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Colonic drug delivery of 5-fluorouracil: an in vitro evaluation

V.R. Sinha [a,*], B.R. Mittal [b], K.K. Bhutani [c], Rachna Kumria [a]

[a] *University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India*
[b] *Postgraduate Institute of Medical Education and Research, Chandigarh 160 014, India*
[c] *National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India*

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Abstract

Compression coating has been found to be useful for colonic drug delivery. The aim of the present investigation was to design a formulation with a considerably reduced coat weight and gum concentration for colonic delivery of 5-fluorouracil for the treatment of colorectal cancer. Rapidly disintegrating core tablets containing 50 mg of 5-fluorouracil were prepared and compression coating with 175 mg of granules containing a mixture of xanthan gum (XG) and guar gum (GG) in varying proportions was done. With this coat weight, a highly retarded drug release was observed. After 24 h of dissolution the mean percent drug release from the compression coated XG:GG 20:20, 20:10 and 10:20 tablets were found to be around 18 ± 1.23%, 20 ± 1.54% and 30 ± 1.77%, respectively. So, the coat weight was further reduced to 150 mg. It was observed that reduction of coat weight did not affect the initial drug release rate in simulated upper gastrointestinal tract (GIT) conditions. At the end of 24 h of dissolution the amount of drug released increased to 25 ± 1.22%, 36.6 ± 1.89% and 42.6 ± 2.22%, respectively in XG:GG 20:20, 20:10 and 10:20 tablets. Studies of XG:GG (10:20) tablets in presence of colonic contents showed an increased cumulative percent drug release of 67.2 ± 5.23% in presence of 2% cecal content and 80.34 ± 3.89% in presence of 4% cecal content after 19 h of incubation.

Keywords: Colon specific drug delivery; Colorectal cancer; 5-Fluorouracil; Xanthan gum; Guar gum

1. Introduction

Site specific delivery of drugs to the receptor site has the potential to reduce side effects and to increase pharmacological response. One of the seemingly interesting areas to target drugs through oral route for systemic drug delivery is the colon, the proximal part of the large intestine. Further, there are a number of local pathologies warranting direct release of drug in the colon. This will not only improve pharmacotherapy but also reduce the potential toxic or side effects. The treatment of disorders of the large intestine, such as irritable bowel syndrome, colitis, Crohn's disease, colon cancer and infectious diseases where it is necessary to attain a high concentration of the active agent, may be efficiently achieved using colon specific delivery systems. Conventional oral dosage forms are ineffective in delivering drugs to the colon due to absorption and/or degradation of the active ingredient in the upper gastrointestinal tract (GIT).

Various approaches have been used for targeting the drugs to the colon including, formation of a prodrug, multicoating time-dependent delivery systems, coating with pH-sensitive polymers, pressure-dependent

* Corresponding author. Tel.: +91-172-534107; fax: +91-172-615430.
E-mail address: vr_sinha@yahoo.com (V.R. Sinha).

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systems, and systems formulated making use of biodegradable polymers (Kinget et al., 1998). Every system has advantage as well as shortcoming. However, biodegradable systems formulated making use of naturally occurring polysaccharides are increasingly being developed (Sinha and Kumria, 2001a,b, 2002). Natural polysaccharides remain undigested in the stomach and the small intestine and are degraded by the vast anaerobic microflora of the colon, for example, *bacteroides*, *bifidobacteria*, *eubacteria*, to smaller monosaccharides, which are then used as energy source by the bacteria. In an earlier study these were used in the from of binders in tablet formulation (Sinha and Kumria, 2002).

The present investigation is aimed at using these inexpensive, naturally occurring and abundantly available polysaccharides for colon delivery of 5-fluorouracil. An attempt was made to formulate a dosage form which consisted of biodegradable polysaccharides as the main constituent, showed minimal release of 5-fluorouracil in the tracts of the upper GIT and rapid release in the tracts of the colon.

5-Fluorouracil is a pyrimidine analogue and is the drug of choice for colon cancer (Calabresi and Chabner, 1992). It inhibits RNA function and/or processing and synthesis of thymidylate. It is administered parenterally since absorption after ingestion is unpredictable and incomplete (Hahn et al., 1975). Targeting of 5-fluorouracil to the colon in cases of colon cancer would not only reduce the systemic toxicity of the drug but would also show the desired action in a lesser dose.

Working on this rationale, a drug release-retarding ingredient falling under the category of polysaccharides, i.e. xanthan gum (XG), which is known to retard drug release considerably, was selected for the study (Talukdar and Kinget, 1995; Sujja-areevath et al., 1998). Guar gum (GG) another polysaccharide being widely used for colon targeting was selected as the other ingredient. Guar gum alone has earlier been used in colon specific drug delivery as matrix forming material and as a compression coat (Wong et al., 1997; Rama Prasad et al., 1998; Krishnaiah et al., 1999). Xanthan gum is known to have a greater drug release retarding property and synergistically enhanced gel properties in presence of galactomannan gums like guar (Melia, 1991). So a combination of these gums was used. This mixture of gums was evaluated for its drug release retarding properties under simulated gastrointestinal tract (GIT) conditions. This mixture was proposed to retard drug release more significantly in conditions of the upper GIT but still retain biodegradability due to the presence of guar gum. Presence of xanthan gum in the compression coat would not only retard the initial drug release from the tablet but due to a higher swelling of xanthan gum the susceptibility of the compression coat to undergo degradation by the microflora would increase as higher the swelling, more the surface area available for microbial action. Starch, which is a usual filler in the tablet dosage form was used as an excipient.

2. Material and methods

2.1. Materials

Guar gum (MW 220,000) was procured from Himedia Laboratories Limited, India. Xanthan gum, USNF and 5-fluorouracil were obtained as gift samples from Dabur Research Foundation, Ghaziabad, India. Cross PVP was obtained as a gift sample from ISP Technologies, Inc., USP. Starch, talc and magnesium stearate used for the preparation of tablets were of Pharmacopoeial grade.

2.2. Preparation of 5-fluorouracil core and compression coated tablets

Rapidly disintegrating core tablets consisting of 5-fluorouracil (50 mg) and a super-disintegrant polyvinyl pyrrolidone (cross-PVP) (2 mg) were prepared. A weighed quantity of 5-fluorouracil required for 20 tablets was mixed thoroughly with the required amount of cross-PVP. The uniformity of mixing was assessed by conducting content uniformity test on the samples of the powder mix. Quantity weighing 52 mg was taken and compressed individual into tablets using 5.1 mm flat plain punches on a single punch tableting machine (Modern Engg. Works, New Delhi, India) using 4000 kg compression force.

The compression coat material was prepared using xanthan gum, guar gum and starch in varying percentages as outlined in Table 1. The ingredients in the quantities mentioned were wet granulated using starch paste (10%). Granules of the above wet mass were

Table 1
Formulation code and the quantities of gums (as percentage) in the tablets

Formulation code	Percent of xanthan gum	Percent of guar gum
XG:GG (20:20)	20	20
XG:GG (20:10)	20	10
XG:GG (10:20)	10	20

prepared by passing through a sieve with a nominal aperture of 1 mm. The granules were dried for 6 h at a temperature of 50 °C. The dried granules were passed through a sieve with a nominal aperture of 1 mm and mixed with talc (1.7%) and magnesium stearate (1.2%). Core tablets were compression coated with different coating mixtures. Initially, 175 mg of coat material was applied on to the core tablets. Further, in an attempt to reduce the coat weight, 150 mg of coat material was applied over the core tablets. For compression coating, 43.33% of coat weight was placed in the die cavity followed by carefully centering the core tablet and addition of the remainder of coat weight. The coating material was compressed around the core tablet at an applied force of 5000 kg using 8.2 mm round concave punches using a single station tableting machine. Compression coated 5-fluorouracil tablets with different compositions were tested for thickness, hardness, drug content and drug release characteristics.

2.3. Determination of drug content

The 5-fluorouracil core and compression coated tablets, both were tested for their drug content. The tablets were finely powdered, and a quantity of powder equivalent to 50 mg of 5-fluorouracil were accurately weighed and transferred to 100 ml volumetric flasks containing approximately 50 ml of buffer pH 6.8. The flasks were shaken to solubilize the drug. The volume was made up with buffer pH 6.8 and mixed thoroughly. The solutions were filtered through a 0.22 μm membrane filter and analyzed for the content of 5-fluorouracil using the HPLC method described below.

2.4. Preparation of rat cecal content medium

Wistar rat weighing 150–200 g and maintained on a normal diet (soaked gram) were used. Forty-five minutes before the commencement of drug release studies, seven rats were killed by spinal traction. The abdomen were opened, the cecal were traced, ligated at both the ends, dissected, and immediately transferred into pH 6.8 buffer previously bubbled with nitrogen. The cecal bags were opened, their contents were individually weighed, pooled, and suspended in the buffer continuously bubbled with nitrogen. These were finally added to the dissolution media to give a final cecal dilution of 2 and 4% w/v, respectively. All the above procedures were carried out under nitrogen in order to maintain anaerobic conditions.

2.5. Drug release studies

The ability of the prepared tablets to retard drug release in the physiological environment of the stomach and the small intestine was assessed by conducting drug release studies in simulated stomach and small intestinal pH, respectively. The changing pH media, Method 1, USP 23, for delayed release tablets was used. Dissolution test was conducted in USP 1 apparatus at 75 rpm and a temperature of 37 °C. Initial drug release studies were conducted in 750 ml of 0.1N HCl for 2 h. Then, 250 ml of 0.2 M trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. Samples were withdrawn after regular intervals of time to evaluate drug release. These were analyzed spectrophotometrically at a wavelength of 266 nm.

Drug release studies in the presence of cecal content were also carried out using USP dissolution test apparatus. However, slight modification in the procedure was done. The experiments were carried out in 250 ml beaker immersed in water maintained in the jars of dissolution test apparatus. Initial studies were carried out in 150 ml of 0.1N HCl (pH of 1.2) for 2 h. After this 50 ml of 0.2 M trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. The study at a pH of 6.8 was continued for 3 h after which cecal content equivalent in cecal content to 4 and 8 g were added to 200 ml of buffer (pH 6.8) to give a final cecal dilution of 2 and 4%, respectively. Dissolution in the cecal content media was carried out till completion of 24 h. The experiments in cecal content media were carried out in presence of a continuous supply of nitrogen. At different time

intervals 1 ml sample was withdrawn from the dissolution medium and 1 ml of cecal content (2 or 4% as the case may be), maintained under anaerobic conditions, was replenished into the dissolution media. The volume of the sample was made upto 10 ml, filtered through sintered glass (G-5) filter and the filtrate was analyzed using HPLC method described below.

2.6. HPLC determination of the 5-fluorouracil content in the tablet formulation and the dissolution fluids containing cecal content

The quantitative determination of 5-fluorouracil was performed by high performance liquid chromatography. The HPLC system consisted of a Shimadzu SCL-6B system controller and a Shimadzu LC-6A pump and a C-R3A Chromatopac integrator (Shimadzu, Japan). A UV detector was used for spectrophotometric analysis. The separations were performed at 25 °C using a 3.9 mm × 150 mm Nova Pak C-18 i.d. 3.9 μm column with a 2.5 cm Nova Pak C-18 i.d. 3.9 μm guard column (Waters, Millipore, USA).

The mobile phase consisted of a mixture of methanol and sodium acetate buffer (pH adjusted to 4.0) in the ratio of 30:70. The mobile phase was filtered and pumped at a flow rate of 0.8 ml/min. The column was maintained at a temperature of 25 °C. The eluent was detected by UV detector at 264 nm. A standard curve was constructed for 5-fluorouracil in the concentration range of 0.10–40 μg/ml. A good linear relationship was observed between the concentration of 5-fluorouracil and peak area ($r^2 = 0.9999$). The retention time was found to be 10.65 min. The standard curve was used for estimating the content of 5-fluorouracil in the tablet and the dissolution medium.

3. Results and discussion

3.1. Drug content

The mean drug content in the 5-fluorouracil tablets (both core and compression coated) was found to be 49.9 ± 0.5 mg. The tablets contained 99.8 ± 1% of 5-fluorouracil.

3.2. Core tablets

The core tablets of 5-fluorouracil were prepared by direct compression of the core mix prepared. The core tablets had a diameter of 5.1 ± 0.01 mm and height of 1.8 ± 0.01 mm. Cross-PVP was added to the tablet core in order to make the core rapidly disintegrating. This would allow the core tablets to disintegrate rapidly once the coat material is digested by the resident microflora of the colon. The hardness of the core tablets was found to be in the range of 3.5–4.0 kg/cm^2. These tablets were found to comply with the friability test since the weight loss was found to be less than 0.5%. The disintegration time of the core tablets was found to be 20 s. This may be due to the presence of cross-PVP in these core tablets.

3.3. Compression coated tablets

The different coat materials were prepared by wet granulation of the prepared mix outlined in Table 1. The granulation was done using starch paste (10%). The hardness of the tablets was found to be in the range of 4.5–5 kg/cm^2. The compression coated tablets had a diameter of 8.2 ± 0.01 mm and height of 4.6 ± 0.01 mm when the weight of the compression coat was 175 mg. Reducing the coat weight to 150 mg reduced the thickness of the compression coated tablet to 4.2 ± 0.1 mm. The effective difference in the diameter and thickness of the core tablet and the compression coated tablet was 3.1 and 2.8 mm, respectively, in the former case. Thereby the thickness of compression coat varied from 1.4 to 1.55 mm in the tablet formulation. In the case of a compression coat of 150 mg, the effective difference in the diameter and thickness of the core tablet and the compression coated tablet was 3.1 and 2.4 mm. Thereby the thickness of compression coat varied from 1.2 to 1.55 mm.

3.4. In vitro drug release studies

For drug delivery systems designed for colon targeting, it is desirable that the system remains intact and shows minimal drug release in the physiological environment of the stomach and the small intestine and triggers drug release in the tracts of the colon. For chemotherapeutic agents, for example, 5-fluorouracil the initial release is required to be drastically

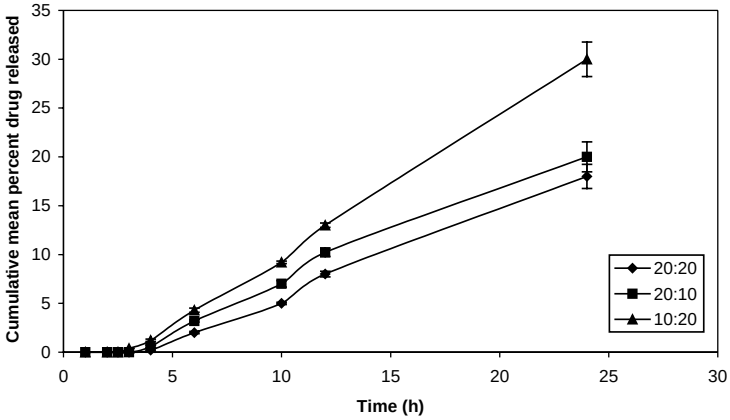

Fig. 1. Mean percent drug release from 5-fluorouracil compression coated XG:GG tablets with a coat weight of 175 mg.

minimized to avoid the side effects associated with these agents. Hence an attempt was made to formulate a dosage form, which showed minimal drug release in conditions mimicking mouth-to-colon transit and ensured maximum drug release in the environments of the colon. The compression coat was designed to undergo bacterial degradation in the colon, exposing the rapidly disintegrating drug containing core in the colon. The core tablets were compression coated with coating mixtures outlined in Table 1. 175 mg of coating material (XG:GG, 20:20; XG:GG, 20:10; XG:GG, 10:20) was applied over the core tablets. Using xanthan gum concentrations less than 10% could not retard the initial drug loss as desired in this study.

The results of the drug release studies carried out on 5-fluorouracil tablets compression coated (175 mg coat weight) with different mixtures of the polysaccharides in simulated gastric (pH 1.2) and small intestinal (pH 6.8) are shown in Fig. 1. The compression coated tablet was found to be intact even after 24 h of the dissolution test. However, there was a considerable swelling in the tablets. At the end of 24 h of dissolution it was observed that tablets having higher xanthan gum content showed higher swelling as compared to those with less XG content. The cumulative mean percentage of 5-fluorouracil released from the tablet compression coated with XG:GG 20:20, 20:10 and 10:20 mixtures during the first 5 h of dissolution were 1.1, 1.9 and 3.01%, respectively. The release of less than 5% in simulated gastric and small intestinal fluids indicates the ability of compression coat of this polysaccharide combination for specific delivery of drugs to the colon. After 24 h of dissolution the mean percent drug release from the compression coated XG:GG 20:20, 20:10 and 10:20 tablets were found to be $18 \pm 1.23\%$, $20.1 \pm 1.54\%$ and $30 \pm 1.77\%$. The AUC values at the end of 24 h were found to be 182.76, 227.95 and 316.42 in XG:GG 20:20, 20:10 and 10:20 tablets, respectively (Fig. 1). After 24 h of dissolution, upon opening the tablet gels, the core tablets could be seen. Since the drug release rate was highly retarded at a coat weight of 175 mg, it was proposed to reduce the coat weight and then evaluate the ability of the compression coating of this polymer mixture for specific drug delivery to the colon. The coat weight of the tablet was reduced to 150 mg.

Drug release studies from compression coated tablets with 150 mg coat weight, showed a drug release in XG:GG tablets 20:20; 20:10 and 10:20, amounting to 3, 3.5 and 4%, respectively, in the first 5 h of dissolution (Fig. 2). This included 2 h dissolution at a pH of 1.2 followed by 3 h dissolution at a pH of 6.8. At the end of 24 h of dissolution the amount of drug released was $25 \pm 1.22\%$, $36.6 \pm 1.89\%$, $42.6 \pm 2.22\%$ and the AUC values were found to be 252.32, 365.05 and 427.46, respectively, in XG:GG 20:20, 20:10 and 10:20 tablets. This showed that by reducing the coat weight, the initial drug release was not significantly increased while the total percent drug released in 24 h was affected. This can be explained on the basis that drug release from the compression coated tablets takes place only upon swelling of the compression coat consisting of gums. The initial delay in drug

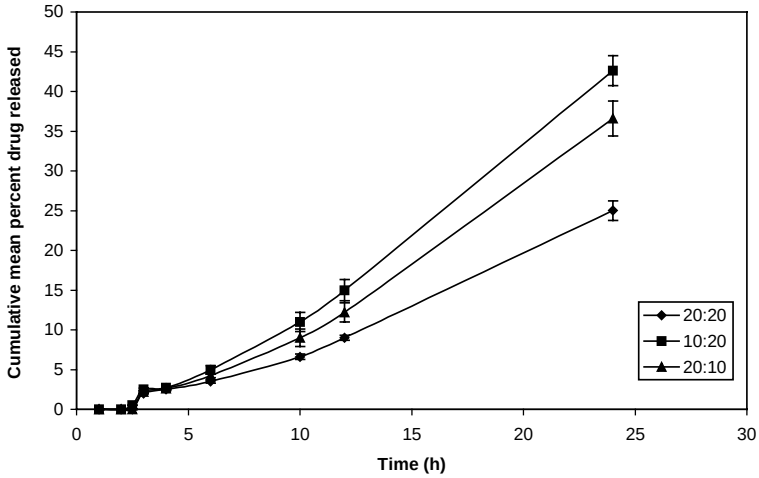

Fig. 2. Mean percent drug release from 5-fluorouracil compression coated XG:GG tablets with a coat weight of 150 mg.

release can also be attributed to the time taken for the glassy to rubbery transition by the gum combination (Colombo et al., 2000). Once the gum combination swells the lesser the thickness of the compression coat the faster the drug release. This explains the higher drug release from 150 mg compression coat as compared to 175 mg at the end of 24 h.

Earlier studies have shown that guar gum (Wong et al., 1997) and starch (Vilivalam et al., 2000) are digested by the colonic bacteria. However, to evaluate the mixtures of these polysaccharides in presence of xanthan gum to carry drug moieties specifically to the colon remains to be studied. Further the rate of drug release from these compression coated tablets in colonic environments needs to be evaluated. So, in order to evaluate the susceptibility of the prepared compression coat to undergo enzymatic action by the colonic bacteria, drug release was carried out in rat cecal content media (2 and 4%) for 19 h after 5 h of dissolution in simulated gastric and small intestinal fluids. The cumulative percent drug released from XG:GG (20:10) tablets was found to increase in presence of rat cecal content in the dissolution media (Fig. 3). Cumulative percent drug released after

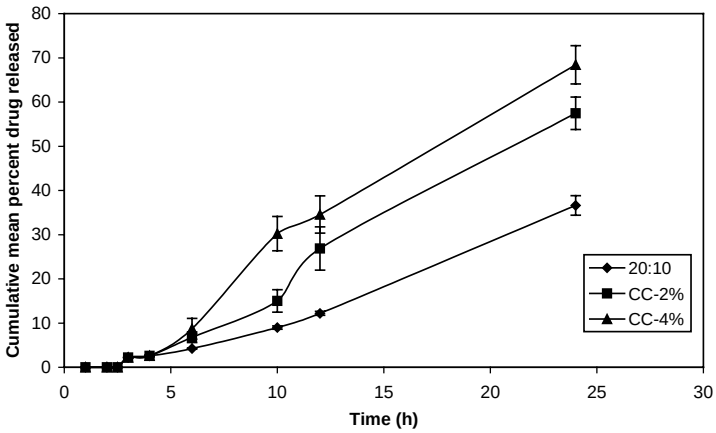

Fig. 3. Mean percent drug release from 5-fluorouracil compression coated tablets XG:GG (20:10) with 150 mg coat weight in absence and in presence of 2 and 4% rat cecal content media.

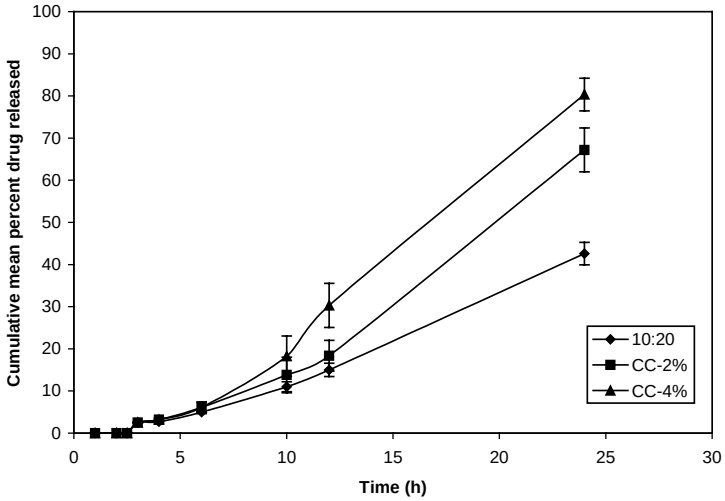

Fig. 4. Mean percent drug released from 5-fluorouracil compression coated tablets XG:GG (10:20) with 150 mg coat weight in absence and in presence of 2 and 4% rat cecal content media.

24 h of dissolution increased from 36.6 ± 1.89% to 57.45 ± 3.66% in 2% cecal content media and further to 68.43 ± 4.34% in presence of 4% cecal content media. AUC values increased to 603.81 in 2% cecal content and to 774.81 in 4% cecal content media at the end of 24 h. Similarly, in case of XG:GG (10:20) tablets the total cumulative percent drug released in 24 h increased from 42.6 ± 2.22% to 67.2 ± 5.23% in presence of 2% cecal content and to 80.34 ± 3.89% in presence of 4% cecal content (Fig. 4). The AUC values in case of XG:GG (10:20) were 593.65 in presence of 2% cecal content media and 773.25 in 4% cecal content media. Considering that the concentration of bacteria present in the colon actually is much higher (as against 2 or 4% taken in this study) complete drug would be released from these tablets. Based on these studies on compression-coated tablets making use of polysaccharides it may be suggested that these compression coated tablets can be used for carrying potent chemotherapeutic agents like antineoplastic drugs specifically to the site of action in case of colon cancer.

XG:GG (10:20), seems to be a better option for colon specific drug delivery because the compression coat in this case consists of a relatively higher concentration of degradable polysaccharide which will facilitate a faster drug delivery to the colon as compared to XG:GG, 20:10 compression coat in which the concentration of xanthan gum is higher.

4. Conclusion

This polysaccharide composition consisting of xanthan gum as a drug release retarding agent in combination with colon degradable polysaccharides, guar and starch, can be successfully used to protect the drug from being released under conditions mimicking mouth-to-colon transit. This compression coat can carry chemotherapeutic agents with upper gastrointestinal toxicity or side effects specifically to the colon. Drug release from these tablets takes place at a highly retarded rate till the compression coat is digested by the microflora of the colon. So, these systems seem to be site specific. Additionally, containing a relatively lower gum concentration, i.e. 30% in XG:GG 10:20, large scale manufacturing may not be

very problematic as in the case of formulations containing higher gum concentration. Studies are planned to assess the relative usefulness of the XG:GG 10:20 formulation to carry drug containing cores specifically to the colon in healthy human volunteers.

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References

Calabresi, P., Chabner, B.A., 1992. Chemotherapy of neoplastic diseases. In: Hardman, J.G., Limbird, L.E., Perry, B.M., Raymond, W.R. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed. McGraw-Hill, pp. 1225–1232.

Colombo, P., Bettini, R., Santi, P., Peppas, N.A., 2000. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanism and optimal performance. Pharm. Sci. Tech. Today 3, 198–204.

Hahn, R.G., Moertel, C.G., Schutt, A.T., 1975. A double-blind comparison of intensive course 5-fluorouracil by oral versus intravenous route in the treatment of colorectal carcinoma. Cancer 35, 1031–1035.

Kinget, R., Kalala, W., Vervoort, L., Mooter, G.V., 1998. Colonic drug targeting. J. Drug Target. 6, 129–149.

Krishnaiah, Y.S.R., Satyanaryana, S., Rama Prasad, Y.R., 1999. Studies of guar gum compression-coated 5-aminosalicylic acid tablets for colon-specific drug delivery. Drug. Dev. Ind. Pharm. 25, 651–657.

Melia, C.D., 1991. Hydrophilic matrix sustained release systems based on polysaccharide carriers. Crit. Rev. Ther. Drug Carrier Syst. 8, 391–421.

Rama Prasad, Y.V., Krishnaiah, Y.S., Satyanarayana, S., 1998. In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. J. Control. Release 51, 281–287.

Sinha, V.R., Kumria, R., 2001a. Colonic drug delivery: prodrug approach. Pharm. Res. 18, 557–564.

Sinha, V.R., Kumria, R., 2001b. Polysaccharides for colon specific drug delivery. Int. J. Pharm. 224, 19–38.

Sinha, V.R., Kumria, R., 2002. Binders for colon specific drug delivery: an in vitro evaluation. Int. J. Pharm. 249, 23–31.

Sujja-areevath, J., Munday, D.L., Cox, P.J., Khan, K.N., 1998. Relationship between swelling, erosion and drug release in hydrophilic natural gum mini-matrix formulations. Eur. J. Pharm. Sci. 6, 207–217.

Talukdar, M.M., Kinget, K., 1995. Swelling and drug release behaviour of xanthan gum matrix tablets. Drug Dev. Ind. Pharm. 120, 63–72.

Vilivalam, V.D., Illum, L., Iqbal, K., 2000. Starch capsules: an alternative system for oral drug delivery. Pharm. Sci. Tech. Today 3, 64–69.

Wong, D., Larrabeo, S., Clifford, K., Tremblay, J., Friend, D.R., 1997. USP dissolution Apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulation. J. Control. Release 47, 173–179.