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2^3 Ful Factorial Design in the development of Fast Dissolving Films of Doxylamine Succinate using Natural Polymer

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ABSTRACT

Aim and Objective: The aim of the present research work is to design and formulate Fast dissolving buccal films that can rapidly provide relief from sudden allergic reactions.

Materials and Methods: Doxylamine succinate films were prepared using natural polymer pullulan through solvent casting technique, using a 2^3 full factorial design with three factors and two responses. The formulation was optimized using Design Expert software. Compatibility studies were carried out using FTIR spectroscopy. The films were assessed for all the parameter.

Results: The results indicated no interaction between the drug and excipients. The optimized batch of fast dissolving films showed a disintegration time of 46 seconds and drug release of 81.3% within 45 minutes. All the physicochemical criteria were satisfied, and *ex-vivo* studies conducted on buccal mucosa demonstrated a cumulative drug release of 79.5% at 60minutes. Stability test was performed on the optimized formulation according to ICH guidelines for one month at a temperature of 30°C±2°C and relative humidity of 65%±5%.

Conclusion: The concept of fast dissolving dosage forms is becoming popular novel delivery system, due to their ability to enhance therapeutic efficacy, improve bioavailability by reducing dosing frequency while bypassing first-pass metabolism for quicker drug absorption and onset of action.

Keywords: Doxylamine succinate, Fast dissolving films, 2^3Full factorial design, Pullulan.

INTRODUCTION:

Fast dissolving films (FDF) drug delivery systems were developed in the 1970's as an alternative to traditional tablets, capsules, and syrups, specifically to aid paediatric and geriatric patients who have trouble swallowing conventional solid oral medications.[1][2] Pharmaceutical researchers focus on developing fast dissolving buccal films (FDF) containing Doxylamine succinate, an antihistamine primarily employed for preventing and treating nausea and vomiting during pregnancy.[3] Doxylamine succinate (DS), acting as a histamine H1 antagonist with notable sedative effects is widely recognized for its potent sedative effects, commonly employed in the treatment of allergies, nausea and insomnia. Its applications extend to veterinary medicine and the management of Parkinson's disease. Frequently used in combination with pain relievers, cough suppressants and other antihistamines, DS effectively mitigates symptoms including body aches, coughing, fever, headaches, runny nose and sneezing typically associated with viral infection. With a half-life ranging from 8 to 10 hours and an oral bioavailability of around 24.7%, the primary focus of this study was to enhance the bioavailability and therapeutic efficacy of DS through the formulation of fast dissolving buccal films (FDBF).[3]

Various hydrophilic polymers serve as film formers for FDF including hydroxypropyl methyl cellulose (HPMC), polyvinyl alcohol (PVA), Pullulan, Kollicoat® IR, Maltodextrin etc.[4] Pullulan is a water-soluble linear polysaccharide composed of maltotriose units linked by α-1,4 and α-1,6 glycosidic bonds, with a molecular weight of approximately 200,000 Dalton consisting of 480 maltotriose units. Pullulan's suitability for FDF due to its distinctive characteristics, including film flexibility, viscosity, solubility in water and biodegradability.[5] The main focus of this research is to improve the bioavailability of Doxylamine succinate (DS), categorized as a BCS Class III drug due to its high solubility and low permeability. This will be achieved by incorporating DS into pullulan-based strip films, along with the addition of the penetration enhancer PEG 400.[6]

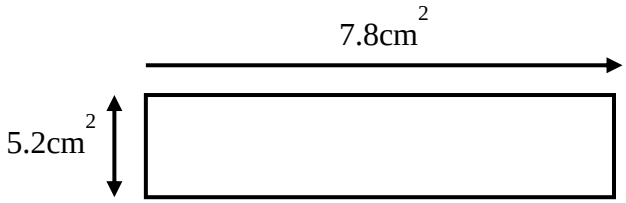
MATERIALS AND METHODS:

Materials

Doxylamine succinate was purchased from Yarrow Chem Products Mumbai, Maharashtra, India. Pullulan was purchased from Kumar Organic Products Ltd, Bangalore, PVP, Mannitol, PEG 400, Polysorbate 80 and Citric acid were obtained from the Department of Pharmaceutics, KLE College of Pharmacy, Hubballi. All the chemicals employed in this research were of analytical grade.

Methods

Dose calculation of Doxylamine succinate:



Each film incorporates 10 mg of DS within an area of 2.25 cm^2. Given that the mould area is 40.56 cm^2 and it yields 18.22 cm^2 of films. S, each mould contains 182 mg of DS and calculated by given formula,

$$\text{Dose per mould} = \frac{\text{Dose per film} \times \text{Area of mould}}{\text{Size of 1 film}}$$

Design of Experiment (DOE):

An experimental design was established with 2^3 full factorial design layouts as shown in (Table1), eight different formulations, each involving three factors at two levels as illustrated in (Table2). The study selected the concentrations of Pullulan (X1), PVP (X2) and PEG 400 (X3) as the independent variables to investigate their effects on the dependent variables: disintegration time measured in seconds (Y1) and cumulative drug permeation measured in % (Y2). This setup aimed to optimize the formulations by examining the influence of each factor.[(7)]

Preformulation studies:

Preformulation studies in pharmaceutical development are crucial for ensuring drug stability and compatibility, utilizing techniques such as UV spectroscopy with calibration curves for accurate drug quantification, FTIR spectroscopy for assessing drug-excipient compatibility through absorption bands analysis and DSC analysis for evaluating thermal properties and detecting incompatibilities. These studies collectively provide essential insights for creating stable and effective pharmaceutical formulations.

Preparation of Standard Stock Solution:

A precise amount of 25 mg of Doxylamine succinate was weighed and placed into a 25 ml volumetric flask containing phosphate buffer at pH 6.8. The mixture was then sonicated to achieve complete dissolution of the drug. After sonication, the volume was adjusted to 25 ml with the same phosphate buffer, resulting in a stock solution-I with a concentration of 1 µg/ml. A volume of 2.5 ml was withdrawn from the stock solution-I and subsequently diluted

to 25 ml with pH 6.8 buffer, resulting in the generation of stock solution-II with a concentration of 10 µg/ml.[8]

a. Detrermination of λ max and Development of Standard Calibration Curve of DS:

Above solution was scanned within the wavelength range of 200-400 nm using a UV spectrophotometer model Shimadzu 1900i.[9]Aliquots of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml and 6 ml were pipetted from the standard stock-II solution into 10 ml volumetric flasks. Each flask was then filled to volume with phosphate buffer of pH 6.8, resulting in final concentrations of 10, 20, 30, 40, 50 and 60 µg/ml, respectively. The absorbance of each concentration was measured at the wavelength of maximum absorption (λmax) at 261 nm using a UV-visible spectrophotometer against a blank consisting of phosphate buffer of pH 6.8.[10]

b. FTIR Compatibility studies:

To assess the compatibility of the chosen polymer with active drugs, it's crucial to investigate drug-polymer interactions using spectroscopic methods like FTIR. Incompatibility often leads to the inactivation of the active drug, either through decomposition or alteration into a less effective form.[11] FTIR spectra for pure Doxylamine succinate, Pullulan,[12] a physical mixture of the drug and polymer, and the optimized formulation were recorded using a Shimadzu IR spectrophotometer from Japan. The drug spectral data was collected and analysed using an FT-IR spectrophotometer to understand any potential interactions between the drug and the polymer.[13] Each spectrum was scanned within the range of 400-4000 cm^{-1} at a spectral resolution with ratio of 1:1, measured against a background interferogram. The collected spectra were then analysed using software provided by Shimadzu.[14]

c. Differential Scanning Calorimetry:

DSC analysis of pure Doxylamine succinate was performed using DSC 60 plus, Shimadzu, Japan. The temperature was increased from 0°C to 150°C at a rate of 10°C per minute to obtain thermograms.[15]

DEVELOPMENT OF FDF:

The formulation of film dosage forms (FDFs) containing DS involved employing the solvent casting technique as shown in (Figure1). Pullulan (polymer)added in the formulation acts as film forming agent. Initially, an aqueous polymeric solution was prepared through agitation using a magnetic stirrer. Subsequently, specific quantities of PVP (plasticizer), PEG 400(penetration enhancer) Mannitol (sweetener), Tween 80 (surfactant agent) and Citric acid (saliva stimulating agent) were added to the solution as described in (Table 3) and the mixture was stirred homogenously for 30 minutes at 300 rpm using a magnetic stirrer until achieving a uniform consistency. Following this, the solutions containing both the drug and the polymer

was poured into a rectangular mould, measuring area of 40.56 cm^2. The solution was then subjected to drying in a hot air oven maintained at 40°C for a duration of 24 hours. Upon completion of the drying process, the films were carefully peeled off from the mould, packaged in aluminium foil and stored in a desiccator for further characterization.[16][17]

EVALUTIONS:

1) Average weight:

Film strips measuring 1.5×1.5 cm^2 were cut from the cast film at three different positions. The weight of each film strip was measured, and weight variation was observed.[15]

2) Film Thickness:

Film thickness was determined for all samples using a screw gauge micrometer. Samples were randomly selected from various positions across the film. The findings are presented as an average value along with the relative standard deviation.[6]

3) Surface pH:

The film was soaked in 2 ml of 6.8 phosphate buffer in a Petri dish for 1minute. Then pH paper was placed on the film's surface for 30 seconds to measure the average pH value based on three readings for each formulation.[18]

4) Percentage moisture loss:

To measure the moisture content in the films, the initially prepared films 1.5×1.5 cm^2 of formulations F1 to F8 were weighed. These films were then placed in desiccators with anhydrous calcium chloride, ensuring airtight conditions. After a period of 3 days, the films were weighed to calculate the percentage of moisture loss.[19]

$$\% \text{ moisture loss} = \frac{\text{Inital weight} - \text{Final weight}}{\text{Intial weight}} \times 100$$

5) Drug content uniformity:

To determine the drug content, a film containing 10 mg of Doxylamine succinate was dissolved in 100 mL of phosphate buffer at pH 6.8, creating a 100 µg/mL solution. A 5 mL sample was then taken and diluted to 10 mL with the same phosphate buffer. This solution was filtered using Whatman filter paper and analysed with a Shimadzu 1900i UV-spectrophotometer at 261 nm. Content uniformity studies were performed in triplicate for each batch of the film, with an acceptable range of 92-108%.[20]

6) Folding Endurance:

Folding endurance of fast dissolving films (FDF) was assessed by repetitively folding each at the same spot until breakage or loss of integrity. Results were averaged from three determinations, providing insight into film durability and flexibility.[21]

7) Disintegration time:

The Center for Drug Evaluation and Research's guidelines for orally disintegrating tablets can be adapted for fast-dissolving films. Although there are no official guidelines for these films, they can be used for quality control and development. In this study, films were immersed in pH 6.8 phosphate buffer in a 500 ml beaker, and disintegration time was recorded with a disintegration apparatus, showing dissolution within three minutes.(14)

8) *In-vitro* diffusion studies:

Dissolution studies for all film formulations were conducted using a Franz diffusion cell apparatus, with simulated salivary fluid (pH 6.8 phosphate buffer) serving as the dissolution medium. The studies were carried out over a period of 45 minutes to ensure complete dissolution and to maintain sink conditions for all formulations. During this period, 0.6 ml aliquots were collected at regular intervals, filtered and analysed using UV Spectrophotometer at a wavelength of 261 nm to determine the concentration of the drug released. The resulting drug release profiles for each film formulation were illustrated in Figures 7, providing a comprehensive comparison of their dissolution characteristics.(22)

9) *Ex-vivo* studies:

An ex-vivo skin permeation study was carried out using a Franz diffusion cell with a receptor compartment holding 13 ml of phosphate buffer at pH 6.8. A porcine buccal mucosa membrane was positioned between the donor and receptor compartments, and a 1.5×1.5 cm formulated film was applied to the dialysis membrane.(23) The donor compartment was secured and the setup was maintained at 37 ± 2ºC on a magnetic stirrer. Samples of 0.6 ml were taken at intervals up to 60 minutes and analysed at 261 nm using a UV spectrophotometer. The receptor phase was replenished with fresh buffer after each sample. Drug release percentages were calculated and plotted over time.(10)

10) Stability studies:

The optimized formulation (OF) underwent stability testing by being stored in aluminum packaging under conditions of 30 ±5°C temperature and 65±5% relative humidity for a period of 30days. During this time, the films was assessed on initial day and 30th day for any changes in their physicochemical and mechanical properties. This testing ensured that the formulation maintained its stability, efficacy and integrity throughout the storage period.(21)

RESULTS AND DISCUSSION:

a. Determination of λ max and Development of Standard Calibration Curve of DS:

The absorption peak for Doxylamine succinate, determined using a 6.8 pH phosphate buffer, was observed at 261 nm. The linearity graph, was plotted with concentration on the x-axis and absorbance on the y-axis, demonstrated a clear linear relationship for Doxylamine succinate. The resulting equation from this graph was $y = mx + c$, demonstrating a strong linear relationship between the concentration and absorbance values with (R^2) regression co-efficient of 0.9978 as shown in (Figure 2).

b. FTIR Compatibility studies:

FTIR spectroscopy was utilized, Spectra were scanned across a frequency range of 500-4000cm^{-1} with a resolution of 4cm^{-1}. No interactions were observed between the selected polymers and the drug Doxylamine succinate. FTIR Spectra of pure drug DS, Pullulan, mixture of drug and polymer and optimized formulation (OF) as shown in the (Figures3, 4, 5 and 6) and the functional groups of the FTIR Spectra is observed in the (Table4).

c. Differential Scanning Calorimetry:

The recorded melting point of 106.78°C aligns with the standard range of 100°C to 108°C.

EVALUTIONS:

1) Average weight:

The weight variation of the formulation was determined to be within the specified range, ranging from 81.6±2.3 mg to 184±0.80 mg. These values satisfied the specified criteria and are detailed in the (Table5).

2) Film thickness:

The thickness was determined by the micrometer screw gauge. The values ranged from 0.11±0.002mm to 0.29±0.002mm. The values are detailed in (Table5).

3) Surface pH:

To assess the potential for any adverse effects resulting from pH changes *in-vivo*, the surface pH of the films was investigated. A film assessed for testing was placed in a petri dish and dissolved in 1ml of 6.8pH buffer, left for 30 seconds. Later, pH paper was brought into contact with the formulation's surface, allowing it to equilibrate for 1 minute having pH ranging from 6-7 as shown in (Table5).

4) Percentage moisture loss:

There was no moisture absorption observed in any of the films, and there were no signs of moisture-related deterioration in the prepared films. The recorded data ranged from 0.2±0.04 to 2.4±0.01, as presented in (Table5).

5) Drug content uniformity:

Drug content uniformity tests were conducted for all 8 formulations, with the results detailed in (Table5). The percentage of Doxylamine succinate content in the fast-dissolving films ranged from 76.4% to 93.5%. These results ranged within the specified range, indicating uniformity across the films.

6) Folding endurance:

The folding endurance values ranged from 0.11±0.002mm to 0.29±0.002mm as shown in (Table5). It was noted that as the concentration of polymer and plasticizer increased, the folding endurance also increased. A higher folding endurance value indicates a reduced likelihood of film rupture.

7) Disintegration time:

The disintegration time of the film ranged from 141±0.8 to 138±2.2 seconds, as detailed in the (Table6). It was observed that with an increase in the concentration Pullulan (polymer) and (PVP) plasticizer, the disintegration time also increased.

8) *In-vitro* diffusion studies:

The cumulative drug permeation for FDF of DS (F1-F8) ranged from 59.9% to 83.5%, with formulation F6 also showing the highest cumulative drug release at 83.5%. The % cumulative drug release (CDR) for all formulations is detailed in (Table6) and (Figure7).

9) *Ex-vivo* Study:

An *ex-vivo* permeation study on the optimized formulation OF1 was conducted using sheep oral mucosa and a modified Franz diffusion cell apparatus for 60 minutes. The percentage of drug permeated was calculated and plotted against time as shown in (Figure11) with the optimized buccal film (OF). At the end of 60 minutes, 79.5% of the drug had permeated through the oral mucosa. These results suggest that the drug has a high capacity to rapidly cross the buccal barrier.

STATISTICAL ANALYSIS

Response 1 (Disintegration time Y1)

Polynomial equation: Y1 = +91.00 + 26 *X1 + 15*X2 + 7.25 *X3

(R^2 = 0.9988)

ANOVA indicated significant effects ($p < 0.05$) on the model and individual response parameters. Surface response and contour plots in (Figure8) showed the influence of independent variables on disintegration time. The quadratic model's F-value of 1070.67 was highly significant ($p < 0.0001$). *In-vitro*, disintegration time increased with increase in concentration of pullulan. *In-vitro*, disintegration time increased with more pullulan, decreased with plasticizer, but increased slightly with excessive polymer making the film brittle.

Response 2 (Cumulative drug permeationY2)

Polynomial equation: $Y2 = +70.23 – 6.70*X1 – 3.88 * X2 –1.35 * X3$

($R^2 = 0.9858$)

After ANOVA estimation, the quadratic model with an F-value of 92.35 indicates the model is significant ($p < 0.0004$). The contour plot and surface response plot in (Figure9) illustrated the effects of different independent variables on drug release. Particularly, Pullulan, recognized for its hydrophilic properties in regulating drug release, played a crucial role. Drug release from the films decreased with rising concentrations of both Pullulan and PVP. Additionally, increasing PEG 400 concentration alongside Pullulan led to reduced drug release from the films. Therefore, an optimal combination featuring lower concentrations of polymer and plasticizer would be preferable to enhance drug release from the films.

Optimization:

Among these solutions, one formulation labelled OF1 stood out with a desirability of 1.000. This formulation was predicted to achieve a disintegration time of 42.75 seconds and a drug release of 82.15% as shown in (Figure10). Consequently, OF1 was selected, and its performance closely matched the software's predictions, fulfilling all the optimization objectives.

10) Stability studies:

The stability study was conducted following the ICH guidelines, indicating no significant changes in the properties of the optimized formulation OF made up of both synthetic and natural polymer. Short-term stability assessments were carried out in a Stability chamber for 1 month on optimized fast dissolving buccal film. Adequate samples were packed in stability containers and stored in the chamber at 30°C±2°C and 65%±5% relative humidity (RH). Samples were withdrawn on the 30^{th} day for drug content estimation, as well as for assessing thickness, weight, folding endurance and *in-vitro* disintegration studies to determine drug release profiles seen in the below (Table12).

FIGURES:

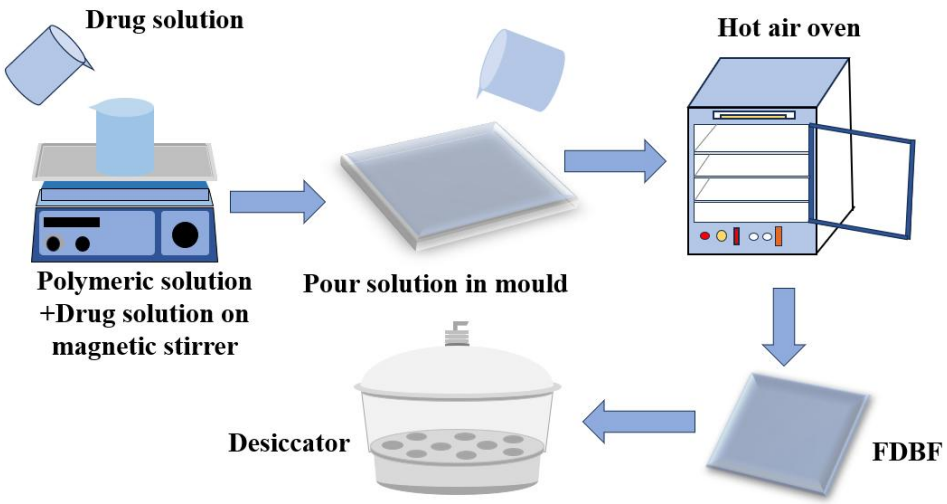


Figure 1: Solvent casting technique used for preparation of FDF

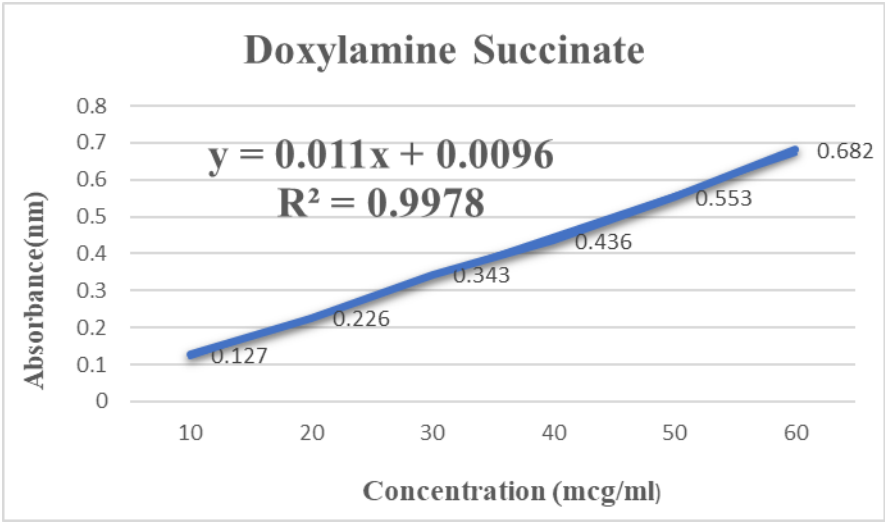


Figure 2: Calibration curve of DS at 261nm

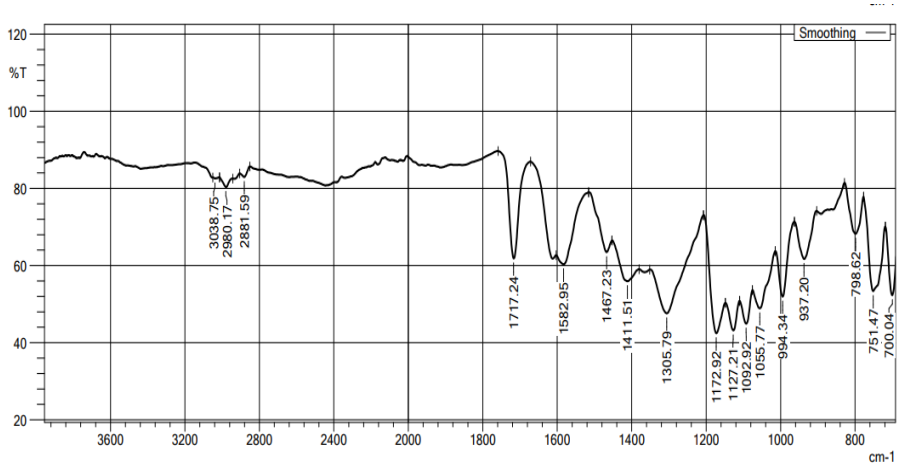


Figure 3: FTIR Spectrum of pure drug DS

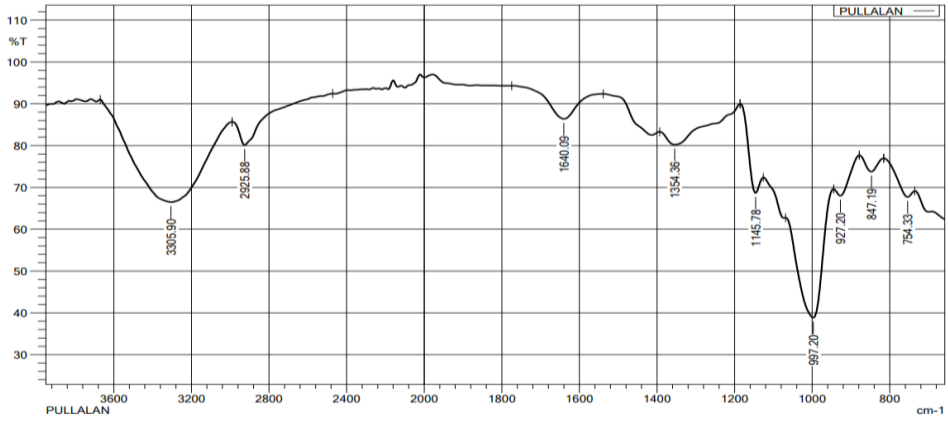


Figure 4: FTIR Spectrum of natural polymer Pullulan

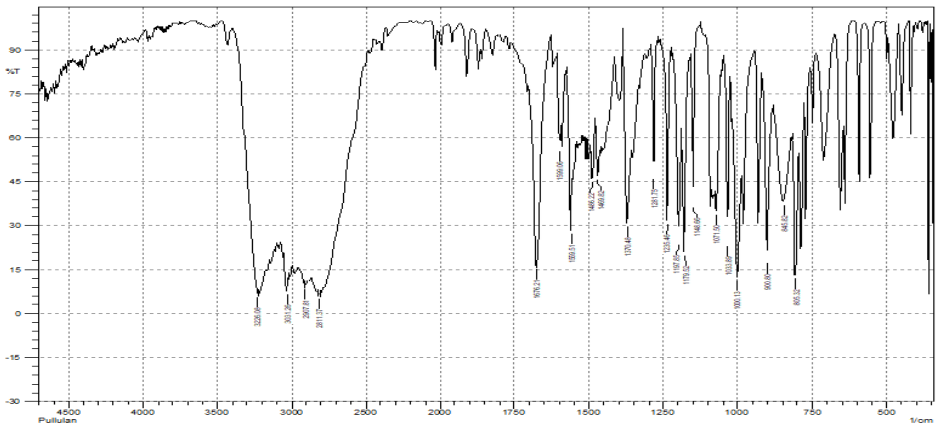


Figure 5: FTIR Spectrum mixture of drug and polymer

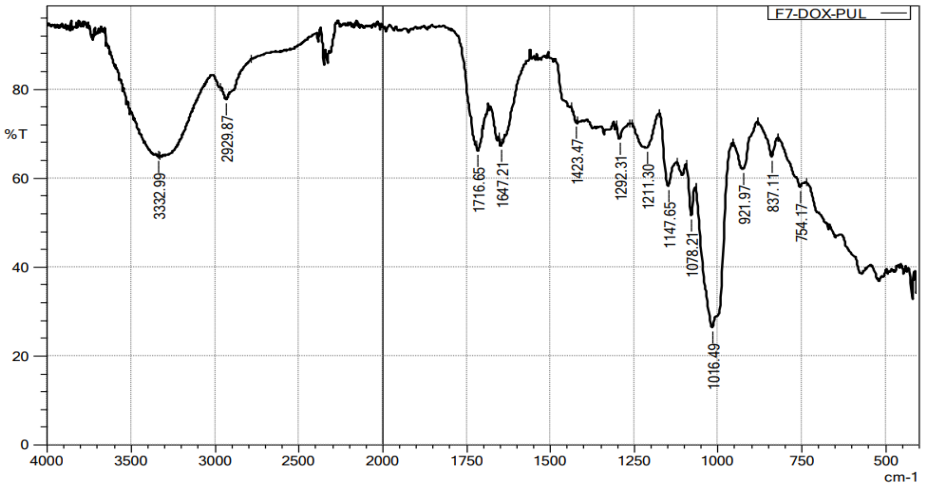


Figure 6: FTIR Spectrum of Optimized formulation

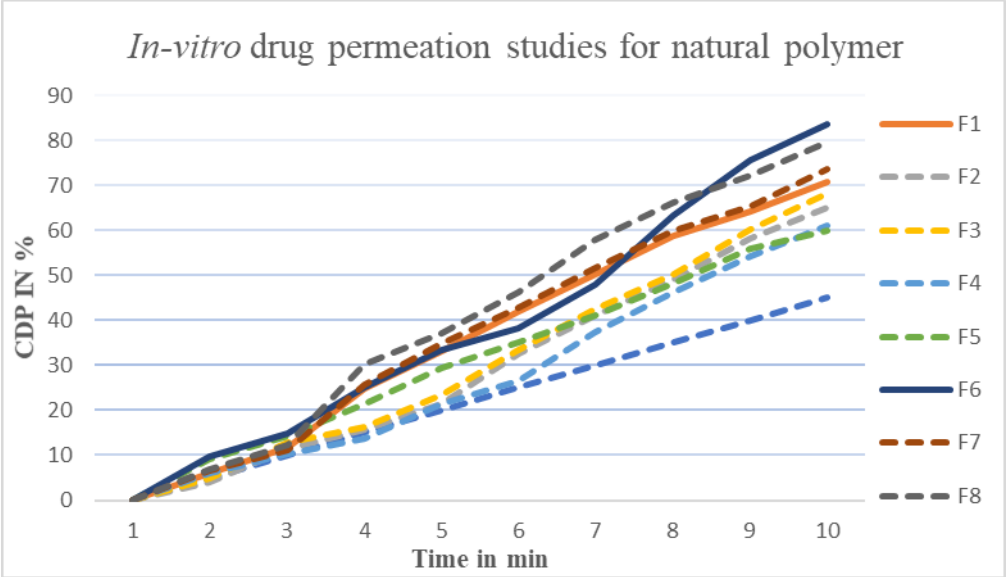


Figure 7: Cumulative drug permeation of DS FDF formulations F1-F8

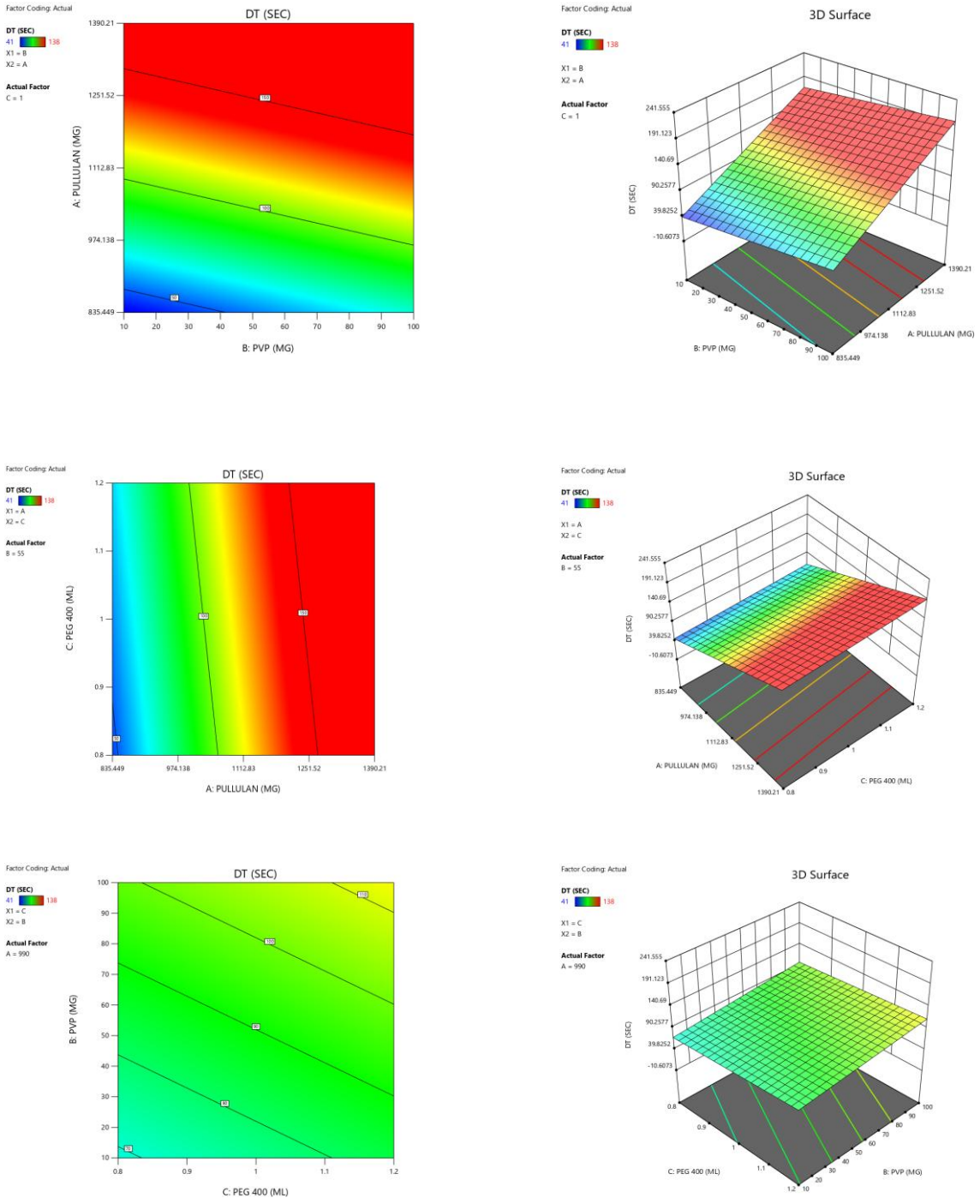
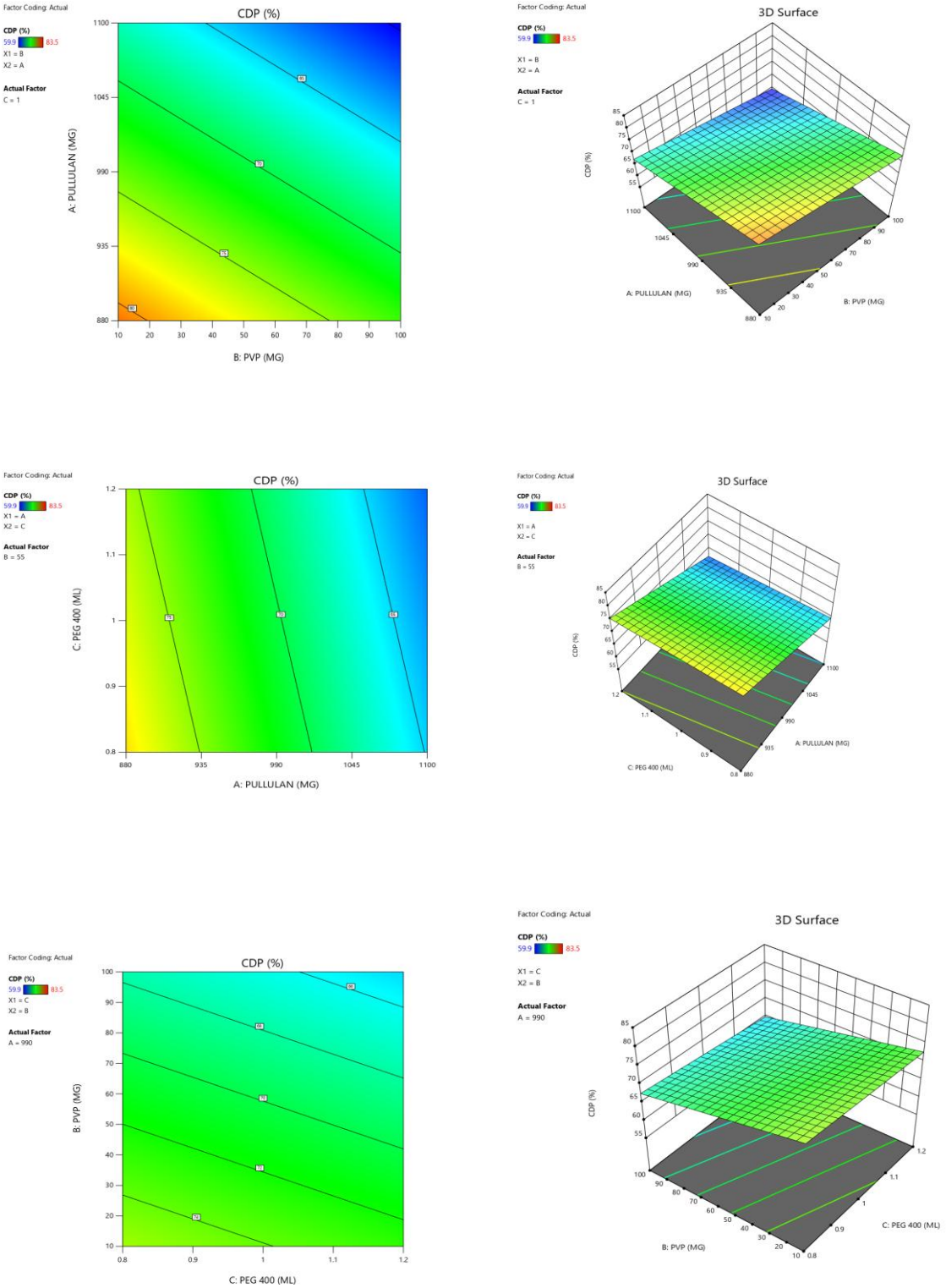


Figure 8: Counter plot and 3D plot of graph of effect of factors X1, X2 and X3 on cumulative drug permeation

Figure 9: Counter plot and 3D plot of graph of effect of factors X1, X2 and X3 on



cumulative drug permeation

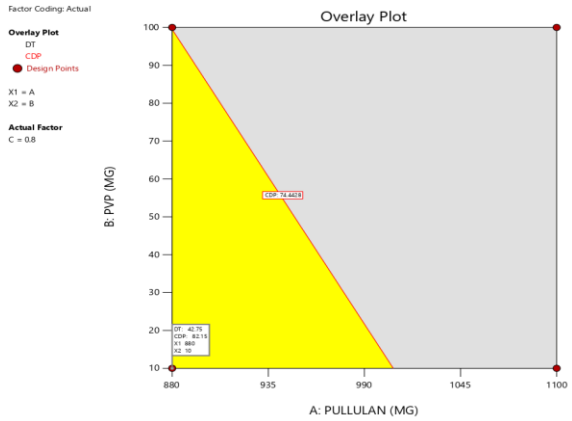


Figure 8: Overlay plot of optimized formulation for natural polymer

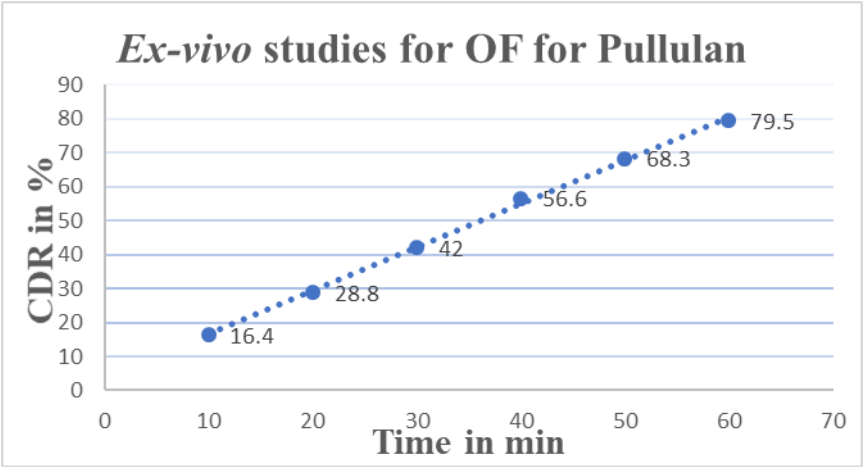


Figure 9: Cumulative drug permeation of optimized formulation

TABLES:

Table 1: 2^3 Full Factorial design layouts for FDF

Batch code	Factor 1 (X1)	Factor 1 (X2)	Factor 1 (X3)
F1	-1	+1	+1
F2	+1	-1	+1
F3	+1	-1	-1
F4	+1	+1	-1
F5	-1	+1	+1
F6	-1	-1	-1
F7	-1	+1	-1
F8	-1	-1	+1

Table 2: Experimental design with three factors, each at two levels.

Independent variables	**Levels used and Coded value**	
X1 = PUL (mg)	(-1) Lower 880 (+1) Upper 1100	**Dependent variables** Y1 = Disintegration time (sec) Y2 = Cumulative drug permeation (%)
X2 = PVP (mg)	(-1) Lower 10	
	(+1) Upper 100	
X3 = PEG 400 (ml)	(-1) Lower 0.8	
	(+1) Upper 1.2	

Table 3: Layout of formulation table 2^3 factorial design using DOE

S.NO	**INGREDIENTS (mg)**	**FORMULATION CODE**							
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1	Doxylamine succinate	182	182	182	182	182	182	182	182
2	Pullulan	880	1100	1100	1100	1100	880	880	880
3	PVP	100	10	10	100	100	10	100	10
4	Citric acid	500	500	500	500	500	500	500	500
5	Mannitol	25	25	25	25	25	25	25	25
6	Polysorbate 80	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml	0.3ml
7	PEG 400	1.2ml	1.2ml	0.8ml	0.8ml	1.2ml	0.8ml	0.8ml	1.2ml
8	Distilled water	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml

Table 4: FTIR spectral data of DS, Pullulan, mixture of DS and Pullulan and Optimized formulation

Functional group	Standard wave no. cm^{-1}	DOX pure drug cm^{-1}	Pullulan polymer cm^{-1}	Mixture of drug and polymer cm^{-1}	Optimized formulation OF1 for natural polymer cm^{-1}
O-H	3050-3350	-	3305.90	3226.06	3332.99
-CH	2800-2900	-	2925.88	2811.37	2929.87
C-O-C	1100-1150	-	1145.78	1148.66	1147.65
C-O	990-1096	1092.92	997.20	1071.50	1078.21
C=N	1550-1675	1582.95	-	1676.21	1647.21
$C-CH_3$	1380-1458	1411.51	-	1446.22	1423.47
C-O-C	1000-1310	1305.79	-	1297.18	1232.51
C=O	1650-1750	1721.53	-	1676.21	1716.65

Table 5: Evaluation parameters of FDF of DS

Formulation code	Weight variation in mg*	Thickness in mm*	Surface pH	% Moisture loss*	Drug content in %*	Folding endurance*
F1	124±1.53	0.18±0.01	6-7	1.88±0.05	80±0.11%	86±3.21
F2	162±0.22	0.25±0.001	6-7	1.98±0.04	78.2±0.24%	75±1.00
F3	148±0.35	0.21±0.002	6-7	2.02±0.01	79±0.16%	83±3.05
F4	170±0.74	0.27±0.001	6-7	2.0±0.04	77.4±0.12%	71±2.30
F5	184±0.80	0.29±0.002	6-7	2.4±0.01	76.4±0.16%	68±2.30
F6	81.6±2.3	0.11±0.002	6-7	0.2±0.04	93.5±0.22%	104±2.15
F7	108±0.50	0.17±001	6-7	1.74±0.05	83.4±0.18%	91±1.5
F8	93.1±1.5	0.15±0.001	6-7	0.48±0.01	88±0.20%	97±2.1

***Mean± SD n=3**

Table 6: Evaluation results of Response 1(Disintegration time) and Response 2 (Cumulative drug release)

Formulation code	Disintegration time in seconds*	Cumulative drug release in %
F1	88±1.2	70.9%
F2	110±0.5	65%
F3	96±1.1	68.2%
F4	124±1.6	61%
F5	138±2.2	59.9%
F6	41±0.8	83.5%
F7	74±1.5	73.6%
F8	57±2.2	79.7%

***Mean± SD n=3**

Table 7: ANOVA for 2^3 factorial design model for response disintegration time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7628.50	3	254.83	1070.67	<0.0001	significant
X1-Pullulan	5408.00	1	5408.00	2277.05	<0.0001	
X2-PVP	1800.00	1	1800.00	757.89	0.0004	
X3-PEG 400	420.50	1	420.50	177.05	0.0002	
Residual	9.50	4	2.37			
Cor Total	7638.00	7				

Table 8: R^2 Regression for 2^3 factorial design model for response disintegration time

Std. Dev.	1.54	**R^2**	0.9988
Mean	91.00	**Adjusted R^2**	0.9978
C.V%	1.69	**Predicted R^2**	0.9950
		Adequate Precision	88.5545

Table 9: ANOVA for 2^3 factorial design model for response cumulative drug permeation

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	493.82	3	164.61	92.35	0.0004	significant
A-Pullulan	359.12	1	359.12	201.47	0.0001	
B-PVP	120.13	1	120.13	67.39	0.0012	
C-PEG 400	14.58	1	14.58	8.18	0.0459	
Residual	7.13	4	1.78			
Cor Total	500.96	7				

Table 10: R^2 Regression for 2^3 factorial design model for response cumulative drug permeation

Std. Dev.	1.34	**R^2**	0.9858
Mean	70.22	**Adjusted R^2**	0.9751
C.V%	1.9	**Predicted R^2**	0.9431
		Adequate Precision	25.2632

Table11: Stability test results for Optimized formulation

Evaluation parameter at 30°C±2°C and 65%±5% relative humidity (RH).			
Testing	**0 day**	**15 days**	**30days**
Weight variation in mg	108±0.20	107±0.20	106±0.20
Thickness in mm	0.17±002	0.17±002	0.16±001
Drug content in %	91%	90.4%	89.8%
% moisture loss	1.26±0.02	1.21±0.04	1.19±0.04
Disintegration time in seconds	46	45	44
CDR in %	81.3	79.8	79.2

***Mean± SD n=3**

CONCLUSION:

In this research, employing Design of Experiment facilitated the identification of how formulation variables affect FDF performance. A film of Doxylamine succinate prepared using the solvent casting method demonstrated the desired drug release percentage and

disintegration time. This film exhibited a very smooth surface, attributed to the use of pullulan, and showed no interactions between the drug and polymer. Optimization of the film was achieved through expert design, with statistical analysis yielding equations that effectively described the impact of selected variables on the studied responses. Formulations that released over 81.3% of the drug within 45 minutes were identified in regions with low pullulan content, facilitating quicker drug release and *ex-vivo* studies conducted on buccal mucosa demonstrated a cumulative drug release of 79.5% at 60minutes. Additionally, formulations with an *in-vitro* disintegration time of less than 46 seconds were found in areas with higher levels of pullulan. The high drug release percentage in simulated saliva (pH 6.8) suggests that this film could be beneficial for treating acute allergies and situations requiring rapid drug bioavailability.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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