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FORMULATION DEVELOPMENT AND INVITRO EVALUATION OF PULSATILE DRUG DELIVERY SYSTEM OF LABETALOL

Ganjaraboina Sai Mahesh, V. Jhansi Priya Marabathuni* and Naidu Narapusetty

Department of Pharmaceutics, Bellamkonda Institute of Technology & Science, Podili. A.P-523240.

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***Corresponding Author**
V. Jhansi Priya Marabathuni
Department of Pharmaceutics, Bellamkonda Institute of Technology & Science, Podili. A.P-523240.

ABSTRACT

The present investigation revealed that the coating of core tablet with 240 mg of HPMC K4M and top coating of PRT with floating layer containing 80 mg HPMC K100M and 25 mg sodium bicarbonate provided desired lag time required for chronotherapy of hypertension. The optimized formulation showed 6 h of pulsatile release lag time, 4.4 min floating lag time and greater than 14.3 h of floating time. *In vitro* drug release study showed 81.21% drug release in 0.1N HCL within 30 min, indicating burst release. From the experimental findings it can be concluded that floating pulsatile tablets of Labetalol can give efficient therapy by reducing dose and dosing frequency and provide chronotherapy for an effective management of morning surge of hypertension.

KEYWORDS: pulsatile drug delivery, Floating delivery system, hypertension, swelling index, dissolution rate.

INTRODUCTION

The purpose of designing by which the drug is released from dosage form depends on the type of coating; insoluble coating under all physiological conditions, pH-dependent coating whose solubility changes dramatically at some point in GI tract, and slowly erodible coating. The method of application and processing conditions may influence the porosity of the coating and consequently the release mechanism.

Less obvious but also important to the kinetics of release are the influences of the core formulation, in terms of both physical properties and amounts of the drug and excipient

materials present, and physiological environment to which the drug is released.

In multiparticulate pulsatile delivery systems, the swelling and rupturing; dissolution or erosion; and changed permeability of the coating membrane are primarily involved in the control of release. The development of low-density floating multiparticulate pulsed release dosage forms possessing gastric retention capabilities has also been addressed with increasing focus on the upcoming multiparticulate-pulsatile technologies being exploited on an industrial scale.[1-4]

Labetalol is a diastereoisomeric mixture of approximately equal amounts of all four possible stereoisomers ((R,S)-labetolol, (S,R)-labetolol, (S,S)-labetalol and (R,R)-labetalol). It is an adrenergic antagonist used to treat high blood pressure. It has a role as an antihypertensive agent, a sympatholytic agent, an alpha-adrenergic antagonist and a beta-adrenergic antagonist. It contains a (R,R)-labetalol, a (S,S)-labetalol, a (R,S)-labetolol and a (S,R)-labetolol.[5]

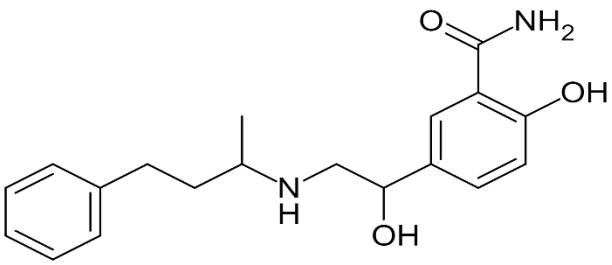

Figure 1: Chemical structurer of Labetalol.

EXPERIMENTAL WORK

MATERIALS AND METHODS

Labetalol gift sample from the spectrum labs Hyderabad, Lactose, Povidone, Sucrose, Crospovidone XL 10, Croscarmellose sodium, Aerosil, HPMC E5, HPMC E15, HPMC K4M, HPMC K100M, Sodium bicarbonate, Magnesium stearate purchased from the SD fine chemical limited, Mumbai. All the instruments used in the work was calibrated.

METHODOLOGY[6]

Preformulation studies

The sample of Labetalol was characterized for its physical state, colour and odour.

The melting point was determined using a capillary tube method. Solubility study was carried

out using different solvents. Fourier transforms infrared (FTIR) spectroscopy studies were used for the evaluation of physicochemical compatibility and interactions among drugs and excipients.

Chemical Compatibility study by FTIR

Infrared spectroscopy can be used to identify a compound and also to investigate the composition of a mixture there by we can study incompatibility with two compounds. Compatibility in between pure drug and compatibility in between both drug and excipient has been investigated by FTIR. The IR spectra of the test samples were obtained by Pressed Pellet technique using Potassium bromide.

Preparation of standard calibration curve of Labetalol[7-9]

Selection of common solvent

The selection of common solvent was made after assessing the solubility of the drug in different solvents. The drug was found to be completely soluble in methanol.

Stock solution

50 mg of Labetalol was solubilized by 50 ml of methanol in a 100 ml volumetric flask, and 0.1N HCL was added to make up the volume.

Standard solution

0.2 ml of stock solution was diluted to 100 ml with 0.1N HCL. Similarly, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml of standard solution was taken and further diluted with the 0.1N HCL to the volume. UV absorbance was taken at the wavelength of 246 nm in UV-Visible spectrophotometer UV-1601, Shimadzu.

Formulation of rapid release core tablets[10-12]

The immediate release tablets will be prepared by wet granulation method and prepared according to the following procedure.

Table 1: Formulation of rapid release core tablets.[10-12]

Ingredients	R1	R2	R3	R4	R5	R6
Labetalol	100	100	100	100	100	100
Croscarmellose sodium	1.3	1.6	1.9	-	-	-
Crospovidone	-	-	-	1.3	1.6	1.9
Lactose	68.375	68.075	67.775	68.375	68.075	67.775
Sucrose	1	1	1	1	1	1

Povidone	2	2	2	2	2	2
Aerosil	3.5	3.5	3.5	3.5	3.5	3.5
Magnesium Stearate	0.7	0.7	0.7	0.7	0.7	0.7
Total (mg)	80	80	80	80	80	80

Preparation of the pulsatile release tablets (PRTs)

The optimized pulsatile release tablets were prepared by using different grades of HPMC (K4M, E15 & E5) at different concentrations. Dry coated tablet was prepared by placing 50% of pulsatile release layer in appropriate die and rapid release core tablet (RRCT) layer was placed on it and then remaining quantity of pulsatile release layer was added in the cavity, so as to cover the RRCT and finally compressed using rotary tablet compression machine.

Table 2: Formulation of PRT of Labetalol.

Polymer used	Concentration in mg/tablet		
HPMC E5	220	240	260
HPMC E15	220	240	260
HPMC K4M	220	240	260

EVALUATIONS

Micromeritic properties

Flow Property Measurements

A. Bulk Density (ρb)

B. Tapped Density (ρt)

C. Angle Of Repose (Ɵ)

D. Carr's Index (Or) % Compressibility

E. Hausner's Ratio

Post compression parameters

A. Thickness

B. Hardness test

C. Weight variation test

A. Friability

10 tablets will be weighed and the initial weight of these tablets will be recorded and placed in Roche friabilator and rotated at the speed of 25 rpm for 100 revolutions. The tablets will be then removed from the friabilator, dusted off the fines and again weighed and the weight is recorded. Percentage friability will be calculated by using the formula.

$$\% \text{ Friability} = \frac{\text{Initial weight of the tablets (W1)} - \text{Final weight of the tablets (W2)}}{\text{Initial weight of the tablets (W1)}} \times 100$$

B. Swelling index

The initial weight of the tablets (W1) will be noted and placed individually into petri dish containing 10 ml of distilled water. The weighed of the tablets (W2) will be noted after 30 minutes after wiping out the excess of water using filter paper. The swelling index will be calculated using the formula

$$\text{Swelling index} = \frac{W2 - W1}{W1} \times 100$$

C. Drug content uniformity

Ten tablets were crushed into powder individually from each batch separately. It was then taken in a volumetric flask dissolved in 15 ml of methanol, the solution was filtered through Watmann filter paper, from this 1 ml of solution was withdrawn and diluted to 10 ml. Again, from this, 1 ml of solution was withdrawn and diluted to 10 ml, absorbance was taken at 246 nm and % drug content was calculated.

D. Disintegration Time

Disintegration test will be carried out as described under procedure for plain coated tablets in USP. One tablet each will be placed in each of six tubes of the basket of the assembly. Apparatus will be operated for one hour using simulated gastric fluid, maintained at 37 ± 2^{o}C as the immersion fluid. After 1 hour the tablet will be examined for disintegration, cracking and softening. Then the apparatus will be operated for specified time. The remaining tests will be carried out with simulated intestinal fluid maintained at 37 ± 2^{o}C as the immersion fluid.

E. In-vitro Dissolution methods for core tablets

In –vitro Dissolution studies will be done with the conventional paddle method of core tablets will be performed at 37^{o}C ± 0.5°C using 0.1N Hcl in USP II paddle method at 50 rpm. 5 ml of filtered aliquot will be manually withdrawn at pre-determined time intervals and replaced with 5 ml of fresh buffer maintained at the same temperature. The samples will be analysed at 246 nm using a UV spectrophotometer. Percentage drug release will be determined for each formulation.

F. In-vitro Dissolution methods for pulsatile release tablets

In-vitro Dissolution studies of Pulsatile delivery systems will be done with the conventional paddle method of press coated tablets were performed at 37^{o}C ± 0.5°C using 0.1N Hcl in USP II paddle method at 50 rpm. 5 ml of filtered aliquot was manually withdrawn at pre-determined time intervals and replaced with 5 ml of fresh buffer maintained at the same temperature. The samples will be analysed at 246 nm using a UV spectrophotometer. The lag time and percentage release will be determined for each formulation.

Assay

Standard stock solution

Standard stock solutions of Labetalol were prepared in methanol. 20 mg of Labetalol was transferred into 100 ml volumetric flask and 60 ml of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. 2 ml of this solution was then diluted to 100 ml volume with methanol. The absorbance of these solutions was measured at 246 nm in U V spectrophotometer. Methanol was used as blank solution.

Sample Solution

Ten tablets of Labetalol were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to 20 mg of Labetalol was transferred into 100 ml volumetric flask and 60 ml of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. 2 ml of this solution was then diluted to 100 ml volume with methanol. The absorbance of these solutions was measured at 246 nm in UV spectrophotometer.

RESULTS AND DISCUSSION

Selection of Drug and Excipients

Formulation development started from selection of API, the cost of efficiency, easy availability and challenging aspects of drug properties made to select the Labetalol. Then the excipients were selected based on the previous studies and compatibility.

Raw material Analysis of Labetalol

Description and solubility

The description of the Active Pharmaceutical Ingredient Labetalol was found comply with BP. Solubility of Labetalol was found with the different solvents. It was slightly soluble in methanol, sparingly soluble in methylene chloride and insoluble in water.

Calibration curve of Labetalol

The drug was analysed by UV spectrophotometer at **246 nm.**

The calibration curve of Labetalol in methanol is given in Table 14 and Fig 3

Table 3: Calibration curve of Labetalol.

Concentration (µg/ml)	Absorbance
1	0.099
2	0.203
3	0.295
4	0.391
5	0.481

Table 14: Calibration curve of Labetalol.

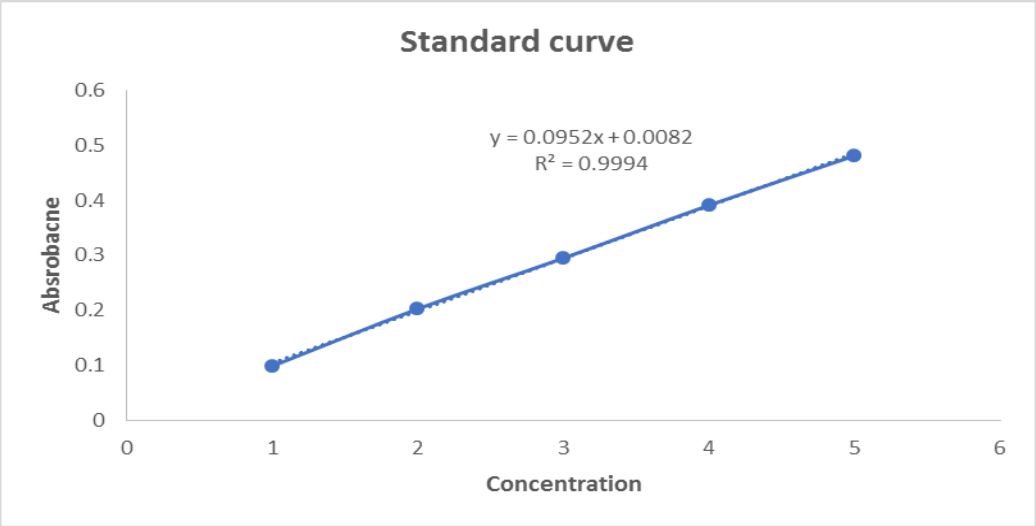

Fig. 3: Standard Curve of Labetalol.

It was found that the solution of Labetalol in methanol show linearity (R^2 = 0.999) in absorbance at concentrations of 1-5 (µg/mL) and obey Beer Lambert Law.

Drug –excipient compatibility (FT-IR) study of Labetalol

The FTIR spectra of the pure drug, excipient and physical mixture of drug and excipients used in floating controlled tablet formulations shown in Fig no 4-7 were recorded in between 400-4000 wave number (cm-1).

Precompression Study

The formulated blends of Labetalol were evaluated for pre compression parameters.

The results are given in Table 20.

Table 4: Evaluation of Flow properties of granules.

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index (%)	Hausner's ratio	Angle of repose (θ)
F1	0.523±0.021	0.645±0.052	18.91±3.6	1.233±0.14	27.68±1.26
F2	0.526±0.021	0.635±0.021	17.17±1.1	1.207±0.12	26.71±1.25
F3	0.536±0.012	0.638±0.034	15.99±2.5	1.190±0.11	28.12±2.14
F4	0.532±0.025	0.627±0.304	15.15±1.0	1.179±0.12	25.62±1.42
F5	0.537±0.012	0.625±0.041	14.08±2.4	1.164±0.13	25.83±1.46
F6	0.543±0.006	0.613±0.032	14.77±1.43	1.189±0.07	25.74±1.63

The bulk density of the Labetalol blend ranged from 0.523 g/mL to 0.543 g/mL and the tapped density ranged from 0.613 g/mL to 0.645 g/mL. The compressibility index of the blend ranged from 15.15% to 18.91% and Hausner's ratio ranged from 1.164 to 1.233. The angle of repose of the ranged from 25.62 to 28.12 Hence the entire formulations blend was found to be good, passable flow property.

Post Compression

Rapid release core tablets

The formulated rapid release core tablets of Labetalol were evaluated for post compression parameters. The results of weight variation, thickness, hardness, friability, assay, disintegration time are given in Table 5.

Table 5: Evaluation of core tablets.

Trial	Weight Variation (%)(±SD)	Thickness (mm) (±SD)	Diameter (mm) (±SD)	Hardness (kg/cm^2) (±SD)	Friability (%)
F1	80±0.13	2.9±0.02	5.5±0.003	3.55±0.08	0.84
F2	80±0.22	3.1±0.06	5.5±0.002	3.7±0.06	0.67
F3	79±0.16	3.0±0.03	5.4±0.002	3.62±0.12	0.71
F4	80±0.18	2.9±0.06	5.5±0.003	3.7±0.15	0.82
F5	80±0.09	3.0±0.05	5.3±0.002	3.7±0.05	0.84
F6	80±0.23	2.9±0.03	5.5±0.001	3.9±0.03	0.86

Table 6: Evaluation of core tablets.

Trial	Assay (%w/w)	Disintegration (±SD)	Content Uniformity(%)
F1	97.5±0.12	250±6	97.45±1.23
F2	97.2±0.20	235±3	98.23±0.74
F3	97.7±0.17	210±7	98.75±2.08
F4	98.7±0.08	193±5	99.21±1.78
F5	98.5±0.04	153±3	98.21±0.63
F6	99.2±0.02	123±6	98.75±0.39

1. Weight variation

The percentage weight variations for all formulations were tabulated in Table 5. The formulated batches passed weight variation test as the Percentage weight variation was within the pharmacopoeial limits.

2. Thickness

The measured thickness of tablets of each batch ranged between 2.9±0.02 to 3.1±0.06 mm. The value shows that formulated tablets have uniform thickness. The parameters were reported in Table.5

3. Hardness

The measured hardness of tablets of each batch ranged between 3.55±0.08 to 3.7±0.15 Kg/cm^2. This ensures good handling characteristics of all batches. The results were shown in Table.5.

4. Friability

The values of friability test were tabulated in Table No. The % friability was less than 1% in all the formulations ensuring that the tablets were mechanically stable.

5. Assay

The assay of the formulations ranged between 97.2±0.20 to 99.2±0.02 w/w. The values are within the pharmacopoeial limits. The results were shown in Table No.6.

6. Disintegration time

The disintegration time of all the batches were found between 123±6 seconds to 250±6 seconds and results were shown in Table No.22. The effects of independent variables on disintegration time were investigated as per optimized response parameters.

7. Content uniformity

The content uniformity of Labetalol buccal tablets was determined and found to be between 97.45±1.23 to 99.21±1.78 and results were shown in table no.6.

Table 7: in-vitro dissolution of RRCT.

Time (mins)	Cumulative drug release (%)					
	F1	F2	F3	F4	F5	F6
5	32.78±0.34	29.23±0.66	31.34±0.33	44.78±0.16	48.57±0.12	50.37±0.67
10	47.56±0.26	43.56±0.88	48.65±0.75	59.31±0.06	63.52±0.48	65.34±0.48
15	66.35±0.13	69.75±0.44	64.98±0.12	69.33±0.19	71.22±0.38	73.76±0.27
20	79.97±0.15	77.43±0.67	74.33±0.77	82.15±0.37	84.44±0.26	86.94±0.43
25	87.59±0.54	88.21±0.27	89.42±0.37	95.02±0.26	94.96±0.13	95.38±0.16
30	90.10±0.16	91.22±0.11	92.72±0.16	98.05±0.35	98.90±0.17	99.95±0.13

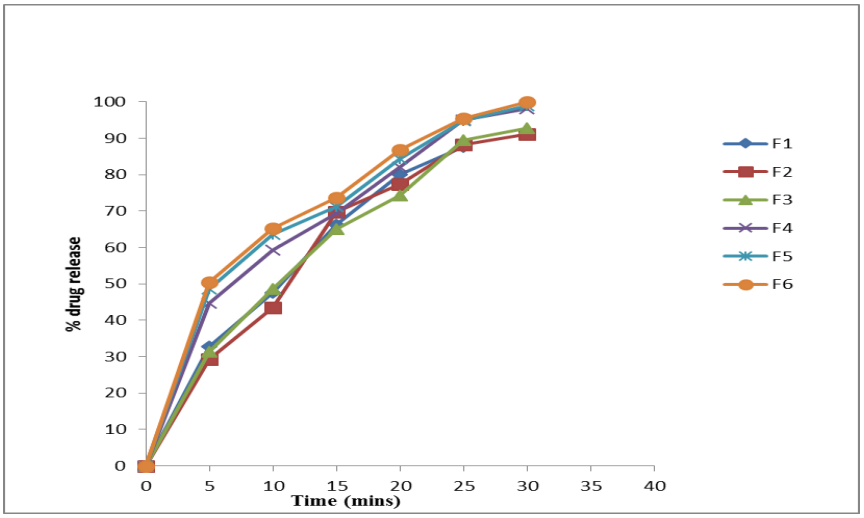

Fig. 3: % Drug release of RRCTs.

In-Vitro dissolution

The dissolution profiles of Labetalol RRCT studied in 0.1N Hcl. The drug release of the formulations were determined. The cumulative drug releases for formulations were found within the range of 90.10±0.16 to 99.95±0.13. The effects of independent variables on cumulative drug release were investigated as per optimized response parameters.

In this study formulations containing crospovidone (RR4-RR6) showed fast drug release than the formulation containing croscarmellose sodium(RR1-RR3) (figure 3). This may be because of the fact that crospovidone probably made larger pores with continuous network or skeleton providing enough pressure for faster disintegration and it also had capability to swell at least twice of its original volume when in contact with dissolution fluid.[38] Among eight formulations of Labetalol RRCTs, it was observed that formulations containing crospovidone in concentration (RR6) showed satisfactory hardness, uniformity of content, lowest disintegration time and highest drug release (table 7). So RR6 was considered as optimized formulation and was taken for further studies.

Pulsatile release tablets

Table 8: Evaluation of PRTs.

Batch codes	Thickness (mm)	Diameter (mm)	Hardness (kg/cm^2)
PRT-E5220	3.67±0.01	10.01±0.01	4.22±0.11
PRT-E5240	3.79±0.008	10.03±0.02	4.38±0.13
PRT-E5260	3.94±0.013	10.05±0.01	4.45±0.21
PRT-E15220	3.73±0.01	10.02±0.011	4.68±0.34
PRT-E15240	3.85±0.13	10.04±0.02	4.73±0.27
PRT-E15260	3.98±0.14	10.02±0.01	4.63±0.25
PRT-K4M220	3.88±0.09	10.00±0.01	4.82±0.16
PRT-K4M240	3.96±0.01	10.03±0.03	4.86±0.36
PRT-K4M260	4.08±0.015	10.03±0.01	4.97±0.21

For PRT characterization, total 9 formulations containing varying concentrateion (220- 260 mg) of HPMC E5, E15 and K4M were evaluated for thickness, diameter, hardness and drug release profile in terms of lag time. It was found that all PRT formulations showed satisfactory features in terms of thickness, diameter and hardness (table 8)

Table 9: Invitro dissolution data of Labetalol from PRT containing E5.

Time (mins)	PRT-E5220	PRT-E5240	PRT-E5260
0	0	0	0
30	0	0	0
60	0	0	0
90	0	0	0
120	0	0	0
150	0	0	0
180	0	0	0
210	40.67±0.76	17.69±0.79	2.17±0.86
240	79.65±1.30	51.34±1.86	37.6.±2.60
270		78.76±0.97	60.6±1.47

Table 10: Invitro dissolution data of Labetalol from PRT containing E15.

Time (min)	PRT-E15220	PRT-E15240	PRT-E15260
0	0	0	0
60	0	0	0
120	0	0	0
180	0	0	0
210	0	0	0
240	0	0	0
270	0	0	0
300	0	0	0
330	31.8±1.02	14.7±0.61	5.21±0.44
360	84.5±1.17	64.7±1.75	43.21±2.1

Table 11: Invitro dissolution data of Labetalol from PRT containing K4M.

Time (mins)	PRT-K4M220	PRT-K4M240	PRT-K4M260
0	0	0	0
60	0	0	0
120	0	0	0
180	0	0	0
240	0	0	0
270	0	0	0
300	0	0	0
360	11.21±0.98	0	0
390	66.2±1.23	16.64±1.08	
420		63.5±1.21	22.15±1.34
450			82.1±2.01

Table 12: Invitro dissolution data of Labetalol from PRT containing K4M.

Time (mins)	PRT-K4M220	PRT-K4M240	PRT-K4M260
0	0	0	0
60	0	0	0
120	0	0	0
180	0	0	0
240	0	0	0
300	0	0	0
330	0	0	0
345	0	0	0
360	12.72±1.28	0	0
375	37.6±2.01	3.5±0.53	0
390	69.2±2.21	18.86±1.33	0
405	86.4±0.7	42.75±2.21	5.21±0.18
420	87.45±1.4	66.93±3.1	24.70±1.42
435		82.65±0.4	53.1±2.01
450			81.2±1.32
465			81.5±1.21

In vitro drug release study of 9 formulations, showed differences in drug release as shown in table. All the formulations coated with HPMC E5 and E15 have given the lag time of less than 3 hr and 5 hr respectively which was considered to be unsuitable for chronotherapeutic objective. The formulations coated with HPMC K4M in concentration of 240 mg showed sufficient lag time as compared to formulations coated with HPMC E5 and E15 with same concentration.

In this study (lag time) as the coated tablet i.e. PRT was placed in the dissolution medium, it was observed that the hydrophilic polymeric layer started erosion, which underwent progressive modification in terms of thickness and consistency. In the second phase of the

dissolution procedure, the coating layer gradually started to erode up to a limiting thickness. After this stage, a shell was ruptured under the pressure applied by the swelling of the core tablet and Labetalol was released.

All of this process contributed to a lag time capable of exhibiting a pulsatile release of the drug. The drug release profiles relevant to the coated tablet showed that a lag phase was followed by the quick delivery of the drug. As the formulation coated with HPMC K4M in concentration of 240 mg showed sufficient lag time as compared to other formulations, this formulation was considered as optimized formulation for FPRT.

Table 13: % swelling index of formulated PRT.

TIME	PRT E5220	PRT E5240	PRT E5260	PRT E15220	PRT E15240	PRT E15260	PRT K4M220	PRT K4M240	PRT K4M260
1 hr	9.25	10.24	18.06	17.64	18.48	19.21	20.11	20.27	26.43
2 hr	24.43	28.12	39.21	28.72	30.12	34.12	33.16	36.09	44.60
3 hr	41.28	45.79	51.56	46.16	47.23	49.56	48.32	51.02	60.57
4 hr				52.09	54.42	60.89	60.06	62.47	72.22
5 hr				60.99	63.15	70.06	66.51	68.34	78.69
6 hr							71.51	72.09	85.11

The swelling behavior of optimized PRT containing HPMC K4M was compared with other PRTs containing HPMC E5 and HPMC E15. The obtained results showed that the swelling front erodes faster for PRTs with HPMC E5 and the swelling front erosion was comparably slower in PRTs with HPMC E15 and K4M due to their marked viscosity properties (figure).

In swelling index study, an increase in thickness of rubbery layer of PRT with HPMC K4M was higher as compared with PRTs with HPMC E5 and HPMC E15. This result may be attributed to complete penetration of solvent and high viscosity of the HPMC K4M [39]. A direct correlation between swelling and lag time was observed and found that the formulations having maximum swelling indices showed higher lag time.

SUMMARY AND CONCLUSION

The present investigation revealed that the coating of core tablet with 240 mg of HPMC K4M and top coating of PRT with floating layer containing 80 mg HPMC K100M and 25 mg sodium bicarbonate provided desired lag time required for chronotherapy of hypertension. The optimized formulation showed 6 h of pulsatile release lag time, 4.4 min floating lag time and greater than 14.3 h of floating time. *In vitro* drug release study showed 81.21 % drug release in 0.1N HCL within 30 min, indicating burst release. From the experimental findings it

can be concluded that floating pulsatile tablets of Labetalol can give efficient therapy by reducing dose and dosing frequency and provide chronotherapy for an effective management of morning surge of hypertension.

Pulsatile drug delivery systems or chronotherapeutic drug delivery systems are designed to release drug as a pulse manner after a pre-determined lag time to increase the drug release at the site of action of a disease according to circadian rhythm at right time and right amount, press coating or compression coating was used. This techniques increase the lag time and shows release according to the need of the pathophysiology of the disease compared to conventional tablets thereby increasing the bioavailability.

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