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Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma

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Abstract

In this study, investigation of an oral colon specific, pulsatile device to achieve time and/or site specific release of theophylline, based on chronopharmaceutical consideration. The basic design consists of an insoluble hard gelatin capsule body, filled with eudragit microcapsules of theophylline and sealed with a hydrogel plug. The entire device was enteric coated, so that the variability in gastric emptying time can be overcome and a colon-specific release can be achieved. The theophylline microcapsules were prepared in four batches, with Eudragit L-100 and S-100 (1:2) by varying drug to polymer ratio and evaluated for the particle size, drug content and *in vitro* release profile and from the obtained results; one better formulation was selected for further fabrication of pulsatile capsule. Different hydrogel polymers were used as plugs, to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. *In vitro* release studies of pulsatile device revealed that, increasing the hydrophilic polymer content resulted in delayed release of theophylline from microcapsules. The gamma scintigraphic study pointed out the capability of the system to release drug in lower parts of GIT after a programmed lag time for nocturnal asthma. Programmable pulsatile, colon-specific release has been achieved from a capsule device over a 2–24 h period, consistent with the demands of chronotherapeutic drug delivery.

Keywords: Pulsatile; Colon-specific device; Chronotherapeutics; Nocturnal asthma; Eudragit microcapsules; Gamma scinitigraphy

1. Introduction

Among modified-release oral dosage forms, increasing interest has currently turned to systems designed to achieve time-specific (delayed, pulsatile) and site-specific delivery of drugs. These systems constitute a relatively new class of devices the importance of which is especially connected with the recent advances in chronopharmacology (Sangalli et al., 2001). In the last decade numerous studies in animals as well as clinical studies have provided convincing evidence, that the pharmacokinetics and/or the drug's effects-side effects can be modified by the circadian time and/or the timing of drug application within 24 h of a day (Lemmer, 1991; Hrushesky, 1994; Bjorn, 1996).

On the other hand, colon-specific drug delivery systems (CDDS) have been developing as one of the site-specific drug delivery systems. Along with many applications in local and systemic delivery of drugs the CDDS would also be advantageous when a delay in absorption is desirable from a therapeutic point of view as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythm, such as nocturnal asthma, angina and rheumatoid arthritis. (Bi-Botti, 2004; Sarasija and Stutie, 2005). So by developing the pulsatile device for specific colonic delivery, plasma peak is obtained at an optimal time, number of doses per day can be reduced; saturable first pass metabolism and tolerance development can also be avoided. (Morta et al., 1998; Richard and Susan, 1998; Gwen, 2002).

The necessity and advantage of CDDS have been well recognized and reviewed recently (Watts and Illum, 1997; Kinget et al., 1998; Libo et al., 2002). There were currently few strategies to achieve colonic specificity such as bacterially triggered, pressure controlled, pH dependent and time dependent CDDS (Libo et al., 2002; Abdul and John, 2003; Chourasia and Jain, 2003).

Recent studies with sensitive and reliable equipment contradict the traditional view and provide evidence of a decrease

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in pH at the gastrointestinal region between the ileum and the colon. Apparently the colon has a lower pH value (6.5) than that of the small intestine (7.0–7.8) (Bajpai et al., 2003). Based on the concept that a formulation on leaving the stomach arrives at the ileocaecal junction in about 6 h after administration and difference in pH throughout GIT, a time and pH dependent pulsatile device proposed for colonic targeting was designed, for achieving the selective delivery of drugs to colon, which is a chronopharmaceutical approach for the better treatment of nocturnal asthma.

The designed capsule device consists of a non-disintegrating capsule body and a soluble cap. The microencapsulated drug formulation prepared by using pH sensitive methacrylic acid copolymers (Eudragit L-100 and S-100) as coat and Theophylline (TPH) as core material, is filled within the capsule body and separated from the water-soluble cap by a hydrogel plug. The entire capsule was enteric coated to prevent variable gastric emptying. The enteric coating prevents disintegration of the soluble cap in the gastric fluid. On reaching the small intestine, the capsule will lose its enteric coating and the water-soluble hydrogel polymer plug inside the capsule swells to create a lag phase that equals the small intestinal transit time. This plug ejects on swelling and releases the microencapsulated drug from the capsule in the colon. Further, the controlled release of theophylline was achieved for up to 24 h as it was microencapsulated in the pH sensitive polymers. In addition, the colon-specific characteristic of modified pulsatile capsule in rabbits was established by gamma scinitgraphic technology. Scitigraphic imaging was performed using radiolabelled units intended for colon-specific release in order to assess their actual time and anatomical site of break-up. The proposed device can be manufactured using currently applicable pharmaceutical technologies and materials recognized as safe. With this system our goal was to avoid drug delivery in the upper GIT and target drugs to the terminal ileum and colonic region. The objective of including the microencapsulated theophylline was to offer a controlled release of drug in this intestinal zone, and to achieve the chronotherapy of nocturnal asthma.

In attempt to simplify the original Pulsincap^{TM} Technology, the complex synthetic hydrogel plug {Desmodur W [bis-(4-isocyanatocyclohexylmethane)] cross linked with hexane triol and polyethylene glycol of different molecular weight is not FDA approved}(Bajpai et al., 2003) has been replaced by pharmaceutically safe hydrophilic hydrogels. Although theophylline (TPH) preparations have generally fallen out of favor for the treatment of asthma, they may be useful in the treatment of nocturnal asthma when administered at specific times in relation to onset of symptoms (Gwen, 2002; Gothaskar et al., 2004). So with the proposed device a *new lease of life* to an existing drug molecule can be achieved.

2. Materials and methods

2.1. Materials

Theophylline was obtained from Cipla India Ltd., Mumbai. pH sensitive methacrylic acid co-polymers (Eudragit® L-100 and S-100) were supplied as gifts by Degussa India Pvt. Ltd., Mumbai. Hydroxypropylmethylcellulose-K_4M (HPMC) was obtained as gift from Colorcon, Goa. Guar gum and sodium alginate were from S.D. Fine Chem. Ltd., Mumbai. Cellulose acetate phthalate (CAP) for enteric coating was purchased from Spectrochem Pvt. Ltd., Mumbai. Elegant Pharmaceuticals, Hubli, India, supplied the hard gelatin capsules (100 and 500 mg capacity). Diethylene triamine penta acetic acid (DTPA) was gifted from Ramsco Chemical Industries, Mumbai, and ^{99m}Technitium solution was kindly supplied by KLES's Hospital and Medical research Center, Belgaum. The rest of the chemicals were obtained from the following commercial supplier and used as received without further purification: heavy liquid paraffin (Ranbaxy fine chemicals Ltd., New Delhi), Span 80 (Research Lab. Mumbai), Dibutylphthalate, acetone, petroleum ether (S.D. Fine Chem. Ltd., Mumbai) were of analytical grade.

2.2. Microencapsulation of TPH

From literature review, it was evident that the pH in the proximal colon ranges from 6.6 to 7.0. Therefore, the Eudragit L-100 and S-100 were combined in different ratios and solubility of these combinations was checked in different pH solutions. From the solubility parameters, it was found that Eudragit L-100 and S-100 in the ratios 1:2 was soluble in pH range of 6.6–7.0. Hence, this combination was selected for preparation of microcapsules.

2.2.1. Preparation method

Accurately weighed Eudragit L-100 and S-100 in 1:2 ratios were dissolved in 10 ml of acetone to form a homogenous polymer solution. Core material, i.e. TPH was dispersed in it and mixed thoroughly. This organic phase was slowly poured at 15 °C into liquid paraffin (100 ml) containing 1% (w/w) of Span-80 with stirring at 1000 rpm to form a uniform emulsion. Thereafter, it was allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth-walled, rigid and discrete microcapsules were formed. The microcapsules were collected by decantation and the product was washed with petroleum ether (40–60 °C), four times and dried at room temperature for 3 h. The microcapsules were then stored in a dessicator over fused calcium chloride (Ahmed et al., 2002).

Four batches were prepared with different proportions of core to coat materials (drug: polymer = 1:0.5, 1:1, 1:1.5 and 1:2 (w/w) named TM-1–4, respectively).

2.2.2. Evaluation of microcapsules

Particle size and external morphology: Determination of average particle size of THP microcapsules was carried out by optical microscopy. SEM studies were carried out by using JEOL JSM T-330 A Scanning microscope (Japan). Dry microcapsules were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microcapsules were taken by random scanning of the stub.

Drug content: In a 100 ml volumetric flask, 25 mg of crushed microcapsules were taken, and volume was made up to mark with pH 6.8. The flask was shaken for 12 h using an orbital shaker incubator. Then the solution was filtered and from the filtrate

appropriate dilutions were made and absorbance was measured at 272 nm by using UV absorption spectroscopy.

In vitro release studies: *In vitro* dissolution profile of each formulation was determined by employing USP XXIII rotating basket method (900 ml of pH 6.8-phosphate buffer, 100 rpm, 37 ± 0.5 °C). Microcapsules equivalent to 100 mg of TPH were filled into dialysis bags (12,000 molecular cutoffs) and loaded into the basket of the dissolution apparatus. Five milliliters of the sample was withdrawn from the dissolution media at suitable time intervals and the same amount was replaced with fresh buffer. The absorbance of the filtrate was determined at wavelength of 272 nm by using UV–vis spectrophotometer, against pH 6.8 as blank. The amount of drug present in the filtrate was then determined from the calibration curve and cumulative percent of drug release was calculated.

2.2.3. Preparation of cross-linked gelatin capsules

Twenty-five milliliters of 15% (v/v) formaldehyde was taken into dessicator and a pinch of potassium permanganate was added to it, to generate formalin vapors. The wire mesh containing the empty bodies of the 100 mg capacity hard gelatin (about 100 in number) capsule was then exposed to formaldehyde vapors. The caps were not exposed leaving them water-soluble. The dessicator was tightly closed. The reaction was carried out for 12 h after which the bodies were removed and dried at 50 °C for 30 min to ensure completion of reaction between gelatin and formaldehyde vapors. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in a polythene bag.

2.2.4. Test for formaldehyde treated empty capsule bodies

Various physical tests such as, identification attributes, visual defects, dimension changes, solubility studies were carried out.

2.2.5. Qualitative chemical test for free formaldehyde

Standard formaldehyde solution used is formaldehyde solution (0.002, w/v) and sample solution is formaldehyde treated bodies (about 25 in number) were cut into small pieces and taken into a beaker containing distilled water. This was stirred for 1 h with a magnetic stirrer, to solubilize the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with the washings. In brief, to 1 ml of sample solution, 9 ml of water was added. One milliliter of resulting solution was taken into a test tube and mixed with 4 ml of water and 5 ml of acetone reagent. The test tube was warmed in a water bath at 40 °C and allowed to stand for 40 min. The solution was not more intensely colored than a reference solution prepared at the same time and in the same manner using 1 ml of standard solution in place of the sample solution. The comparison should be made by examining tubes down their vertical axis.

2.2.6. Formulation of pulsatile drug delivery system

The bodies and caps of formaldehyde treated hard gelatin capsules of were separated manually. Microcapsules (TM-3) equivalent to 150 mg of theophylline were accurately weighed and filled into the treated bodies by hand filling. The capsules containing the microcapsules were then plugged with different amounts (20, 30 and 40 mg) of various polymers, i.e., guar gum, hydroxylpropylmethylcellulose and sodium alginate. The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution. The sealed capsules were completely coated by dip coating method with 5% CAP in 8:2 (v/v) mixture of acetone: ethanol, plasticized with dibutylphthalate (0.75%), to prevent variable gastric emptying. Coating was repeated until an 8–12% increase in weight is obtained. % weight gain of the capsules before and after coating was determined. Composition for modified pulsatile device on the basis of design summary is given in Table 1 (Julie et al., 1996; Seshasayan et al., 2001; Listair et al., 2002).

2.2.7. Evaluation of designed pulsatile capsule

The thickness of the cellulose acetate phthalate coating was measured using screw gauge and was expressed in mm. Ten capsules were selected randomly from each batch and weighed individually for weight variation. The test requirements are met if none of the individual weights are less than 90% or more than 110% of the average.

Table 1
Composition for modified pulsatile device on the basis of design summary

Batch code	Weight of empty body (mg)	Weight of microcapsules[a] (mg)	Weight of gelatin film (mg)	Polymer used	Weight of polymer used (mg)	Total weight with cap (mg)	Weight after CAP coating (mg)
F1	58.7	375	5.82	Guar gum	20	494.52	501.2
F2	57.0	375	5.01	Guar gum	30	501.01	510.3
F3	56.0	375	6.28	Guar gum	40	510.28	521.4
F4	55.5	375	8.16	HPMC	20	493.66	502.5
F5	58.0	375	5.09	HPMC	30	503.09	514.6
F6	57.5	375	6.42	HPMC	40	512.92	522.5
F7	58.0	375	8.11	Sod. Alg.	20	495.11	505.7
F8	57.0	375	7.18	Sod. Alg.	30	501.18	512.6
F9	56.5	375	5.99	Sod. Alg.	40	509.41	520.4

HPMC: hydroxy propyl methylcellulose; Sod. Alg.: sodium alginate.
[a] Microcapsules equivalent to 150 mg of drug used.

2.2.8. In vitro release profile of pulsatile capsule

Dissolution studies were carried out by using USP XXIII dissolution test apparatus (paddle method). Capsules were tied to paddle with a cotton thread so that the capsule should be immersed completely in dissolution media but not float (Chen et al., 2005). In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used, referred to as sequential pH change method (Zahirul Khan et al., 1999; Gang et al., 2004). When performing experiments, the pH 1.2 medium was first used for 2 h (since the average gastric emptying time is 2 h), then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 h (average small intestinal transit time is 3 h), the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hours. Nine hundred milliliters of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37 ± 0.5 °C. Capsules were tied to paddle with a cotton thread in each dissolution vessel to prevent floating. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 272 nm, by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times.

2.2.9. In vivo gamma scintigraphic studies

The radionuclide ^{99m}Technitium was eluted on the study day from Nuclear Medicine Department of KLES's hospital. A suitable amount of radiolabel tracer was complexed with 20 mg of DTPA resin (Diethylenetriamine Penta-Acetic acid) and then the microcapsules were soaked in ^{99m}T complex and dried for 45 min in oven at 50 °C. The microcapsules with 1 MBq of ^{99m}T were filled into an insoluble body of hard gelatin capsule (size # 5), and hydrogel polymer plug (guar gum) was placed above and the joint of the capsule was sealed using ethyl cellulose solution. The capsules were enteric coated with 5% of cellulose acetate Phthalate (Ishibashi et al., 1998; Shunji et al., 2001; Howard et al., 2002; Krishniah et al., 2002; Masataka et al., 2004).

This radio labeled capsule was administered to the overnight fasted healthy rabbit (New Zealand strain) and was immobilized and seated comfortably in the rabbit cage. The rabbit had a small sealed source of 0.05 MBq ^{99m}T firmly taped to the skin at the position of its shoulder joint and hip joint on the same side, which was depicted as an anatomical reference marker. The source was also used for repositioning when the images were taken.

Imaging of the fabricated capsule device, in the GIT was performed using a latest generation SPECT Gamma Camera, connected to Computer (Diacam +Icon). The scintiscans were taken immediately after first dosing and were carried out for 6 h at 30 min time intervals. The acquisition was taken for 60 s.

3. Results and discussion

As indicated in introduction, the aim of the work described here was to design a new pulsatile, colonic drug delivery device, for the better treatment of nocturnal asthma. The pulsatile capsule designed here combines two approaches previously attempted: pH-sensitive delivery and time dependent delivery. The system was fabricated into two steps: first, TPH was entrapped within pH dependent methacrylic acid copolymers (Eudragit L-100 and S-100 soluble at pH above 6 and 7, respectively); second, microcapsules were filled in non-disintegrating capsule body, and sealed with hydrogel plug and the entire capsule was coated with cellulose acetate phthalate for the enteric coating.

3.1. Preparative aspects and physicochemical properties of eudragit microcapsules

To prepare pH dependent microcapsules the O/O (oil in oil) emulsion solvent evaporation technique was used since it yields more uniform particles. The method is correctly referred as O/O instead of W/O (water in oil) since a polymeric solution in organic solvent is considered as oil in microencapsulation terminology. The organic phase containing pH dependent Eudragit L/S-100 in combined ratio of 1:2 and dispersed TPH was emulsified into an external oil phase. Pure acetone did not dissolve Eudragit; however acetone with 2% water fitted the criterion well. Liquid paraffin was used as the dispersion media or external phase. Petroleum ether was used to clean the microparticles since it removes liquid paraffin without affecting the integrity of the microparticles.

The arithmetic mean particle size of the formulations was determined by the optical microscope fitted with an ocular micrometer and stage micrometer. The average mean particle sizes of the microcapsules were found to be 167.33, 190.04, 217.95 and 258.41 μm for formulations TM-1, TM-2, TM-3, and TM-4, respectively. The mean particle size of the microcapsules significantly increased with increase in polymer concentration due to high viscosity of medium at a higher polymer concentration resulting in enhanced interfacial tension and diminished shearing efficiency (Morta et al., 1998). Scanning electron microscopy was performed to characterize the surface of the formed microcapsules. Particles of TM-1 were rough surfaced and crystals of the drug are visible on surface indicated that the concentration of polymeric solution is insufficient for complete encapsulation, whereas TM-2 is also rough but less crystalline as compared to TM-1. The TM-3 and TM-4 were found to be spherical, smooth and discrete. Some aggregates of polymer were found in TM-2. Scanning electron photomicrographs of all the four formulations are shown in Fig. 1.

3.2. Percentage practical yields and drug content

Results are shown in Table 2. The drug content was found to be very high in all the cases probably due to polymer loss by adherence to the container as a result of viscous nature of slurry. Amount of microcapsules to be taken for *in vitro* release studies and further development of pulsatile capsule was calculated based on content of drug present in each formulation.

3.3. In vitro release studies for microcapsules

In vitro release studies were carried out using USP XXIII dissolution assembly. The release profile obtained for all the

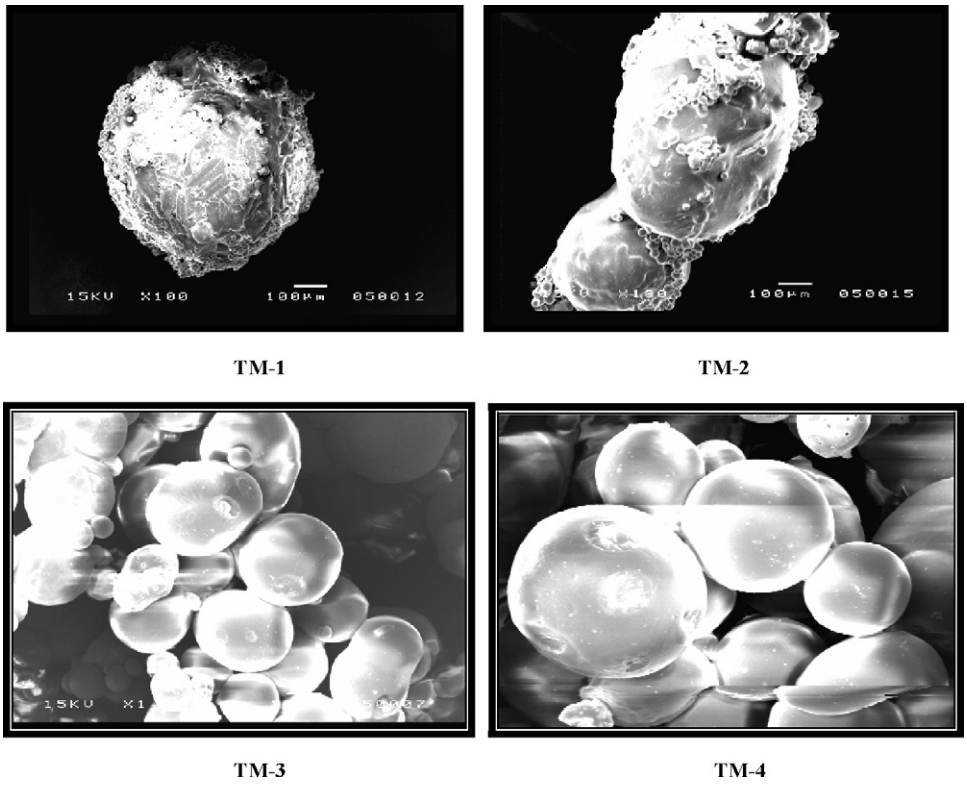

Fig. 1. Scanning electron micrographs of theophylline microcapsules for TM-1–4 formulations.

Table 2
Results of drug content for theophylline microcapsules

Formulation	Absorbance	Concentration (μg/ml)	Practical drug content (μg/ml)	Theoretical drug content (μg/ml)	% Drug content
TM-1	0.816	15	15	16.66	90.03
TM-2	0.624	11.4	11.4	12.5	91.20
TM-3	0.541	9.85	9.85	10	98.5
TM-4	0.78	14.30	14.30	15	95.33

four formulations were shown in Fig. 2. It was observed that the drug release from the formulations decreased with increase in the amount of polymer added in each formulation. The release of drug from polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increase the time required to swell also increase thereby decrease in the drug release. However, the release showed a bi-phasic release with an initial burst effect. In the first 30 min drug release was 32.6%, 28.7%, 20.41% and 16.71% for TM-1, TM-2, TM-3 and TM-4, respectively. The mechanism for the burst release can be attributed to the drug loaded on the microspheres or imperfect entrapment of drug. The overall cumulative % release for TM-1, TM-2, TM-3 and TM-4 were found to be 94.05%, 91.0%, 90.0%, and 84.8% at the end of 12th hour.

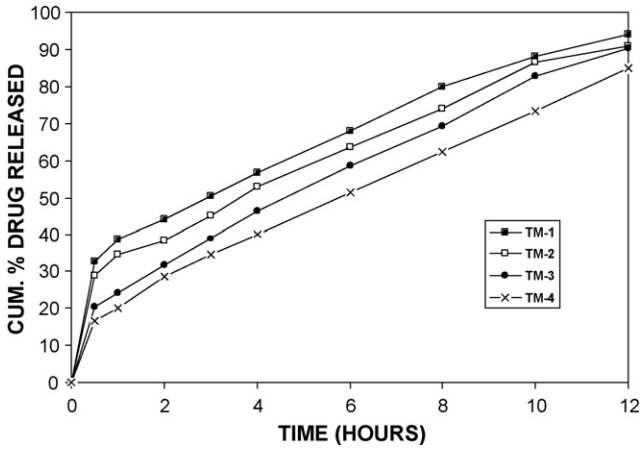

Fig. 2. Comparative *in vitro* release profiles for the Eudragit microcapsules. Results shown are mean of three experiments, errorbars omitted for clarity.

In vitro release study was analyzed using various mathematical models. The regression coefficients for formulation TM-1 to TM-4 of zero-order plot were found to be 0.7080, 0.7811, 0.9126, and 0.9307, respectively. The regression coefficients for formulations TM-1–4 of first-order plot were found to be 0.9796, 0.9821, 0.9821 and 0.9852, respectively. Higuchi matrix plot regression coefficients of formulations TM-1–4 were found to be 0.9795, 0.9896, 0.9940, and 0.9913, respectively. The regression coefficients for formulations TM-1–4 of Hixson Crowell plot were found to be 0.9542, 0.9593, 0.9867 and 0.9855 respec-

tively. The *n* values for TM-1–4 were 0.3437, 0.3703, 0.4860 and 0.5140, respectively.

Overall, the curve fitting into various mathematical models was found to be average. Based on highest regression value (*r*) the best-fit model for TM-1 was Peppas and TM-2, TM-3 and TM-4 was Higuchi matrix, indicating that the release is by diffusion from these formulations. The *n* value obtained from Peppas model for TM-1 was $n = 0.3437$ which is less than 0.45. This indicates that the release mechanism is by non-fickian diffusion (Kulkarni et al., 2001).

3.4. Formaldehyde treatment of hard gelatin capsule

Formalin treatment has been employed to modify the solubility of the gelatin capsules. Exposure to formalin vapors results in an unpredictable decrease in solubility of gelatin owing to the crosslinkage of the amino groups in the gelatin molecular chain with aldehyde groups of formaldehyde by Schiff's base condensation.

In about 100 capsule bodies treated with formaldehyde, about ten were found to be shrunk or distorted. Capsule of 100 mg capacity showed a significant decrease in length and diameter after treatment. The solubility tests were carried out for normal capsules and formaldehyde treated capsules for 24 h. It was observed that in all the case of normal capsules, both cap and body dissolved within 15 min where as in formaldehyde treated capsules, only the cap dissolved within 15 min, while the capsule body remained intact for about 24 h and hence indicates the suitability for colon targeting.

The formaldehyde capsules were tested for the presence of free formaldehyde. The sample solution was not more intensely colored than the standard solution inferring that less than 20 μg of free formaldehyde per 25 capsules, taken for test.

3.5. Evaluation of modified pulsatile capsule

On the basis of drug content, particle size morphology, *in vitro* release and release kinetics, formulation TM-3 was selected as better formulation for designing pulsatile device. *In vitro* release profiles of pulsatile device during 24 h studies were found to have very good sustaining efficacy. The results obtained for all the nine formulations (F1–F9) are shown in Fig. 3, indicating the plots of comparative *in vitro* release profile for formulations F1–F3 (a), F4–F6 (b) and F7–F9 (c), which contains guar gum, HPMC and sodium alginate respectively as hydrogel plugs at different proportions. During dissolution studies, it was observed that, the enteric coat of the cellulose acetate phthalate was intact for 2 h in pH 1.2, but dissolved in intestinal pH, leaving the soluble cap of capsule, which also dissolved in pH 7.4, then the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen matrix. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the eudragit microcapsules into simulated colonic fluid (pH 6.8 phosphate buffer). With all the formulations, there was absolutely no drug release in pH 1.2, thus indicating the efficiency of 5% CAP for enteric coating.

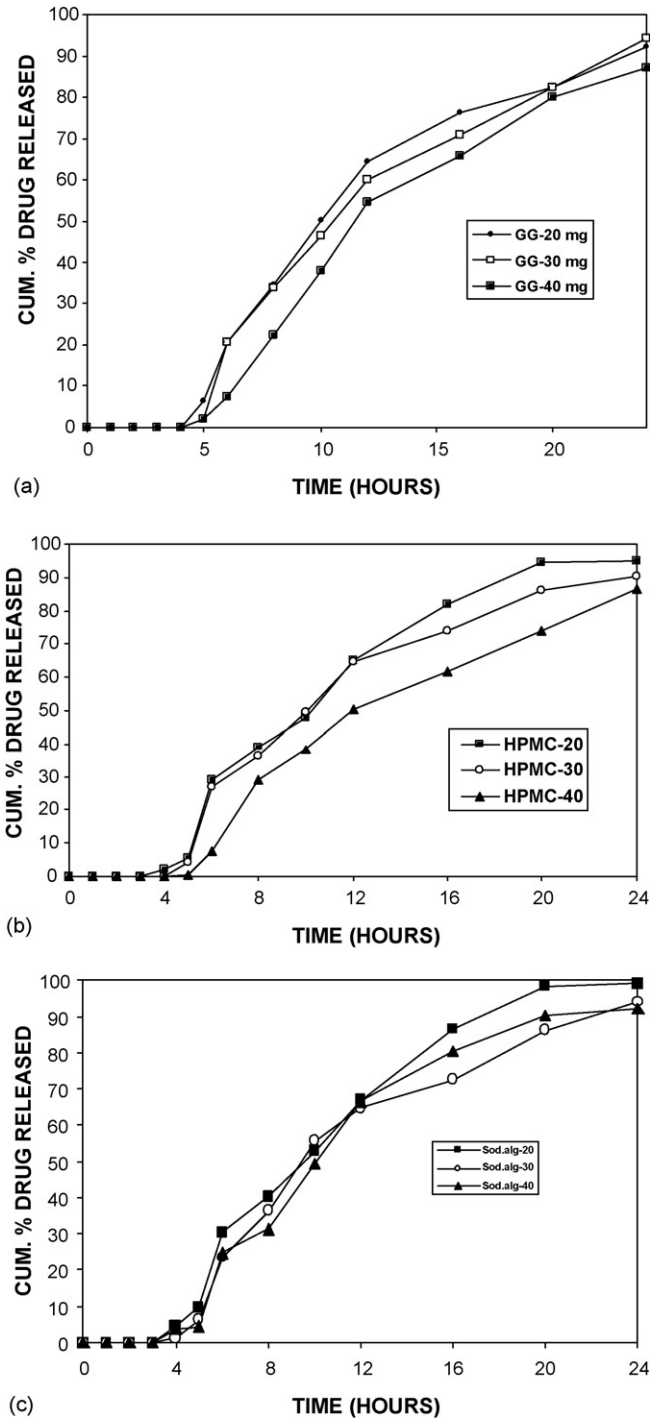

Fig. 3. Comparative *in vitro* release profiles for the pulsatile dosage forms: (a) formulations F1–F3 where guar gum is used as hydrogel polymer plug in 20, 30 and 40 mg, (b) formulations F4–F6 where HPMC is used as hydrogel polymer plug in 20, 30 and 40 mg and (c) formulations F7–F9 where sodium alginate is used as hydrogel polymer plug in 20, 30 and 40 mg.

3.5.1. Formulations with guar gum as hydrogel plug

With formulations F1 (20 mg), F2 (30 mg), at the end of 5th there was 6.38% and 2.15% cumulative drug release was found. In case of F1 and F2 it was observed that polymer concentration was sufficient to retard the drug release in small intestinal fluid and the plug ejected out in colonic fluid, releasing the entire

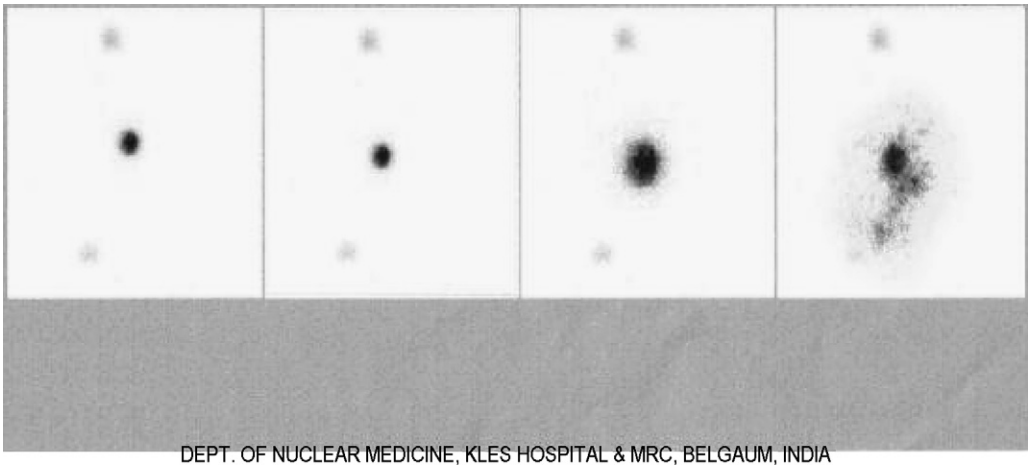

Fig. 4. Scintigraphic images in rabbit.

drug in colonic pH, in a controlled manner. At the end of 24 h, 92.22% and 94.14% of drug release was found in F1 and F2, respectively. With F3, a decrease in expelling power of plug was observed which might be due to inadequate wetting of the polymer. It was observed that plug ejected after 6 h and at the end of 24 h 87.18% of drug release was observed.

3.5.2. Formulation with HPMC as hydrogel plug

With formulation F4 (20 mg), F5 (30 mg), at the end of 5th hour 5.33% and 4.26% of drug was released respectively and at the end of 20th hour F4 formulation had released 94.47% of drug, whereas F5 formulation released 90.42% of drug up to 24 h in controlled manner. In case of F6 (40 mg), hydrogel plug ejected out in between 6th and 8th hour, indicating decrease in expelling power of plug. At the end of 24th hour 86.43% of drug was released.

3.5.3. Formulations with sodium alginate as hydrogel plug

With formulations F7 (20 mg), F8 (30 mg) and F9 (40 mg), at the end of 5th hour around 9.574%, 6.122%, 4.447% of drug release was observed respectively. F7 released 98.97% of drug within 20 h where as F8 and F9 released 94.122% and 92.232% of drug at the end of 24th hour.

From all the above observations, it was found that the order of sustaining capacity of polymer is, guar gum > HPMC > sodium alginate. The hydrophillic polymers like guar gum, HPMC, and sodium alginate can be used as hydrogels to delay the drug release until the formulation reaches the colon and thereafter the drug is released in the colon. The release of drug from modified pulsatile capsule was found to be proportional to the concentration of the polymer in HPMC and sodium alginate, where as with guar gum there is no such relation. With the formulations containing 20 and 30 mg of guar gum there is no significant difference in controlling release of the drug.

3.6. In vivo gamma scintigraphic study

Initially the study was conducted in rats as given in reference (Zahirul Khan et al., 1999). The scintiscans in rats indicated that, the capsule is intact and there was no further moment observed in GIT. The reason may be that (i) capsule size may be so big that it prohibited the further moment, (ii) the frequent sedation of the rat may also be the reason for decreased GI motility and (iii) keeping the animal immobilized by stretching on the mount table in supine position would have altered the normal physiology.

During the study, the expulsion of the capsule by rat on oral administration was also noticed, which made it difficult for repetition of study. Hence the study was further elaborated on rabbits. Fig. 4 shows the gamma scintigraphic images taken on the rabbits. The two spots at corners are the anatomical markers indicating one taped at upper 'shoulder joint' and one at 'lower hip joint'. Image (A) was taken immediately after 1 h of administration, (B) was after 3 h, (C) at 5th hour and (D) is at 6th hour after administration. The region of interest (ROI) for each segment in (A) and (B) indicates the intactness of capsule whereas in image (C) the movement of capsule in GIT can be clearly noticed. (D) shows the drug release in lower part of GIT that might be colon. These results clearly indicate that the capsule also possessed a better *in vivo* colon-specific delivery.

4. Conclusion

The present study demonstrates that the theophylline microcapsules could be successfully colon targeted by design of time and pH dependent modified chronopharmaceutical formulation. In conclusion, pulsatile drug release over a period of 2–24 h, consistent with the requirements for chronopharmaceutical drug delivery was achieved from insoluble gelatin capsules, in which microencapsulated theophylline was sealed by means of a suitable hydrogel plug. Concerning *in vivo* results, the scitigraphic data point out the capability of the system of releasing drugs in the GIT after a programmed lag time, thus allowing pulsatile and in the case of gastroresistant systems, colon-specific drug delivery to be attained. Thus the designed device can be considered as one of the promising formulation technique for preparing colon-specific drug delivery systems and hence in chronotherapeutic management of asthma by opening a "new lease of life" to an existing drug molecule.

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