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Research Article

# Preparation, Evaluation and Optimization of Multiparticulate System of Mebendazole for Colon Targeted Drug Delivery by Using Natural Polysaccharides

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**Abstract**

***Purpose*:** A Multiparticulate system of Mebendazole was developed for colon targeted drug delivery by using natural polysaccharides like Chitosan and Sodium-alginate beads.
***Methods*:** Chitosan microspheres were formulated by using Emulsion crosslinking method using Glutaraldehyde as crosslinking agent. Sodium-alginate beads were formulated by using Calcium chloride as gelling agent. Optimization for Chitosan microspheres was carried out by using $2^3$ full factorial design. $3^2$ full factorial design was used for the optimization of Sodium-alginate beads. The formulated batches were evaluated for percentage yield, particle size measurement, flow properties, percent entrapment efficiency, Swelling studies. The formulations were subjected to Stability studies and In-vitro release study (with and without rat caecal content). Release kinetics data was subjected to different dissolution models.
***Results*:** The formulated batches showed acceptable particle size range as well as excellent flow properties. Entrapment efficiency for optimized batches of Chitosan microspheres and sodium alginate beads was found to be 74.18% and 88.48% respectively. In-vitro release of drug for the optimized batches was found to be increased in presence of rat caecal content. The best-fit models were koresmeyer-peppas for Chitosan microspheres and zero order for sodium-alginate beads.
***Conclusion*:** Chitosan and Sodium-alginate was used successfully for the formulation of Colon targeted Multiparticulate system.

## Introduction

Oral route is considered to be most convenient route for administration of drugs to patients for providing both systemic and local effects in various regions of gastrointestinal tract. Recently greater emphasis has been placed on controlling site and/or release rate of drug from oral formulations to improve the efficacy of treatment.

Colon drug delivery refers to targeted delivery of drugs into the lower GI tract, which occurs primarily in the large intestine (i.e. colon). The colon specific drug delivery system (CDDS) should be capable of protecting the drug in route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon.[1] Colon targeted drug delivery can be achieved by using different approaches like pH sensitive systems, Timed release systems, Bioadhesive systems, Pressure dependent release system, Osmotically controlled system, Microbial triggered system. Natural polysaccharides can be used to formulate the microbially triggered system.[2-5]

Polysaccharides belong to such class of biodegradable materials which are normally metabolized in the colon by bacterial enzymes. This approach is exploited to deliver various drugs using polysaccharides such as pectin, alginate, guar gum, amylase, inulin, dextran, chitosan, chondroitin sulphate etc.[6,7]

The Multiparticulate system were developed in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation. Most commonly investigated multiparticulate formulations for colon specific drug delivery include pellets, granular matrices, beads, microspheres and nanoparticles.[8,9]

Helminthiasis is most widely observed parasitic infection in human. These parasites include Roundworm (*Ascaris lumbricoides*), Hookworm (*Necator americans*), Threadworm (*Enterobius vermicularis*), Whipworm (*Trichuris trichiura*). The heavy load of these worms may irritate the intestinal mucosa, causing inflammation

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and ulceration. They harm the host by depriving him of food, causing blood loss and by secreting toxins. The residence of these parasites is mainly in the colon region. Mebendazole is the drug of choice for treating helminthiasis. For the eradication of these parasites, local action of drug is needed. Moreover systemic absorption of this drug cause toxicities which can be overcome by formulating colon targeted system. This will ensure prolonged local action on colon and complete parasiticidal effect.

Rationale behind the present work was: (a) To achieve targeted delivery of drug to colon to treat helminthiasis. (b) To prolong the release of drug by the use of natural polysaccharides to increase contact time between drug and parasites. (c) To minimize the systemic side effects associated with conventional tablet.

## Materials and Methods

Mebendazole was obtained as gift sample from Indoco Remedies Ltd, Goa, India. Chitosan was obtained as gift sample from Mahtani Chitosan Private Ltd, Veraval, Gujrat, India. Sodium alginate was purchased from Loba Chemie, Mumbai, India. Glutaraldehyde, Calcium chloride was purchased from Loba Chemie, Mumbai, India. All the chemicals used were of analytical grade and were purchased from Loba Chemie, Mumbai, India. The instruments used were as follows: UV-visible double beam spectrophotometer (Shimatzu 1800), Differential scanning calorimeter (JAPE DSC (Perkin elmer), USA), FTIR spectrophotometer (Bruker FTIR spectrophotometer), Dissolution apparatus (Electro lab Dissolution tester (USP) TDT-06L), X-ray diffractometer ($D_8$ advanced model, Bruker Axs), Stability chamber (Thermolab, TH 200S, Mumbai)

### *FT-Infrared Spectroscopy*

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. Drug and drug-polymer mixtures were subjected to FT-IR studies using FTIR Bruker spectrophotometer. The spectra obtained were compared and interpreted for the functional group peaks.

### *Formulation of Chitosan microspheres*

2.5 % W/V Chitosan solution was prepared in 2% v/v acetic acid. 100mg of mebendazole was dispersed in Chitosan solution to give various drug : polymer ratios. This mixture was then dispersed in 50ml of light liquid paraffin containing 1.5%w/v span 80 by using syringe. The speed of rotation was set as 500 rpm. 2.5ml of crosslinking agent glutaraldehyde was added. Stirring was continued for 4 hrs at room temperature. After 4 hrs, formed microspheres were filtered and washed with n-Hexane to remove traces of liquid paraffin. Further, washed microspheres were allowed to dry at room temperature.[10,11] The formula of trial batches and formula by using $2^3$ factorial design is as shown in Table 1.

**Table 1.** Formulation of Chitosan microspheres

| Batch no. | Polymer concentration (mg) | Emulsifier conc. (ml) | Glutaraldehyde conc. (ml) | Stirring speed (rpm) |
|---|---|---|---|---|
| A | 100 | 0.75 | 2.5 | 500 |
| B | 200 | 0.75 | 2.5 | 500 |
| C | 300 | 0.75 | 2.5 | 500 |
| D | 400 | 0.75 | 2.5 | 500 |
| E | 500 | 0.75 | 2.5 | 500 |
| C1 | 200 | 0.75 | 2.5 | 400 |
| C2 | 200 | 0.75 | 2.5 | 600 |
| C3 | 200 | 0.75 | 5 | 400 |
| C4 | 200 | 0.75 | 5 | 600 |
| C5 | 300 | 0.75 | 2.5 | 400 |
| C6 | 300 | 0.75 | 2.5 | 600 |
| C7 | 300 | 0.75 | 5 | 400 |
| C8 | 300 | 0.75 | 5 | 600 |

### *Formulation of Sodium-alginate beads*

The sodium alginate solution comprising of 3%w/v concentration were prepared by dissolving sodium alginate in de-ionized water with gentle heat. 100mg drug was added in sodium alginate solution and stirred continuously to give homogenous dispersion. The above mixture was sonicated for 30 min. to remove the air-bubbles. Then, the mixture was dispersed dropwise in 50 ml of 5% W/V of gelling agent, calcium chloride solution by using 20-guage hypodermic needle fitted with 10 ml syringe. The stirring speed of the magnetic stirrer was set as 200 rpm. The droplets from the dispersion instantaneously gelled into discrete mebendazole-alginate matrices upon contact with the solution of gelling agents. The formed alginate beads were stirred for further 2hrs. After 2 hrs the solution of gelling agent was decanted and the beads were washed with deionized water. The beads were further dried at $80^0$ C for 2 hrs in hot air oven.[12,13] The formula of trial batches and formula by using $2^3$ factorial design is as shown in Table 2.

### *Surface morphology*[14]

The external morphology of microspheres was analyzed by scanning electron microscopy (SEM). The microspheres were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (JEOL JSM-6360A scanning microscope, Tokyo, Japan) at 10 kV. The pictures were taken at 37X, 60X, 85X, 200X.

**Table 2.** Formulation of Sodium alginate beads

| Batch no. | Polymer concentration (mg) | Gelling agent ($CaCl_2$ conc.) (%W/V) | Stirring speed (rpm) | Crosslinking time (hrs) |
|---|---|---|---|---|
| F | 250 | 5 | 200 | 2 |
| G | 300 | 5 | 200 | 2 |
| H | 350 | 5 | 200 | 2 |
| I | 400 | 5 | 200 | 2 |
| J | 450 | 5 | 200 | 2 |
| A1 | 350 | 5 | 200 | 1 |
| A2 | 350 | 5 | 200 | 2 |
| A3 | 350 | 5 | 200 | 3 |
| A4 | 375 | 5 | 200 | 1 |
| A5 | 375 | 5 | 200 | 2 |
| A6 | 375 | 5 | 200 | 3 |
| A7 | 400 | 5 | 200 | 1 |
| A8 | 400 | 5 | 200 | 2 |
| A9 | 400 | 5 | 200 | 3 |

### *Particle size*

Particle size was measured by using microscopy technique. Stage micrometer was mounted in the stage. Eyepiece micrometer was fitted in the eyepiece of microscope for its calibration. Eyepiece micrometer was calibrated by coinciding with stage micrometer scale.

It was observed that, $8^{th}$ division of eyepiece = $10^{th}$ division of stage micrometer

But, each division of stage micrometer = 10 µ

So, 1 division of eyepiece = 100/8 = 12.5 µ

Stage micrometer was removed from the stage and sample was placed on the clean slide. Slide holding sample was mounted on the stage and observed with the help of eyepiece micrometer scale. Divisions of eyepiece micrometer scale was measured for the particle and calculations were carried out by multiplying the divisions with factor 12.5µ.

### *Percentage yield*

Dried microspheres were accurately weighed. Percentage yield was calculated from the formula:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Where, Practical yield = Weight of dried microspheres

Theoretical yield = Weight of drug + Weight of polymer

### *Flow properties*

*Bulk density*

Dried microspheres were accurately weighed. Weighed quantity of microspheres was poured in a graduated measuring cylinder via large funnel and volume occupied by microspheres without tapping was recorded. Bulk density was calculated by using the formula:

$$\text{Bulk density: Db} = \frac{M}{V_b}$$

Where, M = Mass of the microspheres

$V_b$ = Bulk volume of microspheres

*Tapped density*

Weighed quantity of microspheres was poured in graduated measuring cylinder. Measuring cylinder was placed on mechanical tapper to give 100 tapps. Volume was recorded after tapping. Tapped density was calculated by the formula:

$$\text{Tapped density: Dt} = \frac{M}{Vt}$$

Where, M = Mass of the microspheres

Vt = Tapped volume of microspheres

### *Carr's index*

Carr's index was calculated from the formula given below:

$$\text{Carr's index (\%)} = \frac{\text{Dt} - \text{Db}}{\text{Dt}} \times 100$$

Where Dt = Tapped density

Db = Bulk density

*Hausner's ratio*

Hausner's ratio was calculated from the formula given below:

$$\text{Hausner's ratio} = \frac{\text{Dt}}{\text{Db}}$$

Where, Dt = Tapped density

Db = Bulk density

### *Swelling study*

500mg of dried microspheres/beads were accurately weighed and immersed in 200ml of phosphate buffer (pH

7.4). Swelling was allowed to occur at room temperature for 24 hrs. After completion of 24 hrs, microspheres/beads were removed from the medium and blotted with filter paper to remove adsorbed water on the surface and weighed immediately.[15]

Percent degree of swelling was calculated by using following formula

$$\%\ \text{degree of swelling} = \frac{\text{Weight of swollen microspheres/beads} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

### *Percentage Entrapment efficiency[16,17]*

50 mg of microspheres were accurately weighed, crushed by using glass mortar and pestle and then transferred to beaker containing 100 ml of phosphate buffer pH 7.4. It was placed on rotary shaker at 100 rpm for 24 hrs. After 24 hrs, 1ml sample was withdrawn and 1ml of formic acid was added to it and volume was made up to 10 ml using phosphate buffer pH 7.4. Absorbance was taken at 289 nm and concentration was calculated.

$$\%\ \text{Entrapment efficiency} = \frac{\text{Practical content}}{\text{Theoretical content}} \times 100$$

### *Differential scanning calorimetry*

Differential scanning calorimetric (DSC) analyses of both formulations i.e. Chitosan microspheres and alginate beads were carried out by using differential scanning calorimeter equipped with computer analyzer (Shimadzu TA–60 differential scanning calorimeter, Shimadzu Corporation, Kyoto, Japan). Samples (10 mg) were heated under nitrogen atmosphere on an aluminium pan at a heating rate of 10 °C / min over the temperature range of 50 °C -300 °C.

### *Powder X-ray diffraction studies*

XRD studies of the formulation were carried out by using Powder X-ray diffraction (PXRD) patterns were traced by using X-ray diffractometer $D_8$ advanced model of Bruker Axs company fitted with a copper target, a voltage of 40 kV, and a current of 30 mA. The scanning rate was 1°/min over a 2 θ range of 5°-50°.

### *In-vitro drug release studies[18]*

In-vitro drug release studies were carried out by using USP type II dissolution test apparatus (Paddle apparatus). The water bath was thermo stated at 37°C +/- 0.5°C. The paddle was set to rotate at 75 rpm. 200ml of phosphate buffer pH 7.4 was taken as dissolution medium and weighed quantity of microspheres were added to dissolution medium. Study was continued to 12 hrs.[17,18] At the interval of each hour, 1ml of dissolution media was pipetted and added for 10 ml volumetric flask. 1ml of formic acid was added to it and volume was made up with phosphate buffer pH 7.4. Absorbance of the solution was taken using UV spectrophotometer at 289 nm. Each time 1ml of fresh dissolution media was replaced into the jar.

### *Preparation of rat caecal medium[19]*

Albino rats were weighed. Thirty minutes before the commencement of drug release studies, rats were killed by spinal traction. The abdomen was opened, the caecal bags were opened in presence of $C0_2$. Their contents were individually weighed, pooled and suspended in buffer.[19] These were finally added to dissolution media to give final caecal dilution of 2%W/V and 4% W/V. Anaerobic environment was maintained by bubbling $CO_2$ gas in the buffer medium. This part of the study was approved by Institutional Animal Ethics Committee.

### *Release study in rat caecal medium*

The drug release for the optimized formulation was carried out with 200ml of phosphate buffer pH 7.4 with rat caecal content (2%W/V and 4%W/V). At specified intervals, 1ml of sample was withdrawn and 1 ml of fresh dissolution media was replaced. 1ml formic acid was added to sample. Volume was made up to 10 ml with dissolution medium, filtered and absorbance was measured at 289 nm. The experiment was continued for 12 hrs.

### *Stability studies*

According to ICH guidelines, an accelerated stability study has to be carried out on the pharmaceutical dosage form at 40±2°C/75±5% RH. During the present study, developed formulations were subjected to accelerated stability study. The formulations were placed in stability chamber at 40°C/75% RH for period of 30 days. The formulations were withdrawn after 30 days. After withdrawl, percent entrapment efficiency and In-vitro release studies were carried out. The entrapment efficiency and dissolution profiles were compared with the entrapment efficiency and drug release profile of same formulation before conducting stability studies.

### *Dissolution Model Fitting[20]*

To carry out dissolution model fitting different release kinetics parameters were calculated.

Parameter calculated: Square root of time, Log percentage of cumulative drug release, log time, Percent drug remaining, Log percent drug remaining, Cube root of percent drug remaining. By using these parameters, graphs were plotted to find out best fit dissolution model. For, **Zero order model:** cumulative amount of drug released versus time. **First order model:** log cumulative percentage of drug remaining vs. time.**Higuchi model:** cumulative percentage drug release versus square root of time. **Korsmeyer-peppas model:** log cumulative percentage drug release versus log time. **Hixon crowell model:** cube root of percentage of drug remaining versus time.

## Results and Discussion

### *FTIR spectroscopy*

FT-IR studies were carried out to investigate the interaction between drug and polymer. The FT-IR spectra of pure drug when overlapped with FT-IR spectras of physical mixtures of drug + polymer as shown in the Figure 1, it showed characteristic peaks of mebendazole at 1680-1760 $cm^{-1}$ (>C=O), 1500-1600 $cm^{-1}$ (-C=C-), 2210-2260 $cm^{-1}$ (-C≡N), 3010-3100 $cm^{-1}$ (=C-H). It was observed however, that the entire characteristic peak observed for pure drug remained unchanged, and no significant shift or reduction in the intensity of peak of Mebendazole. From the results, it was concluded that there was no interaction with polymer indicating the compatibility of drug and polymer.

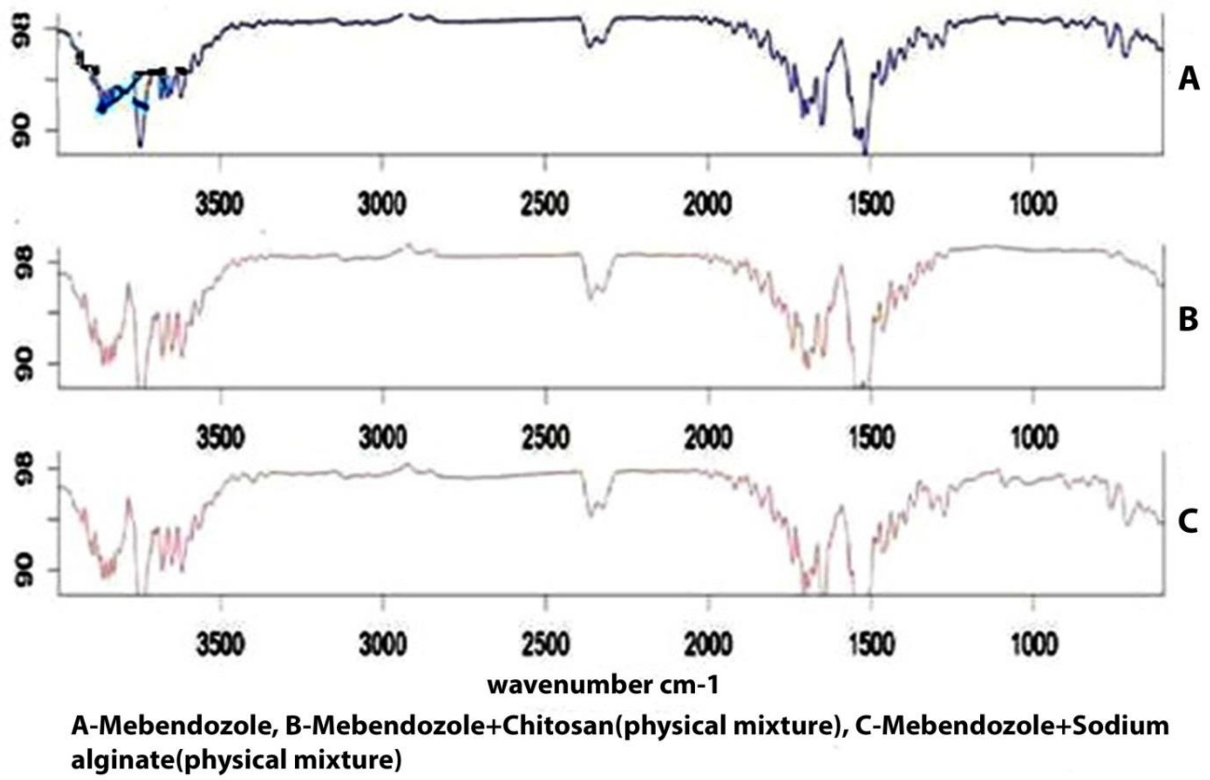


**Figure 1.** FT-IR spectra of Drug and Drug-polymer physical mixture

***Surface morphology***

*Chitosan microspheres*

Surface morphology of Chitosan microspheres is represented by Figure 2. The photographs were taken at 60x (A), 85x (B), 200x(C) magnification. Scanning electron microscopy confirmed the sperical structure of Microspheres. Both A and B showed smooth surface of the microspheres. At 200x (C) surface showed white spots representing the presence of drug at the surface.

*Sodium-alginate beads*

Figure 2 represents the photograph taken at 37x (D) magnification under scanning electron microscope. Scanning electron microscopy confirmed the sphericity of beads. Surface of beads was found to be rough. The rough surface of beads indicates that drug is molecularly dispersed in the polymer matrix.

***Particle size, Percentage yield and Flow properties***

*Chitosan microspheres*

All the formulated batches (Trial as well as Factorial design batches) were evaluated for particle size measurement, Percentage yield and Flow properties (Bulk density, Tapped density, Carr's index, Hausner's ratio). Results are given in Table 3. From the results it was observed that particle size got increased with increase in Drug: Polymer ratio (From 1:1 to 1:5). This was due to increase in polymer concentration. Stirring speed also inverse effect on particle size. As stirring speed was increased from 400rpm to 600rpm, particle size got decreased. The reason behind this can be stated as; the increase in stirring speed causes the breaking of polymer droplet into more fine particles. All the formulated batches showed good percentage yield. Carr's index was found to be in the range of 5-12% and Hausner's ratio was less than 1.2 which indicates that all the formulated batches showed excellent flow properties.

*Sodium-alginate beads*

All the formulated batches (Trial as well as Factorial design batches) were evaluated for particle size measurement, Percentage yield. Results are given in Table 4. Results of the evaluation showed that the bead size was varied with Drug: Polymer ratio (1:2.5 to 1:4.5) and crosslinking time. The bead size was found to be increased with increase in polymer concentration. Increased crosslinking time caused Slight decrease in bead size. Increased contact between polymer droplets and gelling agent (from 1hr to 3hrs) results into the formation of more rigid beads with slight shrinking.

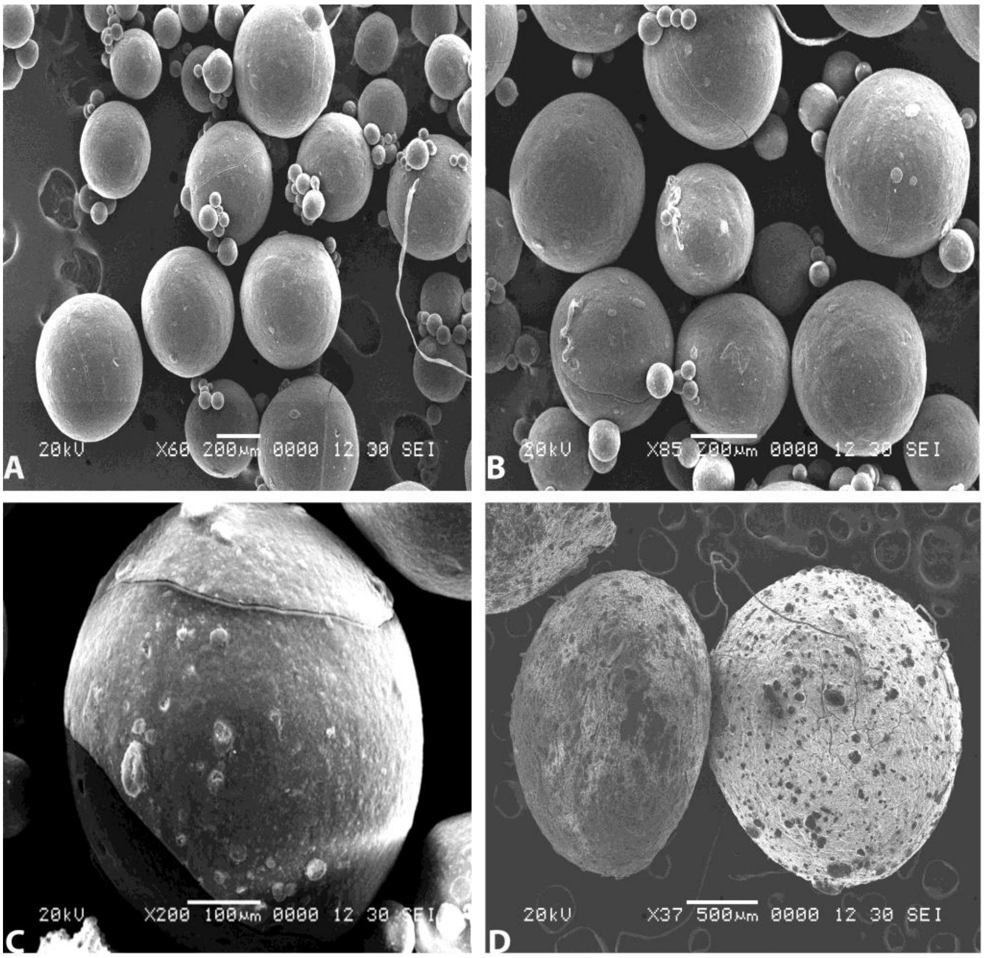


**Figure 2.** Surface morphology of Chitosan microspheres and Sodium-alginate beads

**Table 3.** Evaluation of Chitosan microspheres

| Batch code | Particle size (µm) | Percentage yield (± SD) | Bulk density(±SD) | Tapped density(±SD) | Carr's Index (±SD) | Hausner's ratio (± SD) | %Degree of swelling (±SD) | % Entrapment efficiency (±SD) |
|---|---|---|---|---|---|---|---|---|
| A | 118-312 | 89.46+/-0.341 | 0.658+/-0.033 | 0.730+/-0.034 | 9.86+/-0.163 | 1.109+/-0.245 | 29.48+/-0.338 | 38.4+/-0.321 |
| B | 125 -329 | 94.12+/-0.227 | 0.852+/-0.031 | 0.940+/-0.033 | 9.361+/-0.214 | 1.103+/-0.231 | 33.35+/-0.324 | 52.03+/-0.215 |
| C | 162 -375 | 92+/-0.324 | 0.989+/-0.029 | 1.063+/-0.039 | 6.961+/-0.342 | 1.074+/-0.212 | 35.79+/-0.342 | 69.6+/-0.249 |
| D | 180-475 | 95.46+/-0.217 | 1.016+/-0.035 | 1.139+/-0.039 | 10.79+/-0.189 | 1.121+/-0.283 | 40.52+/-0.338 | 78.95+/-0.367 |
| E | 212- 550 | 93.29+/-0.245 | 1.068+/-0.036 | 1.190+/-0.041 | 10.25+/-0.204 | 1.114+/-0.241 | 44.63+/-0.327 | 84+/-0.342 |
| C1 | 151-382 | 91.68+/-0.318 | 0.991+/-0.028 | 1.068+/-0.029 | 7.20+/-0.235 | 1.07+/-0.165 | 33.96+/-0.356 | 58.14+/-0.378 |
| C2 | 121-318 | 89.12+/-0.342 | 0.689+/-0.034 | 0.768+/-0.033 | 10.28+/-0.258 | 1.11+/-0.193 | 31.84+/-0.348 | 49.58+/-0.412 |
| C3 | 168-396 | 93.71+/-0.251 | 1.074+/-0.039 | 1.188+/-0.037 | 9.59+/-0.187 | 1.10+/-0.226 | 32.49+/-0.352 | 64.47+/-0.432 |
| C4 | 128-336 | 95.24+/-0.214 | 1.038+/-0.037 | 1.148+/-0.038 | 9.58+/-0.176 | 1.10+/-0.146 | 30.28+/-0.367 | 51.94+/-0.468 |
| C5 | 198-424 | 92.51+/-0.336 | 1.132+/-0.038 | 1.218+/-0.036 | 7.06+/-0.321 | 1.07+/-0.168 | 36.71+/-0.362 | 71.64+/-0.514 |
| C6 | 179-418 | 88.46+/-0.347 | 1.124+/-0.037 | 1.208+/-0.036 | 6.95+/-0.315 | 1.07+/-0.202 | 35.42+/-0.359 | 67.46+/-0.439 |
| C7 | 208-475 | 95+/-0.223 | 1.069+/-0.038 | 1.199+/-0.039 | 10.84+/-0.224 | 1.12+/-0.186 | 37.12+/-0.361 | 74.18+/-0.382 |
| C8 | 186-432 | 92.84+/-0.265 | 1.158+/-0.034 | 1.268+/-0.033 | 8.67+/-0.382 | 1.09+/-0.123 | 34.57+/-0.372 | 69.6+/-0.543 |

**Table 4.** Evaluation of Sodium alginate beads.

| Batch code | Particle size (µm) (± SD) | Percentage yield (±SD) | % Degree of swelling (± SD) | % Entrapment efficiency (± SD) |
|---|---|---|---|---|
| F | 1183 +/-1.527 | 91.52+/- 0.272 | 204+/-1.145 | 66.10 +/-0.115 |
| G | 1200 +/-1.643 | 93.73+/- 0.237 | 268+/-1.087 | 71.27 +/-0.128 |
| H | 1221+/-1.678 | 92.98+/-0.289 | 312+/-1.102 | 74.68+/-0.187 |
| I | 1279+/-1.589 | 95.24+/-0.314 | 390+/-1.254 | 86+/-0.231 |
| J | 1286+/-1.681 | 98.82+/-0.254 | 417+/-1.389 | 94+/-0.268 |
| A1 | 1219+/-1.573 | 94.78+/-0.387 | 324+/-1.269 | 73.89+/-0.165 |
| A2 | 1211+/-1.567 | 92.46+/-0.365 | 312+/-1.432 | 72.12+/-0.245 |
| A3 | 1202+/-1.421 | 94.63+/-0.298 | 304+/-1.298 | 70.47+/-0.386 |
| A4 | 1238+/-1.754 | 92.52+/-0.412 | 362+/-1.231 | 78.74+/-0.402 |
| A5 | 1231+/-1.634 | 91.74+/-0.356 | 349+/-1.187 | 78+/-0.189 |
| A6 | 1224+/-1.598 | 95.49+/-0.453 | 336+/-1.159 | 76.89+/-0.342 |
| A7 | 1281+/-1.612 | 96+/-0.378 | 407+/-1.232 | 88.48+/-0.248 |
| A8 | 1275+/-1.756 | 93.78+/-0.497 | 395+/-1.376 | 85.71+/-0.362 |
| A9 | 1271+/-1.421 | 91+/-0.523 | 378+/-1.154 | 81.39+/-0.275 |

### *Swelling studies and percent entrapment efficiency*

#### *Chitosan microspheres*

Results are given in Table 3. Results from swelling studies showed that percent degree of swelling was increased with increase in polymer concentration. Percent entrapment efficiency was calculated to check the amount of drug entrapped in polymer matrix. The effect of the polymer concentration, Amount of crosslinking agent and speed of rotation on percent entrapment efficiency was evaluated by statistical analysis. Results showed that percent entrapment efficiency was increased with increase in polymer concentration and amount of crosslinking agent. As the polymer concentration increases, Viscosity of the solution also increases forming the dense network of polymer which prevents the drug leaving from droplets during the crosslinking process. Faster crosslinking is achieved with increased amount of crosslinking agent forming rigid microspheres and thus prevents loss of drug in external phase during crosslinking process. Speed of rotation had a negative effect on percent entrapment efficiency. Increased speed of rotation results decreased particle size. Thus less amount of polymer matrix is available for entrapment of drug which causes decreased entrapment of drug.

#### *Sodium-alginate beads*

Results are given in Table 4. Results of swelling studies showed that percent degree of swelling was increased with increase in polymer concentration. The effect of polymer concentration and crosslinking time on percent entrapment efficiency was evaluated by statistical analysis Results of the study showed that percent entrapment efficiency was increased with increase in polymer concentration and decreased with increase in the crosslinking time. Hence percent entrapment efficiency get increased with increase in polymer concentration. Percent entrapment efficiency was decreased with increase in crosslinking time which is attributed to loss of drug in dispersion medium due to increased contact time between the polymer droplets and dispersion medium.

### *DSC and XRD*

DSC and XRD patterns of Multiparticulate system are shown in Figure 3 and Figure 4, respectively. As shown in the Figure 3, DSC thermogram of drug (MZ) showed the sharp melting endotherm at 266.58 °C. The DSC thermogram of physical mixture of Mebendazole and Chitosan (MZCS) showed endothermic peak of at 252.93°C for drug. The broad peak observed at 111.52 $^{0}$C is attributed to loss of moisture by evaporation of absorbed water. DSC thermogram of physical mixture of Mebendazole and sodium alginate (MZSA) showed endothermic peak at 246.61°C for drug. The broad peak observed at 117.66 °C is attributed to loss of moisture by evaporation of absorbed water. The change in melting endotherm of drug may be attributed to mixing process which lowers purity of each component. The peak for the drug was diminished in DSC thermograms of formulations of Chitosan microspheres (MZCM) as well as Sodium alginate beads(MZSAM) evidencing the absence of crystalline drug. Therefore, it could be concluded that drug in microspheres was in amorphous phase of a molecular dispersion or solid solution state in polymer matrix.

As shown in Figure 4, XRD spectra of drug (MZ) showed the sharp peaks at 2θ values: 10.4, 12.6, 18.5, 19.9, 20, 20.1, 25, 25.1, 25.2, 27, and 27.2°. These sharp peaks indicate the crystalline nature of drug. XRD spectra of mebendazole loaded Chitosan microspheres (MZCM) showed peaks of drug with decreased intensity at 2θ values 21.6, 25.4, 27.1°. XRD spectra of mebendazole loaded sodium alginate beads (MZSAM) showed peaks of drug with decreased intensity at 2θ values 21.2, 27.2, 32.8°. The XRD spectra of drug loaded formulations with decreased intensity of sharp peaks of drug indicate the amorphous nature of drug entrapped in polymer matrix.

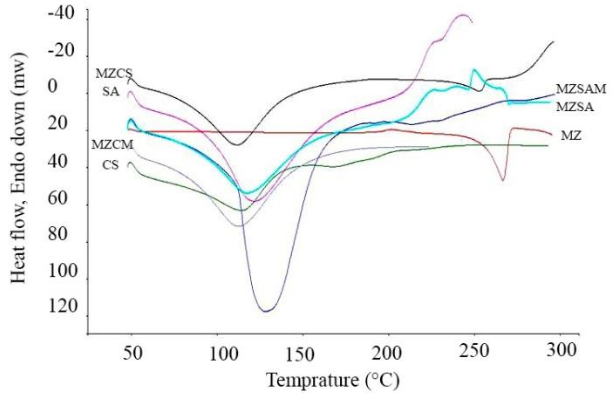


**Figure 3.** DSC spectra of Drug(MZ), Chitosan(CS), Sodium alginate(SA), Drug- Chitosan physical mixture(MZCS), Drug-Sodium alginate physical mixture(MZSA), Mebendazole loaded Chitosan microspheres(MZCM), Mebendazole loaded sodium alginate beads(MZSAM)

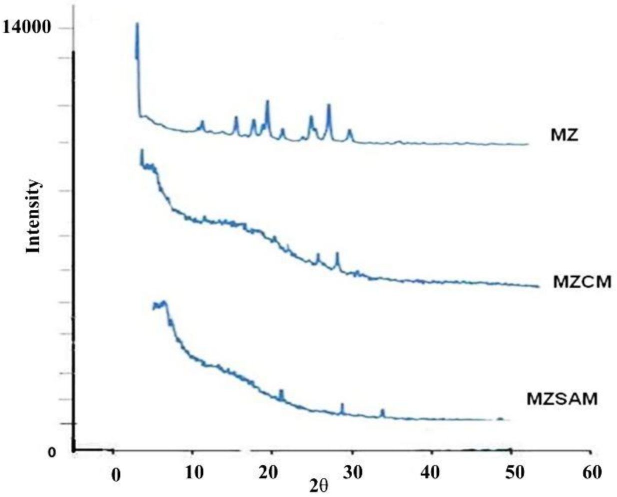


**Figure 4.** XRD spectra of Drug (MZ), Mebendazole loaded Chitosan microspheres (MZCM), Mebendazole loaded sodium alginate beads(MZSAM)

### *In-vitro drug release (Without rat caecal content)*

In-vitro release studies were carried out using phosphate buffer pH-7.4 without rat caecal content. Drug release was continued for 12 hrs. For the drug: polymer ratio 1:1(Batch A), release at the end of 12$^{th}$ hr was found to be 45.12%. For drug: polymer ratios 1:2(Batch B), 1:3(Batch C), 1:4 (Batch D)and 1:5(Batch E),it was found to be 35.79%, 29.1%, 24.12% and 21.37% respectively. Figure 5(A), shows that percent release was found to be as 36.42% for batch C1, 37.12 % for batch C2, 34.27% for batch C3, 34.94% for batch C4, 31.78% for batch C5, 32.54% for batch C6, 28.96% for batch C7, 29.57% for batch C8. The effect of polymer concentration, amount of crosslinking agent and speed of rotation on percent cumulative drug release was evaluated by statical analysis. The percent drug release was found to be decreased with increase in polymer concentration. As the polymer concentration increases, viscocity of the solution also increases forming dense network of polymer preventing the release of drug from the matrix and thus drug release is decreased. Amount of crosslinking agent had a negative effect on drug release. More rigid microspheres are formed with larger amount of crosslinking agent and creates difficulty in release of drug from the rigid matrix. Thus, it was observed that drug release was decreased with increase in amount of crosslinking agent. Percent drug release was increased with increase in speed of rotation. With increase in speed of rotation, particle size decreases and thus having more surface area exposed to dissolution medium. This results in enhanced dissolution and gives more drug release.

Release study was also conducted for sodium-alginate beads. For the drug: polymer ratio 1:2.5(Batch E), release at the end of 12$^{th}$ h was found to be 72.67%. For drug: polymer ratios 1:3(Batch F), 1:3.5(Batch G), 1:4(Batch H) and 1:4.5(Batch I), it was found to be 59.16%, 47.78%, 37.46% and 28.45% respectively. Figure 5(B), shows that percent release was found to be as 45.65% for batch A1, 42.67 % for batch A2, 42.17% for batch A3, 41.23% for batch A4, 40.75% for batch A5, 40.21% for batch A6, 37.58% for batch A7, 35.46% for batch A8, 33.76% for batch A9. The effect of polymer concentration and crosslinking time on percent cumulative drug release is evaluated by statistical analysis. The percent drug release was found to be decreased with increase in polymer concentration as well as increase in crosslinking time. Increase in the crosslinking time allows increase in the contact time between polymer beads and crosslinking agent in the dispersion medium. This causes increased crosslinking and imparts more rigidity. Thus, the drug is released slowly through highly crosslinked polymer matrix.

### *In-vitro release study (In presence of 2%W/V and 4% W/V rat caecal content)*

Batch C7 and A7 were subjected to in-vitro drug release study with different concentrations of rat caecal content. C7 and A7 were selected as optimized batches on the basis of percent entrapment efficiency as these batches possess high percent entrapment efficiency among other respective batches.

Comparative release profiles of batch C7 with and without rat caecal content showed the percent release of 43.18% by the end of 12$^{th}$ hour in presence of 2%W/V rat caecal content whereas in presence of 4%W/V rat caecal content, it was found to be increased up to 66.54%. Similarly, comparative release profiles of batch A7 with and without rat caecal content. showed the percent drug release of 53.32% by the end of 12$^{th}$ hr in the presence of 2%w/v rat caecal content whereas percent drug release was found to be increased upto 73.12% in the presence of 4% W/V rat caecal content by the end of 12$^{th}$ hr. The release in presence of rat caecal content was due to bacterial degradation of polymer matrix. More the concentration of rat caecal content, more will be the bacterial count and hence the degradation rate of polymer matrix will be more which results in enhanced drug release.

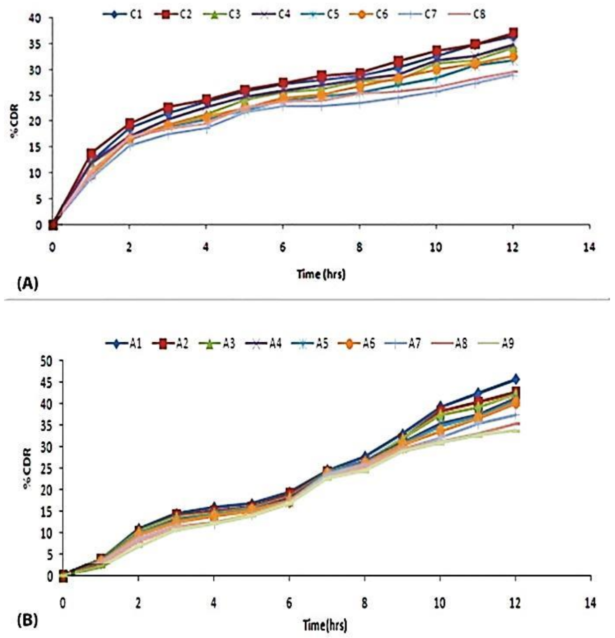


**Figure 5.** Graphical representation of In-vitro release drug release studies of factorial design batches of Chitosan microspheres (C1-C8) and Sodium alginate beads (A1-A9) without rat caecal content

### *Dissolution model fitting*

In-vitro release profile of batch C7 was best explained by korsmeyer peppas model with highest $r^2$ = 0.9481. The respected values for zero-order, first order, higuchi and hixon-crowell model was found to be 0.8621, 0.7549, 0.9408 and 0.8762 respectively. The 'n' value was found to be was found to be less than 0.45 which indicates the fickian diffusion mechanism of drug release. In-vitro release profile of batch A7 was best explained by zero order model with highest $r^2$ = 0.9792. The respected values for first order, Korsmeyer peppas, higuchi and hixon-crowell model was found to be 0.8593, 0.9675, 0.9492 and 0.9762 respectively. The 'n' value was found to be was found to be greater than 0.89 which indicates the Supercase II transport mechanism where drug release occurs by swelling and relaxation of polymer chains.

### *Stability studies*

It was observed that there was no any significant difference in both percent entrapment efficiency and percent drug release before and after the stability study. Hence the both the formulations were found to be stable.

### *Statistical analysis studies*

*Chitosan microspheres*

ANOVA for percent entrapment efficiency: The Model F-value of 58.91 implies the model is significant. There is only a 1.68% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C are significant model terms.

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

%E.E = +63.38 + 7.34 * A+1.67 * B -3.73*C- 0.50*A*B+1.54*A*C………..(1)

Equation 1. represents the quantitative effect of independent variables A ( polymer concentra tion), B (Amount of crosslinking agent), C( Speed of rotation) and their interactions (AB,AC) on the response percent entrapment efficiency. As the coefficient of A bears positive symbol which indicates that increase in polymer concentration causes increase in percent entrapment efficiency. With increase in polymer concentration, viscocity of solution also increases forming a dense polymer network which prevents the drug from leaving the droplet during crosslinking process. The coefficient of B bears positive symbol which indicates that percent entrapment efficiency increases with increase in amount of crosslinking agent. Faster crosslinking is achieved with increased amount of crosslinking agent forming rigid microspheres and thus prevents loss of drug in external phase during crosslinking process. As the coefficient of C bears negative symbol, it indicates that percent entrapment efficiency decreases with increase in speed of rotation. Increase in speed of rotation results into breaking of polymer droplets in fine size particles. Thus less amount of polymer matrix is available for entrapment of drug which causes decreased entrapment of drug.

ANOVA for percent cumulative drug release**:** The Model F-value of 5880.74 implies the model is significant.There is only a 1.00% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms

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

% CDR = + 33.20 - 2.49* A - 1.27 * B + 0.34* C - 0.18*A*B + 0.000*A*C - 0.022*B*C…………(2)

Equation 2. represents the quantitative effect of independent variables A ( polymer concentra tion), B (Amount of crosslinking agent), C( Speed of rotation) and their interactions (AB, AC, BC) on the response percent cumulative drug release. As the coefficient of A bears negative symbol, it indicates decrease in percent cumulative drug release with increase in polymer concentration. As the polymer concentration increases, viscocity of the solution also increases forming dense network of polymer preventing the release of drug from the matrix and thus drug release is decreased. Similarly the coefficient of B bears negative symbol which indicates that cumulative drug release decreases with increase in amount of crosslinking agent. More rigid microspheres are formed with larger amount of crosslinking agent and creates difficulty in release of drug from the rigid matrix. As the coefficient of C bears positive symbol, it indicates that percent cumulative drug release increases with increase in speed of rotation. With increase in speed of rotation, particle size decreases and thus having more surface area exposed to dissolution medium. This results in enhanced dissolution and gives more drug release.

*Sodium alginate beads*

ANOVA for percent entrapment efficiency: The Model F-value of 3123.82 implies the model is significant. There is only a 1.38% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, AB, A2B are significant model terms.

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

$$\%EE = +78.08 + 6.79*A - 0.92*B - 0.92*A*B + 0.80*A^2 - 0.30*B^2 - 1.70*A^2*B - 0.42*A*B^2 \ldots\ldots\ldots(3)$$

Equation 3. represents the quantitative effect of independent variables A ( polymer concentra tion), B (Crosslinking time) and their interactions (AB, $A^2$, $B^2$,$A^2B$, $AB^2$) on the response percent entrapment efficiency. As the coefficient of A bears positive symbol which indicates that percent entrapment efficiency increases with increase in polymer concentration. With increase in polymer concentration, viscocity of solution also increases forming a dense polymer network which prevents the drug from leaving the droplet during crosslinking process. As the coefficient of B bears negative symbol, it indicates that percent entrapment efficiency decreases with increase in crosslinking time which is attributed to loss of drug in dispersion medium due to increased contact time between the polymer droplets and dispersion medium.

ANOVA for percent cumulative drug release: The Model F-value of 61.64 implies the model is significant. There is only a 1.60% chance that a "Model F-Value" this large could occur due to noise. Values of " Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms.

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

$$\%CDR = +40.43 - 3.96*A - 0.51*B - 0.047*A*B - 1.21*A^2 + 0.45*B^2 - 1.30*A^2*B \ldots\ldots\ldots(4)$$

Equation 4. represents the quantitative effect of independent variables A ( polymer concentra tion), B (Crosslinking time) and their interactions (AB, $A^2$, $B^2$,$A^2B$) on the response percent cumulative drug release. As the coefficient of A bears negative symbol, it indicates that percent cumulative drug release decreases with increase in polymer concentration. As the polymer concentration increases, viscocity of the solution also increases forming dense network of polymer preventing the release of drug from the matrix and thus drug release is decreased. The coefficient of B bears negative symbol, it indicates that percent cumulative drug release decreases with increase in crosslinking time. Increase in the crosslinking time allows increase in the contact time between polymer beads and crosslinking agent in the dispersion medium. This causes increased crosslinking and imparts more rigidity. Thus, the drug is released slowly through highly crosslinked polymer matrix.

## Conclusion

Formulation, Optimization and Evaluation of Multiparticulate system was carried out successfully. According to results, batch C7 and A7 showed highest entrapment efficiency among the other batches of Chitosan microspheres and Sodium-alginate beads respectively. Results of in-vitro release study in presence of rat caecal content showed that the optimized formulations were able to prolong the release of drug in colon for more than 12hrs. Thus, it was concluded from the study that both Chitosan (polysaccharide from animal source) and Sodium alginate (Polysaccharide from algal source) can be successfully used for colon targeted drug delivery for once a daily dosage form.

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## Ethical Issues

Not applicable.

## Conflict of Interest

The authors report no conflicts of interest.

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