FORMULATION, OPTIMIZATION AND EVALUATION OF FLOATING PULSATILE BEADS OF CAPTOPRIL

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ABSTRACT

Background: The purpose of this work was to develop a dosage form of an antihypertensive drug for pulsatile release intended for chronotherapy of hypertension. Ionotropic gelation technique was used to develop calcium alginate beads. Methods: A 3^2 Full Factorial Design was employed to study the effect of independent variables (sodium alginate concentration, Sodium bicarbonate concentration) on dependent variables (Floating time, Percentage Cumulative drug release). Optimization was done by fitting the experimental data to software program (Design expert 10). Optimized beads were subjected to different evaluation parameters. Results: The optimized beads show good swelling in basic media compared to acidic media. The entrapment efficiency was 97.59%. The results of invitro drug release in both acidic and phosphate buffer showed minimum% cumulative drug release of 11.13% at 6th hr in acidic buffer and sudden release of 96.49% drug in phosphate buffer within 1 hr. Kinetics of the drug release study follows Higuchi and Kim Fassihi model. Conclusion: The optimized formulation can be used as pulsatile drug delivery system which provide time and site specific sudden drug release in intestine after a lag time of 6hrs in stomach which can be used to treat the morning surge in hypertension in patients with a night time dose itself.
KEYWORDS: Chronotherapeutics; Pulsatile Drug Delivery System; Calcium alginate Bead; Captopril; Ionotropic gelation; $3^2$ Full Factorial Design.

1. INTRODUCTION

Controlled drug delivery systems have acquired very important role in pharmaceutical Research and Development (R&D) business.[1] The oral controlled-release system shows a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period of time, thereby ensuring sustained therapeutic action. But in case of certain diseases that show chronobiological behaviour which demand site and time specific release of drug in a burst manner after a lag time. The above conditions demand release of drug after a lag time that is a time with no drug release.[2] Pulsatile drug delivery system is defined as the rapid and transient release of certain amount of molecules within a short time period immediately after a predetermined off– release period, i.e., lag time. Here release of drug can be controlled by circadian rhythm, which regulates many body functions in human beings.[3]

In this study floating pulsatile multiparticulate drug delivery system is used for the chronotherapy of hypertension. Hypertension is a chronic disease which shows chronobiological behaviour in the severity ie., a surge in systemic Blood Pressure during the earling morning hours around 4-6 am.[4] Multiparticulate systems have several avantages over single unit systems like no risk of dose dumping, reduced risk of local irritation, less inter and intra subject variability, increased bioavailability. Beads are one of the most promising mulatiparticulate system.[5]
Captopril is an ACE inhibitor, antihypertensive drug. It is rapidly and almost completely absorbed after oral administration with peak plasma level reached in about 1hr under a fast condition, and the half life of drug is 2hrs. The bioavailability is 65% due to hepatic metabolism. The currently available formulations of captopril is not able to release the drug when the symptoms of disease were at peak level in the early morning hours in the case of hypertensive patients.

Floating alginate beads were prepared by Ionotropic gelation technique. Sodium alginate is a natural polymer shows pH dependent swelling and degradation which crosslinks with Ca$^{2+}$ ions in Calcium chloride to produce Calcium alginate beads. The buoyancy is imparted by the release of CO$_2$ with the reaction between Sodium bicarbonate and acetic acid. The calcium alginate beads will float in the acidic medium during the lag time after which undergo swelling and release of drug by diffusion or degradation mechanism at intestinal pH.6.

This rationale formulation improves patient compliance by releasing drug at specific time, specific sit and specific amount when the symptoms of hypertension are at peak levels in the early morning hours with a bed time dose itself by maintaining lag phase during floating in stomach followed by burst release in the intestine which is the principle of Floating Pulsatile Drug Delivery System.

2. MATERIALS AND METHODS

2.1. Materials
Captopril was a generous gift sample obtained from Sance Laboratories pvt. Ltd., Kerala. Sodim alginate is purchased form Loba Chemi. All the other Ingredients are purchased form Nice Chemicals, pvt. Ltd, Kerala. All the ingredients are of analytical grade.

2.2. Methods
2.2.1. Preformulation studies of drug and polymer
2.2.1.1. Drug- Polymer interaction study (FTIR spectroscopy)
The FTIR spectrum of pure samples of Captopril, Sodium alginate and its 1:1 physical mixture were recorded by conventional KBr pellet method. The scanning range was 4000-700 cm$^{-1}$ and resolution was 1 cm$^{-1}$.
2.2.1.2. DSC analysis
DSC thermogram of the drug was recorded by heating the sample from 30 °C- 250 °C at a heating rate of 10 °C per min.

2.2.1.3. Solubity study
Solubility of the drug is checked in different solvents. The pH dependent solubility of the drug is also determined in acidic buffer (pH 1.2) and Phosphate buffer (pH 7.4).

2.2.1.4. Preparation of Calibration curve of Captopril
Different dilutions of drug are prepared in both acidic and basic buffer like 2,4,6,8 and 10μg/ml. The absorbance of serial dilutions are analysed by UV spectrophotometry at 202 nm and calibration curve is plotted.

2.2.2. Preparation of floating pulsatile calcium alginate beads
Sodium alginate is added to 10 ml of distilled water in a beaker and stirred well to obtain a clear solution in a magnetic stirrer. To the above solution 100mg of Captopril is dissolved. To the above solution sodium bicarbonate is added and sonicated for 30 min to remove air bubbles. It is kept aside for 30 min. The resultant dispersion is dropped via 23 gauge needle into 100 ml 2% w/v Calcium chloride solution containing 10 % acetic acid. The content was stirred at 100-200 rpm using magnetic stirrer for 15 min. The beads are then filtered, washed with distilled water and dried at room temperature.

2.2.3. Experimental design
A 32 full factorial design was constructed where the concentration of sodium alginate (X1 in%) and Sodium bicarbonate (X2 in %) were selected as the two independent variables. The levels of the two factors were selected on the basis of the literature survey. All other formulation and processing variable were kept constant throughout the study. Optimization of preparation of beads was done by Design Expert Software 10 (Version 10, Stat-Ease Inc., and Minneapolis, MN). The data was inputted to design expert software and polynomial equation was obtained. The responses (dependent variables) studied were cumulative percentage drug release at 6 hr (Y1 in %) and floating time (time for which at least 90% of beads remain floated,Y2 in hrs). Table 5 summarizes the independent and dependent variables along with their levels. 3^2 full factorial design layout, experimental runs and their combinations are listed in the table I.
**Table I: Observed responses in 9 experimental runs**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>X1</th>
<th>X2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>F2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F6</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>F7</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>F8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table II: Dependent and Independent variables**

<table>
<thead>
<tr>
<th>Factors (independent variables)</th>
<th>Coded levels</th>
<th>Responses (dependent variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>X1 = Sodium alginate (mg)</td>
<td>400</td>
<td>500</td>
</tr>
<tr>
<td>X2 = Sodium bicarbonate (mg)</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

2.2.4. Optimization of data analysis[^9]

The targeted response parameters were statistically analyzed by applying one-way ANOVA (analysis of variance), at 5% significance level and the significance of the model was estimated using the statistical package Design-Expert. The individual parameters were evaluated using F-test and mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using multiple linear regression analysis, for determining the levels of factors which yield optimum dissolution responses. A second-order polynomial regression equation that fitted to the data is as follows:

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_2 + b_{22}X_1^2 + b_{22}X_2^2\
\]

Where \(b\) is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; \(b_1, b_2, b_{11}, b_{22}\) are the coefficients computed from the observed experimental values of \(Y\); and \(X_1\) and \(X_2\) stand for the main effects. The terms \(X_1, X_2\) and \(X_i^2\) (\(i = 1\) and 2) represent the interaction and quadratic terms, respectively used to simulate the curvature of the designed sample space.

2.2.5. Evaluation of beads

2.2.5.1. Micromeritic studies

2.2.5.1.1. Particle size and shape

Fifty floating beads were analyzed for their size distribution by optical microscopy.
2.2.5.1.2. Scanning electron microscopy (SEM)

The surface morphology and internal structure of the products were observed by scanning electron microscopy using scanning electron microscope. Pictures of the beads were taken by random scanning of the stub.

2.2.5.2. In-vitro buoyancy study

In-vitro buoyancy studies were done using dissolution test apparatus USP type II (rotating paddle). 50 calcium alginate beads of captopril were taken and added to the dissolution flask containing 0.1 N HCl as medium (900 ml) containing 0.02% tween 80. Temperature was maintained at 37 °C ± 0.5 °C. Paddle maintained at 100±5 rpm. At hourly intervals stirring was stopped for 2 min and number of settled beads was counted visually.

Buoyancy percentage = No. of beads remained floating X 100/Total no. of beads

2.2.5.3. In-vitro drug release

Dissolution studies were performed using the USP dissolution test apparatus-II at 100rpm. The dissolution studies of the beads equivalent to 50mg of captopril were performed using USP type II dissolution test apparatus. The drug release study was carried out in 0.1 N HCl for initial 6 h followed with dissolution in phosphate buffer pH 7.4, each 900ml, maintained at 37 ± 2°C and agitated at 100rpm. 1 ml samples were collected replaced with 1 ml fresh dissolution medium for 8hrs. Absorbance of the sample is taken at regular intervals at 202nm.

2.2.5.4. Percentage entrapment efficiency

Accurately weighed quantities of beads (50mg) of the optimized batch were placed in 100ml phosphate buffer pH 7.4 and mechanically agitated on a shaker at 200 rpm for 24 hrs. Then the resultant dispersion were filtered through Whatt’s man no. 41 filter paper and analysed spectroscopically at 202 nm.

Percentage entrapment efficiency (% EE) =AQ *100/TQ

AQ = actual drug content in the beads
TQ = theoretical drug content in the beads

2.2.5.5. Swelling studies

Beads were studied for swelling characterization. All the prepared formulations were taken and weighed and placed in a beaker containing 100 ml of 0.1 N HCl (pH 1.2) maintained at 37°C. The beads were periodically removed at predetermined intervals for 120min. and
excess moisture is removed using a blotting paper and the immediately weighed. The same procedure was done with using phosphate buffer pH 6.8. Then the swelling ratio was calculated as per the following formula.

\[
\text{Swelling ratio} = \frac{W_t}{W_0}
\]

\[
W_t = \text{weight of beads at time ‘}t’
\]

\[
W_0 = \text{initial weight}
\]

2.2.5.6. Thermal analysis
DSC thermograms of beads were recorded on a disc calibrated with indium and zinc. The DSC runs were performed over a temperature range of 50-250°C at a heating rate of 10°C per minute for bead formulation.

2.2.5.7. Drug release kinetic data\footnote{7}
In order to understand the kinetic and mechanism of drug release, the result of in-vitro drug release study of beads were fitted with various kinetic equation like zero order (cumulative % release vs. time), Kim Fassihi model(log of (cumulative percentage released- overall drug released) Vs log of (time-lag time)), Higuchi’s model (cumulative % drug release vs. square root of time). R² and k values were calculated for the linear curve obtained by regression analysis of the above plots.

2.2.5.8. Exvivo permeation study - Everted sac method\footnote{10}
One end of the isolated everted intestinal segment is fixed to a straight cannula and at the other end tied using a thread to a 1 g weight. The system is filled with Krebs-Ringer solution and is completely immersed into the dissolution vessel of the dissolution test apparatus containing 900 ml of Phosphate buffer pH 7.4 in which accurate quantity of beads (50mg) is dissolved. During the study, assemblies are maintained at 37 ± 0.5°C, and aeration is ensured with a continuous supply of bubbled oxygen. Samples are withdrawn at regular intervals form the medium and cumulative percentage of drug permeated is determined.

2.2.5.9. Stability studies\footnote{11}
The stability studies for beads were done by keeping the sample beads from optimized batches at room temperature (30±2°C, 65±5% RH) for 90 days. The vials were sealed and stored at room temperature only because the polymer used in preparation of beads i.e. sodium alginate is not stable at higher temperature. The samples were put for 90 days. In the interval of each one month the beads were evaluated for different parameters like floating time,
percentage drug entrapment and drug release studies. Methods followed to evaluate these parameters were similar as followed previously.

3. RESULTS AND DISCUSSION

3.1. Preformulation studies

3.1.1. Drug Excipient compatibility study

All the peaks present in the pure samples are seen in the spectrum of the mixture and no additional peaks were obtained. It shows that no incompatibility is present between the drug and excipients. The FTIR spectrum of Captopril, Sodium alginate and mixture are given below.

Figure 1: FT-IR spectrum of Captopril pure drug

Figure 2: FT-IR spectrum of Sodium alginate
3.1.2. DSC analysis of Captopril

A well defined symmetric peak at 109°C before the corresponding melting point of Captopril, is observed with no significant baseline changes implies that the sample is pure and the heat capacity of the sample does not change along the process.

3.1.3. Solubility study of Captopril

Captopril is freely soluble in water and soluble in methanol, ethanol, and chloroform. It has high solubility in basic phosphate buffer compared to acidic buffer.
3.1.3. Solubility study of Captopril Captopril is freely soluble in water and soluble in methanol, ethanol, and chloroform. It has high solubility in basic phosphate buffer compared to acidic buffer.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>1.2 pH</th>
<th>7.4 pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.01131</td>
<td>0.0162</td>
</tr>
<tr>
<td>40</td>
<td>0.02134</td>
<td>0.03092</td>
</tr>
<tr>
<td>60</td>
<td>0.03245</td>
<td>0.04422</td>
</tr>
</tbody>
</table>

3.1.4. Preparation of Calibration curve Captopril

The calibration curve of Captopril in acidic and basic buffer are given below

*Figure 6: Calibration curve of Captopril in acidic buffer pH 1.2*

*Figure 7: Calibration curve of Captopril in Phosphate buffer pH 7.4*
3.2. Preparation of floating pulsatile beads of Captopril
The results of the responses for all the 9 formulations are given below.

*Table IV: Observed responses in 9 experimental runs*

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Independent variables</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X1(%)</td>
<td>X2(%)</td>
</tr>
<tr>
<td>F1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>F2</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>F3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>F5</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>F6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>F7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>F8</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>F9</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

3.3. Data analysis and model validation
The values of all statistical parameters analysed were shown in the table 5,6,7 for each response along with their ANOVA results. Values of p< 0.05 indicates significant model terms. Linear model is found to be significant in this study design confirmed by ANOVA at 5% level of significance.

*Table V: Summary of the model analysis for the responses*

<table>
<thead>
<tr>
<th>Source</th>
<th>Sequential p-value</th>
<th>Lack of Fit p-value</th>
<th>Adjusted R2</th>
<th>Predicted R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y1</td>
<td>Y2</td>
<td>Y1</td>
<td>Y2</td>
</tr>
<tr>
<td>Linear</td>
<td>0.0002</td>
<td>0.0012</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

*Table VI: Summary of the R-square analysis for the responses*

<table>
<thead>
<tr>
<th>Source</th>
<th>Y1</th>
<th>Y2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>Adjusted R2</td>
</tr>
<tr>
<td>Linear</td>
<td>0.9414</td>
<td>0.9218</td>
</tr>
</tbody>
</table>

*Table VII: ANOVA summary for the responses*

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>FValue</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model (Y1)</td>
<td>50.83</td>
<td>2</td>
<td>25.42</td>
<td>48.16</td>
<td>0.0002</td>
<td>Significant</td>
</tr>
<tr>
<td>Model (Y2)</td>
<td>205.29</td>
<td>2</td>
<td>102.64</td>
<td>25.25</td>
<td>0.0012</td>
<td>Significant</td>
</tr>
</tbody>
</table>

A first order polynomial regression equation in terms of coded factors are obtained for all the responses

Floating time (Y1) = +5.67-1.17*A+ 2.67*B
Percentage Cumulative drug release (Y2) = + 11.51-3.84*A+4.42*B
The above model equations carry factors along with coefficients (positive / negative) which quantify response values. A positive sign of Coefficient indicates synergistic effect whereas negative sign indicates antagonistic effect.

3.4. Counter and 3D surface plot analysis
Design expert software generated the counter and three-dimensional response plots which visualized the effects of the process parameters on the response variables. Floating time(Y1) is found to increase with decreased level of sodium alginate (A/X1) and increases with increased level of sodium bicarbonate (B/X2). Percentage Cumulative drug release(Y2) increases with decrease in Sodium alginate (A/X1) and increase with increase in sodium bicarbonate (B/X2).

![Figure 8: contour and 3D surface plot for Floating time](image)

![Figure 9: contour and 3D surface plot for cumulative percentage drug release](image)
3.5. Optimization

A numerical optimization technique using the desirability function approach was employed to generate the optimum formulation. Optimum batch with highest desirability is given below. It was found to satisfy the requisites of an optimum batch when the desirable ranges of responses were restricted to Floating time of 6 hrs and minimum percentage cumulative drug release in a range of 10-15%.

**Table VIII: optimum batch**

<table>
<thead>
<tr>
<th>Number</th>
<th>SODIUM ALGINATE (%)</th>
<th>SODIUM BICARBONATE (%)</th>
<th>CDR (%)</th>
<th>FLOATING TIME (hrs)</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.511</td>
<td>1.674</td>
<td>11.086</td>
<td>6</td>
<td>1.000</td>
</tr>
</tbody>
</table>

3.6. Evaluation of beads

3.6.1. Micrometric studies

3.6.1.1. Particle size analysis

The particle size of the beads was found to be between 756 - 854μm.

3.6.1.2. Scanning Electron Microscopy

Surface morphology of the beads was investigated with a scanning electron microscope. Different magnifications were used while taking these photographs. Particle surface was rough, spherical in shape and discrete. The SEM photographs of the beads are shown in Figure 9.

![Figure 9: SEM photograph of the optimized beads](image)

3.6.2. Differential Scanning Calorimetry

The thermogram of the optimized beads is depicted in Figure 10 which shows three endothermic peaks. The broad peak at 82.12°C which is due to the melting of Captopril and may also be due to the moisture evaporation if any. Sharp peak at 175.64°C may be the endothermal transition peak of calcium alginate usually the transition peak of alginate is around 20°C but gelation with calcium can shift the peak to 180°C. The sharp peak at
186.09°C could be due to the decomposition of polymer bead as such, even though it is weird to see such a peak.

![DSC thermogram of optimized formulation](image1.png)

**Figure 10:** DSC thermogram of optimized formulation

### 3.6.3. Invitro buoyancy study

The beads show excellent buoyancy with no or negligible floating lag time. After 6 hrs only 21 beads were remained floated. All the beads were settled after 7.25 hrs. Time for which 90% of the beads remained floated is taken as floating time and it is 6 hrs from the study. So the beads will provide a lag time of 6 hrs in the stomach which is important for time specific release of the drug. The floating behavior of the beads is shown in Figure 11.

![Invitro buoyancy study of optimized formulation](image2.png)

**Figure 11:** invitro buoyancy study of optimized formulation
3.6.4. Percentage drug entrapment
The drug entrapment efficiency was determined using phosphate buffer pH 7.4 as medium. The beads can entrap 97.59% of the loaded drug which shows that the formulation is highly efficient.

3.6.5. Swelling studies
The beads show high swelling index in phosphate buffer compared to acidic buffer and also the swelling index increases at high rate than acidic buffer. It is clearly depicted in the graph plotted between time and swelling ratio in both buffers shown in Figure 12. Form this it is clear that beads will not release drug in the acidic environment of the stomach but will give a burst release in the intestine with basic pH which is essential for site specific delivery.

![Swelling study](image)

*Figure 12: swelling study of optimized formulation*

3.6.6. In vitro drug release study
In vitro drug release study is performed in USP type II dissolution test apparatus in acidic buffer pH 1.2 for 6hrs and thereafter in phosphate buffer pH 7.4 for 1 hr. The beads show a minimum drug release of 11.13 % after 6 hrs which is the lag time in acidic buffer and thereafter in phosphate buffer 96.48% of the drug is released within 1 hr. The in vitro drug release study proves that the formulation can provide time and site specific drug delivery at the intestine. The graph between time and percentage cumulative drug release shows a sigmoidal curve with initial lag time of 6 hrs and a pulse release for 1 hr which is shown in Figure 13.
Table IX: Invitro drug release study of optimized formulation

<table>
<thead>
<tr>
<th>Medium</th>
<th>Acidic buffer</th>
<th>Phosphate buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>60 120 180 240 300 360</td>
<td>370 380 390 400 410 420</td>
</tr>
<tr>
<td>% CDR</td>
<td>6.64 7.91 8.72 9.84 10.59 11.13</td>
<td>42.993 57.48 69.156 77.39 91.207 96.48</td>
</tr>
</tbody>
</table>

3.6.7. Kinetics of invitro drug release

The high \( r^2 \) value for higuchi model suggests that the release follows diffusion kinetics and good fit to higuchi model. The high \( r^2 \) value for Kim Fassihi plot indicates pulsatile release and the \( n \) value for Kim Fassihi plot is 0.681 which suggests that the release follows anomalous non Fickian transport. The release kinetics of the beads are shown in Figure 14.

3.6.8. Exvivo permeation study

The drug shows 71.23 % of cumulative percentage drug permeated after 2 hrs eventhough the drug has low permeability. From the graph plotted between time and percentage
cumulative drug permeated it is clear that there is gradual increase in the percentage of drug permeated with time. The graph is depicted in Figure 15.

**Figure 15: Ex vivo permeation study of optimized formulation**

### 3.6.9. Stability studies

The stability study results shows that no change in the main evaluation parameters percentage cumulative drug release, floating time, percentage entrapment efficiency of the formulation after the storage of 3 months at room temperature (30±2°C, 65±5% RH). So the formulation is stable for 3 months at the room temperature and can safely be used. The drug release curve for the formulation at 0, 30, 60, 90 days can be seen in the graph shows pulse release after 6 hrs lag time which is shown in Figure 16.

**Table X: Stability study data**

<table>
<thead>
<tr>
<th>DAYS</th>
<th>CUMULATIVE % DRUG RELEASE (%)</th>
<th>FLOATING TIME (h)</th>
<th>% DRUG ENTRAPMENT EFFICIENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96.49</td>
<td>6</td>
<td>97.59</td>
</tr>
<tr>
<td>30</td>
<td>95.87</td>
<td>6</td>
<td>96.86</td>
</tr>
<tr>
<td>60</td>
<td>94.89</td>
<td>6</td>
<td>96.93</td>
</tr>
<tr>
<td>90</td>
<td>94.32</td>
<td>6</td>
<td>96.35</td>
</tr>
</tbody>
</table>

**Figure 16: Invitro drug release profile during stability study period**
4. CONCLUSION
The beads were prepared using Ionotropic gelation technique. The prepared beads were optimized by 3² Full Factorial Design. The optimized beads show floating time of 6hr in acidic media. The beads show good swelling in basic media compared to acidic media. The entrapment efficiency was 97.59%. The results of invitro drug release in both acidic and phosphate buffer showed minimum% cumulative drug release of 11.13% at 6th hr in acidic buffer and sudden release of 96.49% drug in phosphate buffer within 1 hr. The kinetics of release follows higuchi and Kim Fassihi model which suggests a pulsatile release follows diffusion kinetics. The n value of Kim Fassihi plot suggests that release follows anomalous non Fickian transport. The formulation shows good permeation through intestinal tissue.

The results of all the evaluation study demonstrate that the optimized formulation can be used as pulsatile drug delivery system which provide time and site specific sudden drug release in intestine after a lag time of 6hrs in stomach which can be used to treat the morning surge in hypertension in patients with a night time dose itself.

5. ACKNOWLEDGEMENT
We would like to thank CUSAT (KOCHI) for providing facility for SEM and DSC.

6. REFERENCES


