kidneys and faeces expel a large amount of Nebivolol. The half-life of Nebivolol binds to 98 percent of proteins and is mostly metabolized. Nebivolol was discovered to be 12 h in CYP2D6 extensive metabolizers and 19 h in poor metabolizers. In CYP2D6 very high (96%); whereas, it is only about 12% in substantial metabolicizers. The medicine must be given swiftly into the systemic circulation to improve solubility, dissolutions and bioavailability while bypassing first-pass metabolism. It should also be delivered early in the morning when there is a higher risk of hypertension [8].

As an outcome, it is important to develop Nebivolol as a gastro-retentive polymer-coated pulsatile tablet composed of liquid, solid composite, which may enhance Nebivolol release and bioavailability at the required time by delaying drug release and releasing the maximum amount of drug at the time when hypertension is present, where drug release can be controlled by lag time.

INTRODUCTION
Cos of the patient compliance and cost involved in therapy paves the oral route act as the most popular way for medications to be consumed among the many routes of drug administration. However, knowing the exact fate of medications following oral delivery in the body necessitates knowledge of gastrointestinal physiology (GIT). Drug absorption from the GIT is a complex procedure since it is difficult to contain and identify the system inside expected GIT areas and absorption varies depending on GIT conditions [1-3].

For some diseases with circadian rhythms in symptoms, such as cardiovascular disease, arthritis, bronchial asthma, cancer, duodenal ulcers, diabetes and neurological disorders. It is critical to deliver the maximum amount of drug at the time when symptoms are observed, with a lag time controlling drug release. For example, myocardial infarction and cardiac arrest are more likely from morning to noon in cardiovascular disorders; the best anti-hypertensive and anti-angina medicine should be given in the morning [4, 5]. Pulsatile drug delivery systems (PDDS) are better for treating such disorders since they are characterized by a slow initial release of the drug, followed by a rapid and full release of the drug after a brief lag time. The majority of PDDS are reservoir systems with a barrier layer that dissolves, erodes, or ruptures with time, allowing for rapid drug release from the reservoir. Traditional PDDS, on the other hand, release the medication after 5-6 h and in physiological settings, typically in the large intestine. In previous work, we generated timed-release pulsatile formulations for several medications using tablet and capsule technology [6, 7].

Nebivolol binds to 98 percent of proteins and is mostly metabolized in the liver (through CYP2D6), with a half-life of roughly 10 h. The kidneys and faeces expel a large amount of Nebivolol. The half-life of Nebivolol was discovered to be 12 h in CYP2D6 extensive metabolizers and 19 h in poor metabolizers. In CYP2D6 metabolizers with low metabolism, the bioavailability of Nebivolol is very high (96%); whereas, it is only about 12% in substantial metabolicizers. The medicine must be given swiftly into the systemic circulation to improve solubility, dissolutions and bioavailability while bypassing first-pass metabolism. It should also be delivered early in the morning when there is a higher risk of hypertension [8].

As an outcome, it is important to develop Nebivolol as a gastro-retentive polymer-coated pulsatile tablet composed of liquid, solid composite, which may enhance Nebivolol release and bioavailability at the required time by delaying drug release and releasing the maximum amount of drug at the time when hypertension is present, where drug release can be controlled by lag time.

MATERIALS AND METHODS
Aurobindo Pharma Pvt. Ltd. it is provided as a complimentary sample of nebivolol. Propylene glycol, avicel, aerosil, ethylcellulose and crosspovidone are all sourced from Himedia Labs Ltd in Chennai and are utilized in the manufacture of the dosage form.

Formulation of liquid, solid compacts pulsatile compressed coated tablets
A weighed amount of Nebivolol and a liquid vehicle (propylene glycol) are mixed together using a sonication process to achieve a homogeneous combination. The obtained liquid mixture is homogenized at 100 RPM in a prescribed amount of carrier material (Avicel) to guarantee that the liquid medication is uniformly spread throughout the carrier. The required amount of the coating ingredient (Aerosil) is next weighed and mixed together uniformly.

The prepared powder mixture is spread as a homogeneous layer on the surface of a mortar and allowed to stand for 5 min to allow complete absorption of the drug medication into the internal structure of the carrier and coating components. Add the required quantity of disintegrants (Crosspovidone) to the aforementioned mixture to create a final liquid solid system. The resultant liquid solid system was crushed into a tablet using an 8 mm tablet punch in a tablet compression machine. The weighted ethyl cellulose granules were then inserted in a 16 mm die cavity, the previously crushed tablet was kept centrally, and the pulsatile core tablet was
compressed. It should be noted that the tablet’s compression force, weight fluctuation and hardness should all be changed depending on the situation. The formulation and optimization strategy for the Nebivolol solid-liquid pulsatile tablet are shown in Table 1 [9-15].

### Table 1: Absolute values of levels of independent variables employed in 2³ factorial design for optimization of nebivolol pulsatile liquid, solid composite compressed tablet

| Batch No | Nebivolol conc. in vehicle (gsw/wt) X1 | Conc. of carrier (Avicel) (mg) X2 | Disintegrants (Crosspovidone) (mg) X3 | Conc. of coating material (Aerosil) (mg) | Liquid vehicle (propylene glycol) (mg) | Unit dose (mg) | Coating polymer granules |
|----------|---------------------------------------|-------------------------------|------------------------------------|-----------------------------------------|-----------------------------------------|---------------|-------------------------|
| LSC 1    | -1/10                                 | -1/87.5                       | -1/17.5                           | 5.125                                   | 90                                      | 205           | *                       |
| LSC 2    | 1/20                                  | 1/175                         | 1/35                              | 7.75                                    | 80                                      | 310           | 70 (CT1) by HPMC K4M    |
| LSC 3    | 1/20                                  | -1/87.5                       | 1/35                              | 5.5625                                  | 80                                      | 222.5         | 77.5 (CT2) by HPMC K4M  |
| LSC 4    | 1/20                                  | 1/175                         | -1/17.5                           | 7.3125                                  | 80                                      | 292.5         | *                       |
| LSC 5    | 1/20                                  | 1/175                         | -1/17.5                           | 7.3125                                  | 80                                      | 292.5         | *                       |
| LSC 6    | 1/20                                  | -1/87.5                       | 1/35                              | 5.5625                                  | 80                                      | 222.5         | 97.5 (CT3) by EC        |
| LSC 7    | -1/10                                 | 1/175                         | 1/35                              | 7.75                                    | 90                                      | 310           | 80 (CT4) by EC          |
| LSC 8    | -1/10                                 | -1/87.5                       | -1/17.5                           | 5.125                                   | 90                                      | 205           | *                       |
| LSC 9    | 1/20                                  | -1/87.5                       | -1/17.5                           | 5.125                                   | 80                                      | 205           | *                       |
| LSC 10   | -1/10                                 | 1/175                         | -1/17.5                           | 7.3125                                  | 90                                      | 292.5         | *                       |
| LSC11    | -1/10                                 | -1/87.5                       | 1/35                              | 5.5625                                  | 90                                      | 222.5         | *                       |
| LSC12    | -1/10                                 | 1/175                         | 1/35                              | 7.75                                    | 90                                      | 310           | 45 mg+45 mg (CT5) (Both HPMC and EC) |

*The particular liquid solid composite was not selected to be compressed into a pulsatile tablet as per the data

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### Drug polymer interaction studies

#### DSC study

The melting point of samples was determined using DSC testing. It facilitates in the assessment of drug protection and drug-excipients compatibility; crystalline properties of Pulsatile Pellets formulations. DSC investigations were conducted on Nebivolol Pulsatile Pellets dispersion using the DSC-70, Schimadzu model equipment. Approximately 5 mg of the material was weighed and heated in aluminium pans at a rate of 20 °C/min with dry nitrogen as the effluent gas at a temperature range of 20-200 °C. An exothermic or endothermic peak form was used to determine the melting point [17].

#### Evaluation of the Pre-compression parameter of the solid-liquid composite granules

##### Angle of repose

The frictional forces in a granule combination are calculated using the angle of repose. It can be calculated by raising the funnel to a certain height and pouring the mixture through it under gravity, resulting in a pile. The height and surface of the pile, as well as the angle of repose, can be calculated using the equation after constructing a sharp edge on the pile.

\[ \theta = \tan^{-1} \left( \frac{h}{r} \right) \]  
(Eq. 1)

\( \theta \) = angle of repose; \( h \) = height of pile; \( r \) = radius of pile

##### Bulk and tapped density

Pour the granules or pellets (W) into a graduated cylinder after carefully weighing them. The cylinder’s volume (V0) was computed for bulk density and then the cylinder was tapped hundred times on a wooden panel for tap density, and the cylinder’s volume (W) was noted. The following formulas are used to calculate bulk and tapped density.

\[ \text{Bulk density} = \frac{W}{V_0} \]  
(Eq. 2)

\[ \text{Taped density} = \frac{W}{V_t} \]  
(Eq. 3)

\( W \) = Weight of the pellets or granules; \( V_0 \) = Initial volume; \( V_t \) = Final volume

##### Hausner’s ratio

Hausner’s ratio is the ratio of tapped density to bulk density. The lower the value of Hausner’s ratio, the better the flow property. The ratio is calculated using the formula below.

\[ \text{Hausner’s ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \]  
(Eq. 3)

### Carr’s index

By multiplying the difference between tapped and bulk density by 100, the percentage compressibility (Carr’s index) was calculated. It evaluates the particle-particle inter particulate interaction. The following is how the Carr’s index is calculated [18-20].

\[ \text{Carr’s index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \]  
(Eq. 4)

### Evaluation of post-compression parameter of pulsatile tablet

#### Weight variation

Weigh each of the 20 tablets individually. The average weight and percent weight variance must be calculated using the formula. After that, each tablet was weighed and compared to an average weight. The formula below shows the % weight variation of a tablet.

\[ \text{Percentage weight variation} = \frac{W_1 - W_2}{W_1} \times 100 \]  
(Eq. 5)

\( W_1 \) = Individual weight of tablet; \( W_2 \) = Average weight of tablet.

#### Thickness and diameter

To determine the thickness, randomly measure 20 tablets from each batch with a vernier caliper and use the mean, standard deviations to calculate the average thickness.

### Friability

20 tablets (W1) are weighed separately, then spun for 100 revolutions at 25 RPM on the Roche Friabilitator. The tablets are weighed to get the percent friability from the formula (W2). The percentage of friability should not exceed 1%, according to the IP limit. The following formula is used to compute the percent friability.

\[ \text{Percentage friability} = \frac{(W_2 - W_1)}{W_1} \times 100 \]  
(Eq. 6)

\( W_1 \) = Initial weight of tablets; \( W_2 \) = Final weight of tablets

#### Hardness

The force required to break the generated tablets is used to assess crushing strength. A pizer hardness tester is used for the test. The IP limit should be between 4-6 kg/cm².

#### Wetting time

The hydrophilicity of the excipients and the internal structure of the tablet determine this. It’s time to wet the bed. A piece of double-folded tissue paper was put into a Petri plate (internal diameter 6.5 cm).
cm) containing 6 ml of water. The tablet was placed on the paper, and the time it took to completely moisten the tablet was measured in seconds. The procedure was somewhat altered by keeping the water at 37 degrees Celsius.

**Disintegration time**

Six tablets should be put in the tube of the disintegration apparatus. The tube is immersed in a phosphate saline buffer (pH 6.8) and the amount of time it takes for the tablet to dissolve must be noted. Tablets coated with ethyl cellulose have no disintegration time and disintegrate in 60 min in buffer [21, 22].

**In vitro drug release studies**

Tablets are inserted in a six-station USP Type II dissolution test apparatus with 900 ml of phosphate buffer saline standard conditions at room temperature (pH 7.4). Aliquots are withdrawn every 5 min and a new volume of buffer is supplied. The absorbances of aliquots were measured at 280 nm in a UV visible spectrophotometer. By measuring absorbance, samples are withdrawn at different time intervals to quantify the percent of drug release in each interval, and the same quantity of buffer is provided to maintain sink conditions. The cumulative percent in vitro drug release studies were plotted on a graph with time in h on the x-axis and cumulative percent drug release on the y-axis [23-26].

**In vivo pharmacokinetic studies for nebivolol pulsatile tablet in albino wistar rats**

All treatments were involved with Albino Wistar Rats weighing 180-250 g, male and aged 6-8 w were certified by the Institutional Animal Ethical Committee of Sri Venkateswara College of Pharmacy in Chittoor, India, and were documented with Certificates of Conformity: 1044/P0-Re/315/CPCSEA from CPCSEA. They were housed in a controlled setting (25 °C and 12 h/12 h light/dark cycle) with six rats per cage for a week. The animals were given standard laboratory animal food and had free access to drinking water. The care and handling of the animals were taken in accordance with the internationally accepted standard guidelines for the use of animals. The study was submitted to and approved by IAEC ethics committee. The animals were grouped for treatment as follows. A total of 18 male Albino Wistar Rats with each of 180-250 gm were randomly divided into 3 groups for the pharmacokinetics study as Group A (Marketed Nebivolol Tablet Nebicard® 4 mg/kg/oral); Group B (Nebivolol direct compressed coated tablet (CT3) 4 mg/kg). After an overnight fasting (withdrawing food, but not water), group A was given a single dose (4 mg/kg) of Nebicard. Group B was given a Nebivolol direct compressed tablet that was administered orally as a single dose and Group C was given Nebivolol pellet compressed coated tablet (CT3). About 0.5 ml of blood samples were collected from all the groups by retro-orbital venous plexus with heparinized capillary tubes at the time of interval of 0.2, 4, 6, 8 hr. The blood samples were collected in a tube containing anticoagulant ammonium oxalate (1% concentration). Plasma was separated from the collected blood samples by the refrigerated centrifugation at 10,000 rpm for 10 min and analyzed in HPLC reversed-phase (C18) column with isocratic mobile phase for 2 min. The calibration curve was found to be linear over the range of 0.04 to 0.32 µg/ml (R² 0.998). The drug was analyzed by extracting the plasma with trichloromethane [27, 28].

**Sample preparation from nebivolol plasma drug concentration**

About 1 ml of treated animal blood was centrifuged at 5000 RPM for 15 min and 0.5 ml of plasma was collected into a Hertfordoff tube and treated with 150 l of Acentonitrile before vortexing for 20 seconds. The protein precipitation required more than 75 percent of the acetonitrile solution. Centrifuge the medication from plasma for 10 min at 4 °C at 20,000 rpm. To determine the unknown plasma drug concentration from an unknown peak area, the supernatant solution was isolated and injected into HPLC [28].

**Quantification of nebivolol in plasma**

Quantification of Nebivolol in Plasma was carried out by using HPLC (Waters-2695 series, Bangalore, India). Reversed-phase C18 column (250 mm X 4.6 mm i.d., Particle size-5 µm) was used as a column with a mixture of Methanol and water [80:20, V/V] as mobile phase. The flow rate of the mobile phase in the column was adjusted to 1 ml/min with an Injection Volume of 5 µl. The wavelength maxima of Nebivolol were discovered using the following approach, which had a run time of 10–20 min and a sample detection wavelength of 280 nm. UV-Vis detection at 280 nm was used to construct a calibration curve for 8 solutions of Nebivolol in phosphate buffer pH 7.4 concentrations ranging from 0.04-0.32 g/ml [27-29].

**Statistical analysis**

The following data are considered response variables in pharmacokinetic analysis: Cmax, tmax, AUC0-to-

**Stability studies**

This study used the optimized pulsatile compression coated tablet (CT3). Each formulation was divided into two batches and stored for three months at 25 °C±2 °C/60% RH±5% RH; 40 °C±2 °C/75% RH±5%RH for 3 mo. Each sample from both the storage condition were analyzed and reported to determine the compatibility of the drug and excipients used in the formulation. It is also used to determine the polymeric effect in the formulation. It shows that the exothermic melting point of pure nebivolol was found to be 187.34 °C and physical mixture, i.e. the mixture of the drug along with the occupants was found to be 185.35 °C which was reproducible as in pure drug. It indicates the exipients used in the formulation were highly compatible with the drug and there was no polymorphic effect or drug degradation. Thus, the optimal melting point was assumed to be reproducible in the physical mixture as compared to pure Nebivolol. It was verified that the drugs and excipients used in the formulations have been found to be mutually compatible. The crystallinity of the polymer can be measured by heat quantification associated with melting of the polymer. DSC thermogram of pure drug Nebivolol and Physical mixture shows a reproducible melt peak temperature, which means that the identical property of polymer was not disturbed and it has its own ideal melting point, density, permeability and storage modulus. Which confirms that the polymer used in the formulation are highly compatible to the drug Nebivolol.

**Measuring of pre-compression parameters of liquid, solid compacts prepared for the tablet**

The pre-compression parameters for all liquid compacts LSC1 to LSC12 are performed. According to the data, the Angle of repose, Carr’s index and Hausner’s ration data were determined to be good flow properties for LSC6, LSC12 and it is less than The f low properties are excellent when blended with excipients. The granules’ bulk density suggests that they are packed well. The flow property is excellent when blended with excipients. The granule bulk density shows that they have an excellent packaging quality.
For LSC6 and LSC12, the Carr’s index was determined to be less than 15% for all formulations, indicating satisfactory flow characteristics. The Hausner’s ratio was less than 2% for all of the granules. Because the angle of repose and compressibility index measurements demonstrate that granules flow well, direct compression is used for tablet formation.

**Table 2: Optimization of nebivolol liquid compacts for pulsatile tablet formulation 2³ factorial design and effect of independent variable dependent variable**

| Run | Independent variable (Level codes and its concentration) | Dependent variable |
|-----|----------------------------------------------------------|--------------------|
|     | X1            | X2       | X3 | Disintegration time (Sec) Y1 | Wetting time (Sec) Y2 |
| LSC1| -1            | -1       | -1 | 15±2.66                       | 46±2.16               |
| LSC2| 1             | 1        | -1 | 65±2.16                       | 22±2.12               |
| LSC3| 1             | 1        | 1  | 74±2.24                       | 24±2.06               |
| LSC4| 1             | 1        | 1  | 95±2.68                       | 34±2.08               |
| LSC5| 1             | 1        | -1 | 130±2.72                      | 45±2.12               |
| LSC6| 1             | -1       | 1  | 70±2.88                       | 20±2.32               |
| LSC7| -1            | 1        | 1  | 90±2.42                       | 32±2.36               |
| LSC8| -1            | -1       | 1  | 170±2.34                      | 54±2.34               |
| LSC9| 1             | -1       | 1  | 165±2.30                      | 52±2.40               |
| LSC10| -1           | 1        | 1  | 155±2.24                      | 48±2.62               |
| LSC11| -1           | -1       | 1  | 90±2.32                       | 35±2.24               |
| LSC12| -1           | 1        | 1  | 80±2.42                       | 32±2.22               |

*Data are expressed as mean±SD (n=3)

The 2³ factorial optimization design and its result are shown in table L2 revealed about the effect of independent variables like Drug Concentration (Nebivolol) in the liquid vehicle (Propylene glycol) (% w/w); Concentration of Carrier (Avicel) the formulation (% w/w); Concentration of super disintegrants (Crosspovidone) in the formulation (% w/w) in mg on dependent variables like disintegration time and wetting time in sec during the preparation of Nebivolol solid liquid composite compressed tablet. According to the results, there was a substantial relationship between disintegration time and disintegrating agent, as well as a carrier substance. It was demonstrated from the surface response graph that on increasing the disintegrating agent, it shows a reduction in disintegration time of the solid-liquid composite tablet. With ANOVA, the 'p' value for executing the disintegrants vs. disintegrating time in sec was determined to be 0.05, indicating a significant difference in disintegration time when the concentration of Superdisintegrants like crosspovidone is increased. At high+1 level disintegrating agent (i.e. 10% of crosspovidone), LSC6 formulation showed a desired disintegration time of around 70±2.88 sec when compared to other formulations. Increased vehicle concentration in the manufacture of solid liquid compacts resulted in a decrease in the wetting time of the solid liquid compact tablet, confirming the tablet shows rapid disintegration. The 'p' value was found to be 0.05 by establishing it in ANOVA, confirming that raising the concentration of vehicle caused a significant change in wetting time. At high+1 levels of vehicle and disintegrants concentration, with low levels of carrier substance, LSC6 formulation demonstrated a quick wetting property of tablet of about 20±2.32 sec. LSC2, LSC3, LSC6, LSC7 and LSC12 formulations were discovered to be the optimal solid-liquid compacts compressed tablet formulations, which may be ideal candidates for further evaluation parameters like in vitro drug release studies, based on the optimization data. The polynomial equations were created using the coefficient values from optimal design, which were generated by changes in the independent variable depending on the dependent variables:

**Disintegration time (DT) = 110.83-11.66 X1-8.33 X2-33.33 X3 ... (Eq. 7)**

**Wetting time (WT)= 37.25-3.96 X1-1.75 X2-9.25 X3 ... (Eq. 8)**

**Comparative in vitro drug release studies for best LSC compressed core tablets**

![Fig. 2: In vitro drug release of liquid solid compact compressed core tablet LSC2 Vs. LSC3 Vs. LSC6 vs. LSC7 vs. LSC 12 [mean±SD (n=3)]](image)
The selected optimized LCS2, LCS3, LCS6, LCS7 and LCS12 liquid solid compacts compressed tablet was performed to in vitro drug release tests in 6.8 pH phosphate buffer. When comparing the percent cumulative quantity of drug release are shown in fig. 2, it was established that the LSC6 formulation has the highest amount of drug release in a sustained manner (99.82 ± 2.54% in the 12 h time interval). As a result, the formulation containing 10% crosspovidone and 25% avicel carrier exhibits sustained and maximal level of drug release over a 12-h period.

| Evaluation parameters | CT 1               | CT 2               | CT 3               | CT 4               | CT 5               |
|-----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Hardness (Kg/cm²)     | 5.84±0.2           | 5.68±0.2           | 5.52±0.4           | 5.42±0.2           | 5.42±0.12          |
| Friability (%)        | 0.54±0.02          | 0.58±0.02          | 0.62±0.04          | 0.52±0.04          | 0.60±0.02          |
| Thickness (mm)        | 5.4±0±00           | 5.4±0±00           | 5.4±0±00           | 5.4±0±00           | 5.4±0±00           |
| Weight variation (mg) | 375±2.8            | 376±2.6            | 374±2.8            | 376±2.6            | 375±2.2            |
| %Drug content         | 89.7±4.23          | 86.24±2.42         | 97.20±2.66         | 90.64±2.46         | 89.32±1.10         |

*Data are expressed as mean±SD (n=3)

The press coated pulsatile tablet is formulated and compressed as shown in table 1 and evaluated for hardness, thickness, friability, weight variation and %drug content as shown in table 3. The recommended hardness for the compressed coated tablet was found to be 4-8 kg/cm² and the 5 batches from CT1 to CT5 was in this range from 5.42±0.2 to 5.84±0.2 kg/cm². The thickness of all tablets is found to be within limits from 5.42 to 5.48 mm. When compared to core tablet, the compressed coated tablet showed increase slightly in thickness due to coating. The average weight variation of tablets was found to be within the range from 374.6±2.8 mg to 376.6±2.6 mg, i.e.±5% as per USP, which indicates that the weight variation of compressed tablet was within limits. The friability test of prepared tablets was found to be 0.52±0.04 to 0.62±0.04 % and it was found to be within the range i.e.<0.8%. The % drug content for all prepared tablets was also found within the range i.e.>85%. But CT3 tablet shows maximum % drug content when compared to other formulations i.e. 97.20±2.66% when compared to all other formulations. Press-coated tablets were evaluated for drug release in 0.1N HCL, 4.0 pH and 6.8 pH phosphate buffer and the results are shown in fig. 3. From the data it was found that CT1 and CT2 core tablet are coated with two different concentrations of HPMC K4M polymer and it shows less concentration of drug release i.e. 85.23±1.2%, 90.45±1.02 % at 8th time interval. CT3 tablets are coated with ethyl cellulose polymer, which shows maximum and well-controlled drug release from the core tablet i.e., concentration 96.34±1.2% at 8th time interval. It shows there was an efficient delay in drug release form core tablet, i.e., up to 3 h, followed by a maximum amount of drug release 96.34±2.4 at 8h. Which shows the core drug will be more efficiently protect from gastric acid environment i.e pH and duodenal environment 4.0 pH and release the drug only in small intestinal pH. The formulation CT4 shows very less concentration of drug release from the core tablet, i.e.<80% of drug release, it may be due to more control of drug release pattern from core tablet due to concentration and thickness of ethylcellulose coating, and due to this it is not forming pore network in the coated membrane. Varying concentrations of ethylcellulose incorporated controlled the drug release. This may be attributed due to decreased penetration of the solvents molecules in the presence of the hydrophobic polymer coat, leading to reduced diffusion of the drug from the core tablet. As drug release continues, the polymer swells and increases the pore network through which interior drug clusters can diffuse more efficiently in a controlled manner. The CT5 data shows that, although the incorporation of EC controlled drug release to some extent, the combination of this polymer with HPMC decreases furthermore release of the drug i.e. 73.54% in 8 h from Nebivolol core tablet formulation. The reason might be that its large hydrophobic molecules and hydrophilic polymer molecule form more coating on the surface, leads to more control of drug release than expected. The report shows that the extent of polymer swelling and the hydration of the microstructure formed within the gel layer also vary with the degree of polymer interaction with hydrating media. From the above discussed in vitro drug release data, it was found that Formulation CT3 (EC) were found to be an optimized compressed coated pulsatile tablet, based on the drug release pattern limitation that given in United states pharmacopoeia (USP) i.e. USP limit-NMT 10% drug release in 0.1HCl and NLT 75% in 6.8 pH buffer.

The data obtained from dissolution studies of the entire formulations CT-CT5 were fitted to various kinetic equations such as zero order, first order, Higuchi’s model, Korsmeyer Peppas. From table 4, it was observed that the “n” value for all the formulations was found to be greater than 1, which shows that the drug release was found to follow super case 2 transport. Super case 2 transport means the release of drug from core tablet through coated membrane by stress-induced relaxation of polymer, i.e. relaxation takes place at a sharp boundary separating an outer swollen coated polymer shell, essentially at penetration at equilibrium, from unpenetrated core material. As the drug release was best fitted in first-order kinetics, it indicated that the rate of drug release is concentration-dependent. CT2, CT4 and CT5 drug release mechanism was best explained by zero-order equation, as the plots showed the highest linearity (r² = 0.876, 0.9854, 0.9879, 0.8726). As the drug release was best fitted in zero-order kinetics, it indicated that the rate of drug release is concentration-independent. For optimized formulation CT3, “n” value was found to be 1.937, which shows the mechanism of drug release is super case II transport. And also it shows the R² value as 0.9854 as fit to higuchi model, which confirms that the release of drug based on diffusion mechanism.

Fig. 3: In vitro drug release profile of compression coated tablet CCT1 to CCT5
Table 4: *In vitro* release kinetics of compression coated tablet CT1 to CT5

| Formulation | Zero order R² | First order R² | Korsmeyer–peppas n | Higuchi R² | Best-fit model |
|-------------|---------------|----------------|---------------------|----------|----------------|
| CT-1        | 0.8196        | 0.8989         | 0.7511              | 2.012    | 0.9981         | Higuchi       |
| CT-2        | 0.9206        | 0.8894         | 0.7534              | 1.975    | 0.876          | Zero order    |
| CT-3        | 0.8174        | 0.8986         | 0.7566              | 1.937    | 0.9854         | Higuchi       |
| CT-4        | 0.9363        | 0.8926         | 0.7582              | 1.891    | 0.8799         | Zero order    |
| CT-5        | 0.9385        | 0.8809         | 0.7611              | 1.883    | 0.8726         | Zero order    |

Table 5: Comparison of physiochemical properties of optimized pulsatile compression coated tablet after stability studies

| Evaluation parameters | CT 3 | After storage at 25 °C±2 °C/60% RH±5% RH for 3 mo | After storage at 40 °C±2 °C/75% RH±5%RH for 3 mo |
|-----------------------|------|--------------------------------------------------|--------------------------------------------------|
| Hardness (Kg/cm²)     | 5.52±0.4 | 5.52±0.4                                       | 5.52±0.4                                       |
| Weight variation (mg) | 374.6±2.8 | 374.6±2.2                                       | 374.6±2.4                                       |
| %Drug content         | 97.20±2.66 | 97.12±2.68                                       | 96.98±2.42                                       |
| %CDR at 8th h         | 96.34±1.2 | 96.34±1.2                                       | 96.34±1.2                                       |

*Data are expressed as mean±SD (n=3)*

Stability of a drug in a dosage form at different environmental conditions is important as it determines the expiry date of that particular formulation. Among all the 5 (CT1-CT5) formulations, optimized formulations CT5 were selected for stability studies. The stability studies of the optimized formulation CT5 are shown in table 5 and fig. 4. The stability studies are carried out at 40 °C±2 °C/75%±5% RH as per ICH guidelines over a period of 3 mo. There is no significant change in their physical appearance, average weight, hardness of tablets. The release profile and the drug content also did not show any significant changes indicating that there were no changes in the physical as well as chemical characteristics of the formulation. Hence, it can be concluded from the results that the developed tablets were stable and retain their pharmaceutical properties over a period of 3 mo at room temperature (25 °C±2 °C/60% RH±5% RH) as well as stressed temperature (40 °C±2 °C/75% RH±5%RH) condition.

Pharmacokinetic studies of various nebivolol formulations

Table 6: Comparative *in vivo* pharmacokinetic study data between nebivolol treatment groups

| Parameter | Nebicard® (4 mg/kg)-Oral administration (Marketed nebivolol plain formulation) | Nebivolol direct compressed tablet (4 mg/kg) Peroral | Nebivolol pellet compressed coated tablet (CT3) (4 mg/kg) |
|-----------|---------------------------------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------|
| Tmax (h)  | 2                                                                                | 2                                                   | 4                                                      |
| Cmax (µg/ml) | 0.1648                                                                         | 0.1542                                              | 0.218                                                  |
| AUC 0-α (µg/ml/h) | 121.242                                                                       | 126.540                                             | 392.032                                                |
| MRT 0-α (h) | 8                                                                               | 8                                                   | 15                                                     |
| F rel= (AUC)drug/(Dose)rel*(AUC)oral/(Dose)oral |                                   |                                                     | Bioavailability enhanced by 3.11%                     |

*Note: Increase in AUC; MRT; Tmax; Increase in Cmax in Nebivolol Pellet compressed Coated Tablet (CT3) shows better enhancement of bioavailability than other two dosage forms*

The pharmacokinetic parameters of Nebivolol solid-liquid composite compressed Coated Tablet (CT3) are shown in table 6 and fig. 6. *In vivo* pharmacokinetic plasma drug concentration profiles were shown in fig. 6. Concentration of the drug in blood was estimated to 8h using a validated HPLC method. Nebivolol has maximum plasma concentration (Cmax) i.e., 0.1648 µg/ml at Tmax 2h, by administering a marketed dosage form (Nebicard® tablet). The maximum drug concentration declines rapidly in conventional
From the research findings and in vivo pharmacokinetic evidence, it was concluded that the pulsatile press coated tablet loaded with Nebivolol showed improved bioavailability than the conventional marketed dosage form, by improving the plasma drug concentration profile of AUC and MRT. Therefore, for poorly bioavailable BCS class II drugs such as Nebivolol, Nebivolol Pulsatile compressed coated tablet would be a promising drug delivery system and also delay the release of Nebivolol by formulating into Pulsatile liquid solid composite matrix tablet with Ethyl cellulose polymer and also protection of drug from degradation pathways like acid degradation in the stomach, first-pass metabolism and enzymatic degradation. These discussed data prove that, Nebivolol Pulsatile compressed Coated Tablet confirms enhancement of bioavailability by 31.1% when compared to the conventional dosage form. Hence, Nebivolol Pulsatile compressed Coated Tablet was a suitable drug delivery for Nebivolol which enhance the bioavailability.

CONCLUSION

From the research findings and in vivo pharmacokinetic evidence, it was concluded that the pulsatile press coated tablet loaded with Nebivolol showed improved bioavailability than the conventional marketed dosage form, by improving the plasma drug concentration profile of AUC and MRT. Therefore, for poorly bioavailable BCS class II drugs such as Nebivolol, Nebivolol Pulsatile compressed coated tablet would be a promising drug delivery system and also delay the release of Nebivolol by formulating into Pulsatile liquid solid composite compressed coated tablet, do that it will release the Nebivolol drug at early morning hours when there is more risk of hypertension without pill burden on early morning hours. And also this research paves the way to improve the bioavailability of Nebivolol by controlling the drug release through the coated polymer.

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AUTHORS CONTRIBUTIONS

All the authors are involved in the review of literature, collection of data and preparation of the manuscript and also they were involved in reviewing and editing of the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest for this research

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