DESIGN AND EVALUATION OF MUCOADHESIVE ATORVASTATIN CALCIUM TABLET USING SURFACE RESPONSE METHODOLOGY

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ABSTRACT
Objective: Study was aimed at to developed mucoadhesive tablet containing inclusion complex of Atorvastatin calcium with β-cyclodextrin and evaluating the effect of two independent variables (X1) HPMCK4M and (X2) Carbopol 934P using central composite Design.

Method: Mucoadhesive tablets were prepared by kneading method using Factorial design and all prepared tablets were evaluated by for their pre and post compression study, Bioadhesion Studies, Mucoadhesive time, effect of independent variables on dependent variables i.e. (Q):- Percentage release at 2 hr (Q2) percentage drug release at 8 hr. The main effects and the interaction terms were quantitatively evaluated by quadratic model.

Results: All the physical parameters for the tablet were within Pharmacopoeial limits. The bioadhesive strength and in-vitro release of formulation was found to be vary linearly with increasing amount of both polymers. The Q, and Q shows a wide variation (i.e. 18.94-27.54, 69.52-91.54 % respectively). The effects of all the tested independent variables had P-values < 0.05.

Conclusion: A systematically planned study by using a 3 full factorial design revealed that the amount of HPMCK4M (X1) and amount of Carbopol 934P (X2) had a significantly effect on Q and Qs. The formulation F4 was selected as an optimized formulation because it gave the best results in terms of the required bioadhesion study, and drug release in sustained release manner. Dissolution profiles have shown non-fickian drug release mechanism, which indicated that the drug release was by diffusion and erosion mechanism.

Keywords: Atorvastatin Calcium, Mucoadhesive Tablet, HPMC K4M, Carbopol 934P, Factorial Design.

INTRODUCTION
Among the various routes of drug delivery, oral route is the most suitable and most widely accepted one by the patients for the delivery of therapeutically active drugs[1]. Atorvastatin Calcium belongs to anti-hyperlipidemic class. It is normally indicated for the treatment of hyperlipidemias and cardiovascular diseases. Atorvastatin is a competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that convert 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Sterol synthesis is inhibited 1 to 8 hour after single oral dose of atorvastatin. The oral bioavailability of atorvastatin is approximately 14% because of extensive first pass metabolism. It's having half-life of 11-19 h in humans after single dose of 10 mg of Atorvastatin calcium. These pharmacokinetic parameters make Atorvastatin calcium a suitable candidate for buccal delivery [2-3].

Sustained or controlled release delivery systems can achieve predictable and reproducible release rates, extended duration of activity for short half – life drugs, decreased toxicity, and reduction of required dose, optimized therapy and better patient compliance [4].

The application of an optimization technique consisting of statistical design to pharmaceutical formulation development provides an efficient and economical method to acquire the necessary information to understand the relationship between controllable (independent) variables and performance dependent variables or responses[5-7]. The study was aimed to developed mucoadhesive dosage form containing inclusion complex of Atorvastatin calcium with β-cyclodextrin investigate the effect of two independent variables i.e. amount of two polymer: HPMC K4M and Carbopol 934P (CP) on in-vitro release at the end of 2 h and 8 h of mucoadhesive drug delivery system. So an attempt was made by formulating Atorvastatin calcium buccal tablet to reduced dosing frequency and to achieve plasma concentration profile over 10 h.

MATERIAL AND METHOD
Atorvastatin Calcium was obtained as a gift sample by Glenmark Pharmaceuticals, Nasik. (Maharashtra) India, HPMC-K4M and Carbopol 934P were obtained as a gift sample from the Zim Labs. Ltd. Nagpur. India. β-Cyclodextrin was obtained as gift from SDFCL, Mumbai. All other materials and solvents used were of analytical grade.

EXPERIMENTAL
Infrared spectra analysis
Infrared spectrum of Atorvastatin calcium was determined on Fourier Transform Infrared Spectrophotometer (FTIR-4100s) using KBr dispersion technique [8-9]. The line base correction was done using dried potassium bromide. IR spectra for drug and mixture were recorded in a Fourier Transform Infra-Red (FTIR) spectrophotometer with KBr.

Formulation of Mucoadhesive Tablets
Formation of Complex
Accurately weighed quantities of drug and β-cyclodextrin were taken in ratio of (1:1), β-cyclodextrin was added to the mortar, add small quantity of 50% methanol and dichloromethane (1:2) with continuous triturating in clockwise direction to get slurry like incorporated into the slurry and trituration continued for one hour. Slurry was air dried at 25°C for 24 hours, pulverized and passed through sieve No.100 and stored in desiccators over fused calcium chloride [9].

Formulation of Bilayer Mucoadhesive Tablet
The mucoadhesive tablets were formulated by direct compression method. All the ingredients of the formulation were passed through a sieve # 60 and were blended in a mortar with a pestle to obtain uniform mixing. The blended powder of the core was compressed on 8 mm punch with single stroke multi station tablet punching machine [10-11]. After punching the core layer, upper punch was removed and ethyl cellulose 30 mg was added over it and again compressed. Tablets weighing ± 150 mg were obtained. The various compositions of preliminary formulation containing different ratios of polymers were tried are given in the Table 1.
Experimental Design

From the preliminary batches A 3² randomized full factorial design was applied, in this design 2 factors were evaluated, each at 3 levels and experimental trials were performed at all 9 possible combinations. The amount of HPMC K4M (X1) and amount of Carbopol 934P (X2) were selected as independent variables. The percentage drug release at 2 hours (Q₁) and percentage release at 8 hours (Q₂) were selected as dependent variables [5,12-13].

Evaluation of prepared mucoadhesive tablets

Weight variation

Weigh individually 20 units selected at random or, for single dose preparations in individual containers, the contents of 20 units, and calculate the average weight. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table and none deviates by more than twice that percentage [14].

Thickness of Tablets

Six tablets were taken and the thickness was measured using a micrometer screw gauge. The tablet thickness should lie between within a ±5 % variation of a standard value [11].

Hardness

There is a certain requirement of hardness in tablets so as to withstand the mechanical shocks during handling, manufacturing, packaging and shipping. Hardness tester (Monsanto type) was used to measure hardness of tablets[11]. The whole experiment was performed in triplicate. It is expressed in Kg/cm²[11].

Friability

An adequate resistance for powdering and friability are the necessary requisites for consumer acceptance. This test was carried out by using tablet friability test apparatus (Roche). Twenty pre-weighed tablets were rotated at 25 rpm for 4 min. The tablets were then de-dusted and reweighed. The percentage friability was measured using following formula [14].

\[
\% F = \frac{W_0 - w}{W_0} \times 100
\]

Uniformity of Content

Weighed and powdered five tablets accurately. A quantity of powder equivalent to 10mg of Atorvastatin Calcium was weighed accurately and extracted in 100 ml methanol. After shaking for 20 min, sufficient dilution with methanol, samples were analyzed by UV spectrophotometer at 241 nm. This procedure repeated thrice. Amount of drug present was determined from the standard calibration curve Atorvastatin calcium [14].

Surface pH of the Tablet

A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.5 ± 0.05) for 2 h at room temperature. The pH was measured by bringing the electrode in contact with the surface of the tablet and allowing it to equilibrate for 1-8 min [15-17].

Swelling Studies

Tablet was weighed individually (recorded as W1) and placed separately in Petri dish containing 5 ml of phosphate buffer (pH 6.8) solution. At regular intervals for 5 hours, the tablet was removed from the Petri dish and excess surface water was removed carefully using the filter paper [18]. The swollen BADDS was then reweighed (W2), and swelling index (SI) was calculated using formula as

\[
SI = \frac{W_2 - W_1}{W_1}
\]

Swelling Index =

Ex-Vivo Mucoadhesion Time

The Ex-vivo Mucoadhesion time was examined after application of buccal tablet on freshly cut sheep buccal mucosa. The fresh buccal mucosa was tied on the glass slide and tablet wetted with one drop of pH 6.8 phosphate buffers and pasted to mucosa by applying a light force with finger tip. Then the slide was put in beaker containing 200 ml of phosphate buffer and kept at 37 ± 1°C after 2 minute a slow stirring was apply to stimulate buccal cavity environment and tablet adhesion was record for 12 hrs. The time require for detaching tablet from buccal mucosa was recorded as a mucoadhesion time [18-19].

In-vitro Bioadhesion Studies

For in-vitro study, an apparatus designed for determination of mucoadhesive force [19]. The working of balance formed the basis of the fabricated bioadhesion test apparatus. The glass vial was fixed to one side of the balance. The Gout buccal mucosa was tied to this glass vial. The other glass vial was attached to the base of the balance within the bigger size bottle which is filled with phosphate buffer pH 6.8 to maintain the mucosa in moist condition during the study. The balance was balanced at this position by placing weight to the right side pan. The tablet was moistening in the phosphate buffer pH 6.8 and it was placed in between this two vials and press by finger slightly to stuck tablet to the buccal mucosa Then the weight was added slowly to the right side pan till the mucosal surface of the pan detached from the tablet surface. Using this bioadhesion test assembly, the bioadhesive strength expressed in weight, required for detachment of the tablet from the mucosa was determined [19-20].

Ex-Vivo Drug Permeation Study

The in-vivo buccal drug permeation study of Atorvastatin calcium through the sheep mucosa was performed using a modified diffusion cell at 37°C ± 0.2°C. Fresh sheep mucosa was mounted between the donor and receptor compartments of France diffusion cell. The tablet was placed with the core facing the mucosa, and the compartments were clamped together. The donor compartment was filled with 1 ml of phosphate buffer pH 6.8. The receptor compartment was filled with phosphate buffer pH 6.8 and the hydrodynamics in the compartment was maintained by stirring with a magnetic bead at uniform slow speed. 5 ml samples were withdrawn at predetermined time intervals and analyzed for drug content by UV spectrophotometer [21-22].

Accelerated Stability Studies

Accelerated stability testing of prepared formulation batch was carried out to determine the stability of drug and carrier and also to determine the physical stability of formulation under accelerated storage condition at various temperatures [23]. The prepared tablets were placed in bromsulfalein screw capped glass containers. The samples were kept at condition of 45°C+20°C/70%±5RH and were analyzed at 60th, 120th and 180 days for their changes in drug content.
RESULT AND DISCUSSION
Infrared Absorption Spectrum
The FT-IR spectra of the pure Atorvastatin Calcium and physical mixture of drug and polymers were analysed to check for any interaction between drug and polymers. The characteristic peaks of Atorvastatin Calcium were appeared in the spectra without any significant change. This indicated that there was no chemical interaction between Atorvastatin Calcium and polymers. IR spectrum showed all prominent peaks of Atorvastatin which was comparable with standard IR graph. The major IR peaks observed in Atorvastatin were 3055 (Aromatic C-H Stretching), 1572 (C=O Stretching), 2970 (CH3-O Stretching), 2937(C-H Stretching), 3363(N-H stretching vibration), 1454(CH2-O Bending), 1622(Aromatic C=C), 1215(Aromatic C-N Stretching).

Fig. 1: IR spectrum of Atorvastatin Calcium.

Fig. 2: IR Spectrum of Atorvastatin Calcium+ Carbopol

Fig. 3: IR Spectrum of Atorvastatin Calcium + β-Cyclodextrin.

Fig. 4: IR Spectrum of Atorvastatin Calcium+ HPMC-K4M
Optimization Results

A 3² factorial design was constructed to study the effect of the amount of HPMCK4M (X1) and carbopol (X2) on the drug release from buccoadhesive tablet of Atorvastatin respectively. The dependent variables chosen were percentage drug release at 2 hours (Q₂) and percentage drug release at 8 hours (Q₈). A statistical model incorporating interactive and polynomial term was used to evaluate the response eq.1

\[ Y = b0 + b1X1 + b2X2 + b12X1X2 + b11X1X1 + b22X2X2 \]  (1)

Where, Y is dependent variable, b₀ is the arithmetic mean response of the 9 runs, and b₁ (b₁ b₂, b₁₂ b₁₁ and b₂₂) is the estimated coefficient for the factor X₁ the main effect. (X₁ and X₂) represents the average results of changing one factor at a time from its low to high values. The interaction term (X₁ X₂) show how the response changes, when 2 factors are changed simultaneously. The polynomial term (X₁² and X₂²) are included to investigate nonlinearity. The Q₂ and Q₈ for 9 batches (F₁- F₉) showed a wide variation (i.e. 18.94-27.54, 69.52-91.54% respectively). The responses of formulation prepared by 3 factorial designs are indicated in Table 2. The data clearly indicate that the Q₂ and Q₈ were strongly dependent on the selected independent variables. The fitted equation relating the response Q₂ and Q₈ to the transformed factors are, given in eqs.2,3.

% Release at 2 hrs. Q₂ = 26.25 ± 1.95X₁ ± 0.74X₁X₂ + 1.43X₁X₂ ± 0.24X₁² + 3.36X₂² (R² = 0.8599)  (2)

% Release at 8 hrs. Q₈ = 87.45 ± 3.86X₁ ± 3.99X₁X₂ ± 2.60X₁X₂ ± 5.47X₁² + 6.53X₂² (R² = 0.8826)  (3)

Table 2: Dissolution Characteristics of Formulation Batches in 3² Factorial Design

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Coded value</th>
<th>% Release at (Q₂)</th>
<th>% Release at(Q₈)</th>
<th>n</th>
<th>K</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁</td>
<td>X₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
<td>27.54</td>
<td>91.54</td>
<td>0.4911</td>
<td>20.9450</td>
</tr>
<tr>
<td>F2</td>
<td>0</td>
<td>-1</td>
<td>21.22</td>
<td>87.84</td>
<td>0.7567</td>
<td>14.8757</td>
</tr>
<tr>
<td>F3</td>
<td>+1</td>
<td>-1</td>
<td>18.94</td>
<td>74.18</td>
<td>0.6729</td>
<td>19.7260</td>
</tr>
<tr>
<td>F4</td>
<td>-1</td>
<td>0</td>
<td>20.07</td>
<td>86.29</td>
<td>0.7956</td>
<td>13.3931</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
<td>26.25</td>
<td>87.45</td>
<td>0.6199</td>
<td>22.0669</td>
</tr>
<tr>
<td>F6</td>
<td>+1</td>
<td>0</td>
<td>23.90</td>
<td>81.14</td>
<td>0.6998</td>
<td>19.5516</td>
</tr>
<tr>
<td>F7</td>
<td>-1</td>
<td>+1</td>
<td>22.23</td>
<td>76.49</td>
<td>0.7554</td>
<td>14.9075</td>
</tr>
<tr>
<td>F8</td>
<td>0</td>
<td>+1</td>
<td>25.05</td>
<td>78.02</td>
<td>0.7360</td>
<td>17.3378</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>19.33</td>
<td>69.52</td>
<td>0.6300</td>
<td>14.6395</td>
</tr>
</tbody>
</table>

Table 3: Physical Parameters of Mucoadhesive Tablet.

<table>
<thead>
<tr>
<th>Batches</th>
<th>Parameters</th>
<th>Thickness (mm)</th>
<th>% Friability</th>
<th>Avg. Weight</th>
<th>% Wt. Variation</th>
<th>Surface pH</th>
<th>Uniformity of content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Hardness (Kg/cm²)</td>
<td>5.74</td>
<td>±0.18</td>
<td>±0.06</td>
<td>±0.05</td>
<td>±0.66</td>
<td>±1.86</td>
</tr>
<tr>
<td>F2</td>
<td>Thickness</td>
<td>3.75</td>
<td>±0.31</td>
<td>±0.15</td>
<td>±0.04</td>
<td>±0.57</td>
<td>±2.19</td>
</tr>
<tr>
<td>F3</td>
<td>% Friability</td>
<td>3.59</td>
<td>±0.26</td>
<td>±0.09</td>
<td>±0.05</td>
<td>±0.66</td>
<td>±2.02</td>
</tr>
<tr>
<td>F4</td>
<td>Avg. Weight</td>
<td>148.79</td>
<td>±0.28</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.64</td>
<td>±1.92</td>
</tr>
<tr>
<td>F5</td>
<td>% Wt. Variation</td>
<td>148.79</td>
<td>±0.25</td>
<td>±0.21</td>
<td>±0.04</td>
<td>±0.61</td>
<td>±1.87</td>
</tr>
<tr>
<td>F6</td>
<td>Surface pH</td>
<td>150.33</td>
<td>±0.22</td>
<td>±0.09</td>
<td>±0.04</td>
<td>±0.58</td>
<td>±2.58</td>
</tr>
<tr>
<td>F7</td>
<td>Uniformity of content</td>
<td>150.33</td>
<td>±0.27</td>
<td>±0.16</td>
<td>±0.05</td>
<td>±0.59</td>
<td>±1.66</td>
</tr>
<tr>
<td>F8</td>
<td>X₁</td>
<td>3.39</td>
<td>±0.16</td>
<td>±0.04</td>
<td>±0.06</td>
<td>±0.68</td>
<td>±3.11</td>
</tr>
<tr>
<td>F9</td>
<td>X₂</td>
<td>3.36</td>
<td>±0.33</td>
<td>±0.07</td>
<td>±0.06</td>
<td>±0.65</td>
<td>±2.56</td>
</tr>
</tbody>
</table>

Physical Characteristics of Tablet

The prepared formulations were evaluated for the physical characteristics like thickness, hardness, friability, weight variation, uniformity of drug content. The results obtained are shown in Table 3. All the physical parameters values for the tablet were within official and some unofficial tests. The surface pH of all the tablets was within the range of 6.6 to 6.8 which indicated that there is no risk of mucosal damage or irritation.

The values of the correlation coefficient indicate a good fit. (Fig 5-8). The plot of the amount of HPMC K4M (X₁) and amount of Carbopol (X₂) versus Q₂ and Q₈ respectively. The data demonstrate that both X₁ and X₂ affect the drug release (Q₂ and Q₈). It was concluded that the low level of X₁ and the low level of X₂ favour the preparation of buccoadhesive tablets. The high value of X₁X₂ coefficient also suggests that the interaction between X₁ and X₂ has a significant effect on Q₂. An increase in the concentration of HPMC4M (X₁) and
amount of Carbopol (X2), decrease rate of release of buccoadhesive tablet respectively.

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., negative or positive). Table 4 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors. Data were analyzed using Microsoft Excel.

The fitted equations relating the responses, \( Q_2 \), \( Q_8 \) to the transformed factor are shown in the Table 4.

![Fig. 5: Response surface plot for \( Q_2 \)](image)

![Fig. 6: Contour plot for \( Q_2 \)](image)

![Fig. 7: Response surface plot for \( Q_8 \)](image)

![Fig. 8: Contour plot for \( Q_8 \)](image)

**Table 4: Summary of Results of Regression Analysis**

<table>
<thead>
<tr>
<th>Model</th>
<th>( Q_2 ) coefficient</th>
<th>p-value</th>
<th>( Q_8 ) coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>26.25</td>
<td>0.0067</td>
<td>87.45</td>
<td>0.0037</td>
</tr>
<tr>
<td>X1</td>
<td>-1.95</td>
<td>0.0198</td>
<td>-3.86</td>
<td>0.0215</td>
</tr>
<tr>
<td>X2</td>
<td>-0.74</td>
<td>0.2894</td>
<td>-3.99</td>
<td>0.0186</td>
</tr>
<tr>
<td>X1X2</td>
<td>1.43</td>
<td>0.1637</td>
<td>2.60</td>
<td>0.2037</td>
</tr>
<tr>
<td>X1²</td>
<td>-2.24</td>
<td>0.0146</td>
<td>-5.47</td>
<td>0.0059</td>
</tr>
<tr>
<td>X2²</td>
<td>3.36</td>
<td>0.0019</td>
<td>-6.54</td>
<td>0.0023</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.8599</td>
<td></td>
<td>0.8826</td>
<td></td>
</tr>
</tbody>
</table>

\( R^2 \) value for \( Q_2, Q_8 \) are 0.8599 and 0.8826 respectively indicating good correlation between dependent and independent variables. The terms with \( P<0.05 \) were considered statistically significant.
The bioadhesion and drug release profile are dependent upon swelling behavior of the tablets. Swelling index was calculated with respect to time. Swelling index increased as the weight gain by the tablets increased proportionally with the rate of hydration. The formulation batch containing higher Carbopol concentration than HPMC K4M showed higher swelling index. From the results obtained, it was observed that the increased concentration of carbopol 934P and HPMC K4M in the formulation increases the swelling indices as shown in Table 5.

The result from Bioadhesion studies revealed that the highest detachment force was observed in formulation containing carbopol-934P (F1 and F4) in higher indicating that as the concentration of Carbopol 934P in formulation increases, bioadhesion force also increases. From the dissolution study of batch F1 to F9, it was concluded that release from the tablet was largely dependent on the polymer swelling, drug diffusion and matrix erosion. The drug release study was carried out up to 12 hrs as shown in Table 6.

The percentage drug release from batch F1 to F9 vary from 81.52±0.34% to 97.79±0.42%. Large concentration of high viscosity polymer induces the formation of strong viscous gel layer that slowed down the rate of water diffusion into the tablet matrix. Dissolution profiles for all batches were shown in (Fig. 9). The combinations of polymers significantly retard the release for more than 12 hrs. As the concentration of HPMC K4 M increased the release rate decreased. An increase in the polymer This may decrease the effective diffusion coefficient of drug and therefore there is reduction in drug release rate.

![Fig. 9: % Drug Released of Formulations F1 to F9](image-url)

The formulation batch F4 was studied for the in-vivo drug permeation using sheep mucosa, permeation and was found to 94.72±0.45% after the 12 hours there was some decreased at 8 hr may be due to matrix formation.
Accelerated Stability Testing

Stress stability studies (SST) was carried for optimized batch F4 by exposing it to 40°C/75%RH for 1, 3, 6, and 120 days. The sample was analyzed for physical parameters, colour, hardness, IR, uniformity of content, and percentage drug release.

CONCLUSION

Mucoadhesive tablets were prepared by direct compression using central composite method. Formulations batches were evaluated for physical parameter, swelling studies, bioadhesive studies, in-vitro drug release and ex-vivo drug permeation. Bioadhesion studies were carried out to determine mucoadhesive potential of prepared tablets. Tablets were evaluated for in-vitro drug release for 10 hrs, using USP type II method and dissolution profiles has shown non-fickian drug release mechanism, which indicated that the drug release was by diffusion and erosion mechanism. From these results it can be concluded that optimized batch F4 was most promising comprising of 1:2 ratio of HPMC K4M/Carbopol-934p.

REFERENCES