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Research Article

FORMULATION AND IN VITRO EVALUATION OF ROFLUMILAST PULSATILE DRUG DELIVERY

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ABSTRACT:
The purpose of the present study was to design and evaluate an Oral, site specific, Pulsatile drug delivery system containing Roflumilast as a model drug, which can be time dependent manner, to modulate the drug level in synchrony is a member of the drug class known as statins It is used for lowering cholesterol based on chrono pharmaceutical considerations. The basic design consists of an insoluble hard gelatin capsule body, filled with powder blend and sealed with a hydrogel plug. The powder blend containing Roflumilast, Crospovidone, Lycoat, Croscarmellose sodium, MCC and talc was prepared and evaluated for flow properties and FTIR studies. From the obtained results, F12 powder blend formulation was selected for further fabrication of pulsatile capsules. Hydrogel plug was formulated in a lone and in combination of hydrophobic polymer like lactose with hydrophilic polymers like HPMC K15M in 1:1, 2:1, and 1:2 ratios to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. The prepared formulations were evaluated for drug content, weight variation and In vitro release studies. FTIR studies confirmed that there was no interaction between drug and polymers and In vitro release studies of pulsatile device revealed that increasing hydrophilic polymer content resulted in delayed release of Roflumilast from the pulsincap after a predetermined lag time of 6hrs. Based on in vitro studies performed, PC4F12 was found to be optimized formulation.

Keywords: Pulsatile system; time dependent delivery; Roflumilast; Chrono pharmaceutics; In vitro release studies.

INTRODUCTION

COPD is a progressive condition that usually leads to a steady decline in lung function, increased symptoms, and recurrent and worsening exacerbations.[1] The progressive airflow limitation that characterizes COPD is a result of chronic airway inflammation in response to the inhalation of noxious stimuli and resulting parenchymal destruction.[2] Many systemic inflammatory markers have been associated with COPD health status and its extrapulmonary effects.[1,3,4] Inflammatory cells, tissue swelling, and accumulated mucus directly obstruct airways, but also activate innate repair mechanisms that remodel and thicken airway walls over time, leading to parenchymal destruction.[5,6] Parenchymal destruction leads to further decreased gas transfer and increased air trapping. Overall, the combined pathological defects of COPD translate to symptoms of breathlessness, coughing, and variable sputum production that greatly impact a patient's quality of life.

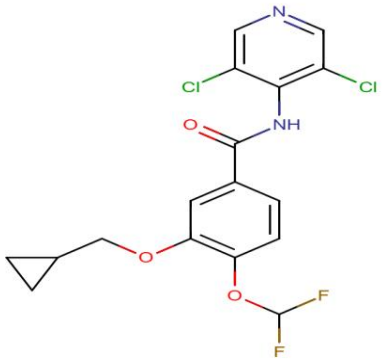

Figure.1 Structure of Roflumilast

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Roflumilast, a potent and selective inhibitor of phosphodiesterase-4 (PDE4), is indicated for treatment to reduce the risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations.[7,8] Selective inhibition of PDE4 inhibits the hydrolysis of cyclic adenosine monophosphate (cAMP) in inflammatory cells.[9] Increased intracellular cAMP results in a wide range of anti-inflammatory effects, including decreased release of inflammatory mediators in neutrophils, decreased release of cytokines, decreased expression of cell surface markers in many cell types, and decreased apoptosis. The suppression of inflammatory mediators and cytokines usually translates into benefits for patients with COPD exacerbations who often have elevated markers of inflammation compared to patients with baseline disease.[10] Roflumilast also reduces allergen-induced inflammation[11] and has been shown to stabilize lipopolysaccharide-induced systemic inflammation.[28]

Chronopharmaceutics is an approach to deliver drugs at a time that match biological requisite for a specified disease treatment or prevention[12,13]. Pulsatile drug delivery systems (PDDS) are the chronopharmaceutical approach intended to release the drug on specific pre-programmed patterns and are characterized by a lag time [14]. Asthma is a chronic inflammatory disease of the respiratory tract, the most common chronic disease among children [15]. Nocturnal asthma follows circadian rhythms where increasing to airway resistance and worsening of lung function are observed during the early morning time. Two-thirds of asthmatics suffer from nocturnal asthma symptoms. The risk of asthma attacks is 100-fold greater during nighttime sleep than during daytime activity. Forced expiratory volume in 1 sec is found to be lower at 4 am [16,17]. Histamine concentrations peak at a level that coincided with the greatest degree of bronchoconstriction at 4 am. Nocturnal bronchoconstriction is driven by circadian changes in epinephrine, cortisol, histamine, AMP, melatonin, vagal tone, body temperature, lower airway secretions, etc[18,19].

MATERIALS & METHODS USED: Roflumilast API was procured from Alain Pharmaceuticals, Hyderabad , and CSS, Crospovidone, Hydrochloric acid, Methanol were procured from S d fine chemical Ltd, Mumbai., Microcrystalline cellulose, Talc, Magnesium stearate were procured from Loba chemie pvt.ltd, Mumbai, Lactose ,Lycoat were procured from Otto Chemicals, Mumbai, Formaldehyde, Potassium permanganate, Potassium dihydrogen Phosphate, Sodium hydroxide pellets were procured from Qualigens fine chemicals,Mumbai.

PULSINCAP DESINGNING

Designing or preparation of pulsincap capsules involves 3 steps:

A. Preparation of cross-linked gelatin capsule.

B. Preparation of powder blends for filling into capsules.

C. Formulation of pulsincap of Roflumilast .

A.PREPARATION OF CROSS-LINKED GELATIN CAPSULE:

Formaldehyde treatment:

About 100 hard gelatin capsules size '0' were taken. Their bodies were separated from the caps and placed on a wire mesh. The bodies which were placed on a wire mesh were spread as a single layer. 25 ml of 15% v/v of formaldehyde solution was prepared and placed in a desiccators. To this 5 g of potassium permanganate was added. The wire mesh containing the bodies of the capsules was kept on the top of desiccators' containing formaldehyde liquid at the bottom in equilibrium with its vapor and immediately the desiccators' was tightly closed and sealed. The bodies of capsules were made to react with formaldehyde vapors by exposing them for varying periods of time viz., 2, 4, 6, 8, 10hrs. Then they were removed and kept on a filter paper and dried for 24 hrs to ensure completion of reaction between gelatin and formaldehyde vapors, afterwards the capsules were kept in an open atmosphere, to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated cap and stored in a polythene bag.

Use of Formaldehyde treatment:

The main aim of formaldehyde treatment was to modify the solubility of hard gelatin capsules. Cross-linking of gelatin molecules was achieved by exposing to formalin vapors. Cross-linking involves the reaction of amino groups in gelatin molecular chain with aldehyde groups of formaldehyde by a "Schiff's base condensation" so that the gelatin becomes water insoluble. Formaldehyde reacts with gelatin forming an irreversible complex. The primary amine group present in gelatin reacts with formaldehyde making it irreversibly bound. Potassium permanganate was added to formaldehyde solution so that formalin vapors were produced. When bodies of hard gelatin capsule were exposed to formaldehyde vapors for different periods of time in a closed desiccator, vapor gets equilibrated with formaldehyde liquid and therefore makes the gelatin water insoluble.

EVALUATION OF FORMALDEHYDE TREATED CAPSULES:

PHYSICAL TESTS:

Identification attributes: Suitable size capsules which are lockable were selected. Generally, the gelatin capsules when touched with wet hand they become sticky but upon formaldehyde treatment the capsules are observed for the stickiness.

Visual defects: Selected 100 treated capsules and observed for visual defects by physical observation and not more than 15-20 capsules must be distorted.

Dimensions: Variations in the dimensions between the formaldehyde treated and untreated capsules were studied. The length and diameter of the capsules were measured before and after formaldehyde treatment by using Vernier calipers.

OPTIMIZATION OF FORMALDEHYDE TREATED CAPSULE BODIES EXPOSED AT VARIOUS TIME INTERVALS VIZ., 2, 4, 6, 8, 10 hrs :-

Formaldehyde treated capsule bodies which were exposed at various time intervals viz., 2, 4, 6, 8, 10hrs were optimized by conducting Disintegration test. The test was performed on both untreated and treated capsules. Formaldehyde treated bodies joined with untreated caps and was tested for disintegration. Disintegration test was carried out by using Hiccon disintegration test apparatus. pH 1.2, pH 7.4, buffers were used as medium and maintained at 37°C throughout the experiment. The time at which the capsules disintegrate are noted.

B.PREPARATION OF ROFLUMILAST TABLET FOR FILLING INTO CAPSULES

All the ingredients were passed through # 60 mesh sieve separately. The drug & MCC were mixed by adding small portion of each at a time and blending it to get a uniform mixture and kept aside.

Then the other ingredients were mixed in geometrical order and passed through coarse sieve (#44 mesh) and the tablets were compressed using hydraulic press. Compression force of the machine was adjusted to obtain the hardness in the range of 3-4 kg/cm2 for all batches. The weight of the tablets was kept constant for all formulations F1 to F12 (100 mg).

Table. 1 Formulae for preparation of blend for filling of Roflumilast pulsincap

Ingredients	F1	F2	F3	F4	F5	F6
Roflumilast	15	15	15	15	15	15
Lycoat	7.5	15	22.5	30	--	--
CSS	--	--	--	--	7.5	15
Crospovidone	--	--	--	--	--	--
MCC	73.5	66	58.5	51	73.5	66
Mg. stearate	2	2	2	2	2	2
Talc	2	2	2	2	2	2
In ratio	1:0.5	1:1	1:1.5	1:2	1:0.5	1:1
Total	100	100	100	100	100	100

Table.2 Formulae for preparation of blend for filling of Roflumilast pulsincap

Ingredients (mg)	F7	F8	F9	F10	F11	F12
Roflumilast	15	15	15	15	15	15
Lycoat	-	-	-	-	-	-
CSS	22.5	30	-	-	-	-
Crospovidone	-	-	7.5	15	22.5	30
MCC	58.5	51	73.5	66	58.5	51
Mg. stearate	2	2	2	2	2	2
Talc	2	2	2	2	2	2
In ratio	1:1.5	1:2	1:0.5	1:1	1:1.5	1:2
Total	100	100	100	100	100	100

C.FORMULATION OF PULSINCAP OF ROFLUMILAST:

The modified release pulsincaps containing 15mg of Roflumilast were prepared by using different excipients and polymers in varying ratios. The formaldehyde treated capsule bodies which were exposed to 6 hrs was optimized and chosen for the pulsincap formulation based on disintegration time. Optimized formulation of Roflumilast tablet was filed into the capsule body. For hydrogel plug formulation, the plug was prepared by using the combination of Lactose : HPMC K15M in varying ratios. Initially the total weight of the plug was taken as 100 mg alone and the ratio of hydrophobic & hydrophilic polymer as 1:1, 2:1, and 1:2.

Method of preparation of Pulsincap dosage form:

Preparation of powder blend: Hard gelatin capsules of 'size 0' which were hardened with formaldehyde treatment for 6hrs were chosen for the formulation. The bodies and caps separated manually. Optimized formulation F12 was fitted at the bottom of the capsule body.

Preparation of Hydrogel plug:

Evaluation of tablets:

Tablet Dimensions: Thickness was measured using a calibrated vernier caliper. Three tablets of each formulation were picked randomly and thickness was measured individually.

Hardness: Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm^2. Three tablets were randomly picked and hardness of the tablets was determined.

Friability test: The friability of tablets was determined by using electro lab Friabilator. It is expressed in percentage (%). Ten tablets were initially weighed (W_I) and transferred into Friabilator. The Friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W_F). The % friability was then calculated by

$$\%F = 100\ (1-W_I/W_F)$$

% Friability of tablets less than 1% was considered acceptable.

Weight Variation Test: Ten tablets were selected randomly from each batch and weighed individually to check for weight variation. A little variation was allowed in the weight of a tablet according to U.S. Pharmacopoeia. The following percentage deviation in weight variation was allowed.

Test for Content Uniformity: Tablet containing 10mg of drug was dissolved in 50ml of 7.4 pH buffer in volumetric flask. The drug was allowed to dissolve in the solvent. The solution was filtered, 2ml of filtrate was taken in 10ml of volumetric flask and diluted up to mark with distilled water and analyzed spectrophotometrically at 213 nm .The concentration of Roflumilast was obtained by using standard calibration curve of the drug. Drug content studies were carried out in triplicate for each formulation batch.

In vitro Disintegration Time: Tablet was added to 900ml of distilled water at 37±0.5ºC. Time required for complete dispersion of a tablet was measured.

In vitro Dissolution Study: In vitro dissolution of Roflumilast tablets was studied in USP XXII dissolution test apparatus. 900ml Phosphate buffer 7.4 (simulated fluid) was used as dissolution medium. The stirrer was adjusted to rotate at 1 0 0 RPM. The temperature of dissolution medium was maintained at 37±0.5ºC throughout the experiment. One tablet was used in each test. Samples of dissolution medium (5ml) were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 213 nm . The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. Cumulative percent Roflumilast released was calculated and plotted against time.

EVALUATION OF PULSINCAP DOSAGE FORM:

In vitro release studies: Dissolution study was carried out to measure the release rate of the drug from the pulsincap formulation. In vitro dissolution profile of each formulation was determined by employing USP I apparatus by rotating basket method. In order to stimulate the pH changes along GI tract 2 different dissolution media with pH 1.2, 7.4, 2 buffers were sequentially used, and therefore referred to as "Sequential pH change method". The dissolution media were maintained at a temperature of 37 ± 0.5ºC throughout the experiment and the speed of rotation of basket maintained at 100 rpm. 900ml of dissolution medium was used at each time. Roflumilast Pulsincaps was placed in basket in each dissolution vessel to prevent floating. While performing experiments, stimulated gastric fluid (SGF) pH 1.2 buffer was first used for 2 hrs (since the average gastric emptying time is 2hrs) and then removed and the fresh stimulated intestinal fluid (SIF) pH 7.4 buffer was added and used for remaining hours. 5 ml samples of dissolution fluid were withdrawn at predetermined time intervals with the help of a syringe. The volume withdrawn at each time interval was replaced with 5ml of fresh dissolution medium maintained at same temperature. The filtered samples were suitably diluted whenever necessary and assayed for Roflumilast by measuring absorbance at 213 nm, by UV absorption spectroscopy. %CDR was calculated over the sampling times.

RELEASE KINETICS: Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. Mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets. The model that best fits the release data is selected based on the correlation coefficient(R) value in various models. The models with high 'R-value is considered as the best fit on the release data. Various mathematical models are:

- **Zero order release model**
- **First order release model**
- **Higuchi release model**
- **Korsmeyer – peppas release model**

RESULTS AND DISCUSSIONS

Solubility: It was determined as per standard procedure.

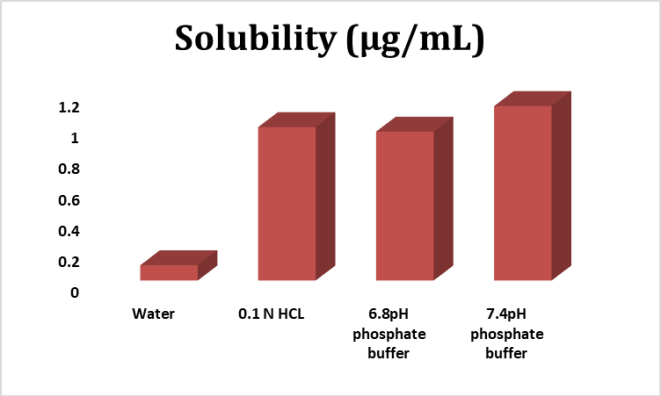

Figure.2 Solubility Studies

Discussion: Roflumilast was found to be more soluble in 7.4 pH phosphate buffer when comparted to other buffers.

Drug-Excipient compatibility studies: The IR spectrum of pure drug was found to be similar to the standard spectrum of Roflumilast.

Pure Drug

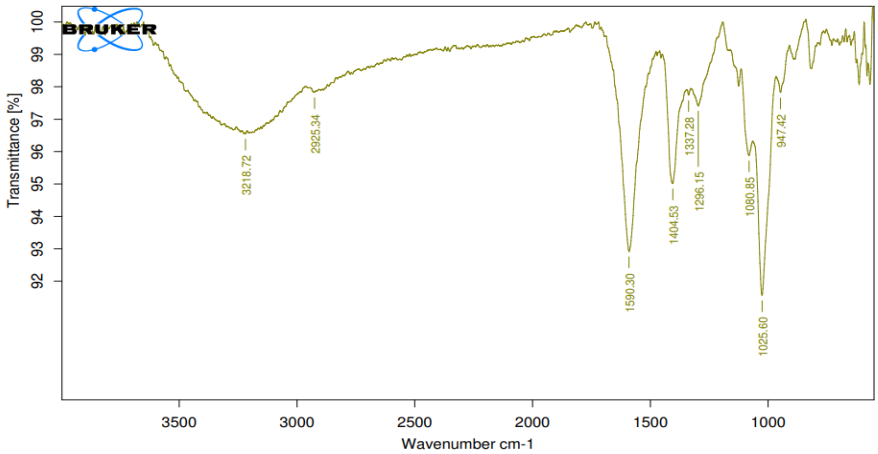

Figure.3 FTIR spectrum of Roflumilast

Optimized Formulation

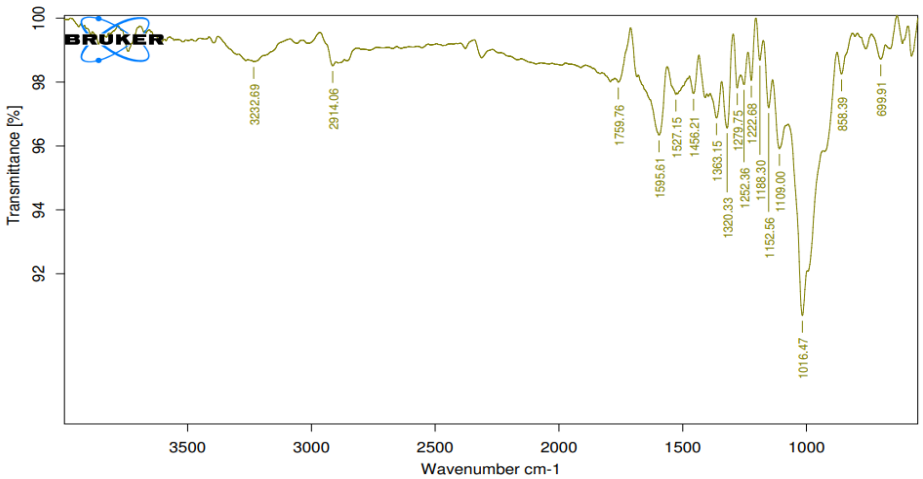

Figure.4 FTIR Spectrum of optimized formulation

Discussion: Chemical interaction between drug and the polymeric material was studied by using FTIR. There was no difference between the IR patterns of Roflumilast, physical mixture of Roflumilast and Roflumilast optimized formulation.

λ max Determination of Roflumilast

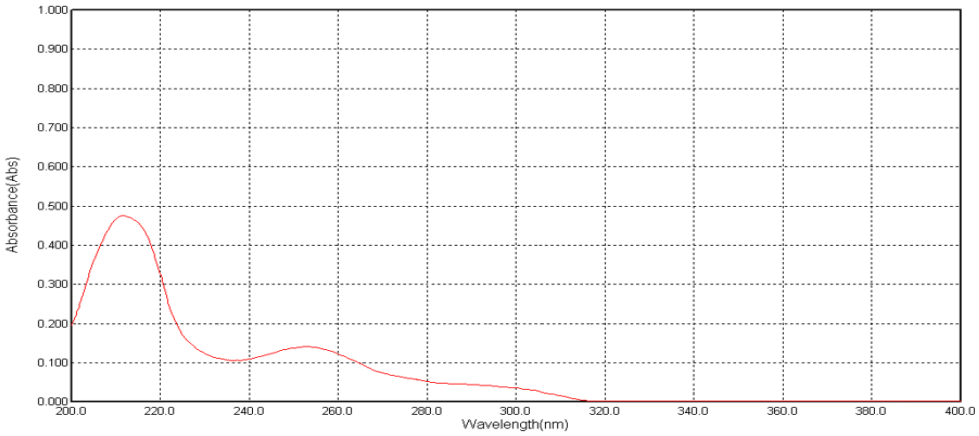

Figure.5 λmax Determination of Roflumilast

Discussion: A solution of Selexipag containing the con8µg/ ml was prepared in 7.4 pH buffer and UV spectrum was taken using Single Beam Spectrophotometer (YIS-294). The solution was scanned in the range of 200 – 400 nm. The maximum absorbance was found to be at 213 nm.

Standard Calibration Curve:

The standard calibration curve of Roflumilast was developed in different pH media such as pH 1.2, and pH 7.4 phosphate buffer. Two buffers were selected in order to mimic the in-vivo conditions of the GIT.

Standard Calibration Curve in 1.2 pH:

Standard graph of Roflumilast showed linearity at the concentration range of 3-18 µg with correlation coefficient of 0.999. Table 7.2 gives the data of the standard graph and Figure 7.5 shows the standard graph in pH 1.2.

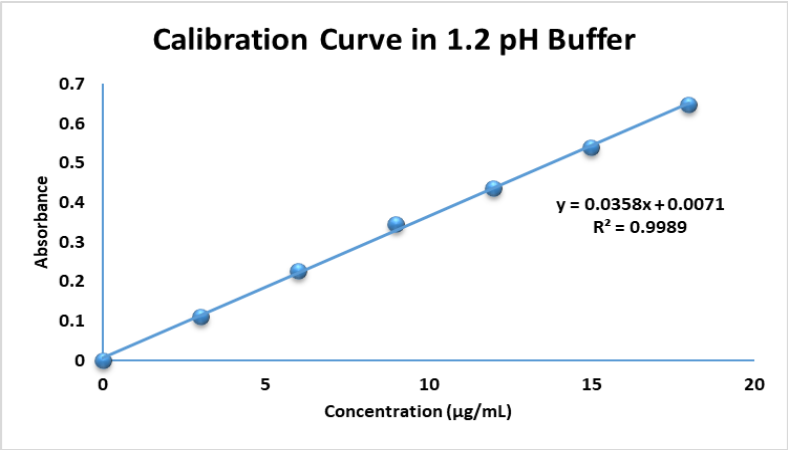

Figure.6 Standard Calibration Curve of Roflumilast in pH 1.2 at 213 nm

Discussion:

The standard calibration curve shown 0.998, through that the drug obeys Beers and Lamberts law in the concentration range of 0 to 18 µg/mL. A standard graph was plotted by keeping the known concentration on X – axis and obtained absorbance on Y – axis.

Standard Calibration Curve in 7.4 pH phosphate buffer:

Standard graph of Roflumilast in pH 7.4 phosphate buffer shows linearity in the concentration range of 3-18 µg with correlation coefficient of 0.999.

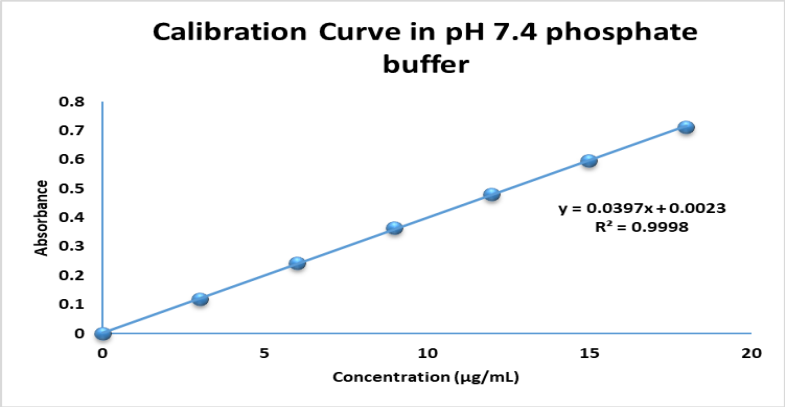

Figure.7 Standard Calibration Curve of Roflumilast in pH 7.4 at 213 nm

Discussion:

The standard calibration curve shown R^2 value 0.999 which ie near to 1 shows the linearity, through that the drug obeys Beers and Lamberts law in the concentration range of 0 to 18 µg/mL. A standard graph was plotted by keeping the known concentration on X – axis and obtained absorbance on Y – axis.

Flow properties of powder blend:

Table.3 Flow properties of powder blend

Formulation Code	Bulk Density (g/ml) ±SD	Tapped Density (g/ml) ±SD	Angle of Repose ±SD	Carr's Index. (%) ±SD	Hausner's ratio ±SD
F1	0.227±0.002	0.317±0.26	29.37±1.24	19.18±1.02	1.18±0.02
F2	0.241±0.005	0.324±0.24	27.84±1.16	18.25±1.52	1.17±0.03
F3	0.259±0.007	0.346±0.15	27.16±1.42	17.41±1.98	1.15±0.01
F4	0.275±0.003	0.357±0.39	26.42±1.25	14.68±1.36	1.14±0.02
F5	0.234±0.004	0.321±0.20	28.37±1.37	18.85±1.42	1.19±0.03
F6	0.245±0.009	0.339±0.16	27.43±1.42	19.55±1.15	1.18±0.02
F7	0.269±0.005	0.357±0.18	26.18±1.09	16.34±1.14	1.16±0.01
F8	0.274±0.007	0.362±0.21	25.37±1.12	15.28±1.24	1.15±0.02
F9	0.234±0.003	0.317±0.20	28.42±1.03	17.44±1.14	1.16±0.03
F10	0.257±0.005	0.349±0.19	27.85±1.43	15.57±1.26	1.15±0.01
F11	0.269±0.004	0.352±0.18	25.45±1.26	14.45±1.38	1.14±0.02
F12	0.281±0.003	0.379±0.21	24.37±1.17	12.21±1.24	1.12±0.01

Discussion: The angle of repose of different formulations was ≤ 29.37±1.24 which indicates that material had good flow property. So it was confirmed that the flow property of blends were free flowing. The bulk density of blend was found between 0.227±0.002 g/cm3 to 0.281±0.003 g/cm3.Tapped density was found between 0.317±0.26 g/cm3 to 0.379±0.21 g/cm3.These values indicate that the blends had good flow property. Carr's index for all the formulations was found to be between 12.21±1.24-19.18±1.02 and Hausner's ratio from 1.12±0.62-1.18±0.02 which reveals that the blends have good flow character

Characterization of Tablets

Post Compression parameters

All the batches of tablet formulations were characterized for official evaluation parameters like Weight variation, Hardness, Friability, Tablet thickness and drug content and results are shown in the table.

Table.4 Characterization Roflumilast Tablets

Formulation code	%Weight variation (mg)	Thickness (mm)	Hardness	Friability (%)	Disintegrating time(sec)	Drug content (%)
F1	102.74±1.42	2.47±1.37	6.75±1.27	0.51±0.07	15±1.24	93.42±1.24
F2	100.81±1.12	2.21±1.42	6.41±1.46	0.24±0.08	17±1.37	94.67±1.18
F3	99.45±1.37	2.42±1.18	6.26±1.39	0.36±0.03	15±1.27	95.51±1.37
F4	98.27±1.28	2.67±1.38	6.47±1.18	0.74±0.05	18±1.45	97.37±1.25
F5	100.65±1.67	2.25±1.45	6.25±1.35	0.25±0.09	13±1.28	95.25±1.49
F6	101.18±1.19	2.39±1.29	6.36±1.47	0.94±0.06	15±1.34	96.69±1.47
F7	99.24±1.32	2.17±1.38	6.44±1.21	0.78±0.03	14±1.45	97.45±1.38
F8	100.15±1.27	2.19±1.45	6.12±1.27	0.25±0.07	18±1.35	98.84±1.18
F9	101.35±1.53	2.24±1.85	6.21±1.39	0.74±0.05	16±1.28	95.51±1.25
F10	97.17±1.18	2.45±1.17	6.62±1.45	0.25±0.02	17±1.57	96.48±1.37
F11	98.14±1.37	2.86±1.24	6.89±1.20	0.75±0.06	18±1.34	97.57±1.24
F12	100.48±1.08	2.25±1.38	6.98±1.74	0.84±0.08	09±1.24	99.42±1.59

Discussion:

Hardness of the tablet was acceptable and uniform from batch-to-batch variation, which was found to be 6.12±1.27-6.98±1.74kg/cm^2. All the formulations passed the weight variation test as the % weight variation was within the pharmacopoeia limits of the tablet weight. Friability values were found to be less than 1% in all the formulations F1–F12 and considered to be satisfactory ensuring that all the formulations are mechanically stable. The drug content values for all the formulations (F1-F12) were found to be in the range of 93.42±1.24-99.42±1.59%.

Dissolution studies of the tablets:

The prepared tablets were subjected to dissolution studies in order to know the amount drug release.

Table.5 % Cumulative drug release of formulations F1-F6

Time (mins)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	17.17±1.48	28.26±1.58	39.45±1.27	52.47±1.02	22.21±1.28	35.57±1.47
10	28.26±1.27	43.84±1.67	45.38±1.49	69.51±1.39	38.23±1.45	47.58±1.25
15	49.54±1.38	59.45±1.48	68.51±1.26	77.24±1.45	55.15±1.27	59.67±1.45
20	65.38±1.45	67.25±1.25	75.57±1.08	88.35±1.20	62.42±1.83	67.28±1.37
30	77.45±1.25	79.75±1.79	81.35±1.45	91.24±1.47	72.26±1.20	75.45±1.58
40	82.29±1.58	85.61±1.67	89.24±1.37	98.86±1.26	79.24±1.47	87.37±1.87
50	90.67±1.49	98.58±1.54	98.17±1.49		88.62±1.06	98.18±14
60	97.27±1.37				96.25±1.35	

Table.6 % Cumulative drug release of formulations F7-F12

Time (mins)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
5	42.89±1.45	56.24±1.48	25.21±1.28	47.15±1.48	59.19±1.07	64.47±1.07
10	55.24±1.79	67.58±1.45	38.46±1.45	55.24±1.50	67.25±1.03	79.68±1.14
15	68.42±1.61	78.47±1.69	43.25±1.36	68.36±1.19	75.75±1.21	85.25±1.25
20	74.19±1.28	85.65±1.12	58.36±1.12	78.45±1.37	85.18±1.15	93.14±1.35
30	87.46±1.45	90.34±1.37	67.18±1.37	85.57±1.45	92.34±1.34	99.15±1.45
40	95.78±1.37	98.45±1.45	75.47±1.45	93.45±1.01	98.18±1.73	
50	98.21±1.42		88.56±1.20	98.25±1.54		
60			98.94±1.49			

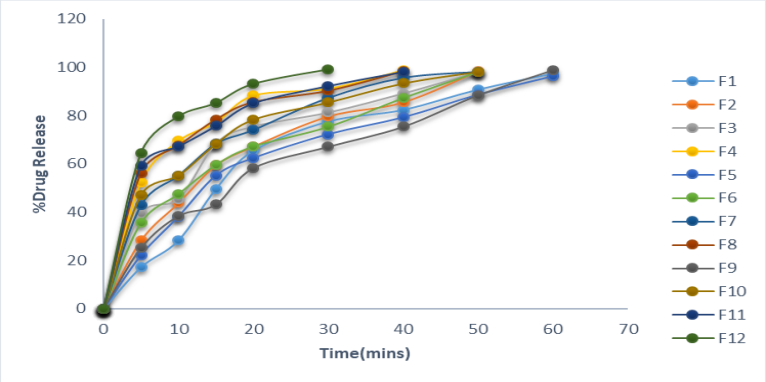

Figure.8 In vitro drug release of formulations F1-F12

Discussion: From the in vitro drug release in studies, it was observed that the formulations containing Lycoat as a super disintegrant in different concentrations like 7.5mg, 15mg, 22.5mg and 30mg in concentrations, reveals that the increased in the super disintegrant concentration decreases the drug release time and the F4 formulation containing Lycoat concentration shows maximum amount of drug release 98.86±1.26% at the end of 40mins.
Whereas formulations containing CCS as a super disintegrant in different concentrations like 7.5mg, 15mg, 22.5mg and 30mg in concentrations reveals that the increased in the super disintegrant concentration decreases the drug release time and the F8 formulation containing CCS with 30mg concentration shows maximum amount of drug release (98.45±1.45%) at the end of 40mins.
And the formulations containing Crospovidone as a super disintegrant in different concentrations like l7.5mg, 15mg, 22.5mg and 30mg in concentrations reveals that the increased in the super disintegrant concentration decreases the drug release time and the F12 formulation containing Crospovidone with 30 mg concentration shows maximum amount of drug release (99.15±1.45%) at the end of 30mins.
So, F12 formulation containing 30mg concentration of Crospovidone shows max. Release 99.15±1.45% within 30mins, so that it is chosen as optimized formulation.

EVALUATION OF FORMALDEHYDE TREATED CAPSULES:

Physical tests:

Identification attributes: The size '0' capsules chosen were opaque, with white colored body and red cap. The normal capsule bodies were soft and sticky when touched with wet hand. After treating with formaldehyde, there were no significant changes in the physical appearance of the capsules except for the stickiness. The body of capsule was hard and non-sticking even when touched with wet hand due to treatment with the formaldehyde.

Visual defects: Among 100 capsules body which were treated with formaldehyde, about 15 to 20 capsule bodies showed visual defects. They were found to be shrunk and distortion into different shapes due to the complete loss of moisture.

Dimensions: Dimensional examination was done by using vernier calipers.

Average capsule length:

Before formaldehyde treatment (untreated cap and body) : 20.5 mm
After formaldehyde treatment(treated body and untreated cap) : 19.1 mm

Average diameter of capsule body:

Before formaldehyde treatment : 7.8 mm
After formaldehyde treatment : 6.8 mm

Average length of capsule body:

Before formaldehyde treatment : 16.2 mm
After formaldehyde treatment : 15.2 mm

Discussion: On formaldehyde treatment, the "0" size capsules bodies showed a significant decrease in length and diameter and attained hardness.

In vitro release studies:

Dissolution study was carried out to measure the release rate of drug from prepared pulsincap formulation using USP I dissolution apparatus at 37^0C using 2 different dissolution media of pH 1.2, pH 7.4 phosphate buffers in order to mimic in vivo GIT conditions. Initially first 2hrs of dissolution was conducted in pH 1.2 buffer, followed by 10hrs of dissolution study in pH 7.4 phosphate buffer.

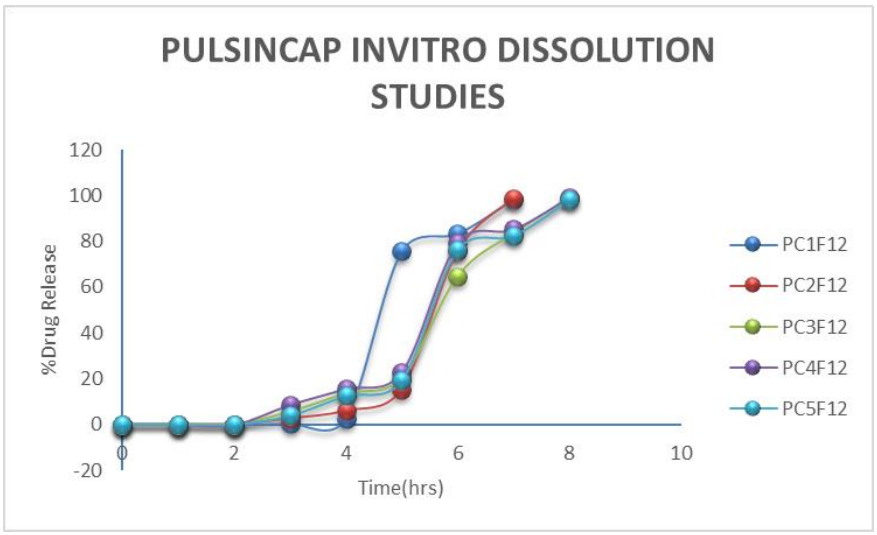

Figure.9 Dissolution plots for formulations PC1F12 to PC5F12

Discussion:

All the 5 formulations of Roflumilast pulsincaps were subjected to dissolution studies. Formulations PC1F12, PC2F12, PC3F12, PC4F12 & PC5F12, contain the hydrogel plug with alone and combination of hydrophobic polymer and Hydrophilic polymer i.e., lactose: HPMC in the ratio of 1:1, 2:1 , 1:2 ,lactose and HPMC of total 100mg weight of the plug. It was observed that a proper lag time of 6 hours was maintained with minimal upper GIT drug release for the combination of Lactose and HPMC K15M hydrogel plug in the 2:1. It was observed that as the concentration of Hydrophilic polymer was increased the release rate of drug was delayed and finally burst release of drug from the formulation occurred after lag time. So basing on these observations, of all the 5 pulsincap formulations, PC4F12 formulation containing hydrogel plug of Lactose & HPMC K15M in 2:1 ratio was selected as optimized pulsincap formulation.

RELEASE KINETICS:

Zero Order

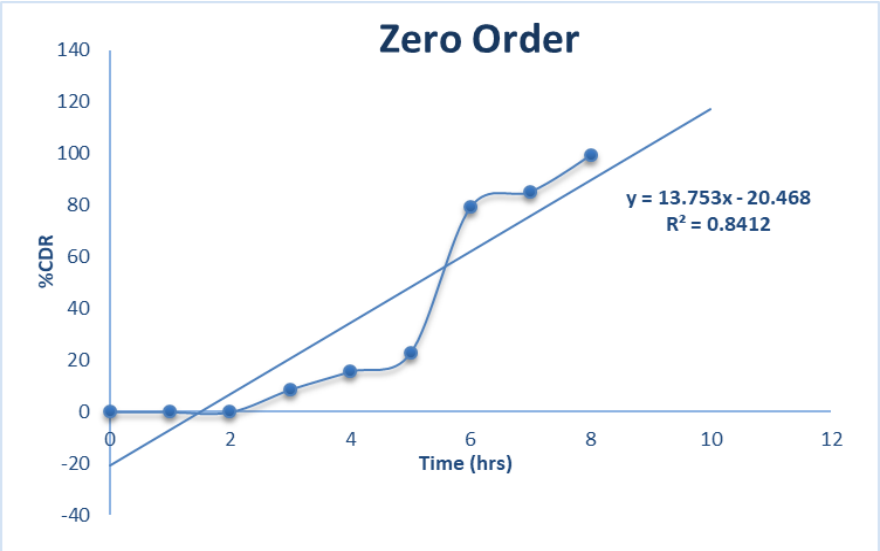

Figure.10 Zero order plot for optimized formulation PC4F12

First Order

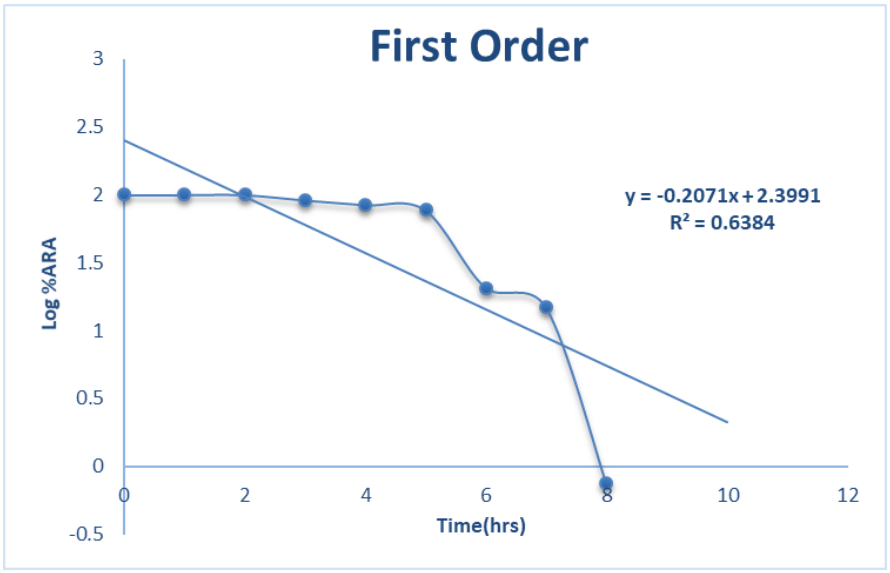

Figure.11 First order plot for optimized formulation PC4F12

Higuchi's order

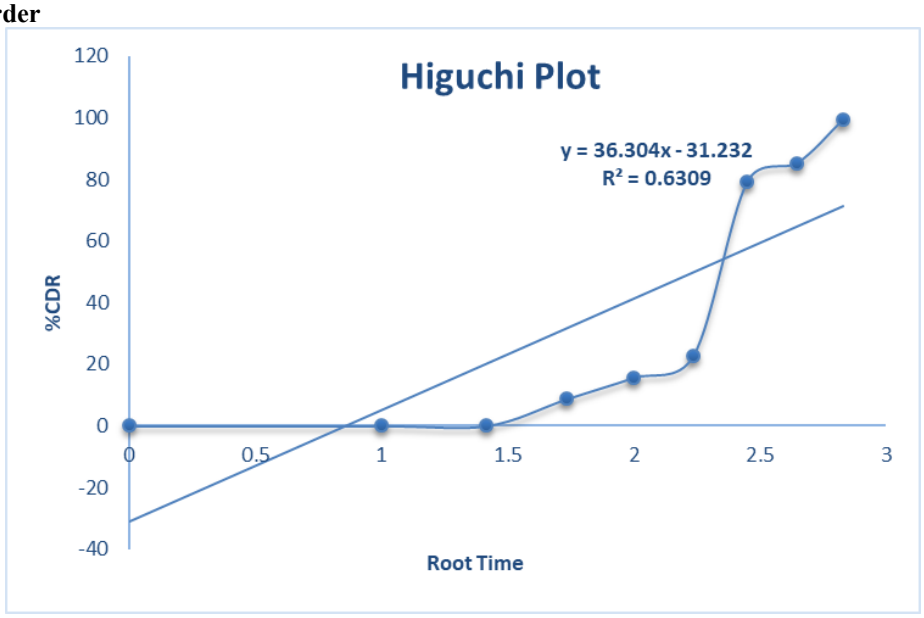

Figure.12 Higuchi's order plot for optimized formulation PC4F12

Koresmayer peppas

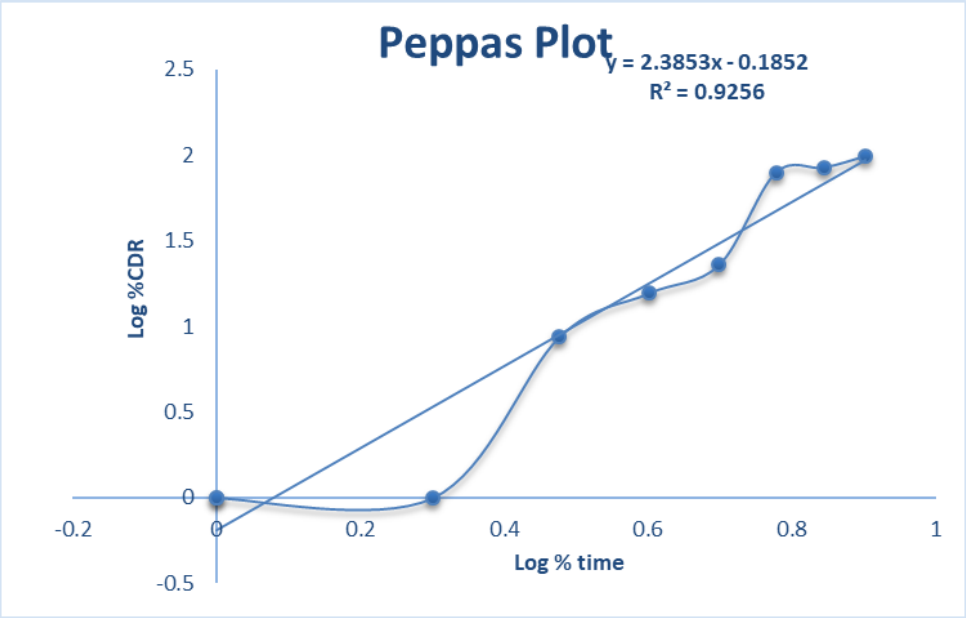

Figure.13 Koresmayer peppas order plot for optimized formulation PC4F12

Discussion:

To analyze the mechanism of drug release from optimized PC4F12 pulsincap formulation, data obtained from the drug release studies was subjected to different kinetic treatments. The correlation coefficient (R) was used as indicator of the best fitting for each of the models considered. The drug release kinetics for the optimized formulation PC4F12 followed the zero order and follows super case II transport mechanism.

SUMMARY:

Over the past two decades there has been a growing appreciation on the importance of circadian rhythms on GIT physiology and on disease states, together with the realization of the significance of the drug administration on resultant pharmacodynamic and pharmacokinetics parameters. The significance of these day-night variations has not been over looked from the drug delivery perspective and pharmaceutical scientists have displayed considerable ingenuity in development of time delayed drug delivery systems to address emerging Chronotherapeutic formulations. Pulsincap technique helps us to deliver the drug at colon which helps to treat chronotherapeutic. The colon is a site where both the local and systemic delivery of drugs can take place; treatment could be more effective if it were possible for drugs to be targeted directly on the colon. In the present study, attempt was made to target the drug to the colon and intentionally delaying the drug absorption from the therapeutic point of view in the treatment of lowering cholesterol. Prior to formulation, Preformulation studies were carried out in order to establish compatibility between Roflumilast and excipients by FTIR spectroscopy. The results revealed that the drug and polymers were satisfactorily compatible, without any significant changes in the chemical nature of Roflumilast. The capsule bodies were made insoluble by formaldehyde treatment by exposing at various time intervals viz., 2, 4, 6, 8, 10hrs and then optimized by using disintegration studies and finally the optimized treated capsule bodies were then subjected to various physical and chemical tests such as identification attributes, visual defects, dimensional studies and qualitative test for free formaldehyde. Total 12 formulations were formulated by using super disintegrant in different ratios by direct compression method. The formulations were subjected to flow properties and FTIR study. Based on the results obtained F12 containing 30mg Crospovidone was considered as the optimum powder blend for fabrication of pulsincap capsule. Different concentration of the polymers like HPMC K4M, Lactose alone and in combination were used for the preparation of hydrogel plug to maintain the suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. The powder blend F12 was filled into the 6th hr formaldehyde treated capsule bodies and plugged with hydrogel polymers, 100mg hydrogel plug. The ratios of hydrophobic polymer like ethyl cellulose and HPMC K4M were taken in alone and 1:1, 2:1 and 1:2. Finally after arranging the plug, the joint of the capsule body and cap was sealed with a small amount of 1% lactose ethanolic solution. The prepared pulsincaps were evaluated for In vitro studies. All the 5 formulations of Roflumilast pulsincaps were subjected to dissolution studies. Formulations PC1F12, PC2F12, PC3F12, PC4F12 & PC5F12, contain the hydrogel plug with alone and in combination of hydrophobic polymer and Hydrophilic polymer i.e., Lactose : HPMC in the ratio of 1:1, 2:1 & 1:2 of total 100mg weight of the plug. It was observed that a proper lag time of

6 hours was maintained with minimal upper GIT drug release for the combination of lactose and HPMC K15M hydrogel plug in the 2:1. It was observed that as the concentration of Hydrophilic polymer was increased the release rate of drug was delayed and finally burst release of drug from the formulation occurred after lag time. So, basing on these observations, of all the 5 pulsincap formulations, PC4F12 formulation containing hydrogel plug of Lactose & HPMC K15M in 2:1 ratio was selected as optimized pulsincap formulation.

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BIBLIOGRAPHY:

1. Agusti AG. COPD, a multicomponent disease: implications for management. Respir Med. 2005;99(6):670–682. doi: 10.1016/j.rmed.2004.11.006. [DOI] [PubMed] [Google Scholar]
2. Global Initiative for Chronic Obstructive Lung Disease (GOLD) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2014. [Accessed July 28, 2015]. Available from: http://www.goldcopd.org/uploads/users/files/GOLD_Report_2014_Jan23.pdf.
3. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax. 2004;59(7):574–580. doi: 10.1136/thx.2003.019588. [DOI] [PMC free article] [PubMed] [Google Scholar]
4. Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? Lancet. 2007;370(9589):797–799. doi: 10.1016/S0140-6736(07)61383-X. [DOI] [PubMed] [Google Scholar]
5. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(26):2645–2653. doi: 10.1056/NEJMoa032158. [DOI] [PubMed] [Google Scholar]
6. Saetta M. Airway inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1999;160(5 Pt 2):S17–S20. doi: 10.1164/ajrccm.160.supplement_1.6. [DOI] [PubMed] [Google Scholar]
7. Daliresp (roflumilast) tablets [package insert] Wilmington, DE: Astra-Zeneca Pharmaceuticals LP; 2015. [Google Scholar]
8. Hatzelmann A, Morcillo EJ, Lungarella G, et al. The preclinical pharmacology of roflumilast – a selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. Pulm Pharmacol Ther. 2010;23(4):235–256. doi: 10.1016/j.pupt.2010.03.011. [DOI] [PubMed] [Google Scholar]
9. Sanz MJ, Cortijo J, Morcillo EJ. PDE4 inhibitors as new anti-inflammatory drugs: effects on cell trafficking and cell adhesion molecules expression. Pharmacol Ther. 2005;106(3):269–297. doi: 10.1016/j.pharmthera.2004.12.001. [DOI] [PubMed] [Google Scholar]
10. Grootendorst DC, Gauw SA, Verhoosel RM, et al. Reduction in sputum neutrophil and eosinophil numbers by the PDE4 inhibitor roflumilast in patients with COPD. Thorax. 2007;62(12):1081–1087. doi: 10.1136/thx.2006.075937. [DOI] [PMC free article] [PubMed] [Google Scholar]
11. Gauvreau GM, Boulet LP, Schmid-Wirlitsch C, et al. Roflumilast attenuates allergen-induced inflammation in mild asthmatic subjects. Respir Res. 2011;12:140. doi: 10.1186/1465-9921-12-140. [DOI] [PMC free article] [PubMed] [Google Scholar]
12. Schick MA, Wunder C, Wollborn J, et al. Phosphodiesterase-4 inhibition as a therapeutic approach to treat capillary leakage in systemic inflammation. J Physiol. 2012;590(Pt 11):2693–2708. doi: 10.1113/jphysiol.2012.232116. [DOI] [PMC free article] [PubMed] [Google Scholar]
13. Hussain SK, Firoz S, Ramya EM, Sarada K, Ramesh B, Jagadesh K. A review of chronopharmaceutical drug delivery system. Int J Med Pharm Res 2015;3:942-7.
14. Kumar JP, Muzib YI, Misra G. Preparation and evaluation of pulsatile drug delivery of fluvastatin sodium. Asian J Pharm Clin Res 2016;9:348-51.
15. Rewar S, Bansal BK, Singh CJ, Sharma AK. Pulsatile drug delivery release technologies: An overview. Int J Res Dev Pharm Life Sci 2015;4:1386-93.
16. Bhuvaneswari S, Manivannan S, Akshay M, Nify F. Formulation and evaluation of gastroretentive microballoons of Acebrophylline for the treatment of bronchial asthma. Asian J Pharm Clin Res 2016;9:105-11.
17. Chandra JR, Arora K, Anuragi N, Kaushal R, Jain V. Pulsatile drug delivery system: A promising delivery system for controlled drug release. Eur J Pharm Med Res 2015;2:564-75.
18. Richard JM. Nocturnal asthma: Circardian rhythms and therapeutic interventions. Am Rev Respir Dis Suppl Airway Dis Update 1993;147:S25-8.
19. Global Strategy for Asthma Management and Prevention. GINA Appendix 2015 Update. Mechanisms of Asthma; 2015. p. 21.