Cross-linked guar gum and sodium borate based microspheres as colon-targeted anticancer drug delivery systems for 5-fluorouracil

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Abstract: The aim was to prepare cross linked polymer of 5-fluorouracil loaded microspheres containing guar gum and sodium borate for colon-targeted drug delivery systems. Micro spheres were prepared using emulsification cross linking method. The influence of drug polymer ratio, cross linker agent concentrations and cross linking timing on *in vitro* drug release and characteristics in terms of drug loading, entrapment efficiency and yielding percentage were investigated. The optimum drug loading, entrapment efficiency and percent yield were obtained from formulations with the lowest content of cross linker agent over 2 h of cross linking timing but with the highest drug to polymer ratio 1:11. The optimum *in vitro* drug release was obvious upon decreasing drug to polymer ratio up to 1:09, resulting in 81.5% drug release over 24 h. In conclusion, micro spheres composed of gaur gum and sodium borate can delay and control the release of 5-fluorouracil over 24 h. Thus, further *in vivo* studies are suggested for final assessment.

Keywords: 5-Fluorouracil, micro spheres, guar gum, colon drug delivery, anticancer delivery, sodium borate.

INTRODUCTION

Colorectal cancer is a common cancer and stands next to lung cancer (Potter, 1999). Surgical intervention, radiation therapy, chemotherapy and biological therapy are the possible treatment of colorectal cancer depending upon the stage of cancer (Krishnaiah *et al*., 2003). For oral chemotherapy treatment, colon targeted drug delivery has gained the importance. Several techniques like pH of the colon, colonic microflora enzymes activity, colon transit time and time dependent release have been used to achieve the colon drug delivery by protecting the drug from it absorption and degradation in the upper gastrointestinal tract (GIT) and then in abrupt release in proximal colon (Chourasia and Jain, 2003). These techniques can be implemented by coating the drug with pH sensitive polymers, used of polymers that are degraded specifically by colonic bacteria and an increase of colon transit time by covalent linking of the drug with the carrier and use of bio-adhesive (Arafat, 2015) and osmotic controlled drug delivery system (Chourasia and Jain, 2003).

Recently, drug loaded biodegradable micro spheres have received increasing effectiveness and potential applications in cancer therapy because of biocompatible, biodegradable, local and control release profiles of the formulation (Seong *et al*., 2003). Several polysaccharides have been deliberated for colon-specific drugs delivery based on this approach. It includes pectin, amylose, dextran, guar gum, insulin, chitosan and chondroitin sulphate (Chourasia and Jain, 2004). As a polysaccharide, they remain resistance to the harsh environment of the stomach and small intestine and retain its integrity as matrices until they reach the colon and exposed to bactericidal activity. Among these polysaccharides, guar gum has been used in pharmaceuticals extensively as a favourable career for colon targeted delivery (Thakur *et al*., 2009). Guar gum is hydrophilic in nature and swells in cold water to form a viscous colloidal dispersion (Johnson and Gee, 1981). It's swelling property control the releases of the drug from the dosage form and protect it from degradation in the colon. Different cross linking agents like trisodium trimetaphosphate were used to modify its swelling properties and to be used as a vehicle in oral dosage form (Chourasia and Jain, 2003). The gelling property of guar gum and its enzymatic degradation has made this polymer as an ideal drug carrier for colon (Sharma *et al*., 2013).

5-Flurouracil (5FU) is the drug of choice in the treatment of colon cancer (Calavresi and Chabner, 1996). The drug belongs to Biopharmaceutical Classification System (BCS) class III drug with high water solubility and poor intestinal permeability (Daniel *et al*., 2003). Chemically, 5FU is a dipodic acid and highly polar in nature with pKa values of 8.0 and 13.0 (Rudy and Senkowski, 1973; Williams and Barry, 1991). After oral administration, the 5FU is poorly absorbed and shows the variable bioavailability up to 80% (Diasio and Harris, 1989). This variation in bioavailability is due to rapid degradation and conversion to dihydrofluorouracil (DHFU) in the gastrointestinal tract (Cao *et al*., 1994) this is contributed by the inter-individual dihydropyrimidine dehydrogenase (DPD) enzyme activity that carried out the catabolism of 5FU. The DPD enzyme primarily found in liver, but also found in the intestine, pancreas, lung, kidney and other

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tissues as well as in different neoplasm (Naguib *et al*., 1985). The DPD activity in intestinal mucosa is particularly variable and probably contributes to the erratic bioavailability of 5FU (Spector *et al*., 1993). Also, DPD enzyme activity is eight folds in cancer patients (Etienna *et al*., 1994) as compared to four folds in healthy individuals (Lu *et al.*, 1993).

At present, standard regimen include the 5FU intravenous (IV) bolus injection that also produces severe systemic toxic effects including gastrointestinal, hematological, neural, cardiac and dermatological origin (Diasio and Harris, 1989). Most of these systemic unwanted effects are due to 5FU after reaching the unwanted sites. Therefore, targeted drug delivery of 5FU in colorectal cancer may reduce the systemic toxic effect with effective and safe therapy when compared with IV and conventional oral administration. Assuming this can lead to reducing systemic toxic effects. The advantage of targeting colon for 5FU delivery is due to the pH range in the colon (5.5-7), low digestive enzymatic activity and long transit time. All these factors can contribute to support drug absorption (Arafat, 2012; Arafat, 2016). In this context, guar gum based micro spheres loaded with 5FU containing sodium borate as cross linker agent was formulated and developed to control the release of the maximum content of drug over 24 h, specifically in the colon site and provide effective therapy for colorectal cancer. It's also assumed to protect the encapsulated 5FU from possibly harsh physiological conditions and thus improves drug stability and duration of action. The present study describes the preparation and development of cross-linked polymer based control release formulation of 5FU-loaded microspheres containing guar gum and sodium borate for colon-targeted oral drug delivery systems.

MATERIALS AND METHODS

Materials

Chemicals

5FU was a gift from Amson pharmaceutical industry (Islamabad, Pakistan). Guar gum (GG) was brought from Sigma-Aldrich (St. Louis, USA). Liquid paraffin, Span-80, Tween-80, Sodium borate (Na-borate), Isopropyl alcohol, Hydrochloric acid, Sulfuric acid and Sodium hydroxide were purchased from Dae-Jung Chemicals & Metal Co., Ltd (Gyeonggi-do, South Korea). Potassium dihydrogen phosphate was obtained by Merck Chemicals, (Darmstadt, Germany). All chemicals used in this work were of high-performance liquid chromatography (HPLC) analytical grade.

Preparation of 5FU -loaded microspheres

Formulation of 5FU-loaded microspheres containing different drug to polymer ratio with the addition of different percentage of crosslinker agent (Na-borate) over various period of crosslinking times were prepared and named accordingly as follow: Fd-1, Fd-2, Fd-3, Fd-4, Fd-5, Fd-6 and Fd-7 (table I, II, III). The preparation of microspheres was based on an O/W emulsification solvent evaporation. method as described previously (Lamprecht *et al*., 2003). Briefly: different ratio of polymer to drug was dissolved in 40 g distilled water containing 0.1% of Tween-80 (w/w). Then, the drug-polymer suspension was emulsified in 100 g liquid paraffin containing 2 % of Span 80 (w/w) using a magnetic stirrer for 4 h. Later, the addition of 0.2 mL of sulfuric acid to the 5 g of distilled water was made followed by the addition of Na-borate (crosslinker agent) by the dropwise method. To confirm the cross linking network formation in the emulsion, an extensive stirring was performed at 500 rpm. The micro spheres were passed thorough filtration (filter, Millipore, pore size 0.45 µm) and washed with isopropyl alcohols three times to remove the remaining amount liquid paraffin. The micro spheres kept overnight at room temperature then dried in an oven for up to 5h at 40^{o}C. For formulation optimization, various parameters like drug-polymer ratios, cross-linking time and content of cross-linker were examined.

Drug loading and entrapment efficiency determination

Drug loading (DL) and entrapment efficiency (EE) of 5FU-loaded micro spheres and yield percentage (YP) were calculated. Micro spheres of 100 mg were added to 10mL of phosphate buffered saline (PBS, pH 7.4). The PBS solution containing micro spheres was stirred for 24 h. The supernatant layer of suspension was collected after centrifugation and the drug concentrations were determined using an ultraviolet (UV) spectrophotometer (Hitachi U2000, Tokyo, Japan) with an absorbance wavelength of 266 nm. The average of YP, DL and EE were calculated in triplicates using the following equations (Golocorbin-Kon *et al*., 2009; Arafat *et al.*, 2016; Arafat *et al*., 2017):

$$\text{DL (\%, w/w)} = \frac{\text{Amount of 5FU in the particles}}{\text{Total amount of 5FU and particles}} \times 100$$

Equation (1)

$$\text{EE (\%)} = \frac{\text{Amount of 5FU in the particles}}{\text{Amount of 5FU used in formulations}} \times 100$$

Equation (2)

$$\text{YP (\%,)} = \frac{\text{Actual yield (g)}}{\text{Theoretical yield (g)}} \times 100$$

Equation (3)

Fourier transform infrared spectroscopy (FTIR) spectraanalysis

Fourier Transform Infrared Spectroscopy (FTIR) was recorded on spectrophotometric analysis. The spectra of pure drug 5FU, pure polymer (GG), microspheres with and without 5FU were obtained from IRAffinity-1S FTIR spectrometer (Shimadzu, Japan). Samples were prepared in potassium bromide discs and the spectra were scanned in the range of 500-4000 cm^{-1} at a resolution of 1 cm^{-1}.

For data analysing FTIR data, Origin Pro software was used.

Scanning electron microscopy

The microspheres image of 5FU-loaded microspheres was obtained by scanning electron microscopy (SEM). The microspheres were places on supports with carbon-glue and coated with gold using a gold sputter module at the voltage of 2-15 kV.

In vitro drug release

The rate of drug releases from polymer-based micro spheres containing GG and Na-borate were determined using USP XXIII basket type dissolution apparatus. 20 mg of micro spheres was filled into capsules and placed in the basket. The experiment was performed in 900mL of phosphate buffer saline (PBS) pH 7.4 as the dissolution medium. The temperature was maintained at 37.0±0.5ºC while the stirring speed was set at 100 rpm. Samples of 5 mL volume each were collected at predetermined time intervals, namely, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13,14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24h using an automated fraction collector. At the end of 24h, the remained un-dissolved samples were crushed in the dissolution vessels and stirring was maintained for another 30 min to ensure that the entire drug was released into the dissolution media. Samples were then collected and analyzed. The drug concentrations were determined using UV-spectrophotometer (Hitachi U2000, Tokyo, Japan) with an absorbance wavelength of 266 nm after appropriate dilutions. For each formulation, the dissolution testing was run in triplicates of six. Besides, various kinetics models were used namely: First order, Zero order, Higuchi, and Korsmeyer-Peppas model, to find out the release kinetics and mechanism of drug release.

STATISTICAL ANALYSIS

A statistical analysis was performed using one-way analysis of variance to compare mean value of each variables considering a result statistically significant when $p<0.05$.

RESULTS

SEM Images and FTIR spectroscopy analysis

To confirm the formation of microspheres, the preparation of cross-linked GG-based microspheres was observed by SEM (fig. 1). The microspheres morphology of 5FU-loaded microspheres made with drug to polymer ratio 1:09 over 2 h timing and with 6.0 % of cross liniking agent were found to be round and smooth in surface with particle size of 258 µm, indicating the successful preparation of cross-linked GG-based microspheres. This finding was supported by the FTIR spectra, in which the microspheres were shown to be cross-linked and no chemical reaction was occurred between the drug and polymer during cross-linking step, indicating the stability of the drug in the formulation.

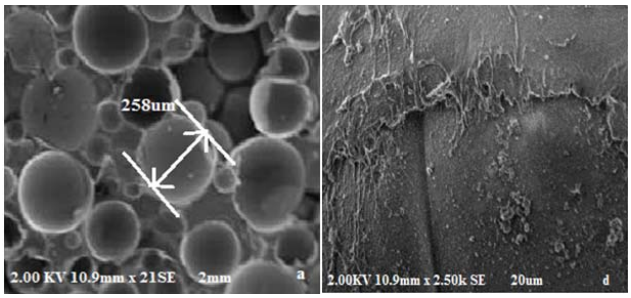

Fig. 1: SEM images of micro spheres (a) and Surface morphology (b) of 5FU-loaded micro spheres made with drug to polymer ratio 1:9 over 2 h timing and with 6.0% cross liniking agent. Calliper indicates 258 µm. Images were obtained by SEM under 10.9 mm x 21k and 2.5k SE respectively, operating at 2.00 kV.

Fig. 2 shows the results of combined FTIR analysis of pure drug powder (5FU), pure polymer (GG) and micro spheres with and without drug. First, pure drug (5FU) shows a peak of (C=O), peak stretching at 1719.65cm^{-1} and peak stretching of (C-N) at 1644.94cm^{-1}, peak stretching of (C-H) at 1242.68 cm^{-1}. The characteristic peak of (–NH) was denoted at 3064.34cm^{-1}. On the other hand, the stretching vibration of O-H in GG appeared at 3308.57cm^{-1} and C-H at 2904.87 cm^{-1} respectively. The bending vibration of C-H and O-H was observed at 1417.95cm^{-1} and 866.29cm^{-1}, whereas sharp peak was present at 1257.50cm^{-1}, suggesting new absorption band has occurred due to the association of O-H and C-H bending vibrations of the polymer. For microspheres with and without drug, the absences of C-H and O-H peaks were observed. However, due to crosslinking complexation between drug and polymer, a new peak appeared at 2923.55 cm^{-1}, characteristic peak of 5FU was also observed in drug loaded microspheres due to carbonyl stretching vibration frequency of ester, indicates the stability of drug as 5FU undergoes no chemical changes leading to a successful cross-linking network achievement between borate and GG in the micro spheres.

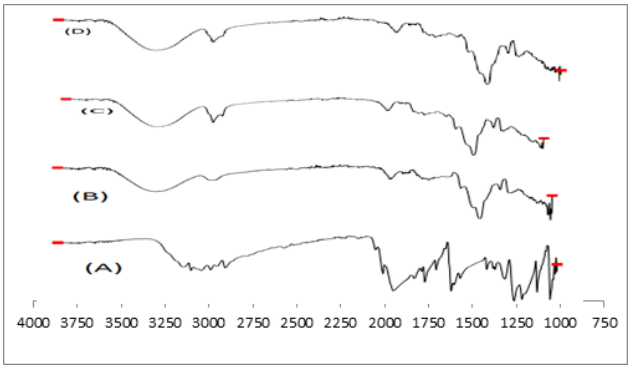

Fig. 2: Combined *FTIR spectra* of (A) 5FU, (B) GG, (C) 5-FU loaded microspheres (D) and Unloaded microspheres.

Table 1: Yield percentage, drug loading and encapsulation efficiency of different formulations of 5FU- loaded microspheres made with different content of cross-linking agents (data are mean, n = 3).

Formulations	(5-FU : GG)	Cross linker (%, w/w)	Cross-linking Time (h)	YP (%)	DL (%)	EE (%)
Fd-1	1:09	10.0	2.0	54.5± 3.5	5.9± 0.5	41.8± 2.5
Fd-2	1:09	8.0	2.0	70.5± 4.7*	6.3± 0.9	51.9± 3.7*
Fd-3	1:09	6.0	2.0	74.3± 3.1*	7.9± 1.6	63.7± 2.8*

* $p<0.05$ vs Fd-1

Table 2:Yield percentage, drug loading and encapsulation efficiency of different formulations of 5FU- loaded microspheres made over different cross-linking timings (data are mean, n = 3).

Formulations	(5-FU : GG)	Cross linker (%, w/w)	Cross-linking Time (h)	YP (%)	DL (%)	EE (%)
Fd-3	1:09	6.0	2.0	74.3± 5.1	7.9± 1.6	63.7± 2.8
Fd-4	1:09	6.0	2.5	70.3± 4.7	5.4± 0.8*	39.5± 3.7*
Fd-5	1:09	6.0	3.0	68.2± 3.6	4.1± 0.9*	30.7± 2.8*

$p<0.05$ vs Fd-3

Table 3:Yield percentage, drug loading and encapsulation efficiency of different formulations of 5FU- loaded microspheres made with a different drug to polymer ratios (data are mean, n = 3).

Formulations	(5-FU : GG)	Cross linker (%, w/w)	Cross-linking Time (h)	YP (%)	DL (%)	EE (%)
Fd-6	1:09	6.0	2.0	74.3± 6.1	7.9± 1.6	63.7± 2.1
Fd-5	1:10	6.0	2.0	78.3± 4.8	8.0± 0.9	67.6± 3.1*
Fd-7	1:11	6.0	2.0	79.2± 5.7	8.4± 1.1	69.7± 2.8*

$p<0.05$ vs Fd-6

Table 4: Kinetic model of drug release data obtained from all tested formulations of 5FU-loaded microspheres.

Formulation	Zero order Kinetic		First order Kinetics		Higuchi Model		Korsmeyer-Peppas' Model	
	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	R^2	n
Fd-1	2.571	0.959	-0.036	0.905	0.135	0.816	0.478	1.04
Fd-2	1.378	0.984	-0.016	0.971	0.074	0.833	0.358	0.74
Fd-3	1.059	0.988	-0.012	0.979	0.057	0.882	0.322	0.70
Fd-4	1.639	0.981	-0.021	0.962	0.087	0.858	0.398	0.84
Fd-5	1.633	0.972	-0.019	0.953	0.086	0.841	0.401	0.88
Fd-6	3.312	0.935	-0.056	0.805	0.172	0.783	0.511	1.08
Fd-7	1.791	0.967	-0.022	0.938	0.095	0.835	0.398	0.81

Characterizations of 5FU-loaded Microspheres

For 5FU-loaded microspheres characterization, DL, YP and EE from various formulations made with different content of cross linker agent of 6.0, 8.0, 10.0%, w/w are shown in table 1. Optimum YP, DL and EE was obtained with 6.0%, w/w of the crosslinker agent, indicating a significant decrease in the content of cross linking agent lead to increase the YP, DL and EE of 5FU-loaded microspheres. A similar observation was obtained upon preparing various formulations of 5FU-loaded microspheres over the different cross linking timing of 2, 2.5 and 3h (table 2). Decreasing the cross linking timing from 3 to 2 h was leading to a significant increase the YP, DL and EE of 5FU-loaded microspheres. Therefore, the optimum combination rate of Na-borate and GG in the formulation had occurred upon the addition of small content of cross linker agent over minimum timing.

On the other hand, table 3 shows the YP, DL and EE obtained from various formulations of 5FU-loaded microspheres were made with different drug polymer ratio of 1:9, 1:10 and 1:11 (5FU:GG). In general, YP, DL and EE of 5FU-loaded microspheres from all the series of the formulation were found to be in the range of 54.5% – 79.2 %, 4.1% - 8.4%, and 30.7% - 69.7%, respectively. The optimum YP, DL and EE of 5FU-loaded microspheres was obtained upon increasing the drug polymer ratio to 1:11.

In-vitro evaluation of 5FU release from microspheres

To investigate the sustainability of the microspheres formulation to control the releases of the drug over prolonged period in PBS media, the *in vitro* release experiment of 5FU loaded microspheres containing GG and Na-borate were carried out over 24 h in PBS media

(pH 7.4) at 37ºC. However, the *in vitro* release experiment in a media with acidic pH of 1.2 and 5.5 were not taken to account because of our preliminary experiments showed that the percentage of drug release was found to be minimal (up to 4%) in acidic pH media. The influence of polymer ratio, cross linking agent content and timing of drug release were showing variable drug release profile, however all formulations were releasing the drug in a sustainable manner.

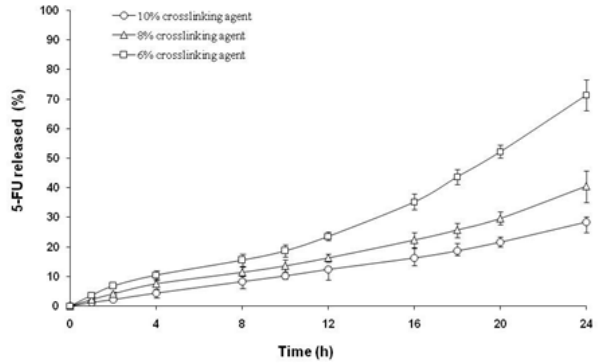

Fig. 3: *In vitro* release of 5FU from the loaded microspheres made with different content of cross linking agent at 37ºC over 24 h in PBS media at pH 7.4.

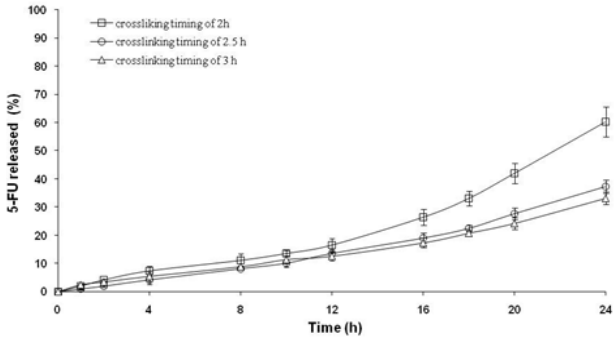

Fig. 4: *In vitro* release of 5FU from the microspheres made over different cross linking timing at 37ºC over 24h in PBS media at pH 7.4.

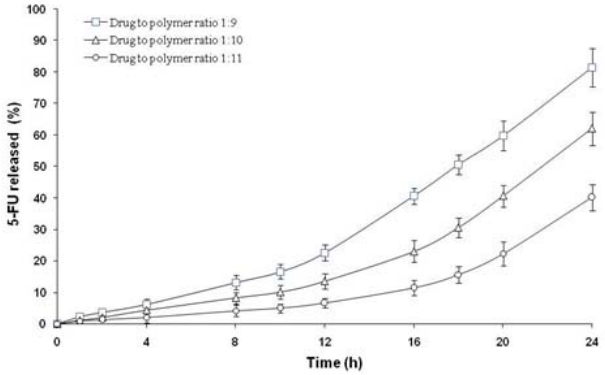

Fig. 5: *In vitro* release of 5FU from the microspheres made with a different drug to polymer ratio at 37ºC over 24h in PBS media at pH 7.4.

Fig. 3 shows that the 5FU release profiles from microspheres containing various contents of the crosslinker agent over 24 h in media with pH adjusted to 7.4. It is evident that the drug release rate was slower as the amount of cross linking agent present in the formulation was progressively increased, as reflected by the slopes of the release profiles. The decrease in the drug release rate profile was in the order of 10% < 8% < 6% of cross linker agent content in the formulation. This indicates that the drug release rate can be varied in a predictable manner by varying the amount of cross linker agent being incorporated. Similarly, fig. 4 shows that the drug release profiles from microspheres under the effect of various cross linking timing. It is evident that the drug release rate was slower as the timing of cross linking agent increased more than 2 h, as reflected by the slopes of the release profiles. The drug release rate profile was in the order of 3 h < 2.5 h < 2 h of cross linker agent timing resulting in 33.2%, 39.3% and 60.3% of 5FU released over 24h, respectively. This indicates that drug release rate can be varied in a predictable manner by varying the timing of cross linking agent being incorporated.

Fig. 5 shows the influences of drug to polymer ratio (5FU: GG) on 5FU release rate from the microspheres over 24 h. It is evident that the drug release rates were slower as the ratios of polymer to the drug in the formulation were progressively increased, as reflected by the slopes of the release profiles. The drug release rate was in the following order: 1:9 > 1:10 > 1:11 of a drug-polymer ratio in the formulation, resulting in a drug release percentage of 81.5%, 62.1% and 40.2%, over 24 h, respectively. This indicates that the release rate can be varied in a predictable manner by varying the drug-polymer ratio being incorporated.

On the other hand, *in vitro* releases of 5FU microspheres were fitted to different kinetics models to predict the mechanism of drug release pattern from the cross linked polymer based microspheres formulation. First and zero order, Higuchi and Korsmeyer-Peppas models of kinetics were applied. The comparison of R^2 and release rate constant (K) was made in the approximation (table 4). All formulations were well fitted and followed zero order kinetics release having a R^2 value from 0.935 to 0.988. Korsmeyer-Peppas model was also used to describe the mechanism of drug release. All formulation were found to release the drug through Super Case II transport (exponent value > 0.89).

DISCUSSION

In the current study, controlled release formulations of GG-based microspheres containing 5FU with different ratio of polymers were prepared using emulsification cross-linking technique. The addition of different content of crosslinker agent in the formulation over different

crosslinking times were evaluated in terms of DL, EE and YP. The formulations with higher results were further subjected to *in vitro* release evaluation. Assuming that formulation of GG based microspheres containing 5FU is expected to further prevent 5FU release in the upper GI track, while a maximum release could occur in colon over determined period of time.

GG was chosen for colon-specific drug delivery system because of the polysaccharide nature of this polymer (Freitas *et al.,* 2009). Usually, polymers matrices from polysaccharides origin such as guar gum are assumed to be physically stable in the stomach and small intestine, but in the colon, polymer disintegration occurs by bacterial polysaccharides, resulting in polymer drug content release (Chourasia and Jain, 2004). Besides, the bio-adhesive and bio-degradable functionality of GG are essential for controlling the drug delivery over prolonged period of time in the colon (Gliko-Kabir *et al*., 2000). Previous studies showed that GG based formulation could release up to 80% of bioactive molecule in the colon, indicating that GG based formulation is promising for colon-specific drug delivery system (Pezron *et al*., 1989). Many previous studies have reported the inclusion of GG in many control release dosage forms containing 5FU such as compression-coated tablets (Sinha *et al*., 2004; Sinha *et al*., 2005; Krishnaiah *et al*., 2002) coated pellets (Krishnaiah *et al*., 2003) microspheres (Ji *et al*., 2007) gelatin capsule coated with GG microspheres (Chandra *et al*., 2011) GG-based multi-unit pellets (Bhat *et al*., 2013). All these formulations were working on protecting the drug from degradation by microbial enzymes in the upper GIT by preventing the drug release in the stomach and small intestine, while a maximum drug release occurred in the colon.

On the other hand, borax was chosen to be incorporated in microspheres formulation because it can be functioned as cross linker agent for polymers, in which hydroxyl groups involved in bonding between the polymeric chains and cross-linker (Chourasia and Jain, 2004) resulting in GG-borax interactions. Or, as a result of the existence of intermolecular linkage between borax ions and polymeric chains, polymer-ion complexes can be formed. This may results in changing the peculiar rheological properties of the polymers (Shibayama *et al*., 1988; Kesavan *et al*., 1992; Tayal *et al*., 1999). A previous NMR spectroscopy study on cross linking reaction between borate ion and polyhydroxy polymers using dilute mixtures of borate ions and GG demonstrated that the values of complexation equilibria are constants (Tayal *et al*., 1999).

In our study, the optimum combination rate of Na-borate and GG in the formulation had occurred upon the addition of small content of cross linker agent over minimum timing. Indicating that higher amount of cross linker agent over long cross linking timing is not recommended as it might cause instant gelling of polymer and squeeze out the aqueous phase from the gel lattice (Rastogia *et al*., 2007).

In terms of characterizations of 5FU-loaded microspheres, a significant increase in DL, EE and YP with increasing the ratio of polymer to the drug from 1:09 to 1:11 could be attributed to the increase in the formation of the cross linking to the system stabilizing the internal aqueous phase and restricting its access to the outer aqueous phase. Therefore, based on various parameters was varied in a series of formulation, the optimum YP, DL and EE were obtained from a formulation with the lowest content of cross linker agent and lowest cross linking timing but with highest drug polymer ratio 1:11 (50 of 5FU:550 of GG). Indicating that best 5FU-loaded microspheres formulation might be related to the highest drug to polymer ratio produced upon decreasing the cross linker agent and timing to a certain level.

Maximum drug release (81.5%) was achieved from a formulation having lower drug polymer ratio while a minimum content of drug was released from a formulation having higher cross linking agent content. This might be attributed to low susceptibility toward cross linked polymer disintegration and delayed swelling property. Similarly, a minimum content of drug was released from a formulation upon increasing the timing of cross-linking agent to 3 h. Therefore, the formulation prepared over short period cross linking timing of 2 h was found to be the optimum formulation in terms of releasing more content of drug (60.3%) compared to less content of drug release (33.2%) over the long cross-linking timing of 3 h. Slow order of drug release was also obtained upon increasing polymer ratio in the formulation, suggesting that the probability of increasing polymer density and subsequently the length of drug diffusion path (Gowda *et al*., 2010).

All formulation were found to release the drug through Super Case II transport as the exponent value was found to be greater than 0.89. Suggesting that in all formulations, diffusion, swelling, and erosion are the main three mechanisms controlling drug release. The first step started with swelling polymers and then swelling as well as diffusion because of the swelling cause polymer chains relaxation and imbibitions of water, leading the polymer to swell and transfer from a glassy to rubbery state, indicating the diffusion mechanism of drug release from the formulation (Patel *et al*., 2011; Siepmam *et al*., 2001; Bhat *et al*., 2013).

Our finding was supported by the previous study on GG microspheres but with grafted-acrylamide as a crosslinking agent, in which only up to 58% of 5FU was released at pH 7.4 (Bhat *et al*., 2013). But in our study, we have achieved up to 81.5% of drug release in PBS, pH 7.4

with the incorporation of Na-borate as across linker agent. Suggesting that drug release rate was related to the existence of cross linker agent which possibly can create cross linking network between the polymeric chains and agent, resulting in potentiating the effectiveness of drug release control pattern. It's obvious that the drug release profile was depending on the formulation composition. Therefore, the inclusion of cross linker agent Na-borate into microspheres formulation can clearly control the release of BCS class III drug 5FU in pH 7.4 over 24 h rendering them as promising candidate for colon-targeted oral drug delivery systems.

CONCLUSION

Microspheres formulation composed of gaur gum and Na-borate was successfully prepared using emulsification cross-linking method. A formulation containing drug to polymer low ratio (1:09) using 6% of the cross linker agent over 2 h was able to control the release of anticancer drug (5-FU) over 24 h. The 5FU-loaded microspheres were found to be stable in the formulation and all microspheres were found to follow zero order kinetics with both degradation and erosion mechanisms. Hence, the preparation of cross-linked polymer based microspheres containing guar gum and Na-borate can delay and control the release of drug assuming to reach the colon with the maximum amount. Thus, *in vivo* studies are suggested to further assess the effectiveness of formulation as promising colon-targeted oral drug delivery systems.

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