Preparation and Characterization of Crosslinked Chitosan Microspheres for the Colonic Delivery of 5-Fluorouracil

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ABSTRACT: In this work, we attempted to develop a simple and inexpensive colon specific pulsatile drug-delivery system using chitosan microspheres loaded with 5-fluorouracil (5-FU) using an enteric-coated soft gelatin capsule. Chemical crosslinking by glutaraldehyde and interactions between the polymer and the drug were determined by Fourier transform infrared spectral study. Scanning electron microscopy of the microspheres revealed spherical shapes with smooth surfaces. Differential scanning calorimetry studies confirmed the molecular dispersion of the drug in the polymer matrix. Three different formulations (i.e., F1, F2, and F3) were prepared by the variation of the amount of 5-FU. Encapsulation efficiencies of 5.5, 10.8, and 17.9% for drug loadings of 10, 20, and 50%, respectively, were obtained. *In vitro* release studies were conducted at pH 1.2 and pH 7.4 (to simulate actual gastrointestinal fluid and gastrointestinal tract conditions, respectively). The results indicate that chitosan microspheres released 5-FU in both acidic (60%) and basic pH (40%) conditions, whereas the capsule (filled with chitosan microspheres) showed only 8–10% release in acidic media and nearly 90% in basic media within 12 h. The newly designed pulsatile capsule device could be used for targeting 5-FU to the colon. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 1736–1744, 2012

Key words: differential scanning calorimetry (DSC); microencapsulation; thermogravimetric analysis (TGA); UV–vis spectroscopy

INTRODUCTION

Designing a pulsatile capsule device for the delivery of anticancer drugs to treat colorectal carcinoma is a major challenge in pharmaceutics. There are many colonic diseases, such as irritable bowel syndrome, Crohn's disease, ulcerative colitis, and colorectal cancer, that require site-specific delivery. Colon cancer is one of the very common internal malignancies in which there is uncontrolled growth of cells inside the colon or rectum. Surgery, chemotherapy, and radiotherapy are the main three types of treatment presently adopted for the treatment of colon cancer. However, attempts have been made to develop a formulation that delivers the anticancer agent to the targeted colon. Because of this reason, polymeric drug-loaded systems with biodegradable polymers have gained more attention because their advantages over conventional dosage forms and because these polymers can be used to target to the delivery site. In the case of colonic delivery, attempts have been made to deliver the drug with polymers that are degradable in the colon. Of these, four kinds of approaches for colonic delivery have been developed; these include time-dependent devices,[1] the coating of the drug with a pH-sensitive polymer,[2] bacterially triggered delivery,[3,4] and osmotic-pressure-controlled delivery.[5]

Chitosan, a natural linear polyaminosaccharide that is the second most abundant organic source next to cellulose,[6] is a deacetylated derivative of chitin, which naturally occurs in the shells of crustaceans, the cuticles of insects, and the cell walls of fungi, which are widely spread among marine and terrestrial invertebrates and lower forms of the plant kingdom.[7,8] It has gained much importance because of its chemical and biological properties, including its particle size, viscosity, density, degree of deacetylation, biodegradable and biocompatible characteristics, and lower toxicity.[9,10] Chitosan is widely used as in sponge and wound-dressing materials,[11] artificial kidney membranes,[12] antacids and antiulcer agents,[13] cosmetics,[14] contact lenses,[15] solid-state batteries,[16] photography,[17] and drug-delivery devices in the form of microspheres,[18–21] nanoparticles,[22,23] beads,[24] tablets,[25] and membranes.[26]

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In this study, we aimed to use a naturally occurring polyaminosaccharide, chitosan, for the development of the colonic delivery of 5-fluorouracil (5-FU). It is a pyrimidine analogue that acts in several ways and inhibits the synthesis of thymidylate synthase. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine,[27] which is a nucleotide required for DNA replication. Our objective in this research was to develop an enteric-coated capsule device filled with microencapsulated drug formulation by the crosslinking of a pH-sensitive matrix loaded with 5-FU to investigate its release behavior for 5-FU. Because the gelatin capsule was enteric coated with cellulose acetate phthalate (CAP), it prevented the disintegration of the capsule in gastric fluid of pH 1.2 (acidic) and, thereby, released the 5-FU in a controlled manner on reaching the small intestine at basic pH 7.4, at which time the capsule lost its enteric coating, swelled slowly, and started to release 5-FU in the colonic region.[28] The targeting of 5-FU to the colon not only would reduce the systemic toxicity and side effects of the drug but would also show the desired action at lower dosages.

EXPERIMENTAL

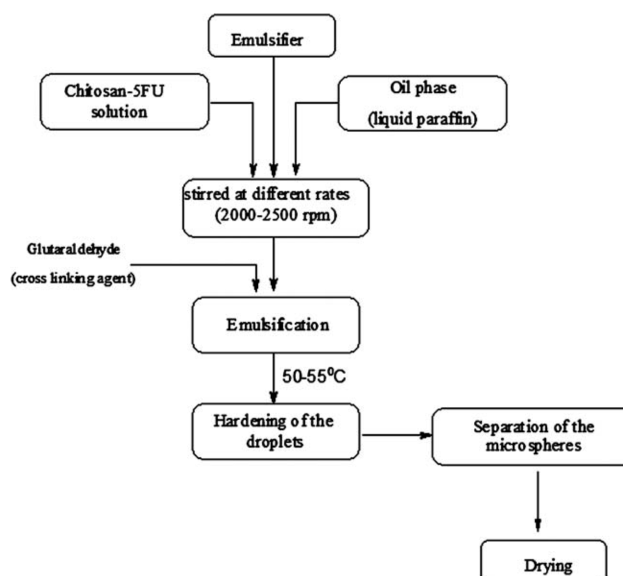
Materials

5-FU was generous gift of Biochem Pharmaceutical Industries (Mumbai, India). Chitosan (85% deacetylated, particle size = 1 ± 3 mm, molecular weight ≈ 1×10^6) was purchased from Sigma-Aldrich Co. (St. Louis, MO). Glutaraldehyde (GA; 25% aqueous solution), used as a crosslinking agent, was procured from Spectrochem Pvt., Ltd. (Mumbai, India). Light liquid paraffin oil containing Span-20 was purchased from S. D. Fine Chemicals (Mumbai, India). All other reagents were analytical grade and were used as received.

Methods

Preparation of the chitosan microspheres

5-FU-loaded chitosan microspheres were prepared with emulsification followed by crosslinking with GA. In a typical procedure,[29] as per Scheme 1, the chitosan solution (1% w/v) was prepared in a 1% acetic acid solution, and 5-FU was dissolved in this solution and mixed well. This solution was dispersed in liquid paraffin (light) containing Span-20 (1% w/v) as an emulsifier. This suspension medium was stirred with a stainless steel, half-moon paddle stirrer at various speeds (≈ 2000–2500 rpm) for 0.5 h, and GA solution was added and stirred for 3 h at about 50–55°C. After the stipulated stirring time, brownish yellow microspheres were obtained, centrifuged, and washed several times with petroleum ether, then with distilled water, and finally with acetone; they were then dried in a vacuum desiccator.



Scheme 1 Preparation of the chitosan microspheres.

Three different formulations were prepared by variation of the amounts of drug and polymer (polymer–drug ratios of 1 : 0.1, 1 : 0.2, and 1 : 0.5). The experiments were repeated many times to obtain a sufficient quantity of microspheres.

Characterization of the microspheres

Scanning electron microscopy (SEM)

The morphological studies of the plain chitosan microspheres and 5-FU-loaded chitosan microspheres were carried out by SEM. Each sample was deposited on a brass holder and sputtered with gold. SEM photographs were taken with a JSM 6400 scanning microscope (Tokyo, Japan) at the required magnification. A working distance of 39 mm was maintained, and the acceleration voltage was 5 kV, with the secondary electron image as a detector. The polymer samples were fixed on a brass holder.

Particle size analysis

The particle size of the microspheres was determined by optical microscopy. An average of 100 microspheres was used for the study, and the mean particle size (arithmetic mean diameter) was considered to be the deciding factor in the selection of optimum formulation conditions for each variable parameter studied.

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra were taken for plain chitosan, crosslinked chitosan microspheres, 5-FU-loaded

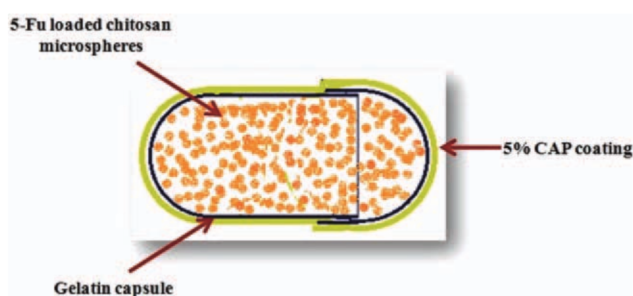


Figure 1 Pictorial representation of the pulsatile capsule device. (Lin = Length in counts). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chitosan microspheres, and 5-FU on a Nicolet model Impact 410 FTIR instrument (Madison, USA). About 2 mg of the sample was ground well with spectroscopic-grade KBr, and the pellets were prepared under a hydraulic pressure of 600 kg/cm^2. The spectra were scanned over the wave-number range 500–4000 cm^{-1}. The instrument used a He–Ne laser (632.8 nm) as an equipment carrier with a deuterated triglycine sulfate detector.

Thermal analysis

Thermogravimetric analysis (TGA), differential thermogravimetric analysis, and differential scanning calorimetry (DSC) were performed on different samples to determine the composition and to predict the thermal stability and molecular dispersion of the drug in the polymer matrix. TGA measurements were done on a Mettler-Toledo TGA unit (Schwerzenbach, Switzerland) and a Universal V4 5A (TA Instruments, Newcastle, USA); samples were heated from 100 to 500°C. DSC measurements were done on a DuPont-2000 micro-calorimeter, and the samples were heated at a rate of 10°C/min. These measurements provided quantitative and qualitative information about the physical and chemical changes that involved endothermic (heat absorbed) or exothermic (heat evolved) processes or changes in the heat capacity.

Powder X-ray diffraction studies (PXRD)

PXRD patterns of the chitosan, plain 5-FU, GA-crosslinked chitosan microspheres, and 5-FU-loaded chitosan microspheres were recorded with a Philips PW 1729 analytical XRD instrument (Almelo, Holland) equipped with Ni-filtered Cu Kα radiation ($\lambda_{Cu} = 1.5418$A°) at a voltage of 40 kV and a current of 30 mA. Dried GA 5-FU-loaded chitosan microspheres of uniform size were mounted on a sample holder, and the samples were analyzed over a 2θ range of 3–60° with scanning step size of 0.029° (2θ) and a scan step time of 77 s.

Drug-loading efficiency

The entrapment efficiency represents the percentage of drug trapped within the crosslinked microspheres with respect to the total amount of drug added to the polymer solution. The concentration of drug in the microspheres was determined with a UV spectrophotometer. An accurately weighed quantity (50 mg) of microspheres was soaked in 10 mL of a 0.1*N* HCl solution for 1 h at 45°C and then sonicated for 20 min. The whole solution was transferred to a 100-mL volumetric flask and diluted up to a mark, with the same solution kept for 24 h for complete extraction. The solution was centrifuged at 5000 rpm, and the supernatant solution was collected analyzed spectrophotometrically at 265 nm with a Shimadzu 1800 UV spectrophotometer (Kyoto, Japan). The percentage drug loading was obtained with the following formula:

$$\text{Drug loading efficiency (\%)} = \frac{M_p}{M_t} \times 100\%$$

where M_p is the actual amount of drug loaded in the various formulations determined by the previous experiment and M_t is the theoretical amount of drug in the formulations.

Percentage swelling

Measurements of the percentage swelling were carried out in distilled water with ocular microscopy on a cavity cover glass; these samples were incubated for several hours to obtain the maximum swelling. The process was repeated three times, and an average reading was calculated.

Development of a pulsatile capsule device

The pulsatile capsule device was prepared by the placement of a known amount of 5-FU-loaded chitosan microspheres inside the gelatin capsule by hand (as shown in Fig. 1). The joint of the capsule body and cap was sealed with a small amount of 5% ethyl cellulose ethanolic solution. The sealed capsules were completely coated by the dip-coating method with 5% CAP in an 8 : 2 (v/v) mixture of acetone and ethanol to prevent variable gastric emptying. The coating was repeated until a 13–15% weight gain was attained. The percentage weight gains of the capsule after and before coating were noted.

In vitro drug-release studies

Dissolution studies were carried out with a USP XXIII digital tablet dissolution test apparatus Six/Eight station (Veego Instruments, Mumbai, India) as per USP standards to determine the amount of drug

released from the pulsatile capsule device. The pulsatile capsule device was inserted into the basket and tied with cotton thread so that the capsule would be completely immersed in the dissolution media and so that it would not float.[30] The drug was dissolved/distributed throughout the matrix and was released only on degradation of the matrix. Dissolution studies were carried out in 900 mL of simulated gastric fluid (0.2*M* HCl/KCl buffer, pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 7.4), both without enzymes, with a dissolution tester equipped with eight pedals. The dissolution rates were measured at 37 ± 0.5°C and a 100-rpm pedal speed. An amount of 900 mL of simulated gastric fluid (pH 1.2) was used during the first 2 h to mimic the gastric emptying time, and then, the medium was drained off and replaced with simulated intestinal fluid (pH 7.4). Aliquots of 5 mL were withdrawn from the vessel at predetermined time intervals: 30 min and 1, 2, 3, 4, 5, 6, 8, and 12 h, and the withdrawn samples were replaced with an equal volume of corresponding fresh dissolution medium. These samples were analyzed at 265 nm by a UV spectrophotometer, and the concentration of the drug was calculated from the calibration curves, which were constructed with reference standards, and also, the cumulative percentage release was calculated over the sampling times.

RESULTS AND DISCUSSION

The microspheres were prepared by the crosslinking of chitosan with GA. Free amino groups of chitosan were reacted with GA to form an imine bond; during the process, 5-FU was entrapped. Drug-loaded microspheres were further placed in an enteric-coated gelatin capsule designed as a pulsatile capsule device.

Characterization of the chitosan microspheres

The SEM photographs of the plain chitosan microspheres and 5-FU-loaded chitosan microspheres are shown in Figure 2. From the SEM photographs of the microspheres, we observed that the particles were spherical in shape with a smooth surface, having a mean particle size range from 3.216 to 4.57 μm. The photographs indicated the absence of free drug on the surfaces of the 5-FU-loaded chitosan microspheres.

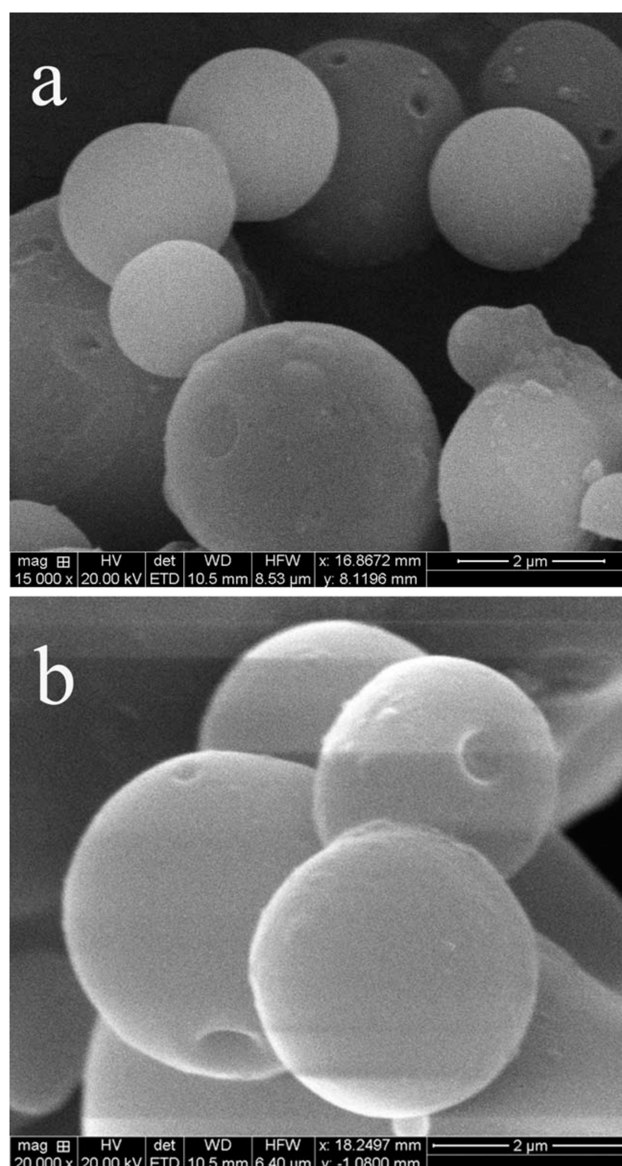


Figure 2 SEM photographs of the (a) GA-crosslinked chitosan microspheres and (b) 5-FU-loaded, GA-crosslinked chitosan microspheres.

FTIR studies

FTIR spectral analyses were carried out to confirm the crosslinking of the chitosan microspheres by GA and to confirm the absence of the chemical reaction between the drug and the polymer. The FTIR spectra of chitosan, crosslinked chitosan microspheres, 5-FU-entrapped chitosan microspheres, and 5-FU are shown in Figure 3. The FTIR spectral data were used to confirm the chemical stability of 5-FU in the chitosan microspheres. In the case of plain chitosan, the characteristic band due to O—H stretching was observed at 3433 cm^{-1}. The N—H stretching and N—H bending bands appeared at 2927 and 1617 cm^{-1}, respectively. After crosslinking with GA, the sharp peak observed at 1664 cm^{-1} was due to the formation of the imine (C=N) group.

5-FU showed the characteristic NH stretching band at 3110 cm^{-1} and C=O and C—N stretching bands at 1720 and 1651 cm^{-1}; CH in-plane and CH out-of-plane bands were observed at 1240 and 892 cm^{-1}, respectively. When the drug was incorporated into the crosslinked chitosan, along with the characteristic

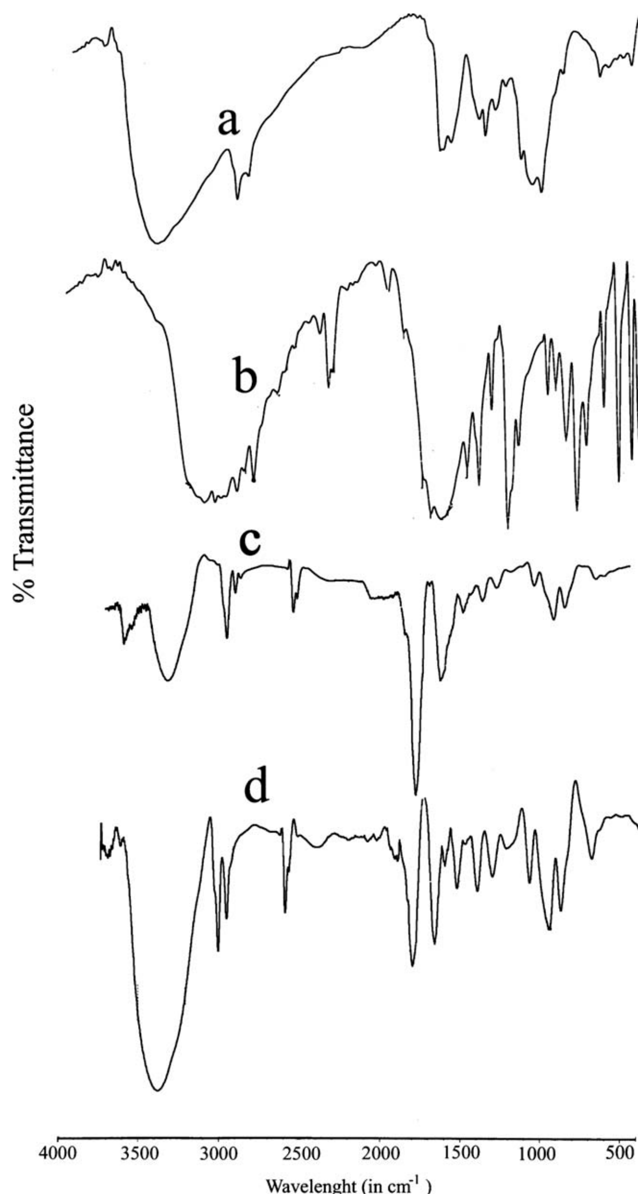


Figure 3 FTIR spectra of the (a) chitosan, (b) 5-FU, (c) GA-crosslinked chitosan microspheres, and (d) 5-FU-loaded, GA-crosslinked chitosan microspheres.

bands of the crosslinked chitosan, additional peaks appeared that were due to the presence of 5-FU in the matrix. The characteristic bands of 5-FU, including C=N— stretching and C=O stretching vibrations, appeared at 1651 and 1720 cm^{-1}, respectively, in the drug-loaded matrix without any change. This indicated that 5-FU did not undergo any chemical changes while producing the microspheres, and this supported the literature data.[31]

DSC studies

The DSC of plain chitosan, GA-crosslinked chitosan microspheres, 5-FU-loaded, GA-crosslinked chitosan microspheres, and 5-FU are shown in Figure 4. An

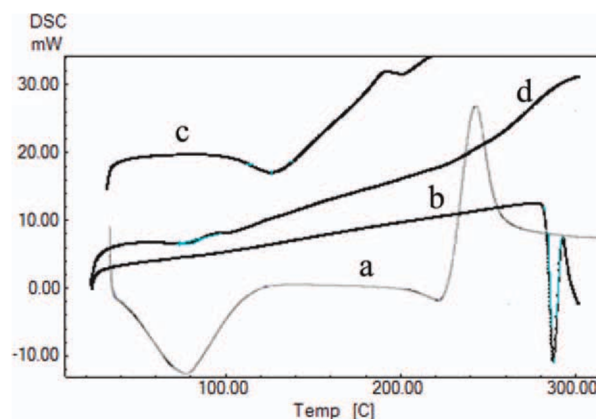


Figure 4 DSC thermograms of the (a) plain chitosan, (b) 5-FU, (c) GA-crosslinked chitosan microspheres, and (d) 5-FU-loaded chitosan microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

endothermic peak of pure chitosan was observed at 95°C, which represented the glass-transition temperature of the chitosan, and the sharp exothermic peak at 305°C corresponded to the decomposition temperature. After crosslinking with GA, the endothermic peak of plain chitosan shifted to 137°C; this indicated that the chemical interaction of polymer with GA and the rigidity of polymer matrix at higher temperatures. 5-FU showed typical peaks at 283.49 and 291.5°C, and these peaks disappeared in the 5-FU-loaded crosslinked chitosan microspheres. This revealed the uniform molecular level dispersion of 5-FU in the chitosan microspheres.

Thermal analysis

Typical TGAs of plain chitosan, 5-FU, GA-crosslinked chitosan microspheres, and 5-FU-loaded, GA-crosslinked chitosan microspheres are shown in Figure 5. From the thermograms, the initial and final

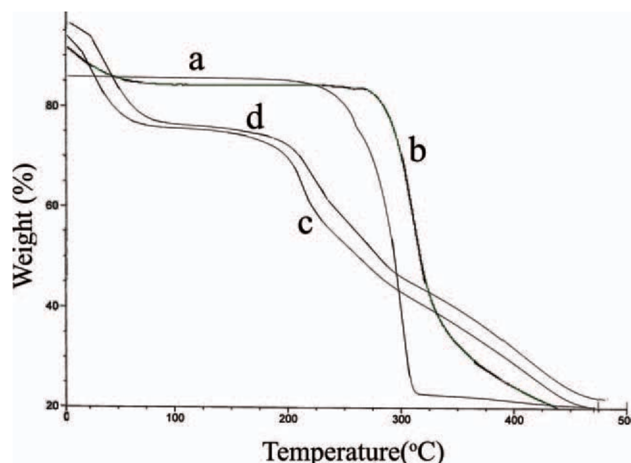


Figure 5 TGA of the (a) chitosan, (b) 5-FU, (c) GA-crosslinked chitosan microspheres, and (d) 5-FU-loaded crosslinked chitosan microspheres.

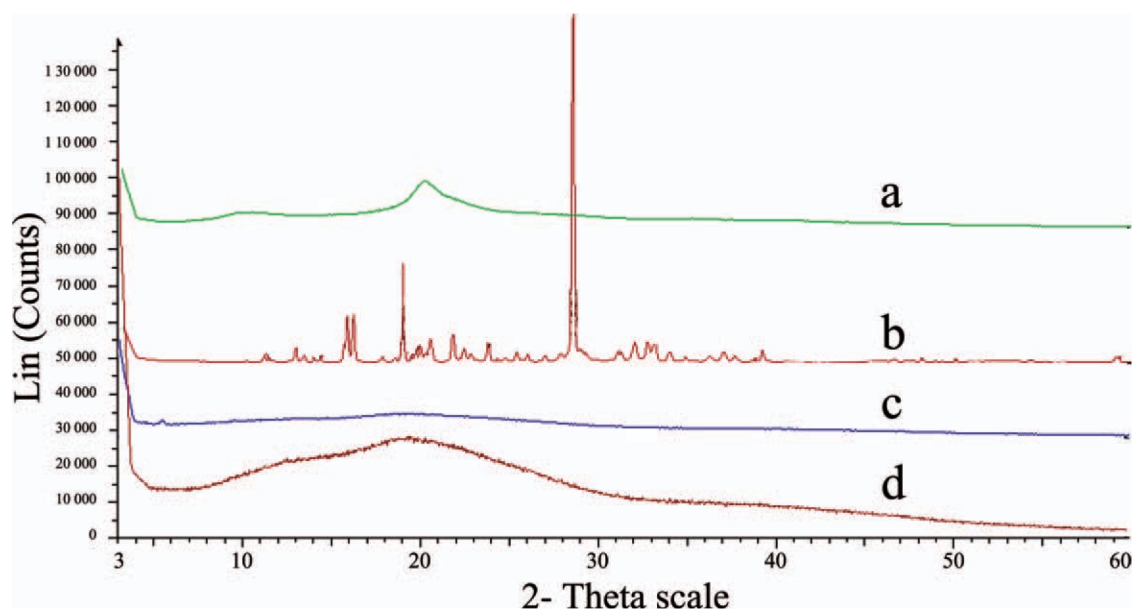


Figure 6 PXRD diffraction patterns of the (a) chitosan, (b) 5-FU, (c) GA-crosslinked chitosan microspheres, and (d) 5-FU-loaded chitosan microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

degradation temperatures were obtained. The weight loss of plain chitosan began at 86°C, and the maximum weight loss was attained at 281°C. It was noted that GA-crosslinked chitosan microsphere showed less weight loss (28%) compared to plain chitosan (43%); this indicated that the crosslinking process decreased the degradation rate and increased the stability. 5-FU started melting at 298°C and completely decomposed at 334°C, whereas the 5-FU-loaded chitosan microspheres showed decomposition temperatures that were almost same as those of 5-FU. From the thermogravimetry curve, we observed that there was no any change in the melting point of 5-FU; this indicated that there was no chemical interaction between the drug and polymer or with the crosslinking agent GA.

PXRD studies

PXRD analyses provided information about the crystallinity of 5-FU in the GA-crosslinked microspheres. The PXRD of plain chitosan, 5-FU, GA-crosslinked chitosan microspheres, and 5-FU-loaded, GA-crosslinked chitosan microspheres are shown in Figure 6. The 5-FU peaks were observed at 2θ values of 16, 19, 22, and 29°; these peaks suggested its crystalline nature. However, these peaks disappeared in 5-FU-loaded chitosan microspheres; this indicated the uniform molecular dispersion of 5-FU in the polymer matrix.

Drug-loading efficiency

Table I shows the percentage drug loading of various 5-FU-loaded chitosan microsphere formulations. As the theoretical drug loading increased, the percentage drug-loading efficiency decreased. This was due to the higher water-soluble property of 5-FU during the crosslinking process and during the water washing process after microsphere preparation. Similar results were also reported in the literature[32,33] with regard to the lower entrapment efficiency of 5-FU formulations. However, the swelling studies indicated that as the theoretical loading increased, the percentage swelling also increased because of the formation of a loose matrix.

TABLE I
Results of Percentage Drug Loading, Percentage Swelling, and Particle Size of the 5-FU Loaded Chitosan Microspheres

Formulation code	Chitosan (% w/v)	5-FU concentration (% w/w)	GA concentration (mL)	Drug loading efficiency (%)	Swelling (%)	Average particle size (μm)
F1	1	10	0.5	5.53 ± 1.10 (55.5)	24.45 ± 2.02	4.13
F2	1	20	0.5	10.82 ± 1.85 (54.1)	30.51 ± 2.45	4.28
F3	1	50	0.5	17.89 ± 2.03 (35.78)	38.23 ± 2.83	4.57

TABLE II
Components of the Designed Pulsatile Capsule Device for Formulations F1, F2, and F3

Formulation code	Weight of the empty body (mg)	100-mg microspheres	Number of coatings	Weight gain (%)
CF1	73.6	F1	2	7.24
CF1	73.6	F1	3	10.8
CF1	74	F1	4	13.8
CF2	75	F2	4	14.1
CF3	74.8	F3	4	13.9

Pulsatile capsule device

In this research, a pulsatile capsule device was developed for the purpose of targeted delivery of 5-FU to the colon. A known quantity of crosslinked chitosan microspheres loaded with 5-FU was filled in a capsule of 118 ± 7 mg capacity by hand. The capsule was dipped into a 5% CAP solution in such a way that the capsule was dipped completely for 5 s. Such dipping was repeated for several cycles, as mentioned in Table II. The results of weight gain of the capsule due to CAP coating are given in Table II. It was observed that as the number of coats was increased, the weight gain of the capsule also increased, and the weight gain could have been enough to extend the release in acidic pH. Preliminary experiments were conducted to optimize the amount of coating, which prevented the capsule from dissolving, so that 5-FU release in acidic pH was almost prohibited for first 2 h and maximum release could be achieved at basic pH 7.4 (simulated actual gastrointestinal tract condition) within 12 h in a controlled manner. A minimum of three to four cycles were necessary to prevent the dissolution of the capsule in the acidic media.

In vitro release studies of the capsule formulation

In vitro release data of 5-FU from all three formulations of various crosslinked chitosan microspheres (F1–F3) and pulsatile capsule devices (CF1–CF3) are shown in Figures 7 and 8, respectively. The chitosan microspheres swelled in acidic media (stomach pH), and the release of the drug took place. However, to develop a successful colon-targeting device formulation, one must keep in mind that the developed formulation must show a minimum drug-release effect in the physiological environment of the stomach and in the small intestine, and it has to show a maximum drug-release effect in the colonic area. For example, if we consider anticancer drugs, they must be delivered exactly to the prefixed targeted site; otherwise, it will lead to adverse effects or may become toxic to other healthy cells. Hence, the capsule was prepared by enteric coating with CAP to prevent the early release of 5-FU.

In vitro release studies were performed with a USP XXIII dissolution apparatus. Release studies were carried out for all three formulations in triplicate. As shown in Figure 7, in the case of the 5-FU-loaded microspheres, a sudden burst release of 55–60% of drug was observed within the first 2 h in the acidic pH; the reason for this was the higher swelling properties of chitosan microspheres in acidic pH so that as the microspheres swelled, the maximum release was attained, and releases of 90.3, 92.4, and 94.7% were observed in F1, F2, and F3, respectively, at the time of 12th h. In the case of the enteric-coated pulsatile capsule device, as shown in Figure 8, the overall percentage cumulative drug releases were found to be 10.24, 6.05, and 7.01% with respect to the CF1, CF2, and CF3 formulations, respectively, for the first 2 h, and burst releases of 65.34, 70.05, and 57.07% corresponding to F1, F2, and F3, respectively, were observed at the 12th h.

All of the dissolution data were treated with five different mathematical models, namely, Higuchi matrices, zero-order kinetics, first-order kinetics, Hixson Crowell model, and Korsemeyer–Peppas

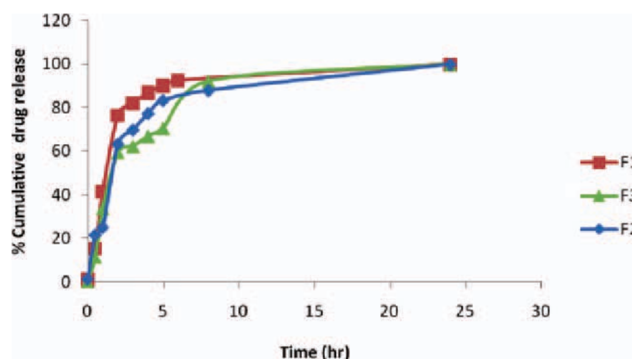


Figure 7 *In vitro* drug-release profiles of the 5-FU-loaded chitosan microspheres for formulations (■) F1, (◆) F2, and (▲) F3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

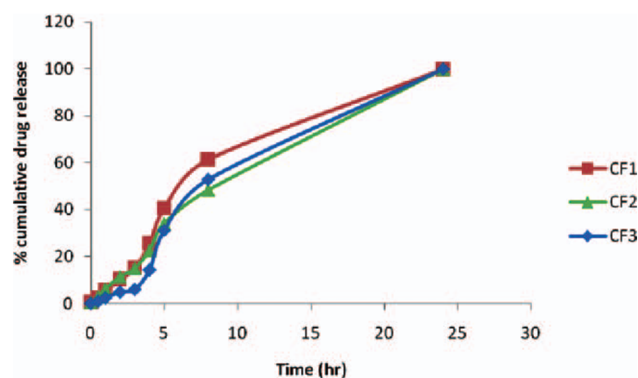


Figure 8 *In vitro* drug-release profiles of the pulsatile capsule device containing 5-FU-loaded chitosan microspheres for formulations (■) F1, (▲) F2, and (◆) F3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III
***In Vitro* Release Kinetics of Various Formulations with Mathematical Models**

Sample	Formulation code	Zero-order R^2	First-order R^2	Higuchi R^2	Hixson Crowell R^2	Korsemeyer–Peppas	
						n	R^2
1	CF1	0.970	0.993	0.985	0.970	1.451	0.955
2	CF2	0.964	0.986	0.981	0.964	1.281	0.958
3	CF3	0.965	0.990	0.981	0.965	1.878	0.933

model; the results of the regression coefficients and correlation coefficients obtained for various formulations with the various mathematical models are shown in Table III. The regression coefficients for formulations F1–F3 of the zero-order plots were found to be 0.970, 0.964, and 0.965, respectively. The regression coefficients for formulations F1–F3 of the first-order plots were found to be 0.993, 0.981, and 0.990, respectively. The Hixson Crowell plot regression coefficients of formulations F1–F3 were found to be 0.970, 0.964, and 0.965, respectively. The Korsemeyer–Peppas plot regression coefficients of formulations F1–F3 were found to be 0.955, 0.958, and 0.933, with the n values being 1.451, 1.281, and 1.878, respectively. The release mechanism indicated that all three formulations (CF1–CF3) followed first-order release patterns. This indicated that the enteric coating prevented various gastric emptying and prevented drug release at acidic pH. As we changed the dissolution media to phosphate buffer at pH 7.4, the pulsatile capsule coating started to dissolve, and simultaneously, the pH-sensitive chitosan microspheres slowly started to release the 5-FU, so that as the time progressed, maximum release was attained within a period of 12 h in the colonic region.

CONCLUSIONS

From these results, we concluded that the developed pulsatile capsule device may become one of the best tools for colonic drug delivery in the future. The gelatin capsules were enteric coated with CAP; this prevented the problem of gastric emptying; thereby, the release of 5-FU from crosslinked chitosan microspheres in acidic media was minimized, and the maximum amount of drug release in a controlled manner in basic media (pH 7.4) was shown to be within 12 h in the mimicking of mouth-to-colon transit. Because the method adopted was simple, inexpensive, and cost-reductive and the materials used for the development of the pulsatile capsule device, that is, the enteric-coating material CAP and gelatin capsule, were nontoxic and biodegradable, this system could be used readily for drug delivery. DSC and XRD studies of the 5-FU-loaded chitosan microspheres showed the uniform molecular level dispersion of 5-FU in the polymer matrix. SEM photographs confirmed the spherical and smooth surface of the microspheres with the absence of any 5-FU particles on the outer surface. In conclusion, the pulsatile capsule device developed in this research could be used as a potential vehicle for the targeted delivery of anticancer drugs to the colon.

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