CHAPTER IV

RESULTS AND DISCUSSION

1. PG Yields from Durian-Fruit Hulls

The extraction and purification of PG was performed according to the method of Pongsamart and Panmaung (1998). The first extraction yielded crude PG approximately 9 %w/w of dried durian-fruit hulls, and the semi-purified one yielded PG approximately 5 %w/w based on the weight of dried durian-fruit hulls in this study. However, PG yield of 7.3 %w/w of dried durian-fruit hulls has been reported by Gerddit (2002).

2. Physico-chemical Properties of PG

The dried PG was pulverized to a fine powder and passed through a 60-mesh sieve, and pale brown powder was obtained (Figure 17).

2.1 pH

The PG powder swelled in distilled water forming a viscous gel. In Figure 18 indicated that the higher the concentration of PG, the lower the pH was obtained. Because according to the previous report, PG composed principally of galacturonic acid (68% of total carbohydrate content) more than the other neutral sugars and due to the dominant feature of PG consisting mainly of a linear chain of 1-4 linked galacturonic acid units (polygalacturonic acids), it was also a pectic polysaccharide (Hokputsa et al., 2004). Therefore, the higher concentration of PG, the lower the pH was found due to its galacturonic acid content.

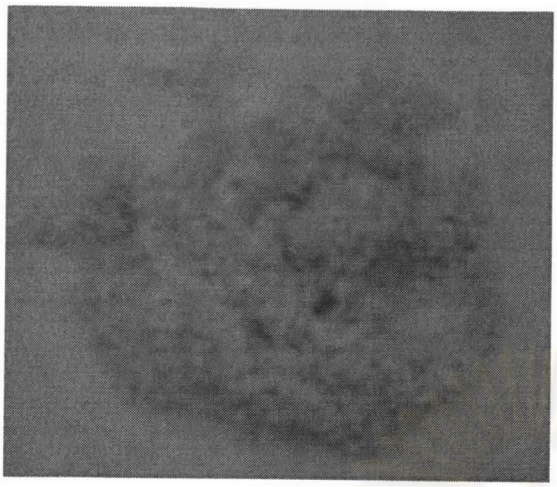

Figure 17 Durian gel powder isolated from dried durian-fruit hulls.

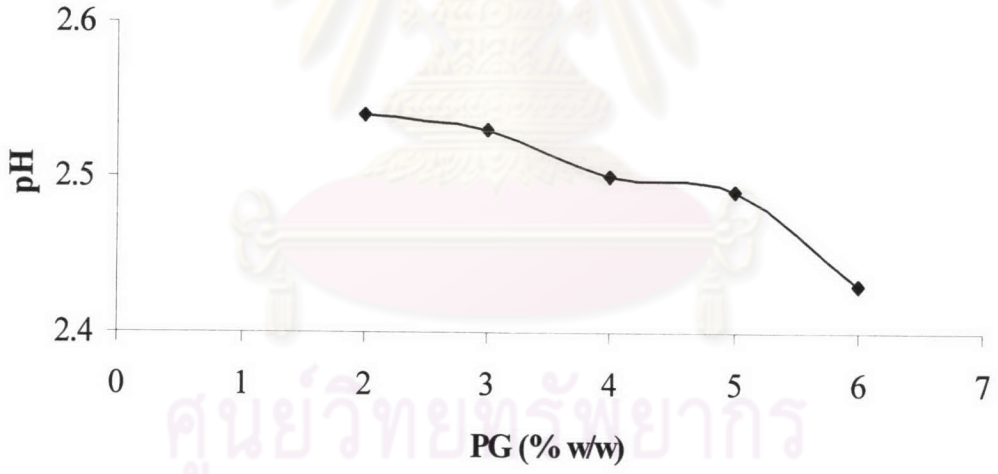

Figure 18 The effect of concentration of the PG gel on pH.

2.2 Viscosity

The effect of concentration of PG on viscosity was studied. The concentration of PG was varied between 2-6 %w/w and the viscosity of PG was recorded (Figure 19). At 2-3 %w/w PG, the liquid was thin. The liquid was more viscous at 3-5 %w/w PG and its viscosity increased abruptly when PG concentration was greater than 5 %w/w. The viscosity was influenced by the hydrodynamic volume of a polymer in solution. The viscous liquid was thick because the polymer chains moved slowly, and this effect distinctly appeared at higher concentration.

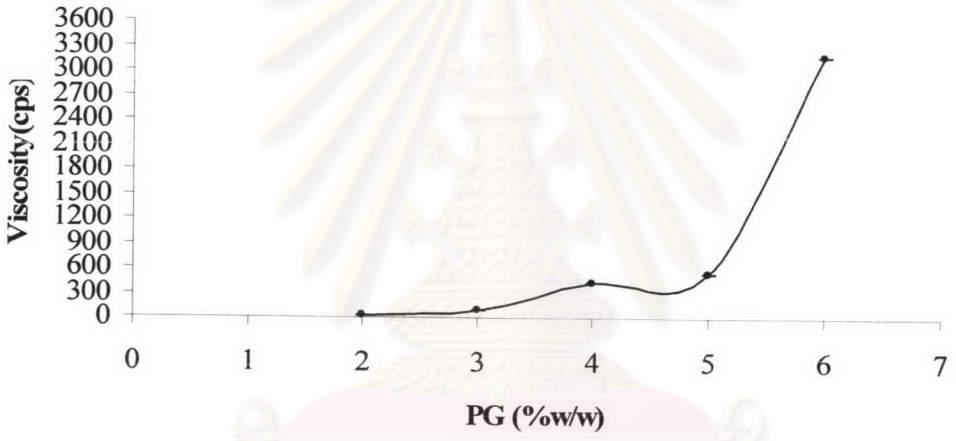

Figure 19 The effect of concentration of PG gel on the viscosity.

3. Selection of Types and Concentrations of Calcium Salts

PG is an anionic polymer that could interact with various cations and thus affected the viscosity. The carboxylate anion of PG could interact with calcium and sodium ions with greater affinity with calcium ion than with sodium ion because the polymer helices being held together by chelate-bound calcium ion and the two-fold helix has been occurred. The binding capacity depended on the degree of ionization of the carboxylic groups. Potassium chloride, magnesium chloride and calcium chloride could also increase the

viscosity of PG gel; calcium chloride had the greatest effect among them (Lertchaiporn, 2003).

In this study, the effect of calcium chloride and calcium gluconate was compared at various concentrations of calcium ions (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 %w/w based on the PG weight). The viscosity of PG dispersions in the presence of calcium chloride and calcium glucoante are shown in Figure 20. The concentration of calcium ion at 0.5 %w/w did not increase the viscosity of PG gel. At 1.0-3.0 %w/w, the greater the concentration of calcium ion, the greater the viscosity of PG gel was obtained. At the same concentration of calcium ion, calcium gluconate had a more prominent effect on the viscosity and gave a more homogeneous gel than calcium chloride. This should be because the gel structure formed by calcium chloride and PG was firm and the gelation rate was fast, thus the gel structure previously formed impeded the diffusion of calcium ion and the later gelation was less than that at the first form. The gelation rate in the case of calcium gluconate, on the other hand, was slower than that in the case of calcium chloride, thus the later gelation the system occurred simultaneously. Therefore, calcium gluconate was chosen in this study for the film formulation.

The minimum concentration of calcium gluconate, which gave appropriate viscosity to cast film was 1.0 %w/w of calcium ion based on the PG weight. At the higher concentrations of calcium (1.5-2 %w/w), PG gel was hard and difficult to cast films and the dried films did not appeared homogeneously. The PG gels with calcium at 2.5-3.0 %w/w were stiff; they were too hard to spread into a very thin film so they were excluded. Therefore, the calcium gluconate at 1.0 %w/w of calcium based on the PG weight was chosen for further study.

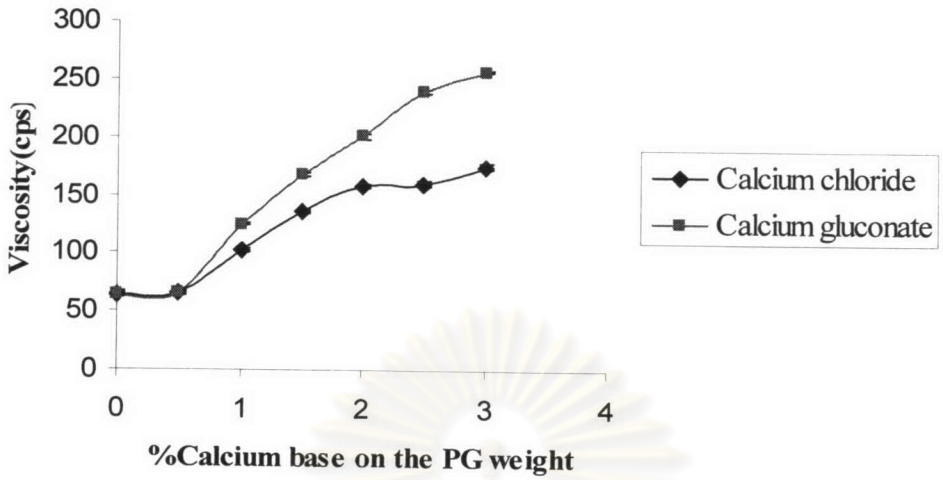

Figure 20 The viscosity of PG dispersions in the presence of calcium chloride and calcium glucoante

4. Preparation of Buccal Mucoadhesive Film Base

4.1 Preparation of Buccal Mucoadhesive Layers

4.1.1 Selection of Plasticizers

PG had a property of film forming according to the study of Gerddit (2002). PG has film forming property similar to cellulose derivatives such as hydroxy propyl methylcellulose, so it is expected to be used as a film forming agent in preparations of dressing patch. The buccal mucoadhesive properties of PG film formulations containing sorbitol 30 %w/w based on PG weight provided the most satisfactory film product according to the test in 32 volunteers (Tachatawepisarn, 2003). However, the film produced from 100% PG was brittle at room temperature and therefore the use of plasticizers was required. The addition of plasticizers to polymeric films could make them both softer and more flexible due to the decrease in the glass transition temperature of the polymer. A preliminary study revealed that a single plasticizer did not provide good physical films, thus combinations of plasticizers including glycerin, PEG 6000, and 70 % sorbitol were studied. The dispersion of PG with calcium gluconate and plasticizers in all tested formulations produced a clear, viscous and homogeneous mixture. All

prepared films (film No. 1-8) were pale brown in color, transparent, smooth and flexible film products (Figure 21). The thickness of the films was in the range of 0.046 ± 0.003 to 0.069 ± 0.003 mm. Formulas of the mucoadhesive films are shown in Table 9. Therefore, *in vitro* mucoadhesion and mechanical properties of all films were further studied.

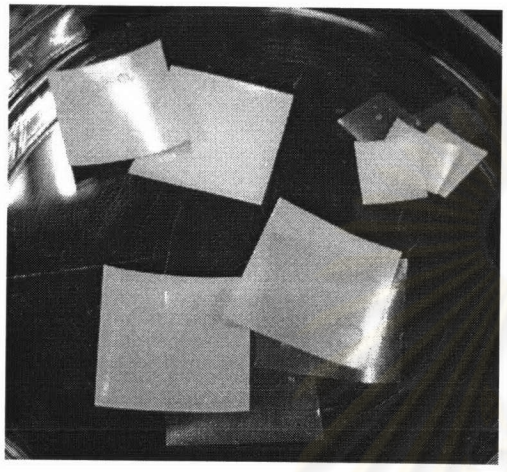

Figure 21 The appearance of mucoadhesive films (film No.6).

Table 9 Formulas of dry mucoadhesive films

Ingredients (% dry weight)	Film No.							
	1	2	3	4	5	**6**	7	8
PG	61.83	58.23	61.45	57.89	58.23	**55.02**	57.89	54.72
Calcium gluconate	6.64	6.25	6.60	6.22	6.25	**5.91**	6.22	5.88
Glycerin	12.37	17.47	12.29	17.37	11.65	**16.51**	11.58	16.42
PEG 6000	0.62	0.58	1.23	1.16	0.58	**0.55**	1.16	1.09
70% sorbitol	12.37	11.65	12.29	11.58	17.47	**16.51**	17.37	16.42
0.5 N NaOH	6.18	5.82	6.14	5.79	5.82	**5.50**	5.79	5.47

4.1.1.1 *In vitro* Mucoadhesive Study

A suitable buccal mucoadhesive film should be flexible and possesses good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration (Wong, Yuen, and Peh, 1999). Various mechanisms have been proposed to explain the *in vitro* mucoadhesive phenomena. Most *in vitro* studies investigating mucoadhesion have provided valuable information on the force of mucoadhesion by measuring tensile and shear strength (Needleman, and Smales, 1995). Determination of the mucoadhesion strength was important in the development of mucoadhesive dosage form since the satisfactory mucoadhesion was essential for successful application of buccal mucoadhesive drug delivery systems.

In this study the adhesive strength evaluated at a predetermined contact time was investigated using porcine buccal mucosa as a model membrane. Two parameters, namely force of maximum detachment force (force of mucoadhesion) and work of adhesion, were used to evaluate the mucoadhesive properties of the films containing the plasticizers. Both were calculated from the measurements taken using a software-controlled program, QMAT 4.10 S-Series-5K, and the values are shown in Table 10-11. In artificial saliva with and without mucin, film No. 6 containing 30% w/w glycerin, 30% w/w sorbitol, and 1 %w/w PEG 6000 based on the PG weight appeared to have the greatest detachment force and work of adhesion. The rank order of detachment force and work of adhesion of films were as follows: No. 6 > No. 8 > No. 4 > No. 3 > No. 1 > No. 2 > No. 7 > No. 5.

Mucin in the artificial saliva significantly increased (t–test, $p < 0.05$) the detachment force of film No. 2-6 and 8 (Table 10 and Figure 22), and increased the work of adhesion of film No. 1-6 and 8 (Table 11 and Figure 23). Mathiowitz, Chickering, and Lehr (1999) proposed the diffusion theory of bioadhesion mechanism. Chains of bioadhesive polymer and the mucus interpenetrate one another to a sufficient depth to create a semipermanent adhesive bond. Incorporation of mucin in the artificial saliva was expected to increase the chain interpenetration, and thus the adhesive bond, so the higher detachment force and work of adhesion were obtained when using artificial saliva with mucin.

Table 10 The effect of mucin in artificial saliva on force of mucoadhesion (mean ± SD, n = 5)

Film No.	Force of mucoadhesion (N)	
	Artificial saliva without mucin	Artificial saliva with mucin
1	0.0359 ± 0.0060	0.0403 ± 0.0023
2	0.0340 ± 0.0034	0.0394 ± 0.0019*
3	0.0373 ± 0.0024	0.0428 ± 0.0016*
4	0.0402 ± 0.0049	0.0462 ± 0.0016*
5	0.0239 ± 0.0043	0.0318 ± 0.0042*
6	0.0480 ± 0.0012	0.0726 ± 0.0023*
7	0.0330 ± 0.0045	0.0342 ± 0.0032
8	0.0451 ± 0.0025	0.0511 ± 0.0029*

* significant difference ($p<0.05$)

Table 11 The effect of mucin in artificial saliva on work of adhesion (mean ± SD, n = 5)

Film No.	Work of adhesion (mJ)	
	Artificial saliva without mucin	Artificial saliva with mucin
1	0.0439 ± 0.0031	0.0549 ± 0.0042*
2	0.0430 ± 0.0014	0.0479 ± 0.0008*
3	0.0441 ± 0.0022	0.0578 ± 0.0010*
4	0.0556 ± 0.0051	0.0659 ± 0.0030*
5	0.0330 ± 0.0047	0.0445 ± 0.0018*
6	0.0660 ± 0.0043	0.0711 ± 0.0022*
7	0.0438 ± 0.0035	0.0457 ± 0.0021
8	0.0568 ± 0.0033	0.0697 ± 0.0027*

* significant difference ($p<0.05$)

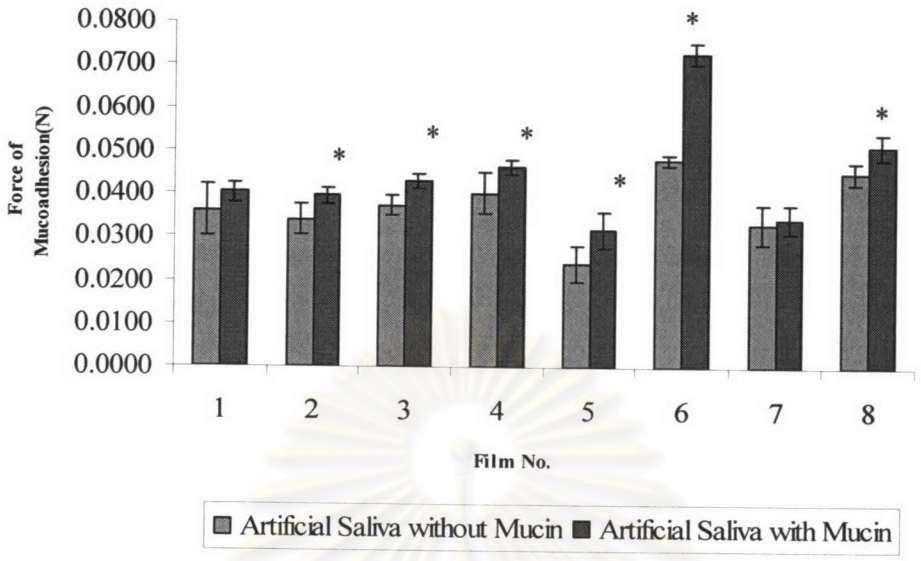

Figure 22 A comparison of force of mucoadhesion of film No. 1-8

* significant difference ($p<0.05$)

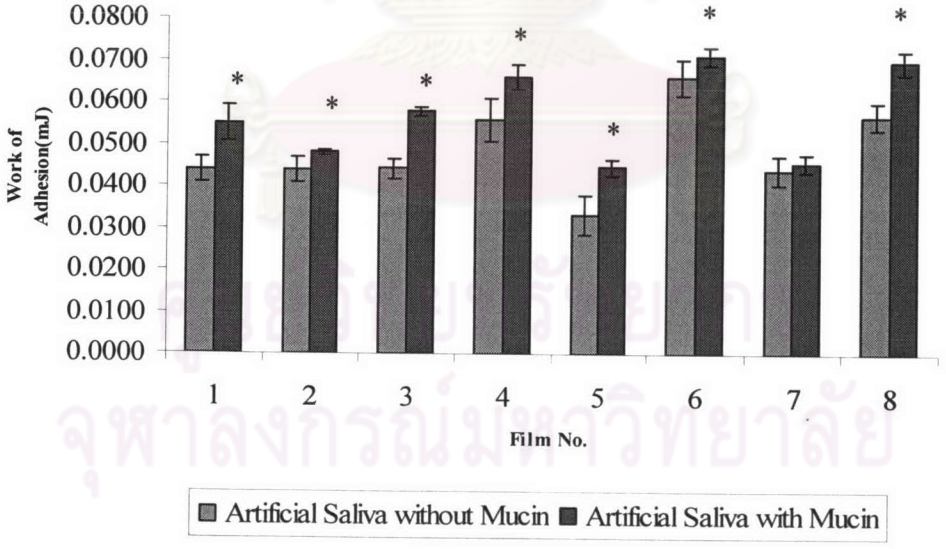

Figure 23 A comparison of work of adhesion of film No. 1-8

* significant difference ($p<0.05$)

The plasticizers significantly influenced both detachment force and work of adhesion in all the films studied ($p < 0.05$) as shown in Table 12-15. The plasticizers could affect mucoadhesive strength by changing the surface properties of the bioadhesive thus enhancing or prolong the formation of an intimate contact between the porcine buccal mucosa and the adhesive surface. An appropriate ratio of plasticizers produced the films with suitable adhesiveness. A combination of 30 %w/w glycerin, 1 %w/w PEG 6000, and 30 %w/w sorbitol based on the PG weight, respectively, was selected for further study because it provided the greatest detachment force and work of adhesion.

The reproducibility of the measurement was confirmed by using the new porcine buccal mucosa from three different pigs. Coefficients of variation were 3.77 % and 3.46 % for detachment force and work of adhesion, respectively. It revealed the validity of the technique (Eouani et al., 2001).

Table 12 Analysis of variance comparing force of mucoadhesion of film No.1-8 in artificial saliva without mucin

Source	Sum of Squares	df	Mean Square	F	Sig.	p-value
Between Groups	0.0020	7	0.0003	18.2065	0.000	0.05
Within Groups	0.0005	32	0.0000			
Total	0.0025	39				

Table 13 Analysis of variance comparing force of mucoadhesion of film No.1-8 in artificial saliva with mucin

Source	Sum of Squares	df	Mean Square	F	Sig.	p-value
Between Groups	0.0058	7	0.0008	119.1706	0.000	0.05
Within Groups	0.0002	32	0.0000			
Total	0.0060	39				

Table 14 Analysis of variance comparing work of adhesion of film No.1-8 in artificial saliva without mucin

Source	Sum of Squares	df	Mean Square	F	Sig.	p-value
Between Groups	0.0038	7	0.0005	40.5710	0.000	0.05
Within Groups	0.0004	32	0.0000			
Total	0.0042	39				

Table 15 Analysis of variance comparing work of adhesion of film No.1-8 in artificial saliva with mucin

Source	Sum of Squares	df	Mean Square	F	Sig.	p-value
Between Groups	0.0041	7	0.0006	97.0541	0.000	0.05
Within Groups	0.0002	32	0.0000			
Total	0.0043	39				

4.1.1.2 Determination of Mechanical Properties of Buccal Mucoadhesive Films

An ideal buccal film should be flexible, elastic, durable and adequately strong to withstand breakage due to stress from oral cavities. So, mechanical properties were critical and needed to be evaluated. A tensile testing gave an indication of the strength and elasticity of the film reflected by the following parameters: tensile strength, % elongation and Young's modulus. A soft and weak polymer was characterized by a low tensile strength, % elongation and Young's modulus; a hard and brittle polymer was defined by a moderate tensile strength, high Young's modulus, and low % elongation; a soft and tough polymer was characterized by a moderate tensile strength, low Young's modulus, and high % elongation; whereas a hard and tough polymer was defined by a high tensile strength, Young's modulus, and % elongation. Therefore, suitable buccal films should have a relatively high tensile strength and % elongation but low Young's modulus (Peh, and Wong, 1999).

According to the study of Gerddit (2002) and Tachatawepisarn (2003); those result indicated that PG film without plasticizer produced the highest Young's modulus (3837 ± 290 MPa) compared to PG film with plasticizer, this value indicated that PG film was very rigid therefore plasticizer make the softer. Added glycerin (5, 10, and 15 %w/w based on the PG weight), sorbitol (10, 20, and 30 %w/w based on the PG weight) and PEG 6000 (1, 2, and 3 %w/w based on the PG weight), respectively into the PG dressing films resulted in increasing hardness and toughness of the films with high values of tensile strength, %elongation values compared to PG dressing film without glycerin. From a preliminary study revealed that a single plasticizer did not provide good physical buccal films, thus combinations of plasticizers including glycerin, PEG 6000, and 70 % sorbitol were studied.

Table 16 and Figure 24-27 depict the mechanical properties of film No.1-8. The PG film containing 30 %w/w glycerin, 1 %w/w PEG 6000, and 30 %w/w sorbitol (film No. 6) gave high tensile strength and percent elongation but relatively low Young's Modulus, thus the film formula No.6 was selected. It also had the greatest bioadhesiveness. A statistically significant difference ($p < 0.05$) was obtained in both the tensile strength and % elongation evaluated in all films.

Table 16 Mechanical property data of film No. 1-8 (mean ± SD, n = 5)

Film No.	Tensile strength (MPa)	% Elongation	Work of failure (mJ)	Young's modulus (MPa)
1	19.366 ± 1.814	28.92 ± 1.43	1.655 ± 0.031	148.960 ± 8.238
2	18.220 ± 1.079	22.99 ± 1.85	1.226 ± 0.176	145.120 ± 2.220
3	11.100 ± 1.793	9.81 ± 0.18	0.371 ± 0.025	202.180 ± 7.162
4	21.874 ± 2.432	14.10 ± 1.45	0.759 ± 0.094	170.540 ± 3.311
5	17.674 ± 1.439	45.40 ± 1.26	2.793 ± 0.298	97.800 ± 4.006
6	31.630 ± 2.854	47.64 ± 1.96	9.391 ± 0.318	140.440 ± 6.479
7	28.590 ± 1.932	17.95 ± 2.81	1.088 ± 0.153	261.500 ± 16.496
8	20.740 ± 1.955	24.35 ± 2.75	1.716 ± 0.114	123.480 ± 2.600

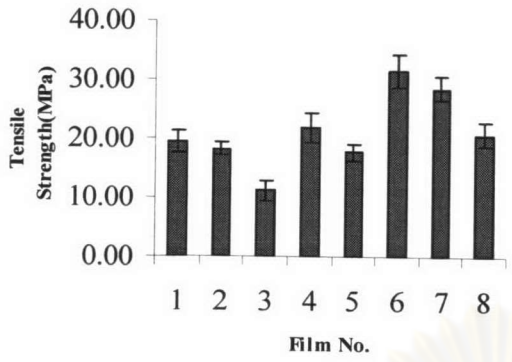

Figure 24 Tensile strength of film No.1-8.

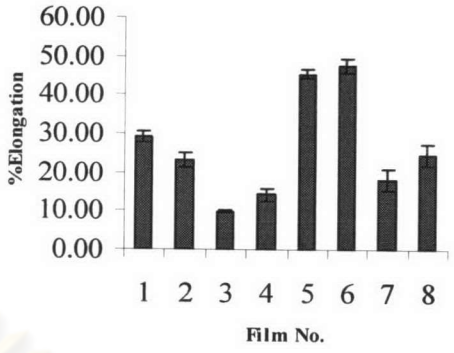

Figure 25 % Elongation of film No.1-8.

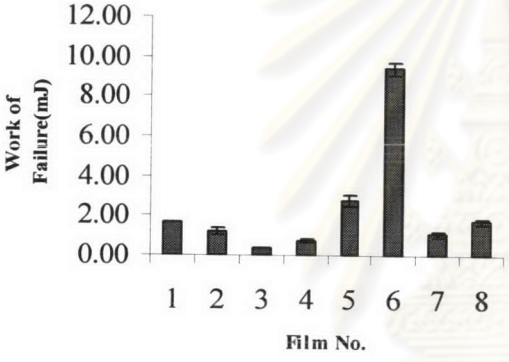

Figure 26 Work of failure of film No.1-8.

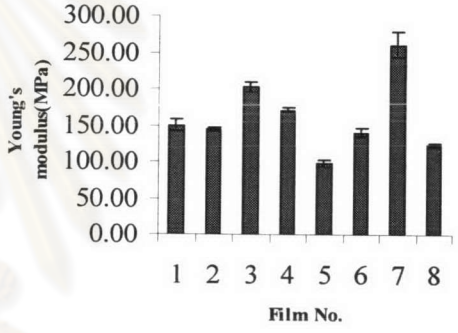

Figure 27 Young's modulus of film No.1-8.

4.1.2 Determination of a Suitable Concentration of PG

Since film No. 6 was chosen, the formulas composed of types and concentration of plasticizers as the following: 30 %w/w glycerin, 1 %w/w PEG 6000, 30 %w/w sorbitol, and 1 %w/w calcium ion (as calcium gluconate) based on the PG weight. Films containing 4, 5, and 6 %w/w PG were prepared and designated as films No. 6, 9, and 10, respectively. The film products were pale brown in color, transparent, smooth and flexible. The thickness of film No. 6, 9, and 10 were 0.069 ± 0.003, 0.082 ± 0.002, and 0.091 ± 0.002 mm, respectively. Therefore, *in vitro* mucoadhesion and mechanical properties of all the three films were further studied.

4.1.2.1 *In vitro* Mucoadhesive Study

The PG concentrations affected the force of mucoadhesion and work of adhesion as shown in Tables 17-18 and Figures 28-29. The force of mucoadhesion and work of adhesion did not depend on the concentration of PG directly as their values dropped at 6 %w/w PG (film No. 10) which was the greatest concentration studied. In concentrated solutions, the coiled molecules became solvent-poor, and the chains available for interpenetration were not numerous (Duchene, Touchard, and Peppas, 1988). Therefore, an optimum concentration of PG, which was 5 %w/w PG (film No. 9) in this study, was necessary in order to provide the maximum adhesive strength of the film product.

Table 17 The force of mucoadhesion of film No. 6, 9, and 10 (mean ± SD, n = 5)

Film No.	Force of mucoadhesion (N)	
	Artificial saliva without mucin	Artificial saliva with mucin
6	0.0480 ± 0.0012	0.0726 ± 0.0023
9	0.0513 ± 0.0043	0.0775 ± 0.0015
10	0.0257 ± 0.0015	0.0221 ± 0.0027

Table 18 The work of adhesion of film No. 6, 9, and 10 (mean ± SD, n = 5)

Film No.	Work of adhesion (mJ)	
	Artificial saliva without mucin	Artificial saliva with mucin
6	0.0660 ± 0.0043	0.0711 ± 0.0022
9	0.0753 ± 0.0025	0.0751 ± 0.0044
10	0.0321 ± 0.0032	0.0506 ± 0.0049

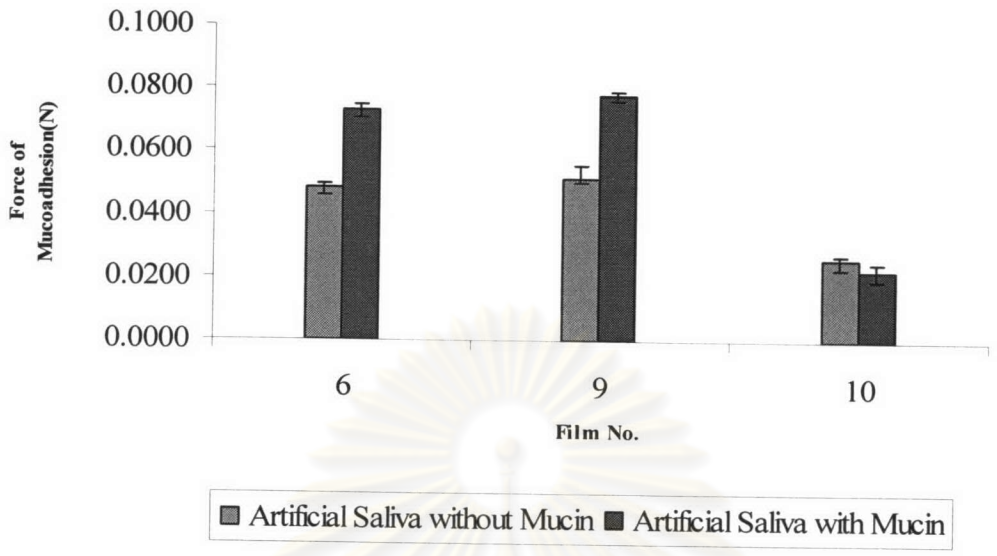

Figure 28 The force of mucoadhesion of film No. 6, 9, and 10.

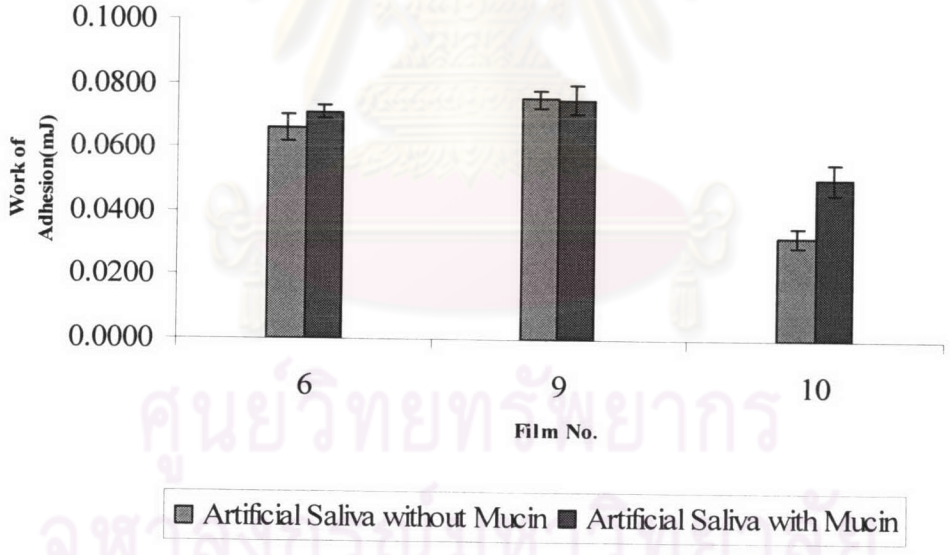

Figure 29 The work of adhesion of film No. 6, 9, and 10.

4.1.2.2 Determination of Mechanical Properties of Buccal Mucoadhesive Films

Table 19 shows that film No. 9 (5 %w/w PG) exhibited the greatest values of tensile strength, % elongation, and Young's modulus, so the film product was relatively strong and tough. The work of failure and Young's modulus values of film No. 6 were less than those of film No.9, and similar value of tensile strength was obtained. The product of film No.6 was more brittle and stiffer than film No. 9. Film No.10 (6 % w/w) was the weakest due to its least tensile strength value and %elongation. In conclusion, film No. 9 was most capable of withstanding breakage in oral cavities.

Table 19 Mechanical property data of film No. 6, 9, and 10 (mean ± SD, n = 5)

Film No.	Tensile strength (MPa)	% Elongation	Work of failure (mJ)	Young's modulus (MPa)
6	31.630 ± 2.854	47.64 ± 1.96	9.391 ± 0.318	140.440 ± 6.479
9	32.046 ± 0.859	77.24 ± 1.78	10.578 ± 0.285	196.002 ± 6.704
10	15.246 ± 1.379	41.72 ± 2.09	5.598 ± 0.169	86.080 ± 2.520

Due to its greatest adhesive strength and ability to withstand breakage, film No. 9 was chosen for further study.

4.1.3 Selection of Water-insoluble Polymers

Since one of the major requirements in developing buccal film systems is the maintenance of the morphology of the film, the film should not dissolve too rapidly (Chun, Kwak, and Choi, 2003). Therefore, water-insoluble polymer was incorporated to retard the dissolution of mucoadhesive layer. These included 12.5-45.5 %w/w Eudragit® RL 100, 0.1-0.3 %w/w Eudragit® RS 100, 0.2-1.0 %w/w Eudragit® NE 30D, and 0.1-1.0 % w/w Kollicoat® SR 30 D based on the PG weight.

Eudragit® RL 100 was a copolymer of acrylic and methacrylic acid esters with 10 % of quaternary ammonium groups (Rowe, Shesky, and Weller, 2003). It did not dissolve in water and could form electrostatic bonds with the carboxyl groups of PG. In this study, PG gel containing Eudragit® RL 100 did not show any signs of PG precipitation, and absolute ethanol used to disperse Eudragit® RL 100 was compatible with PG mixture. Lertchaiporn (2003) has also reported that ethanol at a certain concentration could increase a tendency of PG gelation. Film No. 11-16 contained Eudragit® RL 100. They were pale brown, translucent but the film products were cracked, except film No. 11 which was continuous and pliable with thickness of 0.111 ± 0.003 mm. Therefore, the product of film No.11 was selected for further studies.

Eudragit® RS 100 was a copolymer of acrylic and methacrylic acid esters with 5 % quaternary ammonium groups. It was less water permeable than Eudragit® RL 100. The PG mixtures containing Eudragit® RS 100 were homogeneous and did not show aggregates. However, all films containing Eudragit RS 100® (film No.17-19) were discontinuous, rigid and cracked. This finding agreed with a previous study by Minghetti et al. (1999). The films containing Eudragit® RS 100 were rigid and did not show any adhesive properties. So they were excluded.

Eudragit® NE 30D was a neutral poly (ethylacrylate methylmethacrylate) copolymer prepared by emulsion polymerization (Lehmann, 1989). The PG gel containing Eudragit® NE 30D (No. 20-22) formed visible aggregates. This finding was consistent with the previous study of Wong, Yuen, and Peh (1999). Eudragit® NE 30D was an aqueous colloidal dispersion (latex) and was insoluble. The addition of a hydrophilic polymer into the latex dispersion, which was highly acidic, resulted in latex coagulation. So they were excluded.

Kollicoat® SR 30 D was an aqueous dispersion of polyvinyl acetate stabilized with polyvinylpyrrolidone and sodium lauryl sulfate. The addition of Kollicoat® SR 30 D to PG gel did not show any signs of precipitation and the smooth and homogeneous gel was formed. The film products were pale brown and translucent (film No. 23-28). In addition, all concentrations of Kollicoat SR 30 D® provided good film characteristics because upon evaporation of water, the polymer particles were forced to form a close packing, followed by deformation and coalescence of the particles into a

continuous film. All film products had thickness between 0.118 ± 0.007 to 0.120 ± 0.005 mm. In conclusion, they were included for further studies.

4.1.3.1 *In vitro* Mucoadhesive Study

Tables 20-21 show values of force of mucoadhesion and work of adhesion of film No.11, and 23-28. Film No. 11, which contained 12.5 % w/w Eudragit® RL 100, gave the maximum values (Figures 30-31). It was possible that quaternary ammonium groups in Eudragit® RL 100 could interact with the negative charge of protein in porcine buccal tissue. In 1997, Tirosh et al. reported similarly that polycarbophil discs containing up to 20 %w/w of Eudragit® RL 100 could increase the adhesive force. On the other hand, the acetate groups of Kollicoat® SR 30 D could reduce the interaction of the films with the porcine buccal tissue and thus their adhesive strength were less than those of film No.11.

Table 20 The force of mucoadhesion of film No. 11, and 23-28 (mean ± SD, n = 5)

Film No.	Force of mucoadhesion (N)	
	Artificial saliva without mucin	Artificial saliva with mucin
11	0.0528 ± 0.0041	0.0938 ± 0.0028
23	0.0186 ± 0.0003	0.0267 ± 0.0022
24	0.0190 ± 0.0002	0.0276 ± 0.0013
25	0.0172 ± 0.0036	0.0320 ± 0.0018
26	0.0186 ± 0.0003	0.0314 ± 0.0008
27	0.0179 ± 0.0018	0.0259 ± 0.0010
28	0.0233 ± 0.0010	0.0281 ± 0.0009

Table 21 The work of adhesion of film No. 11, and 23-28 (mean ± SD, n = 5)

Film No.	Work of adhesion (mJ)	
	Artificial saliva without mucin	Artificial saliva with mucin
11	0.0880 ± 0.0049	0.0934 ± 0.0114
23	0.0314 ± 0.0011	0.0529 ± 0.0017
24	0.0332 ± 0.0035	0.0546 ± 0.0018
25	0.0427 ± 0.0010	0.0544 ± 0.0020
26	0.0414 ± 0.0008	0.0539 ± 0.0019
27	0.0224 ± 0.0008	0.0509 ± 0.0084
28	0.0381 ± 0.0009	0.0581 ± 0.0004

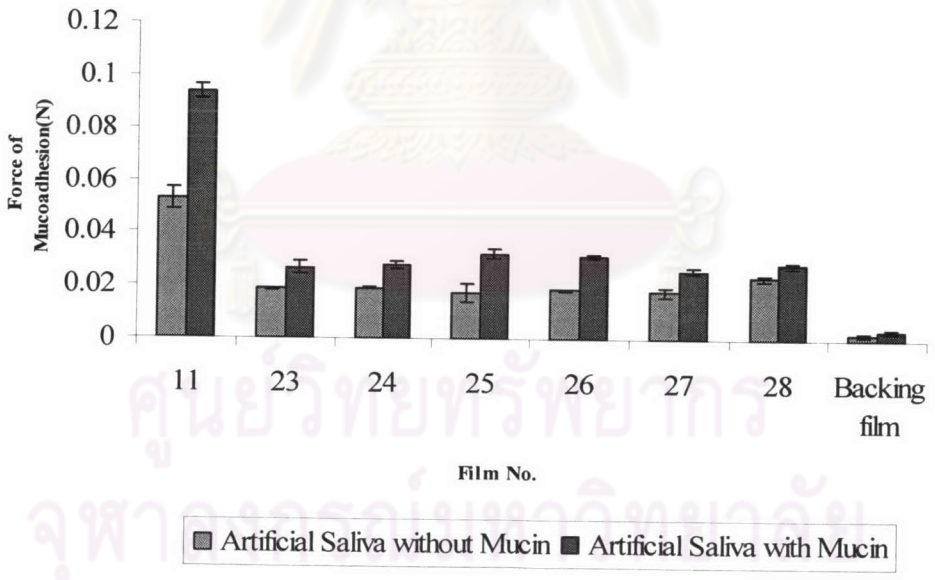

Figure 30 The force of mucoadhesion of film No. 11, and 23-28.

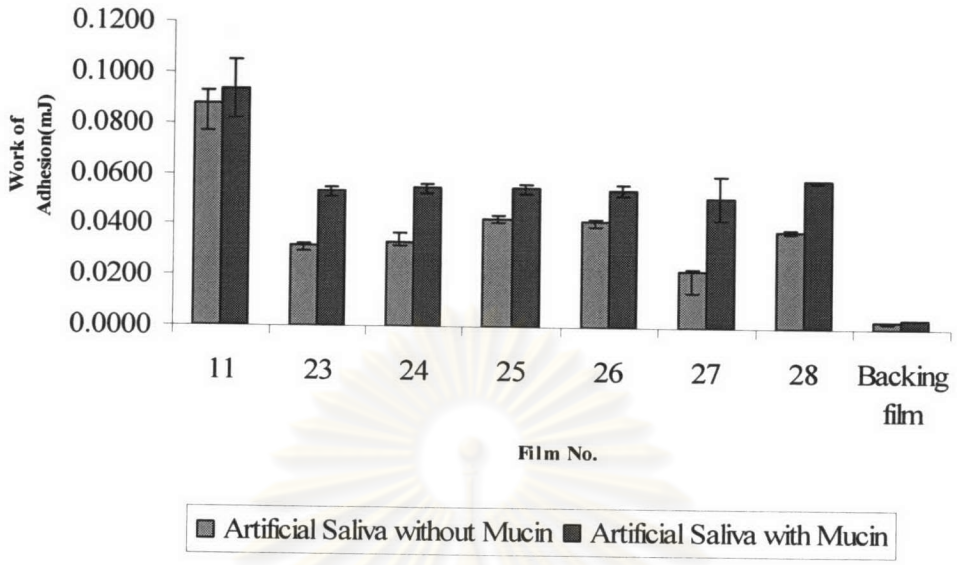

Figure 31 The work of adhesion of film No. 11, and 23-28.

4.1.3.2 A Determination of Mechanical Properties of Buccal Mucoadhesive Films

As shown in Table 22, film No. 11 exhibited the greatest values of tensile strength, % elongation, work of failure, and Young's modulus. This indicated that this film was strongest, toughest and hardest compared with film No. 23-28. This observation was in agreement with those obtained by Tirosh et al. (1997). The incorporation of Eudragit® RL 100 into polycarbophil films also affected their mechanical properties determined by the torsion force and the modulus of elasticity. The more Eudragit® RL 100 in the mixtures of polycarbophil, the larger the values of torsion force and modulus of elasticity. In conclusion, Eudragit® RL 100 could improve mechanical properties of the films.

Table 22 Mechanical property data of film No. 11 and 23-28 (mean ± SD, n = 5)

Film No.	Tensile strength (MPa)	% Elongation	Work of failure (mJ)	Young's modulus (MPa)
11	36.070 ± 2.411	55.08 ± 2.99	4.062 ± 0.080	125.800 ± 2.534
23	3.979 ± 0.237	45.92 ± 3.15	2.747 ± 0.190	80.340 ± 5.302
24	5.638 ± 0.315	27.56 ± 2.37	1.549 ± 0.080	69.040 ± 2.613
25	5.864 ± 0.215	61.26 ± 3.51	2.858 ± 0.074	21.768 ± 4.315
26	10.576 ± 0.524	56.64 ± 3.57	3.913 ± 0.295	68.040 ± 3.097
27	9.242 ± 0.636	27.56 ± 1.45	1.831 ± 0.110	88.800 ± 2.603
28	10.096 ± 0.678	45.44 ± 0.86	3.507 ± 0.113	85.840 ± 2.697

4.1.3.3 Determination of Dissolution Time of Buccal Mucoadhesive Films

Several factors must be taken into consideration in this study since the dissolution of a substance in oral cavity was different from that in the gastrointestinal tract. The oral cavity and its fluid were much smaller in volume. The components in saliva and gastrointestinal fluids were different. The residence time in oral cavity was also much shorter. So, a method of dissolution time determination in gastrointestinal tract was modified in this study and results are shown in Table 23. The rank order of dissolution time obtained was as follows: film No. 28 > 27 > 26 > 11 > 25 > 24 > 23. Tirosh et al. (1997) also got a similar result, i.e., Eudragit® RL 100 could be used to achieve a control over the dissolution of polycarbophil films. In conclusion, Eudragit® RL 100 and Kollicoat® SR 30 D could be used to fabricate the dissolution properties of PG films.

Table 23 Dissolution time data of film No. 11, and 23-28 (mean ± SD, n = 6)

Film No.	Dissolution time (min)
11	181.50 ± 4.76
23	111.17 ± 9.50
24	129.33 ± 9.54
25	156.33 ± 8.82
26	192.17 ± 8.61
27	241.83 ± 9.45
28	327.33 ± 7.47

Although the dissolution times of film No. 26-28 were longer than that of film No. 11, they were not chosen for further studies because their mucoadhesion and mechanical properties were not preferable to that of film No. 11. They would faster detach from the buccal mucosa than film No.11 before they had dissolved completely. In conclusion, film No. 11 was chosen as it was the strongest and toughest film product with the greatest mucoadhesive strength and satisfactory dissolution time.

4.2 Preparation of Bilayered Buccal Mucoadhesive Films

From a technological point of view, the first step in the development of buccal film was the selection and characterization of an appropriate bioadhesive and the second one was the developing of supportive materials in the formulation. An application of impermeable backing layer on buccal films had been considered to prevent drug loss and for the patient's convenience. Backing in the multilayered bioadhesive dosage forms acted as protective layer and prevented adhesion and drug release to the opposite side of the preparation (Mathiowitz, 1999; McQuinn et al., 1995). Guo and Cooklock (1996) had studied the effects of backing layers on the adhesion of buccal films. They found that ethylcellulose, a hydrophobic polymer, had very low water permeability and moderate

flexibility; therefore, it was a good candidate for backing application. It could delay water uptake of the films and prolong the time to reach the maximum adhesive strength. An appropriate design of the impermeable backing layer could prevent the excessive wash-out of the drug by saliva, so a maximum drug activity gradient to the mucosa was established. The wash-out of the adhesive was also diminished which minimized the amount of adhesive necessary to ensure adhesion (Anders and Merkle, 1989; Lopez et al., 1998).

Ethylcellulose film was used as the backing layer and ethycellulose 5 %w/w in ethanol was an optimal concentration used in a casting solution. The backing film showed trivial bioadhesive properties as the force and work of adhesion were very weak (Table 24). The thickness of the bilayered buccal mucoadhesive films consisting of the buccal mucoadhesive layer (film No. 11) and the backing layer was 0.112 ± 0.003 mm. The mucoadhesive layer stuck firmly to the backing layer without any defects or breakages. No disintegration of the backing layer was observed in this investigation. Therefore, this formulation was used to prepare the films containing triamcinolone acetonide.

Table 24 The force of mucoadhesion and work of adhesion of backing film (mean ± SD, n = 5)

	Artificial saliva without mucin	Artificial saliva with mucin
Force of mucoadhesion (N)	0.0021 ± 0.0006	0.0035 ± 0.0004
Work of adhesion (mJ)	0.0026 ± 0.0002	0.0036 ± 0.0002

5. Preparation of Bilayered Buccal Mucoadhesive Film Containing Triamcinolone Acetonide

As it was previously described, film base No.11 with ethylcellulose backing layer was selected to formulate the film containing 0.1 %w/w triamcinolone acetonide (film No. 29). The concentration of triamcinolone acetonide employed in the formulation was 14.2

$\mu g/cm^2$. The medicated film with the backing layer was pale brown and translucent due to Eudragit® RL 100. Its thickness was 0.111 ± 0.004 mm. The other physical appearances of the film were similar to those of film No.11.

Triamcinolone acetonide significantly decreased the adhesive strength as both the force of mucoadhesion and work of adhesion of film No. 29 were less than those of film No. 11 (Tables 25 and 26); using t–test, their p values were less than 0.05 both in the artificial saliva with and without mucin (display as a star in Figures 32 and 33). The effects of drug on the force of mucoadhesion had been reported previously. Anlar et al (1994) found similarly that 15 %w/w morphine sulfate significantly decreased mucoadhesive force in the hydroxy propyl methylcellulose-carbopol buccoadhesive tablets. Minghetti (1999) also found that the presence of miconazole nitrate influenced the adhesive properties of the Eudragit® NE 40D films when compared with placebo films. However, Shojaei, Zhou, and Li (1998) reported that 0.56-7.26 %w/w acyclovir did not significantly affect the force of mucoadhesion of 1.3 %w/w ethylene glycol dimethacrylate films ($p>0.05$). The effect of drug on mucoadhesive force was related to the size and hydrophilicity of the drug as well as the potential interaction between drug and mucoadhesive. The addition of triamcinolone acetonide, which was a hydrophobic drug, to the PG film (hydrophilic matrices) could change the surface properties of the adhesive and thus impeded the intimate contact between the adhesive surface and the buccal mucosa.

Triamcinolone acetonide reduced not only the adhesive strength but also all values of mechanical properties as shown in Table 27. Therefore, the film containing triamcinolone acetonide (film No. 29) was weaker, softer than the film base (film No. 11).

Table 25 The force of mucoadhesion of film No. 11 and 29 (mean ± SD, n = 5)

Film No.	Force of mucoadhesion (N)	
	Artificial saliva without mucin	Artificial saliva with mucin
11	0.0528 ± 0.0041	0.0938 ± 0.0028
29	0.0420 ± 0.0036	0.0552 ± 0.0036

Table 26 The work of adhesion of film No. 11 and 29 (mean ± SD, n = 5)

Film No.	Work of adhesion (mJ)	
	Artificial saliva without mucin	Artificial saliva with mucin
11	0.0880 ± 0.0049	0.0934 ± 0.0114
29	0.0524 ± 0.0031	0.0605 ± 0.0034

Table 27 Mechanical property data of film No.11 and 29 (mean ± SD, n = 5)

Film No.	Tensile strength (MPa)	% Elongation	Work of failure (mJ)	Young's modulus (MPa)
11	36.070 ± 2.411	55.08 ± 2.99	4.062 ± 0.080	125.800 ± 2.534
29	15.704 ± 0.839	35.48 ± 3.32	3.114 ± 0.128	116.100 ± 3.358

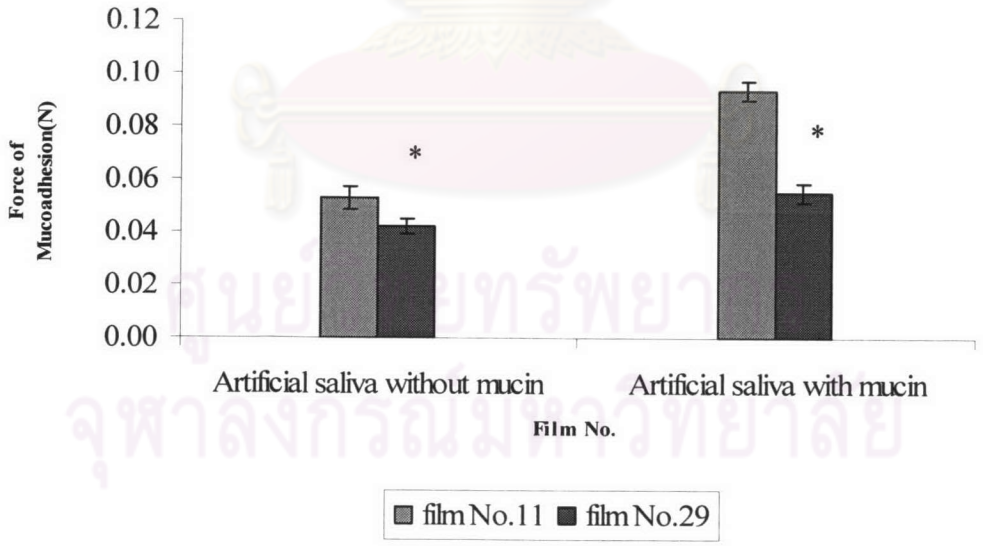

Figure 32 The force of mucoadhesion of film No. 11 and 29.

* significant difference ($p<0.05$)

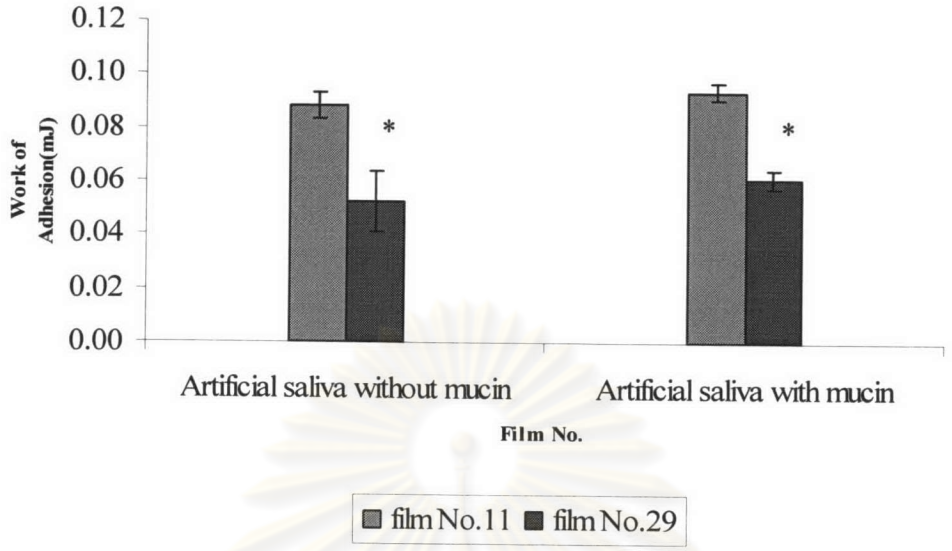

Figure 33 The work of adhesion of film No. 11 and 29.
* significant difference ($p<0.05$)

6. Content Uniformity of Triamcinolone Acetonide in Mucoadhesive Films

The dosage-unit uniformity was determined by assay 10 individual units (1 × 1 cm) using the HPLC method previously described. The limit specified in the USP 27 for the content uniformity of the dosage unit was in the range of 85 - 115 % of the label claim (%LA). Table 28 informs that the drug content in the 10 individual units of prepared mucoadhesive film lied within the limit as specified in the USP 27.

Table 28 The analytical results of the content of triamcinolone acetonide in the product of mucoadhesive films (n=10)

Film unit No.	Peak area ratio	Triamcinolone acetonide (μg)	% LA
1	1.1470	14.47	101.81
2	1.1449	14.43	101.54
3	1.1319	14.28	100.48
4	1.1223	14.16	99.63
5	1.1092	13.99	98.46
6	1.1469	14.47	101.81
7	1.1341	14.31	100.67
8	1.1469	14.47	101.81
9	1.1462	14.46	101.74
10	1.1391	14.37	101.12
Mean	1.1400	14.34	100.91
SD	0.0100	0.16	1.13
%CV	1.12	1.12	1.12

7. Stability Study of Triamcinolone Acetonide Buccal Mucoadhesive Films

Triamcinolone acetonide mucoadhesive films (film No.29) contained in glass vials, which tightly sealed with rubber closures and aluminium caps were stored at ambient conditions, and at 40 °C, 75% RH for three months. The amount of triamcinolone acetonide in mucoadhesive films were analyzed by the HPLC method at 0, 1, 2, and 3 month, respectively. The percentage labeled amount and percentage loss of triamcinolone

acetonide were calculated at each time interval and shown in Table 29 and 30. Since the percentage loss of drug was less than 10 %, it was considered stable (Carstensen, 1990). In addition, no remarkable change in the physical characteristics of the film was observed. The films still retained their flexibility.

Table 29 Percentage labeled amount of triamcinolone acetonide in film No. 29 stored at ambient condition

Time (Month)	Triamcinolone acetonide(μg)	%LA	% Loss of triamcinolone acetonide [a]
0	14.26 ± 0.02	100.34	0.00
1	14.25 ± 0.09	100.26	0.08
2	14.22 ± 0.02	100.07	0.27
3	14.17 ± 0.04	99.69	0.65

[a] $\%Loss\ of\ triamcinolone\ acetonide = \frac{Initial - Final\ \%labeled\ amount}{Initial\ \%labeled\ amount} \times 100$

Table 30 Percentage labeled amount of triamcinolone acetonide in film No. 29 stored at 40 °C and 75% RH

Time (Month)	Triamcinolone acetonide(μg)	%LA	% Loss of triamcinolone acetonide [a]
0	14.26 ± 0.02	100.34	0.00
1	14.17 ± 0.04	99.75	0.59
2	14.05 ± 0.12	98.90	1.44
3	13.93 ± 0.04	98.03	2.30

[a] $\%Loss\ of\ triamcinolone\ acetonide = \frac{Initial - Final\ \%labeled\ amount}{Initial\ \%labeled\ amount} \times 100$

8. *In Vitro* Release Study of Triamcinolone Acetonide from Buccal Mucoadhesive Films and Kenalog® in Orabase

The *In vitro* release of triamcinolone acetonide from buccal mucoadhesive films (film No.29) and Kenalog® in orabase was studied. The release data were fitted according to the following exponential equation (Peppas equation), which was used to describe the drug release behavior from polymeric matrices:

$$M_t / M_\infty = k\, t^n \qquad (9)$$

where M_t / M_∞ was the fraction of drug released, t was the release time, k was a kinetic constant characteristics of the drug polymer system, and n was a release exponent indicative of the release mechanism of the drug. When $n = 0.5$, the drug was released from the polymer with a Fickian diffusion mechanism. For $0.5 < n < 1$, a nonFickian solute diffusion was observed. The condition in which $n = 1$ provided a case II transport mechanism (erosion) with zero-order kinetics (Gohel, and Panchal, 2001).

A plot of log M_t / M_∞ versus log t of triamcinolone acetonide release from Film No. 29 are shown in Figure 34. From this release profile, the correlation coefficient (r^2) was 0.9900, and the corresponding equation was y = 0.6634x - 1.5173. So the release exponent (n) was 0.6634, the nonFickian mechanism or anomalous release was concluded for the release of triamcinolone acetonide from PG film No. 29. Since the hydrophilic PG mucoadhesive films dissolved easily in saliva, the release of drug from the films would depend directly on the ability of the hydrophilic polymer to absorb water, thereby promoted the dissolution, and hence the release of drug. The drug release was also controlled by the diffusion of drug molecules through the gel layer that could dissolve and erode. The PG films dissolved completely at approximately 3 hr.

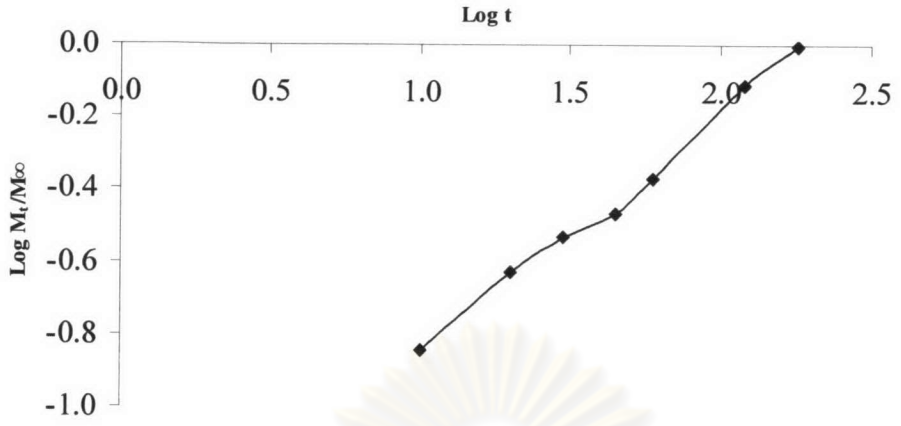

Figure 34 The log M_t / M_∞ versus log t plot of triamcinolone acetonide release from film No.29.

Several theories have been put forward in order to describe the process of release of the drug from inert polymer matrices.A combination of diffusion and hydrodynamic effect was also considered and the pores in the device played a fundamental role. Very often, experimental data have been obtained only for the earlier period of time and the diffusional process has been proved by the square root of law of time dependence with the amount of drug transported. In the case of an insoluble polymer matrix, drug release had generally been expressed by a Fickian diffusion mechanism, i.e., the time dependence of the square root of time. According to this model, a straight line was expected for the percent drug release versus square root of time plot if drug release was based on a diffusion mechanism (Rodriguez et al., 2000).

The square root-of-time plot of triamcinolone acetonide release from kenalog® in orabase is illustrated in Figure 35, and their release rate constants and correlation coefficients calculated between 0-6 hr are shown in Table 31. Kenalog® in orabase was a slow release vehicle and the drug released within 6 hr. The square root-of-time release could be described using the Higuchi equation:

$$M_t / M_\infty = k\, t^{1/2} \qquad (10)$$

where M_t / M_∞ was the fraction of drug released, t was the release time, k was a kinetic constant characteristics of the drug polymer system. This result complied with a previous study of Ungphaiboon, and Maitani (2001).

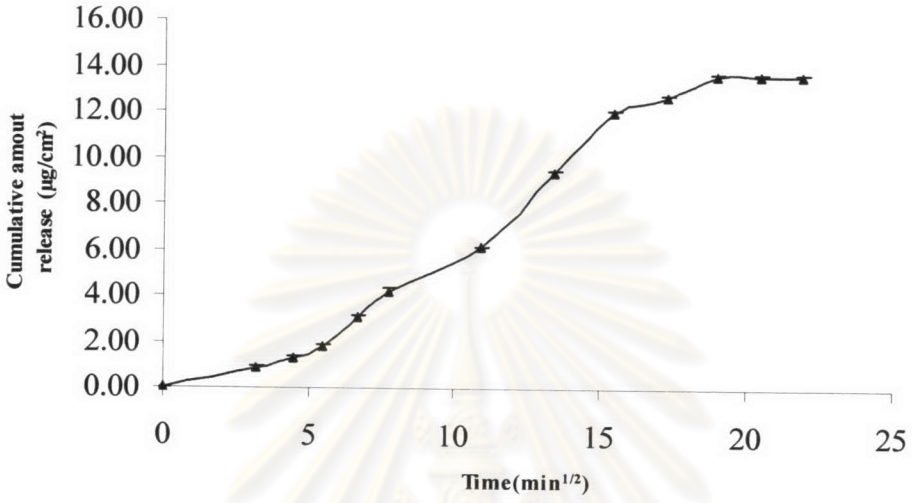

Figure 35 The square root-of-time plot of triamcinolone acetonide release from Kenalog® in orabase.

Table 31 The release rate constants and their correlation coefficients of triamcinolone acetonide release from Kenalog® in orabase

Sample No.	Square root-of-time plot	
	k	r^2
1	0.8158	0.9719
2	0.8165	0.9723
3	0.8144	0.9710
4	0.8166	0.9728
5	0.8140	0.9712
Mean	0.8155	0.9718
SD	0.0012	0.0008

9. Clinical Efficacy of Buccal Mucoadhesive Films with and without Triamcinolone Acetonide

Seventy two subjects with aphthous stomatitis were recruited, 42 women and 30 men, 18-45 years of age. Ulcer sizes were in the range of 1.0-5.5 mm with a mean of 3.3 ± 0.9 mm in diameter. The ulcers were located in lower labial mucosa, upper labial mucosa, and buccal mucosa. Written consent from each subject was allowed. They were grouped randomly into 4 groups; 18 subjects per group. Group No. 1 was a control group; subjects in this group were untreated with any preparations. Subjects in group No. 2, 3, and 4 were treated with Kenalog® in orabase, buccal mucoadhesive film base (film No. 11), and buccal mucoadhesive film containing triamcinolone acetonide (film No. 29), respectively. The efficacy of each treatment was assessed by the determination of curing rate (reduction of ulcer size, mm/day), and time period of ulcer disappearance (day). The PG film base, PG film containing triamcinolone acetonide, and Kenalog® in orabase

significantly cured ($p<0.05$) the subjects in group No. 2-4 before the end of the treatment period (7 days). The subjects were also asked to fill in questionnaires informing their perceptions of taste, convenience, irritation, acceptability, overall satisfaction of PG films, and also duration of film adhesion. Figures 36 and 37 inform that the subjects accepted the PG films well and no severe irritation of buccal mucosa was reported from all subjects treated with PG film with and without triamcinolone acetonide.

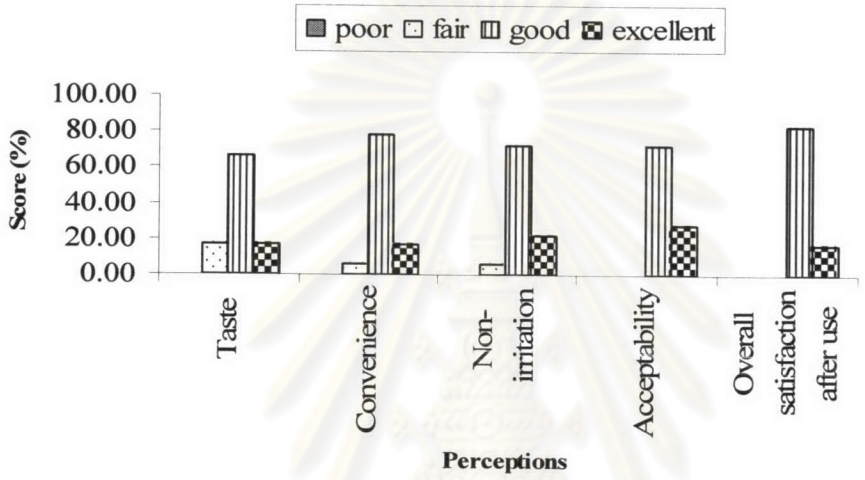

Figure 36 Subjects' perceptions of PG film base (group No. 3).

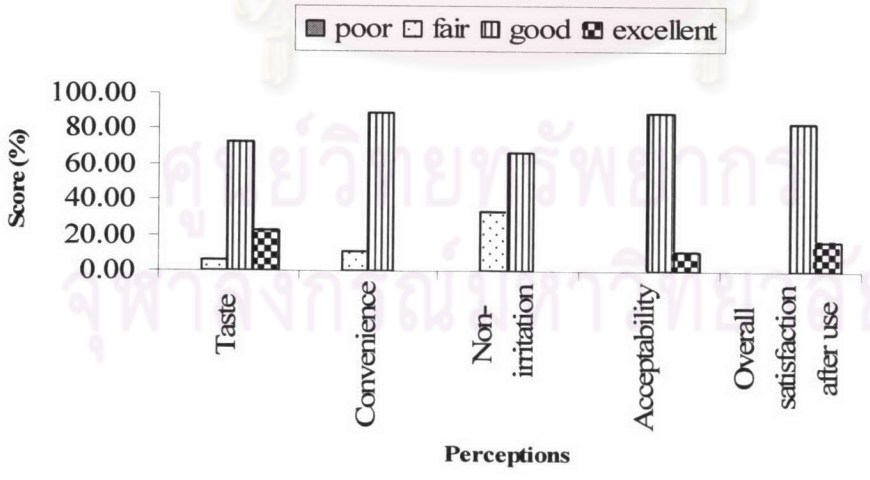

Figure 37 Subjects' perceptions of PG film containing triamcinolone acetonide (group No. 4).

The average reduction rate of ulcer sizes (curing rate) in subject group No. 1, 2, 3, and 4 were 0.4 ± 0.1, 0.7 ± 0.1, 0.7 ± 0.1, and 0.7 ± 0.1 mm/day, respectively (Table 32). From a statistical standpoint (ANOVA), the curing rate of group No. 2, 3, and 4 were not significantly different ($p > 0.05$), but the curing rate of these three groups were higher ($p < 0.05$) than that of group No. 1. In conclusion, PG film with triamcinolone acetonide showed not significant difference of the curing rate ($p>0.05$) compared to PG films.

The time periods for ulcer disappearance of subject group No. 1, 2, 3, and 4 were 6.5 ± 0.9, 5.6 ± 1.1, 4.9 ± 1.0, and 4.9 ± 0.9 days, respectively. The PG film base also gave the shortest curing time. The time period of ulcer disappearance of subjects using the Kenalog® in orabase, PG film base and PG film with triamcinolone acetonide were significantly shorter ($p<0.05$) than those of control group No.1, and the PG film base treated group No.3 showed significantly shorter time of ulcer disappearance ($p<0.05$) than that of Kenalog® in orabase treated group No.2.

The residence times of both PG film products and Kenalog® in orabase were not significantly different ($p > 0.05$).

As reported earlier, PG has a wound healing property in pig skin. The PG film dressing accelerated wound closure by observing the smallest wound area within 12 days treatment compared with the control group (applying 1 % povidone iodine) (Nakchat, 2002). Thus, the PG film base (film No.11) could beneficially treat aphthous stomatitis.

The etiology of aphthous atomatitis involved vitamin B1, B2, B12, C, and calcium deficiencies. Moreover, vitamin C was required for the synthesis of collagen, and vitamin C deficiency might lead to the breakdown of already healed wounds (Mazzotta, 1994). In addition, Ogura et al. (2001) found that lymphocytes from symptomatic individuals were more cytotoxic to epithelial cells in vitro than those of healthy control group. Lymphocytic cytotoxicity and antibody dependent cellular toxicity cause abnormal T-cell activation and migration into ulcers. Levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF) -α and interferon -γ were elevated in these ulcers. TNF was synthesized by T-cells and mast cells, enhancing phagocytosis and neutrophil degranulation. The natural balance existing between pro-inflammatory and anti-inflammatory cytokines was disrupted during ulceration. So, the steroidal anti-inflammatory drug could be used.

The Guideline of the Diagnosis and Management of Recurrent Aphthous Stomatitis Austin (TX) (2003) states that Kenalog® in orabase can be used in patients with minor to major aphthous stomatitis. Triamcinolone acetonide, a synthetic corticosteroid, in Kenalog® has anti-inflammatory properties and therefore can help to limit the progression of aphthous stomatitis's ulceration. The earlier use of Kenalog®, the more quickly the pain can be reduced and the ulcer can be healed. The orabase is a paste designed to adhere to the surface of oral lesions. As used in this formulation, the orabase created a protective film over the aphthous stomatitis and held triamcinolone acetonide in place. However, the use of Kenalog® in orabase has not been shown to decrease the rate of recurrence of aphthous stomatitis.

Table 32 Clinical assessment and *in vivo* residence time of PG films and Kenalog® in orabase

Group No.	Treatment	Mean curing rate (mm/day)	Time period of ulcer disappearance (day)	Mean *in vivo* residence time (min)
1	Control	0.4 ± 0.1^{b}	6.5 ± 0.9^{a}	-
2	Kenalog® in orabase	0.7 ± 0.1^{a}	5.6 ± 1.2^{b}	83.4 ± 6.9
3	Film No.11	0.7 ± 0.1^{a}	4.9 ± 1.0^{c}	80.9 ± 6.7
4	Film No.29	0.7 ± 0.1^{a}	4.9 ± 0.9^{bc}	78.3 ± 5.2

$^{a, b, c}$ significant difference between group ($p<0.05$).

From the preceding discussion, the ideal application of a therapeutic agent to promote healing of ulcer would be to apply it directly to the ulcer and kept the material in contact as long as possible in a manner that complied with patient acceptance. Therefore, the buccal film would be preferred over adhesive tablets in terms of flexibility and comfort. In addition, they could circumvent the relatively short residence time of oral gels on the mucosa which was easily washed away and removed by saliva. Moreover, the buccal film

was able to cover the wound surface, thus reduced pain and could treat oral diseases more effectively. So, the films capable of tissue adhesive could improve patient compliance and shorten duration of symptoms (Ahn et al., 2002).