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RESEARCH ARTICLE

Central Composite Design for the Development of Trimetazidine Dihydrochloride-Loaded Fast Dissolving Film

Swapnil S. CHOPADE[*°], Mangesh A. PAWAR[**], Popat S. KUMBHAR[***], Arehalli S. MANJAPPA[****], John I. DISOUZA[*****], Santosh A. PAYGHAN[******°], Jagruti L. DESAI[*******]

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SUMMARY

A fast-dissolving dosage form is an approach used to improve therapeutic efficacy and bioavailability by avoiding the first-pass metabolism of the drug carrier. Besides, the approach causes rapid drug absorption from the pre-gastric area which may outcome in the quick inception of action. Trimetazidine dihydrochloride (TDC) is an anti-anginal drug, and there is a prerequisite to provide fast onset of action to treat angina. Therefore, the present work aimed to prepare and evaluate fast-dissolving oral films (FDOFs) of TDC to provide fast onset of action. The FDOF is prepared by using the solvent casting method, and it was optimized by employing a central composite statistical design (CCD). The two independent variables such as HPMC K4M (X1) and PEG 400 (X2) are the film-forming polymers that are evaluated at three levels. The dependent variables such as folding endurance (Y1), disintegration time (Y2), and % drug release (Y3). The formulation was prepared and optimized. The batch F-4 showed the least disintegration time (19 s) and the highest drug release (98.55±7.90%). Moreover, the ex-vivo mucus permeation study disclosed better permeation and satisfying physicochemical properties compared to plain drug solution. It was concluded that the prepared formulation could be a novel dosage form to improve drug delivery and patient compliance.

Key Words: *Anti-anginal, CCD, ex-vivo permeation, fast dissolving oral film, solvent casting method, trimetazidine dihydrochloride.*

Merkezi Kompozit Dizaynı ile Trimetazidin Dihidroklorit Yüklü Hızlı Çözünen Film Geliştirilmesi

ÖZ

Hızlı çözünen dozaj formu, ilk geçiş metabolizmasını elimine ederek ilaç taşıyıcı sistemin terapötik etkinliğini ve biyoyararlanımını geliştirmek amacıyla kullanılan bir yaklaşımdır. Ayrıca, bu yaklaşım etkinin hızlı bir şekilde başlamasıyla sonuçlanabilecek olan mide öncesi bölgeden hızlı ilaç emilimine neden olur. Trimetazidin dihidroklorür (TDC) anti-anjinal bir ilaçtır ve anjina tedavisi için hızlı etki başlangıcı sağlanması bir ön koşuldur. Bu nedenle, mevcut çalışmada hızlı etki başlangıcı sağlamak için TDC'nin hızlı çözünen oral filmlerinin (FDOFs) hazırlanması ve değerlendirilmesi amaçlanmıştır. FDOF, çözeltinin dökülmesi metodu ile hazırlanmıştır ve merkezi kompozit istatistiksel tasarım (CCD) uygulanarak optimize edilmiştir. HPMC (K4M) (X1) ve PEG 400 (X2) gibi iki bağımsız değişken, üç seviyede değerlendirilen film oluşturma polimerleridir. Katlanma sayısı (Y1), dağılma süresi (Y2) ve % ilaç salımı (Y3) bağımlı değişkenlerdir. Formülasyon hazırlanmış ve optimize edilmiştir. Seri F-4 en kısa dağılma süresini (19 s) ve en yüksek ilaç salımını (%98,55±7,90) göstermiştir. Ayrıca, ex-vivo mukus permeasyonu çalışması, boş ilaç çözeltisine kıyasla daha iyi permeasyon ve tatmin edici fizikokimyasal özellikler ortaya koymuştur. Hazırlanan formülasyonun ilaç taşınmasını ve hasta uyumunu geliştirmek için özgün bir dozaj formu olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: *Anti-anjinal, CCD, ex-vivo permeasyon, hızlı çözünen oral film, solvan dökme metodu, trimetazidin dihidroklorür.*

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[*] ORCID: 0000-0001-6173-6343 ,Tatyasaheb Kore College of Pharmacy, Warananagar (MS, India)
[**] ORCID: 0000-0002-0108-3149, Tatyasaheb Kore College of Pharmacy, Warananagar (MS, India)
[***] ORCID: 0000-0002-0108-3149, Tatyasaheb Kore College of Pharmacy, Warananagar (MS, India)
[****] ORCID: 0000-0002-8576-6608, Tatyasaheb Kore College of Pharmacy, Warananagar (MS, India)
[*****] ORCID: 0000-0002-8576-6608, Tatyasaheb Kore College of Pharmacy, Warananagar (MS, India)
[******] ORCID: 0000-0002-0653-6784, Vasantidevi Patil Institute of Pharmacy, Kodoli (MS, India).
[*******] ORCID: 0000-0001-7147-5861, Ramanbhai Patel College of Pharmacy, Gujarat (GJ, India)

[°] Corresponding Author;
1. Dr. Santosh A. Payghan, Email: santos14july@gmail.com
2. Mr. Swapnil S. Chopade, Phone: 02328 223526, Fax: 02328 223501, Email: swapnilchopade.tkcp@gmail.com

INTRODUCTION

Among the numerous routes of drug administration, the oral route is considered the most convenient, and preferred route of administration (Al-Ani, 2019). It contributes to 50-60% of total drug formulations. However, the administration of conventional dosage forms *via* the oral route is allied with various shortcomings like presystemic metabolism, poor absorption, bioavailability, non-localized action, and patient noncompliance etc (Masih A, 2017; Al-Mogheraha, 2020). In addition, the administration of dosage forms especially tablet through oral is challenging (dysphasia; difficulty in swallowing) and is a general problem in all age groups, particularly the elderly and pediatrics, because of physiological changes linked with these patients. Therefore, there is an unmet need to find out alternative oral dosage forms to overcome the aforementioned challenges (Logrippo, 2017; Bharti, 2018).

Fast dissolving oral film (FDOF) can be a suitable dosage form that would overcome the above challenges in oral delivery, and it is gaining more attention in the pharmaceutical industries. FDOF is a thin strip or film which is placed on the tongue where it gets disintegrates and dissolves quickly in the presence of saliva and undergoes rapid absorption through oral mucosa (Bose, 2013). FDOF offers various benefits including its suitability for bedridden, mentally disabled, and emetic patients. Besides, FDOF undergoes rapid dissolution or disintegration in the absence of water within a few seconds only and produces a fast onset of action (Binfeng, 2015; Bharti, 2018; Balaa, 2018). The system avoids degradation in the gastrointestinal tract and the first-pass effect as the drug is directly absorbed into the systemic circulation results in maximum bioavailability of the drug. In addition, another chief advantage of FDOF is in cancer chemotherapy patients immediately treat nausea and vomiting-related side effects of chemotherapy by eliciting rapid onset of action, and improving patient compliance towards therapy. Furthermore, it can produce a local effect in toothaches, oral ulcers, cold sores, or teething thereby reducing the side effects. Also, the FDOF increases patient compliance owing to the ease of administration (Ahn, 2015; Bharti, 2018).

Trimetazidine, also known as 1-[(2, 3, 4-trimethoxyphenyl) methyl] piperazine hydrochloride (TDC), is a medically active antiangiogenic agent used to prevent and treat angina pectoris, as well as ischemia of neuron sensory tissue in Meniere's disease (Chaudhary, 2016). Nevertheless, TDC undergoes rapid systemic metabolism after oral administration in the form of a tablet that causes a decrease in bioavailability. The conventional tablet formulations of TDC available in the market may have a slow onset of action and low bioavailability. As it is an anti-anginal drug therefore fast onset of action and better bioavailability is desired to treat angina efficiently. Previous research studies reported that TDC is given at a dosage of 40 to 60 mg per day, and it is rapidly absorbed and eliminated, with a plasma half-life of $t_{1/2}$ 6.0±1.4 hours and a T max of 1.80±7 hours. Because of the rapid absorption, the plasma level is very low at the time of the next dose, resulting in a large difference in peak and trough plasma levels at a steady state. To achieve this, a 20 mg preparation is given two or three times per day to maintain a relatively constant plasma level (Habib, 2014; Dezsi, 2016; Wang, 2016).

The range of hydrophilic polymers employed in the preparation of FDOF is cellulose derivatives (hydroxypropyl methylcellulose (HPMC), methylcellulose), polyethylene glycol (PEG), pullulan, polyvinylpyrrolidone (PVP), etc. The HPMC and PEG are enormously used polymers owing to their good film-forming properties. In addition, both polymers are widely accepted. Furthermore, they form highly transparent, tough, and flexible films with the aid of aqueous solutions (Zayed, 2019).

Nowadays, quality by design (QbD) is a widely used approach in the optimization of pharmaceutical formulation. The optimization of formulation using a design of experiment (DoE), a part QbD can help

in the reduction of batches, cost, and improvement of overall quality of the formulation (Nair, 2018). Thus, the objective of the present research was to develop and optimize the TDC-loaded FDOF for the treatment of angina pectoris effectively. Further, the main effects, interaction effects, and quadratic effects of the formulation excipients on dependent variables (responses) such as folding endurance, disintegration time, and % drug release were investigated using the design of the experiment (DoE).

MATERIALS AND METHODS

Materials

TDC was provided as a gift sample from, Cipla Pvt. Ltd. Verna, Goa, India. HPMC K4M, Sodium starch glycolate was obtained from S. D. Lab chemical center, Mumbai. Cross povidone, Citric acid, Polyethylene glycol (PEG) 400, Tween 80, and Peppermint oil were obtained from Molychem Industries, Mumbai. All other ingredients were used as film base materials in analytical grade without further modifications.

Methods

Drug-excipients compatibility study

Possible physicochemical interaction of the TDC with polymers and other excipients (1:1 ratios) used in the formulation was investigated to assess the compatibility between them in the FDOFs using Fourier Transform Infrared (FTIR) spectroscopy (Karki, 2016).

TDC-loaded FDOF

The solvent casting method was used to prepare the TDC-loaded FDOF. Briefly, the polymeric solution was prepared by dissolving a specific amount of HPMC K4M in 70% of distilled water with continuous stirring **(Solution A)**. Then the desired amount of TDC, PEG 400, and tween 80 was dissolved in 30% of distilled water **(Solution B)**. Next, solution A was added slowly to solution B with continuous stirring and set sideways for 30 min for defoaming. Finally, after defoamation, the solution was poured into petri plates and dried in a hot air oven at 45^0 C. Then the casted film was cut into a suitable size and shape and characterized (Table 1) (Binfeng, 2015; Balaa, 2018; Verma, 2018).

Table 1. Formulation batches of fast dissolving film of TDC

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
TDC (mg)	20	20	20	20	20	20	20	20	20
HPMC K4M (mg)	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0
PEG 400 (mL)	11	12	13	14	15	16	17	18	19
Crospovidone (mg)	3	3.5	4	4.5	5	5.5	6	6.5	7
Citric acid (mL)	2	2	2	2	2	2	2	2	2
Tween 80 (mL)	2	2	2	2	2	2	2	2	2
Peppermint oil (mL)	2	2	2	2	2	2	2	2	2

* All values in the table are expressed in mg and mL

Optimization of FDOFs by using DoE

Central composite design (CCD)

An experimental design is a concept of the careful balance between several variables affecting in the experiment. To decrease the number of trials and attain the highest amount of information, central composite design (CCD) is applied, for further optimization of design responses, and several batches were prepared by using Design-Expert software Version 12.0. A total of 9 runs were presented including centre points. CCD is a popular experimental design for optimizing process variables and determining regression model equations and operating conditions from eligible experiments. It is based on a multivariate nonlinear model. It can also be utilized to look at the inter-

play of the various parameters that affect the process (Khabade, 2017).

In this study, a 3^2 Central composite statistical design was used. Two independent variables are evaluated in this design, each at three levels. HPMC K4M (X_1) and PEG 400 (X_2) concentrations were chosen as factors (independent variables). As responses (dependent variables), folding endurance (Y_1), disintegration time (Y_2), and percent drug release (Y_3) were chosen. The data was processed with the trial software Design-Expert Version 12.0 and statistically analyzed with ANOVA (analysis of variance). The data was also subjected to a 3-D surface response methodology (RSM), which was used to evaluate the effects of HPMC K4M and PEG 400 on dependent variables. Further, the effects of factors on responses were analyzed using the 2FI statistical model developed by 3^2 CCD for optimization (Maheswari, 2014; Navamanisubramaniana, 2018).

$$\mathbf{Y= b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_{21} + b_5X_{22} + b_{12}X_1X_2 + b_{13}X_1X_3}$$

The dependent variable is Y, the intercept is b_0, and the regression coefficients are b_1, b_2, b_3, b_4, and b_5. Individual effects are X_1 and X_2, quadratic effects are X_{21} and X_{22}, and the interaction effect is X_1X_2. To determine the model's and individual response parameters' significance ($P< 0.05$), a one-way ANOVA was used. The effect of an independent variable on the measured responses was investigated using surface response plots (3-D) and contour plots (2-D) (Patel, 2014; Pethe, 2016).

Table 2. Variables used for the optimization of TDC-loaded FDOF

Factors	Name	Unit	Type	Low Actual	High Actual	Low Coded	Medium Coded	High Coded
X_1	HPMC K4M	Mg	Numeric	10	15	-1	0	1
X_2	PEG 400	Mg	Numeric	10	20	-1	0	1
Responses	**Name**	**Units**	**Remark**	**Analysis**		**Min.**	**Medium**	**Max**
Y_1	Folding Endurance	Numbers	9	Polynomial		77	102.5	128
Y_2	DT	Sec	9	Polynomial		19	34.5	50
Y_3	% Drug release	%	9	Polynomial		74	96.5	97

Evaluation of formulated FDOFs

Appearance

The morphological character was studied by the visual inspection of the film's appearance.

Thickness

A digital vernier caliper was used to measure the thickness of the film in five different locations. Finally, each fast-dissolving film formulation's average thickness and standard deviation were calculated (Balaa, 2018).

Weight variation test

The experiment was carried out on drug-loaded films with a diameter of 2×2 cm^2. The films were weighted individually on a digital balance, and the average weight for each batch was recorded (Patel, 2014; Khabade, 2017).

The pH of surface

The film was placed in a petri dish for examination. Briefly, the film was moistened with 0.5 mL of simulated saliva buffer (pH 6.8) and left for 30 seconds. After, the pH is measured with a digital pH meter by immersing the electrode in the formulation and allowing 1 minute for equilibration. The calculation was repeated three times, determining the average value (Khabade, 2017; Bharti, 2018).

Folding endurance

The film was evaluated for folding endurance. Briefly, the film was folded with a consistent cross-sectional area and thickness before breaking. The number

of times the film could be folded at the same location without breaking was calculated as the folding endurance value. This test ensures the film's tensile strength (Shimoda, 2009; Khabade, 2017; Bharti, 2018).

Drug content and content uniformity

The drug content and content uniformity were calculated by taking a (2×2 cm^2) diameter film from each formulation into a 100 mL volumetric flask and filling it with phosphate buffer at pH 6.6. It was left for 1-2 hours before the drug content was measured using a UV spectrophotometer at 269 nm wavelengths. The experiments were repeated three times, and the average results are shown in Table 3 (Singh, 2005).

Percent elongation (%), and tensile strength

The mechanical properties [% elongation at break (% EB) and tensile strength (TS)] of the film were determined by using the Santam testing machine (STM 20, Iran). The maximum deformation of the film without tearing is expressed in percent EB. The TS results indicated that, the film's optimum capacity to resist stress without tearing. This method involves clamping a film of a particular size (2×2 cm^2) between two clamp levers on the equipment and applying a 2 mm/min extension force to the film. At the time of tearing, the load at failure (F) and final length (L) were measured. The percent EB and TS were eventually determined using the following equations (Deswati, 2016):

% Elongation (cm %) = ((L – L0) * 100) / L0
Where, (L: length, L0: Initial length)
Tensile strength (N/cm^2) = (F * 100) / (t * w)
Where, (F: failure, t: thickness, w: width)

Disintegration time (DT)

The DT limit prescribed for orally disintegrating tablets as per CDERs is 30 seconds or less for FDOFs (fda.gov.2018), while no official rules are described for OFDFs. In this study, a Pharmacopoeial disintegrating test device will be used (Sadhukhan, 2016; Bharti, 2018).

In-vitro drug release study

The *in-vitro* drug release study was conducted in 900 mL simulated saliva (pH 6.8) held at 37 ±0.5^{0}C and stirred at 50 rpm using the USP dissolution test apparatus II (paddle apparatus). The film was cut into a 2 x 2 cm^2 patch and submerged into the dissolving apparatus. 1 mL of aliquot samples was withdrawn at 1, 2, 3, 4, and 5 min time intervals, filtered, and analyzed spectroscopically at 269 nm (Bharti, 2018; Zayed, 2019; Al-Mogheraha, 2020).

Ex-vivo permeation study

The goat buccal mucosa was used as a barrier membrane in this research. A local slaughterhouse provided the buccal pouch of a freshly slaughtered goat animal. The buccal mucosa was cut evenly and excised from the sides. Then, it was immediately washed in isotonic phosphate buffer (pH 6.6) and used.

The *ex-vivo* permeation study of a plain drug, optimized fast dissolving film of TDC and Quicobal® film through an excised layer of goat buccal mucosa was carried out using the Franz diffusion cell. The topside of each formulation's (2×2 cm^2) diameter film under analysis was coated with aluminum foil as a backing membrane and put in intimate contact with the excised goat buccal mucosa. The magnetic bead was mounted in a 30 mL pH 7.4 phosphate buffer-filled receptor compartment. A magnetic stirrer was used to stir the contents of the cells, and a temperature of 37±1°C was maintained during the experiment. The samples were taken at regular intervals, filtered, appropriately diluted, and then spectrophotometrically analyzed at 269 nm and, the percent drug permeated was calculated and plotted against time (Semalty, 2008; Tomar, 2012; Xin, 2014; Chonkar, 2016; Suryawanshi, 2021).

Stability study

Under various environmental conditions, the stability of the formulated FDOFs was investigated and the measurements were done by storing the film under controlled conditions at 40±2 °C and 75±5% RH for three months in a stability chamber according to the ICH guideline (Raza, 2019). During the storage period, various evaluating parameters like thickness; morphological properties, disintegration time,

drug content, surface pH, and dissolution behavior are checked (Dandagi, 2013;Izhar, 2015; Khan, 2016; Bharti, 2018).

RESULTS AND DISCUSSIONS

Drug excipients compatibility study

The FTIR spectra of TDC, HPMC K4, and their physical mixture are shown in Figure 1. In the FTIR spectra of pure TDC, characteristic peaks were observed at 3566 cm^{-1}, 2938 cm^{-1}, and 1500-1600 cm^{-1} which are attributed to O-H stretching, C-H stretching and C-O stretching vibrations respectively. The FTIR of HPMC K4 showed characteristic peaks at 1615, 1465, and 1452 cm^{-1} indicating C=C stretching, C-H bending in alkane and C-H bending in aldehyde respectively. The FTIR of the film displayed characteristic peaks at 3292 cm^{-1}, 1738 $cm^{-1}$and 1047 cm^{-1}, indicating–OH and C-O stretching vibrations of TDC. Besides, the peak at 2928 cm^{-1} indicates the C-H bond of alkane compounds. No significant broadening, loss, or presence of functional peaks was observed in the physical mixture of TDC and HPMC K4M, revealing no interactions (compatibility) between TDC and formulation excipients.

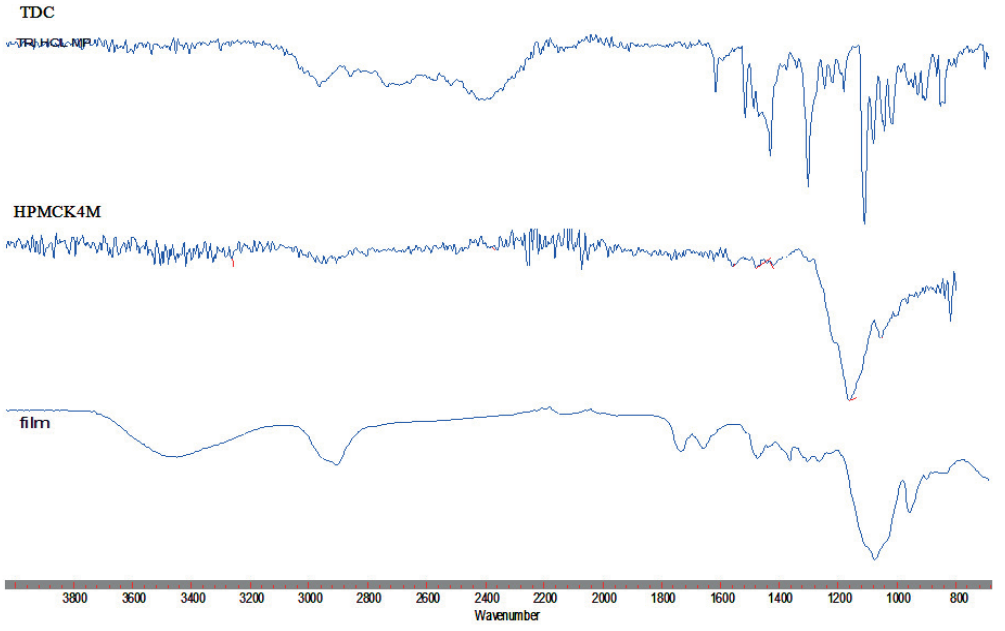

Figure 1. Comparative FTIR spectra of TDC, HPMC K4M, and TDC-loaded fast dissolving film

Optimization of independent variables (Factors)

We have selected the HPMC K4M and PEG 400 as polymers because they are hydrophilic and have good film formation ability. In addition, both polymers are reported to form a highly transparent film. Furthermore, low molecular weight (PEG 400) was selected due to its better dissolution and dispersion ability than high molecular weight PEG polymers. Preliminary study results suggested that 5-30% HPMC K4M and 20-30% PEG 400 cause stickiness in films. However, the concentration of HPMC K4M in prepared films was 10-15% and PEG 400 at 10-20% was non-sticky uniform, and transparent. Based on the results, polymers concentrations (10-15%) and plasticizer concentrations (10-20%) were selected for further formulations. It is also observed that the amount of polymer above 15% showed an increase in DT, below 10% the film did not show flexibility, and above 20% film became sticky.

Optimization of dependent variables (Response)

With the application of the CCD, the (2D contour and 3D RSM) plots were built based on the polynomial functions of the model to evaluate the change in the response surface. These plots can further help to understand the correlation between independent variables (factors) and dependent variables (response).

Response 1 (Y_1=Folding endurance)

ANOVA was employed to ascertain the model's and individual response parameters' significance ($p<0.05$). Contour plots and surface response plots were used to explore the consequence of independent variables on folding endurance (Figures 2 and 3). When the P-value is $p<0.007$, the F-value of 4.32 in the 2FI model suggests that the model is significant. The contour plots and response surface plots displayed the outcome of diverse factors on folding endurance. An increase in the concentration of polymer and plasticizer was found to increase the film's folding endurance.

Response 2 (Y_2= DT)

The ANOVA analysis of outcomes yielded the F-value of 6.34 for the 2FI model which implicates that the model is significant when the p-value is $p<0.03$. The consequence of several independent factors on DT was shown in the contour plots and response surface plots (Figures 2 and 3). As the amount of HPMC K4M in the film rose, the *in-vitro* DT was augmented, and the film became too sticky. Furthermore, augmenting the plasticizer concentration diminishes DT but makes the film brittle.

Response 3 (% Drug release =Y_3)

The ANOVA analysis of outcomes yielded a p-value of less than 0.04 ($p<0.04$) and an F-value of 4.02 which implicates that the model is significant. The contour plot and RSM plots displayed the consequence of diverse independent variables on drug release (Figures 2 and 3). As the amount of plasticizer and polymer in the film increased to 10-20% and 10-15%, respectively, the film's drug release increased. Drug release was reduced when the polymer concentration reached 15% because the drug remained within the polymer matrix.

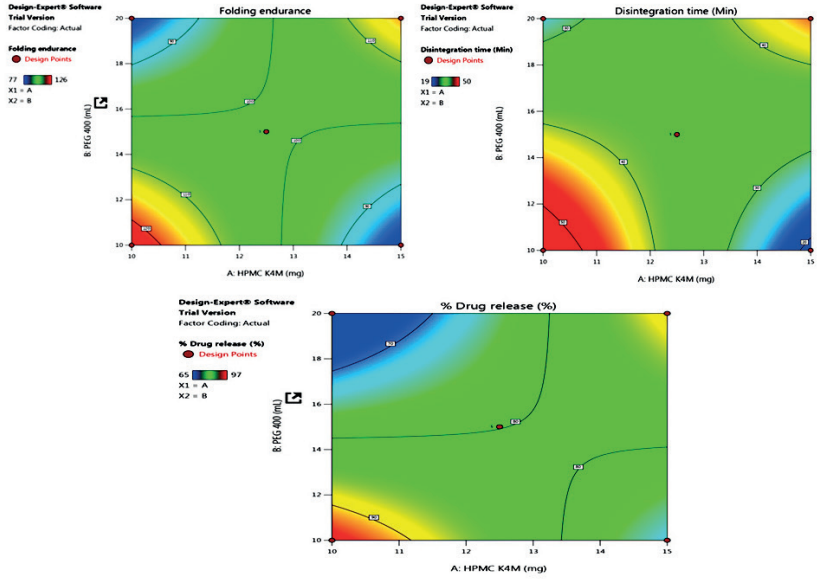

Figure 2. Contour plots showing the effect of HPMC K4M (X_1) and PEG 400 (X_2) on folding endurance (Y_1), Disintegration time (Y_2), and Drug release (Y_3).

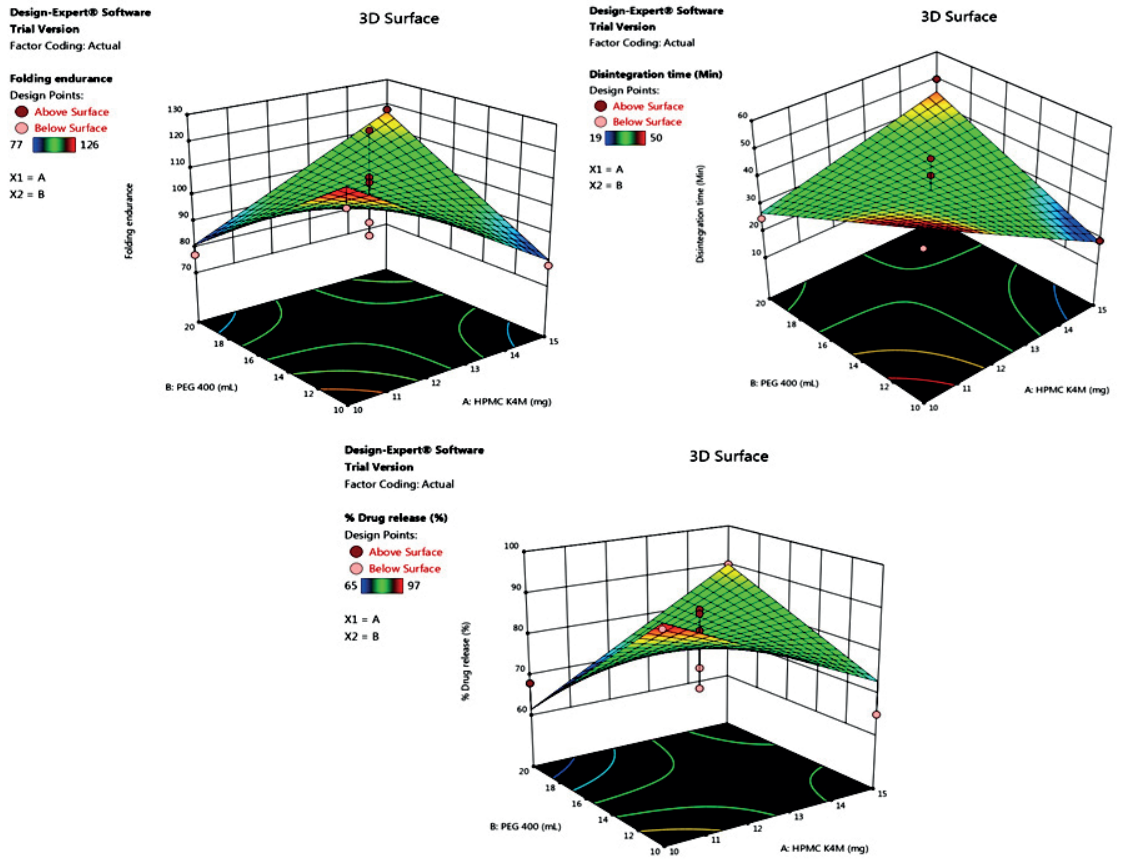

Figure 3. Response surface plots (3D) showing the effect of HPMC K4M (X_1) and PEG 400 (X_2) on Folding endurance (Y_1), Disintegration time (Y_2), and Drug release (Y_3).

Optimization using the desirability functions

There are various methods for optimizing multi-response problems. The desirability feature is one of the most common techniques used for solving multi-response surface problems. The lowest and utmost level must be defined for the apiece parameter employed. Each target can be given a weight to change the shape of its desirability feature. An overall desirability function is created by combining the objectives. Desirability is an objective function with a value of one at the target and zeroes outside of the limits. The software aims to make this role as effective as possible. As a consequence, the foremost intent starts at a random location and progresses up the steepest slope to the highest point. A manifold response means were applied to optimize a combination of two goals (HPMC K4M and PEG 400 concentration). Figure 4 demonstrates the desirability values of HPMC K4M (10-14 mg), and for PEG 400 (10-20 mL) as a "minimum" and "maximum" and within the range to analyze the economically viable optimal condition. At this condition, the model exhibits desirability of 0.999. These optimal values were checked experimentally which resulted in 97.6 %.

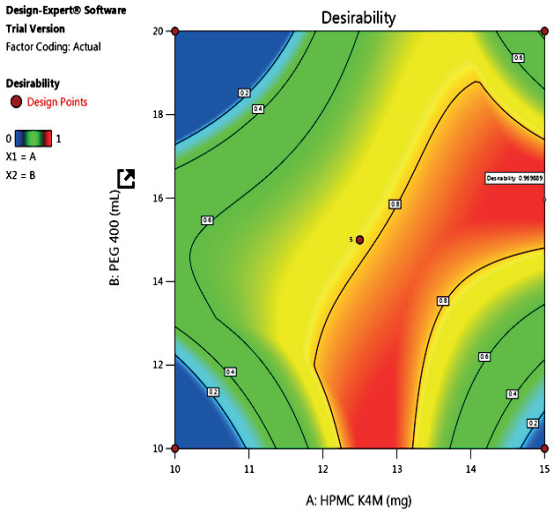

Figure 4. Desirability plot showing the effect of HPMC K4M (X_1) and PEG 400 (X_2).

Evaluation of formulated FDOFs

Appearance

The prepared FDOFs are visually inspected and which is in a whitish transparent appearance.

Thickness

The thicknesses of formulated TDC-loaded FDOFs were in the 0.07 to 0.09 mm range, with a tolerance of 0.01 mm. As shown in Table 3, the mean values were nearly uniform in all F1-F9 formulations. The uniformity in the thickness (due to the low standard deviation obtained) indicated the reproducibility of the method of preparation. According to the findings, increasing film thickness lowers tensile strength while increasing percent elongation. The crystallinity of the film increases as the thickness of the film increases, and the rate of dissolution decreases.

Weight variation test

The percent weight variation of all the formulations is mentioned in Table 3. All the films passed the weight variation test, per the standard Pharmacopoeial limits. The weight of all films was found to be 33.65±1.16 to 39.88±0.95 mg. The weight of the F-4 batch observed is 39.88 ± 0.95 mg.

Determination of surface pH

Table 3 shows the surface pH values of the TDC-loaded film formulations. The surface pH of all of the polymers used in the formulations is neutral. The pH of the surfaces of the film ranged from 6.8 to 7. The neutral surface pH of the films ensured that the mucosal lining of the oral cavity would not be irritated.

Folding endurance

The capacity of a patch to survive a rupture is measured by its folding endurance. The greater the folding endurance, the greater the risk of film rupture, and vice versa. The folding endurance values of all formulations are mentioned in Table 3. The F-4 batch demonstrated the folding endurance of 126±1. The folding endurance of the film increases with increases in the concentration of super disintegrant.

Drug content and content uniformity

The drug content and content uniformity tests were carried out to ensure the uniform distribution of the drug in the film. The % drug content of batch the F-4 is found to be 98.85±1.77. Moreover, the content drug uniformity is observed in all the prepared films.

Percent elongation (%), and tensile strength

The percentage of elongation mainly depends on the tensile strength of the formulated film and which is affected by the nature of polymers used. The % elongation and tensile strength of the F-4 batch are found to be 3.90±0.58%, and 5.58 ± 0.18 respectively.

Disintegration time (DT)

Disintegration time is a very important parameter for OFDFs, to indicate the onset of drug action. The time at which prepared film starts to disintegrate in the solvent is known as DT. The DT of the formulated film was in the range of 19-50 seconds while the F-4 batch exhibited a DT of 19±1 seconds. From the results obtained, it is clear that DT increases with the increased solid contents of the film.

In-vitro drug release study

The simulated saliva was used as a dissolution medium for the dissolution study of TDC fast dissolving film. Amongst, all batches (F-4, F-5, F-8) batch exhibited rapid drug release (Figure 5). The batch F-4 showed significantly more release of TDC (98.55±7.90%) in 5 minutes when compared to the batch F-5 and F-8. This rapid TDC release from all the batches including the F-4 batch may be attributed to the more concentration of PEG 400 and crospovidone which cause augment in the dissolution, and crospovidone (super disintegrating agent). In addition, crospovidone is rapidly wetted into the film by saliva resulting in an expansion of volume and hydrostatic pressure that occurs in rapid disintegration. Moreover, the substantially higher release of TDC from batch 4 could be due to the use of less concentration of HPMC-K4M that yielded thin film formation thereby fast disintegration and dissolution of the film.

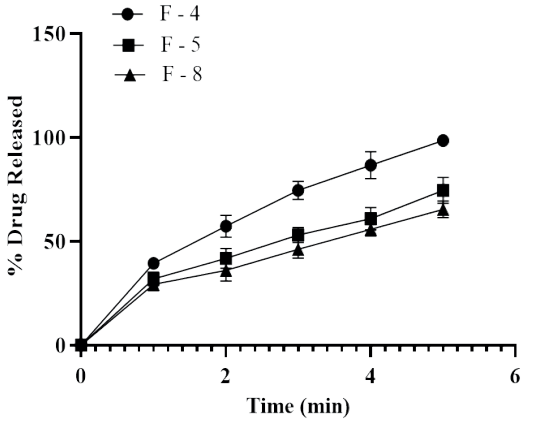

Figure 5. *In-vitro* drug release profile of batch F-4, F-6, and F-8 in simulated saliva (pH 6.8). All the values are expressed as mean ± standard deviation (n=3)

Ex-vivo permeation study

The cumulative percentage of drug permeated through plane drug solution, optimized TDC loaded FDOFs and marketed Quicobal® film was plotted against time as shown in Figure 6. It was found that the slightly increasing concentration of PEG 400 and crospovidone in the film caused a significant increment in the permeation rate. This could be attributed to the concentration-dependent permeation enhancement ability of crospovidone. All the TDC FDOFs showed superior performance *ex-vivo* when compared to plain drug solution. Statistical analysis revealed no significant difference in permeation parameters between optimized film and marketed Quicobal® film. Both the film samples exhibited similar permeation profiles. Moreover, the percent drug permeation was found to be 24.18%, 85.18%, and 85.23% from a plain drug, optimized TDC loaded film and marketed Quicobal® film respectively after 30 minutes.

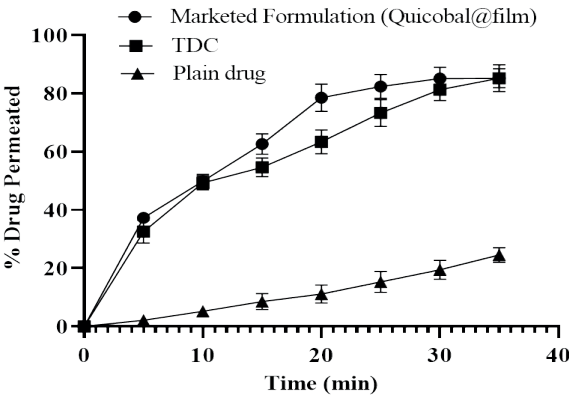

Figure 6. *Ex-vivo* permeation study of plain drug, optimized TDC FDOFs, and marketed Quicobal® film (pH 7.4). All the values are expressed as mean ± standard deviation (n=3)

Table 3. Evaluation of CCD batches of FDOFs

Batch code	Thickness (mm)	Surface pH*	Weight of the film (mg)*	% Elongation	Tensile Strength	Folding endurance	DT (sec)	Drug content per film (2×2 cm)%
F1	0.06±0.008	6.67±0.08	36.68±1.84	1.92±0.82	5.23 ± 0.16	87±0.58	42±1.73	98.79±2.80
F2	0.06±0.007	6.78±0.08	34.55±1.22	1.99±0.67	3.66 ± 0.22	118±1	50±1.73	97.88±2.65
F3	0.08±0.007	6.89±0.08	37.10±1.72	5.94±0.58	5.14 ± 0.20	92±2	25±1.73	98.36±1.89
F4	**0.07±0.008**	**6.97±0.04**	**39.88.±0.95**	**3.90±0.58**	**5.58 ± 0.18**	**126 ±1**	**19±1**	**98.85±1.77**
F5	0.08±0.007	6.90±0.09	38.90±1.14	4.21±0.58	4.37 ± 0.20	118±1.73	24±1	98.73±1.4
F6	0.08±0.008	6.85±0.04	33.65±1.16	1.82±0.58	5.24 ± 0.15	107±1	48±1.7	98.12±1.99
F7	0.08±0.008	6.80±0.08	34.45±1.86	5.34±0.58	4.63 ± 0.25	77±1.73	25±1	97.94±1.92
F8	0.08±0.09	6.86±0.08	37.78±1.84	5.15±0.58	5.11 ± 0.20	118±1.73	24±1	98.79±1.83
F9	0.08±0.009	6.72±0.04	36.78±1.20	2.01±0.58	3.44 ± 0.13	109±1.73	42±1.73	98.18±1.83

*All values are expressed as mean ± S.D. (n=3)

Stability study

The stability of the drug is one of the important parameters considered during the development of a new formulation. During stability studies, the substance is subjected to accelerated temperature and humidity conditions during stability testing. Accelerated stability experiments are those in which a substance is held under high temperatures for a short period. Based on DT, folding endurance, and % cumulative drug release result, batch F-4 is found to be more stable. No significant difference in, the % cumulative drug release, DT, and folding endurance of F-4 batch films were noticed after three months of storage at 40^0±2^0C/, 75 ±5% RH condition (Table 4). Further, the results obtained demonstrated no significant changes observed in the characteristics of the file system.

Table 4. Stability study results of optimized formulation

Formulation (F-4 batch)	Folding endurance	DT	*In-vitro* drug release (%)
Initial	126 ±1	19±1	98.55±1.20
After 1 months	124 ±2.45	21±0.4	96.05±3.50
After 2 months	117 ±0.23	20±0.2	93.78±0.40
After 3 months	109±1.73	24±5.37	93.34±5.02

*All values are expressed as mean ± S.D. (n=3)

CONCLUSION

In this study, novel FDOFs containing the drug TDC were successfully prepared using the solvent casting method. Using a three-level, two-factor (3^2) CCD, the formulations of FDOFs were optimized. Overall, the produced film formulations showed good results in terms of folding endurance, % drug release, and stability. The PEG 400 and crospovidone-driven FDOFs showed immediate disintegration (19 S), improved *in-vitro* dissolution, and faster *ex-vivo* permeation with excellent mechanical properties of TDC from the FDO films. Therefore, these novel FDOFs may provide a potential opportunity for oral delivery of the TDC drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

The investigation, literature research, formulation development, analysis, interpretation of the data, statistics, preparing the study text, and writing the original draft (SSC, MAP, PSK). Developing a hypothesis, reviewing the text, resources, and supervision (ASM, JLD, JID, SAP)

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