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New timed-release tablets of montelukast sodium for the treatment of nocturnal bronchial asthma

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ABSTRACT

Montelukast compressed tablets were prepared by dry blending of excipients and direct compression. The inner layer containing 3 mg of Montelukast to provide a burst release at 6.5 h. A sustained-release layer consisting of 7 mg and different proportions of HPMC K4M was used to optimise the formulation. The different drug: layer ratio, from 3:7 to 5:5 used for F1 to F10 batch, but 20 % of HPMC K4M (F5) with the layer ratio of 3:7, gave the desired release profile within 8 h and achieved burst release from the immediate release layer in 6.5 h. The swelling and erosion properties of optimised formulation were investigated at different pH. *In vivo* pharmacokinetic studies in rabbits confirmed the sustained release with an average T max value at 10 h and C max value of 478.32 ng/mL compared to the conventional tablet, as C max was 490.10 ng/mL attained by 2 h (T max).

Keywords: Bronchial asthma, chronotherapy, Montelukast sodium, timed-release preparation

INTRODUCTION

Chronobiology is the science of biological rhythms and their functions[1-3]. Chronotherapy is a technique that alters the release of drugs according to the body's

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biological rhythms[4]. Chronopharmaceutical technologies currently available in the market are CONTIN®, which drug release depends on selective hydration of polymer. Chronotropic® and Pulsincaps®, in this device, in the form of capsule which release gel-layer either by diffusion and/or erosion. CEFORM®, prepared as microsphere which subjecting biodegradable polymers or bioactive agents to a combination of thermal gradients, mechanical forces, and flow-rates during processing. TIMERx® is the technology enables drugs to be delivered after a predetermined lag-phase that coincides with the circadian rhythm or allows drug to be delivered to various sites within the gastrointestinal tract. OROS®, a technology based on osmotic pump where the drug-loaded compartment, a push compartment and a semi permeable membrane. Egalet®, the drug-loaded core is then coated with a plasticized enteric coating and thereafter coated with a mixture of water insoluble and enteric polymers. Diffucaps®, uses erosion instead of diffusion to control drug release[5,6].

Chronopharmaceutics enables a new approach to drug delivery[7,8]. The sigmoidal release of drug materials is achieved by coating two different layers one after the other to release the drug with the desired release pattern. Thereby, drug's side effects minimized and therapeutic activity of drug molecules increased[9].This drug delivery has been used successfully for the treatment of ulcers and asthma. In the case of heart disease, if prolonged release delivery given at the time of 10 pm and chronotherapy to lower heart rate and increase blood pressure comfortably between 6 pm and 12 am[10,11].

Asthma is a worldwide disease affecting an average of 300 million people. The symptom of asthma mostly in Africa, Eastern Europe, Latin America, and Asia the no of active cases increases. Asthma symptoms are common with the episode of wheezing, chest congestion, continuous cough at night time and early morning as well[7]. Especially antiasthmatic drugs like leukotriene (LT) receptor antagonists (Montelukast, Pranlukast, and Zarfirlukast) and the 5-lipoxygenase inhibitor zileuton benefit to lower the leukotriene level and adjust the airway inflammation and bronchial hyper responsiveness. Thereby improves conditions for bronchial asthma. Montelukast sodium is the drug of choice and the only LT receptor antagonist prescribed at night for bronchial asthma[12,13]. Multi layered multi disc tablets of Theophylline and Diltiazem prepared and reported[14]. Correspondingly, chronotherapeutics Oral Drug Absorption System (CODAS) technology[15] is the multilayer coating technology developed with the concept of administering night time. In this technology, a non-enteric coating technique is applied to the beads to delay the release of the drug for up to 5 h. The drug release control depends on water-soluble and water-insoluble poly-

mers. The hydrophobic polymer act to resist and control the drug release for prolonged period of time[16,17]. The drug release rate independent of pH, posture, and food.

The goal of this study was to develop a once-daily sustained-release dosage form of Montelukast sodium containing an inner layer to provide burst release at the appropriate time (6.5 h). The matrix tablet formulated with Hydroxypropyl Methylcellulose (HPMC) K4M in varying proportions. This chronopharmaceutical dosage from evaluated and characterized by micrometrics, differential scanning calorimetry (DSC), swelling study, erosion rate study, *in-vitro* drug release study and stability study.

METHODOLOGY

Montelukast sodium was obtained from Orchid Health Care Ltd., (Chennai), Hydroxypropyl Methylcellulose purchased from DOW Chemical Company ((HPMC, Methocel® K4M, Midland MI) and Lactose Monohydrate was purchased from DFE Pharma (Borculo, Netherlands). Povidone K30 purchased from ISP Pharmaceuticals, (Plasdone K30 Povidone®, Wayne, NJ, USA) and Croscarmonellose sodium FMC Biopolymer, (Ac-di-sol®, Mandaue City, Philippines), and Sucrose obtained from Lantic Inc (Montréal, QC). Iron oxide red, purchased from BASF India Limited (Sicotrans®,iron oxide pigments, Katipalla, Mangalore), and Magnesium Stearate from Mallinckrodt's Pharmaceutical Lubricants (Coviden, Ireland). All other chemicals used were of analytical grade.

Method of calculation of timed-release dose of Montelukast sodium

Montelukast sodium 10 mg timed-release tablet administered at 10 pm, the formulation releases the drug slowly and elicits a pharmacological response for up to 24 h *in vivo*. Immediate-release layer releases the drug between 4 am and 5 am (6.5 h after the administration of tablet) to control nocturnal bronchial asthma. Therefore, in this study, 7 mg of Montelukast was proposed for slow, prolonged release, while 3 mg of drug was designed for burst release.

Differential Scanning Calorimetry (DSC)

The thermal melting endotherm of montelucast sodium and montelucast sodium with HPMC K4M, HPMC K100M matrix mixture was determined using a differential scanning colorimeter (DSC, Universal V4.7A TA instrument, Elstree, Hertfordshire, UK)[18]. The amount of samples 3-5 mg was scanned in crimped sealed and placed in perforated aluminium pans, under static air atmosphere. An empty pan was used as reference. The heating rate was 10 °C/min, and the temperature interval used was 30.0-250 °C.

Micrometrics properties

The angle of repose was measured by the fixed funnel method. Bulk and tap densities were determined by carefully pouring a pre-weighed powder into a 100 mL graduated cylinder and measuring the volume occupied by the powder. The tapped bulk density was determined by the volume of the powder bed after tapping the cylinder onto a wood surface fifty times from a height of about 2.5 cm at 2 sec intervals, and the ultimate tapped density was calculated after continued tapping caused no further reduction in volume. The compressibility index was calculated using the volume and final tapped bulk density[19].

Preparation of immediate release layer

The burst release tablets were prepared by direct compression method and prepared according to following procedure. All ingredients sieved by 60 mesh in order to get uniform sized particle. The amount of active ingredient as shown in Table 1 along with excipients except red iron oxide and magnesium stearate were accurately weighed and passed through 60 mesh. The above blend was mixed for 10 min. Magnesium stearate and red iron oxide were weighed and passed through 60 mesh. The above blend was mixed for 5 min and subjected to compression using 5.5 mm round flat face bevelled edge.

Table 1. Formulations component used to prepare montelukast sodium tablets (inner layer)

Batch / ingredients	**F1 (mg)**	**F2 (mg)**	**F3 (mg)**	**F4 (mg)**	**F5 (mg)**	**F6 (mg)**	**F7 (mg)**	**F8 (mg)**	**F9 (mg)**	**F10 (mg)**
Montelukast sodium	3	3	3	3	3	4	5	3	4	5
Lactose 11 SD	52.9	52.9	52.9	67.9	67.9	66.9	65.9	67.9	66.9	65.9
Red iron oxide	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	15	15	15	-	-	-	-	-	-	-
Povidone K30	4	4	4	4	4	4	4	4	4	4
Acdisol	4	4	4	4	4	4	4	4	4	4
Magnesium stearate	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Total weight	80	80	80	80	80	80	80	80	80	80
Sustained Release Layer										
Montelukast sodium	7	7	7	7	7	6	5	7	6	5
Lactose 11 SD	175.4	191.4	207.4	191.4	207.4	208.4	209.4	223.4	224.4	225.4
HPMC K4M (methocel)	128	112	96	80	64	64	64	48	48	48
Sucrose	-	-	-	32	32	32	32	32	32	32
Povidone K30	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
Magnesium stearate	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Total weight (mg)	320	320	320	320	320	320	320	320	320	320

Preparation of sustained release layer

The amount of active ingredient along with excipients except magnesium stearate were accurately weighed and passed through 60 mesh (Table 1). The above blend was mixed for 10 min in a polyethylene bag. Magnesium stearate were weighed and passed through 60 mesh. The above blend was mixed for 5 min.

Preparation of Montelukast sodium sustained release tablets

The matrix tablets were prepared by direct compression method. Formulation was made in two parts, inner layer (containing immediate release) and outer layer (containing sustained release). Blend was prepared by direct mixing with previously shifted materials. Soft tablets were compressed with punches and dies of size 5.5 mm round flat faced bevelled edge. Outer layer was prepared with the punches and dies of the size 9.55 mm round flat faced bevelled edge. Soft tablets (80 mg) were placed in dies filled with half of outer layer (160 mg) and the final compression was made after filling the second half of outer layer (160 mg).

In vitro dissolution studies

The *in vitro* drug release studies were performed using a USP dissolution apparatus Il equipped with paddles. Since Montelukast sodium has a pH-dependent solubility[20], 0.5 % sodium dodecyl sulphate (SLS) was included in the medium to maintain sink conditions. Dissolution testing was done using paddle speed of 50 rpm, different dissolution media [0.1 N HCI, simulated gastric fluid, pH 4.0 acetate buffer, pH 6.8 phosphate buffer] with 900 mL were used. Sampling aliquots of 5.0 mL were withdrawn at 0, 1, 2, 4, 6, and 8 h and replaced with an equal volume of the fresh medium maintained at the same temperature. All media were maintained at 37±0.5°C. The amount of dissolved Montelukast sodium was determined by HPLC with UV-detection at the wavelength of 345 nm. Shimadzu prominence quaternary system with dual wavelength detector. The Column (Symmetry®) - C18 150 x 4.6 mm, 5µ operates at ambient temperature. The sample injection volume was 50 µl, the mobile phase was acetonitrile:0.1 M acetate buffer in the ration of 15:85 (v/v) respectively and its flow rate was 1.5 mL/min. Filtered through 0.45 µm membrane filter and degassed for about 10 min. The retention time of Montelukast was 5 min.

Swelling studies

The Previously weighed (W1) tablets were placed individually in the petri dish containing 10 mL of distilled water. The weight of the tablet (W2) was noted after 30 min after wiping out the excess water by filter paper[21]. The swelling index was calculated using the formula,

$$SI = \frac{W2 - W1}{W2} \times 100 \quad \ldots\ldots\ldots (1)$$

Erosion studies

The standard USP/NF dissolution apparatus I (ERWEKA DT800) was used for this purpose. The dry matrix weighed (Wi) into a dissolution basket and placed in the dissolution medium maintained at 37 ± 0.5 °C with the basket spinning at 100 rpm. Periodically basket-matrix assemblies were removed from the dissolution vessels, tablets were dried to a constant weight in a hot-air oven at 50 °C and reweighed (Wt). The separated samples were used for each interval[22]. The experiments were carried out in triplicate. The percentage matrix erosion (E) at time, t, was estimated from the following equation:

$$E = \frac{Wi - Wt}{Wt} \times 100 \quad \ldots\ldots\ldots (2)$$

Release kinetics

Several dissolution models were applied to study the release mechanism of the optimised formulation[23]. The models included zero order, first order, Higuchi's, and Korsmeyer-Peppas model. All formulations were tested with zero order release rate kinetics and then optimised formulations were selected.

Pre-clinical studies (preparation of sustained release (SR) layer for *in vivo* pharmacokinetic study)

The amounts of active ingredients and excipients other than magnesium stearate were accurately weighed and passed through a 60 mesh. The above blend was blended for 10 min. Magnesium stearate were weighed and passed through 60 mesh. The above blend was mixed and blended for 5 min[24]. The above blend was subjected to compression using 5.9 x 3.9 mm oval shaped. Table 2 shown the formula for preparation mini layer of Montelukast sodium for animal study.

Table 2. Formulation of SR layer for *in vivo* animal study

Ingredients	mg/unit
Montelukast sodium	0.517
Lactose 11 SD	26.28
Povidone K 30	0.8
HPMC K4M	8
Sugar	4
Magnesium stearate	0.4
Total weight	40

Pharmacokinetic study procedure

To assess the applicability of the method the study conducted with Male rabbit weigh 1kg. Institutional Ethical committee permission was obtained, IAEC No: IAEC/XLV1/03/CLBMCP/2015.

The animal was housed and free to assess the food and water. Rabbits in the group (N = 6) were orally administered the developed sustained-release tablet formulation (0.50 mg/tablet) by gavage. After single oral administration, 0.5 mL of the blood were collected from marginal ear vein for 0.5, 1, 2, 4, 6, 8, 10, 12, 18 and 24 h after the administration in to the tubes containing EDTA. Plasma concentration of Montelukast analysed by chromatographic technique (Thermo Scientific Accela LC Systems 1250, software versions; ChromQuest 4.2). The mobile phase consists of 250 volume of water, 200 volume of methanol 550 volume of Acetonitrile, and 3 volume of Acetic acid finally adjust the pH 5.5 with Triethylamine. The column was C18, 250 x 4.6 mm, 5µ (YMC-Pack HPLC column, Genetec). The mobile phase was delivered at a flow rate of 1.0 mL/min, the detection wavelength was 240 nm and the ratios of the peak height of Montelukast to that of the concentration of internal standard were calculated for each sample. The concentrations of Montelukast in the plasma samples were then determined from the calibration curve. The assay has been validated, and has a good linearity (-0.999 with slope of 28958 and y intercept of -4276) from 20 to 1000 ng/mL with acceptable reproducibility. All measurements were performed at ambient temperature[25].

RESULTS and DISCUSSION

Differential scanning calorimetry

Differential scanning calorimetry was used to elucidate the physical state of the drug in the system. A sharp melting transition of Montelukast sodium was observed at 140.8 °C (Figure 1) and thermal stability up to 180.3 °C. The mixture shows a broad melting transition with a peak maximum at 140.2 °C. However, reported values was 135.5 °C for Montelukast sodium[26]. From these observations, it can be concluded there was no interaction between the components during heating.

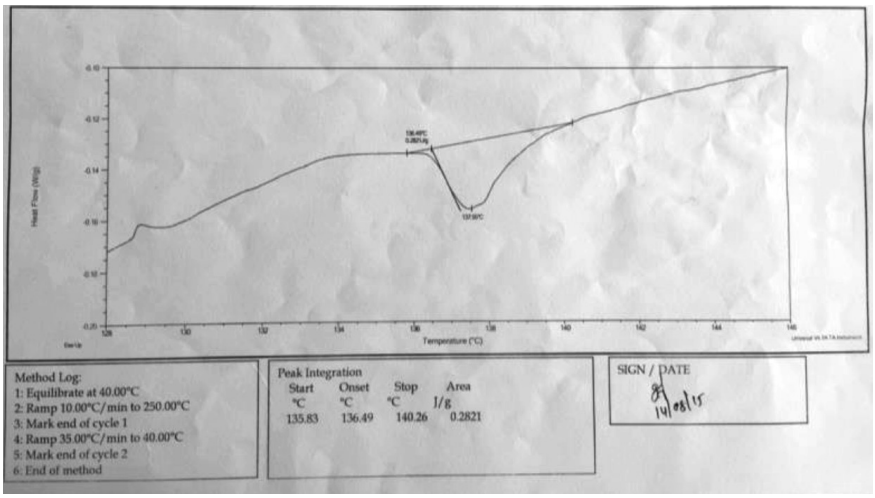

Figure 1. Differential scanning calorimetry of Montelukast:HPMC K4M (1:1) mixtures

Micrometrics

The tapped bulk density measurements of the Montelukast sodium granules for all batches of sustained release (SR) layers (F1-F10) were found to be relatively higher than those of the inner layers. The tapped bulk density of Polymer (HPMC K4M) 1.3, which was lower than that of the drug, and the interlayer density was poor (difference in the density). Further, this value indicating the presence of the comparatively higher number of enclosed voids space. The compressibility index used as an indicator of the change in filler arrangement when the powder was struck, and direct measure of the powder's tendency to consolidate as it undergoes vibration, transportation, and handling[27]. F10, which has the highest compressibility index, indicated diminished flow, as higher values tend to indicate less flow property of granules. The lowest compressibility index was 5-15 % which indicates excellent flow properties. The lo-

west compressibility index 5-15 %, which indicates excellent flow properties. It indicated that 38 % of drug having 400 µ sizes which retained in 40 mesh. Correspondingly, about 26 %, 5 % and 13 % of drug granules having size of 250 µ, 177 µ and 74.11µ which were retained on 60, 80 and 200 mesh respectively. However, it was reported that the spread in particle size had a significant and complex influence on the short-term post-compaction increase in tablet tensile strength[28].

Evaluation of formulated tablets

Montelukast sodium bilayer tablets of F1-F9 were evaluated for various parameters like as hardness, friability, weight variation for inner layer and SR layer, thickness and % drug content. The results of these parameters shown in Table 3. The results were confirming with the official and Organisation of Pharmaceutical Producers of India (OPPI) Standards for tablets[29-31].

In vitro dissolution studies

Effect of polymer concentration on burst release

All powder blends of the inner and polymer layers were successfully compressed into tablets with a total weight of 400 mg (F1-F10). The dissolution profile of all prepared formulation analysed by chromatographic technique. Instead of using calibration curve method, drug release was calculated by assay method using Montelukast standard drug. As Montelukast sodium soluble in water exhibits pH dependent solubility. 0.5% w/v of SLS was used in the dissolution media to maintain sink condition. Dissolution was performed simultaneously at 3 different physiological pH values (1.2, 4.5, and 6.8) without changing the liquid or tablet, and the cumulative drug release was calculated. To study the effect of various concentration of polymer (Methocel K4M), batch of Fl -F4 tested with 40 %, 35 %, 30 % and 25 % (where all polymer concentration calculated in percentage to the total weight of layer in SR layer) was used with the constant layer (inner:sustained) ratio of 3:7. Drug release studies indicated that F1, F2, F3, and F4 (Figure 2) were 6 %, 9 %, 11 %, and 13 % at 1 h, and 32 %, 36 %, 46 %, and 97 % of drug released at 8 h respectively for F7. This was due to the higher concentration of polymer closing the pores on the surface of the matrix tablet, resulting in less diffusion.

Table 3. Physical and chemical parameters of formulated Montelucast sodium compressed tablet formulations (F1 to F10)*

Test	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Thickness (mm)	4.26 ± 0.8	4.28 ± 0.9	4.22 ± 1.3	4.27 ± 1.5	4.25 ± 1.0	4.27 ± 1.2	4.35 ± 1.0	4.26 ± 0.9	4.27 ± 0.8	4.29 ± 1.5
Hardness (kp)	10.5 ± 0.8	10.8 ± 1.5	10.9 ± 1.2	11 ± 0.9	10.2 ± 0.8	10.4 ± 0.8	10.5 ± 1.5	10.1 ± 1.3	10.5 ± 1.2	10.2 ± 1.0
Friability (%)	0.54 ± 0.8	0.53 ± 1.2	0.50 ± 1.0	0.50 ± 0.8	0.57 ± 1.2	0.58 ± 1.5	0.58 ± 0.9	0.6 ± 1.6	0.54 ± 1.3	0.58 ± 1.4
Inner layer (mg) (IR)**	80 ± 0.8	79.5 ± 0.5	79.8 ± 0.7	80 ± 0.5	81 ± 0.4	80.5 ± 0.8	79.2 ± 0.7	80 ± 0.6	80 ± 0.7	80 ± 0.4
Outer layer (mg) (SR)***	320 ± 2.0	320 ± 2.5	320.4 ± 2.1	320.5 ± 2.1	320 ± 2.2	325 ± 2.5	320 ± 2.0	320.2 ± 2.5	320.2 ± 2.5	320 ± 2.3
Total weight (mg)	400 ± 2.5	400 ± 2.3	400.2 ± 1.5	400.5 ± 2.2	401 ± 2.5	401.5 ± 2.0	399.2 ± 2.6	400.2 ± 2.5	400.5 ± 2.5	399.2 ± 1.0
Drug content (%)	100.10	99.5	100.1	100.2	101.2	100.7	99.2	99.5	99.1	99.5

**All values are mean ± SD and % RSD*

*** Inner layer*

**** Sustained release layer*

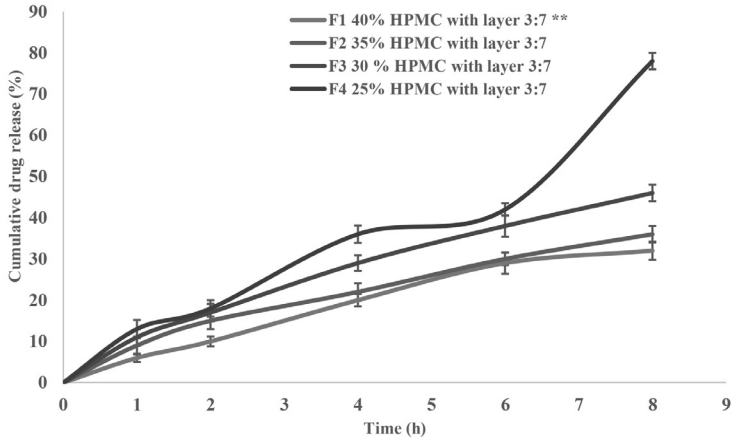

Figure 2. *In vitro* dissolution profile of Montelukast (F1-F4)*

**All values are mean±SD and % RSD for n=3*

*** Drug Inner layer: Sustained release layer (mg)*

As the polymer concentration was inversely proportional to the drug release found in that formulation, it was decided to use 20 %, polymer concentration with drug layer ratio of 3:7, 4:6, and 5:5 for F5, F6, and F7, which gave the release of 15 %, 17 %, and 23 % at 1 h; 90 %, 98 %, and 100 % release was obtained at 8 h (Figure 3). However, desired profile for the inner drug layer for burst release achieved only with F5 and F6 at 6.5 and 6 h. Where as in the formulation F7, burst release obtained at around 5-6 h. Two reasons postulated for this, firstly, the difference in the time for separation of the burst release was due to variations in the swelling index. Deviations of 15-20 % were calculated over the study period and represented as SD in the graph. Secondly, the release rate of the formulation was one of the important criteria for optimization.

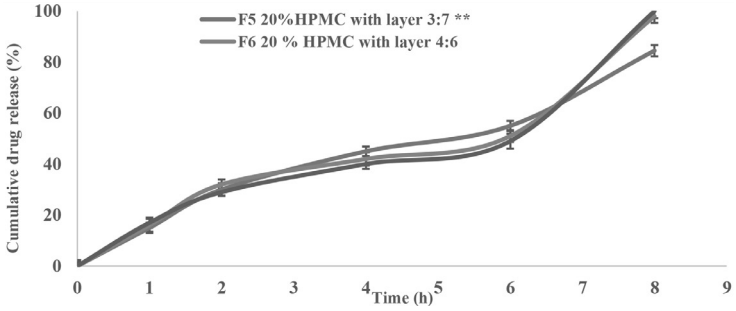

Figure 3. *In vitro* dissolution profile of Montelukast (F5-F7)*

**All values are mean±SD and % RSD for n=3*
*** Drug Inner layer: Sustained release layer (mg)*

Further reducing the polymer concentration to 10 % to maintain layer ratios for F8, F9 and F10 were 3:7, 4:6 and 5:5, respectively (Figure 4).

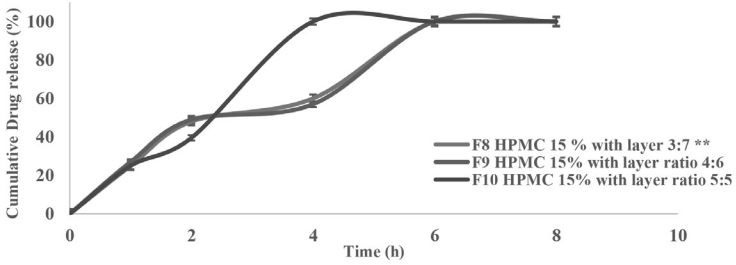

Figure 4. *In vitro* dissolution profile of Montelukast (F8-F10)*

**All values are mean±SD and % RSD for n=3*
*** Drug Inner layer: Sustained release layer (mg)*

The better dissolution profile obtained, where 25 % of the drug released at 1 h and inner layer separated out at around 3-4 h. This results were in agreement with published report where the author stated that increasing the concentration of the blends from 20 to 40 % each, showed a sustaining effect on drug release profile[32-34].

Effect of layer ratio on drug release

To study the effect of drug release on the layer ratio, formulation Fl-F5 was compressed at a ratio of 3:7. In all cases drug release was found to be affected by polymer concentration only where the inner layer was separated at 6.5 h with F5. From Fl-F4, inner layer did not separate until 8 h. When the ratio altered to 4:6 and 5:5 for formulation F6 and F7, inner layer separated out at

around 6 h which were not desirable. Further decreased polymer concentration only effected the drug release. Therefore, the layer ratio did not bring any significant changes to the drug release pattern except for the burst release. Since the inner layer is sandwiched between two SR layers, only changes in the weight ratio or layer pattern that affected the drug release profile[35,36].

Swelling and erosion studies

Measurements of the swelling behaviour of all formulations were carried out to find out their water uptake capacity. Initially, visual inspection of matrix tablets showed that swelling was dominant in all batches, as the tablet surface was smooth and slippery to the touch. This study was proposed to conduct until the sandwiched burst release layer separated from the tablet. Swelling behaviour studied at pH 1.2, 4.5, and 6.8. Figure 5 (X, Y, Z graph) shows the rate of swelling for matrix tablets. Swelling of the matrix, which was indicated by the transition of the polymer from the glassy to the rubbery state[37]. The highest degree of swelling was achieved by F1 to F5 contains 40 %, 35 %, 30 %, 25 %, and 20 % of HPMC K4M. There was about 227, 192, 165, 150 and 120 % weight gain at the end of 8 h due to swelling in these matrices. On the other hand, F6, F7, and F8 contains 20 %, 15 % and 15 % respectively, gave around 120 % only. These matrices could hydrate only up to 6 h (F6, F7) and up to 4 h (F8) after which, there was no further increase in the tablet weight due to water uptake. The F9 and F10 swelling studies could not be performed because the sandwiched inner layers separated at around 3 h. Analogous observations were reported by Nerurkar et al[38] for HPMC containing matrices regarding their inability to hydrate for longer time periods and percentage swelling was directly proportional to the polymer concentration. The swelling behaviour was not affected by any significant changes with respect to pH (1.2, 4.5, 6.8), but the separation of the immediate release (IR) layers was shown in Figure 6. This response was to be the physiochemical characteristic of the polymer especially HPMC K4M, lower Methoxy and Hydroxy propyl content as reported by J. Siepmann et al[39]. In hydrophilic polymeric matrix systems, the overall dissolution rate and, ultimately, drug availability were reported to be controlled by the rate of matrix swelling, and erosion of the outer gel layer[40]. The percent erosion found to be 22 % at the end of 8 h, for F1 whereas F2, F3, F4 and F5, it was found to be 28, 42, 53, and 68 % respectively at the end of the 8 and 6 h (F4, F5). At the end of the dissolution run, the matrix of F6 and F7 was completely eroded. In the case of the F9 and F10, no swelling and erosion studies were performed because of both swelling and erosion were shown to have been occurring at the same time as reported[41].

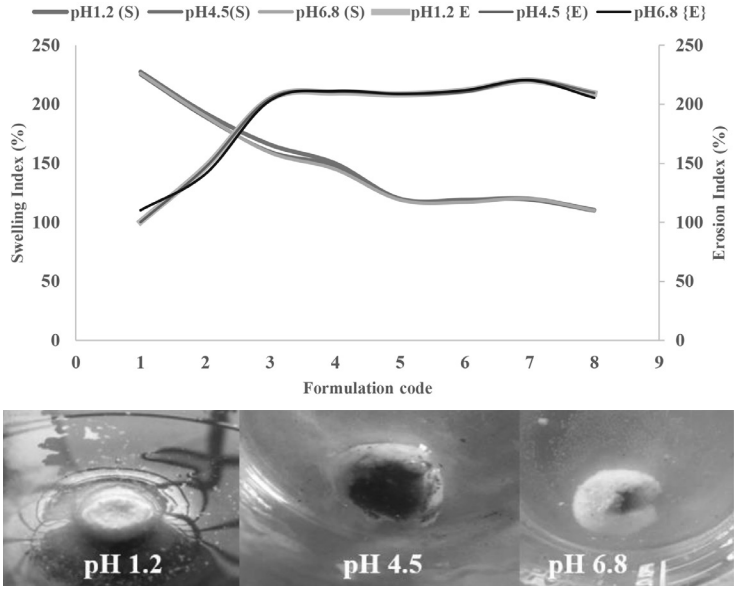

Figure 5. Swelling and Erosion profile of Montelukast formulation (optimized)

Table 4. Fitting with various kinetic model of optimizing formulation (F5)

Parameter	Zero order Release	Higuchi Kinetics	First order Kinetics	Weibull kinetics	Korsemeyer Peppas model
Slope	10.53	40.93	0.099	0.006	0.8335
R_obs-pre	0.9754	0.9619	0.9592	0.9688	0.9885
R^2	0.9482	0.9242	0.9187	0.9381	0.977
R^2adj	0.9482	0.9091	0.9024	0.9072	0.9617
MSE	59.0061	9.0	111.1414	105.6742	33.6876
WSSR	8.0	5.0	555.7069	422.6967	101.0629
AIC	43.0858	47.7452	48.2417	48.3266	33.6945
MSC	2.3106	1.645	1.574	1.5619	2.774
Rate (%/h)	10.5	41.0	0.22799	2.472	16.258
T25 (h)	2.201	1.504	1.6	2.186	0.538
T50 (h)	4.402	3.523	3.647	4.289	2.785
T90 (h)	7.924	9.554	11.772	7.141	7.628

coefficient of determination (R^2), adjusted R^2(R^2_{adj}), mean square error (MSE), Akaike Information criterion (AIC), weighed sum of square of residues (WSSR), model selection criterion (MSC). R-observed-predicted (R_obspre), Time in hours requied to release 90 % of the drug from tablet (T90).

Kinetics of drug release

In order to optimize the formulation, drug release from all batches fitted in to the zero order equation shown in **Table 4.** The *in vitro* release rate (K), which gave the same value for F4 and F5, was further found to separate the burst inner layer at exactly 6.5 h (F5) and was chosen as the optimised formula. Hence, formulation F5 fitted in to the various mathematical models especially using the Peppas and Korsmeyer[42]. The t 25, t 50 and t 90 values of the formulations tested were in the range of 2 to 7 h, indicating a time range for completely releasing the drug from the matrices and further burst release of the IR layer. The value of release exponent *n* was calculated with standing the burst release layer as well. Thus, F5 optimized formulation gave non-fickian release kinetics (*n*= 0.833).

Table 5. Comparison of *in vivo* data of formulation F-5 with market product

AUC summary	Optimized formulation (F5) ($ng*h\ mL^{-1}$)	Market product ($ng*h\ mL^{-1}$)	AUMC summary ($ng*h^2\ mL^{-1}$)	Optimized formulation (F5) ($ng*h^2\ mL^{-1}$)	Market product ($ng*h^2\ mL^{-1}$)
Last conc. value	20.0l	0.9	Last conc. time value	120	10.8
Cumulative observed AUC(0-t)	1628	1185	Cumulative observed AUMC(0-t)	3845	654
Remaining AUC (t-∞)	16	2	Remaining AUMC (t-∞)	110	28
AUC(0-∞)	1644	187	AUMC (0-∞)	3955	682
Cmax (ng/ml)	478.32	490	-	-	-
T max (h)	10	2	-	-	-

Area under plasma concentration curve (AUC), Area under the moment curve (AUMC), The peak time (Tmax), the peak concentration (Cmax)

Pre-clinical evaluation

In order to investigate the *in vivo* performance of the optimized formulation, 200 numbers of sustained release mini tablets with the total weight of 40 mg containing the 0.517 mg of Montelukast sodium was prepared. The *in vivo* study was carried out only for the SR layer due to the low dose of IR (around 0.2 mg). The animal dose calculated based on the surface area method and approved by the Institutional animal ethical committee. Singular tablets containing 10 mg of Montelukast sodium was used for the comparison. Plasma concentration-time curves of Montelukast sodium following a single oral administration of optimized formulation (F5) and Market sample in rabbits were compared Figure 7, and their pharmacokinetic parameters were summarized in Table 5.

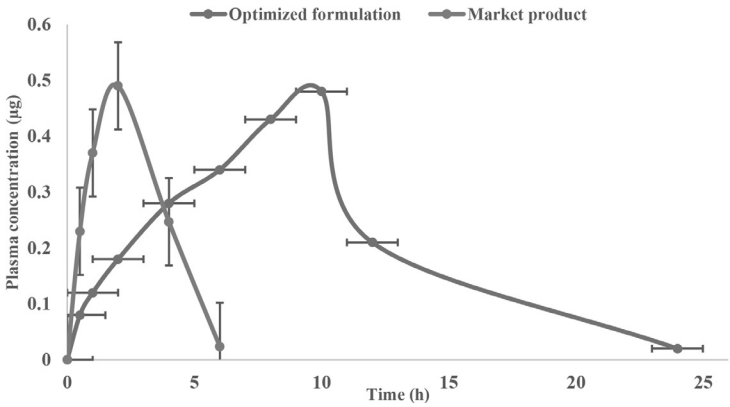

Figure 7. *In vivo* profile of Montelucast Sodium (Optimized F5)

The plasma concentration-time profile was different than expected. When the F5 tablet was administered to 6 rabbits, the time of the first appearance of Montelukast in plasma was 0.5 ± 0.2 h. The plasma concentration increased steadily after administration of the optimised formulation and continued until 24 h after administration. Although the plasma concentration was higher in the market product, it was not detectable after 6 h. The C max, 490.10 ng/mL attained by 2 h (T max) but 478.32 ng/mL of C max only reached by 10 h in an optimized formulation. However, the AUC and AUMC values were profoundly higher for the optimized F-5 formulation. Enrique Muñoz et al[43] reported. C max value of close to the 1 mcg after administering two 10 mg tablet of Montelukast sodium tablet to the human subjects. However, Zaid An et al[44] reported C max value was around 500 ng/mL. Therefore, the long lasting plasma concentration was likely caused from the matrix tablet throughout the gastrointes-

tinal (GI) tract, thereby suitable for chronopharmaceutical delivery.

In conclusion, the new Montelukast sodium timed-release tablet consists of two layers: (a) an inner layer with 3 mg as a burst release at 6.5 h after administration and (b) sustained release of 7 mg released up to 24 h, which could be fabricated as a bilayer tablet. The SR layer made of HPMC K4M was needed to control the release rate by swelling and burst release. Further, *in vivo* plasma concentration profile proved the release of drug over 24 h in comparison with conventional product, this novel bilayer tablet successful approach for Nocturnal Bronchial Asthma.

STATEMENTS OF ETHICS

Animal studies were approved by IAEC of C.L. Baid Metha COP, Chennai-97.

CONFLICT OF INTEREST STATEMENTS

No financial and non-financial competing interests with regards to the publication of this research work.

AUTHOR CONTRIBUTIONS

All authors contribute the work equally throughout.

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