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Preparation, characterization and *in-vitro* release studies of enteric coated gelatin capsules containing guar gum microspheres for targeted delivery of 5fluorouracil to colon

Subramanya K. Bhat¹, J. Keshavayya^{1*}, Venkatrao H. Kulkarni², Venugoapala K. R. Reddy³, Anandrao R. Kulkarni² and Preeti. V. Kulkarni²

¹Kuvempu University, Janna Sahyadri, Shankaragatta, Karnataka, India ²SET's College of Pharmacy, S R Nagar, Dharwad, Karnataka, India ³Sahyadri Science College, Shimoga, Karnataka, India

ABSTRACT

Enteric coated gelatin capsule device containing guar gum (GG) microspheres loaded with 5-fluorouracil (5-FU) were developed. 5-FU loaded GG microspheres prepared by water-in-oil (w/o) emulsification method and loading of 5-FU done by both soaking and in-situ techniques during emulsion cross linking procedure. Absence of interaction of 5-FU with polymeric matrix and the cross linking agent was confirmed by Fourier transform infrared (FTIR) spectral study. Thermal studies confirmed the stability and molecular level dispersion of 5-FU in polymer matrix. Swelling experiments revealed that extended cross-linking led to formation of more rigid microspheres and reduction of swelling behavior. In the present study 5-FU loading was 11.28, 3.55, 12.25 and 4.62% with respect to GG microspheres at 1.5 h and 3 h with soaking and in-situ technique respectively. Enteric coated pulsatile capsule device of 5-FU loaded guar gum microspheres were developed and further, in-vitro release studies were conducted at pH 1.2 and pH 7.4 to simulate actual GSF (gastrointestinal fluid) and GIT (gastrointestinal tract) condition respectively. The results indicated that enteric coated capsule device significantly lowers the initial burst effect when compared to microspheres formulation. Colon-targeted release of enteric coated capsule device was achieved successfully over the time period of 2-8 h.

Key words: Guar gum, 5-fluorouracil, microspheres, targeted drug delivery

INTRODUCTION

Over the past few years, colon targeted drug delivery technology have gained increased interest when compared to other regions of gastrointestinal tract (GIT). The colon has nearly neutral in pH, longer transit time and reduced enzymatic activity which makes drug absorption better. In order to achieve a successful targeted delivery of drug to the colon via GI tract, it needs protection of drug from degradation and release of the drug in stomach and small intestine [1-3]. Present day's colon targeted drug delivery systems are very useful for local treatment of diseases such as ulcerative colitis, crohn's disease, irritable bowel syndrome (IBS) and carcinomas and colon cancer. Among the different methods attempted to deliver drug using polymers which are degradable in the colon, time-dependent devices [4], coating the drug with a pH sensitive polymer [5, 6], bacterially triggered [7,8] and osmotic pressure controlled drug delivery systems [9] have shown promising results. However main drawback with these systems is

their nonspecific nature or burst release of drug in stomach/small intestine leading to toxic effects and increases in cost of treatment. To overcome this problem, various polysaccharides such as Chitosan, pectin, amylase, inulin, GG, chondroitin sulphate, cyclodextrin, dexran, sodium alginates etc. [2] along with their blends or composites [10-12] have been used for colon specific release.

GG is a naturally occurring nonionic polysaccharide derived from the cluster bean seeds, i.e. *Cymompsis* tetraganolobus or *C. psoraloides*. Structurally it comprises long straight chain of D-mannose units, linked together by $(1\rightarrow 4)$ - β -D-mannopyranosyl units with α -D-galactopyranosyl unit joined by an $\alpha(1\rightarrow 6)$ glycoside linkage [13]. It has gained increased importance due to its chemical and biological properties such as non-toxic, easy availability and biodegradability. It has wide range of applications in pharmaceutical sector as thickening agent, binder, disintegrant as well as stabilizing agent [14, 15]. Many reports are available for the use of GG and modified GG for oral delivery of drugs [16-20].

5-FU is a pyrimidine analogue, most commonly used in cancer therapy which inhibits synthesis of thymidylate synthase. Interrupting the action of this enzyme blocks synthesis of the pyrimidine, thymidine [21], which is essential for DNA replication. The goal of the present work was to prepare 5-FU loaded GG microspheres and to develop enteric coated capsule device to investigate its release pattern for 5-FU. 5-FU loaded GG microspheres were placed inside gelatin capsule coated with cellulose acetate phthalate (CAP), so that entire system avoids release of 5-FU in upper GIT and small intestine (pH 7.4), capsule loses its enteric coating and pH sensitive based matrix starts to release 5-FU in a controlled manner.

MATERIALS AND METHODS

MATERIALS

Guar gum (GG), tween 80 and glacial acetic acid were procured from Merck specialties Private Ltd, Mumbai, India. AR grade glutaraldehyde (GA) (25% (v/v) used as a cross linking agent was procured from Spectro Chem Pvt. Ltd. Mumbai, India. Light liquid paraffin oil, span-80, petroleum ether of AR grade were all purchased from S.D Fine chemicals, Mumbai, India. Water used was produced via reverse osmosis water system with double distillation (Merck Millipore). Gift sample of 5-FU was obtained from Biochem Pharmaceutical Industries, Mumbai, India.



Fig. 1. Flow chart for preparation of GG microspheres

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Preparation of GG microspheres

The GG microspheres were prepared by emulsification followed by cross linking method with GA. In a typical procedure (see Fig.1), required quantity of GG was dispersed in tween 80 solution. (1% w/v) and allowed to swell for an hour, then 1ml acetic acid and 1ml dilute sulphuric acid were added, stirred thoroughly to get an uniform mixture. This mixture was dropped to 100 ml light liquid paraffin at 50° C containing span 80 (0.5% w/v) as an emulsifier. This suspension medium was stirred with teflon blade using laboratory stirrer (Remi lab stirrers, Mumbai, India) 1000 rpm for 10 min, 1ml GA (25%) extracted in 2ml toluene was added and stirred for 1.5, 3.0 h. Microspheres obtained were washed with petroleum ether and then distilled water to remove excess GA and tween 80 solution adhered to microsphere surface; To produce 5-FU loaded microspheres, same procedure adopted but 5-FU in solution form was added to GG solution.

Drug loading by soaking technique

Drug loading was also done by soaking technique. Maximum percent upto 50 of 5-FU loading with respect to dry weight of the polymer was performed in order to choose the best method for drug loading. Accurately weighed microspheres were soaked in saturated solution of 5-FU overnight[17] The drug loaded microspheres were filtered and washed with water to remove the adhered drug on surface of microsphere and then dried in a vacuum desiccator and kept in desiccator until use.

Characterization of microspheres

Particle size analysis and morphological study

The mean particle size of microspheres was determined by optical microscopy by measuring nearly 500 randomly selected microspheres. The surface morphology of the microspheres was observed by SEM (JEOL model JSM-840A, Japan). The microspheres were deposited on brass hold and sputtered with gold and acceleration voltage used was 10 kV, images of microspheres were taken by random scanning of the stub.

Swelling Studies

The swelling behavior of cross-linked empty microspheres was determined by measuring the change in diameter of the microspheres in phosphate buffer (pH 7.4). The microscopic technique was used to determine the extent of swelling [22]. In this method, the dry microspheres without containing drug were immersed in a cavity watch glass containing phosphate buffer for an hour; the change in diameter of the microspheres was monitored at regular interval of time, diameter of swollen microsphere was noted. Experiments were repeated thrice and the average values were considered for data treatment and calculation. The percentage swelling of the microspheres was calculated from the formula,

% Swelling =
$$\frac{D_t - D_o}{D_o} \times 100$$

where D_t is the diameter of the swollen microspheres at time t and D_o is the initial diameter of the microspheres.

Fourier transforms infra-red (FTIR) Spectral study

The FTIR spectral studies were performed using a Shimadzu spectrophotometer (model 8400S, Tokyo, Japan). About 2 mg of samples were finely ground with KBr and the pellets were prepared under a hydraulic pressure of 600 Kg/ cm^2 . The spectra were taken in the range 400-4000 cm⁻¹.

Thermogravimetrc analysis

Thermogravimetric analysis of the samples was carried out using a Thermogravimetric Analyzer (Universal V4 5A TA, Instrument Newcastle, USA). Known quantity of sample was placed on an aluminium pan and the measurements were conducted at a heating rate of 10 0 C min⁻¹ from room temperature to 600 0 C. Differential scanning calorimetric (DSC) analysis were performed on different samples to determine dispersion of the drug in polymer matrix. DSC measurements were performed using a DSC-60 (Shimadzu, Tokyo, Japan) and samples were heated at the rate of 10 0 C/min. These measurements provide quantitative and qualitative information about physical and chemical changes that involve endothermic (heat absorbed) or exothermic (heat evolved) processes, or changes in heat capacity.

Content Uniformity

Microspheres were evaluated for 5-FU content. Accurately weighed quantity (50mg) of the microspheres was suspended in 10ml buffer solution (pH 7.4) for an hour at 45 ^oC and the swollen microspheres were crushed in an agate mortar with pestle and the whole solution was transferred to 50 ml volumetric flask, diluted up to mark with same solution, kept for 24 h for complete extraction. Solution was centrifuged at 5000 rpm and supernatant solution was collected, analyzed spectrophotometrically at 266 nm by using LABINDIA UV 3000+, Mumbai, India. Percentage of drug loading and % encapsulation efficiency was obtained by using equations 1 and 2 respectively.

% Drug Loading =
$$\frac{\text{Quantity of drug in the microspheres}}{\text{Quantity of microspheres taken}}$$
 X 100----- (1)
% Encapsulation efficiency = $\frac{\text{Actual drug loaded}}{\text{thearitical loading}}$ X 100 ----- (2)

Development of pulsatile capsule device

The pulsatile capsule device was prepared according to the method adopted earlier [23]. Known amount of 5-FU loaded GG microspheres were placed inside the gelatin capsule by hand filling. 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 enteric coated with 5% cellulose acetate phthalate (CAP) in 8:2 (v/v) mixture of acetone: ethanol by dip coating method. Coating was repeated until a 13 to 15 percent weight gain was attained.

In vitro drug release study

In-vitro drug release studies of 5-FU loaded GG microspheres as well as enteric coated gelatin capsule device was carried out by using digital Tablet Dissolution Test Apparatus (Veego Instruments, Mumbai, India) Six/Eight station, as per USP standards which is operated at 100 rpm and at 37 ^oC. Known amount of 5-FU loaded GG microspheres/ pulsatile capsule device were placed inside the cellulose acetate dialysis membrane bag having a molecular weight cutoff between 12,000 and 14,000 Da. The bag was tied with the cotton thread, placed inside the disso basket having the mesh type pattern in order to avoid the floating of the dialysis bag. Dissolution studies were carried out in 900 ml simulated gastric fluid (SGF, 0.2M HCl/KCl buffer, pH 1.2) for first two hours to mimic gastric emptying time and then the medium was drained off and replaced with SIF (pH 7.4). A 5ml of aliquot was withdrawn at predetermined intervals and replaced with same volume of fresh dissolution medium. The samples were analyzed for drug content by measuring absorbance at 266 nm using UV spectrophotometer (LABINDIA UV 3000+, Mumbai, India).

RESULTS AND DISCUSSION

Characterization of GG microspheres

The SEM photograph of 5-FU loaded GG microsphere is shown in fig. 2. Microspheres produced in this research were almost spherical in shape with spongy structure having average particle size of $125\pm50\mu$ m. The reason for getting almost uniform particle size is due to adopting emulsification technique using over head stirrer with constant stirring speed of 1000 rpm.

Content uniformity and % encapsulation efficiency

Results of % drug loading of various formulations are presented in table 1. Effect of extent of cross-linking, i.e., time duration of exposure on the entrapment efficiency indicated that with increased cross-linking, % encapsulation efficiency decreased. Cross-linked GG microspheres at 1.5 h and 3 h with soaking and in-situ technique showed 11.28 ± 10.37 , $3.55\pm1.07\%$ and 12.25 ± 0.51 , $4.62\pm3.78\%$ drug loading respectively. Lower drug loading were observed for both in-situ and soaking methods at higher cross linking time of 3 h when compared to the 1.5 h cross linked matrices. This is due to high swelling nature of microspheres and water soluble property of 5-FU during water wash process. The results comply with previous reports regarding lower entrapment efficiency of 5-FU formulations [24, 25].



Fig. 2. SEM photograph of 5-FU loaded cross linked GG microspheres

Table 1. Results of % of drug loading, % of swelling of 5-FU loaded cross linked GG microspheres

GG (in w/v)	GA used(ml)	Cross linking time (h)	Method	% 5-FU loading	% swelling
3	1.0	1.5	soaking	11.28±10.37	83.33±25.86
3	1.0	3	soaking	3.55±1.07	65.39±23.71
3	1.0	1.5	In-situ	12.25±0.51	80.48±22.73
3	1.0	3	In-situ	4.62 ± 3.78	64.45±18

Swelling studies of microspheres

Rate of drug release and penetration of water through the polymer depends upon the rigidity of polymer as well as an extent of its cross-linking ability. Swelling behavior of GA cross-linked GG microspheres has been studied by measuring the diameter of the microspheres at a particular time interval. The swelling pattern of cross linked microspheres at two different cross-linking time i.e., 1.5 and 3h at constant temperature is shown in the fig. 3. The plots indicate that microspheres swell as soon they come into contact with phosphate buffer (pH 7.4). The result also indicates that as the cross-linking duration increased, the resultant swelling capacity of the microspheres decreased. This is due to the increased rigidity of the microspheres at longer cross-linking time.



Fig. 3. Plot of % increase in the diameter V/s swelling time for GG microspheres at 1.5 h (•) and 3h (•)

FTIR studies

The microspheres were prepared by emulsion cross linking of GG with GA. Free hydroxyl (-OH) groups of GG reacted with GA to form an ether linkage.

FTIR spectral analysis were carried out to confirm the cross linking of GG microspheres by GA as well as to confirm the absence of chemical reaction between drug and polymer. The FTIR spectra of (a) GG, (b) cross linked GG microspheres, (c) 5-FU entrapped GG microspheres and (d) 5-FU are shown in Fig. 4. In case of plain GG, the

characteristic O-H and C-H stretching vibration was observed at 3425.34 cm⁻¹ 2923.88cm⁻¹ respectively. The C-H and O-H bending vibration appeared at 1419.51 cm⁻¹ and 1018.34 cm⁻¹ respectively. The appearance of a sharp peak appears at 1257.50 cm⁻¹ in the spectra of the cross-linked microspheres confirms the formation of ether linkages due to cross linking reaction.

5-FU showed the characteristic N-H stretching band at 3110 cm^{-1} and C=O and C-N stretch at 1720 cm^{-1} and 1651 cm⁻¹, CH in plane and CH out plane were observed at 1240 and 892 cm⁻¹ respectively. When the drug was incorporated into the cross-linked GG, along with the characteristic bands of the cross-linked GG microspheres, additional peaks have appeared due to the presence of 5-FU in the matrix. The characteristic bands of 5-FU such as C=N- stretching and C=O stretching vibrations appeared at 1644 cm⁻¹ and 1743 cm⁻¹ respectively, in the drug loaded matrix without any change. This indicates that 5-FU did not undergo any chemical changes while producing the microspheres and it supports the literature data [26].



Fig. 4. FTIR spectra of (a) GG, (b) 5-FU (c) cross linked GG microspheres and (d) 5-FU loaded GA cross-linked GG microspheres.

Differential scanning calorimetric studies

The DSC of GG, pure 5-FU, GA cross linked GG microspheres and 5- FU loaded GG microspheres are shown in fig. 5. An endothermic peak of pure GG was observed at 90.56 $^{\circ}$ C, which represents its glass transition temperature (*Tg*) of the GG, and sharp exothermic peak at 355 $^{\circ}$ C corresponds to the decomposition temperature. After cross linking with GA, the endothermic peak was shifted to higher temperature when compared to the pure GG. This indicates cross-linking of polymer with GA and the rigidity of polymer matrix at higher temperature. In case of 5-FU loaded GG microspheres, it showed additional endothermic peak at 297.49 $^{\circ}$ C which suggests the crystalline form of 5-FU in the microspheres.



Fig. 5. DSC of (a) 5-FU, (b) GG (c) cross linked GG microspheres and (d) 5-FU loaded GA cross linked GG microspheres



Fig. 6. Thermogram of (a) GG, (b) 5-FU (c) cross-linked GG microspheres and (d) 5-FU loaded GA cross linked GG microspheres

Thermogravimetric analysis

The therogravimeric analysis of pure GG, 5-FU, cross linked GG microspheres and 5-FU loaded GG microspheres have been shown in fig. 6. Pure GG showed two stage weight loss. The weight loss was observed at around 30 $^{\circ}$ C and up to 120 $^{\circ}$ C due to presence of traces of water molecules in GG and maximum mass loss was observed between

281.73 $^{\circ}$ C to 313.52 $^{\circ}$ C (46.12%) may be due the loss of -OH group of the GG. 5-FU showed a weight loss at 298 $^{\circ}$ C followed melting and decomposition. In case of cross linked GG, lower weight loss (33.64%) attributes to the decreased rate of degradation and increased rigidity compared to that of GG. 5-FU loaded GG microspheres showed higher weight loss of about 40.54% indicating the presence of 5-FU in the GG microspheres. Thermogram also reveals that there is no alteration in the melting point of 5-FU, hence absence of any side reaction between 5-FU and polymer or with cross linking agent.

Pulsatile capsule device

In this research, a pulsatile capsule device was developed for the purpose of targeting the 5-FU to the colon. Known quantity GG microspheres loaded with 5-FU were filled in a zero size gelatin capsule by hand filling method. The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution. The capsule was dipped into 5% CAP solution in such a way that capsule dips completely for 3-5 seconds (as shown in figure 7). The process was repeated few more times until we got the good coating which could avoid dissolution of capsule in acidic media.



Fig. 7. CAP enteric coated gelatin capsule containing 5-FU loaded GG microspheres.

In-vitro drug release studies

In vitro release data of 5-FU loaded cross linked GG microspheres at 1.5 h and 3 h with soaking and in-situ technique and from their pulsatile capsule device have been shown in figure 8a, 8b, 8c and 8d respectively. 5- FU loaded GG microspheres were inserted in the gelatin capsule by hand filling and enteric coated with CAP, in order to provide the protection from the release of 5-FU in upper GIT i.e. acidic environment.

In vitro release studies were performed using a digital Tablet Dissolution Test Apparatus which is operated at 100 rpm at 37^{0} C. The release studies were carried out for all the four formulations in triplicate. As shown in fig. 8a and 8b, in case of 5-FU loaded microspheres, all the four formulations showed higher amount of release within the first 2h. This may be due the enhanced swelling property of GG microspheres. Cumulative release of 66.17, 50.45, 65.74 and 45.75% of the drug has been observed with respect to 5-FU loaded GG microspheres at 1.5 h and 3 h with soaking and in-situ technique during the 2nd h. The reason for this is the high swelling property of GG microspheres in acidic pH so that as the microspheres swells, the increased release of 5-FU has been observed. In case of enteric coated pulsatile capsule device, 3.57, 6.84, 11.86 and 7.08% of cumulative release has been observed for pulsatile capsule device of GG microspheres at 1.5 h and 3 h with soaking and in-situ technique at the time of 2nd h and maximum release of 76 to 84% was observed at the 8th h. % cumulative release of pulsatile capsule device of 5-FU loaded GG microspheres at 1.5 h and 3 h with soaking and in-situ technique at the time of 2nd h and maximum release of 76 to 84% was observed at the 8th h. % cumulative release of pulsatile capsule device of 5-FU loaded GG microspheres at 1.5 h and 3 h with soaking and in-situ technique are shown in fig.8c and 8d.



Fig. 8a. In vitro drug release profile of 5-FU loaded microspheres at 1.5 h (*) and 3 h (*) with soaking method



Fig. 8b. In-vitro drug release profile of 5-FU loaded GG microspheres at 1.5 h (•) and 3 h (•) with in-situ technique



Fig. 8c. *In-vitro* drug release profile of pulsatile capsule device containing 5-FU loaded GG microspheres at 1.5 h (*) and 3 h (**n**) with soaking method

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Fig. 8d. *In vitro* drug release profile of pulsatile capsule device containing 5-FU loaded GG microspheres at 1.5 h (*) and 3 h (m) with insitu technique

CONCLUSION

In the present study, enteric coated capsule containing 5-FU loaded GG microspheres were developed for colon targeted drug delivery. GA cross-linked GG microspheres were prepared by water-in-oil (w/o) emulsification method. Drug was inserted in the matrix by soaking as well as in-situ technique. The gelatin capsules were enteric coated with CAP to overcome the problem of gastric emptying. The effectiveness of the pulsatile capsule device was confirmed by an *in-vitro* release study. Prepared formulations successfully prohibited the drug release in acidic media and showed sustained discharge in the colon. The technique adopted for development of pulsatile capsule device, i.e., coating material CAP and gelatin capsule, were nontoxic and eco-friendly. This system could be used readily for drug delivery. The results showed decreased degree of swelling and lower drug loading with increased cross linking time. FTIR studies confirmed the stability of 5-FU in microspheres, whereas the thermal studies TGA and DSC of microspheres confirmed the rigidity and crystalline nature of the 5-FU in microspheres. The developed pulsatile capsule device may act as potent vehicle for targeted delivery of drug to the colon. However, additional detailed investigations and *in-vivo* studies are required to be carried out to evaluate the efficacy of these formulations.

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REFERENCES

[1] Milabuer M N., Kam Y., and Rubinstein, A. Orally Administered Drug Delivery Systems to the Colon. In: Wen H, Park K, Oral controlled release formulation design and drug delivery: theory and practice. John Wiley & Sons, Inc: Hoboken, New Jersey: **2010**.

[2] Chourasia M K., and Jain S K. Review, Drug. Deliv., 2004, 11, 129.

[3] Prabaharan M. Review, Int. J. Biol. Macromol., 2011, 49, 117.

[4] Maroni A, Curto M D D, Serratoni M, Zema L, Foppoli A, Gazzaniga A, and Sangalli M E, *Eur. J. Pharm. Biopharm.*, 2009, 72, 246.

[5] Makhlof A, Tozuka Y, and Takeuchi H, Eur. J. Pharm. Biopharm., 2009, 71, 1.

[6] Yu C Y, Yin B, Zhang W, Cheng S, Zhang X, and Zhuo R, Colloids Surfaces B., 2009, 68, 245.

[7] McConnell E L, Short M D, and Basit AW, J. Controlled. Release., 2008, 130(2), 154.

[8] Mcconnell E L, Murdan S, and Basit AW, J. Pharm. Sci., 2008, 97(9), 3820.

[9] Chaudhary A, Tiwari N, Jain V and Singh R, Eur. J. Pharm. Biopharm., 2011, 78, 134.

[10] Ravi V, Siddaramaiah T M, and Pramod Kumar, J. Mater. Sci: Mater. Med., 2008, 19, 2131.

[11] Zhou Z, Liu L, Liu Q, Zhao Y, Xu G, Tang A, Zeng W, YI Q, and Zhou J, J. Macromol. Sci. B., 2012, 49, 1030.

[12] Lanjhiyana S K, Bajapayee P, Keshavan K, Lanjhiyana S, and Muthu M S, *Expert. Opin. Drug. Deliv.*, **2013**, 10(1), 5.

[13] Goldstein A M, Alter E N, and Seaman J K, Guar gum, In: R.L. Whistler (Ed.), Industrial Gums, Polysaccharides and Their Derivatives, Academic Press, New York, 1973, **303**.

[14] Yoon S J, Chu D C and Juneja L R, Review, J. Clin. Biochem. Nutr., 2008, 42, 1.

[15] Guo J H, Skinner G W, Harcum W W and Barnum P E, Reviews, Pharm. Sci. Technol. To., 1998, 1(6), 254.

[16] Ji C M, Xu H N and Wu W, J. Biomater. Appl., 2009, 23, 311.

[17] Soppirnath K S and Aminabhavi T M, Eur. J. Pharm. Biopharm., 2002, 53, 87.

- [18] Sarmah J K, Bhattacharjee S K and Mahanta R, J. Incl. Phenom. Macrocycl. Chem., 2009, 65, 329.
- [19] George M and Abraham T E, Int. J. Pharm., 2007, 335, 123.
- [20] Patel M M and Amin A F Carbohydrate Polymers., 2011, 86, 536.

[21] Miller E, J. Surg. Oncol., **1971**, 3(3), 309.

[22] Robert C C R, Bun P A and Peppas N A, J. Appl. Polym. Sci., 1985, 30, 301.

[23] Bhat S K, Keshavayya J, Kulkarni V H, Reddy V K R, Kulkarni P V and Kulkarni A R, J. Appl. Polym. Sci., **2012**, 125: 1736.

[24] Thanoo B C, Sunny M C and Jayakrishnan A, J. Pharm. Pharmacol., 1992, 44, 283.

[25] Akbuga J and Bergisadi N, J. Microencapsul., 1996, 13, 161.

[26] Bayomi S M and Al-Badr A A, In: Florey K, Analytical Profiles of Drug Substances: 5-Fluorouracil; California Academic Press, Inc; San Diego, Vol.18, 599-639. 1989.