CHAPTER III

RESULTS

1. Polysaccharide Gel (PG) From Dried Friut-Hulls of Durian

Polysaccharide gel (PG) of durian was isolated and purified from dried fruit-hulls by the modified method of Pongsamart and Panmaung (1998). The dried polysaccharide gel product was pulverized to a fine powder and passed through 60 mesh sieve, the pale brown powder was obtained. The PG gel dried powder is shown in Figure 10. The PG gel product was swollen and dissolved in water forming a viscous gel, PG at 2% by weight had pH 2.423±0.048 and the viscosity was 49.333± 1.097 cps.

2. Physical Properties of PG

2.1 pH

The pH of PG solution at 2% concentration was 2.423±0.048, addition of 0.1 M $NaHCO_3$ resulted in increasing pH of 2% PG solution. The result was illustrated in Figure 11 together with the effect of pH on viscosity profile. The result indicated that increasing concentration of $NaHCO_3$ as well as increasing pH of PG solution resulted in decreasing the viscosity of PG solution.

2.2 The Viscosity

PG solution at 2% concentration was prepared, 0.1 M of $NaHCO_3$ was added, the pH and the viscosity were measured. The results are shown in Figure 11 that the concentration of $NaHCO_3$ increased resulted in increasing pH and decreasing in viscosity of PG solution. Increasing pH of PG solution at range between 2.5-3 effected rapidly decreasing viscosity, at pH 4-7 showed less effect on decreasing viscosity of PG solution.

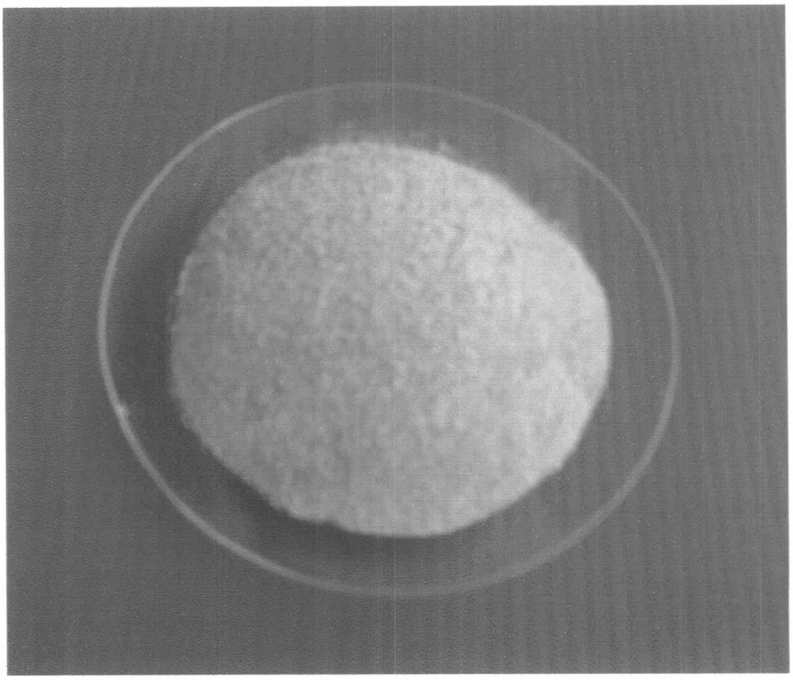

Figure 10 Polysaccharide gel (PG) powder product from dried fruit-hulls of durian

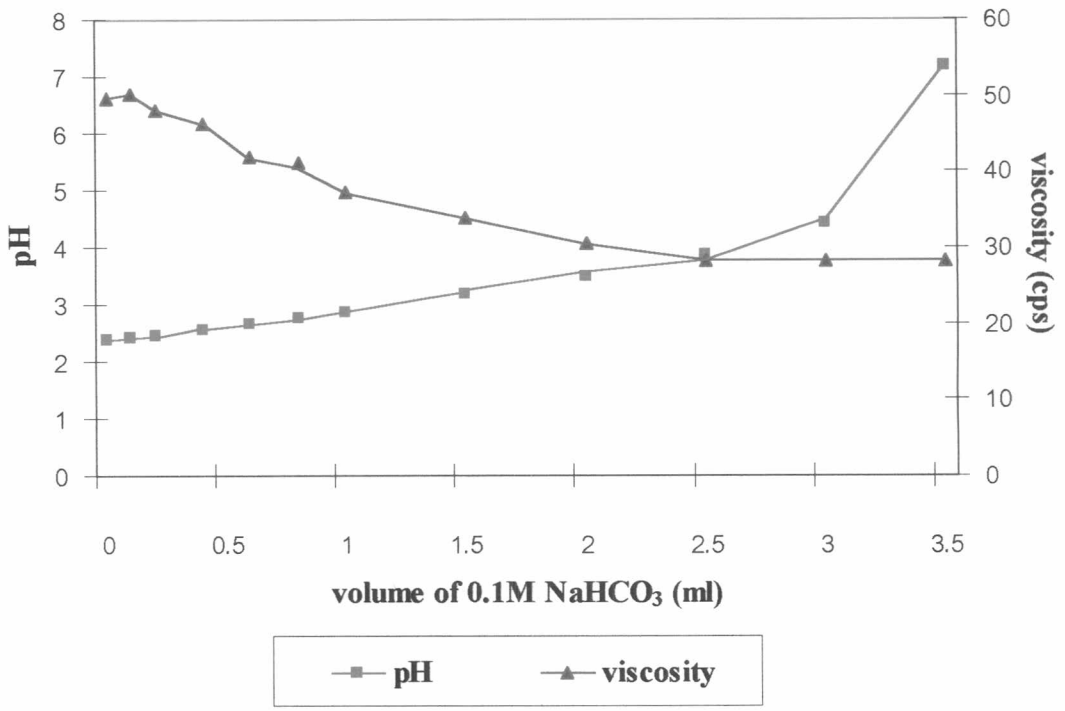

Figure 11 The effect of pH solution on the viscosity of 2% w/v PG in water

3. In Vitro Evaluation of PG Films

Polysaccharide gel of durian fruit-hull has property of film formation according to the study of Gerddit (2002). Sorbitol, PEG 400, PEG 6000 and HPMC were used as plasticizer in this study.

3.1 Physical Characteristics of PG Films

The characteristics of good film preparation should be thin, flexible and not brittle. PG films prepared by casting/solvent evaporation method were pale brown in color, transparent, flexible and easy to peel off from the glass petridish. The film products of PG prepared without plasticizer and the film prepared from PG with HPMC, HPG formula, were very brittle and hard to peel off. The film products prepared with addition of plasticizer in formulas such as sorbitol (SPG), PEG 400 (P4PG), PEG 6000 (P6PG) and sorbitol in 3 layer film (3S30PG) were more flexible and easier to peel off than that of PG film. Products of SPG film at higher concentration of sorbitol gave more flexible film than that of the lower concentration of plasticizer in the PG film formula. However, S40PG and S50PG film products were rather sticky which was due to the humectant property of sorbitol. The formula contained 30% (w/w) sorbitol in S30PG produced a satisfactory film product. Products of 1 layer film was a transparent film except in the formula having HPMC with PG (HPG), while 3 layers film products were a translucent film which was due to the films were cast on ground glass. The products of PG film with drugs in 1TPG, 1MPG, TPG and MPG films were not different in their physical characteristics from their film base. The physical appearances of PG film products are demonstrated in Figure 12-13 and Table 2-4.

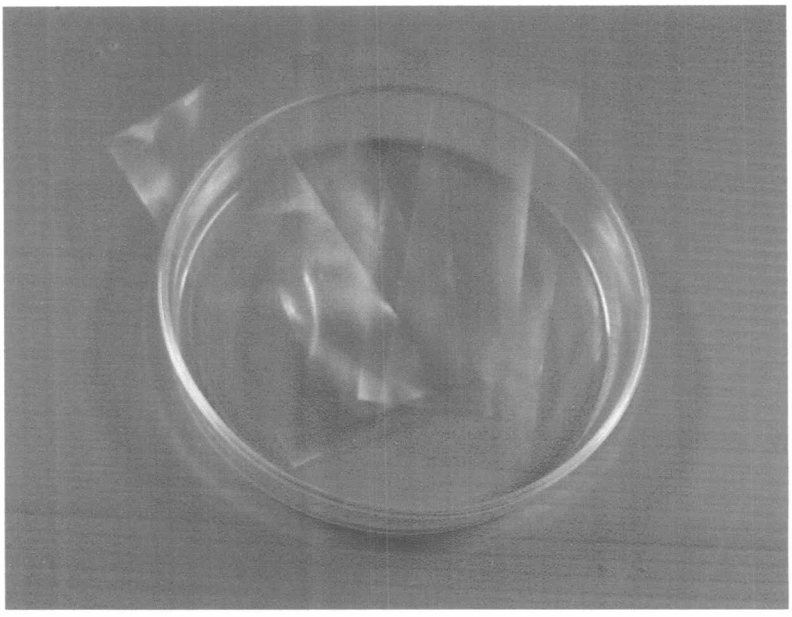

Figure 12 Polysaccharide gel (PG) film prepared by a casting/solvent evaporating technique

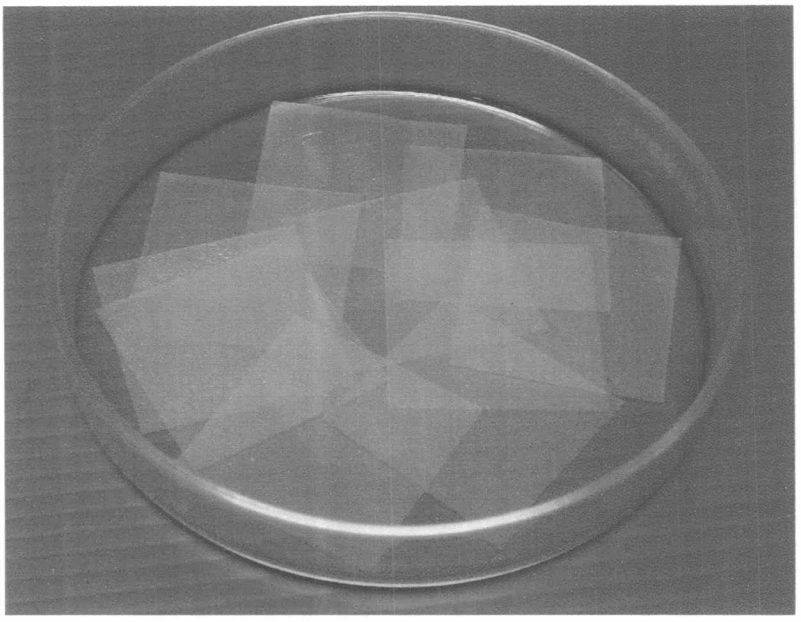

Figure 13 The 3 layer film product of polysaccharide gel (PG)

Table 2 Physical characteristics of the film preparation of PG film and PG film base using different types and %concentration of plasticizer based on PG

Formulas	Active ingredients (%)	Color	Transparency	Flexibility	Ease of peeling
PG	PG 100	Pale brown	TP	-	-
S10PG	PG 100 Sorbitol 10	Pale brown	TP	+	+
S20PG	PG 100 Sorbitol 20	Pale brown	TP	++	++
S30PG	PG 100 Sorbitol 30	Pale brown	TP	++	++
S40PG	PG 100 Sorbitol 40	Pale brown	TP	+++	++
S50PG	PG 100 Sorbitol 50	Pale brown	TP	+++	++
P4/30PG	PG 100 PEG400 30	Pale brown	TP	++	++
P6/1PG	PG 100 PEG6000 1	Pale brown	TP	+	+
P6/1.5PG	PG 100 PEG6000 1.5	Pale brown	TP	+	+
P6/2PG	PG 100 PEG6000 2	Pale brown	TP	++	++

TP = TRANSPARENT TL = TRANSLUCENT
The symbols of (+) and (-) showed the appearance and no appearance, respectively.
The number of the symbol of (+) showed a degree of the appearance.

Table 3 Physical characteristics of the film preparation of HPMC film and PG film with semisynthetic polymer

Formulas	Active ingredients (%)	Color	Transparency	Flexibility	Ease of peeling
HPMC	Water 100 HPMC 2	Clear	TP	++	++
H1PG	PG 100 HPMC 1	Pale brown	TP	-	-
H3PG	PG 100 HPMC 3	Pale brown	TP	-	-
H5PG	PG 100 HPMC 5	Pale brown	TL (+++)	-	-
H10PG	PG 100 HPMC 10	Pale brown	TL (++)	-	-
H20PG	PG 100 HPMC 20	Pale brown	TL (+)	-	-

TP = TRANSPARENT TL = TRANSLUCENT
The symbols of (+) and (-) showed the appearance and no appearance, respectively.
The number of the symbol of (+) showed a degree of the appearance.

Table 4 Physical characteristics of the film preparation of PG film with drug triamcinolone acetonide (T) and miconazole nitrate (M)

Formulas	Active ingredients % based on PG	Color	Transparency	Flexibility	Ease of peeling
S30PG Film base	PG 100 Sorbitol 30	Pale brown	TP	++	++
1TPG 1 layer film	PG 100 Sorbitol 30 TA 0.1	Pale brown	TP	++	++
1MPG 1 layer film	PG 100 Sorbitol 30 PEG6000 2 MN 2	Pale brown	TP	+++	++
3S30PG 3 layer film	TOP: S30PG MIDDLE: 1% PG BOTTOM: 1% HPMC	Pale brown	TL (++)	++	+++
TPG 3 layer film	TOP: 1TPG MIDDLE: 1% PG BOTTOM: 1% HPMC	Pale brown	TL (++)	++	+++
MPG 3 layer film	TOP: 1MPG MIDDLE: 1% PG BOTTOM: 1% HPMC	Pale brown	TL (++)	++	+++

TP = TRANSPARENT TL = TRANSLUCENT
TA = Triamcinolone acetonide MN = Miconazole nitrate
The symbols of (+) and (-) showed the appearance and no appearance, respectively.
The number of the symbol of (+) showed a degree of the appearance.

3.2 Film Thickness

The thickness of the films are indicated in Table 5. The results showed the mean values and SD. The films were prepared to produce thickness in the range of 0.03-0.08 mm. The thickness of PG film without plasticizer (PG film), PG film base (S30PG) and three layer film 3S30PG was 0.032±0.006 mm, 0.043±0.009 mm and 0.07±0.008 mm thick, respectively.

3.3 Mechanical Properties of PG Films

Mechanical properties including, % strain at break, stress at break, Young's modulus and toughness are presented in Table 5 and Figures 14-17. The results demonstrated that PG film with plasticizer showed lower Young's modulus and stress at break than that of PG film without plasticizer (Figures 14-15), the values of Young's modulus and low stress at break of S30PG film indicated that the film was more softer and flexible than that of PG film without plasticizer. The high percentage of sorbitol in formula S40PG and S50PG film showed a higher value at 6.85±1.66 and 10.26±1.27 % strain at break and 1.92±0.72 and 2.92±0.64 Mpa of toughness than that of PG film without plasticizer which was 2.64±0.74 % strain at break and 0.99±0.67 Mpa of toughness (Figures 16-17), however, the moisture was also high due to humactant property of sorbitol. The results indicated that the PG film base was higher in elongation, elasticity and toughness than that of PG film without plasticizer.

In comparison the mechanical properties between PG film base (S30PG) and the film preparation with drugs of formulas 1TPG and 1MPG film showed that 1TPG and 1MPG film produced higher values of toughness and % strain at break but lower in Young's modulus than the PG film base S30PG, the results indicated that 1TPG and 1MPG film were stronger and tougher than that of PG film base. The preparations of 3 layer film of triamcinolone acetonide (TPG) and miconazole nitrate (MPG) demonstrated higher values of toughness and stress at break than that of its film base 3S30PG. Mechanical properties were similar between TPG and MPG.

Table 5 Tensile properties of PG films; data are expressed as means and SD in parentheses; n=5

sample	Mechanical properties				
	Young's modulus (Mpa)	Stress at break (Mpa)	% Strain at break (Mpa)	Toughness (Mpa)	Thickness (mm)
PG	3837 (290)	75.78 (30.65)	2.64 (0.74)	0.99 (0.67)	0.032 (0.006)
S10PG	3373 (683)	53.77 (25.94)	2.03 (0.78)	0.60 (0.39)	0.035 (0.008)
S20PG	2830 (130)	51.60 (13.66)	2.40 (0.54)	0.61 (0.31)	0.041 (0.007)
S30PG	2814 (170)	43.48 (10.95)	2.02 (0.43)	0.41 (0.17)	0.043 (0.009)
S40PG	1622 (127)	37.63 (9.14)	6.85 (1.66)	1.92 (0.72)	0.048 (0.007)
S50PG	1432 (87)	43.15 (2.55)	10.26 (1.27)	2.92 (0.64)	0.053 (0.008)
P4/30PG	590 (112)	3.39 (0.57)	2.15 (0.53)	0.05 (0.02)	0.046 (0.009)
P6/1PG	1138 (198)	5.41 (0.71)	0.80 (0.42)	0.03 (0.02)	0.042 (0.006)
P6/1.5PG	1170 (87)	5.76 (1.04)	0.80 (0.33)	0.03 (0.02)	0.041 (0.006)
P6/2PG	1193 (55)	5.45 (0.59)	0.69 (0.13)	0.02 (0.01)	0.038 (0.005)
HPMC	2193 (80)	101.4 (7.72)	27.09 (1.33)	20.69 (2.08)	0.045 (0.008)
H1PG	1731 (76)	6.11 (1.05)	0.46 (0.08)	0.02 (0.01)	0.036 (0.007)
H3PG	1729 (203)	3.74 (1.18)	0.30 (0.10)	0.01 (0.003)	0.037 (0.006)
H5PG	1378 (137)	2.64 (0.41)	0.27 (0.05)	0.003 (0.001)	0.041 (0.004)
H20PG	1111 (322)	1.39 (0.83)	0.19 (0.06)	0.001 (0.001)	0.043 (0.006)
1TPG	815 (40)	22.52 (1.82)	7.32 (2.44)	0.93 (0.35)	0.063 (0.006)
1MPG	1762 (110)	53.24 (9.12)	5.25 (1.85)	1.75 (1.19)	0.055 (0.008)
3S30PG	1018 (177)	19.99 (5.24)	4.13 (1.47)	0.27 (0.10)	0.070 (0.008)
TPG	1158 (74)	28.84 (3.64)	4.20 (0.53)	0.57 (0.19)	0.076 (0.011)
MPG	1280 (138)	29.58 (3.14)	3.35 (0.56)	0.53 (0.13)	0.084 (0.009)

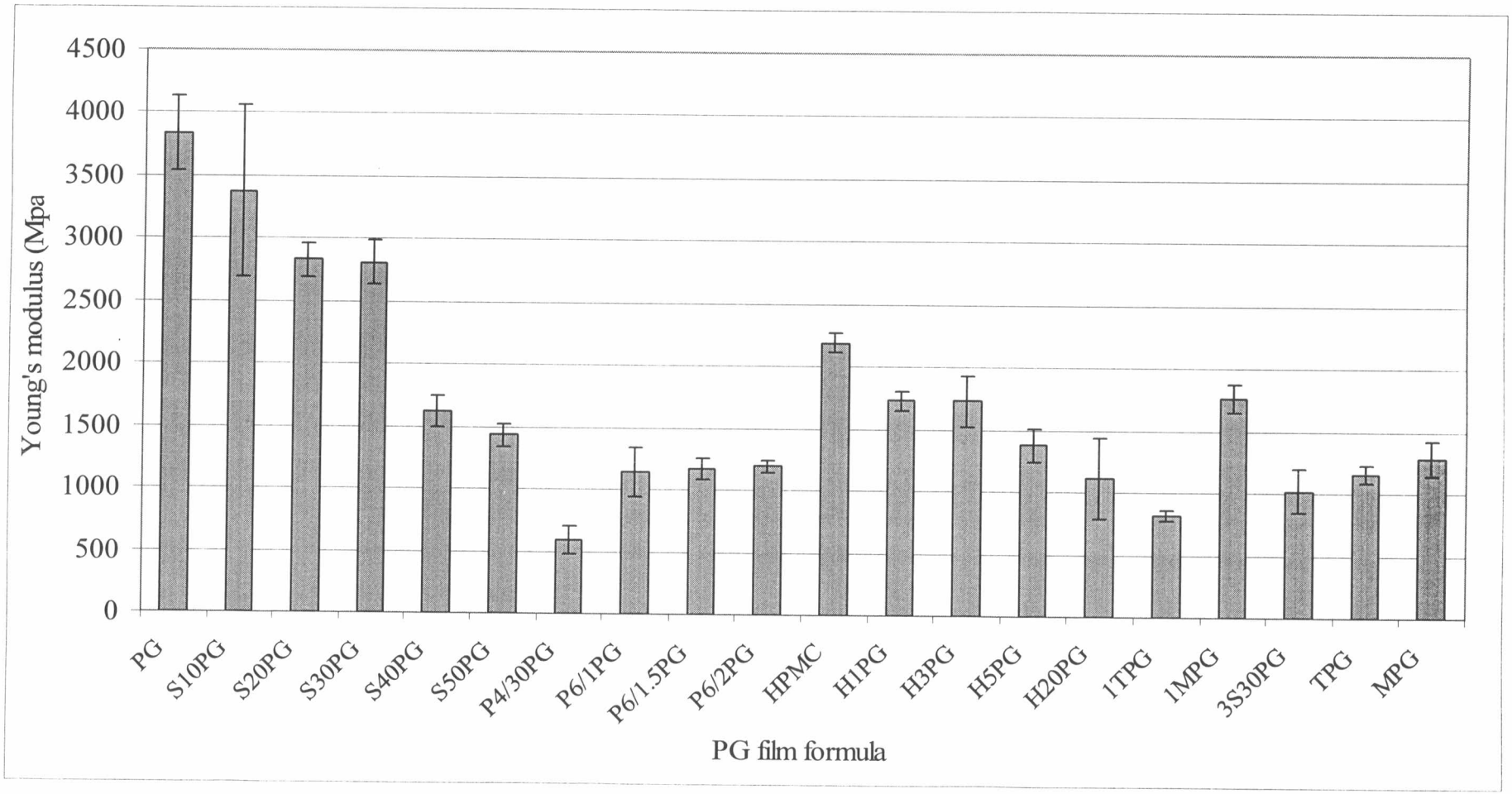

Figure 14 Young's modulus of PG films in each preparation formula. Each bar represents means ± SD

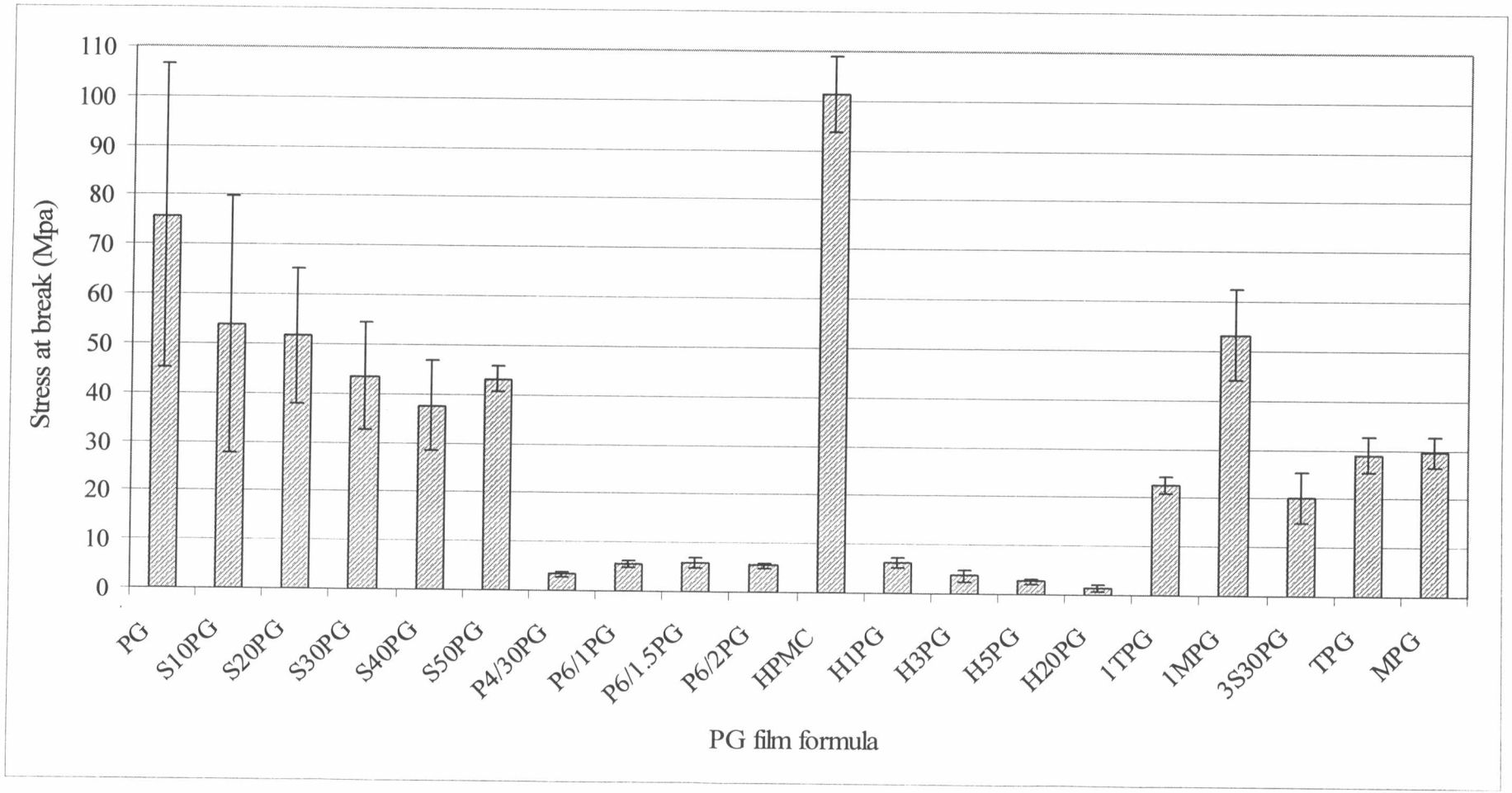

Figure 15 Stress at break of PG films in each preparation formula. Each bar represents means ± SD

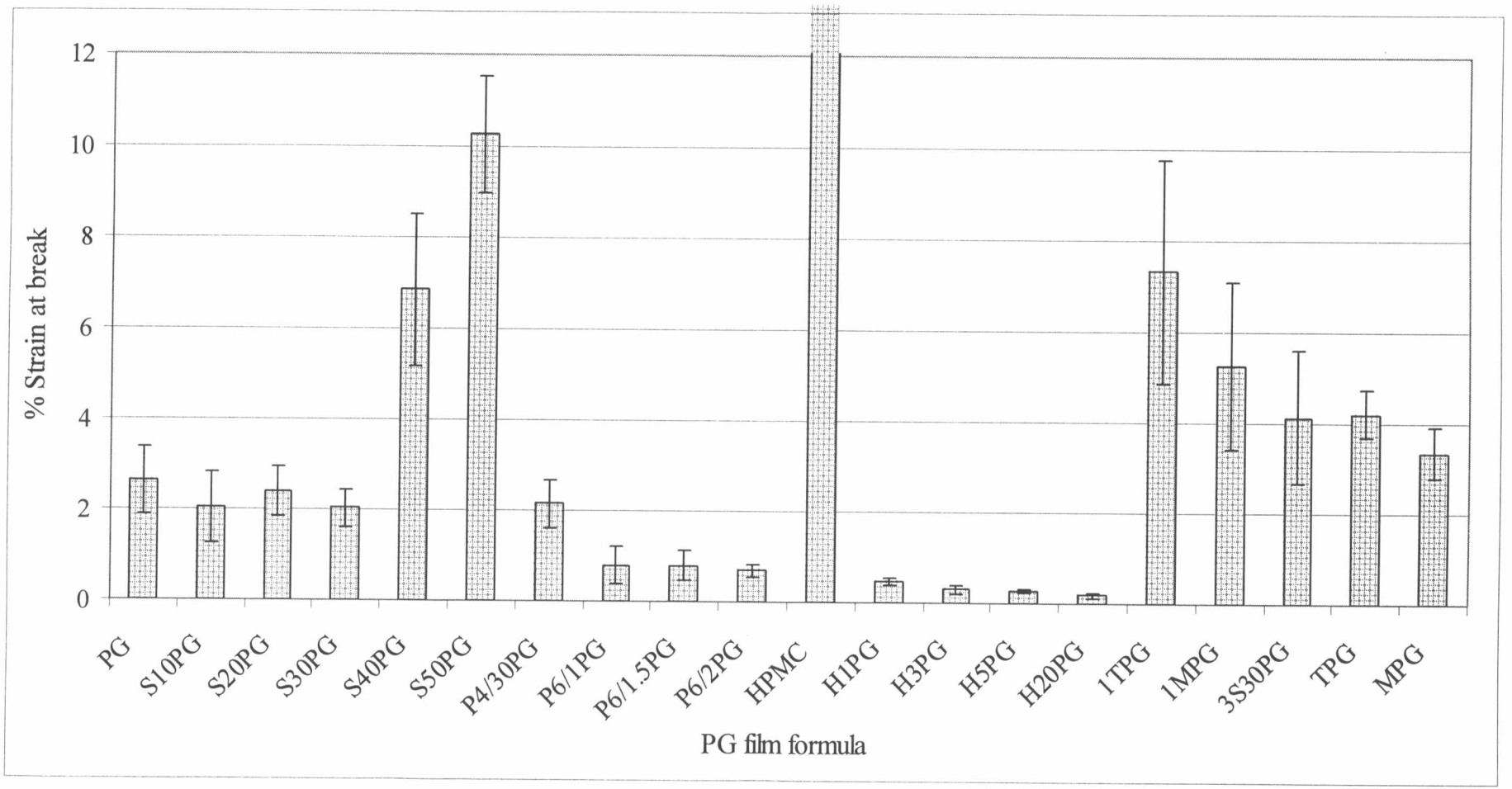

Figure 16 Percent strain at break of PG films in each preparation formula. Each bar represents means ± SD

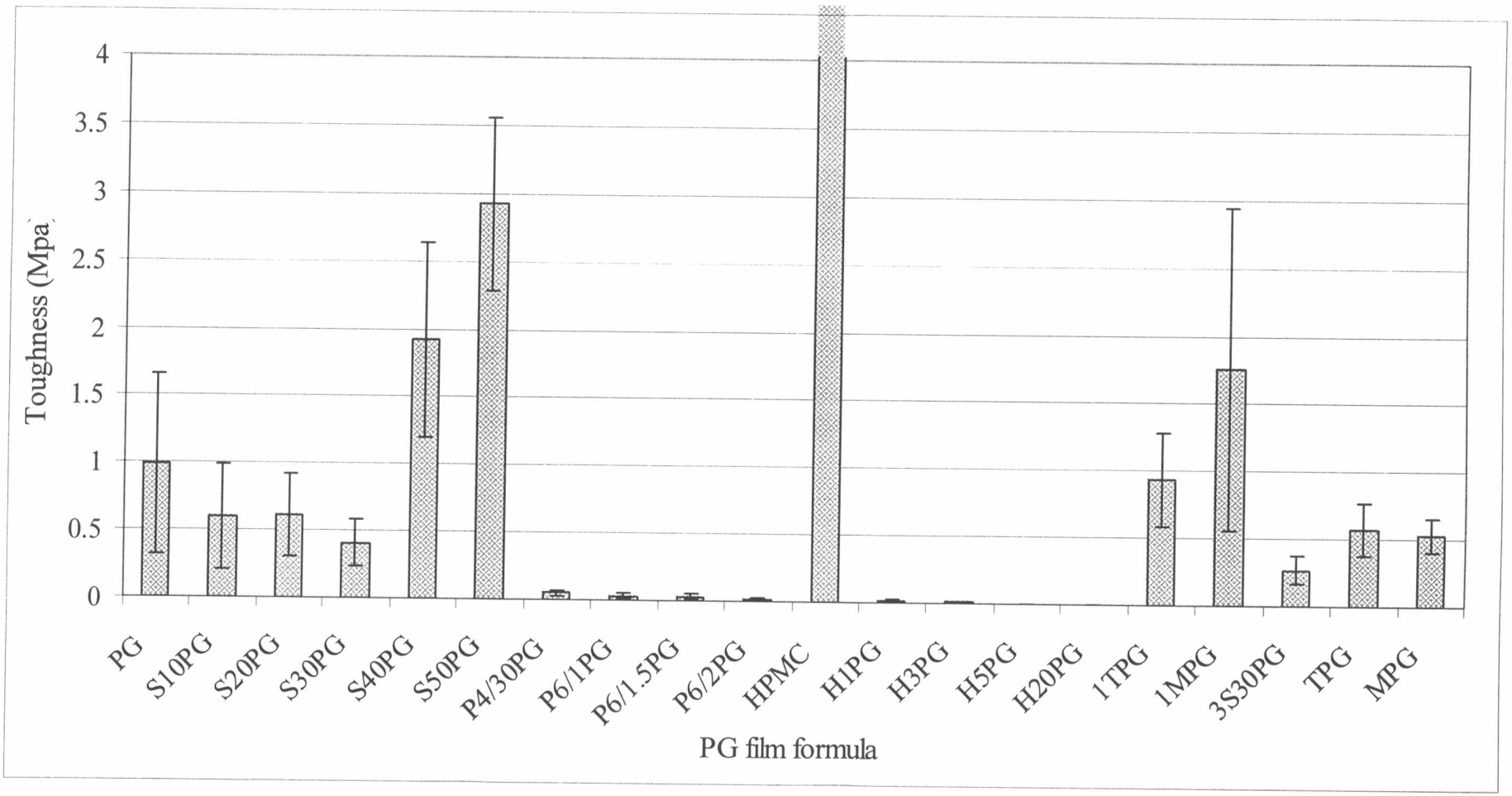

Figure 17 Toughness of PG films in each preparation formula. Each bar represents means ± SD

3.4 Infrared Spectra Patterns

Infrared spectra of PG film showed peaks at 1019 cm^{-1} (C-OH), 1640 cm^{-1} (COO^-) and 1749 cm^{-1} (C=O). The C=O stretching bands of carboxylic acid absorb near 1749 cm^{-1}. One of the characteristic bands in the spectra of carboxylic acid results from the out-of-plane bending of the bonded C-OH. The band appears near 1019 cm^{-1} and is characteristically broad with medium intensity. The OH bending absorption in the region of 3550-3200 cm^{-1}. Aliphatic aldehyde show aldehydic C-H stretching absorption in the 2830-2695 cm^{-1}. The spectrum are illustrated in Figure 18 (A) and Figure 19 (A).

The IR spectra of triamcinolone acetonide powder in Figure 18 (C) showed the peak at 1659 cm^{-1} indicating C=C cyclic stretching overlapped with the C=O stretching (ketone band) at 1708 cm^{-1}. The peak at 2924 cm^{-1} was C-H stretching band and the peak at 3550-3200 cm^{-1} was OH bending.

The IR spectra of miconazole nitrate powder in Figure 19 (C) showed the peak at 1659 cm^{-1} indicating C=C cyclic stretching of the drug. The peak at 2923 cm^{-1} was C-H stretching band and the peak at 1088 cm^{-1} was C-N stretching band. The peak at 3500-3300 cm^{-1} was N-H stretching.

The IR spectra of PG film base, 1TPG film and triamcinolone acetonide are illustrated in Figure 18. The spectrum of 1TPG film did not show peak of new product different from that of triamcinolone acetonide powder or PG film base.

The IR spectra of PG film base, 1MPG film and miconazole nitrate are illustrated in Figure 19.

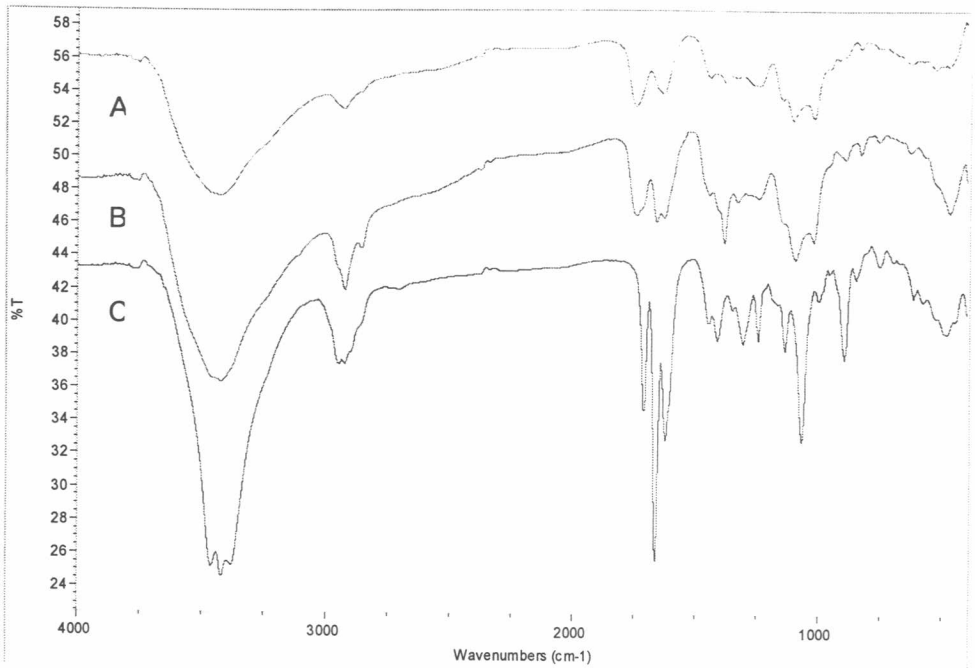

Figure 18 IR spectra of (A) PG film base ; (B) 1TPG film ; (C) Triamcinolone acetonide

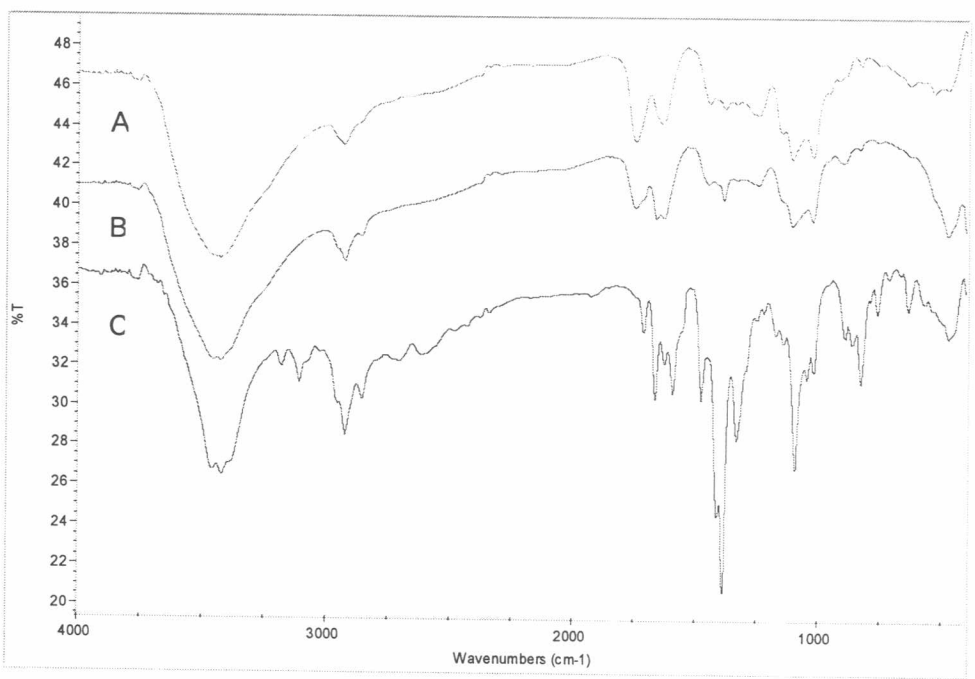

Figure 19 IR spectra of (A) PG film base ; (B) 1MPG film ; (C) Miconazole nitrate

3.5 Moisture Sorption

The moisture sorption of the films is shown in Figure 20. The moisture sorption of PG film base (S30PG) and 3S30PG film showed greater moisture sorption than those of HPMC films. At 75% relative humidity, the moisture sorption of S30PG, 3S30PG and HPMC were at equilibrium within 7 days after moisture exposure. PG films showed no significant difference in moisture sorption when compared with either 1 layer or 3 layers film preparation. Percent moisture sorption of S30PG and 3S30PG film was 19.47 and 18.58 percent, respectively.

3.6 In vitro Drug Release Profile of Triamcinolone PG Films

The penetration-time profile of Triamcinolone-PG film (1TPG) and PG film base (S30PG) through cellulose acetate membrane is illustrated in Figure 21. The PG film base was used as control. Triamcinolone acetonide rapidly penetrated through cellulose acetate membrane into isotonic phosphate buffer (PBS) [137 mM NaCl and 8 mM phosphate buffer at pH 7.4]. Figure 21 showed that the absorbance of Triamcinolone acetonide was interfered with that of PG base. Therefore, the analytical conditions must be further developed. The drug penetration data in this study are presented in Appendix B.

3.7 In vitro Drug Release Profile of Miconazole PG Films

Miconazole nitrate through cellulose acetate membrane were observed. The drug penetration data in this study are presented in Appendix B. The penetration-time profile of Miconazole-PG film (1MPG) and its film base through cellulose acetate membrane is shown in Figure 22. The results showed that the absorbance of miconazole nitrate was interfered with that of PG base. Therefore, the analytical conditions must be further developed.

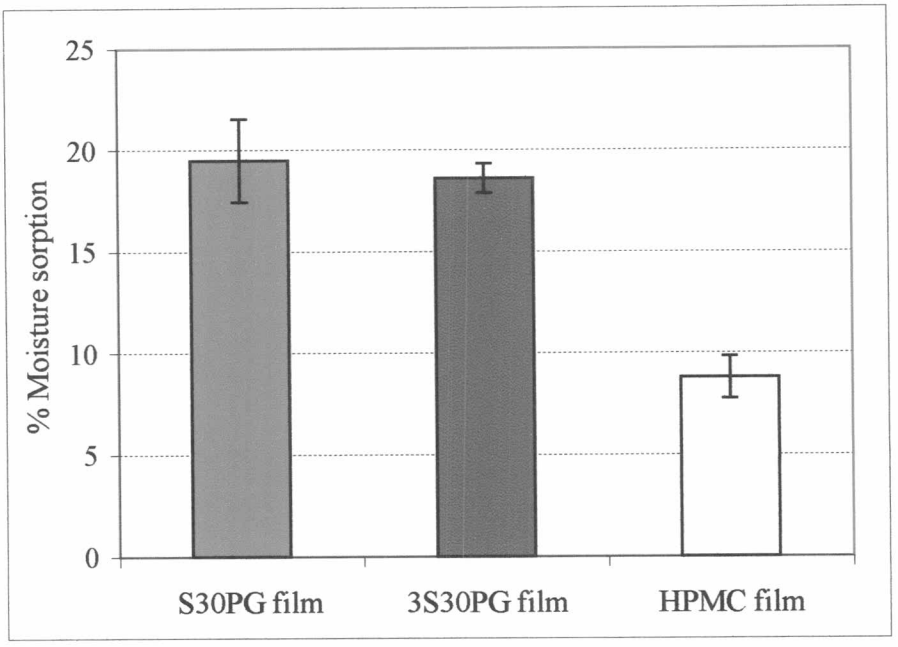

Figure 20 The moisture sorption of film preparations of S30PG and 3S30PG film comparison with HPMC film

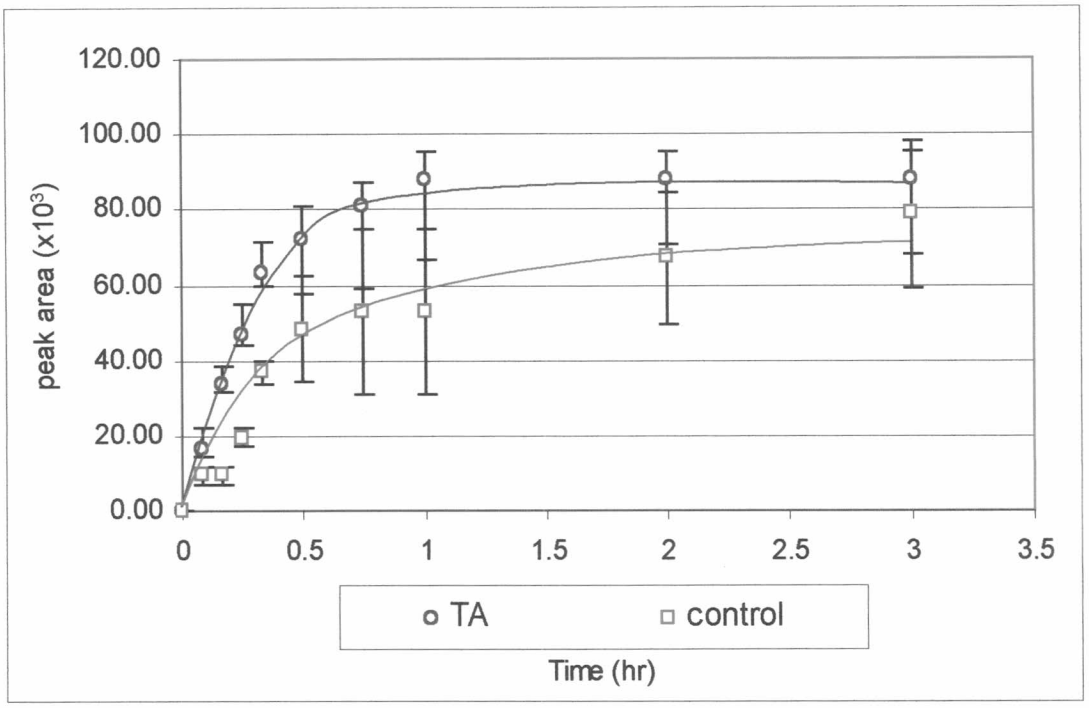

Figure 21 Permeation profiles of Triamcinolone acetonide (TA) released through a cellulose acetate membrane of Triamcinolone-PG film (○), compared to PG film base as a control (□), n=3.

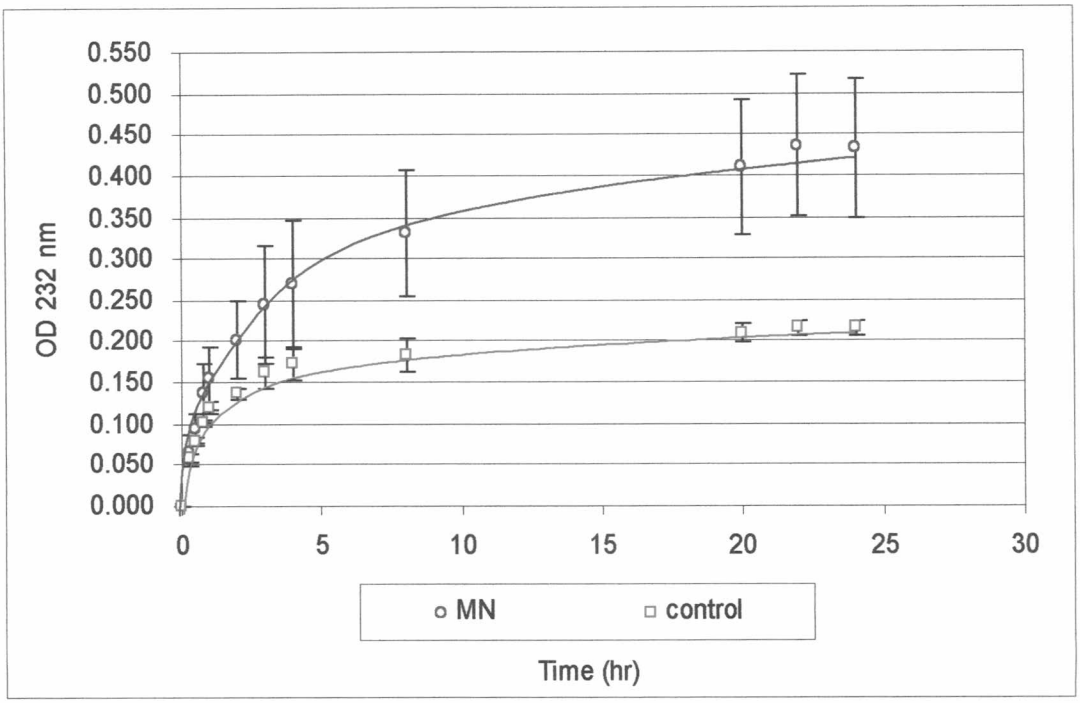

Figure 22 Permeation profiles of Miconazole nitrate (MN) released through a cellulose acetate membrane of Miconazole-PG film (○), compared to PG film base as a control (□), n=3.

3.8 Sensory Analysis of PG Film Base

The results of sensory analysis of PG film base are shown in Figure 23. The instrument used in this study was a questionnaire consisted of listed 7 items : taste, ease of application, adhesiveness, non-irritation, no-residue, non-annoyance, product appearance satisfaction and overall satisfaction after use. 3 layers of PG film base (3S30PG) was improved for this test because of PG film has good adhesive property, then 1 layer film will adhere to oral mucosa with both side of film. HPMC E15 was used as a backing layer for eliminate that problem. The opinions were analyzed by interpreting the percentage of frequency from the scores range from 1-4, 1 = poor, 2 = fair, 3 = good, 4 = excellent. Sum of each scores on each subject in 32 volunteers were evaluated. Total marks on each subject was calculated by multiply with their scores and comparable to 100%.

The values of ease of application, adhesiveness and no-residue showed the highest percent of excellent that means PG film was easy to use, good attachment to oral mucosa and less residue. Furthermore, taste, non-irritation, product appearance satisfaction and overall satisfaction after use showed the highest percent of good that means the film has good satisfaction and less irritation. The only one that the highest percent showed fair was non-annoyance, it means that PG film has less annoyance.

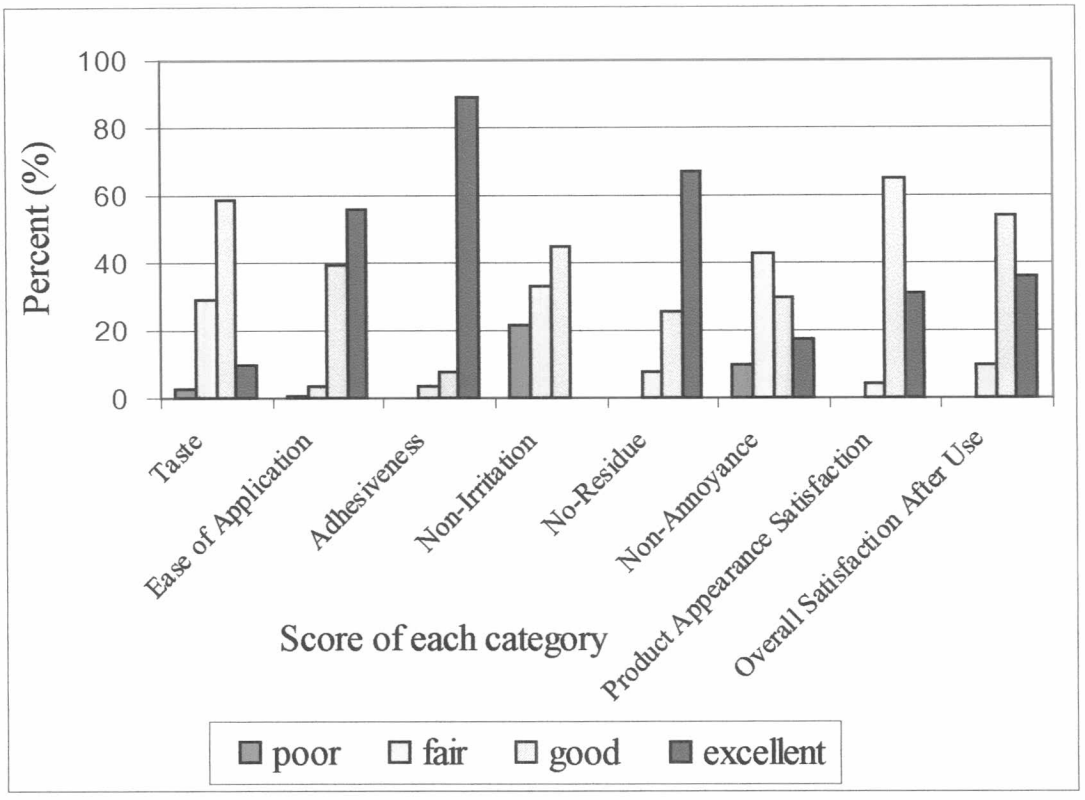

Figure 23 Summary of sensory analysis for the acceptance of PG film base product due to questionnaire in a group of 32 volunteers