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Sublingual Delivery of Ivabradine Via Pullulan-Based Films: Formulation, Characterization, And Optimization

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Abstract
Angina pectoris refers to chest pain or discomfort resulting from insufficient oxygen delivery to the heart muscle, commonly caused by coronary artery disease. Ivabradine (IVB) also known to be effective in managing angina and reducing myocardial ischemia. This study focuses on the development and assessment of sublingual films prepared using various film-forming polymers, including HPMC E5 LV, HPMC E15 LV, HPMC 3 cps, HPMC 5 cps, and pullulan, through the solvent casting technique. Experimental batches containing different concentrations of film-forming polymers were developed and analyzed based on several parameters, such as film thickness, surface area, elongation percentage, disintegration time (in vitro), folding endurance, tensile strength, drug content uniformity, dissolution profile (in vitro), and permeability studies (in vitro). A batch F10 with pullulan 100 mg was found to produce satisfactory results. With the help of minitab software the sublingual films batches were optimized by using 3^2 factorial designs. The 9 factorial batches were formulated, evaluated and validated to prepare the optimized batch. The optimized batch sublingual films found to release 99.5 ± 0.6% of drug within 10 minutes and satisfactory physicochemical properties.

Keywords: *Ivabradine, sublingual film, solvent casting method, 3^2 factorial designs, pullulan, minitab*
Abbreviations: *IVB: Ivabradine, HPMC LV: Hydroxypropyl Methylcellulose with a low viscosity, cps: centipoise, HCN: hyperpolarization-activated cyclic nucleotide*

1. INTRODUCTION

Cardiovascular diseases (CVDs) continue to be a primary cause of death worldwide, necessitating the development of novel drug delivery systems to enhance therapeutic efficacy and patient compliance[1]. Ivabradine (IVB), known for selective inhibiting I_f current, is commonly prescribed for the management of chronic stable angina and heart failure due to its heart rate-lowering effect without compromising cardiac contractility[2]. Despite its clinical value, oral IVB suffers from limitations such as a slow onset and significant first-pass metabolism, which can decrease its systemic availability[3].

To reduce these limitations of oral delivery, sublingual drug delivery has gained attention as a promising route that enables faster drug absorption, bypasses first pass metabolism, and improves bioavailability[4]. Recently sublingual film has become more popular to improve patient compliance and to achieve rapid desired effect of a drug. Sublingual films are also refereed as fast dissolving films or strip. Such films are designed in such way that within one minute it dissolves with saliva when placed in mouth with or without drinking or chewing. These fast dissolving sublingual films offer several advantages over conventional delivery system[5]. Sublingual films provide several benefits, including convenient administration without the need for water, precise dosing, fast therapeutic effects, user-friendly handling, portability, palatable taste, and enhanced patient adherence. These features make them ideal for situations where a prompt medical response is necessary [6, 7]. Considering these advantages, the present study focuses on the development and assessment of IVB sublingual films to improve its bioavailability and therapeutic effectiveness.

IVB belongs to Biopharmaceutical Classification System (BCS) class I, indicating it has high aqueous solubility, approximately 10 mg/ml. The heart's automatic rhythm originates from spontaneous action potentials generated by depolarization of sinoatrial (SA) nodal cells. The process begins with the activation of specialized ion channels that allow a gradual influx of sodium and potassium ions, generating what is

referred to as the pacemaker or "funny" current (If) [8]. IVB selectively blocks hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, primarily affecting the “funny” current (If) in the sinoatrial node. It is primarily prescribed for managing chronic heart failure and stable angina in individuals with elevated resting heart rates. By blocking the I_f current, IVB slows the heart rate without significantly impacting myocardial contractility or systemic blood pressure [9].

This research focuses on optimizing the formulation using different polymers, evaluating physicochemical properties, and assessing in vitro and ex vivo drug release profiles. The findings of this study could contribute to the development of an alternative, patient-friendly dosage form for IVB, potentially improving clinical outcomes in CVD management.

2. *MATERIAL AND METHOD:*

IVB was procured from global calcium, Hosur. Film forming polymers (Pullulan, HPMC 5cps, HPMC 15 cps, HPMC E5 LV, HPMC E15 LV), Plasticizer (Polyrthylene glycol), Sweetner (Aspartame), Saliva stimulating agent (citric acid) were procured from Balaji chemicals, Ahmedabad. All solutions were prepared using deionized water, while the remaining solvents and reagents used were of analytical grade quality.

2.1 Characterization of drug:

2.1.1 Organoleptic and Flow Properties:

IVB was evaluated for its color, odor, bulk density (BD), tapped density (TD), Carr’s index (%), Hausner’s ratio, and angle of repose[10].

2.1.2 *Drug-polymer compatibility study:*

Drug–polymer compatibility was assessed using Fourier Transform Infrared (FTIR) spectroscopy. The samples were mixed with potassium bromide (KBr) in a 1:1 ratio and compressed into discs for analysis. The FTIR spectra were recorded using a Shimadzu FTIR-8400S spectrophotometer (Kyoto, Japan) employing the powder diffuse reflectance method.

2.1.3 *UV Spectrum Analysis of IVB*

A phosphate buffer solution with a pH 6.8 was used to prepare a solution of IVB which was then scanned between 200–400 nm ranges to determine its peak absorbance wavelength. The UV spectrum was subsequently obtained.

2.1.4 *Standard plot of IVB in Phoshphate buffer pH 6.8:*

A precise quantity of IVB (10 mg) was weighed using a digital balance and dissolved in phosphate buffer with pH 6.8. The solution was then diluted to a final volume of 100 ml using the same buffer to obtain a stock solution with a concentration of 100 µg/ml. From this stock, aliquots of 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were each transferred into separate 10 ml volumetric flasks and diluted to volume with phosphate buffer (pH 6.8), resulting in working solutions of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml, respectively. Absorbance readings for each solution were taken at 286 nm, using phosphate buffer (pH 6.8) as the blank. Each measurement was performed three times, and the average absorbance was calculated.

2.2 Method of Preparation of sublingual film of IVB

Fast dissolving films of IVB were prepared by using the solvent casting technique. Various film-forming polymers such as HPMC E5 LV, HPMC E15 LV, HPMC 3 cps, HPMC 5 cps, and Pullulan were employed in the formulation. The selected polymer was first hydrated in a measured volume of purified water for approximately 3–4 hours to achieve a uniform and clear dispersion. Once hydrated, PEG 400 along with other excipients including a sweetening agent and a saliva stimulant were added sequentially and dissolved thoroughly. IVB hydrochloride was dissolved separately in a small quantity of water and then incorporated into the polymer-plasticizer mixture. The entire solution was stirred continuously using a magnetic stirrer at a speed of 80–90 rpm to ensure homogeneity.

The prepared solution was allowed to stand undisturbed to eliminate any entrapped air bubbles, followed by sonication to ensure clarity. A specific volume of the formulation was then carefully poured into a Petri dish and left to dry at room temperature overnight. After drying, the films were trimmed into 4 cm^2 and stored in airtight containers, wrapped in butter paper and aluminium foil, for subsequent evaluation[11, 12].

2.2.1 *Dose Calculation:*

The IVB to be loaded in film was determined by dose calculation by calculating the area of petri plate ($A= \pi r^2 = 3.14 \times 3.1 \times 3.1 = 30.17\ cm^2$). Now 4 cm^2 of film should consist of 5 mg of IVB, so for the area of 30.17 cm^2, the amount of IVB required was determined as 37.71 mg.

2.2.2 *Formulation and optimization of sublingual films*

Material	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
IVB Hydrochloride	5	5	5	5	5	5	5	5	5	**5**
HPMC E5 LV	200	-	-	-	-	100	-	-	-	**-**
HPMC E15 LV	-	200	-	-	-	-	100	-	-	**-**
HPMC 3 cps	-	-	200	-	-	-	-	100	-	**-**
HPMC 5 cps	-	-	-	200	-	-	-	-	100	**-**
Pullulan	-	-	-	-	200	-	-	-	-	**100**
Citric Acid	4	4	4	4	4	4	4	4	4	**4**
Aspartame	5	5	5	5	5	5	5	5	5	**5**
PEG 400 (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	**0.5**
Water (ml)	20	20	20	20	20	20	20	20	20	**20**

Table 1: Trial batches formulation of IVB Hydrochloride Sublingual Films

The trial batches (F1 to F10) were prepared (Table 1). Based upon the results obtained for the above trial batches for the different evaluation parameters, one of the batches was selected for the optimization. Minitab software was employed throughout the optimization process. This included choosing the experimental design, identifying key factors, implementing a response surface methodology, generating the design matrix, creating 3D surface plots and 2D contour diagrams, applying optimization techniques, analysing the outcomes, and ultimately validating the developed method[13,14]. 3^2 full factorial designed was applied.

List of Independent and Dependent Variables	
Independent Variables	X1=Concentration of Pullulan (mg)
	X2= Concentration of PEG 400 (ml)
Dependent Variables	Y1=Drug Release at 2 min
	Y2= Disintegration Time
	Y3=Folding Endurance

Table 2: Independent and Dependent Variables

Levels / Factors	-1	0	1
X1= Pullulan (mg)	80	100	120
X2= PEG 400 (ml)	0.5	0.75	1.0

Table 3: Level and factors of 3^2 full factorial design

List of Dependent and independent variables are as shown in Table 2. Level and factors of 3^2 full factorial are as given in table 3. As per 3^2 full factorial design, the number of IVB sublingual film batches as shown in table 4 were prepared.

2.3 Evaluation

The sublingual films of different batches were evaluated for the different parameters.

2.3.1 *Thickness:*

Film thickness was determined using a digital Vernier caliper (least count: 0.01 mm) by measuring at three distinct points on each film. The average thickness and standard deviation were then calculated from these readings.

Ingredients (mg/film)	I1	I2	I3	I4	I5	I6	I7	I8	I9
IVB Hydrochloride	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Pullulan	80	100	120	80	100	120	80	100	120
PEG 400 (ml)	0.5	0.5	0.5	0.75	0.75	0.75	1.0	1.0	1.0
Citric Acid	4	4	4	4	4	4	4	4	4
Aspartame	5	5	5	5	5	5	5	5	5
Water (ml)	20	20	20	20	20	20	20	20	20

Table 4: Formulation table for factorial batches

2.3.2 ***Average weight***

Film samples of 2 cm2 were excised from three different areas of the cast sublingual film. Each piece was weighed individually, and the average weight was calculated[15].

2.3.3 ***Surface pH***

The surface pH of the films was measured using a digital pH meter (Janki Impex Pvt. Ltd, Ahmedabad, India) to evaluate the potential for oral mucosal irritation. Each film was placed in a sealed Petri dish containing 5 mL of distilled water to allow proper moistening. Once adequately hydrated, the pH probe was gently brought into contact with the film surface, and readings were taken in triplicate. Maintaining a surface pH close to neutral is essential to prevent discomfort or irritation in the oral cavity[16].

2.3.4 ***% Drug Content***

A film section measuring 4 cm2 was finely cut and placed in 100 ml of phosphate buffer (pH 6.8), followed by continuous shaking. The mixture underwent sonication to achieve full dissolution of the film. Following filtration, the solution was appropriately diluted, and the drug concentration was measured using a UV-Visible spectrophotometer (Thermo Fisher Scientific, USA) at 286 nm[17].

2.3.5 ***In-vitro disintegration time***

Disintegration time is defined as the period a film takes to start breaking apart. For in vitro testing, three samples of 4 cm2 each from every formulation were placed in a glass container with 25 mL of phosphate buffer at pH 6.8, maintained at 37°C. The buffer was gently swirled every 10 seconds. Using a stopwatch, the time until the film fully disintegrated, leaving no visible pieces, was recorded. The average disintegration time was then determined[17].

2.3.6 ***Percent elongation***

When a film sample is subjected to stress, it deforms, a change referred to as strain. Strain is calculated as the ratio of the amount of deformation to the film's original length. An increase in plasticizer concentration typically leads to greater film elongation, indicating enhanced flexibility[18].

Percent Elongation= L * 100/ Lo

Where, L = Increase in length of film, Lo = Initial length of film.

2.3.7 ***Folding endurance***

Folding endurance was measured by repeatedly bending the film at the same spot until it broke[15]. Folding endurance was determined by counting how many times the film could be folded repeatedly at one spot without breaking[18].

2.3.8 ***Tensile strength***

Tensile strength indicates the maximum stress a film strip can endure before it breaks. It is calculated by dividing the breaking force by the cross-sectional area of the strip, according to the equation below[18]

$$\textbf{Tensile strength} = \frac{\text{Load at Failure} \times 100}{\text{Strip thickness} \times \text{Strip Width}}$$

2.3.9 ***In-vitro dissolution studies***

The dissolution profile of the fast-dissolving films was assessed using a USP Type II paddle apparatus. The test was conducted in 300 mL of simulated salivary fluid (pH 6.8) maintained at a temperature of 37 ± 0.5 °C, serving as the dissolution medium. The paddle was rotated at a steady speed of 50 rpm. At

two-minute intervals, samples were collected and replaced with an equal volume of fresh dissolution medium. The drug concentration in each sample was measured using a UV spectrophotometer, and the percentage of drug released was plotted against time to construct the dissolution profile[19].

2.3.10 ***In-vitro permeability study using Franz diffusion cell***

The in vitro release of the drug was evaluated using a modified Franz diffusion cell, featuring a receptor chamber with a 20 mL volume. A synthetic cellophane membrane was placed to separate the donor and receptor compartments. Formulated sublingual films, each measuring 4 cm2, were placed on the membrane, and the receptor compartment was filled with phosphate buffer at pH 6.8. The entire setup was placed on a magnetic stirrer, with continuous stirring at 50 RPM using magnetic beads. The temperature of the receptor medium was maintained at 37 ± 0.5 °C throughout the study

Aliquots of 5 mL were collected at predetermined time points—2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 minutes and analyzed for drug content using a UV spectrophotometer at 286 nm, with phosphate buffer serving as the blank. After each sampling, the receptor compartment was refilled with an equal volume of fresh buffer solution to maintain constant volume. The cumulative drug release from the sublingual films was then graphed as a function of time[20].

3. *RESULT AND DISCUSSION:*

3.1 Characterization of drug (IVB HCl)

Organoleptic properties, flow properties, melting point (by using capillary method) and solubility of a drug were determined. The results are presented in table 5.

Sr. No.	**Characteristic Properties**		**Observation/Result**
1	*Organoleptic Characteristics*	*Colour*	White to slightly yellow powder
2		*Odour*	Odorless
3	*Flow Properties*	*Bulk density (g /ml)*	0.244
4		*Tapped density (g /ml)*	0.489
5		*Carr's index (%)*	50.102
6		*Hausner's ratio*	2.004
7		*Angle of repose (θ°)*	45.4 º
8	*Melting Point*	*By Capillary Method*	196.0 ° C
9	*Solubility*	*Water*	Soluble (15.1 mg/ml)
		6.8 pH Phosphate Buffer	Soluble (17.5 mg/ml)

Table 5: Characterization of Drug (IVB HCl)

3.2 ***Compatibility study with FTIR***

The FTIR spectrum of IVB HCl (see figure 1A) and physical mixture of IVB and Pullulan (See figure 1B) individually disclosed characteristic peaks, which are shown respectively. As per the FTIR spectrum, IVB HCl was found to be compatible with pullulan.

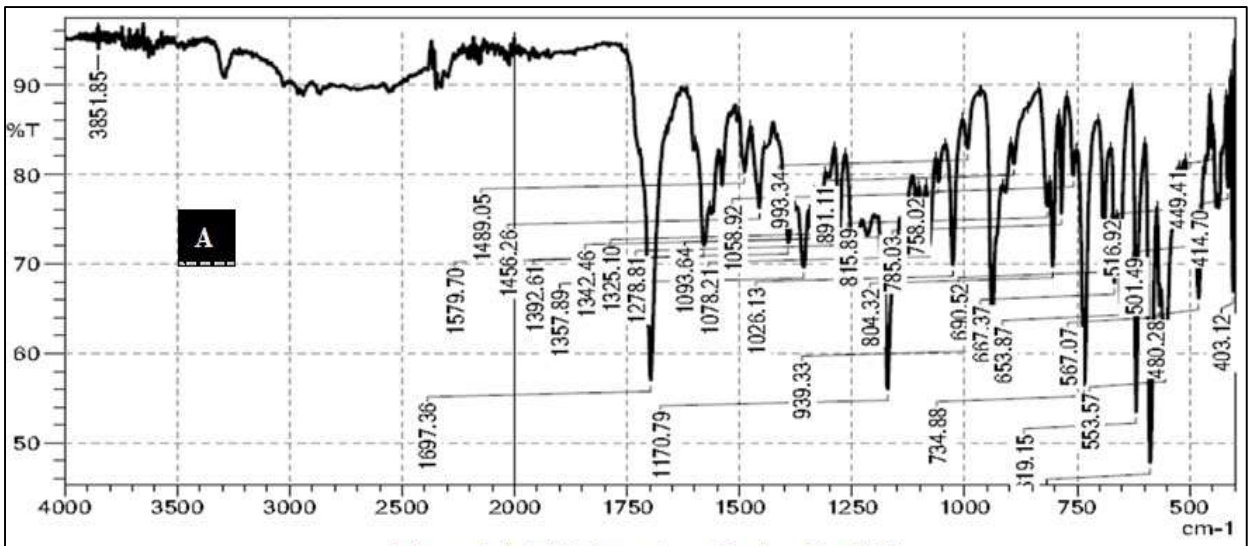

Figure 1 A: FTIR spectra of Ivabradine HCl

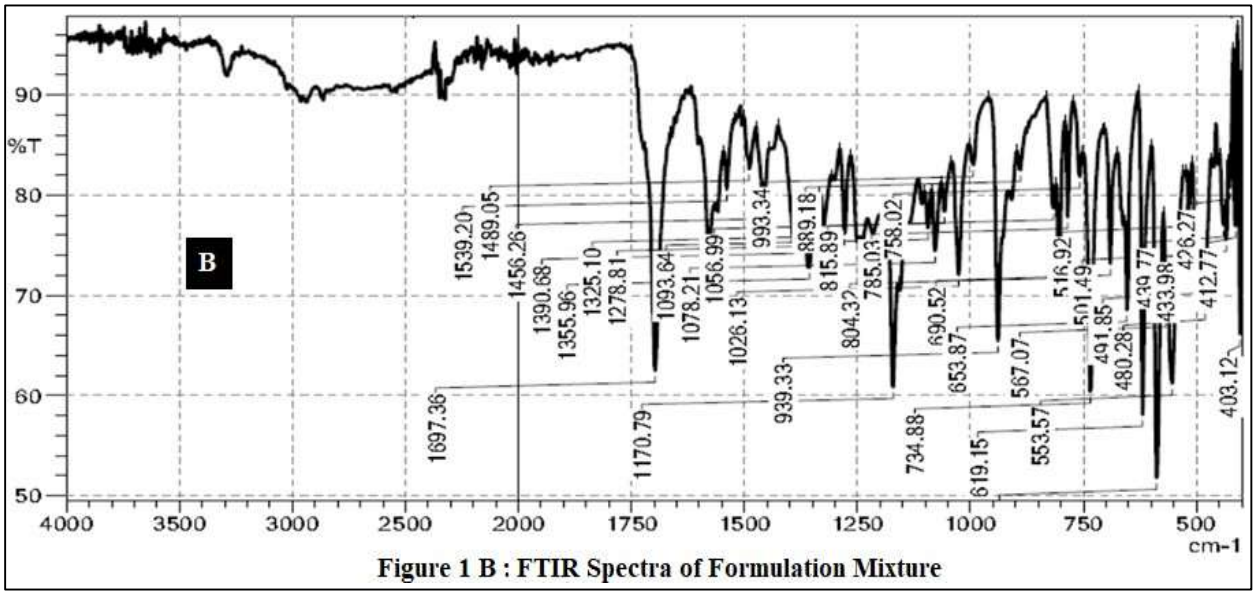

Figure 1 B : FTIR Spectra of Formulation Mixture

3.3 UV Spectrum Analysis of IVB

The λmax of IVB HCl was found to be 286 nm.

3.4 Calibration curve of IVB HCl

Calibration curve for IVB HCl by using different concentration was recorded. A linear graph was found to be recorded for the concentration range of IVB from 10 µg/ml to 50 µg/ml with R^2 value of 0.998 (Figure 2)

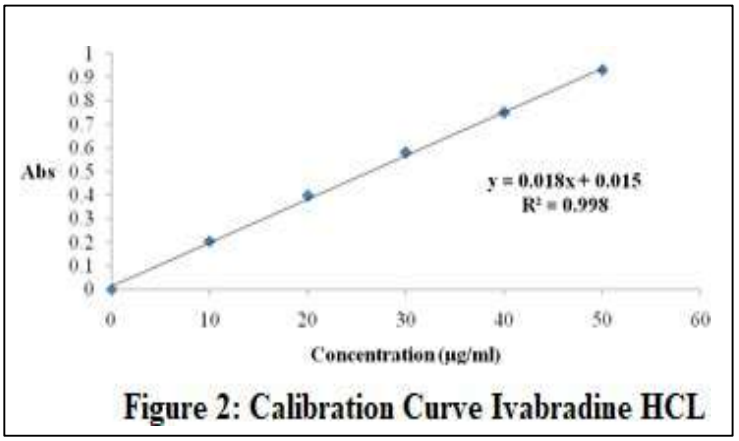

Figure 2: Calibration Curve Ivabradine HCL

3.5 Evaluation of trial batches for different parameters

Different trial batches were formulated as per Table 2; and are then evaluated for different parameters as shown in Table 6 and Table 7.

Trial batches with the varying composition of film forming polymers were evaluated for different parameter. A batch F10, with 100 mg of pullulan found to produce effective results in terms of thickness, surface pH, percent drug content, in-vitro disintegration, percent elongation and folding endurance. Pullulan is a naturally occurring, water-soluble polysaccharide produced by Aureobasidium pullulans.

Thickness

High aqueous solubility of pullulan helps in its even distribution in casting solution which results consistent film thickness during dryness

Surface pH

Pullulan being chemically neutral do not alter the pH of sublingual film.

Batch	Thickness (mm) (n=3)	Average weight (mg) (n=3)	Surface pH (n=3)	% Drug content (n=3)
F1	0.23 ± 0.01	205 ± 5.4	6.9± 0.1	99.6 ± 0.1
F2	0.34 ± 0.05	209 ± 3.4	6.9± 0.4	96.9 ± 0.2
F3	0.22 ± 0.06	211 ± 2.9	7.0± 0.2	98.9 ± 0.7
F4	0.25 ± 0.04	208 ± 3.7	6.9± 0.3	97.1 ± 0.3
F5	0.27 ± 0.07	207 ± 4.9	7.0± 0.2	98.4 ± 0.7
F6	0.26 ± 0.04	115 ± 3.8	7.1± 0.3	96.2 ± 0.4
F7	0.29 ± 0.06	106 ± 2.7	7.0± 0.2	98.6 ± 0.2
F8	0.29 ± 0.07	108 ± 3.1	6.9± 0.4	94.4 ± 0.3
F9	0.28 ± 0.04	107 ± 2.8	6.9±0.3	99.1 ± 0.5
F10	**0.32 ± 0.05**	110 ± 1.8	**7.0 ± 0.2**	**99.6 ±0.4**

Table 6: Evaluation of Trial Sublingual batches (Values ± S. D.)

Batch	In vitro disintegration time (Sec) (n=3)	Percent Elongation (n=3)	Folding Endurance (n=3)	Tensile Strength (gm/cm^2) (n=3)
F1	60 ± 3.6	25.9 ± 2.4	269 ±1.8	58 ± 5.9
F2	65 ± 4.7	26.0 ± 3.9	287 ±2.0	50 ± 3.7
F3	54 ± 9.4	26.6 ± 1.8	284 ±2.6	56 ± 4.9
F4	45 ± 3.5	22.2 ± 2.1	240 ±1.3	60 ± 6.8
F5	35 ± 2.4	24.2 ± 1.7	210 ±1.2	**62 ± 4.7**
F6	28 ± 1.5	20.1 ± 1.9	297 ±1.4	36 ± 2.8
F7	22 ± 2.9	17.9 ± 2.5	196 ±1.8	43 ± 3.7
F8	25 ± 2.0	18.0 ± 2.7	200 ±2.0	43 ± 3.6
F9	62 ± 1.5	26.2 ± 1.9	295 ±1.2	45 ± 2.8
F10	**16 ±2.8**	**36.6 ± 1.7**	**395 ±2.4**	49 ± 3.8

Table 7: Evaluation of Trial Sublingual batches (Values ± S. D.)

Percent drug content

Due to hydrophilic and good compatibility with IVB, pullulan enables uniform drug distribution throughout the film matrix.

In-vitro disintegration time

Pullulan is highly water-soluble and swells rapidly upon contact with saliva. This property promotes fast disintegration of the film in the sublingual area, leading to quick release of the active pharmaceutical ingredient (API), which is crucial for rapid onset of action in sublingual delivery.

Percent elongation

A good plasticity of pullulan due to its molecular structure helps to provide higher percent elongation of a sublingual film

Folding endurance and tensile strength

Pullulan bases sublingual film has shown a good folding endurance and tensile strength which is important for packaging, transport and usage.

In vitro dissolution studies

The outcomes of the in vitro dissolution tests are presented in Figure 3. A batch F10 was found to release maximum 98.5 % of drug in 10 min.

In-vitro permeability study using Franz diffusion cell

The outcomes of the in-vitro dissolution studies are as presented in figure 4. A batch F10 was found to release maximum 92.6 % of drug in 10 min.

Batch F10 with 100 mg Pullulan as a film forming polymer has found to produce better results for the in- vitro dissolution and in-vitro permeability studies.

3.6 Optimization of Sublingual film

As per the results obtained with trial batches, batch F10 was selected for the optimization by using minitab software. Nine batches of sublingual films (Table 4) were prepared by using 3^2 factorial

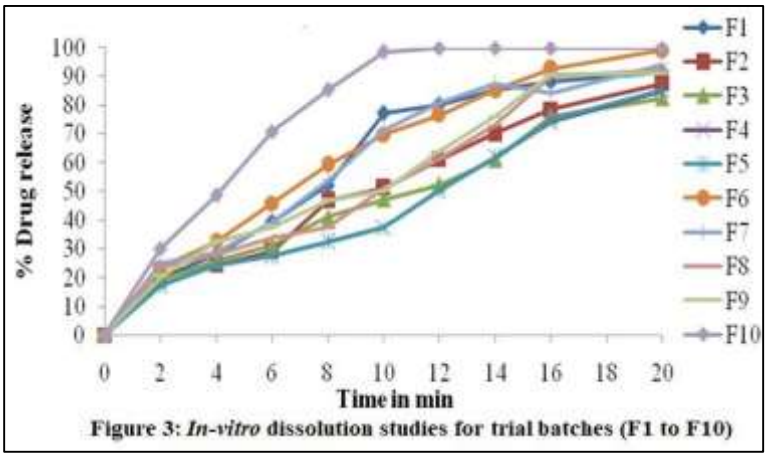

Figure 3: *In-vitro* dissolution studies for trial batches (F1 to F10)

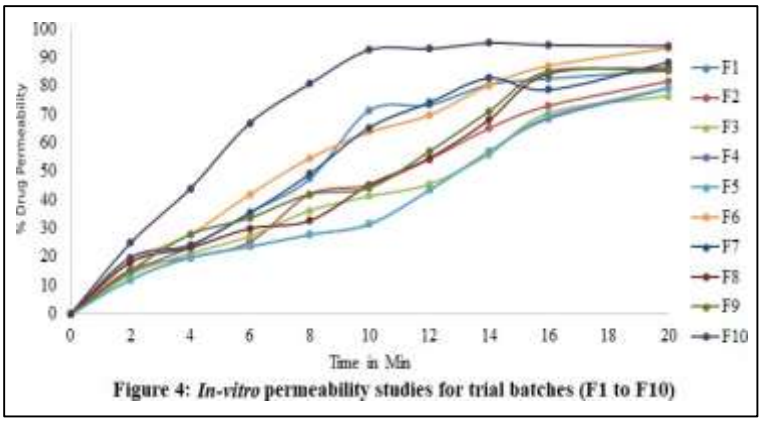

Figure 4: *In-vitro* permeability studies for trial batches (F1 to F10)

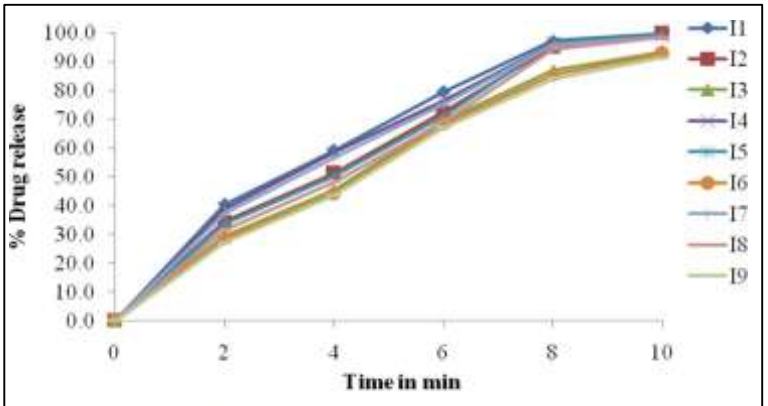

Figure 5: *In-vitro* dissolution studies for factorial batches (I1 to I9)

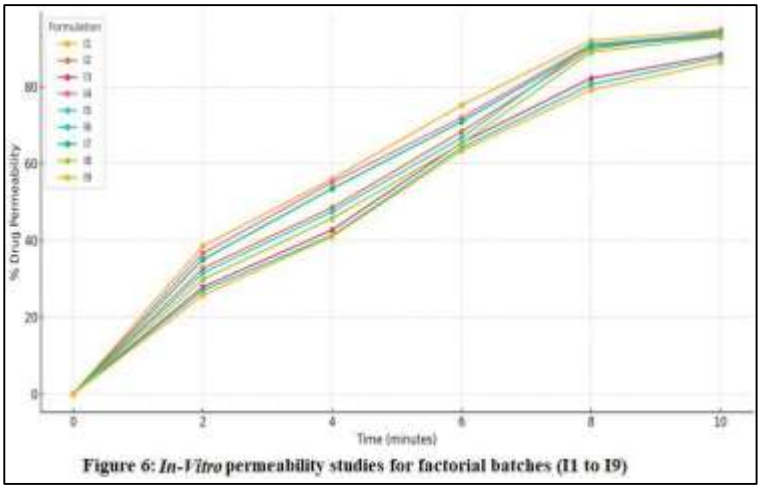

Figure 6: *In-Vitro* permeability studies for factorial batches (I1 to I9)

designs, Table 2 and table 3 shows the "list of dependent and independent variables" and "factors and levels" respectively. Evaluation of factorial batches was carried out and the results are shown in table 8 and table 9.

Dissolution testing for the factorial design formulations (I1 through I9) was performed in vitro, with the outcomes illustrated in Figure 5.

In-vitro permeability study of factorial design batches (I1 to I9) was carried out and the results are shown in figure 6.

Batch	Thickness (mm) (n=3)	Average weight (mg) (n=3)	Surface pH (n=3)	% Drug content (n=3)
I1	0.30 ± 0.01	104 ±2.5	6.5± 0.6	99.1± 0.6
I2	0.32 ± 0.05	128 ±6.5	6.8± 0.2	98.4± 0.8
I3	0.33 ± 0.06	145 ±2.9	7.1± 0.3	98.2± 0.5
I4	0.34 ± 0.04	103± 5.6	6.7± 0.2	98.9± 0.7
I5	0.34 ± 0.07	131 ±6.1	7.2± 0.1	98.9± 0.3
I6	0.34 ± 0.04	141 ±1.9	7.0± 0.3	97.7± 0.4
I7	0.35 ± 0.06	100 ±2.8	6.8± 0.4	99.2± 0.6
I8	0.37 ± 0.07	124±6.6	6.7± 0.5	98.2± 0.8
I9	0.37 ± 0.04	147 ±4.9	6.8±0.4	99.5± 0.9

Table 8: Evaluation of Factorial Batches

Batch	In vitro disintegration time (Sec) (n=3)	Percent Elongation (n=3)	Folding Endurance (n=3)	Tensile Strength (gm/cm^2) (n=3)
I1	12 ± 2.9	26.8 ± 1.9	255 ± 7.8	39± 2.9
I2	18 ± 2.4	36.1 ± 2.4	381 ± 9.1	58± 4.5
I3	30 ± 5.3	39.4 ± 4.6	392 ± 8.4	62± 4.1
I4	17 ± 2.9	29.3 ± 2.5	263 ± 7.6	43± 3.6
I5	20 ± 3.0	39.5 ± 5.4	393 ± 8.3	61± 2.5
I6	39 ± 2.8	42.1 ± 3.4	399 ± 9.7	67± 4.1
I7	23 ± 5.1	31.2 ± 1.8	279 ± 7.3	45± 1.8
I8	25 ± 3.7	34.9 ± 2.5	399 ± 6.8	62± 2.4
I9	45 ± 2.2	43.5 ± 2.4	405 ± 9.0	69± 6.1

Table 9: Evaluation of Factorial Batches

3.7 Analysis of Factorial Design

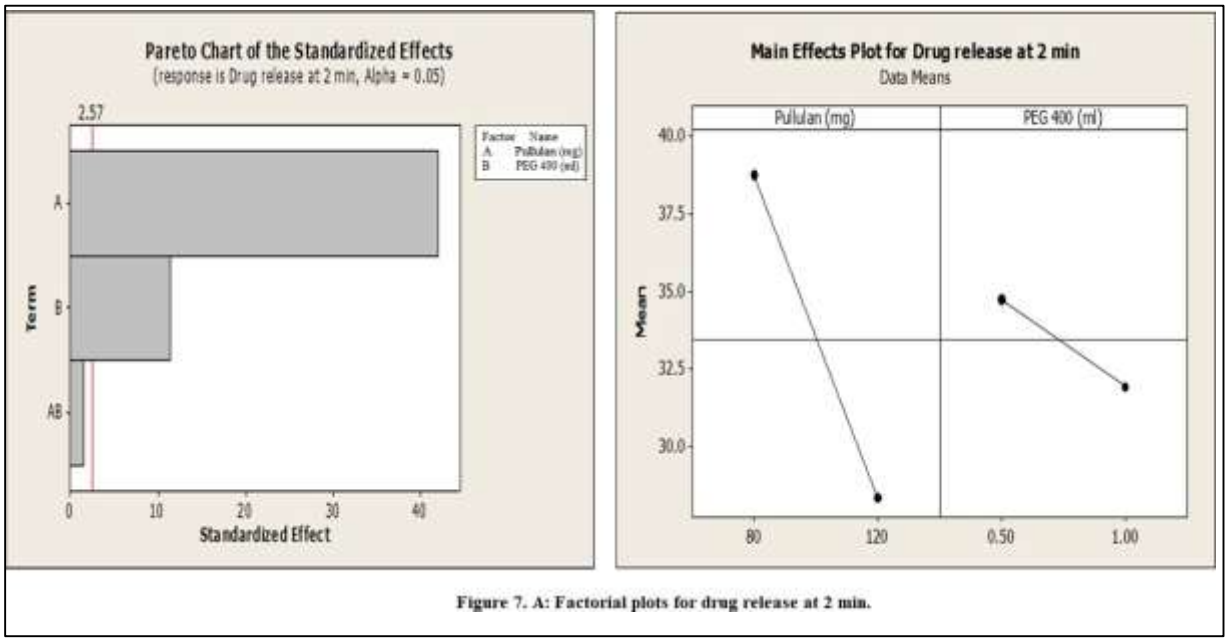

Figure 7. A: Factorial plots for drug release at 2 min.

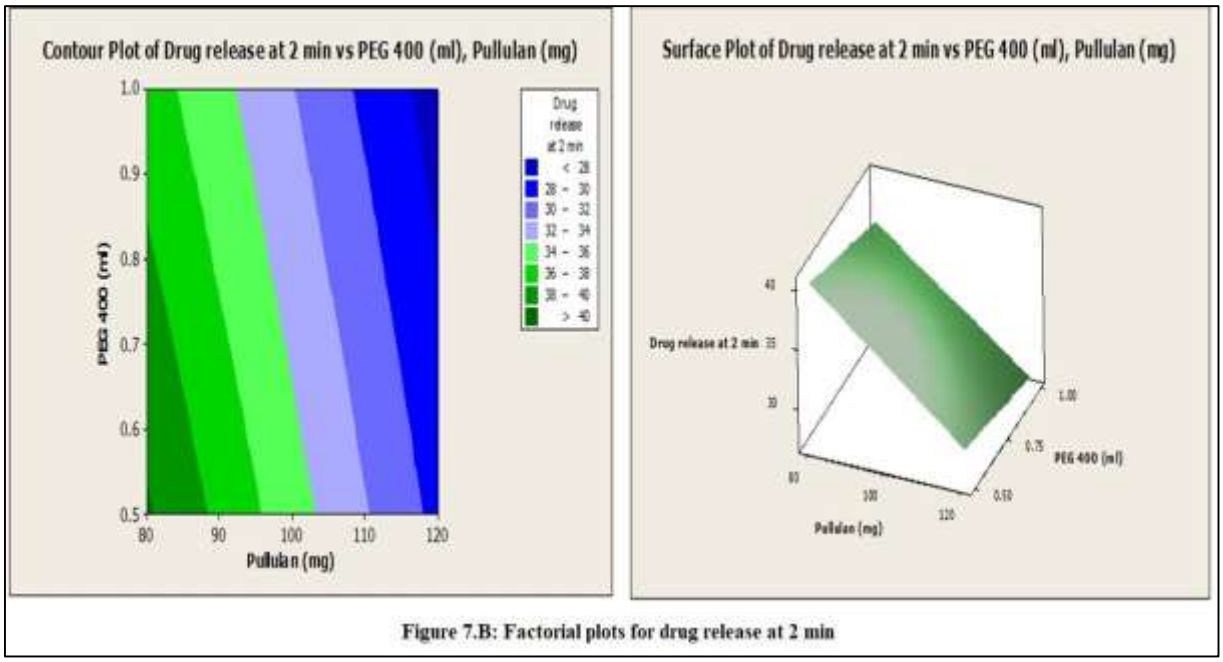

Figure 7.B: Factorial plots for drug release at 2 min

A 3^2 full factorial design layout was prepared as shown in table 10. This resulting data was entered into Minitab and analysed statistically using analysis of variance for drug release at 2 min, disintegration time and folding endurance. To study the effect of two components (Pullulan and PEG) on the given responses, a full factorial design employed using a polynomial equation. The regression equations allow for drawing conclusions based on the size and sign of the coefficients. A positive coefficient in the polynomial model indicates that the response variable increases as the corresponding predictor increases, whereas a negative coefficient suggests a decrease in response. Interaction terms highlight how the response changes when two variables are varied simultaneously.

The response was analyzed using a statistical model that included both interaction effects and polynomial components.

$$Y=\beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2$$

Where: Y = response variable, β_0 = intercept, β_1, β_2 = linear effects, β_{12} = interaction effect β_{11}, β_{22} = quadratic (curvature) effects[14, 21].

3.7.1 Analysis of Variance for Drug release at 2 min (Refer table 11)

Figure 7.A presents the Pareto chart of standardized effects along with the effects plot for drug release at

Batch code	Independent variable		Dependent Variables		
	X1	X2	Y1	Y2	Y3
I1	80	0.5	40.3	12	255
I2	100	0.5	34.5	18	381
I3	120	0.5	29.5	30	392
I4	80	0.75	38.8	17	263
I5	100	0.75	33.6	20	393
I6	120	0.75	28.5	39	399
I7	80	1.0	37.1	23	279
I8	100	1.0	31.6	25	399
I9	120	1.0	27.2	45	405

Translation of coded level in actual unit

Independent variables	Real Value		
	Low(-1)	Medium(0)	High(+1)
X1=Concentration of Pullulan (mg)	80	100	120
X2=Concentration of PEG 400 (ml)	0.5	0.75	1.0
Independent variable: X1= Concentration of Pullulan (mg) X2= Concentration of PEG 400 (ml)	***Dependent variable:*** Y1 = Drug release at 2 min Y2 = Disintegration Time Y3= Folding Endurance		

Table 10: 3^2 Full Factorial Design layout

Analysis of Variance for Drug release at 2 min

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	171.927	171.927	948.71	0.000	***Significant***
Pullulan (mg)	1	160.167	160.167	1767.63	0.000	***Significant***
PEG 400 (ml)	1	11.760	11.760	129.79	0.000	***Significant***
2-Way Interactions	1	0.202	0.202	2.23	0.195	*Non-Significant*
Pullulan (mg)* PEG 400 (ml)	1	0.202	0.202	2.23	0.195	*Non-Significant*
Residual Error	5	0.453	0.453	-	-	-
Total	8	172.582	-	-	-	-

Table 11:Analysis of Variance for Drug release at 2 min

the 2-minute mark. Figure 7.B illustrates the contour and 3D surface plots depicting drug release at 2 minutes in relation to PEG 400 (mL) and Pullulan (mg) concentrations.

Polynomial Equation

Drug release at 2 min = 66.8639 - 0.292083 Pullulan (mg) - 10.1 PEG 400 (ml) + 0.0045 Pullulan (mg)*PEG 400 (ml)

3.7.2 Analysis of variance for disintegration Time (Refer table 12)

Figure 8.A presents pareto chart of the standardized effect along with effects plot for disintegration time. Figure 8.B illustrates contour plot and 3D surface plot of disintegration time versus PEG 400 (ml), Pullulan (mg).

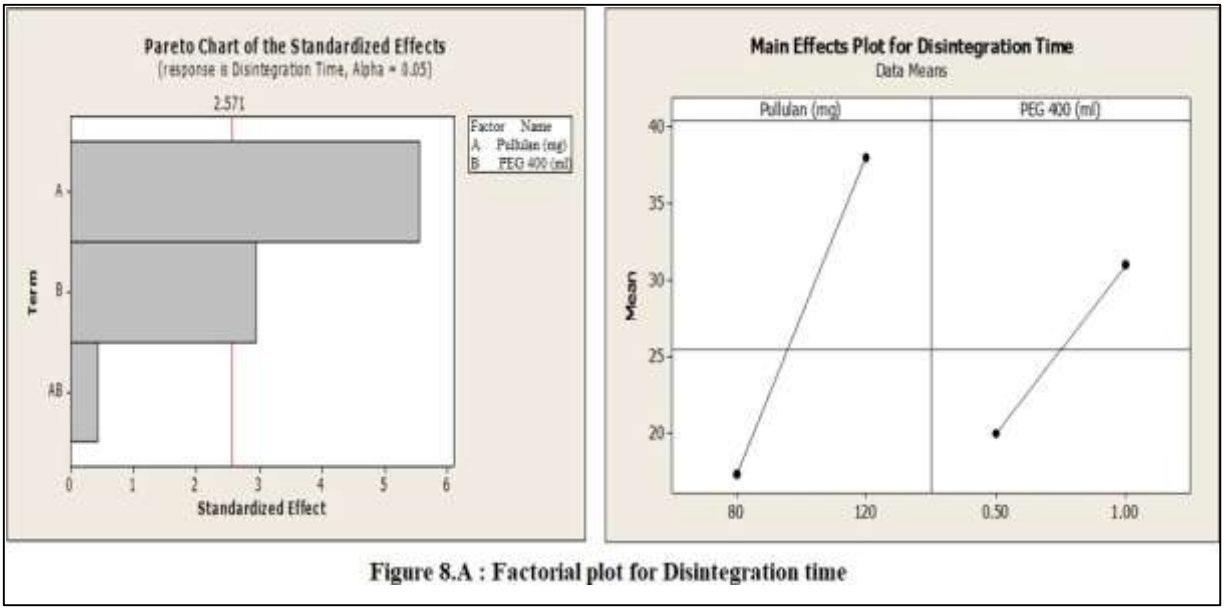

Figure 8.A : Factorial plot for Disintegration time

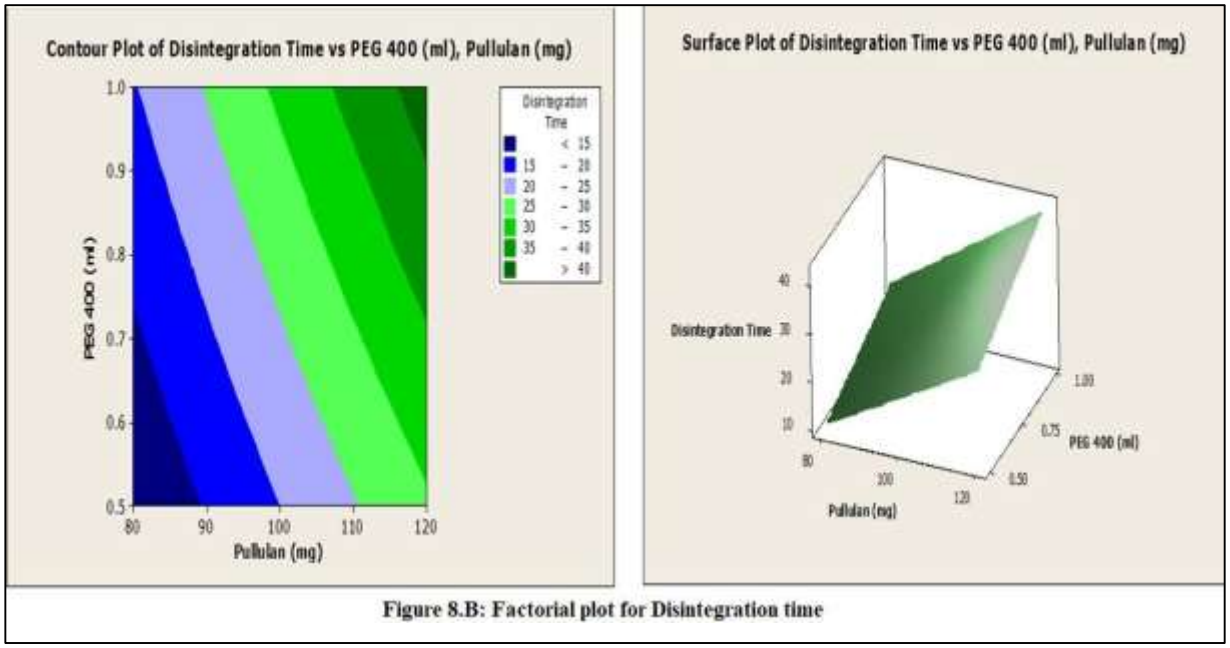

Figure 8.B: Factorial plot for Disintegration time

Polynomial Equation

Disintegration time=-27.7222 +0.0366667 Pullulan (mg) + 2.0 PEG 400 (ml) + 0.20 pullulan (mg)*PEG 400 (ml)

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	822.167	822.167	19.75	0.004	***Significant***
Pullulan (mg)	1	640.667	640.667	30.78	0.003	***Significant***
PEG 400 (ml)	1	181.500	181.500	8.72	0.032	***Significant***
2-Way Interactions	1	4.000	4.000	0.19	0.679	*Non-Significant*
Pullulan (mg)* PEG 400 (ml)	1	4.000	4.000	0.19	0.679	*Non-Significant*
Residual Error	5	104.056	104.056	-	-	-
Total	8	930.222	-	-	-	-

Table 12: Analysis of Variance for Disintegration time

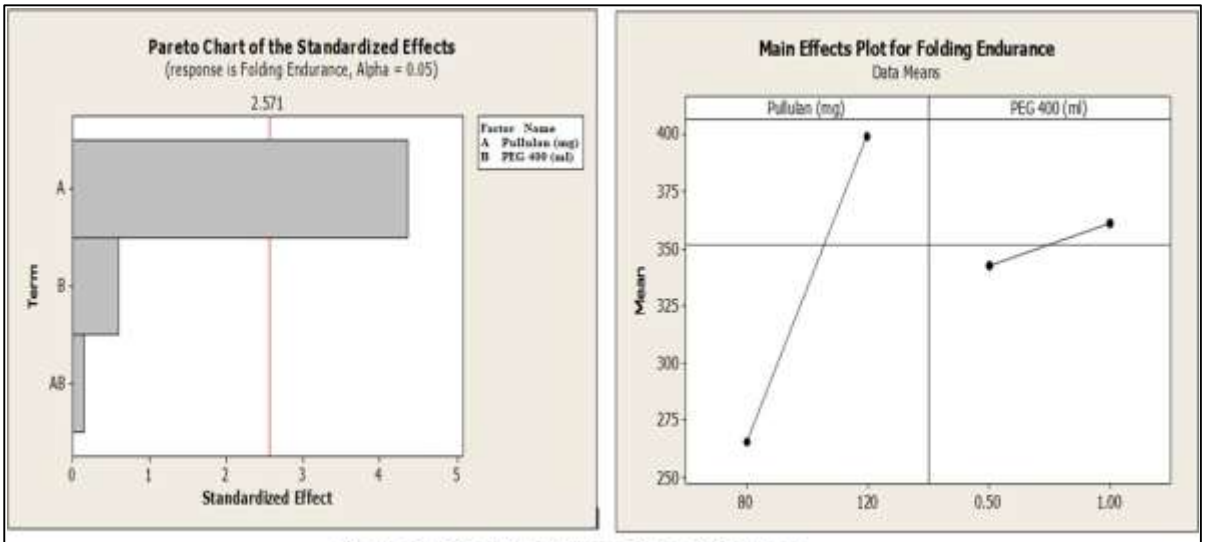

Figure 9.A: Factorial plots for Folding Endurance

3.7.3 Analysis of variance for Folding Endurance (Refer table 13)

Figure 9.A shows pareto chart of the standardized effect and effects plot for folding endurance. Figure 9.B shows contour plot and surface plot of folding endurance versus PEG 400 (ml), Pullulan (mg).

Polynomial Equation

Folding Endurance= -49.472 Constant + 3.73750 Pullulan (mg) + 91.667 PEG 400 (ml) - 0.55 Pullulan (mg)*PEG 400 (ml)

3.8 Formulation and Evaluation of checkpoint Batch

Checkpoint batches I10 and I11 were formulated and evaluated to assess the validity of prediction. The details are summarised in table 14. The contour plot of drug release at 2 min, disintegration time and folding endurance is shown in figure 10.

The response variables obtained from the checkpoint batches were compared to the target response values. The differences between the predicted and actual results were within acceptable limits. The findings are presented in Table 14**.**

3.9 Formulation and Evaluation of Optimized batch

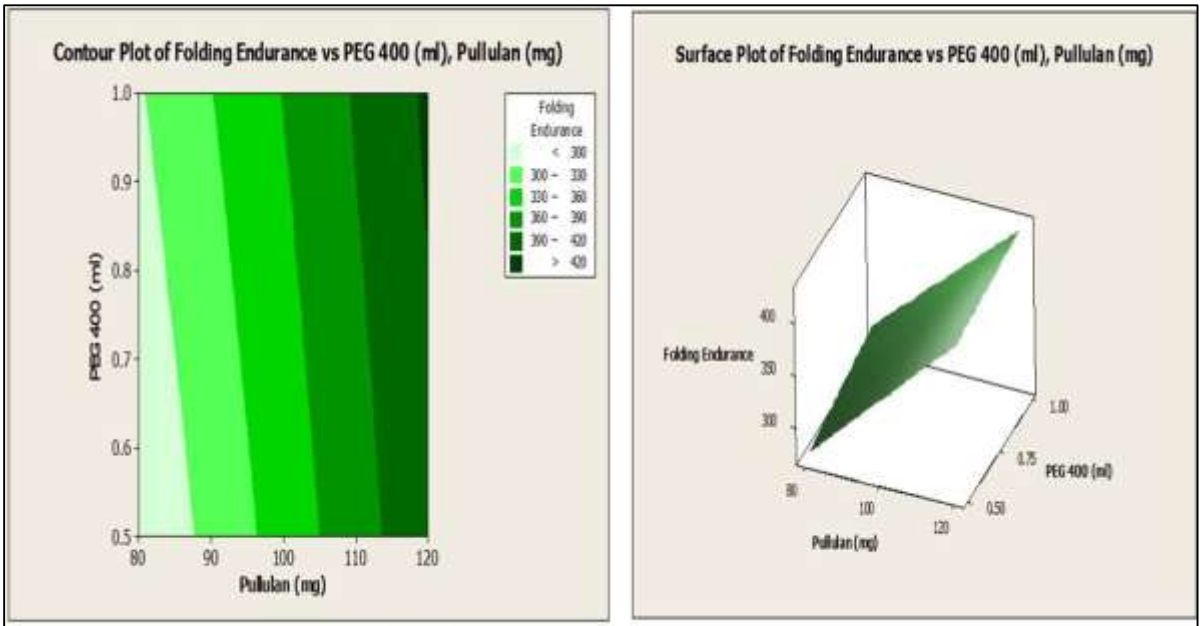

Figure 9.B: Factorial plots for Folding Endurance

A final optimized batch was selected from the contour plot of drug release based on the factorial design data to accomplish the desired disintegration time, folding endurance, and drug release.
The optimized batch was formulated as per table 15 and evaluated for all the parameters, the results of optimized batch are given in table 16. The contour plot of optimized batch for drug release at 2 min, disintegration time and folding endurance is shown in figure 11

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	27037.7	27037.7	9.74	0.019	*Significant*
Pullulan (mg)	1	26533.5	26533.5	19.12	0.007	*Significant*
PEG 400 (ml)	1	504.2	504.2	0.36	0.573	*Non-Significant*
2-Way Interactions	1	30.3	30.3	0.02	0.888	*Non-Significant*
Pullulan (mg)* PEG 400 (ml)	1	30.3	30.3	0.02	0.888	*Non-Significant*
Residual Error	5	6939.6	6939.6	-	-	-
Total	8	34007.6	-	-	-	-

Table 13: Analysis of Variance for Folding Endurance

Batch		**I10**	**I11**
Pullulan (mg)		106.4	90
PEG 400 (ml)		0.9	0.9
Drug release in 2 min	Predicted	30.8	35.1
	Observed	30.1	34.3
	% Bias	1.02	1.02
Disintegration time (sec)	Predicted	32.8	23.4
	Observed	31.2	22.5
	% Bias	1.05	1.04
Folding Endurance	Predicted	379	325
	Observed	375	326
	% Bias	1.01	1.00

Table 14: Formulation and Evaluation of Checkpoint batches

Batch		**I10**	**I11**
Pullulan (mg)		106.4	90
PEG 400 (ml)		0.9	0.9
Drug release in 2 min	Predicted	30.8	35.1
	Observed	30.1	34.3
	% Bias	1.02	1.02
Disintegration time (sec)	Predicted	32.8	23.4
	Observed	31.2	22.5
	% Bias	1.05	1.04
Folding Endurance	Predicted	379	325
	Observed	375	326
	% Bias	1.01	1.00

Table 15: Optimized batch formulation

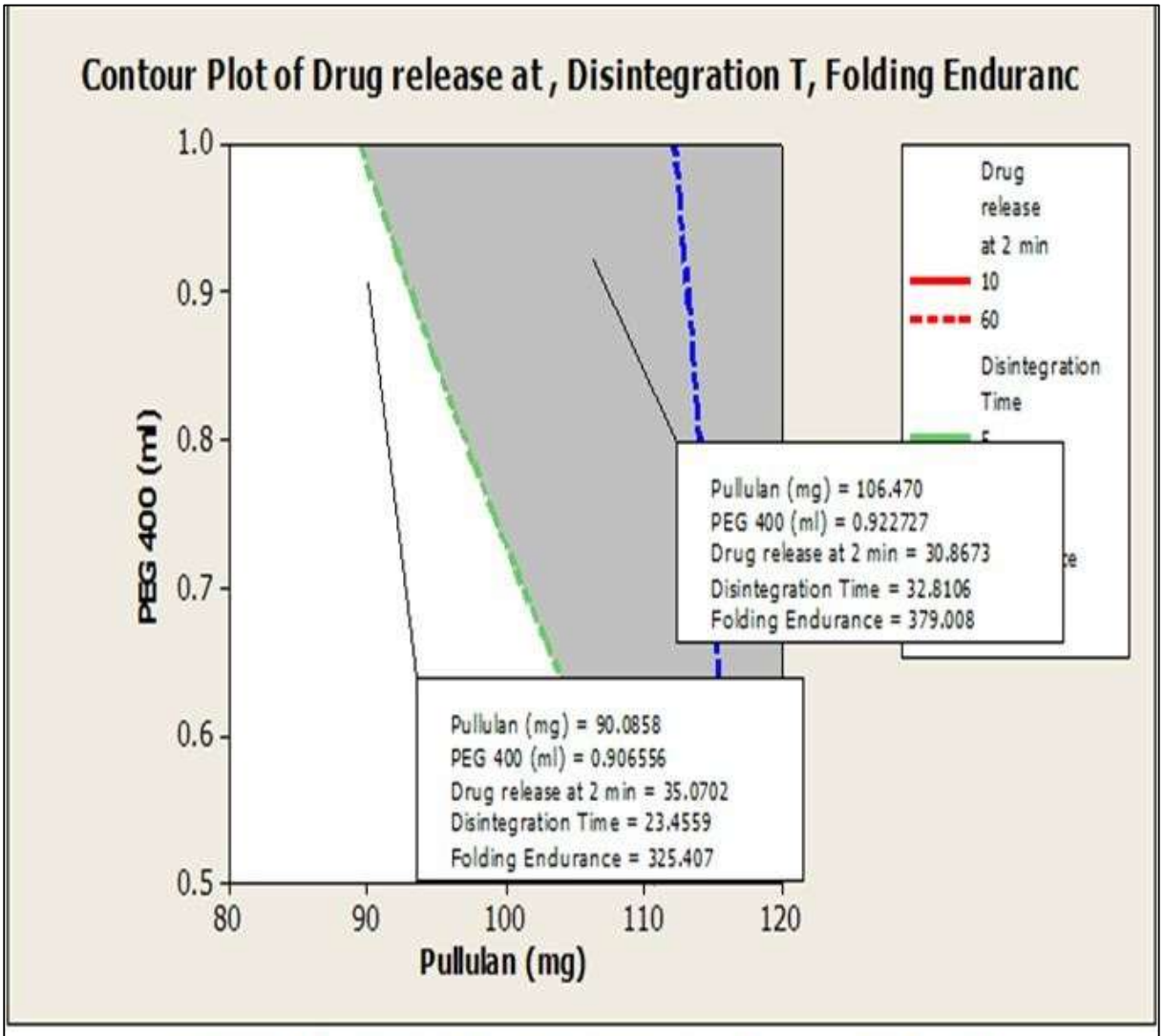

Figure 10: Overaid Contour plot for check point batch

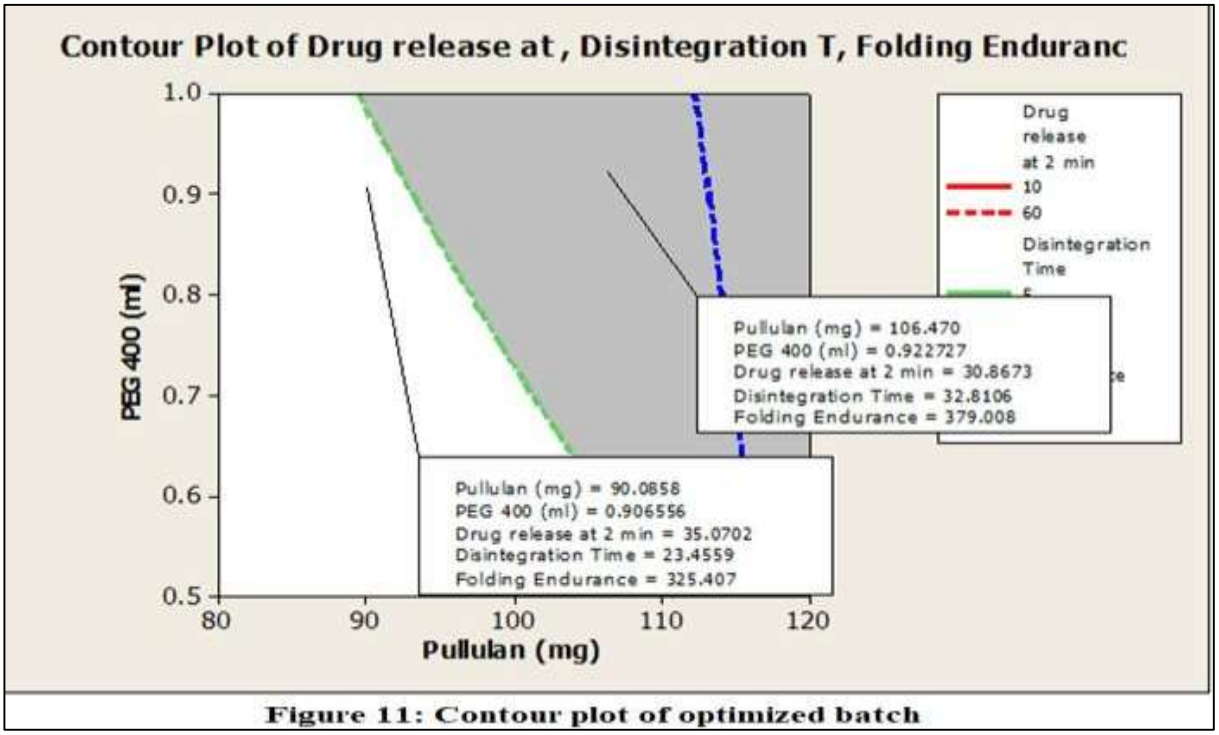

Figure 11: Contour plot of optimized batch

Evaluation Parameter (n=3)	Results	
Appearance	Transparent film	
Texture	Smooth	
Average weight (mg)	106 ± 3.8	
Thickness (mm)	0.31 ± 0.02	
Surface pH	6.9 ± 0.2	
Drug content (%)	99.3 ± 0.8	
Disintegration time (Sec)	13 ± 2.0	
Folding Endurance	280 ± 5.2	
Percent Elongation	25.7 ± 3.6	
Tensile Strength(gm/cm^2)	40 ± 3.4	
% Drug Release	**Time (min)**	**%Drug Release**
	0	0
	2	40.1 ± 4.3
	4	58.6 ± 2.7
	6	80.5 ± 1.8
	8	98.3 ± 1.1
	10	99.5 ± 0.6

Table 16: Evaluation of optimized batch

4. *CONCLUSION*

The purpose of this research was to develop a sublingual IVB film that would improve patient compliance and guarantee a rapid commencement of effect. The current work used a variety of film-forming polymers, including HPMC E5 LV, HPMC E 15 LV, HPMC 3 cps, HPMC 5 cps, pullulan, and varied concentrations of plasticizer, such as PEG 400, to successfully develop and evaluate sublingual films of IVB hydrochloride. Among all these formulation pullulan based sublingual films provided better and satisfactory results in terms of surface pH, percent drug content, disintegration time, percent elongation, folding endurance, tensile strength, in-vitro dissolution studies. These results imply that pullulan-based sublingual films are a viable substitute method of delivering IVB hydrochloride, providing improved therapeutic efficacy and patient compliance in the treatment of heart failure and chronic stable angina. Further in vivo studies are warranted to establish the clinical benefits of this novel formulation

Disclosure statement

The author(s) declare no potential conflict of interest.

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