pH-Enzyme Di-dependent Chronotherapeutic Drug Delivery System of Theophylline for Nocturnal Asthma

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Received August 22, 2010; accepted October 25, 2010; published online November 10, 2010

The purpose of this research work was to develop and evaluate a chronotherapeutic based colon-targeted drug delivery system of theophylline (THEO) exploiting pH-enzyme sensitive property for the prevention of episodic attack of asthma in early morning. Guar gum microspheres of theophylline were prepared by emulsification technique. Coating of microspheres was performed using solvent evaporation method with pH sensitive Eudragit® polymers. The particle size and surface morphology, entrapment efficiency and degree of swelling of microspheres were examined. The *in vitro* drug release studies were performed in pH progression medium and also in the presence of 2% rat caecal content. Theophylline was efficiently microencapsulated in guar gum microspheres at different polymer concentrations (1—4%). Fourier transform infrared (FT-IR)-spectroscopy confirmed the intermolecular interactions between guar gum and glutaraldehyde. Coating of guar gum microspheres by Eudragit led to decelerate the *in vitro* drug release of THEO. Moreover *in vitro* drug release studies also performed with 2% rat caecal content showed marked increment in drug release. The controlled release of THEO after a lag time was achieved with developed formulation for chronotherapeutic delivery. The pH dependent solubility behavior of Eudragit and gelling properties of guar gum are found to be responsible for delaying the release.

Key words chronotherapeutic; colonic delivery; microsphere; guar gum; pH-dependent; Eudragit®

The drug delivery to the colon has value not only in the colonic diseases such as ulcerative colitis, Crohn's diseases, inflammatory bowel disease, colorectal cancer but also improving oral delivery of peptides and other drugs degraded in the upper gastro-intestinal (GI) tract because of the less hostile environment prevailing in the colon compared with the stomach and small intestine. Colonic drug delivery is also useful to improve treatment of diseases depends on diurnal rhythm such as asthma and arthritis.[1—3] The approaches utilized in achieving colonic delivery of drugs include use of prodrugs,[4] pH-sensitive polymer coating[5] and time-dependent formulations.[6] In addition, the use of biodegradable polymers such as azo-polymer and polysaccharide (*e.g.* pectin and dextrin) for colon targeting are also reported in the literature.[7,8] Among the different approaches to achieve colon-selective drug delivery, the use of polymers, specifically biodegraded by colonic bacteria, holds great promise. The pH-dependent systems exploit the generally accepted view that pH of human GI tract increases progressively from the stomach (pH 2—3), small intestine (pH 6.5—7.0) to colon (7.0 to 8.0).[2] Most commonly used pH-dependent coating polymers are methacrylic acid copolymer (*i.e.* Eudragit L100-55, Eudragit L100 and Eudragit S100), which dissolve at pH 5.5, 6.0 and 7.0, respectively. There are several polysaccharides naturally occurring in plant (*e.g.* pectin, guar gum, inulin), animal (*e.g.* chitosan, chondroitin sulfate), algae (*e.g.* alginates), or microbial (*e.g.* dextran) origin were studied for colon targeting.[9]

Guar gum is a naturally occurring polysaccharide obtained from the seeds of two leguminous herbs, *Cyamopsis tetragonolobus* and *Pasoraloides*.[10] It consists linear chains of (1→4)-β-D-manopyranosyl units with α-D-galactopyranosyl units attached by (1→6) linkages.[11] It is hydrophilic in nature and swells in cold water forming viscous colloidal dispersions or sols.[12] This gelling property retards release of the drug from the dosage form as well as it is susceptible to degradation in the colonic environment. The pH of 1% w/v aqueous dispersion varies from 5 to 7 and it is stable over wide pH range. It is non-ionic and hence the viscosity of dispersion is unaffected by pH and remain same in both acidic and alkaline medium. In the pharmaceutical formulations, guar gum is used as a binder, disintegrant, suspending agent, thickening agent and stabilizing agent.[13] Guar gum was found to be a colon-specific drug carrier in the form of matrix and compression coated tablets,[13—15] microspheres,[9,16] and hydrogels.[17,18]

The circadian timing system has a powerful influence on the regulation of sleep–wake state and recent evidence suggests that it can also affect the respiratory control system. This has led to the suggestion that the circadian timing system may influence the occurrence or severity of some types of sleep-related breathing disorders.[19] The role of circadian rhythms in the pathogenesis and treatment of asthma has been extensively studied and indicates that airway resistance increases progressively at early morning in asthmatic patients. Hetzel and Clark[20] have reported that 70% of sudden deaths and 80% of cases of respiratory arrest in active asthma occur during sleep-related hours. A new therapeutic method called chronotherapy has been recently considered for the treatment of asthma. Chronotherapy of asthma aims to provide a more rational approach to treatment and also reduce side effects. Asthma is well suited for chronotherapy because broncho-constriction and exacerbation of symptoms vary in a circadian fashion.[21,22]

In the present study, it was attempted to achieve the chronotherapy for bronchial asthma. We utilized dual approach for the effective colonic delivery of theophylline (THEO) using pH dependent solubility behavior of Eudragit

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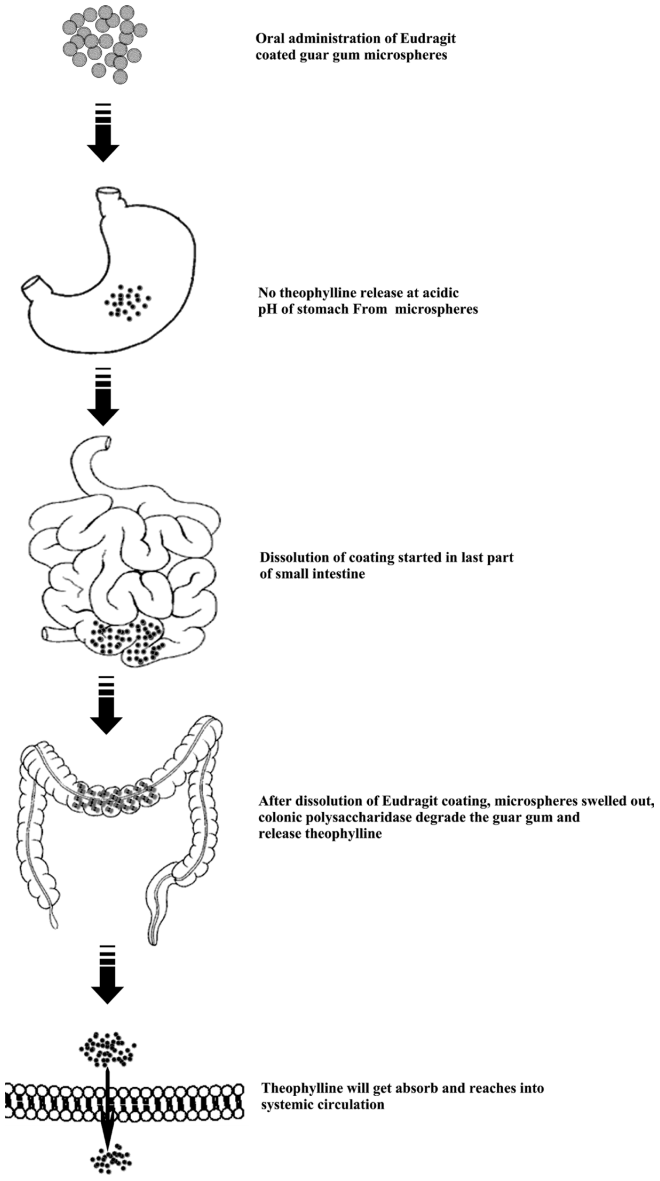

Fig. 1. Schematic Showing Proposed Mechanism of Action of Designed System

and susceptibility of guar gum to colonic environment. Eudragit coated guar gum microspheres containing THEO were prepared and characterized. Proposed mechanism of action of designed formulation is shown in Fig. 1.

Experimental

Materials Theophylline was obtained as a gift sample from M/s IPCA Laboratories Ltd., Ratlam, India. Guar gum was procured from HiMedia Laboratories Pvt. Ltd., Mumbai India. Light liquid paraffin, Glutaraldehyde, Span 80, Span 85 and Tween 80 were procured from Central Drug House Pvt. Ltd., Mumbai, India. Eudragit S-100 and L-100 were obtained as a gift sample from M/s Roehm Chemische, Fabrik, GmBH, Germany. All other chemicals used were of analytical reagent grade and were used as received.

Preparation of Guar Gum Microspheres Guar gum microspheres were prepared by using the method reported by Chaurasia *et al.*[16] with slight modification. Guar gum dispersion was prepared by mixing of guar gum with tween 80 (0.2% w/w) followed by the addition of distilled water in which THEO was previously dissolved and allowed to swell for 2 h. This aqueous phase was dispersed in light liquid paraffin (100 ml) containing span 80 (0.5% w/w) and maintained at 60 °C. This dispersion was stirred employing a mechanical agitation with propeller stirrer (Remi, Mumbai, India) at 2000 rpm. After 10 min, concentrated sulphuric acid (0.2 ml) was added to the dispersion followed by the addition of glutaraldehyde (0.5 ml) and stirring was continued for 2 h at constant speed. The microspheres were centrifuged (CFC-FREE, C-24, Cooling centrifuge, REMI, India), washed with *n*-hexane, methanol and acetone to remove the oil and dried under vacuum oven (Dolphin, India). Guar gum microspheres were prepared using different drug : polymer ratios *i.e.* 1 : 1, 1 : 2, 1 : 3, and 1 : 4.

Fourier Transform Infrared Spectroscopy (FT-IR) FT-IR spectroscopy was carried out to confirm the cross-linking reaction between guar gum and glutarldehyde. The KBr discs of uncoated guar gum microspheres (without drug) and pure guar gum were prepared and scanned in an FT-IR spectrophotometer (Perkin Elmer-Spectrum RX-I, Lambda, U.S.A.). The scanning range and resolution was 400—4000 cm^{-1} and 4 cm^{-1}, respectively.

Encapsulation of Guar Gum Microspheres Guar gum microspheres (core) were coated with Eudragit polymer by oil-in-oil solvent evaporation method.[23] Coating solution (5%) was prepared by dissolving 1 : 1 mixture of Eudragit S-100 (ES) and Eudragit L-100 (EL) in 10 ml of organic solvent (acetone : ethanol, 1 : 1). Guar gum microspheres (50 mg) were dispersed in this organic phase and poured in 70 ml light liquid paraffin containing 1% w/v Span 85. The system was stirred at 1000 rpm speed using a mechanical stirrer at room temperature for 3 h to allow the evaporation of solvent. Finally, the coated microspheres were collected by centrifugation, washed with *n*-hexane, freeze dried overnight (Heto Drywinner, Denmark) and kept in airtight container for further studies.

Surface Morphology and Particle Size The morphology and appearance of microparticles were examined by scanning electron microscopy (SEM). The prepared microspheres were freeze dried at −30 °C for 48 h and coated with gold palladium under an argon atmosphere for 150 s to achieve a 20 nm film (Sputter coater, SCD 004, BAL-TEC, Balzers, Furstentum, Lienchestein). The coated samples were then examined with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan). The particle size of prepared microspheres was determined by optical microscope using a calibrated ocular micrometer (Leica, Germany).

Entrapment Efficiency Entrapment efficiency was determined by using the method reported by Chaurasia *et al.*[16] An accurately weighed quantity of guar gum microspheres (equivalent to 50 mg of THEO) was incubated in 10 ml of phosphate buffered saline (PBS) (pH 7.4). The sample was ultrasonicated for 3 consecutive periods of 5 min each, with a resting period of 5 min each and kept for 48 h for complete extraction of THEO. The solution was centrifuged and supernatant was assayed for THEO spectrophotometrically (UV-1800 Spectrophotometer, Shimadzu, Japan) at 271.5 nm.[24] Each determination was made in triplicate.

Swellability A known weight (100 mg) of guar gum microspheres and Eudragit coated guar gum microspheres were placed in enzyme free simulated intestinal fluid (SIF) and allowed to swell up to constant weight at 37±0.5 °C in the dissolution apparatus (USP XXIII Model DT-06, Erweka, Germany). The microspheres were periodically removed, blotted with filter paper and their change in weight was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using formula:

$$SR = \frac{\omega_g - \omega_0}{\omega_0}$$

Where, SR=swelling ratio, ω_0=initial weight of microspheres, ω_g=final weight of microspheres.

***In Vitro* Drug Release Study in Simulated GI Fluids** The release of THEO from guar gum microspheres and Eudragit coated guar gum microspheres was investigated using rotating paddle dissolution apparatus at rotation speed of 100 rpm. The system was thermostated at 37 °C. Drug release was measured from accurately weighed amount of microspheres, equivalent to 100 mg of THEO, added to 500 ml of dissolution medium. For microspheres, simulation of gastrointestinal transit conditions was achieved by pH progression medium.[8,25,26] The pH of the dissolution medium was kept 1.2 for 2 h with 0.1 N HCl. Then 1.7 g of KH_2PO_4 and 2.225 g of $Na_2HPO_4 \cdot 2H_2O$ were added adjusting the pH 4.5 with 1.0 M NaOH and release rate study was continued for further 2 h. After 4 h the pH of dissolution medium was adjusted to 7.0 and maintained up to end of study. The final volume in all cases was 500 ml. The samples were withdrawn from dissolution medium at various time intervals using a pipette fitted with a micro filter and analyzed spectrophotometrically at 271.5 nm. All dissolution studies were performed in triplicate.

***In Vitro* Drug Release Study in the Presence of Rat Caecal Content** Rat caecal content was prepared by the method, reported by Van Der Mooter *et al.*[27] and Paharia *et al.*[8] Albino rats (Sprague-Dawley strains) of uniform

body weight (180—200 g) with no prior drug treatment were used for all the present *in vivo* studies, were weighed, maintained on normal diet and administered 1 ml of 2% dispersion of guar gum/EL/ES in water and this treatment was continued for 7 d to induce enzymes acting on polymers. Thirty minutes before starting the study, the rat was sacrificed, the abdomen was opened, caecal were traced, legated at both end dissected and immediately transferred into pH 6.8 (PBS), which was previously bubbled with CO_2. The caecal bag was opened; contents were weighed, homogenized, and then suspended in PBS (pH 7.4) to give the desired concentration (2%) of caecal content, which was used as simulated colonic fluid. The suspension was filtered through cotton wool and ultrasonicated for 10 min in an ice bath at 40% voltage frequency using a probe sonicator (Frontline Electronics, India) at 4 °C to disrupt the bacterial cells. After sonication, the mixture was centrifuged (Remi, India) at 2000 rpm for 20 min.

A weighed amount of microspheres was placed in 200 ml of dissolution media (PBS, pH 7.4) containing 2% w/v rat caecal content. The experiment was carried out with continuous CO_2 supply into the dissolution medium. At different time intervals, the samples were withdrawn and replaced with fresh PBS. The experiment was continued up to 24 h. The withdrawn samples were pipetted into a series of 10 ml volumetric flasks and volumes were made up to the mark with PBS and centrifuged. The supernatant was filtered through 0.45 μm membrane filter (Millipore, U.S.A.) and the filtrate analyzed for THEO content at 271.5 nm using UV spectrophotometer. All the experiments were performed in triplicate.

Results and Discussion

Guar gum microspheres of THEO were successfully prepared by emulsification technique. Microspheres were prepared by using different drug: polymer ratios. Four formulations of microspheres, *i.e.* GTM-1, GTM-2, GTM-3 and GTM-4 containing THEO : Guar gum in ratios of 1 : 1, 1 : 2, 1 : 3 and 1 : 4 were prepared. These guar gum microspheres were coated with ES and EL (1 : 1) by oil-in-oil solvent evaporation method.

Fourier Transform Infrared Spectroscopy Intermolecular interactions between guar gum and glutaraldehyde were confirmed by FT-IR-spectroscopy (Fig. 2). During cross-linking, glutaraldehyde might have reacted with the –OH groups of the guar gum through the formation of ether linkage. The appearance of a sharp intense peak at *ca.* 1104.5 cm^{-1} in the spectrum of the cross-linked guar gum microspheres (Fig. 2b) was found due to the formation of more ether linkages. Also the intensity of –OH peak is decreased as compared with spectrum of plain guar gum polymer (Fig. 2a) because free hydroxyl groups were engaged with ether linkage formation. This could be further supported by the presence of a sharp high intensity peak at 2927.7 cm^{-1} due to $-CH_2$ group of the alkyl chain formed by cross-linking. Soppimath and Aminabhavi[28] also reported the cross-linking of guar gum and glutaraldehyde.

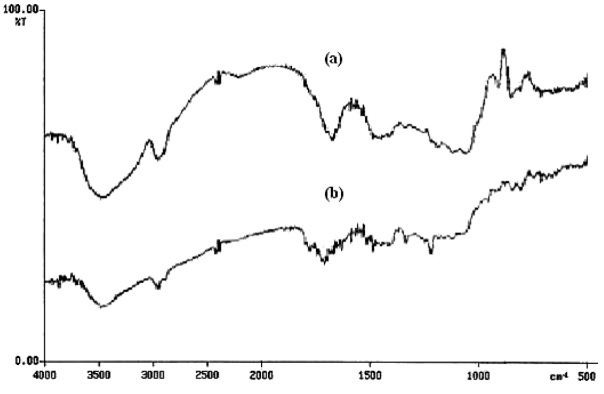

Fig. 2. FT-IR Spectra of (a) Guar Gum and (b) Glutaraldehyde Cross-Linked Guar Gum Microspheres

Surface Morphology and Particle Size Uniform, surface cross-linked and almost spherical microspheres were obtained as shown in scanning electron photomicrographs (Fig. 3A). The coated microspheres were found to be of spherical shape as observed in SEM photomicrographs (Fig. 3B). The mean diameter of guar gum microspheres varied from 20.99±2.51 μm to 27.30±2.05 μm with varying guar gum concentration from 1 to 4% weight per volume (wt/vol) (Table 1). When guar gum concentration increased from 1 to 4%, particle size was increased. Higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres as reported by Pongpaibul *et al.*[29] The particle size of Eudragit coated guar gum microspheres was considerably increased from 41.37±3.95 to 50.17±4.55 μm (Table 2).

Entrapment Efficiency and Swellability The entrapment efficiency of guar gum microspheres varied from 60.42±2.12 to 71.46±2.46% with varying guar gum concentration from 1 to 4% w/v. The highest entrapment efficiency was found with microspheres prepared using 4% guar gum (Table 1). This significant increment in entrapment efficiency could be attributed due to higher mass of guar gum for distribution.

Native guar gum swells 100—120-fold in GI buffers. Cross-linking with glutaraldehyde does not interfere with the nonionic nature of the polymer therefore; swelling is inde-

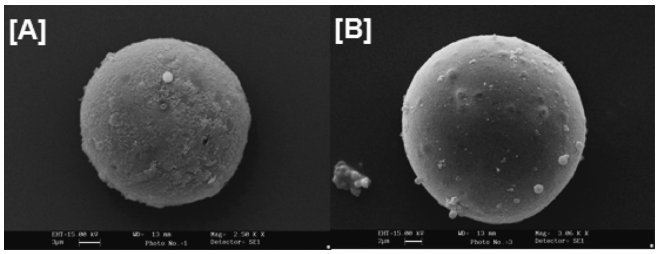

Fig. 3. Scanning Electron Micrographs of (A) Cross-Linked Guar Gum Microsphere and (B) Eudragit® Coated Cross-Linked Guar Gum Microsphere

Table 1. Effect of Drug, Polymer Ratio on the Particle Size, Percentage Drug Entrapment and Degree of Swelling of Various Guar Gum Microspheres

S. No.	Drug : Polymer ratio	Formulation code	Mean diameter (μm)	Drug entrapment (%)	Degree of swelling
1	1 : 1	GTM-1	20.99±2.51	60.42±2.12	0.88±0.06
2	1 : 2	GTM-2	22.65±1.26	63.68±1.75	1.21±0.12
3	1 : 3	GTM-3	25.02±2.58	67.29±1.83	1.27±0.16
4	1 : 4	GTM-4	27.30±2.05	71.46±2.46	1.31±0.15

GTM1—4-Guar gum microspheres containing theophylline. Values are average of three readings±standard deviation.

pendent of the pH of medium.[17] Cross-linking interferes with free access of water to the guar gum hydroxyl group, which in turn reduces the swelling properties of the cross-linked polymer. While microspheres prepared using 1 to 4% of guar gum showed increased swelling (Table 1). It may be results of increased concentration of hydroxyl group. No significant swelling was observed with Eudragit coated guar gum microspheres as compared to uncoated guar gum microspheres (data not shown). Thus ensuring the better resistance of Eudragit coated guar gum microspheres in upper GI tract to swelling and preventing subsequent drug release at the non-target site.[8]

***In Vitro* Drug Release Study in pH Progression Medium** *In vitro* THEO release study of guar gum microspheres and Eudragit coated guar gum microspheres was performed in pH progression medium at 37±0.5 °C to mimic the physiological condition. The results showed that the rate of release of THEO from guar gum microspheres was mainly influenced by polymer concentration. THEO release from guar gum microspheres in simulated GI fluids followed the order GTM-1>GTM-2>GTM-3>GTM-4 (Fig. 4). The initial higher release of THEO from microspheres might have resulted from the dissolution of drug crystals on the surface of microspheres. The results indicated the more sustained effect produced with increase in guar gum concentration. Swelling and gel strength of the guar gum microspheres might have prevented the release of drug from formulation.

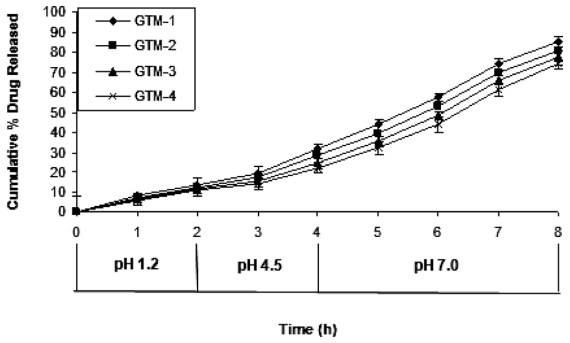

Fig. 4. *In Vitro* THEO Release Pattern from Guar Gum Microspheres Containing Different Drug : Guar Gum Ratios (1 : 1 to 1 : 4) in pH Progression Medium

GTM1—4-Guar gum microspheres. Values are average of three readings±standard deviation.

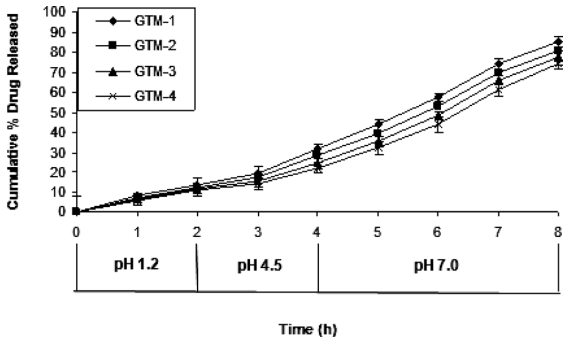

Fig. 5. *In Vitro* THEO Release Profile from Eudragit Coated Guar Gum Microspheres in pH Progression Medium

EGTM1—4-Eudragit coated guar gum microspheres. Values are average of three readings±standard deviation.

The cumulative % drug release from Eudragit coated guar gum microspheres showed the desired rate as there was no measurable drug release observed up to 2 h in simulated gastric fluid (SGF, pH 1.2) while at pH 4.5, the THEO release was quite insignificant (<2%) up to 4 h. THEO release from Eudragit coated guar gum microspheres in simulated GI fluids followed the order EGTM-1>EGTM-2>EGTM-3>EGTM-4 (Fig. 5). Drug release from microspheres before and after coating indicated that there was significant difference in the release rates before and after coating. It was observed from Eudragit coated guar gum microspheres that THEO release was protected from acidic environment by Eudragit coating.

***In Vitro* Drug Release Study in the Presence of Rat Caecal Content** The *in vitro* release of THEO from optimized Eudragit coated guar gum microspheres in presence of 2% rat's caecal content in simulated colonic fluid showed faster drug release at different time periods when compared to release study without rat caecal content (Fig. 6). *In vitro* drug release in simulated colonic fluid without rat caecal material was 63.3%, but drug release in simulated colonic fluid with 2% rat caecal material after 8 h study period was 83%. This improvement in drug release could be explained by susceptibility of guar gum to colonic enzymes after solubilization of coating where microspheres were degraded and results higher percentage of drug release.

Conclusion

The present study demonstrates successfully chronopharmaceutics based colonic drug delivery system for nocturnal asthma. The experimental results demonstrated that Eudragit coated guar gum microspheres have potential to be used as a drug carrier for an effective colon targeted delivery system.

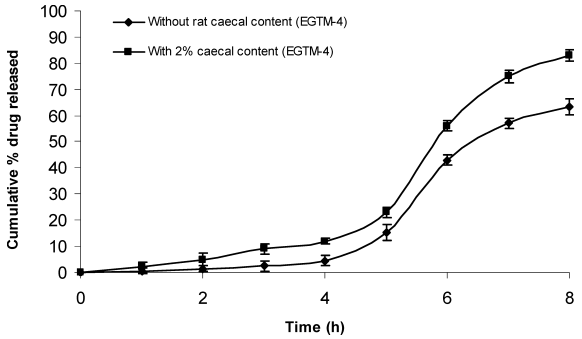

Fig. 6. Effect of Caecal Content on Percent THEO Release from Optimized Eudragit Coated Guar Gum Microspheres (EGTM-4) in Simulated Colonic Fluid (pH 7.4) with and without 2% Caecal Content

Values are average of three readings±standard deviation.

Table 2. Particle Size of Eudragit Coated Guar Gum Microspheres

S. No.	Core : Coating ratio	Formulation code	Mean diameter (μm)
1	1 : 5	EGTM-1	41.37±3.95
2	1 : 5	EGTM-2	43.20±2.89
3	1 : 5	EGTM-3	48.25±5.34
4	1 : 5	EGTM-4	50.17±4.55

EGTM1—4-Eudragit coated guar gum microspheres containing theophylline. Values are average of three readings±standard deviation.

Acknowledgement The authors gratefully acknowledge Head, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur, India, for providing the necessary facilities to carry out the research work.

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