ORIGINAL ARTICLE

# Design, Optimization, and Evaluation of Capecitabine-loaded Chitosan Microspheres for Colon Targeting

**Goutam Kumar Jena[1], Sunit Kumar Sahoo[2], Ch. Niranjan Patra[1], Kahnu Charan Panigrahi[1], Swatilekha Sahu[1], Prasanna Kumar Dixit[3]**

*[1]Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, Khodashingi, Berhampur - 10, Ganjam, Odisha, India, [2]University Department of Pharmaceutical Sciences (UDPS), Utkal University, Vani Vihar, Bhubaneswar, Odisha, India, [3]Department of Zoology, Berhampur University, Bhanja Bihar, Berhampur, Ganjam, Odisha, India*

## Abstract

**Aims:** Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of advanced stage of colorectal cancers (CRC). The present research was to formulate and optimize capecitabine-loaded microspheres for CRC targeting to enhance bioavailability, reduce dose, minimize side effect, and sustain drug release for 24 h. **Materials and Methods**: Capecitabine-loaded microspheres were prepared by emulsion solvent evaporation method. Nine formulations of microspheres with different ratios of capecitabine and chitosan were prepared. A central composite design with design expert software version 10.0.3.1 was employed in formulating and optimizing the microspheres to maximize entrapment efficiency and minimize particle size. The optimized microspheres were coated with Eudragit S100, a pH sensitive polymer and were evaluated. **Results and Discussion**: Fourier transform infrared spectroscopy study revealed the compatibility of drug with excipients while differential scanning calorimetry study confirmed the complete drug entrapment in polymer matrix and scanning electron microscopy revealed spherical shape of microspheres. The release profile of capecitabine from Eudragit S100-coated chitosan microspheres was found to be pH dependent. *In vitro* dissolution studies of Eudragit S100-coated microspheres revealed negligible released in simulated gastric as well as intestinal fluid, followed by 100% released in simulated colonic fluid, in 24 h. The optimized microspheres showed colon-specific controlled release properties, and thus could be effective for CRC treatment. **Conclusion:** Capecitabine-loaded microspheres can be prepared using chitosan and Eudragit S100 as sustained release with mucoadhesion and pH sensitive polymer, respectively, for CRC targeting.

**Key words:** Capecitabine, central composite design, Eudragit S100, mucoadhesion, pH-dependent release

## INTRODUCTION

Capecitabine is a novel oral anticancer drug that is mostly referred for the treatment of advanced stage of colorectal cancer (CRC). It is converted to the cytotoxic moiety fluorouracil in target tumor tissue by thymidine phosphorylase.[1] When capecitabine is administered orally at dose of 1250 mg/m$^2$ it is rapidly and extensively absorbed from gastrointestinal tract. It has a relatively short elimination half-life ($t_{1/2}$) of 0.55-0.89 h. CRC is a major cause of death worldwide. It is a heterogeneous disease that occurs in the colon and rectum, which are parts of the gastrointestinal tract. The colon has four sections, namely, ascending, transverse, descending, and sigmoid colon. Most CRC arise from sigmoid colon and develop slowly from adenomatous polyps or adenomas.[2] Colon targeting is most challenging for the orally administered drugs which are degraded by digestive enzymes of the stomach and small intestine.[3] Colon targeting may improve local concentration of drug in colon region to a level which is not feasible by unmodified oral drug delivery. This may improve efficacy of drug treatment and open up the possibility to switch to oral

**Address for correspondence:**
Goutam Kumar Jena,
Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, Engineering School Road, Berhampur, Ganjam - 760 010, Odisha, India.
E-mail: goutam2902@gmail.com

**Received:** 14-07-2017
**Revised:** 11-08-2017
**Accepted:** 16-08-2017

instead of parenteral administration.[4] Targeted drug delivery to the colon is highly desirable for the local treatment of variety of bowel diseases such as ulcerative colitis, cirrhosis disease, amebiasis, and local treatment of CRC. There are several approaches, which are utilized in achieving colon targeting such as use of pH-sensitive polymers, time-dependent drug release, bacterial degrading coating materials, biodegradable polymer matrix, hydrogels, and prodrugs.[5]

Targeted delivery to the colon is an attempt to delay the release of the drug in the GI tract to achieve a high local concentration while reducing the dose, and thus undue side effects of the drug. Researchers have reported alkaline pH (7.0-8.0) of colonic contents in patients suffering from CRC.[6-8] These specific pH conditions of the colonic region may enhance the chances to successfully deliver the drug to the region by developing a suitable pH-dependent system. In addition, increased residence time of the formulation in colon will significantly improve the therapeutic efficacy of selected drug. Among the various attempts made to increase the retention of an oral dosage form, it seems that mucoadhesive systems are preferred because of their effectiveness to maintain the desired drug concentration in the targeted site, inhibiting the dilution of drugs in the body fluids and allowing targeting and localization of drugs at a specific site.[9] In comparison to the single-unit systems, which are characterized by an all-or-nothing process, the multiple-unit dosage forms (microspheres) have been shown to reduce inter- and intra-subject variability. Chitosan (derived from chitin) is a unique biopolymer that exhibits outstanding properties, beside biocompatibility, and biodegradability. Most of these peculiar properties arise from the presence of primary amines along the chitosan backbone.[10,11] To extend the release of drug, mucoadhesion, and residence time of microspheres in colon; chitosan can be used as a polymer of choice. The pH-dependent release systems can be prepared using suitable grade of Eudragit.[12,13]

Mucoadhesive microparticles coated with a pH-dependent polymer are proposed to initiate the release of the drug at the putative colonic pH 7-8. Hence, the objective of the present research is to develop and optimize capecitabine microspheres for colon targeting by central composite design using Eudragit S100 as pH sensitive polymer, chitosan as mucoadhesive, and sustain-release polymer.

## MATERIALS AND METHODS

Capecitabine was received as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India. Eudragit S100 was procured from Evonik India Pvt. Ltd., Mumbai, India, Chitosan was procured from HiMedia, India. Solvents and reagents used were of laboratory reagent (LR) grade.

### Method of preparation of microspheres

Microspheres of capecitabine in varying drug polymer ratios ($F_1$-$F_9$), as shown in Table 1, were prepared following previously published methods with slight modification.[14-16] In a typical experiment, a defined quantity of capecitabine was dispersed in aqueous solutions of 1% v/v acetic acid of varying concentrations of chitosan in accordance with a drug polymer ratio as in Table 1. Subsequently, the dispersion was emulsified in light liquid paraffin containing 2% v/v tween 80, with the help of a mechanical stirrer (Remi Instruments Ltd, Mumbai, India) at 1500 rpm for 30 min. Approximately 1.5 ml of the toluene saturated glutaraldehyde was then added to the emulsion and then left for cross-linking and stabilization for 6 h. Then, emulsion was centrifuged at 4000 rpm and the sediment was washed with petroleum ether thrice for removing the residual liquid paraffin. Microspheres were then dried in hot air oven at 50°C and kept in desiccator until further used.

### Method of coating of microspheres

Selected formulations of core microspheres were coated with Eudragit S100 with core coat ratio of 1:4 by emulsion solvent evaporation method. Thus, core microspheres were dispersed in Eudragit S100 solution (10% w/v) in methanol at room temperature followed by emulsification in light liquid paraffin containing 1% tween 80 in a beaker, with the help of a mechanical stirrer (1500 rpm). Stirring was continued for 3 h at room temperature to evaporate the solvent completely. Encapsulated microspheres were filtered and washed with petroleum ether to remove the liquid paraffin and dried in hot air oven at 50°C and kept in desiccator until further used.[17]

### Experimental design

To design the colon-specific microspheres, it was essential to recognize the major parameters in the formulation since these variables could affect the properties of the desired formulation. Hence, for optimization of the formulation, a concept of design of experiments was used.[18] Different batches of microspheres were planned based on central composite design (Design Expert®, Version 10.0.3.1, Stat-Ease Inc., USA). For these experiments, three factors were evaluated, each at two levels, and experimental trials were performed for all nine possible combinations. The independent variables selected were the concentration of chitosan (A), concentration of tween 80 (B), and the stirring speed (C). Percent drug entrapment efficiency (Y1), particle size (Y2), and extended period of drug release (Y3) were taken as response parameters (dependent variables). The design layout for capecitabine-loaded microspheres for optimization was given in Table 2. The lack of fit tests and model summary statistics were given in Tables 3 and 4, respectively. AVOVA for response surface Quadratic model for responses R1, R2, and R3 were given in Tables 5-7, respectively. The three-dimensional (3D) response surface curves were given in Figure 1.

**Table 1:** Formulation for microspheres

| Formulation code | Capecitabine (CAP) (mg) | Chitosan (mg) | CAP: Chitosan | Tween 80 (ml) | Glutaraldehyde (ml) |
|---|---|---|---|---|---|
| F1 | 100 | 400 | 1:4 | 0.5 | 0.5 |
| F2 | 100 | 400 | 1:4 | 1 | 1 |
| F3 | 100 | 400 | 1:4 | 1.5 | 1.5 |
| F4 | 100 | 600 | 1:6 | 0.5 | 0.5 |
| F5 | 100 | 600 | 1:6 | 1 | 1 |
| F6 | 100 | 600 | 1:6 | 1.5 | 1.5 |
| F7 | 100 | 800 | 1:8 | 0.5 | 0.5 |
| F8 | 100 | 800 | 1:8 | 1 | 1 |
| F9 | 100 | 800 | 1:8 | 1.5 | 1.5 |

**Table 2:** Design layout of capecitabine microspheres for optimization

| Run | $X_1$ (Chitosan) | $X_2$ (tween 80) | $X_3$ (RPM) | $Y_1$ (%EE) | $Y_2$ (particle size [μm]) | $Y_3$ (time period) |
|---|---|---|---|---|---|---|
| 1 | 600 | 1 | 2500 | 61 | 198 | 16 |
| 2 | 600 | 1 | 2000 | 64 | 201 | 16 |
| 3 | 600 | 1 | 2000 | 63 | 200 | 18 |
| 4 | 600 | 1.5 | 2000 | 62 | 202 | 17 |
| 5 | 600 | 0.5 | 2000 | 63 | 203 | 24 |
| 6 | 400 | 0.5 | 1500 | 58 | 188 | 16 |
| 7 | 400 | 1.5 | 2500 | 56 | 186 | 16 |
| 8 | 800 | 1.5 | 1500 | 70 | 210 | 24 |
| 9 | 800 | 0.5 | 2500 | 71 | 210 | 24 |
| 10 | 400 | 1 | 2000 | 59 | 185 | 16 |
| 11 | 600 | 1 | 2000 | 62 | 200 | 16 |
| 12 | 800 | 1 | 2000 | 72 | 208 | 24 |
| 13 | 600 | 1 | 2000 | 68 | 191 | 16 |
| 14 | 600 | 1 | 1500 | 65 | 189 | 14 |
| 15 | 600 | 1 | 2000 | 67 | 187 | 12 |

**Table 3:** Lack of fit tests

| Source | Sum of squares | Df | Mean squares | F value | *P* value *P*>F | Interpretation |
|---|---|---|---|---|---|---|
| Linear | 18.63 | 7 | 2.66 | 0.40 | 0.8646 | Suggested |
| 2FI | 14.38 | 4 | 3.60 | 0.54 | 0.7193 | |
| Quadratic | 2.17 | 1 | 2.17 | 0.32 | 0.6001 | |
| Cubic | 0.000 | 0 | | | | Aliased |
| Pure error | 26.80 | 4 | 6.70 | | | "Lack of fit tests": Want the selected model to have insignificant lack-of-fit |

## Characterization of capecitabine microspheres

### *Drug-polymer interaction by Fourier transform infrared (FT-IR) study*

Drug-polymer interactions were studied using FT-IR spectroscopy. The studies were performed for free drug, blank microspheres, and drug-loaded microspheres. The FT-IR spectra of the pellets, obtained by pressing the sample-potassium bromide powder mixture by a press, were recorded using an alpha FT-IR spectrophotometer (Bruker Optik GmBH, Germany). The FT-IR spectra were given in Figure 2.

**Table 4:** Model summary statistics

| Source | SD | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Press | Interpretation |
|---|---|---|---|---|---|---|
| Linear | 2.03 | 0.8575 | 0.8187 | 0.7881 | 67.57 | Suggested |
| 2FI | 2.27 | 0.8709 | 0.7740 | 0.6679 | 105.93 | |
| Quadratic | 2.41 | 0.9092 | 0.7457 | –0.6395 | 522.90 | |
| Cubic | 2.59 | 0.9160 | 0.7059 | | + | Aliased+Case (s) with leverage of 1.0000: PRESS statistic not defined<br>"Model summary statistics": Focus on the model maximizing the "adjusted $R^2$" and the "predicted $R^2$" |

**Table 5:** ANOVA for response surface quadratic model for R1 (EE)

**Analysis of variance table [partial sum of squares - Type III]**

| Source | Sum of squares | df | Mean square | F value | *P* value *P*>F | Interpretation |
|---|---|---|---|---|---|---|
| Model | 289.97 | 9 | 32.22 | 5.56 | 0.0367 | Significant |
| A-Chitosan | 84.50 | 1 | 84.50 | 14.59 | 0.0124 | |
| B-Tween 80 | 0.50 | 1 | 0.50 | 0.086 | 0.7807 | |
| C-RPM | 8.00 | 1 | 8.00 | 1.38 | 0.2928 | |
| AB | 4.08 | 1 | 4.08 | 0.70 | 0.4394 | |
| AC | 0.083 | 1 | 0.083 | 0.014 | 0.9092 | |
| BC | 0.083 | 1 | 0.083 | 0.014 | 0.9092 | |
| $A^2$ | 6.12 | 1 | 6.12 | 1.06 | 0.3512 | |
| $B^2$ | 5.66 | 1 | 5.66 | 0.98 | 0.3685 | |
| $C^2$ | 2.46 | 1 | 2.46 | 0.43 | 0.5431 | |
| Residual | 28.97 | 5 | 5.79 | | | |
| Lack of fit | 2.17 | 1 | 2.17 | 0.32 | 0.6001 | Not significant |
| Pure error | 26.80 | 4 | 6.70 | | | |
| Cor total | 318.93 | 14 | | | | |

**Table 6:** ANOVA for response surface quadratic model of $R^2$ (particle size)

**Analysis of variance table (partial sum of squares - Type III)**

| Source | Sum of squares | df | Mean square | F value | *P* value *P*>F | Interpretation |
|---|---|---|---|---|---|---|
| Model | 935.34 | 9 | 103.93 | 3.15 | 0.1098 | Not significant |
| A-Chitosan | 264.50 | 1 | 264.50 | 8.01 | 0.0366 | |
| B-Tween 80 | 0.50 | 1 | 0.50 | 0.015 | 0.9068 | |
| C-RPM | 40.50 | 1 | 40.50 | 1.23 | 0.3184 | |
| AB | 33.33 | 1 | 33.33 | 1.01 | 0.3611 | |
| AC | 0.000 | 1 | 0.000 | 0.000 | 1.0000 | |
| BC | 0.000 | 1 | 0.000 | 0.000 | 1.0000 | |
| $A^2$ | 0.057 | 1 | 0.057 | 1.713E-003 | 0.9686 | |
| $B^2$ | 89.60 | 1 | 89.60 | 2.71 | 0.1604 | |
| $C^2$ | 25.90 | 1 | 25.90 | 0.78 | 0.4163 | |
| Residual | 165.06 | 5 | 33.01 | | | |
| Lack of fit | 2.26 | 1 | 2.26 | 0.055 | 0.8253 | Not significant |
| Pure error | 162.80 | 4 | 40.70 | | | |
| Cor total | 1100.40 | 14 | | | | |

**Table 7:** ANOVA for response surface quadratic model of $R^3$ (extended time period)

**Analysis of variance table (partial sum of squares - Type III)**

| Source | Sum of squares | df | Mean square | F value | *P* value *P*>F | Interpretation |
|---|---|---|---|---|---|---|
| Model | 198.48 | 9 | 22.05 | 4.17 | 0.0652 | Not significant |
| A-Chitosan | 32.00 | 1 | 32.00 | 6.05 | 0.0573 | |
| B-Tween 80 | 24.50 | 1 | 24.50 | 4.63 | 0.0840 | |
| C-RPM | 2.00 | 1 | 2.00 | 0.38 | 0.5655 | |
| AB | 1.33 | 1 | 1.33 | 0.25 | 0.6370 | |
| AC | 16.33 | 1 | 16.33 | 3.09 | 0.1392 | |
| BC | 2.842E-014 | 1 | 2.842E-014 | 5.373E-015 | 1.0000 | |
| $A^2$ | 21.73 | 1 | 21.73 | 4.11 | 0.0985 | |
| $B^2$ | 29.92 | 1 | 29.92 | 5.66 | 0.0633 | |
| $C^2$ | 11.73 | 1 | 11.73 | 2.22 | 0.1967 | |
| Residual | 26.45 | 5 | 5.29 | | | |
| Lack of fit | 7.25 | 1 | 7.25 | 1.51 | 0.2864 | Not significant |
| Pure error | 19.20 | 4 | 4.80 | | | |
| Cor total | 224.93 | 14 | | | | |

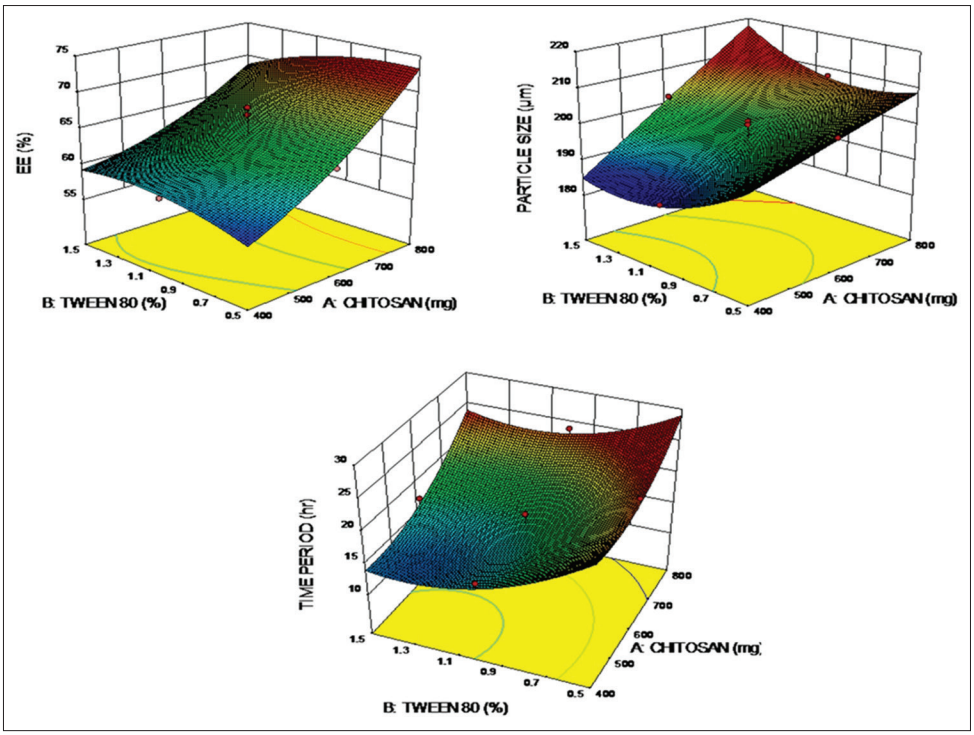


**Figure 1:** Response surface plot (three dimensional) showing effect of Tween 80 and chitosan concentration on; (A) % entrapment

## Differential scanning calorimetry (DSC)

Thermal analysis of pure drug capecitabine ad selected microspheres selected microspheres were performed using a DSC-TA system (Perkin Elmer). All samples were sealed in a crimped aluminum pan by application of the minimum possible pressure and heated at a rate of 10°C/min from 40°C to 260°C in a nitrogen atmosphere. An empty aluminum pan was utilized as the reference pan. The DSC spectra were given in Figure 3.

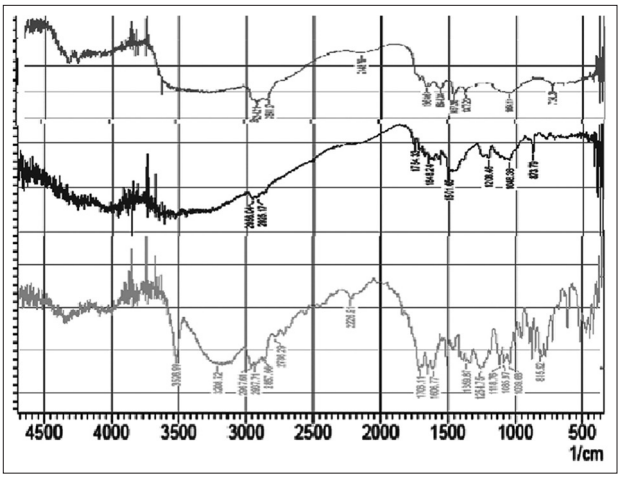


**Figure 2:** Fourier transform infrared spectra of capecitabine, chitosan, and optimized microspheres

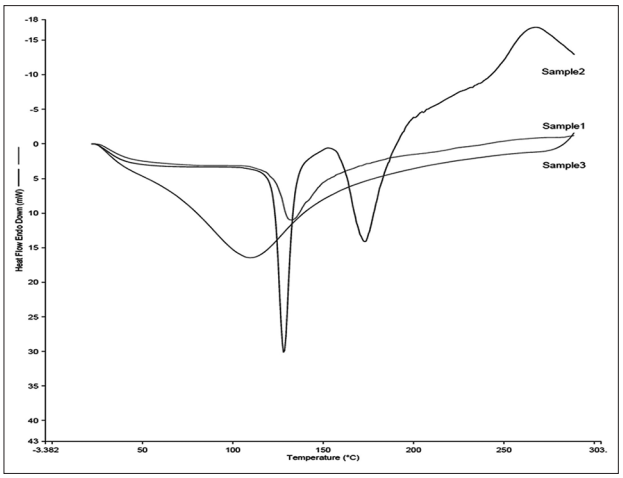


**Figure 3:** Differential scanning calorimetry thermograms of capecitabine, chitosan, and optimized microspheres

## Percentage yield

The microspheres were collected and weighted. The actual weight of microspheres divided by the total amount of all materials that involved in the preparation of the microspheres was calculated.

$$\%\ \text{yield} = \frac{\text{Actual weight of microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

## Micromeritic properties of microspheres

The flow properties of microspheres were investigated by determining the angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio. The angle of repose was determined by the fixed-based funnel method. Bulk and tapped densities were measured in 10 mL of a graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. The volume occupied by the sample after tapping was recorded and bulk density, tapped density, Carr's index, and Hausner's ratio was calculated using the following formula. The calculated data was presented in Table 8.

$$\text{Angle of repose } (ø) = \tan^{-1}(h/r)$$

Where
ø = Angle of repose
h = height of cone
r = average radius of the cone base.

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Bulk volume}}$$

$$\text{Tapped density} = \frac{\text{Weight of sample}}{\text{Tapped volume}}$$

$$\text{Carr's index} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped ensity}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

## Entrapment efficiency

Microspheres containing equivalent to 10 mg of drug was allowed to equilibrate in 100 mL of phosphate buffer pH 7.4 for 24 h. The solution was filtered using Whatman filter paper (44). The resulting solution was analyzed using an ultraviolet (UV) spectrophotometric method at 237 nm in the presence of a blank prepared from microspheres containing all materials except the drug. The percentage drug entrapment efficiency of all formulations was given in Table 9.

$$\%\ \text{Entrapment efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

## Swelling index

The swelling index was studied by measuring percentage water uptake by the microspheres. Accurately weighed 100 mg of microspheres were allowed to swell in phosphate buffer pH 6.8 and pH 7.4 at 37°C for 12 h. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed again. This study was carried out for all batches in triplicate and the swelling indices of all batches were calculated using the following equation.[17] The result of swelling index was given in Table 9.

$$\text{Swellig index} = \frac{[\text{Weight of swollen microspheres} - \text{Initial weight}]}{\text{Initial weight}} \times 100$$

**Table 8:** Flow properties of different formulations

| Formulation code | *Angle of repose | *Bulk density | *Tapped density | *Carr's index | *Hausner's ratio |
|---|---|---|---|---|---|
| F1 | 23.54±1.23 | 0.585±0.00 | 0.725±0.01 | 23.93±1.25 | 1.24±0.00 |
| F2 | 22.56±1.15 | 0.655±0.01 | 0.721±0.01 | 10.07±1.01 | 1.10±0.01 |
| F3 | 22.67±1.02 | 0.678±0.00 | 0.724±0.02 | 6.78±1.28 | 1.06±0.01 |
| F4 | 23.25±1.23 | 0.595±0.01 | 0.726±0.01 | 22.01±1.52 | 1.22±0.00 |
| F5 | 22.55±1.10 | 0.658±0.01 | 0.729±0.01 | 10.79±1.00 | 1.11±0.02 |
| F6 | 21.65±1.05 | 0.679±0.00 | 0.726±0.01 | 6.92±0.01 | 1.06±0.01 |
| F7 | 24.76±1.18 | 0.589±0.01 | 0.728±0.01 | 23.59±1.85 | 1.23±0.01 |
| F8 | 18.45±1.01 | 0.609±0.01 | 0.718±0.01 | 17.89±1.04 | 1.17±0.01 |
| F9 | 22.45±1.05 | 0.615±0.01 | 0.734±0.00 | 19.34±1.28 | 1.19±0.01 |

*Mean±SD, *n*=3

**Table 9:** Results of swelling and mucoadhesion of different batches of uncoated microspheres

| Formulation code | Swelling index in phosphate buffer pH 6.8* | Swelling index in phosphate buffer pH 7.4* | % Mucoadhesion | % Entrapment efficiency |
|---|---|---|---|---|
| F1 | 234±2.55 | 420±3.55 | 72 | 55.55 |
| F2 | 260±2.56 | 480±3.45 | 74 | 63.25 |
| F3 | 280±2.55 | 540±3.58 | 75 | 64.34 |
| F4 | 325±3.55 | 580±3.95 | 78 | 65.22 |
| F5 | 345±2.55 | 610±3.85 | 80 | 73.56 |
| F6 | 348±2.55 | 640±3.95 | 84 | 74.02 |
| F7 | 420±3.55 | 680±3.95 | 86 | 85.76 |
| F8 | 560±3.95 | 820±3.55 | 90 | 86.35 |
| F9 | 580±3.95 | 840±3.55 | 90 | 87.32 |

*Mean±SD, *n*=3

### Mucoadhesivity study by *in vitro* wash-off test

Goat colonic mucosa was used for this study. The mucosa was removed, cut into 2 $cm^2$ pieces, and rinsed with phosphate buffer pH 7.4. Pieces of wet goat colonic mucosa were mounted on glass slides with acrylate glue. One hundred microspheres were counted and spread over the surface of the wet mucosa. The glass slide was then connected to a support and hung on the arm of a USP tablet disintegration test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beaker containing phosphate buffer at pH 6.8. The temperature was maintained at 37±2°C throughout the study. The microspheres still adhering to the tissue were counted at the end of 6 h and percentage mucoadhesion was calculated as per the following equation. The result of mucoadhesivity was given in Table 9.

$$\% \text{ Mucoadhesion} = \frac{\text{Number of adhered microspheres}}{\text{Number of applied microspheres}} \times 100$$

### Particle size and surface morphology analysis

The shape and surface morphology of the microspheres were studied using scanning electron microscopy (SEM). Microspheres were fixed with carbon tape, mounted on metal stubs and then coated with platinum, and keeping the acceleration voltage at 10 kV. Photographs were taken using SEM (6390, Jeol JSM). The SEM of optimized batch was given in Figure 4.

### Drug release profile

#### *Core microspheres*

Accurately weighed core microspheres equivalent to 10 mg of capecitabine were suspended in 20 ml of phosphate buffer pH 7.4 containing 1.5% w/v span 80. The mixture was stirred magnetically at 37°C at 100 rpm. Samples were withdrawn at specified time intervals with replacement of same volume of phosphate buffer 7.4. The withdrawn samples were centrifuged at 2000 rpm; supernatant was filtered through 0.45 µm membrane filter, diluted to 10 ml with phosphate

**Table 10:** Kinetics and mechanism of drug release from optimized formulation (F8) of microspheres

| Release model | Equation | $R^2$ | *n* | Release mechanism |
|---|---|---|---|---|
| Zero order | At=Kt | 0.9879 | | (*n*=0.5): Fickian diffusion |
| 1st order | $\ln[1-\left(\frac{At}{A0}\right)=-Kt$ | 0.8875 | | (0.5<*n* <1.0): Non-Fickian |
| Higuchi | $At=K\sqrt{t}$ | 0.7845 | | Diffusion |
| Hixon–Crowel | $\sqrt[3]{A0}-\sqrt[3]{At=Kt}$ | 0.7985 | | (*n*=1.0): Case II (zero order) |
| Korsmeyer–peppas | $\ln\left(\frac{At}{A0}\right)=\ln K+n\ln t$ | 0.7898 | 2.4512 | (*n*>1.0): Super case II transport |

A0: Initial amount of drug present, At: Amount of drug released at time t, K: Release rate constant, n: Diffusion exponent

buffer pH 7.4, and analyzed for drug content by measuring absorbance at 237 nm in a UV spectrophotometer. All the experiments were conducted in triplicate. The kinetic and drug release mechanism were given in Table 10.

### Coated microspheres

Accurately weighed coated microspheres equivalent to 10 mg of drug were placed in 20 ml 0.01 N Hydrochloric acid (pH 2.0) and stirred magnetically at 50 rpm for 2 h. The samples were centrifuged and supernatant filtered through 0.45 µm membrane filter and analyzed for the drug content as described previously. In a similar experiment, coated microspheres equivalent to 10 mg of drug were placed in 100 ml of phosphate buffer containing 1.5% w/v span 80 and stirred magnetically at 100 rpm. The initial pH of the buffer was maintained at 5.5 for 2 h, which was increased by the addition of $Na_2HPO4$ to 6.8 and maintained for 2 h. Subsequently, the pH of the buffer was raised by further addition of $Na_2HPO4$ to 7.4 and maintained until the completion of study. Hourly, 5 ml of the sample was withdrawn, and each withdrawn sample was replaced with fresh release medium. The samples were centrifuged and the supernatant was passed through a 0.45 µm filter and analyzed for drug content as described previously. All the experiments were run in triplicate. The dissolution profiles of all formulations were given in Figure 5.

### X-ray diffraction (XRD)

X-ray diffractograms of the selected microspheres were recorded using an X-ray diffractometer (Model- Ultima-III Rigaku Make [Japan] Cu target slit 10 mm) using nickel filtered CuKα radiation (λ = 1.540598A°) generated at 40 kV and 30 mA and scanning rate 2°/min over a 2θ range of 10-80°. The XRD spectra were presented in Figure 6.

### Stability studies

To assess accelerated stability, the optimized batch of formulation (F5) was subjected to stability studies as per International Conference on Harmonization guidelines. Coated microspheres were wrapped in aluminum foil laminated on the inside with polyethylene. The samples were kept at 40°C ± 2°C/75% ± 5% RH in a stability chamber (Scope Enterprises, Delhi, India) for 3 months. Samples were withdrawn after an interval of 15 days, 30 days, and 90 days and were analyzed for drug content. The result was presented in Table 11.

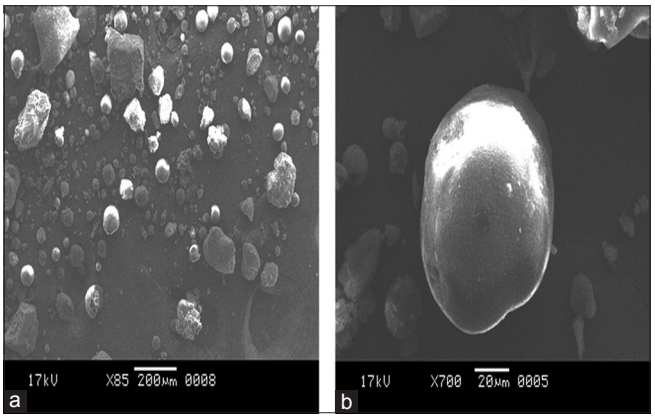


**Figure 4:** (a and b) Scanning electron microscopy images of optimized formulation

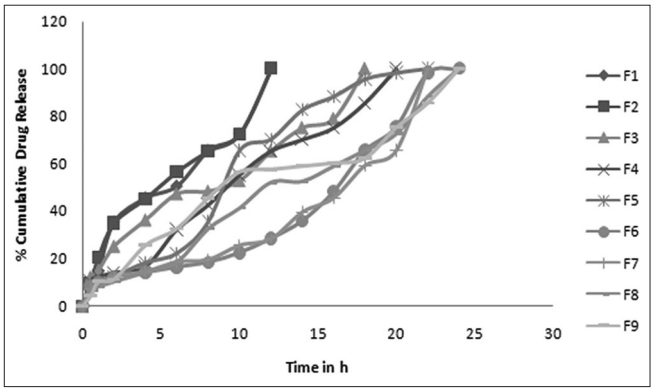


**Figure 5:** *In vitro* drug release profile of all formulations

## RESULTS AND DISCUSSION

Mucoadhesive polymers as carriers that sustain the drug release seem to be the most promising polymers for colonic

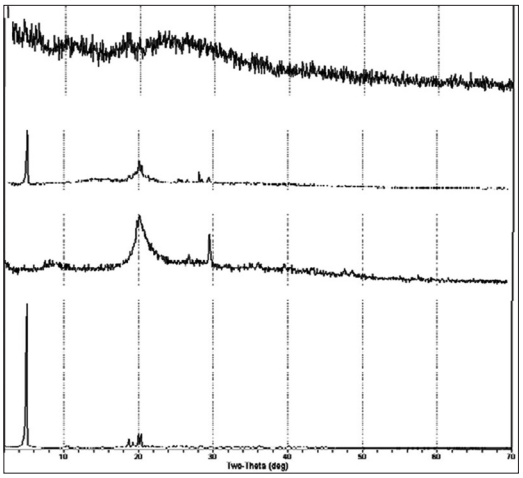


**Figure 6:** X-ray diffraction spectra of capecitabine, chitosan, Eudragit S100, and optimized microspheres

delivery of drugs.[19,20] Among such polymers, Chitosan is widely used natural polymer for mucoadhesion, but it is unable to effectively prevent the drug release during transit through upper gastrointestinal tract.[21] To overcome this difficulty, chitosan polymers are cross-linked using glutaraldehyde as a cross-linking agent, and the microspheres were coated with pH sensitive polymer, that is, Eudragit S100. The Eudragit S100 is an enteric polymer and its threshold pH is 7 which is not soluble in pH below 7. Hence, it does not allow the drug to release in the stomach and upper gastrointestinal tract but allows the drug release in pH 7 and above, that is, in colorectal region only. Hence, Eudragit S100-coated chitosan microspheres can be used as carrier for capecitabine in the treatment of CRC. For measuring the responses, three parameters were selected percentage EE and percentage drug release and particle size of microspheres. The 3D surface-response curves were given in Figure 1.

The influences of both factors on the responses were elucidated by the response surface method. This method is a widely used approach during development and optimization of formulations to study the effects of factors and their levels on a model to predict the responses within the domain. The response surface method generally gives two-dimensional contour graphs and 3D response surface graphs. The latter is more useful for understanding main effects and the interaction effects of the factors on responses.[22,23] Therefore, 3D graphs were generated for both responses. In case of Y1, it was found that on increasing chitosan concentration, the entrapment efficiency increased up to an optimum value, and then with further increase in chitosan concentration, it decreased. Due to cross-linking of chitosan by glutaraldehyde, a network of chitosan is formed which maximize the drug entrapment. In addition, electrostatic attraction between the negatively charged capecitabine and the positively charged chitosan also becomes stronger, promoting the drug entrapment.[24]

As shown by the response surface plot for $Y_2$ [Figure 1] chitosan concentration was increased, the percentage drug release decreased. To obtain the optimized formulation, numerical optimization was performed in the software. Desirability was fed into the software as constraints for the responses. The optimum formulation was based on set criteria of maximum drug entrapment and minimum drug release. Figure 1 shows the desirability of the optimized formulation predicted by the software; the predicted values were 1:10 ratio of capecitabine and chitosan and 1% glutaraldehyde concentration. This new batch of microspheres was formulated as per the same procedure and the responses were measured. The observed values of the responses are shown in Table 2 along with the percentage error to validate the method. The observed values were close to the predicted values of the software which proves the validity of the optimization method of the software. The results of micromeritic studies indicated that an increase in chitosan concentration led to an increase in particle size. The morphological evaluation of the optimized batch was done by SEM analysis. These images confirmed that the formulated microspheres were spherical in shape with a relatively smooth surface texture. The chemical compatibility between capecitabine and the other formulation components (excipients) was ensured using an FT-IR study. In the spectrum of capecitabine, the major peaks assigned to capecitabine confirmed the peak at 3526.99 $cm^{-1}$, 2967.61 $cm^{-1}$,1706.11 $cm^{-1}$, 1606.77 $cm^{-1}$, 1510 $cm^{-1}$, and 1320-1359.81 $cm^{-1}$ due to the presence of O-H (stretching), N-H (stretching), N-C=O-N (IMIDE) and (C=O, stretching) NH-C=O, C=O (stretching, amide), NH (bending), and C-N (stretching), respectively. It was confirmed that the peak of 3208 $cm^{-1}$ became wider and flatter, indicating that hydrogen bond was enhanced.[23] These peaks were absent in the blank beads while they were found in the drug-loaded beads. This confirms that there was no chemical interaction found between the drug and polymers, thus confirming the drug compatibility with these excipients. Thermal analysis of the capecitabine bulk powder and capecitabine-loaded beads were conducted using DSC. These experiments measure the heat gain or loss from chemical or physical changes within a sample as a function of temperature. In the DSC thermogram of capecitabine, there was a sharp endothermic peak at 128.08°C which nearly corresponded to the melting point of capecitabine (118-121°C). This peak was absent in the thermogram of the loaded beads formulation, confirming the complete drug entrapment in the polymer matrix. Swelling of the microspheres was studied and the swelling index was calculated for all batches. When the microspheres come in contact with aqueous media, the polymers imbibed water and swelled as a result of the presence of physical-chemical cross-links in the hydrophilic polymer network. These cross-links prevented the dissolution of the polymers, thus maintaining the physical integrity of the microspheres. The swelling behavior of the polymers in the microspheres has been reported as one of the significant factors for controlling the drug release in drug delivery systems.[25] An optimum

**Table 11:** Accelerated stability predicted data of optimized formulation

| Time (months) | Drug content | | % Cumulative drug release at 12th h | |
|---|---|---|---|---|
| | 25±2°C/60±5% RH | 40±2°C/75±5% RH | 25±2°C/60±5% RH | 40±2°C/75±5% RH |
| 0 | 63.50 | 62.95 | 52.45 | 51.52 |
| 1 | 60.54 | 61.65 | 51.54 | 50.43 |
| 2 | 59.85 | 60.55 | 51.05 | 49.88 |
| 3 | 58.65 | 59.45 | 50.55 | 48.55 |

amount of cross-linking is required to maintain a balance between swelling and dissolution.[26]

Moreover, the swelling behavior of the polymers in the beads depends on the pH, ionic strength, and ionic composition of the medium.[27] Therefore, swelling studies were carried out in different pH conditions. It was found that an increase in chitosan concentrations in the microspheres resulted in decreased swelling. The reason may be that the swelling of dry microspheres is mainly attributed to hydration of hydrophilic groups of the chitosan. In higher pH (pH 7.4 media), significantly higher swelling was found for all batches as compared to pH 6.8 (Table 5). Chitosan exhibits properties of adhesion with mucous membranes, and hence a mucoadhesivity study was performed for all formulation batches and mucoadhesion was found to be 90% for the optimized batch. The basis of the mucoadhesion could be described in terms of electronic theory; electron transfer occurred between the positively charged chitosan and the negatively charged mucus glycoprotein network. This led to formation of an electrical double layer that resulted in adherence to the microspheres for longer time. Moreover, increased polymer concentrations resulted in increased viscosity of the gel that was formed and ultimately led to higher adhesion. This helps in release of the drug in a sustained manner before the microspheres were eroded away.[28]

The formulated microspheres were tested in an *in vitro* dissolution study under conditions mimicking the stomach, small intestine, and colon to evaluate the potential of this formulation for colon-specific drug delivery.[29] The results showed that due to the enteric microspheres, drug release was effectively suppressed in SGF pH 1.2 with no release. Further, in SIF pH 6.8, the enteric-coated microspheres started to dissolve and the microspheres came in contact with the physiological fluid with near neutral pH. This release might be due to the presence of drug on the outer surface of the microspheres. Subsequently, controlled release was observed up to 24 h for batches containing higher amounts of chitosan. This was expected since chitosan reacts with glutaraldehyde and gets more cross-linked. At physiological pH, some of the amine groups might be deprotonated and those that remain protonated acts as a barrier for the penetration of media and drug release. This resulted in drug release at a slow rate, and hence higher chitosan led to controlled drug release. The release of drug followed zero order and super case II transport mechanisms (Table 11). In the case of the currently marketed sample, the tablet released up to 99.62% of the drug within an hour due to the absence of any matrix or other system that could control the release. The result of the stability studies showed that there was no significant change in percentage drug content and *in vitro* drug release of capecitabine-loaded microspheres between 0 and 3 months of storage before the tests (Table 9), indicating the developed formulations were stable and the XRD study of pure capecitabine, chitosan, Eudragit S100, and microspheres revealed that coated microspheres exhibited neither crystallinity of polymers nor drug.

## CONCLUSIONS

To target colon and rectum associated with sustained drug release for 24 h, microspheres were successfully prepared and optimized with maximum drug entrapment and minimum particle size. The optimized microspheres coated with Eudragit S100, prevented drug release both in the stomach and small intestines evidenced by no drug release in 0.1 N HCl and SIF pH 6.8. The formulation with capecitabine and chitosan in the ratio of 1:10 and glutaraldehyde concentration of 1% w/v was considered optimum based on their most desirable *in vitro* characteristics, namely, 62.5% entrapment efficiency and 100% drug release in phosphate buffer pH 7.4 in 24 h. The results suggested the suitability of the capecitabine-loaded microspheres as a colon-targeted delivery system. However, pharmacokinetic, targeting, and cytotoxicities studies are still needed to give us a better idea of the performance of the formulated microspheres *in vivo*.

## ACKNOWLEDGMENT

All authors are thankful to Roland institute of pharmaceutical sciences, Ambapua, Khodasingi, Berhampur 10, Ganjam, Odisha, India, for providing necessary facilities to carry out the research work. Authors are sincerely thankful to Jadavpur University, Kolkata, West Bengal, for providing facilities for SEM, XRD in sampling.

## REFERENCES


1. Arunprasath B, Sreenivas SA, Kousar S, Sailaja S. Capecitabine microspheres-targeted drug delivery of colorectal cancer. Int J Life Biotechnol 2015;1:62-8.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al*. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-6.
4. Cohen A, Minsky B, Schilsky P. Colorectal cancer, in cancer: Principles and Practice of Oncology. In: DeVita VT, Hellman S, Rosenberg SA, editors. Philadelphia, PA: Lippincott Williams & Wilkins; 2005. p. 929-77.
5. Sharma S, Saltz LB. Oral chemotherapeutic agents for colorectal cancer. Oncologist 2000;5:99-107.
6. DeMario MD, Ratain MJ. Oral chemotherapy: Rationale and future directions. J Clin Oncol 1998;16:2557-67.
7. Rao RN, Kumar MS, Kumar KD, Vineeth P. Formulation and evaluation of capcitabine microspheres. Int J Pharm Technol 2011;3:2598-632.
8. Latha A, Selvamani P, Naveenkumar P, Ayyanar P, Silambarasi T. Formulation and evaluation of capecitabine nanoparticles for cancer therapy. Int J Biol Pharmaceut Res 2012;3:477-87.
9. Chowdary KP, Rao YS. Design and *in vitro* and *in vivo* evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. AAPS PharmSciTech 2003;4:E39.
10. Agnihotri SA, Aminabhavi TM. Novel interpenetrating network chitosan poly(ethylene oxide-g-acrylamide) hydrogel microspheres for the controlled release of capecitabine. Int J Pharm 2006;324:103-15.
11. Laroui H, Dalmasso G, Nguyen HT, Yan Y, Sitaraman SV, Merlin D. Drug loaded nanoparticles targeted to the colon with polysaccharide hydrogel reduce colitis in a mouse model. Gastroenterology 2010;138:843-53.
12. Kumar P, Mishra B. Colon targeted drug delivery systems – An overview. Curr Drug Deliv 2008;5:186-98.
13. Van den Mooter G. Colon drug delivery. Expert Opin Drug Deliv 2006;3:111-25.
14. Calis S, Arica B, Kas HS, Hincal AA. 5-Fluorouracil-loaded alginate microspheres in chitosan gel for local therapy of breast cancer. Chitosan in Pharmacy and Chemistry. In: Muzzarelli RA, Muzzarelli C, editors. Grottammare, Italy: Atec; 2002. p. 65-9.
15. Rahman Z, Kohli K, Khar RK, Ali M, Charoo NA, Shamsher AA. Characterization of 5-fluorouracil microspheres for colonic delivery. AAPS PharmSciTech 2006;7:E47.
16. Thakral NK, Ray AR, Majumdar DK. Eudragit S-100 entrapped chitosan microspheres of valdecoxib for colon cancer. J Mater Sci Mater Med 2010;21:2691-9.
17. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery. Int J Pharm 2001;224:19-38.
18. Ahuja M, Yadav M, Kumar S. Application of response surface methodology to formulation of ion tropically gelled gum cordia/gellan beads. Carbohydr Polym 2010;80:161-7.
19. Sriamornsak P. Investigation of pectin as a carrier for oral delivery of proteins using calcium pectinate gel beads. Int J Pharm 1998;169:213-20.
20. Shu XZ, Zhu KJ. Controlled drug release properties of ionically cross-linked chitosan beads: The influence of anion structure. Int J Pharm 2002;233:217-25.
21. Aydin Z, Akbuga J. Preparation and evaluation of pectin beads. Int J Pharm 1996;137:133-6.
22. Rajesh KS, Khanrah A, Biswanath S. Release of ketoprofen from alginate microparticles containing film forming polymers. J Sci Ind Res 2003;62:985-9.
23. Iliescu RI, Andronescu E, Ghitulica CD, Voicu G, Ficai A, Hoteteu M. Montmorillonite-alginate nanocomposite as a drug delivery system - Incorporation and *in vitro* release of irinotecan. Int J Pharm 2014;463:184-92.
24. Olukman M. Release of anticancer drug 5-fluorouracil from different ionically cross linked alginate beads. J Biomater Nanobiotechnol 2012;3:469-79.
25. Kobayashi M, Sakane M, Abe T, Ikoma T, Ochinai N. Anti-tumor effect of a local delivery system; hydroxyapatite-alginate beads of paclitaxel. Bioceram Dev Appl 2012;2:1-3.
26. Deming SN, Morgan SL. Data handling in science and technology. Experimental Design: A Chemometric Approach. 2nd ed., Vol. XV. Amsterdam, New York: Elsevier; 1993. p. 437.
27. Takka S, Gurel A. Evaluation of chitosan/alginate beads using experimental design: Formulation and *in vitro* characterization. AAPS Pharm SciTech 2010;11:460-6.
28. Prashanthi D, Raj Kumar B, Nagarajan G, Methusala RJ, Prasanthi A. A new analytical method development and validation of capecitabine in pure and tablet dosage forms by UV spectroscopy. Int J Med Nanotechnol 2014;1:48-53.
29. Liu L, Fishman ML, Kost J, Hicks KB. Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials 2003;24:3333-43.


**Source of Support:** Nil. **Conflict of Interest:** None declared.