Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

DEVELOPMENT AND EVALUATION OF ENZYMATICALY TRIGGERED MULTIPARTICULATE COLON TARGETED DRUG DELIVERY SYSTEM

Mohapatra Santosh K*, Kshirsagar Sanjay J, Bhalekar Magesh R, Shukla Gajendra N, Patil Amol V
Department of Pharmaceutics, AISSMS College of Pharmacy, Kennedy Road, Near R.T.O., Pune-411001, Maharashtra, India

Received on: 14/12/2010 Revised on: 21/01/2011 Accepted on: 03/02/2011

ABSTRACT
The most critical challenge in oral colon specific drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine. Microbial enzyme-triggering mechanisms seem to be promising to provide more reliable colonic delivery .The objective of the present study was to develop biodegradable colon targeted multiparticulate system by using guar gum. In this study drug (Budesonide) loaded pellets were coated with aqueous guar gum solution and subjected to In-vitro drug release studies simulating GIT with and without enzyme as well as coating properties were evaluated by SEM. In-vitro release studies indicates that drug release after 4.5 h lag time in presence of enzyme and lag time increase in absence of enzyme which indicated the enzyme triggered system for colonic release. This Multiparticulate system can be effectively used for colonic drug delivery for effective treatment of colonic diseases.
KEYWORDS: Budesonide, Multiparticulate system, Enzyme activity, Colonic drug delivery, In-vitro study

***Address for correspondence**
Mr. Santosh Kumar Mohapatra, M. Pharm 3rd Sem, Department of Pharmaceutics, AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune 411 001, Maharashtra, India
E-mail: santosh.pharmaicon@gmail.com

INTRODUCTION

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome but also for the potential it holds for the systemic delivery of proteins and therapeutic peptides[1,2]. The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine [3]. Various systems employing pH-sensitive, time-dependent and microbial enzyme-triggering mechanisms have been studied[3,4,5,6]. However, due to the complexity in gastrointestinal physiology and high individual variability, colon-specificity of these systems was not always reliable. And for time-dependent systems, variation in gastric and intestinal transit time may lead to premature drug release in small intestine or delayed release far down the colon[9]. For pH sensitive systems, the limited difference in pH between distal intestine and colon sometimes does not allow for reproducible colonic delivery[3]. But due to the simplicity of the formulation of this device many marketed preparations utilize this approach. On prolonged use, these polymers may accumulate in the body so the use of biodegradable polymers is essential. Among CSDDSs, those employing microbial enzyme-triggering mechanisms seem to be promising to provide more reliable colonic delivery[6,10]. The most interested enzyme-degradable carriers for such systems are natural polysaccharides[11,12,13], which are specifically hydrolyzed by the colonic microflora. The upper part of GIT, that is the stomach and the duodenum has a microflora of less than 103–104 CFU/ml. These are mainly gram-positive facultative bacteria[14]. The microflora of colon on the other side is in the range of 1011–1012 CFU/ml consisting mainly of anaerobic bacteria, e.g. Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci and Enterobacteria[15] etc. This vast microflora fulfils its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, for example di-and tri-saccharides, polysaccharide[10,16]. For this fermentation, the microflora produces a vast number of enzymes like glucuronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, azoreductase, deaminase and urea dehydroxylase[17]. Because of the presence of these biodegradable enzymes

only in the colon, the use of bacterial degradable polymers for colon specific drug delivery seems to be a more site specific approach as compared to other approaches. The ability of natural polymers i.e. the polysaccharides, from algal origin (e.g. alginates), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum) and animal origin (chitosan, chondroitin) to act as substrates for the bacterial inhabitants of the colon together with their properties, such as swelling, film forming and their biocompatibility, biodegradability invites their use as colon-carriers. Single unit colon targeted drug delivery system may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying[18]. Multiparticulate approaches tried for colonic delivery include formulations in the form of pellets, granules, microparticles and nanoparticles. The use of multiparticulate drug delivery systems in preference to single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy and co-workers[20] showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability. Moreover, multiparticulate systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption[20, 21].Therefore the objective of the present study was to develop biodegradable colon targeted multiparticulate system by using guar gum.

MATERIALS AND METHODS

Neutral pellets were obtained as a gift sample from Murlikrishnan Pharmaceuticals Pvt. Ltd.Pune, India. Budesonide was obtained as gift sample from Mepro Pharmaceuticals Pvt. Ltd. (unit II), Wadhawan, India, and Guar gum (MW 220,000) was procured from Himedia Laboratories Limited, India. Other excipients used were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Preparation of coating solution for drug loading

Budesonide was incorporated on non-pareils seeds by spraying budesonide in a solution in dichloromethane containing polyvinyl pyrrolidone (PVP 30K) as a binder and talc as antisticking agent. The coating solution parameters are stated in **Table 2.** The stated amounts of budesonide and PVP 30 K (**Table 1**) were dissolved in methanol solution separately. After mixing each solution for 30 minutes, these two solutions were mixed together and methanol was added up to the 100 ml volume. The coating solution was sprayed over the non-pareils seed by using INSTA COAT R & D Coater. During preliminary studies various combination of above mentioned composition were tried.

Preparation of aqueous guar gum coating system

To prepare the guar gum coating dispersion a guar gum (4% w/v) in ethanol/water or guar gum (1% w/v) in water/isopropanol (7/3) was used. Glyceryl monostearate and PEG400 were added to mixture as a glidant. Mixture was stirred 10 min until a fine homogenous dispersion was obtained.

Coating of guar gum polymer coating solution to budesonide loaded pellets

The non pareils for guar gum aqueous dispersion coating in 6-inch coating pan. The various coating parameters controlled during coating process are given in **Table 2.** Hundred grams of budesonide loaded pellets were coated with guar gum polymer coating solution by using the INSTA-COAT R & D coating machine. Samples of coated pellets were removed from apparatus at 10%, 20%, 30%, 40%, weight gain coating level. Based on the *in-vitro* drug dissolution study and desired lag time the final coating level 40% weight gain was selected.

Evaluation of guar gum coated budesonide pellets

The guar gum coated budesonide pellets were evaluated for drug content, physical properties, scanning electron microscopic study and in-vitro dissolution testing.

***In-vitro* drug release study for guar gum coated pellets**

The dissolution studies of budesonide loaded guar gum coated pellets were carried out in a USP XXIII dissolution apparatus II (DA 6D Veego, TDT 08L Electrolab) at a rotation speed of 100 rpm in a 933.3 ml medium at 37^0 C. In order to simulate enzyme in GIT pepsin 0.32%w/v was added in the dissolution medium. The capsules (n=3) are transfer to dissolution medium and samples were taken at selected time intervals, filtered through Whatman filter paper no. 41 and analyzed by UV spectrophotometer (V-530 Jasco) at 247 nm for acidic medium and at 245 nm for phosphate buffer. The continuous dissolution method USP XXIII was used by simulating conditions of the GI tract. In this

study capsules were added in 700 ml of 0.1 N HCl (pH 1.2) for 2 h. At the end of 2 h 233.3 ml of tribasic sodium phosphate was added to all the dissolution vessels and the pH was adjusted to 6.5 (1h), 6.8 (2 h) and 7.2 (till end of test) by using 2 M NaOH or 2 M HCl. To evaluate enzyme-triggered drug release of guar gum-coated pellets, at the end of 3 hours pancreatin, a rich product of colonic microflora in a concentration of 1%w/w was added into pH 6.5 phosphate buffers to simulate the degradation of polysaccharide by micro flora in the colon.

Drug and excipients compatibility studies

The drug with excipient guar gum (1:1 ratio) was subjected to storage at room temperature and elevated temperature of 40^0C and 75% RH for one month. Sampling was done at a predetermined time intervals of 7, 14, 21 and 30days. The mixtures of drug and excipient was then evaluated by %assay by using double beam UV spectrophotometer (Jasco V-550) and IR spectra by FTIR spectrometer (Jasco 460 plus).

RESULTS AND DISCUSSION

Coating of guar gum coating solution to drug loaded pellets

Coating with guar gum was done with different weight gain level. Depending on in-vitro study finally 40% weight gain was selected which was showing maximum drug release in 9 hours with 4.5 hours lag time. 40% weights gained coated pellets were further taken for evaluation.

Drug content

Drug content of all formulations prepared was 96% to 98% and was found to be within limit.

Physical properties

Various physical properties were evaluated such as bulk density, tapped density, angle of repose, hausner ratio and loss on drying. Each formulation showed all physical properties within excellent flow properties limits.

SEM study of guar gum coated pellets

The morphology of the guar gum coated pellet was examined by the help of SEM analysis which is clearly shown in **figure 2**.

***In-vitro* evaluation of guar gum coated pellets**

An outer guar gum coating can significantly slowdown the release rate, which negatively correlated to weight gain. Release profiles of the guar gum coated pellets are biphasic, typical of an initial constrained release and a later incremental release. Highly hydrophilic in nature, film coating with guar gum seemed to be able to retard initial budesonide release significantly, even at a relatively small weight gain of 40%. In-vitro evaluation of guar gum coated budesonide pellets was carried out by changing pH dissolution method with enzyme and without enzyme at different pH conditions like pH 1.2, 6.5, 6.8 and 7.2 for 2, 1, 2 h and till end of study respectively.

Without enzyme

Study carried out without enzyme shows the drug release which is non uniform and in very low concentration. The retardation of drug release was due to absence of microbial flora and their degradation enzymatic products which are responsible for increase in redox potential to cause the degradation of biodegradable polymers. Drug release of 40% weight gain pellets was found to be below 10% and gradually increased up to 64% in 9 hours.

With enzyme

In presence of enzyme pancreatin which was a rich product of enzymes in colon and the release rate was accelerated due to degradation of guar gum layer. Hence as soon as the addition of enzyme was done the drug release was markedly increased and finally about 96% drug releases was observed at end of 9 hours which was indicative of obvious enzyme-triggering mechanisms(**Figure 3**). The in-vitro evaluation clearly indicates that in absence of the enzyme drug release was retarded and in the presence of enzyme drug release was accelerated significantly. The variation in drug release pattern was due to the variation in microbial enzyme in dissolution medium which is the major disadvantage of the microbial controlled colonic delivery.

CONCLUSION

In the present study, Multiparticulate system for colonic drug delivery was developed and tested *in vitro* with enzyme simulating the GI condition without the use of rat faecal matter and scarification of animals as previously done in some studies. The coated multiparticulate shows release of the drug specifically at target site after suitable lag time and may contribute in the effective treatment of colonic diseases which need to be further proven by *in vivo* study.

ACKNOWLEDGMENTS

The authors thankful to Degussa India Pvt. Ltd. Mumbai for providing Eudragit S100 and Dr. A. R. Madgulkar, Principal, AISSMS College of pharmacy, Pune for providing the facilities.

REFERENCES

1. Davis SS. Overcoming barriers to the oral administration of peptide drugs. Trends Pharm Sci 1990; 11: 353-355.
2. Vanden MG, Kinget R. Oral colon-specific drug delivery a review. Drug Deliv 1995; 2: 81-93.
3. Ashford M, Fell JT. Targeting drugs to the colon delivery systems for oral administration. J Drug Target 1994; 2: 241-258.
4. Pozzi F, Furlani P, Gazzaniga A, Davis SS, Wilding IR. A new oral dosage form for fast and complete release of drug after predetermined lag time. J Control Release 1994; 31:99–108.

5. Gupta VK, Beckert TE, Price JC. A novel pH and time based multi-unit potential colonic drug delivery system. Int J Pharm 2001; 213:83–91.
6. Krishnaiah YSR, Styanarayana S, Rama Prasad YV. Evaluation of guar gum as a compression coat for drug targeting to colon. Int J Pharm 1998; 171:137–146.
7. Krishnaiah YSR, Veer Raju P, Dinesh Kumar B, Bhaskar P, Satyanarayana V. Development of colon targeted drug delivery systems for mebendazole. J Control Release 2001; 77:87–95.
8. Krishnaiah YSR, Styanarayana V, Dinesh Kumar B, Karthikeyan RS.In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of indomethacin. Eur J Pharm Sci 2002;16:185–192.
9. Lee WW, Wilson CG, Mukherji G. Time-dependent systems for colonic delivery and Modified-release drug delivery technology. New York: Marcel Dekker; 2003; 243–248.
10. Rubinstein A, Radai R, Ezra M, Pathak S, Rokem JS. In vitro evaluation of calcium pectinate: A Potential colon-specific drug delivery carrier. Pharm Res 1993; 10:258–263.
11. Milojevic S, Newton JM, Cummings JH et al. Amylose as a coating for drug delivery to the colon: Preparation and in vitro evaluation using 5-aminosalicylic acid pellets. J Control Release 1996; 38:75–84.
12. Tozaki H, Komoike J, Tada C et al. Chitosan capsules for colonspecific drug delivery, Improvement of insulin absorption from the rat colon. J Pharm Sci 1997; 86:1016–1021.
13. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery. Int J Pharm 2001; 224:19–38.
14. Gorbach SL. Intestinal Microflora: Gastroenterology 1971; 60: 1110–1129.
15. Moore WEC, Holdeman LV. Discussion of current bacteriological investigations of the relationship between intestinal flora, diet and colon cancer. Cancer Res 1975; 35: 3418–3420.
16. Cumming JH, Englyst HN. Fermentation in the human large intestine and the available substrates. Am. J.Clin. Nutr 1987; 45: 1243–1255.
17. Scheline RR. Metabolism of foreign compounds by gastrointestinal micro-organisms. Pharmacol. Rev 1973; 25: 451–523.
18. Perkins AC. Regional differences in quinine absorption from the undisturbed human colon assessed using a timed release delivery system. Pharm. Res 1999; 16:1087–1092.
19. Kramer A, Turk S, Vrecer F. Statistical optimization of diclofenac sodium sustained release pellets coated with polymethacrylic films. Int J Pharm 2003; 256: 43-52.
20. Hardy JG, Wilson CG, Wood E. Drug delivery to the proximal colon. J. Pharmacol 1985; 37: 874-877.
21. Davis SS. Assessment of gastrointestinal transit and drug absorption: Novel Drug Delivery and its Therapeutic Application. 1989; 89-101.
22. Chongmin ji, Huinan xu. In vitro evaluation and pharmacokinetics in dogs of guar gum and Eudragit FS30D-coated colon-targeted pellets of indomethacin. Journal of Drug Targeting, February 2007;15(2): 123–131.
23. Smita Rane, Vinita Kale. Evaluation of modified Guar Gum as film coating material International Journal of Chem Tech Research; April-June 2009; 1: No.2, 180-182.
24. Joshi MR, Misra AN. Spectrophotometric determination of budesonide: Indian journal of Pharmaceutical science 2000; 62(2):92-96.
25. Peter J Wilson, Abdul W Basit .Exploiting gastrointestinal bacteria to target drugs to colon: An in-Vitro study using amylose coated tablets. International journal of pharmaceutics 2005; 300:89-94.

Table 1: Composition for solution layering of drug

Sr.No.	Ingredient	Quantities
1	Budesonide	0.5 g
2	PVP K30	3.5 g
3	Talc	2 g
4	Methanol	100 ml

Table 2: Coating parameters for drug loading and guar gum coating

Parameters	Drug Specification	Guar gum Specification
Batch size	50 g	10 g (~100 capsules)
Spray rate	0.5 ml/m	1.2 ml/m
Nozzle diameter	1mm	1 mm
Atomizing air pressure	2 – 3 lb/inch^2	1 bar
Air inlet temperature	55°C	60-65^0 C
Pan speed	30RPM	30 RPM
Coating efficiency	82.00 to 85.00 %	50-60%

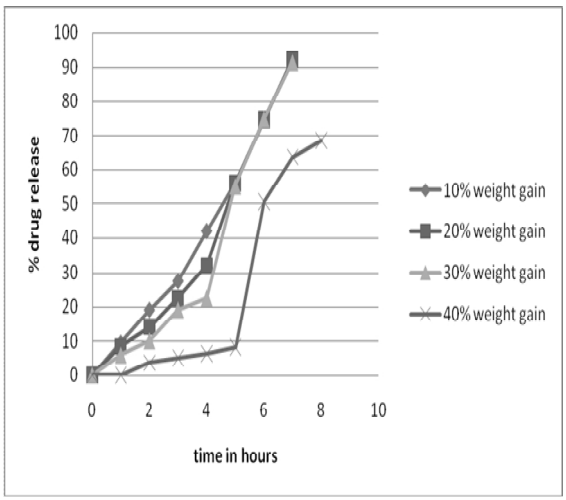
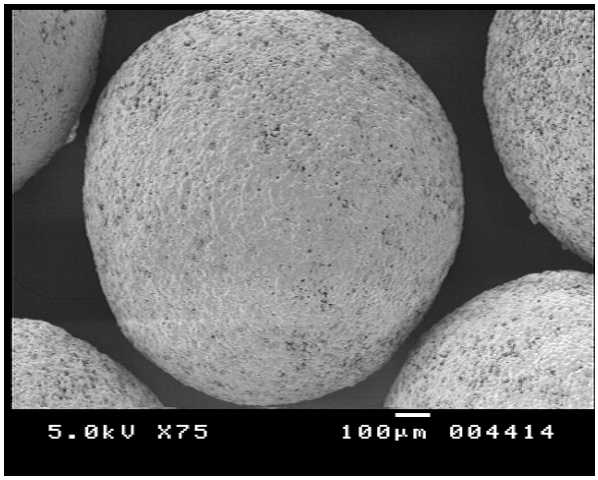


Fig 1: Dissolution profile of pellets coated with different percentage coating of guar gum.



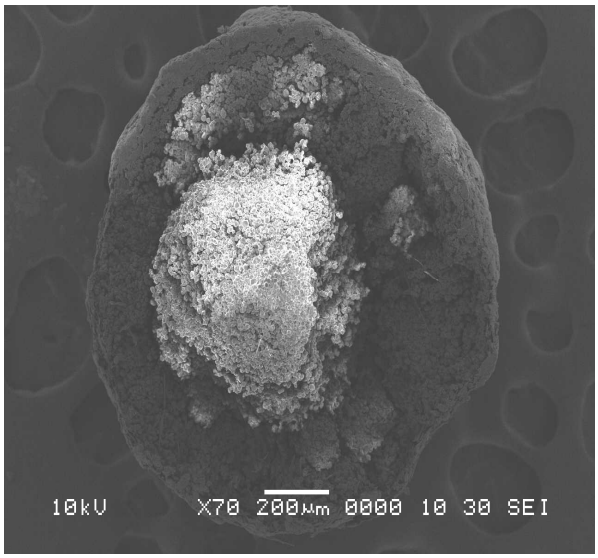


Fig 2: SEM photograph of guar gum coated pellets.

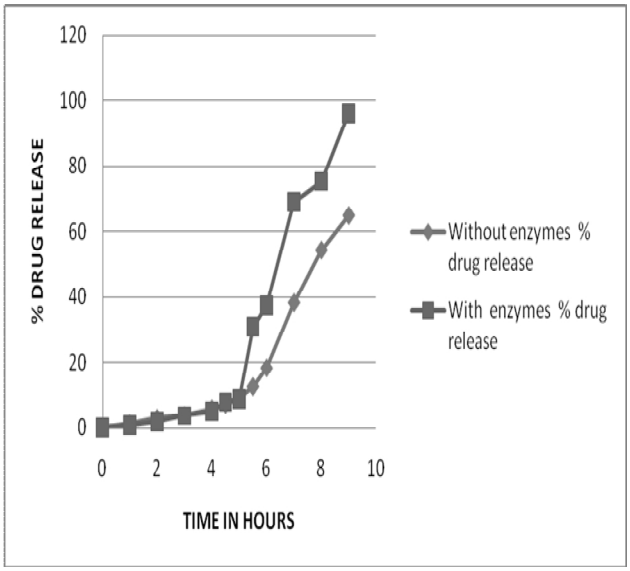


Fig 3: In-vitro drug release of guar gum coated budesonide pellets with and without enzyme.

Source of support: Nil, Conflict of interest: None Declared