# DESIGN AND CHARATERIZATION OF VAGINAL MUCHOADHESIVE FILMS CONTAINING VALACYCLOVIR HYDROCHLORIDE: A FACTORIAL STUDY

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**KEYWORDS**
Vaginal films, Valacyclovir Hydrochloride, independent variables, dependent variables, 2-Mercaptorethanol.

**ABSTRACT**

Designing and characterizing the mucoadhesive vaginal film of valacyclovir hydrochloride is the primary goal of this work. 23 factorial designs are used in the formulation of the vaginal film. PEG 400, 2-Mercaptocthanol, and Ethyl Cellulose concentration were selected as independent factors, and thickness, tensile strength, and dissolution were selected as dependent variables. The improved batch was tested using FTIR and DSC, and its thickness, tensile strength, folding endurance, surface PH, percentage of drug release, and swelling index were further assessed. The F-OPT formulation and the pure drug's FTIR and DSC data show that the drug and polymer are compatible. The F-OPT formulation, out of all the formulations created, has remarkable results, with the highest percentage of drug release (96.92%) in 12 hours and the lowest swelling index (45.62%). Studies on the F-OPT formulation's short-term stability showed that it was stable. Therefore, valacyclovir HCL mucoadhesive vaginal films may be an appropriate formulation for treating genital herpes with a practical administration method.

## INTRODUCTION

Genital Herpes (GH), which is characterized by recurrent outbreaks of painful sores in the genital area, is in fact a serious public health issue. This disorder is caused by the herpes simplex virus (HSV), of which HSV-2 is the conventional cause. Sexual transmission of HSV-2 is significantly influenced by asymptomatic viral shedding. When engaging in sexual activity, HSV-2 seroprevalence rates rise during adolescence and early adulthood before leveling off at age thirty. Three oral antiviral drugs are now authorized for the treatment of GH. The first medication created to treat HSV infection was acyclovir. These medications are competitive inhibitors of viral DNA polymerase, which suppresses the synthesis of viral DNA. Valacyclovir is the precursor of the medicine Acyclovir, while Famciclovir is the precursor of Guanosine.[1,2,3] Valacyclovir hydrochloride is an antiviral drug, being the L-valine ester of aciclovir, comes under the category of nucleoside analog DNA polymerase enzyme inhibitors. The therapeutical effects of drug primarily come from aciclovir, a purine nucleoside analog metabolite. [4,5]

Mucoadhesive delivery systems, which use formulations including one or more hydrophilic polymers in conjunction with the medicine for continuous delivery, were developed a few decades ago. This demonstrates the positive impact of polymers, which are in charge of the development of mucoadhesive preparation.[6] It is true that using the vagina to administer drugs has been done for a very long time. Compared to the oral first-pass impact, the vaginal route has a number of advantages. Some medications can penetrate the vagienal mucosa and enter the bloodstream in sufficient concentrations to cause systemic effects, even though they are typically employed for local activity. Furthermore, topical application to the vagina results in increased drug concentrations and improved efficacy for medications that target the female reproductive tract locally. At the moment, vaginal products come in a variety of forms, including creams, gels, tablets, capsules, ovules, foams, and solutions. The qualities of the drug, the intended effect, the patient's preference, the simplicity of administration, and the ailment being treated all play a role in selecting the right vaginal product form. [7]

Another cutting-edge type of drug delivery method is vaginal films, which are made using a variety of polymers to produce mucoadhesion and the required release profiles of the active components. They can overcome a number of obstacles in vaginal drug distribution, including permeability problems, cervical production, and pH fluctuations, thanks to this special formulation. These films have benefits like discreetness, convenience of administration, and the possibility of controlled drug release. Long-term interaction with the vaginal mucosa is made possible by the films' mucoadhesive qualities, which improve drug absorption and bioavailability. For a targeted and efficient treatment of a variety of gynecological disorders, vaginal films may be a viable local drug delivery method to the female reproductive tract. [8]

**Materials and method:**

Zydus Lifesciences Ahmedabad provided a gift sample of valacyclovir hydrochloride, while Loba Chemie Pvt. Ltd. in Maharashtra and SD Fine Chemical in Mumbai provided ethylcellulose, propylene glycol, and 2-Mercaptoethanol.

**Materials utilized in this study:**

**Table 1: Materials utilized with their source**

| Sl. No | Materials | Source |
|---|---|---|
| 1 | Valacyclovir Hydrochloride | Pure drug |
| 2 | Ethylcellulose | Film former |
| 3 | Propylene glycol | Plasticizer |
| 4 | 2-Mercptoethanol | Permeation enhancer |
| 5 | Distilled water | solvent |

**Instruments**

**Following are the instruments used in the present work**

**Table 2 Instruments used in this work**

| Sl. No | Name of the Instruments |
|---|---|
| 1 | Electronic weighing balance |
| 2 | Magnetic stirrer |
| 3 | Dissolution apparatus (USP) |
| 4 | UV-Visible Spectrophotometer |
| 5 | FTIR |
| 6 | Tensile strength |
| 7 | Hot air oven |

**Methods:**

**Preformulation studies:**

A crucial series of studies known as preformulation focuses on examining the physical and chemical characteristics of a novel medicinal ingredient that may affect its effectiveness and the creation of an appropriate dosage form. These studies offer crucial data for formulating formulations or assessing whether molecular changes are required. The inherent chemical and physical characteristics of the medicine must be taken into account before creating a pharmaceutical product. The way the medication interacts with pharmaceutical components when the dosage form is being created is determined by these qualities. In preformulation investigations, important characteristics such as drug solubility and polymorphic forms are crucial because they direct the formulation process for the best possible drug administration and efficacy.[9]

**Solubility studies:**

Valacyclovir hydrochloride's solubility test was conducted utilizing a variety of mediums and solvents. A small amount of methanol, acetone, ethanol chloroform, and dichloromethane are used to dissolve

valacyclovir hydrochloride individually. 0.1N HCL, 7.4, 6.8, and SVF in various media by storing them in a horizontal shaker at 370C for 48 hours.

**Melting point determination:**

Utilizing the Thales tube apparatus, the melting point was determined. When a little quantity of the medicine is placed in a capillary tube that is connected to a melting point equipment, the temperature can be detected and the melting point is noted.[10]

**Determination of Valacyclovir Hydrochloride by analytical method:**

The medication was analyzed using the UV spectrophotometric method with a double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).

**Determination of $\lambda_{max}$ of drug:**

After precisely weighing 100 mg of valacyclovir hydrochloride, it was put to a 100 ml volumetric flask. It was filtered after being dissolved in pH 4.2 Simulated Vaginal Fluid. As a stock solution A, the final solution had 1 µg/ml of medication. The finished solution is scanned using a twin beam UV spectrophotometer in the 200–400 nm range. (UV-1800 Shimadzu Corporation, Japan) in order to achieve optimal absorption.

**Calibration curve of Valacyclovir Hydrochloride in Simulated vaginal fluid pH 4.2:**

Starting with a stock solution, a sequence of dilutions are required to prepare the Valacyclovir HCL solutions. First, stock solution 1 is made by dissolving 100 mg of the medication in 100 ml of simulated vaginal fluid (pH 4.2). This yields a concentration of 1 mg/ml. 10 of stock solution 1 was added to this stock solution to further dilute it. Stock solution 2 with a concentration of 0.1 mg/ml is then produced by adding the same SVF (pH 4.2) to the volume. from the stock solution 2 known volume of aliquots 5ml, 10ml, 15ml, 20 and 25 ml are taken and diluted with SVF. Final volumes are thus 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml, respectively. A UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) was also used to detect absorbance at 255 nm. Each concentration's absorbance was measured at 255 nm with a blank of Simulated Vaginal Fluid (pH 4.2). In accordance with Beer-Lambert's law, the method's efficacy in measuring absorbance, complying linearity, precision, and accuracy within the designated concentration range of 2–10 µg/ml was validated.

**Formulation of mucoadhesive vaginal films:**

The solvent casting procedure, which entails a number of successive steps, was used to produce mucoadhesive vaginal films. First, 10 milliliters of distilled water were used to dissolve ethylcellulose, which was then agitated to create a transparent solution. To make sure that all air bubbles are gone, the solution is then allowed to stand for an hour. The medication, plasticizer, and permeation enhancer are all dissolved in the required volume of distilled water to generate a second solution at the same time. For one and a half hours,

these two solutions are mixed together and agitated. The resultant solution is then put into a 9 cm petri plate and left to dry overnight at 370C in a hot air oven. After drying, a 3x3 cm 2 film separates from the petri plate. To guarantee their quality and effectiveness, the formed films are tested for weight homogeneity, tensile strength, thickness, drug content, and dissolving behavior. [11]

**$2^3$ factorial designs**

A two-level factorial design was used to examine the effects of the independent variables X1 (ethylcellulose), X2 (polyethylene glycol 400), and X3 (2-meraptoethanol) on the dependent variables, including thickness, dissolution, and tensile strength. Eleven experimental batches were created in total since each of the three components was investigated at two levels (-1 and +1) in this experimental design. Table 3 lists the composition of each of the eleven potential valacyclovir HCL mucoadhesive vaginal film combinations using the $2^3$ complete factorial design.

**Table 3: List of factors employed in $2^3$ factorial designs**

| Factors | Levels | |
|---|---|---|
| | Low(-1) | High(+1) |
| Valacyclovir Hydrochloride (mg) | 320.25 | |
| Ethylcellulose(mg) | 300 | 500 |
| PEG 400(ml) | 4 | 6 |
| 2-Mercaptoethanol(ml) | 1 | 3 |
| Distilled water (ml) | 10 | |

**Table 4: preparation of Mucoadhesive vaginal films on the basis of $2^3$ factorial designs**

| Formulation Code | Ethylcellulose (mg) | PEG 400 (ml) | 2 Mercaptoethanol (ml) | Ethylcellulose (mg) | PEG 400 (ml) | 2 Mercaptoethanol (ml) |
|---|---|---|---|---|---|---|
| | Levels | | | Actual amount | | |
| **F1** | +1 | +1 | -1 | 500 | 6 | 1 |
| **F2** | 0 | 0 | 0 | 400 | 5 | 1.5 |
| **F3** | -1 | -1 | +1 | 300 | 4 | 2 |
| **F4** | -1 | +1 | -1 | 300 | 6 | 1 |
| **F5** | 0 | 0 | 0 | 400 | 5 | 1.5 |
| **F6** | +1 | +1 | +1 | 500 | 6 | 2 |
| **F7** | +1 | -1 | +1 | 500 | 4 | 2 |
| **F8** | -1 | -1 | -1 | 300 | 4 | 1 |
| **F9** | -1 | +1 | +1 | 300 | 6 | 2 |
| **F10** | 0 | 0 | 0 | 400 | 5 | 1.5 |
| **F11** | +1 | -1 | -1 | 500 | 4 | 1 |

*Amount of drug and distilled water was kept constant

**Evaluation of Formulated Mucoadhesive Vaginal film:**

The prepared vaginal films were subjected for their physical, mechanical properties and drug uniformity followed by *In-vitro* and stability studies.

**Physical appearance:**

Film appearance was evaluated by observing the colour, elegance, stickiness and texture.[12]

**Weight variation:**

Nine centimeters (3 × 3 cm) of film were cut at three distinct locations. For weight variation, individual film weights were recorded. [13]

**Thickness:**

The thickness of the patch was measured at several locations on the film using a Digital Vernier, and the average and standard deviation were computed.[14]

**Surface pH:**

The pH measurement of the film's surface indicates whether irritating effects on the vaginal mucosa are present. It is envisaged that the film's surface pH will react with typical human vaginal fluid.[15]

**Folding endurance:**

The vaginal film was repeatedly cut and folded in the same spot until it snapped. The folding endurance value is determined by folding the vaginal film at the same spot without breaking the number of times.[16]

**Tensile strength:**

To evaluate the Tensile quality of each film formulation, Tensile strength was measured. The elasticity was computed using the appropriate formula,

$$\textbf{Tensile strength} = \frac{\textbf{load at failure} \times \textbf{100 Film}}{\textbf{Thickness} \times \textbf{Film Width}}$$ [17]

**Swelling index:**

The films were left to swell on the agar plate surface at 37 ± 0.2°C. The weight growth of the films (n=3) was measured at intervals of 1–6 hours.

$$\textbf{Percentage swelling index (\%S)} = \frac{W_t - W_0}{W_0} \textbf{ x 100}$$

Where $W_t$ is the weight of the swollen film after time t, $W_0$ is the weight of the film at zero time. [18]

**Drug content uniformity of films:**

In beaker containing 100ml of SVF, a piece of 3x3 $cm^2$ Patch was dissolved using ultrasonicator. Pipette Suitable aliquots and filter. The filtered solution is measured for absorbance by UV-visible spectrophotometer at $\lambda_{max}$ 255nm.

***In-vitro* Drug Dissolution study:**

USP Type-I equipment was used to measure the medication release rate of Valacyclovir Hydrochloride's mucoadhesive vaginal film. 900 cc of pH 4.2 simulated vaginal fluid was used in the dissolving investigation, which was carried out at 37.50C and 50 rpm basket speed. Aliquot of 5 ml solution

was collected and replace with 5 ml or same amount of fresh dissolution medium at the same time. Using Whatman filter paper aliquots were filtered and the absorbance measured at 255 nm. [19]

**Stability studies:**

According to ICH recommendations, the optimized film formulation was held in the appropriate container (usually aluminum) at 25 °C and 65% relative humidity. Accelerated stability could also be investigated for six months at 40% °C and 75% relative humidity. The films are taken out of the stability chamber with the primary packages at specific intervals, such as 1, 3, 6, and 12 months, and are then characterized. [20]

## RESULTS AND OBSERVATION:

**Preformulation studies:**

**Solubility studies of pure drug in different solvents:**

Valacyclovir Hydrochloride was freely soluble in waster, soluble in Ethanol & poorly soluble in chloroform and methanol.

**Solubility studies of pure drug in different media:**

Valacyclovir Hydrochloride shows highest solubility in SVF, phosphate buffer solution pH 7.4, 6.8 and 0.1HCL

**Table 5:** Solubility parameter of Valacyclovir Hydrochloride in different medias. (n=3)

| Drug | water mg/ ml | 0.1N HCL (pH 1.2) mg/ml | phosphate Buffer (pH 6.8) mg/ml | Phosphate Buffer (pH 7.4) mg/ml | SVF (pH 4.2) mg/ml |
|---|---|---|---|---|---|
| Valacyclovir Hydrochloride | 174.5 | 13.5 | 113 | 115 | 152 |

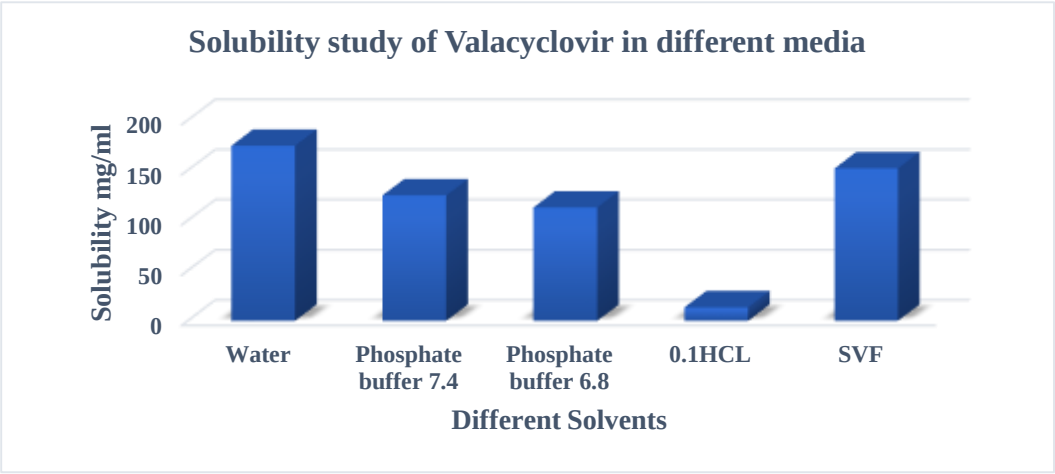


**Figure 1:** Solubility study of Valacyclovir in different media

**Determination of Melting Point:**

Using a Thales tube instrument, the melting point of valacyclovir hydrochloride was determined to be $170^0$ C.

**Determination of Valacyclovir Hydrochloride by analytical method:**

The medication was analyzed using the spectrophotometric method using a twin beam UV-visible spectrophotometer. (Shimadzu, Japan, UV-1800)

**Determination of $\lambda_{max}$ of drug:**

A twin beam UV-visible spectrophotometer was used to assess the drug using the spectrophotometric approach. (UV-1800, Shimadzu, Japan)

**Calibration curve of Valacyclovir Hydrochloride:**

In SVF pH 4.2, the standard plot for valacyclovir hydrochloride was created. After measuring the absorbance at 255 nm, a concentration vs absorbance graph was produced.

**Table 6:** Calibration cure of Valacyclovir Hydrochloride in SVF (pH 4.2) (n=3)

| Sl.No. | Valacyclovir Hydrochloride (µg/ml) | Absorbance at 255nm | |
|---|---|---|---|
| | | -- | RSD (%) |
| 1 | 0 | 0 | 0 |
| 2 | 5 | 0.168 | 0.168±0.126 |
| 3 | 10 | 0.323 | 0.323±0.123 |
| 4 | 15 | 0.491 | 0.491±0.152 |
| 5 | 20 | 0.654 | 0.654±0.141 |
| 6 | 25 | 0.836 | 0.836±0.114 |

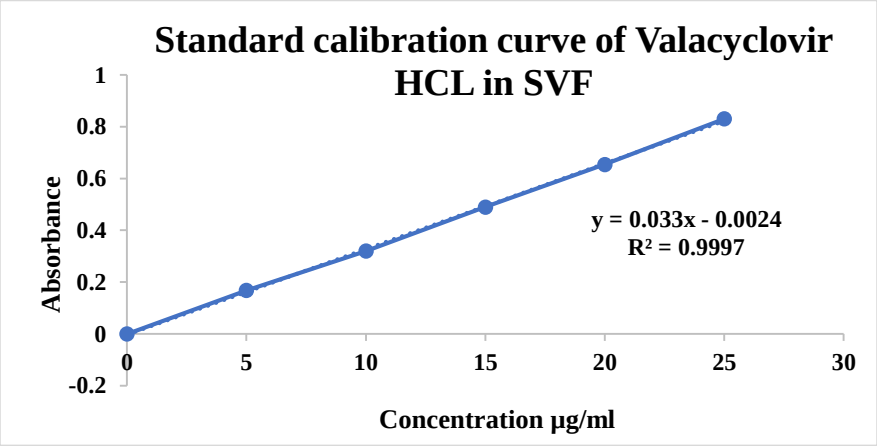


**Figure 2:** Standard calibration curve of Valacyclovir HCL in SVF

**Characterization of pure Drug and Physical mixture**

**Fourier Transform Infrared Spectroscopy:**

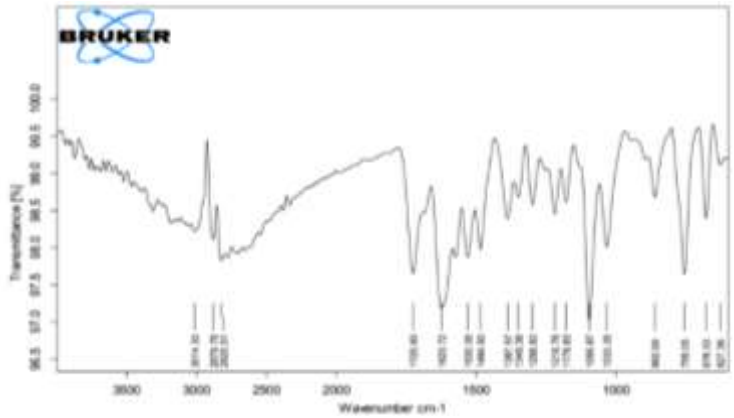


**Figure 3:-** Spectra of Valacyclovir Hydrochloride

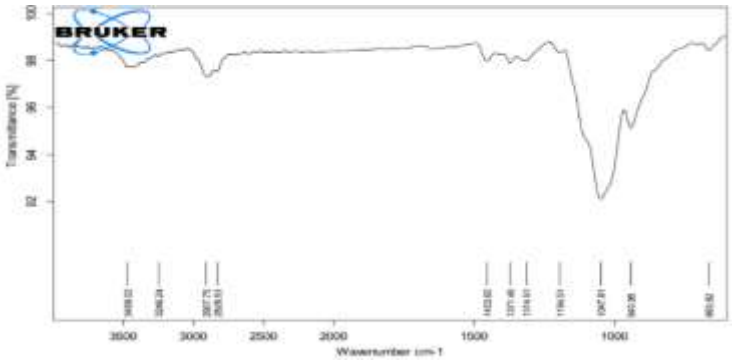


**Figure 4:-**FTIR of Ethylcellulose

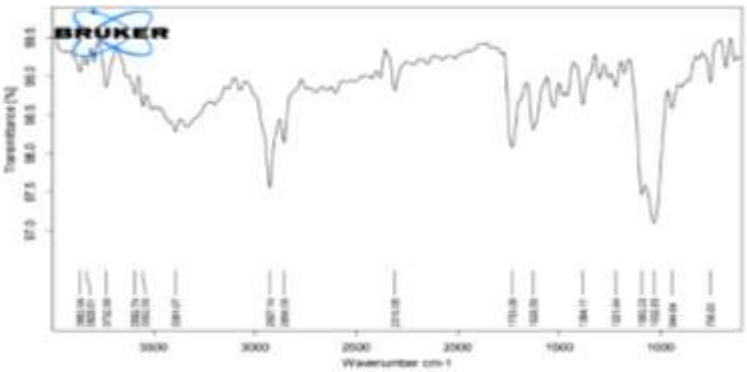


**Figure 5:-**FTIR Spectra of Physical mixture

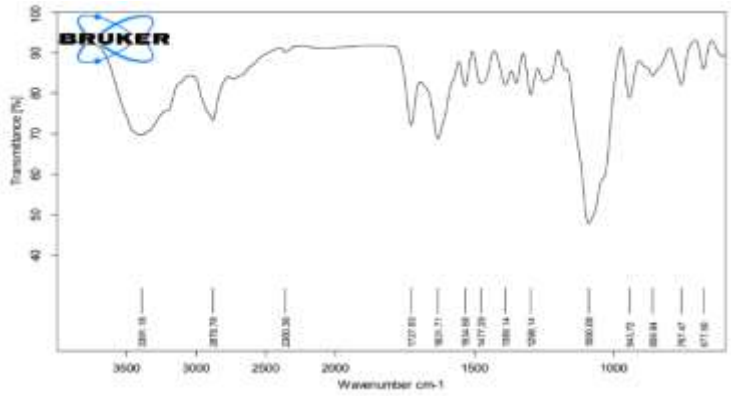


**Figure 6:-** FTIR Spectra of optimized Formulation F-OPT

**Differential Scanning Calorimetry:**

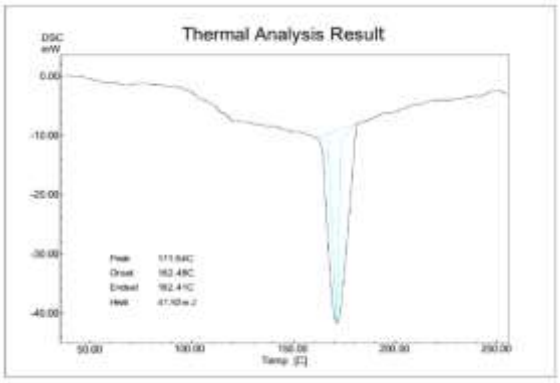


**Figure 7:** DSC thermogram of drug Valacyclovir Hydrochloride

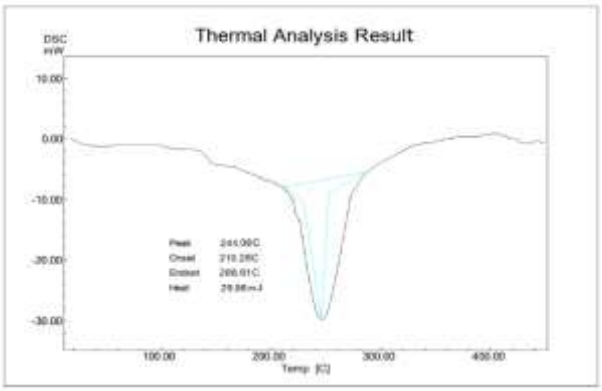


**Figure 8:** DSC thermogram of Ethyl Cellulose

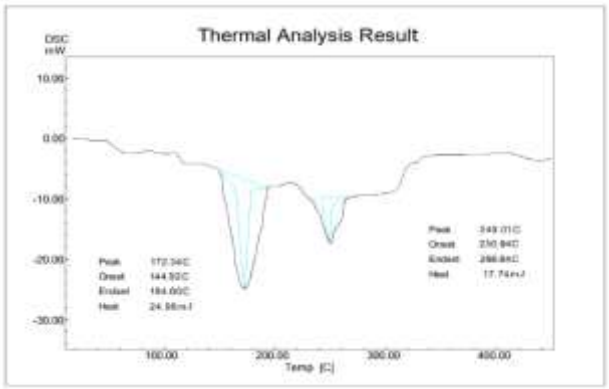


**Figure 9:** DSC thermogram of Formulation (F-OPT)

## Physicochemical evaluation of Mucoadhesive Vaginal films

**Table 8:** Weight Uniformity, Thickness and Surface pH (n=3)

| Formulation Code | Weight uniformity x̄ ±RSD (%) | Thickness (mm) x̄ ±RSD (%) | Surface pH x̄ ±RSD (%) | Folding Endurance x̄ ±RSD (%) |
|---|---|---|---|---|
| F1 | 55.25±0.18 | 0.496 | 4.2 | 139 |
| F2 | 49.20±0.13 | 0.367 | 4 | 136 |
| F3 | 50.09±0.12 | 0.246 | 3.9 | 121 |
| F4 | 51.11±0.10 | 0.311 | 4.3 | 134 |
| F5 | 50.18±0.11 | 0.381 | 4.1 | 132 |
| F6 | 50.22±0.12 | 0.515 | 4 | 134 |
| F7 | 51.15±0.18 | 0.49 | 4 | 122 |
| F8 | 45.06±0.15 | 0.211 | 4.2 | 120 |
| F9 | 45.08±0.13 | 0.315 | 4 | 131 |
| F10 | 54.15±0.11 | 0.372 | 4.3 | 133 |
| F11 | 54.11±0.10 | 0.477 | 4.3 | 122 |
| F-OPT | 49.22±0.11 | 0.452 | 4.2 | 140 |

**Table 9:** Folding Endurance, Tensile Strength and Drug Content Uniformity (n=3)

| Formulation code | Tensile Strength ($N/mm^2$) x̄ ±RSD (%) | Swelling index % | Drug Content Uniformity x̄ ±RSD (%) |
|---|---|---|---|
| F1 | 2.21 | 60.67 | 97.03 |
| F2 | 1.49 | 52 | 95.98 |
| F3 | 1.12 | 46.33 | 97.88 |
| F4 | 1.38 | 47.32 | 97.79 |
| F5 | 1.44 | 50.12 | 98.27 |
| F6 | 2.57 | 59.2 | 96.84 |
| F7 | 1.79 | 60.12 | 98.76 |
| F8 | 1 | 49.6 | 97.58 |
| F9 | 1.27 | 48.3 | 95.52 |
| F10 | 1.38 | 50.1 | 96.14 |
| F11 | 1.73 | 58.3 | 97.56 |
| F-OPT | 2.46 | 45.62 | 98.62 |

## ANOVA- Analysis of variance

**Table 10:** Summary of ANOVA applied to $2^3$ factorial designs for Thickness (YI) Response

| Source | Sum of Squares | F-value | p-value | |
|---|---|---|---|---|
| **Model** | 0.1064 | 99.43 | < 0.0001 | **Significant** |
| A-Ethylcellulose | 0.1001 | 280.64 | < 0.0001 | |
| B-PEG 400 | 0.0057 | 15.89 | 0.0053 | |
| C-2-Mercaptoethanol | 0.0006 | 1.77 | 0.2255 | |
| **Residual** | 0.0025 | | | |
| Lack of Fit | 0.0024 | 9.52 | 0.0977 | **Not significant** |

**Table 11:** Summary of ANOVA applied to $2^3$ factorial designs for Tensile strength(Y2) Response

| Source | Sum of Squares | F-value | p-value | |
|---|---|---|---|---|
| **Model** | 2.02 | 27.68 | 0.0003 | **Significant** |
| A-Ethylcellulose | 1.56 | 63.89 | < 0.0001 | |
| B-PEG 400 | 0.4005 | 16.43 | 0.0049 | |
| AB | 0.0666 | 2.73 | 0.1423 | |
| **Residual** | 0.1707 | | | |
| Lack of Fit | 0.1646 | 10.85 | 0.0865 | **Not significant** |

**Table 12:** Summary of ANOVA applied to $2^3$ factorial designs for Dissolution(Y3) Response

| Source | Sum of Squares | F-value | p-value | |
|---|---|---|---|---|
| **Model** | 323.20 | 116.00 | < 0.0001 | **Significant** |
| A-Ethylcellulose | 300.37 | 323.42 | < 0.0001 | |
| B-PEG 400 | 13.89 | 14.95 | 0.0062 | |
| C-2-Mercaptoethanol | 8.95 | 9.63 | 0.0172 | |
| **Residual** | 6.50 | | | |
| Lack of Fit | 1.59 | 0.1293 | 0.9705 | **Not significant** |

**Model Summary Statistics**

**Table 13:** Model summary statistics:- Influence of formulation variables on the response factors

| Response factors | Std. Dev | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Adequate precision |
|---|---|---|---|---|---|
| **Y1** | 0.0189 | 0.9771 | 0.9672 | 0.9246 | 25.8770 |
| **Y2** | 0.1561 | 0.9223 | 0.8889 | 0.7983 | 14.1253 |
| **Y3** | 0.9637 | 0.9803 | 0.9718 | 0.9654 | 29.2616 |

## Verification of Model adequacy

Figure 10 displays the projected vs real (experimental data) charts for Y1, Y2, and Y3, which were used to assess the regression model's suitability.

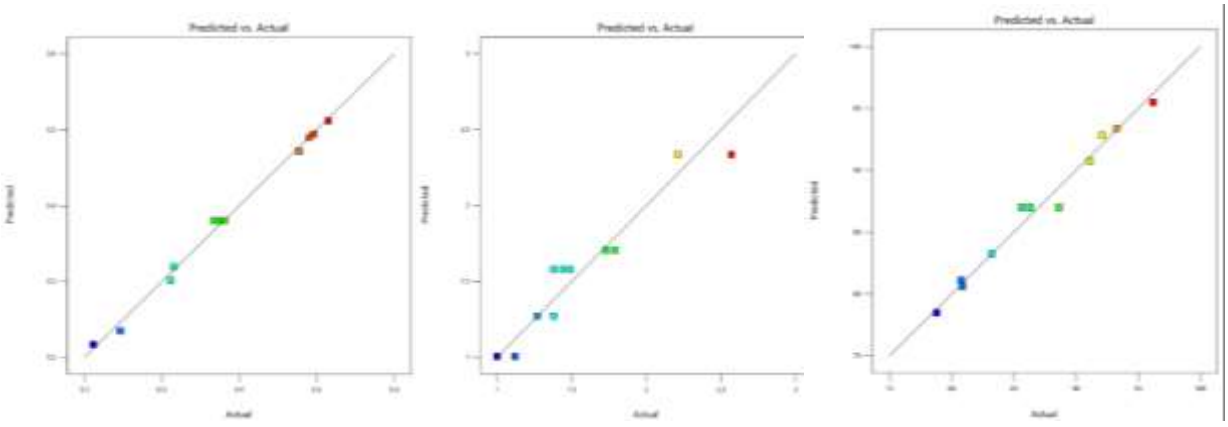

**Figure 10:** Distribution of actual (experimental) v/s predicted values of the regression model of Thickness (Y1), Tensile strength (Y2) and Dissolution (Y3)

## Response Surface Analysis

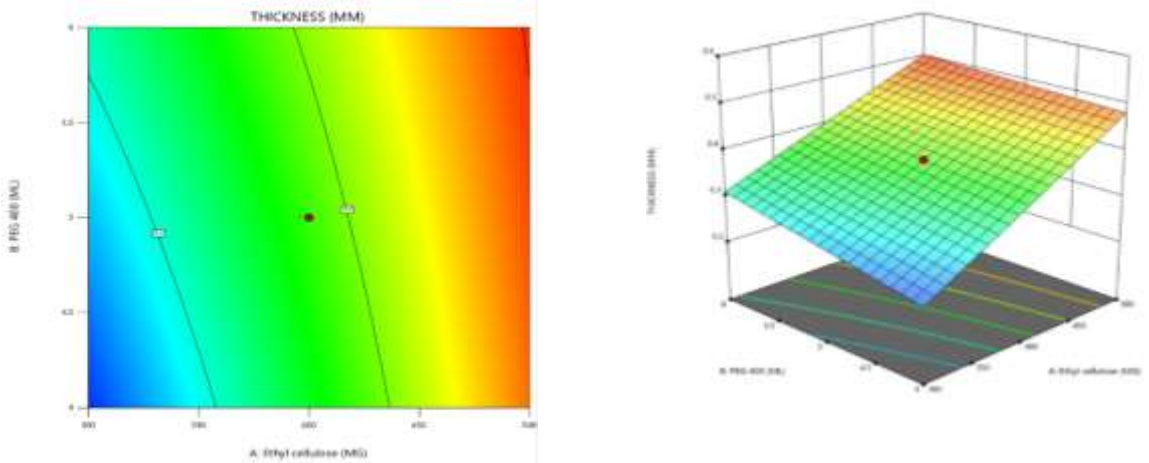


**Figure 11:** Contour plot and 3D response surface showing the effect of two factors on Thickness (Y1)

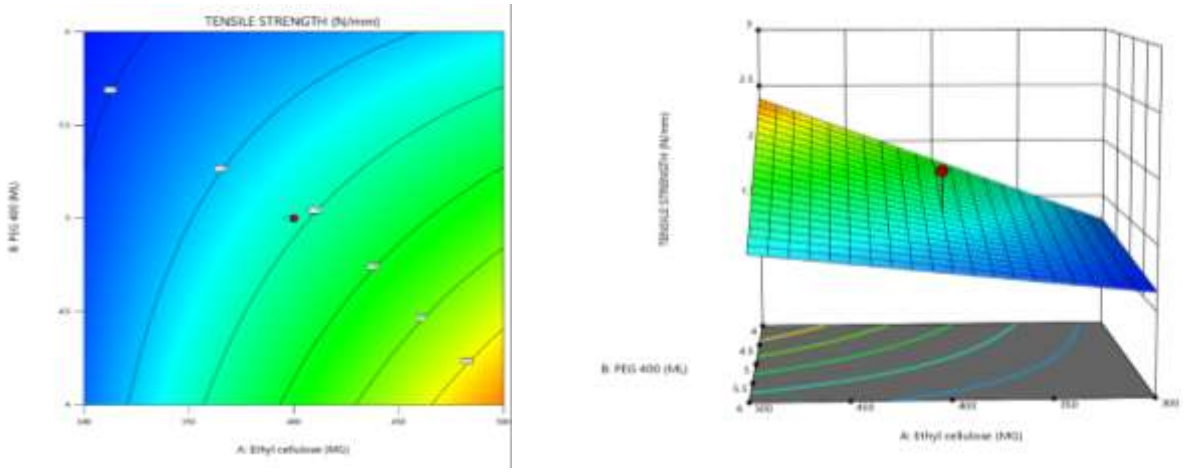


**Figure 12:** Contour plot and 3D response surface showing the effect of two factors on Tensile strength (Y2)

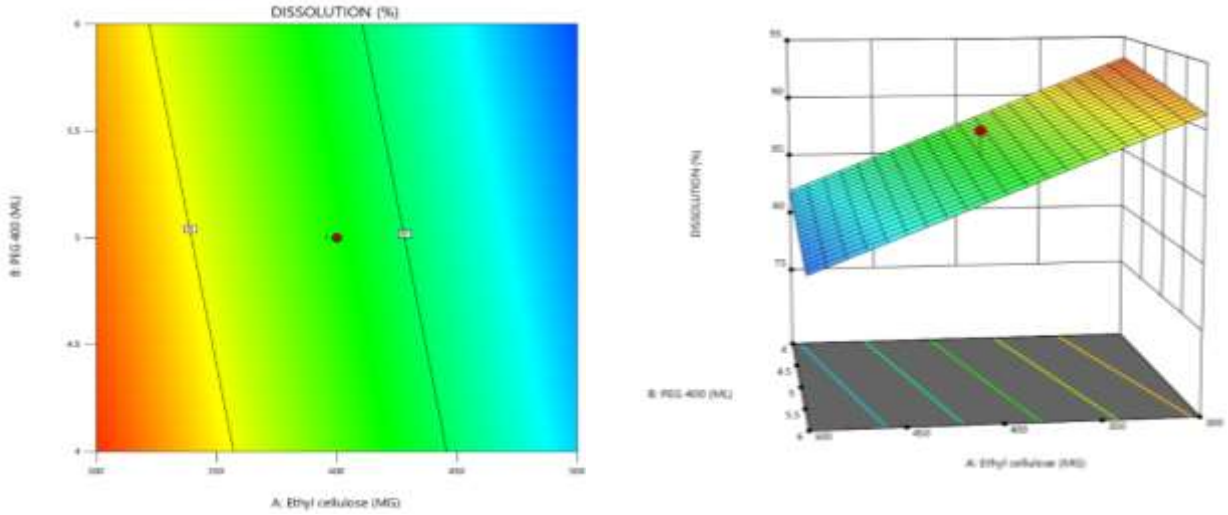


**Figure 13:** Contour plot and 3D response surface showing the effect of two factors on Dissolution (Y3)

## Formulation Optimization

**Table 14:** Comparison of predicted and observed responses for the statistically optimized formulation

| Formulation code | Response | Observed value | Predicted value | Desirability |
|---|---|---|---|---|
| F-OPT | Y1: Thickness(mm) | 0.252 | 0.477 | 1.00 |
| | Y2: Tensile strength($N/mm^2$) | 2.46 | 2.89 | 1.00 |
| | Y3: Dissolution | 96.92 | 95.215 | 1.00 |

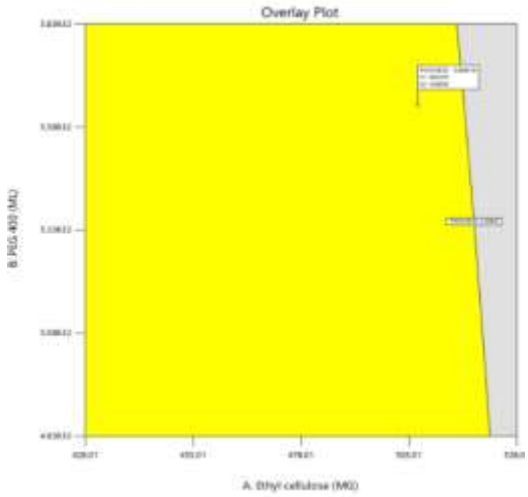


**Figure 14:** Design space of optimized formulation

**Table 15-** *In-vitro* drug release of Valacyclovir vaginal film from F1 to F6

| Time (hrs) | % Drug Release | | | | | |
|---|---|---|---|---|---|---|
| | x̄± RSD (%) (n=6) | | | | | |
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 7.15±0.12 | 6.85±0.10 | 7.49±0.22 | 5.78±0.10 | 6.23±0.14 | 6.48±0.22 |
| 0.5 | 8.12±0.14 | 9.12±0.11 | 10.72±0.25 | 6.89±0.12 | 8.56±0.16 | 9.48±0.12 |
| 0.75 | 10.57±0.17 | 10.49±0.15 | 11.79±0.14 | 15.79±0.14 | 12.23±0.12 | 12.89±0.18 |
| 1 | 18.45±0.18 | 16.42±0.23 | 17.46±0.17 | 18.43±0.15 | 22.15±0.13 | 16.14±0.19 |
| 1.5 | 27.56±0.16 | 25.78±0.22 | 23.61±0.19 | 24.82±0.22 | 28.23±011 | 20.49±0.21 |
| 2 | 38.79±0.18 | 36.13±0.21 | 37.43±0.12 | 42.82±0.26 | 37.65±0.15 | 30.12±0.22 |
| 2.5 | 43.19±0.19 | 41.86±0.21 | 42.73±0.23 | 48.61±0.15 | 42.67±0.12 | 35.78±0.17 |
| 3 | 48.50±0.15 | 51.86±0.12 | 51.78±0.25 | 55.19±0.19 | 52.53±0.14 | 42.89±0.12 |
| 4 | 56.61±0.16 | 63.49±0.15 | 62.19±0.32 | 62.48±0.18 | 60.39±0.16 | 55.13±0.21 |
| 5 | 66.45±0.16 | 70.16±0.17 | 73.49±0.11 | 70.83±0.11 | 69.22±0.10 | 62.79±0.23 |
| 6 | 72.89±0.11 | 81.46±0.15 | 82.79±0.10 | 76.13±0.10 | 76.73±0.11 | 72.46±0.41 |
| 12 | 80.89±0.10 | 88.63±0.11 | 92.33±0.18 | 91.57±0.22 | 85.64±0.12 | 78.82±0.15 |

**Table 16-** *In-vitro* drug release of Valacyclovir vaginal film from F7 to F-OPT

| Time (hrs) | % Drug Release | | | | | |
|---|---|---|---|---|---|---|
| | x̄± RSD (%) (n=6) | | | | | |
| | F7 | F8 | F9 | F10 | F11 | F-OPT |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 6.23±0.14 | 5.124±0.12 | 6.22±0.25 | 6.48±0.12 | 7.12±0.10 | 4.46±0.022 |
| 0.5 | 9.45±0.15 | 7.568±0.15 | 7.49±0.21 | 9.47±0.13 | 10.44±0.22 | 10.49±0.23 |
| 0.75 | 11.63±0.16 | 9.468±0.14 | 10.96±0.12 | 13.78±0.11 | 14.23±0.45 | 17.46±0.12 |
| 1 | 13.56±0.18 | 14.786±0.13 | 22.79±0.18 | 21.89±0.10 | 18.55±0.15 | 26.14±0.18 |
| 1.5 | 26.83±0.11 | 28.468±0.12 | 26.31±0.24 | 28.47±0.25 | 26.78±0.22 | 37.46±0.17 |
| 2 | 35.73±0.15 | 36.89±0.15 | 35.71±0.21 | 36.77±0.22 | 38.49±0.23 | 44.73±0.15 |
| 2.5 | 40.72±0.12 | 41.58±0.13 | 44.66±0.22 | 41.98±0.21 | 42.99±0.25 | 54.12±0.21 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 50.39±0.22 | 45.78±0.12 | 56.71±0.36 | 52.92±0.13 | 51.86±0.26 | 60.46±0.22 |
| 4 | 58.63±0.23 | 52.89±0.15 | 62.73±0.21 | 60.89±0.33 | 58.96±0.21 | 70.13±0.11 |
| 5 | 64.71±0.12 | 64.78±0.14 | 71.93±0.12 | 74.92±0.25 | 69.43±011 | 79.12±0.10 |
| 6 | 70.23±0.15 | 78.63±0.16 | 78.23±0.17 | 80.56±0.21 | 78.89±0.10 | 86.56±0.19 |
| 12 | 80.87±0.25 | 93.92±0.15 | 91.12±0.19 | 86.38±0.22 | 83.22±0.19 | 96.92±0.17 |

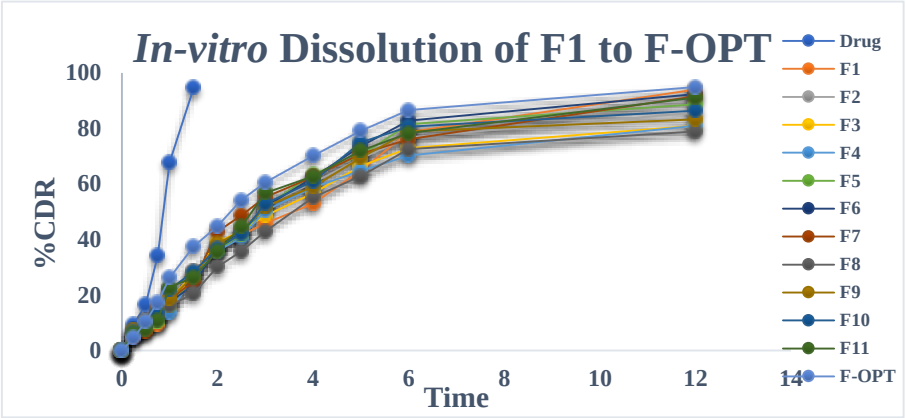


**Figure 15-** *In-vitro* drug release of Valacyclovir vaginal film from F1 to F-OPT

**Table 17-** Regression co efficient ($R^2$) values and Release exponential (n) values of Valacyclovir Vagin Film according to the different kinetic models

| Formulation code | Zero order | | First order | | Higuchi | Peppas | |
|---|---|---|---|---|---|---|---|
| | $R^2$ | n | $R^2$ | n | $R^2$ | $R^2$ | n |
| **F1** | 0.7842 | 7.305 | 0.9133 | 0.152 | 0.9388 | 0.9424 | 0.7571 |
| **F2** | 0.8033 | 8.218 | 0.939 | 0.201 | 0.9354 | 0.9561 | 0.7913 |
| **F3** | 0.8205 | 8.478 | 0.9692 | 0.231 | 0.8422 | 0.9634 | 0.7617 |
| **F4** | 0.8022 | 8.183 | 0.9838 | 0.214 | 0.9457 | 0.9439 | 0.8148 |
| **F5** | 0.7946 | 7.728 | 0.9373 | 0.176 | 0.9441 | 0.9542 | 0.7727 |
| **F6** | 0.8197 | 7.233 | 0.9118 | 0.145 | 0.9411 | 0.9748 | 0.7374 |
| **F7** | 0.792 | 7.32 | 0.9244 | 0.15 | 0.9389 | 0.9541 | 0.768 |
| **F8** | 0.8661 | 8.421 | 0.9906 | 0.237 | 0.9613 | 0.9618 | 0.8495 |
| **F9** | 0.8059 | 8.266 | 0.9754 | 0.214 | 0.9434 | 0.9462 | 0.8113 |
| **F10** | 0.7843 | 7.923 | 0.9065 | 0.187 | 0.9344 | 0.963 | 0.7597 |
| **F11** | 0.781 | 7.577 | 0.895 | 0.168 | 0.9353 | 0.9655 | 0.7292 |

**Short term stability studies:**

**Table 18:** Optimized formulation vaginal films subjected to stability studies at 5 $^0$C ± 3 $^0$C

| Time | Visual appearance | Percentage Drug Content | Surface pH | Folding Endurance | % CDR |
|---|---|---|---|---|---|
| Initial | White | 98.62% | 4.2 | 136 | 96.92 |
| After 3 Months | White | 97.76 | 4.1 | 134 | 96.74 |

**Table 19:** Optimized formulation subjected to stability study at 25 $^0$C ± 2 $^0$C

| Time | Visual appearance | Percentage Drug Content | Surface pH | Folding Endurance | % CDR |
|---|---|---|---|---|---|
| Initial | White | 98.62% | 4.2 | 136 | 96.92 |
| After 3 Months | White | 97.58 | 4 | 132 | 96.52 |

**Comparison of *In-vitro* Dissolution Studies of Optimized Mucoadhesive vaginal film of Valacuclovir Hydrochloride(F-OPT) with the pure drug and Marketed Product (Valcivir).**

| Time (hrs) | % Drug Release (n= 3) X̄± RSD (%) | | |
|---|---|---|---|
| | Pure drug | F-OPT | Marketed products |
| 0 | 0 | 0 | 0 |
| 0.25 | 9.43 | 0.25 | 9.43 |
| 0.5 | 16.44 | 0.5 | 16.44 |
| 0.75 | 34.27 | 0.75 | 34.27 |
| 1 | 67.75 | 1 | 67.75 |
| 1.5 | 94.56 | 1.5 | 94.56 |
| 2 | - | 2 | - |
| 2.5 | - | 2.5 | - |
| 3 | - | 3 | - |
| 4 | - | 4 | - |
| 5 | - | 5 | - |
| 6 | - | 6 | - |
| 12 | - | 12 | - |

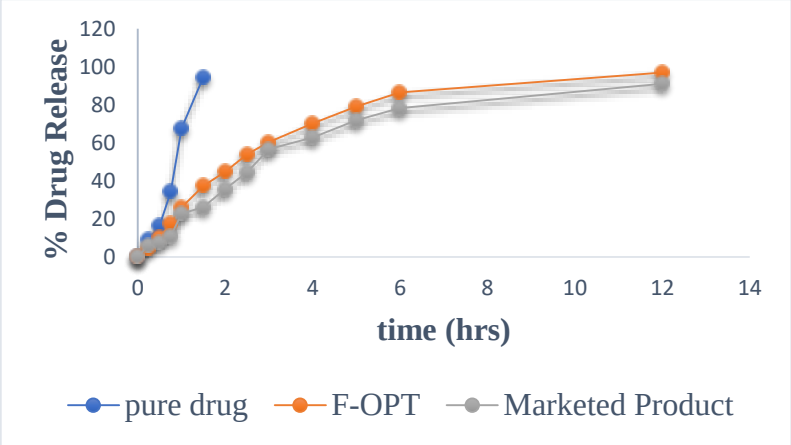


**Figure 16:** Comparative study of *In-vitro* dissolution of drug, F-OPT and marketed product

**Discussion:**

Using $2^3$ factorial designs, valacyclovir hydrochloride vaginal films were prepared by solvent method using Ethyl Cellulose, PEG 400, and 2-Mercaptoethanol in varying concentrations. The formulations were assessed for tensile strength, folding endurance, thickness, and surface pH, among other characteristics.

**Determination of $\lambda_{max}$ of Drug:**

Using SVF pH 4.5 as a blank, a diluted solution of Valacyclovir hydrochloride was scanned for λmax in the 200–400 nm range using a UV-Visible Spectrophotometer (UV1800, Shimadzu, Japan). At 255 nm, the highest absorption was detected.

**Calibration curve of Valacyclovir Hydrochloride:**

Valacyclovir hydrochloride's calibration curve was created at a wavelength of 255 nm and ranged from 5 to 25 µg/ml. Beer-Lambert's law is followed by the standard calibration curve, which produces a linear curve with an $R^2$ value between 0.18 and 0.83 that is not higher than 0.999. Table 6 and Figure 2 present the findings.

**Organoleptic properties**

The drug's amorphous nature, which indicates its solid-state structure, which is important for its stability and solubility, and its white color are all revealed by the organoleptic evaluation of Valacyclovir Hydrochloride. The drug's bitter taste, which is a common characteristic of many active pharmaceutical ingredients, and its odorless nature, which encourages patient compliance because unpleasant smells can discourage proper usage, are all properties that collectively influence the drug's acceptability and formulation considerations.

**Solubility:**

Figure 81 shows the solubility curve of Valacyclovir Hydrochloride in several solvents and media, including distilled water, ethanol, chloroform, and methanol. SVF, phosphate buffer (pH 6.8 and 7.4), and 0.1 N HCl. The molecule is more soluble in aqueous and somewhat acidic conditions, making it potentially appropriate for vaginal drug delivery. The maximum solubility is seen in water and SVF (around 174 and 152 mg/mL, respectively). The reduced solubility in organic solvents is reflected in the poorer solubility in methanol and chloroform.

**Melting point:**

An accurate melting point further confirms the identity of the substance and suggests minimal contamination or degradation in the sample analyzed. Valacyclovir Hydrochloride's observed melting point was recorded at 170 °C. This close alignment with the expected range indicates the purity and stability of the compound, as impurities would typically cause deviations in melting behavior.

**Drug polymer compatibility study:**

By utilizing FT-IR Spectroscopy (BRUKER ALPHA E) drug polymer compatibility is studied for drug, Ethyl Cellulose, Physical mixture and Optimized formulation) which are shown in **Figure 3-6.** The IR spectra of drug show sharp peak at 1349$cm^{-1}$ is due to presence of -CO stretching. 3592 $cm^{-1}$ is due to presence of NH stretching. 2879 $cm^{-1}$ is due to presence of C-H stretching. 1725 $cm^{-1}$ is due to presence of C=O stretching. These agreed with the previously reported monograph for Valacyclovir Hydrochloride. The characteristic peaks were also found in the Physical mixture and Optimized formulation and there is no significant shift in the peak position. Hence polymer was compatible with the drug.

**Differential Scanning Calorimetry:**

Figure 7-9 displayed the medication and formed film DSC thermograms. With onset and endset temperatures of 162.48°C and 182.41°C, respectively, that correspond to its melting point, the drug's DSC thermogram displayed an endothermic peak at 171.64°C. It was discovered that the latent heat of fusion was

41.92 mJ. With onset and endset temperatures of 210.28°C and 288.61°C, respectively, the polymer's DSC thermogram displayed an endothermic peak at 244.08°C, which is its melting point. It was determined that the latent heat of fusion was 29.96 mJ. Two endothermic peaks were visible in the optimized vaginal film formulation F-OPT's DSC thermogram at 172.34°C, with onset and endset temperatures of 138.26°C and 206.84°C, respectively. With onset and endset temperatures of 210.94°C and 263.84°C, respectively, the latent heat of fusion was determined to be 34.06 mJ, with an additional endothermic peak at 233.64°C. It was discovered that the latent heat of fusion was 24.91 mJ. The melting endoderm of formulation F-OPT does not significantly alter, suggesting that the medication is amorphous as described in the literature.

**Experimental Design**

Tables 3 and 4 show the effects of independent factors, such as the concentrations of ethylene cellulose (X1), PEG 400 (X2), and 2-meraptoethanol (X3), on thickness (Y1), tensile strength (Y2), and dissolution (Y3). Table 4 lists the levels of the parameters under study.

**ANOVA - Analysis of variance.**

Factorial design models for thickness (Y1), tensile strength (Y2), and dissolution (Y3) were found to be significant and appropriate without a significant lack of fit, according to an ANOVA. The data for the analysis of variance is predicted by Tables 10, 11, and 12. Model forms are considered significant when the p-value (degree of significance) is less than 0.05. As indicated in Tables 10, 11, and 12, X2, X3, X1X2 X3, X12, X22, and X32 are significant model forms in this instance due to their p-values being less than 0.05. The model forms are not significant if the p-value is bigger than 0.1. F-value for the lack of Fit of Y1, Y2 & Y3 responses are 0.09, 0.08 and 0.9 correspondingly which indicates lack of fit is not significant i.e. our model is statistically accurate. We want the model to fit, thus a non-significant lack of fit is ideal.

**Model summary statistics:**

A high $R^2$ value, greater than 0.8, indicates that the regression model fits the data well. The selected significant models is shown in **Table 13**. The high $R^2$ value for all responses suggest a strong correlation between the experimental and predicted values. Adequate precisions measure the signal to noise ratio greater. A ratio greater than 4 is desirable.

The predicted $R^2$ value of Y1, Y2 & Y3 are 0.9672, 0.8889 & 0.9718 respectively. In addition, the predicted $R^2$ value is in good agreement with the adjusted $R^2$ value because the difference between predicted and adjusted $R^2$ value is less than 0.2 which resulting in reliable models. Adequate precision of Y1, Y2 & Y3 are 25.87, 14.1 & 29.26 respectively i.e., greater than 4, which indicate on adequate signal. Hence this model can be used to navigate the design space.

Multiple linear regression analysis was done to estimate the effect of factors on responses by generating a polynomial equation:

$Y=b_o+b_1X_1+b_2X_2+b_3X_3+b_1X_1X_2+b_2X_2X_3+b_3X_2X_3+b_{123}X_1X_2X_3$…...

In the polynomial equation, Y represents the response parameter, β0 is the intercept, and A,B and C are the coded levels of independent variables, where positive and negative coefficients indicate synergistic and antagonistic effects, respectively.

**The polynomial linear equation generated for responses are as follows.**

Y1= 0.3801 + 0.119A + 0.0266B + 0.0089C -0.0156 AB

By reducing interactions equations becomes

Y1 = 0.3801 + 0.119A + 0.0266B + 0.0089C

Y2 = 1.66 + 0.355A - 0.265B + 0.1975AB

By reducing interactions equations

Y2 = 1.66 + 0.355 A - 0.265B

Y3 = 86.59 - 5.64A - 0.9925B - 0.8075

**Verification of Model Adequacy:**

The predicted versus actual plots for Y1, Y2, and Y3 demonstrated that the predicted values closely matched the actual data, and all points on the scatter plot followed a straight line, demonstrating that the quadratic regression model accurately represented the experimental range under study. These findings validated the adequacy of the regression model.

**Response surface analysis:**

The 3-D response surface plots and the Studied corresponding contour plots for response parameters, Thickness the tensile strength & Dissolution are depicted in **Figure 11-13**

**For response Y1 (Thickness)** the 3-D response surface plots and the corresponding contour plot revealed that increasing the conc.of Ethyl Cellulose and PEG 400 is expected to increase the thickness. The results range from **0.1 to 0.3mm**. The film thickness can be generally tuned by changing the concentration of the polymer solution and the spreading area.

**For response Y2 (Tensile strength)** the 3-D response surface plots and the corresponding contour plot revealed that higher concentration of plasticizer showed lesser tensile strength**.** The reason for decreased tensile strength was due to the addition of plasticizer which relaxes the polymer internal chain and thereby increasing free space in the polymer internal structure. Though, formulations with larger concentration of Ethyl Cellulose showed higher Tensile strength. Results ranged from **2.57 to 1.12 N/mm$^2$.**

**For response Y3 (Dissolution)** the 3-dimensional response surface plots and the corresponding contour plots revealed that concentration of Ethyl Cellulose has changed the Physical properties of the vaginal films. At higher polymer concentration, the viscosity of the dispersion increases & at lower concentrations the interactions b/w particles are not significant whereas, at higher concentration there is an increase in the hydrodynamic interactions which increases viscosity. On the contour plot it has been detected that increased

concentration of Ethyl Cellulose decreases the dissolution rate. This is due to formation of a relatively strong matrix layer because of deformability which leads to poor water permeability for drug diffusion.

**Formulation optimization**

In order to assess the reliability of the proposed optimized model, the optimized formula (F-OPT) was developed and the dependent variables (Y1, Y2, and Y3) were reassessed. The predicted and observed responses value for the statistically optimized formulation was displayed in Table 14. The lower magnitude of % relative errors (0.052, 0.18, and 0.0030) for Y1, Y2, and Y3 respectively could indicate reasonable agreements and no marked differences between the predicted and observed responses value. The independent variables constraints were set within their minimum and maximum ranges. The desirability and design space of the optimized formulation is shown in Figure 14, with a desirability of 1, indicating that the optimized formulation has the most desirable responses.

**Evaluation of Mucoadhesive Vaginal Films:**

1. **Physical appearance:**

The developed films' appearance was visually assessed. It was discovered that all of the prepared films with varying polymer concentrations were homogeneous, flexible, smooth, translucent, and non-sticky. The distribution of the polymer and medication was consistent.

2. **Weight uniformity:**

As demonstrated in Table 18, the weight of the films was found to be consistent within a batch with low standard deviation values; the weight of the films ranged from 145.06 mg to 155.25 mg, and the weight of the film increased as the concentration of polymer increased.

3. **Surface pH:**

The surface pH of the film should be similar to that of vaginal fluid i.e. 4.2 as it is being kept in the vagina for dissolution for avoiding the irritation. The pH of films was measured in triplicate for each sample and found in the range from **3.9-4.3** within average of around pH 4.20 which, indicated that pH range was well within the targeted pH. The results are shown in **Table 8.**

4. **Folding endurance:**

The folding endurance was less than 300 times which indicates the flexibility of the films. The results range from **120-140** times. Highest folding endurance was shown by film F4 and lowest was found to be for film F7. Folding endurance was directly proportional to quantity of plasticizer because as the amount of polymer increases folding endurance decreases. The results are shown in **Table 8.**

5. **Swelling index:**

Swelling index of the polymer directly affects the residence time of the formulation. The excessive swelling of the films can lead to the rapid clearance of the formulation from the vaginal cavity, which may

retard the effective drug release within the vagina. Optimized formulation F-OPT was found to be 45.62%. which is shown in **Table 9.**

**6. Drug Content Uniformity:**

Drug content uniformity test was carried out, in order to make sure about the uniform dispersion of drug in the films. The drug content was analysed using UV- Vis Spectrophotometer at predetermined $\lambda_{max}$ of 255 nm. The results are shown in **Table 9.** The drug was uniformly dispersed in prepared solution which gives the reproducible results ranging from **95.52% to 98.62%.**

**% Relative error = (predicted value-experimental value) / (predicted value)**

***In-vitro* Drug release:**

The medium was simulated vaginal fluid with a pH of 4.2. because the films ought to stick to the mucosa of the vagina. Tables 15 and 16 display the findings of in vitro dissolution investigations. The CDR of the formulation in Tables 15 and 16 varies between 78.82% and 96.92%. 96.92% of the optimized formulation exhibits the maximum drug release compared to other formulations. As Table 17 makes clear, Peppas appears to fit the release data of formulation best when the correlation coefficient values are at their maximum. All formulations' drug release kinetics were more linear in Peppas when compared to zero order kinetics. This is an illustration of the drug's liberation from the polymeric structure. When the Korsmeyer-Peppas equation is fitted, the drug's release kinetics follow non-Fickian kinetics. Since the Valacyclovir vaginal films' "n" values in the Korsmeyer-Peppas equation fall between 0.45 and 0.89, this indicates that the release kinetics are not Fickian but rather non-Fickian. Table 17 lists the "n" values and $R^2$ values for all 11 and optimized formulation F-OPT vaginal films.

**Short term stability studies:**

After three months of storage at two different temperatures—5±3˚C and 25˚C ± 2˚C—and with a relative humidity of 65%±5% RH, the improved formulation was put through tests. Tables 27 and 28 present information on appearance, drug concentration, surface pH, and folding endurance % CDR. The formulation F-OPT showed no changes in appearance at 5±3˚C. The drug content decreased from 98.85% to 98.76% following a 90-day study. The pH of the formulation's surface also altered. CDR as a percentage changed from 96.92 to 96.74%. Additionally, the formulation's pH was altered. The percentage of CDR shifted from 96.92 to 96.52%. The findings are discussed in Tables 18 and 19.

**Comparison of *In-vitro* Dissolution Studies of Optimized Mucoadhesive vaginal film of Valacyclovir Hydrochloride(F-OPT) with the pure drug and Marketed Product (Valcivir).**

In order to assess the dissolution efficiency, an in-vitro drug release study of the pure drug and the F-OPT formulation was conducted using the commercially available product (Valcivir) in SVF as a dissolution medium. The findings are displayed in Figure 16. The F-OPT formulation demonstrated 96.92% drug release in 12 hours, the marketed product demonstrated 91.12% in 12 hours, and the pure medication's dissolving profile revealed 94.56% in 1.5 hours. Compared to marketed products and pure drugs, the F-OPT formulation

has the highest proportion of drug release. This is because, compared to tablets, thin films exhibit more disintegration because of their larger surface area. In contrast to tablets, which are brittle and encourage drug disintegration followed by increased permeability, films are more robust and flexible.

**Conclusion:**

According to the findings of this study, the manufacturing process and formulation excipients can control the permeability and dissolution of oral conventional solid dosage forms of valacyclovir hydrochloride. PEG 400, 2-Mercaptoethanol, and Ethyl Cellulose can all be effectively utilized to increase the bioavailability and solubility of the low permeability medication Valacyclovir in an easy and affordable way. Given the decrease in dosage quantity and frequency as well as the fact that valacyclovir is not available as a film, it can be said that prepared vaginal film is a good way to get around the issue with traditional dosage forms. Additionally, the investigations provided the creation of promising mucoadhesive dose forms with enhanced bioavailability to increase patient compliance and possible commercial

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**References:**

1. Barbosa AI, Costa Lima SA, Reis S. Application of pH-responsive fucoidan/chitosan nanoparticles to improve oral quercetin delivery. Molecules. 2019 Jan 18;24(2):346.
2. Kinghorn GR. Genital herpes: natural history and treatment of acute episodes. Journal of medical virology. 1993;41(S1):33-8.
3. Schiffer JT, Corey L. New concepts in understanding genital herpes. Current infectious disease reports. 2009 Nov;11(6):457-64.
4. https://en.wikipedia.org/wiki/Valaciclovir
5. https://www.drugs.com/valacyclovir.html
6. Yadav VK, Gupta AB, Kumar R, Yadav JS, Kumar B. Mucoadhesive polymers: means of improving the mucoadhesive properties of drug delivery system. J. Chem. Pharm. Res. 2010;2(5):418-32.
7. Vermani K, Garg S. The scope and potential of vaginal drug delivery. Pharmaceutical science & technology today. 2000 Oct 1;3(10):359-64.
8. Mesquita L, Galante J, Nunes R, Sarmento B, das Neves J. Pharmaceutical vehicles for vaginal and rectal administration of anti-HIV microbicide nano systems. Pharmaceutics. 2019 Mar 26;11(3):145.
9. Vilegave K, Vidyasagar G, Chandankar P. Preformulation studies of pharmaceutical new drug molecule and products: An Overview. The American Journal of Pharmacy. 2013;1(3):1-20.

10. Lade PD, Patil SV, Kadam SS, Tipugade OB, Nakhare PG. Analytical method development and validation of valacyclovir to estimate from its formulation. Journal of Current Pharma Research. 2019 Apr 1;9(3):3086-94.
11. Sudeendra BR, Umme H, Gupta RK, Shivakumar HG. Development and characterization of bioadhesive vaginal films of clotrimazole for vaginal candidiasis. Acta Pharmaceutica Sciencia. 2010;52(4).
12. Gurumurthy V, Deveswaran R, Bharath S, Basavaraj BV, Madhavan V. Development of bioadhesive controlled release ketoconazole vaginal films. Research Journal of Pharmacy and Technology. 2012;5(3):376-82.
13. Younus Pasha M, Bhat SR, Hani U. Formulation design and evaluation of bioadhesive vaginal films of metronidazole for vaginal candidiasis. Latin American Journal of Pharmacy. 2012;31.
14. Zaman M, Hanif M. In vitro and ex vivo assessment of hydrophilic polymer-and plasticizer-based thin buccal films designed by using central composite rotatable design for the delivery of meloxicam. Advances in Polymer Technology. 2018 Oct;37(6):1823-36.
15. Pandey GS, Kumar R, Sharma R, Singh Y, Teotia UV. Development and optimization of oral fast dissolving film of salbutamol sulphate by design of experiment. Am. J. PharmTech Res. 2013;3(4):407-23.
16. Mohite B, Patel R, Kayande N, Thenge R. Vaginal Mucoadhesive Drug Delivery System. Journal of Pharmaceutical Research International. 2021 Nov 22;33(51A):123-33.
17. Abdul Aziz BI, Rajab NA. Preparation and in-vitro evaluation of mucoadhesive clotrimazole vaginal hydrogel. Iraqi J Pharm Sci. 2017;23:1.
18. Bawane S, Telrandhe R, Pande SD. Formulation and evaluation of oral fast dissolving film of bisoprolol fumarate. International Journal of Pharmaceutics and Drug Analysis. 2018;6(2):105-15.
19. Dedeloudi A, Siamidi A, Pavlou P, Vlachou M. Recent advances in the excipients used in modified release vaginal formulations. Materials. 2022 Jan 3;15(1):327.
20. Osmałek T, Froelich A, Jadach B, Tatarek A, Gadziński P, Falana A, Gralińska K, Ekert M, Puri V, Wrotyńska-Barczyńska J, Michniak-Kohn B. Recent advances in polymer-based vaginal drug delivery systems. Pharmaceutics. 2021 Jun 15;13(6):884.