CHAPTER III

RESULTS AND DISCUSSION

1. Effect of Concentration of PG on Viscosity

An apparent viscosity of PG gel solutions at various concentrations is shown in Figure 24. PG at high concentration had high viscosity; in particular, the viscosity value of PG at a concentration of more than 4% showed a sharp increase in viscosity. In comparison to the other gelling agents such as carboxymethylcellulose (CMC) and Carbomer 940, we found that increasing their concentration resulted in increase in viscosity. The viscosity of Carbomer 940 at the same concentration with PG and CMC was very high. The viscosity value of 4% of CMC and 1% of carbomer 940 was more than 10,000 cps. (shown in table B2 in appendix B). Therefor, PG could be used as a thickening or gelling agent and use increase in PG concentration would lead to an increase in viscoscity as shown in Figure 2.

2. Effect of Concentration of PG on pH

The PG was a water-soluble acidic polymer and pH value of 1-6 % PG was about 2.2-2.3 (Figure 25). The pH value of 1-6 % CMC was about 6-7, the increasing concentration had little effect on its pH value, the increasing concentration of Carbomer 940 had no effect on its pH value.

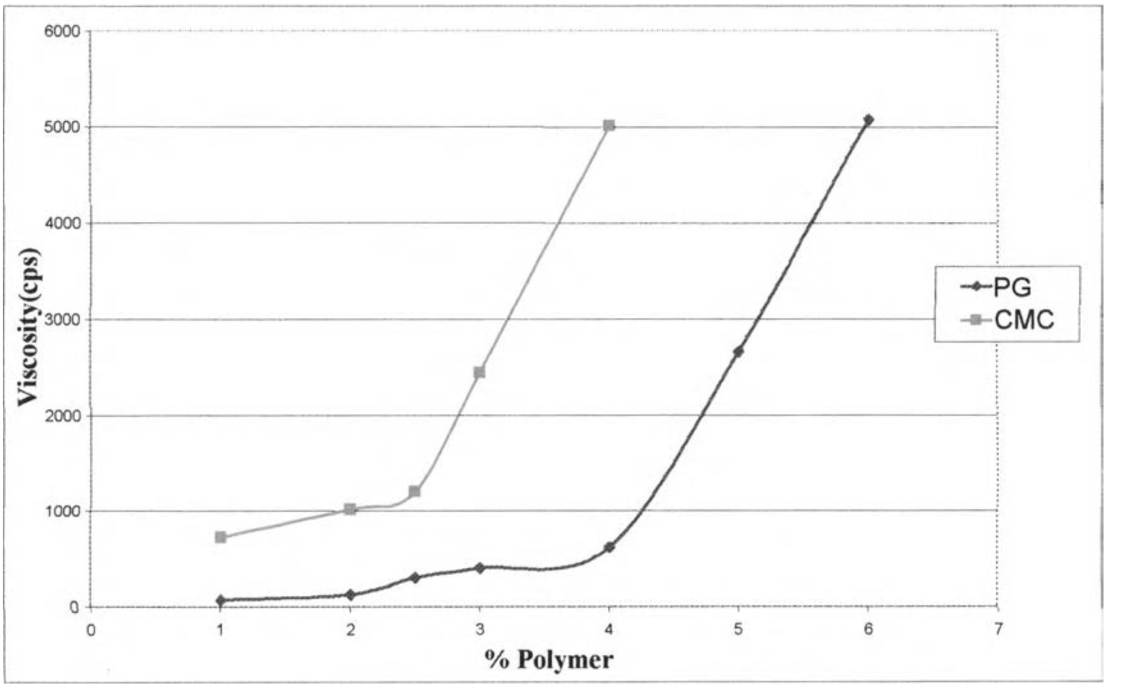


Figure 24 The apparent viscosity profile of polysaccharide gel (PG) and Carboxymethyl cellulose (CMC).

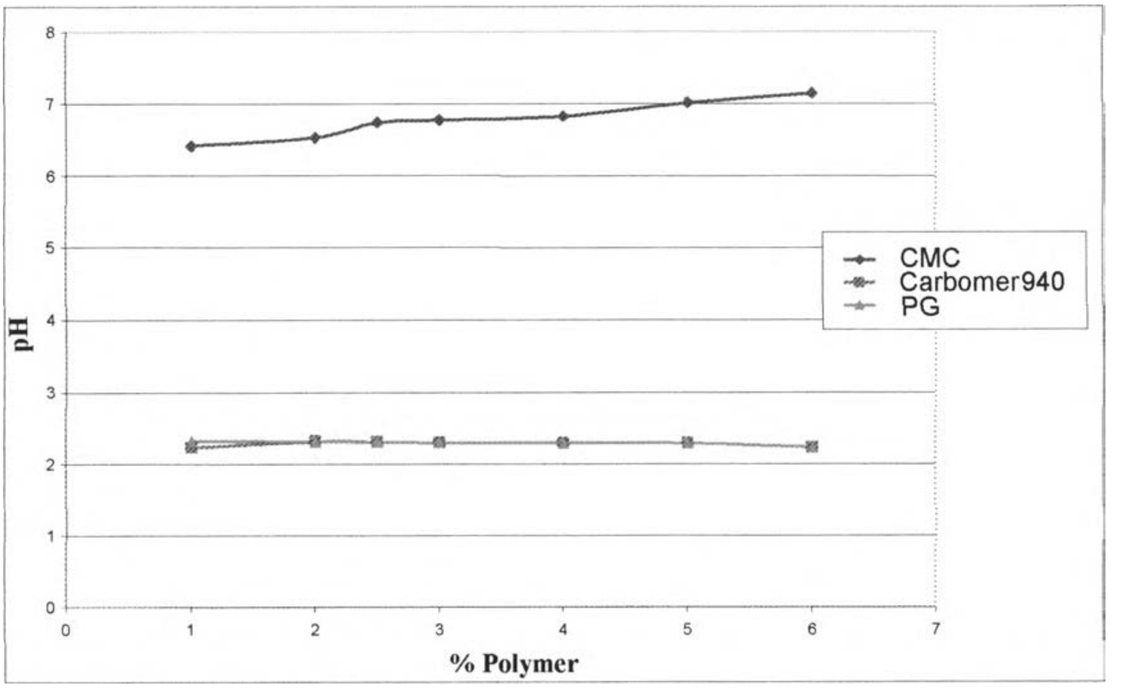


Figure 25 The pH profile of polysaccharide gel (PG), CMC and Carbomer 940

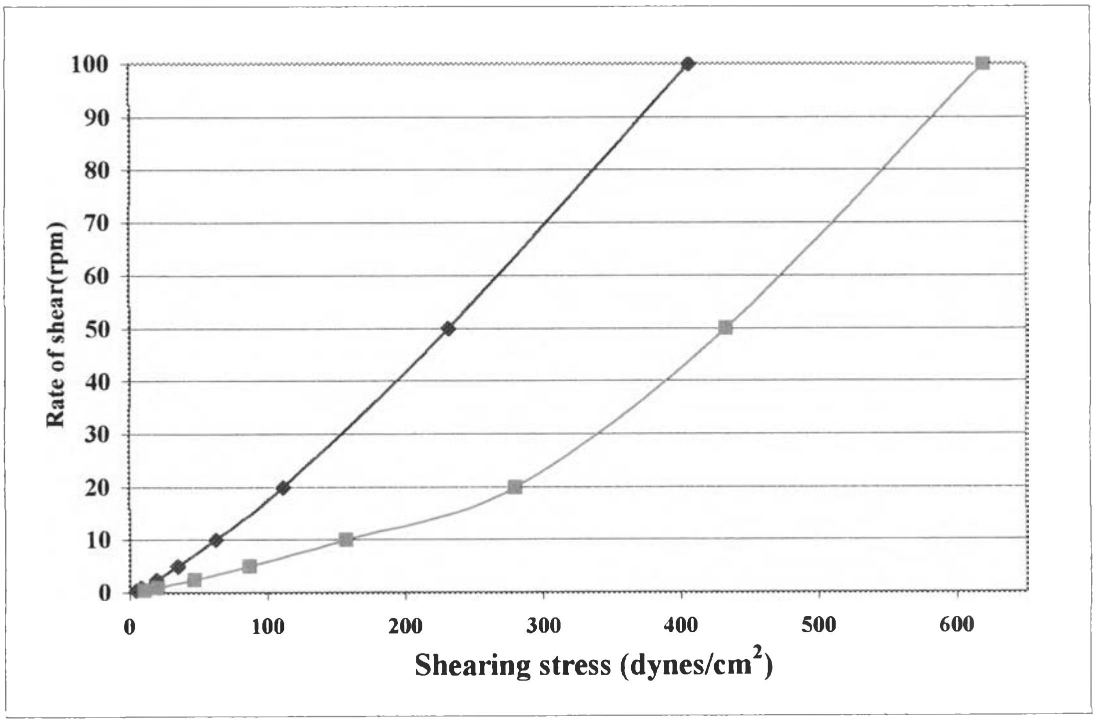


Figure 26 Rheological characterization of polysaccharide gel (PG). 3%PG(♦), 4%PG(■)

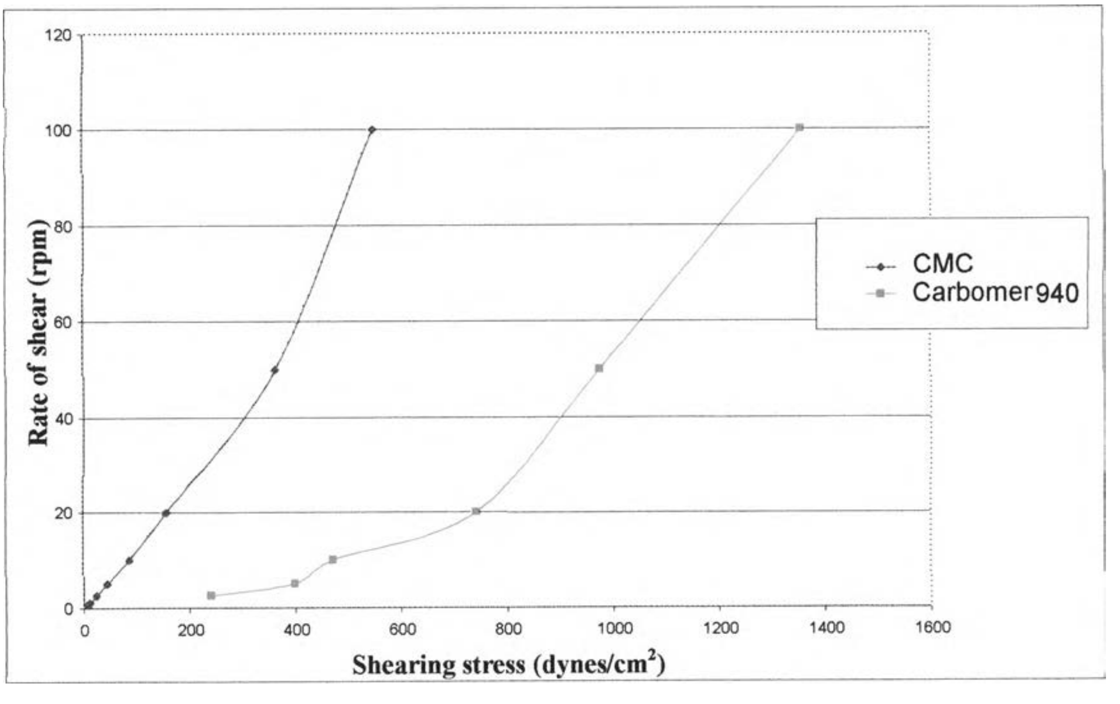


Figure 27 Rheological characterization of CMC and Carbomer 940

3. Rheological Properties

When the rate of shear was increased, the viscosities of 3% and 4% PG decreased. The result shows in Figure 26. The graph plotted between rate of shear and the shearing stress showed the same profile even at different concentration was used. The characteristic of non-newtonian pseudoplastic behavior was obtained. The viscosity decreased after agitation. The flow characteristics of CMC and Carbomer were similar as shown in Figure 27. The flow behavior of PG was the same as other polysaccharide such as gum, hydroxypropyl methylcellulose that were mostly used as thickening, gelling and suspending agent in cosmetic industries (Kabre, 1964).

4. Factors that effect the viscosity of PG

4.1 Effect of pH

The pH value above 5.0 decreased the viscosities of CMC and Carbomer 940. Lower pH value (2.0-4.0) showed more effect of decreasing viscosity of CMC and Carbomer 940. Figure 28 showed that pH values between 2.0-4.0 had less effect on PG viscosity. From this experiment we learned that PG was acid resistant at low pH value, therefore, PG had benefit for using in low pH formulations.

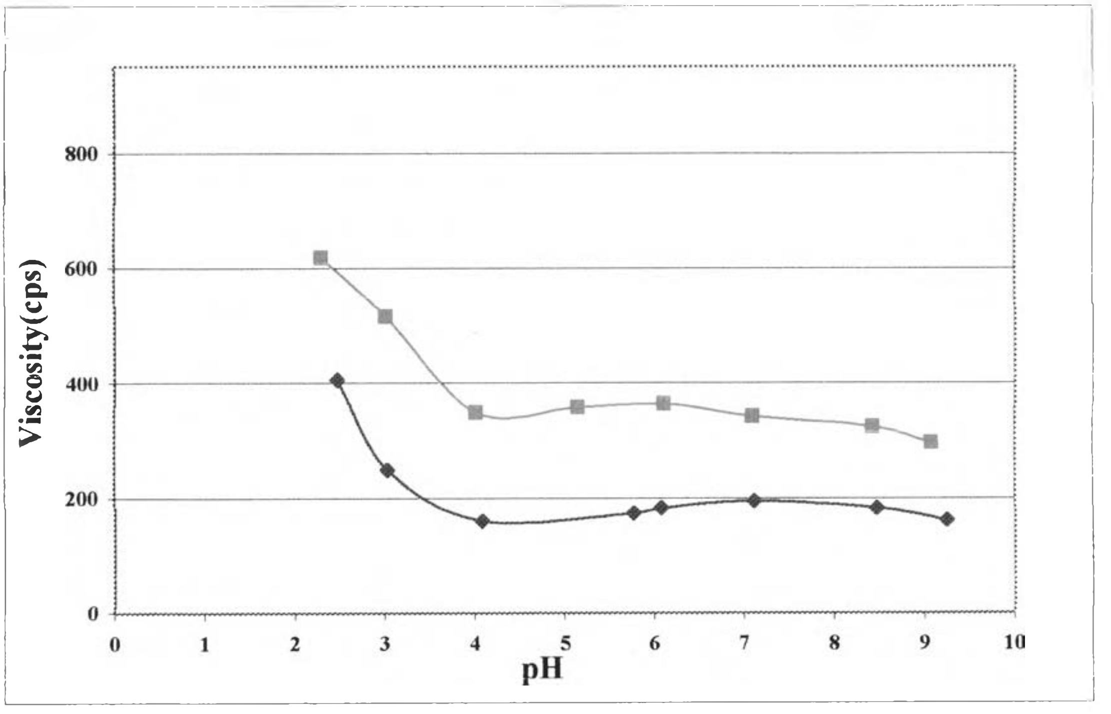


Figure28 Effect of pH on the apparent viscosity of Polysaccharide gel (PG).3%(♦), 4%(■)

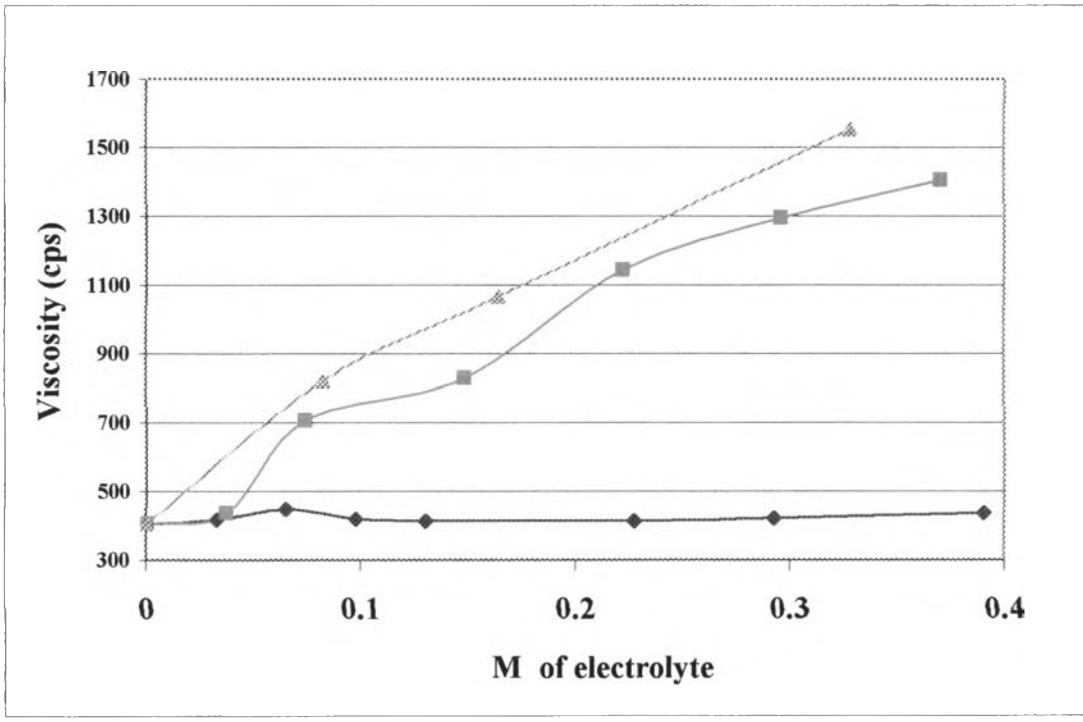


Figure 29 Effect of Electrolyte on the apparent viscosity of Polysaccharide gel (PG).
NaCl(♦), KCl(■), $MgCl_2$(▲)

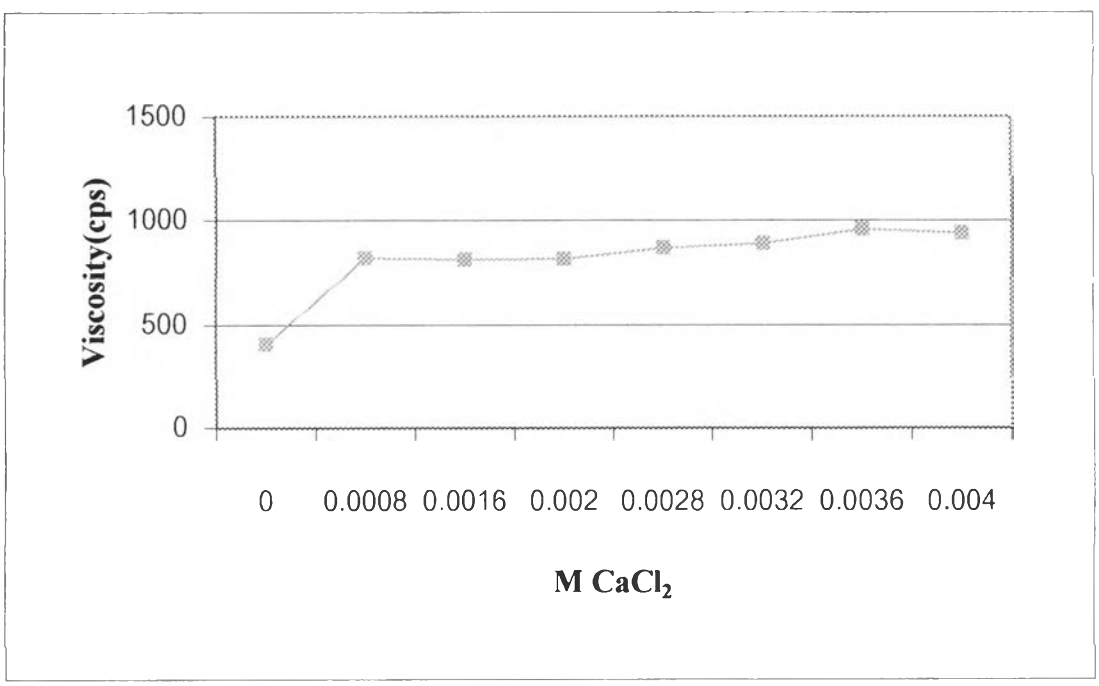


Figure 30 Effect of $CaCl_2$ on the apparent viscosity of polysaccharide gel (PG).

4.2 Effect of Electrolyte

The results of electrolytes on the viscosity of PG are shown in figures 29 and 30, respectively. The viscosities were increased when di-valent cations ($MgCl_2$ and $CaCl_2$) and high concentrations of mono-valent cation (KCl) were present. But NaCl did not have any effects. The increment of viscosity of PG when the divalent cations were added, which was similar to case of alginic acid bonding to Ca^{2+} as shown in Figure 11 (Cardinali, S. *et al.* 1997). However, all 4 electrolytes caused CMC and Carbomer 940 gels precipitated.

4.3 Effect of Solvent

The results of solvents on the viscosity of PG are shown in figure 31. Ethyl alcohol and isopropyl alcohol increased the viscosity of PG solution, but butyl alcohol did not have any effects. In the case of CMC , ethyl alcohol, isopropyl alcohol and butyl alcohol decreased the viscosity of CMC solution as shown in figure 32. All solvents decreased the viscosity of Carbomer 940 gel a little.

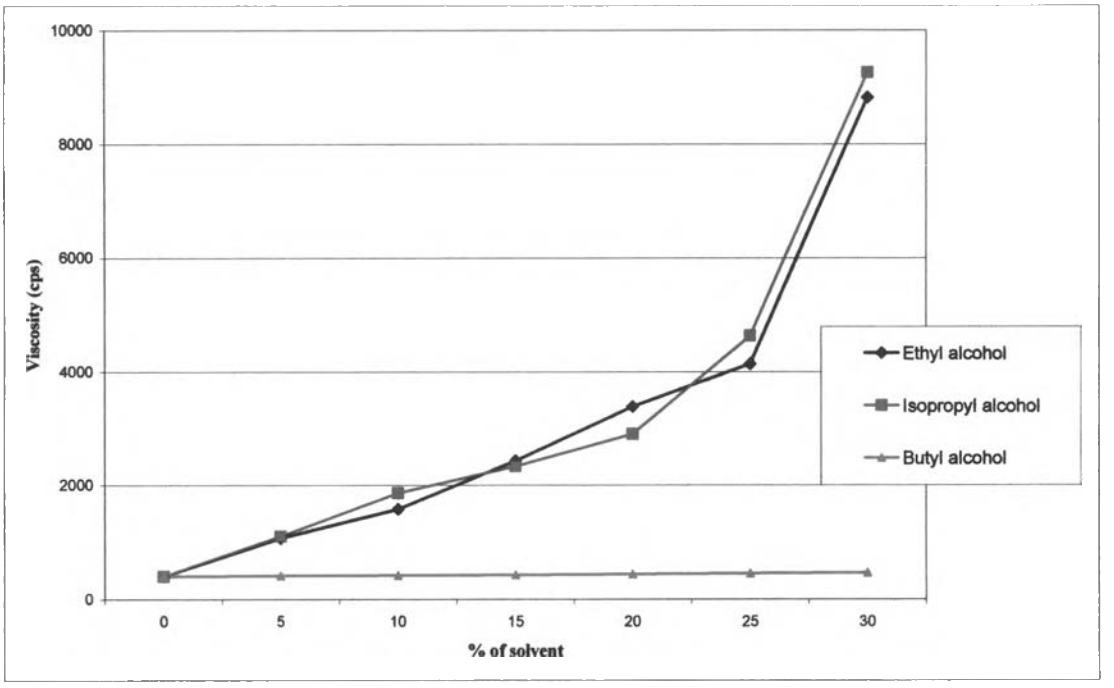


Figure 31 Effect of solvent on the apparent viscosity of polysaccharide gel (PG). ethyl alcohol(♦), isopropyl alcohol(■), butyl alcohol(▲)

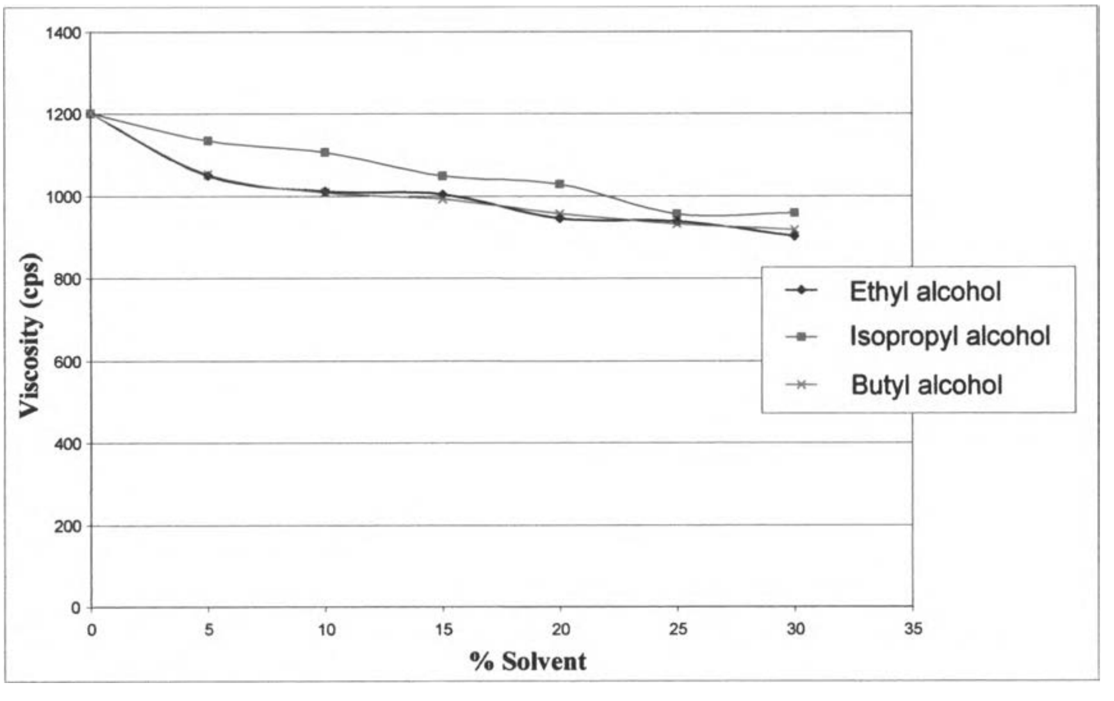


Figure 32 Effect of solvent on the apparent viscosity of CMC. ethyl alcohol(♦), isopropyl alcohol(■), butyl alcohol(x)

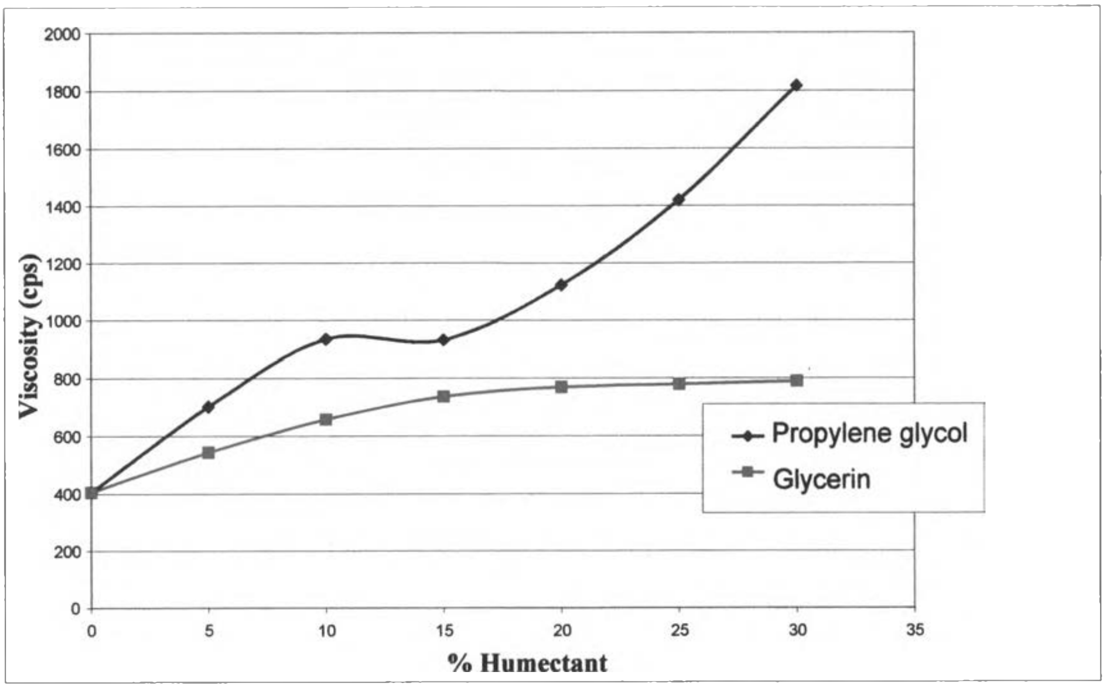


Figure 33 Effect of humectant on the apparent viscosity of Polysaccharide gel(PG). propylene glycol (♦), glycerin (■)

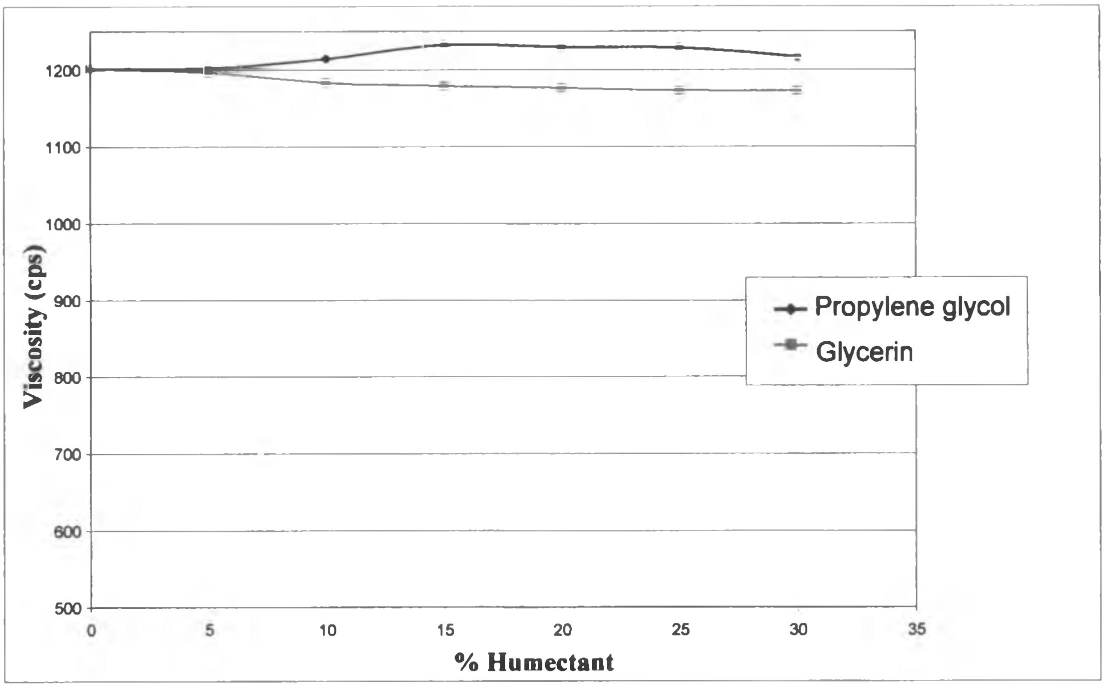


Figure 34 Effect of humectant on the apparent viscosity of CMC. propylene glycol (♦), glycerin (■)

4.4 Effect of Humectant

Two humectants, propylene glycol and glycerin at various concentration levels will have the effect to the viscosity as shown in Figure 33. When increase concentration level of propylene glycol in CMC solution, the viscosity will increase. When increase concentration level of glycerin, the viscosity will go down as shown in Figure 34. When increase the concentration of propylene glycol and glycerin in Carbomer 940 solution, the viscosity of Carbomer 940 will decrease a little as shown in Table B8 in appendix B

4.5 Effect of Preservative

Preservatives which are normally used in gel and lotion are 1% Paraben concentrate, 1-2% benzoic acid and 1-2% sorbic acid. At this level of concentration we found that they had no effect on PG viscosity. At a higher concentration, 2% Paraben concentrate had some effect on PG viscosity. Benzonic acid and sorbic acid at the same concentration decreased the PG viscosity as shown in Figure 35. Since the paraben concentrate contained propylene glycol, propylene glycol might increase the viscosity of PG solution. When the paraben concentrate was added to the PG solution, it might increase the viscosity of PG solution as well. In the case of CMC and Carbomer 940 as shown in table B7 and appendix B, only paraben concentrate had no effect on PG viscosity. Benzonic acid and sorbic acid wre not compatible to CMC and Carbomer gels.

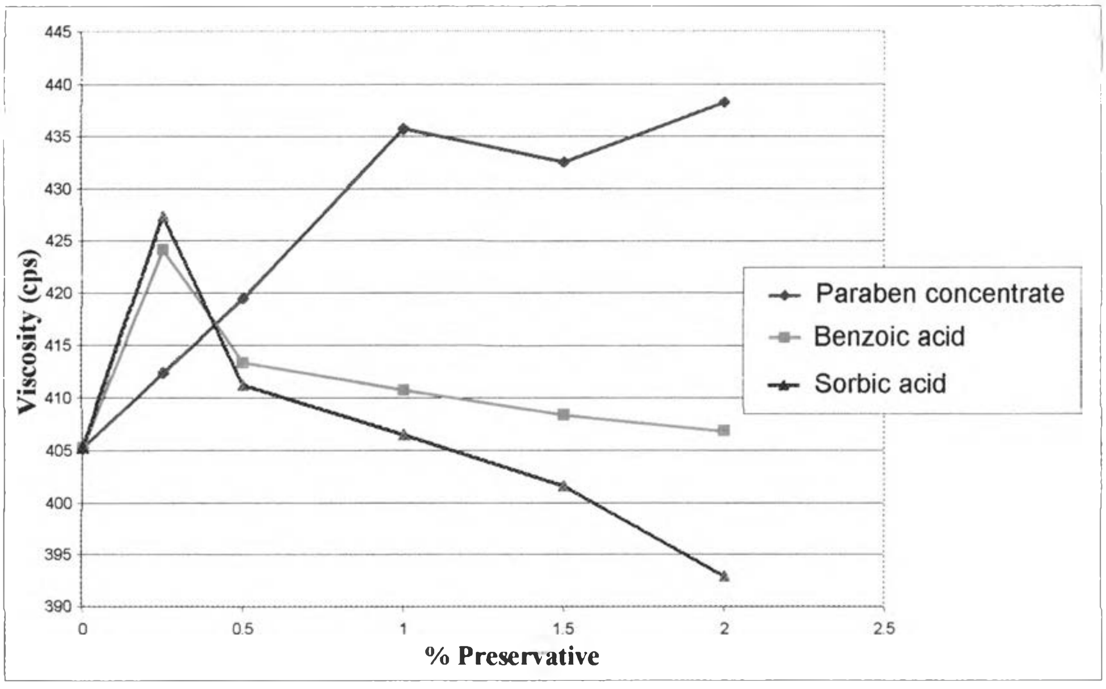


Figure 35 Effect of preservative on the apparent viscosity of Polysaccharide gel (PG). paraben concentrate (♦), benzoic acid (■), sorbic acid (▲)

Table 4 Effect of temperature on viscosity of Polysaccharide gel(PG) .

%PG	Viscosity (cps) at test temperature						
	Ambient Temperature	50°C		70°C		100°C	
		Heated Temperature	Cool down to ambient	Heated Temperature	Cool down to ambient	Heated Temperature	Cool down to ambient
3	405.23±7.48	355.44±3.54	392.66±2.38	170.21±5.33	395.48±2.17	78.09±2.11	120.90±5.81
4	617.80±9.84	535.86±8.43	593.89±4.42	455.46±7.17	483.27±3.28	173.10±3.98	191.28±6.57

Table 5 Effect of temperature on viscosity of CMC and Carbomer 940

% Gelling agent	Viscosity (cps) at test temperature						
	Ambient Temperature	50°C		70°C		100°C	
		Heated Temperature	Cool down to ambient	Heated Temperature	Cool down to ambient	Heated Temperature	Cool down to ambient
CMC2.5%	1201.11±95.31	1054.96±51.65	1035.23±23.84	966.12± 45.70	874.22±82.93	824.22±66.41	727.85±52.44
Carbomer1%	>10,000	>10,000	>10,000	7345.72±156.79	6439.12±136.82	5741.45±145.12	3658.49±198.25

4.6 Effect of temperature

The results of temperature effect on the viscosity of 3% and 4% PG is shown in Table 4. Their viscosities decreased at higher temperature especially at the temperature of 70 °C and 100 °C. However, at 50-70 °C the viscosity increased to the original viscosity after PG was cooled down to ambient temperature. At 100 °C, the viscosity d ecreased p ermanently. T he e ffect o f h eat o n t he v iscosity of C MC a nd Carbomer 940 is shown in Table 5. CMC viscosity was less affected at 50 °C and 70°C than that at 100°C as its viscosity dropped permanently a lthough it was cool down. While the viscosity of Carbomer changed a lot at 70°C and 100 °C as shown in Figure 14. This was similar to the case of carrageenans (Guiseley, KB. *et al.* 1980). Permanent lost of PG viscosity after heated to 100 °C for 30 minutes was the result of structural deformation of PG at high temperature.

5. Preparation of PG Gel

Solubility Test of VitaminE Acetate in Solubilizers

Vitamin E acetate, which was andoil soluble vitamin, was used as an active ingredient. In order to be able to prepare a transparent gel, some solubilizers were studied. These included Tween 20, Pluronic F 68 and Cremophor RH 40. Each solubilizer was mixed with vitamin E acetate and propylene glycol. Only Cremophor RH 40 could dissolve vitamin E acetate completely and gave a clear solution. Tween 20 and Pluronic F68 gave white muddy. Therefore, Cremophor RH 40 was used as the solubilizer in the formulation of Vitamin E-PG gel as shown in table B11 in appendix B.

6. Evaluation of PG Preparation

From the Table B 12-16 in appendix B described the characteristics of Vitamin –E PG gels at varieties of surrounding. Most ingredients in the gels were the same. The gel were clear with a little brown. The brown color is the natural color of PG as shown in Figure 36. After testing at many conditions, the characteristics of gel were not changed. The viscosity of some formulations is higher than those freshly prepared. Figure 37 showed that when the gel concentration is higher, the viscosity of gel is also higher. The result of concentration between $CaCl_2$ and PG were shown in Figures 38 and 39, respectively when pH were higher than 5, the viscosity of gel decreased a little bit. The physical stability tests of vitamin C-PG gel and vitamin E&C shown that the color of gels changed to yellow and the characteristics of gel had not changed.

7. Sensory analysis of Vitamin E PG Gel Preparation

The sensory analysis of vitamin E PG gel by volunteers revealed that most of them were satisfied with the product. The first and second ranks of satisfaction were its spreadability and coolness, repectively. After finishing the test, the volunteers were also satisfied with the physical appearance of vitamin E gel. Furthermore, they were interested in using PG due to its natural origin.

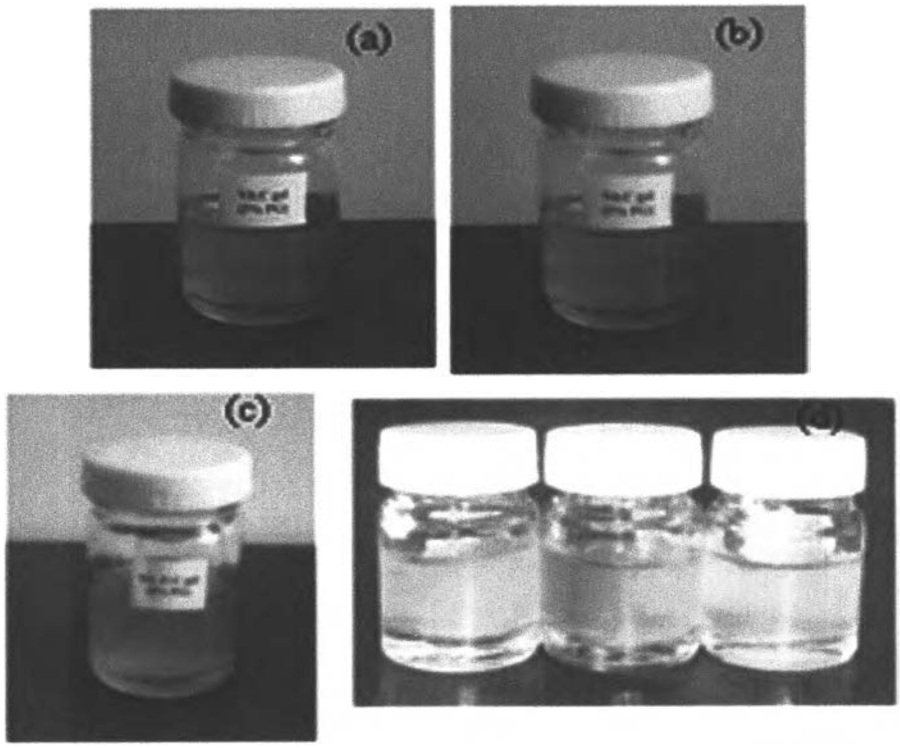


Figure 36 (a) Vitamin E PG preparation
(b) Vitamin C PG preparation
(c) Vitamin E and C PG preparation
(d) Validation of Vitamin E PG preparation

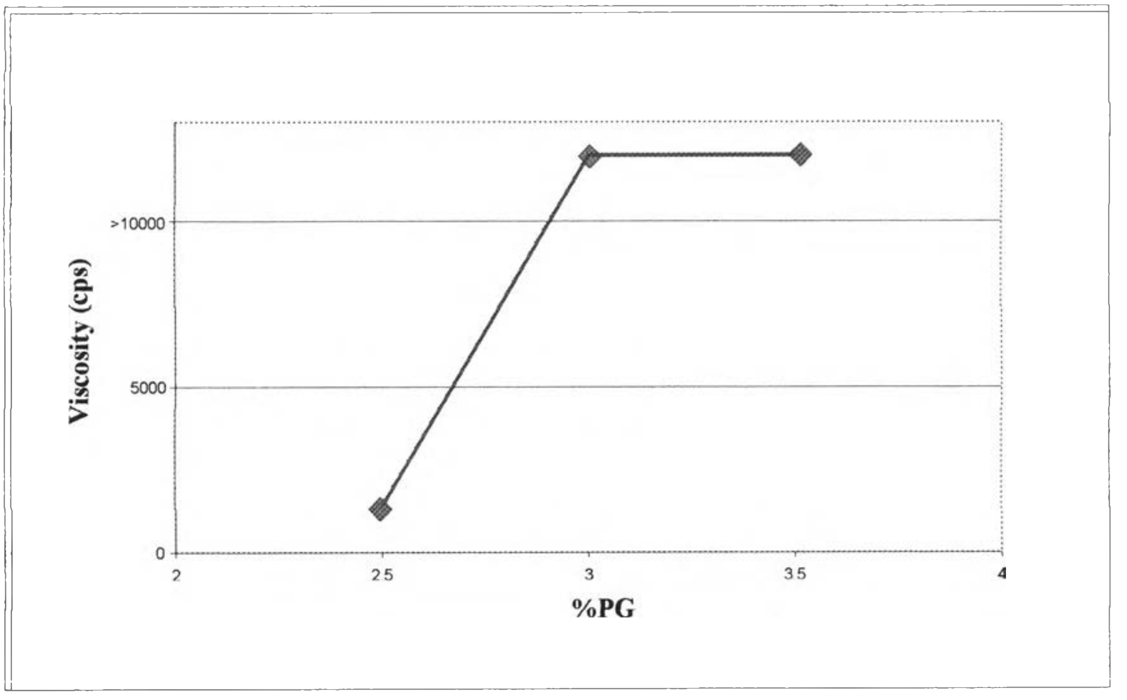


Figure 37 Effect of PG concentration on viscosity of vitamin E gel

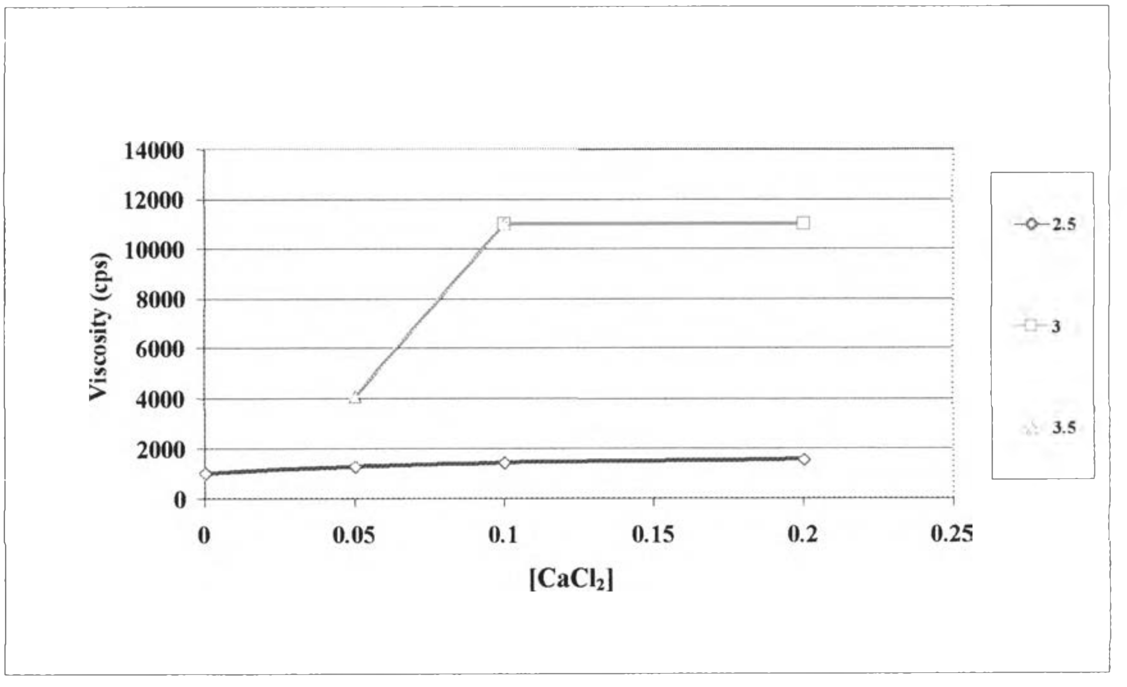


Figure 38 Effect of $CaCl_2$ on viscosity of vitamin E gel

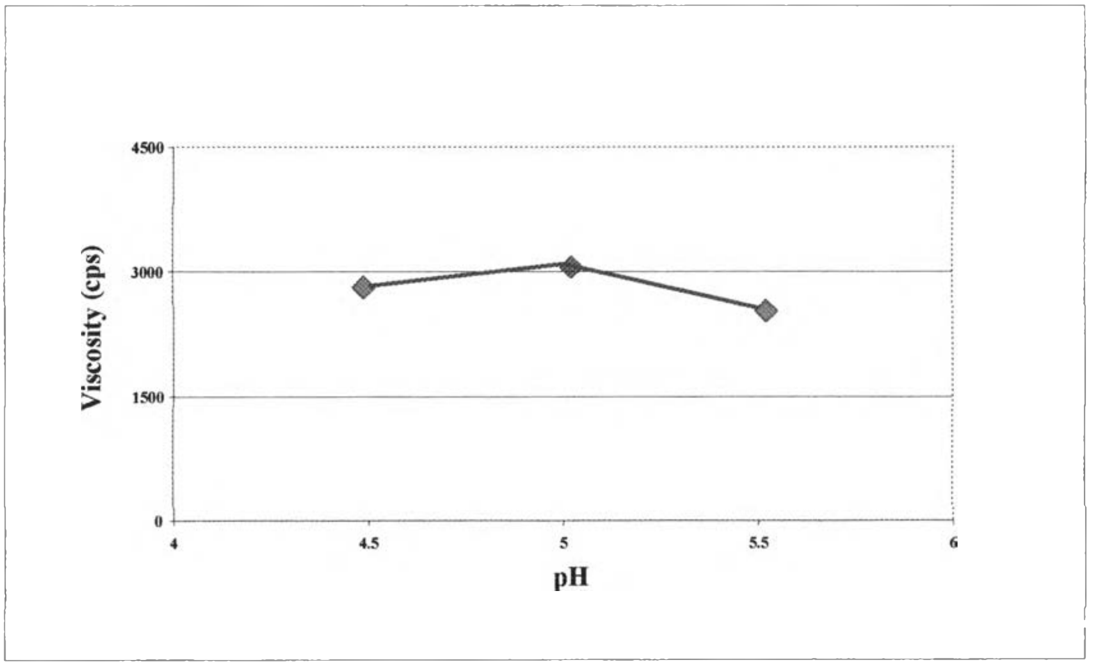
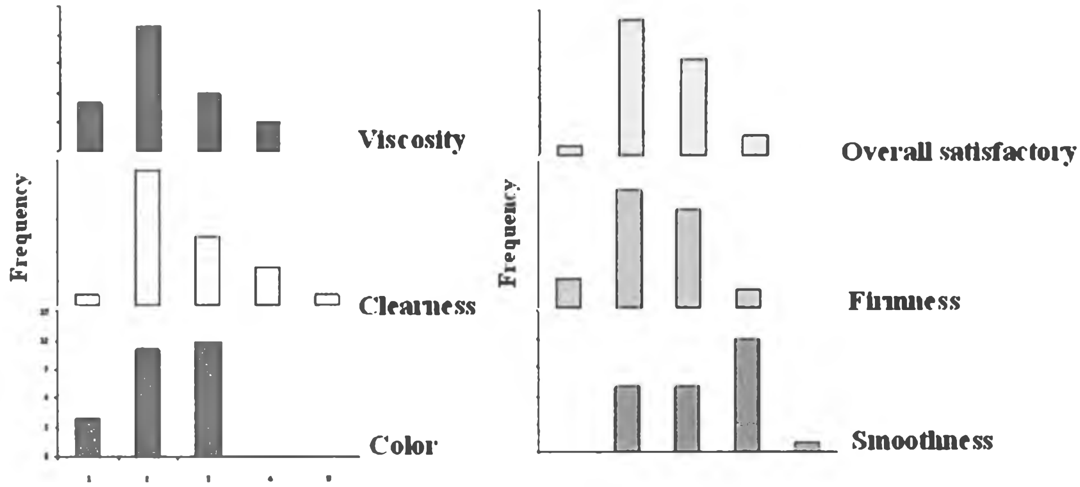
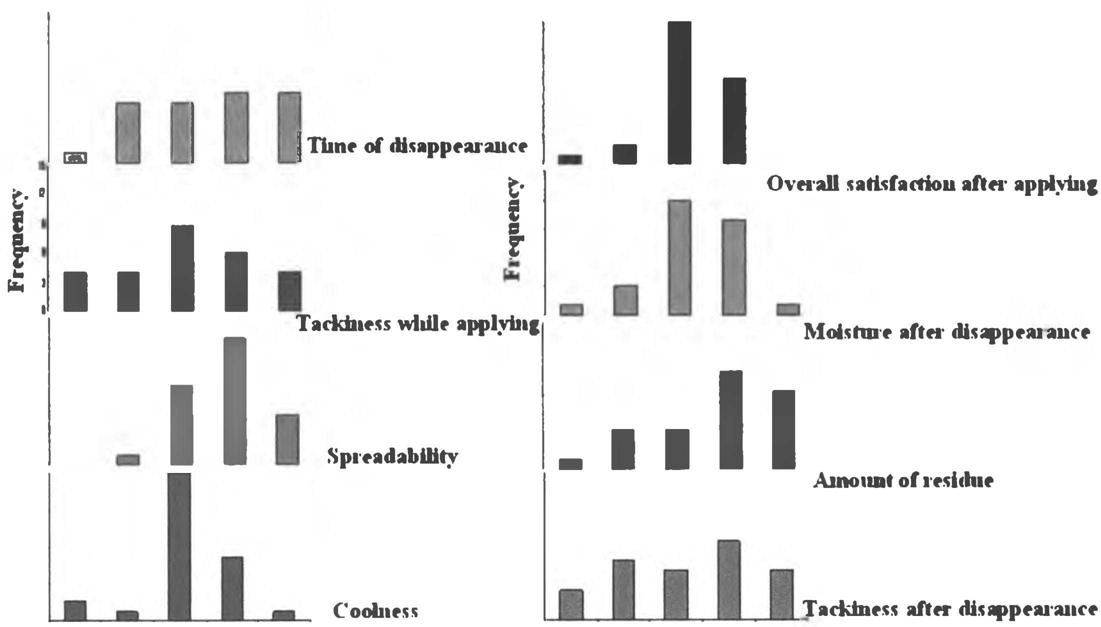


Figure 39 Effect of pH on viscosity of vitamin E gel



a) Physical appearance



b) After testing

Figure 40 A summary of sensory analysis of vitamin E PG preparation by a group of volunteer, (a) Physical appearance (b) After testing, scores ranging from 1 = least satisfy, 2 = less satisfy , 3 = satisfy, 4 = more satisfy, 5 = most satisfy

8. Film-forming properties of PG

The PG has an additional quality to be interesting. It can be formed itself as a film. The characteristics of this film are the transparent plate, light blown, hard and brittle. In additional, it is not easy to separate the film from the plate. The objective of adding a plasticizer is to produce a film plate with required qualification for further applications. The study of PG film that has an addition of 3 different plasticizers at 3% concentration per weight of PG by comparing with a PG film without plasticizer. The experiment started by using a film and letting it pass the mechanical test with a tensometer. The study of film's tensile monitors 3 factors: the percentage of strain at breakdown point, young's modulus and ultimate tensile strength. Value of film's characteristic shown the brittleness, hardness, softness and shear strength etc. This experiment required the film refresher to be soft, flexible and easy to dissolve, not brittle, hard and easily peelable from the glass plate. Table 6 found that the unadded plasticizer film, the strain at break point is 7.50 ±2.25 %. Ultimate strength of 5.83 ±0.96 MPa and young 's modulus of 526.21 ± 41.46 MPa. The film was a little hard ,brittle, easy to break and hard to peel from the plate. After added the plasticizer , the characteristic had changed signicficantly. By adding Propylene glycol and glycerin we found that even the percentage of strain at break point of the plasticized film will lower than the PG film without plasticizer but when compared with the ultimate tensile strength and young's modulus we found that were higher. The characteristics of film with plasticizer are more flexible than PG without plasticizer, tough, easy to peel from the plate. The propylene glycol is better than glycerin. But at 10% propylene glycol, if we increase propylene glycol will result in decrease percentage of strain at break point. In sobitol, we found that it's had high value of percentage of strain at break point and low value of young's modulus. This shown that the film is soft and strength, able to peel out from the plate in a good shape.

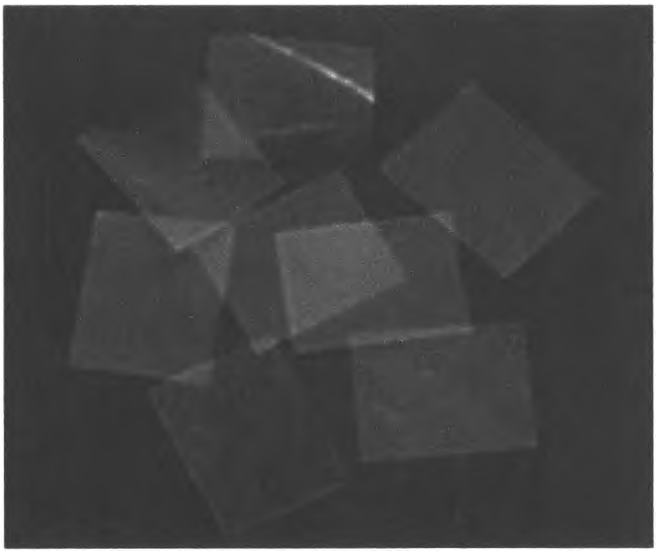


Figure 41 Product of PG mouth refreshing films

Table 6 Effect of plasticizers and concentrations on properties of PG film. (PG content 1.24 mg/strip)

PG film with plasticizer content (%w/v) based on PG weight (2x2.5 cm./strip)	Thickness ± SD (μm)	%Strain at point of break ± SD (%)	Young's modulus ± SD (MPa)	Ultimate tensile strength ± SD (MPa)
0%plasticizer (0)	47.86±2.99	7.50±2.15	526.21±41.46	5.83±0.96
5%propylene glycol (P5)	46.13±3.31	1.53±0.18	1392.00±58.65	10.40±1.83
10% propylene glycol (P10)	49.60±2.83	2.10±0.52	740.90±105.89	1.57±0.32
15%propylene glycol (P15)	48.60±3.88	1.50+0.92	1335.00±203.49	12.70±2.73
5% glycerin (G5)	46.13±3.80	0.90±0. 03	1121.00±77.70	9.00±1.24
10% glycerin (G10)	44.46±3.73	2.60±0.58	867.00±39.93	4.50±1.00
15% glycerin (G15)	45.80±3.70	1.90±0.64	1056.00±55.28	3.48±0.73
5% sorbitol (S5)	43.53±2.82	12.48±1.46	322.50±31.18	13.98±4.72
10% sorbitol (S10)	46.86±2.45	8.84±4.45	246.30±36.79	18.69±5.39
15% sorbitol (S15)	47.20±3.61	7.00±2.18	394.81±36.90	15.47±4.57

9. Mechanical Evaluation of PG Films

The mechanical properties of PG films are demonstrated in Table 6 and Figures 42, 43 and 44. The profiles of Thickness, % Strain at break, Young's modulus and Tensile strength (MPa) were evaluated, respectively. The results compared between the formulations studied. However, PG films without plasticizer product was clear, translucent, but hard and very brittle. Addition of plasticizers effected the mechanical properties of PG films, significant difference values of %strain at break, Young's modulus and tensile strength (MPa) were obtained.

Tensile strength of PG film were found increase with respected to increase plasticizer concentration, except that of propylene glycol. Increase propylene glycol resultes in decreaseing tensile strength and G10 ,G15 formula plasticized with glycerin. %Strain at break which is defined as the distance at break related to original distance of free film. The% strain at break of PG film were found decrease with respected to increase plasticizer concentration, expect that of S5 formula plasticized with sorbitol. Young's modulus reported hardness of a PG film showed and inclination for this value as the level of plasticizer increase.

The addition of plasticizer increased the ductility of the film, but this is often accompanied by a reduction in its tensile strength and modulus of elasticity. Plasticization therefore results in a soft, tough film. Increasing plasticizer concentration enhances this effect (Aulton, M.E., 1982).

Figure 42, Table 6 showed mechanical properties of films P5, P10, and P15 adding propylene glycol into the films formulation of PG at concentration 5%, 10%, and 15% w/w based on PG polymer, resulted in increasing hardness and strongness of films with moderate values of % Strain at break and high values of tensile strength and Young's modulus values compared to PG films without plasticizer. These results indicated that propylene glycol, a water soluble plasticizer, was the component plasticizer for the PG films, at the 10% propylene glycol based on PG polymer resulted decreasing the tensile strength and Young's modulus less than the PG films plasticized with propylene glycol at 5% and 15% plasticizer based on PG polymer. From the present results, the films plasticized with 10%propylene glycol produced lowest value of tensile strength and Young's modulus.

Figure 43, Table 6 showed mechanical properties of films G5, G10, and G15 adding glycerin into the films formulation of PG at concentration 5%, 10%

and 15% w/w based on PG polymer, resulted in increasing hardness with moderate values of % strain at break and high values of Tensile strength and Young's modulus values compared to PG films without plasticizer. These results indicated that glycerin, a water soluble plasticizer, was the component plasticizer for the PG films, at the 5% glycerin based on PG polymer resulted increasing the tensile strength and Young's modulus more than the PG films plasticized with glycerin at 10% and 15% plasticizer based on PG polymer. From the present results, the films plasticized with 5% glycerin produced highest value of tensile strength and Young's modulus.

Figure 44, Table 6 showed mechanical properties of films S5, S10, and S15 adding sorbitol into the films formulation of PG at concentration 5%, 10%, and 15% w/w based on PG polymer, resulted softness and toughness of film with high values of % strain at break, tensile strength and low values of Young's modulus compared to PG films without plasticizer. These results indicated that sorbitol, a water soluble plasticizer, was the component plasticizer for the PG films, at the every concentration based on PG polymer resulted increasing the tensile strength and decreasing Young's modulus more than the PG films without sorbitol

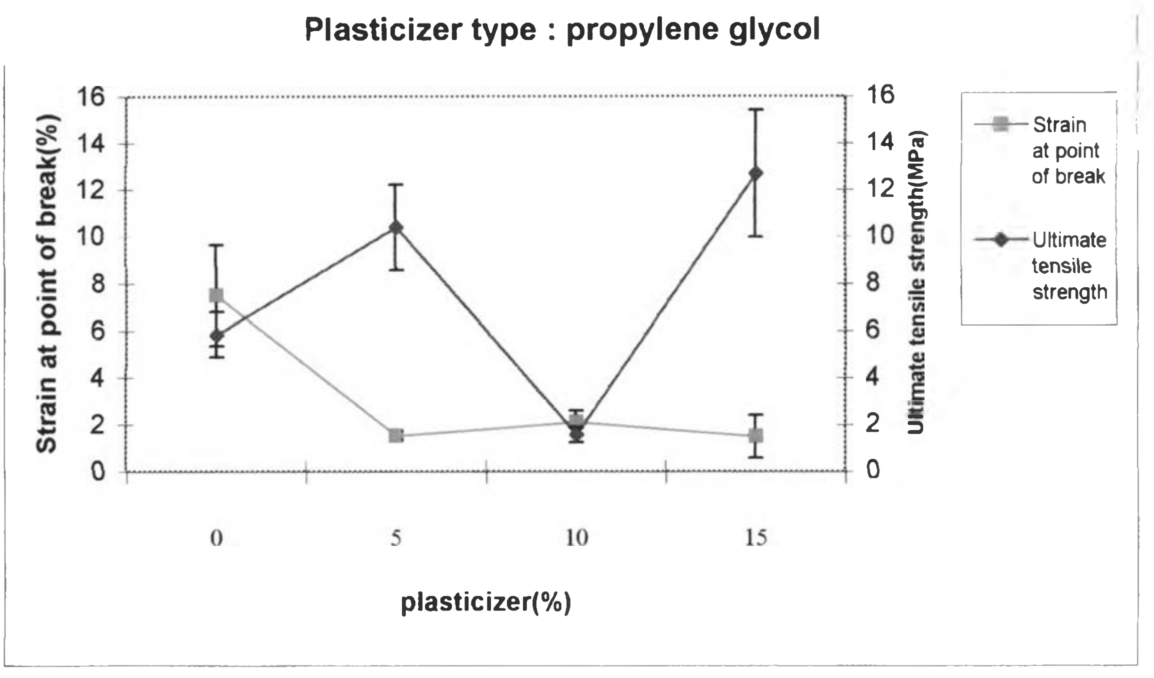


Figure 42 Effect of propylene glycol concentrations on strain and strength profiles of PG films

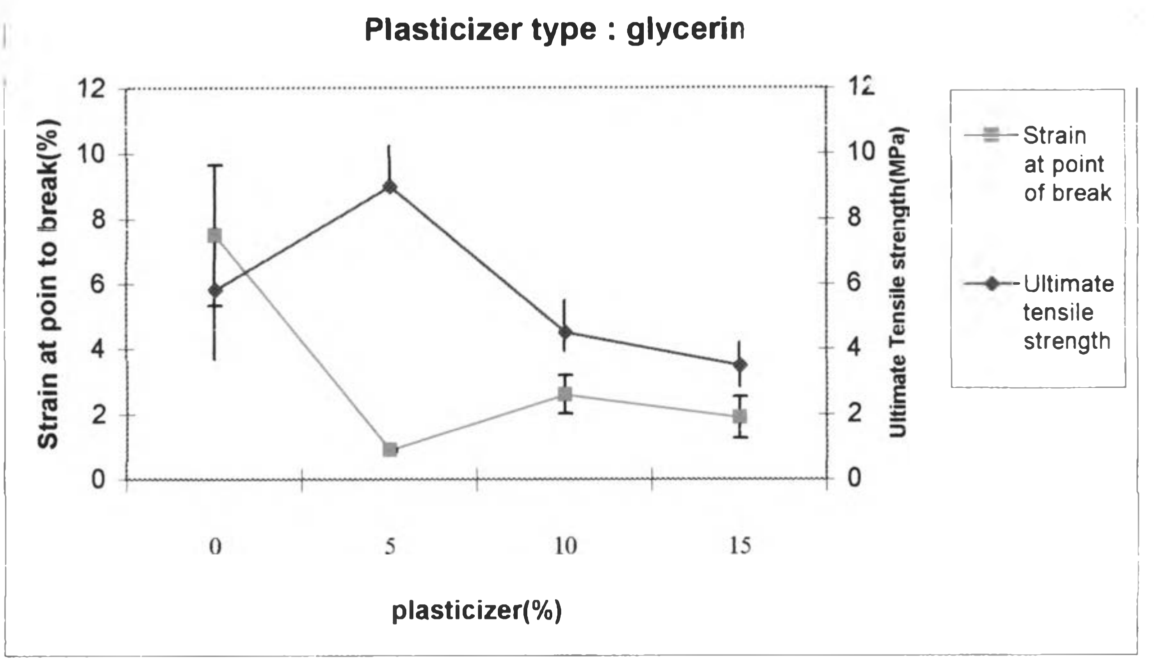


Figure 43 Effect of glycerin concentrations on strain and strength profiles of PG films

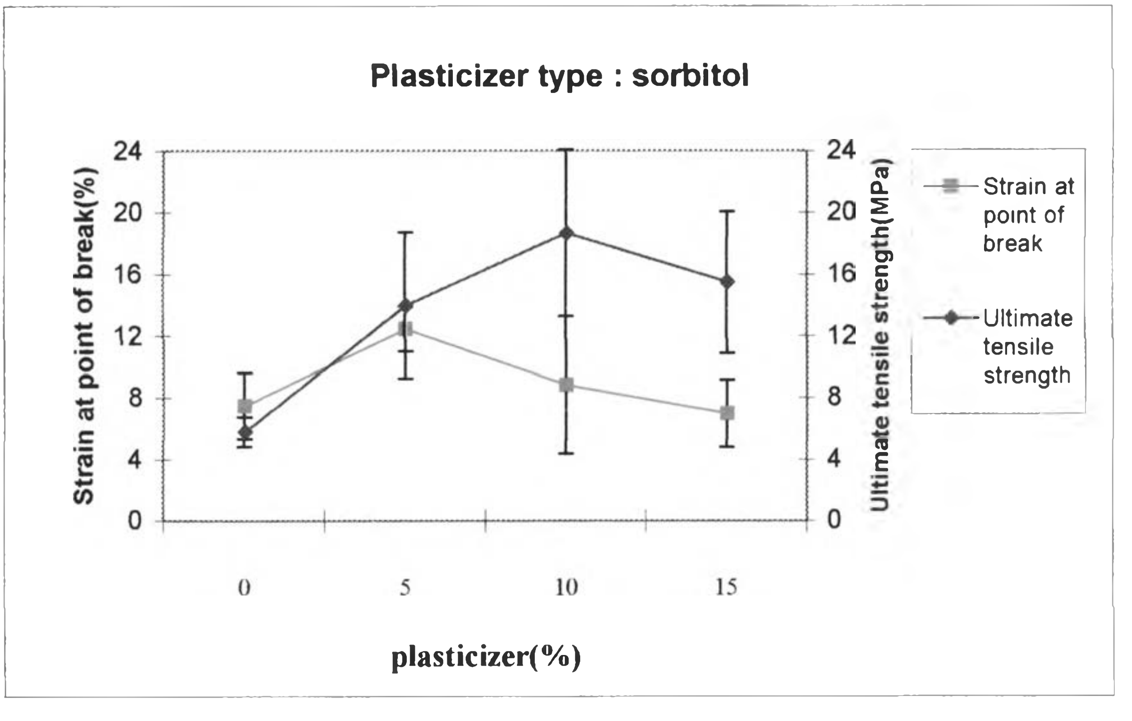


Figure 44 Effect of sorbitol concentrations on strain and strength profiles of PG films

Table 7 Effect of HPMC on mechanical properties of PG refreshing film (PG content 1.24 mg/strip)

PG film with HPMC content (%w/v) based on PG weight (2x2.5cm./strip)	Thickness ± SD (μm)	%Strain at point of break ± SD (%)	Young's modulus ± SD (MPa)	Ultimate tensile strength ± SD (MPa)	Solvation Time± SD (s)
0	54.80±1.58	10.21±1.44	162.98±41.02	7.53±1.88	88.33±6.50
6.5	56.40±1.06	14.23±2.57	244.20±31.57	15.12±1.50	65.67±8.73
13.0	54.53±1.10	14.41±3.02	191.81±7.00	18.82±2.57	70.33±5.68
19.5	53.60±1.44	15.75±1.40	103.92±13.85	5.91±1.42	73.67±4.04
26.0	52.67±0.90	13.44±1.79	141.57±34.76	8.65±2.32	65.33±2.51
33.0	51.40±2.64	14.77±2.82	254.94±31.93	2.18±0.76	71.67±2.88

10. Effect of HPMC on Mechanical Properties of PG Mouth Refreshing Film.

The objective of this research is to develop the film that can rapidly dissolve in mouth, from PG forming. Normally, PG film can slowly dissolve whereas the mouth-film development is necessary to dissolve the film quickly when it is in the mouth. In order to increase the dissolvability of PG film, we can add an eatable polymer, which has been used in the pharmaceutics for a long time. We select HPMC and added into PG solution before the process of film forming will be happened. From the Table 7, we found that by adding of HPMC at appropriate concentration into PG film could change the mechanical property and the dissolvability of PG film. Therefore, PG film solution with HPMC will take shorter time to dissolve than PG film without HPMC. While the values of %strain at point of break, Young's modulus, tensile strength increase, hardness and toughness were increased of lower concentration (6.5 and 13.0%) of HPMC, while at higher concentration (19.5-33.0%) HPMC result in reduction of tensile strength, hardness and brittleness of the film product. From this experiment, we can select the suitable concentration of HPMC for the PG solution at HPMC content of 19.5 %w/v based on PG weight. This condition changes values are: %strain at point of break is high; toughness and tensile strength are low, Young's modulus is less than free film. Therefore, the softness and strictness of the film will be enough to separate from the plate and can dissolve faster.

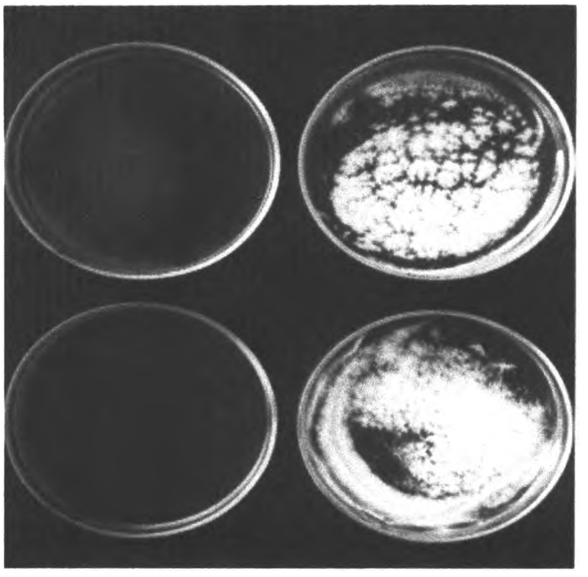


Figure 45 Picture of dried films on the plate of petri dish., mouth refreshing film using mixture of polymer. (Left) HPMC+PG (Right) HPMC.

Figure 46 The picture should immiscible oil drop in the film of mouth refreshing film formula using HPMC polymer.

11. Assessment of Mouth Refreshing Film of PG

The forming of mixture film, PG and HPMC can be formulated with adding the substances on testing and smelling in order to freshen. These added substances are menthol and peppermint oil. They are non-solution substances. However, the most components of mouth refreshing film are water. From Figure 45 and Figure 46, we found that the formulation that has the mixtures of PG and HPMC, when prepare the film. The obtained film has stick well to each other, with no separation in the part of oil phase. It can be separated to be a beautiful film. For the formulation with only HPMC as a film forming agent, we found that the forming of the film in the part of oil phase will be white hazy on the film and there are small drops on the film as well. Therefore, the film could not be separated from the plate. After finishing the experiment, we can conclude that PG may include the water phase and oil phase without the separation.

12. Moisture Sorption Study

The effect of humidity on PG mouth refreshing film at 75% relative humidity and different time from the Figure 47 was found that % moisture sorption of PG mouth refreshing film at the variety quantities of PG film with HPMC 0.08, 0.245 mg/strip will have a little bit of % moisture sorption and the film with HPMC 0, 0.165, 0.33, 0.41 mg/strip is less than.

11. Film Swelling Study

Figure 48 shown the swelling in distilled water and phosphate buffer saline solution, we found that film with HPMC can swell in the distilled water and phosphate buffer saline solution very well. The percentage of swelling is as follows: 0.33, 0.165, 0.245 and 0.08, 0.41 mg/strip and free film.

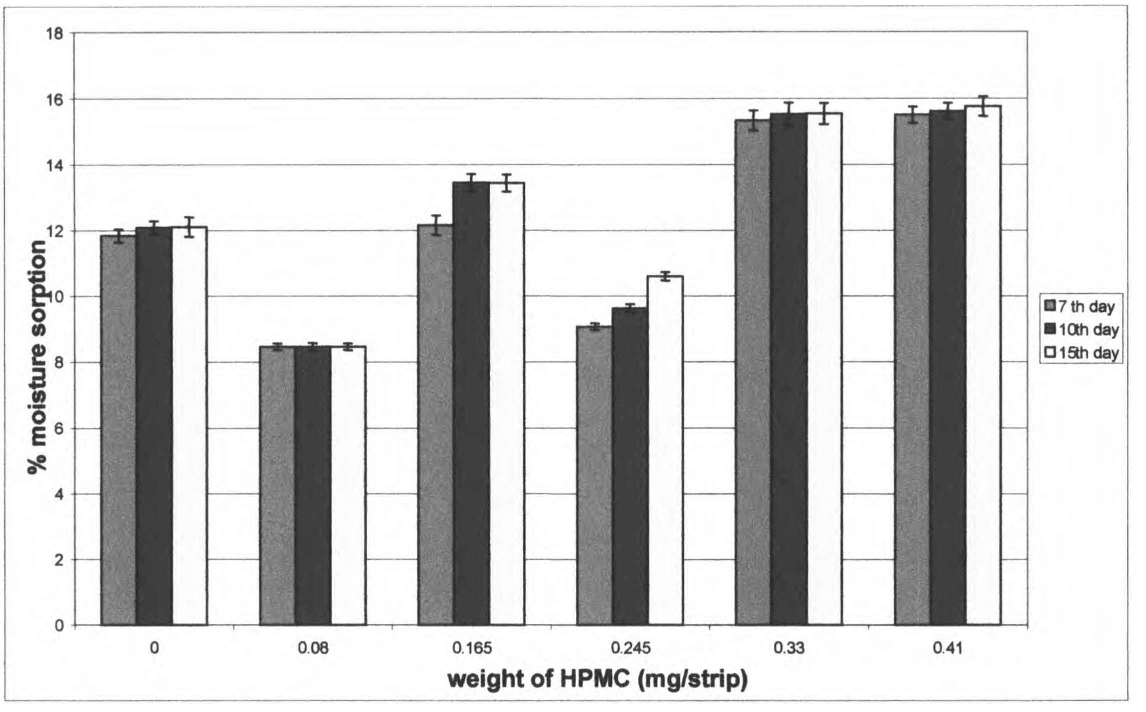


Figure 47 Effect of 75% relative humidity on moisture sorption of PG mouth refreshing film with different concentration of HPMC

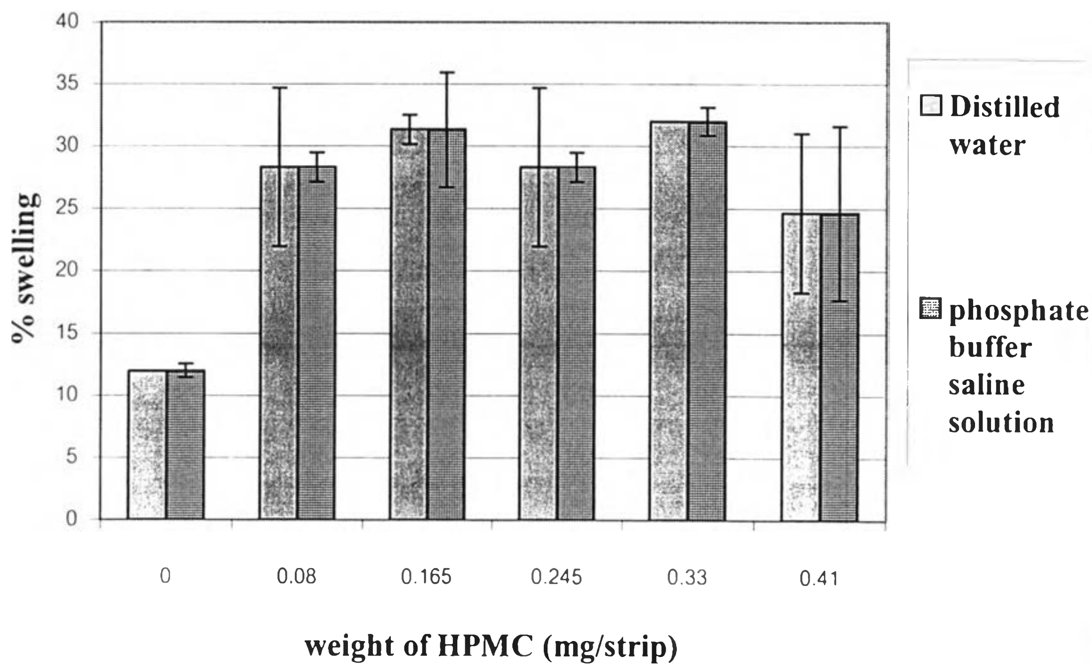


Figure 48 Effect of distilled water and phosphate buffer saline solution on swelling of PG mouth refreshing film. Each bar represents mean $\pm$ S.D.

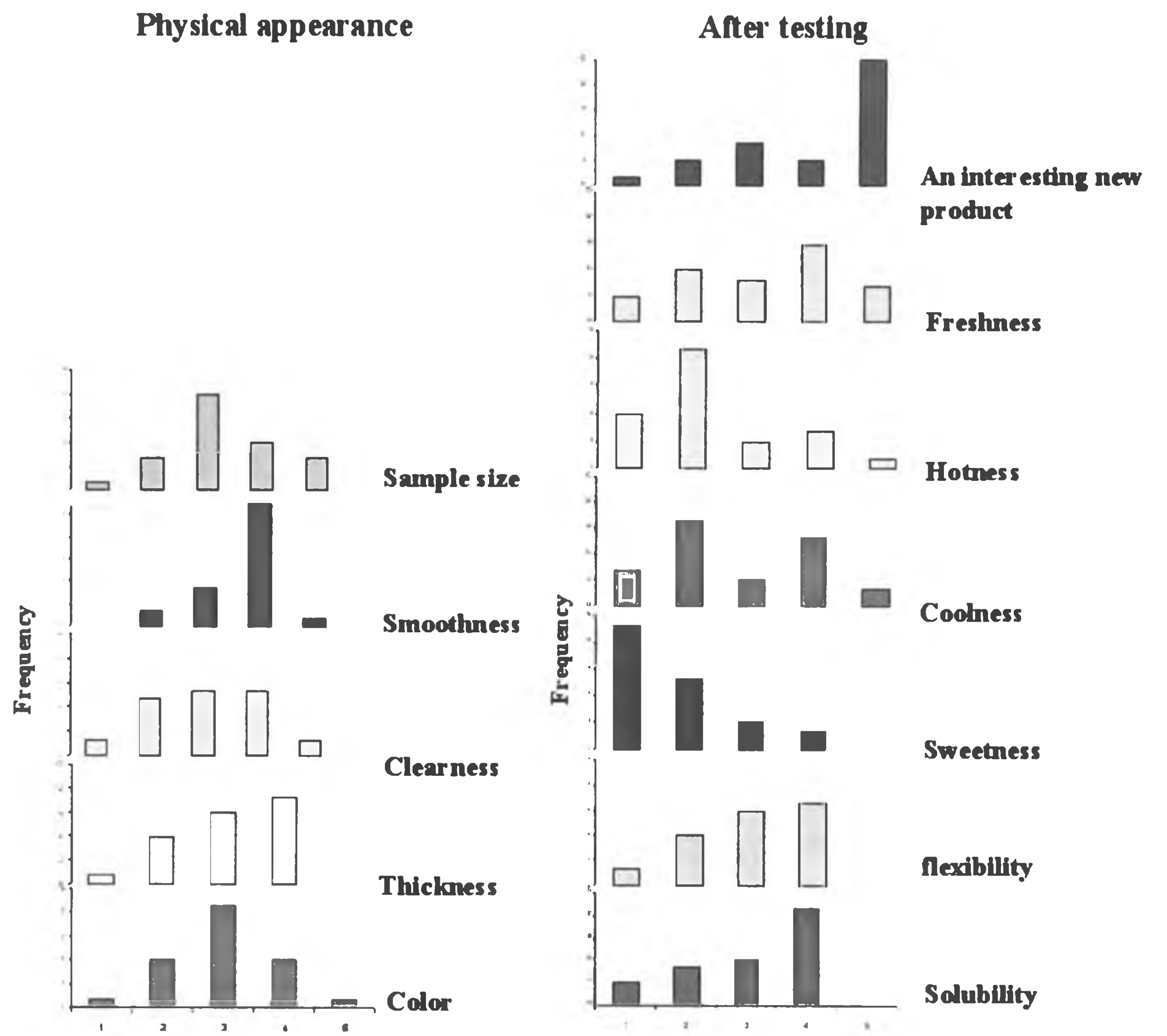


Figure 49 A summary of sensory analysis of PG mouth refreshing film by a group of volunteer, scores ranging from 1 = least satisfy, 2 = less satisfy, 3 = satisfy, 4 = more satisfy, 5 = most satisfy.

13. Sensory Analysis of PG Mouth Refreshing Film

The sensory analysis of PG mouth-refreshing film by volunteers revealed that most of them were satisfied with the product. The first and second ranks of satisfaction were its smoothness and solubility, repectively. After finishing the test, the volunteers were also satisfied with the physical appearance of PG mouth-refreshing film. Furthermore, they were interested in using PG due to its natural origin