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FORMULATION AND EVALUATION OF MANGO GUM MICROSPHERES AS A TARGETED DRUG DELIVERY TO COLON.

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ABSTRACT

Present work was aimed for the design of phosphated cross-linkedmicrospheres ofmongo gum (MG) by emulsification method using sodium-tri-meta phosphate (STMP) as a cross-linking agent for treatment of colon cancer using Methotrexate (MTX) as model drug.At a stirring speed of 1000rpm and about 5h of stirring time was found to be optimal to obtain reproducible microspheres.The particle size was increased as there was increase in polymer concentration and decrease in particle size take place as there is increase in stirring speed. Emulsification method was employed for the preparation of Cross-linked MG microspheres. At an surfactant concentration of 2% reproducible microspheres were obtained. SEM studies showed that the drug-loaded microspheres were non-aggregated and in spherical shape. DSC and FTIR studies showed that drug and excipients are compatible. Release studies showed that drug release was more profound in cecal medium induced with enzymes causing degradation of the MG than that of the release showed in SIF. Stability studies showed that there were no significant changes in the drug content and physical appearance of microspheres.

Keywords: **Mango Gum (MG), Tri-sodium tri-metaphosphate (STMP), Cross-linked microspheres, Colon cancer, Methotrexate (MTX).**

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INTRODUCTION

The oral colon targeting system refers to the system, in which orally administered medications are kept from releasing in the upper digestive tract until they are transited to the cecum or colon so that they can exert a local effect on the diseased region to improve their therapeutic effect and Eliminate their toxic or adverse actions at the same time[1,2]. The colon specific drug delivery is valuable in t he topical treatment of colonic disorders such as irritable bowel syndrome, Cronh's disease, Ulcerative colitis and colon carcinomas. The delivery of drugs to colon is also useful for systemic absorption of drugs especially proteins and peptides which are degraded in upper GIT[3]. Most of the conventional drug delivery systems for treating the colonic disorders fail, as the drug do not reach the site of action in appropriate concentration. Thus, an effective and safe therapy of these colonic disorders, using site specific drug delivery is a challenging task. The enzymatic activity associated with the microflora of colon can be used as a tool for colon specific drug delivery. In addition the colon has a longer retention time and appears to be highly responsive to agents that Enhance the absorption of poorly water soluble drugs.The bacterial population present in the colon is a unique feature that allows the site specific drug delivery using polysaccharides. A number of carriers have been investigated like Guargum[4,5],pectin[6],chitosan[7],and dextrin[8]for digestion by colonic bacteria for colon specific drug delivery.During past few decades, utilization of natural polymers for the development of various drug delivery systems has been the subject of great interest[9]. Natural polymers primarily remain. Attractive because of their easy availability, cost effectiveness, biodegradability and biocompatibility[9,10]. One cheap and naturally derived biopolymer is mongo gum (MG) obtained from tree mangiferaindica, Family: Anacardiaceae. Mango gum is a dried gummy exudate polysaccharide obtained from the bark of *Mangiferaindica*, belongs to the family Anacardiaceae. Physical, thermal, sorption and functional properties of a mango gum were characterized. The results obtained in this study establish the fundamental characteristics of mango gum[11].Gum of Mangiferaindica (mango) as a tablet binder employingparacetamol as a model drug[12],resin ofmangiferaindica (mango) as a tablet retardant polymer in the formulation development of sustained release ofdrugs, employing diclofenac sodium as a model drug was studied[13].Mouth dissolving tablets of metforminhydrochloride was prepared using mango gum powder as disintegrant[14].However, no report is available in literature on the formulation of phosphate cross-linked MGmicrospheres.The drug chosen for the preparation of microspheres is Methotrexate (MTX). Methotrexate (MTX) acts as an antagonist of folic acid, which is necessary for DNA synthesis, and has a therapeutic effect on many types of cancer cells that overexpress folate receptors on their surfaces[15]. MTX is currently widely used as a major chemotherapeutic agent for human malignancies such as acute lymphoblastic leukemia, malignant lymphoma, osteosarcoma, breast cancer and head and neck cancer[16]. However, its clinical efficacy is often compromised by the acquisition of resistance in cancer cells, due to cellular efflux of the molecule[17].The encapsulation of antitumor drugs in nanoparticulated systems like polymeric NPs, which retain a higher drug concentration within the cell, might overcome the shortcomings associated with conventional drug delivery strategies[18]. MTX-loaded CS-based NPs have been developed using especially modified forms of chitosan, to improve controlled drug delivery to tumors[19].

MATERIALS AND METHODS

The Methotrexate was purchased from Hi-media, Mumbai. Mongo gum was obtained from local market.Sodium tri meta phosphate was procured from Sigma Aldrich, Mumbai. All other reagents were of analytical grade and were purchased from Loba chemicals, Mumbai.

2.1. Preparation of Cross-Linked MG Microspheres

Preparation of cross-linked MG microspheres were carried out in two stages: Firstly making an aqueous phase, secondly preparation of organic phase. This was subsequently followed by slow addition of aqueous phase into organic phase with magnetic stirring. The following step-by-step preparation is given as follows:

2.1.1 Aqueous Phase

Solution of MG was prepared by dispersing (1 to 4% w/v) of MG in a beaker containing 10ml of a 2M sodium hydroxide (NaOH) aqueous solution. Solution of STMP (1-4% w/v) was prepared by dissolving STMP in a beaker containing 10ml of de-ionized water. The aqueous phase was obtained by mixing the dispersed MG solution and STMP solution and stirring the mixture for 2min.

2.1.2 Organic Phase

Liquid paraffin (150ml) was taken in a beaker to which 2% w/v span 80 was added and stirred at 50°C. Aqueous phase was added drop wise into the beaker under mechanical stirring (1200 rpm) to obtain the w/o emulsion. The cross-linking reaction took place at 50°C with a constant
stirring speed of 1200 rpm. After 5h of reaction, the microspheres were isolated and washed with acetone thrice. Finally, the cross-linked MG microspheres were dried at 40°C for 12h and kept in closed containers for further studies. Formulation chart of prepared cross-linked MG microspheres were given in Table 1.

Table 1
Formulation chart of prepared cross-linked MG microspheres

Formulation code	MTX(mg)	MG (mg)	STMP(mg)	Liquid paraffin(ml)	rpm
F1	50	100	100	150	1000
F2	50	100	200	150	1000
F3	50	100	300	150	1000
F4	50	200	100	150	1000
F5	50	200	300	150	1000
F6	50	300	100	150	1000
F7	50	300	200	150	1000
F8	50	400	100	150	1000
F9	50	400	300	150	1000

1000 rpm was maintained throughout the preparation

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EVALUATION OF MICROSPHERES

3.1Particle Size Analysis

Particle size analysis[19]was performed by optical microscopy using a compound microscope. A small amount of dry microspheres was suspended in purified water (10ml). The suspension was ultra sonicated for 1min. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and particles of diameter of atleast 300 particles were measured using a calibrated ocular micrometer.

3.2 *Micromeritic Properties*

Micromeritic properties such as tap density, Carr index, angle of repose were calculated. Tap density of the pre-pared microspheres was determined using tap density tester and percentage Carr index (%CI) was calculated. Angle of repose (h) was assessed to know the flow ability of themicrospheres, by a fixed funnel method.

3.3 *Scanning Electron Microscopic (SEM)*

SEM photographs were taken with a scanning electron microscope Model Joel-LV-5600, USA, at the required magnification at room temperature. Prepared microspheres were deposited on a glass disc applied on ametallic stub and evaporated under a vacuum overnight. Before the SEM
analysis, the samples were metallized under an argon atmosphere with a 10-nm gold

palladium thickness (EMITECH-K550 Sputter Coater, Houston, TX).

3.4 *Differential Scanning Calorimetry (DSC)*

All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC module. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10°C/min. The runs were made in triplicate.

3.5 *Fourier Transform Infrared Radiation Measurements (FT-IR)*

FT-IR analysis was carried out for pure drug and for microspheres obtained, using KBr pellet method on FTIR spectrophotometer type Shimadzu model 8033, USA.

3.6 *Sphericity of the Microsphere*

To determine the sphericity[20] the tracings of MTXloaded cross-linked microspheres (magnification 45x) weretaken on a black paper using camera lucida, (Model–Prismtype, Rolex, India). Circulatory factor (S) was calculated as:

$$S = \frac{p^2}{1256 \times A} \quad (1)$$

where A is area (cm^2) and, P is the perimeter of the circulartracing.

3.7 *Swellability*

First, 100mg of microspheres was placed in distilled water and allowed to swell until a constant weight is attained in each medium. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The formula for calculation of degree of swelling(α) is as follows:

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

W_o=Initial weight of the microspheres, W_g=Final Weight of the microspheres.

3.8 *Process yield*

The yield was determined by weighing the microspheres and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and cross-linking agent. The formula for calculation of percentage yield[21]is as follows;

$$\% \text{ yield} = \frac{\text{wt of microspheres}}{\text{wt of polymers} + \text{wt of drug}} \times 100 \quad (3)$$

3.9 *Drug Loading and Encapsulation Efficiency*

10mg of microspheres was dispersed in 10ml of pH 7.4 phosphate buffer. The sample was ultra-sonicated for 3 con-secutive periods of 5min. Solution was filtered and from the filtrate obtained, 1ml of solution was transferred to 10ml volumetric flask and diluted up to the mark. Absorbance was measured at 307nm at UV absorption spectrophot-ometer (Shimadzu 1801, USA). The drug content was calculated by using the formula:

$$\text{Amount of drug} = \frac{\text{conc. from standard graph} \times \text{dilution factor}}{1000} \quad (4)$$

Percent drug loading and encapsulation efficiency were calculated using the following equations:

$$\% \, drug \; loading = \frac{weight \; of \; drug}{weight \; of \; microparticles} \times 100 \quad (5)$$

$$Encapsulation \; efficacy = \frac{actual \; drug \; content}{theoretical \; drug \; content} \times 100 \quad (6)$$

3.10 *In Vitro Drug Release Studies*

The release studies of MTX from cross-linked MGmicrospheres was performed using a U.S. pharmacopoeia dissolution rate test apparatus (Basket type, 100 rpm, 37±0.1°C) in pH progression method i.e. in SGF of pH 1.2 for 2h and SIF of pH 4.5 for 3h and SIF of pH 7.4for next 3h. At pre-determined time intervals, 1ml of samples were withdrawn and sink conditions were adjusted by replacing an equal volume of fresh medium. Withdrawal samples were analyzed for drug release measuring at 307nm by UV absorption spectrophotometer (Shimadzu1801, USA).

3.11 In-vivo Studies

The project proposal has been cleared and approved by Institutional animal ethical committee, J.S.S. College of pharmacy, Mysore (Code: 108/2012).

Preparation of Enzymes Induced Rat Cecal Content Medium

Rats weighing 150 to 200 gm were kept in normal diet and administered 1ml of 1% w/v solution of MGin water. This treatment was continued for 7 days. Rats were sacrificed humanely and the cecum was isolated and ligated at both ends. Further ligated cecum was cut loosed and was immediately transferred into SIF (pH 7.4) previously bubbled with carbon dioxide to maintain anaerobic condition. The above solution was kept in an incubator for 24h to ensure that the bacteria present should sufficiently multiply and enzymes will be produced. Later the suspension is filtered through Whatmann filter paper no. 42 and suspended in buffer to produce a final concentration of 4% w/v[22]. This solution is used for dissolution studies as simulated colonic fluid. To analyze the drug release mechanism, *in vitro* release data were fitted into a various drug release models like zero-order, first order, Higuchi, Hixon-Crowell cube root law and Korsmeyer-peppas model.

3.11 *Stability Studies of the Optimized Formulation*

Optimized formulation of the microspheres was selected for stability studies[23]according to ICH guidelines by storing at 25°C/60% RH and 40°C/75% RH for 90 days.Samples were withdrawn on the 15th, 45th and 90th days and checked for changes in physical appearance and drug content spectrophotometrically at 307nm.

RESULTS AND DISCUSSION

STMP cross-linked MG microspheres were successfully prepared by using an emulsification method. Crosslinking reaction takes place due to heat i.e at 50°C and also due to emulsion formation.

4.1 *Micromeritic Properties*

The values of angle of repose (h) ranged from22.4 to 26.6indicating that the obtained values were well within the limits. Result clearly shows that the prepared microsphereshave reasonably good flow potential. The value of CI was found to be in the range of 10.6 to 16.2%. The values of tapped density ranged between 0.3851 to 0.6413 g/cm^3. Sphericity of all the prepared cross-linked MG microspheres was found to be near to 1 confirming the spherical shape of prepared micropsheres.

4.2 *Effect of Stirring Speed and Mixing Time on Prepared Cross-Linked MG Microspheres*

Two important factors that influence the size distribution and yield of microspheres are the optimum stirring speed and stirring time. Studies showed that a stirring speed of 1000 rpm and stirring time of 5h were found tobe optimal to obtain reproducible microspheres.It was found that as we increase the stirring speed from1000 to 1200 rpm, there was a decrease in the average sizeof the microspheres and a low recovery of microspheres had been observed as shown in Table 2. It is due to the smallsize of microspheres, which were lost during successivewashings during filtration.Trials were done with stirring speed lower than1000 rpm. It was found that when the stirring speed waslower than 1000 rpm, larger particles were formed. Increasein stirring time, from 5 to 7h (at stirring speed of1000 rpm) caused decrease in the recovery yield and hardening of the microspheres, resulting in reduced release of thedrug. When the stirring time lower than 5h, it was observed that some

amount of microspheres particles adhered to the sides of the beaker resulting in the lower recovery. Repeat batches treated at an optimized rate of 1000 rpm and for 5h proved to produce reproducible sizes, showing that stirring speed and stirring time were well controlled.

Table 2
Effect of stirring speed on % yield of cross-linked MG microspheres

Rotational Speed	% yield ± SD*
800	72±1.89
900	56±1.62
1000	87±0.38
1100	64±0.47
1200	57±1.42

Mean±SD, n=3.

4.3 *Effect of Emulsifier Concentration on Prepared Cross-Linked MG Microspheres*

Span 80 was used as an emulsifier to facilitate the stable dispersion of the polymer in oil. To obtain an optimal surfactant concentration, various concentrations ranging from 0.5 to 2.0% w/w of total formulations were tested. Discrete microspheres with good flow properties using an optimum concentration of surfactant 2% w/w of span-80 were obtained. Below this concentration, the dispersed globules/droplets tend to fuse and produce larger globules because of insufficient lowering in interfacial tension and did not give reproducible microspheres.

4.4 *Particle Size Determination*

Size of prepared cross-linked MG microspheres ranges from 281 to 534µm. It was found that there is an increase in particle size as polymer concentration is increased whereas a reduction in particle size was observed as there is increase in stirring speed. At stirring speeds above 1500 rpm, the turbulence caused frothing and adhesion of the microspheres to the container walls and propeller blade surfaces, resulting in high shear and a smaller size of the dispersed droplets. Spherical microspheres were obtained at a stirring speed of 1000 rpm; therefore, this speedwas used duringmanufactureof all microspheres. For instance, as the amount of polymerincreases from 1:1 to 4:3 (polymer: cross-linking) particle size has increased from 281 to 534µm. This can be explained to the fact that at higher concentration of polymer theviscosity of polymer solution increased, thereby producing bigger droplets during emulsification.

4.5 SEM

SEM studies shown in Fig. 1 clearly show the spherical nature of prepared cross-linked microspheres. Non-aggregated microspheres were observed. Absence of crystalline structures on the surface of the microspheres indicates that MTX is well dispersed inside the carrier. Due to the cross-linking of the polymer the surface of the microspheres were found to be rough with slight ridges on the surface.

Figure 1
SEM pictures of microspheres formulations. SEM of F5 at 50x

4.6 DSC and FTIR Studies

The DSC thermo gram of pure Methotrexateshowed a sharp and large melting point at 195.2°C and 198.1°C, indicating the absence of drug and polymer interactions as shown in Fig. 2. Fluorouracil pure drug and optimized formulation (F5) were subjected to FT-IR spectroscopic analysis for compatibility studies to ascertain whether there is any interaction between the drug and cross-linked polymer. The IR spectra of MTX and drug-loaded micro-spheres (F5) were found to be identical indicating that characteristics peaks of MTX were not altered in their position after successful entrapment in the microspheres as shown in Fig. 3. The characteristic IR absorption peaks of MTX at 1643 cm-1 (-CO-NH), 1604 cm-1 (C=C benzene backbone stretching), 1500 cm-1 (aryl systems) and 831 cm-1 (aromatic ring system) were present in drug-loaded microspheres.

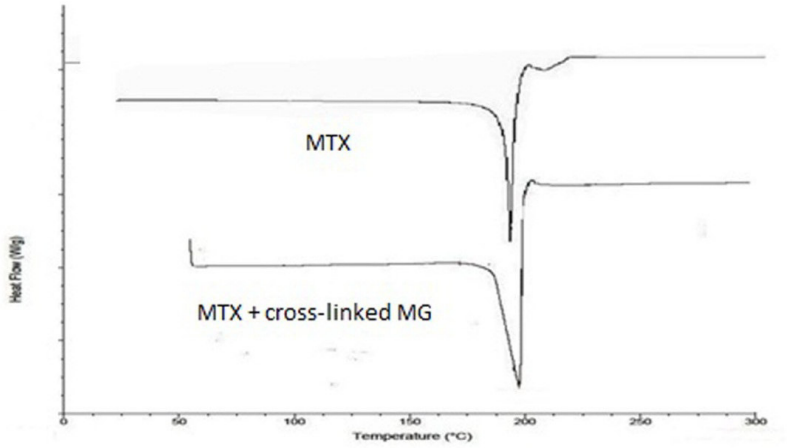

Figure 2
DSCoverlap spectra of pure drug and formulation F5.

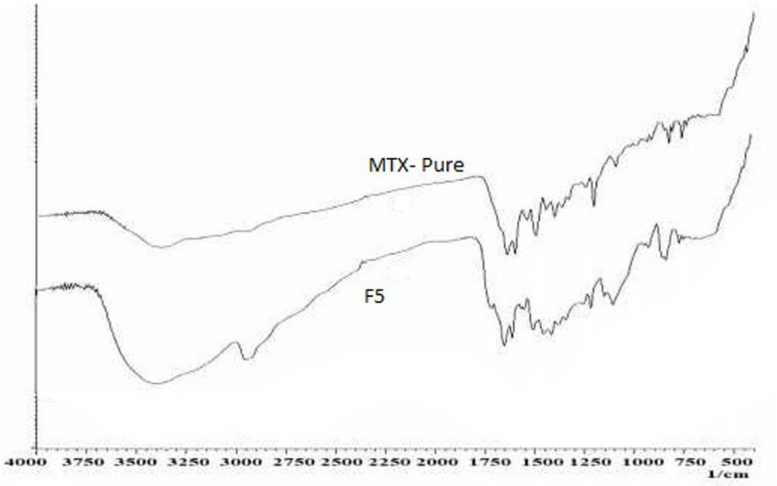

Figure 3
FT-IR overlap spectra of pure drug and formulation F5.

4.7 *Swelling Studies*

Results of swelling studies were shown in Fig. 4. Native MG swells 80 to 100 fold in gastric and intestinal fluid which results in retarded drug release. As a result of cross-linking with STMP the overall swelling of polysaccharide decreased significantly thereby enhancing the release of drug. Cross-linking of MG with STMP interferes with free access of water to the MG hydroxyl group, which in turn reduced the swelling properties of cross-linked polymer.

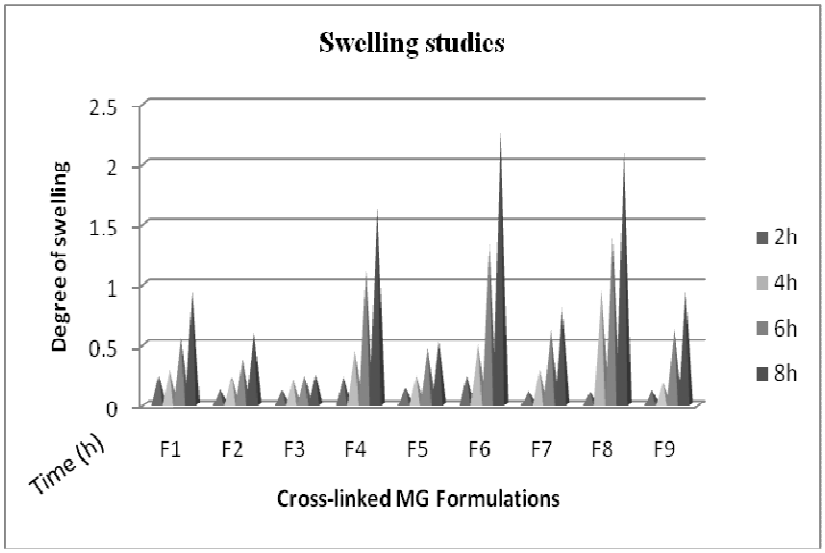

Figure 4
Swelling studies of prepared cross linked MG microspheres

4.9 *Process yield, Drug Loading and Encapsulation Efficiency*

Percentage yield of prepared cross-linked MG microspheres varies from 73.8±1.11 to 92.18±0.49. High encapsulation efficiency was observed for all microsphere formulations. The encapsulation efficiency ranged between 48.78±0.81% and 83.53±1.21%. F5, F7, showed relativelyhigher encapsulation efficiency as these formulations com-posed of high concentration of polymer. Among all formulations, F5 and F7 showed maximum percentage yield anddrug loading.

4.10 In Vitro Drug Release Studies

In vitro release studies for all prepared microspheres formulations were shown in Fig. 5. In all the formulations the % CDR is<33% till 5th and the release was increased from 6th in SIF of pH 7.4. From 1st to 5th the release is <33% due to the leaching and dissolution of drug particlesadsorbed at the surface. The release is also restricted due to the % cross-linking agent used. At 6th, 7th, 8th the formulations get slowly degraded by the pancreatic and bacterial colonic enzymes, thus releasing the drug, depending on the ratio of the gum to cross-linking agent.F5 containing 2:3 ratio of MG and STMP showed 82.61% release of drug in SIF. As cross-linking of MG with STMP in this ratio completely interferes with the free access of waterto the hydroxyl group of MG swelling of the MG is reduced, thus preventing the retardness of the drug release. F2 andF7 containing 1:2 and 3:2 ratio of MG and STMP showed 72.41% and 78.09% release of the drug at the end of 8th. F3 containing 1:3 ratio of MG and STMP showed 38.17% release of drug as MG is cross-linked with a very high amount of STMP. F6 and F8 containing 3:1 and 4:1 ratio of MG and STMP showed 52.72% and 55.33% release of drug. The release of the drug in these formulations in the 6th is very high followed by very less amount of release in the 7th and 8th as required amount of STMP is not used to cross-link all the molecules of MG thus causing immense swelling of the gum thereby retarding the release of the drug. Thus, the formulation F5 showed high % CDR in 8h in SIF of pH 7.4. The dissolution of F5 is done in medium containing 4% rat cecal content (with enzymes induced) for 3h to compare the release in the presence of MG degrading enzymes as shown in Fig. 6. Only 27.370.19% of drug released in SGF and SIF of pH 1.2 and 4.5. After 6h the percentage drug released in ratcecal content medium was 43.810.14% and release was further increased to 95.200.18% at 8h because of the digestion of the polysaccharide by the enzymes induced by colonic microbial flora in the enzyme induced rat cecal medium.

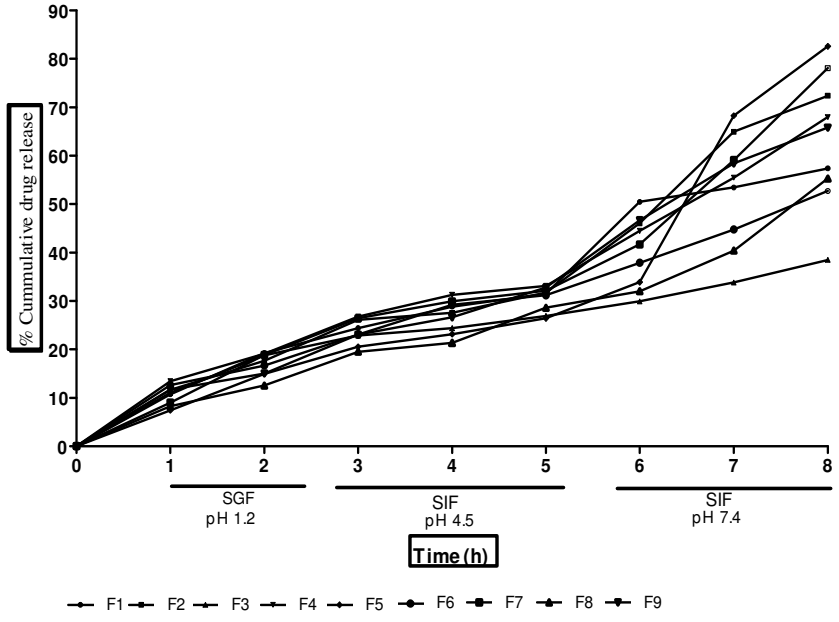

Figure 5
In vitro dissolution studies

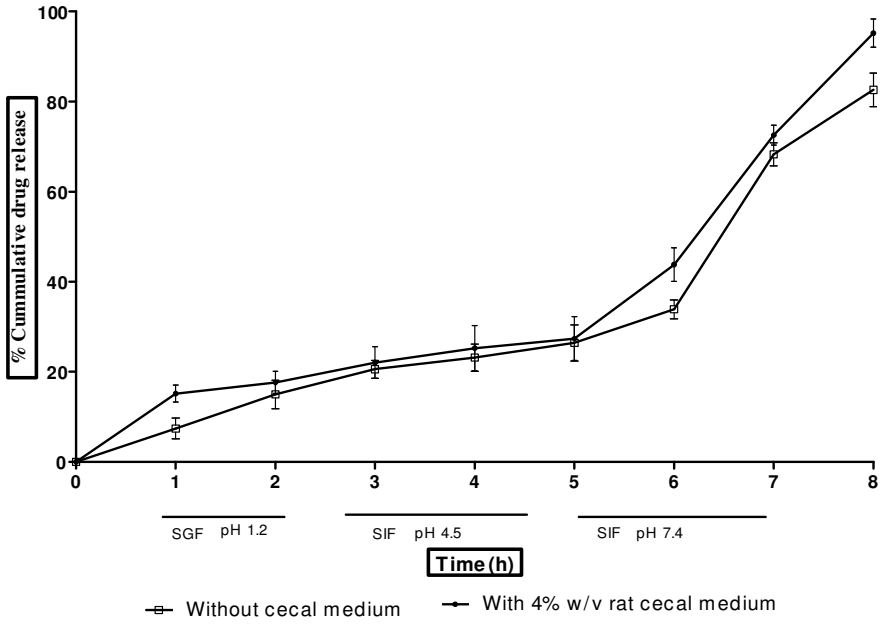

Figure 6
In vitro drug release profile of cross linked MG Microspheres in pH buffer and 4% Cecal

4.13 ***Stability Studies***

Samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. From the stability data it was found that there were no significant differences between drug content within desired stability period. There was no change in physical appearance in the microspheres. It is clear that the formulation did not undergo any chemical changes/interaction during the study period.

DISCUSSION

Prepared multiparticulate delivery system of MTX loaded cross-linked MG microspheres can be used as a novel delivery system for the targeted release of MTX for treatment of colon cancer. Method used for microspheres preparation was most suitable for water soluble drugs. From the results of particle size analysis, it is clear that all the process variables were within the limits and the process is reproducible. The prepared microspheres exhibited good micromeritic properties. The mean size was ranging between to 281 to 534mm. From the SEM studies, it was observed that the drug loaded microspheres were non-aggregated with spherical shape. The microspheres showed rough surface due to the cross-linking of the MG and the temperature used during emulsification. The DSC and FTIR studies revealed drug and polymer compatibility indicating absence of any interactions between drug and polymer. Release studies showed that drug release was more profound in cecal medium induced with enzymes causing degradation of the MG than that of the release showed in SIF of pH 7.4. The *in vitro* drug release studies showed that, the release of drug was found to follow peppas model. The *in-vitro* and *in-vivo* correlation between the % drug released and % drug absorbed was found to be good having regression coefficient of R^2=0.96. Results of the stability studies showed that there were no significant changes in the drug content and physical appearance. Thus 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 MG and allow drug release to occur at the desired site.

CONCLUSION

From the above results it can be concluded that the MG was a promising polysaccharide in designing the formulation of cross-linked MG microspheres as an targeted delivery of MTX to colon.

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