.9832 | 0.9858 | 0.9782 | 0.9867 | 0.9740 | 0.9764 |
| $K_h$ | 21.9911 | 21.4922 | 21.4775 | 20.2747 | 19.4126 | 17.6292 | 17.2725 | 16.0700 | 14.1215 |
| k-Peppas | | | | | | | | | |
| $R_p$ | 0.9931 | 0.9981 | 0.9941 | 0.9958 | 0.9973 | 0.9977 | 0.9915 | 0.9935 | 0.9905 |
| $K_p$ | 27.2602 | 26.9692 | 26.9447 | 26.5308 | 25.5030 | 25.2808 | 24.2931 | 24.9344 | 21.6135 |
| $n_p$ | 0.5199 | 0.5219 | 0.5275 | 0.5319 | 0.5387 | 0.5412 | 0.5425 | 0.5478 | 0.5476 |
| Hix.Crow. | | | | | | | | | |
| $R_h$ | 0.9818 | 0.9700 | 0.9688 | 0.9382 | 0.9350 | 0.8775 | 0.9280 | 0.8657 | 0.8304 |
| $K_h$ | -0.0334 | -0.0304 | -0.0307 | -0.0261 | -0.0241 | -0.0201 | -0.0198 | -0.0175 | -0.0145 |

0.9981), followed by Higuchi's equation, ($R_h$ = 0.9852) and First order ($R_1$ = 0.9944). This indicates that the drug was diffused from polymeric matrix. The drug release was found to be closed to Higuchi kinetics which indicates that the drug diffuses at a comparatively slower rate as the distance of diffusion increases.

**Stability study of the optimized factorial design batch formulation of glipizide:**

The optimized formulation (C2) 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 $^0$C ± 2 $^0$C and 75% ± 5% RH), the results of which are shown in Table 8 and Figure 9. 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 8) 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.

**Table 8:** Evaluation of CS-BR microspheres (C2) after 3 months of storage at 40 $^0$C ± 2 $^0$C and 75% RH ± 5% RH

| Parameter | Initial | One month | Two months | Three months |
|---|---|---|---|---|
| $f_1$ value* | --- | 2.45 | 4.13 | 5.26 |
| $f_2$ value* | --- | 86.58 | 79.57 | 74.22 |

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

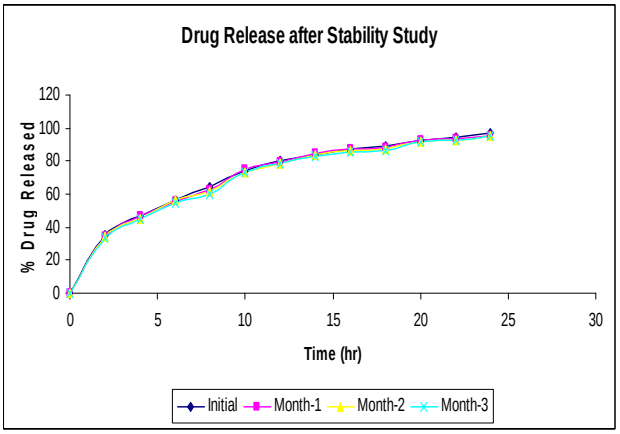


**Fig. 9:** *In vitro* dissolution profile of the optimized formulation (C2) after subjected to stability study

## 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 stirring rate influence particle size of microspheres, sphericity and flow property of microspheres. 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 C2 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.

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