Design and Optimization of Bioadhesive Vaginal Tablets of Acyclovir

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ABSTRACT

Genital herpes is a common, highly infectious, contagious disease caused by a virus that infects genital areas. Oral and topical acyclovir formulations are available in the market but its bioavailability is reported to be 10 to 20%, hence there is a need to develop vaginal drug delivery. Vaginal tablets of acyclovir were prepared by direct compression method using carbopol 934P and xanthan gum as bioadhesive polymers. The effect of process variables, amount of carbopol 934P, xanthan gum and sodium bicarbonate on the responses percent swelling index and percent drug release were studied using D-Optimal design. Parameters like swelling index, surface pH, In-vitro drug release, In-vitro drug permeation and bioadhesion strength were studied. The vaginal tablets were found to sustain the release of acyclovir for 12 hrs in simulated vaginal fluid. The maximum bioadhesion strength was observed in the tablets formulated with increased concentration of xanthan gum. The possible drug release mechanism for the optimized vaginal tablet of acyclovir was observed to be Korsemeyer - Peppas. In-vitro absorption studies showed that the drug absorption is low and the amount of the drug in the absorption medium at the end of 8 hour was found to be 26.03%. Stability studies indicated that there were no significant changes in drug content for period of 6 months. Thus stable safe vaginal tablets of acyclovir can be formulated to impose maximum bioavailability of drug, by sustaining the drug delivery thereby improving the patient compliance.

Keywords: Acyclovir, Bioadhesion, Vaginal tablets, D-Optimal design, In-vitro release, In-vitro permeation, In-vitro absorption

INTRODUCTION

Bioadhesion has been the subject of interest in recent years because mucoadhesion is the solution for bioavailability problems, resulting from too short stay of pharmaceutical dosage form at the absorption site¹. Bioadhesion is described as the adhesion of drug to the biological substrates such as skin or other tissues⁵. Bioadhesive vaginal dosage forms releases the active ingredient slowly, so that the vagina would not be immediately exposed to the entire dose of the active ingredient thereby minimizing toxic effects on the vaginal epithelium¹. The vagina, in addition to being a genital organ with functions related to conception, serves as a potential route for drug administration, mainly used for local action in the cervico-vaginal region. It has potential of delivering drugs for systemic effects and uterine targeting because of its large surface area, rich blood supply and permeability to a wide range of compounds¹. In vaginal drug delivery, the physiological conditions imposed by the protective mechanisms of the vagina often lead to the limited contact time of administered drugs with vaginal mucosa and short duration of therapeutic efficacy, making a frequent dosing regimen necessary. Vaginal therapy would be thus significantly improved if an intravaginally administered drug can retain at the site of administration for a prolonged period of time².

Genital herpes is a highly infectious disease caused by the Herpes Simplex Virus (HSV). They are of two types namely, HSV-1 and HSV-2. Most genital herpes infections are caused by HSV-2. Genital herpes infections look like small blisters or ulcers on the genitals. The ulcers or blisters found around the genitals (the perineum), in and around the anus. Genital herpes infection is treated with acyclovir. Acyclovir is an anti-viral drug, a synthetic nucleoside analogue, which is active against herpes viruses. Acyclovir is activated via monophosphorylation by virus induced thymidine kinase. Acyclovir undergoes two additional phosphorylation to acyclovir triphosphate. It binds to HSV DNA polymerase in competition with guanosine, incorporated into viral DNA and prevents further chain elongation⁶. Topical acyclovir has been used but it is less effective. The molecular formula for acyclovir is C₇H₇N₄O₆. The chemical name is 9-[(2-Hydroxy)methyl] guanine; 2-Amino-1, 9-dihydro-9-(2-hydroxyethoxymethyl)-6H-purin-6-one. Its molecular weight is 225.2; Acyclovir is slightly soluble in water, very slightly soluble in alcohol; freely soluble in dimethyl sulfoxide; soluble in dilute solutions of alkali hydroxides and mineral acids. The pKa is 2.27 and 9.25, half life 2-3 hours; protein binding 9-33%, melting point is 256.5°C⁷. So there is always a need to focus on development of bioadhesive vaginal tablets of acyclovir that is designed to prolong the residence time and consequently to obtain a long therapeutic concentration at the site of infection. This study addresses the possible use of mixture of xanthan gum and carbopol 934P in varying ratio with sodium bicarbonate for the preparation of bioadhesive tablets of acyclovir using D-Optimal design.

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MATERIALS AND METHODS

Materials

Acyclovir was a gift sample from Remidex Pharma Pvt Ltd, Bangalore, Carbopol 934P and aerosil was obtained from Himedia laboratories Pvt. Ltd, Mumbai, Xanthan gum and microcrystalline Cellulose was obtained from Yarrow Chem Products, Mumbai. Sodium bicarbonate was obtained from RFCL Limited, New Delhi. All other materials used in the current study were of analytical grade.

Methods

Formulation of vaginal tablets of acyclovir

Vaginal tablets of acyclovir were prepared by direct compression method. Accurately weighed quantity of acyclovir was triturated with appropriate quantities of carbopol 934P and xanthan gum (Table 1) in a mortar and pestle. To this sodium bicarbonate, microcrystalline cellulose, aerosil and talc were added, triturated and passed through BSS# 80. The resulting mixture were compressed using 12 mm caplet punches in rotary tablet punching machine (Rimek RSB-4 Mini Press).

Optimization of acyclovir vaginal tablets by D-optimal design

The runs based on D- Optimal design using response surface methodology, were utilized to evaluate the response variables. The formulation variables are 1) Amount of xanthan gum (X1) 2) Amount of carbopol (X2) and 3) Amount of sodium bicarbonate (X3). The responses subjected for the analysis was; 1] Swelling index (Y1) 2] Time taken for 90% drug release (t_{90}) in hours (Y2). Each variable was studied at two different levels (-1, +1) and center point (0) which is the midpoint of each factor range. The minimum and maximum range of variables investigated for the variables were 10 mg to 40 mg for Xanthan gum; 50 mg to 100 mg for carbopol 934P and 15 mg to 30 mg for sodium bicarbonate. The responses were subjected to multiple regression analysis to find out the relationship between the factors used and the responses obtained. The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at 0.05 level using a commercially available software package Design Expert 8.04 trial version (Stat Ease, USA). The optimization of the bioadhesive vaginal tablets was carried out by taking into consideration the amount of polymer as formulation variables whose operating range is mentioned in the Table 1. All experiments were carried out in triplicate.

Compatibility studies

Differential scanning calorimetry

Acyclovir, carbopol 934P, xanthan gum and physical mixture of polymers with drug were subjected for DSC studies using differential scanning calorimeter (Mettler-7, Germany). 5mg of sample was placed in a 50 µl perforated aluminium pan and sealed. Heat runs for each sample were set from 5°C to 300°C using nitrogen as purging gas and the samples were analyzed.

Analytical method development of acyclovir

A new, simple, accurate, environmental friendly, cost effective, safe, and sensitive spectrophotometric method for

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<th>Carbopol (mg)</th>
<th>Sodium bicarbonate (mg)</th>
<th>Acyclovir (mg)</th>
<th>Micro crystalline cellulose (mg)</th>
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estimation of acyclovir using equal volume of 4M urea and 25% sodium acetate as hydrotropic solution was developed.

**Preliminary solubility studies of the drug**

Solubility of acyclovir was determined by saturation aqueous solubility method in equal volume of 4M urea and 25% sodium acetate in distilled water. An excess amount of drug was added to the 50ml beakers containing equal volume of 4M urea and 25% sodium acetate in distilled water. The beakers were shaken at 27±0.5°C for 24 hours. The solution was then filtered through Whatmann filter paper # 41. Then the filtrate was suitably diluted and analyzed spectrophotometrically against corresponding solvent blank at a wavelength of 255nm (Shimadzu UV-1700).

**Preparation of standard stock and calibration curve**

The standard stock solution of acyclovir (1mg/ml) was prepared by dissolving 50mg of drug in 50 ml of equal volume of 4M Urea and 25% Sodium acetate. From this stock solution working standard solutions having concentrations 5, 10, 15, 20, 25, 30 and 40 µg/ml was prepared with distilled water. The absorbance of resulting solutions were measured spectrophotometrically at 255nm and a calibration curve was plotted to get the linearity and regression equation.

**Analysis of Acyclovir in tablets using 4M Urea and 25% Sodium Acetate**

Twenty tablets (commercially available product) were weighed and powdered. Powder equivalent to 200 mg acyclovir was weighed and transferred to a 100 ml volumetric flask containing 80 ml of equal volume of 4M urea and 25% Sodium acetate. The flask was shaken for about 5 min to solubilize the drug. Then volume was made up to the mark with distilled water. Solution was filtered through Whatmann filter paper #41. A part of filtrate was taken and kept at room temperature for 24 hours to check the effect on stability of drug in presence of urea and also to note precipitation. The remaining part of filtrate was appropriately diluted with distilled water and analyzed for drug content measured at a wavelength of 255nm. There was no precipitation in the filtrate for 48 hours.

**Validation of the proposed method**

The proposed analytical method was validated for parameters such as linearity, percent recovery, precision, robustness, LOD and LOQ.

**Evaluation of bioadhesive vaginal tablets**

**Surface pH**

One tablet was placed in 10 ml of simulated vaginal fluid (SVF) in small beakers and it was allowed to swell and the pH was measured at time intervals of 1, 2, 3, and 4 up to 8 h by placing the electrode in contact with the surface of the tablet. Average of three determinations was taken and calculated.

**Swelling study**

One tablet was weighed (W₁) and placed in a petri dish containing 5ml of SVF. At interval of 1hr, the tablet was removed from the petri dish and wiped off with a tissue paper. The swollen tablet was then reweighed (W₂) and the swelling index were calculated by taking average of three determinations using the formula given below.

Swelling Index = \[
\frac{W₂ \times W₁}{W₁} \times 100
\]

**Ex-vivo bioadhesion strength**

Mucoadhesive strength of the tablet was determined by using modified balance method. Fresh sheep vaginal mucosa was obtained from local slaughter house and suitable dimension of the mucosa was fixed to the apparatus using cyanoacrylate adhesive. One end of the tablet is adhered on to the mucosa with little amount of simulated vaginal fluid there by creating adhesive bond between the tablet and the membrane. The other end of the tablet is connected to the pan for the addition of weights. The weights were added slowly to the pan until the tablet gets detached from the mucosal surface, from which mucoadhesive strength of the tablet in grams was determined. The following parameters were also calculated by using the bioadhesion strength.

\[
\text{Force of Adhesion (N)} = \frac{\text{Bioadhesion Strength} \times 9.81}{1000}
\]

\[
\text{Bond Strength (N/m²)} = \frac{\text{Force of Adhesion (N)}}{\text{(Surface Area)}}
\]

**In-vitro dissolution studies**

The studies were carried out in USP dissolution apparatus Type II containing 600ml of Simulated Vaginal Fluid pH4.5 maintained at 37°C± 0.5°C and rotated at 50 rpm. 2ml of solution was withdrawn from the dissolution apparatus at regular predetermined time intervals and same volume of sample was replaced with fresh dissolution medium. The collected samples were diluted suitably and their absorbances were measured spectrophotometrically at 253 nm using UV-visible Spectrophotometer (UV-1601, Shimadzu).

**Kinetic modeling of drug release mechanism**

The dissolution data of all formulations were fitted to zero-order, first-order, Hixson-Crowell, Higuchi and Korsemeyer and Peppas models to predict the drug release mechanism.

**In-vitro drug Permeation**

**In-vitro** permeation was studied using the vagina of freshly sacrificed sheep. Tablets to be tested were placed with in the mucus membrane of sheep vagina and hanged on to the burette stand in such a way that the tablet was immersed into a
beaker containing 100ml of SVF and placed on the magnetic stirrer, stirred at 50 rpm by maintaining the temperature at 37°C. Samples of 2ml were withdrawn at predetermined intervals and amount of acyclovir was analyzed spectrophotometrically at 253 nm. Average of three determinations was taken and calculated.

In-vitro absorption studies
The dissolution-absorption studies were performed using Continuous Dissolution absorption system. The optimized batch of acyclovir vaginal tablet was used in the absorption studies. The medium consisted of 600ml of SVF of pH 4.5 maintained at 37 ± 0.5°C. A freshly sacrificed sheep vaginal mucosa was obtained from the slaughter house and a segment was clamped to the perfusion apparatus. The total volume of the absorption compartment (tube A and tube B of perfusion apparatus) was 30ml of SVF. The drug diffused from the dissolution medium (mucosal side) to the serosal side (absorption compartment). The tablet was placed in the dissolution basket of the designed system and rotated at 50 rpm. Dissolution samples (2 ml) were withdrawn at regular intervals of time and analyzed spectrophotometrically at 253 nm. The transported drug from the absorption compartment was sampled at 3 min later than their corresponding dissolution samples and analyzed spectrophotometrically at 253 nm. The whole experiment was repeated in triplicate (n=3) using fresh dissolution medium as well as fresh vaginal mucosa each time.

Stability studies
Accelerated stability studies were carried out as per ICH guidelines (Q1A) where the conditions were maintained 40°C±2°C with 75± 5% RH for a period of 6 months. The formulations were closely packed in aluminum foils and stored in stability chamber and evaluated for their physical appearance, drug content and in-vitro drug release studies at intervals of 2 months. The shelf life period of the prepared vaginal tablets is determined by using similarity factor.

RESULTS AND DISCUSSION
Vaginal tablets of acyclovir were prepared by direct compression method. In the present study 16 formulations of acyclovir with varying concentration of Carbopol 934P and Xanthan gum used as bioadhesive agents were prepared, each tablet weighing 400mg.

Differential scanning calorimetry
The DSC analysis of pure drug, polymers and the physical mixture were carried out to evaluate any possible interaction between drug and polymer. The DSC thermogram of pure drug acyclovir showed a characteristic endothermic peak at 256°C as in figure 1a which is the melting point of acyclovir. DSC thermogram of carbopol 934P revealed a bulge at 216.82°C (1b) confirming the amorphous nature of the polymer. DSC thermogram of xanthan gum revealed an exothermic peak at 271°C (1c). Similar sharp endothermic peaks at 256°C (figures 1d, 1e) was observed in physical mixture of acyclovir and polymers. This study confirmed that there was no interaction between the drug and the polymer.

Analytical method development
The solubility studies indicated that aqueous solubility of acyclovir was enhanced in hydrotropic solution of equal volume of 4M urea and 25% sodium acetate as compared to solubility in distilled water. The Beer-Lambert’s concentration range was found to be 5-40 μg/ml for acyclovir at the wavelength of 255 nm. The solubility of pure acyclovir in distilled water was found to be 10.34mg/ml, whereas in the equal volume of 4M urea and 25% sodium acetate, the solubility was found to be 26.4mg/ml. There was a marked increase in solubility of acyclovir in the hydrotropic solution used. So it was optimized to employ this solution in the analysis of the tablet formulation. A part of the solution was kept at room temperature for 24 hours to check the effect on stability of drug in presence of urea and for precipitation. The study revealed that estimations of acyclovir can be done within 24 hours without any detrimental effect on drug stability. The drug showed good regression value at this wave length. It was evident that there was good correlation between the amounts estimated and the label claim. The estimated label claim was found to be 99.33±0.57mg with a standard error of 0.389. Accuracy and reproducibility of the proposed method were further confirmed by the recovery studies. The results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method (Table 2). The method was found to be robust within the Beer's range of 5-40 μg ml⁻¹. The low values of LOD and LOQ indicated good sensitivity of proposed method. Repeatability results indicated the precision under the same operating conditions over a short

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<sup>a</sup> mean ±S.D (n= 6)
interval time and inter-assay precision. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for the method co-efficient of variation the values were 0.2014 and 0.2890 that were not more than 1.0% which confirmed the good intermediate precision. The molar absorptivity was found to be 13.67 X 10^3, whereas the correlation co-efficient and slope were found to be 0.987 and 0.059 respectively. The values of LOD were 0.3664 μg ml^{-1} and LOQ were 0.6789 μg ml^{-1} respectively. This developed and validated analytical method for acyclovir was used in the evaluation of drug content of the prepared vaginal tablet formulations.

**Evaluation of prepared tablets**

**Ex-vivo Bioadhesive strength**

There is a strong prophylactic and clinical need to develop vaginal products with desired characteristics such as product dispersion throughout the vagina and retention for intended intervals. Retention of a dosage form in vaginal cavity for prolonged intervals is desirable for therapeutic efficacy and minimizes the need of frequent dosing intervals. The bioadhesive strength of the tablets was found to be a function of nature and concentration of polymer. The tablets showed bioadhesive strength between 125 to 390 g. As the polymer concentration increased bioadhesive strength of the prepared tablets increased. Vaginal tablets containing high amount of xanthan gum were found to have increased bioadhesive strength. The bioadhesive strength exhibited by the xanthan gum and tablets were considered satisfactory for maintaining them in the vaginal cavity.

**Force of adhesion and Surface pH**

The force of adhesion for all 16 formulations was found to be in the range of 1.22 N for formulation VT 1 and 3.82 N for formulation VT 16. This study demonstrated as the polymer concentration increased the force of adhesion increased. The surface pH of all 16 formulations was determined in SVF pH 4.5. There was no changes in pH and all the formulations possess the pH In the range of 4.52-4.88.

**Swelling Study**

The swelling studies for all 16 formulations were performed and it was observed that as the polymer concentration of carbopol 934P increased, there was a marked increase in the swelling index. This was observed in all the formulations. The maximum swelling index was observed to be 156 % in formulation VT 6 at the end of 8th hour and the least swelling index was observed to be 71 % in formulation VT 11 after 8 hours.

**In-vitro drug release studies**

The simulated dynamic vaginal system used in this study mimics the physic dynamic conditions of the vagina. As evident from the diverse nature of dissolution profiles the influence of polymer seems to be vital in regulating the drug release. In-vitro drug release studies were performed in SVF pH 4.5 for all the prepared formulations by using USP dissolution test apparatus-type II, rotating paddle method. The graphs showing drug release profile for formulations were shown in the figure 2a & 2b. In-vitro dissolution studies showed that the formulation containing carbopol 934P and xanthan gum (VT1, VT3, VT7, and VT8) showed 100% drug release with in a period of 6 hours. In formulations VT4, VT9, VT10, VT12, VT13, VT14, VT15, an increase in concentration of xanthan gum was found to delay the drug release over an extended period of time. Formulations VT2, VT6 and VT11 sustained the drug release over a period of 8 hours.

**In-vitro drug release mechanism**

Models with highest correlation coefficient were judged to be most appropriate model for dissolution data. The drug release data when fitted into various kinetic equations, showed Koresmeyer - Peppas release pattern for all formulations except Vt7, that showed zero order with r^2 value of 0.9826, VT15 showed zero order release with r^2 of 0.9784 and VT13 showed hixon-crowell release with r^2 value of 0.9879 respectively.

**In-vitro drug permeation studies**

In-vitro drug permeation was performed using fabricated
apparatus at 37±0.5°C. The graphs showing drug release profile for formulations were shown in the figure 3a & 3b. In-vitro permeation studies revealed that acyclovir vaginal tablet formulations containing increased concentration of Carbopol 934P and less concentration of xanthan gum in the range of 10 - 20mg showed maximum drug permeation of 45%. Tablet formulations containing Xanthan gum in the range of 20 - 40mg showed permeation in the range of 20-25% indicating as the concentration of xanthan gum is increased permeation is delayed.

Optimization of vaginal tablet formulations

Results of design experiments for acyclovir vaginal tablets by D-Optimal design are shown in table 3. The 3-dimensional response surface graphs for the factors percent swelling index and T 90% were plotted shown in figures 4a and 4b respectively. From the numerical optimization results, one of the solutions was selected randomly as the optimized formula and coded as VTGM1 and this optimized formulation was characterized further. The generated optimization study was conducted to study the constraints on the design space and the vulnerability of the experimental model. This is important, since it suggest factors, responses and the goal for each variable with respect to the measured response. The results were shown in the table 4 which confirmed there is a correlation of the predicted results with that of the observed results. It was observed that the response was almost similar to the response predicted by the design expert software. All the further studies were carried out using this optimized batch for swelling and In-vitro studies.

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<th>Factor:3 C:sodium bicarbonate (mg)</th>
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<th>Response 2 T 90% (h)</th>
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<tr>
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<td>40.00</td>
<td>75.00</td>
<td>22.50</td>
<td>110</td>
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</tr>
<tr>
<td>18</td>
<td>17.50</td>
<td>75.00</td>
<td>22.50</td>
<td>85</td>
<td>7</td>
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</tbody>
</table>

Table 4: Results of optimized Acyclovir vaginal tablet formulation

<table>
<thead>
<tr>
<th>Observation</th>
<th>Xanthan gum (mg)</th>
<th>Carbopol 934P (mg)</th>
<th>Sodium bicarbonate (mg)</th>
<th>Swelling index (%)</th>
<th>T 90% (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothetical values (Predicted)</td>
<td>13.88</td>
<td>91.11</td>
<td>22.13</td>
<td>95</td>
<td>12</td>
</tr>
<tr>
<td>Practical values for VTGM 1 (Actual)</td>
<td>13.5</td>
<td>91</td>
<td>22</td>
<td>94.32±0.92*</td>
<td>11.8±0.52*</td>
</tr>
</tbody>
</table>

* mean ±S.D (n= 3)
**In-vitro drug release studies**

The *in-vitro* drug release data for optimized formulation and the drug release mechanism showed 90% drug release in 12th hour with an $r^2$ value of 0.9931. The curve fits for various release systems for optimized formulation were shown in table 5. The n-value was found to be 0.1392 and the k-value was found to be 56.5185 for the optimized formulation.

**Kinetic modeling of drug release**

Models with the highest correlation coefficient were judged to be the most appropriate model for the dissolution data. The optimized formulation has shown correlation coefficient value 0.9931 that showed Peppas would be the most appropriate drug release mechanism where the drug could be released by diffusion process.

**Swelling Studies**

The swelling studies for optimized formulation showed maximum swelling at the end of the study. The optimized formula showed 95% of swelling in 12 hours. Hence there is a correlation between hypothetical and practical values.

**In-vitro absorption studies**

The vaginal mucus membrane is the rate-limiting in the proposed absorption system. As the drug released in the dissolution medium, this is available for absorption through the mucus membrane. The concentration between the two sides of the tissue helps to maintain sink conditions. The absorption profile of the optimized formulation showed that the amount of the drug in the absorption medium is 26.7% at the end of 8th hour.

**Stability studies**

Stability of the tablets is crucial during storage. Stability studies revealed that the tablets kept at elevated temperature...
of 40°C and 75% RH showed maximum stability. The similarity factor value was found to be 62.22, which confirmed that it has an acceptable shelf life up to 3 years.

CONCLUSION

Vaginal tablets of acyclovir were prepared using carbopol 934P and xanthan gum as bioadhesive polymers. The vaginal tablets were found to sustain the release of acyclovir for 12 hrs in simulated vaginal fluid. The present study signifies the utility of vaginal delivery system in retarding the drug release. This may in turn reduce the frequency of dosing, thereby improving the patient compliance.

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REFERENCES