Research Article

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Applying design of experiments (DoE) on the properties of buccal film for nicotine delivery

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Abstract: Design of experiments is used to optimize ratios between deproteinized natural rubber latex, Eudragit® NM 30 D, and pectin for nicotine buccal film with dependent variables as moisture content, moisture uptake, and swelling index in simulated saliva 3 and 5 h. Mathematical models were linear for moisture content and moisture uptake, while swelling index in simulated saliva 3 and 5 h was a quadratic model. Optimized polymer ratio was 0.319:0.362:0.319, respectively. Experimental values were $13.17 \pm 0.92\%$, $3.96 \pm 0.84\%$, $112.58 \pm 22.63\%$, and $124.69 \pm 8.01\%$ for dependent variables, respectively. The buccal film showed high swelling at pH 7 and swelling–deswelling behaviors in a water/ethanol environment. The surface pH, weight, and thickness were 8.11, 63.28 ± 6.18 mg, and 219.87 ± 44.28 μm , respectively. Nicotine content was found as 10.22 ± 0.46 mg/4 cm². Maximum cumulative nicotine release was 9.82 ± 0.94 mg/4 cm². Kinetic model fitted to the Korsmeyer-Peppas model and release exponent was 0.36, representing that release mechanism was controlled by Fickian diffusion release.

Keywords: 3D response surface, contour plot, design of experiments, buccal film, nicotine delivery

1 Introduction

Nicotine is an active alkaloid drug found in tobacco smoke. Most of the toxicity of smoking is mortality and morbidity caused by other components in tobacco products; however, the nicotine may induce to addiction of tobacco (1,2). Nicotine replacement therapy is used for withdrawal of the behavior of taking the tobacco that affects both the physiological and psychomotor functions (3,4). The scientific evidence and clinical guideline accept and recommend nicotine replacement therapy as the first choice for people seeking help to stop smoking (4). Many formulations for nicotine replacement therapy are currently developed and used such as transdermal patches (5–9), film-forming polymeric solutions (10,11), nasal sprays (12,13), chewing gums (14,15), oral inhalers (16,17), and tablets (18,19). The transdermal patch is the best dosage form for nicotine replacement therapy products compared with other dosage forms because it is widely and easily used to facilitate the cessation of smoking and is applied once a day, usually used at the same time each day. However, it may induce skin irritation from the adhesive tape or any ingredients (20).

The delivery of nicotine via oral mucosa is increasingly accepted and arising interest due to high vascularity, no sensitivity to irritation, and low enzyme activity. Moreover, this can avoid gastric acid, the enzymes in the small intestine, and the first-pass metabolism in the liver (21,22). The release of nicotine is controlled by matrix film and oral mucosal. Thus, the desired polymer used as a matrix film should have high adhesion, good film-forming abilities, water-solubility, good wetting, neutrality, non-toxicity, non-immunogenicity, biodegradability, etc. Many polymers, such as hydroxypropyl methylcellulose, sodium alginate (23), and maltodextrin (24), can be prepared and utilized in the buccal nicotine delivery systems that have been investigated as film and wafer formulations. Their functional properties can be improved when different types of polymers were blended.

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Natural rubber latex presents interesting physical properties such as high tensile strength, high elongation at break, and easy film-forming. It can be used as controlled release matrix films (6,25) and matrix tablets (26,27), and also in biomedical applications (28,29). Deproteinized natural rubber latex (DNRL) is a rubber latex that removed the allergic protein. DNRL has high flexibility and easily produces the film. Eudragit® NM 30 D is an aqueous colloidal dispersion of a neutral polymethacrylate used for pharmaceutical dosage form for controlled release products. Its property is high flexibility after producing the film (30). Pectin is a hydrophilic natural polymer that has been widely used in the pharmaceutical development of buccal drug delivery systems as a mucoadhesive polymer (31,32). It is a major component of a complex heterogeneous polysaccharide found in the primary cell walls and middle lamella in plant tissues. It has flexibility and strong mechanical properties. Therefore, DNRL, Eudragit® NM 30 D, and pectin are interesting to produce the buccal film for nicotine delivery. The blending of three polymers has not been previously investigated and reported for nicotine delivery.

Therefore, the aim of this project was a preparation of buccal film for nicotine delivery using the blending between three polymers, DNRL, Eudragit® NM 30 D, and pectin, as a polymer matrix film, and glycerin was used as a plasticizer at a concentration of 30% w/w depending on the polymer amount. The optimum ratio of the amount of three polymers was predicted by the design of experiments (DoE) method using Design-Expert® program version 11 (Stat-Ease, Inc, USA) in terms of moisture content, moisture uptake, and swelling index in simulated saliva solution. The obtained optimized formula was evaluated and studied the *in vitro* release of nicotine from the buccal film. The kinetics of *in vitro* nicotine release was calculated from the DDSolver program and reported.

2 Experimental

2.1 Preparation of buccal film for nicotine delivery

Concentrated nicotine solution (Merck, Germany) was diluted in distilled water (0.2% w/w) and then slowly dropped in polymeric solution. The glycerin (P C Drug Center Co., Ltd., Thailand) was used as a plasticizer at 30% w/w depending on the polymer content that was a control variable for the buccal film. The polymeric

mixture of the buccal film was composed of DNRL (prepared from pichayakorn group (33,34)), Eudragit® NM 30 D (Jebesen & Jessen Ingredients (T) Ltd., Thailand), and pectin (VR Bioscience Co., Ltd, Thailand), which were used as independent variables of the response surface methodology (Table 1). Briefly, the fresh NRL collected from the rubber tree (*Hevea brasiliensis*) is deproteinized by 0.2 phr alcalase enzyme, stabilized by 1% sodium dodecyl sulfate, preserved by 2% Uniphen P-23, and incubated at $37 \pm 2^\circ\text{C}$ for 48 h. The DNRL is washed with distilled water and centrifuged 3 times. Finally, the DNRL is redispersed in distilled water. The prepared DNRL is safe for the skin as confirmed by our research group (5,33). The mixture polymer solution was poured in a petri dish at 25 g. The dried films were produced at $80 \pm 2^\circ\text{C}$ in a hot air oven. Moisture content (Y_1), moisture uptake (Y_2), and swelling index in simulated saliva solution (Y_3 and Y_4) were optimized and predicted by the Design-Expert® program (Stat-Ease, Inc, USA).

2.2 Optimization of properties of buccal film for nicotine delivery

2.2.1 Moisture content measurement (Y_1)

The sample of the buccal film was accurately weighed about 1.0 g in an aluminum pan. Each sample was initially heated at 120°C using a moisture analyzer (MAC 50/NH, Poland). The percentage of moisture content was measured and calculated according to Eq. 1. The results were tested in five replicates with the obtained mean result.

Table 1: The independent variables of the response surface methodology

Run	Independent variables		
	X_1	X_2	X_3
	A = DNRL	B = Eudragit® NM 30 D	C = Pectin
1	1.00	0	0
2	0	1.00	0
3	0	0	1.00
4	0.50	0.50	0
5	0.50	0	0.50
6	0	0.50	0.50
7	0.67	0.17	0.17
8	0.17	0.67	0.17
9	0.17	0.17	0.67
10	0.33	0.33	0.33

$$\text{Percentage of moisture content} = \frac{W_{\text{in}} - W_{\text{dr}}}{W_{\text{dr}}} \times 100 \quad (1)$$

where W_{in} and W_{dr} were the weight of the buccal film at an initial and dried sample.

2.2.2 Moisture uptake measurement (Y_2)

The 2 cm × 2 cm square size of the buccal film sample was initially weighed and stored in a desiccator at room temperature under 75% RH environment that equilibrated with sodium chloride solution. The percentage of moisture uptake was calculated according to Eq. 2 (35). The results were tested in five replicates with the obtained mean result.

$$\text{Percentage of moisture uptake} = \frac{W_{\text{co}} - W_{\text{in}}}{W_{\text{in}}} \times 100 \quad (2)$$

where W_{in} and W_{co} were the weight of the buccal film at an initial and constant sample.

2.2.3 Swelling index in simulated saliva solution (Y_3 and Y_4)

The 2 cm × 2 cm square size of the buccal film sample was initially weighed. Each sample was immersed in simulated saliva solution at room temperature. The simulated saliva solution was prepared from 0.19 g of potassium dihydrogen phosphate [KH_2PO_4], 2.38 g of disodium hydrogen phosphate [Na_2HPO_4], and 8.00 g of sodium chloride [NaCl] dissolve in distilled water up to 1 liter and adjusted the pH to 6.8 by phosphoric acid (36,37). The percentage of the swelling index was calculated according to Eq. 3 (38,39). The results were tested in five replicates with the obtained mean result.

$$\text{Percentage of swelling index} = \frac{W_{\text{sw}} - W_{\text{in}}}{W_{\text{in}}} \times 100 \quad (3)$$

where W_{in} and W_{sw} were the weight of the buccal film at an initial and swollen sample.

2.3 Swelling measurement of nicotine buccal film

The 2 cm × 2 cm square size of the buccal film sample was initially weighed. Each sample was immersed in various pH solutions. Sodium hydroxide and hydrogen chloride solution at a concentration of 1 mol/L were used to adjust

the pH solution to 2, 4, 7, and 10. The ratio of water absorption amount was calculated according to Eq. 4 (40,41). The results were tested in five replicates with the obtained mean result.

$$\text{Ratio of water absorption amount} = \frac{W_{\text{sw}} - W_{\text{dr}}}{W_{\text{dr}}} \quad (4)$$

where W_{dr} and W_{sw} were the weight of the buccal film at a dried and swollen sample.

2.4 Study of water absorption properties of nicotine buccal film

The 2 cm × 2 cm square size of the buccal film sample was initially weighed and transferred into a test tube that was filled with distilled water until the swollen buccal film sample (W_s) was obtained. The swollen buccal film sample was subsequently moved to immerse in ethanol at room temperature until the equilibrium point of the buccal film sample (W_n) was obtained. The relative gel volume was presented as swelling and deswelling behaviors of the buccal film sample following Eq. 5 (42). The results were tested in five replicates with the obtained mean result.

$$\text{Relative gel volume} = \left(\frac{W_n}{W_s} \right)^3 \quad (5)$$

2.5 Surface pH measurement of nicotine buccal film

The 1 cm × 1 cm square size of the buccal film sample was initially contacted with distilled water 1 mL in glass tubes. The excess distilled water was removed. The pH of buccal film at the surface area was determined by pH meter and maintained the electrode on the wetted surface of the buccal film to equilibrate for 1 min (43,44). The results were recorded in five replicates with the obtained mean result.

2.6 Weight measurement of nicotine buccal film

The 1 cm × 1 cm square size of the buccal film sample was weighed by analytical balance. The results were recorded in five replicates with the obtained mean result.

2.7 Thickness measurement of nicotine buccal film

The 1 cm × 1 cm square size of the buccal film sample had measured the thickness using a micrometer. The results were recorded in five replicates with the obtained mean result.

2.8 Determination of nicotine content in the buccal film

The 2 cm × 2 cm square size was cut from five different sites on the buccal film sample. Each buccal film sample was cut in small sizes and transferred into a test tube filled with 5 mL of distilled water. The buccal film sample was sonicated for 30 min to extract the nicotine content. The solution was diluted and analyzed by UV spectrophotometer (UV-1800, SHIMADZU) using wavelength of maximum absorbance ($\lambda_{\max}$) at 260 nm. The obtained absorbance values were compared with the calibration curve of nicotine standard ($y = 0.148x + 0.0876$, $R^2 > 0.9992$).

2.9 *In vitro* release of nicotine from buccal film

The 2 cm × 2 cm square size of the buccal film sample was applied on the diffusion cell of the modified Franz-type cell (Hanson[®] 57-6 M, USA). The area of the donor compartment for the diffusion of the drug was 1.77 cm². The partition layer between the donor compartment and receptor compartment was a cellulose membrane (CelluSep[®] T4, Membrane Filtration Product, Inc., USA). The receptor medium was 12 mL of simulated saliva solution. The receptor medium was equilibrated at 37 ± 0.5°C and stirred constantly at 100 rpm. One mL of simulated saliva solution pH 6.8 was withdrawn from the receptor compartment at 0.25, 0.50, 0.75, 1, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 h, and each withdrawn sample was replaced by fresh simulated saliva solution pH 6.8. The amount of nicotine release was measured by UV spectrophotometer (UV-1800, SHIMADZU) using a wavelength of maximum absorbance ($\lambda_{\max}$) at 260 nm. The release profile of nicotine from the buccal film was done in triplicate with the obtained mean result.

3 Results and discussion

The buccal film for nicotine delivery was optimized and predicted by the Design-Expert[®] program. The 3D response surface and contour plot of nicotine buccal film formulations are shown in Figure 1. When the amount of DNRL in the buccal film was increased, the moisture content (Y_1), moisture uptake (Y_2), swelling index in simulated saliva solution 3 h (Y_3), and swelling index in simulated saliva solution 5 h (Y_4) decreased. This was due to the hydrophobicity of DNRL (X_1) similar to other studies that indicated the effect of decreased hydrophilicity of the film (7,8,33). While increasing the amount of Eudragit[®] NM 30 D (X_2) and pectin (X_3), the moisture content (Y_1), moisture uptake (Y_2), swelling index simulated saliva solution 3 h (Y_3), and swelling index in simulated saliva solution 5 h (Y_4) increased. They might increase the hydrophilicity of the buccal film. Eudragit[®] NM 30 D is the polymethacrylate-based emulsion polymerization that is in the concentration and nature of emulsifier and plasticizer (30). Pectin is a natural polymer that widely occurs in nature and is extracted from plants or animals (31,32). Thus, the buccal film that increased the Eudragit[®] NM 30 D (X_2) and pectin (X_3) could easily absorb the moisture, water, or fluid in the film structure, presenting high hygroscopic films.

The statistic of analysis of variance from the Design-Expert[®] program found that three polymers affected all dependent variables. The mathematical models and actual equations of optimization are shown in Table 2. The mathematical models of the moisture content (Y_1) and moisture uptake (Y_2) were linear, while mathematical models of the swelling index in simulated saliva solution (Y_3 and Y_4) were quadratic models. The linear model of the moisture content (Y_1) and moisture uptake (Y_2) could be explained from the relationship of a constant rate of change of independent variables. The DNRL (X_1), Eudragit[®] NM 30 D (X_2), and pectin (X_3) had a significant positive effect on the dependent variables: the moisture content (Y_1) and moisture uptake (Y_2). A two-factor interaction mathematical model was found for the moisture uptake (Y_2) that assigned X_1X_2 , X_1X_3 , and X_2X_3 , describing possible interesting combinations between the DNRL (X_1), Eudragit[®] NM 30 D (X_2), and pectin (X_3). It was found that the X_1X_2 and X_1X_3 had significant positive effect, while the X_2X_3 had a significant negative effect. A quadratic mathematical model was the relationship between the independent variables and was a parabola when plotted on a graph. It was found that all factor interaction mathematical models had a significant positive effect except X_1X_2 and $X_1X_2X_3^2$, which had a

Figure 1: 3D response surface and contour plot of nicotine buccal film formulations with different dependent variables: moisture content (Y_1), moisture uptake (Y_2), swelling index in artificial saliva 3 h (Y_3), and swelling index in artificial saliva 5 h (Y_4).

significant negative effect on the swelling index in simulated saliva solution (Y_3 and Y_4).

In summary, the optimized buccal film for nicotine delivery based on the high desirability value that composed of the ratio of DNRL:Eudragit[®] NM 30 D:pectin as 0.319:0.362:0.319, respectively. The prediction values were 14.59%, 3.17%, 104.07%, and 103.95% for the moisture content (Y_1), moisture uptake (Y_2), swelling index simulated saliva solution 3 h (Y_3), and swelling index in simulated saliva solution 5 h (Y_4), respectively. The formulation of buccal film for nicotine delivery obtained as 0.319:0.362:0.319 of DNRL:Eudragit[®] NM 30 D:pectin with 30% w/w of glycerin depending on the polymer amount was prepared again. The experimental values were $13.17 \pm 0.92\%$, $3.96 \pm 0.84\%$, $112.58 \pm 22.63\%$, and $124.69 \pm 8.01\%$ and the percent error of the prediction [(experimental value-predicted value/

experimental value) $\times 100$] was -11.16% , 17.30% , 5.27% , and 16.41% for the moisture content (Y_1), moisture uptake (Y_2), swelling index simulated saliva solution 3 h (Y_3), and swelling index in simulated saliva solution 5 h (Y_4), respectively. Thus, the obtained percent error of the prediction was less than 20% of that accepted for preparation.

The obtained optimized formulation of buccal film for nicotine delivery was evaluated for the swelling measurement, water absorption properties, surface pH, weight, thickness, nicotine content, and *in vitro* release of nicotine. The swelling measurement and water absorption properties are shown in Figure 2. Both blank buccal film and the nicotine-loaded buccal film showed a high ratio of water absorption amount at pH 7 (Figure 2a). Thus, the obtained optimized formulation of buccal film for nicotine delivery might highly swell in the mouth

Table 2: Mathematical models and actual equations

Mathematical models	Equations
Linear	Y_1 : Moisture content (%) = $6.45X_1 + 19.72X_2 + 16.89X_3$
Linear	Y_2 : Moisture uptake (%) = $2.98X_1 + 64.67X_2 + 22.13X_3 - 128.53X_1X_2 + 0.81X_1X_3 - 116.89X_2X_3$
Quadratic	Y_3 : Swelling index at 3 h (%) = $20.54X_1 + 101.77X_2 + 101.77X_3 - 199.16X_1X_2 + 169.55X_1X_3 + 7.08X_2X_3 + 3950.05X_1^2X_2X_3 + 1025.59X_1X_2^2X_3 - 2292.78X_1X_2X_3^2$
Quadratic	Y_4 : Swelling index at 5 h (%) = $25.02X_1 + 101.71X_2 + 101.71X_3 - 199.18X_1X_2 + 160.22X_1X_3 + 6.84X_2X_3 + 3797.72X_1^2X_2X_3 + 1036.81X_1X_2^2X_3 - 2197.82X_1X_2X_3^2$

Figure 2: (a) Ratio of water absorption amount at different pH values and (b) relative gel volume of blank and nicotine buccal films.

because the pH in the oral cavity is near neutrality (45). The nicotine might be freely released from the buccal film in the oral cavity. At pH below 7, the nicotine-loaded buccal film showed a low ratio of water absorption amount, while the ratio of water absorption amount increased at pH above 7, compared to blank buccal film. This was due to the hygroscopic property of nicotine that very readily absorbs and retains the water by forming hydrogen bonds between the pyridine structure of nicotine and the water (19,22). The water absorption properties presented as swelling and deswelling behaviors of blank buccal film and nicotine-loaded buccal film in terms of the relative gel volume (Figure 2b), which was evaluated in a water/ethanol environment. Both blank buccal film and the nicotine-loaded buccal film could form strong hydrogen bond with water and swell, while they rapidly deswelled in ethanol due to the greater polarity and dielectric constant of water than ethanol (46). The molecules of ethanol had a greater tendency to replace molecules of water and then the water could be removed from the swollen buccal film, representing the decrease in the swelling of buccal film. Therefore, it could be concluded that the presence of alcohols might directly decrease the release pattern of the drug from

Figure 3: *In vitro* release of nicotine from buccal film.

the buccal film. Thus, the patients should be advised to avoid concomitant administration of the buccal film with alcohol.

The pH on the surface of the blank buccal film and nicotine-loaded buccal film was 6.84 and 8.11, respectively, that closed to neutral. The nicotine-loaded buccal film could be applied in the mouth without irritation on the oral mucosa (47). The weight of the blank buccal film and nicotine-loaded buccal film was 59.34 ± 3.44 and 63.28 ± 6.18 mg, respectively. The thickness of the blank buccal film and nicotine-loaded buccal film was 201.33 ± 33.76 and 219.87 ± 44.28 μ m, respectively.

The nicotine content in the buccal film was found as 10.22 ± 0.46 mg/4 cm² which closed to the required amount in the film. The *in vitro* release profile of nicotine is shown in Figure 3. It was found that the maximum cumulative release of nicotine was 9.82 ± 0.94 mg/4 cm² or the percentage cumulative release of $96.12 \pm 9.21\%$

Table 3: <i>In vitro</i> release kinetic models and their parameters obtained from the DDSolver program		
Zero-order model	R^2	0.9075
	k_0	2.037
First-order model	R^2	0.9122
	k_1	0.022
Higuchi model	R^2	0.9838
	k_H	4.297
Korsmeyer-Peppas model	R^2	0.9956
	k_{KP}	5.056
Hixson-Crowell model	n	0.36
	R^2	0.9107
	k_{HC}	0.007

The R^2 was the coefficient of determination. The k_0 , k_1 , k_H , k_{KP} , and k_{HC} were released of the nicotine at a constant rate following the zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models, respectively. The n was the release exponent.

within 6 h. The buccal film showed the high nicotine release from the matrix; thus, this release behavior could be found in the oral cavity after being applied in the mouth. The kinetic models, zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models, and their parameters of *in vitro* release of nicotine from the buccal film are shown in Table 3. *In vitro* release of nicotine from buccal film fitted to the Korsmeyer–Peppas model showed the highest R^2 value. Korsmeyer–Peppas model was the kinetic model used to describe drug release from the polymeric system (48). The release exponent (n value) from the Korsmeyer–Peppas model was 0.36 which was less than 0.5, representing the release mechanism.

4 Conclusion

The ratios between DNRL (X_1), Eudragit® NM 30 D (X_2), and pectin (X_3) were optimized by Design-Expert® program version 11 for preparation of the buccal film for nicotine delivery. The hydrophilicity of three polymers affected these dependent variables: moisture content (Y_1), moisture uptake (Y_2), swelling index in artificial saliva solution 3 h (Y_3), and swelling index in artificial saliva solution 5 h (Y_4). The DNRL decreased the hydrophilicity of the buccal film, while the Eudragit® NM 30 D and pectin increased the hydrophilicity of the buccal film. The mathematical models were linear for Y_1 and Y_2 , while Y_3 and Y_4 were quadratic models. The obtained optimized ratio of polymer blend was 0.319:0.362:0.319. The prediction values were 14.59%, 3.17%, 104.07%, and 103.95% and the experimental values were $13.17 \pm 0.92\%$, $3.96 \pm 0.84\%$, $112.58 \pm 22.63\%$, and $124.69 \pm 8.01\%$ for Y_1 – Y_4 , respectively. Both blank buccal film and the nicotine-loaded buccal film showed the highest ratio of water absorption amount at pH 7 and had swelling and deswelling behaviors in water/ethanol environment. The surface pH, weight, and thickness of blank buccal film were 6.84, 59.34 ± 3.44 mg, and 201.33 ± 33.76 μ m, respectively, while the surface pH, weight, and thickness of nicotine-loaded buccal film were 8.11, 63.28 ± 6.18 mg, and 219.87 ± 44.28 μ m, respectively. The nicotine content was found as 10.22 ± 0.46 mg/4 cm² in the buccal film. The maximum cumulative release of nicotine from the buccal film was 9.82 ± 0.94 mg/4 cm² within 6 h. The kinetic model fitted to the Korsmeyer–Peppas model showed the highest R^2 value and the release exponent was 0.36, representing that release mechanism was controlled by a Fickian diffusion release mechanism.

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