Original Research Article

Microcapsule-based chronomodulated drug delivery systems of montelukast sodium in the treatment of nocturnal asthma

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Abstract

Background: Asthma is a chronic disease characterized by repeated attacks of breathlessness and wheezing, which occurs principally during night. This is due to circadian changes in ventilation and airway responsiveness. Hence, the chronomodulated therapy is more considering in the treatment of nocturnal asthma.

Objective: The objective of the study was to prepare microcapsule-based chronomodulated drug delivery system of montelukast sodium (MLS) for the treatment of nocturnal asthma.

Materials and Methods: Five batches of MLS microcapsules were prepared using pH-dependent polymer combination of Eudragit® S-100 with L-100. The optimized batch microcapsules were enclosed in a capsule to obtain chronomodulated pulsincap systems (CMPSs). In this system, natural gums were used as hydrogel plugs to achieve the required time lag. Microcapsules and CMPSs have been evaluated for their characteristic properties.

Results: The developed microcapsules are spherical in shape with smooth surfaces. *In vitro* release studies predicted that M4 and M5 batches could control the drug release for a period of 12 h. The optimized batch microcapsules (M4) were used in the preparation of CMPSs. The natural hydrogel plugs, i.e. xanthan gum, gum kondagogu, and guargum used in CMPSs showed approximate lag phase of 5, 6, and 5.5 h and drug releases up to 16, 24, and 24 h, respectively.

Conclusion: CMPSs containing Eudragit® microcapsules of MLS with programmable lag phase and prolonged drug release were successfully developed using natural gums as hydrogel plugs, which congregates the demands of chronomodulated therapy in asthma.

Keywords: Chronomodulated pulsincap systems, chronotherapy, cross-linked gelatin capsules, hydrogel plug, lag phase, pH-dependent polymer

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INTRODUCTION

The current statistics of WHO states that there are 235 million people suffering from asthma worldwide. It is a chronic disease characterized by repeated attacks of breathlessness and wheezing. These symptoms are getting worse during dark.[1-4] The main contributing factors of that state are circadian changes in ventilation, airway responsiveness and inflammation, mucociliary clearance, and changes in hormonal levels.[5] Hence, the chronotherapy associated delivery systems are booming in the treatment of nocturnal asthma which are designed to release the drug and elicit the action after the programmable lag time at the time of necessity.[6,7] Chronotherapy refers to a treatment method in which *in vivo* drug availability is correlated with the circadian rhythm of disease to optimize therapeutic outcomes and to limit the side effects.[8,9] Among various delayed release systems, pulsatile drug delivery systems proved successful because of their spatial and temporal drug delivery in their inherent excellence in achieving chronotherapeutic goals.[10] Chronotherapeutic goals can be achieved by designing of colon-specific delayed release systems.[11,12] For the design of colon-specific chronomodulated systems, a range of strategies are available; among them, time dependent, pH dependent, bacterial triggered, and pressure controlled are thoroughly studied.[13-15] By challenging the traditional view, recent studies confirm that the colon has lower pH value (6.8) than the small intestine (7.0–7.8),[16] and it takes about 5–6 h to reach the dosage form to an ileocecal junction. In view of delayed time and variations of pH in the Gastro Intestinal Tract (GIT), the mutual pH- and time-dependent pulsatile device was designed for colon-specific delivery which is a chronopharmaceutical approach to treat the diseases which follow circadian rhythm like asthma. Hence, the present study was aimed to fabricate chronomodulated pulsincap systems (CMPSs) which consist of insoluble, nondisintegrating capsule body and soluble cap.[17,18] The body of the capsule is filled with microcapsules of the montelukast sodium (MLS) which were prepared by using pH-sensitive Eudragits®; the prior release of drug core in the small intestine was prevented by a swellable hydrogel plugs which separate the body and cap. The entire capsule was enteric coated to prevent its dissolution in the stomach. After administration, the enteric coat of the capsule will dissolve in the small intestine, and hydrogel plug starts swelling. On complete swelling, the hydrogel plug will be ejected from the capsule body and facilitates the release of MLS microcapsules into the colon. The free microcapsules will release the drug for an extended period of time for the prolonged treatment in nocturnal asthma. Prolonged release is essential to prevent the signs and symptoms of asthma and associated early morning rhinitis.

Chemically, MLS is R-(E)]-1-[[[1-[3-[2-(7-chloro -2-quinolinyl)ethenyl]phenyl]-3- [2-(1-hydroxy -1-methylethyl) phenyl] propyl] thio] methyl] cyclopropaneacetic acid monosodium salt.[19] It is an orally effective antiasthmatic drug for adults and children. It acts by inhibiting the physiological actions of cysteinyl leukotrienes (LTC4, LTD4, and LTE4). The properties of MLS such as extensive presystemic metabolism and higher absorption in alkaline pH allow to design into CMPSs to meet the objective of delivery of drug to the colon for the treatment of nocturnal asthma. Moreover, the microencapsulation of MLS in the present study aids in increasing the stability of hygroscopic MLS along with achieving prolonged release.[20]

MATERIALS AND METHODS

Materials

MLS was gifted from RA Chem Pharma Ltd., Hyderabad, India. Eudragit® L-100 and Eudragit® S-100 were obtained as a gift sample from Strides Arcolab Ltd, Bangalore. Xanthan gum and guar gum were procured from Finoso Pharma Private Limited, Hyderabad. Cellulose acetate phthalate (CAP) for enteric coating was purchased from Hi media, Mumbai. All other chemicals were procured from Qualigens, Mumbai, which include heavy liquid paraffin, Span-80, dibutyl phthalate, acetone, and petroleum ether were of analytical grade.

Methods

Fourier-transform infrared and differential scanning calorimetry studies

Fourier transform infrared (FT-IR) and differential scanning calorimetry (DSC) studies were performed to study the interactions between the drug and excipient mixtures. FT-IR spectral studies were performed using Bruker alpha M15 FT-IR spectrophotometer using OPUS 6.5 software. DSC analysis was conducted using Mettler Toledo 822E calorimeter.

Preparation of microcapsules

MLS microcapsules were prepared by emulsion solvent evaporation method developed by Alavi *et al*., 2002.[21] Briefly, weighed quantities of Eudragit® L-100, Eudragit® S-100, and drug were dissolved in 10 ml of acetone and emulsify the solution in liquid paraffin containing 1% (W/W) of Span-80 as emulsifying agent. Five batches of microcapsules were prepared by using different core to coat ratios and were coded as M1, M2, M3, M4, and M5 and stored at desiccator for further evaluation.

Particle size and morphology

The particle size of MLS microcapsules was examined using optical microscopy. The shape and surface textures were studied using scanning electron microscopy (SEM); Carl Zeiss EVO MA-15 instrument with 20 kW power and photographs were taken by random scanning method.

Microcapsule wall thickness

The average thickness of the wall that surrounds the drug particles was determined by the method of Luu *et al.*,[22] using the following equation:

$h = (\bar{r}\ [1\text{-}p]\ d_1/3\ [pd_2 + [1\text{-}p]\ d_1)$, where, h is the wall thickness, $\bar{r}$ is the mean radius of the microcapsules, d_1 is the density of the core material, d_2 is the density of the coat material, and P is the portion of the medicament in the microcapsules.

Drug content and microencapsulation efficiency

Drug content was determined by crushing of microcapsules followed by estimating the concentration of drug in phosphate buffer of pH 6.8 using ultraviolet (UV)-visible spectrophotometry. Encapsulation efficiency was calculated using the following equation:

$$\text{Percentage of encapsulation efficiency} = \frac{\text{Quantity of drug present in the microcapsules}}{\text{Total quantity of drug added during the preparation of microcapsules}}$$

In vitro drug release studies

In vitro drug release studies were conducted by employing USP XXXIII rotating basket apparatus; microcapsules equivalent to 10 mg of MLS were taken in a basket by packing them in a dialysis bag (12,000 molecular weight cutoff) in 900 ml of pH 6.8 phosphate buffer as medium at 37°C with a basket speed of 100 rpm and samples were analyzed by using UV spectrophotometer. Cumulative amounts of drug release were calculated and dissolution profiles were constructed.

Formulation of chronomodulated pulsincap systems

Preparation of cross-linked gelatin capsules

Cross-linked gelatin capsule bodies were prepared by treating the separated capsule bodies with formaldehyde to make them water insoluble.[23] Over 100 gelatin capsule bodies of 350 mg capacity were made insoluble by cross linking of gelatin with aldehyde. The treated capsule bodies were joined with soluble caps and kept safe for further studies. Treated microcapsules were tested for free formaldehyde content according to the method reported by Mastiholimath *et al.*[24]

Development of chronomodulated pulsincap systems

The cross-linked capsule bodies were separated manually and were filled with Eudragit® microcapsules equivalent to 10 mg of MLS. Filled capsule bodies were closed with natural hydrogel-forming gums such as xanthan gum, gum kondagogu, and guargum and the capsules were locked using 5% w/v ethanolic solution of ethyl cellulose. Meanwhile, the obtained capsules were enteric coated by dipping in 5% w/v solution of CAP in acetone to avoid disintegration of capsule in the stomach. The percentage of weight gain of capsules after the CAP coating was calculated.[18,25] By considering the characterization studies of microcapsules, M4 batch microcapsules were chosen in fabrication of CMPSs. Nine batches of CMPSs (F1, F2, F3, F4, F5, F6, F7, F8, and F9) were prepared using three concentrations, namely 10, 20, and 30 mg of gums mixed with 10 mg of lactose monohydrate as a common filler. Fabrication details of the CMPS systems are given in Table 1.

Evaluation of fabricated chronomodulated pulsincap systems

Ten modified pulsincap capsules were taken randomly and evaluated for weight variation. Thickness of the CAP coating was determined using digital screw gauge.

In vitro drug release studies of developed chronomodulated pulsincap systems

In vitro drug release studies were performed using USP XXXIII rotating paddle apparatus. Capsules were tied to paddle with a cotton thread to facilitate the capsule immersion to avoid the floating on dissolution media.[26] Three different buffers of pH 1.2, 7.4, and 6.8 were used sequentially as dissolution media to simulate the variable pH of the GIT, designated as a sequential pH change media[27-29] at 37°C with paddle rotation of 100 rpm using 900 ml of media. Samples were analyzed spectrophotometrically by measuring the absorbance at 346 nm and constructed the dissolution profiles.

RESULTS

Drug excipient compatibility studies

FT-IR spectrum of drug exhibited broad peak around 3300 cm^{-1} indicated the presence of tertiary hydroxyl groups, strong peak near 1700 cm^{-1} indicates the presence of carboxylic acid group, and aromatic C-H peaks are observed between 2900 and 3000 cm^{-1}. FT-IR spectra of drug, placebo, and drug excipient mixture are shown in Figure 1a-c. The DSC thermogram of pure drug showed the endothermic peak at 122.96°C, and the thermogram of drug excipient mixture showed the endothermic peak at 126.33°C. DSC thermograms of drug, placebo, and drug excipient mixture are shown in Figure 2a-c.

Table 1: Composition of chronomodulated pulsincap systems

Formulation code	Weight of empty body (mg)	Weight of microcapsules* (mg)	Polymer used	Weight of polymer (mg)	Total weight with cap (mg)	Weight after CAP coating (mg); mean±SD**
F1	57.2	240	Xanthan gum	10	341.2	350.49±0.23
F2	56.8	240	Xanthan gum	20	351.8	360.64±0.35
F3	58.2	240	Xanthan gum	40	348.2	359.32±0.54
F4	57.0	240	Gum kondagogu	10	341.0	352.12±0.58
F5	57.5	240	Gum kondagogu	20	351.5	358.18±0.21
F6	58.2	240	Gum kondagogu	40	371.2	380.48±0.68
F7	57.3	240	Guargum	10	342.3	353.81±0.98
F8	56.7	240	Guargum	20	350.7	359.99±0.97
F9	57.0	240	Guargum	40	345.4	354.69±0.65

*Microcapsules equivalent to 10 mg of drug, **Mean of three replicates. SD: Standard deviation, CAP: Cellulose acetate phthalate

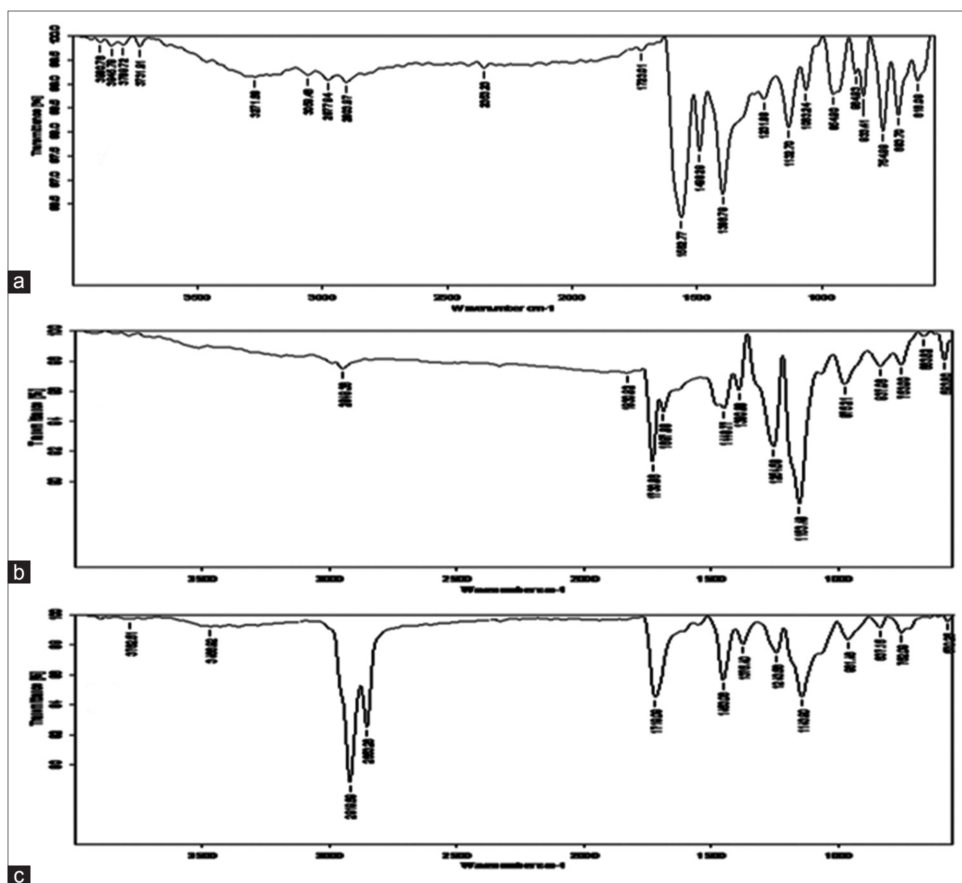

Figure 1: Fourier-transform infrared spectrum of (a) pure montelukast sodium (b) placebo mixture (c) drug and excipient mixture

Characterization of Eudragit® microcapsules

Optical microscopic studies revealed that the size of microcapsules was ranging from 40 to 160 μm, wall thickness was from 13.8 to 40.2 μm, drug content was >90%, and microencapsulation efficiency was found to be >79%. SEM photographs are shown in Figure 3.

In vitro drug release studies of montelukast sodium microcapsules

In vitro drug release studies on MLS microcapsules were performed using USP XXXIII dissolution apparatus (rotating basket). The weight of microcapsules was considered to conduct dissolution studies which were calculated on the basis of drug content values. Dissolution profiles of microcapsules are shown in Figure 4. In order to understand the release kinetics and its mechanisms, the *in vitro* dissolution profiles were analyzed by means of various kinetic models such as of zero order, first order, Higuchi, and Peppas kinetics. In the first 30 min, the percentage of drug release was 40.8%, 37.4%, 33.2%, 26.7%, and 20.33% for M1, M2, M3, M4, and M5, respectively. At the end of 24 h, the cumulative percent of drug release was 98.61 ± 0.35, 99.31 ± 0.2, 99.00 ± 0.44, 98.23 ± 0.4, and 94.45 ± 0.12 form M1 in 6 h, M2 in 8 h, M3 in 10 h, M4 in 12 h, and M5 in 12 h, respectively. The correlation coefficient

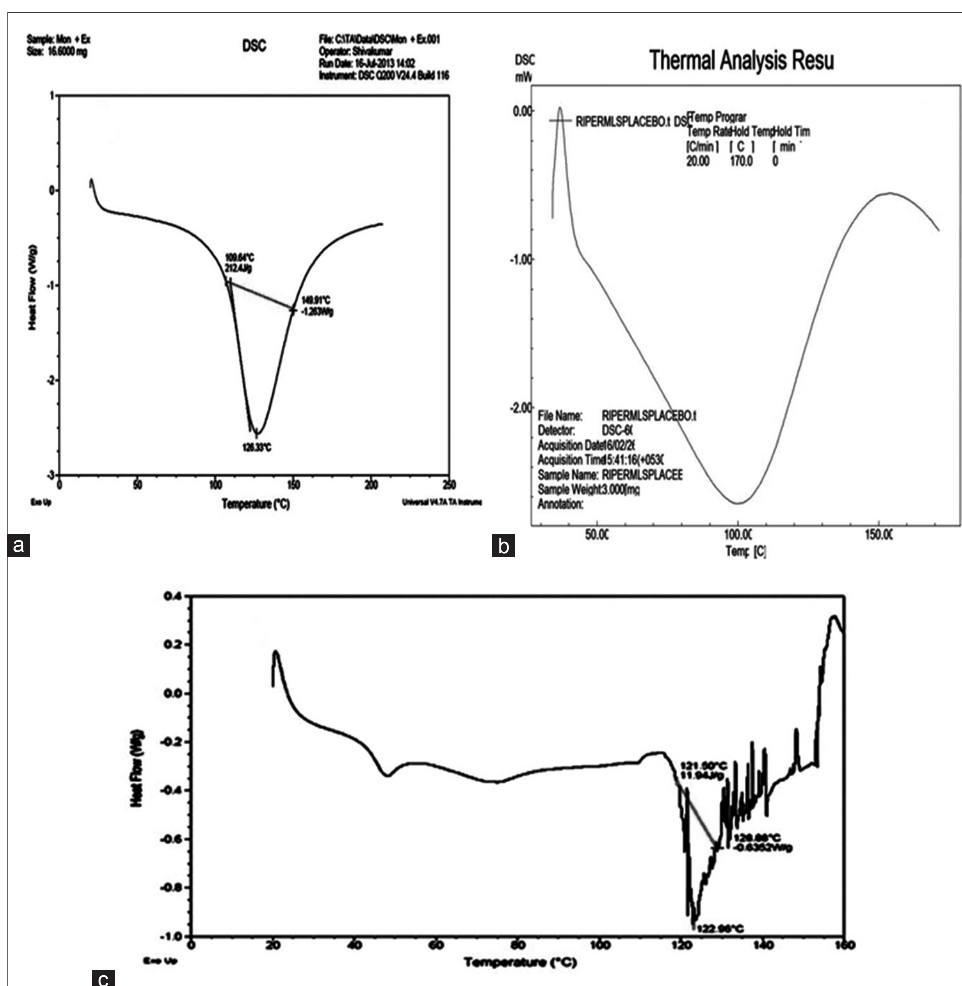

Figure 2: Differential scanning calorimetry thermograms of (a) pure montelukast sodium (b) placebo mixture (c) drug and excipient mixture

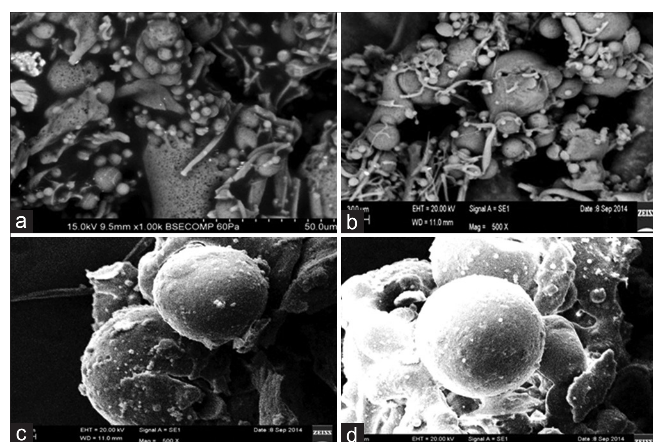

Figure 3: Scanning electron microscopy pictures of montelukast sodium microcapsules for (a) M1, (b) M2, (c) M4, and (d) M5 formulations

values of kinetic models and "*n*" value of Peppas are given in Table 2.

Evaluation of chronomodulated pulsincap systems

The weight of the capsules was ranging from 300 to 350 mg and the thickness of the CAP coat on the capsule was ranging from 1.5 to 2.5 μm. The results of content of free formaldehyde testing on cross-linked capsule bodies have shown lesser color intensity of the test solution when compared with the standard formaldehyde solution. The *in vitro* drug release profiles of CMPs, F1, F2, F3, F4, F5, F6, F7, F8, and F9 are displayed in Figure 5a-c. At the end of 5 h, F1, F2, F3, F4, F5, F7, F8, and F9 showed 15.2%, 10.8%, 6.4%, 4.8%, 2.5%, 7.2%, 2.8%, and 0.9% of drug release whereas F6 did not show any drug release at that time.

DISCUSSION

It was quite obvious to state that lung function is maximum at 4 pm and minimum at 4 am.[30] When administering this prepared capsule during bedtime, it facilitates the release of drug after 5–6 h lag period and extend the drug release for prolonged period of time to avoid the signs and symptoms of asthma and associated early morning rhinitis.

Appearance of the characteristic peaks in FT-IR spectrum of Figure 1a confirms the structure of MLS. The strong

Table 2: Results of drug content and encapsulation efficiency, microcapsule size, and wall thickness of montelukast sodium microcapsules

Formulation code	Mean±SD*				
	Practical drug content (μg/ml)	Drug content (%)	Microencapsulation efficiency (%)	Average particle size (μm)	Wall thickness (μ)
M1	16.9±0.53	91.35±1.3	86.4±0.85	40±4	13.8±0.85
M2	19.9±0.54	90.45±1.5	79.8±1.2	76±2	16.2±0.92
M3	18.09±0.59	92.76±2.0	83.4±1.3	88±5	28.2±0.84
M4	18.28±0.69	91.4±1.5	89.8±1.8	136±6	33.1±0.78
M5	15.59±1.0	94.4±1.0	86.5±1.8	160±7	40.2±0.81

*Mean of three replicates. SD: Standard deviation

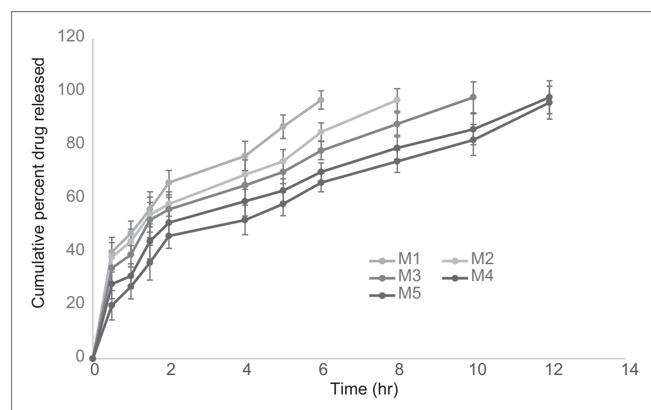

Figure 4: Comparative *in vitro* release profiles of prepared montelukast sodium microcapsules

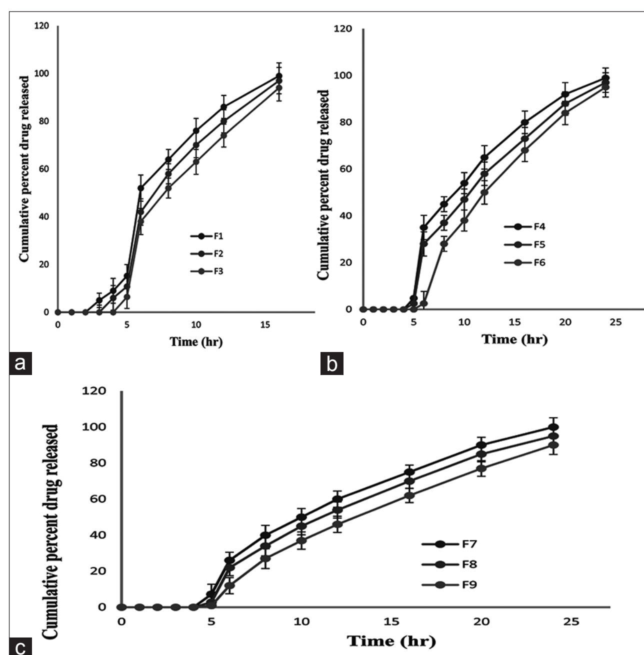

Figure 5: Comparative *in vitro* release profiles of designed chronomodulated pulsincap systems: (a) F1, F2, F3 xanthan gum is used as hydrogel plug (b) F4, F5, F6 gum kondagogu is used as hydrogel plug (c) F7, F8, F9 guargum is used as hydrogel plug

absorption peaks at 3400 and 1700 cm^{-1} along with the functional groups of the excipients in spectrum of drug excipient mixture [Figure 1c] indicates that neither the drug nor the excipients have undergone any chemical reactions. This clearly signifies the compatibility of drug with the selected excipients.[31,32] The temperature at which the peak was observed in the thermogram of pure drug is attributed to its melting point [Figure 2a]. When compared to the thermal peaks of drug and drug excipient mixture [Figure 2a and c], It was found the identicalness in the peak size, area and temperature of the peaks and hence, confirmed the absence of physical interaction of drug with the excipients.[33]

The size of the average microcapsule was increased with increase in concentration of the polymer; this is due to the high surface tension offered by viscous solution and causes reduction of shearing efficiency.[34] Smaller particles have thinner walls and showed faster drug release. Results of the characterization studies of microcapsules are given in Table 3. SEM images of M1 and M2 have clearly demonstrated that the particles are spherical in shape with rough surfaces and found in aggregates, which may be due to insufficient concentration of added polymer.[35] However, the SEM images of M4 and M5 showed discrete and spherical particles with smooth surface textures [Figure 3a-d].

Dissolution profiles of microcapsules demonstrated that, the drug release pattern was biphasic, which is designated by an initial burst effect followed by constant release. The burst release was due to the release of unentrapped drug which was adhered on the surface of microcapsules. Profiles also signified the considerable decrease of drug release with increased concentration of polymers.[36] From the results of kinetic study, it is evident that the drug release from the microcapsules follows first-order kinetics with Fickian diffusion as controlled release mechanism (n = 0.37–0.44) [Table 3].[37,38] By considering the above studies, M4 microcapsule formulation was chosen for the preparation of CMP systems.

After treatment with formaldehyde, few capsules were shrunken, and all capsules exhibited a significant reduction in length and diameter. The untreated capsule bodies were dissolved before 15 min whereas treated capsule

Table 3: Results of analysis of release data of montelukast sodium microcapsules as per various kinetic models

Formulation code	Correlation coefficient values				"*n*" value of peppas	t_{50} (h)	(h^{-1})
	Zero order	First order	Higuchi	Peppas			
M1	0.834	0.961	0.974	0.981	0.39	1.2	0.36
M2	0.841	0.952	0.975	0.985	0.37	1.4	0.27
M3	0.842	0.972	0.978	0.988	0.37	1.6	0.25
M4	0.856	0.983	0.983	0.982	0.39	2.0	0.20
M5	0.885	0.986	0.990	0.980	0.44	4.0	0.16

bodies were stayed; this remains undamaged for 24 h. The reduction in solubility of formaldehyde-treated capsule bodies were due to cross-linking of aldehyde groups of formaldehyde with the amino acids of gelatin by Schiff's base condensation. Hence, the treated capsule bodies were used in the fabrication of CMPSs to carry the drug to hold the release for specific period of time. Study inferred the insignificant traces of free formaldehyde present in cross-linked capsule bodies.

The prepared CMP systems were elegant with smooth surfaces and fulfilling the pharmacopeial limits of weight variation for capsules. The *in vitro* release profiles [Figure 5a-c] indicated that the drug release was controlled up to 24 h with varied lag times. The lag time before the drug release depends on the composition and thickness of the hydrogel plug.[39] *In vitro* dissolution studies revealed that the capsules were remained intact despite acidic environment of simulated gastric fluids during the first 2 h of study. This is stated that, the optimum concentration of CAP was being used in the coating of capsules. In the simulated intestinal fluids (pH 7.4 phosphate buffer), the enteric coat and cap of the capsule were dissolved. Swelling of the hydrogel plug was started due to the slow fluid uptake and converted into gelatinous mass. Later, this gelatinous mass was expelled from the capsule body to release the Eudragit® microcapsules containing MLS into the simulated colonic fluids (pH 6.8 phosphate buffer).

Chronomodulated pulsincap with xanthan gum as hydrogel plug

From the drug release studies [Figure 5a], it is evident that F1 and F2 were completely failed in preventing the drug release in the intestine, but F3 was found successful in preventing the drug release to maximum extent as it showed nearly 6 h time lag. All the three plugs were expelled in simulated colonic fluid. Easy expulsion of the plugs was observed due to proper swelling and softening of the plugs which was due to the addition lactose monohydrate in the preparation of plugs. After the expulsion of plugs, controlled release of drug was observed up to 16 h.

Chronomodulated pulsincap with gum kondagogu as hydrogel plug

Formulations prepared by using gum kondagogu as hydrogel plugs showed negligible quantity of drug release [Figure 5b] at the end of 5 h, 4.8%, 2.5%, and 0.0% from F4, F5, and F6, respectively. Hence, it is evident that the concentrations of the gum used in the study were adequate to prevent the early drug release to obtain time lag. Whereas F6 showed no drug release up to 6 h. After the time lag, plugs were expelled very slowly and showed the controlled drug release up to 24 h.

Chronomodulated pulsincap with guargum as hydrogel plug

In vitro dissolution studies [Figure 5c] depicted that the hydrogel plugs of the three formulations, F7, F8, and F9, were ejected in the simulated colonic fluids. F9 formulations have taken nearly 6 h for complete ejection. All the formulations could control the drug release up to 24 h.

From the above discussion, it can be resolved that the anticipated lag phase was observed with gum kondagogu followed by guargum effectively when compared with xanthan gum used as plugs. Xanthan gum plugs were unable to clench the drug release from the capsule for longer period, which may be due to faster erosion of plugs in the selected concentrations.[40]

CONCLUSION

In the present research, microcapsule-based CMPSs of MLS were successfully developed and evaluated. *In vitro* release studies of CMPSs revealed that the fabricated systems could release the drug for an extended period of time after a programmable lag phase. The CMP systems, designed using gum kondagogu as hydrogel plugs, were succeeded in preventing drug release in the physiological pH environment of stomach and small intestine. Thus, the microcapsule-based CMPSs of MLS can be predicted as one of the promising formulation approaches for chemotherapeutic management of nocturnal asthma by giving a new outlook to an existing drug molecule. However, future pharmacokinetic and dynamic studies

need to be conducted to evaluate the effects of developed microcapsule-based CMPSs of MLS for the treatment of nocturnal asthma.

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Conflicts of interest

There are no conflicts of interest.

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