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Original Article

FORMULATION AND OPTIMIZATION OF MUCOADHESIVE BUCCAL FILM FOR NICOTINE REPLACEMENT THERAPY

AADITAYA PRAKASH, PRAKASH K. SONI, SURESH K. PASWAN*, T. R. SAINI

Industrial Pharmacy Research Lab, Department of Pharmacy, Shri G. S. Institute of Technology and Science, 23, Park Road Indore-452003 (M. P.), India
Email: skpaswan@gmail.com

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ABSTRACT

Objective: Nicotine replacement therapy (NRT) is a widely used method for reducing the desire to smoke and managing withdrawal symptoms during smoking cessation efforts. The research work aims to prepare and optimize a mucoadhesive nicotine buccal film by solvent casting method to provide the rapid onset and prolonged effects of cigarette smoking.

Methods: Mucoadhesive nicotine buccal films were developed from the polymers Carbopol 934, Eudragit RLPO, and HPMC E15 by solvent casting method. The optimization of the mucoadhesive nicotine buccal film was performed using a three-factor, three-level Box-Behnken design where Carbopol 934, Eudragit RLPO, and HPMC E15 were selected as independent variables, while the swelling index, adhesion time, mucoadhesive strength, and cumulative % drug release were selected as response variables.

Results: The optimized mucoadhesive nicotine buccal film showed uniform thickness and drug content. It had a swelling index of 188.21%, adhesion time of 7 h 45 min, and mucoadhesive strength of 0.23 N. The film showed a burst release followed by a steady release of 76.55 % over 360 min. It exhibited a 2-fold enhancement in buccal mucosal permeation as compared to a lozenge.

Conclusion: The mucoadhesive nicotine buccal film prepared by the solvent casting method provides a rapid onset of action and prolonged effect for an extended period which replicates the effects of cigarette smoking. The findings show that it will reduce the frequency of administration, as a result of decreased nicotine cravings and reduced withdrawal symptoms, compared to currently available NRTs, ultimately helping individuals quit smoking.

Keywords: Mucoadhesive buccal film, Box-behnken design, Nicotine replacement therapy, Rapid onset, Prolonged release

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INTRODUCTION

Nicotine is a naturally occurring alkaloid found in tobacco plants (*Nicotiana tabacum*) and tobacco products like cigarettes, cigars, and chewing tobacco. It acts as a stimulant, affecting the central nervous system and increasing the release of various neurotransmitters, including dopamine, norepinephrine, and acetylcholine. The increase in these neurotransmitters results in a pleasurable and euphoric sensation, leading to the development of addiction [1]. Nicotine addiction is a chronic disease caused by the prolonged use of tobacco products containing nicotine. It increases the risk of many health issues, such as cancer, heart disease, and respiratory illnesses. The addicted individuals continue to seek tobacco products despite the detrimental effects on their health. The addictive nature of nicotine makes it challenging to quit tobacco use as it leads to withdrawal symptoms [2].

Nicotine replacement therapy (NRT) is a widely used treatment for nicotine addiction to help people gradually reduce their nicotine cravings and dependence while they work on smoking cessation. NRT products contain lower doses of nicotine than tobacco products and are designed to ease withdrawal symptoms while offering a safer nicotine delivery method [3-5]. Commercially available NRT products include transdermal patches, chewing gums, lozenges, oral inhalers, and nasal sprays [6, 7]. Although NRT products are effective, they often have a slower onset of action and lower drug bioavailability than cigarette smoking. Nicotine absorption is primarily through the buccal mucosa and is excreted through the urine after being metabolized by the liver, lungs, and kidneys [8].

Buccal drug administration is a type of local or systemic drug delivery through the mucosal membrane of the cheek and gums [9]. This route of drug delivery has several advantages over traditional oral and parenteral routes, including quick and direct absorption of the drug into the systemic circulation, bypassing the liver's first-pass metabolism resulting in higher bioavailability and faster onset of the drug's action [10-13]. Furthermore, buccal administration is non-invasive, and the drug is easy to administer, which makes it more convenient for patients having difficulty in swallowing or in cases of frequent dosing [14]. The buccal route of drug administration is commonly used for drugs with a short half-life, such as nicotine, which is rapidly metabolized and requires frequent administration to maintain therapeutic levels [15].

Buccal films are a recent development in buccal drug delivery and are a preferred option over adhesive tablets due to their flexibility and comfort. Mucoadhesive buccal films, in particular, have the advantage of adhering to the mucosa and providing prolonged drug release, which can extend the duration of the therapeutic effect [16, 17]. These films are composed of a polymeric matrix that can be optimized to control the drug release kinetics and the adhesion properties of the film to the mucosal tissue [18]. As a result, mucoadhesive buccal films may be an effective option for nicotine replacement therapy, which can provide a quick onset of action to alleviate withdrawal symptoms and prolonged release to help reduce cravings over time [19, 20].

In the current study, we aimed to formulate a mucoadhesive nicotine buccal film using a solvent casting method that can provide quick onset of action followed by a prolonged release as a promising NRT. The film was formulated using different mucoadhesive polymers, i.e., Carbopol 934, Eudragit RLPO, and HPMC E15. The optimization of the mucoadhesive nicotine buccal film was carried out by using a statistical approach of the Box-Behnken experimental design [21]. The software suggested quantities of independent variables i.e., mucoadhesive polymers that influence the response variables i.e., swelling index, adhesion time, mucoadhesive strength, and in vitro drug release study at different time intervals. To assess its efficacy in providing prolonged nicotine release and mucoadhesion to the buccal mucosa, an ex-vivo permeation study was performed. This mucoadhesive nicotine buccal film is expected to be a safer alternative for existing NRTs.

MATERIALS AND METHODS

Material

Nicotine was obtained as a gift sample from Gelnova Laboratories Pvt. Ltd. (Navi Mumbai), Carbopol 934, Eudragit RLPO, and HPMC

E15 were procured from Lubrizol, Evonik, and Colorcon respectively. Ethanol was purchased from E-Merck. All other chemicals and reagents were of analytical grade.

Preparation of mucoadhesive buccal films

The mucoadhesive nicotine buccal film was prepared by the solvent casting method [22]. A weighed amount of HPMC E15 was dissolved in 10 ml ethanol: water solvent blend (1:1), and the polymeric solution was slowly added to the ethanolic solution of carbopol 934 and eudragit RLPO (10 ml) under constant stirring, followed by the addition of 0.5 % w/w propylene glycol as a plasticizer. The pH was adjusted by sodium hydroxide, and nicotine (25 mg) was added to the above polymeric solution. A thin film was cast on a glass petri dish with help of the above solution. The film was formed by gradually drying the solvent at room temperature for 12 h. The dried films were stored in aluminium foil for further evaluation.

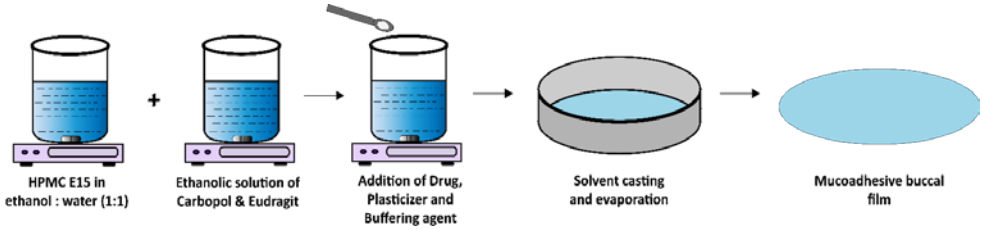

Fig. 1: Schematic diagram of a mucoadhesive buccal film prepared by solvent casting method

Optimization of mucoadhesive nicotine buccal films

The optimization was carried out to minimize the number of experimental trials. The mucoadhesive nicotine buccal films were prepared by solvent casting method. In the current study, a three-factor, three-level Box-Behnken design of response surface methodology was employed [21]. The study included the independent variables i.e., the quantity of Carbopol 934 (A), Eudragit RLPO (B), HPMC E15 (C), and the response variable selected such as swelling index (R1), adhesion time (R2), mucoadhesive strength (R3), cumulative % drug release at 5 min (R4), 15 min (R5), 30 min (R6), 60 min (R7), 120 min (R8), 240 min (R9), 360 min (R10). The amount of drug (25 mg) and concentration of plasticizer (0.5 % w/w) were kept constant throughout the batches. The variables and their levels are recorded in table 1. The Design-Expert® software (version 12, stat-Ease, Inc., Minneapolis, MN) was used to analyze the experimental data. A 3D response surface was generated to illustrate the independent and response variables. Fifteen experimental runs were planned as per the Box-Behnken design. The ANOVA studies show the significance of the response variable (p-value of 0.05). The correlation coefficients (R^2) and adjusted R^2 were used to assess the model's applicability. The experimental results were compared to the predicted values after the optimized formulation had been prepared.

Table 1: Variable and their levels in box-behnken design

Independent variables				
Factors	**Units**	**Levels**		
		Low	**Medium**	**High**
A: Carbopol 934	%	1.5	2	2.5
B: Eudragit RLPO	%	1.5	2	2.5
C: HPMC E15	%	3.75	5	6.25
Response variables				
	Units		Constraints	
(R1) Swelling index	%		Maximize	
(R2) Adhesion time	H		Maximize	
(R3) Mucoadhesive strength	N		Maximize	
(R4) Cumulative % drug release at 5 min	%		Minimize	
(R5) Cumulative % drug release at 15 min	%		Minimize	
(R6) Cumulative % drug release at 30 min	%		Minimize	
(R7) Cumulative % drug release at 60 min	%		Minimize	
(R8) Cumulative % drug release at 120 min	%		Minimize	
(R9) Cumulative % drug release at 240 min	%		Minimize	
(R10) Cumulative % drug release at 360 min	%		Minimize	

Table 2: Box-behnken design for optimization of mucoadhesive nicotine buccal film

Batch No.	**Carbopol 934 (%)**	**Eudragit RLPO (%)**	**HPMC E 15 (%)**
NBF1	2.0	2.5	3.75
NBF2	1.5	2.0	3.75
NBF3	2.0	2.0	5.00
NBF4	2.0	2.0	5.00
NBF5	2.0	2.0	5.00
NBF6	2.5	2.0	3.75
NBF7	1.5	1.5	5.00
NBF8	2.0	1.5	6.25
NBF9	2.5	1.5	5.00
NBF10	1.5	2.5	5.00
NBF11	2.5	2.0	6.25
NBF12	2.0	2.5	6.25
NBF13	2.5	2.5	5.00
NBF14	2.0	1.5	3.75
NBF15	1.5	2.0	6.25

Characterization of mucoadhesive nicotine buccal film

Determination of surface pH

The alkaline or acidic pH of the film may irritate the buccal mucosa. The objective was to keep the surface pH near neutral. The surface pH of the films was measured using a pH meter (Cyberscan 510) by placing a buccal film in a small beaker having 5 ml of water. The observations were recorded in triplicate and their mean value was determined.

Film weight and thickness

The weight and thickness of the film are essential factors in ensuring the content uniformity of the drug and excipients. The drug is very potent, so the uneven thickness and weight variation of the film may result in dose variation. Three equal sections of 2 cm x 2 cm of films were taken, weighed, and their average weight was recorded. The thickness of the film was evaluated using a digital micrometer (Digimatic micrometer, Mitutoyo, Tokyo, Japan). The film was evaluated in five different areas, and the average thickness reading was recorded.

Folding endurance

The objective of the folding endurance test is to assess if the film can sustain the mechanical stress that occurs during its handling. The value of folding endurance was determined by repeatedly folding the film at a point, till any crack or break was observed. The mean value of three observations was recorded.

Tensile strength and extensibility

Tensile strength refers to the maximum amount of stress a material can withstand before breaking, while extensibility is the ability of a material to be stretched or extended without breaking. The tensile strength and extensibility were determined to quantify the mechanical stress induced by jaw movement in the buccal cavity. The tensile strength and extensibility of the mucoadhesive nicotine buccal film were evaluated using a texture analyzer (Stable microsystems ltd., Surrey, UK) with probe tensile grips (A/TG). In this method, the buccal film was placed between the mounting cards. The test speed was kept at 0.5 mm/s and the results were recorded.

Swelling index

A mucoadhesive polymer must be hydrated to expand and form an adequate macromolecular mesh, enabling the polymer chains to become more mobile and exposing bioadhesive sites for hydrogen bonding or electrostatic contact between the polymer and the mucosal network, thus facilitating mechanical entanglement. Mucoadhesive nicotine buccal film (2 cm x 2 cm) was weighed and then immersed in phosphate buffer pH 6.8 for a predetermined time, further it was blotted on filter paper, and weighed[23]. The following formula was used to get the percent swelling index.

$$\% \text{ SI} = \frac{W2-W1}{W1} \text{ x } 100$$

Where W1 = weight of the dry film

W2 = weight of the hydrated film

Adhesion time

The adhesion time of the mucoadhesive buccal film is determined by measuring the duration it takes for the film to separate from a stuck surface. Therefore, to increase the bioavailability and extend the retention time of the delivery system, mucoadhesive films should maintain substantial adhesive contact with the membrane. A longer adhesion time is generally desirable, as it can lead to more consistent and prolonged drug delivery. The adhesion time of mucoadhesive nicotine buccal film was determined manually by attaching the film to a moist surface to simulate buccal mucosa and the time of detachment was recorded.

Mucoadhesive strength

The mucoadhesive strength is determined by force required to detach the film from the buccal mucosa. It was measured using a texture analyzer (Stable microsystems ltd., Surrey, UK) and goat buccal mucosa as the substrate. The buccal mucosa was affixed to the stationary stage of the analyzer and a 2 cm x 2 cm film was attached to the probe. The movable probe was gradually brought down until it made contact with the mucosa, which was maintained for 1 min at the hold position. The probe was released at a pre-specified speed (0.5 mm/s) and the force required to detach the film from the mucosa was recorded.

Drug content uniformity

Drug content is an important factor in ensuring the availability and uniformity of drug in a film. A 2 cm x 2 cmpiece of the developed film was cut and placed in a beaker containing 100 ml of phosphate buffer pH 6.8. The film was allowed to dissolve, the resulting solution was filtered and analyzed using a UV spectrophotometer (Shimadzu, 1700) at 260 nm.

$$\text{Drug content} = \frac{\text{Actual amount of drug in the film}}{\text{Theoretical amount of drug in the film}} \text{ X } 100$$

***In vitro* drug release**

The *in vitro* drug release studies of the formulated mucoadhesive nicotine buccal film were carried out using a paddle-over disc (65 mm) dissolution apparatus (Electrolab dissolution apparatus) to determine the amount of nicotine released into the medium. In the dissolution vessel, the mucoadhesive buccal film was placed beneath the disc containing 900 ml phosphate buffer pH 6.8. The test was conducted at 37 °C with a 50-rpm speed, and aliquots of 5 ml were taken out at predetermined intervals for 360 min. After being filtered through a membrane filter (0.45 µm), an equal volume of fresh, pre-warmed (37±0.5 °C) phosphate buffer media was added to the dissolution vessel. The absorbance of aliquots was measured using a UV spectrophotometer (Shimadzu, 1700) at 260 nm, and the cumulative % drug release was calculated.

***Ex-vivo* adhesion time**

The *ex-vivo* adhesion time of the mucoadhesive nicotine buccal film was determined by evaluating its adhesion to fresh goat buccal mucosa. The mucosa was affixed to a glass slide using adhesive. The film was adhered to buccal mucosa by pressing lightly with a fingertip after it had been moistened on one side with phosphate buffer pH 6.8. The glass slide was placed in a beaker containing 50 ml of phosphate buffer pH 6.8. The retention of the buccal film was observed after two minutes when the contents of the beaker were gently agitated to simulate the conditions of the buccal cavity. The time required for the film to detach from the mucosal surface was used to assess the mucoadhesion time [24].

***Ex-vivo* drug permeation**

In this study, the goat buccal mucosa was used as a barrier membrane. The buccal mucosa from a freshly sacrificed goat was purchased from a nearby slaughterhouse and rinsed in isotonic phosphate buffer pH 6.8. The goat buccal mucosa (total exposed area 2.54 cm^2) was mounted on a diffusion cell between the donor and receptor compartment. The mucoadhesive film then adhered to the goat buccal mucosa. The receptor compartment was filled with phosphate buffer pH 6.8 and maintained at a temperature of 37±0.5 °C. The buffer solution was constantly stirred using a magnetic stirrer set at a low speed of 50±5 rpm. Aliquots of 5 ml were taken at predetermined intervals for 360 min, filtered through a 0.45 µm membrane filter, and analyzed using a UV spectrophotometer (Shimadzu, 1700) at 260 nm. Pre-warmed (37±0.5 °C) dissolution medium was added to the diffusion cell after each sample withdrawal. The experiment was carried out in triplicate (n=3), and the mean value was calculated for the determination of *ex-vivo* drug permeation.

RESULTS AND DISCUSSION

Optimization of mucoadhesive nicotine buccal film

The optimization of mucoadhesive nicotine buccal film was performed using box behnken response surface design with three independent variables such as carbopol 934 (A), Eudragit RLPO (B), and HPMC E15 (C). The ranges of independent variables were selected for optimization were 1.5 %-2.5 % of carbopol 934, 1.5 %-2.5 % of eudragit RLPO and 3.75 %-6.25 % of HPMC E15. The swelling index (R1), adhesion time (R2), mucoadhesive strength (R3), cumulative % drug release at 5 min (R4), 15 min (R5), 30 min (R6), 60 min (R7), 120 min (R8), 240 min (R9), 360 min (R10) of prepared mucoadhesive

nicotine buccal film were rendered as response variables, and the effects of independent variables studied are shown in table 4.

It was found that the swelling index increased with an increase in the concentration of HPMC E15 and carbopol 934 but there was no significant effect of eudragit RLPO as shown in fig. 3 and fig. 4. It is because HPMC E15 and carbopol 934 are hydrophilic polymers that absorb water from the surrounding environment, increasing the size and volume of the film which results in slower drug release [25]. It was found that the adhesion time increased with an increase in the concentration of HPMC E15 and carbopol 934 (fig. 5 and fig. 6). The HPMC E15 forms a gel in an aqueous environment that adher to mucosal membrane for a longer period, whereas carbopol 934 adhere to the mucosal membrane due to increased surface charge of the film, the concentration of eudragit RLPO showed no substantial effect on adhesion of film [26]. It was observed that the mucoadhesive strength increased with an increase in the concentration of HPMC E15 and carbopol 934(fig. 7 and fig. 8). The HPMC E15 increases the mechanical strength and flexibility of the film, while carbopol 934 shows better interaction with mucosal tissue due to increased surface charge creating a stronger bond, [27]. It was observed that the concentration of carbopol 934, eudragit RLPO, and HPMC E15 affected the cumulative % drug release (R4-R10) at 5 min, 15 min, 30 min, 60 min, 120 min, 240 min, and 360 min respectively. The drug release decreased with an increase in the concentration of the polymers (fig. 9 to fig. 22). The HPMC E15 forms a matrix with the drug, prolonging its release [25, 27]. The carbopol 934 and eudragit RLPO are pH-sensitive polymers due which the release of nicotine was slightly decreased with an increase in their concentration [26, 28]. The initial burst release of the mucoadhesive buccal film varied depending on the viscosity and solubility of the polymers, while the prolonged release was solely determined by the quantity of polymers used [29]. Therefore, the optimization process helped in identifying the optimal concentration of polymers that provide the desired properties of the mucoadhesive buccal film as shown in table 6.

Table 3: Polynomial equation for the response variable

Response variables	Polynomial equation
Swelling index (R1)	$182.18–2.94A–1.21B+21.05C–0.4075AB+7.82AC+0.9150BC+0.0663A^2–2.55B^2–10.77C^2$
Adhesion time (R2)	7.30+0.1250A+0.0937B+0.4063C
Mucoadhesive strength (R3)	$0.1669+0.0080A+0.0012B+0.0631C+0.0012AB+0.0000AC+0.0012BC–0.0013A^2+0.0024B^2+0.0061C^2$
Cumulative % drug release at 5 min (R4)	$6.73–2.79A–0.7958B–3.98C+4.38AB+2.79AC–1.99BC+9.35A^2+8.55B^2–1.79C^2$
Cumulative % drug release at 15 min (R5)	$15.95–2.00A–0.9993B-8.58C+5.59AB–1.58AC+1.18BC+10.20A^2+7.41B^2+0.1890C^2$
Cumulative % drug release at 30 min (R6)	$38.28–4.39A+1.19B–6.41C+5.60AB–1.60AC+1.60BC+0.4540A^2+4.42B^2–5.17C^2$
Cumulative % drug release at 60 min (R7)	$52.72–3.82A–1.19B–9.77C+4.01AB+2.40BC+6.56A^2+0.6321B^2–7.80C^2$
Cumulative % drug release at 120 min (R8)	$65.54+1.37A–2.39B–7.41C+2.40AB+0.3737AC-0.7825BC+6.60A^2+3.79B^2–0.6428C^2$
Cumulative % drug release at 240 min (R9)	$74.10+0.0080A–4.39B–4.82C+3.20AB+0.7980AC+0.7915BC+6.53A^2+4.13B^2+1.72C^2$
Cumulative % drug release at 360 min (R10)	$93.25–0.7959A–2.81B–7.59C–0.7777AB–0.7915AC–2.38BC–0.1688A^2–0.6405B^2–3.04C^2$

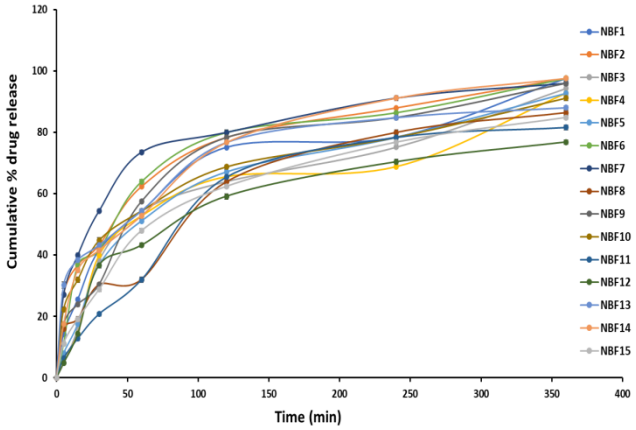

Fig. 2: *In vitro* drug release profile of mucoadhesive nicotine buccal film (NBF1 to NBF15). All the data shown were measured in mean±SD where n = 3

Table 4: Observed response of the optimization batches of mucoadhesive nicotine buccal film produced by Box-Behnken design

Batch No.	Swelling index (R1)	Adhesion time (R2)	Mucoadhesive strength (R3)	Cumulative % drug release						
				5 min (R4)	15 min (R5)	30 min (R6)	60 min (R7)	120 min (R8)	240 min (R9)	360 min (R10)
NBF1	144.11±0.94	7.00±0.23	0.11±0.01	14.32±0.12	25.54±0.19	41.52±0.17	54.34±0.09	75.10±0.10	78.40±0.08	97.51±0.10
NBF2	163.69±1.41	6.75±0.13	0.09±0.01	27.05±0.06	36.75±0.09	43.17±0.09	62.30±0.18	78.32±0.20	87.96±0.12	97.56±0.10
NBF3	181.82±1.89	7.25±0.15	0.16±0.01	4.77±0.10	15.94±0.14	38.28±0.18	54.32±0.34	63.96±0.32	75.15±0.44	94.31±0.30
NBF4	180.17±1.41	7.25±0.09	0.16±0.01	6.36±0.23	14.35±0.27	39.86±0.36	52.74±0.23	65.54±0.39	68.79±0.59	92.68±0.38
NBF5	184.55±1.38	7.25±0.13	0.16±0.02	7.95±0.73	17.55±0.43	36.70±0.56	51.13±0.63	67.12±0.56	78.35±0.56	92.74±0.55
NBF6	142.22±1.82	7.00±0.09	0.11±0.01	11.14±0.69	36.66±0.71	41.58±0.53	63.80±0.68	79.92±0.80	86.38±0.51	97.56±0.65
NBF7	184.40±0.84	7.25±0.22	0.16±0.01	27.05±0.65	39.38±0.71	54.33±0.49	73.51±0.57	79.98±0.61	91.15±0.44	95.99±0.64
NBF8	191.79±1.22	7.50±0.30	0.23±0.02	15.19±0.70	19.18±0.61	30.34±0.56	31.99±0.86	63.83±0.71	79.92±0.77	86.38±0.72
NBF9	179.30±0.85	7.50±0.35	0.17±0.01	17.50±0.59	23.97±0.73	30.37±0.60	57.46±0.62	78.30±0.73	84.78±0.68	95.95±0.71
NBF10	180.91±1.39	7.25±0.25	0.16±0.02	22.28±0.82	31.95±0.93	44.73±0.85	54.35±0.74	68.76±0.64	78.36±0.80	91.14±0.77
NBF11	194.91±1.15	7.75±0.23	0.24±0.02	6.36±0.37	12.76±0.66	20.76±0.63	31.94±0.62	65.42±0.65	78.34±0.65	81.60±0.76
NBF12	193.08±1.66	8.00±0.15	0.24±0.03	4.87±0.42	14.35±1.07	36.68±0.83	43.17±0.70	59.12±0.88	70.35±0.78	76.78±0.66
NBF13	174.18±1.27	7.50±0.53	0.17±0.01	30.23±1.01	38.36±0.81	43.18±0.80	54.35±0.57	76.69±0.40	84.77±0.78	88.00±0.66
NBF14	146.48±1.29	6.75±0.23	0.11±0.01	17.50±0.62	35.11±0.73	41.57±1.22	52.75±0.56	76.68±0.57	91.14±0.79	97.58±0.64
NBF15	185.10±1.30	7.50±0.23	0.22±0.02	11.14±0.66	19.16±0.72	28.75±0.70	47.90±0.72	62.33±0.48	76.73±0.80	84.77±0.56

All the data shown were measured in mean±SD where n = 3.

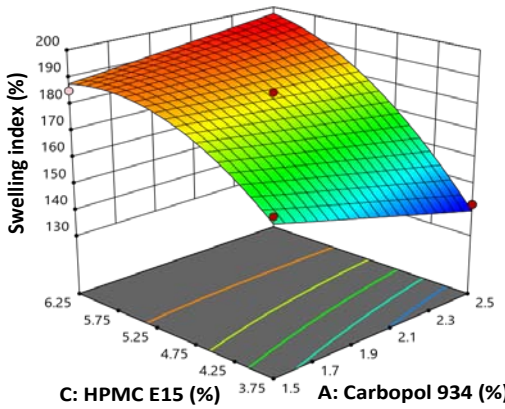

Fig. 3: 3D plot showing the effect of carbopol 934 and HPMC E15 on the swelling index

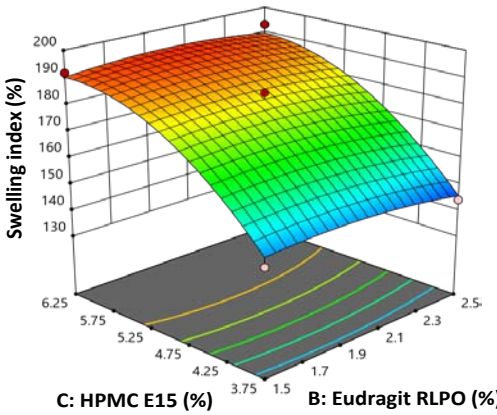

Fig. 4: 3D plot showing the effect of eudragit RLPO and HPMC E15 on the swelling index

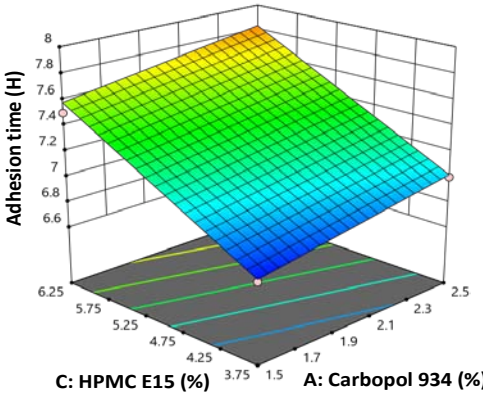

Fig. 5: 3D plot showing the effect of carbopol 934 and HPMC E15 on adhesion time

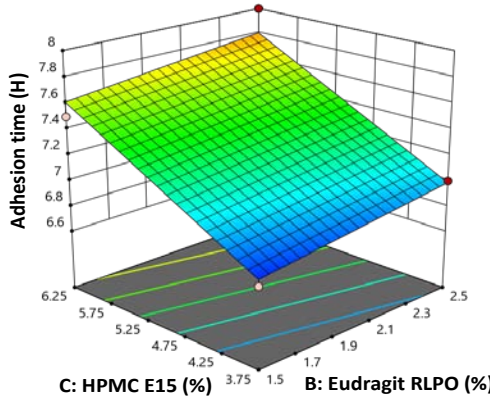

Fig. 6: 3D plot showing the effect of eudragit RLPO and HPMC E15 on adhesion time

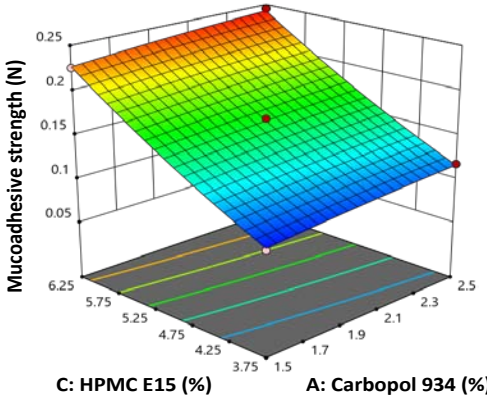

Fig. 7: 3D plot showing the effect of carbopol 934 and HPMC E15 on mucoadhesive strength

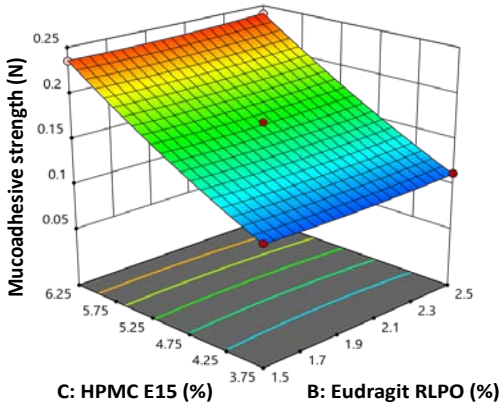

Fig. 8: 3D plot showing the effect of eudragit RLPO and HPMC E15 on mucoadhesive strength

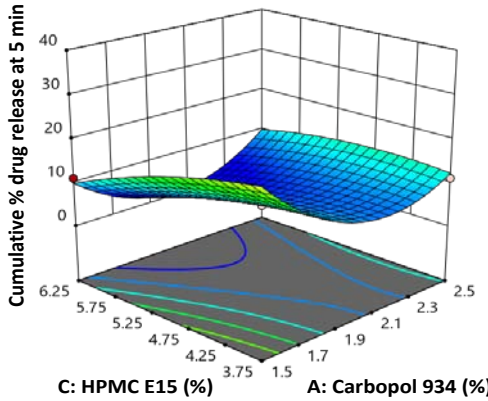

Fig. 9: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 5 min

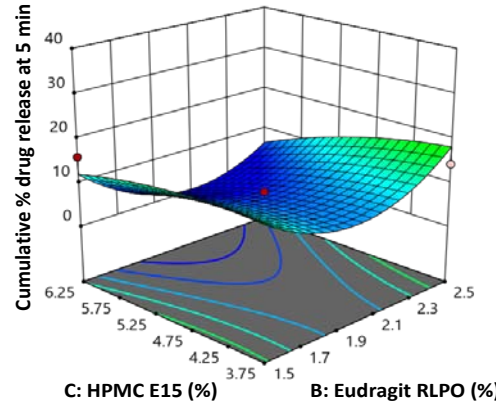

Fig. 10: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 5 min

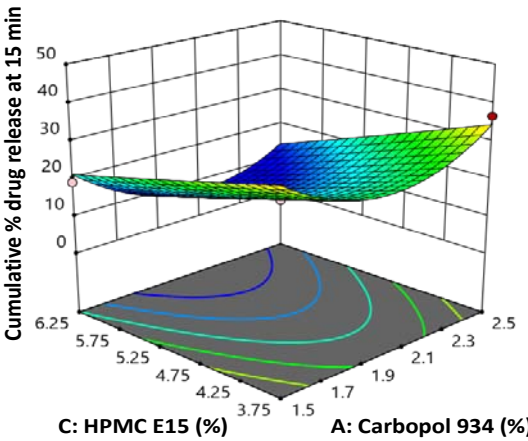

Fig. 11: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 15 min

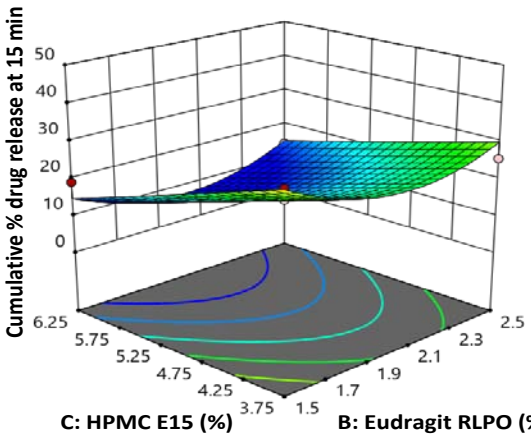

Fig. 12: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 15 min

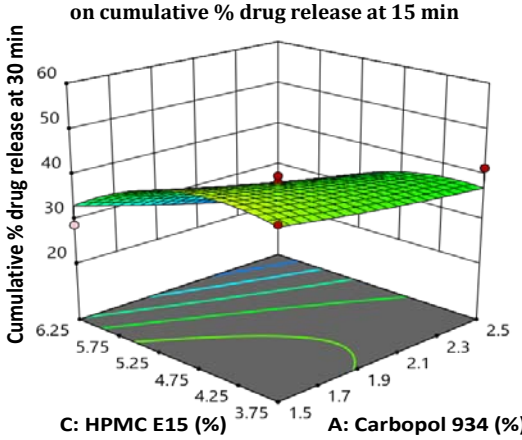

Fig. 13: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 30 min

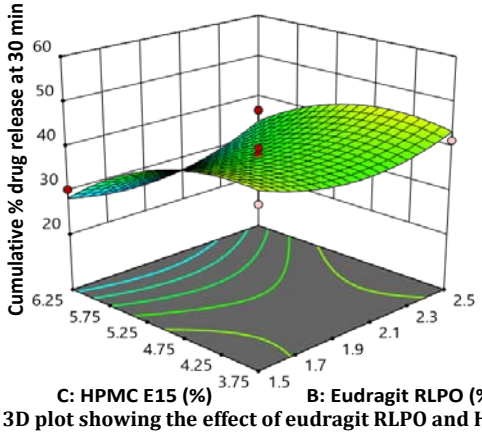

Fig. 14: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 30 min

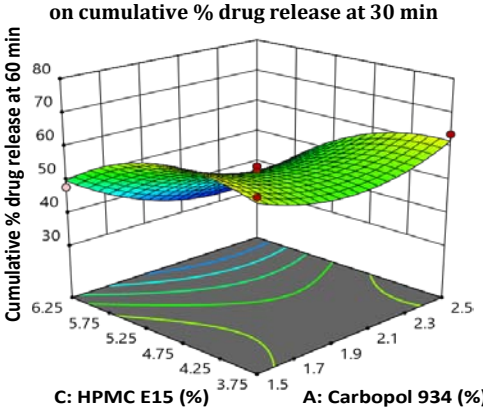

Fig. 15: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 60 min

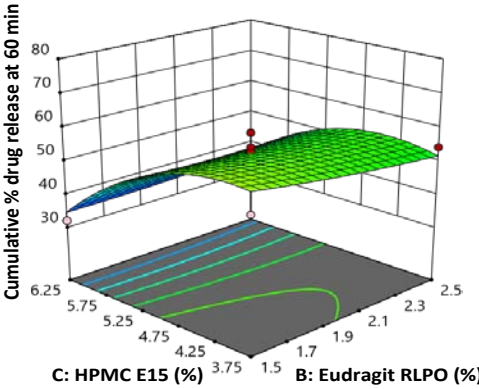

Fig. 16: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 60 min

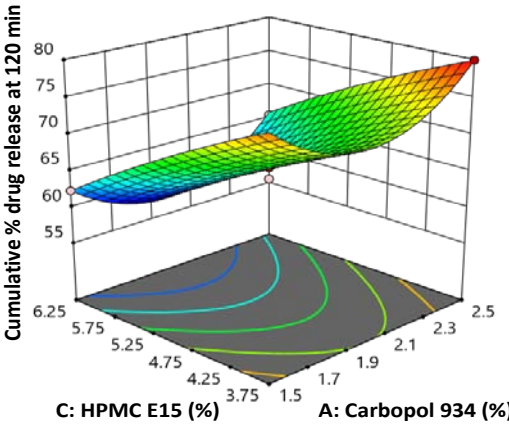

Fig. 17: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 120 min

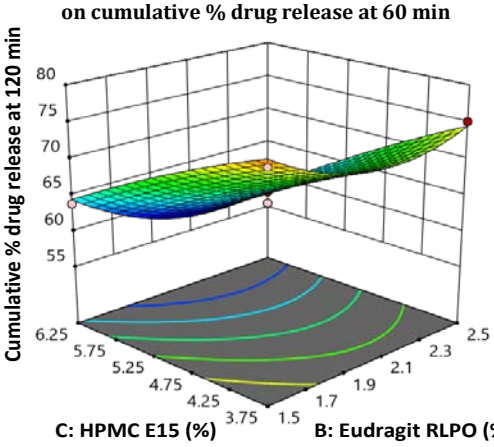

Fig. 18: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 120 min

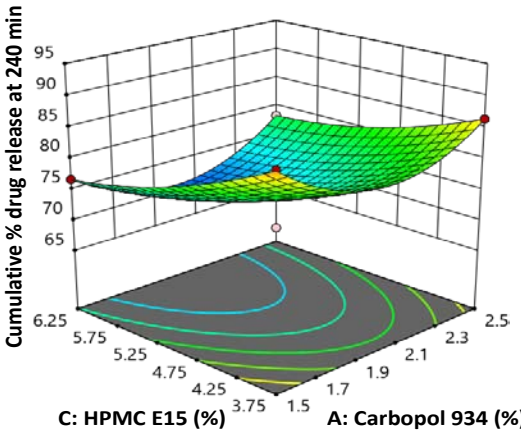

Fig. 19: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 240 min

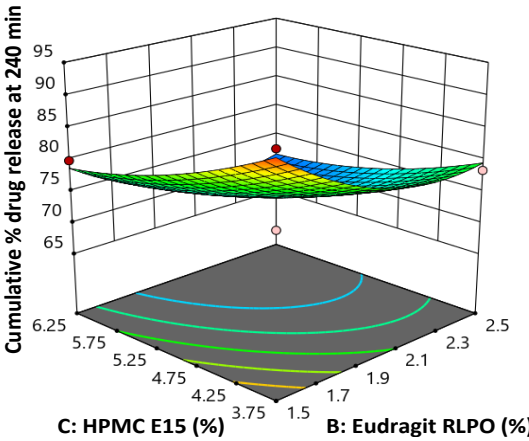

Fig. 20: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 240 min

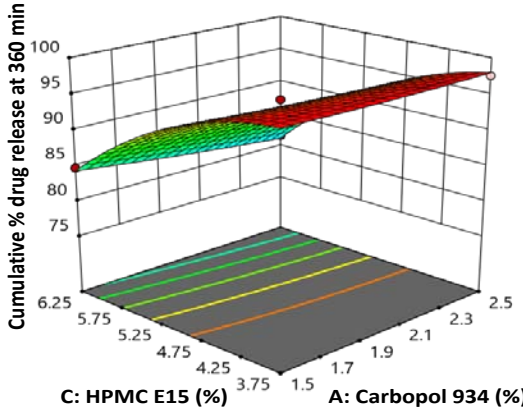

Fig. 21: 3D plot showing the effect of carbopol 934 and HPMC E15 on cumulative % drug release at 360 min

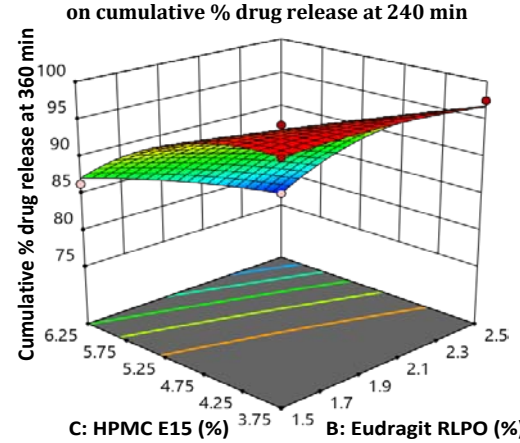

Fig. 22: 3D plot showing the effect of eudragit RLPO and HPMC E15 on cumulative % drug release at 360 min

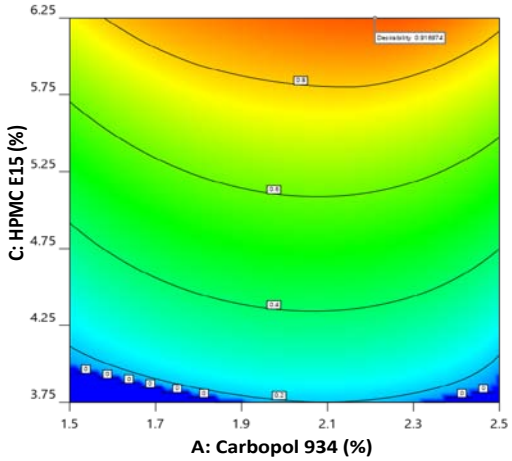

Fig. 23: Contour plot showing the maximum desirability of mucoadhesive nicotine buccal film

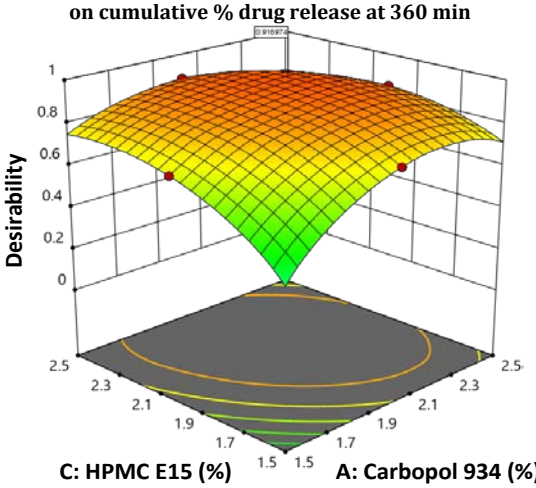

Fig. 24: 3D-response surface curve showing the maximum desirability of mucoadhesive nicotine buccal film

Table 5: ANOVA summary of response variables of mucoadhesive nicotine buccal film

Response variable	Model	Sequential *p*-value	Lack of fit *p*-value	Adjusted R^2 value	Predicted R^2 value
Swelling index (R1)	Quadratic	0.0124	0.2086	0.9567	0.7833
Adhesion time (R2)	Linear	<0.0001	-	0.8963	0.8301
Mucoadhesive strength (R3)	Quadratic	0.0354	0.3972	0.9964	0.9845
Cumulative % drug release at 5 min (R4)	Quadratic	0.0243	0.0606	0.6617	-0.8651
Cumulative % drug release at 15 min (R5)	Quadratic	0.0130	0.0873	0.8283	0.0686
Cumulative % drug release at 30 min (R6)	Quadratic	0.0969	0.0882	0.7419	-0.3992
Cumulative % drug release at 60 min (R7)	Quadratic	0.0938	0.0411	0.6881	-0.7400
Cumulative % drug release at 120 min (R8)	Quadratic	0.0011	0.5979	0.9587	0.8563
Cumulative % drug release at 240 min (R9)	Quadratic	0.0416	0.9153	0.7490	0.5606
Cumulative % drug release at 360 min (R10)	Quadratic	0.0071	0.5434	0.9803	0.9267

Prediction of optimized formulation of mucoadhesive nicotine buccal film

Statistical analysis was performed using the Design Expert software. The software provided a final optimized formula for the mucoadhesive nicotine buccal film with a desirability of 0.917. This formula met all the requirements for the properties of the final batch, such as maximum swelling index, adhesion time, mucoadhesive strength, and prolonged release of the drug. The mucoadhesive nicotine buccal films were prepared using predicted values and then evaluated. The results of this evaluation are shown in table 6. The graph of cumulative % drug release between the predicted and the observed responses showed that the predicted and observed responses were in close agreement with each other. The drug release profile of the predicted and observed batch of optimized mucoadhesive nicotine buccal film is shown in fig. 25. A linear regression plot between the predicted and observed drug release profile was also plotted for studying the correlation between them, as shown in fig. 26. The regression coefficient (R^2) was calculated as 0.9982 and the correlation coefficient was 0.9989, both of which were close to 1.

Table 6: Software predicted and experimentally observed variable response data of optimized mucoadhesive nicotine buccal film

Optimized formulation composition		Response			
Component	**Quantity**	**Evaluation parameter**	**Software predicted**	**Experimentally observed**	**Relative error (%)**
A = Carbopol 934	2.21 %	Swelling index	193.95	188.21±1.93	2.96
B = Eudragit RLPO	2.19 %	Adhesion time	7.79	7.75±0.22	0.59
C = HPMC E 15	6.25 %	Mucoadhesive strength	0.24	0.23±0.01	2.32
		Cumulative % drug release at 5 min	3.20	3.34±0.10	4.40
		Cumulative % drug release at 15 min	10.00	9.83±0.75	1.67
		Cumulative % drug release at 30 min	26.96	28.57±0.90	5.97
		Cumulative % drug release at 60 min	34.11	36.14±0.97	5.94
		Cumulative % drug release at 120 min	59.12	54.10±1.09	8.49
		Cumulative % drug release at 240 min	72.25	70.11±0.99	2.96
		Cumulative % drug release at 360 min	79.72	76.55±0.99	3.97

All the data shown were measured in mean±SD where n = 3

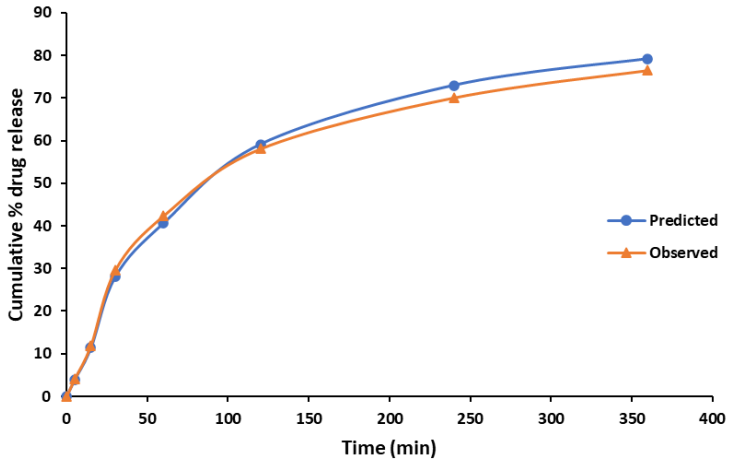

Fig. 25: Predicted and observed drug release profile of optimized mucoadhesive nicotine buccal film. All the data shown were measured in mean±SD where n = 3

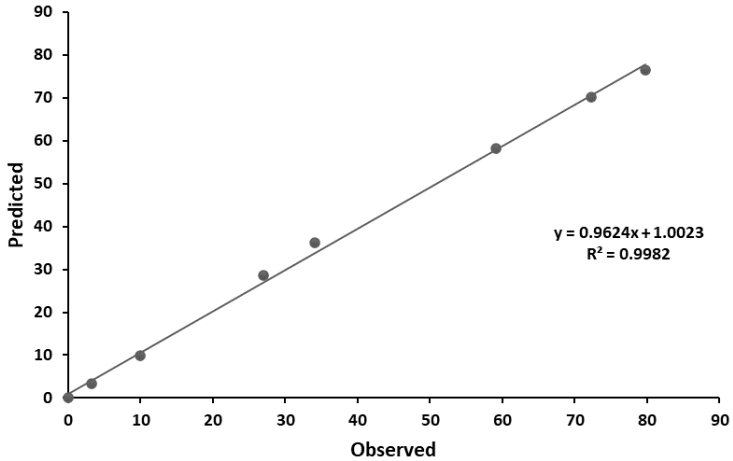

Fig. 26: Linear regression plot between predicted and observed drug release profile

Surface pH

The surface pH of the film was measured to determine the effect of pH on the buccal mucosa. It was found that the pH of the optimized buccal film was 7.54±0.10, which is neutral and suitable for the buccal mucosa, as it does not irritate. This indicates that the pH of the film is well-suited for the buccal mucosa and does not cause any discomfort [30].

Film weight and thickness

A mucoadhesive buccal film with consistent thickness and weight ensures a uniform drug concentration throughout the film. The optimized mucoadhesive nicotine buccal film was found to have a thickness of 0.18±0.01 mm, which is an optimal thickness for a buccal film because a film that is too thick may not dissolve as intended, resulting in slow drug release, while films that are too thin may dissolve too quickly and may not deliver an adequate amount of drug to be effective [25]. The weight of the optimized mucoadhesive nicotine buccal film (2 cm x 2 cm) was found to be 106.28±0.37 mg, which is uniform for buccal mucosa application and homogeneity [31].

Folding endurance

High folding endurance is a desirable characteristic in films as it ensures that they do not easily displace from the application site or break while being administered. The optimized mucoadhesive nicotine buccal film was found to have a folding endurance of 540-folds, indicating that it has high mechanical strength and resistance to breaking [32].

Tensile strength and extensibility

Soft and durable inserts that can withstand the continuous mechanical stress caused by jaw movement in the buccal cavity are preferred for buccal application. The tensile strength of the optimized mucoadhesive nicotine buccal film was observed to be 87.20 N and its extensibility was observed to be 23.69 mm as seen in fig. 27. The formulation exhibited high tensile strength and optimal elongation due to the high concentration of HPMC E15 and the presence of carbopol 934, respectively [33]. As reported in the literature for oral thin films of nicotine [34], the mucoadhesive buccal film showed higher mechanical strength, as indicated by its higher tensile strength and greater flexibility and elongation without breaking, as shown by its higher extensibility. These properties suggest that the film has better integrity and mechanical properties, making it a more durable and robust option for buccal drug delivery.

Swelling index

The swelling index is an important factor in drug delivery through mucoadhesive buccal film due to its ability to affect the rate of drug release. This is because water absorption-induced swelling allows initially twisted, stretched, or entangled bioadhesive polymers to relax, resulting in the rapid disentanglement of individual polymer chains and the formation of a specific-size macromolecular network that increases the porosity of the film and initiates drug release [35]. The swelling index of all the batches was found to be in the range of 142.22 % to 194.91 %, as recorded in table 4. The 3D response surface plots in fig. 3 and fig. 4 show the effect of independent variables on the swelling index of all batches. It was observed that the swelling index increased with an increase in the concentration of HPMC E15 and carbopol 934, as they are hydrophilic polymers that absorb water from the surrounding environment, increasing the size and volume of the film, but there was no significant effect of eudragit RLPO on the swelling index of the film. The swelling index of the optimized mucoadhesive nicotine buccal film was found to be 188.21±1.93 %, indicating a slow drug release profile, as a high swelling index is generally associated with a slower drug release, while a low swelling index is associated with a more rapid release. The results indicate that the developed mucoadhesive nicotine buccal film has a higher swelling index than nicotine thin films as reported in the literature [36]. The higher swelling index leads to a slower drug release profile and stronger mucoadhesion, suggesting that the mucoadhesive nicotine buccal film has better water uptake and film integrity. In contrast, nicotine thin films tend to disintegrate quickly and have a faster release rate.

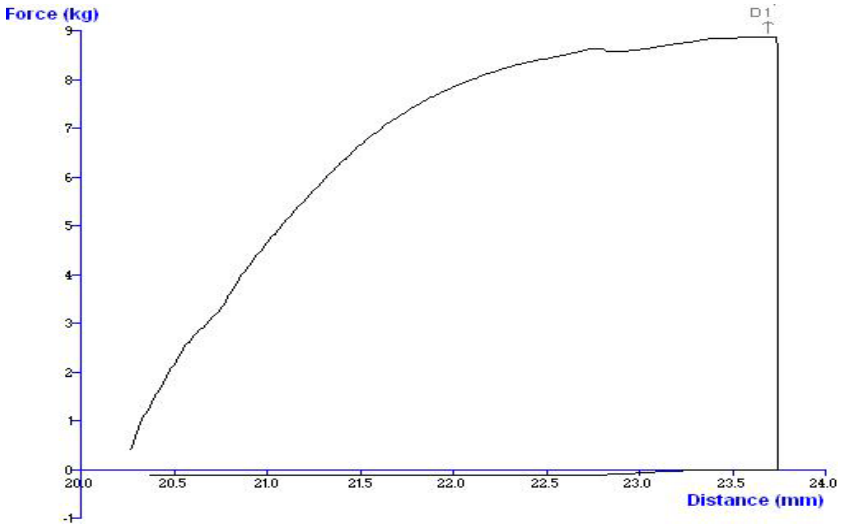

Fig. 27: Tensile strength and extensibility of optimized mucoadhesive nicotine buccal film

Adhesion time

Adhesion time is a measure of the effectiveness of mucoadhesive properties of film and can impact the rate and duration of drug delivery. A longer adhesion time allows for a slower release of the drug, while a shorter adhesion time results in a rapid release [37]. The adhesion time of all the batches was found to be in the range of 6 h to 8 h, as recorded in table 4. The 3D response surface plots in fig. 5 and fig. 6 show the effect of independent variables on the adhesion time of all batches. The results showed that the adhesion time increased with an increase in the concentration of HPMC E15 and carbopol 934 because HPMC E15 formed a gel that adhered for a longer period, whereas carbopol 934 increased the surface charge of the film, allowing it to adhere better. However, the varied concentration of eudragit RLPO had no substantial effect. The adhesion time of the optimized mucoadhesive nicotine buccal film was found to be 8 h approximately, indicating that the mucoadhesive film sustained a strong bond for an extended period, leading to a prolonged release of the drug. The developed mucoadhesive nicotine buccal film exhibits a higher adhesion time compared to 2-3 h adhesion time of nicotine mucoadhesive tablets and other mucoadhesive buccal films as reported in various literatures [16,

38]. This improved adhesion leads to enhanced efficacy, patient compliance, and convenience of nicotine replacement therapy by reducing the frequency of drug administration.

Mucoadhesive strength

Mucoadhesive strength is a measure of the adhesive force between a mucoadhesive buccal film and a mucous membrane. The mucoadhesive strength of all the batches was found to be in the range of 0.09 N to 0.24 N, as recorded in table 4. The 3D response surface plots in fig. 7 and fig. 8 show the effect of independent variables on the mucoadhesive strength of all batches. It was observed that the mucoadhesive strength increased with an increase in the concentration of HPMC E15 and carbopol 934. This is because HPMC E15 increases the mechanical strength and flexibility of the film, while carbopol 934 increases the surface charge on the film, thus allowing better interaction with the negatively charged mucosal tissue and creating a stronger bond [39]. However, the concentration of eudragit RLPO did not have a significant impact on the mucoadhesive strength. The mucoadhesive strength of the optimized mucoadhesive nicotine buccal film was found to be 0.23±0.01 N, which is higher than nicotine wafer and film formulations as reported in literature [40]. This allows it to maintain high drug concentration and prevent displacement, resulting in more effective nicotine delivery, as well as enhancing patient compliance and convenience by reducing the frequency of drug administration.

Drug content uniformity

The uniformity of drug content is an important aspect of pharmaceutical quality control to ensure that the drug is distributed evenly throughout the formulation. The drug content of the optimized mucoadhesive nicotine buccal film was found to be 96.13±1.71 %. The result indicates that the nicotine was dispersed uniformly throughout the buccal film, with a very low and acceptable standard deviation [35].

***In vitro* drug release studies**

The *in vitro* drug release studies of formulated batches were carried out in dissolution apparatus using phosphate buffer pH 6.8 as the dissolution medium. The cumulative % drug release of all batches ranged from 4.77 % to 30.23 % at 5 min and 76.78 % to 97.58 % at 360 min, as recorded in table 4 and shown graphically in fig. 2. All formulated batches showed an initial burst release of nicotine, which was followed by a prolonged release. The initial burst effect varied depending on the viscosity of the polymers. The 3D response surface plots from fig. 9 to fig. 22 show the effect of the independent variables on the cumulative % drug release for different time intervals for all batches. It was observed that the drug release decreased with an increase in the concentration of HPMC E15, this is because the matrix formed by the polymer entrapped the drug and prolonged its release [25]. However, the drug release slightly decreased with an increase in the concentration of carbopol 934 and eudragit RLPO. It is because carbopol 934 absorbs water and swells, which affects the release of the drug from the film, and eudragit RLPO alters the release of nicotine as it is a pH-sensitive polymer. The *in vitro* drug release study of the optimized batch of mucoadhesive nicotine buccal film showed a cumulative % drug release of 76.55±0.99 % at 360 min as shown in fig. 28. This infers that it has an initial burst release followed by a prolonged release. The *in vitro* drug release studies of nicotine lozenges and transdermal patches have been reported in various literature [41, 42]. However, the developed mucoadhesive nicotine buccal film exhibited both rapid onset of action and prolonged release, setting it apart from the lozenge and transdermal patch formulations which provide only either fast or prolonged action.

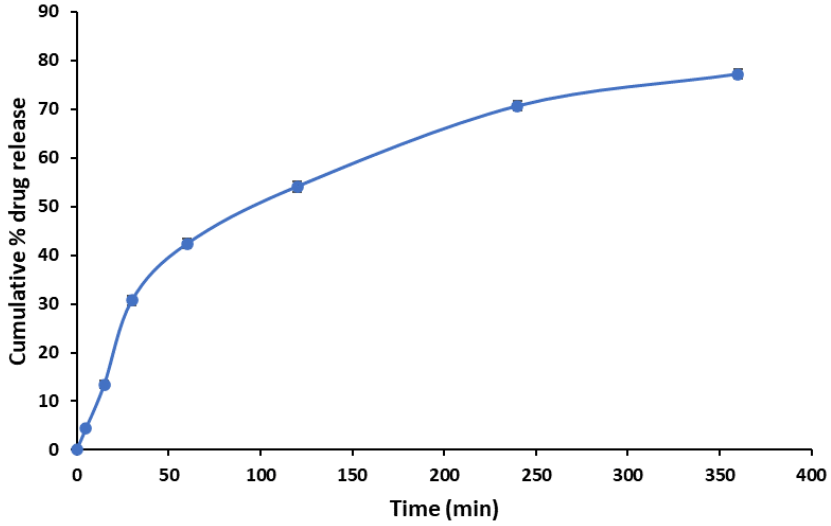

Fig. 28: *In vitro* drug release profile of optimized mucoadhesive nicotine buccal film. All the data shown were measured in mean±SD where n = 3

***Ex-vivo* adhesion time**

The *ex-vivo* adhesion time of the optimized mucoadhesive nicotine buccal film was measured to be 6 h approximately, which was found to be in concurrence with the previously reported studies on mucoadhesive buccal films [29]. This is significant as prolonged adhesion time is desirable in nicotine replacement therapy, allowing for consistent delivery of nicotine over an extended period which can increase its effectiveness in aiding smoking cessation. This is due to the properties of HPMC E15, which is a hydrophilic polymer that swells significantly upon absorbing water, and is less affected by changes in hydration, thereby maintaining its structure well and prolonging the release of the entrapped drug. The increased viscosity of HPMC also resulted in the formation of a surface gel that remained in place for a long time, contributing to the extended adhesion time of the film. Further, higher carbopol content increases the surface charge on the film, allowing it to interact better with the negatively charged mucosal tissue, creating a stronger bond [27, 43].

***Ex-vivo* permeation**

Ex-vivo permeation studies were conducted to determine the kinetics of drug absorption through a biological membrane. The result indicates that the optimized mucoadhesive nicotine buccal film had a 2-fold higher drug permeation rate (0.56 mg or 37.38 %) compared to the lozenge (0.28 mg or 19.29 %) over 360 min. The optimized film also permeated a higher percentage of the drug (95.09 %) compared to the lozenge (49.07 %) over the same

period. The swelling properties of HPMC E15, the surface charge of carbopol, and the pH-sensitive nature of eudragit RLPO contributed to the higher drug permeation of the optimized film, as shown in fig. 29. The *ex-vivo* permeation study of marketed lozenge is reported in various literature [44]. However, the use of mucoadhesive nicotine buccal film has an advantage over the marketed lozenge in terms of higher drug delivery efficiency through the biological membrane.

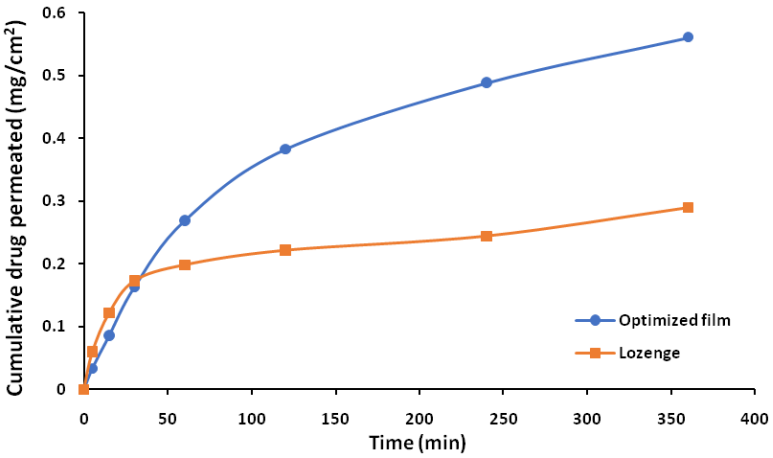

Fig. 29: Comparison of cumulative drug permeated from optimized mucoadhesive nicotine buccal film and lozenge. All the data shown were measured in mean±SD where n = 3

Kinetic release assessment

The *in vitro* drug release data for the optimized mucoadhesive nicotine buccal film were analyzed using different mathematical models such as zero order, first order, Higuchi, Korsmeyer-peppas, and Hixson-crowell. The best fit in the drug release kinetic model was selected based on the highest regression coefficient (R^2) value. It was found that *in vitro* drug release kinetic followed the Higuchi model with the highest regression coefficient (R^2) of 0.9679 as recorded in table 7 and plotted in fig. 30. This implies that the drug release mechanism from the film follows a diffusion-controlled process, where the rate of drug release is directly proportional to the surface area available for dissolution, providing a rapid onset of drug release followed by a prolonged release [45]. The results were found in agreement with previously reported work done on nicotine fast-dissolving film and nicotine mucoadhesive tablets which respectively provided either a fast onset of action or prolonged release [38, 46]. However, the developed mucoadhesive nicotine buccal film combines the benefits of both fast onset and prolonged action, making it a better option in terms of drug release kinetics.

Table 7: Drug release kinetic data and model fitting of optimized mucoadhesive nicotine buccal film

S. No.	Drug release kinetic model	Equation	K	R^2
1.	Zero-order	$Q_0 - Q_t = k_0 t$	2.09 x 10^{-1} mol/min	0.8452
2.	First order	$\log Q = \log Q_0 - kt/2.303$	-1.80 x 10^{-3} mol/min	0.9494
3.	Higuchi	$Q_0 = Q_t = kt^{1/2}$	4.43 x 10 ° mol/min	0.9679
4.	Korsmeyer-Peppas	$\log (Q_0 - Q_t) = \log k - n \log t$	7.72 x 10^{-1} mol/min	0.9640
5.	Hixson-Crowell	$Q_0^{1/3} - Q_t^{1/3} = kt$	5.0 x 10^{-3} mol/min	0.9196

Q_o is initial drug concentration; Q_t is the amount of drug remaining at a specific time; k is rate constant; t is time.

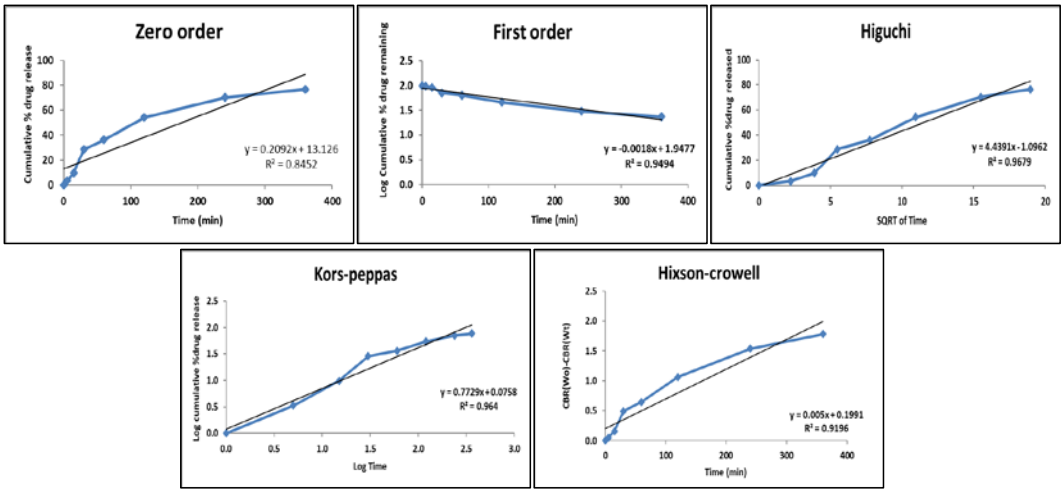

Fig. 30: Kinetic graphs of *in vitro* drug release of optimized mucoadhesive nicotine buccal film

CONCLUSION

In the present study, the mucoadhesive nicotine buccal film was formulated by solvent casting method and optimized by box-behnken experimental design. The film was evaluated for its swelling index, adhesion time, mucoadhesive strength, and cumulative % drug release. The effect of independent variables on response variables was determined using 3D surface plots and polynomial equations. The optimized formulation was prepared based on the evaluation data and desired constraints of response variables, resulting in the highest desirability. The optimized film demonstrated a prolonged adhesion time and better contact with the buccal mucosa, resulting in a rapid onset of action followed by prolonged release. The *in vitro* drug release study showed a release of 76.55 % in 6 h. The *ex-vivo* study of developed mucoadhesive nicotine buccal film revealed a 2-fold enhancement in buccal mucosal permeation compared to a lozenge. This inferred that the optimized mucoadhesive nicotine buccal film can be an effective tool for smokers seeking to quit, increasing the likelihood of achieving and maintaining a smoke-free lifestyle.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

The authors hereby declare that there is no conflict of interest and other support.

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