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Original Article

IMPLEMENTING CENTRAL COMPOSITE DESIGN FOR THE DEVELOPMENT OF TACROLIMUS FILM FOR SUBLINGUAL ADMINISTRATION

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ABSTRACT

Objective: The study aimed to develop fast-dissolving films (FDFs) of the immunosuppressant drug tacrolimus monohydrate for sublingual administration, employing central composite design (CCD), to improve its bioavailability.

Methods: Tacrolimus: β-cyclodextrin inclusion complexes prepared earlier were formulated into FDFs. CCD was used for developing optimal film formulation with the desired characteristics. The solvent casting method was used for the preparation of films. For optimization, the independent variables selected were the concentration of hydroxy propyl methyl cellulose E5 (HPMC E5) (X_1) and concentration of croscarmellose sodium (CCS) (X_2) and the responses were disintegration time (Y_1) and percentage drug release at 5 min (Y_2). The suggested optimal films were subjected to further characterization.

Results: All the formulations showed good mechanical properties. The composition of optimized FDF constituted 3.016% w/v of HPMC and 11.731%w/w of CCS and its average disintegration time was 27.28s and showed 83.13% mean drug release at 5 min. Differential Scanning calorimetry (DSC) analysis showed complete dispersion and partial conversion into the amorphous form of the drug, which was also confirmed by X-ray diffraction (XRD) studies. Scanning Electron Microscopy (SEM) revealed the smooth and porous nature of the film.

Conclusion: The developed FDF may be used sublingually for delivering tacrolimus efficiently, avoiding its oral bioavailability problems.

Keywords: Tacrolimus, Croscarmellose sodium, Sublingual, Fast dissolving films, Central composite design

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INTRODUCTION

The sublingual route of administration has the advantage of bypassing first-pass metabolism and thereby increasing the bioavailability of drugs. Also, the sublingual mucosa is having relatively increased permeability due to the thin membrane and supply of vascular and lymphatic drainage. Additionally, reduced drug dosage to achieve similar target levels compared with oral administration may result in lower medical costs and patient compliance will be more especially in patients facing difficulty in swallowing and chewing [1, 2].

The commonly used sublingual dosage forms are orodispersible films and tablets. As the fast-dissolving film (FDF) is administered through the sublingual route, rapid absorption of the drug is possible, which finally leads to the quick onset of drug action [3]. They are taken without water and due to their ability to disintegrate within a few minutes, they quickly release medication in the mouth [4, 5]. Various superdisintegrants are also employed in the formulation of fast-dissolving films [6].

Tacrolimus is an immunosuppressant having poor and erratic bioavailability mainly due to low solubility, the influence of food intake and concomitant medication, and heapto first-pass metabolism. Tacrolimus has played a key role in today's high success rate of solid organ transplantation. A variety of clinical situations requiring nonoral medication delivery arise, presenting the need for reliable alternative routes of tacrolimus administration. Many clinical studies suggest the use of sublingual administration of tacrolimus producing comparable blood-drug levels with similar or even lower doses than the oral route in the lung as well as liver transplant recipients [7–9].

Response surface methodology (RSM) is an efficient technique for formulation optimization by efficiently exploring the relationships between the investigated factors and measured responses using the minimum number of experimental trials. Central composite design (CCD) is one of the most pertinent designs of RSM, which consists of factorial and axial points as well as one point at least at the center of the experimental region that provides properties like orthogonality and rotatability for fitting quadratic polynomials. This diversity of points is useful for providing complete knowledge of responses using the least number of experiments [10].

In the present work, an attempt was made to formulate tacrolimus into FDF formulation for sublingual application. CCD was employed to analyze the effect of the independent variables on the selected responses and to suggest the optimized production parameters for attaining a film formulation with desirable responses.

MATERIALS AND METHODS

Materials

Tacrolimus monohydrate was purchased from Yarrowchem, Mumbai, India. Hydroxy propyl methyl cellulose E5 (HPMC E5) and saccharine sodium were procured from Loba Chemie, Mumbai, India, croscarmellose sodium (CCS) from Astron Chemicals, Ahmedabad, India, and Menthol from the Sisco Research Laboratories, India. Polyethylene glycol 400 (PEG 400) and methanol were purchased from Kanton Laboratories, India.

Methods

Formulation of sublingual ODFs

To increase the solubility of tacrolimus, it was converted to an inclusion complex with β-cyclodextrin as the carrier by the kneading method. The drug and the carrier were triturated with a mixed solution of 1:1 (v/v) methanol: water in a glass mortar to obtain a thin slurry. The slurry was kneaded thoroughly for around 40 min. After the evaporation of the solvents, the complexes were dried at room temperature for a period of 1 d [11].

FDF formulations loaded with β-cyclodextrin inclusion complex of tacrolimus monohydrate were formulated with HPMC E5 as the film-forming polymer, PEG 400 as the plasticizer, and CCS as the superdisintegrant [12]. Menthol was used as the flavouring agent to give a mouth refreshment feeling and saccharine sodium was used as the sweetening agent.

The films were prepared by solvent casting method. An accurately weighed quantity of HPMC E5 was soaked in distilled water for 30 min for proper dispersion of the polymer. Added PEG 400, inclusion complex of the drug and other excipients, and the final volume was adjusted with methanol. The dispersion was sonicated to remove the air-entrapped bubbles.

The casting solution (10 ml) was poured into a glass mold (area =32 cm^2, every 2 cm^2 contains 1 mg of drug) and the solvent was allowed to evaporate by placing the glass mold in an oven for 24 h. The dried films were stored in a desiccator at room temperature.

Experimental design using CCD

CCD was used for the formulation and optimization of FDF using design expert software (version 13, Statease Inc, Minneapolis, USA) to investigate the influence of two independent variables i.e., the concentration of HPMC E5 (X_1) and concentration of CCS (X_2) on two responses, disintegration time (Y_1) and *in vitro* percentage drug release at 5 min (Y_2). A total of thirteen experimental runs were generated by the software, including 5 replicates of the center points, 4 axial, and 4 factorial points, and were carried out in randomized order. The different levels of variables are presented in table 1 and the actual composition of each formulation batch (F1-F13) is given in table 2.

Table 1: Variables used in the CCD

	Levels				
	-α	**-1**	**0**	**+1**	**+α**
Independent variables					
X_1= Conc. of HPMC E5(%w/v)	2.58579	3	4	5	5.41421
X_2= Conc. of CCS (%w/w)	6.75736	8	11	14	15.2426
Dependent variables Desirability constraints					
Y_1= Disintegration time (s)			Minimise		
Y_2= *In vitro* drug release at 5 min (%)			Maximise		

Table 2: Composition of different batches of formulations*

Run	Batch	HPMC E5 (%w/v)	CCS (%w/w)	PEG 400 (%v/v)	Menthol (mg)	Saccharine sodium (mg)	Distilled water (ml)	Methanol q. s. (ml)
1	F1	3	8	2	100	80	3	10
2	F2	4	11	2	100	80	3	10
3	F3	3	14	2	100	80	3	10
4	F4	5	8	2	100	80	3	10
5	F5	4	6.75736	2	100	80	3	10
6	F6	4	11	2	100	80	3	10
7	F7	2.58579	11	2	100	80	3	10
8	F8	4	15.2426	2	100	80	3	10
9	F9	4	11	2	100	80	3	10
10	F10	4	11	2	100	80	3	10
11	F11	5	14	2	100	80	3	10
12	F12	4	11	2	100	80	3	10
13	F13	5.41421	11	2	100	80	3	10

*note: the dose of the drug-β-cyclodextrin inclusion complex was calculated to contain 1 mg of the drug in a single film of 2 cm^2 area

Evaluation of sublingual films

Physical and mechanical properties

The prepared films were evaluated for physical characteristics, weight uniformity, thickness, and folding endurance.

The prepared films were tested visually for their physical appearance and surface texture as well as for any drug precipitation and air bubble entrapment. Weight variation was calculated by weighing the film formulations in electronic analytical balance. The films of 2 × 1 cm^2 size were cut from three different positions of each batch prepared and analyzed. The film thickness was measured using a micrometer screw gauge [13]. Thickness was measured at different locations of a film and the average value was determined. Folding endurance was measured manually by repeated folding of the films at the same place till they broke. The folding endurance value is the number of times the film is folded without breaking [14, 15]. All the evaluations were done in triplicate and the results were expressed as average values and SD.

Surface pH

The surface pH of the film and the sublingual p^H (6.8) should be closer so that the film dissolves quickly and be compatible. The surface pH is determined to evaluate the possible irritative effects of the formulations on the mucosae.

The film was placed in a Petri dish and was moistened with 1 ml of distilled water and kept for 30 seconds. The p^H was noted after bringing the electrode of the p^H meter in contact with the surface of the formulation and allowing equilibration for 1 min [16, 17].

Drug content uniformity

The film was placed in a 100 ml volumetric flask and then dissolved in 50 ml methanol using a magnetic stirrer. The volume was completed with methanol and the solution was filtered and drug content was estimated by HPLC (instrument model: HPLC Agilent 1260 infinity).

The estimation of tacrolimus in the samples was done by the HPLC method according to USP. The mobile phase was constituted from different compositions of 6 mmol phosphoric acid and acetonitrile and tertiary butyl methyl ether (81:19). The diluent used was acetonitrile and water (7:3). The column employed was of dimension 15 cm X 4.6 mm and the column temperature, 60 °C. 20 µl of injection volumes were used and the eluents were examined at 220 nm by a stream rate of 1.5 ml/minute [16, 18].

***In vitro* disintegration time**

No official guidelines are available for the determination of *in vitro* disintegration time of sublingual films. So, a non-official method was adopted. The disintegration test was performed by placing a film of

size 2 × 1 cm^2 in a glass Petri dish containing 10 ml of phosphate buffer pH 6.8. The time required for the film to disintegrate was recorded [14, 19].

***In vitro* drug release studies**

The drug release study from the prepared FDFs was performed in beakers, each containing 100 ml of phosphate buffer at pH 6.8 [4, 14]. The beakers were placed in a shaking water bath set at 37±0.5 °C and 100 rpm. Samples were removed at predetermined time intervals and filtered through a Whatman filter paper and the drug content of sample solutions was determined after suitable dilutions by HPLC. The aliquots withdrawn were replaced with fresh medium to maintain sink condition.

The determinations were conducted in triplicates. The amount of drug released at each time interval as well as the cumulative amount of drug released, was calculated as a function of time and the drug release profile graphs were constructed.

Statistical analysis

The experimental data obtained from the evaluations of all the film formulations (13 batches) were analyzed using Design Expert software. The optimum concentration of the independent variables, concentrations of HPMC E5 and CCS, was chosen based on the condition of the responses in obtaining maximum *in vitro* drug release at 5 min and minimum disintegration time. The response surface behavior was investigated for the response function (Y) employing the polynomial equation (1) and the generalized response surface model;

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_{12}+\beta_{22}X_{22}+\beta_{12}X_1X_2 \ldots. (1)$$

Where Y is the predicted response; β_0 is constant; β_1 and β_2 are the linear and quadratic coefficients and β_{11}, β_{22}, and β_{12} are the interaction coefficients.

The analysis of variance (ANOVA) was used to identify significant effects of factors on response regression coefficients. All significant independent variable effects ($p<0.05$) were included in the reduced model. To visualize the interaction effect of the variables on the responses, three-dimensional (3D) response surface plots were composed.

With the application of desirability constraints on *in vitro* drug release at 5 min and disintegration time, a numerical optimization analysis was then employed by Design-Expert® software to develop the optimal ODF formulation with the desired responses. The disintegration time and drug release studies were carried out on the prepared optimized formulation to verify the theoretical prediction. The relative errors (%) between the predicted and observed values for each response were calculated [20].

Differential scanning calorimetry (DSC)

The prepared optimized formulation was subjected to DSC analysis (instrument model Q20 TA instruments) and compared with the thermogram of pure tacrolimus. Film strips weighing 10–12 mg were hermetically sealed in a standard aluminium pan and were heated at a scanning rate of 10 °C/min from room temperature to 225 °C under nitrogen gas flow.

X-ray diffraction (XRD) analysis

The roentgen graphic analyses were conducted with bruker model D8 Advance X-ray diffract meter (The samples were analyzed utilizing monochromatic Cu K-radiation at 40 mA and 40 kV within the region of $2.5° \leq 2\theta \leq 40°$ at 25 °C in continuous scan mode with a step size of 0.02°. The diffractogram of the film was compared to the pure tacrolimus.

Surface morphology by scanning electron microscopy (SEM)

SEM studies of optimized FDF and that of pure tacrolimus were done with the Scanning Electron Microscope (JOEL JSM-6390; JAPAN), samples were mounted with double-sided carbon tape on aluminium stubs, and all the samples were sputtered with a thin layer of gold in auto fine coater JEOL JFC 1600 and the images were examined under vacuum at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Physical and mechanical properties

FDFs should possess suitable mechanical strength to resist handling during processing, packing, and transit without being damaged.

All the films were smooth, transparent, homogeneous, and flexible. The results of evaluations of weight variation, film thickness, folding endurance, surface p^H, and drug content uniformity are presented in table 3. The weight variation was found to be acceptable as indicated by the small standard deviations. This shows the uniform distribution of the ingredients in the prepared films. All the films were of uniform thickness, in the range of 0.090 to 0.113 mm. Folding endurance is used to estimate the mechanical properties of a film and all the batches showed satisfactory flexibility. The surface p^H of all the formulations was in the sublingual p^H range; therefore, no possibility of irritation may be expected when administered sublingually and thus, patient acceptance will not be affected. The drug content of the ODFs was in the range of 95.71–99.12%, implying uniform distribution of the drug in the films.

Table 3: Evaluation of weight variation, film thickness, folding endurance, surface pH, and drug content uniformity of films

Batch	Weight variation(mg)	Film thickness (mm)	Folding endurance	Surface p^H	Drug content uniformity (%)
F1	19.77±1.2494	0.097±0.0058	30±3.5498	6.89±0.0582	98.16±0.9301
F2	23.10±2.7118	0.107±0.0058	33±5.4654	6.64±0.0747	97.89±1.5396
F3	20.56±0.7409	0.010±0.0100	28±3.8173	6.71±0.0151	97.66±0.7723
F4	27.67±1.3255	0.113±0.0058	38±4.8875	6.65±0.0473	96.82±1.8630
F5	22.58±0.9754	0.103±0.0058	32±7.5878	6.85±0.0354	98.47±1.0792
F6	23.14±1.3230	0.107±0.0058	28±2.5473	6.72±0.0620	95.91±0.8334
F7	18.98±2.1204	0.090±0.0100	25±10.554	6.69±0.0645	99.12±1.0414
F8	24.43±1.1412	0.107±0.0058	28±6.4979	6.73±0.0745	98.44±1.4462
F9	23.87±1.4390	0.107±0.0058	30±5.3556	6.67±0.0954	98.48±0.7456
F10	24.91±1.2871	0.107±0.0058	29±7.9854	6.76±0.0453	97.65±2.0165
F11	26.24±2.4746	0.113±0.0058	39±9.2591	6.81±0.0980	98.44±1.4166
F12	23.31±1.2146	0.107±0.0058	34±10.655	6.64±0.0655	95.67±0.8747
F13	28.82±1.6756	0.113±0.0058	38±4.9645	6.84±0.0873	96.96±1.6874

Data are expressed as mean±SD, n=3

***In vitro* disintegration time**

The basic requisite of FDF is its short disintegration time in the saliva when placed in the mouth. But none of the official guidelines states the acceptable range of disintegration time. In the present study, almost all the batches have shown a disintegration time of fewer than 60 seconds, and the results obtained are presented in table 5. This response was selected as one of the independent variables, X_1, for the optimization of the formulation.

***In vitro* drug release studies**

No official compendial methods have been reported for release studies of FDF dosage forms. The literature reports various

approaches, including the use of different volumes from 5 to 900 ml, different apparatus (pharmacopoeial or custom-made), and different media [19].

A fast release of the drug from the sublingual film is necessary so that the dose is not swallowed into the stomach. Moreover, since tacrolimus is a BCS class II drug, once the drug is released into the sublingual mucosa it will be rapidly absorbed and the higher permeability of the sublingual mucosa also aids the absorption process. So, the greater the drug release, the better we can expect bioavailability.

The cumulative percentage of drug release from all the prepared batches is shown in fig. 1a, 1b, and 1c. For all the batches, more than 95% of drug release was obtained in 30 min and almost 80% of drug content was released at around 5 min and this % drug release at 5 min was selected as one of the independent variables (X_2) for the optimization of the formulation.

The fast drug release from the film can be attributed to the hydrophilic nature of the used polymer, HPMCE5, which absorbs water, dissolves rapidly and, thus introducing porosity into the film. The external solvent then diffuses into the film, thereby accelerating the drug dissolution [21].

Sublingual administration of tacrolimus FDF is expected to improve the drug's bioavailability by bypassing first-pass hepatic metabolism. In addition, the inclusion of the drug into β-cyclodextrin complexes before its formulation as a film enhances the drug's solubility too.

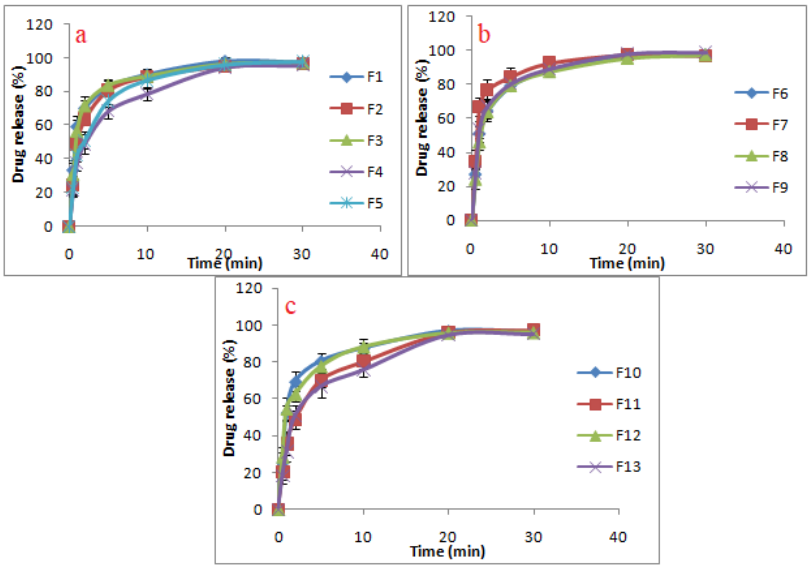

Fig. 1: *In vitro* drug release profiles of sublingual films a) from batches F1-F5 b) from batches F6-F9 and 1c) from batches F10-F13 (mean±SD, n=3)

Table 4: ANOVA analysis of responses

Variables	Disintegration time (s)		*In vitro* drug release at 5 min (%)	
	Regression coefficients	P-value	Regression coefficients	P-value
Intercept	37.82	<0.0001	79.62	<0.0001
X_1-HPMC E5	11.08	<0.0001	-6.68	<0.0001
X_2-CCS	-5.56	<0.0001	1.19	0.0232
$X_1 X_2$	-5.94	<0.0001	0.1000	0.8682
X_1^2	-1.39	0.0089	-2.03	0.0025
X_2^2	2.57	0.0003	-1.45	0.0131
R^2	0.9949		0.9773	
Adjusted R^2	0.9913		0.9611	
Predicted R^2	0.9828		0.9252	
Std. Dev.	1.02		1.16	
CV%	2.65		1.50	

Statistical analysis

Based on CCD analysis, the effect of concentration of HPMC E5 (X1) and concentration of CCS (X2) on two responses disintegration time (Y1) and percentage drug release at 5 min (Y2) was studied and evaluated the main effects, interaction effects, and quadratic effects of the process variables on selected responses.

Fitting model to the data

The Design expert software suggested a quadratic model fitting best for both responses (disintegration time and percentage drug release at 5 min) as this model maximized adjusted R^2 and the predicted R^2 values.

ANOVA analysis

Analysis of variance (ANOVA) was performed to assess the significance of the quadratic polynomial models developed. The regression coefficients and p-values obtained are given in table 4. The small p-value ($p<0.05$) of the terms in the models indicated a significant influence on the response variables.

Sufficiency of models

Diagnostic plots (such as predicted vs. actual) are useful graphic tools to observe the adequacy of developed models. It shows the relationship between predicted and experimental values and describes model suitability. Table 5 shows the predicted and

experimental values of disintegration time and *in vitro* drug release of the films and fig. 2a and 2b show the predicted vs. actual plots for all responses. The closer the points are to the diagonal line, the better the model fits the experimental data [22].

Table 5: Predicted and experimental values of disintegration time and *in vitro* drug release of the films

Batch	Independent variables		Response variables			
	X_1	X_2	Y_1		Y_2	
			Actual	Predicted	Actual	Predicted
F1	3	8	27.53	27.02	82.60	81.73
F2	4	11	39.20	37.82	80.80	79.62
F3	3	14	28.64	28.30	83.90	83.90
F4	5	8	62.14	63.84	68.20	68.17
F5	4	6.75736	50.60	50.81	74.40	75.03
F6	4	11	37.38	37.82	78.80	79.62
F7	2.58579	11	19.31	19.37	84.40	85.01
F8	4	15.2426	34.42	35.10	79.00	78.39
F9	4	11	36.34	37.82	80.20	79.62
F10	4	11	37.61	37.82	80.50	79.62
F11	5	14	39.47	38.58	69.90	70.75
F12	4	11	38.56	37.82	77.80	79.62
F13	5.41421	11	49.88	50.72	66.70	66.12

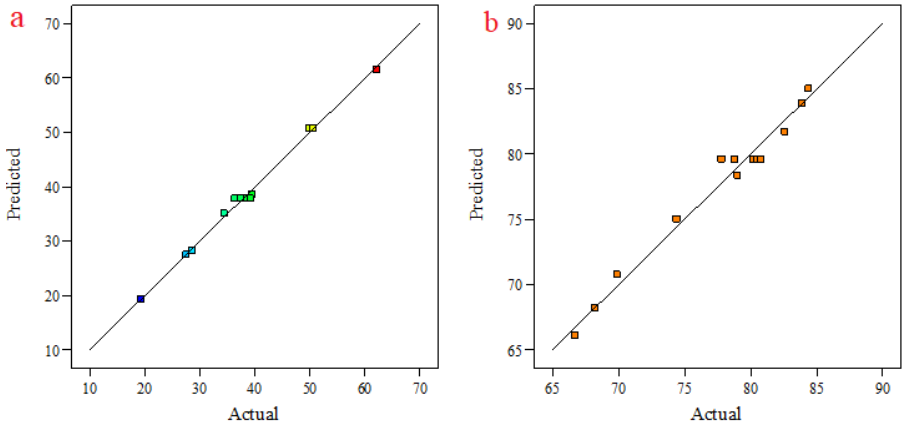

Fig. 2: Predicted and experimental values of a) disintegration time and b) drug release

Effect of factors on responses

The 3D response surface plots were used to study the effects of independent variables on responses. These plots are known to study the interaction effects of the factors on the responses [23]. The 3D graphs for both responses are given in fig. 3a and 3b

Effect on disintegration time (Y1)

The following polynomial equation was proposed for the disintegration time of the FDFs:

$37.818+11.0841X_1-5.55525\ X_2-5.945X_1\ X_2-1.38838X_1^2+2.56912\ X_2^2$

The Model F-value of 275.76 implies the model is significant. P-values less than 0.05 indicate model terms are significant. In this case X_1, X_2, X_1X_2, X_1^2, X_2^2 are significant model terms. The Lack of Fit F-value of 0.66 implies the Lack of Fit is not significant.

Fig. 3a shows the response surface plot of the effect of different independent variables on the disintegration time of the FDFs. From the figure, it can be seen that the disintegration time (Y1) was prolonged with the increase in the concentration of film-forming polymer, HPMC E5 (X_1)because, with an increase in the amount used in the formulation, the viscous gel produced after the film's swelling in water and subsequent retardation of penetration of water resulted in the delay of disintegration. With the increase of the super disintegrant (CCS) concentration, there were changing tendencies in the disintegration time which first descended and then ascended. The reasons might be that CCS was swelled in water due to its good water uptake ability, which is beneficial to disintegration, but an excess quantity would lead to high viscosity with swelling, which would suppress the water penetration.

Effect on *in vitro* drug release at 5 min (Y2)

The polynomial equation proposed for the % *in vitro* drug release at 5 min was:

$79.62-6.67895X_1+1.18817X_2+0.1X_1X_2-2.02875X_1^2-1.45375X_2^2$

The Model F-value was 60.31 implies that the model is significant. In this case, the significant model terms are X_1, X_2, X_1^2, and $X_2^2$and their P-values were less than 0.05. The Lack of Fit F-value of 0.61 implies the Lack of Fit is not significant.

Fig. 3b is the 3-D surface plot of the effect of the two independent variables on the cumulative % release of the drug from the films within 5 min. Response surface 3D plot reveals that *in vitro* drug release at 5 min (Y2) significantly decreases with the increase in HPMC (X1) concentration. On contact with the release medium, the viscous nature of the film-forming polymer (HPMC) creates thick matrix gel fluid, which governs drug release by resisting drug diffusion or matrix erosion and may lead to a delay in the drug release from the films. Another reason contributing to decreased drug release at increased polymer concentration may be the increase in the time required for wetting and dissolving the drug molecules present in the polymer matrices [15].

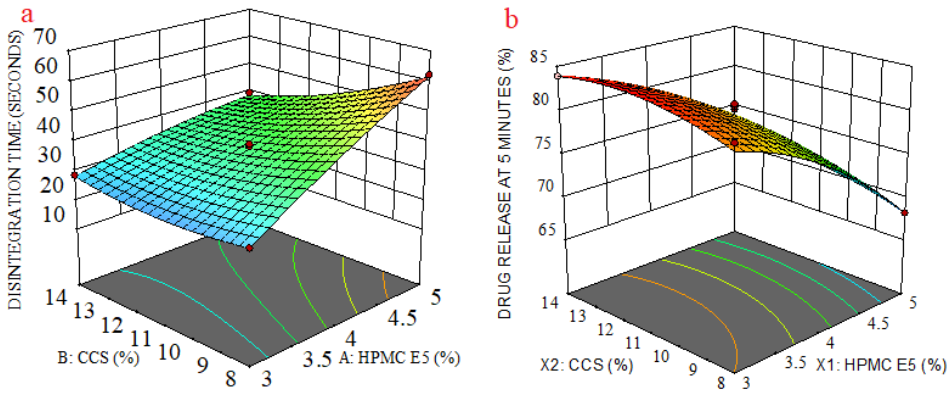

Fig. 3: 3D response surface plots of FDFS for the effect of independent variables on a) disintegration time and b) drug release at 5 min

Selection of optimum formulation

The optimized formulation was selected with the following criteria: minimum disintegration time and the maximum cumulative drug release of the FDFs and from among the independent variables' concentration solutions suggested by the software, the following composition was selected for the preparation of the optimal formulation: 3.016% w/v of HPMC E5 (X_1) and 11.731%w/w CCS (X_2) with the desirability of 1. With these values, 25.79 sec for disintegration time (Y_1) and 84.41% for *in vitro* drug release at 5 min (Y_2) were the responses predicted.

Validation of optimum formulation

To validate these predicted values generated according to the results of the CCD design, the optimum formulation batches were prepared according to the optimized concentrations of the factors HPMC E5 and CCS and subjected to the disintegration and drug release tests. The data means of 27.7 sec for disintegration time and 83.13% for *in vitro* drug release at 5 min were obtained. These differences in the film's responses were not significantly deviated from the predicted responses of the optimized formula (table 6). From the confirmational analysis, it was shown that the above data means were within the range of 95% low and high prediction intervals. The results indicate the effective use of DOE for investigating variables' effects and optimization.

DSC analysis of optimized FDF

DSC thermograms of pure tacrolimus and optimized film are shown in fig. 4. DSC of pure tacrolimus showed a broad endothermic event attributed to dehydration happening at temperatures between 75 °C and 122 °C followed by melting of the anhydrate form with a sharp endothermic peak at 129.46 °C which corresponds to its melting point and indicating its crystalline nature. The optimized film formula showed the disappearance of the characteristic peak of tacrolimus which might be due to the homogenous dispersion of the drug in the formed film.

Table 6: Comparison of predicted and observed response values of FDFs prepared at optimal conditions

Response	Predicted values	Observed values	Prediction error (%)
Disintegration time (sec)	25.79	27.7	-6.9
In vitro drug release at 5 min (%)	84.41	83.13	1.54

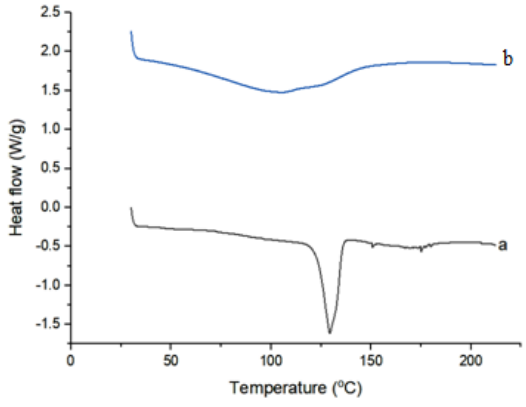

Fig. 4: DSC thermogram of a) tacrolimus monohydrate b) optimized film

XRD

To obtain further information on the solid-state changes, XRD spectra were carried out on the optimized FDF formulation. The diffractograms of the drug and the optimized films are shown in fig. 5. The presence of numerous distinct peaks in the spectrum of tacrolimus indicates that the drug is present as a crystalline material. The FDF formulation revealed a decrease in the intensity of diffraction peaks, indicating that the drug completely dispersed in the film matrix. This corresponds with the results obtained from DSC testing. Apart from the reduction of peak intensities, they were significantly broadened, which indicated that due to the inclusion complex formation, the drug was converted to a partial amorphous form.

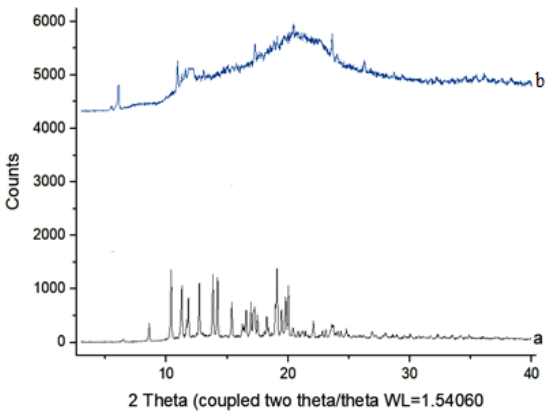

Fig. 5: XRD pattern of a) tacrolimus monohydrate b) optimized film

SEM

The SEM image of the pure drug as well as the morphology of the optimized FDF formula, is given in fig. 6a and 6b. From fig. 6a, it was found that tacrolimus occurred as cuboidal-shaped crystals, and the SEM image of the film revealed a smooth, slightly porous surface, indicating complete and uniform embedment of the drug: β-cyclodextrin complexes in the polymeric film matrix. The porous nature indicates a uniform distribution of the drug complexes that help the medium to get penetrate inside the film. Very few drug crystals were found in the film, probably formed during the drying of the film.

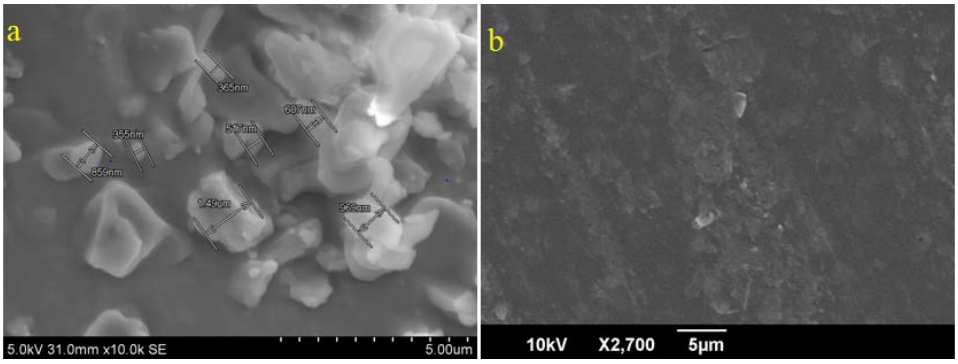

Fig. 6: Scanning electron micrograph of a) tacrolimus monohydrate, b) optimized film (magnification 2700×)

CONCLUSION

In the present work, FDF formulations containing tacrolimus-β-cyclodextrin inclusion complexes for sublingual application were developed and optimized employing the central composite statistical design. The multiple regression analysis of the results provided equations describing the influence of the selected variables on the responses under study. The desirability function led to the optimum values of the factors at which the prepared film showed minimum disintegration time and maximum drug release. DSC evaluation suggested the homogenous dispersion of the drug in the FDFs. The XRD analysis suggested that the drug in the FDF_S was in a partial amorphous state. SEM analysis revealed the smooth and porous nature of the film. Accordingly, it could be concluded that FDFs, optimized using CCD, may provide a novel sublingual delivery system of tacrolimus for avoiding its oral bioavailability problems.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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