Cross-Linked Guar Gum Microspheres: A Viable Approach for Improved Delivery of Anticancer Drugs for the Treatment of Colorectal Cancer

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ABSTRACT

In the present work, guar gum microspheres containing methotrexate (MTX) were prepared and characterized for local release of drug in the colon, which is a prerequisite for the effective treatment of colorectal cancer. Guar gum microspheres were prepared by the emulsification method using glutaraldehyde as a cross-linking agent. Surface morphological characteristics were investigated using scanning electron microscopy. Particle size, shape, and surface morphology were significantly affected by guar gum concentration, glutaraldehyde concentration, emulsifier concentration (Span 80), stirring rate, stirring time, and operating temperature. MTX-loaded microspheres demonstrated high entrapment efficiency (75.7%). The in vitro drug release was investigated using a US Pharmacopeia paddle type (type II) dissolution rate test apparatus in different media (phosphate-buffered saline [PBS], gastrointestinal fluid of different pH, and rat cecal content release medium), which was found to be affected by a change to the guar gum concentration and glutaraldehyde concentration. The drug release in PBS (pH 7.4) and simulated gastric fluids followed a similar pattern and had a similar release rate, while a significant increase in percent cumulative drug release (91.0%) was observed in the medium containing rat cecal content. In in vivo studies, guar gum microspheres delivered most of their drug load (79.0%) to the colon, whereas plain drug suspensions could deliver only 23% of their total dose to the target site. Guar gum microspheres showed adequate potential in achieving local release of drug in in vitro release studies, and this finding was further endorsed with in vivo studies.

KEYWORDS: Polysaccharides for colon drug delivery, NDDS, guar gum microspheres, methotrexate, colorectal cancer, glutaraldehyde cross-linking.

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INTRODUCTION

Cancer of the colon and rectum is one of the most common internal malignancies. Colorectal cancer is the second-leading cause of cancer deaths in the United States. In 2005, an estimated 145 290 new cases of colon cancer were diagnosed in the United States. The majority of people with colorectal cancer are over the age of 50. Dietary factors such as low folate intake are thought to increase the risk of colorectal cancer by 2 to 5 times.[1,2] The incidence of colorectal cancer, however, could be reduced dramatically by preventative methods such as colonoscopy and detection of mutations in fecal DNA.[3,4] Almost all cases of colorectal cancer begin with the development of benign or noncancerous polyps. When colon cancer cells spread outside the colon or rectum to lymph nodes, they may also spread to other lymph nodes, the liver, or other organs. Surgery is still a mainstay of the treatment of colorectal cancer.[5] Before and after surgery, neoadjuvant therapy and adjuvant therapy are given to patients to shrink large tumors, destroy any remaining cancer cells, and prevent the cancer from recurring.

Chemotherapy is also used to treat advanced colorectal cancer. However, conventional chemotherapy is not as effective in colorectal cancer as it is in other cancers, as the drug does not reach the target site in effective concentrations.[6,7] Thus, effective treatment demands increased dose size, which may lead to undue consequences. To improve this situation, pharmaceutical technologists have been working on ways to deliver the drug more efficiently to the colon, where it can target the tumor tissues. Ciftci and Groves[8] showed that it is possible for a colon-targeted delivery system to selectively deliver drug *to* tissues, not through tissues. It is possible that delivery of small quantities of antineoplastic agent to the inner surface of the colon could destroy small tumors that arise spontaneously in this region, reducing the need for surgery.

Several strategies can be used to selectively target the drug release to the colon. Drugs are commonly delivered to the large bowel by coating them with polymeric substances such as cellulose derivatives or acrylic polymers.[9-11] However, the performance of such colonic delivery systems may be limited by gastrointestinal motility and pH variations. Multiparticulate systems have been developed to overcome these

limitations.[12,13] Other strategies have been based on the assumption that the high enzymatic activity of the rich microbial flora in the colon would act as a release trigger.[14]

Polysaccharides, the polymers of monosaccharides, retain their integrity in the upper gastrointestinal tract because they are resistant to the digestive action of gastrointestinal enzymes. The matrices of polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but once they reach the colon, they are acted upon by the bacterial polysaccharidases and they degrade. This family of natural polymers is appealing for use in drug delivery because it comprises many polymers with a large number of derivatizable groups, a wide range of molecular weights, varying chemical compositions, and, for the most part, low toxicity and biodegradability yet high stability.[15,16] Another favorable property of these materials is that they have already been approved as pharmaceutical excipients.

The present investigation is aimed at using the inexpensive and naturally and abundantly available guar gum for colon-targeted delivery of methotrexate (MTX). Guar gum is a natural nonionic polysaccharide derived from the seeds of *Cymompsis tetraganolobus* (family: Leguminaceae).[17] Guar gum is hydrophilic and swells in cold water, forming viscous colloidal dispersions or sols. This gelling property retards release of the drug from the dosage form, making it more likely that degradation will occur in the colon. Guar gum was found to be a colon-specific drug carrier in the form of matrix and compression-coated tablets as well as microspheres.[18,19] The present research focused on the development and characterization of novel guar gum microspheres for colon-targeted drug delivery of MTX.

Materials and Methods

Materials

MTX (98%-102% pure compared with standard reference sample) was received as a gift sample from Unimed Technologies Ltd (Gujarat, India). Guar gum (particle size 150 μm and viscosity in the range of 3500-4000 cps) was procured from Central Drug House (New Delhi, India). Pancreatin (from pig pancreas), pepsin (bovine), and glutaraldehyde were purchased from Sigma Chemicals (St. Louis, MO). All other chemicals were of analytical grade.

Preparation of Guar Gum Microspheres

Drug-loaded guar gum microspheres were prepared by the emulsification method.[20] An aqueous dispersion of guar gum (40 g) containing 2% wt/wt of guar gum (an accurately weighed amount of gum was dispersed in a specified volume of cold water containing the drug and allowed to swell for 2 hours) was dispersed in 100 g of castor oil containing 3 g of Span 80 using a mechanical stirrer at 4000 rpm. After complete mixing, 0.2 mL of concentrated sulfuric acid and 1.5 mL of glutaraldehyde were added to the dispersion, followed by stirring at a constant speed for 4 hours at 50°C. The microspheres formed were collected by sedimentation followed by decantation of oil, then washed with several fractions of isopropyl alcohol. The final preparation was a free-flowing powder consisting of spherical micron-sized particles.

Various formulation and process variables that could affect the preparation and properties of the microspheres were identified and optimized to get small, discrete, uniform, smooth-surfaced, and spherical microspheres. (The underlined values were used as constants.) The formulation variables included concentration of guar gum (% wt/wt): 1.0, 2.0, 3.0, 4.0; concentration of emulsifier Span 80 (% wt/wt): 1, 2, 3, 4; and concentration of cross-linking agent glutaraldehyde (mL): 0.5, 1.0, 1.5, 2.0. The process variables included stirring speed: 2000, 3000, 4000, and 5000 rpm; stirring time: 1 hour, 2 hours, 3 hours, 4 hours, 5 hours; and temperature of the system: 30°C, 40°C, 50°C, and 60°C.

Particle Size Analysis

Particle size was determined by using a laser diffraction particle size analyzer (Cilas 1064L, Orleans, France). Microspheres were suspended in the chamber of the particle size analyzer containing distilled water, and the particle size was determined using the software provided by the manufacturer.

Shape and Surface Morphology

Surface and shape characteristics of microspheres were evaluated by means of scanning electron microscopy. The scanning electron microscopy samples were prepared by lightly sprinkling the microsphere powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å using a sputter coater, and the photographs of samples were taken (Figure 1).

Drug-Polymer Interaction

The extent of interaction of the drug with the polymer was determined using an equilibrium dialysis technique. MTX (20 mg) was dissolved in phosphate-buffered saline (PBS) (pH 7.4), and a 2.0% wt/wt solution of polymer was prepared by continuous stirring of the polymer in the drug solution for 1.5 hours. Polymer solution (10 mL) containing 20 mg of drug was introduced in a moistened cellulose dialysis tube (donor compartment) that was suspended in 200 mL of PBS (pH 7.4) (recipient compartment). It was gently stirred using a magnetic stirrer at 37 ± 1°C for 24 hours, samples (2 mL) were withdrawn from the recipient compartment

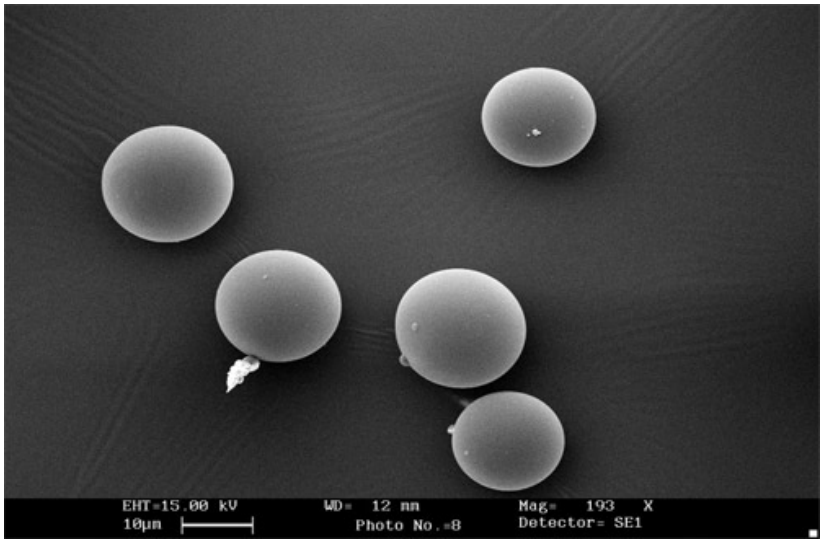

Figure 1. Scanning electron photomicrograph showing placebo guar gum microspheres.

diluted with PBS (7.4), and the amount of drug was quantified using high-performance liquid chromatography (HPLC).[21] The HPLC analysis was performed with a Lichrosorb RP-18 (7 μm) column (Alltech Associates, Deerfield, IL) that was maintained at 25ºC. The mobile phase consisted of 0.05M ammonium acetate buffer-acetone-methanol mixture (174:13:13, pH 5.0), which was pumped at a flow rate of 1.8 mL/min, and detection was performed at 254 nm using a UV detector. A control medium was created using pure polymer solution. The extent of drug-polymer interaction (β) was expressed by the ratio of Sb to St:

$$\beta = \frac{Sb}{St} = \frac{(S_t - S_f)}{(S_T - S_R)}, \qquad (1)$$

where Sb and S_T are the amount of bound drug molecules in the donor compartment and the total amount of drug used, respectively. S_t is the amount of drug in the donor compartment after equilibrium is accomplished, and S_R is the amount of drug in the receptor compartment after equilibrium is accomplished. S_f is the amount of free drug in the donor compartment.

Equilibrium Swelling Studies of Microspheres

A preweighed amount (100 mg) of microspheres was placed in simulated intestinal fluid (SIF) (pH 7.4) and allowed to swell up to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (α) was then calculated from the following formula:

$$\alpha = \frac{W_g - W_o}{W_o}, \qquad (2)$$

where w_o is the initial weight of the microspheres and w_g is the weight of the microspheres at equilibrium swelling in the medium.

Drug-Loading Capacity and Encapsulation Efficiency

Loading capacity is the maximum amount of drug that can be incorporated in the microspheres. Loading capacity was determined to be the maximum amount of MTX found in 100 mg of microspheres. Encapsulation efficiency is the amount of added drug (in percent) that is encapsulated in the formulation of microspheres. Encapsulation efficiency was calculated in terms of the ratio of drug in the final formulation to the amount of added drug.

An accurately weighed amount (100 mg) of the formulation of microspheres was dispersed in 100 mL of PBS (pH 7.4). The sample was ultrasonicated for 3 consecutive periods of 5 minutes each, with a resting period of 5 minutes each. It was left to equilibrate for 24 hours at room temperature, and the suspension was then centrifuged at 3000 rpm for 15 minutes. The supernatant was diluted appropriately with PBS (pH 7.4) and analyzed for concentration of MTX using HPLC, as described previously. The drug content of each sample was determined in triplicate, and results were averaged.

In Vitro Digestion

An in vitro digestion study of guar gum microspheres was performed to ensure complete delivery of loaded drug in the colon. This was determined by incubating 200 mg of the microsphere preparation in 100 mL of rat cecal content release medium (6% wt/vol) for 48 hours. A sample (2 mL) was withdrawn and diluted appropriately. The amount of MTX released was estimated by HPLC.

Dissolution Studies

The release studies of MTX from guar gum microspheres were performed in PBS (pH 7.4), in simulated gastrointestinal fluids, and in a medium containing rat cecal content (with and without enzyme induction). Simulation of gastrointestinal transit conditions was achieved by using different dissolution media. Simulated gastric fluid (SGF) pH 1.2 consisted of NaCl (2.0 g), HCl (7 mL), and pepsin (3.2 g), and pH was adjusted to 1.2 ± 0.5. SIF pH 7.5 consisted of KH_2PO_4 (6.8 g), 0.2N NaOH (190 mL), and pancreatin (10.0 g), and pH was adjusted to 7.5 ± 0.1. SIF pH 4.5 was prepared by mixing SGF pH 1.2 and SIF pH 7.5 in a ratio of 39:61. The drug release studies were conducted in SGF for the first 2 hours and in mixed SGF and SIF for 3 hours. The dissolution medium was then replaced with SIF and tested for the next 3 hours.

The release studies of drug from guar gum microspheres were performed using a US Pharmacopeia dissolution rate test apparatus (paddle apparatus, 100 rpm, 37 ± 0.1°C). Samples (2 mL) were withdrawn at appropriate intervals and estimated for MTX using HPLC. Sink conditions were adjusted with the addition of an equal volume of fresh release medium at the same temperature. The percentage of drug released was expressed with respect to the drug content of the microspheres.

The ability of the most promising formulation of guar gum microspheres ($DPGGC_3$) to release MTX in the physiological environment of the colon was assessed by carrying out release studies in the rat cecal content release medium. This medium was prepared by the method reported by Van den Mooter and Kinget.[22] Rats weighing 150 to 200 g were kept on a normal diet and administered 1 mL of 1% wt/vol solution of guar gum in water. This treatment was continued for 7 days (to induce the specific enzyme responsible for degradation of guar gum in vivo). Thirty minutes before the drug release studies began, the rat was sacrificed, the abdomen was opened, ligatures were made before and after the cecum, and the cecum was removed under anaerobic conditions. The cecum bag was opened and its contents were weighed and homogenized, then suspended in PBS (pH 7.0) to give the desired concentrations (0%, 2%, 4%, and 6%) of cecal contents. The suspension was filtered through glass wool and sonicated (50 W) for 20 minutes at 4°C to disrupt the bacterial cells. After sonication, the mixture was centrifuged at 2000 rpm for 20 minutes. Because the cecum's environment is naturally anaerobic, all the operations were performed in a CO_2 atmosphere.

The drug release studies were performed in sealed glass vials at 37 ± 0.1°C. The previously weighed amount of microspheres (an amount equivalent to 10 mg MTX) was placed in the 20 mL dissolution media PBS (pH 7.0) containing 0%, 2%, 4%, and 6% rat cecal contents. The PBS (pH 7.0) containing a similar concentration of cecal content with placebo microspheres served as a blank. The vials were shaken, samples (0.2 mL) were withdrawn after a fixed time interval of 1 hour for analysis, and the volume of dissolution media was replaced with fresh dissolution media. The studies were performed for 24 hours; samples were diluted appropriately with PBS (pH 7.0) and centrifuged at 2000 rpm for 10 minutes. The supernatant was filtered through Whatman filter paper, and the filtrate was analyzed for MTX content using HPLC.

In Vivo Studies

Twelve healthy albino rats (males and females) were selected for the in vivo study, which was approved by the university's committee on the ethical treatment of animals. The formulation $DPGGC_3$ was selected in order to study in vivo performance of the preparation, on the basis of in vitro release studies.

Albino rats of similar weight were selected for in vivo studies, kept in well-spaced ventilated cages, and maintained on a normal diet (grams soaked in water). The animals were divided into 3 groups of 4 animals each. The first group served as controls, and the second group received the plain drug suspension, which was prepared using 1% gum acacia (dose calculated in relation to body weight of the animal). The third group was given the formulation of guar gum microspheres. The doses were given orally with the help of cannula, and after 2-, 4-, 6-, and 8-hour intervals animals were humanely killed and the stomach, the small intestine, and the colon were isolated. These organs were homogenized with a small amount of PBS (pH 7.4), followed by addition of 1 mL of acetonitrile, and kept for 30 minutes. The contents were centrifuged, and the supernatant liquid was separated and diluted appropriately; the drug content was determined using HPLC.

Results and Discussion

Evaluation of Preparation Method

Guar gum microspheres were prepared by the emulsification method. Hardening of microspheres was performed by chemical cross-linking with glutaraldehyde as well as with temperature-induced cross-linking. The particle size of the microspheres was determined using a particle size analyzer. The mean diameter of glutaraldehyde cross-linked guar gum microspheres increased from 12.4 ± 1.02 μm to 16.5 ± 1.22 μm with increasing polymer concentration from 1% to 4% wt/wt. In the present investigation a 2% guar gum concentration was found to be optimal, ensuring the optimal size of microspheres. The average particle size of microspheres increased with increasing polymer concentration, since at higher concentrations the polymer solution dispersed into

larger droplets. At concentrations lower than the optimum the solution became less viscous and dispersed into numerous fine droplets that easily coalesced, resulting in larger microspheres. The mean particle size of microspheres decreased from 29.7 ± 1.27 μm to 14.6 ± 0.95 μm with increasing mixer rotational speed, from 2000 rpm to 5000 rpm (Table 1). Results revealed that the average diameter of microspheres was controlled by rotational speed. The ultimate mean diameter of microspheres was determined by the size of dispersion of the polymer solution, which decreased with increasing mixer rotational speed. Results also suggested that there was a mixing rate limit for a particular polymer concentration. A higher mixing rate did not further reduce the mean diameter. The mixing speed of 4000 rpm was found to be optimal for guar gum microspheres. The effect of stirring time at a particular rotational speed was also observed, and it was recorded that stirring time influenced the shape as well as the size distribution of microspheres, possibly because of variable shear force experienced by the particulate system. A mixing time of 4 hours was found to be optimal (Table 1).

The mean diameter of microspheres was found to range from 17.1 ± 1.33 to 10.4 ± 0.93 μm, with varying concentrations of Span 80 from 1% to 4% wt/wt. Span 80 was used to facilitate the stable dispersion of the polymer in oil. With a fixed rotational speed, the stability of the dispersion of a particular polymer system depends on the concentration of emulsifier. An optimal concentration of emulsifier is required to produce the finest stable dispersion. Below this concentration the dispersed globules/droplets tend to fuse and produce larger globules because of insufficient lowering in interfacial tension, while above the optimal concentration no significant decrease in particle size is observed, because a high amount of emulsifying agent increases the viscosity of the dispersion medium. The optimal concentration of Span 80 was found to be 3%. No remarkable effect on particle size and size distribution was observed with varying concentration of the cross-linking agent; rather, this concentration showed a significant effect on surface smoothness (Figure 1), swellability (Table 1), and in vitro digestion (Table 2). An optimal concentration of cross-linking agent (1.5 mL) was a compromise between degree of swelling and in vitro digestion of the microsphere preparation.

The preparation of guar gum microspheres involved heating the dispersion at an elevated temperature. The mean diameter of guar gum microspheres varied from 25.8 ± 2.19 μm

Table 1. Composition and Characteristics of Various Formulations*

Formulation Code	Variables	Average Particle Size (μm)	Drug-Loading Capacity (mg/100 mg)	Encapsulation Efficiency (%)	Degree of Swelling
MD_O	Methotrexate (0%, 10%, 20%,	12.15 ± 1.04	—	—	0.79
MD_1	30%, and 40% wt/wt)	13.39 ± 1.23	6.9 ± 0.5	69.0 ± 5.0	0.84
MD_2		14.12 ± 1.23	14.7 ± 0.6	73.5 ± 3.2	0.99
MD_3		15.63 ± 1.23	22.7 ± 0.7	75.7 ± 2.1	1.08
MD_4		16.14 ± 1.57	23.0 ± 0.6	57.5 ± 1.6	1.20
MG_1	Guar gum (1%, 2%, 3%,	12.40 ± 1.02	19.7 ± 0.9	65.7 ± 2.9	1.27
MG_2†	and 4% wt/wt)	12.70 ± 1.23	22.1 ± 0.7	73.7 ± 2.1	1.19
MG_3		14.60 ± 1.39	22.6 ± 1.3	75.3 ± 4.2	1.36
MG_4		16.57 ± 1.22	22.9 ± 0.8	76.3 ± 2.8	1.57
MC_1	Glutaraldehyde	13.43 ± 2.32	21.7 ± 1.7	72.3 ± 2.2	1.86
MC_2	(0.5, 1, 1.5, and 2 mL)	12.62 ± 6.23	21.9 ± 0.3	73.0 ± 1.1	1.01
MC_3†		11.59 ± 1.05	22.3 ± 0.9	74.3 ± 3.2	0.59
MC_4		11.53 ± 2.55	22.7 ± 0.7	75.7 ± 2.4	0.41
MS_1	Stirring speed (2000, 3000, 4000,	33.9 ± 2.02	23.2 ± 1.1	73.4 ± 2.4	1.31
MS_2	and 5000 rpm)	32.3 ± 1.70	22.5 ± 0.9	72.3 ± 2.2	1.24
MS_3†		18.7 ± 1.98	21.7 ± 1.1	74.5 ± 2.8	1.13
MS_4		10.8 ± 1.15	21.5 ± 0.9	73.8 ± 2.1	1.06
ME_1	Span 80 (1%, 2%, 3%	17.1 ± 1.74	22.4 ± 1.3	70.8 ± 1.4	1.38
ME_2	and 4% wt/wt)	14.3 ± 2.12	21.8 ± 1.4	72.4 ± 2.6	1.22
ME_3†		11.6 ± 1.54	21.4 ± 0.8	74.2 ± 2.2	1.18
ME_4		10.4 ± 1.34	20.6 ± 0.9	73.3 ± 2.5	1.09
MT_1	Temperature (30°, 40°,	25.8 ± 2.46	21.8 ± 0.9	70.5 ± 2.1	1.54
MT_2	50°, and 60°C)	15.4 ± 2.23	22.9 ± 1.2	71.3 ± 2.5	1.36
MT_3†		13.4 ± 1.06	23.1 ± 1.1	73.6 ± 2.9	1.22
MT_4		22.4 ± 2.56	22.2 ± 0.8	75.4 ± 2.3	1.08

*M indicates microspheres; D, drug (methotrexate); P, pectin; C, cross-linking agent (glutaraldehyde); S, stirring speed; E, emulsifier (Span 80); T, temperature.

†Parameters taken for optimal formulation. Data are expressed as mean ± SD of at least triplicate.

Table 2. In Vitro Digestion of Different Microsphere Preparations in Rat Cecal Contents*

Formulation Code	Glutaraldehyde Concentration	Percent Drug Release in 48 Hours
GG-C_1	0.5 mL	98.6
GG-C_2	1.0 mL	98.5
GG-C_3	1.5 mL	98.7
GG-C_4	2.0 mL	69.8

*GG indicates guar gum; C, cross-linking agent (glutaraldehyde).

to 13.4 ± 1.17 μm when the temperature was increased from 30°C to 50°C, whereas a further increase in temperature, to 60°C, led to an increase in mean diameter to 20.7 ± 1.72 μm. This suggested that the temperature of the system determines the size of the microspheres. Various drug-loaded formulations were prepared by using optimized parameters. Formulations were studied microscopically for shape and surface smoothness, size, and size distribution and observed for effect of drug concentration. The particle size varied from 12.1 ± 1.04 μm to 16.1 ± 1.57 μm, with increasing drug concentration from 0% to 40% wt/wt.

Shape and surface morphology were investigated using scanning electron microscopy. Photographs (Figure 1) indicated that cross-linked placebo microspheres possessed a nearly smooth surface and spherical shape.

Drug-Loading Capacity and Encapsulation Efficiency

Encapsulation efficiency was calculated as the ratio of the weight of MTX content in the final microspheres (100 mg) and the MTX introduced in the process. Percent encapsulation efficiency increased up to 76.3% ± 2.8% with increasing polymer concentrations up to 4%. The concentration of the cross-linking agent had no significant effect on percent encapsulation efficiency (Table 1).

Swellability

Native guar gum swells 100- to 120-fold in gastric and intestinal fluids. As a result of cross-linking with glutaraldehyde the overall swelling of polymer decreased significantly. Cross-linking interferes with free access of water to the guar gum hydroxyl group, which in turn reduces the swelling properties of the cross-linked polymer. The cross-linking of the modified guar gum formulation depended on the glutaraldehyde concentration, but the optimal concentration of the cross-linking agent was a compromise between swellability and in vitro digestion of microsphere preparation in the presence of rat cecal content.

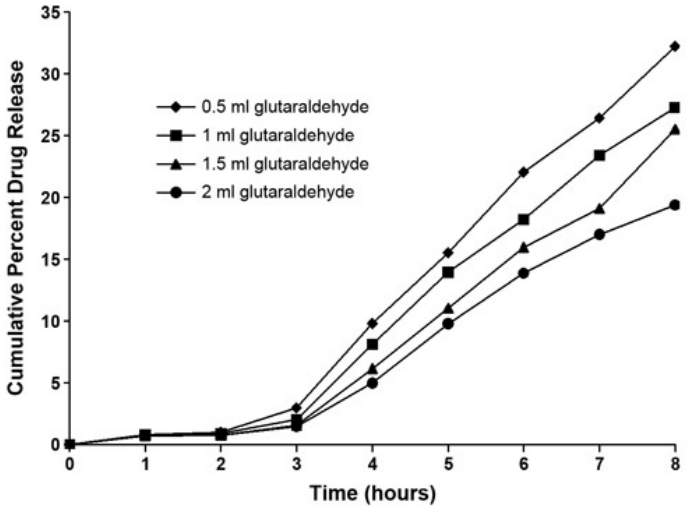

Figure 2. Dissolution profile of methotrexate from glutaraldehyde cross-linked guar gum microspheres in phosphate-buffered solution (pH 7.4) showing effect of glutaraldehyde concentration on rate and extent of methotrexate release.

Drug Release

The mean in vitro dissolution profiles of MTX for investigational preparations (Figures 2 and 3) in PBS and simulated gastrointestinal fluids (SGF and SIF) showed similar patterns and rates of release. Only 0% to 15.3% of the drug was released in the upper gastrointestinal tract environment. This might have been due to dissolution of drug particles adsorbed at the surface and subsequent diffusion from the matrix. Thus, guar gum microspheres can prevent the drug from being released in the physiological environment of the stomach and the small intestine. These results are

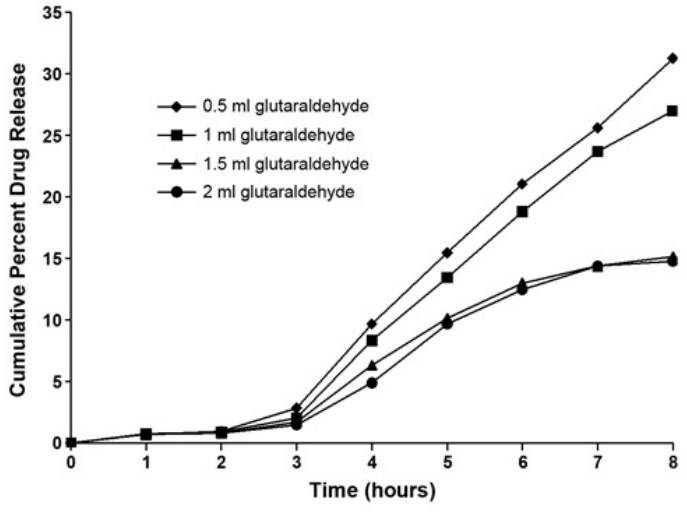

Figure 3. Dissolution profile of methotrexate from glutaraldehyde cross-linked guar gum microspheres in simulated gastrointestinal fluids of different pH. Figure shows effect of increasing glutaraldehyde concentration on rate and extent of methotrexate release.

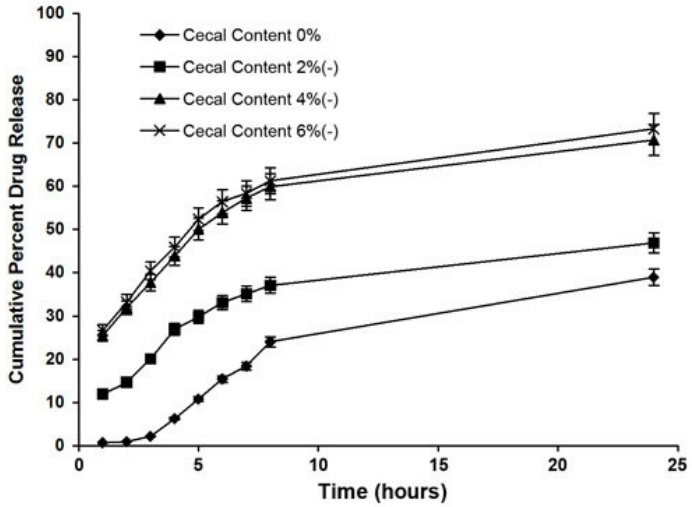

Figure 4. Percent cumulative methotrexate release from glutaraldehyde cross-linked microspheres in media containing rat cecal contents at different percentages. (-) indicates that cecal content was collected without enzyme induction in the rat.

concordant with the results of Krishnaiah et al,[23] who have used compression-coated tablets of guar gum for colon-targeted delivery of 5-fluorouracil. After 5 hours of testing in 0.1M HCl and pH 7.4 Sorensen's phosphate buffer, compression-coated tablets with 60% and 70% guar gum released 4.0% and 3.6% of the drug, respectively, because of the strong shielding effect of the compression coat of guar gum. The release profile of guar gum microspheres in both media clearly indicates that glutaraldehyde slows MTX release from microspheres. Glutaraldehyde causes cross-linking by reacting with the hydroxyl group of galactose and the mannose unit of guar gum, thus interfering with the free access of water to the hydroxyl group of guar gum. This significantly reduces the swelling rate of the microspheres and consequently the penetration of the solvent into the microspheres. Cross-linking also reduces polymer chain mobility, increases glass transition temperature, and decreases diffusivity.[24] MTX release from cross-linked microspheres was found to be glutaraldehyde concentration–dependent; percent cumulative release in PBS decreased from 32.2% ± 1.3% to 19.3% ± 1.2% (in 8 hours) with an increase in glutaraldehyde concentration from 0.5% to 2.0% wt/wt. Depending on the amount of polymer added, such gels tend to hinder the outward diffusion of MTX by blocking it mechanically.

A colon-targeted drug delivery system should not only protect its load from being released in the physiological environment of the stomach and the small intestine but also deliver its load to the colon. Conventional dissolution testing is less likely to accurately predict in vivo performance of colon delivery systems triggered by bacteria residing in the colon (because aspects of the colon's environment [ie, scarcity of fluid, reduced motility, and presence of microflora] cannot be simulated in conventional dissolution methods). Hence, release studies were performed in an alternate release medium (Sorensen's buffer containing rat cecal content at different concentrations) called rat cecal content release medium.

In every case the cumulative drug release was significantly higher in the presence of rat cecal contents than in the control system. In 24 hours the percent cumulative release in the control medium was 38.9% ± 2.0% compared with 73.2% ± 2.3% obtained with the medium containing 6% cecal content without enzyme induction (Figure 4), and that was further increased to 91.0% ± 1.6% with 6% cecal matter obtained after enzyme induction (Figure 5). This could

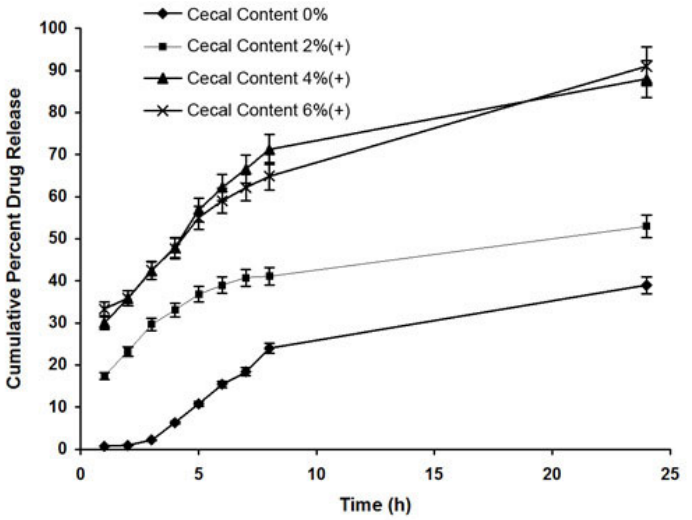

Figure 5. Percent cumulative methotrexate release from glutaraldehyde cross-linked microspheres in media containing rat cecal contents at different percentages. (+) indicates that cecal content was collected after enzyme induction in the rat.

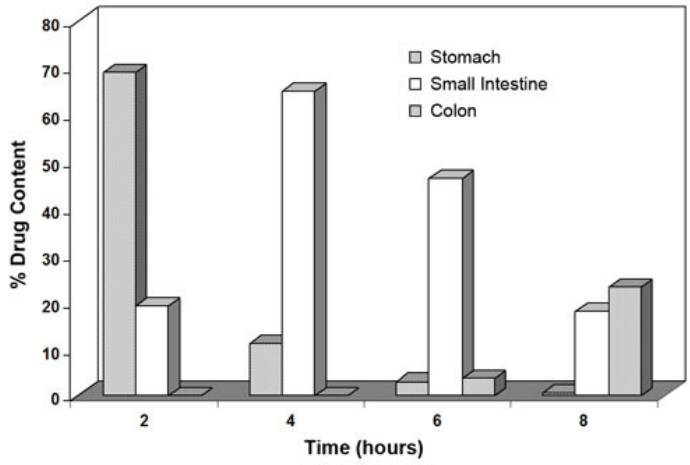

Figure 6. Percentage of administered dose in isolated parts of GIT (stomach, small intestine, and colon) of albino rat after 2, 4, 6, and 8 hours of oral administration of plain methotrexate (as suspension).

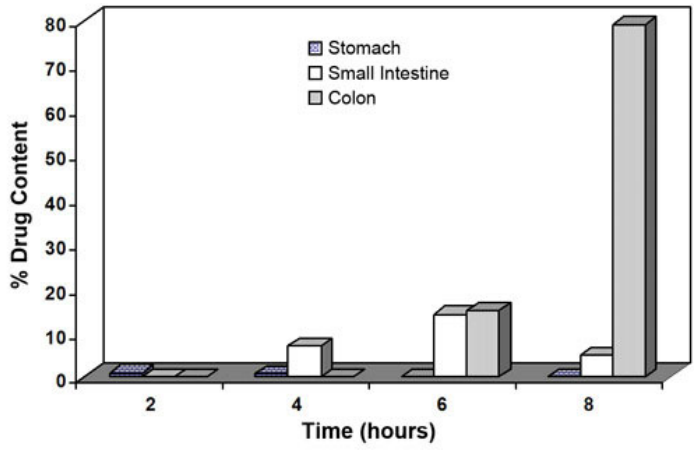

Figure 7. Percentage of administered dose in isolated parts of GIT (stomach, small intestine, and colon) of albino rat after 2, 4, 6, and 8 hours of oral administration of glutaraldehyde cross-linked guar gum microspheres.

have been due to the enzymes present in cecum (secreted by various anaerobic bacteria), which are responsible for digestion/degradation of guar gum in order to release the drug from the microspheres. The rate and extent of drug release were higher in the medium containing a higher concentration of cecal content. The percent cumulative amount of MTX released in the presence of cecal content was found to be increased with a higher concentration of cecal content. The release rate was higher in the medium containing the same amount of cecal content obtained after enzyme induction. Similar results have been reported by Sinha et al,[25] who prepared rapidly disintegrating core tablets containing 50 mg of 5-fluorouracil and compression coating with 175 mg of granules containing a mixture of xanthan gum (XG) and guar gum (GG) in varying proportions. After 24 hours of dissolution the mean percent drug release from the compression-coated XG:GG 20:20, 20:10, and 10:20 tablets was found to be 18% ± 1.2%, 20% ± 1.5%, and 30% ± 1.8%, respectively. Furthermore, the presence of rat cecal content in the dissolution media increased the drug release. Studies of XG:GG (10:20) tablets in the presence of cecal matter showed an increased cumulative percent drug release of 67.2% ± 5.2% in the presence of 2% cecal content and 80.3% ± 3.9% in the presence of 4% cecal content after 19 hours.

In Vivo Studies

For in vivo studies, formulations were orally administered to albino rats and the amount of released drug at different time intervals was estimated in various parts of GIT (ie, stomach, small intestine, colon). The results indicate that after oral administration of plain MTX suspension, a 69.4% ± 3.5% concentration of MTX was observed in the stomach after 2 hours, and in subsequent hours, a far smaller percentage of drug reached the small intestine and colon (Figure 6). Only 23.5% ± 1.3% of the drug load of the conventional dosage form reached the colon after 8 hours. The microsphere formulation was observed to be relatively intact in the upper part of the gastrointestinal tract. Only 0% to 16% of the total load was released during transit through the upper gastrointestinal tract (2-6 hours) because of the leaching process. After 6 to 8 hours the maximum percentage of drug was observed in the colon, and no drug was found in the stomach and the small intestine (Figure 7). The amount of drug recovered from the colon after 6 hours was found to be 14.9% ± 0.9%. That was further increased to 79.0% ± 3.5% after 8 hours, because of the digestion of the polysaccharide by the colon's microbial flora.

CONCLUSIONS

Spherical and free-flowing glutaraldehyde cross-linked microspheres were successfully prepared by the emulsification method. The release profile from glutaraldehyde cross-linked guar gum microspheres was affected by guar gum concentration and glutaraldehyde concentration. Results of release studies demonstrated that microspheres are capable of retarding the release of MTX until it reaches the colon, an environment rich in bacterial enzymes that degrade the guar gum and allow drug release to occur at the desired site. Thus, guar gum microspheres are a potential system for colon delivery of MTX for chemotherapy of colorectal cancer.

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