

FORMULATION, OPTIMIZATION AND EVALUATION OF SUBLINGUAL FILM OF ENALAPRIL MALEATE USING 3² FULL FACTORIAL DESIGN

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ABSTRACT

Objective: The purpose of this investigation was to formulate, optimize and evaluate sublingual film of Enalapril maleate for rapid management of Hypertension.

Methods: Sublingual films were prepared by solvent casting method. Present investigation were formulated by using HPMC E 15 (X₁) as polymer and Polyethylene glycol (X₂) as plasticizer were chosen as independent variables in 3² full factorial design while Tensile strength (TS), Disintegration time (DT) and % Cumulative drug release at 10 min. (% CDR) were taken as dependent variables. The various physical parameters were evaluated for sublingual films such as thickness, tensile strength, folding endurance, disintegration time, surface pH and % CDR.

Results: From the experimental study, it was concluded that the optimized batch F₈ showed 98.6 %, the highest release of the drug. Stability study was performed by taking an optimized formulation and it was observed stable. The sublingual films showed acceptable results in all studies such as thickness, tensile strength, folding endurance, disintegration time, surface pH and % CDR at 10 min. R² values for Tensile Strength (Y₁), Disintegration time (Y₂) and % cumulative drug release at 10 min. of Enalapril maleate (Y₃) found to be 0.9852, 0.9829 and 0.9641 respectively. Thus, a good correlation between dependent and independent variables was developed.

Conclusion: 3² full factorial design was successfully applied during preparation, optimization and evaluation of sublingual films of Enalapril maleate. The present investigation showed quick disintegration and fast release of the drug for rapid management of Hypertension.

Keywords: Sublingual film, Hypertension, Enalapril maleate, HPMC E 15, Polyethylene glycol, 3² full factorial designs

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INTRODUCTION

Development of a formulation involves a great deal of study and experimental work to get optimum results. First pass metabolism can be overcome by sublingual drug delivery and quick drug delivery into the systemic circulation can be obtained. Sublingual administration can offer an attractive alternative route of administration. The advantage of the sublingual drug delivery is that the drug can be directly absorbed into systemic circulation bypassing enzyme degradation in the gut and liver. These formulations are particularly beneficial to pediatric and geriatric patients. In addition sublingual mucosa and abundance of blood supply at the sublingual region allow excellent drug penetration to achieve high plasma drug concentration with rapid onset of an action [1]. Oral mucosal drug delivery is an alternative method of systemic drug delivery that offers several advantages over both injectable and enteral methods. Because the oral mucosa is highly vascularised, drugs that are absorbed through the oral mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver [2]. Sublingual means literally 'under the tongue' refers to a method of administering substances via the mouth in such a way that the substances are rapidly absorbed via the blood vessels under the tongue rather than via the digestive tract [3]. Medically, sublingual drug administration is applied in the field of cardiovascular drugs, steroids, some barbiturates and enzymes. It has been a developing field in the administration of many vitamins and minerals which are found to be readily and thoroughly absorbed by this method [4]. The delivery of drugs in oral mucosal cavity is classified into two categories such as local delivery and systemic delivery [5]. Consequently, permeability decreases in the order: sublingual > buccal > palatal. Transcellular and Paracellular routes are the two possible routes for drug absorption [6]. The sublingual route can produce rapid onset of action due to high permeability and rich blood supply [7]. Factors affecting the sublingual absorption are Lipophilicity of drug, Solubility in salivary secretion, pH and pKa of

the saliva, binding to oral mucosa, Thickness of oral epithelium and Oil-to-water partition coefficient [8-11].

Solvent Casting Method is the most preferred method to manufacture fast dissolving film [12]. Hypertension is a common cardiovascular disorder, which is an important risk factor for coronary artery diseases. Hypertension (HTN) is the term used to denote elevated blood pressure (BP). It is defined as the condition in which BP remains consistent to systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure (DBP) > 90 mmHg [13]. Hypertension as worldwide recognized public health problem is one of the leading causes of death influenced by cardiovascular diseases (CVD) like heart failure (HF), coronary heart disease (CHD), myocardial infarction (MI) and stroke. According to World Health Organization (WHO) and International Society of Hypertension (ISH) from 2003 it is estimated that hypertension cause 4.5% of global disease burden and is a prevalent in many developing countries as in the developed world [14]. Under a threshold of 140/90 mmHg, the World Health Organization estimates that nearly 1 billion people in developed and developing countries are affected with hypertension. About 1 in 8 deaths worldwide is due to hypertension and 4 million people die annually thus making it the third largest killer in the world. Thus, sublingual film of Enalapril maleate can be administered for rapid management of Hypertension [15].

MATERIALS AND METHODS

Materials and reagents

Enalapril Maleate was obtained from West Coast Pharmaceuticals, India. HPMC E5 and HPMC E15 were obtained from Colorcon, Goa. Polyethylene glycol, Propylene glycol were purchased from SAVA Fine Chemical, Mumbai, Maharashtra. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Drug excipients compatibility study

Drug-Excipients interaction plays a vital role in achieving stability of drug in dosage form. Fourier transform infrared spectroscopy (FT-

IR) was used to study the physical and chemical interactions between drug and excipients. FT-IR spectra of Enalapril maleate, HPMC E15 and Polyethylene glycol and their mixture were recorded using potassium bromide mixing method on FT-IR spectrophotometer. (FTIR-1700, Shimadzu, Kyoto, Japan) [16].

Formulation of sublingual film containing enalapril maleate

Sublingual film of Enalapril maleate was prepared using hydrophilic polymers by solvent casting method. In this method, polymer was dissolved in 10 ml water and kept for 30 min in a sonicator. Drug, polyethylene glycol and aspartame were dissolved in 5 ml ethanol to form a clear solution. Both the mixtures were mixed to form homogenous viscous solution and placed in a sonicator for 30 min to remove entrapped bubbles if any. Then, the resultant viscous

solution was casted in petridish and it was dried in the oven at 40 °C at 5 h. The film was carefully removed from the petridish by forceps and cut into 2 cm×2 cm in size. Each film contained 10 mg of Enalapril maleate. The sample was stored in a desiccator maintained at a temperature of 30 ±1 °C and relative humidity 60±5% [17].

Preliminary screening of sublingual film with different polymers

Preliminary study of different polymers were carried out to check its effect on release profile of sublingual film formulation. The preliminary trial batches T₁ to T₁₂ were formulated and evaluated for disintegration time, Tensile Strength and Cumulative % drug release at 10 min and their composition and results were shown in table 1.

Table 1: Preparation of trial batches

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Enalapril maleate	159	159	159	159	159	159	159	159	159	159	159	159
HPMC E 5	200	300	400	500	-	-	-	-	-	-	-	-
HPMC E 15	-	-	-	-	200	300	400	500	-	-	-	-
Polyethylene glycol (%)	-	-	-	-	-	-	-	-	0.5	1.0	1.5	2.0
Aspartame	25	25	25	25	25	25	25	25	25	25	25	25
Citric acid	30	30	30	30	30	30	30	30	30	30	30	30
Preservative	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethanol	5	5	5	5	5	5	5	5	5	5	5	5
Water	10	10	10	10	10	10	10	10	10	10	10	10

Based on results obtained in trial batches the Factors and level of factors were decided. It was observed that HPMC E 15 alone was not able to produce fast disintegration. So, it was combined with Polyethylene Glycol to increase the fast disintegration of the prepared film. The main characteristic of sublingual film is to dissolve quickly. In order to achieve rapid disintegration of film, combination of these two polymers play a crucial role in formulation of sublingual film. Hence, the two factors for Factorial design were:

- i) Concentration of HPMC E 15 (X₁)
- ii) Concentration of Polyethylene Glycol (X₂)

Two levels for each factor were selected to study the effect of X₁ and X₂.

Experimental design of sublingual film of enalapril maleate containing HPMC E 15 and polyethylene glycol

A 3² full factorial design was used in the present study. On the basis of preliminary results, the amount of HPMC E 15 (X₁) and the amount of Polyethylene Glycol (X₂) were chosen as independent variables in 3² full factorial design, while Tensile strength (TS), Disintegration time (DT) and % Cumulative drug release after 10 min (% CDR) were taken as dependent variables. Thus to achieve the formulation with desired Tensile strength (TS), Disintegration time (DT) and % Cumulative drug release after 10 min. (% CDR), the formulation prepared by using different combination of HPMC E 15

and Polyethylene Glycol were optimized and evaluated using 3²-full factorial design.

Full factorial design

This design is useful when a detailed analysis of higher order interactions among the factors is needed. Runs are made at all possible combinations of factor levels. As the number of runs required increases rapidly as the number of factors increases, full factorials are usually used when a relatively small set of factors that are known to be important are available or when collecting a large number of observations is feasible. More information is obtained with less work and effects are measured with maximum precision.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y) is measured for each trial.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

In The 3²-full factorial design 2 independent factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The design layout of 3²-full factorial design as shown in table 2 and table 3.

Two independent variables were selected as below:

X₁ = % w/v concentration of HPMC E 15

X₂ = % w/v concentration of Polyethylene Glycol

Table 2: Variables with coded and exact values for 3² full factorial design

Independent variables	Low	Medium	High
Coded values	(-1)	(0)	(+1)
X ₁ = concentration of HPMC E 15 (mg/ml)	250	350	450
X ₂ = concentration of Polyethylene Glycol (mg/ml)	0.75	1.25	1.75
Dependent variables			
Y ₁ = Tensile strength			
Y ₂ = Disintegration time			
Y ₃ = Cumulative drug release			

Table 3: Formulations showing factors optimized by 3² full factorial design, (Formulation = EMSF) (n = 9)

Formulation code	Factor 1	Factor 2
	Concentration of HPMC E 15 (mg/ml)	Concentration of polyethylene glycol (mg/ml)
EMSF 1	250	0.75
EMSF 2	350	1.25
EMSF 3	450	1.75
EMSF 4	250	0.75
EMSF 5	350	1.25
EMSF 6	450	1.75
EMSF 7	250	0.75
EMSF 8	350	1.25
EMSF 9	450	1.75

Weight uniformity of the film

One square inch film was cut at five different places in the caste film. The weight of each filmstrip was taken and the weight variation was calculated [18].

Thickness of the film

The thickness of the film was measured using micro meter screw gauge and the average thickness of all films was calculated [19].

Tensile strength

Tensile strength of the film was determined with digital tensile tester, which consists of two load cell grips. The lower one is fixed and upper one is movable. The test film of specific size 2×2 cm can be fixed between these two cell grips and force will be gradually applied till the film breaks. Results of tensile strength in kg will be taken [20].

$$\text{Tensile strength (Ncm}^2\text{)} = \frac{\text{force at break (Kg)} \times 9.8}{\text{Initial cross sectional area of sample (cm}^2\text{)}}$$

Folding endurance

The folding endurance of the film was determined by repeatedly folding a small strip of the film at the same place till it broke and the average folding endurance of all films was measure [21].

Disintegration time

The disintegration time limit of 30s or less for orally disintegrating described in CDER guidance can be applied to sublingual film. Although no official guidance is available for sublingual film, this may be used as a qualitative guideline for quality control test or at development stage. Pharmacopoeia disintegrating test apparatus may be used for study. Typical disintegration time for film 5-50s. Test was performed using disintegration test apparatus. 2×2 cm film was placed in the basket; it was raised and lowered it in such a manner that the complete up and down movement at a rate equivalent to thirty times a minute [22].

Drug content uniformity

Sublingual film prepare with various polymer were subjected to the uniform dispersion of drug throughout the film, in each case three films were used and the average drug content was calculated. Suggesting that drug was uniformly dispersed throughout all film.

Films were cut in 2 × 2 cm size, dissolved in Phosphate buffer pH 6.8 and volume was made up to 100 ml. Solution was diluted if necessary. Absorbance was measure at 270 nm [23].

$$\% \text{Drug content} = \frac{\text{Actual amount of drug in film}}{\text{Thoritical amount of drug present in film}} \times 100$$

In vitro drug release

The *in vitro* dissolution studies were conducted using simulated saliva fluid (300 ml). The dissolution studies were carried out using USP dissolution apparatus at 37±0.5 °C and at 50 rpm. Each film with dimension (2×2 cm²) was placed on a stainless-steel wire mesh with sieve opening 700 μm. The film sample placed on the sieve was submerged into dissolution media. 5 ml samples were withdrawn at time intervals of 1, 2,5,8, and 10, min, filtered through 0.45 μm Whatman filter paper and were analyzed spectrophotometrically at 270 nm. To maintain the volume, an equal volume of fresh dissolution medium was added after withdrawing samples [24].

Accelerated stability study

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors. The optimized formulation was wrapped in aluminum foil and stored at 45±0.5 °C and 50%RH for period of one month. After the period of one month, film was tested for weight Uniformity of film, Thickness of film, Tensile strength, Folding endurance, Disintegration time, Content uniformity and *in vitro* drug release study. Both the data were compared and change was observed if any [25].

RESULTS AND DISCUSSION

Drug excipients compatibility study

Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drug and excipients. FT-IR spectra of Enalapril maleate, HPMC E 15, Polyethylene glycol and their mixture of Enalapril maleate, HPMC E 15, Polyethylene glycol were recorded by using KBr mixing method on FT-IR instrument. The drug exhibited peaks due to Carboxylic group, C-N, Aromatic ring, C-O and C=O stretching. It was observed that there were no or very minor changes in drug main peaks in the IR spectra of the mixture and pure drug. The FTIR study revealed no physical or chemical interaction of Enalapril maleate, HPMC E 15, Polyethylene glycol [26].

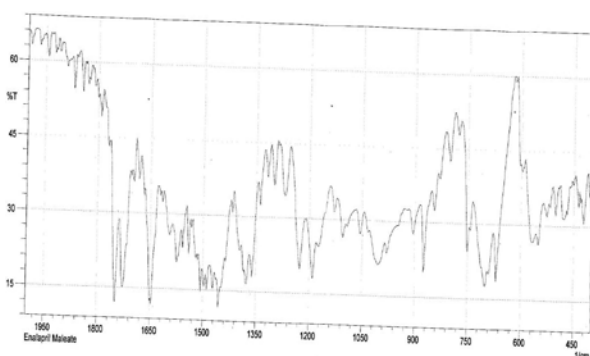


Fig. 1.1: FT-IR spectra of enalapril maleate

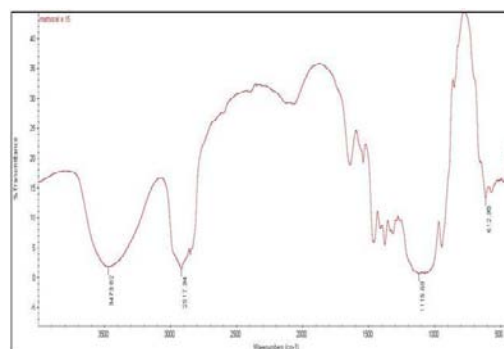


Fig. 1.2: FT-IR spectra of HPMC E 15

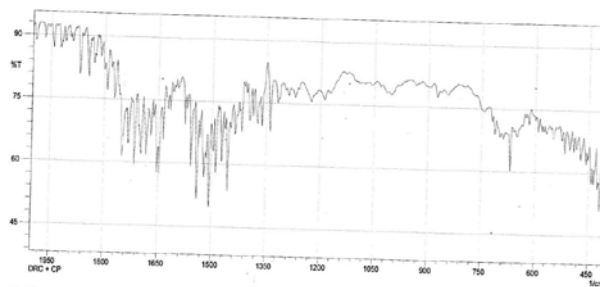


Fig. 1.3: FT-IR spectra of enalapril maleate and HPMC E 15

Evaluation parameter

Thickness of film

The average thickness of all the formulations was between 2.7 to 2.9 mm.

Weight variation

The weight of film formulations were within the range of 9.82 ± 2.08 to 10.05 ± 0.36 mg. All sublingual films passed weight variation test as per pharmacopoeia limits.

Tensile strength

The measured tensile strength of all batches lies between 11.4 ± 0.15 to 13.8 ± 0.05 kg/cm². This ensure good handling characteristics of all batches.

Folding endurance

Folding endurance of batch EMSF1 to EMSF9 was found to be in the range of 110 ± 2.05 to 170 ± 1.80 . It was found that polymer and plasticizer concentration markedly affect the folding endurance of film.

Disintegration time

The disintegration time of all the batches was between 48 to 61 sec. It was observed that as the concentration of HPMC E 15 increases, then disintegration time decreases. On the other hand, as the concentration of Polyethylene glycol increases, then disintegration time increases.

Surface pH

Surface pH of factorial batches EMSF1 to EMSF9 was found to be in therange of 6.6 ± 0.04 to 6.9 ± 0.02 . It was observed within limits.

Drug content

The percentage drug content of the all batches was between 93.80% to 98.60%, which is within acceptable limits indicate dose uniformity in each batch.

In vitro dissolution study

From dissolution study it was concluded that as concentration of HPMC E 15 increases amount of drug released decreases and as the concentration of Polyethylene glycol increases amount of drug released increases.

Table 4: *In vitro* dissolution of batch EMSF1 to EMSF9

Time (in sec)	% Drug release								
	EMSF1	EMSF2	EMSF3	EMSF4	EMSF5	EMSF6	EMSF7	EMSF8	EMSF9
0	0	0	0	0	0	0	0	0	0
60	19.7±0.18	18.2±0.12	17.5±0.16	17.4±0.06	16.9±0.12	14.6±0.16	15.2±0.18	14.6±0.06	13.8±0.12
120	35.6±0.15	33.4±0.16	31.2±0.15	31.4±0.18	25.6±0.16	24.8±0.15	25.8±0.14	24.5±0.15	24.2±0.18
180	56.9±0.12	52.6±0.18	51.8±0.06	45.9±0.14	38.9±0.06	36.5±0.05	39.5±0.12	35.8±0.04	35.4±0.14
240	78.2±0.06	74.5±0.08	72.9±0.12	59.6±0.12	49.8±0.15	48.2±0.18	50.2±0.05	47.2±0.06	45.8±0.14
300	86.9±0.14	83.2±0.05	82.1±0.14	71.2±0.16	60.2±0.05	58.6±0.12	61.6±0.16	59.5±0.14	56.5±0.05
360	91.9±0.16	90.2±0.14	89.6±0.05	77.8±0.15	71.5±0.08	69.3±0.14	72.2±0.15	68.4±0.08	67.9±0.16
420	96.8±0.08	97.2±0.15	95.2±0.18	82.6±0.14	78.2±0.14	76.8±0.05	79.8±0.05	76.6±0.18	74.5±0.15
480	-	-	-	89.2±0.08	86.7±0.14	81.5±0.18	85.4±0.08	85.2±0.04	81.8±0.06
540	-	-	-	93.6±0.06	92.8±0.05	88.6±0.14	89.5±0.14	91.5±0.08	87.6±0.14
600	-	-	-	94.6±0.14	95.4±0.08	93.8±0.05	96.8±0.06	98.6±0.16	95.8±0.18

*Data represented in mean±SD (n=3)

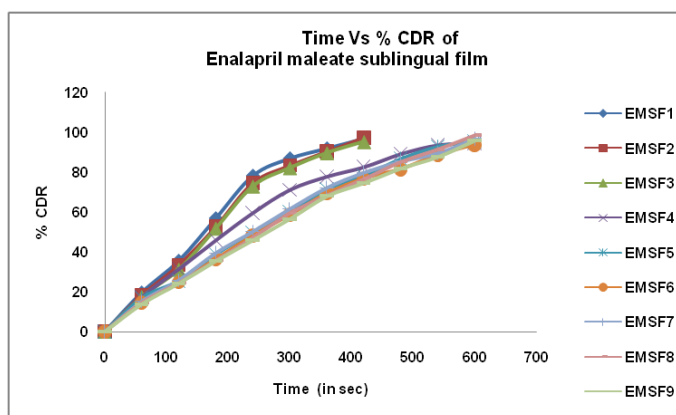


Fig. 2: Drug release profile of batch EMSF1-EMSF9

Statistical analysis

The results of 3² full factorial design were analyzed. A considerable information was gathered by using statistical design to optimize the formulation. All the responses were fitted to a quadratic model and compatibility of the model was verified by ANOVA, lack of fit and coefficient of determination (R²). To optimize the responses, every response should be interconnected with each other and the most

supportive zone must be required for every response to exclude bias. Desirability function was supported by much literature to optimize the multiple responses [27, 28].

The statistical analysis of the factorial design batches was performed by multiple linear regression analysis. The Tensile Strength (Y₁), Disintegration time (Y₂) and % cumulative drug release at 10 min. of Enalaprilmaleate (Y₃) were selected as dependent variables.

Table 5: Optimization of enalapril maleate sublingual films using 3² full factorial design (Formulation-EMSF) (n = 9)

Formulation code	Response 1 (Y ₁) tensile strength (kg/cm ²)	Response 2 (Y ₂) disintegration time (sec)	Response 3 (Y ₃) % cumulative drug release at 10 min.
EMSF1	11.4±0.10	48±0.20	96.8±0.10
EMSF 2	11.8±0.50	51±1.06	97.20.25
EMSF 3	12.1±0.24	54±0.40	95.2±0.20
EMSF 4	11.7±0.36	51±1.28	94.6±0.10
EMSF 5	12.6±0.40	58±0.20	95.4±0.30
EMSF 6	13.0±0.25	61±1.30	93.8±0.50
EMSF 7	12.8±0.64	48±0.10	96.8±0.40
EMSF 8	13.5±0.80	53±0.50	98.6±0.10
EMSF 9	13.8±0.18	59±1.80	95.8±0.24

*Data represented in mean±SD (n=3)

The fitted equations (full model) relating the responses that is, Tensile Strength (Y₁), Disintegration time (Y₂) and % cumulative drug release at 10 min of Enalapril maleate (Y₃) to the transformed factor are shown in table 5. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). Data were analyzed using Design of Expert version 9.

R² values for Tensile Strength (Y₁), Disintegration time (Y₂) and % cumulative drug release at 10 min of Enalapril maleate (Y₃) were 0.9852, 0.9829 and 0.9641 respectively indicating good correlation between dependent and independent variables. There was no need

to develop reduced models because response variable were significant i.e. P<0.05. The terms with P<0.05 were considered statistically significance and retained in the full model.

The results of ANOVA suggested that F values calculated for Tensile Strength (Y₁), Disintegration time (Y₂) and % cumulative drug release at 10 min of Enalapril maleate (Y₃) were 39.93, 34.43 and 18.10 respectively. Calculated F values were greater than tabulated for all dependent variables therefore factors selected have shown significant effects. From the results of multiple regression analysis, it was found that both factors had statistically significant influence on all dependent variables as p<0.05 (table 6).

Table 6: Summary of regression analysis of the responses (EMSF)

Quadratic model	R ²	Adjusted R ²	SD	Adequate precision	p-value
Tensile Strength (kg/cm ²)	0.9852	0.9605	0.17	19.267	0.0060
Disintegration time (sec)	0.9829	0.9543	1.01	16.410	0.0075
% Cumulative Drug release at 10 min.	0.9641	0.9144	0.43	13.198	0.0189

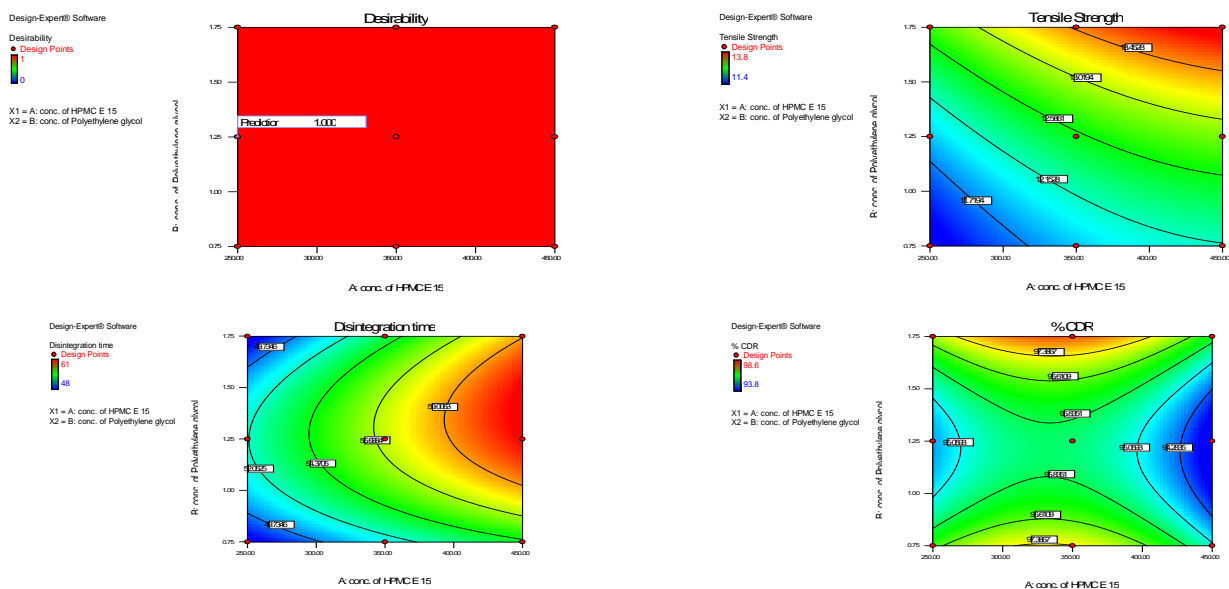


Fig. 3: 2D Response surface contour showing desirability between factors and responses

Polynomial equation with intercept and coded factors

$$Y_1 = +12.54 + 0.50A (*P>0.05) + 0.80B (*P<0.05) + 0.75AB (*P>0.05) - 0.17A^2 (*P>0.05) + 0.13B^2 (*P>0.05).$$

$$Y_2 = +57.00 + 4.50A (*P<0.05) + 1.17B (*P>0.05) + 1.25AB (*P>0.05) - 0.50A^2 (*P>0.05) - 4.50B^2 (*P<0.05)$$

$$Y_3 = 95.67 - 0.53A (*P>0.05) + 0.37B (*P>0.05) + 0.10AB (*P>0.05) - 1.60A^2 (*P<0.05) + 2.10B^2 (*P<0.05)$$

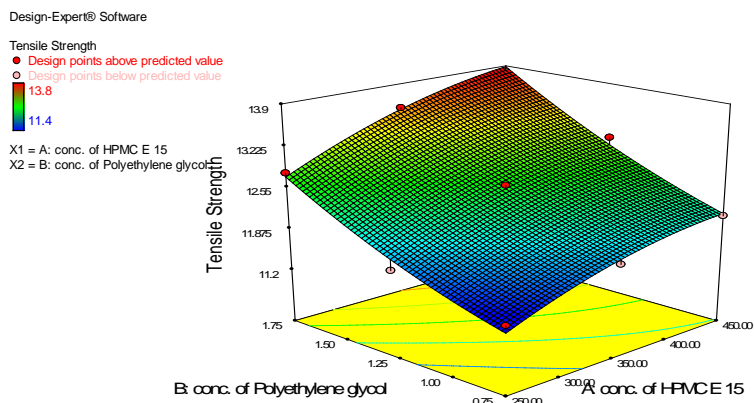


Fig. 4: Response surface plot showing the effect of HPMC E 15 (X₁) and polyethylene glycol (X₂) on tensile strength (Y₁)

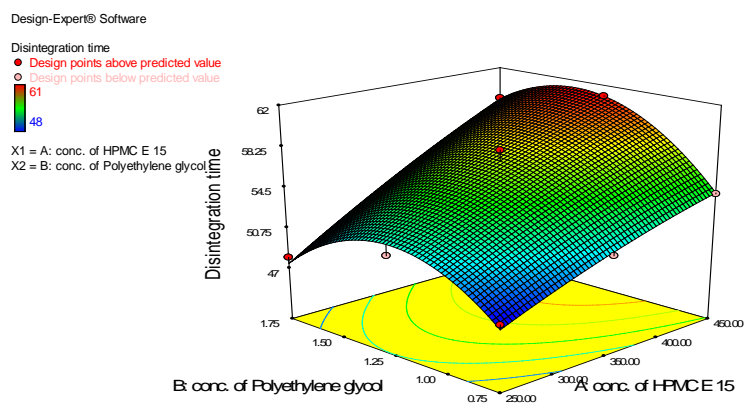


Fig. 5: Response surface plot showing the effect of HPMC E 15 (X₁) and polyethylene glycol (X₂) on disintegration time (Y₂)

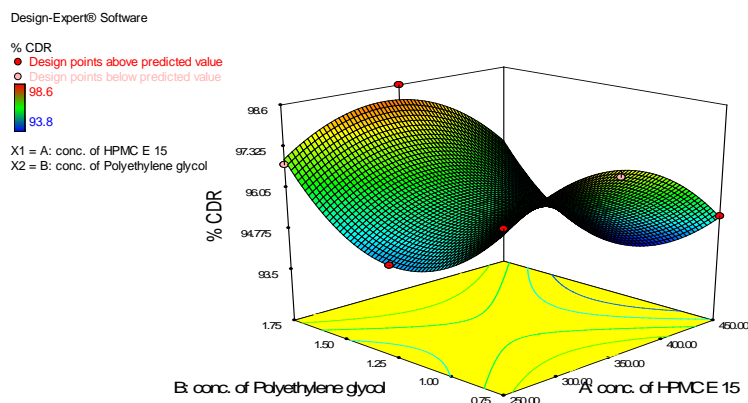


Fig. 6: Contour plot showing the effect of HPMC E 15 (X₁) and polyethylene glycol (X₂) on %CDR (Y₃)

Table 7: Comparison of predicted and observed values of EMSF

Confirmation location	Conc. of HPMC E 15 (X ₁)	Conc. of polyethylene glycol (X ₂)	*Bias %
	375.88	1.12	
Response	Predicted value	Observed value (n=3)	
Tensile Strength (kg/cm ²)	12.46	12.80±0.40	-2.656
Disintegration time (sec)	57.4462	58±0.10	-0.954
% CDRat 10 min.	95.4601	94.54±0.50	+0.973

*Bias % = (Predicted value–Observed value) *100/Observed value, *Data from each response for the observed values is presented in mean±SD(n=3)

Full and reduced model for tensile strength of enalapril maleate

The contour plot and 3D response surface graph for tensile strength was observed in fig. 3 and fig. 4 respectively and revealed that a corresponding increase of tensile strength was observed with increase in concentration of Polyethylene glycol (X₂). Moreover, the results also indicated that the effect of Polyethylene glycol (X₂) was more significant. From regression, it was observed that X₁ and X₂ was significant model term which affect the flexibility and elasticity of film. Interaction and non-linearity was not observed.

For Tensile Strength, the significant levels of the coefficients b₁₂, b₁₁ and b₂₂ were found to have P value of 0.4310, 0.2491 and 0.3367. So, it was omitted from the full model to generate a reduced model. The coefficients b₀, b₁ and b₂ were found to be significant at P<0.05. Hence, they were retained in the reduced model.

The reduced model for tensile strength was:

$$\text{Tensile Strength} = +12.54 + 0.50 * X_1 + 0.80 * X_2$$

Full and reduced model for disintegration time of enalapril maleate

The contour plot and 3D response surface graph for Disintegration time was observed in fig. 3 and fig. 5 respectively and revealed that a corresponding decrease in the disintegration time of tablet was observed with increase in concentrations of HPMC E 15. Moreover, the regression coefficient values of both factors can be concluded that the disintegration time appeared to decrease more with an increasing amount of the HPMC E 15 and decreasing the amount of Polyethylene glycol. Interaction and non-linearity was not observed.

For disintegration time, the significant levels of the co-efficients b₂, b₁₁ and b₂₂ were found to have P value of 0.0668, 0.0904 and 0.5357. So, it was omitted from the full model to generate a reduced model. The coefficients b₀, b₁ and b₁₂ were found to be significant at P<0.05. Hence, they were retained in the reduced model.

The reduced model for Disintegration time was:

$$\text{Disintegration time} = +57.00 + 4.50 * X_1 - 4.50 * X_2^2$$

Full and reduced model for % CDR at 10 min. of Enalapril maleate

The contour plot and 3D response surface graph for % CDR at 10 min. was observed in fig. 3 and fig. 6 respectively and revealed that a corresponding decrease in the % drug release of tablet was observed with increase in concentrations of Polyethylene glycol and decrease in concentration of HPMC E 15. Interaction and non-linearity was not observed.

For disintegration time, the significant levels of the coefficients b₁, b₂ and b₁₂ were found to have P value of 0.0554, 0.1706 and 0.5568. So, it was omitted from the full model to generate a reduced model. The coefficients b₀, b₁₁ and b₂₂ were found to be significant at P<0.05. Hence, they were retained in the reduced model.

The reduced model for % CDR was:

$$\% \text{CDR} = +95.64 - 1.57 * X_1^2 + 2.13 * X_2^2$$

Validation by check point batch

A check point batch was prepared to confirm the validity of response surface plot and equation generated by multiple regression analysis which was shown in table 10. An overlay plot was obtained by adding desired range of evaluation parameters from Design Expert 9. The overlay plot is shown in fig. 7. Yellow color area in overlay plot showed optimum concentration range for desired result. A batch was prepared by taking concentration of HPMC E 15 (X₁) and concentration of Polyethylene glycol (X₂) observed in overlay plot and the actual responses were evaluated from the prepared check point batch. The overlay plot indicated that optimum concentration which showed the best result. The practically obtained values were closer to the predicted values as shown in table 11. Thus, it justified the validation of design.

Accelerated stability study

The stability study indicated that the optimized formula was physically and chemically stable with no significant changes in any of the evaluated parameters when stored at the 40 °C and at 75%±5 RH conditions. From stability studies it was concluded that the sublingual films of Enalapril maleate was stable.

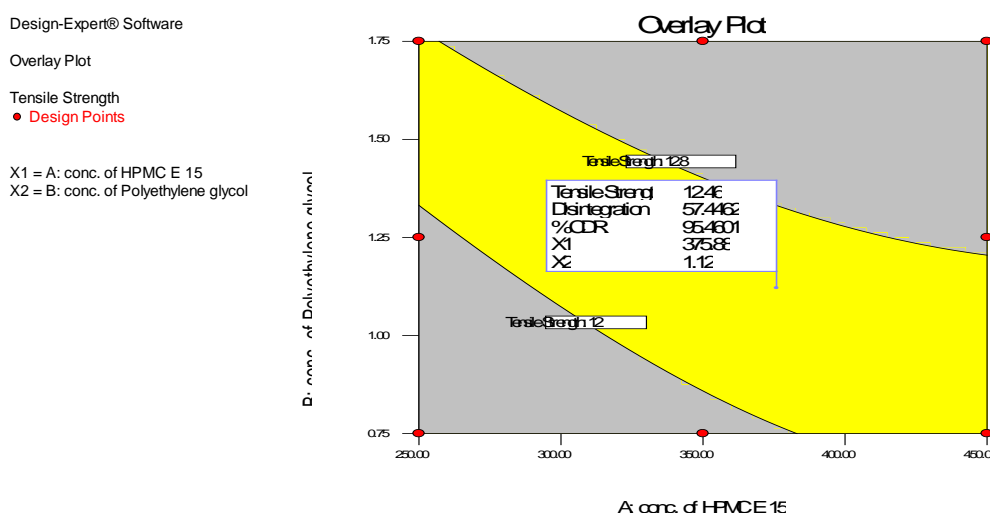


Fig. 7: Overlay plot of check point batch

Table 8: Result of short term stability study of optimized batch

Evaluation parameters	Before stability period	After stability period
Tensile Strength (kg/cm ²)	13.5±0.80	13.1±0.60
Disintegration time(sec)	53±0.50	56±0.80
% CDR at 10 min.	98.6	98.20

*Data represented in mean±SD (n=3)

The results of 3² full factorial design were analyzed. The utility of this statistical design resulted in providing considerable information to optimize the formulation. All the responses were fitted to a quadratic model and compatibility of the model was verified by ANOVA, lack of fit and co-efficient of determination (R²). Surface pH varies in the range of 6.6±0.04 to 6.9±0.02 the formulation (F₈) shows pH range 6.8±0.050. The value was nearest to the saliva pH (i.e. 6.8) there was no irritation produced during the administration of films. Folding endurance for all the formulation was found to be more than 100 folds. It was revealed that all formulation showed good film properties. In the present study, the following constraints were arbitrarily used for the selection of an optimized batch: DT<53 sec, Tensile Strength 13.5 kg/cm² and %CDR>98.6. Batches F₁, F₂, F₇ and F₈ met the selection criteria. Batch F₈ showed the highest % cumulative drug release (98.6) at 10 min. Also, on the basis of Desirability approach, formulation containing HPMC E15 and Polyethylene Glycol in the amount of 450 mg and 0.75 %/batch was selected as an optimized batch. From the *in vitro* study, it was found that the developed formulation provided fast release of the drug at 10 min. by formulating in the form of sublingual Enalapril maleate films. Thus, Batch F₈ was selected as an optimized batch. The optimized formulation was subjected to accelerated stability study.

As reported in previous studies by Harshada Deoghare *et al.*, prepared fast dissolving films of enalapril maleate containing HPMC K15 polymer showed 97.5 % after 25 min. When it was compared with our present study, then drug release showed 98.6 % which is higher than Harshada Deoghare *et al.* study. Also, in present study it was observed that drug was released at 10 min. which showed faster onset of action compared to Harshada Deoghare *et al.* study [29].

Also reported in previous studies by Irene Thomas *et al.* developed the formulation of fast dissolving buccal films of Enalapril maleate using hydroxyl propyl methyl cellulose as polymer which showed 99.8 % release of drug at 10 min. It is found to be same as compared to our present study but it is known to us that sublingual route of administration is preferred as compared to buccal route of administration [30].

CONCLUSION

The sublingual films of Enalapril maleate were successfully prepared, optimized and evaluated using Design Expert software by 3² full factorial design. The present investigation showed quick disintegration and fast release of the drug for rapid management of Hypertension. HPMC E15 and Polyethylene Glycol were used as film forming polymer that showed rapidly disintegration time of film in saliva fluid. These formulations were evaluated for the parameters like drug excipient compatibility study, uniformity of weight, thickness, tensile strength, content uniformity, folding endurance, *in vitro* drug release and accelerated stability studies. On the basis of preliminary results, the amount of HPMC E 15 (X₁) and the amount of Polyethylene Glycol (X₂) were selected as independent variables in 3² full factorial design, while Tensile strength (TS), Disintegration time (DT) and Cumulative % drug release at 10 min. (%CDR) were taken as dependent variables. Multiple linear regression analysis, ANOVA and graphical representation of the influence of factor by contour plots and 3D response surface graphs were performed using Demo version of Design Expert. Check point batch was prepared to validate the evolved model. Batch F₈ was selected as an optimized batch. The optimized formulation was subjected to accelerated stability study. The optimized batch F₈ was found to be stable in the stability evaluation. Also, when the studies of Harshada Deoghare *et al.* and Irene Thomas *et al.* were compared with our present study then it was concluded that our present study gives better promising action during the management of hypertension.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Authors declare no conflict of interest.

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