

# Formulation and Development of Colon Specific Multiparticulate System of Capecitabine

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## Abstract

**Aim:** In the present study, the main objective was to develop a multiparticulate system containing chitosan microspheres for colon-specific drug delivery of capecitabine for the treatment of colorectal cancer. **Materials and Methods:** This study was based on the microbial degradability of chitosan microspheres. The microspheres were prepared with chitosan by emulsion cross linking method. A factorial design was applied to optimize the formulation. The effect of concentration of chitosan and drug: Polymer ratio was studied on particle size, % entrapment efficiency, and % drug release using 3<sup>2</sup> factorial designs. **Results and Discussion:** The prepared microspheres also analyzed for percentage yield, flow properties, and surface morphology. The results of analysis of variance test for responses measured indicated that the test is statistically significant. **Conclusion:** *In vitro* drug release studies were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract showed a fast drug release initially demanded microencapsulation.

**Keywords:** Chitosan microspheres, colon-targeted drug delivery, multiparticulate system

## INTRODUCTION

Colon-specific drug delivery systems (CDDS) have been the focus of increasing interest for the last decade, as it is ineffective in delivering drugs to the colon which provides therapeutic concentrations of anticancer agent at the site of action. At present, the specific drug delivery to the colon is considered an important alternative for the treatment of serious local diseases such as Crohn's disease, ulcerative colitis, carcinomas, and infection. Colonic residence time is 2-3 days, whereas food remains in small intestine for as little as 5 h.<sup>[1-3]</sup> This long colonic residence time provides a significant opportunity for the slow absorption of drugs and other materials, drugs which would be unstable in the small intestine may be released in the colon safely and absorbed there to act systemically.<sup>[4-6]</sup>

In recent times, much emphasis is made on the development of multiparticulate approaches (pellets, granules, beads, microspheres, and nanoparticles) in comparison to single unit systems to increase the bioavailability, reduce toxicity and local irritations, vary resident time, etc., Multiparticulate systems tend to

be more uniformly dispersed in gastrointestinal tract and uniform absorption.<sup>[7-11]</sup> Drug-specific or formulation-specific approaches have been used and systems have been designed for the purpose of achieving colon targeting, such as pH-dependent delivery; time dependent delivery; pressure-dependent delivery; and bacteria-dependent delivery.<sup>[12-16]</sup>

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of colorectal cancer and metastatic breast cancer. Capecitabine is a prodrug that is enzymatically converted to fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue since it is readily absorbed from the gastrointestinal tract. The recommended daily dose is large, i.e., 2.5 g/m<sup>2</sup> and it have a short elimination half-life of 0.5-1 h.<sup>[17]</sup> The adverse effects associated with capecitabine include bone-marrow depression, cardiotoxicity, diarrhea, nausea and vomiting,

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stomatitis, and dermatitis. Thus, formulating capecitabine as a controlled release multiparticulate system would provide greater and safe effect.<sup>[18]</sup>

Chitosan [poly ( $\beta$ -(1 $\rightarrow$ 4)-2-amino-2-deoxy-D-glucose)] is a high molecular weight, polycationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation. Chemically, it is a poly (N-glucosamine).

Chitosan has favorable biological properties such as nontoxicity, biocompatibility, and biodegradability.<sup>[19]</sup> It is a linear polyamine containing number of free amine groups that are readily available for crosslinking, its cationic nature allows for ionic crosslinking with multivalent anions, it has mucoadhesive character, which increases the residual time at the site of absorption.<sup>[20]</sup> Chitosan however is soluble in dilute acid and precipitates at a pH above 7. Because of the solubility of chitosan at low pH ranges, its successful use in colon-specific delivery requires an enteric layer over the chitosan which would protect it against the acidity of the stomach. As the formulation reaches the intestine, the pH increases and the enteric layer dissolves releasing the chitosan-coated core.<sup>[21,22]</sup> Development of successful colon targeted drug delivery system requires the protection of drug from degradation, release, and absorption in stomach and small intestine and then ensures controlled release in proximal colon.<sup>[3]</sup>

In the present study, an attempt was made to develop a multiparticulate system of capecitabine by utilizing microbial degradation of the chitosan in the colon. A 3<sup>2</sup> factorial design model was employed to investigate the effect of the selected variables on the properties of microspheres and *in vitro* drug release characteristics.

## MATERIALS AND METHODS

The capecitabine was obtained as gift sample from Cipla Laboratories Ltd. (Mumbai, India). The Chitosan was obtained as gift sample from Aarti Drugs, Mumbai. hydrochloric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, methanol, petroleum ether, acetone, n-hexane, glacial acetic acid, Gluteraldehyde, toluene, and span 80 were purchased from Yash Chemicals, Pune. All other chemicals and reagents used in the study were of analytical grade.

### Preparation of chitosan microspheres<sup>[23,24]</sup>

The chitosan microspheres were prepared by emulsion cross-linking method. Chitosan solution was prepared in aqueous acetic acid solution by overnight stirring in a magnetic stirrer. The drug was dispersed in this solution and mixed well. Resultant mixture was then injected through a syringe into 20 ml of oil phase; mixture of heavy and light liquid paraffin (1:1 ratio), containing Span 80 (1% w/v) and stirring was

performed by mechanical stirrer at 1500 rpm to form w/o emulsion.

After 30 min of homogenization period, gluteraldehyde saturated toluene (GST) was added to it stage by stage. GST was prepared by mixing gluteraldehyde and toluene (1:1 ratio). Gluteraldehyde and toluene were placed in a beaker and stirred at 1000 rpm for 1 h using a magnetic stirrer. Then, the solvent mixture was kept overnight for the stabilization after which the upper toluene layer saturated with gluteraldehyde was decanted and used as GST. It was then left for stabilization and cross-linking for a period of 7 h. Microspheres thus obtained were centrifuged at 4000 rpm and the sediment was then washed with petroleum ether and acetone and then dried in a hot air oven at 50°.

### Design of experiments

Capecitabine-loaded chitosan microspheres were optimized using 3<sup>2</sup> factorial designs for different formulation variables [Table 1]. For this design independent variables (X) were concentration of chitosan in internal phase (X<sub>1</sub>) and drug: Polymer ratio (X<sub>2</sub>) whereas dependent variables (Y) were the average size of chitosan microspheres (Y<sub>1</sub>), the entrapment efficiency (Y<sub>2</sub>), % drug release after 2 h (Y<sub>3</sub>), and % drug release after 5 h (Y<sub>4</sub>).

### Determination of percentage yield<sup>[25]</sup>

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres (W<sub>1</sub>) divided by the total amount of all non-volatile material (W<sub>2</sub>) that was used for the preparation of the microspheres. The percentage (%) yield of microspheres was calculated using the following equation:

$$\% \text{Yield} = \frac{W_1}{W_2} \times 100$$

### Particle size analysis<sup>[26]</sup>

The size of all the microspheres were evaluated using optical microscope fitted with a calibrated eyepiece micrometer. The average size was determined by the Edmondson's Equation:

$$D_{\text{mean}} = \frac{\sum nd}{\sum n}$$

Where, n=Number of microspheres observed,

d=Mean size range.

### Surface morphology<sup>[27]</sup>

The shape and surface morphology of the microspheres was studied using a scanning electron microscope (Model-SU-SEM-Probe, Camecha, France).

**Table 1:** 3<sup>2</sup> factorial design for optimization of chitosan microspheres

Batch code	Factor		Average microsphere size ( $\mu\text{m}$ )	Entrapment efficiency (% w/w)	Cumulative % drug release	
	X1:Chitosan concentration	X2: D-P ration			After 2 hrs	After 5 hrs
CMF1	-1	-1	12.52	65.41 $\pm$ 0.87	62.32 $\pm$ 1.74	84.34 $\pm$ 1.67
CMF2	-1	0	13.61	67.38 $\pm$ 0.83	56.86 $\pm$ 1.23	81.54 $\pm$ 0.74
CMF3	-1	1	18.12	69.23 $\pm$ 0.37	52.32 $\pm$ 1.56	78.31 $\pm$ 1.65
CMF4	0	-1	12.67	72.14 $\pm$ 0.61	60.77 $\pm$ 1.05	83.78 $\pm$ 1.71
CMF5	0	0	16.61	73.98 $\pm$ 0.89	54.44 $\pm$ 1.11	78.19 $\pm$ 1.06
CMF6	0	1	19.88	76.98 $\pm$ 0.56	49.75 $\pm$ 1.43	78.88 $\pm$ 1.30
CMF7	1	-1	17.55	75.87 $\pm$ 0.13	49.11 $\pm$ 1.00	71.64 $\pm$ 1.44
CMF8	1	0	19.39	79.67 $\pm$ 0.78	48.91 $\pm$ 0.89	73.71 $\pm$ 1.67
CMF9	1	1	21.42	84.77 $\pm$ 0.44	46.13 $\pm$ 1.23	64.34 $\pm$ 1.09

Chitosan concentration: (-1) level=0.5% w/v, (0) level=1% w/v, (1) level=1.5% w/v. Drug-Polymer ratio: (-1) level=1:2, (0) level=1:4, (1) level=1:6

### Flow properties<sup>[28,29]</sup>

The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr's index, and Hauser's ratio.

#### Angle of repose

Angle of repose ( $\theta$ ) was determined by fixed funnel method. Accurately weighed microspheres were poured in the glass funnel. The height of funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of microspheres. The microspheres were allowed to flow through a glass funnel freely onto a clean surface. The diameter of the microspheres heap so formed was measured and angle of repose was calculated using the following equation:

$$\tan \theta = h/r, \text{ Therefore; } q = \tan^{-1} (h/r)$$

Where, h is the height of microspheres heap and r is the radius of the microspheres heap.

#### Density

Tapping cylinder method was used for determining bulk density ( $\rho_b$ ) and tapped density ( $\rho_t$ ) using bulk density apparatus. Microspheres were taken in a 50 ml measuring cylinder and the initial volume (bulk volume) and the volumes after 50 tapping were measured.

Bulk density ( $\rho_b$ ) and tapped density ( $\rho_t$ ) were calculated using the following equations:

$$\rho_b = \text{Weight of the powder (W)/volume of the packing (V}_b)$$

$$\rho_t = \text{Weight of the powder (W)/tapped volume of the packing (V}_t)$$

#### Carr's compressibility index and Haussner's ratio

Carr's compressibility index and Haussner's ratio were calculated from the following equations.

$$\text{Carr's compressibility index (\%)} = [(\rho_t - \rho_b) \times 100] / \rho_t$$

$$\text{Haussner's ratio} = \rho_t / \rho_b$$

#### Entrapment efficiency<sup>[30]</sup>

Microspheres were accurately weighed and triturated with methanol to break up the microparticles and kept overnight for extraction of drug for the determination of entrapment efficiency. The solution was then filtered and appropriate dilution with methanol the absorbance was measured with ultraviolet (UV) spectrophotometer (Jasco-4100) at 240 nm. The drug entrapment efficiency (E) was calculated using the following formula:

$$E (\%) = (\text{ADL}/\text{TDL}) \times 100$$

Where, ADL is actual drug loading,

TDL is theoretical drug loading

#### In vitro drug release study<sup>[31]</sup>

The *in vitro* drug release study of colon targeting microspheres were carried out in pH progression medium using rotating basket method using apparatus I USP XXIII (TDT-08L,

electro lab India, Mumbai) with 100 rpm speed at  $37 \pm 0.5^\circ$ . The weighed amount of microspheres was wrapped in cellophane membrane and kept in baskets. The simulation of gastrointestinal transit conditions was achieved by altering the pH of the dissolution medium at various time intervals. The drug release studies were carried in 900 ml of the dissolution medium at pH 1.2 (consisted of NaCl 2.0 g, 0.1N HCl 7 ml, in 1000 ml distilled water) for 2 h (as average gastric transit time is about 2 h). Then, the dissolution medium was replaced with pH 6.8 phosphate buffer (consisted of Na<sub>2</sub>HPO<sub>4</sub> 28.80 g, KH<sub>2</sub>PO<sub>4</sub> 11.45 g in 1000 ml distilled water) and study carried out for next 3 h (as average small intestinal transit time is about 3 h). The release study was continued in pH 7.4 phosphate buffer (consisted of KH<sub>2</sub>PO<sub>4</sub> 6.8 g, 0.2 N NaOH 190 ml in 1000 ml distilled water).

At various time intervals, 5 ml of samples was withdrawn from the dissolution medium and replaced with fresh dissolution medium. The samples were then analyzed by UV spectrophotometer at 240 nm.

### Statistical analysis

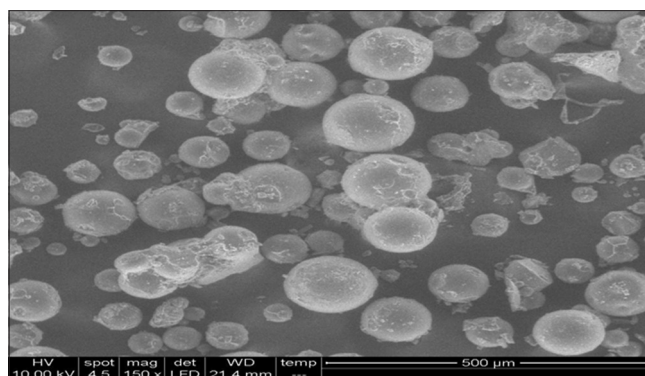
To select the factors showing the most effects on the properties of microsphere, a screening based on the factorial design was done, using Design Expert<sup>®</sup> software (8.0.7.1). Then, these factors were analyzed according to response surface. Statistical evaluation of data was performed using an analysis of variance (ANOVA) and the significance conformed by the outcome of the ANOVA, a value of  $P < 0.05$  was accepted as significant.

## RESULTS AND DISCUSSIONS

The chitosan microspheres were successfully prepared by emulsion cross linking method.

The percentage yield of different formulations was calculated [Table 2], the results were found in the range of 83.87%-91.57% for all the formulations [CMF1-CMF9]. The results indicated that the emulsion cross-linking method gives chitosan microspheres with satisfactory percentage of capecitabine containing. The SEM studies of chitosan microspheres showed that spherical shape and smooth surface of microspheres [Figure 1]. The values of angles of repose were in the range of  $21.39^\circ$ - $24.65^\circ$ , the values of Carr's index were in the range of 10.76%-14.24%, and the values of Hausner's ratio were ranged from 1.12 to 1.17 for all the formulations. Values of angle of repose  $\leq 30^\circ$  and Carr's index below 20% usually indicate a free flowing material. As results indicates an overall free flowing nature of microspheres of all batches, also supported by lower values of Hausner's ratio [Table 2].

Microscopic analysis was performed to determine the average particle size of chitosan microspheres. The average particle size of different chitosan microsphere formulations was found to be in the range of 12.52-21.42  $\mu\text{m}$  [Table 1]. The effects of process variables such as chitosan concentration



**Figure 1:** Scanning electron microphotographs of Chitosan microspheres

**Table 2:** Evaluation of microspheres

Batch code	Flow properties				Hausner's ratio <sup>†</sup>	Percentage yield
	Angle of repose ( $\theta$ ) <sup>*</sup>	Bulk density ( $\text{gm}/\text{cm}^3$ ) <sup>*</sup>	Tapped density ( $\text{gm}/\text{cm}^3$ ) <sup>*</sup>	Carr's index (%) <sup>*</sup>		
CMF1	23.04 $\pm$ 1.12	0.347 $\pm$ 0.018	0.443 $\pm$ 0.007	14.24	1.17	84.11
CMF2	22.19 $\pm$ 1.37	0.412 $\pm$ 0.014	0.492 $\pm$ 0.009	13.08	1.15	85.21
CMF3	21.45 $\pm$ 0.87	0.388 $\pm$ 0.017	0.461 $\pm$ 0.013	11.39	1.15	83.87
CMF4	23.67 $\pm$ 1.19	0.409 $\pm$ 0.023	0.485 $\pm$ 0.018	12.57	1.13	88.87
CMF5	24.65 $\pm$ 1.04	0.405 $\pm$ 0.016	0.496 $\pm$ 0.011	11.45	1.15	88.52
CMF6	22.45 $\pm$ 0.77	0.433 $\pm$ 0.008	0.514 $\pm$ 0.009	12.81	1.13	89.05
CMF7	21.39 $\pm$ 1.02	0.397 $\pm$ 0.023	0.479 $\pm$ 0.005	12.65	1.15	91.24
CMF8	25.36 $\pm$ 0.36	0.419 $\pm$ 0.009	0.488 $\pm$ 0.012	10.76	1.13	91.01
CMF9	23.23 $\pm$ 1.06	0.428 $\pm$ 0.010	0.493 $\pm$ 0.003	11.07	1.12	91.57

\*All values represented as mean $\pm$ SD (n=3). <sup>†</sup>Indicates SD is $\pm$ 0.1

( $X_1$ ) and drug-polymer ratio ( $X_2$ ) on average size of chitosan microspheres ( $Y_1$ ) was explained with response curve [Figure 2a] and contour plot [Figure 2b]. As the chitosan concentration and drug-polymer ratio increases the average particle size of chitosan microspheres increases may be due to the higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres were formed.

The drug entrapment efficiency of different formulations was found to be between 65%-85%. The effects of chitosan concentrations ( $X_1$ ) and drug: Polymer ratios ( $X_2$ ) were studied and the entrapment efficiency ( $Y_2$ ) was higher ( $84.77\% \pm 0.52\%$ ) for formulation CMF9 which contain 1.5% chitosan concentration and 1:6 drug-polymer ratio, explained with response curve [Figure 3a] and contour plot [Figure 3b].

This showed that high drug entrapment at higher chitosan concentration and higher drug: Polymer ratio [Table 1].

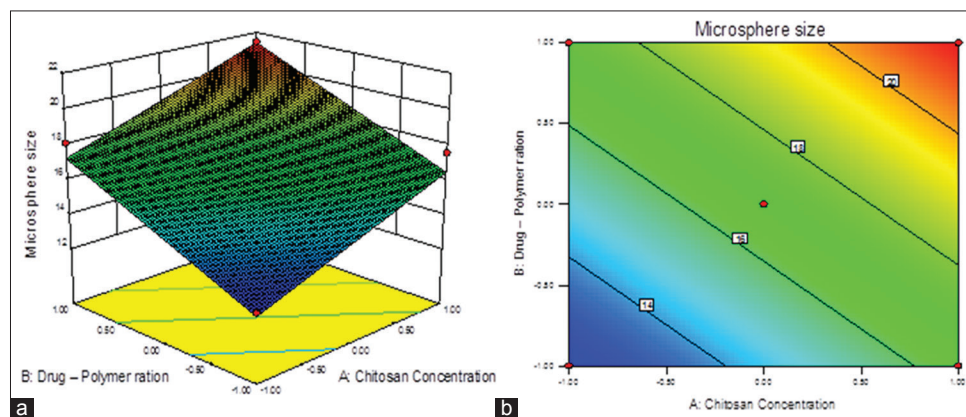
The effects of chitosan concentrations ( $X_1$ ) and drug: Polymer ratios ( $X_2$ ) were studied on the cumulative % drug release after 2 h ( $Y_3$ ), explained with response curve [Figure 4a] and contour plot [Figure 4b] and after 5 h ( $Y_4$ ), explained with response curve [Figure 5a] and contour plot

[Figure 5b]. The *in vitro* drug release studies [Figure 6] in the dissolution medium at pH 1.2 showed fast drug release in the initial 2 h. A release of  $62.32\% \pm 1.74\%$  was observed from the formulation CMF1, which contain 0.5% w/v chitosan concentration and 1:2 drug polymer ratio; whereas a release of  $46.13 \pm 1.23$  was observed from CMF9, which contain 1.5% w/v chitosan concentration and 1:6 drug polymer ratio. Within 5 h, 64%-85% of drug was released from the formulations, these results indicated burst release and observed that with lesser drug-polymer ratio and chitosan concentration shows higher drug release [Table 1].

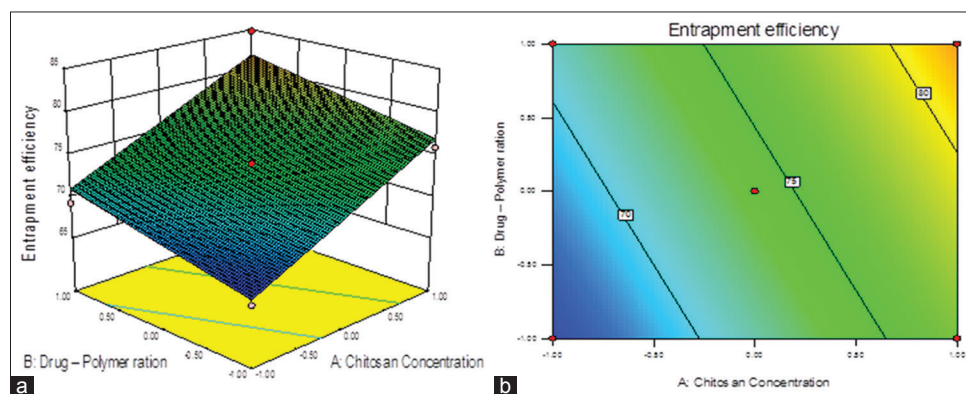
The significance of the various responses was statistically confirmed by ANOVA test,  $P < 0.05$  [Table 3]. All of the variables and their interactions had significant effects. Moreover, the calculated F-values for all the responses concluded that the variables selected contributed significantly in the regression of measured responses.

## CONCLUSION

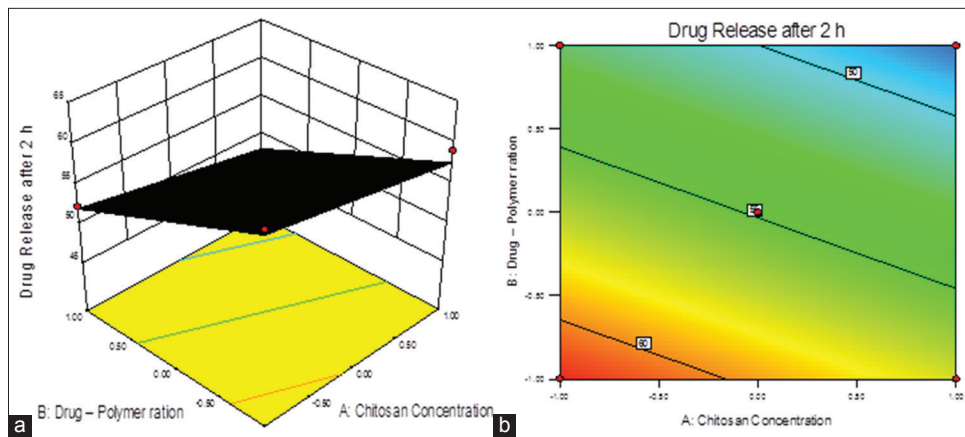
The results confirmed that chitosan microspheres can be optimized and prepared by emulsion cross linking method.



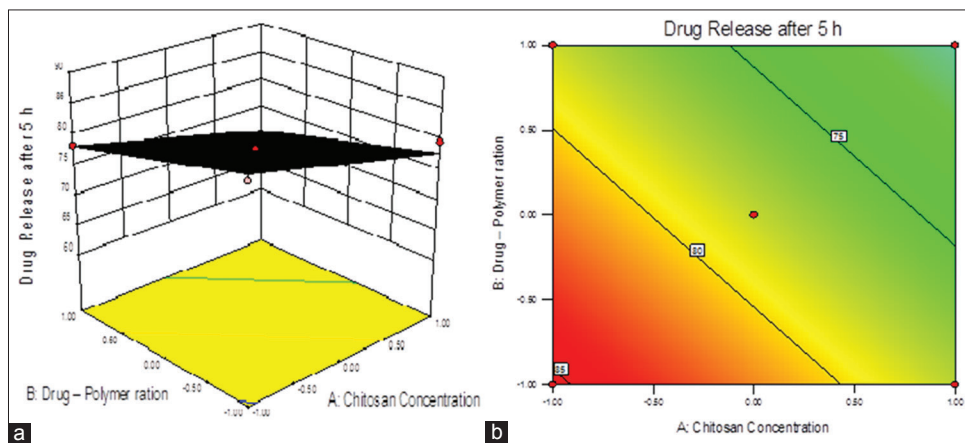
**Figure 2:** Effect of chitosan concentration and drug: Polymer ratio on microsphere size. (a) Response surface curve, (b) contour plot



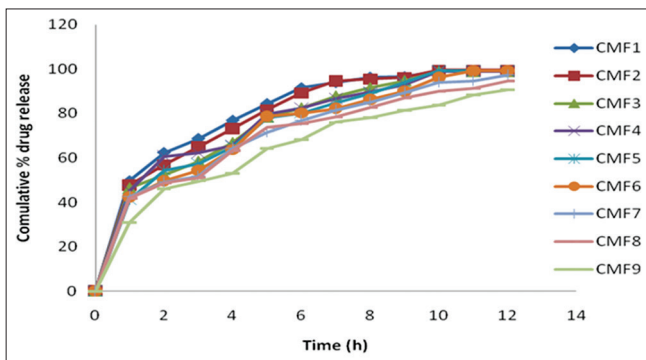
**Figure 3:** Effect of chitosan concentration and drug: Polymer ratio on % entrapment efficiency. (a) Response surface curve, (b) contour plot



**Figure 4:** Effect of chitosan concentration and drug: Polymer ratio on % drug release after 2 h. (a) Response surface curve, (b) contour plot



**Figure 5:** Effect of chitosan concentration and drug: Polymer ratio on % drug release after 5 h. (a) Response surface curve, (b) contour plot



**Figure 6:** Cumulative % drug release profile of chitosan microspheres

Application of factorial design demonstrates the useful method of optimization of microspheres as chitosan concentration and drug/polymer ratios affected the microspheres characteristics while keeping the other variables constant. High drug release in stomach and small intestine is not satisfactory for a formulation, which is supposed to release its contents in the colon. The burst release may be due to the solubility of chitosan in the acidic pH. In order to prevent the drug release in stomach and small intestine need to encapsulate, these

**Table 3:** Statistical analysis data for measured response

Coefficient	Microsphere size	Entrapment efficiency	Drug release after 2 h	Drug release after 5 h
Significance	0.0001	0.0003	0.0001	0.0234
F- value	82.38	40.01	61.15	7.49
R <sup>2</sup>	0.9649	0.9303	0.9532	0.7140

chitosan microspheres. As per the result shown, batch CMF9 was selected as optimum formulation for further studies like microencapsulation of microspheres and its evaluation.

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