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DESIGN AND *INVIVO* EVALUATION OF METOPROLOL TARTARATE PULSINCAP DRUG DELIVERY SYSTEM

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ABSTRACT

The chronological behaviour of Hypertension confirms increased blood pressure at early morning hours, need a preferable dosage form which will provide desired concentration of the drug at pre-determined time points especially in early morning hours. Pulsatile dosage forms are designed to mimic the circadian rhythm by releasing the drug at the desired time, by means of an internal preprogrammed designed dosage form that is initiated when the dosage form comes in contact with gastrointestinal fluids especially in colon. The prepared dosage forms were optimized and evaluated both in vitro and in vivo evaluation. Metoprolol tartarate, a selective beta blocker pulsincaps were formulated using, body of the capsules with modified solubility by coating with formaldehyde and polymer plug of Eudragit L 100 was fitted in mouth of body of the capsule. The results of the final two optimized formulation P1 and P2 was found to ideal for pulsatile release. The maximum in vitro drug release of 101.24± 1.24 and 101.08± 1.54 % (first pulse Metoprolol tartarate granules immediately release the drug after dinner from the uncoated cap of the capsule), 94.84±1.4% and 97.74±1.8 (Second pulse at early morning hours after lag time in Colonic pH). The selected formulations of Metoprolol tartarate pulsincaps (P1 and P2) were subjected for pharmacokinetic studies using male albino rats. From the Pharmacokinetic studies, in-vivo studies shown that increased AUC, relative bioavailability and delayed time of Metoprolol tartarate pulsincap proves the Sustainability and targetability of drug in colon.

Key Words: Colon targeting, Pulsatile drug delivery system, Metoprolol tartarate, Chronomodulated drug delivery system.

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INTRODUCTION

The overall goal for optimum therapy is to match the needs of the patient while improving the efficiency and safety of the administered drugs. Various drug delivery approaches have always played a challenging and crucial role in ensuring and predicting the delivery of promising and successful drugs to the target site of delivery in the human body. Colon specific drug delivery has gained increased importance not just for the delivery of drugs for the treatment of local diseases associated with the colon but also as potential site for the systemic delivery of therapeutic peptide and

proteins(1). To achieve successful colon targeted drug delivery, a drug needs to be protected from degradation, release and/or absorption in the upper portion of the GI tract and then ensure abrupt or controlled release in the proximal colon. Drug modifications through covalent linkages with carrier or prodrug approach and formulation based approaches can be used for colonic delivery (2).

Pulsatile systems are designed according to the circadian rhythm of the body. Circadian rhythm regulates many body functions in humans, viz., metabolism, physiology, behavior, sleep patterns, hormone production, etc. It has been reported that more shocks and heart attacks occur during morning hours. The patients suffering from diabetes are reported to have high blood sugar levels after meals compared to other timings. The pulsatile effect, i.e., the release of drug as a pulse after a lag time has to be designed in such a way that a complete and rapid drug release should follow the lag time. Such systems are also called time controlled as the drug released is independent of the environment. These systems beneficial for drugs having high first-pass effect, drugs administered for diseases that follow chronopharmacological behavior, drugs having specific absorption site in GIT, targeting to colon, and cases where night time dosing is required

Pulsatile drug release system, allows the release of active pharmaceutical material in single or successive pulses at precise and well controlled time periods. Assuming that physiological processes and biological functions display constancy over time, much effort had been devoted in the past in developing the drug delivery systems that maintain a flatter plasma level for an extended period of time. Pulsed or pulsatile drug release is defined as the rapid and transient release of a certain amount of drug molecules within a short time-period immediately after a predetermined off-release period called the lag time (3).

However, chronotherapy belief this concept. Along with many applications in local and systemic delivery of drugs, pulsatile release system would also be advantageous when a delay in absorption is desirable from a therapeutic point of view as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythm, such as nocturnal asthma, angina and rheumatoid arthritis.

So by developing the pulsatile device, plasma peak is obtained at an optimal time, number of doses per day can be reduced, saturable first pass metabolism and tolerance development can also be avoided. Chronotherapeutics refers to a clinical practice of synchronizing drug delivery in a manner consistent with the body's circadian rhythm including disease states to produce maximum health benefit and minimum harmful effects (4).

Such novel drug delivery has been attempted for: (i) chronopharmacotherapy of diseases which show circadian rhythms in their pathophysiology ; (ii) avoiding degradation of active ingredients in upper GI tract, e.g. proteins and peptides (iii) for time programmed administration of hormones and many drugs such as isosorbide dinitrate, respectively to avoid suppression of normal secretion of hormones in body that can be hampered by constant release of hormone from administered dosage form and development of resistance (iv) to avoid pharmacokinetic drug–drug interactions between concomitantly administered drugs etc

The chronological behavior of Hypertension confirms increased blood pressure at early morning hours, so there is a need of preferable dosage form which will provide desired concentration of the drug at pre-determined time points especially early in the morning. These dosage forms are designed to mimic the circadian rhythm by releasing the drug at the correct time, by means of an internal pre programmed designed dosage form that is initiated when the dosage form comes in contact with gastrointestinal fluids especially in colon(5)

MATERIALS AND METHODS

The materials used in the projects like Metoprolol tartarate are received as gift sample from Actavis ltd., Chennai, Sodium alginate from media lab, Chennai., Eudragit L 100 form Merck ltd., Mumbai, and other solvents used are in analytical standards. Softwares like Kinetica and Prism are used for Pharmacokinetic and statistical calculation.

Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It is defined as an investigation of physical and chemical properties of a drug substance. The overall objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms.

Formulation of First pulse immediate release Metoprolol tartarate granules

The first pulse Metoprolol tartarate immediate release (IR) granules were formulated using sodium starch glycolate, crosspovidone by wet granulation method. All the powders were passed through 60 mesh sieve.

Required quantity of drugs, polymers and diluents were mixed thoroughly and a sufficient quantity of granulating agent starch mucilage was added slowly to get dough mass. The mass was sieved through 22/40 mesh and dried at 50° for 2h.The dried granules retained on 40 mesh were mixed with 2% talc and 1% magnesium stearate. The representative proportions for all the formulations were given in table 1 (6)

Formulation of Second pulse Microbeads-Ionotropic Gelation Technique

The microbeads were prepared by the ionotropic gelation technique. The sodium alginate solution was prepared by dispersing the sodium alginate in de-ionized water under continuous stirring for 30 minutes. The weighed amount of the drug was thoroughly mixed with sodium alginate dispersion. By following the same procedure the alginate beads of different ratios of drug: polymer were prepared. The resulted homogeneous dispersion was extruded in to the 5% calcium chloride solution through hypodermic syringe with flat tip needle (20G) and stirred for 15 minutes at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for

30 minutes in the calcium chloride solution to complete the gelation reaction. The microbeads were then filtered and dried in hot air oven at 60ºC for 3 hr (7).

Designing of pulsincap dosage form

The formaldehyde coated body of the capsule was taken and filled with second pulse metoprolol tartarate microbeads and then plug in the mouth of the capsule with polymer plug. Polymer plug are formed by forming a mass of Eudragit L 100 with the help of suitable solvent and make into a handmade plug and allow drying at 40˚C. After that leave it in room temperature upto drying and the fill the uncoated cap portion of the capsule with immediate release optimized granules and then undergo for sealing the capsule (8) Figure- 9, 10.

EVALUATION PARAMETERS

Compatibility studies

Fourier transformed infrared (FTIR) spectroscopic analysis

Fourier transform infrared (FT-IR) spectra of the samples were obtained in the range of 400 to 4,000 cm−1. IR spectral analysis of pure drug and physical mixture of formulation were carried out. The peaks and patterns produced by the pure drug were compared with physical mixture.

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) analysis was performed using Nietzsche DSC 200PC (Nietzsche, Selb, Germany). The instrument was calibrated with indium (calibration standard, > 99.999%) for melting point and heat of fusion.

A heating rate of 100C/min was employed in the range of 25–2000ºC. Analysis was performed under nitrogen purge (20 mL/min). The samples were weighted into standard aluminium pans and an empty pan was used as reference (9).

Flow properties of IR granules & Microbeads

The flow properties of microparticles were characterized in terms of angle of repose, Carr's index and Hausner's ratio. Bulk Density: Apparent bulk density was determined by placing pre-sieved drug excipients blend in to a graduated cylinder. Measure the weight of the powder. 10 g of pre- weighed microparticles were transferred into a graduated measuring cylinder. The Thermonik bulk density apparatus was used to measure bulk density. The volume of the powder is measured. Bulk density of the powder can be determined by the formula given below (10).

LBD = Weight of Powder / Volume occupied by the Powder

Tapped Density: Tapped density was determined by USP method II. Formulation blend was filled in 100 ml graduated cylinder of tapped density tester which was operated for 100 number of tappings, thus was calculated by following formula.

$$Dt = M/Vb$$

Where, M = Weight of powder taken; Vb = tapped volume.

Compressibility Index and Hausner's Ratio:

This was measured for the property of a powder to be compressed, as such they are measured for relative importance of inter particulate interactions. Compressibility index and was calculated by following equation.

$$\text{Compressibility index} = [(Dt - Db)] / Dt \times 100$$

Where, Dt = tapped density; Db = bulk density;

Hausner's ratio was calculated by following equation

$$\text{Hausner's ratio} = Dt/ Do$$

Where, Dt = tapped density; Do = bulk density

Angle of repose: A funnel is fixed at a particular height 'h' cm on a burette stand. A white paper is placed below the funnel on the tablet. The given powdered drug whose angle is to be determined is passed slowly through the funnel, until it forms a pile, care is taken to see that the drug particles slip and roll over each other through the sides of the funnel. Further addition of drug is stopped as soon as the drug pile touches the tip of the funnel.

Circumference of the pile of drug is drawn with a pencil and measure the height of the pile without disturbing the pile. The radius of the pile is noted down as 'r' cm. and is calculated by following formula (11).

$$\text{Tan } \theta = h/r$$

$$\theta = \text{Tan}^{-1} h/r$$

Where, h = height of the pile: r = radius of the pile

Evaluation of Microbeads

Particle Size Determination

The particle size was measured using an optical microscope. The microparticles were dispersed in water, the size of the microparticles were determined by using calibrated eye piece micrometer. The particle size distribution was plotted and the average size was determined.

Surface morphology /Scanning Electron Microscopy (SEM)

The external morphology of the microparticles was studied by scanning electron microscopy. The sample of the SEM analysis was prepared by sprinkling the microparticles onto one side of the double adhesive stub. The stubs were then coated with gold using polaran SC 500 sputter coater, to neutralize the electrons and to obtain a clear morphology of the microparticles. The SEM was performed on microparticles after and before dispersing it in 0.1N HCl (12)

Micrometric properties of the beads:

The mean particle size of the alginate microbeads was determined by optical microscopic method using a pre-calibrated stage micrometer.

Flow properties of Beads:

The flow properties of prepared Microbeads were investigated by measuring the Angle of Repose by using fixed funnel method.[73] Depends upon these values, the flow properties of the Microbeads can be assumed. The Angle of Repose Values was mentioned in the Table-3. The value of angle of repose was calculated by using the following formula,

$$\textbf{Angle of repose } (\theta) = \tan^{-1} h/r$$

h = cone height, r = radius of circular base

The bulk and tapped densities were measured in a 10ml graduated cylinder as a measure of packability of the Microbeads. Each experiment was carried out in triplicate.

Drug entrapment efficiency or incorporation efficiency

To determine the drug entrapment efficiency or incorporation efficiency, the microparticles were crushed in glass mortar and powered, then suspended in 10 ml of methanol, after 24 h the solution was filtered and filtrate was analyzed for drug content spectrophotometrically at 274nm for Metoprolol tartarate. The drug incorporation efficiency was calculated by the following formula (13).

$$\text{Incorporation efficiency} = b/a \times 100$$

b = calculated amount of drug present in the formulation,

a = theoretical amount of drug present in the formulation

Drug content

Four portions each containing 200 mg were randomly picked from the prepared samples and were crushed with help of mortar and pestle. Then it was stirred continuously for 3h with simulated gastric fluid (pH 1.2). After 3 h, the samples were filtered, suitably diluted and estimated spectrophotometrically at 274nm for Metoprolol tartarate. The estimation was done in 3 replicates to determine the uniformity of drug in microparticles (14).

Production yield

The production yield of the microparticles can be determined by calculating accurately the initial weight of the raw materials and the last weight of the microparticles obtained (15).

$$\text{Production Yield (Y)} = \frac{\text{Practical mass of Microparticles}}{\text{Theoretical Mass (Polymer+drug)}} \times 100$$

Invitro drug release of first pulse immediate release Metoprolol tartarate granules

The first pulse Invitro release studies of metoprolol tartarate from IR granules was carried out in pH 1.2 phosphate buffer using USP XXII apparatus at 50 rpm maintained at a temperature of 37+1ºC for a period of 1h as the transit time in gastric pH. At periodic time intervals 5ml of sample was withdrawn suitably diluted and the extent drug released was determined spectrophotometrically at 274 nm (16).

Invitro drug release of Colon targeting second pulse - Pulsincap Metoprolol Tartarate Microbeads

The second pulse release of metoprolol tartarate was investigated using the USP rotating basket type dissolution apparatus at 50 rpm and maintain the bath with 37+1ºC.

The simulation of gastrointestinal transit conditions was achieved by altering the pH of the dissolution medium at various time intervals. The pH of the dissolution medium was kept at 1.2 for 2 h with 0.1 N HCl. Then, 1.7 g of KH2PO4 and 2.225 g of Na2HPO4.2H2O were added, adjusting the pH to 4.5 by adding 1.0 M NaOH. A release rate study was continued for another 2 h. After 4 h, the pH of the dissolution medium was adjusted to 7.0 and maintained for 24 h. The final volume in all cases was 500 ml. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a micro filter, and the filtrate was subjected to UV analysis at 274nm. All dissolution studies were performed in triplicate (17).

Invitro drug release kinetics

The release data obtained was fitted into various mathematical models using PCP disso-V2.08 software. The parameters 'n' and time component 'k', the release rate constant and 'R', the regression coefficient were determined by korsmeyer-Peppas equation to understand the release mechanism.To examine the release mechanism of drug from microparticles, the release data was fitted into the Peppa's equation.

$$Mt / M\infty = Ktn$$

Where, Mt / M∞ is the fractional release of drug, 't' denotes the release time, 'K' represents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusion exponent and characterize the type of release mechanism during the release process. If $n < 0.5$, the polymer relaxation does not affect the molecular transport, hence diffusion is Fickian.If $0.5<n<1.0$, the solid transport will be non – Fickian and will be relaxation controlled (18)

Evaluation of Metoprolol tartarate Pulsincap

Weight variation test

Weight variation test was carried out for plain Metoprolol tartarate pulsincaps. In this test twenty Metoprolol tartarate pulsincaps were weighed and the average weight was calculated. Then they were weighed individually. The percentage deviation of individual tablet or pulsincap from the average weight was calculated (19)

Drug content

Drug content study was carried out for Metoprolol tartarate pulsincaps. Standard preparation, chromatographic condition and the procedure as given in content uniformity determinations were applied for this drug content study also. For the Sample preparation twenty Metoprolol tartarate pulsincaps were weighed and powdered are taken out i/e both the granules and microbeads are taken out. Weighed accurately the powdered sample equivalent to 25 mg of Metoprolol tartarate and transferred in to a 25 ml standard flask. The sample was dissolved with 5 ml of water and diluted to 25 ml with acetonitrile.

Then the standard and samples were injected and the amount of Metoprolol tartarate present was calculated by measuring peak areas of standard and sample (20).

Pharmacokinetic Parameter Studies

RP-HPLC Assay of Metoprolol tartarate

Chromatographic conditions

Instrument- Shimadzu

Stationary phase- C18 column

Mobile phase- buffer: acetonitrile (1:4)

Flow rate-2ml / mt

Injection volume-20 µl

Detection wave length-274nm

Procedure

The stock solution of Metoprolol tartarate was prepared by accurately weighing 25 mg Metoprolol tartarate, transferring to 25 ml volumetric flask, dissolving in 5 ml of water and diluting it up to the mark with acetonitrile. Appropriate aliquot of this solution was further diluted to 10 ml with acetonitrile to obtain final standard solution of 100 µg/ ml of Metoprolol tartarate. Resultant solution was filtered through Whatman filter paper No.1. All the stock solutions were refrigerated (2- 80˚C when not in use).

The mobile phase consisted of sodium dihydrogen phosphate: acetonitrile (85:15, v/v) in Milli-Q water and the pH adjusted to 7.0 using sodium dihydrogen phosphate. The flow rate was fixed to 2ml/min with sample volume 20 µl and the mobile phase was filtered through a 0.22µ membrane and degassed using ultra sonicator injected into. The wave length used to read the values was 274 nm. The run time was set at 10 minutes. The concentrations of Metoprolol tartarate were quantified using HPLC technique. Consequently, based on the drug concentrations the AUC, Cmax and other pharmacokinetic parameters were calculated by using ―KINETICA software. The results were compared statistically by using ―graph pad prism software (21).

ANTI-HYPERTENSIVE ACTIVITY OF FORMULATION P1 & P2 AGAINST ADRENALINE INDUCED HYPERTENSION IN MALE ALBINO WISTAR RATS

Experimental animals

Male Wistar albino rats (n=6) of weighing 230-280 g were used for the present study. The animals were procured from animal house, Department of Pharmacology, Sun Institute of Pharmaceutical Education and Research, Nellore, Andhra Pradesh, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water *ad libitum (*22)

Adrenaline induced hypertension

Rats were anaesthetized with diethyl ether and 0.1 ml of adrenaline was injected into rats by intraperitoneal (I.P) injection using a 1 ml disposable syringe for 10 consecutive days to induce hypertension. To confirm the induction of hypertension, systolic and diastolic blood pressure was measured by using Non-invasive tail cuff method with Biopac Student Lab PRO 3.7 software (Model No. MP35) make BIOPAC Systems, Inc. 42 Aero Camino, Goleta, CA 93117.

The following procedure was adopted for administering the mini capsules to fully conscious rats. The oral administration tube was designed and long enough to reach the stomach. After insertion of tube to animals, the minicapsule was placed and blows the tube. So, the mini capsule was reached directly to the stomach. Animals were trained for 2 days using the dummy tube before starting the capsule administration. In this latter experiment rats received only water after the minicapsule administration (23).

Experimental design

Male albino wistar rats were divided into the five groups each group contain six animals

Control: Placebo mini pulsincap

Disease control: Adrenaline (0.5 mg/kg/100 µL, i.p) + Placebo mini pulsincap

Test sample 1: Adrenaline (0.5 mg/kg/100 µL, i.p) + Mini pulsincap formulation (P1) contain metoprolol 5mg/kg, p.o

Test sample 2: Adrenaline (0.5 mg/kg/100 µL, i.p) + Mini pulsincap formulation (P2) contain metoprolol 5mg/kg, p.o

Standard: Adrenaline (0.5 mg/kg/100 µL, i.p) + Pure drug of Metoprolol 5mg/kg, p.o

Method to measure Systolic and diastolic blood pressure using Biopac Student Lab with tail cuff apparatus

After administration of dose to animals, blood pressure was measured by Non-invasive Tail cuff method using pressure meter (NIBP250 and NIBP200). The rat was kept in strainer and the tail cuff was applied on the tail of rat for determination of blood pressure. Normal blood pressures of all the rats were recorded as baseline blood pressure. After that the animals were treated with respective treatment and again blood pressure was recorded as after drug treatment. The blood pressure as SBP (systolic blood pressure), DBP (Diastolic blood pressure), were displayed on monitor were recorded.

To evaluate anti-hypertensive effect of drugs, adrenaline was injected after 5 minutes. Again the blood pressure was recorded and the difference between baseline blood pressure and blood pressure after adrenaline treatment were calculated and compared (24).

RESULTS AND DISCUSSION

Compatibility study using IR and DSC

In the IR spectrum of Metoprolol tartarate standard consists of characteristics band values at 2933 cm-1(C-H-stretching) ,3344 cm-1 (N-H-stretching), 3340 cm-1 (OH- alcohol). These characteristic band values were observed in all the recorded IR spectra. i.e., for the Physical mixture band values are shown at 2989 cm-1(C-H- stretching), 3400 cm-1 (N-H-stretching), 3300 cm-1 (OH- alcohol).DSC of Metoprolol tartarate showed a sharp endothermic peak at 146.390C (melting point). The physical mixture of Metoprolol tartarate and other excipients also showed the same thermal behavior as the individual component i.e., a blunt peak at 149.090C (melting point). Results are shown in Fig 1-4. DSC results also revealed that the physical mixture of Metoprolol tartarate with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of Metoprolol tartarate and excipients. From the IR and DSC studies, it was found that there were no interaction took place between Metoprolol tartarate and the other ingredients used in the formulation of pulsincaps (25).

Evaluation of Formaldehyde Treated body of the Capsules

Solubility test

The solubility study results for the formaldehyde treated capsules showed that, only the cap dissolved within 5 mts but the body of the capsules remained intact for about 24 hrs in different pH 1.2, 4, 6.8 and 7. It shows that the immediate release first pulse granules which was filled in the cap of the capsule will be released within 5 minutes, but the Eudragit plug with body, filled by second pulse microbeads will be carried to the colon without degradation of shell of the body of the capsule by alteration in pH of the GIT

Chemical test for free formaldehyde

The chemical test was carried out to check the presence of free formaldehyde in body of the capsules. The results of chemical test for free formaldehyde showed that the intensity of colour produced in the sample solution was not more intensely colored than the colour produced in the

Standard solution. This result confirmed that less than concentration of free formaldehyde was present in the capsules body (26).

Micromeritics Parameters of first pulse Metoprolol tartarate Immediate Release granules

Among the Six formulations of IR granules, all the formulation showed good flow properties as shown in table 4. For bulk density was found within the range of 0.51±0.01 to 0.55±0.05, tapped density is within range of 0.60±0.02 to 0.60±0.02, it shows good packing efficiency of granules. Hausner's ratio is within the range of 1.1±0.06 to 1.18±0.03, which indicate good flow character, when compared to limits (USP Limits 1.12-1.18).Compressibility Index (%) is within range of 11.35±0.95 to 14.12±1.88, which showed good compressibility of the powder. When compared to limits (USP Limits 10-15). Angle of repose was found within range of 22˚80'±0 ˚ 85' to 26 ˚52'±1 ˚42, which indicates that powder exhibit good flow properties, when compared to limits (USP Limits 25° to 30°) (27).

Invitro drug release

The in vitro drug release profiles of all IR formulations containing SSG, CP superdisintegrants have been shown in (Figure 5). The release of IR Metoprolol mainly depends upon the concentration and type of super disintegrants. Metoprolol release from all the

formulations was found to be fast and immediate within 30 min, especially GF1 possess maximum release of 99.74± 2.24 in 10th minutes itself. So, GF1 selected as the best Optimized formulation among the first pulse Metoprolol tartarate IR granules in Pulsincap dosage form. The release rate of the drug from the IR granules was found to be maximum with less concentration of SSG (28).

Evaluation of Second Pulse Metoprolol Microbeads

Particle size

The particle size and surface morphology was determined with the help of scanning electron microscopy (SEM). Spherical shaped Microbeads were observed. Among the five formulations of Microbeads MF2 possess small particle size 100.52 and also uniform particle size distribution because of increased concentration of polymer and optimum concentration of cross ling agent which leads to coat the drug effectively and uniformly through the surface .The particle size ranges of formulations were shown in (Figure 6,7,8)

Drug Entrapment Efficient

On increasing the concentration the Sodium alginate and gelatin, the amount of drug entrapped with the polymers coat also increased, as it was observed maximum 85.31 ± 1.25 % in MF2 and less 73.89 ± 2.30 in MF6 .This shows that the best polymer for entrapping metoprolol tartarate in pulsincap microbeads was sodium alginate and optimum concentration of polymer is about 4% with 5% of cross linking agent calcium chloride. The results are shown in Figure 8.

Product yield

The percentage yield of Microbeads was more than 70 % for all the formulation and among the prepared batches, batch MF2 show highest percentage yield of 89.34% due to increased concentration of the polymer and cross linking agent. The results of production yield for all the batches were shown in Figure 7.

Drug content

The drug content in the micro beads was found to be in the range of 79.85 ± 2.25 to 98.59 ± 1.15 mg based on the polymer and cross linking agent ratio. Increase in concentration of the polymer and cross linking agent there will be increase in drug content of the formulation. The formulation MF2 shows maximum drug content and the values are given in Figure 8.

In-vitro drug release

These studies show the effect of environment of the body fluids on the drug release pattern from the prepared Pulsincaps Microbeads. It was found that the release rate from the all formulations was found to be different for the polymer proportion and concentration of cross linking agent used in the all formulations. All the formulation shows a good initial lag time of around 4 hr in pH 1.2,4.5, that shows that the release pattern of drug is delayed .

Formulation MF2 with polymer Sodium alginate concentration and Calcium chloride cross linking agent showed maximum Sustained release 98.25±0.5 in 16th hr in a cumulative pattern. MF2 shows maximum drug release because increase concentration of cross linking agent (29). The values are as shown in Figure 9&10.

Effect of lag time

The lag time for all the formulation i.e. the delay of release of drug in different ph are shown between 30 minutes to 4 hrs 40 minutes as in the figure no: . It shows that all the formulation shows good lag time to target the release of metoprolol tartarate in colonic pH especially early time in the morning hours. But order of best lag time results are as follows MF1>MF2> MF4>MF5>MF6> MF7> MF8> MF3.

In- vitro release kinetics

By applying the time vs. % CDR in release kinetics data, for best formulations MF1 & MF2 shows a R2 value 0.995 and 0.947 in Zero order and applying time in hr vs. %log ARR shows a R2 value 0.861 and 0.851 in First order represents an ideal release in order to prolonged pharmacological action. The results are shown in Table 5.And according to the peppas fitting both the formulation obeys nonfickian diffusion type of release mechanism (29).

Evaluation of Pulsincap dosage form

Solubility test: The empty formaldehyde treated capsules were stirred in a 250 ml beaker containing 100 ml of buffer solutions. The different buffer solutions such as pH 1.2, pH 4.5 and pH 7.0 were used for this study. The time taken for capsule to dissolve or form soft mass was noted. It soluble only in the pH 7.0 Colonic pH.

Average weight, content uniformity and drug content results of Metoprolol tartarate Pulsincaps (P1-P2)

There were no significant changes in the average weight, content uniformity and drug content of the prepared Metoprolol pulsincaps in the all formulations (P1- P2). The average weight, content uniformity, drug content results for the prepared Metoprolol pulsincaps were found to be within the limits. The content uniformity and drug content results for all the formulations were nearly 100% which indicated that there was no drug loss by manufacturing process or by additives used in the preparation Table-6.

Pharmacokinetics studies

The mean pharmacokinetic parameters of pure drug Metoprolol tartarate and Metoprolol tartrate pulsincap formulation were summarized in the table 7. The Cmax, Tmax of pure drug Metoprolol and Metoprolol pulsincap formulation was 30.24 µg/mL, 1hr and 26.44 µg/mL and 27.82 µg/mL, 5hr respectively for P1 and P2. The Tmax of Metoprolol tartarate pulsincap formulation was higher than pure drug. It indicates that the drug release started only in the colonic pH after the lag time. The statistical analysis of pharmacokinetic parameters of Metoprolol tartarate determined by ANOVA (FCal < FTab Accepted at 5% level of significance) and proved that there was no significant difference between the pure drug Metoprolol tartarate and Metoprolol tartrate nanobeads formulation indicated that, the absorption of Metoprolol tartrate was not influenced by the in-vivo behavior of the prepared Metoprolol tartarate pulsincap formulation. The pharmacokinetic study of Metoprolol tartarate pulsincap formulation shows the delayed and sustained release by reducing high peak plasma concentration (Cmax) than pure drug and prolong the time (Tmax) required to reaching the maximum plasma concentration there by reducing the T1/2 especially at early in the morning. The studies proved that T1/2 increased four folds higher than pure drug. The values of T1/2, Tmax and MRT of the Metoprolol tartarate pulsincap formulation showed higher than pure drug due to sustained release action. The increased AUC and relative bioavailability of Metoprolol tartarate pulsincap formulation might be attributed due to sustained release action or avoidance of first pass metabolism (30).

Table- 1 Formulation of Metoprolol tartarate IR granules

Formulation	Metoprolol tartarate in mg	Lactose in mg	Sodium Starch Glycolate(SSG) in mg	Cross Povidone (CP)	Magnesium Stearate	Talc
GF1	20	10	2	-	1%	1%
GF2	20	10	4	-	1%	1%
GF3	20	10	-	2	1%	1%
GF4	20	10	-	4	1%	1%
GF5	20	10	1	1	1%	1%
GF6	20	10	2	2	1%	1%

Table- 2 Formulation of Metoprolol tartarate Pulsincap Microbeads

Formulation code	**Amount of Metoprolol tartarate (mg)**	**Amount of Gelatin**	**Amount of Sodium alginate**	**Amount of calcium chloride**
MF1	50	-	2%	5%
MF2	50	-	4%	5%
MF3	50	2%	-	5%
MF4	50	4%	-	5%
MF5	50	-	2%	10%

MF6	50	-	4%	10%
MF7	50	2%	-	10%
MF8	50	4%	-	10%
Plug formulation				
Eudragit L100	60 mg	60 mg	60 mg	60 mg

Table- 3 Release Kinetics

Release Exponent 'n'	Drug Transport Mechanism	Rate as a function of time
0.5	Fickian Diffusion (Higuchi Matrix)	$t^{n-0.5}$
0.5<n<1.0	Non-Fickian Diffusion	t^{n-1}
1.0	Case – II Transport (Zero Order Release)	Zero Order Release
Higher Release (n>1)	Super Case – II Transport	t^{n-1}

Table-4 Micromeritics Properties of IR metoprolol tartarate granules

Formulation code	Parameters				
	Angle of repose(θ)	**Bulk Density(g/ml)**	**Tapped density(g/ml)**	**Carr's Index (%)**	**Hausner's ratio**
GF1	22˚80'±0 ˚ 85'	0.55±0.03	0.65±0.05	11.35±0.95	1.12±0.01
GF2	23 ˚28'±0 ˚ 15'	0.55±0.05	0.60±0.02	14.12±1.88	1.16±0.03
GF3	23 ˚ 60'±0 ˚ 22'	0.53±0.01	0.62±0.05	13.81±0.77	1.16±0.01
GF4	24 ˚ 53'±0 ˚ 21'	0.51±0.01	0.60±0.02	13.25±0.75	1.10±0.06
GF5	26 ˚52'±1 ˚42 '	0.55±0.05	0.64±0.05	15.36±1.03	1.18±0.03
GF6	23 ˚ 64'±0 ˚ 22'	0.53±0.03	0.62±0.02	13.42±0.77	1.10±0.01

Table- 5 *In- vitro* release kinetics studies

Formulation code	Release kinetics			
	Zero order R^2	**First order R^2**	**Peppa's R^2**	**'n' value for Peppa's**
MF1	0.947	0.861	0.978	0.478
MF2	0.995	0.851	0.975	0.469

Table- 6 Results of average weight, content uniformity and drug content of Metoprolol pulsincaps

Formulation	**Average weight (mg)**	**Content uniformity (%)**	**Drug content (%)**
P1	485.26± 1.44	99.52± 2.16	92.84± 1.14
P 2	490.54± 1.32	99.83± 2.14	94.62± 1.18

Table-7 Pharmacokinetic parameters of Metoprolol tartarate after oral administration pure drug Metoprolol and Metoprolol pulsincap formulation (Kinetica software)

S.NO	PARAMETER	PURE DRUG	P1	P2
1	T_{max} (h)	1.5± 0.1	5.0 ± 0.5	5.0 ± 0.2
2	C_{max} (µg/mL)	30.24±0.64	26.44±0.24	27.82±0.54
3	$AUC_{0\rightarrow\infty}$ (µg. hr/mL)	30.0613±0.540	144.6854±0.542	145.7013±0.432
4	$AUMC_{0\rightarrow\infty}$ (µg. hr/mL)	24.0434±0.800	544.0044±0.242	606.422±0.544
5	$T_{1/2}$ (h)	1.622±0.430	4.82±0.44	4.51±0.54
6	K_A	1.222±0.42	0.3572±0.54	0.2576±0.56
7	MRT(h)	1.022 ±0.004	7.5432±0.52	7.6428±0.34
8	V_F (mg)	24.0834±0.24	40.430±0.74	42.024±0.82
9	CL_F (mg(hr. µg/mL))	54.22±0.532	12.466±0.64	12.475±0.68

Table-8 *In vitro* release study for IR Metoprolol tartarate granules GF1-GF6 in pH 1.2

Time (min)	GF1	GF2	GF3	GF4	GF5	GF6
0	12.37 ± 2.68	5.35± 2.22	4.99± 2.64	8.82± 2.40	9.37± 2.68	10.37± 2.80
5	76.03± 2.24	36.22± 1.25	40.77± 2.24	56.97± 1.25	59.07± 2.40	54.27± 2.50
10	99.74± 2.24	82.26± 1.25	86.27± 2.68	98.44± 2.64	94.80± 2.64	92.86± 2.60
15	101.56± 1.00	85.72± 2.64	94.98± 1.25	100.11± 1.00	98.44± 1.25	96.12± 2.20
20	102.25± 0.28	93.25± 1.00	98.21± 1.25	101.23± 1.25	99.68± 1.24	99.53 ± 2.22
30	102.51± 1.24	93.74± 1.40	98.68± 1.28	101.34± 1.32	99.82± 1.42	99.74± 2.24

Table-9 *In vitro* release study for Metoprolol Microbeads MF1-MF4 in pH 1.2, 4.5 followed by pH 7.0

Time (hrs)	Cumulative % drug release			
	Formulation code			
	MF1	MF2	MF3	MF4
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	10.5±1.5	14.5±1.5	12.52±1.5	14.32±2.1
5	25.65±1.3	26.54±1.4	24.56±1.3	14.52±2.5
6	35.8±1.4	35.98±1.6	33.45±1.2	15.86±1.2
7	55.89±±1.1	56.98±1.2	42.56±1.5	33.45±1.1
8	59.83±1.2	61.52±1.2	49.56±1.4	45.95±2.1
9	62.83±1.3	64.32±1.1	51.24±1.5	47.56±2.3
10	65.32±1.2	69.54±1.2	52.35±1.2	55.63±1.2
11	70.58±1.4	75.63±1.3	59.54±1.1	58.65±1.3
12	79.54±2.1	79.58±1.4	61.75±21	61.52±1.5

13	81.5±1.8	82.45±2.1	65.84±2.5	65.79±1.2
14	86.32±1.6	85.32±2.4	69.89±2.1	71.25±2.4
15	93.54±1.2	96.54±2.4	83.65±2.4	81.48±2.1
16	93.86±1.2	96.35±0.5	85.13±2.8	82.59±2.5

Table-10 *In vitro* release study for Metoprolol Microbeads MF5-MF8 in pH 1.2, 4.5 followed by pH 7.0

Time (hrs)	**Cumulative % drug release**			
	Formulation code			
	MF5	**MF6**	**MF7**	**MF8**
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	12.54±2.4	13.5±1.5	11.52±1.5	14.00±2.0
5	26.54±2.1	26.54±1.4	23.36±1.3	16.08±2.0
6	36.52±1.3	56.98±1.2	40.16±1.6	35.40±1.2
7	39.56±2.5	61.52±1.2	48.45±1.6	42.05±2.2
8	48.21±2.4	64.32±1.1	50.26±1.4	45.50±2.2
9	56.29±2.1	75.63±1.3	57.56±1.4	54.65±1.4
10	59.45±1.2	79.58±1.4	60.45±1.24	58.24±1.8
11	61.25±1.3	82.45±2.1	62.44±2.5	60.04±1.6
12	64.25±2.1	82.36±2.8	65.66±1.2	68.26±2.0
13	68.51±2.3	85.32±2.4	68.66±1.2	70.44±2.0
14	72.85±2.4	91.52±2.7	74.84±1.4	72.54±1.4
15	75.23±1.2	96.24±2.4	80.54±1.2	81.54±2.0
16	79.2±1.3	96.55±0.5	84.55±1.8	81.98±2.0

Table -11 Selection of Optimized Batch

Pulse		**Optimized Pulsincap formulation code**
First	**Second**	
GF1 (2mg of SSG)	MF1 (2% of Sodium alginate with 5% $Cacl_2$)	P1
GF4 (4 mg of CP)	MF2(4% of Sodium alginate with 5% $Cacl_2$)	P2

Table-12 *In vitro* release study for Metoprolol Pulsincap P1-P2 in pH 1.2 followed by 4.5, pH 7.0

Time (hrs)	**Buffer pH**	**Cumulative % drug release**	
		Formulation code	
		P1	**P2**
0 to 30 min	1.2	101.24± 1.24	101.08± 1.54
1	4.5	0	0

2	LAG	0	0
3	TIME	0	0
4	7.0	8.5±1.8	10.5±1.8
5		18.65±1.6	16.52±1.6
6		23.8±1.4	25.90±1.6
7		55.89±±1.8	56.90±1.6
8		59.83±1.8	61.44±1.8
9		62.83±1.4	66.54±1.8
10		65.32±1.8	70.62±1.2
11		70.58±1.6	74.70±1.8
12		78.24±2.0	78.88±1.4
13		81.45±1.6	80.44±2.0
14		86.34±1.8	86.54±2.0
15		92.04±1.8	98.42±2.0
16		94.84±1.4	97.74±1.8

INVIVO PHARMACOLOGICAL ACTIVITY

Table-13 Anti-Hypertensive Activity of Formulation P1 & P2 against Adrenaline Induced Hypertension in Male Albino Wistar Rats

Groups	**Arterial blood pressure before induction of hypertension**	**After 3hrs**	**After 7hrs**	**After 10hrs**
Systolic Blood Pressure (mmHg)				
Normal control (Placebo mini pulsincap)	113.32±6.22	115.56±4.12**	112.33±5.14**	114.42±5.24*
Disease control: Adrenaline + Placebo	112.33±5.41	178.24±7.57	167.21±6.14	175.27±6.52
Adrenaline + **Formulation P1** (5mg/kg, *p.o*)	114.41±5.22	132.15±6.52*	129.28±5.24*	134.12±4.36**
Adrenaline + **Formulation P2 (**5mg/kg, *p.o*)	110.41±5.42	136.19±5.22**	130.12±6.39**	136.28±5.41**
Pure drug of Metoprolol 5mg/kg, *p.o*	115.54±4.24	109.21±6.19**	132.47±8.17*	148.42±6.34
Diastolic Blood Pressure (mmHg)				
Normal control (Placebo mini pulsincap)	79.24±5.33	77.29±5.68**	75.24±6.18**	79.21±5.41**

Disease control: Adrenaline + Placebo	81.22±6.24	105.26±4.52	106.41±6.13	102.21± 6.36
Adrenaline + **Formulation P1 (**5mg/kg, *p.o*)	80.14±5.19	87.46±4.52**	84.66±5.33**	86.28±5.24**
Adrenaline + **Formulation P2 (**5mg/kg, *p.o*)	79.52±4.69	89.24±4.22**	85.28±6.33**	89.54±5.41**
Pure drug of Metoprolol 5mg/kg, *p.o*	78.33±4.29	72.46±5.51*	88.12±5.36*	97.33±6.52

SBP and DBP were significantly increased in adrenaline treated rats as compared to normal rats in tail-cuff with BIOPAC instrument. According to mechanisms of adrenaline, β-adrenergic blocker-metoprolol blocking the action of adrenaline and significantly reduced the Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) in wistar rats. The delayed and sustained release of Metoprolol tartarate pulsincap showed sustained the antihypertensive effect in hypertensive rats.

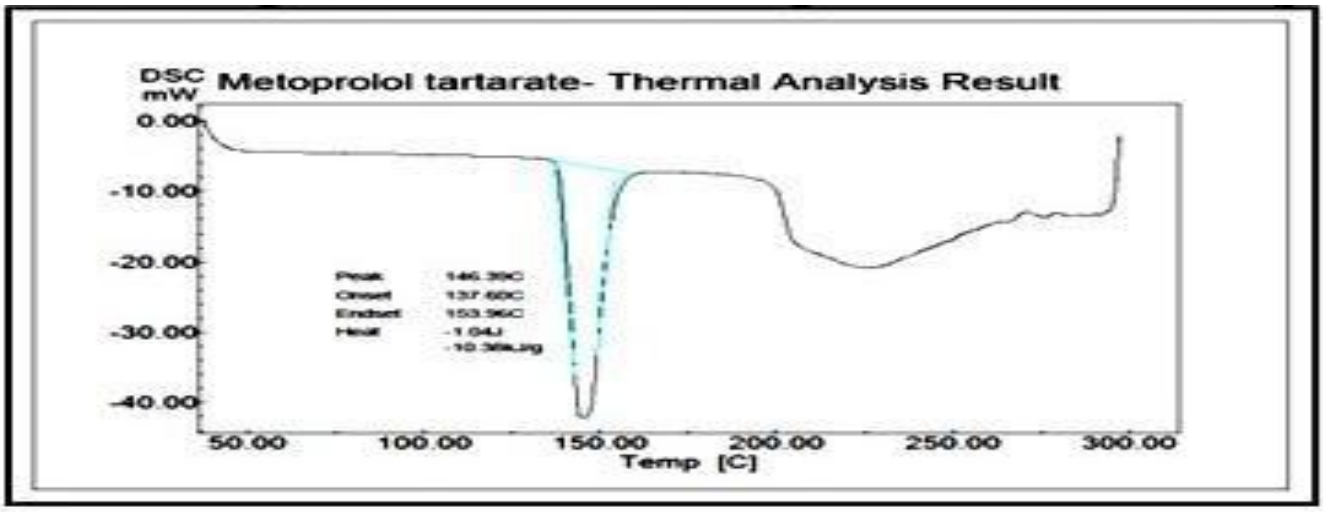


Fig-1 DSC Spectrum of Metoprolol tartarate pure drug

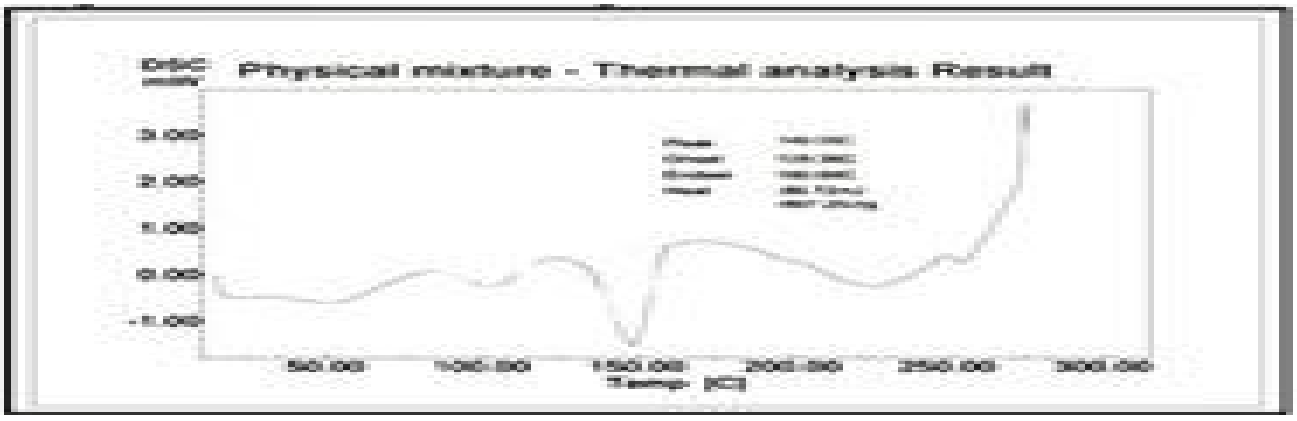


Fig-2 DSC Spectrum of Metoprolol tartarate and other excipients

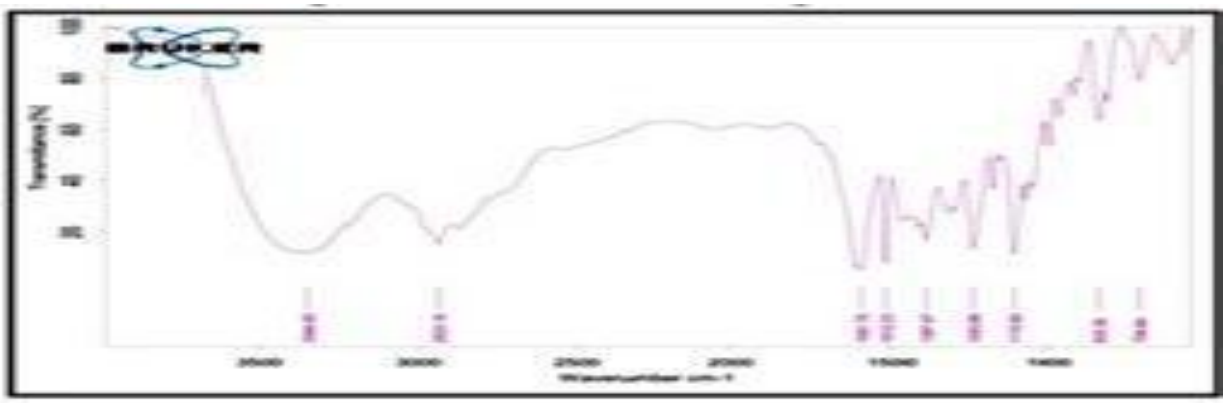


Fig-3 IR Spectrum of Metoprolol tartarate pure drug

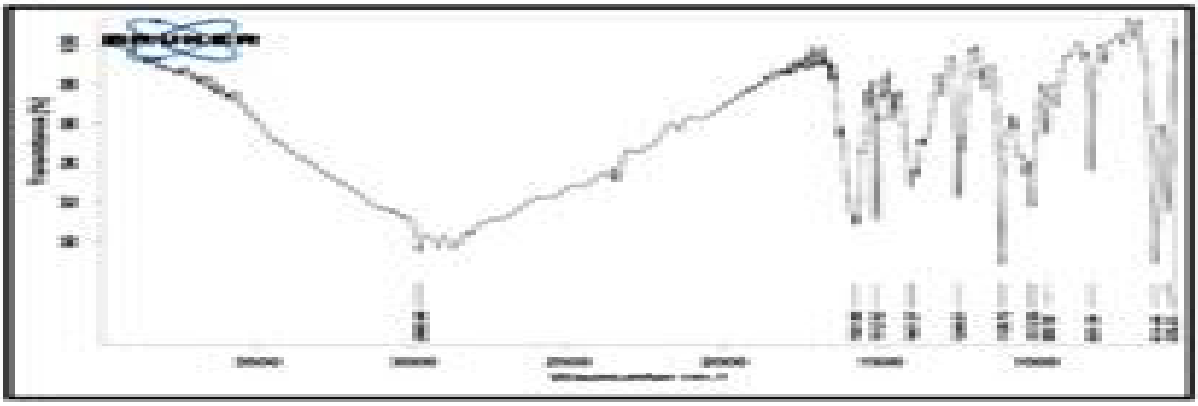


Fig-4 IR Spectrum of Metoprolol tartarate and other excipients

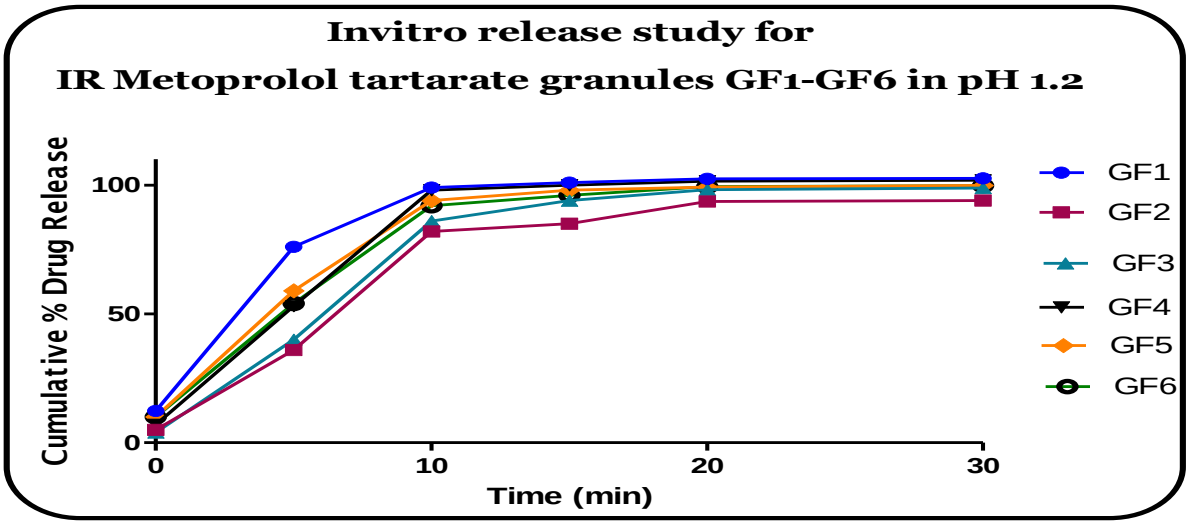


Fig-5 Cumulative % drug release profile for GF1-GF6 Metoprolol tartarate IR granules

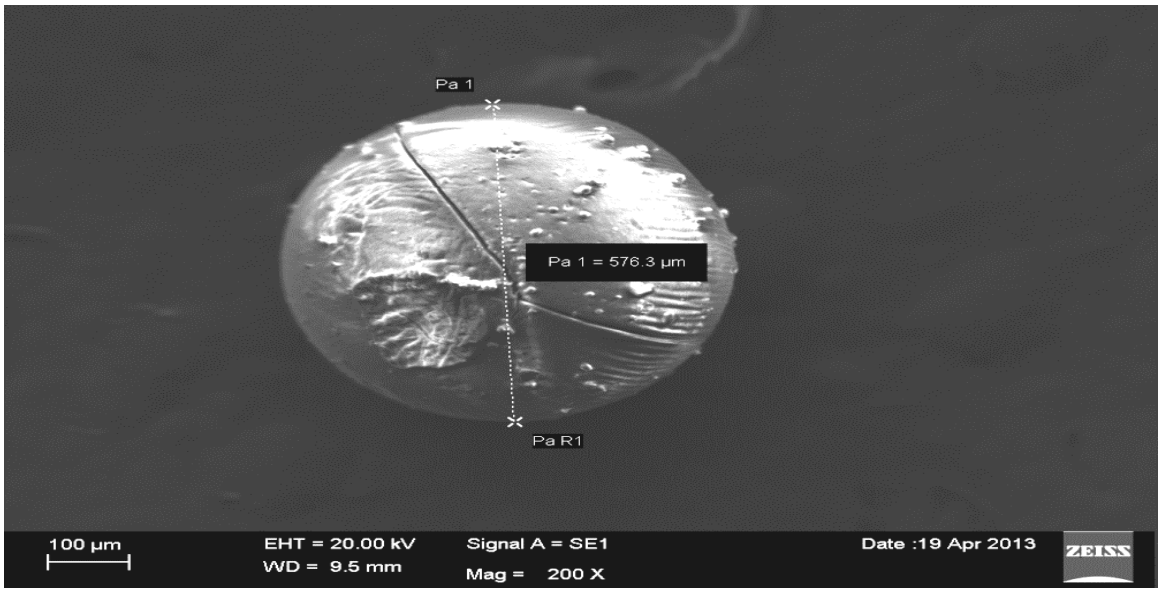


Fig-6 SEM Photography of Microbeads

Figure 7:Particle size(nm) and Production yield(%) of Metoprolol tartarate Microbeads

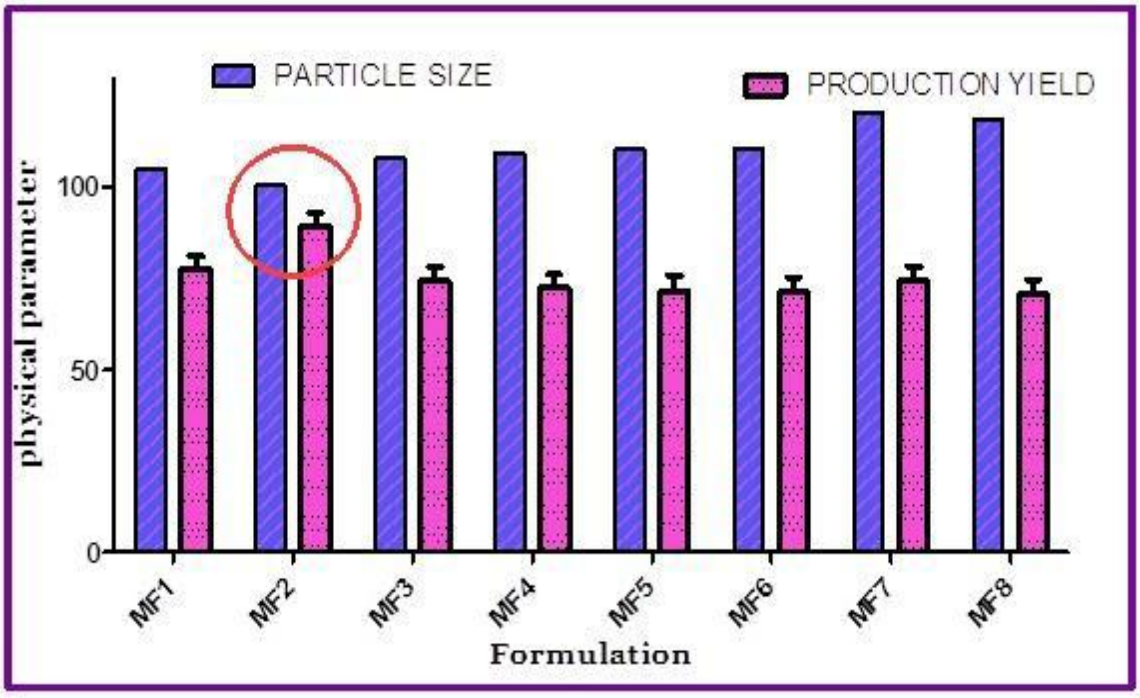
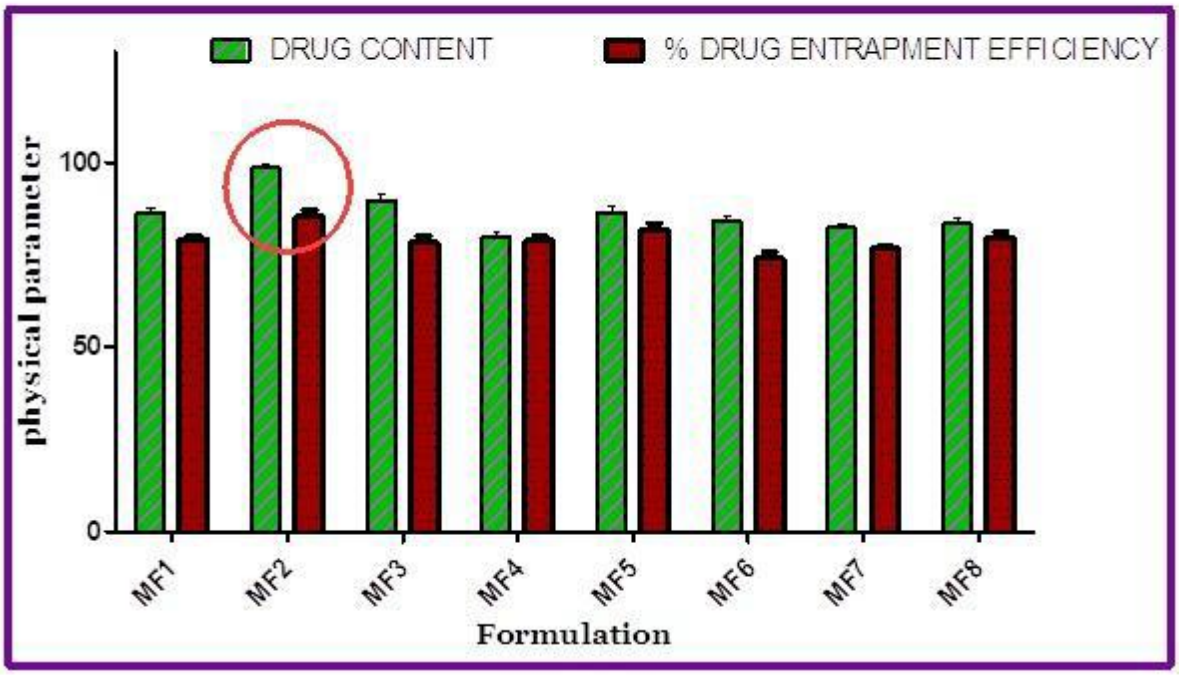


Figure 8:Particle size(nm) and Production yield(%) of Metoprolol tartarate Microbeads



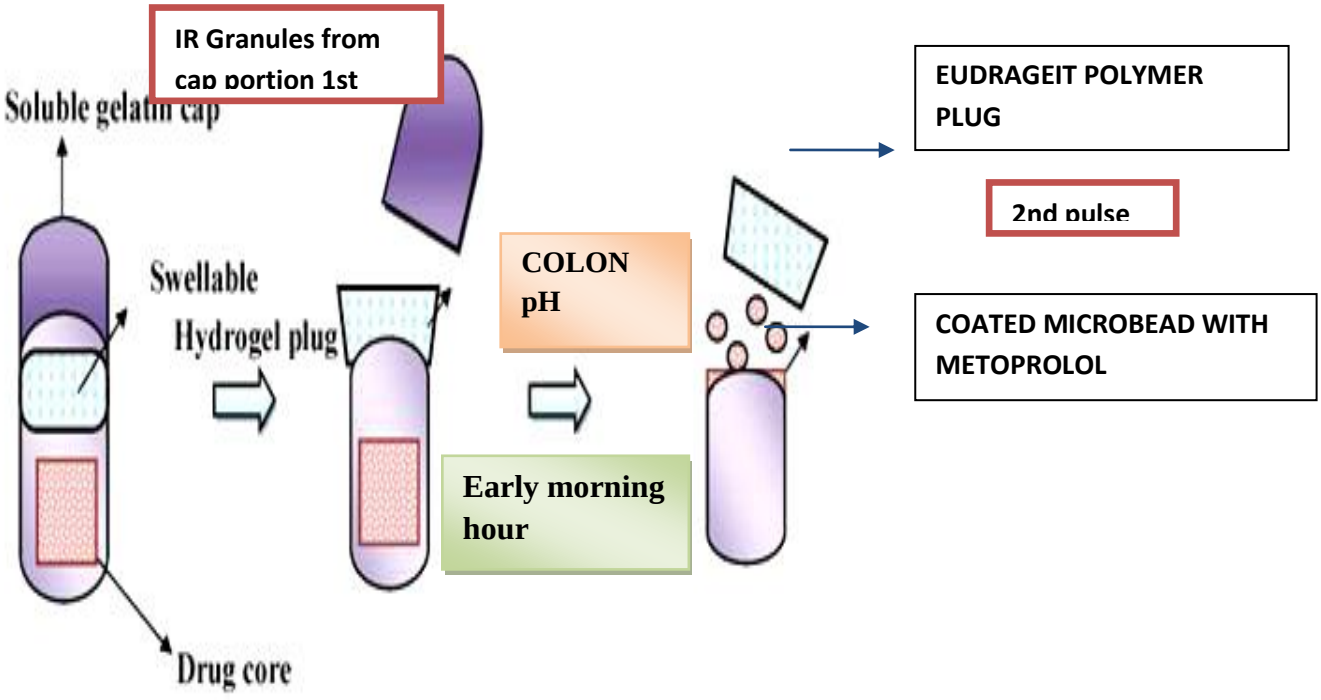


Fig-9 Design of Pulsatile Dosage Form

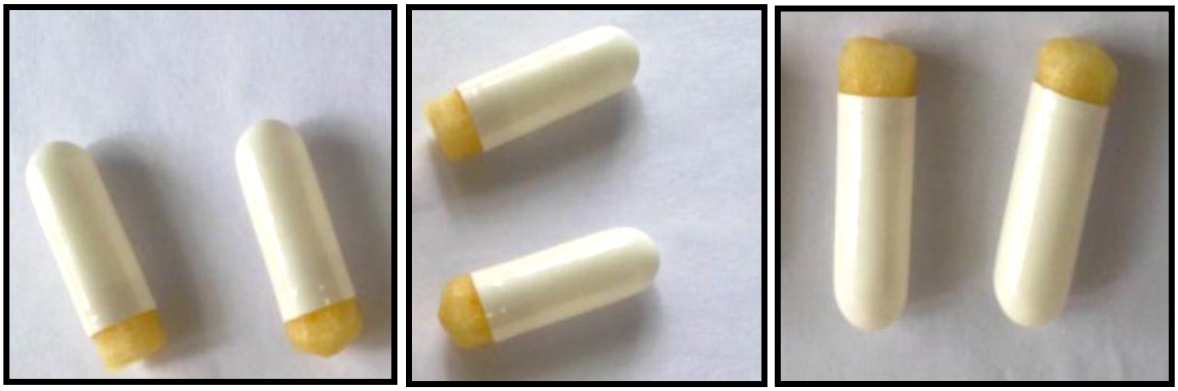


Fig-10 Metoprolol tartarate Pulsincap
(Body of the Capsule with plug loaded with microbeads later load the granules in cap of the capsule)

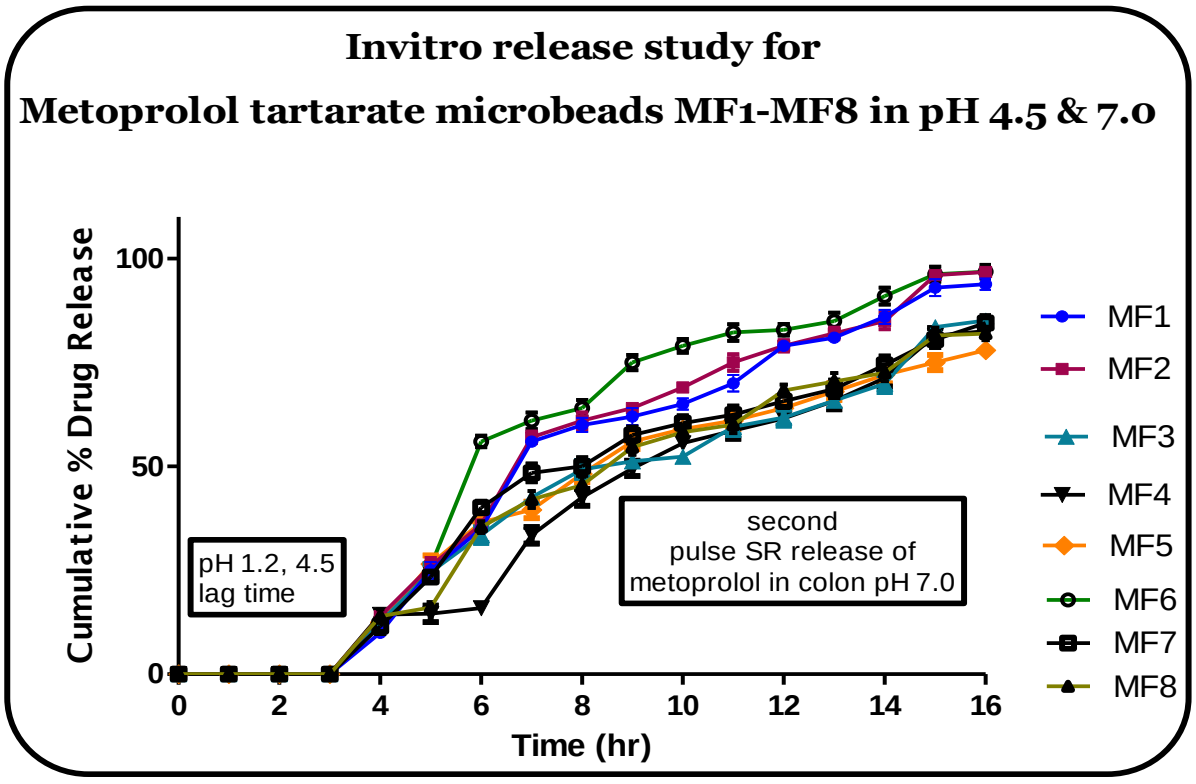


Fig-11 Cumulative % drug release profile for Metoprolol Tartrate Microbeads MF1 – MF8 in pH 1.2, 4.5 & 7.0

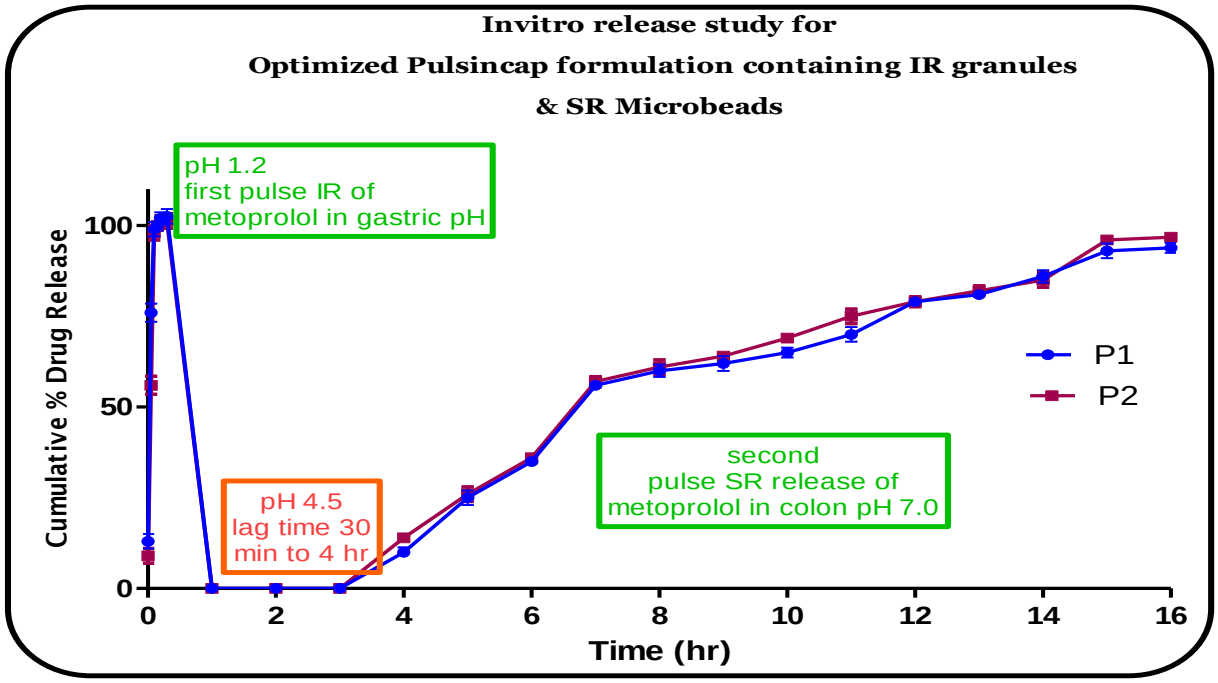


Fig-12 Cumulative % drug release profile for Optimized Metoprolol Tartrate Pulsincap formulation

CONCLUSION

From this research work it was made to develop the pulsatile drug delivery of Metoprolol tartarate and they formulated and evaluated. Metoprolol tartarate pulsincap prepared by two pulse one immediate release granules first pulse and another one sustained release colon targeting microbeads. The *in vitro* dissolution studies revealed that the formulated pulsincap shows desired concentration of the drug release at pre-determined time points. The animal studies confirmed that the pharmacokinetic and Pharmacodynamic parameters of Metoprolol tartarate pulsincap formulations were performed and it shows the better desired results. Hence it may be concluded that the newly formulated pulsatile drug delivery systems of Metoprolol tartarate produce effective control of hypertension after intake of meals and also at early morning risk hours by allowing the drug to release after a lag time at colonic pH. The chronotherapeutic effect of Formulation P1 & P2 was confirmed with results of SBP and DBP after 3, 7 & 10 h. This effect showed the potent and significant antihypertensive action of Formulation P1 & P2 than pure drug metoprolol.

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